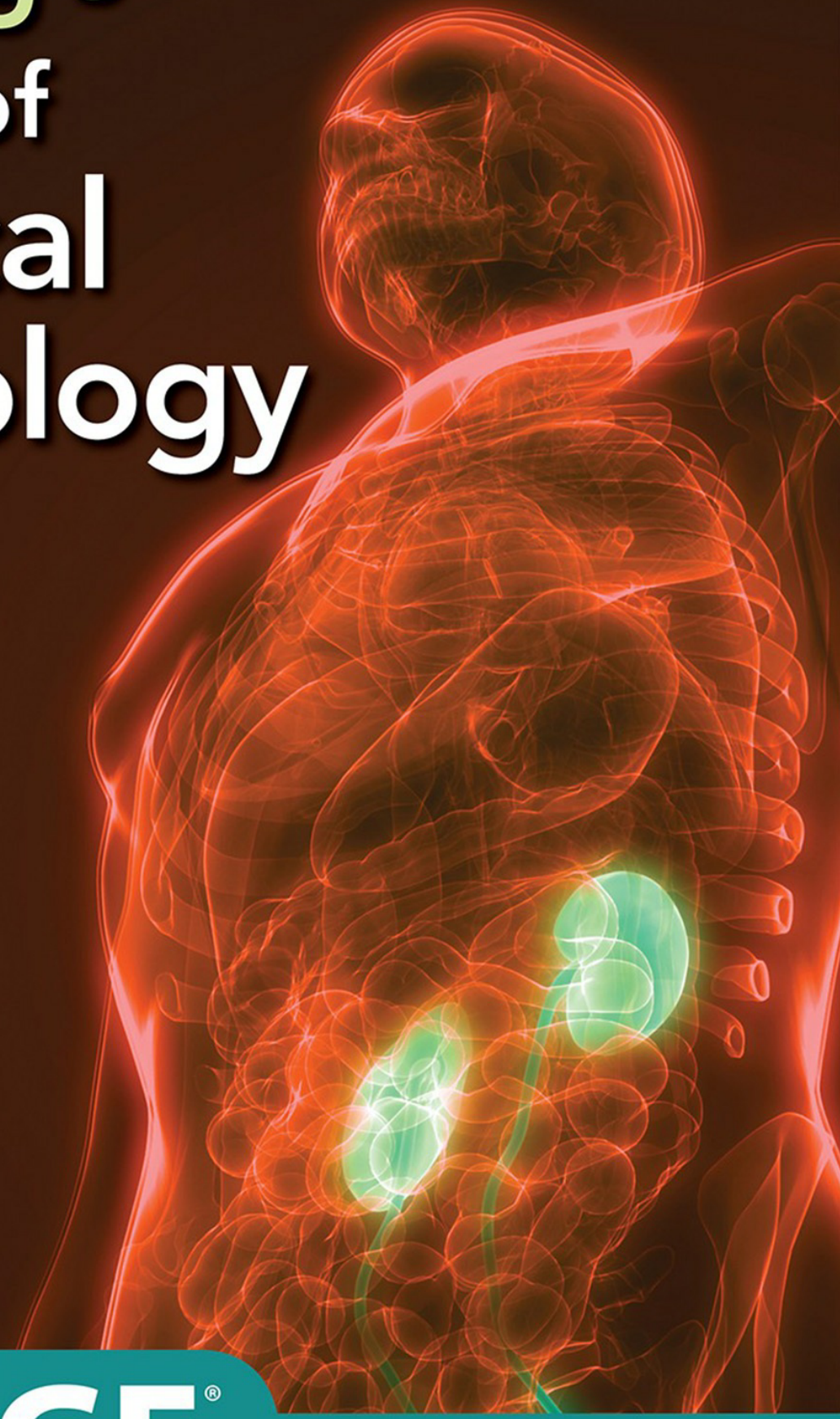
Ganong's Review of Medical Physiology

26th Edition



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Ganong's Review of Medical Physiology

TWENTY-SIXTH EDITION

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Dedication to

William Francis Ganong

William Francis ("Fran") Ganong was an outstanding scientist, educator, and writer. He was completely dedicated to the field of physiology and medical education in general. Chairman of the Department of Physiology at the University of California, San Francisco, for many years, he received numerous teaching awards and loved working with medical students.

Over the course of 40 years and some 22 editions, he was the sole author of the best selling *Review of Medical Physiology*, and a co-author of 5 editions of *Pathophysiology of Disease: An Introduction to Clinical Medicine*. He was one of the "deans" of the Lange group of authors who produced concise medical text and review books that to this day remain extraordinarily popular in print and now in digital formats. Dr. Ganong made a gigantic impact on the education of countless medical students and clinicians.

A general physiologist par excellence and a neuroendocrine physiologist by subspecialty, Fran developed and maintained a rare understanding of the entire field of physiology. This allowed him to write each new edition (every 2 years!) of the *Review of Medical Physiology* as a sole author, a feat remarked on and admired whenever the book came up for discussion among physiologists. He was an excellent writer and far ahead of his time with his objective of distilling a complex subject into a concise presentation. Like his good friend, Dr. Jack Lange, founder of the Lange series of books, Fran took great pride in the many different translations of the *Review of Medical Physiology* and was always delighted to receive a copy of the new edition in any language.

He was a model author, organized, dedicated, and enthusiastic. His book was his pride and joy and like other best-selling authors, he would work on the next edition seemingly every day, updating references, rewriting as needed, and always ready and on time when the next edition was due to the publisher. He did the same with his other book, *Pathophysiology of Disease: An Introduction to Clinical Medicine*, a book that he worked on meticulously in the years following his formal retirement and appointment as an emeritus professor at UCSF.

Fran Ganong will always have a seat at the head table of the greats of the art of medical science education and communication. He died on December 23, 2007. All of us who knew him and worked with him miss him greatly.

About the Authors

KIM E. BARRETT

Kim Barrett received her PhD in biological chemistry from University College London in 1982. Following postdoctoral training at the National Institutes of Health, she joined the faculty at the University of California, San Diego, School of Medicine in 1985, rising to the rank of Professor of Medicine in 1996, and was named Distinguished Professor of Medicine in 2015. From 2006 to 2016, she also served the University as Dean of the Graduate Division. Her research interests focus on the physiology and pathophysiology of the intestinal epithelium, and how its function is altered by commensal, probiotic, and pathogenic bacteria as well as in specific disease states, such as inflammatory bowel diseases. She has published more than 250 articles, chapters, and reviews, and has received several honors for her research accomplishments including the Bowditch and Davenport Lectureships from the American Physiological Society (APS), the Bayliss-Starling Lectureship from The Physiological Society of the UK and Ireland, and the degree of Doctor of Medical Sciences, honoris causa, from Queens University, Belfast. She has been very active in scholarly editing, serving currently as the Editor-in-Chief of the *Journal of Physiology*. She is also a dedicated and award-winning instructor of medical, pharmacy, and graduate students, and has taught various topics in medical and systems physiology to

these groups for more than 30 years. Her efforts as a teacher and mentor were recognized with the Bodil M. Schmidt- Nielson Distinguished Mentor and Scientist Award from the APS in 2012, and she also served as the 86th APS President from 2013 to 2014. Her teaching experiences led her to author a prior volume (*Gastrointestinal Physiology*, McGraw-Hill, 2005; second edition published in 2014) and she was honored to have been invited to take over the helm of Ganong in 2007 for the 23rd and subsequent editions, including this one.

SUSAN M. BARMAN

Susan Barman received her PhD in physiology from Loyola University School of Medicine in Maywood, Illinois. Afterward she went to Michigan State University (MSU) where she is currently a Professor in the Department of Pharmacology/Toxicology and the Neuroscience Program. She is also Chair of the Institutional Animal Care and Use Committee and serves on the College of Human Medicine (CHM) Curriculum Development Group for medical school education. She has had a career-long interest in neural control of cardiorespiratory function with an emphasis on the characterization and origin of the naturally occurring discharges of sympathetic and phrenic nerves. She has published about 150 research articles, invited review articles, and book chapters. She was a recipient of a prestigious National Institutes of Health MERIT (Method to Extend Research in Time) Award. She is also a recipient of an MSU Outstanding University Woman Faculty Award, a CHM Distinguished Faculty Award, a Distinguished Service Award from the Association of Chairs of Departments of Physiology, and the Carl Ludwig Distinguished Lecture Award from the Neural Control of Autonomic Regulation section of the American Physiological Society (APS). She is also a Fellow of the APS and served as its 85th President. She has also served as a Councilor of APS and Chair of the Women in Physiology and Section Advisory Committees of the APS. She is also active in the Michigan Physiological Society, a chapter of the APS.

HEDDWEN L. BROOKS

Heddwen Brooks received her PhD from Imperial College, University of London and is a Professor in the Departments of Physiology and Pharmacology at the University of Arizona (UA). Dr. Brooks is a renal physiologist and is best known for her development of microarray technology to address in vivo signaling pathways involved in the hormonal regulation of renal function. Dr. Brooks' many awards include the American Physiological Society (APS) Lazaro J. Mandel Young Investigator Award, which is for an individual demonstrating outstanding promise in epithelial or renal physiology. In 2009, Dr. Brooks received the APS Renal Young Investigator Award at the annual meeting of the Federation of American Societies for Experimental Biology. Dr. Brooks served as Chair of the APS Renal Section (2011–2014) and currently serves as Associate Editor for the *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, and on the Editorial Board for the *American Journal of Physiology-Renal Physiology* (since 2001). Dr. Brooks has served on study sections of the National Institutes of Health, the American Heart Association, and served as a member of the Nephrology Merit Review Board for the Department of Veterans' Affairs.

JASON X.-J. YUAN

Jason Yuan received his medical degree from Suzhou Medical College (Suzhou, China) in 1983, his doctoral degree in cardiovascular physiology from Peking Union Medical College (Beijing, China), and his postdoctoral training at the University of Maryland at Baltimore. He joined the faculty at the University of Maryland School of Medicine in 1993 and then moved to the University of California, San Diego in 1999, rising to the rank of Professor in 2013. His research interests center on pathogenic roles of membrane receptors and ion channels in pulmonary vascular disease. He has published more than 300 articles, reviews, editorials and chapters, and has edited or co-edited nine books. He has received several honors for his research accomplishments including the Cournand and Comroe Young Investigator Award, the Established Investigator Award and the Kenneth D. Bloch Memorial Lectureship from the American Heart Association; the Guggenheim Fellowship Award from the John Simon Guggenheim Memorial Foundation; the Estelle Grover Lectureship from the American Thoracic Society; and the Robert M. Berne Memorial Lectureship from The American Physiological Society. He is an elected Fellow of the American Association for the Advancement of Science and an elected Member of the American Society for Clinical Investigation and the Association of American Physicians. He has served on many advisory committees including Chair of the Respiratory Integrative Biology and Translational Research study section of the National Institutes of Health and Chair of the Pulmonary Circulation Assembly of the American Thoracic Society. He has also been very active in scholarly editing serving currently as the Editor-in-Chief of the journal *Pulmonary Circulation* and Associate Editor of the *American Journal of Physiology-Cell Physiology*. He is a leading editor of the *Textbook of Pulmonary Vascular Disease* (Springer, 2011).

Contents

Preface

It is difficult to believe that this preface signifies the fourth edition *of Ganong's Review of Medical Physiology* that our author group has overseen, and the 26th edition overall of this important reference work aimed at medical and other health professional students. As always, we have tried to maintain the highest standards of excellence that were promulgated by the original author, Fran Ganong, over the 46 years where he served, remarkably, as the sole author of the textbook.

In this new edition, we have cast a fresh eye on the pedagogical approach taken in each chapter and section, and have focused particularly on including only material that is of the highest yield. We have thoroughly revised the learning objectives for every chapter, reorganized and updated the text to ensure that all objectives are clearly addressed in a logical order, aligned chapter summaries so that the take-home messages quickly address each learning objective in turn, and expanded the number of review questions so that readers also have the ability to check their understanding and retention of every objective covered. As a discipline evolves and new information emerges, there is a tendency simply to concatenate these concepts such that chapter structure degrades inevitably over time. With in-depth discussions amongst the author team and significant "spring-cleaning," we believe we have freshened and simplified the volume while also making sure that important new developments are incorporated. We are immensely thankful to Erica Wehrwein, PhD, Assistant Professor of Physiology and an award-winning instructor at Michigan State University, who took on the task of reviewing the book as a whole and providing specific and detailed feedback to us on each chapter.

This new edition also welcomes a new member to the author team. We are delighted to have been able to recruit Jason X.-J. Yuan, MD, PhD, Professor of Medicine and Physiology as well as Chief of the Division of Translational and Regenerative Medicine and Associate Vice President for Translational Health Sciences at the University of Arizona, who has assumed responsibility for some

cell physiology and cardiovascular topics, as well as the respiratory physiology section. We are particularly excited to have a physician-scientist on the team, who can guide us overall to focus on material that is of most benefit to those preparing for a career incorporating patient care. We are most grateful for the past contributions of Scott Boitano, PhD, whose other obligations meant that he could no longer serve as an author.

We continue to be gratified by the many colleagues and students who contact us from all over the world to request clarification of material covered in the text, or to point out errors or omissions. We are especially grateful to Rajan Pandit, Lecturer in Physiology at Nepal Medical College, who has painstakingly offered dozens of suggestions for revision over the years. His efforts, and those of the many others whom we have not named, allow us to engage in a process of continual improvement. While, as always, any errors that remain in the book (inevitable in a complex project such as this) are the sole responsibility of the authorship team, we greatly value critical input, and urge readers once again to contact us with any suggestions or critiques. We thank you in advance both for such feedback, and also for your support of this new edition.

This edition is a revision of the original works of Dr. Francis Ganong.

SECTION I

Cellular & Molecular Basis for Medical Physiology

Study of physiological system structure and function, as well as pathophysiological alterations, has its foundations in physical and chemical laws and the molecular and cellular makeup of each tissue and organ system. *Ganong's Review of Medical Physiology* is structured into seven sections. This first section provides an overview of the basic building blocks or bases that provide the important framework for human physiology. It is important to note here that the seven chapters in this initial section are not meant to provide an exhaustive understanding of biophysics, biochemistry, or cellular and molecular physiology; rather, they are to serve as a reminder of how the basic principles from these disciplines contribute to medical physiology discussed in later sections associated with physiological functions of organs and systems.

In the first two chapters of this section, the following basic building blocks are introduced and discussed: electrolytes; carbohydrates, lipids, and fatty acids; amino acids and proteins; and nucleic acids. Students are reminded of some of the basic principles and building blocks of biophysics and biochemistry and how they fit into the physiologic environment. Examples of direct clinical applications are provided in the clinical boxes to help bridge the gap between basic principles and human cell, tissue, and organ functions. These basic principles are followed up with a discussion of the generic cell and its components. It is important to realize the cell is the basic functional unit within the body, and it is the collection and fine-tuned interactions among and between these fundamental units that allow for proper tissue, organ, and organism function.

In the third to seventh chapters of this introductory section, we take a cellular

approach to lay a groundwork of understanding groups of cells that interact with many of the systems discussed in future chapters. The first group of cells presented contribute to inflammatory reactions in the body. These individual players, their coordinated behavior, and the net effects of the “open system” of inflammation in the body are discussed in detail. The second group of cells discussed are responsible for the excitatory responses in human physiological function and include both neuronal and muscle cells. A fundamental understanding of the inner workings of these cells, and how they are controlled by their neighboring cells, helps the student to understand their eventual integration into individual systems discussed in later sections.

This first section serves as an introduction, refresher, and quick source of material to best understand organ functions and systems physiology presented in the later sections. For detailed understanding of any of the chapters within this section, several excellent and current textbooks that provide more in-depth reviews of principles of biochemistry, biophysics, cell physiology, and muscle and neuronal physiology are provided as resources at the end of each individual chapter. Students are encouraged to visit these texts for a more thorough understanding of these basic principles.

CHAPTER 1

General Principles & Energy Production in Medical Physiology

OBJECTIVES

After studying this chapter, you should be able to:

- Define functional units used in measuring physiological properties.
- Define pH and buffering.
- Understand electrolytes and define diffusion, osmosis, and tonicity.
- Define and explain the significance of resting membrane potential.
- Understand in general terms the basic building blocks of the cell (eg, nucleotides, amino acids, carbohydrates, and fatty acids) to cell metabolism, proliferation, and function.
- Understand higher-order structures of the basic building blocks of the cell (eg, DNA, RNA, proteins, and lipids) to cell replication, proliferation, and signal transduction.
- Understand the basic contributions of the basic building blocks of the cell to its structure, function, and energy balance.

INTRODUCTION

In unicellular organisms, all vital processes occur in a single cell. As the evolution of multicellular organisms progressed, various cell groups organized into tissues, and organs have taken over particular functions. In humans and other vertebrate animals, there are a number of specialized collections of cells that consist in organ systems serving for different functions. For example, a gastrointestinal system to digest and absorb food; a respiratory system to take up O_2 and eliminate CO_2; a urinary system to remove wastes; a cardiovascular system to distribute nutrients, O_2, and the products of metabolism; a reproductive system to perpetuate the species; and nervous and endocrine systems to coordinate and integrate the functions of the other systems. This book is concerned with the way these systems function and the way each contributes to the functions of the body as a whole. This first chapter lays a foundation for the discussion of these organ systems with a review of basic biophysical and biochemical principles at the cellular level and the introduction of the molecular building blocks that contribute to cell physiological function within these organ systems.

GENERAL PRINCIPLES

THE BODY AS ORGANIZED "SOLUTIONS"

In the average young adult male, 18% of the body weight is protein and related substances, 7% is mineral, and 15% is fat. The remaining 60% is water. The distribution of the body water is shown in **Figure 1–1A**.

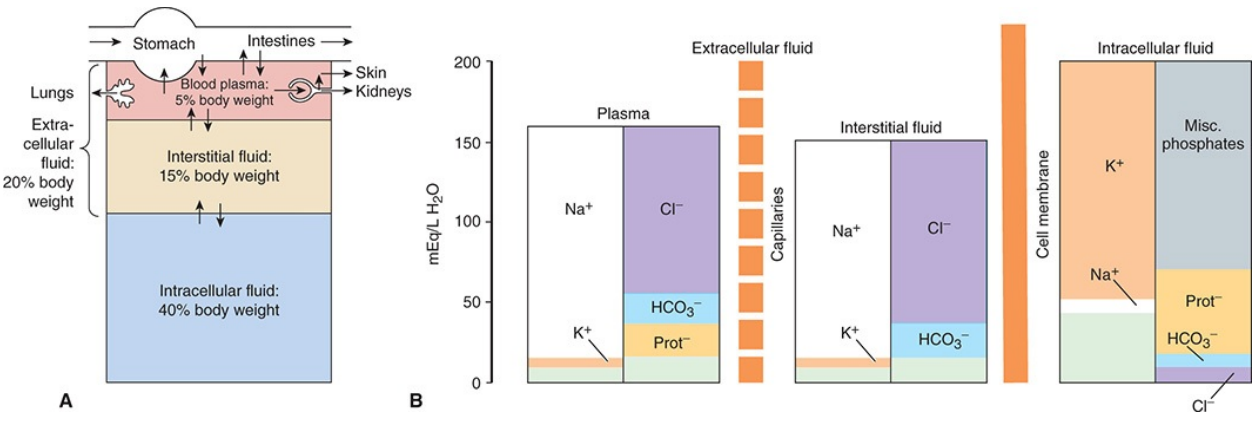


FIGURE 1–1 Organization of body fluids and electrolytes into compartments. A) Body fluids can be divided into intracellular and extracellular fluid compartments (ICF and ECF, respectively). Their contribution

to percentage body weight (based on a healthy young adult male; slight variations exist with age and gender) emphasizes the dominance of fluid makeup of the body. Transcellular fluids, which constitute a very small percentage of total body fluids, are not shown. Arrows represent fluid movement between compartments. **B)** Electrolytes and proteins are unequally distributed among the body fluids. This uneven distribution is crucial to physiology. $Prot^-$, protein, which tends to have a negative charge at physiologic pH.

The cells that make up the bodies of all but the simplest multicellular animals, both aquatic and terrestrial, exist in an "internal sea" of **extracellular fluid (ECF)** enclosed within the integument of the animal. From this fluid, the cells take up O_2 and nutrients; into it, they discharge metabolic waste products. The ECF is more dilute than present-day seawater, but its composition closely resembles that of the primordial oceans in which, presumably, all life originated.

In animals with a closed vascular system, the ECF is divided into the **interstitial fluid,** the circulating **blood plasma,** and **the lymph fluid that bridges these two domains.** The interstitial fluid is the part of the ECF that is outside the vascular and lymph systems, bathing the cells. The plasma and the cellular elements of the blood, principally red blood cells, fill the vascular system, and together they constitute the **total blood volume.** About one-third of the **total body water** is extracellular; the remaining two-thirds is intracellular **(intracellular fluid).** Inappropriate compartmentalization of the body fluids can result in edema **(Clinical Box 1–1)**.

CLINICAL BOX 1–1

Edema

Edema is the buildup of body fluids extracellularly or interstitially in tissues. The increased fluid is related to an increased leak from the blood and/or reduced removal by the lymph system. Edema is often observed in the feet, ankles, and legs, but can happen in many areas of the body in response to disease, including those of the heart, lung, liver, kidney, or thyroid.

THERAPEUTIC HIGHLIGHTS

The best treatment for edema includes reversing the underlying disorder. Thus, proper diagnosis of the cause of edema is the primary first step in therapy. More

general treatments include restricting dietary sodium to minimize fluid retention and using appropriate diuretic therapy.

The intracellular component of the body water accounts for about 40% of body weight and the extracellular component for about 20%. Approximately 25% of the extracellular component is in the vascular system (plasma = 5% of body weight) and 75% outside the blood vessels (interstitial fluid = 15% of body weight). The total blood volume is about 8% of body weight. Flow between these compartments is tightly regulated.

UNITS FOR MEASURING CONCENTRATION OF SOLUTES

In considering the effects of various physiologically important substances and the interactions among them, the number of molecules, electrical charges, or particles of a substance per unit volume of a particular body fluid are often more meaningful than simply the weight of the substance per unit volume. For this reason, physiological concentrations are frequently expressed in moles, equivalents, or osmoles.

Moles

A mole is the gram-molecular weight of a substance, that is, the molecular weight (MW) of the substance in grams. Each mole (mol) consists of 6×10^{23} molecules. The millimole (mmol) is 1/1000 of a mole, and the micromole (µmol) is 1/1,000,000 of a mole. Thus, 1 mol of NaCl = 23 g + 35.5 g = 58.5 g and 1 mmol = 58.5 mg. The mole is the standard unit for expressing the amount of substances in the SI unit system.

The molecular weight of a substance is the ratio of the mass of one molecule of the substance to the mass of one-twelfth the mass of an atom of carbon-12. Because molecular weight is a ratio, it is dimensionless. The dalton (Da) is a unit of mass equal to one-twelfth the mass of an atom of carbon-12. The kilodalton (1 kDa = 1000 Da) is a useful unit for expressing the molecular mass of proteins. Thus, for example, one can speak of a 64-kDa protein or state that the **molecular mass** of the protein is 64,000 Da. However, because molecular weight is a dimensionless ratio, it is incorrect to say that the molecular weight of the protein

is 64 kDa.

Equivalents

The concept of electrical equivalence is important in physiology because many of the solutes in the body are in the form of charged particles. One equivalent (Eq) is 1 mol of an ionized substance divided by its valence. One mole of NaCl dissociates into 1 Eq of Na^+ and 1 Eq of Cl^-. One equivalent of Na^+ = 23 g, but 1 Eq of Ca^{2+} = 40 g ÷ 2 = 20 g. The milliequivalent (mEq) is 1/1000 of 1 Eq.

Electrical equivalence is not necessarily the same as chemical equivalence. A gram equivalent is the weight of a substance that is chemically equivalent to 8.0 g of oxygen. The normality (N) of a solution is the number of gram equivalents in 1 liter (L). A 1 N solution of hydrochloric acid (HCl) contains both H^+ (1.0 g) and Cl^- (35.5 g) equivalents, = (1.0 g + 35.5 g)/L = 36.5 g/L.

WATER, ELECTROLYTES, & ACID/BASE

The water molecule (H_2O) is an ideal solvent for physiological reactions. H_2O has a **dipole moment** where oxygen slightly pulls away electrons from the hydrogen atoms and creates a charge separation that makes the molecule **polar.** This allows water to dissolve a variety of charged atoms and molecules. It also allows the H_2O molecule to interact with other H_2O molecules via hydrogen bonding. The resulting hydrogen bond network in water allows for several key properties relevant to physiology: (1) water has a high surface tension, (2) water has a high heat of vaporization and heat capacity, and (3) water has a high dielectric constant. In layman's terms, H_2O is an excellent biological fluid that serves as a solute; it provides optimal heat transfer and conduction of current.

Electrolytes (eg, NaCl) are molecules that dissociate in water to their cation (Na^+) and anion (Cl^-) equivalents. Because of the net charge on water molecules, these electrolytes tend not to reassociate in water. There are many important electrolytes in physiology, notably Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, and HCO_3^-. It is important to note that electrolytes and other charged compounds (eg, proteins) are unevenly distributed in the body fluids **(Figure 1–1B)**. These separations play an important role in physiology, for example, in the establishment of membrane potential and generation of action potential.

pH & BUFFERING

The maintenance of a stable hydrogen ion concentration ($[H^+]$) in body fluids is essential to life. The **pH** of a solution is defined as the logarithm to the base 10 of the reciprocal of the H^+, that is, the negative logarithm of the $[H^+]$. The pH of water at 25°C, in which H^+ and OH^- ions are present in equal numbers, is 7.0 **(Figure 1–2)**. For each pH unit less than 7.0, the $[H^+]$ is increased 10-fold; for each pH unit above 7.0, it is decreased 10-fold. In the plasma of healthy individuals, pH is slightly alkaline, maintained in the narrow range of 7.35–7.45 **(Clinical Box 1–2)**. Conversely, gastric fluid pH can be quite acidic (on the order of 3.0) and pancreatic secretions can be quite alkaline (on the order of 8.0). Enzymatic activity and protein structure are frequently sensitive to pH; in any given body or cellular compartment, pH is maintained to allow for maximal enzyme/protein efficiency.

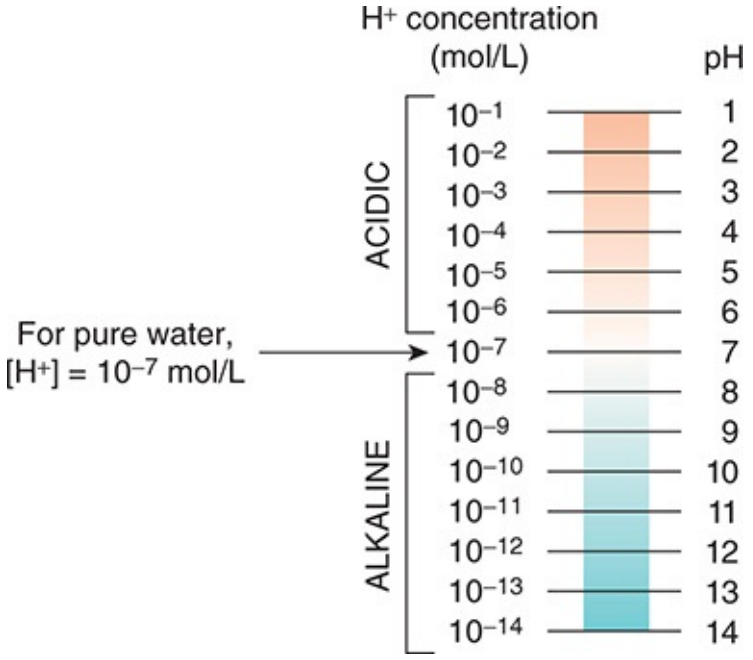


FIGURE 1–2 Proton concentration and pH. Relative proton (H^+) concentrations for solutions on a pH scale are shown.

Molecules that act as H^+ donors in solution are considered acids, while those that tend to remove H^+ from solutions are considered bases. Strong acids (eg, HCl) or bases (eg, NaOH) dissociate completely in water and thus can most change the $[H^+]$ in solution. In physiological compounds, most acids or bases are considered “weak,” that is, they contribute or remove relatively few H^+ from

solution. Body pH is stabilized by the **buffering capacity** of the body fluids. A **buffer** is a substance that has the ability to bind or release H^+ in solution, thus keeping the pH of the solution relatively constant despite the addition of considerable quantities of acid or base. Of course there are a number of buffers at work in biological fluids at any given time. All buffer pairs in a homogenous solution are in equilibrium with the same $[H^+]$; this is known as the **isohydric principle.** One outcome of this principle is that by assaying a single buffer system, we can understand a great deal about all of the biological buffers in that system.

When acids are placed into solution, there is dissociation of some of the component acid (HA) into its proton (H^+) and free acid (A^-). This is frequently written as an equation:

$$HA \rightleftharpoons H^+ + A^-$$

According to the laws of mass action, a relationship for the dissociation can be defined mathematically as:

$$K_a = [H^+][A^-]/[HA]$$

CLINICAL BOX 1–2

Acid–Base Balance and Disorders

Excesses of acid (acidosis) or base (alkalosis) exist when the blood or blood plasma is outside the normal pH range (7.35–7.45). Such changes impair the delivery of O_2 to and removal of CO_2 from tissues. There are a variety of conditions and diseases that can interfere with pH control in the body and cause blood pH to fall outside of healthy limits. Acid–base disorders that result from respiration to alter CO_2 concentration are called respiratory acidosis and respiratory alkalosis. Respiratory acidosis is often caused by respiratory failure or ventilator failure, while respiratory alkalosis is caused by alveolar hyperventilation and often found in patients with chronic liver disease. Nonrespiratory disorders that affect HCO_3^- concentration are referred to as metabolic acidosis and metabolic alkalosis. Metabolic acidosis or alkalosis can be caused by electrolyte disturbances, severe vomiting or diarrhea, ingestion of certain drugs and toxins, kidney disease, and diseases that affect normal metabolism (eg, diabetes).

THERAPEUTIC HIGHLIGHTS

Proper treatments for acid–base disorders are dependent on correctly identifying the underlying causal process(es). This is especially true when mixed disorders are encountered. Treatment of respiratory acidosis should be initially targeted at restoring ventilation, whereas treatment for respiratory alkalosis is focused on the reversal of the primary causes (eg, alveolar hyperventilation associated with head injury and anxiety, hypoxemia due to peripheral chemoreceptor stimulation, pulmonary embolism, and edema). Bicarbonate (via intravenous injection) is typically used as a treatment for acute metabolic acidosis. An adequate amount of a chloride salt can restore acid–base balance to normal over a matter of days for patients with a chloride-responsive metabolic alkalosis whereas chloride-resistant metabolic alkalosis requires treatment of the underlying disease.

where K_a is a constant, and the brackets represent concentrations of the individual species (elements). In layman's terms, the product of the proton concentration ($[H^+]$) and the free acid concentration ($[A^-]$) divided by the bound acid concentration ([HA]) is a defined constant (K). This can be rearranged to read:

$$[H^+] = K_a\,[HA]/[A^-]$$

If the logarithm of each side is taken:

$$\log[H^+] = \log K_a + \log[HA]/[A^-]$$

Both sides can be multiplied by –1 to yield:

$$-\log[H^+] = -\log K_a + \log[A^-]/[HA]$$

This can be written in a more conventional form known as the **Henderson-Hasselbalch equation:**

$$pH = pK_a + \log[A^-]/[HA]$$

This relatively simple equation is quite powerful. One thing that can be discerned right away is that the buffering capacity of a particular weak acid is

best when the pK_a of that acid is equal to the pH of the solution, or when:

$$[A^-] = [HA], pH = pK_a$$

Similar equations can be set up for weak bases. An important buffer in the body is carbonic acid (H_2CO_3). Carbonic acid is a weak acid, and thus is only partly dissociated into H^+ and HCO_3^-:

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

If H^+ is added to a solution of carbonic acid, the equilibrium shifts to the left and most of the added H^+ is removed from solution. If OH^- is added, H^+ and OH^- combine, taking H^+ out of solution. However, the decrease is countered by more dissociation of H_2CO_3, and the decline in H^+ concentration is minimized. A unique feature of HCO_3^- is the linkage between its buffering ability and the ability for the lungs to remove CO_2 from the body. Other important biological buffers include phosphates and proteins.

DIFFUSION

The particles (molecules or atoms) of a substance dissolved in a solvent are in continuous random movement. Diffusion is the process by which a gas or a substance in a solution expands or moves from a region to another, because of the motion of its particles, to fill all the available volume. A given particle is equally likely to move into or out of an area in which it is present in high concentration. However, because there are more particles in the area of high concentration, the total number of particles moving to areas of lower concentration is greater; that is, there is a **net flux** of solute particles from areas of high concentration to areas of low concentration. The time required for equilibrium by diffusion is proportional to the square of the diffusion distance. The magnitude of the diffusing tendency from one region to another separated by a boundary (eg, cell membrane, blood-gas barrier) is directly proportional to the cross-sectional area across which diffusion is taking place and the **concentration gradient,** or **chemical gradient,** which is the difference in concentration of the diffusing substance divided by the thickness of the boundary **(Fick's law of diffusion).** Thus,

$$J = -DA\frac{\Delta c}{\Delta x}$$

where J is the net rate of diffusion, D is the diffusion coefficient, A is the area, and $\Delta c/\Delta x$ is the concentration gradient. The minus sign indicates the direction of diffusion. When considering movement of molecules from a higher to a lower concentration, $\Delta c/\Delta x$ is negative, so multiplying by –DA gives a positive value. The permeabilities of the boundaries across which diffusion occurs in the body vary, but diffusion is still a major force affecting the distribution of water and solutes.

OSMOSIS

When a substance is dissolved in water, the concentration of water molecules in the solution is less than that in pure water, because the addition of solute to water results in a solution that occupies a greater volume than does the water alone. If the solution is placed on one side of a membrane that is permeable to water but not to the solute, and an equal volume of water is placed on the other, water molecules diffuse down their concentration (chemical) gradient into the solution **(Figure 1–3)**. This process—the diffusion of **solvent** molecules into a region in which there is a higher concentration of a **solute** to which the membrane is impermeable—is called **osmosis.** It is an important factor in physiological processes. The tendency for movement of solvent molecules to a region of greater solute concentration can be prevented by applying pressure to the more concentrated solution. The pressure necessary to prevent solvent migration is the **osmotic pressure** of the solution.

Osmotic pressure—like vapor pressure lowering, freezing-point depression, and boiling-point elevation—depends on the number rather than the type of particles in a solution; that is, it is a fundamental colligative property of solutions. In an **ideal solution,** osmotic pressure (P) is related to temperature and volume in the same way as the pressure of a gas:

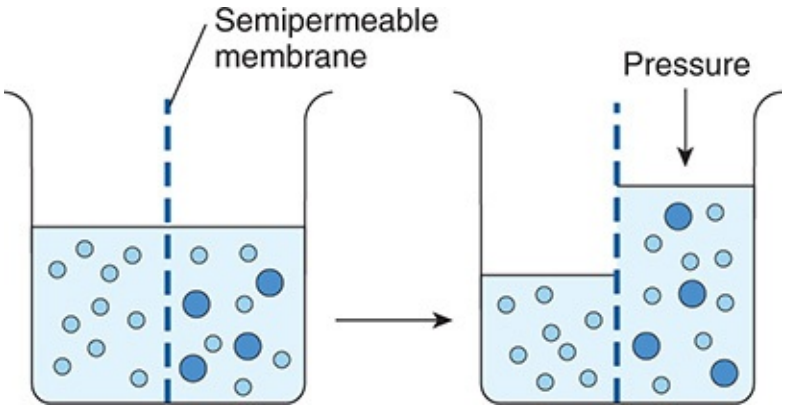


FIGURE 1–3 Diagrammatic representation of osmosis. Water molecules are represented by small open circles, and solute molecules by large solid circles. In the diagram on the left, water is placed on one side of a membrane permeable to water but not to solute, and an equal volume of a solution of the solute is placed on the other. Water molecules move down their concentration (chemical) gradient into the solution, and, as shown in the diagram on the right, the volume of the solution increases. As indicated by the arrow on the right, the osmotic pressure is the pressure that would have to be applied to prevent the movement of the water molecules.

$$P = \frac{nRT}{V}$$

where n is the number of particles, R is the gas constant, T is the absolute temperature, and V is the volume. If T is held constant, it is clear that the osmotic pressure is proportional to the number of particles in solution per unit volume of solution. For this reason, the concentration of osmotically active particles is usually expressed in **osmoles.** One osmole (Osm) equals the gram-molecular weight of a substance divided by the number of freely moving particles that each molecule liberates in solution. For biological solutions, the milliosmole (mOsm; 1/1000 of 1 Osm) is more commonly used.

If a solute is a nonionizing compound such as glucose, the osmotic pressure is a function of the number of glucose molecules present. If the solute ionizes and forms an ideal solution, each ion is an osmotically active particle. For example, NaCl would dissociate into Na^+ and Cl^- ions, so that each mole in solution would supply 2 Osm. One mole of Na_2SO_4 would dissociate into Na^+, Na^+, and SO_4^{2-} supplying 3 Osm. However, the body fluids are not ideal solutions, and although the dissociation of strong electrolytes is complete, the number of

particles free to exert an osmotic effect is reduced owing to interactions between the ions. Thus, it is actually the effective concentration **(activity)** in the body fluids rather than the number of equivalents of an electrolyte in solution that determines its osmotic capacity. This is why, for example, 1 mmol of NaCl per liter in the body fluids contributes somewhat less than 2 mOsm of osmotically active particles per liter. The more concentrated the solution, the greater the deviation from an ideal solution.

The osmolal concentration of a substance in a fluid is measured by the degree to which it depresses the freezing point, with 1 mol of an ideal solution depressing the freezing point by 1.86°C. The number of milliosmoles per liter in a solution equals the freezing point depression divided by 0.00186. The **osmolarity** is the number of osmoles per liter of solution (eg, plasma), whereas the **osmolality** is the number of osmoles per kilogram of solvent. Therefore, osmolarity is affected by the volume of the various solutes in the solution and the temperature, while the osmolality is not. Osmotically active substances in the body are dissolved in water, and the density of water is 1, so osmolal concentrations can be expressed as osmoles per liter (Osm/L) of water. In this book, osmolal (rather than osmolar) concentrations are considered, and osmolality is expressed in milliosmoles per liter (of water).

Note that although a homogeneous solution contains osmotically active particles and can be said to have an osmotic pressure, it can exert an osmotic pressure only when it is in contact with another solution across a membrane permeable to the solvent but not to the solute.

OSMOLAL CONCENTRATION OF PLASMA: TONICITY

The freezing point of normal human plasma averages −0.54°C, which corresponds to an osmolal concentration in plasma of 290 mOsm/L. This is equivalent to an osmotic pressure against pure water of 7.3 atmospheres (atm). The osmolality might be expected to be higher than 290 mOsm/L, because the sum of all the cation and anion equivalents in plasma is over 300 mOsm/L. It is not this high because plasma is not an ideal solution and ionic interactions reduce the number of particles free to exert an osmotic effect. Except when there has been insufficient time after a sudden change in composition for equilibrium to occur, all fluid compartments of the body are in (or nearly in) osmotic equilibrium. The term **tonicity** is used to describe the osmolality of a solution

relative to plasma. Solutions that have the same osmolality as plasma are said to be **isotonic;** those with greater osmolality are **hypertonic;** and those with lesser osmolality are **hypotonic.** All solutions that are initially isosmotic with plasma (ie, that have the same actual osmotic pressure or freezing-point depression as plasma) would remain isotonic if it were not for the fact that some solutes diffuse into cells and others are metabolized. Thus, a 0.9% saline solution remains isotonic because there is no net movement of the osmotically active particles in the solution into cells and the particles are not metabolized. On the other hand, a 5% glucose solution is isotonic when initially infused intravenously, but glucose is metabolized, so the net effect is that of infusing a hypotonic solution.

It is important to note the relative contributions of the various plasma components to the total osmolal concentration of plasma. All but about 20 of the 290 mOsm in each liter of normal plasma are contributed by Na^+ and its accompanying anions, principally Cl^- and HCO_3^-. Other cations and anions make a relatively small contribution. Although the concentration of the plasma proteins is large when expressed in grams per liter, they normally contribute less than 2 mOsm/L because of their very high molecular weights. The major nonelectrolytes of plasma are glucose and urea, which in the steady state are in equilibrium with cells. Their contributions to osmolality are normally about 5 mOsm/L each but can become quite large in hyperglycemia or uremia. The total plasma osmolality is important in assessing dehydration, overhydration, and other fluid and electrolyte abnormalities **(Clinical Box 1–3)**.

NONIONIC DIFFUSION

Some weak acids and bases are quite soluble in cell membranes in the undissociated form, whereas they cannot cross membranes in the charged (ie, dissociated) form. Consequently, if molecules of the undissociated substance diffuse from one side of the membrane to the other and then dissociate, there is appreciable net movement of the undissociated substance from one side of the membrane to the other. This phenomenon is called **nonionic diffusion.**

DONNAN EFFECT

When an ion on one side of a membrane cannot diffuse through the membrane, the distribution of other ions to which the membrane is permeable is affected in a

predictable way. For example, the negative charge of a nondiffusible anion hinders diffusion of the diffusible cations and favors diffusion of the diffusible anions. Consider the situation shown in **Figure 1–4**, in which the membrane (M) between compartment X and compartment Y is impermeable to charged proteins ($Prot^-$) but freely permeable to K^+ and Cl^-.

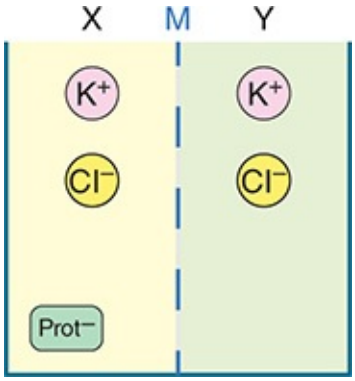


FIGURE 1–4 Equilibrium across a cell membrane. Diagram showing two compartments (X and Y) separated by the membrane (M). Charged K^+ and Cl^- are distributed in both compartments, while charged protein ($prot^-$) is only in X compartment.

CLINICAL BOX 1–3

Plasma Osmolarity & Disease

Unlike plant cells, which have rigid walls, animal cell membranes are flexible. Therefore, animal cells swell when exposed to extracellular hypotonicity and shrink when exposed to extracellular hypertonicity. Cells contain ion channels and pumps that can be activated to offset moderate changes in osmolarity; however, these can be overwhelmed under certain pathological conditions. Hyperosmolality can cause coma (hyperosmolar coma), a prolonged state of deep unconsciousness. Because of the predominant role of the major solutes and the deviation of plasma from an ideal solution, one can ordinarily approximate the plasma osmolarity within a few mOsm/L by using the following formula, in which the constants convert the clinical units to millimoles of solute per liter:

$$\text{Osmolarity (mOsm/L)} = 2[Na^+]\ \text{(mEq/L)} + 0.055[\text{Glucose}]\ \text{(mg/dL)} + 0.36[\text{BUN}]\ \text{(mg/dL)}$$

BUN is the blood urea nitrogen. The formula is also useful in calling attention to abnormally high concentrations of other solutes. An observed plasma osmolarity (measured by freezing-point depression) that greatly exceeds the value predicted by this formula indicates the presence of a foreign substance such as ethanol, mannitol (sometimes injected to shrink swollen cells osmotically), or poisons such as ethylene glycol (component of antifreeze) or methanol (alternative automotive fuel).

Assume that the concentrations of the anions (eg, Cl^-) and of the cations (eg, K^+) on the two sides are initially equal. Cl^- diffuses down its concentration gradient from Y to X, and some K^+ moves with the negatively charged Cl^- because of its opposite charge. Therefore,

$$[K^+_X] > [K^+_Y]$$

Furthermore,

$$[K^+_X] + [Cl^-_X] + [Prot^-_X] > [K^+_Y] + [Cl^-_Y]$$

that is, more osmotically active particles are on side X (or compartment X) than on side Y (or compartment Y).

Donnan and Gibbs showed that in the presence of a nondiffusible ion, the diffusible ions distribute themselves so that at equilibrium their concentration ratios are equal:

$$\frac{[K^+_X]}{[K^+_Y]} = \frac{[Cl^-_Y]}{[Cl^-_X]}$$

Cross-multiplying,

$$[K^+_X][Cl^-_X] = [K^+_Y][Cl^-_Y]$$

This is the **Gibbs–Donnan equation.** It holds for any pair of cations and anions of the same valence.

The Donnan effect on the distribution of ions has three effects in the body introduced here and discussed below. First, because of charged proteins ($Prot^-$) in cells, there are more osmotically active particles in cells than in interstitial or intercellular fluid, and because animal cells have flexible walls, osmosis would

make them swell and eventually rupture if it were not for the sodium-potassium adenosine triphosphatase (**Na, K ATPase)** pumping ions back out of cells. Thus, normal cell volume and pressure largely depend on Na, K ATPase, also known as the Na^+/K^+ pump. Second, because at equilibrium the distribution of permeant ions across the membrane (m, in the example shown in Figure 1–4) is asymmetric, an electrical difference exists across the membrane whose magnitude can be determined by the **Nernst equation** (see below). In the example used here (Figure 1–4), side X will be negative relative to side Y. The charges line up along the membrane, with the concentration gradient for Cl^- exactly balanced by the oppositely directed electrical gradient, and the same holds true for K^+. Third, because there are more proteins in plasma than in interstitial fluid, there is a Donnan effect on ion movement across the capillary wall.

FORCES ACTING ON IONS

The forces acting across the cell membrane on each ion can be analyzed mathematically. Chloride ions (Cl^-) are present in higher concentration in the ECF than in the cell interior, and they tend to diffuse along this **concentration gradient** into the cell. The interior of the cell is negative relative to the exterior, and chloride ions are pushed out of the cell along this **electrical gradient.** An equilibrium is reached between Cl^- influx and Cl^- efflux. The membrane potential at which this equilibrium exists is the **equilibrium potential.** Its magnitude can be calculated from the Nernst equation, as follows:

$$E_{Cl} = \frac{RT}{FZ_{Cl}} \ln \frac{[Cl_o^-]}{[Cl_i^-]}$$

where

E_{Cl} = equilibrium potential for Cl^-

R = gas constant

T = absolute temperature

F = the Faraday number (number of coulombs per mole of charge)

Z_{Cl} = valence of Cl^- (–1)

$[Cl_o^-]$ = Cl^- concentration outside the cell

$[Cl_i^-]$ = Cl^- concentration inside the cell

Converting from the natural log to the base 10 log and replacing some of the constants with numeric values holding temperature at 37°C, the equation becomes:

$$E_{Cl} = 61.5 \log \frac{[Cl_i^-]}{[Cl_o^-]} \text{ at } 37^\circ C$$

Note that in converting to the simplified expression the concentration ratio is reversed because the –1 valence of Cl^- has been removed from the expression.

The equilibrium potential for Cl^- (E_{Cl}) in the mammalian spinal neuron, calculated from the standard values listed in **Table 1–1**, is −70 mV, a value identical to the typical measured resting membrane potential of −70 mV. Therefore, no forces other than those represented by the chemical and electrical gradients need to be invoked to explain the distribution of Cl^- across the membrane.

A similar equilibrium potential can be calculated for K^+ (E_K; again, at 37°C):

$$E_K = \frac{RT}{FZ_K} \ln \frac{[K_o^+]}{[K_i^+]} = 61.5 \log \frac{[K_o^+]}{[K_i^+]} \qquad (\text{at } 37^\circ C)$$

where

E_K = equilibrium potential for K^+

Z_K = valence of K^+ (+1)

$[K_o^+]$ = K^+ concentration outside the cell

$[K_i^+]$ = K^+ concentration inside the cells R, T, and F as above

In this case, the concentration gradient is outward and the electrical gradient inward. In mammalian spinal motor neurons, E_K is −90 mV (Table 1–1). Because the resting membrane potential is −70 mV, there is somewhat more K^+ in the neurons that can be accounted for by the electrical and chemical gradients.

TABLE 1–1 Concentration of some ions inside and outside mammalian spinal motor neurons.

Ion	Concentration (mmol/L of H_2O)		Equilibrium Potential (mV)
	Inside Cell	Outside Cell	
Na^+	15.0	150.0	+60
K^+	150.0	5.5	−90
Cl^-	9.0	125.0	−70

Resting membrane potential = −70 mV.

The situation for Na^+ in the mammalian spinal motor neuron is quite different from that for K^+ or Cl^-. The direction of the chemical gradient for Na^+ is inward, to the area where it is in lesser concentration, and the electrical gradient is in the same direction. E_{Na} is +60 mV (Table 1–1). Because neither E_K nor E_{Na} is equal to the membrane potential, one would expect the cell to gradually gain Na^+ and lose K^+ if only passive electrical and chemical forces were acting across the membrane. However, the intracellular concentrations of Na^+ and K^+ remain constant because of selective permeability of the membrane to different ions (Na^+, K^+) and the action of the Na, K ATPase that actively transports Na^+ out of the cell and K^+ into the cell (against their respective electrochemical gradients).

ESTABLISHMENT OF THE MEMBRANE POTENTIAL

The distribution of ions across the cell membrane and the nature of this membrane provide the explanation for the membrane potential. The concentration gradient for K^+ facilitates its movement out of the cell via K^+ channels, but its electrical gradient is in the opposite (inward) direction. Consequently, an equilibrium is reached in which the tendency of K^+ to move out of the cell is balanced by its tendency to move into the cell, and at that equilibrium there is a slight excess of cations on the outside and anions on the inside. This condition is maintained by Na, K ATPase, which uses the energy of ATP to pump K^+ back into the cell and keeps the intracellular concentration of Na^+ low. Because the Na, K ATPase moves three Na^+ out of the cell for every two K^+ moved in, it also contributes to the membrane potential, and thus is termed an **electrogenic pump.** It should be emphasized that the number of ions

responsible for the membrane potential is a minute fraction of the total number present and that the total concentrations of positive and negative ions are equal everywhere except along the membrane.

ENERGY PRODUCTION

ENERGY TRANSFER

Energy used in cellular processes and cell function is primarily stored in bonds between phosphoric acid residues and certain organic compounds. Because the energy of bond formation in some of these phosphates is particularly high, relatively large amounts of energy (10–12 kcal/mol) are released when the bond is hydrolyzed. Compounds containing such bonds are called **high-energy phosphate compounds.** Not all organic phosphates are of the high-energy type. Many, like glucose 6-phosphate, are low-energy phosphates that on hydrolysis liberate 2–3 kcal/mol. Some of the intermediates formed in carbohydrate metabolism are high-energy phosphates, but the most important energy-rich phosphate compound is **adenosine triphosphate (ATP).** This ubiquitous molecule, ATP **(Figure 1–5)**, is the energy storehouse of the body. On hydrolysis to adenosine diphosphate (ADP), it liberates energy directly to such processes as muscle contraction, active transport, and the synthesis of many chemical compounds. Loss of another phosphate to form adenosine monophosphate (AMP) releases more energy.

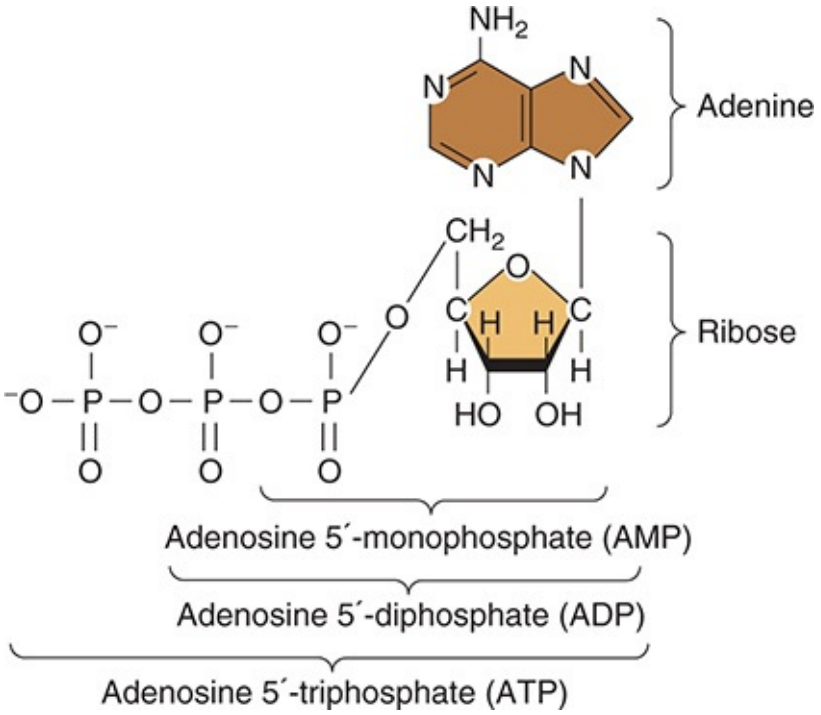


FIGURE 1–5 Energy-rich adenosine derivatives. Adenosine triphosphate is broken down into its backbone purine base and sugar (at right) as well as its high-energy phosphate derivatives (across bottom). (Reproduced with permission from Murray RK, et al: *Harper's Biochemistry*, 28th ed. New York, NY: McGraw-Hill; 2009.)

Another group of energy-rich, or high-energy, compounds are the thioesters, the acyl derivatives of mercaptans. **Coenzyme A (CoA)** is a widely distributed mercaptan-containing adenine, ribose, pantothenic acid, and thioethanolamine **(Figure 1–6)**. Reduced CoA (usually abbreviated HS-CoA) reacts with acyl groups (R–CO–) to form R–CO–S–CoA derivatives. A prime example is the reaction of HS-CoA with acetic acid to form acetylcoenzyme A (acetyl-CoA), a compound of pivotal importance in intermediary metabolism. Because acetyl-CoA has a much higher energy content than acetic acid, it combines readily with substances in reactions that would otherwise require outside energy. Acetyl-CoA is therefore often called "active acetate." From the point of view of energetics, formation of 1 mol of any acyl-CoA compound is equivalent to the formation of 1 mol of ATP.

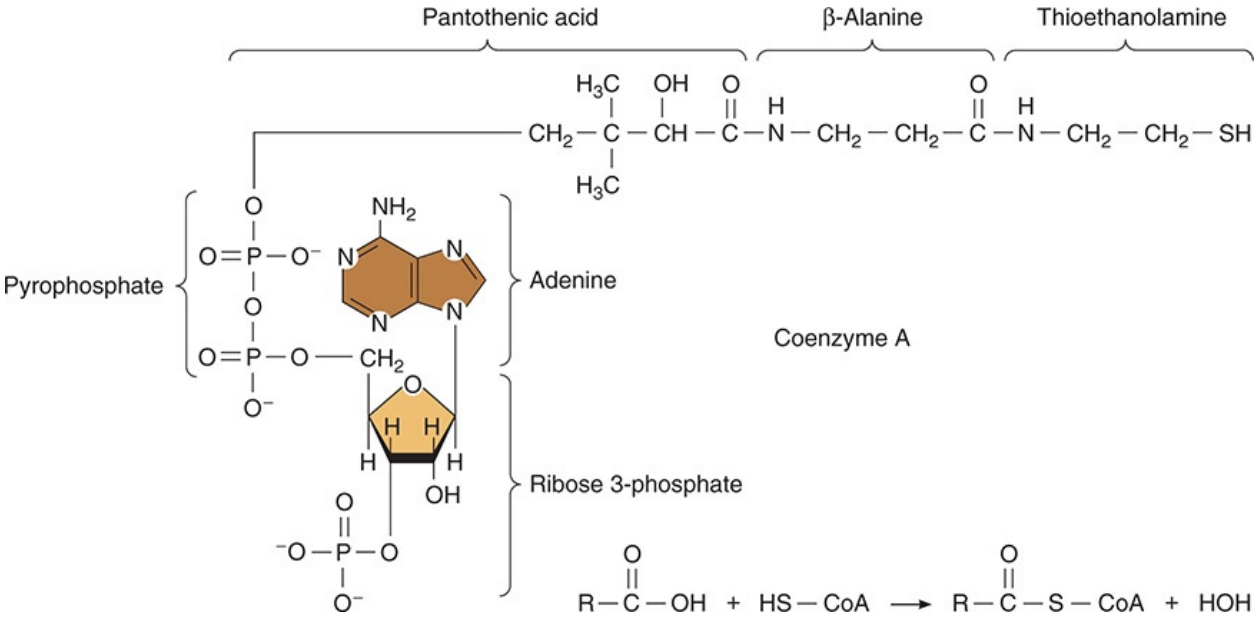


FIGURE 1–6 Coenzyme A (CoA) and its derivatives. Left: Formula of reduced coenzyme A (HS-CoA) with its components highlighted. **Right:** Formula for reaction of CoA with biologically important compounds to form thioesters. R, remainder of molecule.

BIOLOGICAL OXIDATIONS

Oxidation is the combination of a substance with O_2, or loss of hydrogen, or loss of electrons. The corresponding reverse processes are called **reduction.** Biological oxidations are catalyzed by specific enzymes. Cofactors (simple ions) or coenzymes (organic, nonprotein substances) are accessory substances that usually act as carriers for products of the reaction. Unlike the enzymes, the coenzymes may catalyze a variety of reactions.

A number of coenzymes serve as hydrogen acceptors. One common form of biological oxidation is removal of hydrogen from an R–OH group, forming R=O. In such dehydrogenation reactions, nicotinamide adenine dinucleotide (NAD^+) and dihydronicotinamide adenine dinucleotide phosphate ($NADP^+$) pick up hydrogen, forming dihydronicotinamide adenine dinucleotide (NADH) and dihydronicotinamide adenine dinucleotide phosphate (NADPH) **(Figure 1–7)**. The hydrogen is then transferred to the flavoprotein–cytochrome system, reoxidizing the NAD^+ and $NADP^+$. Flavin adenine dinucleotide (FAD) is formed when riboflavin is phosphorylated, forming flavin mononucleotide (FMN). FMN then combines with AMP, forming the dinucleotide. FAD can accept hydrogens

in a similar fashion, forming its hydro (FADH) and dihydro ($FADH_2$) derivatives.

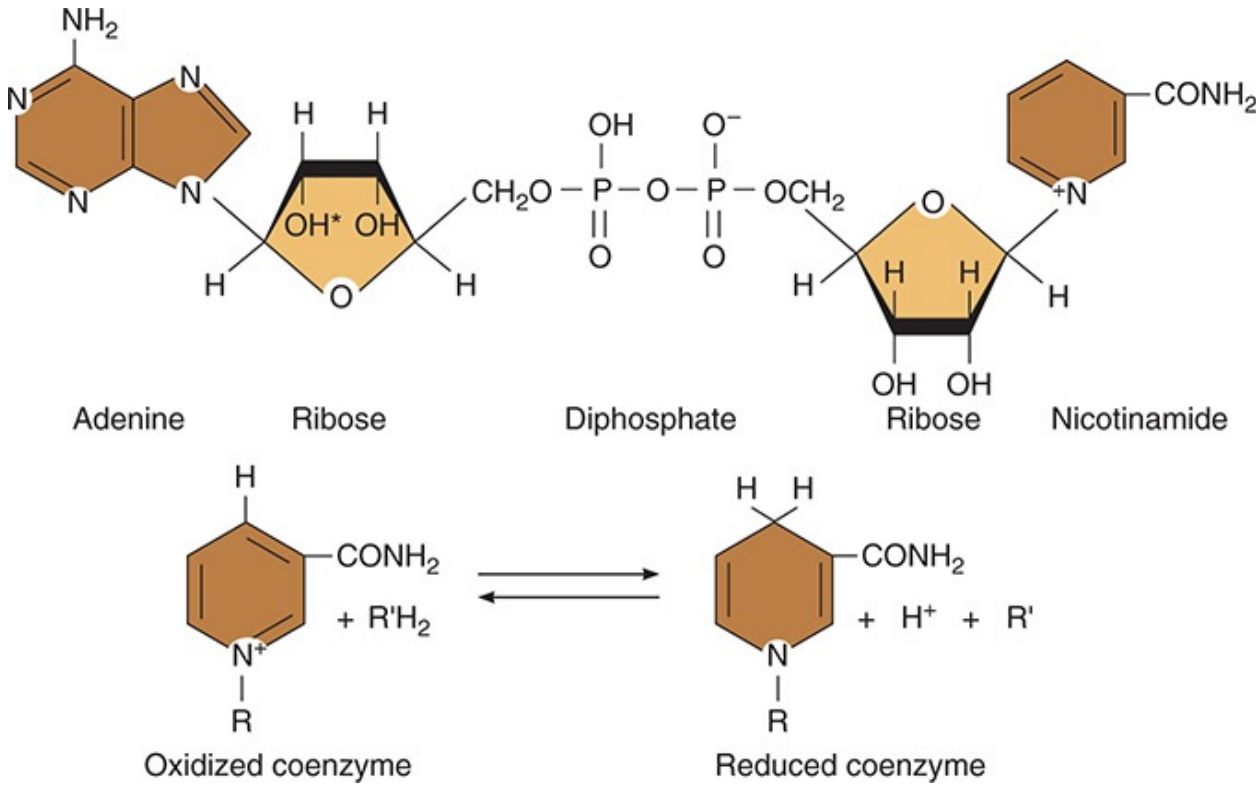


FIGURE 1–7 Structures of molecules important in oxidation–reduction reactions to produce energy. Top: Formula of the oxidized form of nicotinamide adenine dinucleotide (NAD^+). Nicotinamide adenine dinucleotide phosphate ($NADP^+$) has an additional phosphate group at the location marked by the asterisk. **Bottom:** Reaction by which NAD^+ and $NADP^+$ become reduced to form NADH and NADPH. R, remainder of molecule; R′, hydrogen donor.

The flavoprotein–cytochrome system is a chain of enzymes that transfers hydrogen to oxygen, forming water. This process occurs in the mitochondria. Each enzyme in the chain is reduced and then reoxidized as the hydrogen is passed down the line. Each of the enzymes is a protein with an attached nonprotein prosthetic group. The final enzyme in the chain is cytochrome c oxidase, which transfers hydrogens to O_2, forming H_2O. It contains two atoms of Fe and three of Cu and has 13 subunits.

The principal process by which ATP is formed in the body is **oxidative phosphorylation.** This process harnesses the energy from a proton gradient across the mitochondrial membrane to produce the high-energy bond of ATP

(see Figure 2–4 for more detail). Ninety percent of the O_2 consumption, the amount of oxygen used by the body per minute, in the basal state is in mitochondria, and 80% of the mitochondrial O_2 consumption is coupled to ATP synthesis.

ATP is utilized throughout the cell, with the bulk used in a handful of processes: approximately 27% is used for protein synthesis, 24% by Na, K ATPase to help set membrane potential, 9% by gluconeogenesis, 6% by Ca^{2+} ATPase to maintain a low cytosolic Ca^{2+} concentration, 5% by myosin ATPase, and 3% by ureagenesis.

MOLECULAR BUILDING BLOCKS

NUCLEOSIDES, NUCLEOTIDES, & NUCLEIC ACIDS

Nucleosides contain a sugar linked to a nitrogen-containing base. The physiologically important bases, **purines** and **pyrimidines,** have ring structures **(Figure 1–8)**. These structures are bound to a sugar, either ribose or 2-deoxyribose, to complete the nucleoside. When inorganic phosphate is added to the nucleoside, a **nucleotide** is formed **(Figure 1–9)**. Nucleosides and nucleotides form the backbone for RNA and DNA, as well as a variety of coenzymes and regulatory molecules of physiological importance (eg, NAD^+, $NADP^+$, and ATP) **(Table 1–2)**. Nucleic acids in the diet are digested and their constituent purines and pyrimidines absorbed, but most of the purines and pyrimidines are synthesized from amino acids, principally in the liver. The nucleotides and RNA and DNA are then synthesized. RNA is in dynamic equilibrium with the amino acid pool, but DNA, once formed, is metabolically stable throughout life. The purines and pyrimidines released by the breakdown of nucleotides may be reused or catabolized. Minor amounts are excreted unchanged in the urine.

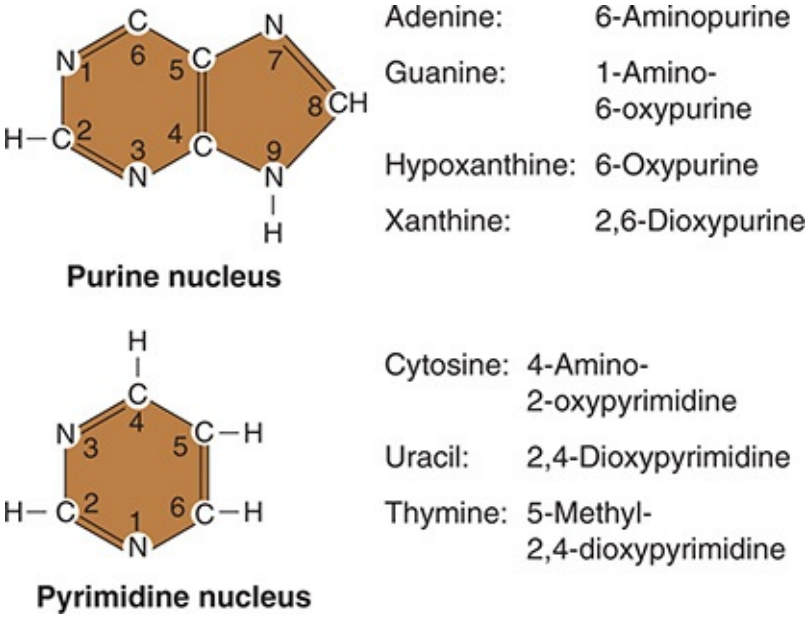


FIGURE 1–8 Principal physiologically important purines and pyrimidines. Purine and pyrimidine structures are shown next to representative molecules from each group. Oxypurines and oxypyrimidines may form enol derivatives (hydroxypurines and hydroxypyrimidines) by migration of hydrogen to the oxygen substituents.

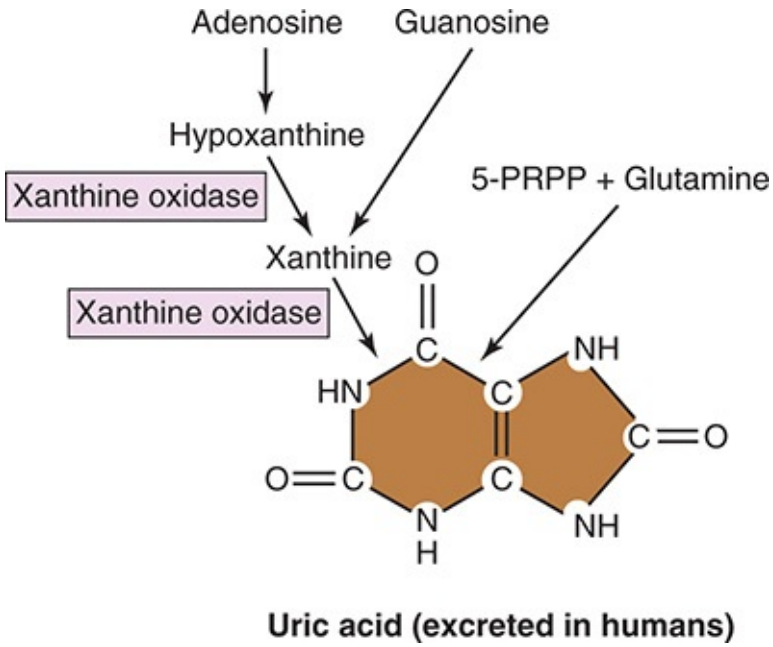


FIGURE 1–9 Synthesis and breakdown of uric acid. Adenosine is converted to hypoxanthine, which is then converted to xanthine, and xanthine is converted to uric acid. The latter two reactions are both catalyzed by xanthine oxidase.

Guanosine is converted directly to xanthine, while 5-PRPP and glutamine can be converted to uric acid.

TABLE 1–2 Purine- and pyrimidine-containing compounds.

Type of Compound	Components
Nucleoside	Purine or pyrimidine plus ribose or 2-deoxyribose
Nucleotide (mononucleotide)	Nucleoside plus phosphoric acid residue
Nucleic acid	Many nucleotides forming double-helical structures of two polynucleotide chains
Nucleoprotein	Nucleic acid plus one or more simple basic proteins
Contain ribose	RNA
Contain 2-deoxyribose	DNA

The pyrimidines are catabolized to the **β-amino acids,** β-alanine, and β-aminoisobutyrate. These amino acids have their amino group on β-carbon, rather than the α-carbon typical to physiologically active amino acids. Because β-aminoisobutyrate is a product of thymine degradation, it can serve as a measure of DNA turnover. The β-amino acids are further degraded to CO_2 and NH_3.

Uric acid is formed by the breakdown of purines and by direct synthesis from 5-phosphoribosyl pyrophosphate (5-PRPP) and glutamine (Figure 1–9). In humans, uric acid is excreted in the urine, but in other mammals, uric acid is further oxidized to allantoin before excretion. The normal blood uric acid level in humans is approximately 4 mg/dL (0.24 mmol/L). In the kidney, uric acid is filtered, reabsorbed, and secreted. Normally, 98% of the filtered uric acid is reabsorbed and the remaining 2% makes up approximately 20% of the amount excreted. The remaining 80% comes from the tubular secretion. The uric acid excretion on a purine-free diet is about 0.5 g/24 h and on a regular diet about 1 g/24 h. Excess uric acid in the blood or urine is a characteristic of gout **(Clinical Box 1–4)**.

CLINICAL BOX 1–4

Gout

Gout is a disease characterized by recurrent attacks of arthritis; urate (a salt derived from uric acid) deposits in the joints, kidneys, and other tissues; and elevated blood and urine uric acid levels. The joint most commonly affected initially is the metatarsophalangeal joint of the great toe. There are two forms of "primary" gout. In one, uric acid production is increased because of various enzyme abnormalities. In the other, there is a selective deficit in renal tubular transport of uric acid. In "secondary" gout, the uric acid levels in the body fluids are elevated as a result of decreased excretion or increased production secondary to some other disease process. For example, excretion is decreased in patients treated with thiazide diuretics and those with renal disease. Production is increased in leukemia and pneumonia because of increased breakdown of uric acid-rich white blood cells.

THERAPEUTIC HIGHLIGHTS

The treatment of gout is aimed at relieving the acute arthritis with drugs such as colchicine or nonsteroidal anti-inflammatory drugs (NSAIDs) and decreasing the uric acid level in the blood. Colchicine does not affect uric acid metabolism, and it apparently relieves gouty attacks by inhibiting the phagocytosis of uric acid crystals by leukocytes, a process that in some way produces the joint symptoms. Phenylbutazone and probenecid inhibit uric acid reabsorption in the renal tubules. Allopurinol, which directly inhibits xanthine oxidase in the purine degradation pathway, is used to decrease uric acid production.

DNA

DNA is found in bacteria, in the nuclei of eukaryotic cells, and in mitochondria. It is made up of two extremely long nucleotide chains containing the bases adenine (A), guanine (G), thymine (T), and cytosine (C) **(Figure 1–10)**. The chains are bound together by hydrogen bonding between the bases, with A bonding to T and G to C. This stable association forms a double-helical structure **(Figure 1–11)**. The double helical structure of DNA is compacted in the cell by association with **histones,** and further compacted into **chromosomes.** A diploid

human cell contains 46 chromosomes.

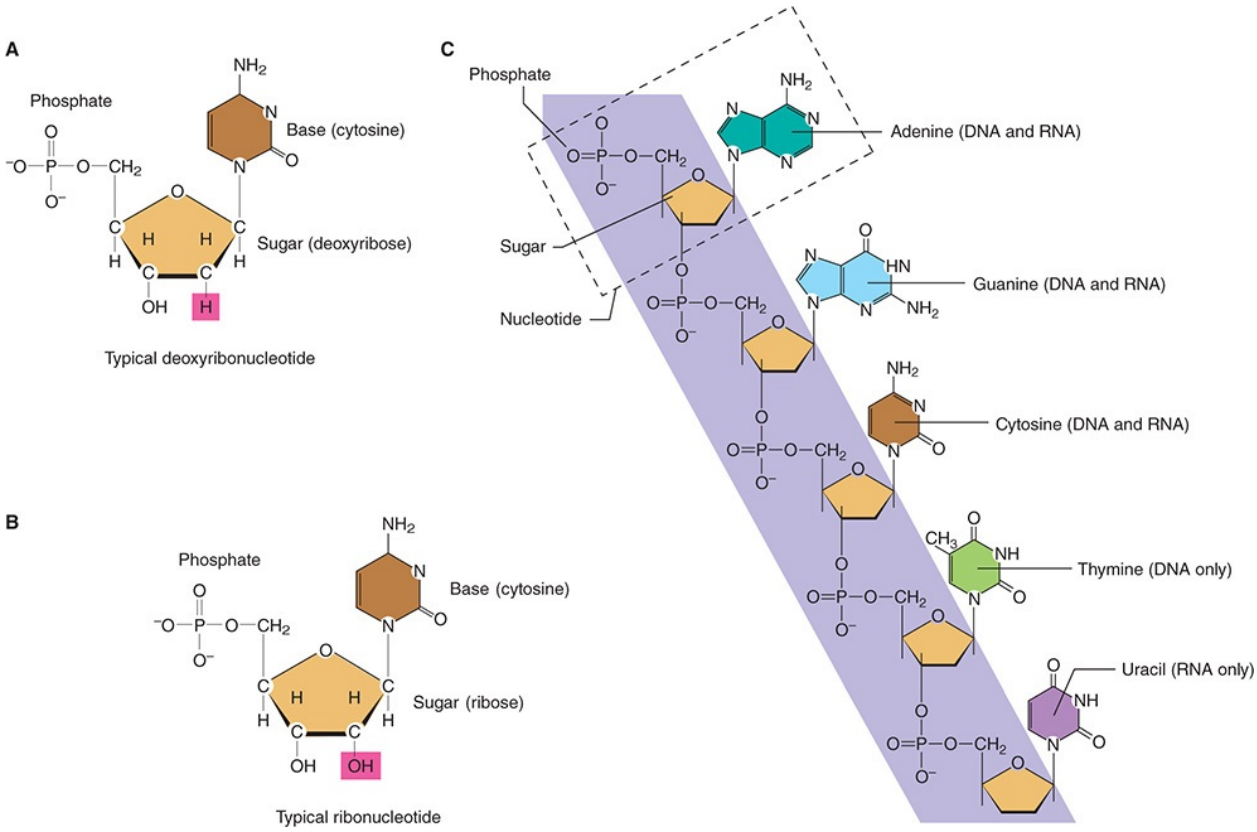


FIGURE 1–10 Basic structure of nucleotides and nucleic acids. A and B) The nucleotide cytosine is shown with deoxyribose and with ribose as the principal sugar. **C)** Purine bases adenine and guanine are bound to each other or to pyrimidine bases, cytosine, thymine, or uracil via a phosphodiester backbone between 2′-deoxyribosyl moieties attached to the nucleobases by an N-glycosidic bond. Note that the backbone has a polarity (ie, a 5′ and a 3′ direction). Thymine is only found in DNA, while uracil is only found in RNA.

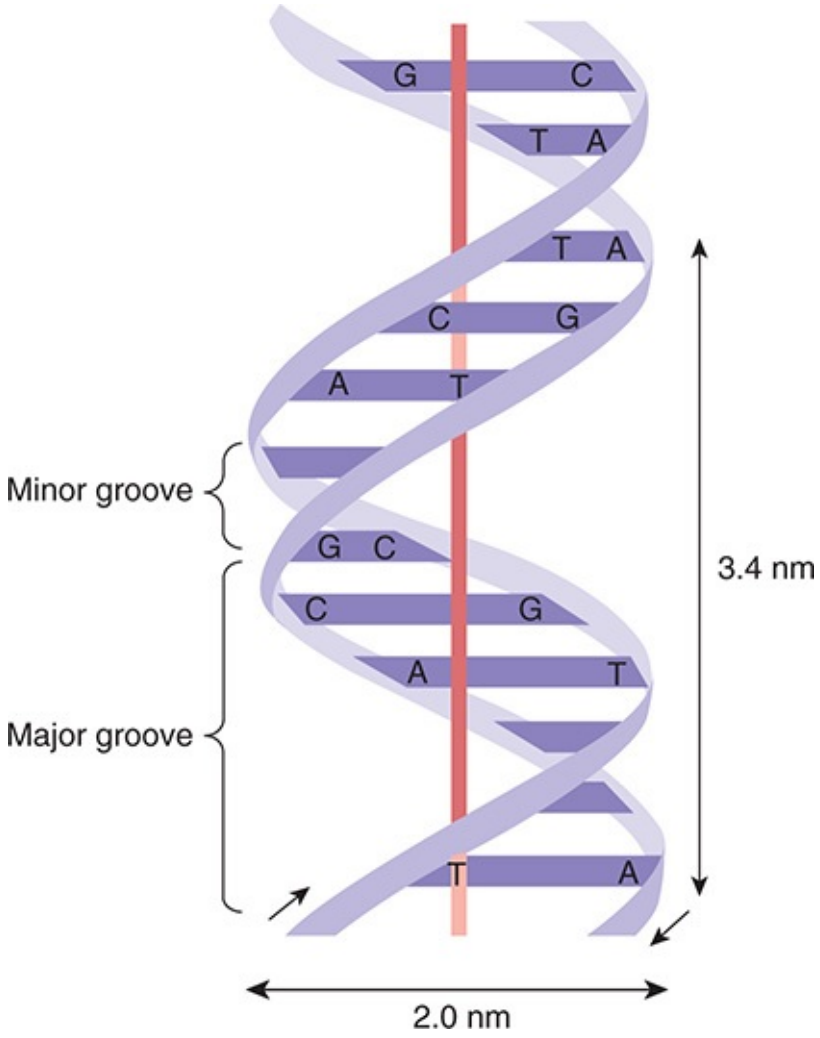


FIGURE 1–11 Double-helical structure of DNA. The compact structure has an approximately 2.0 nm thickness and 3.4 nm between full turns of the helix that contains both major and minor grooves. The structure is maintained in the double helix by hydrogen bonding between purines and pyrimidines across individual strands of DNA. Adenine (A) is bound to thymine (T) and cytosine (C) to guanine (G). (Reproduced with permission from Murray RK et al: *Harper's Biochemistry*, 28th ed. New York, NY: McGraw-Hill; 2009.)

A fundamental unit of DNA, or a **gene,** can be defined as the sequence of DNA nucleotides that contain the information for the production of an ordered amino acid sequence for a single polypeptide chain. Interestingly, the protein encoded by a single gene may be subsequently divided into several different physiologically active proteins. Information is accumulating at an accelerating rate about the structure of genes and their regulation. The basic structure of a typical eukaryotic gene is shown in diagrammatic form in **Figure 1–12**. It is

made up of a strand of DNA that includes coding and noncoding regions. In eukaryotes, unlike prokaryotes, the portions of the genes that dictate the formation of proteins are usually broken into several segments **(exons)** separated by segments that are not translated **(introns).** Near the transcription start site of the gene is a **promoter,** which is the site at which RNA polymerase and its cofactors bind. It often includes a thymidine–adenine–thymidine–adenine (TATA) sequence **(TATA box),** which ensures that transcription starts at the proper point. Farther out in the 5′ region are **regulatory elements,** which include enhancer and silencer sequences. It has been estimated that each gene has an average of five regulatory sites. Regulatory sequences are sometimes found in the 3′-flanking region as well. In a diploid cell, each gene will have two **alleles,** or versions of that gene. Each allele occupies the same position on the homologous chromosome. Individual alleles can confer slightly different properties of the gene when fully transcribed. It is interesting to note that changes in single nucleotides within or outside coding regions of a gene **(single nucleotide polymorphisms; SNPs)** can have great consequences for gene function. The study of SNPs in human disease is a growing and exciting area of genetic research.

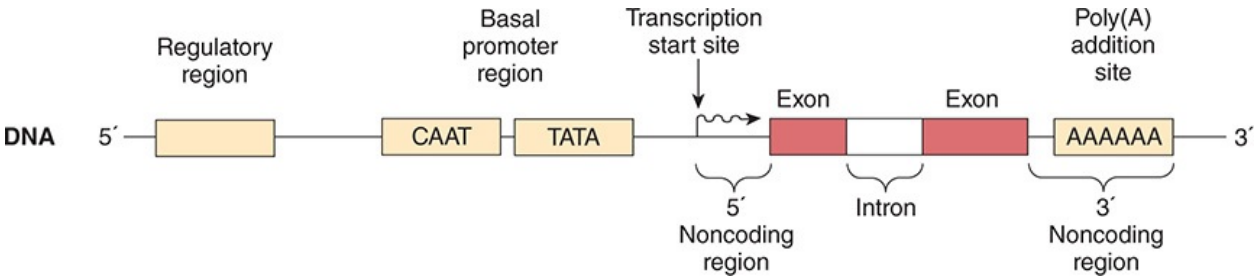


FIGURE 1–12 Diagram of the components of a typical eukaryotic gene. The region that produces introns and exons is flanked by noncoding regions. The 5′-flanking region contains stretches of DNA that interact with proteins to facilitate or inhibit transcription. The 3′-flanking region contains the poly(A) addition site. (Modified with permission from Murray RK et al: *Harper's Biochemistry*, 28th ed. New York, NY: McGraw-Hill; 2009.)

Gene mutations occur when the base sequence in the DNA is altered from its original sequence. Alterations can be through insertions, deletions, or duplications. Such alterations can affect protein structure and be passed on to daughter cells after cell division. **Point mutations** are single base substitutions. A variety of chemical modifications (eg, alkylating or intercalating agents, or ionizing radiation) can lead to changes in DNA sequences and mutations. The

collection of genes within the full expression of DNA from an organism is termed its **genome.** An indication of the complexity of DNA in the human haploid genome (the total genetic message) is its size; it is made up of 3×10^9 base pairs that can code for approximately 30,000 genes. This genetic message is the blueprint for the heritable characteristics of the cell and its descendants. The proteins formed from the DNA blueprint include all the enzymes, and these in turn control the metabolism of the cell.

Each nucleated somatic cell in the body contains the full genetic message, yet there is great differentiation and specialization in the functions of the various types of adult cells. Only small parts of the message are normally transcribed. Thus, the genetic message is normally maintained in a repressed state. However, genes are controlled both spatially and temporally. The double helix requires highly regulated interaction by proteins to unravel for **replication, transcription,** or both.

REPLICATION: MITOSIS & MEIOSIS

At the time of each somatic cell division **(mitosis),** the two DNA chains separate, each serving as a template for the synthesis of a new complementary chain. DNA polymerase catalyzes this reaction. One of the double helices thus formed goes to one daughter cell and one goes to the other, so the amount of DNA in each daughter cell is the same as that in the parent cell. The life cycle of the cell that begins after mitosis is highly regulated and is termed the **cell cycle** **(Figure 1–13)**. The G_1 (or Gap 1) phase represents a period of cell growth and divides the end of mitosis from the DNA synthesis (or S) phase. Following DNA synthesis, the cell enters another period of cell growth, the G_2 (Gap 2) phase. The ending of this stage is marked by chromosome condensation and the beginning of mitosis (M stage).

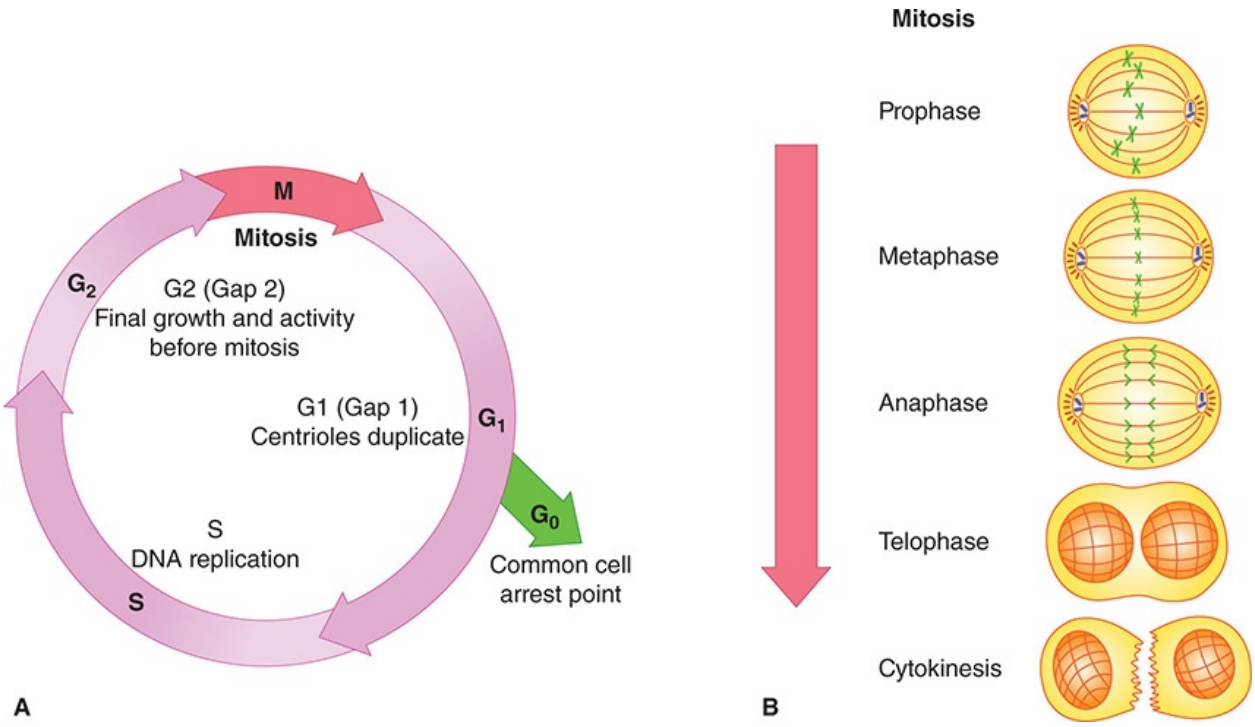


FIGURE 1–13 Sequence of events during the cell cycle. A) Immediately following mitosis (M) the cell enters a gap phase (G_1). At this point many cells will undergo cell arrest (G_0 phase). G_1 is followed by a DNA synthesis phase (S) a second gap phase (G_2) and back to mitosis. **B)** Stages of mitosis are highlighted.

In germ cells, reductive division **(meiosis)** takes place during maturation. The net result is that one of each pair of chromosomes ends up in each mature germ cell; consequently, each mature germ cell contains half the amount of chromosomal material found in somatic cells. Therefore, when a sperm unites with an ovum, the resulting zygote has the full complement of DNA, half of which came from the father and half from the mother. The term “ploidy” is sometimes used to refer to the number of chromosomes in cells. Normal resting diploid cells are **euploid** and become **tetraploid** just before division. **Aneuploidy** is the condition in which a cell contains other than the haploid number of chromosomes or an exact multiple of it, and this condition is common in cancerous cells.

RNA

The strands of the DNA double helix not only replicate themselves but also serve

as templates by lining up complementary bases for the formation in the nucleus of **RNA.** RNA differs from DNA in that it is single-stranded, has **uracil** (U) in place of thymine (T), and its sugar moiety is ribose rather than 2′-deoxyribose (Figure 1–10). The production of RNA from DNA is called **transcription.** Transcription can lead to several types of RNA including: **messenger RNA (mRNA), transfer RNA (tRNA), ribosomal RNA (rRNA),** and other RNAs. Transcription is catalyzed by various forms of **RNA polymerase.**

Typical transcription of an mRNA is shown in **Figure 1–14**. When suitably activated, transcription of the gene into a pre-mRNA starts at the **cap site** and ends about 20 bases beyond the AATAAA sequence. The RNA transcript is capped in the nucleus by addition of 7-methylguanosine triphosphate to the 5′ end; this cap is necessary for proper binding to the ribosome. A **poly(A) tail** of about 100 bases is added to the untranslated segment at the 3′ end to help maintain the stability of the mRNA. The pre-mRNA formed by capping and addition of the poly(A) tail is then processed by elimination of the introns, and once this posttranscriptional modification is complete, the mature mRNA moves to the cytoplasm. Posttranscriptional modification of the pre-mRNA is a regulated process where differential splicing can occur to form more than one mRNA from a single pre-mRNA. The introns of some genes are eliminated by **spliceosomes,** complex units that are made up of small RNAs and proteins. Other introns are eliminated by **self-splicing** by the RNA they contain. Because of introns and splicing, more than one mRNA can be formed from the same gene.

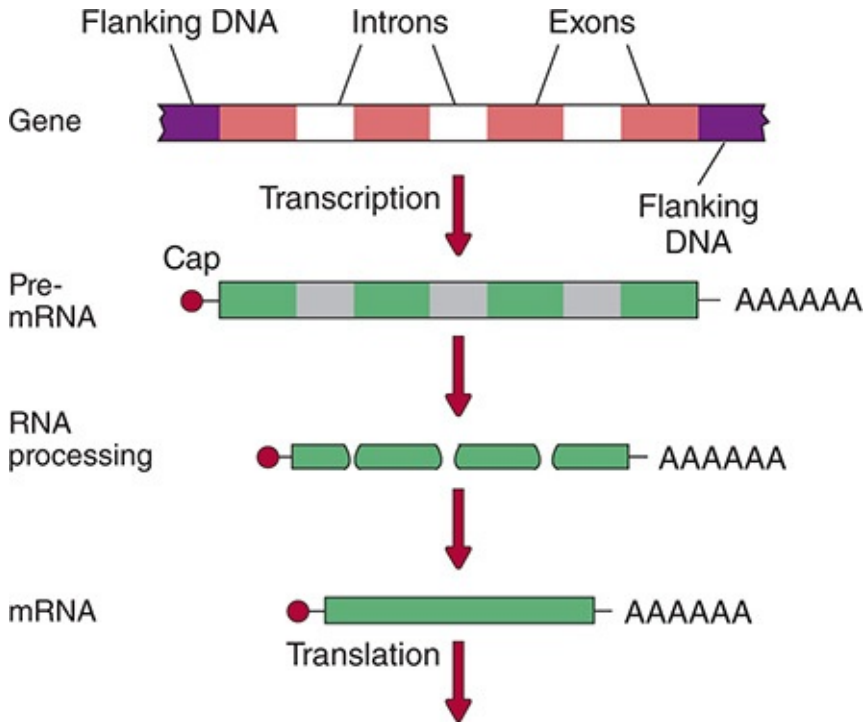


FIGURE 1–14 Transcription of a typical mRNA. Steps in transcription from a typical gene to a processed mRNA are shown. Cap, cap site; AAAAAA, poly(A) site.

Most forms of RNA in the cell are involved in **translation,** or protein synthesis. A brief outline of the transition from transcription to translation is shown in **Figure 1–15**. In the cytoplasm, ribosomes provide a template for tRNA to deliver specific amino acids to a growing polypeptide chain based on specific sequences in mRNA. The mRNA molecules are smaller than the DNA molecules, and each represents a transcript of a small segment of the DNA chain. For comparison, the molecules of tRNA contain only 70–80 nitrogenous bases, compared with hundreds in mRNA and 3 billion in DNA. A newer class of RNA, **microRNAs,** have recently been reported. MicroRNAs measure approximately 21–25-nucleotides in length and have been shown to negatively regulate gene expression at the posttranscriptional level. It is expected that roles for these small RNAs will continue to expand as research into their function continues.

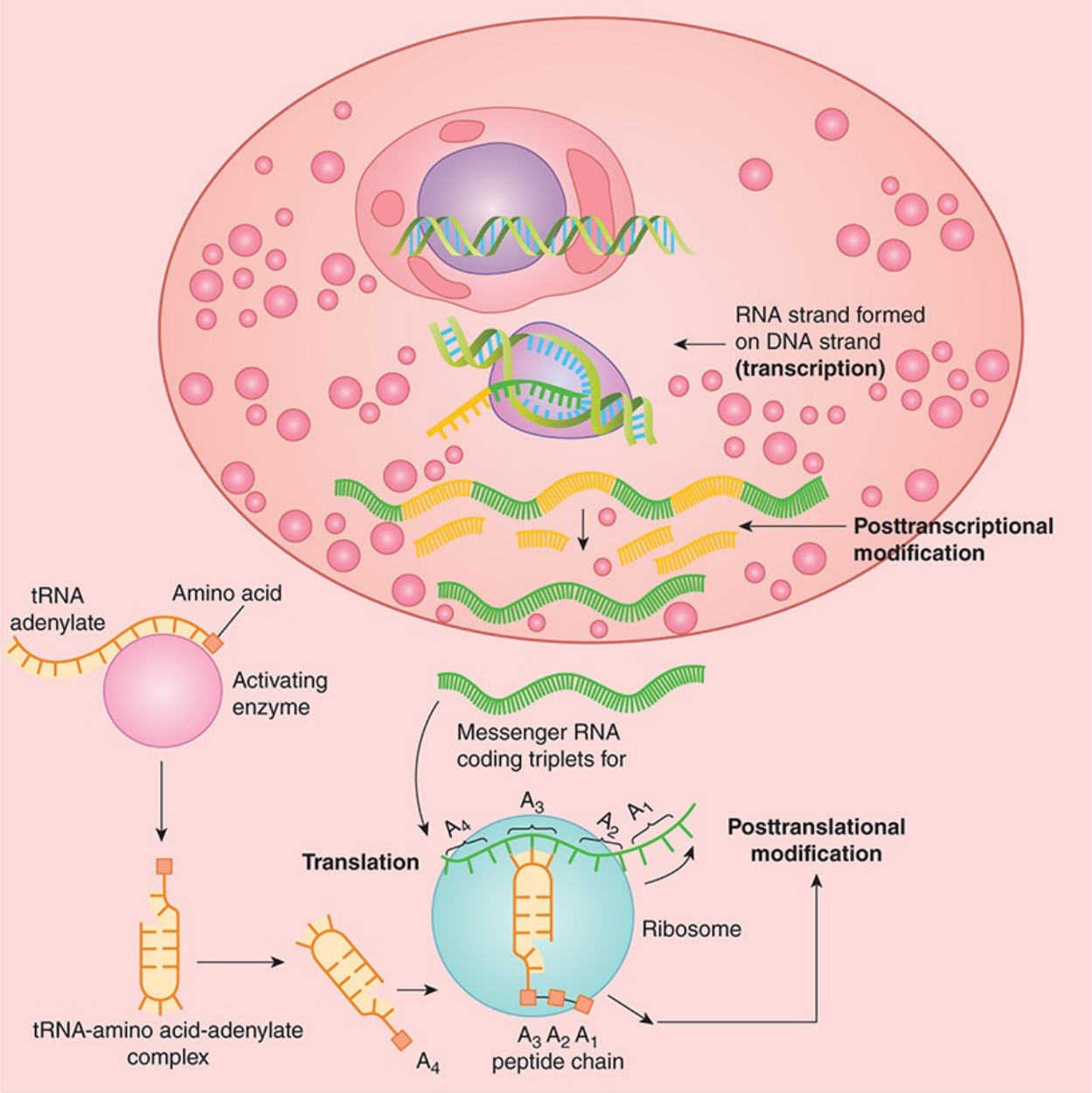


FIGURE 1–15 Diagrammatic outline of transcription to translation. In the nucleus, a messenger RNA is produced from the DNA molecule. This messenger RNA is processed and moved to the cytosol where it is presented to the ribosome. It is at the ribosome where charged tRNA match up with their complementary codons of mRNA to position the amino acid for growth of the polypeptide chain. The lines with multiple short projections in DNA and RNA represent individual bases. Small boxes labeled A represent individual amino acids.

AMINO ACIDS & PROTEINS

AMINO ACIDS

Amino acids that form the basic building blocks for proteins are identified in Table 1–3. These amino acids are often referred to by their corresponding three-letter, or single-letter abbreviations (eg, Ala or A for alanine). Various other important amino acids such as ornithine, 5-hydroxytryptophan, L-dopa, taurine, and thyroxine (T_4) are present in the body but are not found in proteins. In higher animals, the L isomers of the amino acids are the only naturally occurring forms in proteins. The L isomers of hormones such as thyroxine are much more active than the D isomers. The amino acids are acidic, neutral, or basic, depending on the relative proportions of free acidic (–COOH) or basic ($–NH_2$) groups in the molecule. Some of the amino acids are **nutritionally essential amino acids,** that is, they must be obtained in the diet, because they cannot be made in the body. Arginine and histidine must be provided through diet during times of rapid growth or recovery from illness and are termed **conditionally essential.** All others are **nonessential amino acids** in the sense that they can be synthesized in vivo in amounts sufficient to meet metabolic needs.

TABLE 1–3 Amino acids found in proteins.

Amino acids with aliphatic side chains
- Alanine (Ala, A)
- **Valine** (Val, V)
- **Leucine** (Leu, L)
- **Isoleucine** (Ile, I)

Hydroxyl-substituted amino acids
- Serine (Ser, S)
- **Threonine** (Thr, T)

Sulfur-containing amino acids
- Cysteine (Cys, C)
- **Methionine** (Met, M)
- Selenocysteine[a]

Amino acids with aromatic ring side chains
- **Phenylalanine** (Phe, F)
- Tyrosine (Tyr, Y)
- **Tryptophan** (Trp, W)

Amino acids with acidic side chains, or their amides
- Aspartic acid (Asp, D)
- Asparagine (Asn, N)
- Glutamine (Gln, Q)
- Glutamic acid (Glu, E)
- γ-Carboxyglutamic acid[b] (Gla)

Amino acids with side chains containing basic groups
- ***Arginine***[c] (Arg, R)
- **Lysine** (Lys, K)
- Hydroxylysine[b] (Hyl)
- ***Histidine***[c] (His, H)

Imino acids (contain imino group but no amino group)
- Proline (Pro, P)
- 4-Hydroxyproline[b] (Hyp)
- 3-Hydroxyproline[b]

Those in bold type are the nutritionally essential amino acids or conditionally essential amino acids. The generally accepted three-letter and one-letter abbreviations for the amino acids are shown in parentheses.

[a]Selenocysteine is a rare amino acid in which the sulfur of cysteine is replaced by selenium. The codon UGA is usually a stop codon, but in certain situations it codes for selenocysteine.

[b]There are no tRNAs for these four amino acids; they are formed by posttranslational modification of the corresponding unmodified amino acid in peptide linkage. There are tRNAs for selenocysteine and the remaining 20 amino acids, and they are incorporated into peptides and proteins under direct genetic control.

[c]Arginine and histidine are sometimes called "conditionally essential"—they are not necessary for maintenance of nitrogen balance, but are needed for normal growth (especially for children).

THE AMINO ACID POOL

Although small amounts of proteins are absorbed from the gastrointestinal tract and some peptides are also absorbed, most ingested proteins are digested into their constituent amino acids before absorption. Traditionally, peptides are

defined as molecules that consist of 2–100 amino acids, while proteins are made up of 100 or more amino acids. The body's proteins are being continuously hydrolyzed to amino acids and resynthesized. The turnover rate of endogenous proteins averages 80–100 g/day, being highest in the intestinal mucosa and practically nil in the extracellular structural protein, collagen. The amino acids formed by endogenous protein breakdown are identical to those derived from ingested protein. Together they form a common **amino acid pool** that supplies the needs of the body **(Figure 1–16)**.

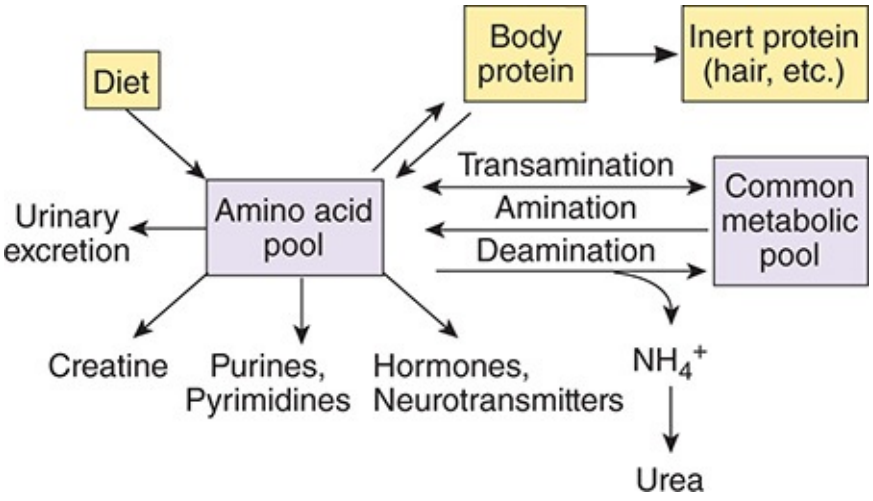


FIGURE 1–16 Amino acids in the body. There is an extensive network of amino acid turnover in the body. Boxes represent large pools of amino acids and some of the common interchanges are represented by arrows. Note that most amino acids come from the diet and end up in protein; however, a large portion of amino acids are interconverted and can feed into and out of a common metabolic pool through amination reactions.

PROTEINS

Proteins are made up of large numbers of amino acids linked into chains by **peptide bonds** joining the amino group of one amino acid to the carboxyl group of the next **(Figure 1–17)**. In addition, some proteins contain carbohydrates (glycoproteins) and lipids (lipoproteins). Smaller chains of amino acids are called **peptides** or **polypeptides.** The boundaries between peptides, polypeptides, and proteins are not well defined. For this text, amino acid chains containing 2–10 amino acid residues are called peptides, chains containing more than 10 but fewer than 100 amino acid residues are called polypeptides, and

chains containing 100 or more amino acid residues are called proteins.

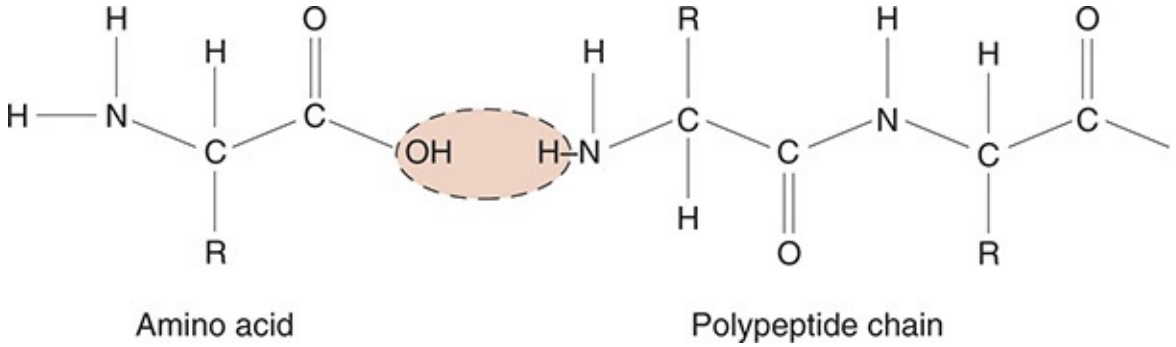


FIGURE 1–17 Amino acid structure and formation of peptide bonds. The dashed line shows where peptide bonds are formed between two amino acids. The highlighted area is released as H_2O. R, remainder of the amino acid. For example, in glycine, R = H; in glutamate, R = —$(CH_2)_2$—COO^-.

The order of the amino acids in the peptide chains is called the **primary structure** of a protein. The chains are twisted and folded in complex ways, and the term **secondary structure** of a protein refers to the spatial arrangement produced by the twisting and folding. A common secondary structure is a regular coil with 3.7 amino acid residues per turn (α-helix). Another common secondary structure is a β-sheet. An antiparallel β-sheet is formed when extended polypeptide chains fold back and forth on one another and hydrogen bonding occurs between the peptide bonds on neighboring chains. Parallel β-sheets between polypeptide chains also occur. The **tertiary structure** of a protein is the arrangement of the twisted chains into layers, crystals, or fibers. Many protein molecules are made of several proteins, or subunits (eg, hemoglobin), and the term **quaternary structure** is used to refer to the arrangement of the subunits into a functional structure.

PROTEIN SYNTHESIS

The process of protein synthesis, **translation,** is the conversion of information encoded in mRNA to a protein (Figure 1–15). As described previously, when a definitive mRNA reaches a ribosome in the cytoplasm, it dictates the formation of a polypeptide chain. Amino acids in the cytoplasm are activated by combination with an enzyme and AMP (adenylate), and each **activated amino acid** then combines with a specific molecule of tRNA. There is at least one

tRNA for each of the 20 unmodified amino acids found in large quantities in the body proteins of animals, but some amino acids have more than one tRNA. The tRNA–amino acid–adenylate complex is next attached to the mRNA template, a process that occurs in the ribosomes. The tRNA "recognizes" the proper spot to attach on the mRNA template because it has on its active end a set of three bases that are complementary to a set of three bases in a particular spot on the mRNA chain. The genetic code is made up of such triplets **(codons),** sequences of three purine, pyrimidine, or purine and pyrimidine bases; each codon stands for a particular amino acid.

Translation typically starts in the ribosomes with an AUG (transcribed from ATG in the gene), which codes for methionine. The amino terminal (or N-terminal) amino acid is then added, and the chain is lengthened one amino acid at a time. The mRNA attaches to the 40S subunit of the ribosome during protein synthesis, the polypeptide chain being formed attaches to the 60S subunit, and the tRNA attaches to both. As the amino acids are added in the order dictated by the codon, the ribosome moves along the mRNA molecule like a bead on a string. Translation stops at one of three stop, or nonsense, codons (UGA, UAA, or UAG), and the polypeptide chain is released. The tRNA molecules are used again. The mRNA molecules are typically reused approximately 10 times before being replaced. It is common to have more than one ribosome on a given mRNA chain at a time. The mRNA chain plus its collection of ribosomes is visible under the electron microscope as an aggregation of ribosomes called a **polyribosome.**

POSTTRANSLATIONAL MODIFICATION

After the polypeptide chain is formed, it "folds" into its biological form and can be further modified to the final protein by one or more of a combination of reactions that include hydroxylation, carboxylation, glycosylation, or phosphorylation of amino acid residues; cleavage of peptide bonds that converts a larger polypeptide to a smaller form; and the further folding, packaging, or folding and packaging of the protein into its ultimate, often complex configuration. Protein folding is a complex process that is dictated primarily by the sequence of the amino acids in the polypeptide chain. In some instances, however, nascent proteins associate with other proteins called **chaperones,** which prevent inappropriate contacts with other proteins and ensure that the final "proper" conformation of the nascent protein is reached.

Proteins also contain information that helps direct them to individual cell

compartments. Many proteins that are destined to be secreted or stored in organelles and most transmembrane proteins have at their amino terminal a **signal peptide (leader sequence)** that guides them into the endoplasmic reticulum. The sequence is made up of 15–30 predominantly hydrophobic amino acid residues. The signal peptide, once synthesized, binds to a **signal recognition particle (SRP),** a complex molecule made up of six polypeptides and 7S RNA, one of the small RNAs. The SRP stops translation until it binds to a **translocon,** a pore in the endoplasmic reticulum that is a heterotrimeric structure made up of Sec 61 proteins. The ribosome also binds, and the signal peptide leads the growing peptide chain into the cavity of the endoplasmic reticulum **(Figure 1–18)**. The signal peptide is next cleaved from the rest of the peptide by a signal peptidase while the rest of the peptide chain is still being synthesized. SRPs are not the only signals that help direct proteins to their proper place in or out of the cell; other signal sequences, posttranslational modifications, or both (eg, glycosylation) can serve this function.

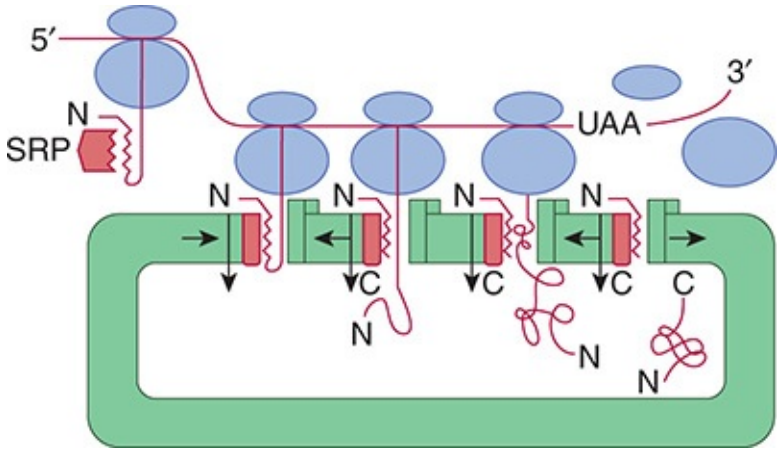


FIGURE 1–18 Translation of protein into the endoplasmic reticulum according to the signal hypothesis. The ribosomes synthesizing a protein move along the mRNA from the 5′ to the 3′ end. When the signal peptide of a protein destined for secretion, the cell membrane, or lysosomes emerges from the large unit of the ribosome, it binds to a signal recognition particle (SRP), and this arrests further translation until it binds to the translocon on the endoplasmic reticulum. C, carboxyl end of protein; N, amino end of protein. (Reproduced with permission from Perara E, Lingappa VR: Transport of proteins into and across the endoplasmic reticulum membrane. In: *Protein Transfer and Organelle Biogenesis*. Das RC, Robbins PW (editors). Academic Press, 1988.)

UBIQUITINATION & PROTEIN DEGRADATION

Like protein synthesis, protein degradation is a carefully regulated, complex process. It has been estimated that overall, up to 30% of newly produced proteins are abnormal, such as can occur during improper folding. Aged normal proteins also need to be removed as they are replaced. Conjugation of proteins to the 74-amino-acid polypeptide **ubiquitin** marks them for degradation. This polypeptide is highly conserved and is present in species ranging from bacteria to humans. The process of binding ubiquitin is called **ubiquitination,** and in some instances, multiple ubiquitin molecules bind **(polyubiquitination).** Ubiquitination of cytoplasmic proteins, including integral proteins of the endoplasmic reticulum, can mark the proteins for degradation in multisubunit proteolytic particles, or **proteasomes.** Ubiquitination of membrane proteins, such as the growth hormone receptors, also marks them for degradation; however, these can be degraded in lysosomes as well as via the proteasomes. Alteration of proteins by ubiquitin or the small ubiquitin-related modifier **(SUMO),** however, does not necessarily lead to degradation. More recently it has been shown that these posttranslational modifications can play important roles in protein–protein interactions and various cellular signaling pathways.

There is an obvious balance between the rate of production of a protein and its destruction, so ubiquitin conjugation is of major importance in cellular physiology. The rates at which individual proteins are metabolized vary, and the body has mechanisms by which abnormal proteins are recognized and degraded more rapidly than normal body constituents. For example, abnormal hemoglobins are metabolized rapidly in individuals with congenital hemoglobinopathies (see Chapter 31).

CATABOLISM OF AMINO ACIDS

The short-chain fragments produced by amino acid, carbohydrate, and fat catabolism are very similar (see below). From this **common metabolic pool** (Figure 1–16) of intermediates, carbohydrates, proteins, and fats can be synthesized. These fragments can enter the citric acid cycle (or tricarboxylic acid cycle or Krebs cycle), a final common pathway of catabolism, in which they are broken down to hydrogen atoms and CO_2. Interconversion of amino acids involves transfer, removal, or formation of amino groups. **Transamination** reactions, conversion of one amino acid to the corresponding keto acid with simultaneous conversion of another keto acid to an amino acid, occur in many

tissues:

$$\text{Alanine} + \alpha\text{–Ketoglutarate} \rightleftharpoons \text{Pyruvate} + \text{Glutamate}$$

Oxidative deamination of amino acids occurs in the liver. An imino acid is formed by dehydrogenation, and this compound is hydrolyzed to the corresponding keto acid, with production of NH_4^+:

$$\text{Amino acid} + NAD^+ \rightarrow \text{Imino acid} + NADH + H^+$$

$$\text{Imino acid} + H_2O \rightarrow \text{Keto acid} + NH_4^+$$

Interconversions between the amino acid pool and the common metabolic pool are summarized in **Figure 1–19**. Leucine, isoleucine, phenylalanine, and tyrosine are said to be **ketogenic** because they are converted to the ketone body acetoacetate (see below). Alanine and many other amino acids are **glucogenic** or **gluconeogenic;** that is, they give rise to compounds that can readily be converted to glucose.

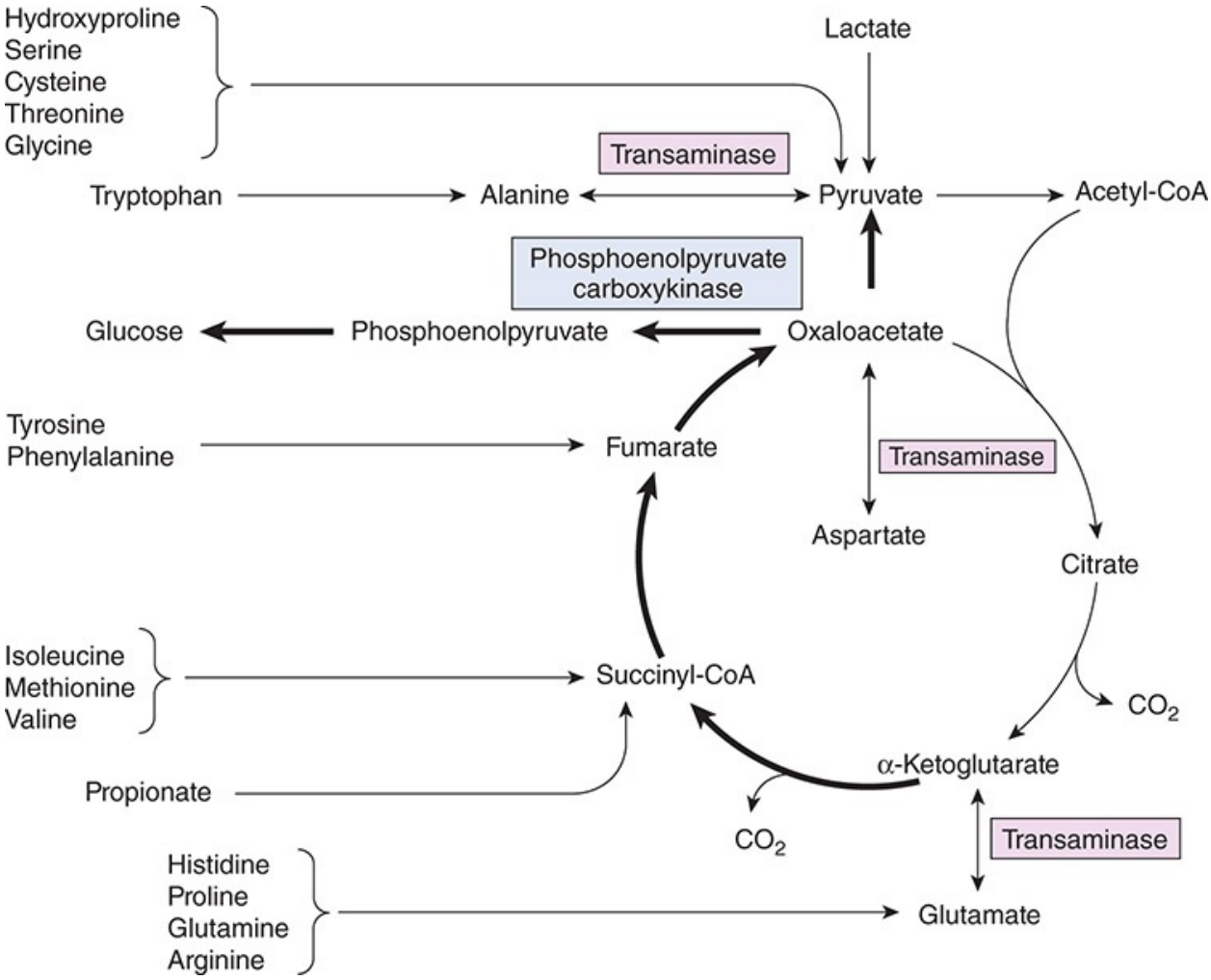


FIGURE 1–19 Involvement of the citric acid cycle in transamination and gluconeogenesis. The bold arrows indicate the main pathway of gluconeogenesis. Note the many entry positions for groups of amino acids into the citric acid cycle. (Reproduced with permission from Murray RK et al: *Harper's Biochemistry*, 28th ed. New York, NY: McGraw-Hill; 2009.)

UREA FORMATION

Most of the NH_4^+ formed by deamination of amino acids in the liver is converted to urea, and the urea is excreted in the urine. The NH_4^+ forms carbamoyl phosphate, and in the mitochondria it is transferred to ornithine, forming citrulline. The enzyme involved is ornithine carbamoyltransferase. Citrulline is converted to arginine, after which urea is split off and ornithine is regenerated (urea cycle; **Figure 1–20**). The overall reaction in the urea cycle

consumes 3 ATP (not shown) and thus requires significant energy. Most of the urea is formed in the liver, and in severe liver disease, the blood urea nitrogen (BUN) falls and blood NH_3 rises (see Chapter 28). Congenital deficiency of ornithine carbamoyltransferase can also lead to NH_3 intoxication.

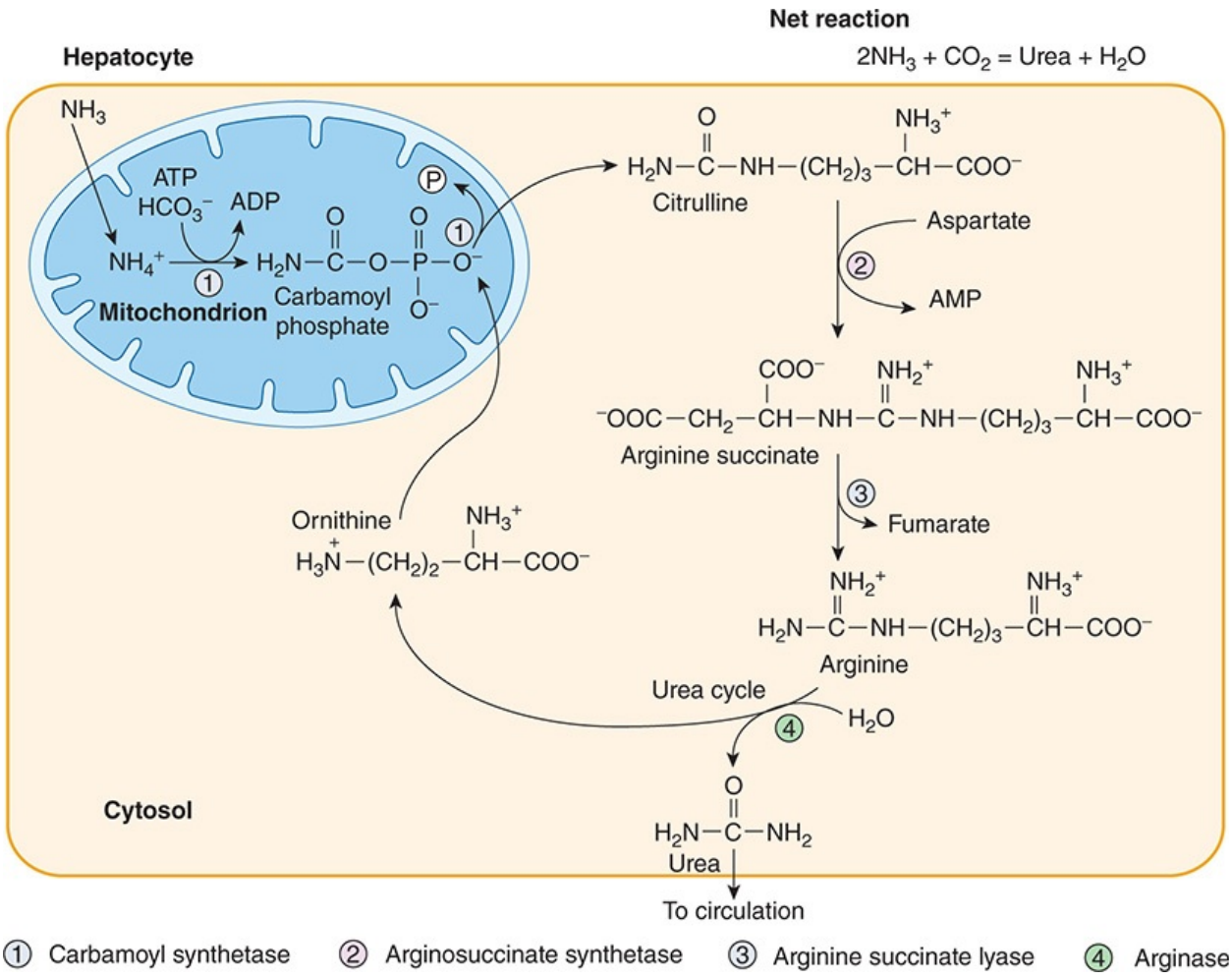


FIGURE 1–20 Urea cycle. The processing of NH_3 to urea for excretion contains coordinative steps in both the cytosol and the mitochondrion of a hepatocyte. Note that the production of carbamoyl phosphate and its conversion to citrulline occurs in the mitochondria, whereas other processes are in the cytoplasm.

METABOLIC FUNCTIONS OF AMINO ACIDS

In addition to providing the basic building blocks for proteins, amino acids also have metabolic functions. Thyroid hormones, catecholamines, histamine,

serotonin, melatonin, and intermediates in the urea cycle are formed from specific amino acids. Methionine and cysteine provide the sulfur contained in proteins, CoA, taurine, and other biologically important compounds. Methionine is converted into S-adenosylmethionine, which is the active methylating agent in the synthesis of compounds such as epinephrine.

CARBOHYDRATES

Carbohydrates are organic molecules made of equal amounts of carbon and H_2O. The simple sugars, or **monosaccharides,** including **pentoses** (five carbons; eg, ribose) and **hexoses** (six carbons; eg, glucose) perform both structural (eg, as part of nucleotides discussed previously) and functional (eg, inositol 1,4,5 trisphosphate acts as a cellular signaling molecule) roles in the body. Monosaccharides can be linked together to form disaccharides (eg, sucrose), or polysaccharides (eg, glycogen). The placement of sugar moieties onto proteins (glycoproteins) aids in cellular targeting, and in the case of some receptors, recognition of signaling molecules. In this section, the major role of carbohydrates in the production and storage of energy will be discussed.

Dietary carbohydrates are for the most part polymers of hexoses, of which the most important are glucose, galactose, and fructose. Most of the monosaccharides occurring in the body are the D isomers. The principal product of carbohydrate digestion and the principal circulating sugar is glucose. The normal fasting level of plasma glucose in peripheral venous blood is 70–110 mg/dL (3.9–6.1 mmol/L). In arterial blood, the plasma glucose level is 15–30 mg/dL higher than in venous blood.

Once it enters cells, glucose is normally phosphorylated to form glucose-6-phosphate. The enzyme that catalyzes this reaction is **hexokinase.** In the liver, there is an additional enzyme, **glucokinase,** which has greater specificity for glucose and which, unlike hexokinase, is increased by insulin and decreased in starvation and diabetes. The glucose-6- phosphate is either polymerized into glycogen or catabolized. The process of glycogen formation is called **glycogenesis,** and glycogen breakdown is called **glycogenolysis.** Glycogen, the storage form of glucose, is present in most body tissues, but the major supplies are in the liver and skeletal muscle. The breakdown of glucose to pyruvate or lactate (or both) is called **glycolysis.** Glucose catabolism proceeds via cleavage through fructose to trioses or via oxidation and decarboxylation to pentoses. The pathway to pyruvate through the trioses is the **Embden–Meyerhof pathway,** and that through 6-phosphogluconate and the pentoses is the **direct oxidative**

pathway (hexose monophosphate shunt). Pyruvate is converted to acetyl-CoA. Interconversions between carbohydrate, fat, and protein include conversion of the glycerol from fats to dihydroxyacetone phosphate and conversion of a number of amino acids with carbon skeletons resembling intermediates in the Embden–Meyerhof pathway and citric acid cycle to these intermediates by deamination. In this way, and by conversion of lactate to glucose, nonglucose molecules can be converted to glucose **(gluconeogenesis).** Glucose can be converted to fats through acetyl-CoA, but because the conversion of pyruvate to acetyl-CoA, unlike most reactions in glycolysis, is irreversible, fats are not converted to glucose via this pathway. There is therefore very little net conversion of fats to carbohydrates in the body because, except for the quantitatively unimportant production from glycerol, there is no pathway for conversion.

CITRIC ACID CYCLE

The **citric acid cycle** (Krebs cycle, tricarboxylic acid cycle) produces ATP through a sequence of reactions in which acetyl-CoA is metabolized to CO_2 and H atoms. Acetyl-CoA is first condensed with the anion of a four-carbon acid, oxaloacetate, to form citrate and HS-CoA. In a series of seven subsequent reactions, 2 CO_2 molecules are split off, regenerating oxaloacetate **(Figure 1–21)**. Four pairs of H atoms are transferred to the flavoprotein–cytochrome chain, producing 12 ATP and 4 H_2O, of which 2 H_2O is used in the cycle. The citric acid cycle is the common pathway for oxidation to CO_2 and H_2O of carbohydrate, fat, and some amino acids. The major entry into it is through acetyl CoA, but a number of amino acids can be converted to citric acid cycle intermediates by deamination. The citric acid cycle requires O_2 and does not function under anaerobic conditions.

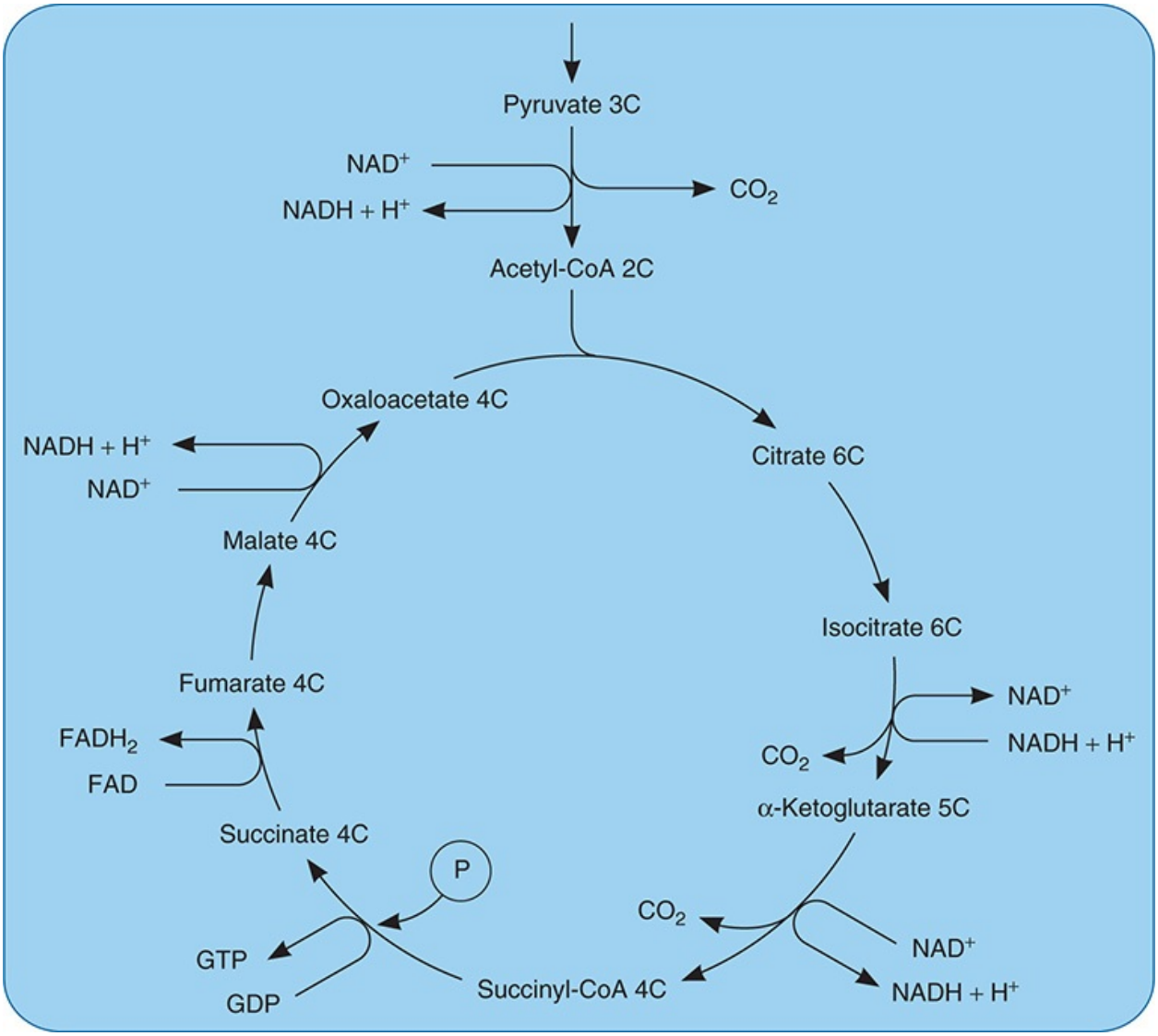


FIGURE 1–21 Citric acid cycle. The numbers (6C, 5C, etc.) indicate the number of carbon atoms in each of the intermediates. The conversion of pyruvate to acetyl-CoA and each turn of the cycle provide four NADH and one $FADH_2$ for oxidation via the flavoprotein-cytochrome chain plus formation of one GTP that is readily converted to ATP.

ENERGY PRODUCTION

The net production of energy-rich phosphate compounds during the metabolism of glucose and glycogen to pyruvate depends on whether metabolism occurs via the Embden–Meyerhof pathway or the hexose monophosphate shunt. By oxidation at the substrate level, the conversion of 1 mol of

phosphoglyceraldehyde to phosphoglycerate generates 1 mol of ATP, and the conversion of 1 mol of phosphoenolpyruvate to pyruvate generates another. Because 1 mol of glucose-6-phosphate produces, via the Embden–Meyerhof pathway, 2 mol of phosphoglyceraldehyde, 4 mol of ATP is generated per mole of glucose metabolized to pyruvate. All these reactions occur in the absence of O_2 and consequently represent anaerobic production of energy. However, 1 mol of ATP is used in forming fructose 1,6-diphosphate from fructose 6-phosphate and 1 mol in phosphorylating glucose when it enters the cell. Consequently, when pyruvate is formed anaerobically from glycogen, there is a *net* production of 3 mol of ATP per mole of glucose-6-phosphate; however, when pyruvate is formed from 1 mol of blood glucose, the net gain is only 2 mol of ATP.

A supply of NAD^+ is necessary for the conversion of phosphoglyceraldehyde to phosphoglycerate. Under anaerobic conditions (anaerobic glycolysis), a block of glycolysis at the phosphoglyceraldehyde conversion step might be expected to develop as soon as the available NAD^+ is converted to NADH. However, pyruvate can accept hydrogen from NADH, forming NAD^+ and lactate:

$$\text{Pyruvate} + \text{NADH} \rightleftharpoons \text{Lactate} + \text{NAD}^+$$

In this way, glucose metabolism and energy production can continue for a while without O_2. The lactate that accumulates is converted back to pyruvate when the O_2 supply is restored, with NADH transferring its hydrogen to the flavoprotein–cytochrome chain.

During aerobic glycolysis, the net production of ATP is 19 times greater than the 2 ATPs formed under anaerobic conditions. Six ATPs are formed by oxidation, via the flavoprotein–cytochrome chain, of the 2 NADHs produced when 2 molecules of phosphoglyceraldehyde are converted to phosphoglycerate (Figure 1–21), 6 ATPs are formed from the 2 NADHs produced when 2 molecules of pyruvate are converted to acetyl-CoA, and 24 ATPs are formed during the subsequent two turns of the citric acid cycle. Of these, 18 are formed by oxidation of 6 NADHs, 4 by oxidation of 2 $FADH_2$s, and 2 by oxidation at the substrate level, when succinyl-CoA is converted to succinate (this reaction actually produces guanosine triphosphate [GTP], but the GTP is converted to ATP). Thus, the net production of ATP per mol of blood glucose metabolized aerobically via the Embden–Meyerhof pathway and citric acid cycle is $38 = 2 + [2 \times 3] + [2 \times 3] + [2 \times 12]$.

Glucose oxidation via the hexose monophosphate shunt generates large amounts of NADPH. A supply of this reduced coenzyme is essential for many

metabolic processes. The pentoses formed in the process are building blocks for nucleotides (see below). The amount of ATP generated depends on the amount of NADPH converted to NADH and then oxidized.

“DIRECTIONAL-FLOW VALVES” IN METABOLISM

Metabolism is regulated by a variety of hormones and other factors. To bring about any net change in a particular metabolic process, regulatory factors obviously must drive a chemical reaction in one direction. Most of the reactions in intermediary metabolism are freely reversible, but there are a number of “directional-flow valves,” that is, reactions that proceed in one direction under the influence of one enzyme or transport mechanism and in the opposite direction under the influence of another. Five examples in the intermediary metabolism of carbohydrate are shown in **Figure 1–22**. The different pathways for fatty acid synthesis and catabolism (see below) are another example. Regulatory factors exert their influence on metabolism by acting directly or indirectly at these directional-flow valves.

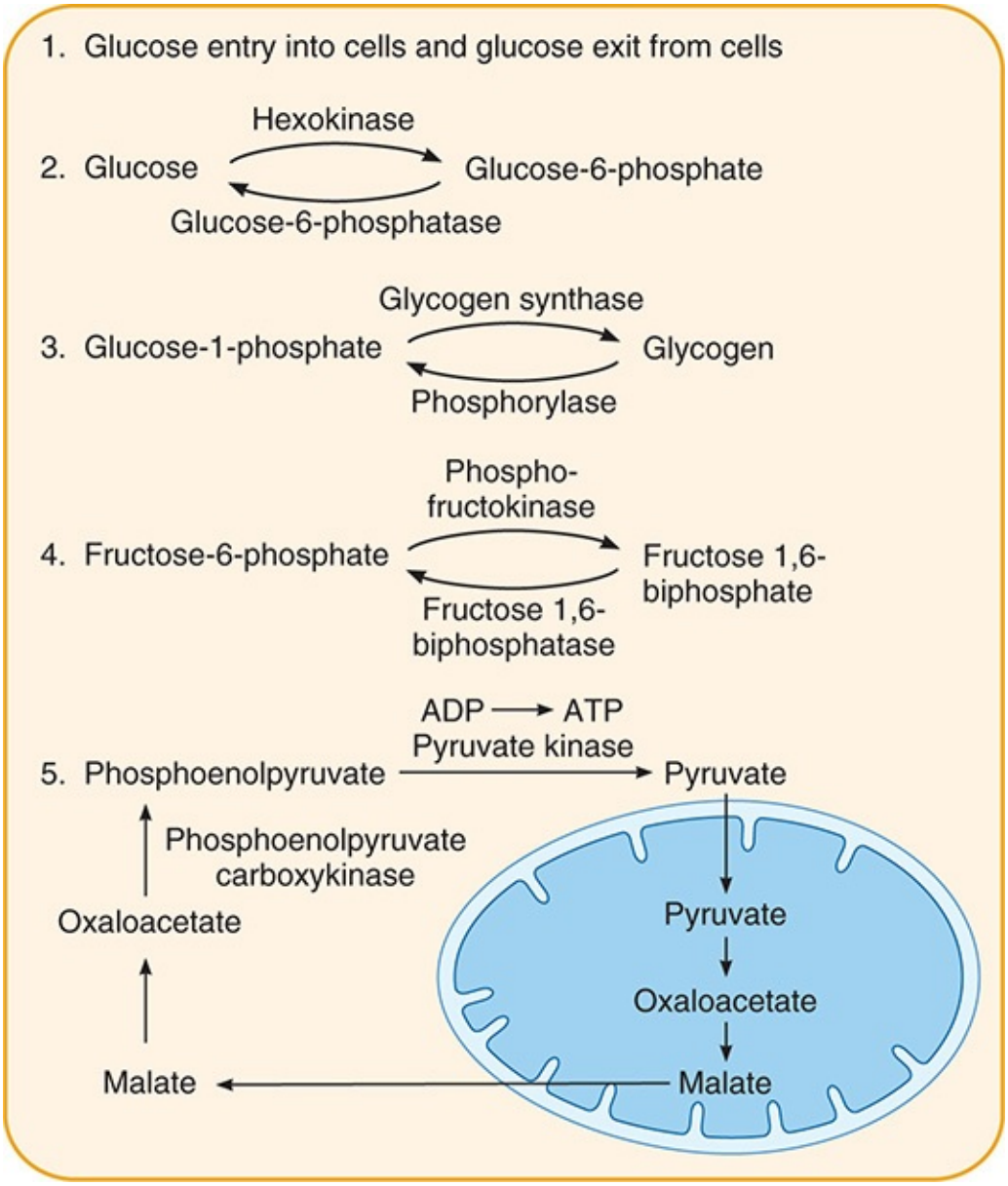


FIGURE 1–22 Directional-flow valves in energy production reactions. In carbohydrate metabolism there are several reactions that proceed in one direction by one mechanism and in the other direction by a different mechanism, termed "directional-flow valves." Five examples of these reactions are illustrated (numbered at left). The double line in example 5 represents the mitochondrial membrane. Pyruvate is converted to malate in mitochondria, and the malate diffuses out of the mitochondria to the cytosol, where it is converted to phosphoenolpyruvate.

GLYCOGEN SYNTHESIS & BREAKDOWN

Glycogen is a branched glucose polymer with two types of glycoside linkages: 1:4α and 1:6α **(Figure 1–23)**. It is synthesized on **glycogenin,** a protein primer, from glucose-1-phosphate via uridine diphosphoglucose (UDPG). The enzyme **glycogen synthase** catalyses the final synthetic step. The availability of glycogenin is one of the factors determining the amount of glycogen synthesized. The breakdown of glycogen in 1:4α linkage is catalyzed by phosphorylase, whereas another enzyme catalyzes the breakdown of glycogen in 1:6α linkage.

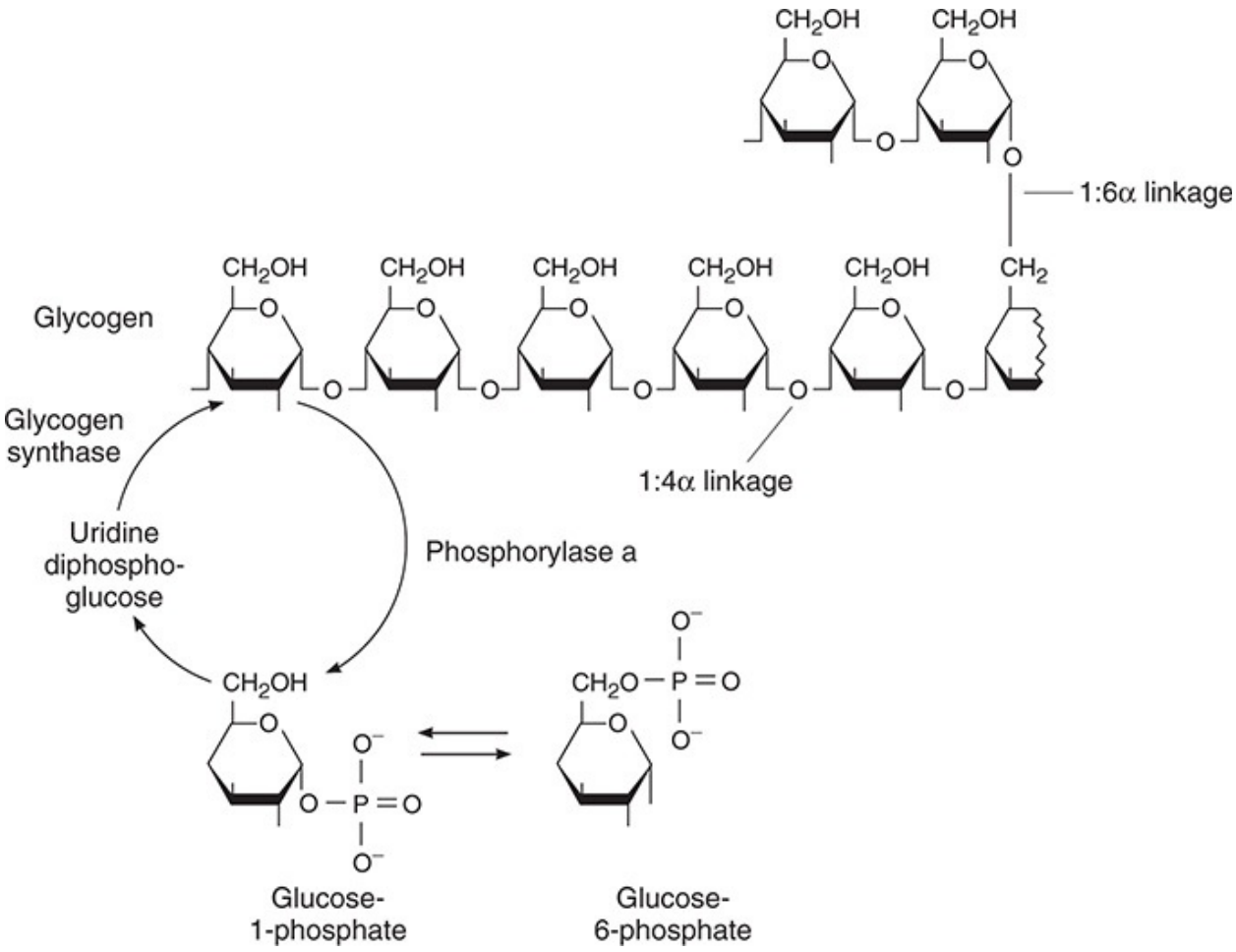


FIGURE 1–23 Glycogen synthesis and breakdown. Glycogen is the main storage for glucose in the cell. It is cycled: built up from glucose-6-phosphate when energy is stored and broken down to glucose-6-phosphate when energy is required. Note the intermediate glucose-1-phosphate and enzymatic control by phosphorylase a and glycogen kinase.

FACTORS DETERMINING THE PLASMA GLUCOSE LEVEL

The blood plasma glucose level at any given time is determined by the balance between the amount of glucose entering the bloodstream and the amount of glucose leaving the bloodstream. The principal determinants are therefore the dietary intake; the rate of entry into the cells of muscle, adipose tissue, and other organs; and the glucostatic activity of the liver **(Figure 1–24)**. Five percent of ingested glucose is promptly converted into glycogen in the liver, and 30–40% is converted into fat. The remainder is metabolized in muscle and other tissues. During fasting, liver glycogen is broken down and the liver adds glucose to the bloodstream. With more prolonged fasting, glycogen is depleted and there is increased gluconeogenesis from amino acids and glycerol in the liver. Plasma glucose declines modestly to about 60 mg/dL during prolonged starvation in normal individuals, but symptoms of hypoglycemia do not occur because gluconeogenesis prevents any further fall.

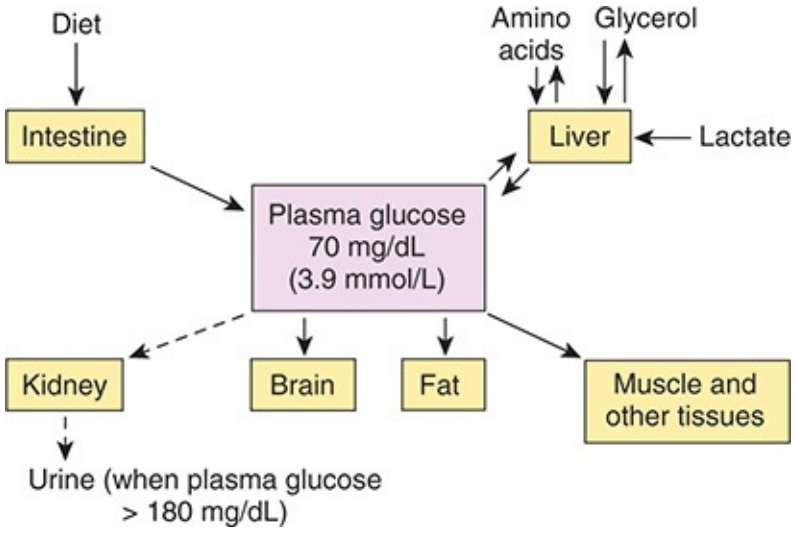


FIGURE 1–24 Plasma glucose homeostasis. Note the glucostatic function of the liver, as well as the loss of glucose in the urine when the renal threshold is exceeded (dashed arrows).

METABOLISM OF HEXOSES OTHER THAN GLUCOSE

Other hexoses that are absorbed from the intestine include galactose, which is

liberated by the digestion of lactose and converted to glucose in the body; and fructose, part of which is ingested and part produced by hydrolysis of sucrose. After phosphorylation, galactose reacts with UDPG to form uridine diphosphogalactose. The uridine diphosphogalactose is converted back to UDPG, and the UDPG functions in glycogen synthesis. This reaction is reversible, and conversion of UDPG to uridine diphosphogalactose provides the galactose necessary for formation of glycolipids and mucoproteins when dietary galactose intake is inadequate. The utilization of galactose, like that of glucose, depends on insulin. The inability to make UDPG can have serious health consequences **(Clinical Box 1–5)**.

CLINICAL BOX 1–5

Galactosemia

In the inborn error of metabolism known as **galactosemia**, there is a congenital deficiency of galactose-1-phosphate uridyl transferase, the enzyme responsible for the reaction between galactose-1-phosphate and UDPG, so that ingested galactose accumulates in the circulation; serious disturbances of growth and development result.

THERAPEUTIC HIGHLIGHTS

Treatment with galactose-free diets improves galactosemia without leading to galactose deficiency. This occurs because the enzyme necessary for the formation of uridine diphosphogalactose from UDPG is present.

Fructose is converted in part to fructose 6-phosphate and then metabolized via fructose 1,6-diphosphate. The enzyme catalyzing the formation of fructose 6-phosphate is hexokinase, the same enzyme that catalyzes the conversion of glucose to glucose-6-phosphate. However, much more fructose is converted to fructose 1-phosphate in a reaction catalyzed by fructokinase. Most of the fructose 1-phosphate is then split into dihydroxyacetone phosphate and glyceraldehyde. The glyceraldehyde is phosphorylated, and it and the dihydroxyacetone phosphate enter the pathways for glucose metabolism. Because the reactions proceeding through phosphorylation of fructose in the 1 position can occur at a normal rate in the absence of insulin, it had been

recommended that fructose be given to diabetics to replenish their carbohydrate stores. However, most of the fructose is metabolized in the intestines and liver, so its value in replenishing carbohydrate elsewhere in the body is limited.

Fructose 6-phosphate can also be phosphorylated in the 2 position, forming fructose 2,6-diphosphate. This compound is an important regulator of hepatic gluconeogenesis. When the fructose 2,6-diphosphate level is high, conversion of fructose 6-phosphate to fructose 1,6-diphosphate is facilitated, and thus breakdown of glucose to pyruvate is increased. A decreased level of fructose 2,6-diphosphate facilitates the reverse reaction and consequently aids gluconeogenesis.

FATTY ACIDS & LIPIDS

The biologically important lipids are the fatty acids and their derivatives, the neutral fats (triglycerides), the phospholipids and related compounds, and the sterols. The triglycerides are made up of three fatty acids bound to glycerol **(Table 1–4)**. Naturally occurring fatty acids contain an even number of carbon atoms. They may be saturated (no double bonds) or unsaturated (dehydrogenated, with various numbers of double bonds). The phospholipids are constituents of cell membranes and provide structural components of the cell membrane, as well as an important source of intracellular and intercellular signaling molecules. Fatty acids also are an important source of energy in the body.

TABLE 1–4 Lipids.

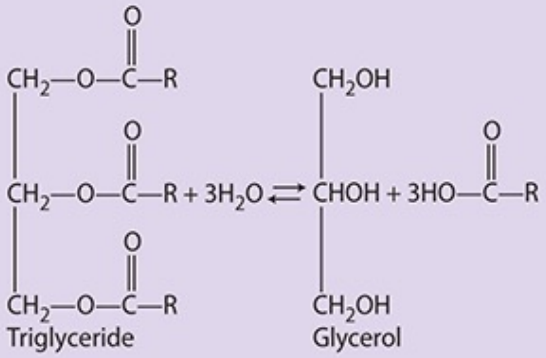
Typical fatty acids:

Palmitic acid: $CH_5(CH_2)_{14}—C(=O)—OH$

Stearic acid: $CH_5(CH_2)_{16}—C(=O)—OH$

Oleic acid: $CH_5(CH_2)_7CH{=}CH(CH_2)_7—C(=O)—OH$ (Unsaturated)

Triglycerides (triacylglycerols): Esters of glycerol and three fatty acids.



R = Aliphatic chain of various lengths and degrees of saturation.

Phospholipids:

A. Esters of glycerol, two fatty acids, and
   1. Phosphate = phosphatidic acid
   2. Phosphate plus inositol = phosphatidylinositol
   3. Phosphate plus choline = phosphatidylcholine (lecithin)
   4. Phosphate plus ethanolamine = phosphatidyl-ethanolamine (cephalin)
   5. Phosphate plus serine = phosphatidylserine

B. Other phosphate-containing derivatives of glycerol.

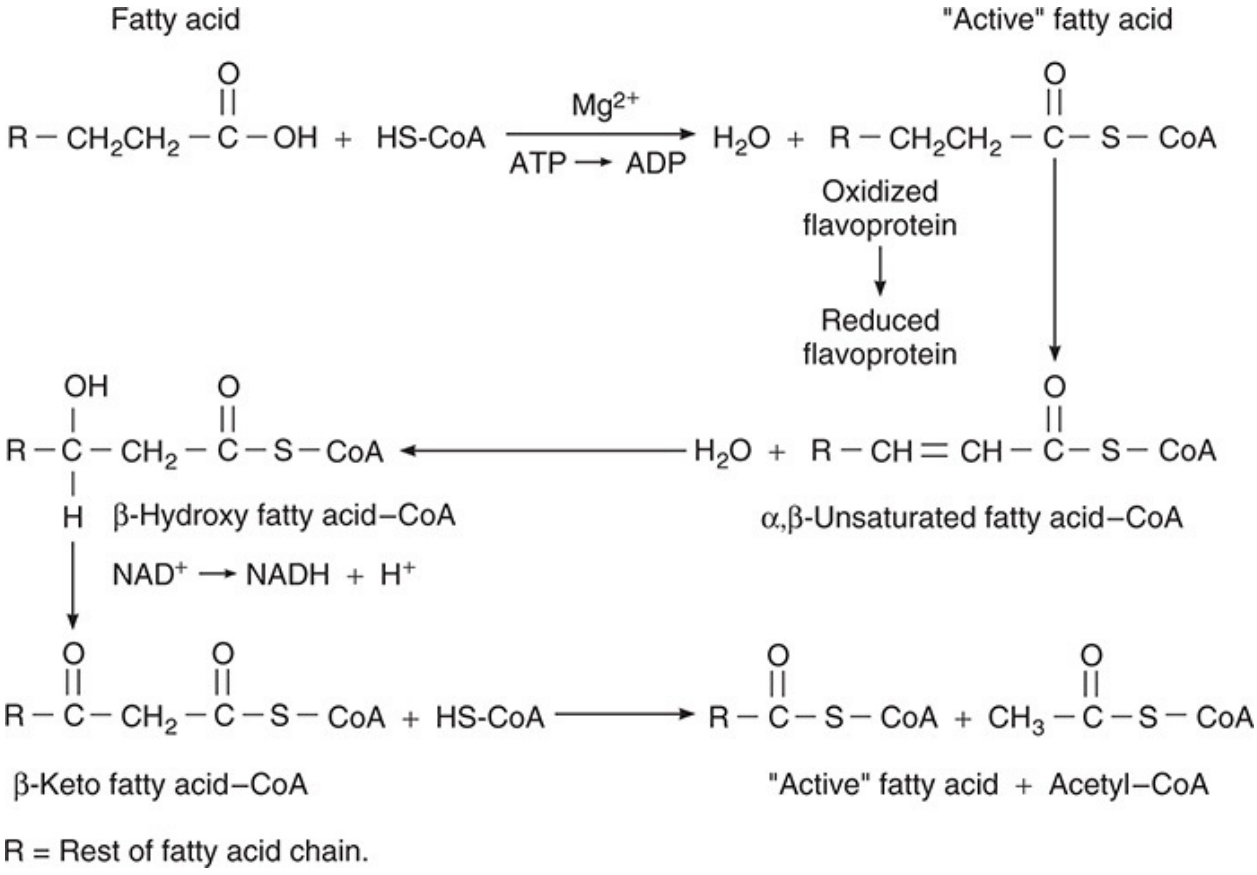
C. Sphingomyelins: Esters of fatty acid, phosphate, choline, and the amino alcohol sphingosine.

Cerebrosides: Compounds containing galactose, fatty acid, and sphingosine.

Sterols: Cholesterol and its derivatives, including steroid hormones, bile acids, and various vitamins.

FATTY ACID OXIDATION & SYNTHESIS

In the body, fatty acids are broken down to acetyl-CoA, which enters the citric acid cycle. The main breakdown occurs in the mitochondria by β-oxidation. Fatty acid oxidation begins with activation (formation of the CoA derivative) of the fatty acid, a reaction that occurs both inside and outside the mitochondria. Medium- and short-chain fatty acids can enter the mitochondria without difficulty, but long-chain fatty acids must be bound to **carnitine** in ester linkage before they can cross the inner mitochondrial membrane. Carnitine is β-hydroxy-γ-trimethylammonium butyrate, and it is synthesized in the body from lysine and methionine. A translocase moves the fatty acid–carnitine ester into the matrix space. The ester is hydrolyzed, and the carnitine recycles. β-Oxidation proceeds by serial removal of two carbon fragments from the fatty acid **(Figure 1–25)**. The energy yield of this process is large. For example, catabolism of 1 mol of a six-carbon fatty acid through the citric acid cycle to CO_2 and H_2O generates 44 mol of ATP, compared with the 38 mol generated by catabolism of 1 mol of the six-carbon carbohydrate glucose.



R = Rest of fatty acid chain.

FIGURE 1–25 Fatty acid oxidation. This process, splitting off two carbon fragments at a time, is repeated to the end of the chain.

KETONE BODIES

In many tissues, acetyl-CoA units condense to form acetoacetyl- CoA **(Figure 1–26)**. In the liver, which (unlike other tissues) contains a deacylase, free acetoacetate is formed. This β-keto acid is converted to β-hydroxybutyrate and acetone, and because these compounds are metabolized with difficulty in the liver, they diffuse into the circulation. Acetoacetate is also formed in the liver via the formation of 3-hydroxy-3-methylglutaryl-CoA, and this pathway is quantitatively more important than deacylation. Acetoacetate, β-hydroxybutyrate, and acetone are called **ketone bodies.** Tissues other than liver transfer CoA from succinyl-CoA to acetoacetate and metabolize the "active" acetoacetate to CO_2 and H_2O via the citric acid cycle. Ketone bodies are also metabolized via other pathways. Acetone is discharged in the urine and expired air. An imbalance of ketone bodies can lead to serious health problems **(Clinical Box 1–6)**.

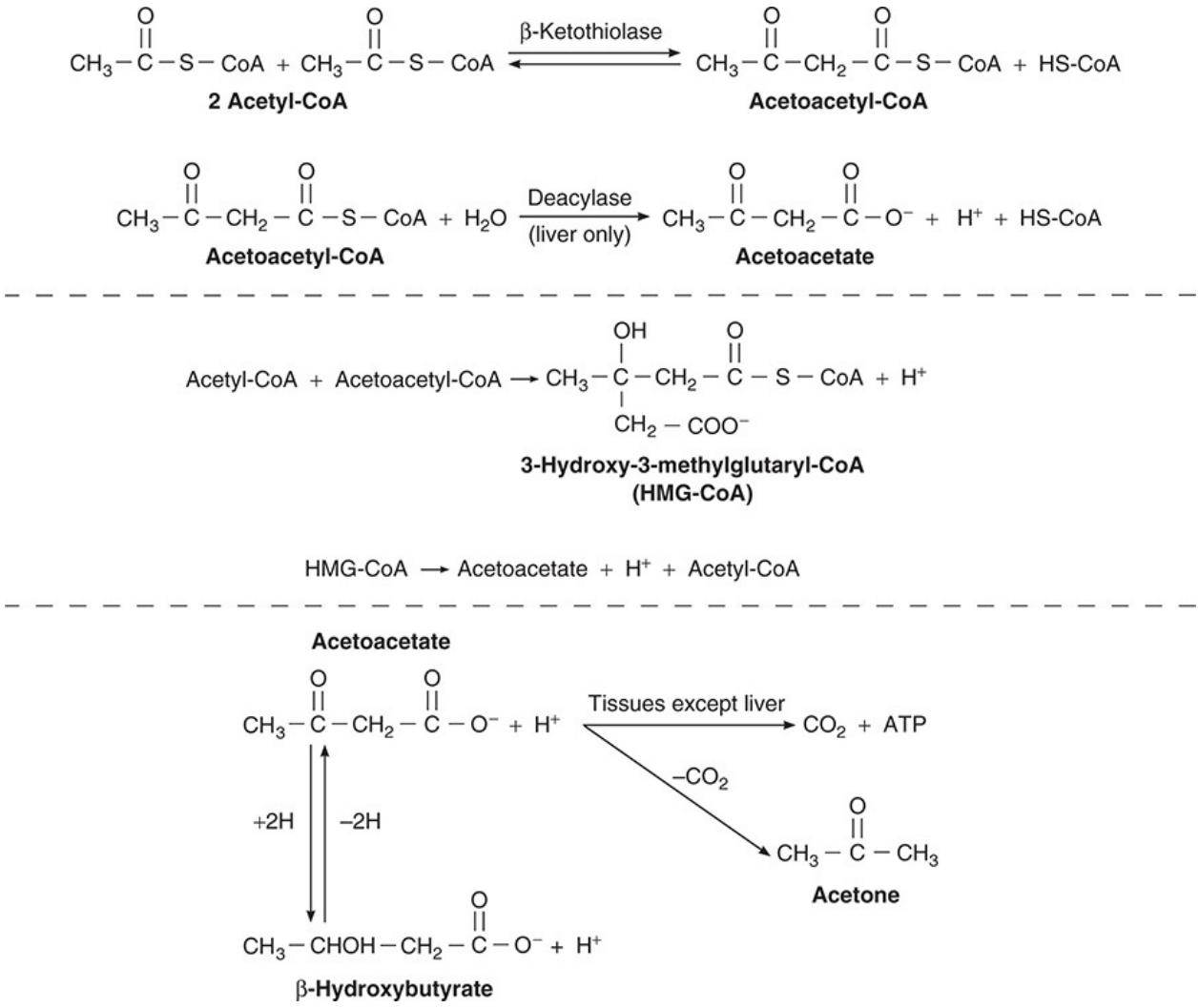


FIGURE 1–26 Formation and metabolism of ketone bodies. Note the two pathways for the formation of acetoacetate.

CLINICAL BOX 1–6

Diseases Associated with Imbalance of β-oxidation of Fatty Acids

Ketoacidosis

The normal blood ketone level in humans is low (about 1 mg/dL) and less than 1 mg is excreted per 24 h, because the ketones are normally metabolized as rapidly as they are formed. However, if the entry of acetyl-CoA into the citric acid cycle is depressed because of a decreased supply of the products of glucose metabolism, or if the entry does not increase when the supply of

acetyl-CoA increases, acetyl-CoA accumulates, the rate of condensation to acetoacetyl-CoA increases, and more acetoacetate is formed in the liver. The ability of the tissues to oxidize the ketones is soon exceeded, and they accumulate in the bloodstream (ketosis). Two of the three ketone bodies, acetoacetate and β-hydroxybutyrate, are anions of the moderately strong acids acetoacetic acid and β-hydroxybutyric acid. Many of their protons are buffered, reducing the decline in pH that would otherwise occur. However, the buffering capacity can be exceeded, and the metabolic acidosis that develops in conditions such as diabetic ketosis can be severe and even fatal. Three conditions lead to deficient intracellular glucose supplies, and hence to ketoacidosis: starvation; diabetes mellitus; and a high-fat, low-carbohydrate diet. The acetone odor on the breath of children who have been vomiting is due to the ketosis of starvation. Parenteral administration of relatively small amounts of glucose abolishes the ketosis, and it is for this reason that carbohydrate is said to be antiketogenic.

Carnitine Deficiency

Deficient β-oxidation of fatty acids can be produced by carnitine deficiency or genetic defects in the translocase or other enzymes involved in the transfer of long-chain fatty acids into the mitochondria. This causes cardiomyopathy. In addition, it causes **hypoketonemic hypoglycemia** with coma, a serious and often fatal condition triggered by fasting, in which glucose stores are used up because of the lack of fatty acid oxidation to provide energy. Ketone bodies are not formed in normal amounts because of the lack of adequate CoA in the liver.

THERAPEUTIC HIGHLIGHTS

The primary treatment for diabetic ketoacidosis is to replace the lost fluids and electrolytes, while insulin is generally given to reverse the processes that cause diabetic ketoacidosis. The main treatment for primary carnitine deficiency is lifelong use of L-carnitine, a natural substance that helps body cells make energy and get rid of harmful wastes. L-carnitine can also reverse the heart problems and muscle weakness caused by carnitine deficiency. Pediatric patients with carnitine deficiency also benefit from a low-fat, high carbohydrate diet and drinking more fluids.

CELLULAR LIPIDS

The lipids in cells are of two main types: **structural lipids,** which are an inherent part of the membranes and can serve as progenitors for cellular signaling molecules; and **neutral fat,** stored in the adipose cells of the fat depots. Neutral fat is mobilized during starvation, but structural lipid is preserved. The fat depots obviously vary in size, but in nonobese individuals they make up about 15% of body weight in men and 21% in women. They are not the inert structures they were once thought to be but, rather, active dynamic tissues undergoing continuous breakdown and resynthesis. In the depots, glucose is metabolized to fatty acids, and neutral fats are synthesized. Neutral fat is also broken down, and free fatty acids (FFAs) are released into the circulation.

A third, special type of lipid is **brown fat,** which makes up a small percentage of total body fat. Brown fat, which is somewhat more abundant in infants but is present in adults as well, is located between the scapulas, at the nape of the neck, along the great vessels in the thorax and abdomen, and in other scattered locations in the body. In brown fat depots, the fat cells as well as the blood vessels have an extensive sympathetic innervation. This is in contrast to white fat depots, in which some fat cells may be innervated but the principal sympathetic innervation is solely on blood vessels. In addition, ordinary lipocytes have only a single large droplet of white fat, whereas brown fat cells contain several small droplets of fat. Brown fat cells also contain many mitochondria. In these mitochondria, an inward proton conductance that generates ATP takes places as usual, but in addition there is a second proton conductance that does not generate ATP. This “short-circuit” conductance depends on a 32-kDa uncoupling protein (UCP1). It causes uncoupling of metabolism and generation of ATP, so that more heat is produced.

PLASMA LIPIDS & LIPID TRANSPORT

The major lipids are relatively insoluble in aqueous solutions and do not circulate in the free form. **FFAs** are bound to albumin, whereas cholesterol, triglycerides, and phospholipids are transported in the form of **lipoprotein** complexes. The complexes greatly increase the solubility of the lipids. The six families of lipoproteins **(Table 1–5)** are graded in size and lipid content. The density of these lipoproteins is inversely proportionate to their lipid content. In general, the lipoproteins consist of a hydrophobic core of triglycerides and cholesteryl esters surrounded by phospholipids and protein. These lipoproteins

can be transported from the intestine to the liver via an **exogenous pathway,** and between other tissues via an **endogenous pathway.**

TABLE 1–5 The principal lipoproteins.[a]

Lipoprotein	Size (nm)	Composition (%)					Origin
		Protein	Free Cholesteryl	Cholesterol Esters	Triglyceride	Phospholipid	
Chylomicrons	75–1000	2	2	3	90	3	Intestine
Chylomicron remnants	30–80	…	…	…	…	…	Capillaries
Very low-density lipoproteins (VLDL)	30–80	8	4	16	55	17	Liver and intestine
Intermediate-density lipoproteins (IDL)	25–40	10	5	25	40	20	VLDL
Low-density lipoproteins (LDL)	20	20	7	46	6	21	IDL
High-density lipoproteins (HDL)	7.5–10	50	4	16	5	25	Liver and intestine

[a]The plasma lipids include these components plus free fatty acids from adipose tissue, which circulate bound to albumin.

Dietary lipids are processed by several pancreatic lipases in the intestine to form mixed micelles of predominantly FFA, **2-monoacylglycerols,** and cholesterol derivatives (see Chapter 26). These micelles additionally can contain important water-insoluble molecules such as **vitamins A, D, E,** and **K**. These mixed micelles are taken up into cells of the intestinal mucosa where large lipoprotein complexes, **chylomicrons,** are formed. The chylomicrons and their remnants constitute a transport system for ingested exogenous lipids (exogenous pathway). Chylomicrons can enter the circulation via the lymphatic ducts. The chylomicrons are cleared from the circulation by the action of **lipoprotein lipase,** which is located on the surface of the endothelium of the capillaries. The enzyme catalyzes the breakdown of the triglyceride in the chylomicrons to FFA and glycerol, which then enter adipose cells and are reesterified. Alternatively, the FFA can remain in the circulation bound to albumin. Lipoprotein lipase, which requires heparin as a cofactor, also removes triglycerides from circulating **very low-density lipoproteins (VLDL).** Chylomicrons depleted of their triglyceride remain in the circulation as cholesterol-rich lipoproteins called **chylomicron remnants,** which are 30–80 nm in diameter. The remnants are carried to the liver, where they are internalized and degraded.

The endogenous system, made up of VLDL, **intermediate-density lipoproteins (IDL), low-density lipoproteins (LDL),** and **high-density lipoproteins (HDL),** also transports triglycerides and cholesterol throughout the

body. VLDL are formed in the liver and transport triglycerides formed from fatty acids and carbohydrates in the liver to extrahepatic tissues. After their triglyceride is largely removed by the action of lipoprotein lipase, they become IDL. The IDL give up phospholipids and, through the action of the plasma enzyme **lecithin-cholesterol acyltransferase (LCAT),** pick up cholesteryl esters formed from cholesterol in the HDL. Some IDL are taken up by the liver. The remaining IDL then lose more triglyceride and protein, probably in the sinusoids of the liver, and become LDL. LDL provide cholesterol to the tissues. The cholesterol is an essential constituent in cell membranes and is used by gland cells to make steroid hormones.

FREE FATTY ACID METABOLISM

In addition to the exogenous and endogenous pathways described above, FFA are also synthesized in the fat depots in which they are stored. They can circulate as lipoproteins bound to albumin and are a major source of energy for many organs. They are used extensively in the heart, but probably all tissues can oxidize FFA to CO_2 and H_2O.

The supply of FFA to the tissues is regulated by two lipases. As noted above, lipoprotein lipase on the surface of the endothelium of the capillaries hydrolyzes the triglycerides in chylomicrons and VLDL, providing FFA and glycerol, which are reassembled into new triglycerides in the fat cells. The intracellular **hormone-sensitive lipase** of adipose tissue catalyzes the breakdown of stored triglycerides into glycerol and fatty acids, with the latter entering the circulation. Hormone-sensitive lipase is increased by fasting and stress and decreased by feeding and insulin. Conversely, feeding increases and fasting and stress decrease the activity of lipoprotein lipase.

CHOLESTEROL METABOLISM

Cholesterol is the precursor of the steroid hormones and bile acids and is an essential constituent of cell membranes. It is found only in animals. Related sterols occur in plants, but plant sterols are poorly absorbed from the gastrointestinal tract. Most of the dietary cholesterol is contained in egg yolks and animal fat.

Cholesterol is absorbed from the intestine and incorporated into the chylomicrons formed in the intestinal mucosa. After the chylomicrons discharge

their triglyceride in adipose tissue, the chylomicron remnants bring cholesterol to the liver. The liver and other tissues also synthesize cholesterol. Some of the cholesterol in the liver is excreted in the bile, both in the free form and as bile acids. Some of the biliary cholesterol is reabsorbed from the intestine. Most of the cholesterol in the liver is incorporated into VLDL and circulates in lipoprotein complexes.

The biosynthesis of cholesterol from acetate is summarized in **Figure 1–27**. Cholesterol feeds back to inhibit its own synthesis by inhibiting **HMG-CoA reductase,** the enzyme that converts 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) to mevalonic acid. Thus, when dietary cholesterol intake is high, hepatic cholesterol synthesis is decreased, and vice versa. However, the feedback compensation is incomplete, because a diet that is low in cholesterol and saturated fat leads to only a modest decline in circulating plasma cholesterol. The most effective and most commonly used cholesterol-lowering drugs are lovastatin and other **statins,** which reduce cholesterol synthesis by inhibiting HMG-CoA. The relationship between cholesterol and vascular disease is discussed in **Clinical Box 1–7**.

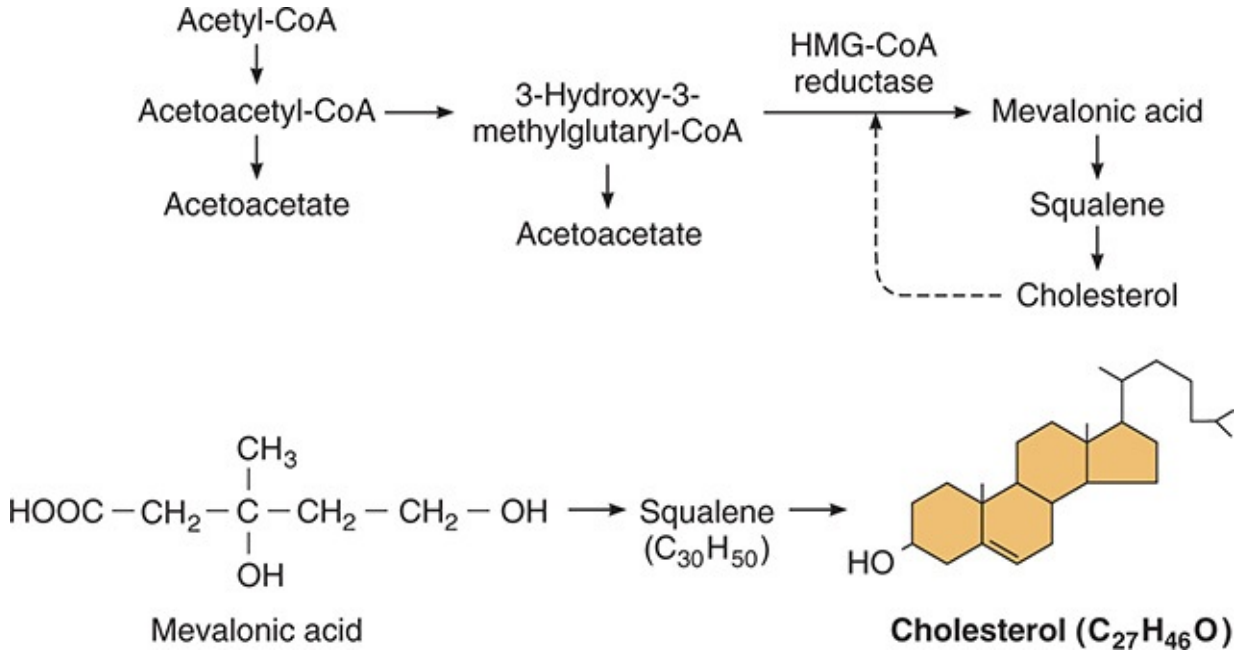


FIGURE 1–27 Biosynthesis of cholesterol. Six mevalonic acid molecules condense to form squalene, which is then hydroxylated to cholesterol. The dashed arrow indicates feedback inhibition by cholesterol of HMG-CoA reductase, the enzyme that catalyzes mevalonic acid formation.

ESSENTIAL FATTY ACIDS

Animals fed a fat-free diet fail to grow, develop skin and kidney lesions, and become infertile. Adding linolenic, linoleic, and arachidonic acids to the diet cures all the deficiency symptoms. These three acids are polyunsaturated fatty acids and because of their action are called **essential fatty acids.** Similar deficiency symptoms have not been unequivocally demonstrated in humans, but there is reason to believe that some unsaturated fats are essential dietary constituents, especially for children. Dehydrogenation of fats is known to occur in the body, but there does not appear to be any synthesis of carbon chains with the arrangement of double bonds found in the essential fatty acids.

EICOSANOIDS

One of the reasons that essential fatty acids are necessary for health is that they are the precursors of prostaglandins (including prostacyclin, thromboxanes), lipoxins, leukotrienes, and related compounds. These substances are called **eicosanoids,** reflecting their origin from the 20-carbon (eicosa-) polyunsaturated fatty acid **arachidonic acid (arachidonate)** and the 20-carbon derivatives of linoleic and linolenic acids.

The **prostaglandins** are a series of 20-carbon unsaturated fatty acids containing a cyclopentane ring. They were first isolated from semen but are synthesized in most and possibly in all organs in the body. Prostaglandin H_2 (PGH_2) is the precursor for various other prostaglandins, thromboxanes, and prostacyclin. Arachidonic acid is formed from tissue phospholipids by **phospholipase A_2**. It is converted to prostaglandin H_2 (PGH_2) by **prostaglandin G/H synthases** 1 and 2. These are bifunctional enzymes that have both cyclooxygenase and peroxidase activity, but they are more commonly known by the names cyclooxygenase 1 **(COX1)** and cyclooxygenase 2 **(COX2).** Their structures are very similar, but COX1 is constitutive whereas COX2 is induced by growth factors, cytokines, and tumor promoters. PGH_2 is converted to prostacyclin, thromboxanes, and other prostaglandins (eg, PGE_2, $PGF_{2\alpha}$, PGD_2) by various tissue isomerases. The effects of prostaglandins are multitudinous and varied. They are particularly important in the female reproductive cycle, in parturition, in the cardiovascular system, in inflammatory responses, and in the causation of pain. Drugs that target production of prostaglandins are among the most common over-the-counter drugs available **(Clinical Box 1–8)**.

CLINICAL BOX 1–7

Cholesterol & Atherosclerosis

The interest in cholesterol-lowering drugs stems from the role of cholesterol in the development and progression of **atherosclerosis**. This extremely widespread disease predisposes to myocardial infarction, cerebral thrombosis, ischemic gangrene of the extremities, and other serious illnesses. It is characterized by infiltration of cholesterol and oxidized cholesterol into macrophages, converting them into foam cells in lesions of the arterial walls. This is followed by a complex sequence of changes involving platelets, macrophages, and smooth muscle cells, as well as growth factors and inflammatory mediators that produces proliferative lesions that eventually ulcerate and may calcify. The lesions distort the vessels and make them rigid. In individuals with elevated plasma cholesterol levels, the incidence of atherosclerosis and its complications is increased. The normal range for plasma cholesterol is said to be 120–200 mg/dL, but in men, there is a clear, tight, positive correlation between the death rate from ischemic heart disease and plasma cholesterol levels above 180 mg/dL. Furthermore, it is now clear that lowering plasma cholesterol by diet and drugs slows and may even reverse the progression of atherosclerotic lesions and the complications they cause.

In evaluating plasma cholesterol levels in relation to atherosclerosis, it is important to analyze the LDL and HDL levels as well. LDL delivers cholesterol to peripheral tissues, including atheromatous lesions, and the LDL plasma concentration correlates positively with myocardial infarctions and ischemic strokes. On the other hand, HDL picks up cholesterol from peripheral tissues and transports it to the liver, thus lowering plasma cholesterol. It is interesting that women, who have a lower incidence of myocardial infarction than men, have higher HDL levels. In addition, HDL levels are increased in individuals who exercise and those who drink one or two alcoholic drinks per day, whereas they are decreased in individuals who smoke, are obese, or live sedentary lives. Moderate drinking decreases the incidence of myocardial infarction, and obesity and smoking are risk factors that increase it. Plasma cholesterol and the incidence of cardiovascular diseases are increased in **familial hypercholesterolemia**, due to various loss-of-function mutations in the genes for LDL receptors.

THERAPEUTIC HIGHLIGHTS

Although atherosclerosis is a progressive disease, it is also preventable in many cases by limiting risk factors, including lowering “bad” cholesterol through a healthy diet and exercise. Drug treatments for high cholesterol, including the statins among others, provide additional relief that can complement a healthy diet and exercise. If atherosclerosis is advanced, invasive techniques, such as angioplasty and stenting, can be used to unblock arteries.

CLINICAL BOX 1–8

Pharmacology of Prostaglandins

Because prostaglandins play a prominent role in the genesis of pain, inflammation, and fever, pharmacologists have long sought drugs to inhibit their synthesis. Glucocorticoids inhibit phospholipase A_2 and thus inhibit the formation of all eicosanoids. A variety of NSAIDs inhibit both cyclooxygenases, inhibiting the production of PGH_2 and its derivatives. Aspirin is the best-known of these, but ibuprofen, indomethacin, and others are also used. However, there is evidence that prostaglandins synthesized by COX2 are more involved in the production of pain and inflammation, and prostaglandins synthesized by COX1 are more involved in protecting the gastrointestinal mucosa from ulceration. Several novel NSAIDs have been introduced in an attempt to specifically target COX enzymes. However, in many cases, significant side effects, including increased incidence of stroke and heart attack, have led to drug withdrawals from the market. More research is underway to better understand all the effects of the COX enzymes, their products, and their inhibitors. Some prosglandins can however be used as drugs for cardiovascular disease based on the vasodilative and antiproliferative effects. For example, intravenous perfusion of prostacyclin is an effective treatment for idiopathic pulmonary arterial hypertension, a progressive and fatal disease that predominantly affects young women.

Arachidonic acid also serves as a substrate for the production of several physiologically important **leukotrienes** and **lipoxins.** The leukotrienes, thromboxanes, lipoxins, and prostaglandins have been called local hormones.

They have short half-lives and are inactivated in many different tissues. They undoubtedly act mainly in the tissues at sites in which they are produced. The leukotrienes are mediators of allergic responses and inflammation. Their release is provoked when specific allergens combine with IgE antibodies on the surfaces of mast cells (see Chapter 3). They produce bronchoconstriction, constrict arterioles, increase vascular permeability, and attract neutrophils and eosinophils to inflammatory sites. Diseases in which they may be involved include asthma, psoriasis, acute respiratory distress syndrome, allergic rhinitis, rheumatoid arthritis, Crohn disease, and ulcerative colitis.

CHAPTER SUMMARY

- Cells contain approximately two-thirds of the body fluids, while the remaining extracellular fluid is found between cells (interstitial fluid) or in the circulating lymph and blood plasma.
- The number of molecules, electrical charges, and particles of substances in solution are important in physiology.
- Biological buffers including bicarbonate, proteins, and phosphates can bind or release protons in solution to help maintain pH. Biological buffering capacity of a weak acid or base is greatest when pK_a = pH.
- Although the osmolality of solutions can be similar across a plasma membrane, the distribution of individual molecules and distribution of charge across the plasma membrane can be quite different. The separation of concentrations of charged species sets up an electrical gradient at the plasma membrane (inside negative). The electrochemical gradient is in large part maintained by the Na, K ATPase. These are affected by the Gibbs–Donnan equilibrium and can be calculated using the Nernst equation.
- Cellular energy can be stored in high-energy or energy-rich phosphate compounds, including adenosine triphosphate (ATP). Coordinated oxidation–reduction reactions allow for the production of a proton gradient at the inner mitochondrial membrane that ultimately yields to the production of ATP in the cell.
- Nucleotides made from purine or pyrimidine bases linked to ribose or 2-deoxyribose sugars with inorganic phosphates are the basic building blocks for nucleic acids, DNA, and RNA. The fundamental unit of DNA is the gene, which encodes information to make proteins in the cell. Genes are transcribed into messenger RNA, and with the help of ribosomal RNA and

transfer RNAs, translated into proteins.

- Amino acids are the basic building blocks for proteins in the cell and can also serve as sources for several biologically active molecules. Translation is the process of protein synthesis. After synthesis, proteins can undergo a variety of posttranslational modifications prior to obtaining their full function in the cell.
- Carbohydrates are organic molecules that contain equal amounts of carbon (C) and H_2O. Carbohydrates can be attached to proteins (glycoproteins) or fatty acids (glycolipids) and are critically important for the production and storage of cellular and body energy. The breakdown of glucose to generate energy, or glycolysis, can occur in the presence or absence of O_2 (aerobically or anaerobically). The net production of ATP during aerobic glycolysis is 19 times higher than anaerobic glycolysis.
- Fatty acids are carboxylic acids with extended hydrocarbon chains. They are an important energy source for cells and fatty acid derivatives—including triglycerides, phospholipids, and sterols—have additional important cellular applications.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. The membrane potential of a particular cell is at the K^+ equilibrium. The intracellular concentration for K^+ is at 150 mmol/L and the extracellular concentration for K^+ is at 5.5 mmol/L. What is the resting potential?

   A. −70 mV

   B. −90 mV

   C. +70 mV

   D. +90 mV

2. The difference in concentration of H^+ in a solution of pH 2.0 compared with one of pH 7.0 is

   A. 5-fold

   B. 1/5 as much

   C. 10^5-fold

   D. 10^{-5} as much

3. Transcription refers to
   A. the process where an mRNA is used as a template for protein production.
   B. the process where a DNA sequence is copied into RNA for the purpose of gene expression.
   C. the process where DNA wraps around histones to form a nucleosome.
   D. the process of replication of DNA prior to cell division.
4. The primary structure of a protein refers to
   A. the twist, folds, or twist and folds of the amino acid sequence into stabilized structures within the protein (ie, α-helices and β-sheets).
   B. the arrangement of subunits to form a functional structure.
   C. the amino acid sequence of the protein.
   D. the arrangement of twisted chains and folds within a protein into a stable structure.
5. Fill in the blanks: Glycogen is a storage form of glucose. _____ refers to the process of making glycogen and _____ refers to the process of breakdown of glycogen.
   A. Glycogenolysis, glycogenesis
   B. Glycolysis, glycogenolysis
   C. Glycogenesis, glycogenolysis
   D. Glycogenolysis, glycolysis
6. The major lipoprotein source of the cholesterol used in cells is
   A. chylomicrons.
   B. intermediate-density lipoproteins (IDL).
   C. albumin-bound free fatty acids.
   D. low-density lipoproteins (LDL).
   E. high-density lipoproteins (HDL).
7. Which of the following produces the most high-energy phosphate compounds?
   A. Aerobic metabolism of 1 mol of glucose
   B. Anaerobic metabolism of 1 mol of glucose
   C. Metabolism of 1 mol of galactose
   D. Metabolism of 1 mol of amino acid
   E. Metabolism of 1 mol of long-chain fatty acid
8. When LDL enters cells by receptor-mediated endocytosis, which of the

following does not occur?

A. Decrease in the formation of cholesterol from mevalonic acid
B. Increase in the intracellular concentration of cholesteryl esters
C. Increase in the transfer of cholesterol from the cell to HDL
D. Decrease in the rate of synthesis of LDL receptors
E. Decrease in the cholesterol in endosomes

CHAPTER 2

Overview of Cellular Physiology

OBJECTIVES

After studying this chapter, you should be able to:

- Name the prominent cellular organelles and state their functions in cells.
- Name the building blocks of the cellular cytoskeleton and state their contributions to cell structure and function.
- Name the intercellular connections and intracellular to extracellular connections.
- Define the processes of exocytosis and endocytosis, and describe the contribution of each to normal cell function.
- Define proteins that contribute to membrane permeability and transport.
- Recognize various forms of intercellular communication and describe ways in which chemical messengers (including second messengers) affect cellular functions.

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INTRODUCTION

The cell is the fundamental working unit of all organisms. In humans, cells can be highly specialized in both structure and function; alternatively, cells from

different organs can share features and function. In the previous chapter, some basic principles of biophysics and the catabolism and metabolism of building blocks found in the cell were examined. In some of those discussions, how the building blocks could contribute to basic cell function (eg, DNA replication, transcription, and translation) were discussed. In this chapter, more of the fundamental aspects of cellular and molecular physiology will be reviewed. Additional aspects that concern specialization of cellular and molecular function are considered in the next chapters concerning immune function and excitable cells and within the sections that highlight each physiological system.

FUNCTIONAL MORPHOLOGY OF THE CELL AND HOMEOSTASIS

The actual environment of the cells of the body is the interstitial component of the extracellular fluid (ECF). Because normal cell function depends on the constancy of this fluid, it is not surprising that in multicellular animals, an immense number of regulatory mechanisms have evolved to maintain it. To describe “the various physiologic arrangements which serve to restore the normal state, once it has been disturbed,” W.B. Cannon coined the term **homeostasis.** The buffering properties of the body fluids and the renal and respiratory adjustments to the presence of excess acid or alkali are examples of homeostatic mechanisms. There are countless other examples, and a large part of physiology is concerned with regulatory mechanisms that act to maintain the constancy of the internal environment. Many of these regulatory mechanisms operate on the principle of negative feedback; deviations from a given normal set point are detected by a sensor, and signals from the sensor trigger compensatory changes that continue until the set point is again reached.

A basic knowledge of cell function and structure is essential to an understanding of the homeostasis, the organ systems and the way they function in the body. A key tool for examining cellular constituents is the microscope. A light microscope can resolve structures as close as 0.2 μm, while an electron microscope can resolve structures as close as 0.002 μm. Although cell dimensions are quite variable, this resolution can provide a good look at the inner workings of the cell. The advent of common access to phase contrast, fluorescent, confocal, and many other microscopy techniques along with specialized probes for both static and dynamic cellular structures further expanded the examination of cell structure and function. Equally revolutionary advances in modern biophysical, biochemical, and molecular biological

techniques have also greatly contributed to our knowledge of the cell.

The specialization of the cells in the various organs is considerable, and no cell can be called “typical” of all cells in the body. However, a number of cell structures **(organelles)** are common to most cells. These structures are shown in **Figure 2–1**. Many of them can be isolated by ultracentrifugation combined with other techniques. When cells are homogenized and the resulting suspension is centrifuged, the nuclei sediment first, followed by the mitochondria. High-speed centrifugation that generates forces of 100,000 times gravity or more causes a fraction made up of granules called the **microsomes** to sediment. This fraction includes organelles such as the **ribosomes** and **peroxisomes.**

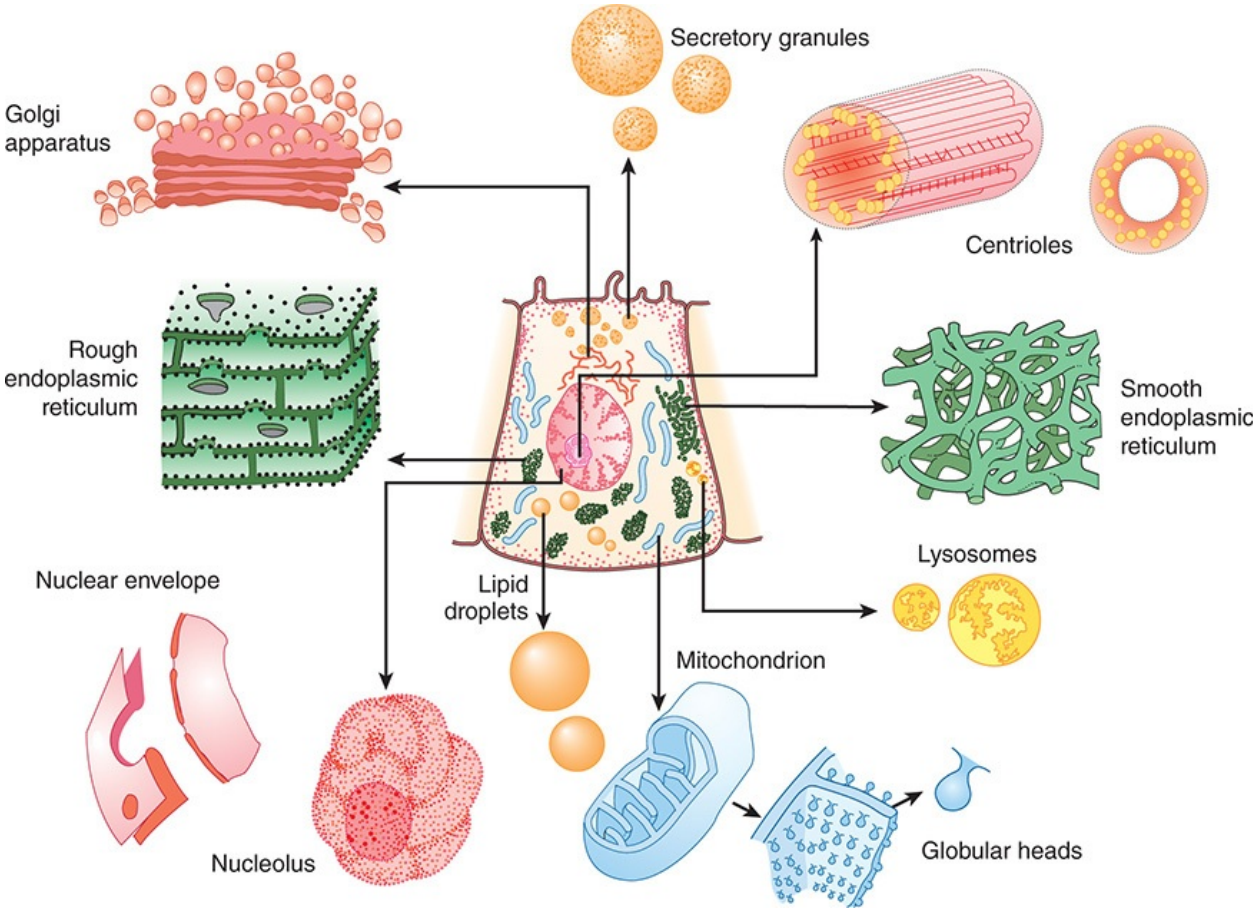


FIGURE 2–1 Cross-sectional diagram of a hypothetical cell as seen with the light microscope. Individual organelles are expanded for closer examination. (Adapted with permission from Bloom and Fawcett. Reproduced with permission from Junqueira LC, Carneiro J, Kelley RO: *Basic Histology*, 9th ed. New York, NY: McGraw-Hill; 1998.)

CELL MEMBRANES

The membrane that surrounds the cell is a remarkable structure. It is made up of lipids and proteins and is semipermeable, allowing some substances to pass through it and excluding others. However, its permeability can also be varied because it contains numerous regulated ion channels and other transport proteins that can change the amounts of substances moving across it. It is generally referred to as the **plasma membrane.** The nucleus and other organelles in the cell are bound by similar membranous structures.

Although the chemical structures of membranes and their properties vary considerably from one location to another, they have certain common features. They are generally about 7.5 nm (75 angstroms [Å]) thick. The major lipids are phospholipids such as phosphatidylcholine, phosphatidylserine, and phosphatidylethanolamine. The shape of the phospholipid molecule reflects its solubility properties: the "head" end of the molecule contains the phosphate portion and is relatively soluble in water (polar, **hydrophilic**) and the "tail" ends are relatively insoluble (nonpolar, **hydrophobic**). The possession of both hydrophilic and hydrophobic properties makes the lipid an **amphipathic** molecule. In the membrane, the hydrophilic ends of the molecules are exposed to the aqueous environment that bathes the exterior of the cells and the aqueous cytoplasm; the hydrophobic ends meet in the water-poor interior of the membrane **(Figure 2–2)**. In **prokaryotes** (ie, bacteria in which there is no nucleus), the membranes are relatively simple, but in **eukaryotes** (ie, cells containing nuclei), cell membranes contain various glycosphingolipids, sphingomyelin, and cholesterol in addition to phospholipids and phosphatidylcholine.

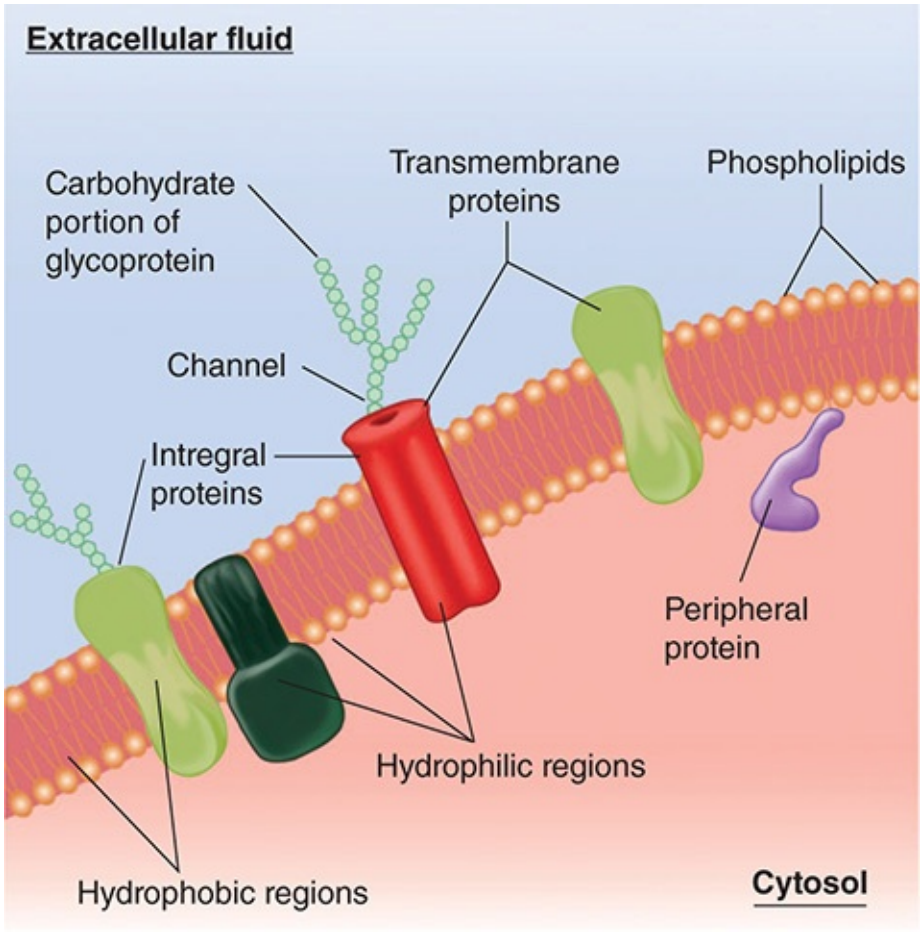


FIGURE 2–2 Organization of the phospholipid bilayer and associated proteins in a biologic membrane. The phospholipid molecules that make up the membrane each have two hydrophobic fatty acid chains attached to a hydrophilic phosphate head. Individual proteins take on different shapes and positions in the cell. Many are integral proteins, extending into the membrane or peripheral proteins that are attached to the inside or outside (not shown) of the membrane. Proteins can be modified (eg, with carbohydrate chains). Many specific protein attachments and cholesterol that are commonly found in the bilayer are omitted for clarity.

Many different proteins are embedded in the membrane. The membrane proteins are classified into two categories: **integral proteins** or intrinsic membrane proteins and **peripheral proteins** or extrinsic membrane proteins. They exist as separate globular units and many pass through or are embedded in one leaflet of the membrane (eg, integral proteins), whereas peripheral proteins are associated with the inside or outside of the membrane (Figure 2–2). The amount of protein varies significantly with the function of the cell but makes up

on average 50% of the mass of the membrane; that is, there is about one protein molecule per 50 of the much smaller phospholipid molecules. The proteins in the membrane carry out many functions. Some are **cell adhesion molecules** (CAMs) that anchor cells to their neighbors or to basal laminas. Some proteins function as **pumps,** actively transporting ions across the membrane. Other proteins function as **carriers,** transporting substances down electrochemical gradients by facilitated diffusion. Still others are **ion channels,** which, when activated, permit the passage of ions into or out of the cell. The role of the pumps, carriers, and ion channels in transport across the cell membrane is discussed below. Proteins in another group function as **receptors** that bind **ligands** or messenger molecules, initiating physiological changes inside the cell. Proteins also function as **enzymes,** catalyzing reactions at the surfaces of the membrane. Examples from each of these groups are discussed later in this chapter.

The hydrophobic portions of the proteins are usually located in the interior of the membrane, whereas the charged, hydrophilic portions are located on the surfaces. Peripheral proteins are attached to the surfaces of the membrane in various ways. One common way is attachment to glycosylated forms of phosphatidylinositol. Proteins held by these **glycosylphosphatidylinositol (GPI) anchors** **(Figure 2–3)** include enzymes such as alkaline phosphatase, various antigens, a number of CAMs, and proteins that combat cell lysis by complement. Over 250 GPI-linked cell surface proteins have now been described in humans. Other proteins are **lipidated,** that is, they have specific lipids attached to them (Figure 2–3). Proteins may be **myristoylated, palmitoylated,** or **prenylated** (ie, attached to geranylgeranyl or farnesyl groups).

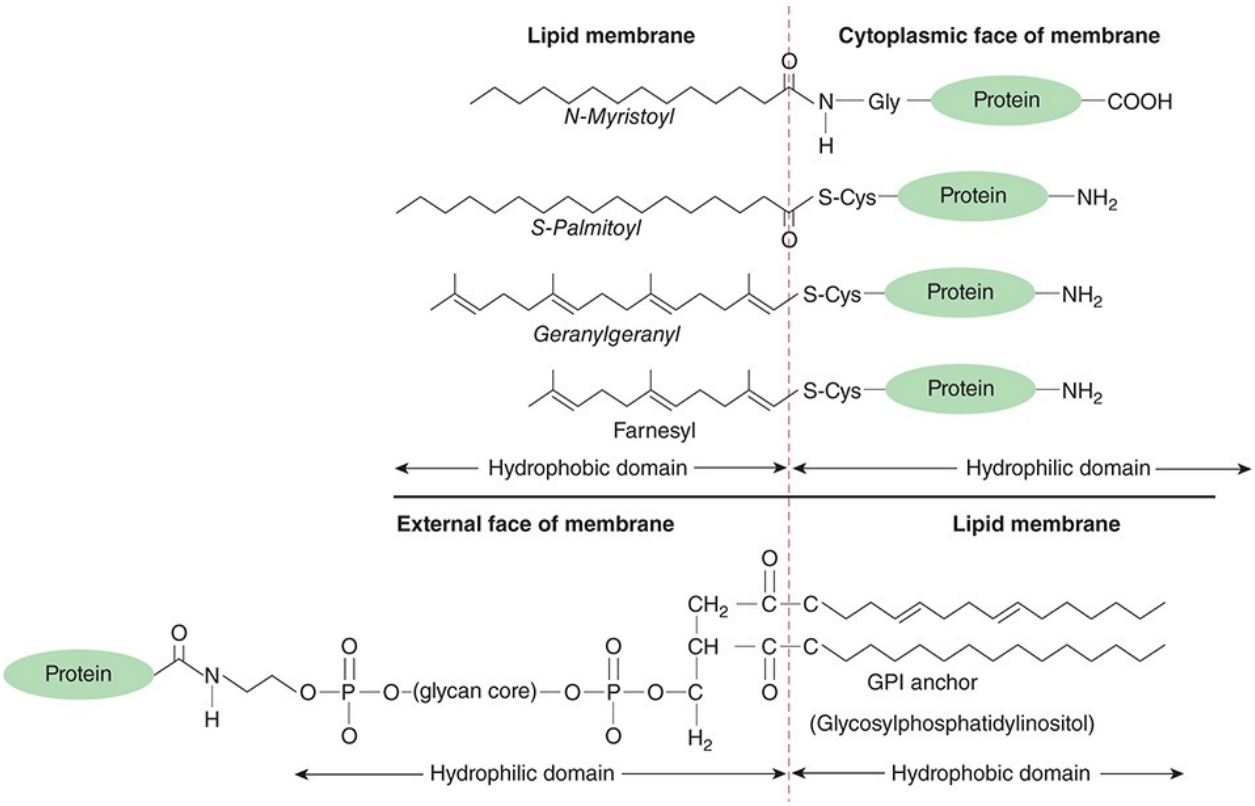


FIGURE 2–3 Protein linkages to membrane lipids. A variety of lipid modifications can occur at amino or carboxy terminals of proteins attached to cytosolic side of the plasma membrane. Many proteins associated with the external side of the plasma membrane can be attached via glycosylated forms of phosphatidylinositol (eg, GPI anchors).

The protein structure—and particularly the enzyme content—of biologic membranes varies not only from cell to cell, but also within the same cell. For example, some of the enzymes embedded in cell membranes are different from those in mitochondrial membranes. In epithelial cells, the enzymes in the cell membrane on the mucosal surface differ from those in the cell membrane on the basal and lateral margins of the cells; that is, the cells are **polarized.** Such polarization makes directional transport across epithelia possible. The membranes are dynamic structures, and their constituents are being constantly renewed at different rates. Some proteins are anchored to the cytoskeleton, but others move laterally in the membrane.

Underlying most cells is a thin, “fuzzy” layer plus some fibrils that collectively make up the **basement membrane** or, more properly, the **basal lamina.** The basal lamina and, more generally, the extracellular matrix are made up of many proteins that hold cells together, regulate their development, and

determine their growth. These include collagens, laminins, fibronectin, tenascin, and various proteoglycans.

MITOCHONDRIA

Over a billion years ago, aerobic bacteria were engulfed by eukaryotic cells and evolved into **mitochondria,** providing the eukaryotic cells with the ability to form the energy-rich compound ATP by **oxidative phosphorylation.** Mitochondria perform other functions, including a role in the regulation of **apoptosis** (programmed cell death), but oxidative phosphorylation is the most crucial. Each eukaryotic cell can have hundreds to thousands of mitochondria. In mammals, they are generally depicted as sausage-shaped organelles (Figure 2–1), but their shape can be quite dynamic. Each has an outer membrane, an intermembrane space, an inner membrane, which is folded to form shelves **(cristae),** and a central matrix space. The enzyme complexes responsible for oxidative phosphorylation are lined up on the cristae **(Figure 2–4)**.

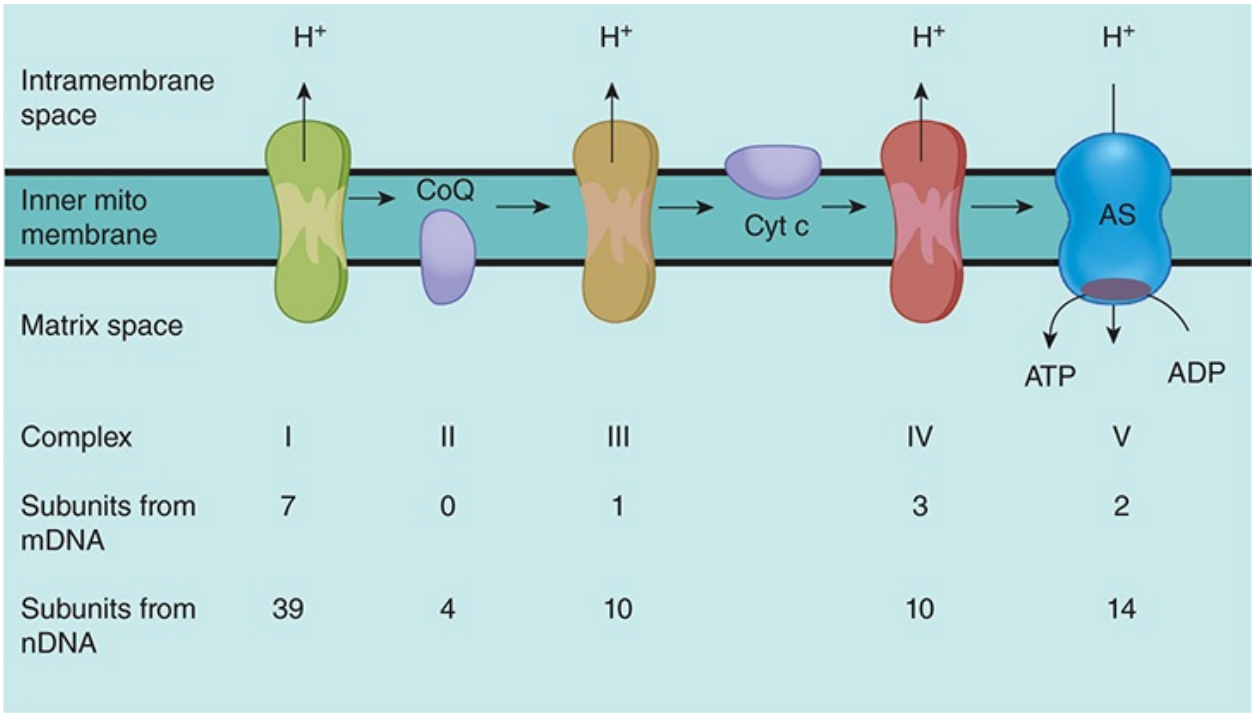


FIGURE 2–4 Components involved in oxidative phosphorylation in mitochondria and their origins. As enzyme complexes I through IV convert 2-carbon metabolic fragments to CO_2 and H_2O, protons (H^+) are pumped into the intermembrane space. The protons diffuse back to the matrix space via complex

V, ATP synthase (AS), in which ADP is converted to ATP. The enzyme complexes are made up of subunits coded by mitochondrial DNA (mDNA) and nuclear DNA (nDNA), and the figures document the contribution of each DNA to the complexes.

Consistent with their origin from aerobic bacteria, the mitochondria have their own genome. There is much less DNA in the mitochondrial genome than in the nuclear genome, and 99% of the proteins in the mitochondria are the products of nuclear genes, but mitochondrial DNA is responsible for certain key components of the pathway for oxidative phosphorylation. Specifically, human mitochondrial DNA is a double-stranded circular molecule containing approximately 16,500 base pairs (compared with over a billion in nuclear DNA). It codes for 13 protein subunits that are associated with proteins encoded by nuclear genes to form four enzyme complexes plus two ribosomal and 22 transfer RNAs that are needed for protein production by the intramitochondrial ribosomes.

CLINICAL BOX 2–1

Mitochondrial Diseases

Mitochondrial diseases encompass at least 40 diverse disorders that are grouped because of their links to mitochondrial failure. These diseases can occur following inheritance or spontaneous mutations in mitochondrial or nuclear DNA that lead to altered functions of the mitochondrial proteins (or RNA). Depending on the target cell and/or tissues affected, symptoms resulting from mitochondrial diseases may include altered motor control; altered muscle output; gastrointestinal dysfunction; altered growth; diabetes; seizures; visual/hearing problems; lactic acidosis; developmental delays; and susceptibility to infection or cardiac, liver, and respiratory disease. Although there is evidence for tissue-specific isoforms of mitochondrial proteins, mutations in these proteins do not fully explain the highly variable patterns or targeted organ systems observed with mitochondrial diseases.

THERAPEUTIC HIGHLIGHTS

With the diversity of disease types and the overall importance of mitochondria in energy production, it is not surprising that there is no single cure for mitochondrial diseases and focus remains on treating the symptoms when possible. For example, in some mitochondrial myopathies (ie, mitochondrial

diseases associated with neuromuscular function), physical therapy may help extend the range of movement of muscles and improve dexterity.

The enzyme complexes responsible for oxidative phosphorylation illustrate the interactions between the products of the mitochondrial genome and the nuclear genome. For example, complex I, reduced nicotinamide adenine dinucleotide dehydrogenase (NADH), is made up of seven protein subunits coded by mitochondrial DNA and 39 subunits coded by nuclear DNA. The origin of the subunits in the other complexes is shown in Figure 2–4. Complex II, succinate dehydrogenase- ubiquinone oxidoreductase; complex III, ubiquinonecytochrome c oxidoreductase; and complex IV, cytochrome c oxidase, act with complex I, coenzyme Q, and cytochrome c to convert metabolites to CO_2 and H_2O. Complexes I, III, and IV pump protons (H^+) into the intermembrane space during this electron transfer. The protons then flow down their electrochemical gradient through complex V, ATP synthase, which harnesses this energy to generate ATP.

As zygote mitochondria are derived from the ovum, their inheritance is maternal. This maternal inheritance has been used as a tool to track evolutionary descent. Mitochondria have an ineffective DNA repair system, and the mutation rate for mitochondrial DNA is over 10 times the rate for nuclear DNA. A large number of relatively rare diseases have been traced to mutations in mitochondrial DNA. These include disorders of tissues with high metabolic rates in which energy production is defective as a result of abnormalities in the production of ATP, as well as other disorders **(Clinical Box 2–1)**.

LYSOSOMES

In the cytoplasm of the cell there are large, somewhat irregular structures surrounded by membranes (Figure 2–1). The interior of these structures, which are called **lysosomes,** is more acidic than the rest of the cytoplasm, and external material such as endocytosed bacteria, as well as worn-out cell components, are digested in them. The interior is kept acidic by the action of a **proton pump,** or **H^+ ATPase.** This integral membrane protein uses the energy of ATP to move protons from the cytosol up their electrochemical gradient and keep the lysosome relatively acidic, near pH 5.0. Lysosomes can contain over 40 types of hydrolytic enzymes, some of which are listed in **Table 2–1**. Not surprisingly,

these enzymes are all acid hydrolases, in that they function best at the acidic pH of the lysosomal compartment. This can be a safety feature for the cell; if the lysosomes were to break open and release their contents, the enzymes would not be efficient at the near neutral cytosolic pH 7.2, and thus would be unable to digest cytosolic targets they may encounter. Diseases associated with lysosomal dysfunction are discussed in **Clinical Box 2–2**.

TABLE 2–1 Some of the enzymes found in lysosomes and the cell components that are their substrates.

Enzyme	Substrate
Ribonuclease	RNA
Deoxyribonuclease	DNA
Phosphatase	Phosphate esters
Glycosidases	Complex carbohydrates: glycosides and polysaccharides
Arylsulfatases	Sulfate esters
Collagenase	Collagens
Cathepsins	Proteins

CLINICAL BOX 2–2

Lysosomal Diseases

When a lysosomal enzyme is congenitally absent, the lysosomes become engorged with the material the enzyme normally degrades. This eventually leads to one of the **lysosomal diseases** (also called lysosomal storage diseases). There are over 50 such diseases currently recognized. For example, Fabry disease is caused by a deficiency in α-galactosidase; Gaucher disease is caused by a deficiency in β-galactocerebrosidase; and Tay-Sachs disease, which causes mental retardation and blindness, is caused by the loss of hexosaminidase A, a lysosomal enzyme that catalyzes the biodegradation of gangliosides (fatty acid derivatives). Such individual lysosomal diseases are rare, but they are serious and can be fatal.

THERAPEUTIC HIGHLIGHTS

Since there are many different lysosomal disorders, treatments vary considerably and “cures” remain elusive for most of these diseases. Much of the care is focused on managing symptoms of each specific disorder. Enzyme replacement therapy has shown to be effective for certain lysosomal diseases, including Gaucher disease and Fabry disease. However, the long-term effectiveness and the tissue-specific effects of many of the enzyme replacement treatments have not yet been established. Recent alternative approaches include bone marrow or stem cell transplantation. Again, medical advances are still necessary to fully combat this group of diseases.

PEROXISOMES

Peroxisomes are 0.5 µm in diameter (Figure 2–1), are surrounded by a membrane, and contain enzymes that can either produce H_2O_2 **(oxidases)** or break it down **(catalases).** Proteins are directed to the peroxisome by a unique signal sequence with the help of protein chaperones, **peroxins.** The peroxisome membrane contains a number of peroxisome-specific proteins that are concerned with transport of substances into and out of the matrix of the peroxisome. The matrix contains more than 40 enzymes, which operate in concert with enzymes outside the peroxisome to catalyze a variety of anabolic and catabolic reactions (eg, breakdown of lipids). Peroxisomes can form by budding of the endoplasmic reticulum, or by division. A number of synthetic compounds were found to cause proliferation of peroxisomes by acting on receptors in the nuclei of cells. These **peroxisome proliferator-activated receptors (PPARs)** are members of the nuclear receptor superfamily. When activated, they bind to DNA, producing changes in the production of mRNAs. The known effects for PPARs are extensive and can affect most tissues and organs.

CYTOSKELETON

All cells have a **cytoskeleton,** a system of fibers that not only maintains the structure of the cell but also permits it to change shape and move. The cytoskeleton is made up primarily of **microtubules, intermediate filaments,** and **microfilaments (Figure 2–5)**, along with proteins that anchor them and tie

them together. In addition, proteins and organelles move along microtubules and microfilaments from one part of the cell to another, propelled by molecular motors.

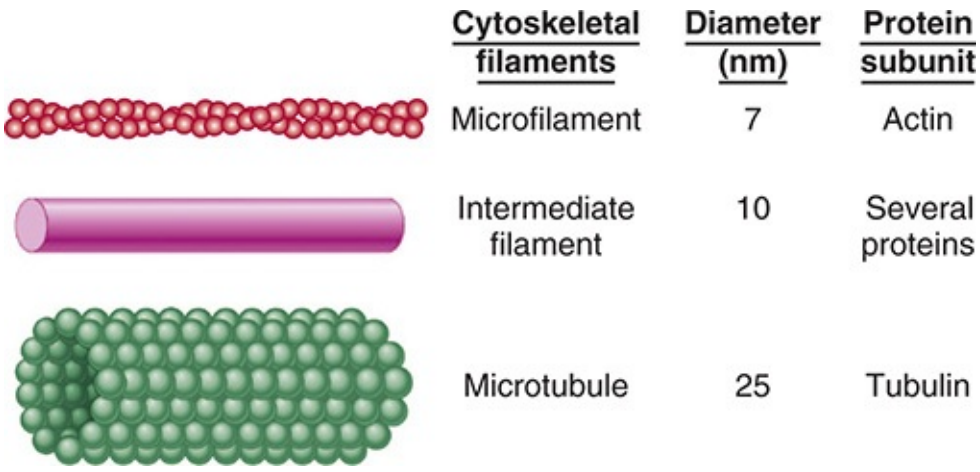


FIGURE 2–5 Cytoskeletal elements of the cell. Artistic impressions that depict the major cytoskeletal elements are shown on the left, with approximate diameters and protein subunits of these elements are listed for comparison. (Reproduced with permission from Widmaier EP, Raff H, Strang KT: *Vander's Human Physiology: The Mechanisms of Body Function*, 11th ed. New York, NY: McGraw-Hill; 2008.)

Microtubules (Figure 2–5 and **Figure 2–6**) are long, hollow structures with 5 nm walls surrounding a cavity 15 nm in diameter. They are made up of two globular protein subunits: α- and β-tubulin. A third subunit, γ-tubulin, is associated with the production of microtubules by the centrosomes. The α and β subunits form heterodimers, which aggregate to form long tubes made up of stacked rings, with each ring usually containing 13 subunits. The tubules interact with guanosine triphosphate (GTP) to facilitate their formation. Although microtubule subunits can be added to either end, microtubules are polar with assembly predominating at the plus ("+") end and disassembly predominating at the minus ("–") end. Both processes occur simultaneously in vitro. The growth of microtubules is temperature sensitive (disassembly is favored under cold conditions) as well as under the control of a variety of cellular factors that can directly interact with microtubules in the cell.

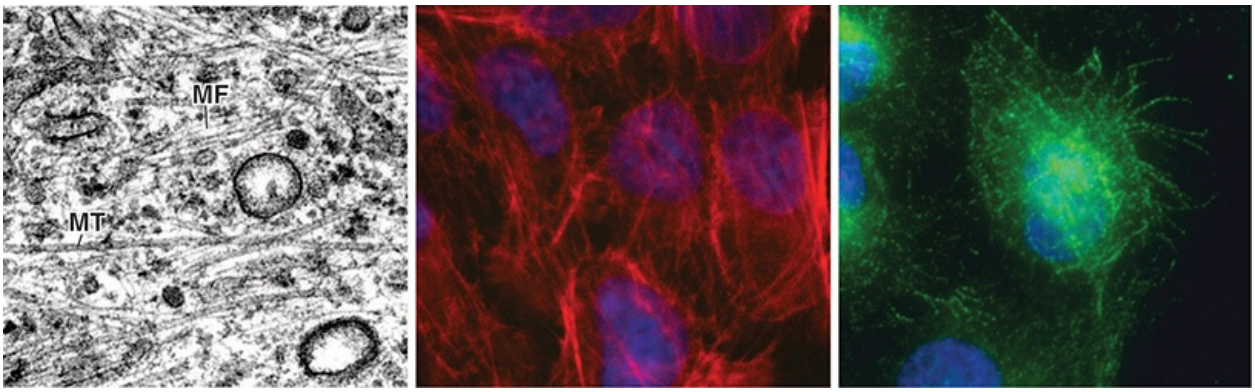


FIGURE 2–6 Microfilaments and microtubules. Electron micrograph **(Left)** of the cytoplasm of a fibroblast, displaying actin microfilaments (MF) and microtubules (MT). Fluorescent micrographs of airway epithelial cells displaying actin microfilaments stained with phalloidin **(Middle)** and microtubules visualized with an antibody to β-tubulin **(Right)**. Both fluorescent micrographs are counterstained with Hoechst dye (blue) to visualize nuclei. Note the distinct differences in cytoskeletal structure. (Electron 70 micrograph used with permission of E Katchburian; fluorescent micrographs used with permission of Stephanie Manberg.)

Because of their constant assembly and disassembly, microtubules are a dynamic portion of the cytoskeleton. They provide the tracks along which several different molecular motors move transport vesicles, organelles such as secretory granules, and mitochondria from one part of the cell to another. They also form the spindle, which moves the chromosomes in mitosis. Cargo can be transported in either direction on microtubules.

There are several drugs available that disrupt cellular function through interaction with microtubules. Microtubule assembly is prevented by colchicine and vinblastine. The anticancer drug **paclitaxel (Taxol)** binds to microtubules and makes them so stable that organelles cannot move. Mitotic spindles cannot form, and the cells die.

Intermediate filaments (Figure 2–5) are 8–14 nm in diameter and are made up of various subunits. Some of these filaments connect the nuclear membrane to the cell membrane. They form a flexible scaffolding for the cell and help it resist external pressure. In their absence, cells rupture more easily, and when they are abnormal in humans, blistering of the skin is common. The proteins that make up intermediate filaments are cell-type specific, and are thus frequently used as cellular markers. For example, vimentin is a major intermediate filament in fibroblasts, whereas cytokeratin is expressed in epithelial cells.

Microfilaments (Figures 2–5 and 2–6) are long solid fibers with a 5–9 nm diameter that are made up of **actin.** Although actin is most often associated with muscle contraction, it is present in all types of cells. It is the most abundant protein in mammalian cells, sometimes accounting for as much as 15% of the total protein in the cell. Its structure is highly conserved; for example, 88% of the amino acid sequences in yeast and rabbit actin are identical. Actin filaments polymerize and depolymerize in vivo, and it is not uncommon to find polymerization occurring at one end of the filament while depolymerization is occurring at the other end. **Filamentous (F) actin** refers to intact microfilaments and **globular (G) actin** refers to the unpolymerized protein actin subunits. F-actin fibers attach to various parts of the cytoskeleton and can interact directly or indirectly with membrane-bound proteins. They reach to the tips of the microvilli on the epithelial cells of the intestinal mucosa. They are also abundant in the lamellipodia that cells put out when they crawl along surfaces. The actin filaments interact with integrin receptors and form **focal adhesion complexes,** which serve as points of traction with the surface over which the cell pulls itself. In addition, some molecular motors use microfilaments as tracks.

MOLECULAR MOTORS

The molecular motors that move proteins, organelles, and other cell parts (collectively referred to as "cargo") to all parts of the cell are 100–500 kDa ATPases. They attach to their cargo at one end of the molecule and to microtubules or actin polymers with the other end, sometimes referred to as the "head." They convert the energy of ATP into movement along the cytoskeleton, taking their cargo with them. There are three super families of molecular motors: **kinesin, dynein,** and **myosin.** Examples of individual proteins from each superfamily are shown in **Figure 2–7**. It is important to note that there is extensive variation among superfamily members, allowing for the specialization of function (eg, choice of cargo, cytoskeletal filament type, and/or direction of movement).

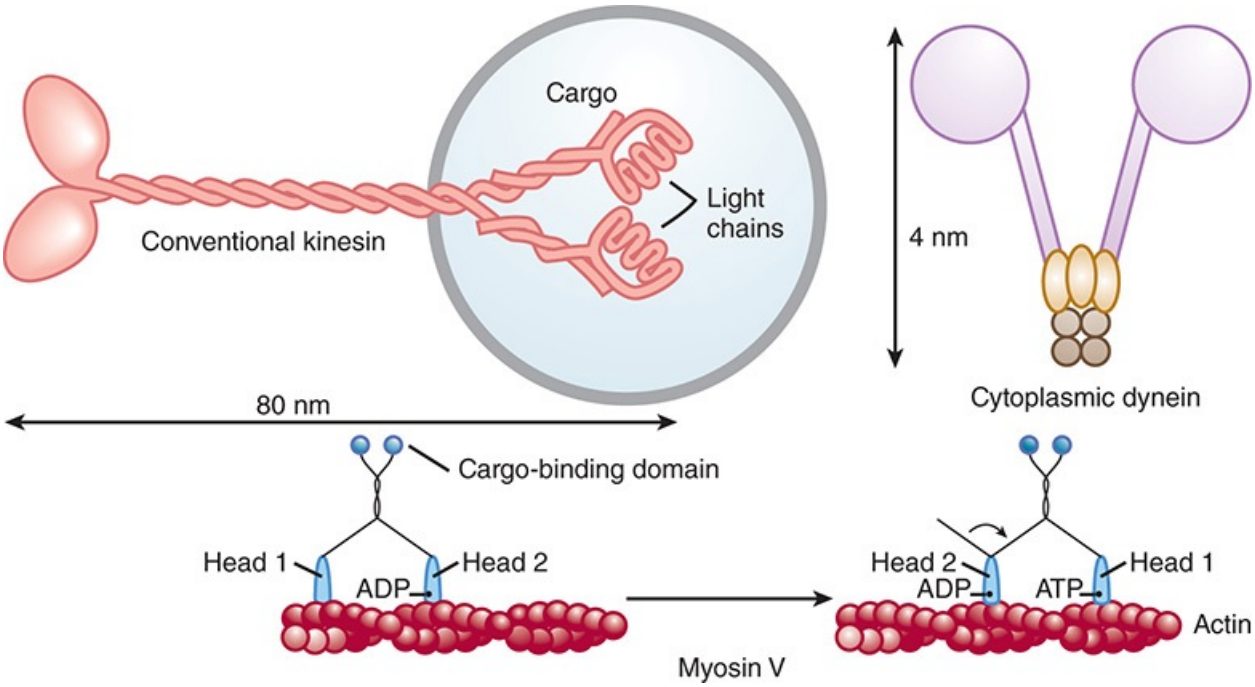


FIGURE 2–7 Examples of molecular motors. Conventional kinesin is shown attached to cargo, in this case a membrane-bound organelle (light blue). Cytoplasmic dynein is shown in isolation. Myosin V and its ability to "walk" along a microfilament are displayed in a two-part sequence. Note that the "heads" of each of the motors hydrolyze ATP and use the energy to produce motion.

The conventional form of **kinesin** is a double-headed molecule that tends to move its cargo toward the plus ("+") ends of microtubules. One head binds to the microtubule and then bends its neck while the other head swings forward and binds, producing almost continuous movement. Some kinesins are associated with mitosis and meiosis. Other kinesins perform different functions, including, in some instances, moving cargo to the minus ("–") end of microtubules. **Dyneins** have two heads, with their neck pieces embedded in a complex of proteins. **Cytoplasmic dyneins** have a function like that of conventional kinesin, except they tend to move particles and membranes to the minus (–) end of the microtubules. The multiple forms of **myosin** in the body are divided into 18 classes. The heads of myosin molecules bind to actin and produce motion by bending their neck regions (myosin II) or walking along microfilaments, one head after the other (myosin V). In these ways, they perform functions as diverse as contraction of muscle and cell migration.

CENTROSOMES

Near the nucleus in the cytoplasm of eukaryotic animal cells is a **centrosome.** The centrosome is made up of two **centrioles** and surrounding amorphous **pericentriolar material.** The centrioles are short cylinders arranged so that they are at right angles to each other. Microtubules in groups of three run longitudinally in the walls of each centriole (Figure 2–1). Nine of these triplets are spaced at regular intervals around the circumference.

The centrosomes are **microtubule-organizing centers (MTOCs)** that contain γ-tubulin. The microtubules grow out of this γ-tubulin in the pericentriolar material. When a cell divides, the centrosomes duplicate themselves, and the pairs move apart to the poles of the mitotic spindle, where they monitor the steps in cell division. In multinucleate cells, a centrosome is near each nucleus.

CILIA

Cilia are specialized cellular projections that are used by unicellular organisms to propel themselves through liquid and by multicellular organisms to propel mucus and other substances over the surface of various epithelia. Additionally, virtually all cells in the human body contain a primary cilium that emanates from the surface. The primary cilium serves as a sensory organelle that receives both mechanical and chemical signals from other cells and the environment. Cilia are functionally indistinct from the eukaryotic flagella of sperm cells. Within the cilium there is an **axoneme** that comprises a unique arrangement of nine outer microtubule doublets and two inner microtubules (“9+2” arrangement). Along this cytoskeleton is **axonemal dynein.** Coordinated dynein– microtubule interactions within the axoneme are the basis of ciliary and sperm movement. At the base of the axoneme and just inside lies the **basal body.** It has nine circumferential triplet microtubules, like a centriole, and there is evidence that basal bodies and centrioles are interconvertible. A wide variety of diseases and disorders arise from dysfunctional cilia **(Clinical Box 2–3)**.

CELL ADHESION MOLECULES

Cells are attached to the basal lamina and to each other by **CAMs** that are prominent parts of the intercellular connections described below. The unique structural and signaling functions of these adhesion proteins have been found to

be important in embryonic development and formation of the nervous system and other tissues, in holding tissues together in adults, in inflammation and wound healing, and in the metastasis of tumors. Many CAMs pass through the cell membrane and are anchored to the cytoskeleton inside the cell. Some bind to like molecules on other cells (homophilic binding), whereas others bind to nonself molecules (heterophilic binding). Many bind to **laminins,** a family of large cross-shaped molecules with multiple receptor domains in the extracellular matrix.

Nomenclature in the CAM field is somewhat chaotic, partly because the field is growing so rapidly and partly because of the extensive use of acronyms, as in other areas of modern biology. However, the CAMs can be divided into four broad families: (1) **integrins,** heterodimers that bind to various receptors; (2) adhesion molecules of the **IgG superfamily** of immunoglobulins; (3) **cadherins,** Ca^{2+}-dependent molecules that mediate cell-to-cell adhesion by homophilic reactions; and (4) **selectins,** which have lectin-like domains that bind carbohydrates.

CLINICAL BOX 2–3

Ciliary Diseases

Primary ciliary dyskinesia refers to a set of inherited disorders that limit ciliary structure and/or function. Disorders associated with ciliary dysfunction have long been recognized in the conducting airway. Altered ciliary function in the conducting airway can slow the mucociliary escalator and result in airway obstruction and increased infection. Dysregulation of ciliary function in sperm cells has also been well characterized to result in loss of motility and infertility. Ciliary defects in the function or structure of primary cilia have been shown to have effects on a variety of tissues/organs. As would be expected, such diseases are quite varied in their presentation, largely due to the affected tissue, and include mental retardation, retinal blindness, obesity, polycystic kidney disease, liver fibrosis, ataxia, and some forms of cancer.

THERAPEUTIC HIGHLIGHTS

The severity in ciliary disorders can vary widely, and treatments targeted to individual organs also vary. Treatment of ciliary dyskinesia in the conducting airway is focused on keeping the airways clear and free of infection. Strategies

include routine washing and suctioning of the sinus cavities and ear canals and liberal use of antibiotics. Other treatments that keep the airway from being obstructed (eg, bronchodilators, mucolytics, and corticosteroids) are also commonly used.

The CAMs not only fasten cells to their neighbors, but they also transmit signals into and out of the cell. For example, cells that lose their contact with the extracellular matrix via integrins have a higher rate of apoptosis than anchored cells, and interactions between integrins and the cytoskeleton are involved in cell movement.

INTERCELLULAR CONNECTIONS

Intercellular junctions that form between the cells in tissues can be broadly split into two groups: junctions that fasten the cells to one another and to surrounding tissues, and junctions that permit transfer of ions and other molecules from one cell to another. The types of junctions that tie cells together and endow tissues with strength and stability include **tight junctions,** which are also known as the **zonula occludens** **(Figure 2–8)**. The **desmosome** and **zonula adherens** also help hold cells together, and the **hemidesmosome** and **focal adhesions** attach cells to their basal laminas. The **gap junction** forms a cytoplasmic “tunnel” for diffusion of small molecules (< 1000 Da) between two neighboring cells.

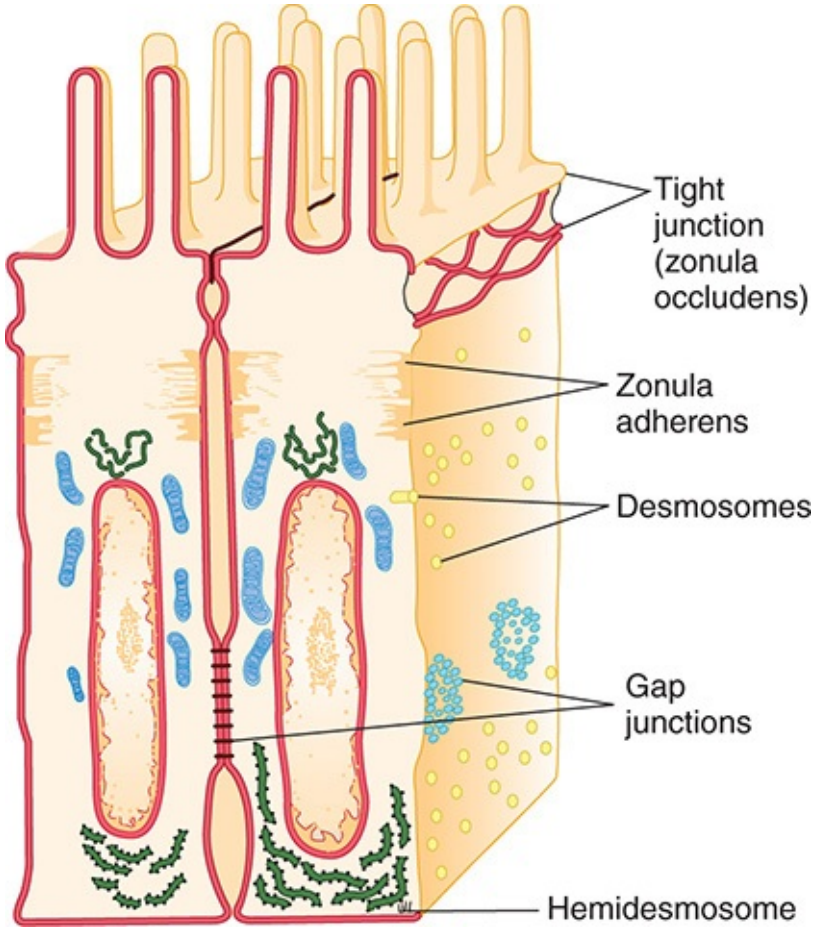


FIGURE 2–8 Intercellular junctions in the mucosa of the small intestine. Tight junctions (zonula occludens), adherens junctions (zonula adherens), desmosomes, gap junctions, and hemidesmosomes are all shown in relative positions in a polarized epithelial cell.

Tight junctions characteristically surround the apical margins of the cells in epithelia such as the intestinal mucosa, the walls of the renal tubules, and the choroid plexus. They are also important to endothelial barrier function and endothelium-dependent vasodilation. They are made up of ridges—half from one cell and half from the other—which adhere so strongly at cell junctions that they almost obliterate the space between the cells. There are three main families of transmembrane proteins that contribute to tight junctions: **occludin, junctional adhesion molecules,** and **claudins;** there are several more proteins that interact from the cytosolic side. Tight junctions permit the passage of ions, solute and intracellular signaling molecules in between adjacent cells **(paracellular pathway)** and the degree of this “leakiness” varies, depending in part on the protein makeup of the tight junction. Extracellular fluxes of ions and solute

across epithelia at these junctions are a significant part of overall ion and solute flux. In addition, tight junctions prevent the movement of proteins in the plane of the membrane, helping maintain the different distribution of transporters and channels in the apical and basolateral cell membranes that make transport across epithelia possible.

In epithelial cells, each zonula adherens is usually a continuous structure on the basal side of the zonula occludens, and it is a major site of attachment for intracellular microfilaments. It contains cadherins.

Desmosomes are patches characterized by apposed thickenings of the membranes of two adjacent cells. Attached to the thickened area in each cell are intermediate filaments, some running parallel to the membrane and others radiating away from it. Between the two membrane thickenings, the intercellular space contains filamentous material that includes cadherins and the extracellular portions of several other transmembrane proteins.

Hemidesmosomes look like half-desmosomes that attach cells to the underlying basal lamina and are connected intracellularly to intermediate filaments. However, they contain integrins rather than cadherins. Focal adhesions also attach cells to their basal laminas. As noted previously, they are labile structures associated with actin filaments inside the cell, and they play an important role in cell movement.

GAP JUNCTIONS

The intercellular space is up to 4 nm at gap junctions. Here, units called **connexons** in the membrane of each cell are lined up with one another to form the dodecameric gap junction **(Figure 2–9)**. Each connexon is made up of six protein subunits called **connexins.** They surround a channel that, when lined up with the channel in the corresponding connexon in the adjacent cell, permits substances to pass between the cells without entering the ECF. The pore diameter in the channel is estimated between 0.8 and 1.4 nm, which permits the passage of ions, sugars, amino acids, and other solutes with molecular weights up to about 1000 Da. Gap junctions thus permit the rapid propagation of electrical activity from cell to cell, as well as the exchange of various chemical messengers and intracellular signaling molecules. However, the gap junction channels are not simply passive, nonspecific conduits. At least 20 different genes code for connexins in humans, and mutations in these genes can lead to diseases that are highly selective in terms of the tissues involved and the type of communication between cells produced **(Clinical Box 2–4**). Experiments in

which particular connexins are deleted by gene manipulation or replaced with different connexins confirm that the particular connexin subunits that make up connexons determine their permeability and selectivity. It should be noted that connexons can also provide a conduit (ie, connexin semichannels) for regulated passage of small molecules between the cytoplasm and the ECF. Such movement can allow additional signaling pathways between and among cells in a tissue.

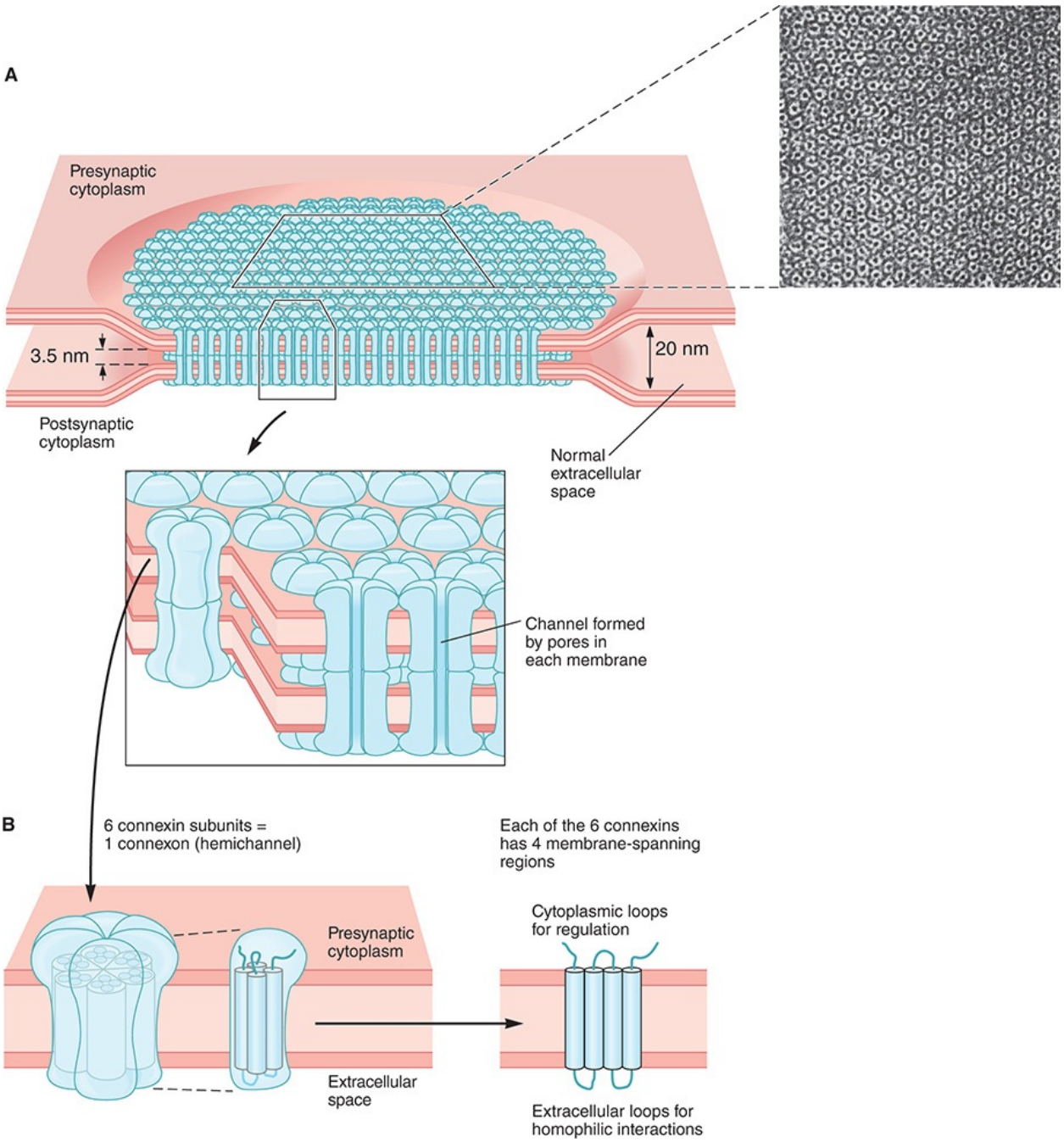


FIGURE 2–9 Gap junction connecting the cytoplasm of two cells. A) A gap

junction plaque, or collection of individual gap junctions, is shown to form multiple pores between cells that allow for the transfer of small molecules. Inset is an electron micrograph from rat liver. **B)** Topographic depiction of individual connexon and corresponding six connexin proteins that traverse the membrane. Note that each connexin traverses the membrane four times. (Reproduced with permission from Kandel ER, Schwartz JH, Jessell TM, Siegelbaum SA, Hudspeth AJ (editors): *Principles of Neural Science*, 5th ed. New York, NY: McGraw-Hill; 2013.)

CLINICAL BOX 2–4

Connexins in Disease

There is extensive information related to the in vivo functions of connexins, growing out of work on connexin knockouts in mice and the analysis of mutations in human connexins. The mouse knockouts demonstrated that connexin deletions lead to electrophysiological defects in the heart and predisposition to sudden cardiac death, female sterility, abnormal bone development, abnormal growth in the liver, cataracts, hearing loss, and a host of other abnormalities. Information from these and other studies has allowed for the identification of several connexin mutations known to be responsible for almost 20 different human diseases. These diseases include several skin disorders such as Clouston syndrome (a connexin 30 (Cx30) defect) and erythrokeratoderma variabilis (Cx30.3 and Cx31); inherited deafness (Cx26, Cx30, and Cx31); predisposition to myoclonic epilepsy (Cx36); predisposition to arteriosclerosis (Cx37); cataract (Cx46 and Cx50); idiopathic atrial fibrillation (Cx40); and X-linked Charcot-Marie-Tooth disease (Cx32). It is interesting to note that each of these target tissues for disease contains other connexins that do not fully compensate for loss of the crucial connexins in disease development. Understanding how loss of individual connexins alters cell physiology to contribute to these and other human diseases is an area of intense research.

NUCLEUS & RELATED STRUCTURES

A nucleus is present in all eukaryotic cells that divide (Figure 2–1). The nucleus is made up in large part of the **chromosomes,** the structures in the nucleus that

carry a complete blueprint for all the heritable species and individual characteristics of the animal. Except in germ cells, the chromosomes occur in pairs, one originally from each parent. Each chromosome is made up of a giant molecule of **DNA.** The DNA strand is about 2 m long, but it can fit in the nucleus because at intervals it is wrapped around a core of histone proteins to form a **nucleosome.** There are about 25 million nucleosomes in each nucleus. Thus, the structure of the chromosomes has been likened to a string of beads. The beads are the nucleosomes, and the linker DNA between them is the string. The whole complex of DNA and proteins is called **chromatin.** During cell division, the coiling around histones is loosened, probably by acetylation of the histones, and pairs of chromosomes become visible, but between cell divisions only clumps of chromatin can be discerned in the nucleus. The ultimate units of heredity are the **genes** on the chromosomes. As discussed in Chapter 1, each gene is a portion of the DNA molecule.

The nucleus of most cells contains a **nucleolus** (Figure 2–1), a patchwork of granules rich in **RNA.** In some cells, the nucleus contains several of these structures. Nucleoli are most prominent and numerous in growing cells. They are the site of synthesis of ribosomes, the structures in the cytoplasm in which proteins are synthesized.

The interior of the nucleus has a skeleton of fine filaments that are attached to the **nuclear membrane,** or **envelope** (Figure 2–1), which surrounds the nucleus. This membrane is a double membrane, and spaces between the twofolds are called **perinuclear cisterns.** The membrane is permeable only to small molecules. However, it contains **nuclear pore complexes.** Each complex has eightfold symmetry and is made up of about 100 proteins organized to form a tunnel through which transport of proteins and mRNA occurs. There are many transport pathways; many proteins that participate in these pathways, including **importins** and **exportins,** have been isolated and characterized. Much current research is focused on transport into and out of the nucleus, and a more detailed understanding of these processes should emerge in the near future.

ENDOPLASMIC RETICULUM

The **endoplasmic reticulum** is a complex series of tubules in the cytoplasm of the cell (Figure 2–1; **Figure 2–10**; and **Figure 2–11**). The inner limb of its membrane is continuous with a segment of the nuclear membrane, so in effect this part of the nuclear membrane is a cistern of the endoplasmic reticulum. The tubule walls are made up of membrane. In **rough (granular) endoplasmic**

reticulum, ribosomes are attached to the cytoplasmic side of the membrane, whereas in **smooth (agranular) endoplasmic reticulum,** ribosomes are absent. Free ribosomes are also found in the cytoplasm. The rough endoplasmic reticulum is concerned with protein synthesis and the initial folding of polypeptide chains with the formation of disulfide bonds. The smooth endoplasmic reticulum is the site of steroid synthesis in steroid-secreting cells and the site of detoxification processes in other cells. A modified endoplasmic reticulum, the sarcoplasmic reticulum, plays an important role in skeletal muscle, cardiac muscle, and smooth muscle cells. In particular, the endoplasmic or sarcoplasmic reticulum can sequester cytosolic Ca^{2+} ions and function as an intracellular Ca^{2+} store, which then allow for Ca^{2+} release into the cytosol as signaling molecules to stimulate cell contraction, proliferation, and migration.

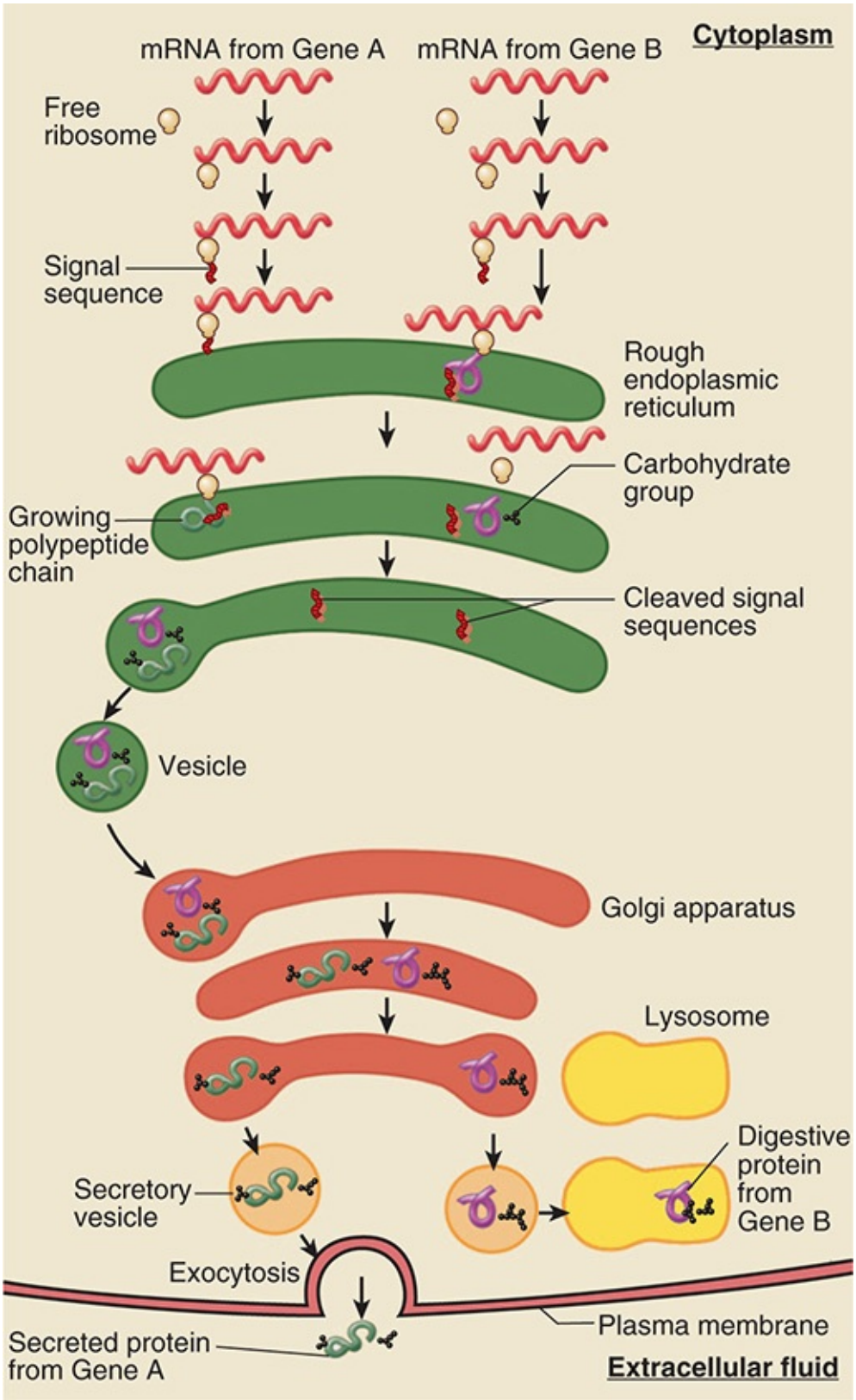


FIGURE 2–10 Rough endoplasmic reticulum and protein translation.

Messenger RNA and ribosomes meet up in the cytosol for translation. Proteins that have appropriate signal peptides begin translation, and then associate with the endoplasmic reticulum (ER) to complete translation. The association of ribosomes is what gives the ER its “rough” appearance. (Reproduced with permission from Widmaier EP, Raff H, Strang KT: *Vander’s Human Physiology: The Mechanisms of Body Function*, 11th ed. New York, NY: McGraw-Hill; 2008.)

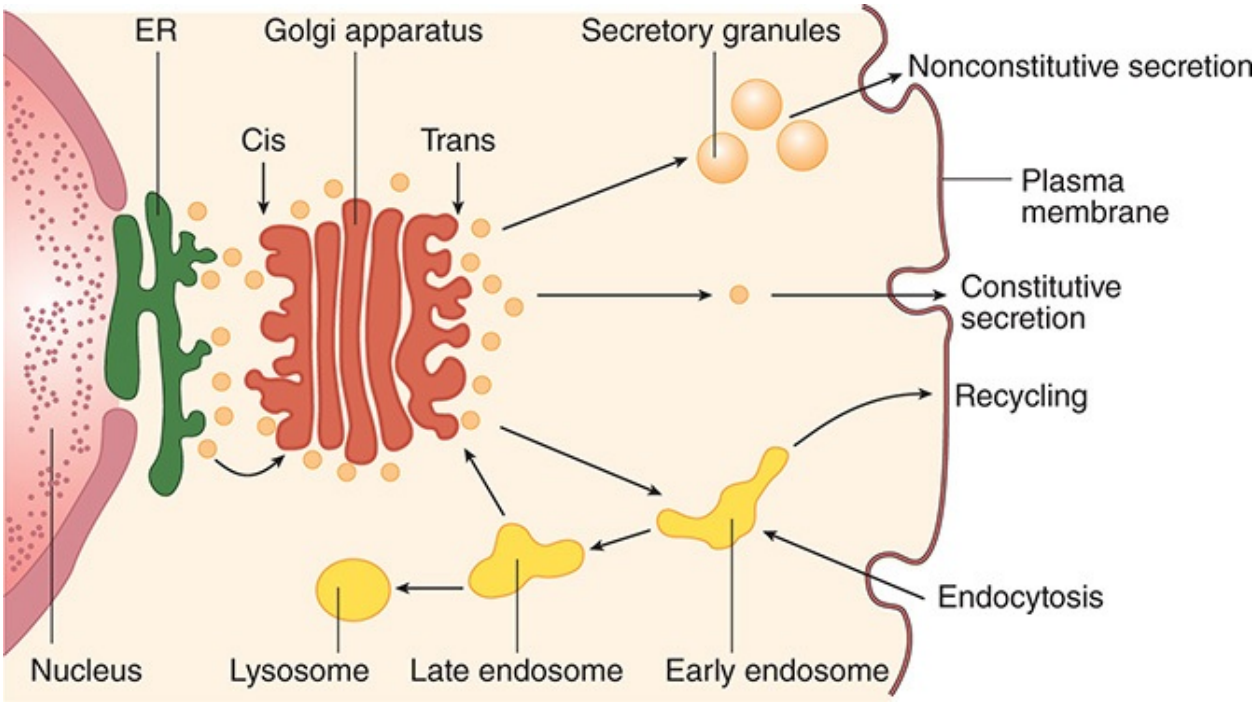


FIGURE 2–11 Cellular structures involved in protein processing. Structures involved in protein processing, from transcription to secretion, are shown. See text for details.

RIBOSOMES

The ribosomes in eukaryotes measure approximately 22 × 32 nm. Each is made up of a large and a small subunit called, on the basis of their rates of sedimentation in the ultracentrifuge, the 60S and 40S subunits. The ribosomes are complex structures, containing many different proteins and at least three ribosomal RNAs. They are the sites of protein synthesis. The ribosomes that become attached to the endoplasmic reticulum synthesize all transmembrane proteins, most secreted proteins, and most proteins that are stored in the Golgi apparatus, lysosomes, and endosomes. These proteins typically have a

hydrophobic **signal peptide** at one end (Figure 2–10). The polypeptide chains that form these proteins are extruded into the endoplasmic reticulum. The free ribosomes synthesize cytoplasmic proteins such as hemoglobin and the proteins found in peroxisomes and mitochondria.

GOLGI APPARATUS & VESICULAR TRAFFIC

The Golgi apparatus is a collection of membrane-enclosed sacs (cisternae) that are stacked like dinner plates (Figure 2–1). One or more Golgi apparati are present in all eukaryotic cells, usually near the nucleus. Much of the organization of the Golgi is directed at proper glycosylation of proteins and lipids. There are more than 200 enzymes that function to add, remove, or modify sugars from proteins and lipids in the Golgi apparatus.

The Golgi apparatus is a polarized structure with two distinct sides or faces, cis and trans sides (Figures 2–1, 2–10, 2–11). The cis face is near the endoplasmic reticulum, while the opposite trans face is near the plasma membrane (Figure 2–11). Membranous vesicles containing newly synthesized proteins bud off from the rouph endoplasmic reticulum and fuse with the cistern on the cis side of the Golgi apparatus. The proteins are then passed via other vesicles to the middle cisterns and finally to the cistern on the trans side, from which vesicles branch off into the cytoplasm. From the trans side of Golgi, vesicles shuttle to the lysosomes and to the cell exterior via constitutive and nonconstitutive pathways, both involving **exocytosis.** Conversely, vesicles are pinched off from the cell membrane by **endocytosis** and pass to endosomes. From there, they are recycled.

Vesicular traffic in the Golgi, and between other membranous compartments in the cell, is regulated by a combination of common mechanisms along with special mechanisms that determine where inside the cell they will go. One prominent feature is the involvement of a series of regulatory proteins controlled by GTP or guanosine diphosphate (GDP) binding **(small G-proteins)** associated with vesicle assembly and delivery. A second prominent feature is the presence of proteins called **SNAREs** (for soluble N-ethylmaleimide-sensitive factor attachment receptor). The v- (for vesicle) SNAREs on vesicle membranes interact in a lock-and-key fashion with t- (for target) SNAREs. Individual vesicles also contain structural protein or lipids in their membrane that help target them for specific membrane compartments (eg, Golgi sacs, cell membranes).

QUALITY CONTROL

The processes involved in protein synthesis, folding, and migration to the various parts of the cell are so complex that it is remarkable that more errors and abnormalities do not occur. The fact that these processes work as well as they do is because of mechanisms at each level that are responsible for "quality control." Damaged DNA is detected and repaired or bypassed. The various RNAs are also checked during the translation process. Finally, when the protein chains are in the endoplasmic reticulum and Golgi apparatus, defective structures are detected and the abnormal proteins are degraded in lysosomes and proteasomes. The net result is a remarkable accuracy in the production of the proteins needed for normal cell function.

APOPTOSIS

In addition to dividing and growing under genetic control, cells can die and be absorbed under genetic control. This process is called **programmed cell death,** or **apoptosis** (Gr. apo "away" + ptosis "fall"). It can be called "cell suicide" in the sense that the cell's own genes play an active role in its demise. It should be distinguished from necrosis ("cell murder"), in which healthy cells are destroyed by external processes such as inflammation.

Apoptosis is a very common process during development and in adulthood. In the central nervous system (CNS), large numbers of neurons are produced and then die during the remodeling that occurs during development and synapse formation. In the immune system, apoptosis gets rid of inappropriate clones of immunocytes and is responsible for the lytic effects of glucocorticoids on lymphocytes. Apoptosis is also an important factor in processes such as removal of the webs between the fingers in fetal life and regression of duct systems in the course of sexual development in the fetus. In adults, it participates in the cyclic breakdown of the endometrium that leads to menstruation. In epithelia, cells that lose their connections to the basal lamina and neighboring cells undergo apoptosis. This is responsible for the death of the enterocytes sloughed off the tips of intestinal villi. Abnormal apoptosis probably occurs in autoimmune diseases, neurodegenerative diseases, and cancer. It is interesting that apoptosis occurs in invertebrates, including nematodes and insects. However, its molecular mechanism is much more complex than that in vertebrates.

One final common pathway bringing about apoptosis is activation of **caspases,** a group of cysteine proteases. Many of these have been characterized

to date in mammals; 14 have been found in humans. They exist in cells as inactive proenzymes (procaspases) until activated by the cellular machinery. The net result is DNA fragmentation, cytoplasmic and chromatin condensation, and eventually membrane bleb formation, with cell breakup and removal of the debris by phagocytes **(Clinical Box 2–5)**.

CLINICAL BOX 2–5

Cellular and Molecular Medicine

Fundamental research on molecular aspects of genetics, regulation of gene expression, and protein synthesis has been paying off in clinical medicine at a rapidly accelerating rate.

One early dividend was an understanding of the mechanisms by which antibiotics exert their effects. Almost all act by inhibiting protein synthesis at one or another of the steps described previously. Antiviral drugs act in a similar way; for example, acyclovir and ganciclovir act by inhibiting DNA polymerase. Some of these drugs have this effect primarily in bacteria, but others inhibit protein synthesis in the cells of other animals, including mammals. This fact makes antibiotics of great value for research as well as for treatment of infections. Single genetic abnormalities that cause over 600 human diseases have been identified. Many of the diseases are rare, but others are more common and some cause conditions that are severe and eventually fatal. Examples include the defectively regulated Cl^- channel in cystic fibrosis and the unstable **trinucleotide repeats** in various parts of the genome that cause Huntington disease, the fragile X syndrome, and several other neurologic diseases. Abnormalities in mitochondrial DNA can also cause human diseases such as Leber hereditary optic neuropathy and some forms of cardiomyopathy. Not surprisingly, genetic aspects of cancer are probably receiving the greatest current attention. Some cancers are caused by **oncogenes,** genes that are carried in the genomes of cancer cells and are responsible for producing their malignant properties. These genes are derived by somatic mutation from closely related **proto-oncogenes,** which are normal genes that control growth. Over 100 oncogenes have been described. Another group of genes produce proteins that suppress tumors, and more than 10 of these **tumor suppressor genes** have been described. The most studied of these is the p53 gene on human chromosome 17. The p53 protein produced by this gene triggers apoptosis. It is also a nuclear transcription factor that

appears to increase production of a 21-kDa protein that blocks two cell cycle enzymes, slowing the cycle and permitting repair of mutations and other defects in DNA. The p53 gene is mutated in up to 50% of human cancers, with the production of p53 proteins that fail to slow the cell cycle and permit other mutations in DNA to persist. The accumulated mutations eventually cause cancer.

TRANSPORT ACROSS CELL MEMBRANES

There are several mechanisms of transport across cellular membranes. Primary pathways include exocytosis, endocytosis, membrane permeability and ion channels, and primary and secondary active transport. Each of these is discussed below.

EXOCYTOSIS

Vesicles containing material for export, such as secretory granules, are targeted to the cell membrane (Figure 2–11), where they bond in a similar manner to that discussed in vesicular traffic between Golgi stacks, via the v-SNARE/t-SNARE arrangement. The area of fusion then breaks down, leaving the contents of the vesicle outside and the cell membrane intact. This is the Ca^{2+}-dependent process of **exocytosis** **(Figure 2–12)**.

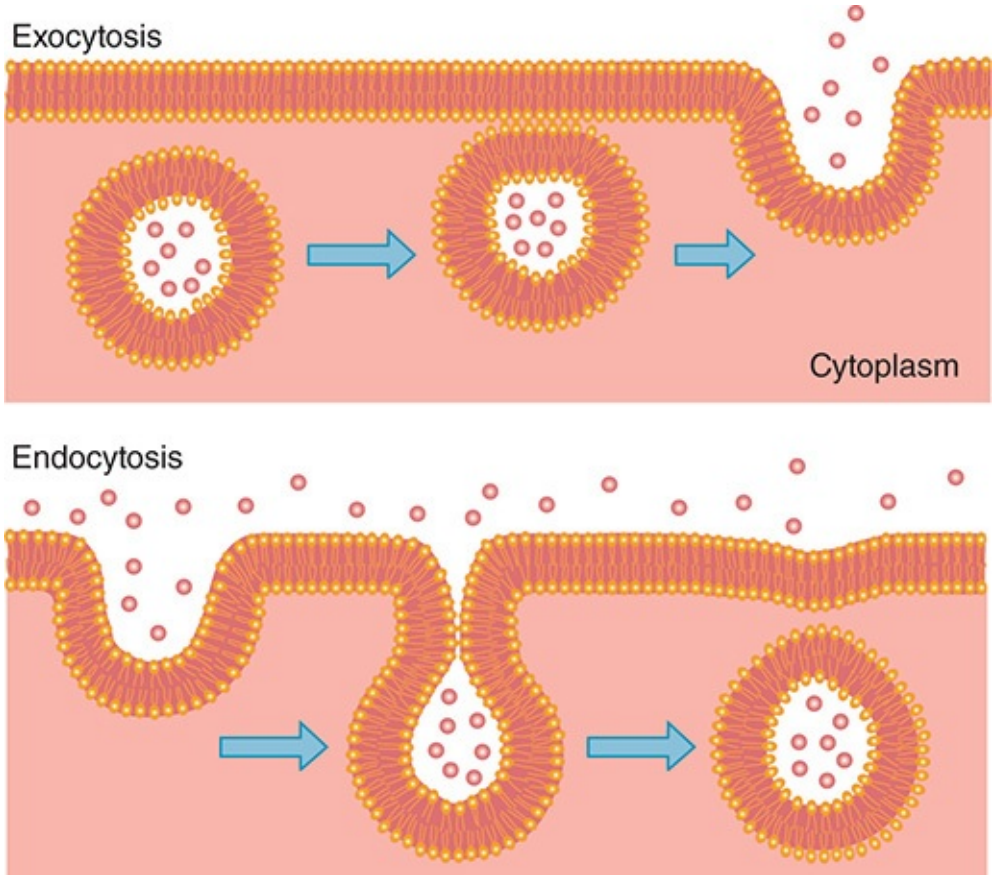


FIGURE 2–12 Exocytosis and endocytosis. Note that in exocytosis the cytoplasmic sides of two membranes fuse, whereas in endocytosis two extracellular sides fuse.

Note that secretion from the cell occurs via two pathways (Figure 2–11). In the **nonconstitutive (or regulated) pathway,** proteins from the Golgi apparatus initially enter secretory granules, where processing of prohormones to the mature hormones occurs before exocytosis. The other pathway, the **constitutive pathway,** involves the prompt transport of proteins to the cell membrane in vesicles, with little or no processing or storage. The nonconstitutive pathway is sometimes called the **regulated pathway,** but this term is misleading because the output of proteins by the constitutive pathway is also regulated.

ENDOCYTOSIS

Endocytosis is the reverse of exocytosis. There are various types of endocytosis named for the size of particles being ingested as well as the regulatory requirements for the particular process. These include **phagocytosis,**

pinocytosis, clathrin-mediated endocytosis, caveolae-dependent uptake, and **nonclathrin/noncaveolae endocytosis.**

Phagocytosis ("cell eating") is the process by which bacteria, dead cells, or other bits of microscopic material are engulfed by cells such as the polymorphonuclear leukocytes of the blood. The material makes contact with the cell membrane, which then invaginates. The invagination is pinched off, leaving the engulfed material in the membrane-enclosed vacuole and the cell membrane intact. **Pinocytosis** ("cell drinking") is a similar process with the vesicles much smaller in size and the substances ingested are in solution. The small size membrane that is ingested with each event should not be misconstrued; cells undergoing active pinocytosis (eg, macrophages) can ingest the equivalent of their entire cell membrane in just 1 h.

Clathrin-mediated endocytosis occurs at membrane indentations where the protein **clathrin** accumulates. Clathrin molecules have the shape of triskelions, with three "legs" radiating from a central hub **(Figure 2–13)**. As endocytosis progresses, the clathrin molecules form a geometric array that surrounds the endocytotic vesicle. At the neck of the vesicle, the GTP-binding protein **dynamin** is involved, either directly or indirectly, in pinching off the vesicle. Once the complete vesicle is formed, the clathrin falls off and the three-legged proteins recycle to form another vesicle. The vesicle fuses with and dumps its contents into an **early endosome** (Figure 2–11). From the early endosome, a new vesicle can bud off and return to the cell membrane. Alternatively, the early endosome can become a **late endosome** and fuse with a lysosome (Figure 2–11) in which the contents are digested by the lysosomal proteases. Clathrin-mediated endocytosis is responsible for the internalization of many receptors and the ligands bound to them—including, for example, nerve growth factor (NGF) and low-density lipoproteins. It also plays a major role in synaptic function.

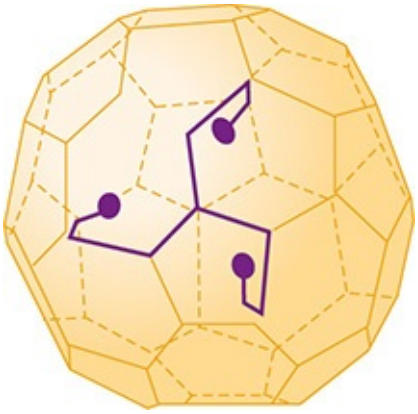


FIGURE 2–13 Clathrin molecule on the surface of an endocytotic vesicle.

Note the characteristic triskelion shape and the fact that with other clathrin molecules it forms a net supporting the vesicle.

It is apparent that exocytosis adds to the total amount of membrane surrounding the cell, and if membrane were not removed elsewhere at an equivalent rate, the cell would enlarge. However, removal of cell membrane occurs by endocytosis, and such exocytosis–endocytosis coupling maintains the surface area of the cell at its normal size.

RAFTS & CAVEOLAE

Some areas of the cell membrane are especially rich in cholesterol and sphingolipids and have been called **rafts.** These rafts are probably the precursors of flask-shaped membrane depressions called **caveolae** (little caves) when their walls become infiltrated with a protein called **caveolin** that resembles clathrin **(Figure 2–14)**. There is considerable debate about the functions of rafts and caveolae, with evidence that they are involved in cholesterol regulation and transcytosis. It is clear, however, that cholesterol can interact directly with caveolin, effectively limiting the protein's ability to move around in the membrane. Internalization via caveolae involves binding of cargo to caveolin and regulation by dynamin. Caveolae are prominent in endothelial cells, where they help in the uptake of nutrients from the blood.

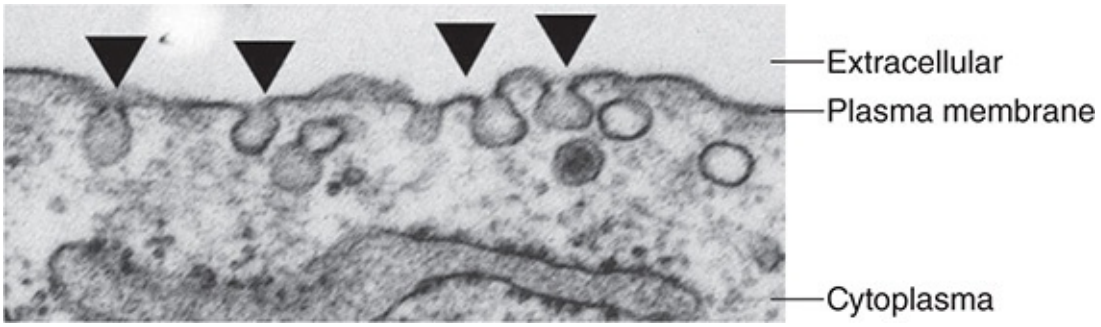


FIGURE 2–14 Caveolae in vascular smooth muscle cell. Ultrastructure of the plasma membrane of a human pulmonary arterial smooth muscle cell is assessed by electron microscopy. Arrow heads indicate individual caveolae.

COATS & VESICLE TRANSPORT

It now appears that all vesicles involved in transport have protein coats. In

humans, over 50 coat complex subunits have been identified. Vesicles that transport proteins from the trans Golgi to lysosomes have **assembly protein 1 (AP-1)** clathrin coats, and endocytotic vesicles that transport to endosomes have AP-2 clathrin coats. Vesicles that transport between the endoplasmic reticulum and the Golgi have coat proteins I and II (COPI and COPII). Certain amino acid sequences or attached groups on the transported proteins target the proteins for particular locations. For example, the amino acid sequence Asn–Pro–any amino acid–Tyr targets transport from the cell surface to the endosomes, and mannose-6-phosphate groups target transfer from the Golgi to mannose-6-phosphate receptors (MPR) on the lysosomes.

Various small G-proteins of the Rab family are especially important in vesicular traffic. They appear to guide and facilitate orderly attachments of these vesicles. To illustrate the complexity of directing vesicular traffic, humans have 60 Rab proteins and 35 SNARE proteins.

MEMBRANE PERMEABILITY & MEMBRANE TRANSPORT PROTEINS

Small, nonpolar molecules (including O_2 and N_2) and small uncharged polar molecules such as CO_2 diffuse across the lipid membranes of cells. However, the membranes have very limited permeability to other substances. Instead, they cross the membranes by endocytosis and exocytosis and by passage through highly specific **transport proteins,** transmembrane proteins that form channels for ions or transport substances such as glucose, urea, and amino acids. The limited permeability applies even to water, with simple diffusion being supplemented throughout the body with various water channels **(aquaporins).** For reference, the sizes of ions and other biologically important substances are summarized in **Table 2–2**.

TABLE 2–2 Size of hydrated ions and other substances of biologic interest.

Substance	Atomic or Molecular Weight	Radius (nm)
Cl^-	35	0.12
K^+	39	0.12
H_2O	18	0.12
Ca^{2+}	40	0.15
Na^+	23	0.18
Urea	60	0.23
Li^+	7	0.24
Glucose	180	0.38
Sucrose	342	0.48
Inulin	5000	0.75
Albumin	69,000	7.50

Data from Moore EW: *Physiology of Intestinal Water and Electrolyte Absorption.* American Gastroenterological Association, 1976.

Some transport proteins are simple aqueous **ion channels,** though many of these have special features that make them selective for a given substance such as Ca^{2+} or, in the case of aquaporins, for water. These membrane-spanning proteins (or collections of proteins) have tightly regulated pores that can be **gated** opened or closed in response to local changes **(Figure 2–15)**. Some are gated by alterations in membrane potential **(voltage-gated),** whereas others are opened or closed in response to a ligand **(ligand-gated).** The ligand is often external (eg, a neurotransmitter, a hormone, or an agonist). However, it can also be internal; intracellular Ca^{2+}, cyclic adenosine 3′,5′-monophosphate (cAMP), lipids, or one of the G-proteins produced in cells can all bind directly to channels and activate them. Some channels are also opened by mechanical stretch, and these mechanosensitive channels play an important role in cell movement.

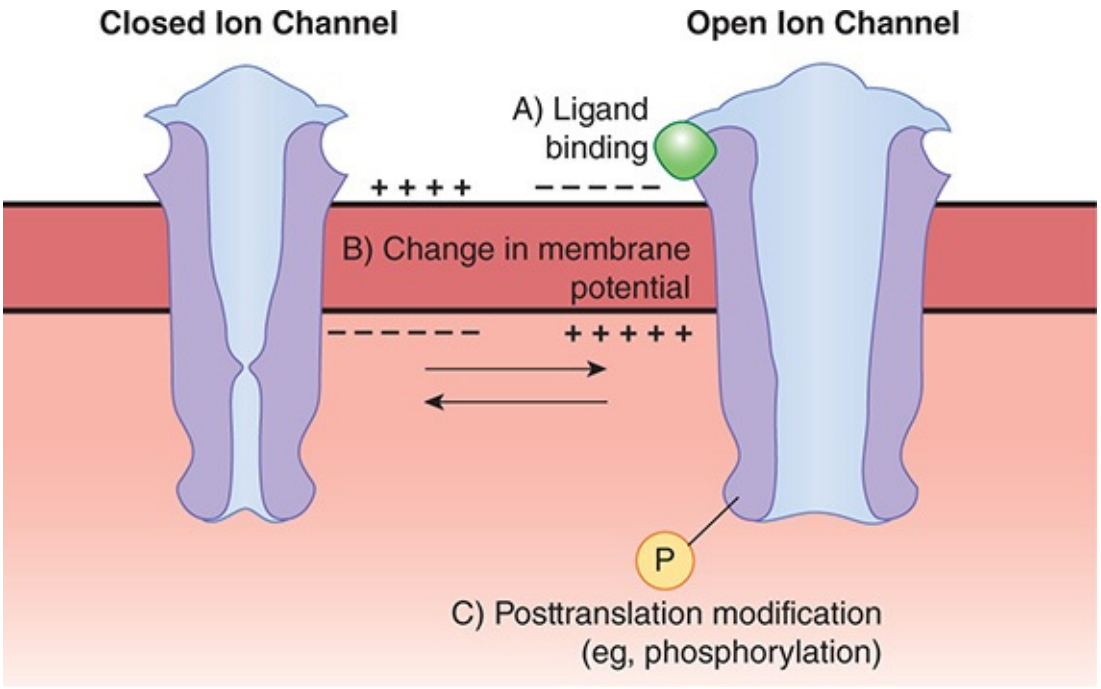


FIGURE 2–15 Regulation of gating in ion channels. Ion channels can gate open or closed in response to several environmental signals. Some typical examples are shown in an idealized channel. **A)** Ligand gating: Channel opens in response to ligand binding. **B)** Voltage gating: Channel opens in response to a change in membrane potential. **C)** Posttranslational modification: Channel gates in response to modification such as phosphorylation.

Other transport proteins are **carriers** that bind ions and other molecules and then change their configuration, moving the bound molecule from one side of the cell membrane to the other. Molecules move from areas of high concentration to areas of low concentration (down their **chemical gradient)**, and cations move to negatively charged areas whereas anions move to positively charged areas ("down" their **electrical gradient)**. When carrier proteins move substances in the direction of their chemical or electrical gradients, no energy input is required and the process is called **facilitated diffusion.** A typical example is glucose transport by the glucose transporter, which moves glucose down its concentration gradient from the ECF to the cytoplasm of the cell. Other carriers transport substances against their electrical and chemical gradients. This form of transport requires energy and is called **active transport.** In animal cells, the energy is provided almost exclusively by hydrolysis of ATP. Not surprisingly, therefore, many carrier molecules are ATPases, enzymes that catalyze the hydrolysis of ATP. One of these ATPases is **sodium–potassium adenosine triphosphatase (Na, K ATPase),** which is also known as the **Na, K pump.**

There are also H, K ATPases in the gastric mucosa and the renal tubules. Ca^{2+} ATPase pumps Ca^{2+} out of cells. Proton ATPases acidify many intracellular organelles, including parts of the Golgi complex and lysosomes.

Some of the transport proteins are called **uniports** because they transport only one substance. Others are called **symports** because transport requires the binding of more than one substance to the transport protein and the substances are transported across the membrane together. An example is the symport in the intestinal mucosa that is responsible for the cotransport of Na^+ and glucose from the intestinal lumen into mucosal cells. Other transporters are called **antiports** because they exchange one substance for another.

ION CHANNELS

There are ion channels specific for K^+, Na^+, Ca^{2+}, and Cl^-, as well as channels that are nonselective for cations or anions. Each type of channel exists in multiple forms with diverse properties. Most are made up of identical or very similar subunits. **Figure 2–16** shows the multiunit structure of various channels in diagrammatic cross-section.

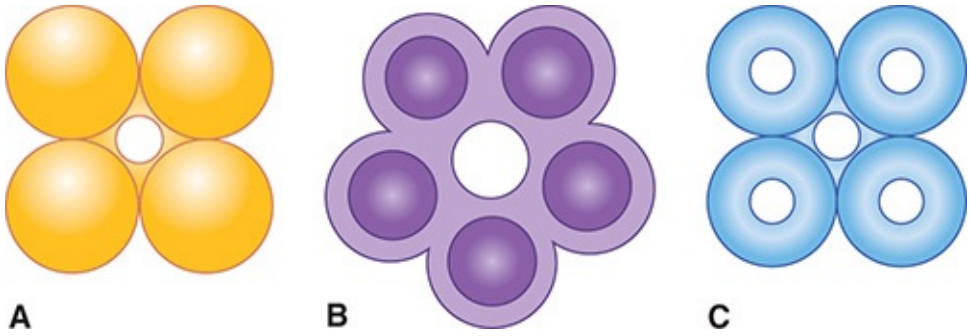


FIGURE 2–16 Different ways in which ion channels form pores. A) Four subunits form a central pore in typical K^+ channels. **B)** Some ligand-gated channels such as the acetylcholine receptor contain five similar subunits that form a central pore in the channel. **C)** Aquaporin, or water channels, contain four subunits that each contain a pore that can allow for water movement. Additionally, these subunits form a central pore that can allow for ion movement. It should be noted that other formations also occur.

Most K^+ channels are tetramers, with each of the four subunits forming part of the pore through which K^+ ions pass. Structural analysis of a bacterial

voltage-gated K^+ channel indicates that each of the four subunits has a paddle-like extension containing four charges. When the channel is closed, these extensions are near the negatively charged interior of the cell. When the membrane potential is reduced, the paddles containing the charges bend through the membrane to its exterior surface, causing the channel to open. The bacterial K^+ channel is very similar to the voltage-gated K^+ channels in a wide variety of species, including mammals and humans. In the acetylcholine ion channel and other ligand-gated cation or anion channels, five subunits make up the pore. Members of the ClC family of Cl^- channels are dimers, but they have two pores, one in each subunit. Finally, aquaporins are tetramers with a water pore in each of the subunits. Recently, a number of ion channels with intrinsic enzyme activity have been cloned. More than 30 different voltage-gated or cyclic nucleotide-gated Na^+ and Ca^{2+} channels of this type have been described. Representative Na^+, Ca^{2+}, and K^+ channels are shown in extended diagrammatic form in **Figure 2–17**.

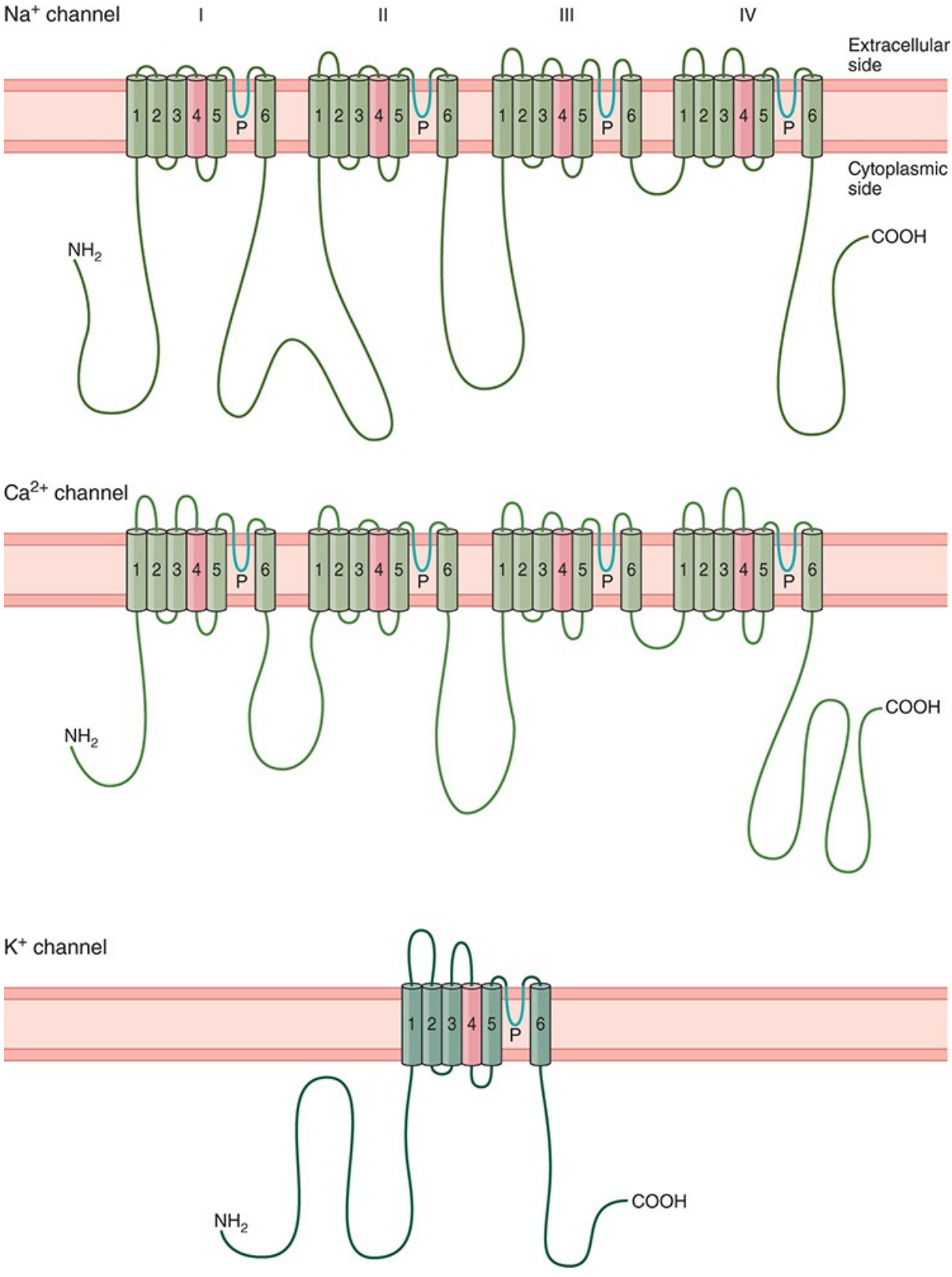

Na+ channel
I
II
III
IV
Extracellular side
Cytoplasmic side
1 2 3 4 5 P 6
NH2
COOH
Ca2+ channel
K+ channel

FIGURE 2–17 Diagrammatic representation of the pore-forming subunits of three ion channels. The α subunit of the Na^+ and Ca^{2+} channels traverses the membrane 24 times in four repeats of six membrane-spanning units. Each repeat has a "P" loop between membrane spans 5 and 6 that does not traverse the membrane. These P loops are thought to form the pore. Note that span 4 of each repeat is colored in red, representing its net "+" charge. The K^+ channel has only a single repeat of the six spanning regions and P loop. Four K^+ subunits are assembled for a functional K^+ channel. (Reproduced with permission from Kandel ER, Schwartz JH, Jessell TM, Siegelbaum SA, Hudspeth AJ (editors): *Principles of Neural Science*, 5th ed. New York, NY: McGraw-Hill; 2013.)

Another family of Na^+ channels with a different structure has been found in the apical membranes of epithelial cells in the kidneys, colon, lungs, and brain. The **epithelial sodium channels (ENaCs)** are made up of three subunits encoded by three different genes. Each of the subunits probably spans the membrane twice, and the amino terminal and carboxyl terminal are located inside the cell. The α subunit transports Na^+, whereas the β and γ subunits do not. However, the addition of the β and γ subunits increases Na^+ transport through the α subunit. ENaCs are inhibited by the diuretic amiloride, which binds to the α subunit, and they used to be called **amiloride inhibitable Na^+ channels.** The ENaCs in the kidney play an important role in the regulation of ECF volume by aldosterone. ENaC knockout mice are born alive but promptly die because they cannot move Na^+, and hence water, out of their lungs.

Humans have several types of Cl^- channels. The ClC dimeric channels are found in plants, bacteria, and animals, and there are nine different ClC genes in humans. Other Cl^- channels have the same pentameric form as the acetylcholine receptor; examples include the γ-aminobutyric acid A ($GABA_A$) and glycine receptors in the CNS. The cystic fibrosis transmembrane conductance regulator (CFTR) that is mutated in patients with cystic fibrosis is also a Cl^- channel. Ion channel mutations cause a variety of **channelopathies**—diseases that mostly affect muscle and brain tissue and produce episodic paralyses or convulsions, but are also observed in nonexcitable tissues **(Clinical Box 2–6)**.

PATCH CLAMP TECHNIQUE

Activity of ion channels and transporters in the plasma membrane plays an important role in the regulation of cell excitability and muscle contraction, as

well as cell proliferation, migration, and apoptosis. An important technique that has permitted major advances in our knowledge about ion transport proteins (channels and transporters) is **patch clamp technique.** The technique is used to measure and study ionic currents through membrane ion channels and via electrogenic membrane transporters in isolated living cells and tissue sections. Depending on the research interest, there are four recording configurations using patch clamp technique. A micropipette or glass pipette is placed on the membrane of a cell and forms a tight seal to the membrane. The patch of membrane under the pipette tip usually contains only a few transport or channel proteins or a single channel protein (eg, a K^+ channel). The cell can be left intact to record single-channel activity using the **cell-attached patch** mode **(Figure 2–18)**. Alternatively, the membrane patch sealed to the pipette can be pulled loose from the cell, forming an **inside-out patch** to record single-channel currents. If the membrane patch is then disrupted by briefly applying a strong suction, the interior of the pipette becomes continuous with the cytoplasm. This **whole-cell recording** configuration is used to record the whole-cell currents from the entire cell (Figure 2–18) and to record electrical potentials (eg, the resting membrane potential and action potentials) in the whole cell. The pipette can be retracted during the whole-cell recording to cause a rupture and reformation of the membrane to form an **outside-out patch** to study the effect of extracellular agonists on the single-channel currents.

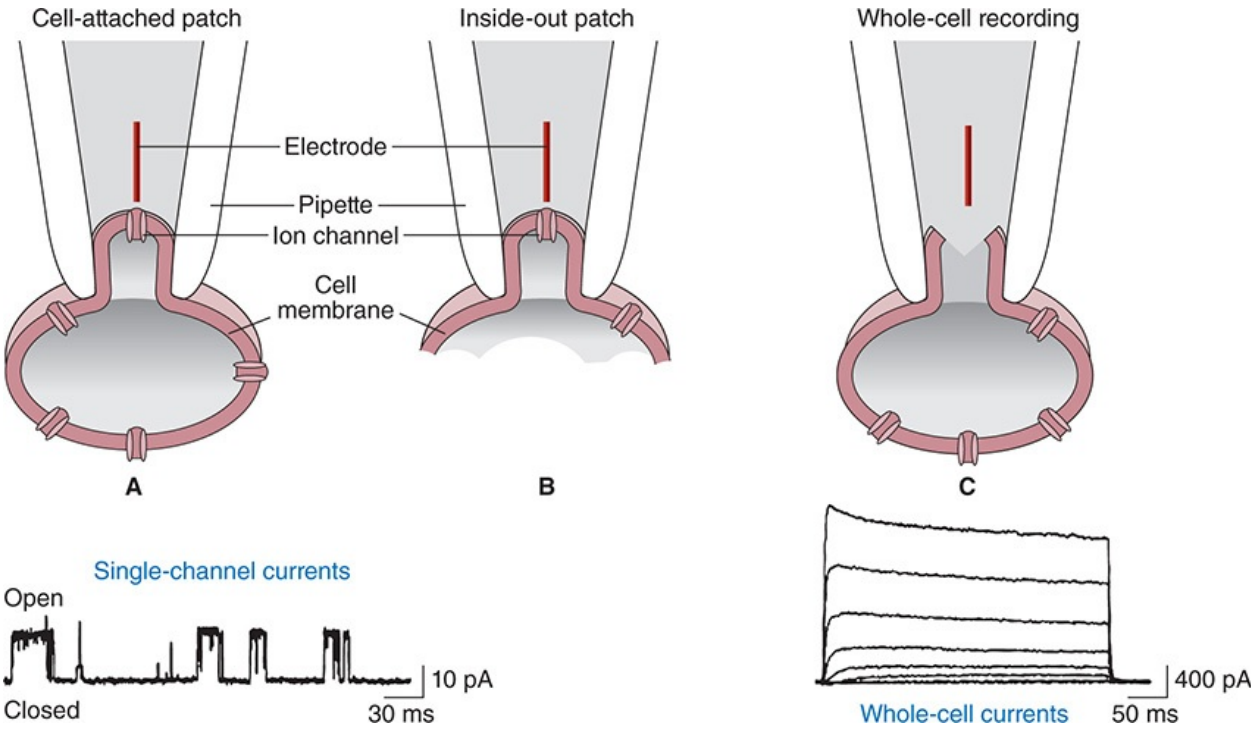


FIGURE 2–18 Patch clamp technique for studying ion transport. In a patch clamp experiment, a small glass pipette filled with extracellular (or intracellular) solution is carefully maneuvered to seal off a portion of a cell membrane (cell-attached patch, **A**). The pipette filled with an appropriate solution has an electrode that allows for recording of electrical changes through any pore or channel in the membrane patch. A typical single-channel recording of the Ca^{2+}-activated K^+ currents in cell-attached patch is shown below in panel **A**. The inside-out patch configuration is achieved by rapidly pulling up the pipette from the attached cell, without breaking the seal (**B**). The patch sealed to the pipette can also be ruptured off by a brief suction to form the whole-cell recording mode (**C**). This mode allows to record ionic currents in the entire cell. A typical whole-cell recording of the superimposed voltage-gated K^+ currents is shown in panel **C**.

CLINICAL BOX 2–6

Channelopathies

Channelopathies include a wide range of diseases that can affect both excitable (eg, neurons and muscle) and nonexcitable cells. Using molecular genetic tools, many of the pathologic defects in channelopathies have been traced to mutations in single ion channels. Examples of channelopathies in excitable cells include periodic paralysis (eg, Kir2.6/KCNJ18, a K^+ channel, $Ca_V1.1$/CACNA1S, a Ca^{2+} channel, or $Na_V1.4$/SCN4A, a Na^+ channel), myasthenia (eg, nicotinic acetyl choline receptor, a ligand-gated nonspecific cation channel), myotonia (eg, Kir1.1/KCNJ1, a K^+ channel), malignant hyperthermia (ryanodine receptor/RYR1, a Ca^{2+} channel), long QT syndrome (both Na^+ and K^+ channels, such as $K_V7.1$/KCNQ1, $K_V11.1$/KCNH2/hHERG, Kir2.1/KCNJ2 and $Na_V1.5$/SCN5A) and several other disorders. Examples of channelopathies in nonexcitable cells include the underlying cause for cystic fibrosis (CFTR/ABCC7, a Cl^- channel) and a form of Bartter syndrome (Kir1.1/KCNJ1, a K^+ channel). Importantly, advances in treatment of these disorders can come from the understanding of the basic defect and tailoring drugs that act to alter the mutated properties of the affected channel.

Na, K ATPase

As noted previously, Na, K ATPase catalyzes the hydrolysis of ATP to adenosine diphosphate (ADP) and uses the energy to extrude three Na^+ from the cell and take two K^+ into the cell for each molecule of ATP hydrolyzed. It is an **electrogenic pump** in that it moves three positive charges ($3Na^+$) out of the cell for each two ($2K^+$) that it moves in, and it is therefore said to have a **coupling ratio** of 3:2. It is found in all parts of the body. Its activity is inhibited by ouabain and related digitalis glycosides used in the treatment of heart failure. It is a heterodimer made up of an α subunit with a molecular weight of approximately 100,000 and a β subunit with a molecular weight of approximately 55,000. Both extend through the cell membrane **(Figure 2–19)**. Separation of the subunits eliminates activity. The β subunit is a glycoprotein, whereas Na^+ and K^+ transport occur through the α subunit. The β subunit has a single membrane-spanning domain and three extracellular glycosylation sites, all of which appear to have attached carbohydrate residues. These residues account for one-third of its molecular weight. The α subunit probably spans the cell membrane 10 times, with the amino and carboxyl terminals both located intracellularly. This subunit has intracellular Na^+- and ATP- binding sites and a phosphorylation site; it also has extracellular binding sites for K^+ and ouabain. The endogenous ligand of the ouabain-binding site, or endogenous ouabain, is identified in blood plasma and various organs and tissues of humans, especially in patients with essential hypertension. When Na^+ binds to the α subunit, ATP also binds and is converted to ADP, with a phosphate being transferred to Asp 376, the phosphorylation site. This causes a change in the configuration of the protein, extruding Na^+ into the ECF. K^+ then binds extracellularly, dephosphorylating the α subunit, which returns to its previous conformation, releasing K^+ into the cytoplasm.

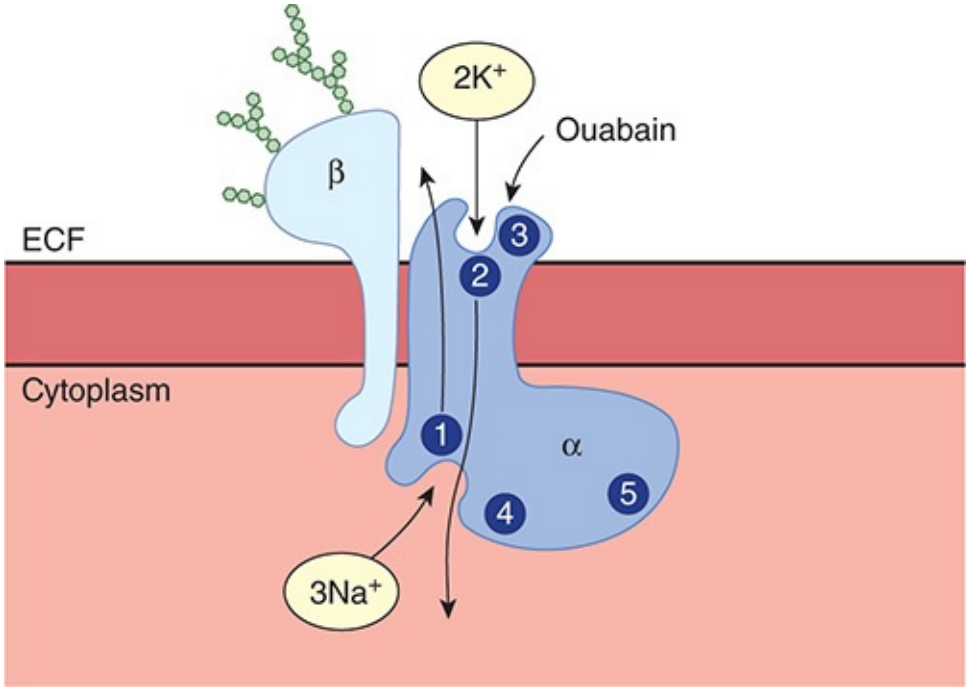


FIGURE 2–19 Na, K ATPase. The intracellular portion of the α subunit has a Na^+-binding site **(1)**, a phosphorylation site **(4)**, and an ATP-binding site **(5)**. The extracellular portion has a K^+-binding site **(2)** and an ouabain-binding site **(3)**. (Reproduced with permission from Horisberger JD, Lemas V, Kraehenbühl JP et al: Structure–function relationship of Na, K-ATPase. Annu Rev Physiol 1991;53:565-584.)

The α and β subunits are heterogeneous, with α_1, α_2, and α_3 subunits and β_1, β_2, and β_3 subunits described so far. The α_1 isoform is found in the membranes of most cells, whereas α_2 is present in muscle, heart, adipose tissue, and brain, and α_3 is present in heart and brain. The β_1 subunit is widely distributed but is absent in certain astrocytes, vestibular cells of the inner ear, and glycolytic fast-twitch muscles. The fast-twitch muscles contain only β_2 subunits. The different α and β subunit structures of Na, K ATPase in various tissues probably represent specialization for specific tissue functions.

REGULATION OF Na, K ATPase

The amount of Na^+ normally found in cells is not enough to saturate the pump, so if the Na^+ increases, more is pumped out. Pump activity is affected by second messenger molecules (eg, cAMP and diacylglycerol [DAG]). The magnitude and

direction of the altered pump effects vary with the experimental conditions. Thyroid hormones increase pump activity by a genomic action to increase the formation of Na, K ATPase molecules. Aldosterone also increases the number of pumps, although this effect is probably secondary. Dopamine in the kidney inhibits the pump by phosphorylating it, causing a natriuresis. Insulin increases pump activity, probably by a variety of different mechanisms.

SECONDARY ACTIVE TRANSPORT

In many situations, the active transport of Na^+ is coupled to the transport of other substances **(secondary active transport).** For example, the luminal membranes of mucosal cells in the small intestine contain a symport that transports glucose into the cell only if Na^+ binds to the protein and is transported into the cell at the same time. From the cells, the glucose enters the blood. The electrochemical gradient for Na^+ is maintained by the active transport of Na^+ out of the mucosal cell into ECF. Other examples are shown in **Figure 2–20**. In the heart, Na, K ATPase indirectly affects Ca^{2+} transport. An antiport in the membranes of cardiac muscle cells normally exchanges intracellular Ca^{2+} for extracellular Na^+.

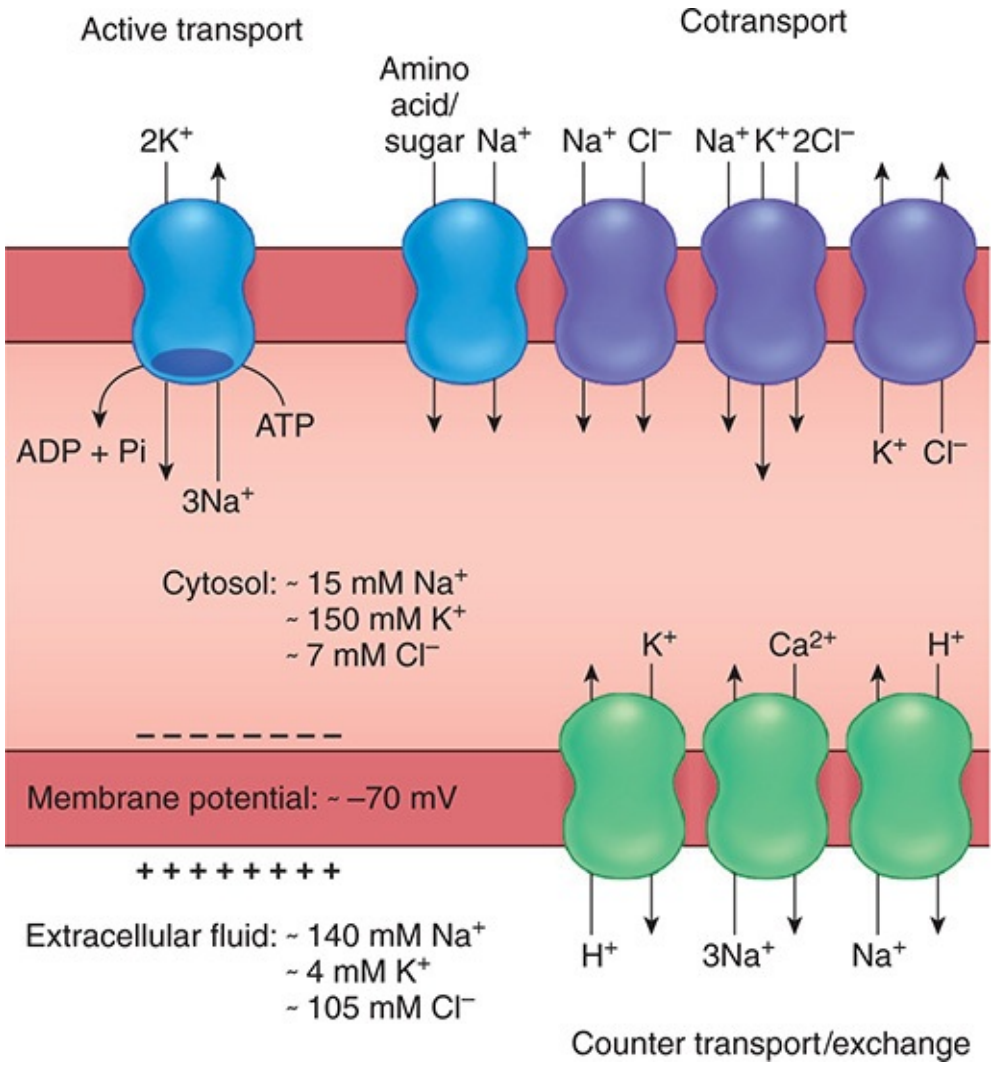


FIGURE 2–20 Composite diagram of main secondary effects of active transport of Na^+ and K^+. Na, K ATPase converts the chemical energy of ATP hydrolysis into maintenance of an inward gradient for Na^+ and an outward gradient for K^+. The energy of the gradients is used for countertransport, cotransport, and maintenance of the membrane potential. Some examples of cotransport and countertransport that use these gradients are shown.

Active transport of Na^+ and K^+ is one of the major energy-using processes in the body. On the average, it accounts for about 24% of the energy utilized by cells, and in neurons it accounts for 70%. Thus, it accounts for a large part of the basal metabolism. A major payoff for this energy use is the establishment of the electrochemical gradient in cells.

TRANSPORT ACROSS EPITHELIA

In the gastrointestinal tract, the pulmonary airways, the renal tubules, and other structures lined with polarized epithelial cells, substances enter one side of a cell and exit another, producing movement of the substance from one side of the epithelium to the other (Figure 2–8). For transepithelial transport to occur, the cells need to be bound by tight junctions and, obviously, have different ion channels and transport proteins in different parts of their membranes. Most of the instances of secondary active transport cited in the preceding paragraph involve transepithelial movement of ions and other molecules.

SPECIALIZED TRANSPORT ACROSS THE CAPILLARY WALL

The capillary wall separating plasma from interstitial fluid is different from the cell membranes separating interstitial fluid from intracellular fluid because the pressure difference across it makes **filtration** a significant factor in producing movement of water and solute. By definition, filtration is the process by which fluid is forced through a membrane or other barrier because of a difference in pressure on the two sides.

The structure of the capillary wall varies from one vascular bed to another. However, near skeletal muscle and many other organs, water and relatively small solutes are the only substances that cross the wall with ease. The apertures in the junctions between the endothelial cells are too small to permit plasma proteins and other colloids to pass through in significant quantities. The colloids have a high molecular weight but are present in large amounts. Small amounts cross the capillary wall by vesicular transport, but their effect is slight. Therefore, the capillary wall behaves like a membrane impermeable to colloids, and these exert an osmotic pressure of about 25 mmHg. The colloid osmotic pressure due to the plasma colloids is called the **oncotic pressure.** Filtration across the capillary membrane as a result of the hydrostatic pressure head in the vascular system is opposed by the oncotic pressure. The way the balance between the hydrostatic and oncotic pressures controls exchanges across the capillary wall is considered in detail in Chapter 31.

TRANSCYTOSIS

Vesicles are present in the cytoplasm of endothelial cells, and tagged protein molecules injected into the bloodstream have been found in the vesicles and in the interstitium. This indicates that small amounts of protein are transported out of capillaries across endothelial cells by endocytosis on the capillary side followed by exocytosis on the interstitial side of the cells. The transport mechanism makes use of coated vesicles that appear to be coated with caveolin and is called **transcytosis, vesicular transport,** or **cytopempsis.**

INTERCELLULAR COMMUNICATION

Cells communicate with one another via chemical messengers. Within a given tissue, some messengers move from cell to cell via gap junctions without entering the ECF. In addition, cells are affected by chemical messengers secreted into the ECF, or by direct cell–cell contacts. Chemical messengers typically bind to protein receptors on the surface of the cell or, in some instances, in the cytoplasm or the nucleus, triggering sequences of intracellular changes that produce their physiologic effects. Three general types of intercellular communication are mediated by messengers in the ECF: (1) **neural communication,** in which neurotransmitters are released at synaptic junctions from nerve cells and act across a narrow synaptic cleft on a postsynaptic cell; (2) **endocrine communication,** in which hormones and growth factors reach cells via the circulating blood or the lymph; and (3) **paracrine communication,** in which the products of cells diffuse in the ECF to affect neighboring cells that may be some distance away **(Figure 2–21)**. In addition, cells secrete chemical messengers that in some situations bind to receptors on the same cell, that is, the cell that secreted the messenger **(autocrine communication).** The chemical messengers include amines, amino acids, steroids, polypeptides, and in some instances, lipids, purine nucleotides, and pyrimidine nucleotides. It is worth noting that in various parts of the body, the same chemical messenger can function as a neurotransmitter, a paracrine mediator, a hormone secreted by neurons into the blood (neural hormone), and a hormone secreted by gland cells into the blood.

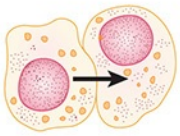
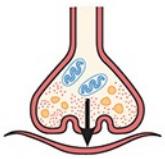
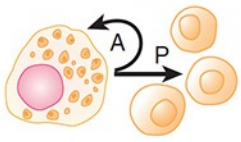
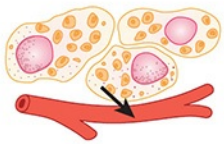
	GAP JUNCTIONS	SYNAPTIC	PARACRINE AND AUTOCRINE	ENDOCRINE
				
Message transmission	Directly from cell to cell	Across synaptic cleft	By diffusion in interstitial fluid	By circulating body fluids
Local or general	Local	Local	Locally diffuse	General
Specificity depends on	Anatomic location	Anatomic location and receptors	Receptors	Receptors

FIGURE 2–21 Intercellular communication by chemical mediators. Several common forms of cellular communication are illustrated. A, autocrine; P, paracrine.

An additional form of intercellular communication is called **juxtacrine communication.** Some cells express multiple repeats of growth factors such as **transforming growth factor alpha (TGF-α)** extracellularly on transmembrane proteins that provide an anchor to the cell. Other cells have TGF-α receptors. Consequently, TGF-α anchored to a cell can bind to a TGF-α receptor on another cell, linking the two. This could be important in producing local foci of growth in tissues. Notch-mediated juxtacrine signaling between adjacent cells is another good example of juxtacrine communication. Notch ligands, such as Jagged (Jag1/2) and Delta-like (DLL1/3/4), in signal sending cells first bind to Notch receptors, such as Notch1-4, in signal receiving cells, through cell-to-cell contact. The activated Notch receptors mediate a series of signal transduction cascades involved in regulating cell fate decisions, cell proliferation and differentiation, and tissue and organ development.

RECEPTORS FOR CHEMICAL MESSENGERS

The recognition of chemical messengers by cells typically begins by interaction with a receptor at that cell. There have been over 20 families of receptors for chemical messengers characterized. These proteins are not static components of the cell, but their numbers increase and decrease in response to various stimuli, and their properties change with changes in physiologic conditions. When a hormone or neurotransmitter is present in excess, the number of active receptors generally decreases **(downregulation),** whereas in the presence of a deficiency

of the chemical messenger, there is an increase in the number of active receptors **(upregulation).** In its actions on the adrenal cortex, angiotensin II is an exception; it increases rather than decreases the number of its receptors in the adrenal. In the case of receptors in the membrane, receptor-mediated endocytosis is responsible for downregulation in some instances; ligands bind to their receptors, and the ligand-receptor complexes move laterally in the membrane to coated pits, where they are taken into the cell by endocytosis **(internalization).** This decreases the number of receptors in the membrane. Some receptors are recycled after internalization, whereas others are replaced by de novo synthesis in the cell. Another type of downregulation is **desensitization,** in which receptors are chemically modified in ways that make them less responsive.

MECHANISMS BY WHICH CHEMICAL MESSENGERS ACT

Receptor–ligand interaction is usually just the beginning of the cell response. This event is transduced into secondary responses within the cell that can be divided into four broad categories: (1) ion channel activation, (2) **G-protein** activation, (3) activation of enzyme activity within the cell, or (4) direct activation of transcription. Within each of these groups, responses can be quite varied. Some of the common mechanisms by which chemical messengers exert their intracellular effects are summarized in **Table 2–3.** Ligands such as acetylcholine bind directly to ion channels in the cell membrane, changing their conductance. Thyroid and steroid hormones, 1,25-dihydroxycholecalciferol, and retinoids enter cells and act on one or another member of a family of structurally related cytoplasmic or nuclear receptors. The activated receptor binds to DNA and increases transcription of selected mRNAs. Many other ligands in the ECF bind to receptors on the surface of cells and trigger the release of intracellular mediators such as cAMP, inositol trisphosphate (IP_3), and DAG that initiate changes in cell function. Consequently, the extracellular ligands are called **“first messengers”** and the intracellular mediators are called **“second messengers.”** Second messengers bring about many short-term changes in cell function by altering enzyme function, triggering exocytosis, and so on, but they also can lead to the alteration of transcription of various genes. A variety of enzymatic changes, protein–protein interactions, or second messenger changes can be activated within a cell in an orderly fashion following receptor recognition of the primary messenger. The resulting **cell signaling pathway** provides amplification

of the primary signal and distribution of the signal to appropriate targets within the cell. Extensive cell signaling pathways also provide opportunities for feedback and regulation that can fine-tune the signal for the correct physiologic response by the cell.

TABLE 2–3 Common mechanisms by which chemical messengers in the ECF bring about changes in cell function.

Mechanism	Examples
Open or close ion channels in cell membrane	Acetylcholine on nicotinic cholinergic receptor; norepinephrine on K^+ channel in the heart; capsaicin on transient receptor potential cation channel vanilloid member 1 (TRPV1)
Act via cytoplasmic or nuclear receptors to increase transcription of selected mRNAs	Thyroid hormones, retinoic acid, steroid hormones
Activate phospholipase C with intracellular production of DAG, IP_3, and other inositol phosphates	Angiotensin II, norepinephrine via α_1-adrenergic receptor, vasopressin via V_1 receptor
Activate or inhibit adenylyl cyclase, causing increased or decreased intracellular production of cAMP	Norepinephrine via β_1-adrenergic receptor (increased cAMP), norepinephrine via α_2-adrenergic receptor (decreased cAMP)
Increase cGMP in cell	Atrial natriuretic peptide, nitric oxide
Increase tyrosine kinase activity of cytoplasmic portions of transmembrane receptors	Insulin, EGF, PDGF, M-CSF
Increase serine or threonine kinase activity	TGF-β, activin, inhibin

cAMP, cyclic adenosine 3′,5′-monophosphate; cGMP, cyclic guanosine monophosphate; DAG, diacylglycerol; ECF, extracellular fluid; EGF, epidermal growth factor; IP_3, inositol triphosphate; M-CSF, monocyte colony-stimulating factor; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor β.

The most predominant posttranslation modification of proteins, phosphorylation, is a common theme in cell signaling pathways. Cellular phosphorylation is under the control of two groups of proteins: **kinases,** enzymes that catalyze the phosphorylation of tyrosine or serine and threonine residues in proteins (or in some cases, in lipids); and **phosphatases,** proteins that remove phosphates from proteins (or lipids). Some of the larger receptor families are themselves kinases. Tyrosine kinase receptors initiate phosphorylation on tyrosine residues on complementary receptors following ligand binding. Serine/threonine kinase receptors initiate phosphorylation on serines or threonines in complementary receptors following ligand binding. Cytokine receptors are directly associated with a group of protein kinases that are activated following cytokine binding. Alternatively, second messenger changes can lead to phosphorylation further downstream in the signaling pathway. More than 500 protein kinases have been described. Some of the principal ones that are important in mammalian cell signaling are summarized in **Table 2–4**. In general, addition of phosphate groups changes the conformation of the proteins, altering their functions and consequently the functions of the cell. The close relationship between phosphorylation and dephosphorylation of cellular proteins allows for a temporal control of activation of cell signaling pathways. This is sometimes referred to as a **"phosphate timer."** The dysregulation of the phosphate timer and subsequent cellular signaling in a cell can lead to disease **(Clinical Box 2–7)**.

TABLE 2–4 Sample protein kinases.

Phosphorylate serine or threonine residues, or both
Calmodulin-dependent
Myosin light-chain kinase
Phosphorylase kinase
Ca^{2+}/calmodulin kinase I
Ca^{2+}/calmodulin kinase II
Ca^{2+}/calmodulin kinase III
Calcium-phospholipid-dependent
Protein kinase C (more than 10 subtypes)
Cyclic nucleotide-dependent
cAMP-dependent kinase (protein kinase A; two subtypes)
cGMP-dependent kinase
Phosphorylate tyrosine residues
Insulin receptor, EGF receptor, PDGF receptor, and M-CSF receptor

cAMP, cyclic adenosine 3′,5′-monophosphate; cGMP, cyclic guanosine monophosphate; EGF, epidermal growth factor; M-CSF, monocyte colony-stimulating factor; PDGF, platelet-derived growth factor.

STIMULATION OF TRANSCRIPTION

The activation of transcription, and subsequent translation, is a common outcome of cellular signaling. There are three distinct pathways for primary messengers to alter transcription of cells. First, as is the case with steroid or thyroid hormones, the primary messenger is able to cross the cell membrane and bind to a nuclear receptor, which then can directly interact with DNA to alter gene expression. A second pathway to gene transcription is the activation of cytoplasmic protein kinases that can move to the nucleus to phosphorylate a latent transcription factor for activation. This pathway is a common end point of signals that go through the **mitogen-activated protein (MAP) kinase** cascade. MAP kinases can be activated following a variety of receptor–ligand interactions through second messenger signaling. They comprise a series of three kinases that coordinate a stepwise phosphorylation to activate each protein in series in the cytosol. Phosphorylation of the last MAP kinase in series allows it to migrate to the nucleus where it phosphorylates a latent transcription factor. A third common pathway is the activation of a latent transcription factor in the cytosol, which

then migrates to the nucleus and alters transcription. This pathway is shared by a diverse set of transcription factors that include **nuclear factor kappa B (NF-κB;** activated following tumor necrosis family receptor binding and others) and **signal transducers of activated transcription (STATs;** activated following cytokine receptor binding). In all cases, the binding of the activated transcription factor to DNA increases (or in some cases, decreases) the transcription of mRNAs encoded by the gene to which it binds. The mRNAs are translated in the ribosomes, with the production of increased quantities of proteins that alter cell function.

CLINICAL BOX 2–7

Kinases in Cancer: Chronic Myeloid Leukemia

Kinases frequently play important roles in regulating cellular physiology outcomes, including cell growth and cell death. Dysregulation of cell proliferation or cell death is a hallmark of cancer. Although cancer can have many causes, a role for kinase dysregulation is exemplified in chronic myeloid leukemia (CML). CML is a pluripotent hematopoietic stem cell disorder characterized by the Philadelphia (Ph) chromosome translocation. The Ph chromosome is formed following a translocation of chromosomes 9 and 22, resulting in a shortened chromosome 22 (Ph chromosome). At the point of fusion, a novel gene (*bcr-abl*) encoding the active tyrosine kinase domain from a gene on chromosome 9 (Abelson tyrosine kinase; c-Abl) is fused to novel regulatory region of a separate gene on chromosome 22 (breakpoint cluster region; bcr). The *bcr-abl* fusion gene encodes a cytoplasmic protein with constitutively active tyrosine kinase. The dysregulated kinase activity in bcr-abl protein effectively limits white blood cell death signaling pathways while promoting cell proliferation and genetic instability. Experimental models have shown that translocation to produce the fusion bcr-abl protein is sufficient to produce CML in animal models.

THERAPEUTIC HIGHLIGHTS

The identification of *bcr-abl* as the initial transforming event in CML provided an ideal target for drug discovery. The drug imatinib was developed to specifically block the tyrosine kinase activity of the bcr-abl protein. Imatinib has proven to be an effective agent for treating chronic phase CML.

INTRACELLULAR CA^{2+} AS A SECOND MESSENGER

Ca^{2+} regulates a very large number of physiological processes that are as diverse as proliferation, neural signaling, learning, contraction, secretion, and fertilization, so regulation of intracellular Ca^{2+} is of great importance. The free Ca^{2+} concentration in the cytoplasm at rest is maintained at about 100 nmol/L. The Ca^{2+} concentration in the interstitial fluid is about 12,000 to 18,000 times the cytoplasmic concentration (ie, 1,200,000 to 1,8,00,000 nmol/L), so there is a marked inwardly directed concentration gradient as well as an inwardly directed electrical gradient. Much of the intracellular Ca^{2+} is stored at relatively high concentrations in the endoplasmic reticulum (approximately 100,000 to 1,200,000 nmol/L) and other organelles **(Figure 2–22)**, and these organelles provide a store from which Ca^{2+} can be mobilized via ligand-gated channels in the endoplasmic reticulum (eg, ryanodine receptors and inositol trisphosphate receptors) to increase the concentration of free Ca^{2+} in the cytoplasm. Increased cytoplasmic Ca^{2+} binds to and activates calcium-binding proteins. These proteins can have direct effects in cellular physiology, or can activate other proteins, commonly protein kinases, to further cell signaling pathways.

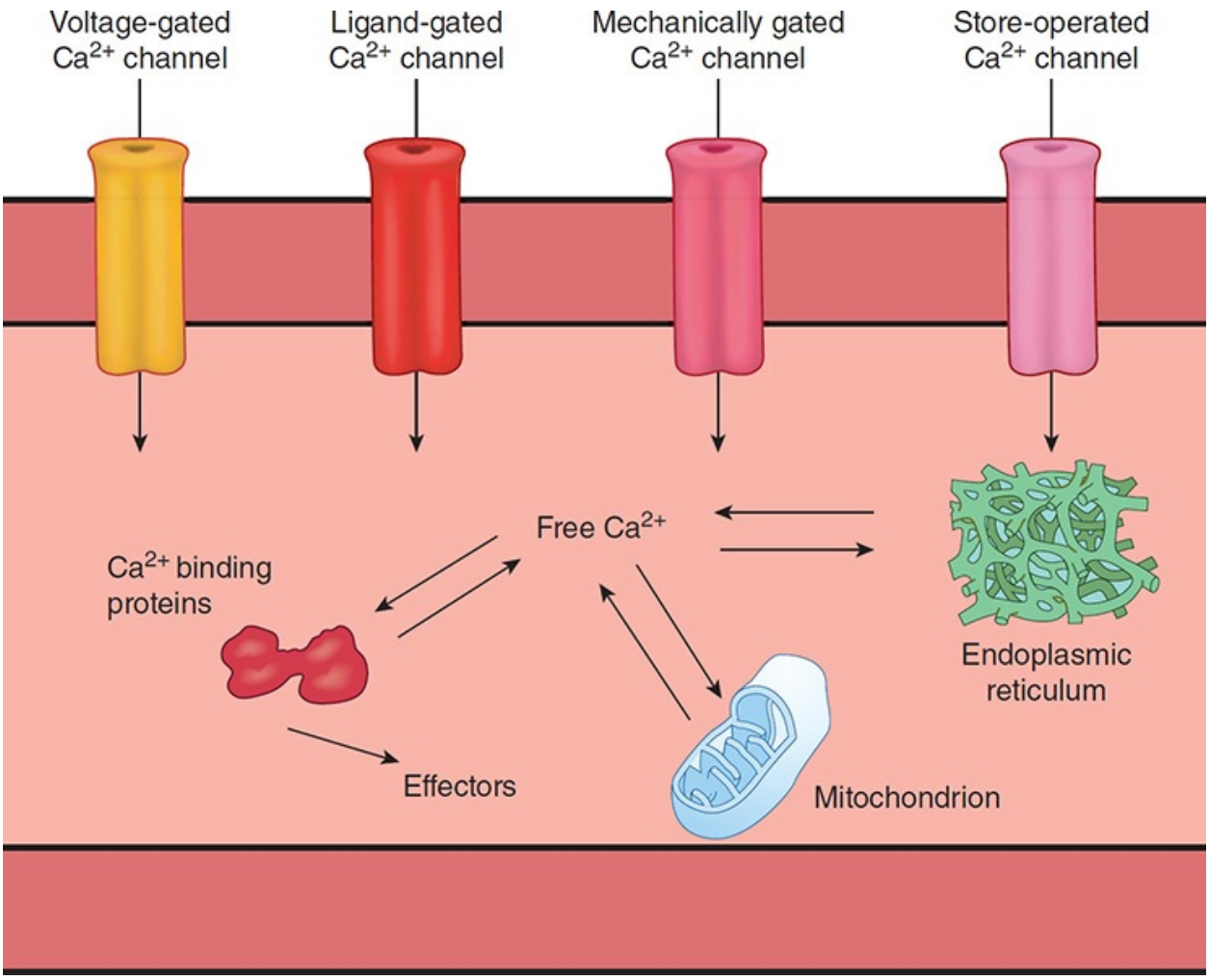


FIGURE 2–22 Ca^{2+} handling in mammalian cells. Ca^{2+} can enter the cell via a variety of channel types. In addition, Ca^{2+} is stored in the endoplasmic reticulum (and, to a lesser extent in the mitochondrion) where it can be released to alter free Ca^{2+} concentration in the cytoplasm. Free Ca^{2+} can be bound by proteins that then have a variety of downstream physiologic effects. Ca^{2+} can be removed from the cytoplasm by ATPases in the endoplasmic reticulum or at the plasma membrane, or via Na, Ca exchangers (not shown).

Ca^{2+} can enter the cell from the ECF, down its electrochemical gradient, through many different Ca^{2+} channels. Some of these are ligand-gated and others are voltage-gated. Stretch-activated channels exist in some cells as well.

Many second messengers act by increasing the cytoplasmic Ca^{2+} concentration. The increase is produced by releasing Ca^{2+} from intracellular stores—primarily the endoplasmic reticulum—or by increasing the entry of Ca^{2+}

into cells, or by both mechanisms. IP_3 is the major second messenger that causes Ca^{2+} release from the endoplasmic reticulum through the direct activation of a ligand-gated channel, the IP_3 receptor. In effect, the generation of one second messenger (IP_3) can lead to the release of another second messenger (Ca^{2+}). In many tissues, transient release of Ca^{2+} from internal stores into the cytoplasm triggers opening of a population of Ca^{2+} channels in the cell membrane **(store-operated Ca^{2+} channels; SOCCs).** The resulting Ca^{2+} influx replenishes the total intracellular Ca^{2+} supply and refills the endoplasmic reticulum. Recent research has identified the physical relationships between SOCCs (eg, Orai channels) and regulatory interactions of proteins (eg, stromal interaction molecules 1 and 2) from the endoplasmic reticulum that facilitate the recruitment of Orai to form SOCCs.

As with other second messenger molecules, the increase in Ca^{2+} within the cytosol is rapid, and is followed by a rapid decrease. Because the movement of Ca^{2+} outside of the cytosol (ie, across the plasma membrane or the membrane of the internal store) requires that it move up its electrochemical gradient, it requires energy. Ca^{2+} movement out of the cell is facilitated by the plasma membrane Ca^{2+} ATPase. Alternatively, it can be transported by an antiport (ie, Na^+/Ca^{2+} exchanger) that exchanges three Na^+ for each Ca^{2+} driven by the energy stored in the Na^+ electrochemical gradient. Ca^{2+} movement into the internal stores is through the action of the **sarcoplasmic** or **endoplasmic reticulum Ca^{2+} ATPase,** also known as the **SERCA pump.**

CALCIUM-BINDING PROTEINS

Many different Ca^{2+}-binding proteins have been described, including **troponin, calmodulin,** and **calbindin.** Troponin is the Ca^{2+}-binding protein involved in contraction of skeletal muscle (Chapter 5). Calmodulin contains 148 amino acid residues **(Figure 2–23)** and has four Ca^{2+}-binding domains. It is unique in that amino acid residue 115 is trimethylated, and it is extensively conserved, being found in plants as well as animals. When calmodulin binds Ca^{2+}, it is capable of activating five different calmodulin-dependent kinases (CaMKs; Table 2–4), among other proteins. One of the kinases is **myosin light-chain kinase,** which phosphorylates myosin. This brings about contraction in smooth muscle. CaMKI and CaMKII are concerned with synaptic function, and CaMKIII is concerned with protein synthesis. Another calmodulin-activated protein is **calcineurin,** a

phosphatase that inactivates Ca^{2+} channels by dephosphorylating them. It also plays a prominent role in activating T cells and is inhibited by some immunosuppressants.

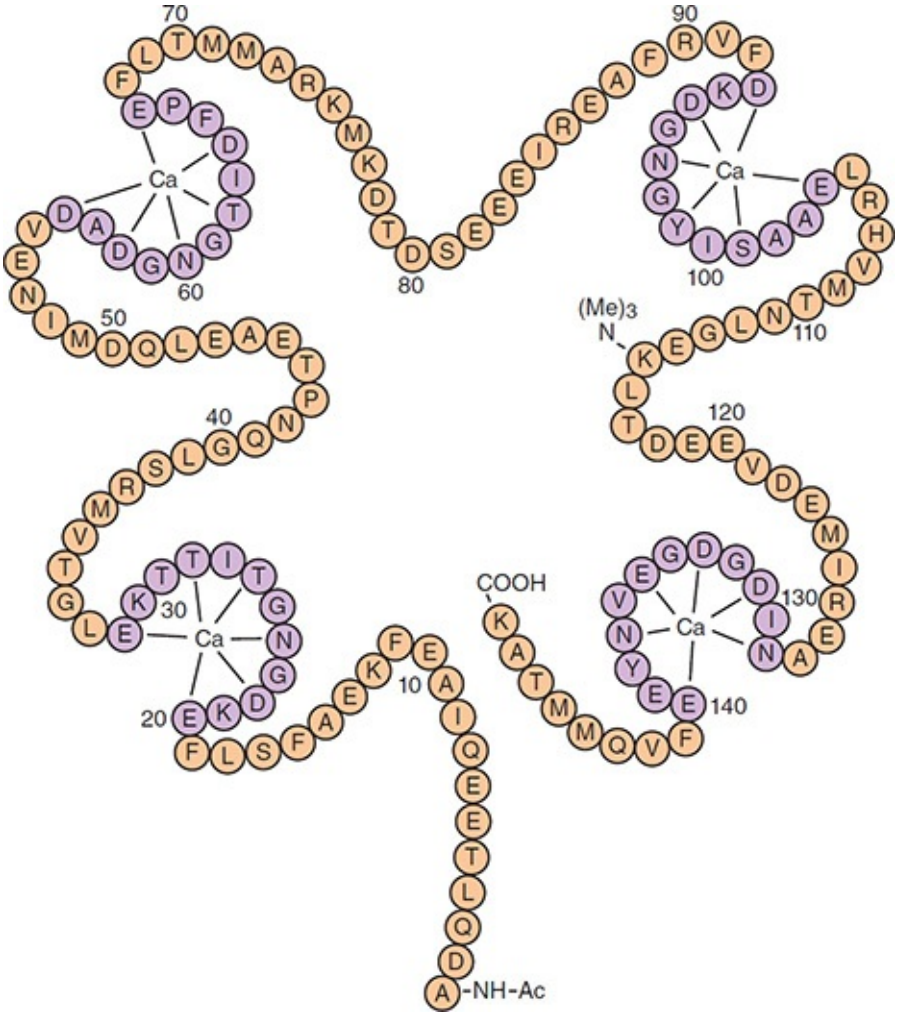


FIGURE 2–23 Secondary structure of calmodulin from bovine brain. Single-letter abbreviations are used for the amino acid residues. Note the four calcium domains (purple residues) flanked on either side by stretches of amino acids that form α-helices in tertiary structure. (Reproduced with permission from Cheung WY: Calmodulin: An overview. Fed Proc 1982;May;41(7):2253-2257.)

MECHANISMS OF DIVERSITY OF Ca^{2+} ACTIONS

It may seem difficult to understand how intracellular Ca^{2+} can have so many

varied effects as a second messenger. Part of the explanation is that Ca^{2+} may have different effects at low and at high concentrations. The ion may be at high concentration at the site of its release from an organelle or a channel **(Ca2+ sparks)** and at a subsequent lower concentration after it diffuses throughout the cell. Some of the changes it produces can outlast the rise in intracellular Ca^{2+} concentration because of the way it binds to some of the Ca^{2+}-binding proteins. In addition, once released, intracellular Ca^{2+} concentrations frequently oscillate at regular intervals, and there is evidence that the frequency and, to a lesser extent, the amplitude of those oscillations codes information for effector mechanisms. Finally, increases in intracellular Ca^{2+} concentration can spread from cell to cell in waves, producing coordinated events such as the rhythmic beating of cilia in airway epithelial cells.

G-PROTEINS

A common way to translate a signal to a biological effect inside cells is by way of nucleotide regulatory proteins that are activated after binding GTP **(G-proteins).** When an activating signal reaches a G-protein, the protein exchanges GDP for GTP. The GTP–protein complex brings about the activating effect of the G-protein. The inherent GTPase activity of the protein then converts GTP to GDP, restoring the G-protein to an inactive resting state. G-proteins can be divided into two principal groups involved in cell signaling: **small G-proteins** and **heterotrimeric G-proteins.** Other groups that have similar regulation and are also important to cell physiology include elongation factors, dynamin, and translocation GTPases.

There are several different families of small G-proteins (or **small GTPases**) that are all highly regulated. **GTPase activating proteins (GAPs)** tend to inactivate small G-proteins by encouraging hydrolysis of GTP to GDP in the central binding site. **Guanine exchange factors (GEFs)** tend to activate small G-proteins by encouraging exchange of GDP for GTP in the active site. Some of the small G-proteins contain lipid modifications that help anchor them to membranes, while others are free to diffuse throughout the cytosol. Small G-proteins are involved in many cellular functions. Members of the Rab family regulate the rate of vesicle traffic between the endoplasmic reticulum, the Golgi apparatus, lysosomes, endosomes, and the cell membrane. Another family of small GTP-binding proteins, the Rho/Rac family, mediates interactions between the cytoskeleton and cell membrane. The Ras family regulates growth by transmitting signals from the cell membrane to the nucleus.

Another family of G-proteins, the larger **heterotrimeric G-proteins,** couple cell surface receptors to catalytic units that catalyze the intracellular formation of second messengers or couple the receptors directly to ion channels. Despite the knowledge of the small G-proteins described above, the heteromeric G-proteins are frequently referred to in the shortened "G-protein" form because they were the first to be identified. Heterotrimeric G-proteins are made up of three subunits designated α, β, and γ **(Figure 2–24)**. Both the α and the γ subunits have lipid modifications that anchor these proteins to the plasma membrane. The α subunit is bound to GDP. When a ligand binds to a G-protein-coupled receptor (GPCR, discussed below), this GDP is exchanged for GTP and the α subunit separates from the combined β and γ subunits. The separated α subunit brings about many biologic effects. The β and γ subunits are tightly bound in the cell and together form a signaling molecule that can also activate a variety of effectors. The intrinsic GTPase activity of the α subunit then converts GTP to GDP, and this leads to reassociation of the α with the βγ subunit and termination of effector activation. The GTPase activity of the α subunit can be accelerated by a family of **regulators of G-protein signaling (RGS).**

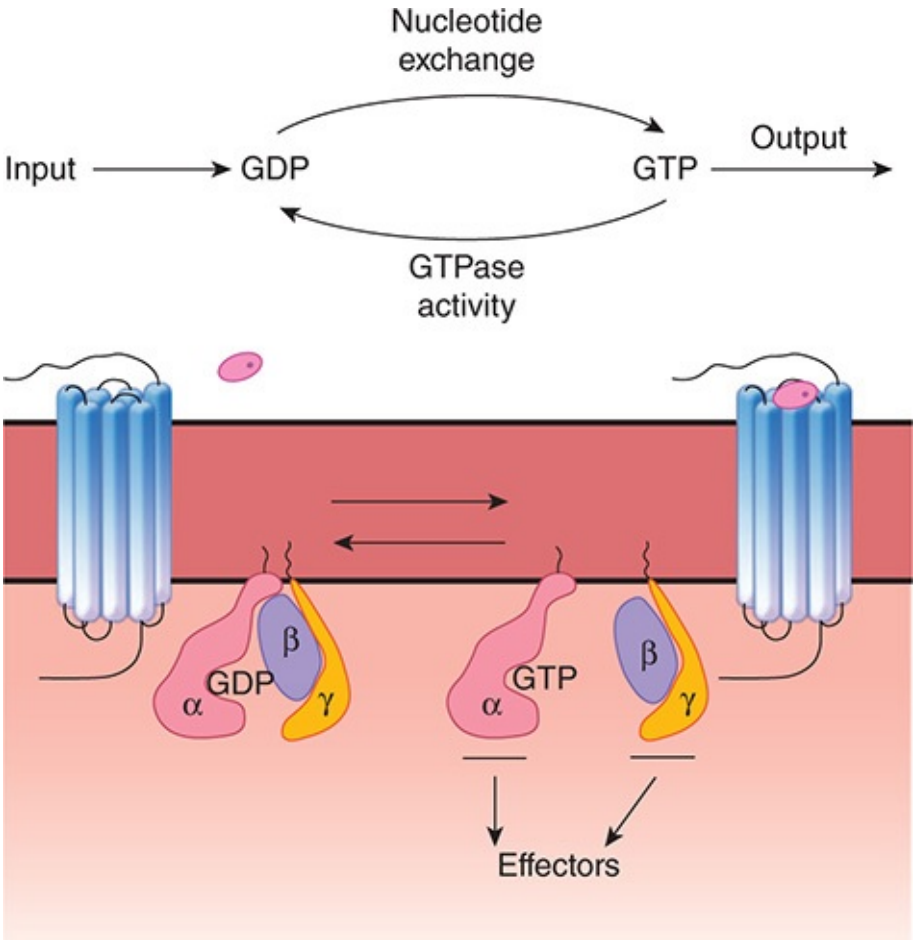


FIGURE 2–24 Heterotrimeric G-proteins. Top: Summary of overall reaction that occurs in the Gα subunit. **Bottom:** When the ligand (red oval) binds to the G-protein-coupled receptor in the cell membrane, GTP replaces GDP on the α subunit. GTP-α separates from the βγ subunit and GTP-α and βγ both activate various effectors, producing physiologic effects. The intrinsic GTPase activity of GTP-α then converts GTP to GDP, and the α, β, and γ subunits reassociate.

Heterotrimeric G-proteins relay signals from over 1000 GPCRs, and their effectors in the cells include ion channels and enzymes. There are 20 α, 6 β, and 12 γ genes, which allow for over 1400 α, β, and γ combinations. Not all combinations occur in the cell, but over 20 different heterotrimeric G-proteins have been well documented in cell signaling. They can be divided into five families, each with a relatively characteristic set of effectors.

G-PROTEIN-COUPLED RECEPTORS

All the **GPCRs** that have been characterized to date are proteins that span the cell membrane seven times. Because of this structure they are alternatively referred to as **seven-helix receptors** or **serpentine receptors.** A very large number have been cloned, and their functions are multiple and diverse. This is emphasized by the extensive variety of ligands that target GPCRs **(Table 2–5)**. The structures of four GPCRs are shown in **Figure 2–25**. These receptors assemble into a barrel-like structure. Upon ligand binding, a conformational change activates a resting heterotrimeric G-protein associated with the cytoplasmic leaf of the plasma membrane. Activation of a single receptor can result in 1, 10, or more active heterotrimeric G-proteins, providing amplification as well as transduction of the first messenger. Bound receptors can be inactivated to limit the amount of cellular signaling. This frequently occurs through phosphorylation of the cytoplasmic side of the receptor. Because of their diversity and importance in cellular signaling pathways, GPCRs are prime targets for drug discovery **(Clinical Box 2–8)**.

TABLE 2–5 Examples of ligands for G-protein-coupled receptors.

Class	Ligand
Neurotransmitters	Epinephrine Norepinephrine Dopamine 5-Hydroxytryptamine Histamine Acetylcholine Adenosine Opioids
Tachykinins	Substance P Neurokinin A Neuropeptide K
Other peptides	Angiotensin II Arginine vasopressin Oxytocin VIP, GRP, TRH, PTH
Glycoprotein hormones	TSH, FSH, LH, hCG
Arachidonic acid derivatives	Thromboxane A_2
Other	Odorants Tastants Endothelins Platelet-activating factor Cannabinoids Light Cations (eg, Ca^{2+}, Mg^{2+})

FSH, follicle-stimulating hormone; GRP, gastrin-releasing hormone; hCG, human chorionic gonadotropin; LH, luteinizing hormone; PTH, parathyroid hormone; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone; VIP, vasoactive intestinal peptide.

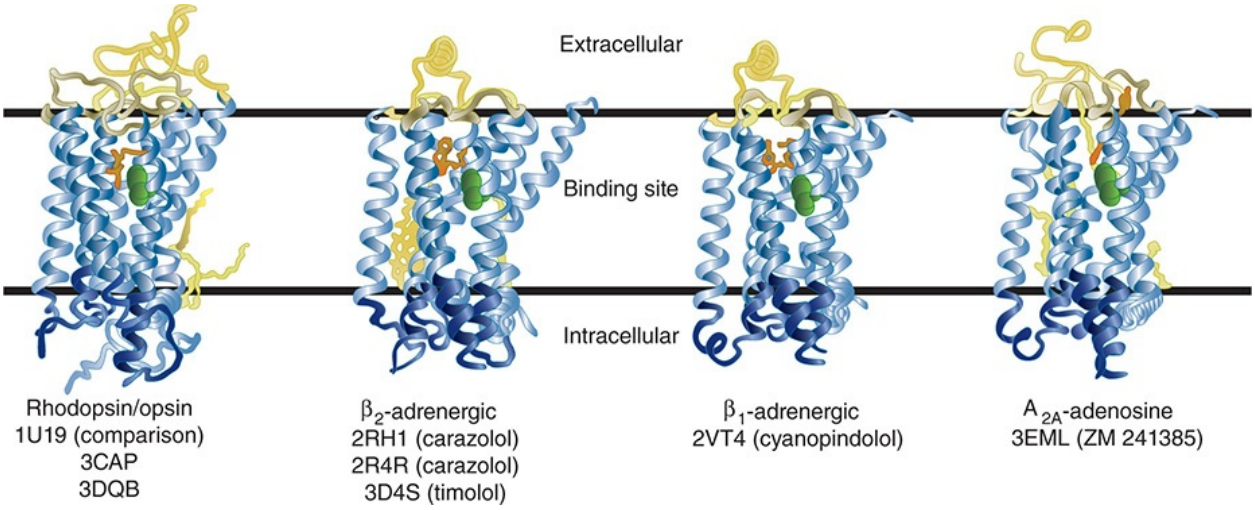


FIGURE 2–25 Representative structures of four G-protein-coupled receptors from solved crystal structures. Each group of receptors is represented by one structure, all rendered with the same orientation and color scheme: transmembrane helices are colored light blue, intracellular regions are colored darker blue, and extracellular regions are brown. Each ligand is colored orange and rendered as sticks, bound lipids are colored yellow, and the conserved tryptophan residue is rendered as spheres and colored green. This figure highlights the observed differences seen in the extracellular and intracellular domains as well as the small differences seen in the ligand binding orientations among the four GPCRs various ligands. (Reproduced with permission from Hanson MA, Stevens RC: Discovery of new GPCR biology: one receptor structure at a time. Structure 1988;Jan 14;17(1):8-14.)

INOSITOL TRISPHOSPHATE & DIACYLGLYCEROL AS SECOND MESSENGERS

The link between membrane binding of a ligand that acts via Ca^{2+} and the prompt increase in the cytoplasmic Ca^{2+} concentration is often **IP_3**. When one of these ligands binds to its receptor, activation of the receptor produces activation of phospholipase C (PLC) on the inner surface of the membrane. Ligands bound to GPCR can do this through the G_q heterotrimeric G-proteins, while ligands bound to tyrosine kinase receptors can do this through other cell signaling pathways. PLC has at least eight isoforms; PLC_β is activated by heterotrimeric G-proteins, while PLCγ forms are activated through tyrosine kinase receptors. PLC isoforms can catalyze the hydrolysis of the membrane lipid

phosphatidylinositol 4,5-diphosphate (PIP_2) to form IP_3 and **DAG** **(Figure 2–26)**. The IP_3 diffuses to the endoplasmic reticulum where it triggers the release of Ca^{2+} into the cytoplasm by binding the IP_3 receptor, a ligand-gated Ca^{2+} channel **(Figure 2–27)**. DAG is also a second messenger; it stays in the cell membrane where it activates one of several isoforms of **protein kinase C** and activates receptor-operated Ca^{2+} channels (ROCCs) in the plasma membrane to further increase intracellular Ca^{2+} concentration.

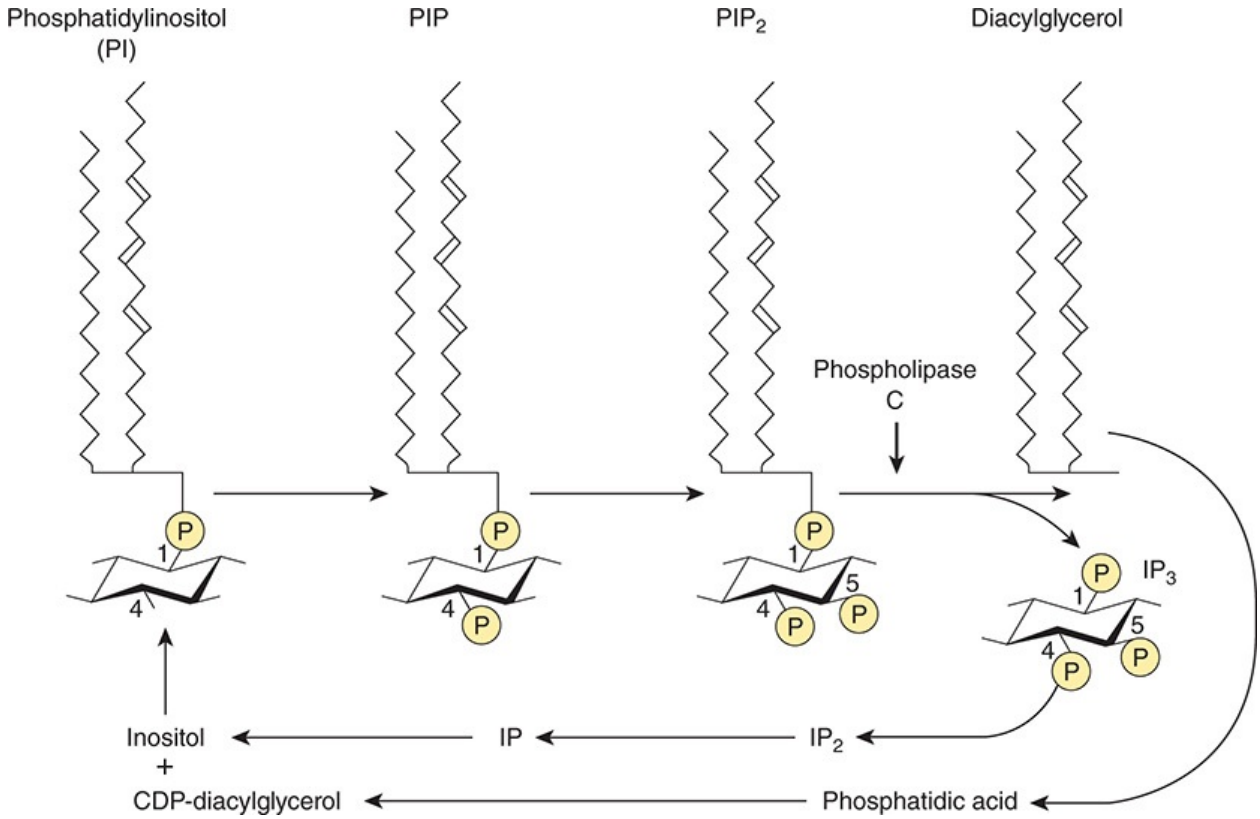


FIGURE 2–26 Metabolism of phosphatidylinositol in cell membranes. Phosphatidylinositol is successively phosphorylated to form phosphatidylinositol 4-phosphate (PIP), then phosphatidylinositol 4,5-bisphosphate (PIP_2). Phospholipase C_β and phospholipase $C\gamma$ catalyze the breakdown of PIP_2 to inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol. Other inositol phosphates and phosphatidylinositol derivatives can also be formed. IP_3 is dephosphorylated to inositol, and diacylglycerol is metabolized to cytosine diphosphate (CDP)-diacylglycerol. CDP-diacylglycerol and inositol then combine to form phosphatidylinositol, completing the cycle. (Modified with permission from Berridge MJ: Inositol triphosphate and diacylglycerol as second messengers.

Biochem J 1984;June 1;220(2):345-360.)

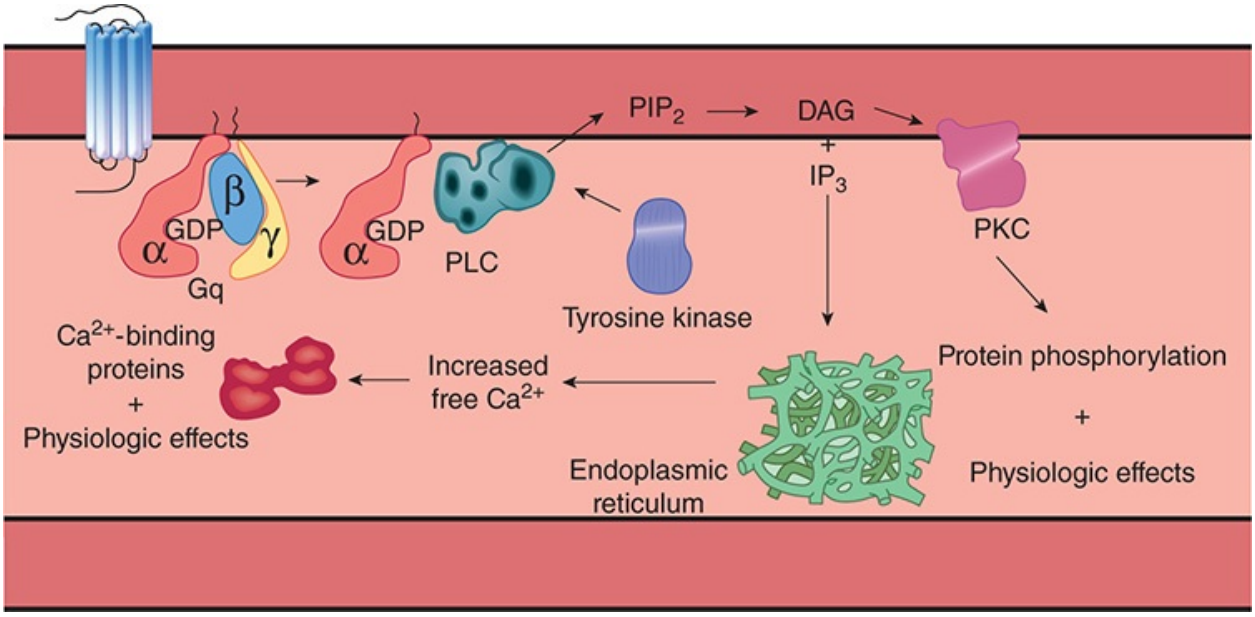


FIGURE 2–27 Diagrammatic representation of release of inositol trisphosphate (IP_3) and diacylglycerol (DAG) as second messengers. Binding of ligand to G-protein-coupled receptor activates phospholipase C (PLC). Alternatively, activation of receptors with intracellular tyrosine kinase domains can activate PLCγ. The resulting hydrolysis of phosphatidylinositol 4,5-diphosphate (PIP_2) produces IP_3, which releases Ca^{2+} from the endoplasmic reticulum (ER), and DAG, which activates protein kinase C (PKC).

CLINICAL BOX 2–8

Drug Development: Targeting the G-Protein-Coupled Receptors (GPCRs)

GPCRs are among the most heavily investigated drug targets in the pharmaceutical industry, representing approximately 40% of all the drugs in the marketplace today. These proteins are active in just about every organ system and present a wide range of opportunities as therapeutic targets in areas including cancer, cardiac dysfunction, diabetes, central nervous system disorders, obesity, inflammation, and pain. Features of GPCRs that allow them to be drug targets are their specificity in recognizing extracellular ligands to initiate cellular response, the cell surface location of GPCRs that

make them accessible to novel ligands or drugs, and their prevalence in leading to human pathology and disease.

Specific examples of successful GPCR drug targets are noted with two types of **histamine receptors.**

Histamine-1 receptor (H_1-receptor) antagonists: allergy therapy. Allergens can trigger local mast cells or basophils to release histamine in the airway. A primary target for histamine is the H_1-receptor in several airway cell types and this can lead to transient itching, sneezing, rhinorrhea, and nasal congestion. There are a variety of medications with improved peripheral H_1-receptor selectivity that are currently used to block histamine activation of the H_1-receptor and thus limit allergen effects in the upper airway. H_1-receptor antagonists on the market include loratadine, fexofenadine, cetirizine, and desloratadine. These "second" and "third" generation medications have improved specificity and reduced adverse side effects (eg, drowsiness and central nervous system dysfunction) associated with some of the "first" generation drugs first introduced in the late 1930s and widely developed over the next 40 years.

Histamine-2 receptor (H_2-receptor) antagonists: treating excess stomach acid. Excess stomach acid can result in gastroesophageal reflux disease or even peptic ulcer symptoms. The parietal cell in the stomach can be stimulated to produce acid via histamine action at the H_2-receptor. Excess stomach acid results in heartburn. Antagonists or H_2-receptor blockers, reduce acid production by preventing H_2-receptor signaling that leads to production of stomach acid. There are several drugs (eg, ranitidine, famotidine, cimetidine, and nizatidine) that specifically block the H_2-receptor and thus reduce excess acid production.

CYCLIC AMP

Another important second messenger is **cAMP** (Figure 2–28). cAMP is formed from ATP by the action of the enzyme **adenylyl cyclase** and converted to physiologically inactive 5' AMP by the action of the enzyme **phosphodiesterase.** Some of the phosphodiesterase isoforms that break down cAMP are inhibited by methylxanthines such as caffeine and theophylline. Consequently, these compounds can augment hormonal and transmitter effects

mediated via cAMP. cAMP activates one of the cyclic nucleotide-dependent protein kinases **(protein kinase A, PKA)** that, like protein kinase C, catalyzes the phosphorylation of proteins, changing their conformation and altering their activity. In addition, the active catalytic subunit of PKA moves to the nucleus and phosphorylates the **cAMP-responsive element-binding protein (CREB).** This transcription factor then binds to DNA and alters transcription of a number of genes.

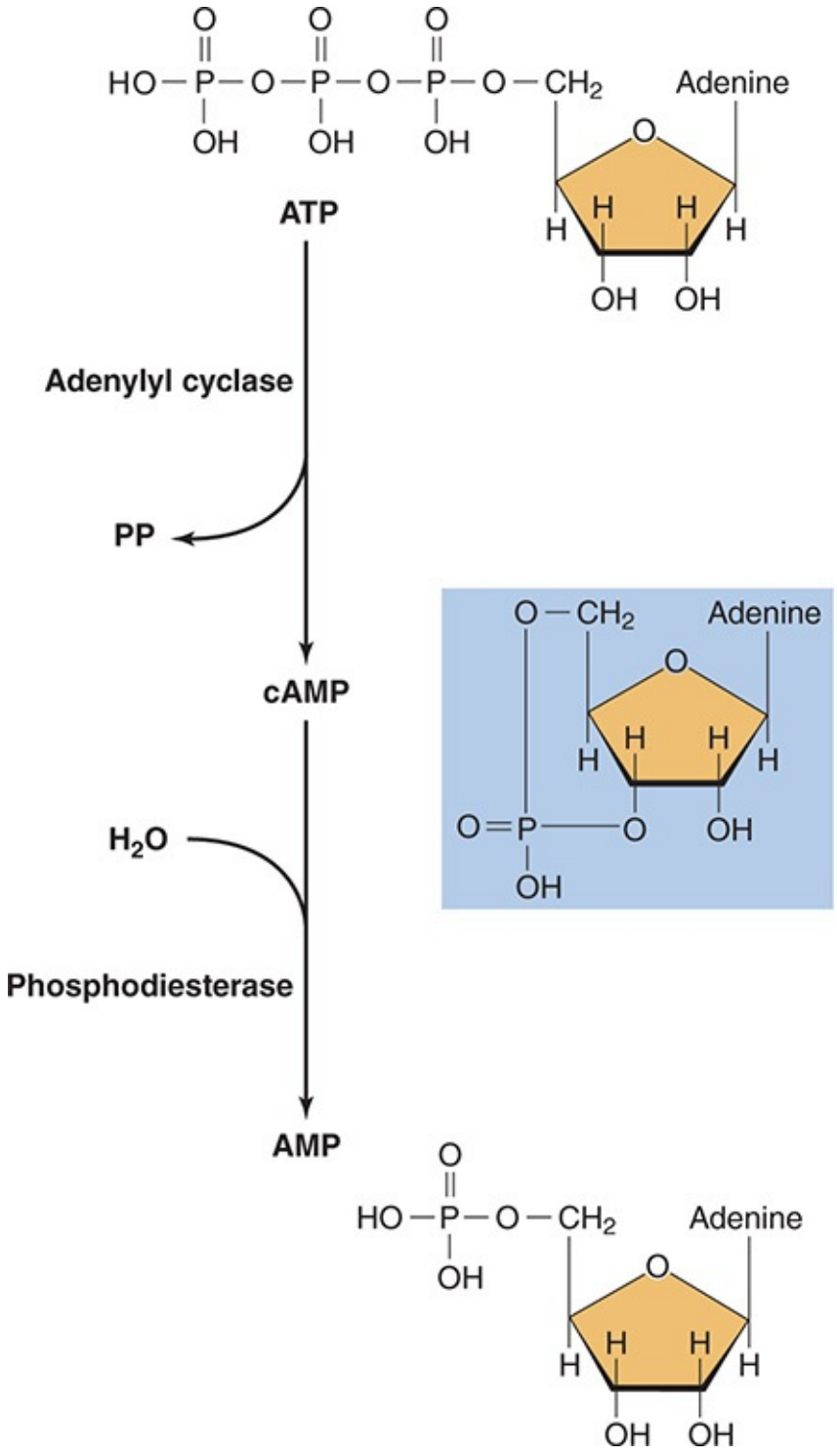


FIGURE 2–28 Formation and metabolism of cAMP. The second messenger cAMP is made from ATP by adenylyl cyclase and broken down into AMP by phosphodiesterase.

PRODUCTION OF cAMP BY ADENLYL CYCLASE

Adenylyl cyclase is a membrane bound protein with 12 transmembrane regions. Ten isoforms of this enzyme have been described and each can have distinct regulatory properties, permitting the cAMP pathway to be customized to specific tissue needs. Notably, stimulatory heterotrimeric G-proteins (G_s) activate, while inhibitory heterotrimeric G-proteins (G_i) inactivate adenylyl cyclase **(Figure 2–29)**. When the appropriate ligand binds to a stimulatory receptor, a G_s α subunit activates one of the adenylyl cyclases. Conversely, when the appropriate ligand binds to an inhibitory receptor, a G_i α subunit inhibits adenylyl cyclase. The receptors are specific, responding at low threshold to only one or a select group of related ligands. However, heterotrimeric G-proteins mediate the stimulatory and inhibitory effects produced by many different ligands. In addition, cross-talk occurs between the phospholipase C system and the adenylyl cyclase system, as several of the isoforms of adenylyl cyclase are stimulated by calmodulin. Finally, the effects of protein kinase A and protein kinase C are very widespread and can also affect directly, or indirectly, the activity at adenylyl cyclase. The close relationship between activation of G-proteins and adenylyl cyclases also allows for spatial regulation of cAMP production. All of these events, and others, allow for fine-tuning the cAMP response for a particular physiologic outcome in the cell.

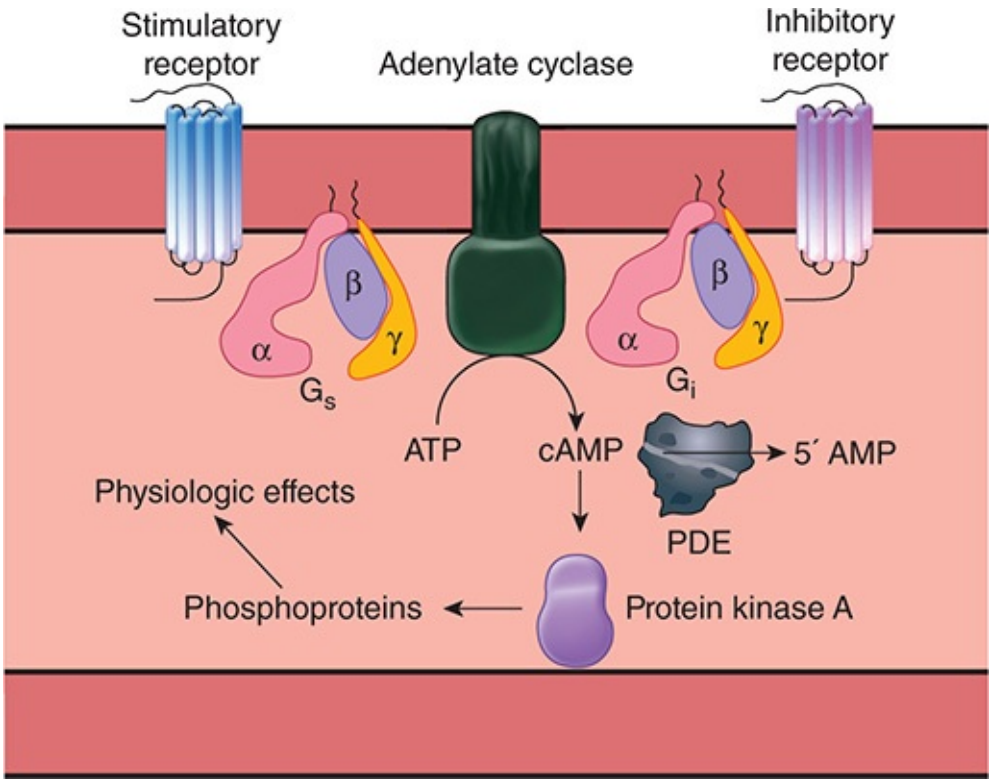


FIGURE 2–29 The cAMP system. Activation of adenylyl cyclase catalyzes the conversion of ATP to cAMP. Cyclic AMP activates protein kinase A, which phosphorylates proteins, producing physiologic effects. Stimulatory ligands bind to stimulatory receptors and activate adenylyl cyclase via G_s. Inhibitory ligands inhibit adenylyl cyclase via inhibitory receptors and G_i.

Two bacterial toxins have important effects on adenylyl cyclase that are mediated by G-proteins. The A subunit of **cholera toxin** catalyzes the transfer of ADP ribose to an arginine residue in the middle of the α subunit of G_s. This inhibits its GTPase activity, producing prolonged stimulation of adenylyl cyclase. **Pertussis toxin** catalyzes ADP-ribosylation of a cysteine residue near the carboxyl terminal of the α subunit of G_i. This inhibits the function of G_i. In addition to the implications of these alterations in disease, both toxins are used for fundamental research on G-protein function. The compound forskolin also stimulates adenylyl cyclase activity by a direct action on the enzyme, and is commonly used in research studies to evaluate adenylyl cyclase/cAMP contributions to cellular physiology.

GUANYLYL CYCLASE

Another cyclic nucleotide of physiologic importance is **cyclic guanosine monophosphate (cyclic GMP** or **cGMP).** cGMP is important in vision in both rod and cone cells. In addition, there are cGMP-regulated ion channels, and cGMP activates cGMP-dependent kinase, producing a number of physiologic effects.

Guanylyl cyclases are a family of enzymes that catalyze the formation of cGMP. They exist in two forms **(Figure 2–30)**. One form has an extracellular amino terminal domain that is a receptor, a single transmembrane domain, and a cytoplasmic portion with guanylyl cyclase catalytic activity. Several such guanylyl cyclases have been characterized. Two are receptors for atrial natriuretic peptide (ANP; also known as atrial natriuretic factor), and a third binds an *Escherichia coli* enterotoxin and the gastrointestinal polypeptide guanylin. The other form of guanylyl cyclase is soluble (sGC), contains heme, and is not bound to the membrane. There appear to be several isoforms of the intracellular enzyme. They are activated by nitric oxide (NO) and NO-containing compounds.

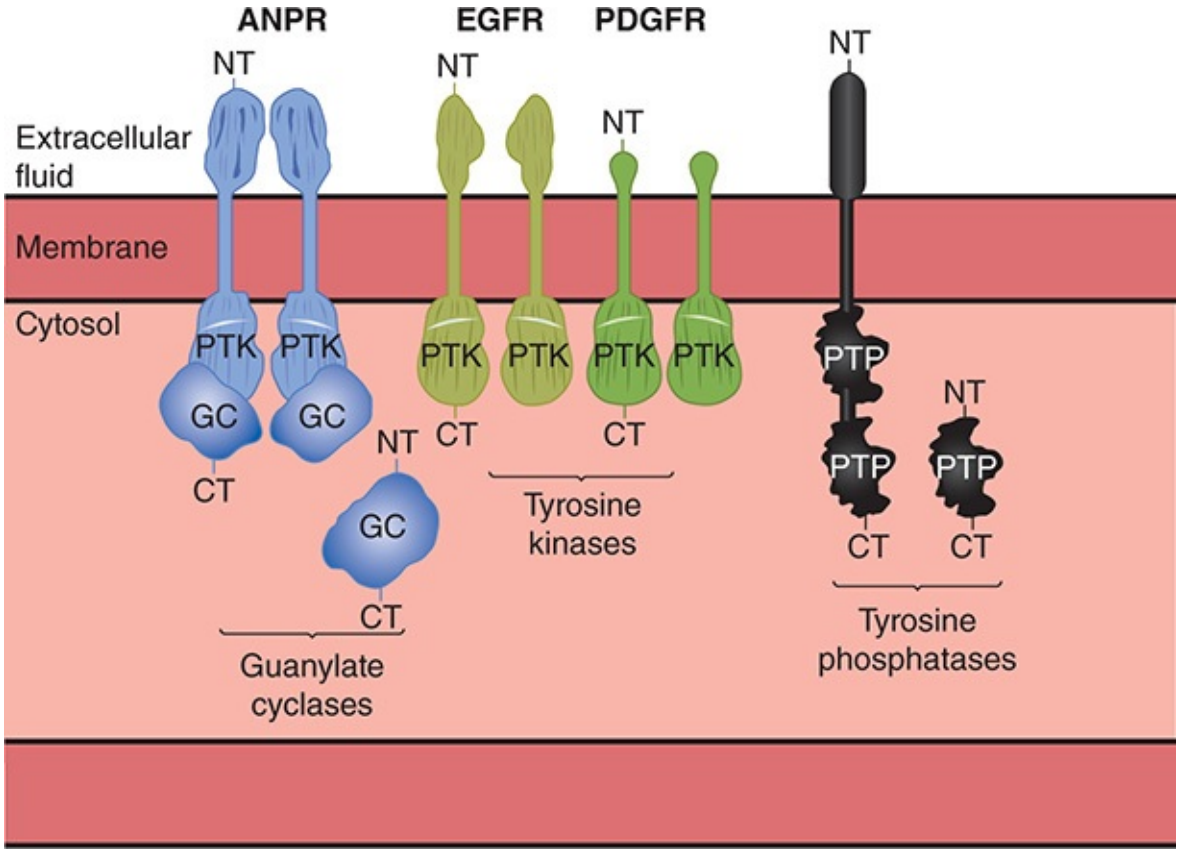


FIGURE 2–30 Diagrammatic representation of guanylyl cyclases, tyrosine kinases, and tyrosine phosphatases. NT refers to the amino (NH_2) terminus

and CT to the carboxyl terminus of each protein. Individual molecules are as follows: ANP, atrial natriuretic peptide; GC, guanylyl cyclase domain; EGFR, epidermal growth factor receptor; PDGFR, platelet-derived growth factor receptor; PTK, protein tyrosine kinase domain (PTK is inactive in guanylyl cyclase); PTP, tyrosine phosphatase domain.

GROWTH FACTORS

Growth factors have become increasingly important in many different aspects of physiology. They are polypeptides and proteins that are conveniently divided into three groups. One group is made up of agents that foster the multiplication or development of various types of cells; NGF, insulin-like growth factor I (IGF-I), activins and inhibins, and epidermal growth factor (EGF) are examples. More than 20 have been described. The cytokines are a second group. These factors are produced by macrophages and lymphocytes, as well as other cells, and are important in regulation of the immune system (see Chapter 3). Again, more than 20 have been described. The third group is made up of the colony-stimulating factors that regulate proliferation and maturation of red and white blood cells.

Receptors for EGF, platelet-derived growth factor (PDGF), and many of the other factors that foster cell multiplication and growth have a single membrane-spanning domain with an intracellular tyrosine kinase domain (Figure 2–29). When ligand binds to a tyrosine kinase receptor, it first causes a dimerization of two similar receptors. The dimerization results in partial activation of the intracellular tyrosine kinase domains and a cross-phosphorylation to fully activate each other. One of the pathways activated by phosphorylation leads, through the small G-protein Ras, to MAP kinases, and eventually to the production of transcription factors in the nucleus that alter gene expression **(Figure 2–31)**.

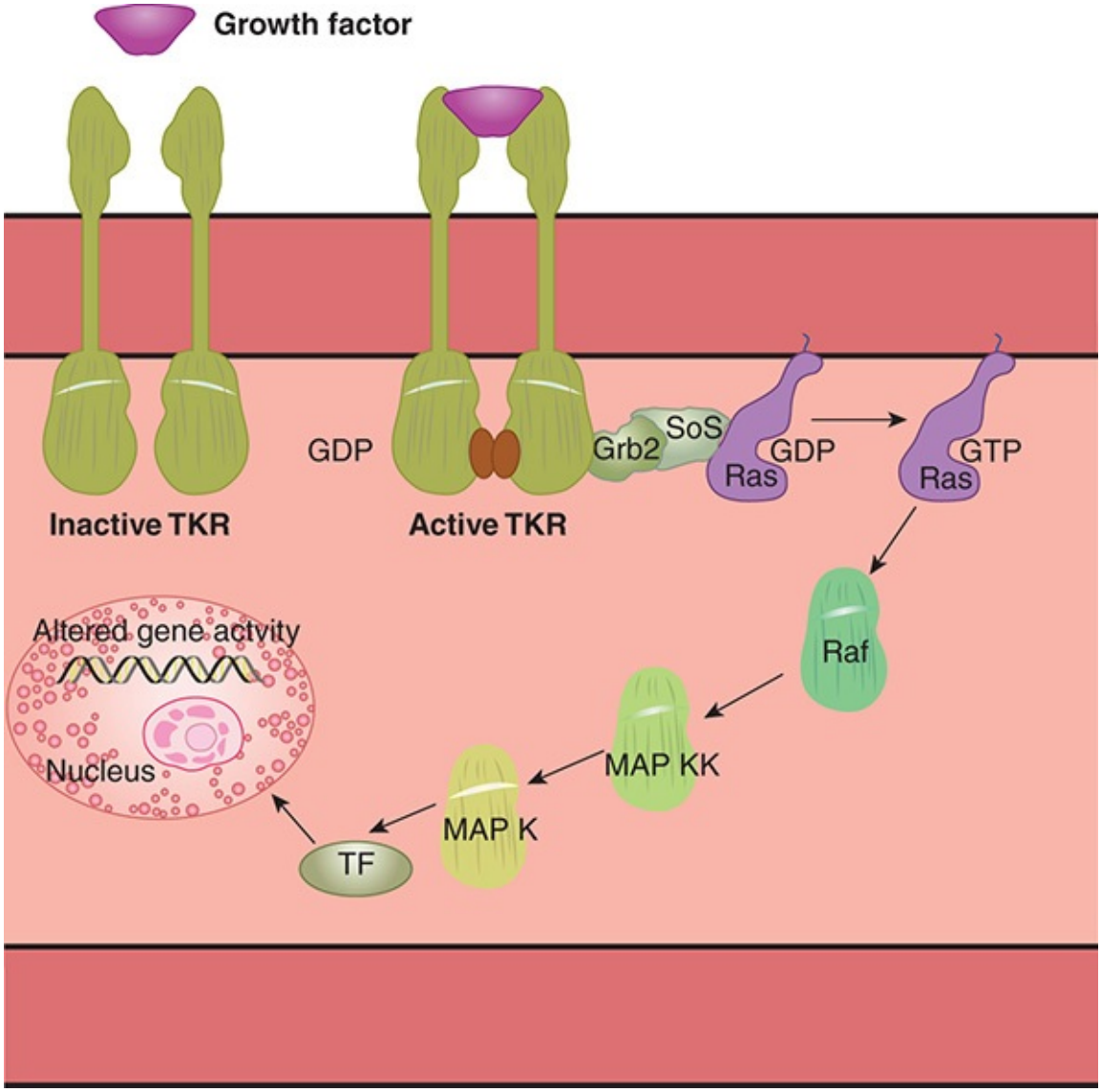


FIGURE 2–31 One of the direct pathways by which growth factors alter gene activity. Grb2, Ras activator/controller; MAP K, mitogen-activated protein kinase; MAP KK, MAP kinase kinase; Ras, product of the *ras* gene; Sos, Ras activator; TF, transcription factors; TKR, tyrosine kinase domain. There is a cross-talk between this pathway and the cAMP pathway, as well as a cross-talk with the IP_3–DAG pathway.

Receptors for cytokines and colony-stimulating factors differ from the other growth factors in that most of them do not have tyrosine kinase domains in their cytoplasmic portions and some have little or no cytoplasmic tail. However, they initiate tyrosine kinase activity in the cytoplasm. In particular, they activate the so-called Janus tyrosine kinases **(JAKs)** in the cytoplasm **(Figure 2–32)**. These

in turn phosphorylate **STAT** proteins. The phosphorylated STATs form homo- and heterodimers and move to the nucleus, where they act as transcription factors. There are four known mammalian JAKs and seven known STATs. Interestingly, the JAK–STAT pathway can also be activated by growth hormone and is another important direct path from the cell surface to the nucleus. However, it should be emphasized that both the Ras and the JAK–STAT pathways are complex and there is cross-talk between them and other signaling pathways discussed previously.

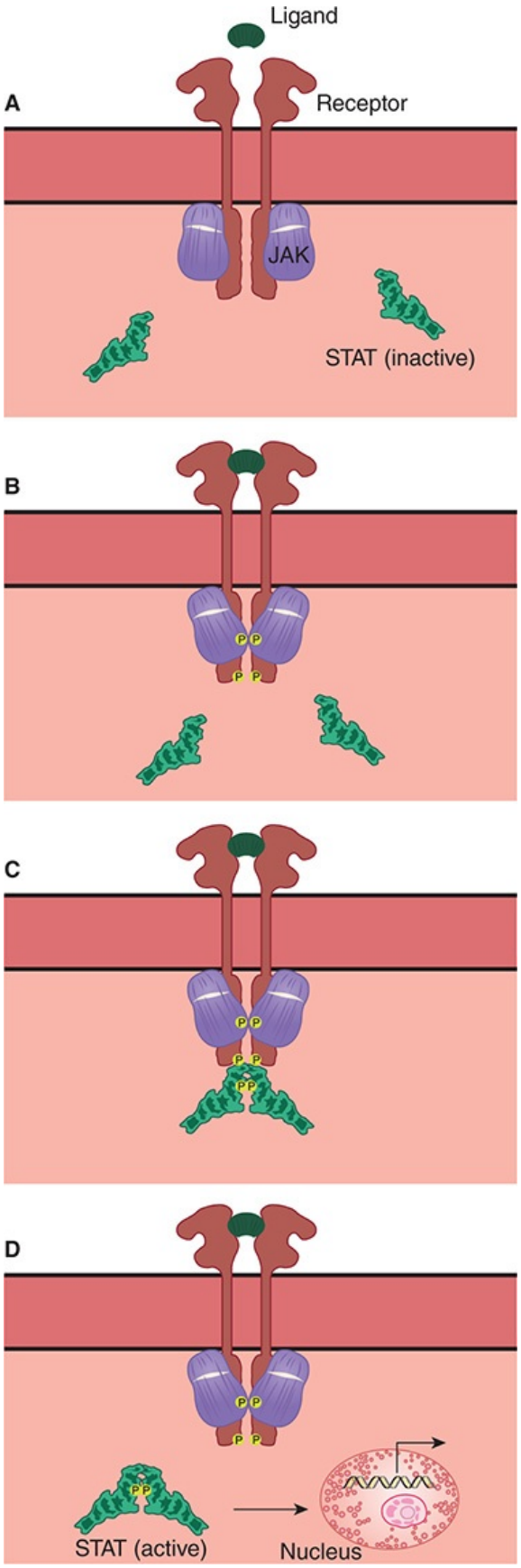

Ligand
A
Receptor
JAK
STAT (inactive)
B
P
C
D
STAT (active)
Nucleus

FIGURE 2–32 Signal transduction via the JAK–STAT pathway. A) Inactive JAKs are associated with individual receptors. **B)** Ligand binding leads to dimerization of receptor and activation JAKs that phosphorylate tyrosine residues on opposing receptors and their associated JAK. **C)** STATs then associate with the phosphorylated receptors and JAKs in turn phosphorylate these STATs. **D)** Phosphorylated STATs dimerize and move to nucleus, where they bind to response elements on DNA.

Finally, note that the whole subject of second messengers and intracellular signaling has become immensely complex, with multiple pathways and interactions. It is only possible in a book such as this to list highlights and present general themes that will aid the reader in understanding the rest of physiology **(Clinical Box 2–9).**

CLINICAL BOX 2–9

Receptor & G-Protein Diseases

Many diseases are being traced to mutations in the genes for receptors. For example, loss-of-function receptor mutations that cause disease have been reported for the 1,25-dihydroxycholecalciferol receptor and the insulin receptor. Certain other diseases are caused by production of antibodies against receptors. Thus, antibodies against thyroid-stimulating hormone (TSH) receptors cause Graves disease, and antibodies against nicotinic acetylcholine receptors cause myasthenia gravis.

An example of loss of function of a receptor is the type of nephrogenic diabetes insipidus that is due to loss of the ability of mutated V_2 vasopressin receptors to mediate concentration of the urine. Mutant receptors can gain as well as lose function. A gain-of-function mutation of the calcium-sensing receptor (CaSR) causes excess inhibition of parathyroid hormone secretion and familial hypercalciuric hypocalcemia. G-proteins can also undergo loss-of-function or gain-of-function mutations that cause disease **(Table 2–6)**. In one form of pseudohypoparathyroidism, a mutated $G_s\alpha$ fails to respond to parathyroid hormone, producing the symptoms of hypoparathyroidism without any decline in circulating parathyroid hormone. Testotoxicosis is an interesting disease that combines gain and loss of function. In this condition, an activating mutation of $G_s\alpha$ causes excess testosterone secretion and

prepubertal sexual maturation. However, this mutation is temperature-sensitive and is active only at the relatively low temperature of the testes (33°C). At 37°C, the normal temperature of the rest of the body, it is replaced by loss of function, with the production of hypoparathyroidism and decreased responsiveness to TSH. A different activating mutation in $G_s\alpha$ is associated with the rough-bordered areas of skin pigmentation and hypercortisolism of the McCune–Albright syndrome. This mutation occurs during fetal development, creating a mosaic of normal and abnormal cells. A third mutation in $G_s\alpha$ reduces its intrinsic GTPase activity. As a result, it is much more active than normal, and excess cAMP is produced. This causes hyperplasia and eventually neoplasia in somatotrope cells of the anterior pituitary. Forty percent of somatotrope tumors causing acromegaly have cells containing a somatic mutation of this type.

TABLE 2–6 Examples of abnormalities caused by loss- or gain-of-function mutations of heterotrimeric G-protein-coupled receptors and G-proteins.

Site	Type of Mutation	Disease
Receptor		
Cone opsins	Loss	Color blindness
Rhodopsin	Loss	Congenital night blindness; two forms of retinitis pigmentosa
Vasopressin receptor 2 (V2R) or arginine vasopressin receptor 2	Loss	X-linked nephrogenic diabetes insipidus
Adrenocorticotropic hormone (ACTH) receptor or melanocortin receptor 2	Loss	Familial glucocorticoid deficiency
Luteinizing hormone receptor (LHR)	Gain	Familial male precocious puberty
Thyrotropin receptor (TSH receptor)	Gain	Familial nonautoimmune hyperthyroidism
TSH receptor	Loss	Familial hypothyroidism
Calcium-sensing receptor (CaSR)	Gain	Familial hypercalciuric hypocalcemia
Thromboxane A_2 receptor (TP) or prostanoid TP receptor	Loss	Congenital bleeding
Endothelin receptor B (ET_B)	Loss	Hirschsprung disease
G-protein		
G_s α	Loss	Pseudohypothyroidism type 1a
G_s α	Gain/loss	Testotoxicosis
G_s α	Gain (mosaic)	McCune-Albright syndrome
G_s α	Gain	Somatotroph adenomas with acromegaly
G_i α	Gain	Ovarian and adrenocortical tumors

ACTH, adrenocorticotropic hormone; LH, luteinizing hormone; TSH, thyroid-stimulating hormone.

CHAPTER SUMMARY

- The cell and the intracellular organelles are surrounded by semipermeable membranes. Biological membranes have a lipid bilayer core that is populated by structural and functional proteins. These proteins contribute greatly to the semipermeable properties of biological membrane.
- Cells contain a variety of organelles that perform specialized cell functions. The nucleus is an organelle that contains DNA and is the site of gene transcription. The endoplasmic reticulum and the Golgi apparatus are important in protein processing and the targeting of proteins to correct compartments within the cell. Lysosomes and peroxisomes are membrane-bound organelles that contribute to protein and lipid processing. Mitochondria are organelles that allow for oxidative phosphorylation in eukaryotic cells and also are important in specialized cellular signaling.
- The cytoskeleton is a network of three types of filaments that provide structural integrity to the cell as well as a means for trafficking of organelles and other structures around the cell. Actin filaments are important in cellular contraction, migration, and signaling. Actin filaments also provide the backbone for muscle contraction. Intermediate filaments are primarily structural. Microtubules provide a dynamic structure in cells that allows for the movement of cellular components around the cell.
- There are three superfamilies of molecular motor proteins in the cell that use the energy of ATP to generate force, movement, or both. Myosin is the force generator for muscle cell contraction. Cellular myosins can also interact with the cytoskeleton (primarily thin filaments) to participate in contraction as well as movement of cell contents. Kinesins and cellular dyneins are motor proteins that primarily interact with microtubules to move cargo around the cells.
- Cellular adhesion molecules aid in tethering cells to each other or to the extracellular matrix as well as providing for initiation of cellular signaling. There are four main families of these proteins: integrins, immunoglobulins, cadherins, and selectins.
- Cells contain distinct protein complexes that serve as cellular connections to other cells or the extracellular matrix. Tight junctions provide intercellular connections that link cells into a regulated tissue barrier and also provide a barrier to movement of proteins in the cell membrane. Gap junctions

provide contacts between cells that allow for direct passage of small molecules between two cells. Desmosomes and adherens junctions are specialized structures that hold cells together. Hemidesmosomes and focal adhesions attach cells to their basal lamina.

- Exocytosis and endocytosis are vesicular fusion events that allow for movement of proteins and lipids between the cell interior, the plasma membrane, and the cell exterior. Exocytosis can be constitutive or nonconstitutive; both are regulated processes that require specialized proteins for vesicular fusion. Endocytosis is the formation of vesicles at the plasma membrane to take material from the extracellular space into the cell interior.
- Cells can communicate with one another via chemical messengers. Individual messengers (or ligands) typically bind to a plasma membrane receptor to initiate intracellular changes that lead to physiological changes. Plasma membrane receptor families include ion channels, G-protein-coupled receptors, or a variety of enzyme-linked receptors (eg, tyrosine kinase receptors). There are additional cytosolic receptors (eg, steroid receptors) that can bind membrane-permeant compounds. Activation of receptors leads to cellular changes that include changes in membrane potential, activation of heterotrimeric G-proteins, increase in second messenger molecules, or initiation of transcription.
- Second messengers are molecules that undergo a rapid concentration changes in the cell following primary messenger recognition. Common second messenger molecules include Ca^{2+}, cyclic adenosine monophosphate (cAMP), and cyclic guanine monophosphate (cGMP), inositol trisphosphate (IP_3).

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. The electrogenic Na, K ATPase plays a critical role in cellular physiology by

A. using the energy in ATP to extrude 3 Na^+ out of the cell in exchange for taking two K^+ into the cell.

B. using the energy in ATP to extrude 3 K^+ out of the cell in exchange for taking two Na^+ into the cell.

C. using the energy in moving Na^+ into the cell or K^+ outside the cell to make

ATP.

D. using the energy in moving Na^+ outside of the cell or K^+ inside the cell to make ATP.

2. Cell membranes

A. contain relatively few protein molecules.

B. contain many carbohydrate molecules.

C. are freely permeable to electrolytes but not to proteins.

D. have variable protein and lipid contents depending on their location in the cell.

E. have a stable composition throughout the life of the cell.

3. Second messengers

A. are substances that interact with first messengers outside cells.

B. are substances that bind to first messengers in the cell membrane.

C. are hormones secreted by cells in response to stimulation by another hormone.

D. mediate the intracellular responses to many different hormones and neurotransmitters.

E. are not formed in the brain.

4. The Golgi complex

A. is an organelle that participates in the breakdown of proteins and lipids.

B. is an organelle that participates in posttranslational processing of proteins.

C. is an organelle that participates in energy production.

D. is an organelle that participates in transcription and translation.

E. is a subcellular compartment that stores proteins for trafficking to the nucleus.

5. Endocytosis

A. includes phagocytosis and pinocytosis, but not clathrin-mediated or caveolae-dependent uptake of extracellular contents.

B. refers to the merging of an intracellular vesicle with the plasma membrane to deliver intracellular contents to the extracellular milieu.

C. refers to the invagination of the plasma membrane to uptake extracellular contents into the cell.

D. refers to vesicular trafficking between Golgi stacks.

6. G-protein-coupled receptors

A. are intracellular membrane proteins that help regulate movement within the cell.

B. are plasma membrane proteins that couple the extracellular binding of primary signaling molecules to exocytosis.

C. are plasma membrane proteins that couple the extracellular binding of primary signaling molecules to the activation of heterotrimeric G-proteins.

D. are intracellular proteins that couple the binding of primary messenger molecules with transcription.

7. Gap junctions are intercellular connections that

A. primarily serve to keep cells separated and allow for transport across a tissue barrier.

B. serve as a regulated cytoplasmic bridge for sharing of small molecules between cells.

C. serve as a barrier to prevent protein movement within the cellular membrane.

D. are cellular components for constitutive exocytosis that occurs between adjacent cells.

8. F-actin is a component of the cellular cytoskeleton that

A. provides a structural component for cell movement.

B. is defined as the “functional” form of actin in the cell.

C. refers to the actin subunits that provide the molecular building blocks of the extended actin molecules found in the cell.

D. provides the molecular architecture for cell to cell communication.

CHAPTER 3

Immunity, Infection, & Inflammation

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OBJECTIVES

After studying this chapter, you should be able to:

- Understand the significance of immunity, particularly with respect to defending the body against microbial invaders.
- Delineate the role and mechanisms of innate immunity.
- Identify the roles of the humoral and cellular arms of acquired immunity, the cellular mediators of these responses, and the molecular basis for the recognition of a diverse set of antigens.
- Understand the general principles governing regulation of immunity by soluble cytokines, chemokines and hematopoietic growth factors, as well as the complement system.
- Define the roles of additional circulating and tissue cell types that contribute to immune and inflammatory responses, including granulocytes, mast cells, monocytes, and platelets. Describe how phagocytes are able to kill internalized bacteria.
- Understand the basis of inflammatory responses and wound healing.

INTRODUCTION

As an open system, the body is continuously called upon to defend itself from potentially harmful invaders such as bacteria, viruses, and other microbes. This is accomplished by the immune system, which is subdivided into innate and adaptive (or acquired) branches. The immune system is composed of specialized effector cells that sense and respond to foreign antigens and other molecular patterns not found in human tissues, as well as various regulatory molecules, including the large collection of soluble mediators known as cytokines. Likewise, the immune system clears the body's own cells that have become senescent or abnormal, such as cancer cells. Finally, normal host tissues occasionally become the subject of inappropriate immune attack, such as in autoimmune diseases or in settings where normal cells are harmed as innocent bystanders when the immune system mounts an inflammatory response to an invader. It is beyond the scope of this volume to provide a full treatment of all aspects of modern immunology. Nevertheless, the student of physiology should have a working knowledge of immune functions and their regulation, due to a growing appreciation for the ways in which the immune system can contribute to normal physiologic regulation in a variety of tissues, as well as contributions of immune effectors to pathophysiology.

IMMUNITY

OVERVIEW

All multicellular organisms, including invertebrates and plants, express the ancient protective mechanism of **innate immunity.** This system is triggered by receptors that bind sequences of sugars, lipids, amino acids, or nucleic acids that are common in bacteria and other microorganisms, but are not found in eukaryotic cells. These receptors, in turn, activate various defense mechanisms. The receptors are coded in the germ line, and their fundamental structure is not modified by exposure to antigen. The activated defenses include, in various species, release of cytokines known as interferons, phagocytosis, production of antibacterial peptides, activation of the complement system, and several proteolytic cascades. This primitive immune system is important in vertebrates, particularly in the early response to infection. However, innate immunity is also complemented in vertebrates by **adaptive** or **acquired immunity,** a system in which T and B lymphocytes are activated by specific antigens. Activated B lymphocytes form clones that produce secreted antibodies, which attack foreign proteins. T cells bear receptors that are related to antibody molecules, but which

remain cell-bound. When these receptors encounter their cognate antigen, the T cell is stimulated to proliferate and produce cytokines that orchestrate the immune response, including that of B cells. After the invasion is repelled, small numbers of lymphocytes persist as memory cells so that a second exposure to the same antigen provokes a prompt and magnified immune attack. The genetic event that led to acquired immunity occurred 450 million years ago and was probably insertion of a transposon into the genome in a way that made possible the generation of the immense repertoire of T cell receptors and antibodies that can be produced by the body.

In vertebrates, including humans, innate immunity provides the first line of defense against infections, but it also triggers the slower but more specific acquired immune response **(Figure 3–1)**. In vertebrates, natural and acquired immune mechanisms also attack tumors and tissue transplanted from other animals.

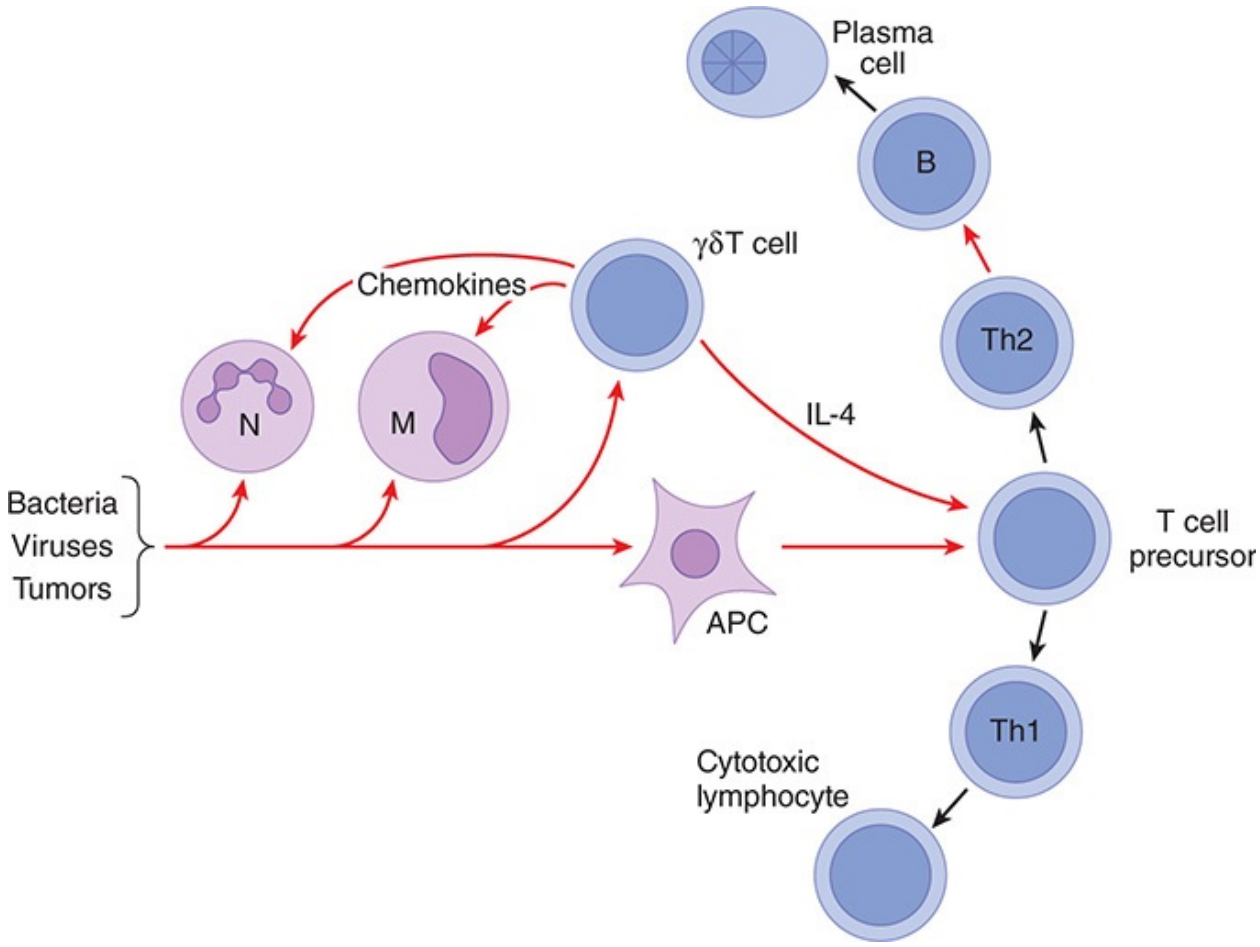


FIGURE 3–1 How bacteria, viruses, and tumors trigger innate immunity and initiate the acquired immune response. Red arrows indicate

mediators/cytokines that act on the target cell shown; black arrows show pathways of differentiation. APC, antigen-presenting cell; M, monocyte; N, neutrophil; Th1 and Th2, type 1 and type 2 helper T cells, respectively.

Once activated, immune cells communicate by means of cytokines and chemokines. They kill viruses, bacteria, and other foreign cells by secreting other cytokines and activating the complement system.

INNATE IMMUNITY

A wide variety of innate immune cells respond to molecular patterns produced by bacteria and to other substances characteristic of viruses, tumors, and transplanted cells. Many cells that are not professional immunocytes contribute to innate immune responses, such as endothelial and epithelial cells. The activated cells produce their effects via the release of cytokines, as well as, in some cases, complement and other systems.

Innate immunity in *Drosophila* centers around a receptor protein named **toll,** which binds fungal antigens and triggers activation of genes coding for antifungal proteins. An expanding list of toll-like receptors (TLRs) has now been identified in humans and other vertebrates. One of these, TLR4, binds bacterial lipopolysaccharide and a protein called CD14, and this initiates intracellular events that activate transcription of genes for a variety of proteins involved in innate immune responses. This is important because bacterial lipopolysaccharide produced by gram-negative organisms is the cause of septic shock. TLR2 mediates the response to microbial lipoproteins, TLR6 cooperates with TLR2 in recognizing certain peptidoglycans, TLR5 recognizes a molecule known as flagellin in bacterial flagellae, and TLR9 recognizes bacterial DNA. TLRs are referred to as **pattern recognition receptors (PRRs)** because they recognize and respond to the molecular patterns expressed by pathogens. Other PRRs may be intracellular, such as the so-called NOD proteins. One NOD protein, NOD2, has received attention as the product of a candidate gene leading to the intestinal inflammatory condition, Crohn disease **(Clinical Box 3–1)**.

CLINICAL BOX 3–1

Crohn Disease

Crohn disease is a chronic, relapsing, and remitting disease that involves

transmural inflammation of the intestine that can occur at any point along the length of the gastrointestinal tract but most commonly is confined to the distal small intestine and colon. Patients with this condition suffer from changes in bowel habits, bloody diarrhea, severe abdominal pain, weight loss, and malnutrition. Evidence is accumulating that the disease reflects a failure to downregulate inflammatory responses to the normal gut commensal microbiota. In genetically susceptible individuals, mutations in genes controlling innate immune responses (eg, *NOD2*) or regulators of acquired immunity appear to predispose to disease when individuals are exposed to appropriate environmental factors, which can include a change in the microbiota, or stress.

THERAPEUTIC HIGHLIGHTS

During flares of Crohn disease, the mainstay of treatment remains high-dose corticosteroids to suppress inflammation nonspecifically. Surgery is often required to treat complications such as strictures, fistulas, and abscesses. Some patients with severe disease also benefit from ongoing treatment with immunosuppressive drugs, or from treatment with antibodies targeted against tumor necrosis factor-α (TNF-α) or other inflammatory cytokines. Probiotics, therapeutic microorganisms designed to restore a “healthy” microbiota, may have some role in prophylaxis. The pathogenesis of Crohn disease, as well as the related inflammatory bowel disease, ulcerative colitis, remains the subject of intense investigation, and therapies that target specific facets of the inflammatory cascade that may be selectively implicated in individual patients with differing genetic backgrounds are under development.

ACQUIRED IMMUNITY

As noted previously, the key to acquired immunity is the ability of lymphocytes to produce antibodies (in the case of B cells) or cell-surface receptors (in the case of T cells) that are specific for one of the many millions of foreign agents that may invade the body. The antigens stimulating production of T cell receptors or antibodies are usually proteins and polypeptides, but antibodies can also be formed against nucleic acids and lipids if these are presented as nucleoproteins and lipoproteins. Antibodies to small molecules can also be produced experimentally if the molecules are bound to protein. Acquired

immunity has two components: humoral immunity and cellular immunity. **Humoral immunity** is mediated by circulating immunoglobulin antibodies in the γ-globulin fraction of the plasma proteins. Immunoglobulins are produced by differentiated forms of B lymphocytes known as **plasma cells,** and they activate the complement system and attack and neutralize antigens (Figure 3–1). Humoral immunity is a major defense against bacterial infections. **Cellular immunity** is mediated by T lymphocytes. It is responsible for delayed allergic reactions and rejection of transplants of foreign tissue. Cytotoxic T cells attack and destroy cells bearing the antigen that activated them. They kill by inserting proteins known as perforins that form pores in the membrane of target cells, and by initiating apoptosis. Cellular immunity constitutes a major defense against infections due to viruses, fungi, and a few bacteria such as the tubercle bacillus. It also helps defend against tumors.

LYMPHOCYTES

Lymphocytes are the key elements in the production of acquired immunity. After birth, some lymphocytes are formed in the bone marrow. However, most are formed in the lymph nodes **(Figure 3–2)**, thymus, and spleen from precursor cells that originally came from the bone marrow and were processed in the thymus (T cells) or bone marrow (B cells, see below). Lymphocytes enter the bloodstream for the most part via the lymphatics. At any given time, only about 2% of the body lymphocytes are in the peripheral blood. Most of the rest are in the lymphoid organs.

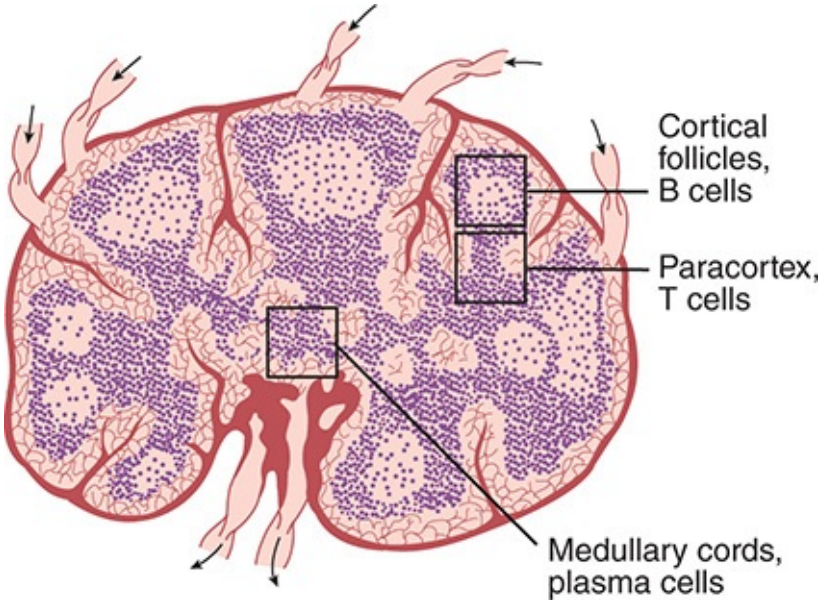


FIGURE 3–2 Anatomy of a normal lymph node. (Reproduced with permission from Chandrasoma. Reproduced with permission from McPhee SJ, Lingappa VR, Ganong WF [editors]: *Pathophysiology of Disease*, 4th ed. New York, NY: McGraw-Hill; 2003.)

During fetal development, and to a much lesser extent during adult life, lymphocyte precursors come from the bone marrow. Those that populate the thymus **(Figure 3–3)** become transformed by the environment in this organ into T lymphocytes. Another lymphoid subset that forms in the thymus is the NKT cell, so-called because it shares features of both T lymphocytes and **natural killer** (NK) cells. Transformation to B lymphocytes, on the other hand, occurs in the fetal liver and, after birth, the bone marrow. NK cells also form in these sites. After residence in the thymus, liver, or bone marrow, many of the T and B lymphocytes migrate to the lymph nodes.

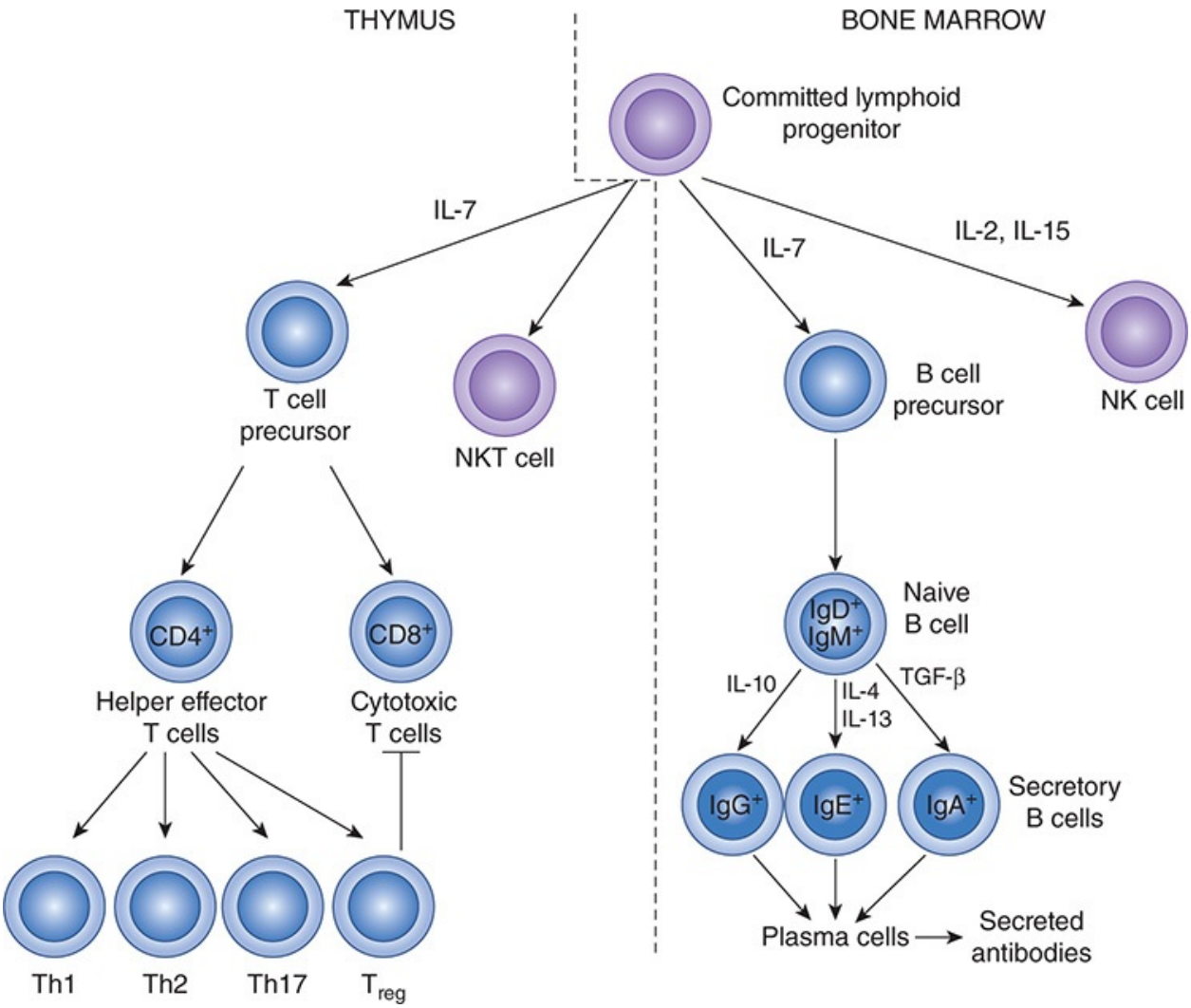


FIGURE 3–3 Development of the system mediating acquired immunity. Committed lymphoid progenitors arise in the bone marrow. Maturation into the B and NK cell lineages occurs in this site, whereas development into T and NKT cells takes place after lymphoid progenitors migrate to the thymus. NK, natural killer.

T and B lymphocytes are morphologically indistinguishable but can be identified by markers on their cell membranes. B cells differentiate sequentially into cells capable of producing the various classes of immunoglobulins and thereafter into **plasma cells.** There are two major types of T cells: **cytotoxic T cells** and **helper/effector T cells.** There are at least four subtypes of helper T cells: T helper 1 (Th1) cells secrete interleukin (IL)-2 and γ-interferon and are concerned primarily with stimulating cellular immunity; Th2 cells secrete IL-4 and IL-5 and interact primarily with B cells in relation to humoral immunity. Th17 cells are induced in response to bacterial infections, produce IL-6 and IL-17, and help recruit neutrophils. They are also implicated in the generation of harmful inflammatory responses that occur in autoimmune diseases. Finally, T_{reg} cells produce IL-10 to dampen T cell–driven responses. Cytotoxic T cells destroy transplanted cells and those expressing foreign antigens (eg, virally infected targets), with their development aided and directed by helper T cells.

Markers on the surface of lymphocytes are assigned CD (clusters of differentiation) numbers on the basis of their reactions to a panel of monoclonal antibodies. Most cytotoxic T cells display the glycoprotein CD8, and helper T cells display the glycoprotein CD4. These proteins are closely associated with the T cell receptors and may function as coreceptors. On the basis of differences in their receptors and functions, cytotoxic T cells are divided into αβ and γδ types (see below). NK and NKT cells (see above) are also cytotoxic lymphocytes. Thus, there are four main types of cytotoxic lymphocytes in the body: αβ T cells, γδ T cells, NK cells, and NKT cells.

MEMORY B CELLS & T CELLS

After exposure to a given antigen, a small number of activated B and T cells persist as memory B and T cells. These cells are readily converted to effector cells by a later encounter with the same antigen. This ability to produce an accelerated response to a second exposure to an antigen is a key characteristic of acquired immunity. The ability persists for long periods of time, and in some instances (eg, immunity to measles) it can be life-long.

After activation in lymph nodes, lymphocytes disperse widely throughout the body and are especially plentiful in areas where invading organisms enter the body (eg, the mucosa of the respiratory and gastrointestinal tracts). This puts memory cells close to sites of reinfection and may account in part for the rapidity and strength of their response. Chemotactic cytokines known as **chemokines** (see below) are involved in guiding activated lymphocytes to these locations.

ANTIGEN RECOGNITION

The number of different antigens recognized by lymphocytes in the body is extremely large. The repertoire develops initially without exposure to the antigen. Stem cells differentiate into many millions of different T and B lymphocytes, each with the ability to respond to a particular antigen. When the antigen first enters the body, it can bind directly to the appropriate receptors on B cells. However, a full antibody response requires that the B cells contact helper T cells. In the case of T cells, the antigen is taken up by an antigen-presenting cell (APC) and partially digested. A peptide fragment of it is presented to the appropriate receptors on T cells. In either case, the cells are stimulated to divide, forming **clones** of cells that respond to this antigen **(clonal selection).** Effector cells are also subject to **negative selection,** during which lymphocyte precursors that are reactive with self-antigens are normally deleted. This results in immune **tolerance.** It is this latter process that presumably goes awry in autoimmune diseases, where the body reacts to and destroys cells expressing normal proteins, with accompanying inflammation that may lead to tissue destruction.

ANTIGEN PRESENTATION

APCs include specialized cells called **dendritic cells** in the lymph nodes and spleen and the Langerhans dendritic cells in the skin. Macrophages and B cells themselves, and likely many other cell types, can also function as APCs. For example, in the intestine, the epithelial cells that line the tract are likely important in presenting antigens derived from commensal bacteria. In APCs, polypeptide products of antigen digestion are coupled to the HLA protein products of the **major histocompatibility complex (MHC)** genes and presented on the surface of the cell.

The genes of the MHC encode glycoproteins that are divided into two classes

on the basis of structure and function. Class I antigens are composed of a 45-kDa heavy chain associated noncovalently with β_2-microglobulin encoded by a gene outside the MHC **(Figure 3–4)**. They are found on all nucleated cells. Class II antigens are heterodimers made up of a 29- to 34-kDa α chain associated noncovalently with a 25- to 28-kDa β chain. They are present in "professional" APCs, including B cells, and in activated T cells.

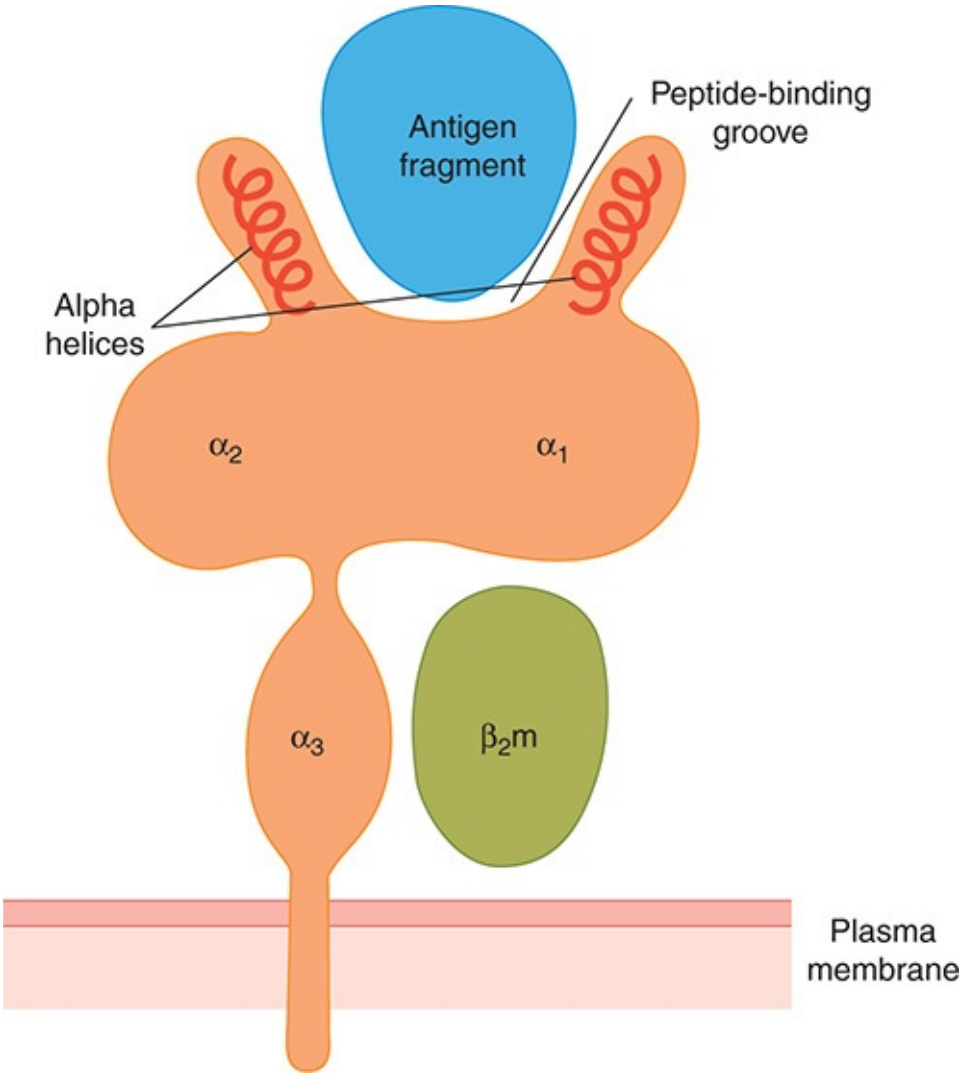


FIGURE 3–4 Schematic structure of a class I MHC protein bound to an antigen fragment, based on the structure of the human histocompatibility antigen HLA-A2. The antigen-binding pocket is at the top and is formed by the α_1 and α_2 parts of the molecule. The α_3 portion and the associated β_2-microglobulin (β_2m) are close to the membrane. MHC, major histocompatibility complex. (Reproduced with permission from Bjorkman PJ, et al: Structure of the

human histocompatibility antigen HLA-A2, Nature 1987;October 8-14;329(6139):506-512.)

The class I MHC proteins (MHC-I proteins) are coupled primarily to peptide fragments generated from proteins synthesized within cells. Peptides to which the host is not tolerant (eg, those from mutant or viral proteins) are recognized by T cells. The digestion of these proteins occurs in complexes of proteolytic enzymes known as **proteasomes,** and the peptide fragments bind to MHC proteins in the endoplasmic reticulum. The class II MHC proteins (MHC-II proteins) interact primarily with peptide products of extracellular antigens, such as bacteria, that enter the cell by endocytosis and are digested in the late endosomes.

T CELL RECEPTORS

The MHC protein–peptide complexes on the surface of the APCs bind to appropriate T cells. Therefore, receptors on the T cells must recognize a very wide variety of complexes. Most of the receptors on circulating T cells are made up of two polypeptide units designated α and β. They form heterodimers that recognize the MHC proteins and the antigen fragments with which they are combined **(Figure 3–5)**. These cells are called αβ T cells. On the other hand, about 10% of circulating T cells have two different polypeptides designated γ and δ in their receptors, and they are called γδ T cells. These T cells are prominent in the mucosa of the gastrointestinal tract, and there is evidence that they form a link between the innate and acquired immune systems by way of the cytokines they secrete (Figure 3–3).

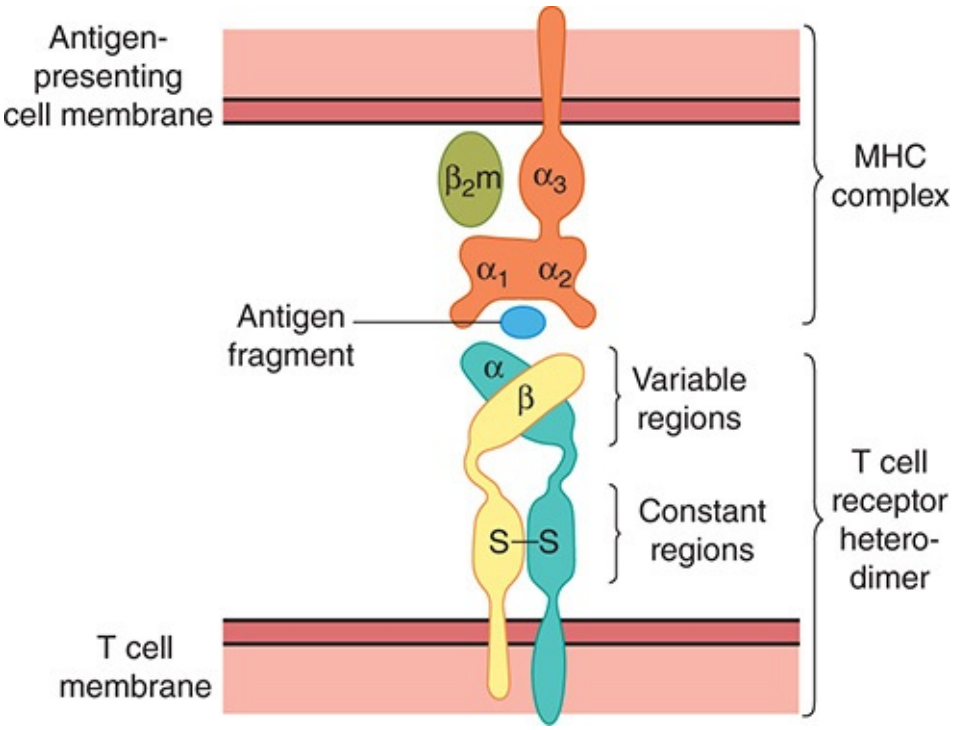


FIGURE 3–5 Interaction between antigen-presenting cell (top) and αβ T lymphocyte (bottom). The MHC proteins (in this case, MHC-I) and their peptide antigen fragment bind to the α and β units that combine to form the T cell receptor. Some experts refer to this set of protein–protein interactions as the "immune synapse." MHC, major histocompatibility complex.

CD8 occurs on the surface of cytotoxic T cells that bind MHC-I proteins, and CD4 occurs on the surface of helper T cells that bind MHC-II proteins **(Figure 3–6)**. The CD8 and CD4 proteins facilitate the binding of the MHC proteins to the T cell receptors, and they also foster lymphocyte development. The activated CD8 cytotoxic T cells kill their targets directly, whereas the activated CD4 helper T cells secrete cytokines that activate other lymphocytes.

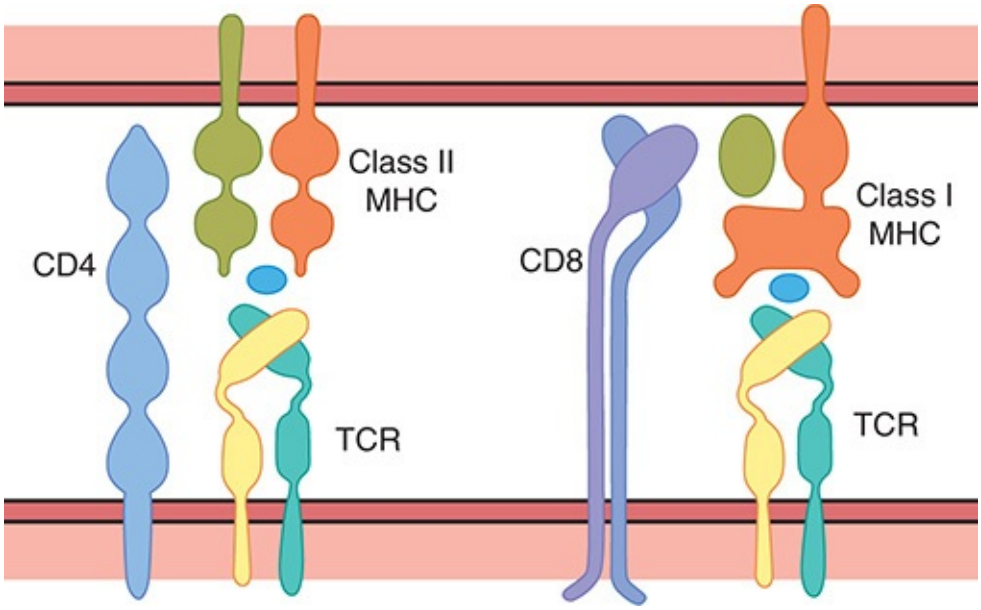


FIGURE 3–6 Diagrammatic summary of the structure of CD4 and CD8, and their relation to MHC-I and MHC-II proteins. Note that CD4 is a single protein, whereas CD8 is a heterodimer. MHC, major histocompatibility complex.

The T cell receptors are surrounded by adhesion molecules and proteins that bind to complementary proteins in the APC when the two cells transiently join to form the "immunologic synapse" that permits T cell activation to occur **(Figure 3–7)**. It is now generally accepted that two signals are necessary to produce activation. One is produced by the binding of the digested antigen to the T cell receptor. The other is produced by the joining of the surrounding proteins in the "synapse." If the first signal occurs but the second does not, the T cell is inactivated and becomes unresponsive.

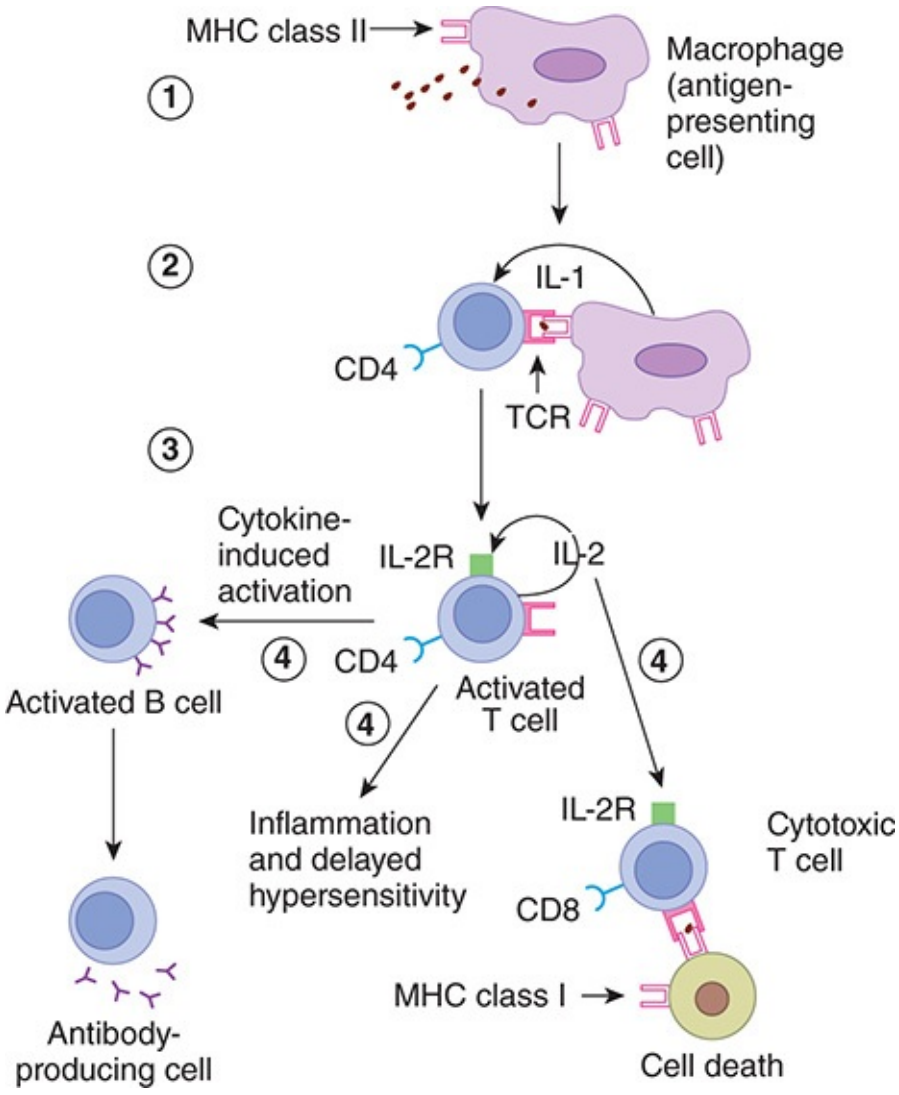


FIGURE 3–7 Summary of acquired immunity. (1) An antigen-presenting cell (depicted here as a macrophage, but other cell types, including dendritic cells, are important) ingests and partially digests an antigen, then presents part of the antigen along with MHC peptides (in this case, MHC II peptides on the cell surface). **(2)** An "immune synapse" forms with a naive CD4 T cell, which is activated to produce IL-2. **(3)** IL-2 acts in an autocrine fashion to cause the cell to multiply, forming a clone. **(4)** The activated CD4 cell may promote B cell activation and the proliferation of plasma cells that produce antibodies or it may activate a cytotoxic CD8 cell. The CD8 cell can also be activated by forming a synapse with an MCH I antigen-presenting cell. MHC, major histocompatibility complex. (Reproduced with permission from McPhee SJ, Lingappa VR, Ganong WF [editors]: *Pathophysiology of Disease*, 6th ed. New York, NY: McGraw-Hill; 2010.)

B CELLS

As noted above, B cells can bind antigens directly, but they must contact helper T cells to produce full activation and antibody formation. It is the Th2 subtype that is mainly involved. Helper T cells develop along the T_h2 lineage in response to IL-4 (see below). On the other hand, IL-12 promotes the Th1 phenotype. IL-2 acts in an autocrine fashion to cause activated T cells to proliferate. The role of various cytokines in B cell and T cell activation is summarized in Figure 3–7.

The activated B cells proliferate and transform into **memory B cells** (see above) and **plasma cells.** The plasma cells secrete large quantities of antibodies into the general circulation. The antibodies circulate in the globulin fraction of the plasma and, like antibodies elsewhere, are called **immunoglobulins.** The immunoglobulins are actually the secreted form of antigen-binding receptors on the B cell membrane.

IMMUNOGLOBULINS

Circulating antibodies protect their host by binding to and neutralizing some protein toxins, by blocking the attachment of some viruses and bacteria to cells, by opsonizing bacteria (see below), and by activating complement (see below). Five general types of immunoglobulin antibodies are produced by plasma cells. The basic component of each is a symmetric unit containing four polypeptide chains **(Figure 3–8)**. The two long chains are called **heavy chains,** whereas the two short chains are called **light chains.** There are two types of light chains, k and λ, and nine types of heavy chains. The chains are joined by disulfide bridges that permit mobility, and there are intrachain disulfide bridges as well. In addition, the heavy chains are flexible in a region called the hinge. Each heavy chain has a variable (V) segment in which the amino acid sequence is highly variable, a diversity (D) segment in which the amino acid segment is also highly variable, a joining (J) segment in which the sequence is moderately variable, and a constant (C) segment in which the sequence is constant. Each light chain has a V, J, and C segment. The V segments form part of the antigen-binding sites (Fab portion of the molecule [Figure 3–8]). The Fc portion of the molecule is the effector portion, which mediates the reactions initiated by antibodies.

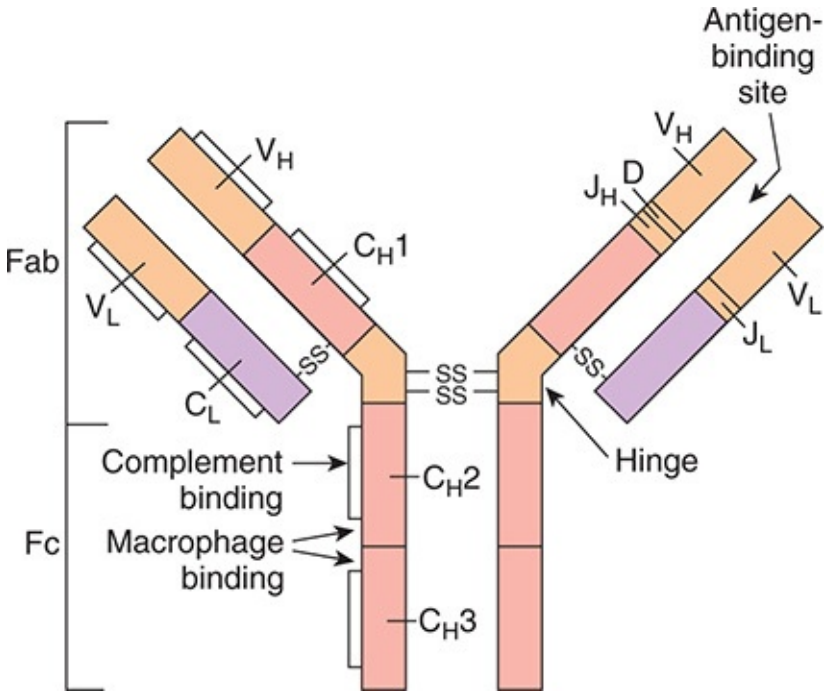


FIGURE 3–8 Typical immunoglobulin G molecule. Fab, portion of the molecule that is concerned with antigen binding; Fc, effector portion of the molecule. The constant regions are pink and purple, and the variable regions are orange. The constant segment of the heavy chain is subdivided into CH1, CH2, and CH3. SS lines indicate intersegmental disulfide bonds. On the right side, the C labels are omitted to show regions J_H, D, and J_L.

Two of the classes of immunoglobulins contain additional polypeptide components **(Table 3–1)**. In IgM, five of the basic immunoglobulin units join around a polypeptide called the J chain to form a pentamer. In IgA, the **secretory immunoglobulin,** the immunoglobulin units form dimers and trimers around a J chain and a polypeptide that comes from epithelial cells, the secretory component (SC).

TABLE 3–1 Human immunoglobulins.[a]

Immunoglobulin	Function	Heavy Chain	Additional Chain	Structure	Plasma Concentration (mg/dL)
IgG	Complement activation	$\gamma_1, \gamma_2, \gamma_3, \gamma_4$		Monomer	1000
IgA	Localized protection in external secretions (tears, intestinal secretions, etc.)	α_1, α_2	J, SC	Monomer; dimer with J or SC chain; trimer with J chain	200
IgM	Complement activation	μ	J	Pentamer with J chain	120
IgD	Antigen recognition by B cells	δ		Monomer	3
IgE	Reagin activity; releases histamine from basophils and mast cells	ε		Monomer	0.05

[a]In all instances, the light chains are k or γ.

In the intestine, bacterial and viral antigens are taken up by M cells (see Chapter 26) and passed on to underlying aggregates of lymphoid tissue **(Peyer patches),** where they activate naive T cells. These lymphocytes then form B cells that infiltrate mucosa of the gastrointestinal, respiratory, genitourinary, and female reproductive tracts and the breast. There they secrete large amounts of IgA when exposed again to the antigen that initially stimulated them. The epithelial cells produce the SC, which acts as a receptor for, and binds to, IgA. The resulting secretory immunoglobulin passes through the epithelial cell and is secreted by exocytosis. This system of **secretory immunity** is an important and effective defense mechanism at all mucosal surfaces. Because IgA is secreted into the breast milk, it also accounts for the immune protection that is conferred by the breastfeeding of infants whose immune systems are otherwise immature.

CLINICAL BOX 3–2

Autoimmunity

Sometimes, the processes that eliminate antibodies against self-antigens fail and a variety of different **autoimmune diseases** are produced. These can be B cell– or T cell– mediated and can be organ-specific or systemic. They include type 1 diabetes mellitus (antibodies against pancreatic islet B cells), myasthenia gravis (antibodies against nicotinic cholinergic receptors), and multiple sclerosis (antibodies against myelin basic protein and several other components of myelin). In some instances, the antibodies are against receptors and are capable of activating those receptors; for example, antibodies against TSH receptors increase thyroid activity and cause Graves disease (see Chapter 20). Other conditions are due to the production of

antibodies against invading organisms that cross-react with normal body constituents **(molecular mimicry)**. An example is rheumatic fever following a streptococcal infection; a portion of cardiac myosin resembles a portion of the streptococcal M protein, and antibodies induced by the latter attack the former and damage the heart. Some conditions may be due to **bystander effects,** in which inflammation sensitizes T cells in the neighborhood, causing them to become activated when otherwise they would not respond.

THERAPEUTIC HIGHLIGHTS

The therapy of autoimmune disorders rests on efforts to replace or restore the damaged function (eg, provision of exogenous insulin in type 1 diabetes) as well as nonspecific efforts to reduce inflammation (using corticosteroids) or to suppress immunity. Recently, agents that deplete or dampen the function of B cells have been shown to have some efficacy in a range of autoimmune disorders, including rheumatoid arthritis, most likely by interrupting the production of autoantibodies that contribute to disease pathogenesis.

GENETIC BASIS OF DIVERSITY IN THE IMMUNE SYSTEM

The genetic mechanism for the production of the immensely large number of different configurations of immunoglobulins produced by human B cells, as well as T cell receptors, is a fascinating biologic problem. Diversity is brought about in part by the fact that in immunoglobulin molecules there are two kinds of light chains and nine kinds of heavy chains. As noted previously, there are areas of great variability **(hypervariable regions)** in each chain. The variable portion of the heavy chains consists of the V, D, and J segments. In the gene family responsible for this region, there are several hundred different coding regions for the V segment, about 20 for the D segment, and four for the J segment. During B cell development, one V coding region, one D coding region, and one J coding region are selected at random and recombined to form the gene that produces that particular variable portion. A similar variable recombination takes place in the coding regions responsible for the two variable segments (V and J) in the light chain. In addition, the J segments are variable because the gene segments join in an imprecise and variable fashion (junctional site diversity) and nucleotides are sometimes added (junctional insertion diversity). It has been

calculated that these mechanisms permit the production of about 10^{15} different immunoglobulin molecules. Additional variability is added by somatic mutation.

Similar gene rearrangement and joining mechanisms operate to produce the diversity in T cell receptors. In humans, the α subunit has a V region encoded by 1 of about 50 different genes and a J region encoded by 1 of another 50 different genes. The β subunits have a V region encoded by 1 of about 50 genes, a D region encoded by 1 of 2 genes, and a J region encoded by 1 of 13 genes. These variable regions permit the generation of up to an estimated 10^{15} different T cell receptors (**Clinical Box 3–2** and **Clinical Box 3–3**).

More than 300 primary immunodeficiency states are now known to arise from defects in these various stages of B and T lymphocyte maturation **(Clinical Box 3–4)**. A few important ones are shown in **Figure 3–9**.

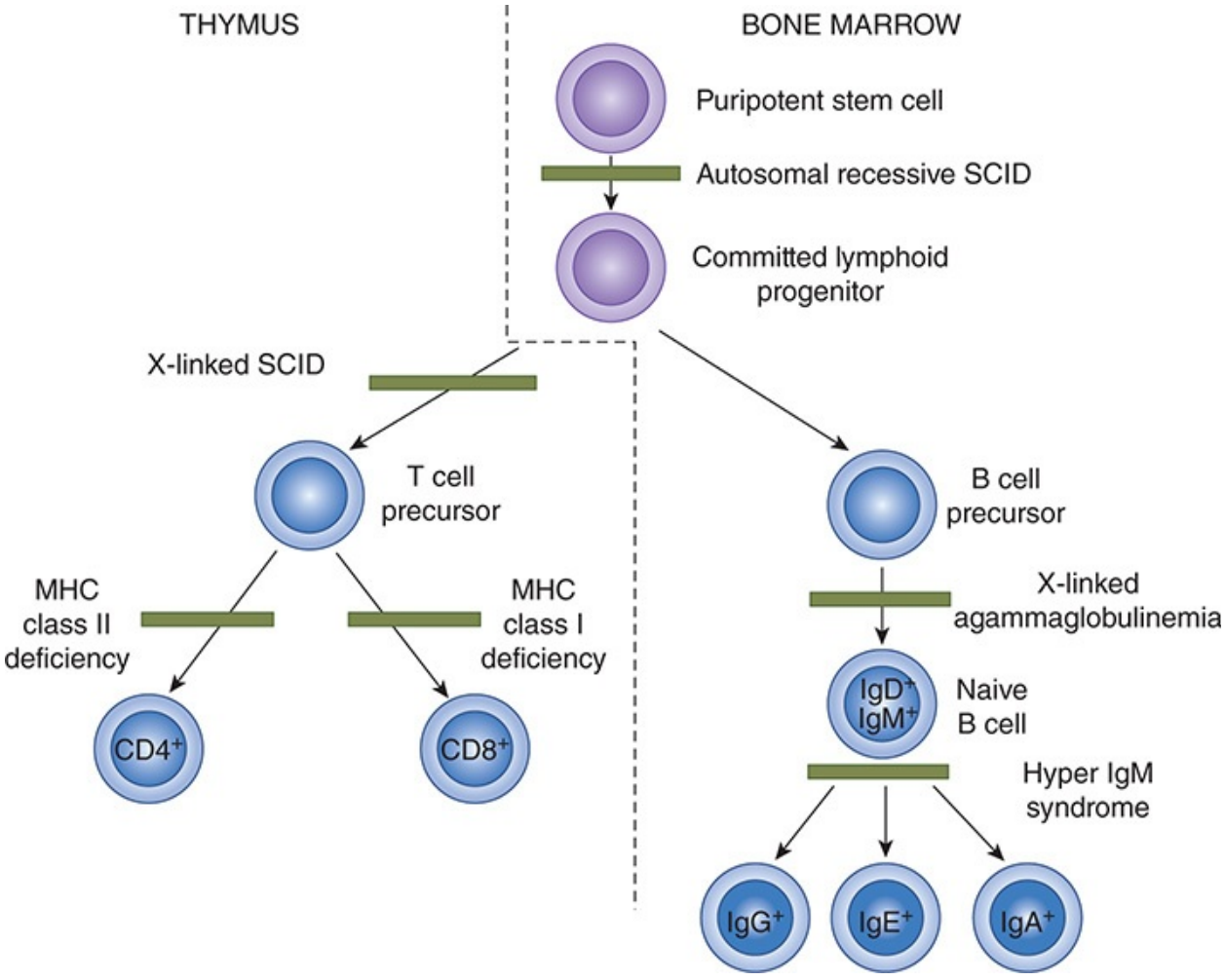


FIGURE 3–9 Sites of congenital blockade of B and T lymphocyte maturation in various primary immunodeficiency states. SCID, severe

combined immune deficiency.

SOLUBLE REGULATORS OF IMMUNITY

CYTOKINES

Cytokines are hormone-like molecules that act—generally in a paracrine fashion—to regulate immune responses. They are secreted not only by lymphocytes and macrophages but also by endothelial cells, neurons, glial cells, and other types of cells. Most of the cytokines were initially named for their actions, for example, B cell–differentiating factor, or B cell–stimulating factor 2. However, the nomenclature has since been rationalized by international agreement to that of the **interleukins.** For example, the name of B cell–differentiating factor was changed to interleukin-4. A number of cytokines selected for their biologic and clinical relevance are listed in Table 3–2, but it would be beyond the scope of this text to list all cytokines, which now number more than 100.

TABLE 3–2 Examples of cytokines and their clinical relevance.

Cytokine	Cellular Sources	Major Activities	Clinical Relevance
IL-1	Macrophages	Activation of T cells and macrophages; promotion of inflammation	Implicated in the pathogenesis of septic shock, rheumatoid arthritis, and atherosclerosis
IL-2	Type 1 (Th1) helper T cells	Activation of lymphocytes, natural killer cells, and macrophages	Used to induce lymphokine-activated killer cells; used in the treatment of metastatic renal cell carcinoma, melanoma, and various other tumors
IL-4	Type 2 (Th2) helper T cells, mast cells, basophils, and eosinophils	Activation of lymphocytes, monocytes, and IgE class switching	As a result of its ability to stimulate IgE production, plays a part in mast-cell sensitization and thus in allergy and in defense against nematode infections
IL-5	Type 2 (Th2) helper T cells, mast cells, and eosinophils	Differentiation of eosinophils	Monoclonal antibody against interleukin-5 used to inhibit the antigen-induced late-phase eosinophilia in animal models of allergy
IL-6	Type 2 (Th2) helper T cells and macrophages	Activation of lymphocytes; differentiation of B cells; stimulation of the production of acute-phase proteins	Overproduced in Castleman disease; acts as an autocrine growth factor in myeloma and in mesangial proliferative glomerulonephritis
IL-8	T cells and macrophages	Chemotaxis of neutrophils, basophils, and T cells	Levels are increased in diseases accompanied by neutrophilia, making it a potentially useful marker of disease activity
IL-11	Bone marrow stromal cells	Stimulation of the production of acute-phase proteins	Used to reduce chemotherapy-induced thrombocytopenia
IL-12	Macrophages and B cells	Stimulation of the production of interferon γ by type 1 (Th1) helper T cells and by natural killer cells; induction of type 1 (Th1) helper T cells	May be useful as an adjuvant for vaccines
IL-17	T cells	Promotion of inflammatory cell chemotaxis and inflammation	Implicated in many immune/autoimmune diseases such as rheumatoid arthritis, asthma, and psoriasis
Tumor necrosis factor-α	Macrophages, natural killer cells, T cells, B cells, and mast cells	Promotion of inflammation	Treatment with antibodies against tumor necrosis factor-α beneficial in rheumatoid arthritis and Crohn disease
Lymphotoxin (tumor necrosis factor-β)	Type 1 (Th1) helper T cells and B cells	Promotion of inflammation	Implicated in the pathogenesis of multiple sclerosis and insulin-dependent diabetes mellitus
Transforming growth factor-β	T cells, macrophages, B cells, and mast cells	Immunosuppression	May be useful therapeutic agent in multiple sclerosis and myasthenia gravis
GM-CSF	T cells, macrophages, natural killer cells, and B cells	Promotion of the growth of granulocytes and monocytes	Used to reduce neutropenia after chemotherapy for tumors and in ganciclovir-treated patients with AIDS; used to stimulate cell production after hematopoietic stem cell transplantation
Interferon-α	Virally infected cells	Induction of resistance of cells to viral infection	Used to treat AIDS-related Kaposi sarcoma, melanoma, chronic hepatitis B infection, and chronic hepatitis C infection
Interferon-β	Virally infected cells	Induction of resistance of cells to viral infection	Used to reduce the frequency and severity of relapses in multiple sclerosis
Interferon-γ	Type 1 (Th1) helper T cells and natural killer cells	Activation of macrophages; inhibition of type 2 (Th2) helper T cells	Used to enhance the killing of phagocytosed bacteria in chronic granulomatous disease

Modified with permission from Delves PJ, Roitt IM: The immune system. First of two parts, N Engl J Med 2000; July 6:343(1):37-49.

CLINICAL BOX 3–3

Tissue Transplantation

The T lymphocyte system is responsible for the rejection of transplanted tissue. When tissues such as skin and kidneys are transplanted from a donor to a recipient of the same species, the transplants "take" and function for a while but then become necrotic and are "rejected" because an immune response to the transplanted tissue develops in the recipient. This is generally true even if the donor and recipient are close relatives, and the only transplants that are never rejected are those from an identical twin. Nevertheless, organ transplantation remains the only viable option in a number of end-stage diseases.

THERAPEUTIC HIGHLIGHTS

A number of treatments have been developed to overcome the rejection of transplanted organs in humans. The goal of treatment is to stop rejection without leaving the patient vulnerable to massive infections. One approach is to kill T lymphocytes by killing all rapidly dividing cells with drugs such as azathioprine, a purine antimetabolite, but this makes patients susceptible to infections and cancer. Another is to administer corticosteroids, which inhibit cytotoxic T cell proliferation by inhibiting production of IL-2, but these cause osteoporosis, mental changes, and the other facets of Cushing syndrome (see Chapter 19). More recently, immunosuppressive drugs such as **cyclosporine** or **tacrolimus (FK-506)** have found favor. Activation of the T cell receptor normally increases intracellular Ca^{2+}, which acts via calmodulin to activate calcineurin. Calcineurin dephosphorylates the transcription factor NF-AT, which moves to the nucleus and increases the activity of genes coding for IL-2 and related stimulatory cytokines. Cyclosporine and tacrolimus prevent the dephosphorylation of NF-AT. However, these drugs inhibit all T cell–mediated immune responses, and cyclosporine causes kidney damage and cancer. A new and promising approach to transplant rejection is the production of T cell unresponsiveness by using drugs that block the costimulation that is required for normal activation (see text). Clinically effective drugs that act in this fashion could be of great value to transplant surgeons.

CLINICAL BOX 3–4

Primary Immunodeficiencies

Mutations in enzymes, transcription factors, receptors, and other regulators of acquired or innate immunity lead to primary immunodeficiency disorders. Typically, patients experience frequent infections or have an increased susceptibility to unusual infections, but they may also be subject to autoimmune problems, spontaneous inflammation, and malignancy. The implications depend on the level at which the immune system is disrupted, with mutations that prevent the development of many lymphoid lineages having the most serious consequences (Figure 3–9).

THERAPEUTIC HIGHLIGHTS

Mild immunodeficiencies may require only minimal or supportive therapies, but the most serious cause substantial morbidity and early mortality. Patients suffering from the latter conditions, therefore, are candidates for early and definitive therapy, and many have benefitted greatly from the transplant of allogeneic hematopoietic stem cells (HSCs), provided they can tolerate the procedure and a suitable donor can be found. On the other hand, the monogenic nature of many serious primary immunodeficiencies has made them an attractive target for trials of gene therapy approaches. There have been a number of notable successes with transplant of HSCs transduced with retroviral or lentiviral vectors carrying a wild-type copy of the affected gene, such as in X-linked severe combined immune deficiency (SCID), which results in mutations in the gamma chain of the receptor for IL-2.

Many of the receptors for cytokines and hematopoietic growth factors (see above), as well as the receptors for prolactin (see Chapter 22), and growth hormone (see Chapter 18) are members of a cytokine-receptor superfamily that has three subfamilies **(Figure 3–10)**. The members of subfamily 1, which includes the receptors for IL-4 and IL-7, are homodimers. The members of subfamily 2, which includes the receptors for IL-3, IL-5, and IL-6, are heterodimers. The receptor for IL-2 (and several other cytokines) consists of a heterodimer plus an unrelated protein, the so-called Tac antigen. The other members of subfamily 3 have the same γ chain as the IL-2 receptor. The extracellular domain of the homodimer and heterodimer subunits all contain four conserved cysteine residues plus a conserved Trp-Ser-X-Trp-Ser domain, and although the intracellular portions do not contain tyrosine kinase catalytic

domains, they activate cytoplasmic tyrosine kinases when ligand binds to the receptors.

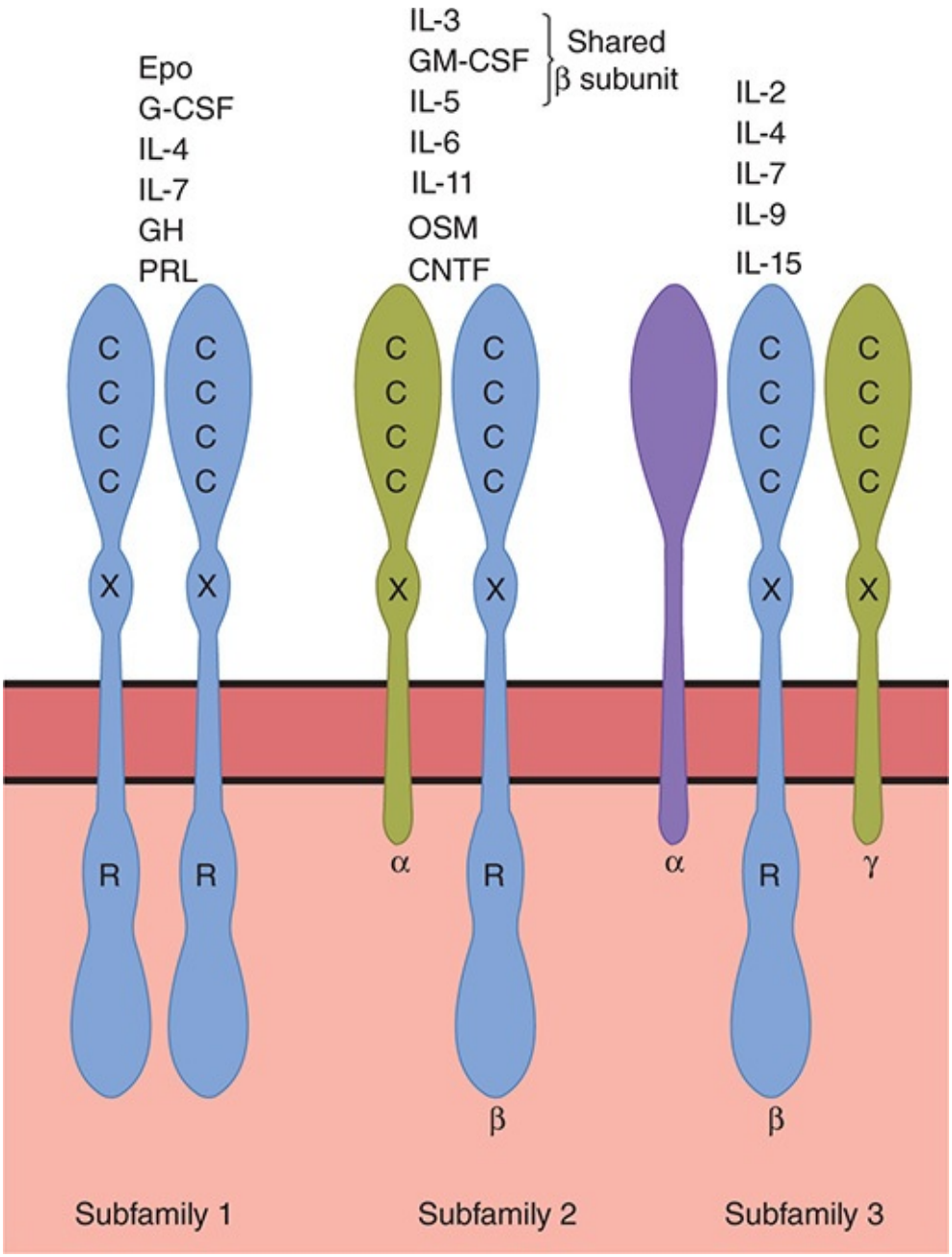


FIGURE 3–10 Members of an important cytokine receptor superfamily, showing shared structural elements. Note that all subunits except the α subunit in subfamily 3 have four conserved cysteine residues (C) and a Trp-Ser-X-Trp-Ser motif (X). Many subunits also contain a critical regulatory domain in their cytoplasmic portions (R). CNTF, ciliary neurotrophic factor; Epo, erythropoietin; GH, growth hormone; OSM, oncostatin M; PRL, prolactin.

The effects of the principal cytokines are listed in Table 3–2. Some of them have systemic as well as local paracrine effects. For example, IL-1, IL-6, and TNF-α cause fever, and IL-1 increases slow-wave sleep and reduces appetite.

Another superfamily of cytokines is the **chemokine** family. Chemokines are substances that attract neutrophils and other immune effector cells to areas of inflammation or an immune response. Over 40 have now been identified, and it is clear that they also play a role in the regulation of cell growth and angiogenesis. The chemokine receptors are G-protein-coupled receptors that cause, among other things, extension of pseudopodia with migration of the cell toward the source of the chemokine.

HEMATOPOIETIC GROWTH FACTORS

The production of immune effector cells is regulated with great precision in healthy individuals, and the production of granulocytes, in particular, is rapidly and dramatically increased in infections. Many cytokines participate in this regulation, together with other soluble mediators, many of which are referred to as colony-stimulating factors (CSF) because they stimulate progenitors to grow as colonies in soft agar. The overall proliferation and self-renewal of HSCs depends on **stem cell factor (SCF).** The proliferation and maturation of the cells that enter the blood from the marrow are then regulated by growth factors that cause cells in one or more of the committed cell lines to proliferate and mature **(Table 3–3)**. The factors stimulating the production of committed stem cells include granulocyte/macrophage **(GM)-CSF, granulocyte CSF (G-CSF),** and **macrophage CSF (M-CSF).** Interleukins **IL-1** and **IL-6** followed by **IL-3** (Table 3–3) act in sequence to convert pluripotential uncommitted stem cells to committed progenitor cells. IL-3 is also known as **multi-CSF.** Each of the CSFs has a predominant action, but all the CSFs and interleukins also have other overlapping actions. In addition, they activate and sustain mature blood cells. In fact, it is interesting that basal hematopoiesis is normal in mice in which the GM-CSF gene is knocked out, indicating that loss of one factor can be compensated for by others. On the other hand, the absence of GM-CSF causes accumulation of surfactant in the lungs (see Chapter 34).

TABLE 3–3 Hematopoietic growth factors.

Cytokine	Cell Types Stimulated	Source
IL-1	Erythrocyte Granulocyte Megakaryocyte Monocyte	Multiple cell types
IL-3	Erythrocyte Granulocyte Megakaryocyte Monocyte	T lymphocytes
IL-4	Basophil	T lymphocytes
IL-5	Eosinophil	T lymphocytes
IL-6	Erythrocyte Granulocyte Megakaryocyte Monocyte	Endothelial cells, fibroblasts, macrophages
IL-11	Erythrocyte Granulocyte Megakaryocyte	Fibroblasts, osteoblasts
Erythropoietin	Erythrocyte	Kidney Kupffer cells of liver
SCF	Erythrocyte Granulocyte Megakaryocyte Monocyte	Multiple cell types
G-CSF	Granulocyte	Endothelial cells, fibroblasts, monocytes
GM-CSF	Erythrocyte Granulocyte Megakaryocyte	Endothelial cells, fibroblasts, monocytes, T lymphocytes
M-CSF	Monocyte	Endothelial cells, fibroblasts, monocytes,
Thrombopoietin	Megakaryocyte	Liver, kidney

CSF, colony-stimulating factor; G, granulocyte; IL, interleukin; M, macrophage; SCF, stem cell factor.

Reproduced with permission from McPhee SJ, Lingappa VR, Ganong WF (editors): *Pathophysiology of Disease*, 6th ed. New York, NY: McGraw-Hill; 2010.

The other factors listed are produced by macrophages, activated T cells, fibroblasts, and endothelial cells. For the most part, the factors act locally in the bone marrow.

THE COMPLEMENT SYSTEM

The cell-killing effects of innate and acquired immunity are mediated in part by a system of more than 30 plasma proteins originally named the **complement system** because they "complemented" the effects of antibodies. Three different pathways or enzyme cascades activate the system: the **classic pathway,** triggered by immune complexes; the **mannose-binding lectin pathway,** triggered when this lectin binds mannose groups in bacteria; and the **alternative** or **properdin pathway,** triggered by contact with various viruses, bacteria, fungi, and tumor cells. The proteins that are produced have three functions: they help kill invading organisms by opsonization (coating of the organism), chemotaxis, and eventual lysis of the cell; they serve in part as a bridge from innate to acquired immunity by activating B cells and aiding immune memory; and they help dispose of waste products after apoptosis. Cell lysis, one of the principal ways the complement system kills cells, is brought about by inserting proteins called **perforins** into their cell membranes. These create holes, which permit free flow of ions and thus disruption of membrane polarity.

OTHER IMMUNE/INFLAMMATORY EFFECTOR CELLS

Many additional immune effector cells circulate in the blood as the white blood cells. In addition, the blood is the conduit for the precursor cells that eventually develop into the immune cells of the tissues. The circulating immunologic cells include **granulocytes (polymorphonuclear leukocytes, PMNs),** comprising **neutrophils, eosinophils,** and **basophils;** lymphocytes (discussed above); and **monocytes.** Immune responses in the tissues are further amplified by these cells following their extravascular migration, as well as tissue **macrophages** (derived from monocytes) and **mast cells** (related to basophils). Acting together, these cells provide the body with powerful defenses against tumors and viral, bacterial, and parasitic infections.

GRANULOCYTES

All granulocytes have cytoplasmic granules that contain biologically active substances involved in inflammatory and allergic reactions.

The average half-life of a neutrophil in the circulation is 6 h. To maintain the normal circulating blood level, it is necessary to produce over 100 billion neutrophils per day. Many neutrophils enter the tissues, particularly if triggered to do so by an infection or by inflammatory cytokines. They are attracted to the endothelial surface by cell adhesion molecules known as selectins, and they roll along it. They then bind firmly to neutrophil adhesion molecules of the integrin family. They next insinuate themselves through the walls of the capillaries between endothelial cells by a process called **diapedesis.** Many of those that leave the circulation enter the gastrointestinal tract and are eventually lost from the body.

Invasion of the body by bacteria triggers the **inflammatory response.** The bone marrow is stimulated to produce and release large numbers of neutrophils. Bacterial products interact with plasma factors and cells to produce chemokines and other agents that attract neutrophils to the infected area **(chemotaxis).** The chemotactic agents include a component of the complement system (C5a); leukotrienes; and polypeptides from lymphocytes, mast cells, and basophils. Other plasma factors act on the bacteria to make them “tasty” to the phagocytes **(opsonization).** The principal opsonins that coat the bacteria are IgG immunoglobulins and complement proteins. The coated bacteria then bind to G-protein-coupled receptors on the neutrophil cell membrane. This triggers increased motor activity of the cell, exocytosis, and the so-called respiratory burst. The increased motor activity leads to prompt ingestion of the bacteria by endocytosis **(phagocytosis).** By **exocytosis,** neutrophil granules discharge their contents into the phagocytic vacuoles containing the bacteria and also into the interstitial space **(degranulation).** The granules contain various proteases plus antimicrobial proteins called **defensins.** In addition, the cell membrane-bound enzyme **nicotinamide adenine dinucleotide phosphate (NADPH) oxidase** is activated, with the production of toxic oxygen metabolites. The combination of the toxic oxygen metabolites and the proteolytic enzymes from the granules makes the neutrophil a very effective killing machine. Disorders that affect the neutrophil’s killing ability increase the susceptibility to infections **(Clinical Box 3–5)**.

Activation of NADPH oxidase is associated with a sharp increase in O_2 uptake and metabolism in the neutrophil (the **respiratory burst**) and generation

of superoxide (O_2^-) by the following reaction:

$$NADPH + H^+ + 2O_2^+ \rightarrow NADP^+ + 2H^+ + 2O_2^-$$

O_2^- is a **free radical** formed by the addition of one electron to O_2. Two O_2^- react with two H^+ to form H_2O_2 in a reaction catalyzed by the cytoplasmic form of superoxide dismutase (SOD-1):

$$2O_2^- + 2H^+ \xrightarrow{\text{SOD-1}} H_2O_2 + O_2$$

O_2^- and H_2O_2 are both oxidants that are effective bactericidal agents, but H_2O_2 is converted to H_2O and O_2 by the enzyme **catalase.** The cytoplasmic form of SOD-1 contains both Zn and Cu. It is found in many parts of the body. It is defective as a result of genetic mutation in a familial form of **amyotrophic lateral sclerosis** (ALS; see Chapter 15). Therefore, it may be that O_2^- accumulates in motor neurons and kills them in at least one form of this progressive, fatal disease. Two other forms of SOD encoded by at least one different gene are also found in humans.

Neutrophils also discharge the enzyme **myeloperoxidase,** which catalyzes the conversion of Cl^-, Br^-, I^-, and SCN^- to the corresponding acids (HOCl, HOBr, etc). These acids are also potent oxidants. Because Cl^- is present in greatest abundance in body fluids, the principal product is HOCl.

In addition to myeloperoxidase and defensins, neutrophil granules contain elastase, metalloproteinases that attack collagen, and a variety of other proteases that help destroy invading organisms. These enzymes act in a cooperative fashion with O_2^-, H_2O_2, and HOCl to produce a killing zone around the activated neutrophil. This zone is effective in killing invading organisms, but in certain diseases (eg, rheumatoid arthritis) the neutrophils may also cause local destruction of host tissue.

Like neutrophils, **eosinophils** have a short half-life in the circulation, are attracted to the surface of endothelial cells by selectins, bind to integrins that attach them to the vessel wall, and enter the tissues by diapedesis. Like neutrophils, they release proteins, cytokines, and chemokines that produce inflammation but are capable of killing invading organisms. However, eosinophils have some selectivity in the way in which they respond and in the killing molecules they secrete. Their maturation and activation in tissues is particularly stimulated by IL-3, IL-5, and GM-CSF. They are especially

abundant in the mucosa of the gastrointestinal tract, where they defend against parasites, and in the mucosa of the respiratory and urinary tracts. Circulating eosinophils are increased in allergic diseases such as asthma and in various other respiratory and gastrointestinal diseases.

CLINICAL BOX 3–5

Disorders of Phagocytic Function

More than 15 primary defects in neutrophil function have been described, along with at least 30 other conditions in which there is a secondary depression of the function of neutrophils. Patients with these diseases are prone to infections that are relatively mild when only the neutrophil system is involved, but which can be severe when the monocyte-tissue macrophage system is also involved. In one syndrome (neutrophil hypomotility), actin in the neutrophils does not polymerize normally, and the neutrophils move slowly. In another, there is a congenital deficiency of leukocyte integrins. In a more serious disease (chronic granulomatous disease), there is a failure to generate O_2^- in both neutrophils and monocytes and consequent inability to kill many phagocytosed bacteria. In severe congenital glucose-6-phosphate dehydrogenase deficiency, there are multiple infections because of failure to generate the NADPH necessary for O_2^- production. In congenital myeloperoxidase deficiency, microbial killing power is reduced because hypochlorous acid is not formed.

THERAPEUTIC HIGHLIGHTS

The cornerstones of treatment in disorders of phagocytic function include scrupulous efforts to avoid exposure to infectious agents, and antibiotic and antifungal prophylaxis. Antimicrobial therapies must also be implemented aggressively if infections occur. Sometimes, surgery is needed to excise and/or drain abscesses and relieve obstructions. HSC transplantation may offer the hope of a definitive cure for severe conditions, such as chronic granulomatous disease. Sufferers of this condition have a significantly reduced life expectancy due to recurrent infections and their complications, and so the risks of bone marrow transplantation may be acceptable. Gene therapy, on the other hand, remains a distant goal.

Basophils also enter tissues and release proteins and cytokines. They resemble but are not identical to mast cells, and like mast cells they contain histamine (see below). They release histamine and other inflammatory mediators when activated by binding of specific antigens to cell-fixed IgE molecules, and participate in immediate-type hypersensitivity (allergic) reactions. These range from mild urticaria and rhinitis to severe anaphylactic shock. The antigens that trigger IgE formation and basophil (and mast cell) activation are innocuous to most individuals and are referred to as allergens.

MAST CELLS

Mast cells are heavily granulated cells of the connective tissue that are abundant in tissues that come into contact with the external environment, such as beneath epithelial surfaces. Their granules contain proteoglycans, histamine, and many proteases. Like basophils, they degranulate when allergens bind to cell-bound IgE molecules directed against them. They are involved in inflammatory responses initiated by immunoglobulins IgE and IgG. The inflammation combats invading parasites. In addition to this involvement in acquired immunity, they release TNF-α in response to bacterial products by an antibody-independent mechanism, thus participating in the nonspecific **innate immunity** that combats infections prior to the development of an adaptive immune response (see following text). Marked mast cell degranulation produces clinical manifestations of allergy up to and including anaphylaxis.

MONOCYTES

Monocytes enter the blood from the bone marrow and circulate for about 72 h. They then enter the tissues and become **tissue macrophages** **(Figure 3–11)**. Their life span in the tissues is unknown, but bone marrow transplantation data in humans suggest that they persist for about 3 months. It appears that they do not reenter the circulation. Some may end up as the multinucleated giant cells seen in chronic inflammatory diseases such as tuberculosis. The tissue macrophages include the Kupffer cells of the liver, pulmonary alveolar macrophages (see Chapter 34), and microglia in the brain, all of which come originally from the circulation.

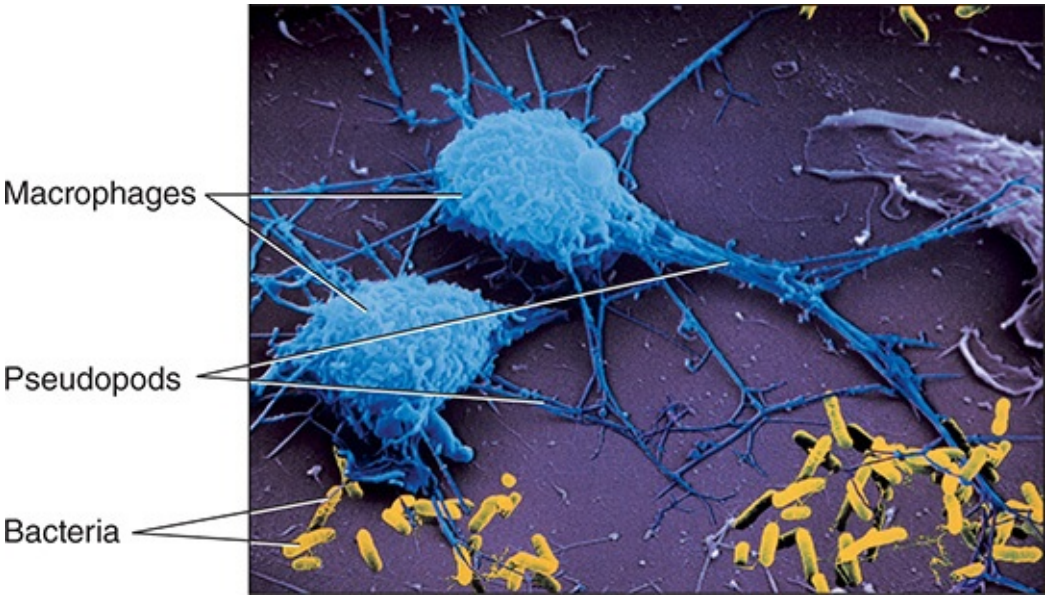


FIGURE 3–11 Macrophages contacting bacteria and preparing to engulf them. Figure is a colorized version of a scanning electron micrograph.

Macrophages are activated by cytokines released from T lymphocytes, among others. Activated macrophages migrate in response to chemotactic stimuli and engulf and kill bacteria by processes generally similar to those occurring in neutrophils. They play a key role in innate immunity (see below). They also secrete up to 100 different substances, including factors that affect lymphocytes and other cells, prostaglandins of the E series, and clot-promoting factors.

PLATELETS

Platelets are non-nucleated circulating blood elements that are important mediators of hemostasis. While not immune cells, per se, they often participate in the response to tissue injury in cooperation with inflammatory cell types. They form in the bone marrow as pinched-off pieces of precursor cells known as megakaryocytes. **Thrombopoietin** facilitates megakaryocyte maturation (see Figure 31–3) and is produced constitutively by the liver and kidneys, and there are also thrombopoietin receptors on platelets. Consequently, when the number of platelets is low, less is bound and more is available to stimulate production of platelets. Conversely, when the number of platelets is high, more is bound and less is available, producing a form of feedback control of platelet production.

Platelets have a ring of microtubules around their periphery and an extensively invaginated membrane with an intricate canalicular system in

contact with the extracellular fluid. Their membranes contain receptors for collagen, ADP, vessel wall von Willebrand factor (see below), and fibrinogen. Their cytoplasm contains actin, myosin, glycogen, lysosomes, and two types of granules: (1) dense granules, which contain nonprotein substances that are secreted in response to platelet activation, including serotonin, ADP, and other adenine nucleotides; and (2) α-granules, which contain secreted proteins. These proteins include clotting factors and **platelet-derived growth factor (PDGF).** PDGF stimulates wound healing and is a potent mitogen for vascular smooth muscle. Blood vessel walls as well as platelets contain von Willebrand factor, which, in addition to its role in adhesion, regulates circulating levels of the clotting factor, factor VIII (see below).

When a blood vessel wall is injured, platelets adhere to the exposed collagen and **von Willebrand factor** in the wall via receptors on the platelet membrane (see also Chapter 31). von Willebrand factor is a large circulating molecule that is produced by endothelial cells. Binding produces platelet activation, which releases the contents of their granules. The released ADP acts on the ADP receptors in the platelet membranes to produce further accumulation of more platelets **(platelet aggregation).** Humans have at least three different types of platelet ADP receptors: $P2Y_1$, $P2Y_2$, and $P2X_1$. These are obviously attractive targets for drug development, and several new inhibitors have shown promise in the prevention of myocardial infarctions and strokes. Aggregation is also fostered by **platelet-activating factor (PAF)**, a cytokine secreted by neutrophils and monocytes as well as platelets. This compound also has inflammatory activity. It is an ether phospholipid, 1-alkyl-2-acetylglyceryl-3-phosphorylcholine, which is produced from membrane lipids. It acts via a G-protein-coupled receptor to increase the production of arachidonic acid derivatives, including thromboxane A_2. The role of this latter compound in the balance between clotting and anticlotting activity at the site of vascular injury is discussed in Chapter 31.

When the platelet count is low, clot retraction is deficient and there is poor constriction of ruptured vessels. The resulting clinical syndrome **(thrombocytopenic purpura)** is characterized by easy bruisability and multiple subcutaneous hemorrhages. Purpura may also occur when the platelet count is normal, and in some of these cases, the circulating platelets are abnormal **(thrombasthenic purpura).** Individuals with thrombocytosis are predisposed to thrombotic events.

INFLAMMATION & WOUND HEALING

LOCAL INJURY

Inflammation is a complex localized response to foreign substances such as bacteria or, in some instances, to internally produced substances. It includes a sequence of reactions initially involving cytokines, neutrophils, adhesion molecules, complement, and IgG. PAF also plays a role. Later, monocytes and lymphocytes are involved. Arterioles in the inflamed area dilate, and capillary permeability is increased (see Chapters 32 and 33). When the inflammation occurs in or just under the skin **(Figure 3–12)**, it is characterized by redness, swelling, tenderness, and pain. Elsewhere, it is a key component of asthma, ulcerative colitis, Crohn disease (Clinical Box 3–1), rheumatoid arthritis, and many other diseases.

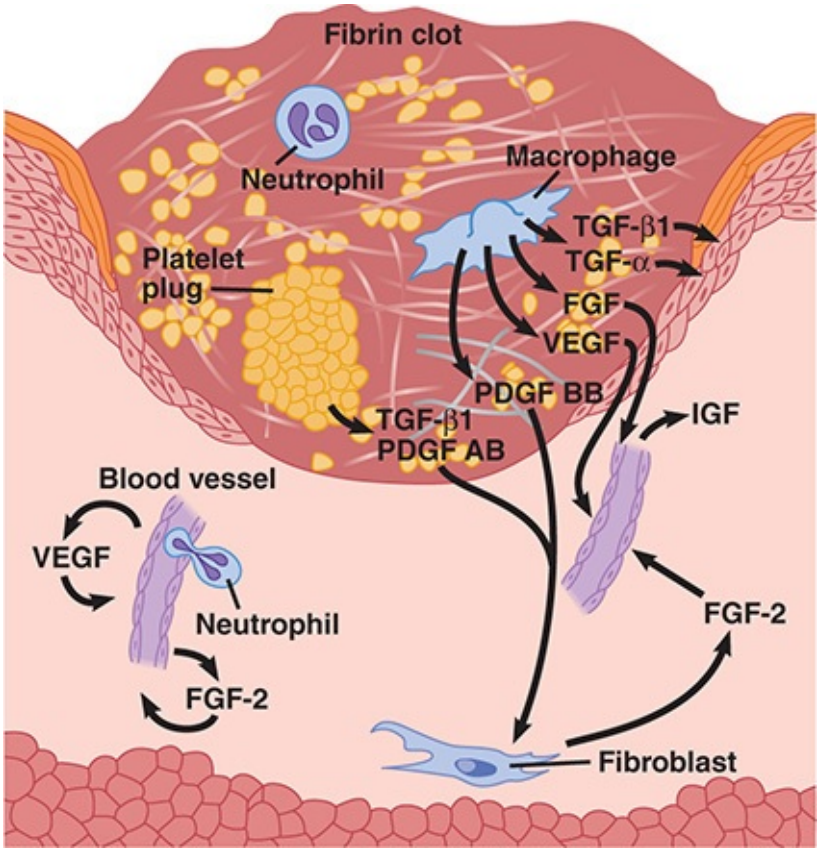


FIGURE 3–12 Cutaneous wound 3 days after injury, showing the multiple cytokines and growth factors affecting the repair process. FGF, fibroblast growth factor; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor; VEGF, vascular endothelial growth

factor. Note the epidermis growing down under the fibrin clot, restoring skin continuity. (Modified from Singer AJ, Clark RAF: Cutaneous wound healing. N Engl J Med 1999;September 2;341(10):738-746.)

A transcription factor known as **nuclear factor-κB** (NF-κB) plays the pivotal role in orchestrating the inflammatory response. NF-κB is a heterodimer that normally exists in the cytoplasm of cells bound to IκBα, which renders it inactive. Stimuli such as cytokines, viruses, and oxidants induce signals that allow NF-κB to dissociate from IκBα, which is then degraded. NF-κB moves to the nucleus, where it binds to the DNA of the genes for numerous inflammatory mediators, resulting in their increased production and secretion. Corticosteroids inhibit the activation of NF-κB by increasing the production of IκBα, and this is probably the main basis of their anti-inflammatory action (see Chapter 19).

SYSTEMIC RESPONSE TO INJURY

Cytokines produced in response to inflammation and other injuries, as well as disseminated infection, also produce systemic responses. These include alterations in plasma **acute phase proteins,** defined as proteins whose concentration is increased or decreased by at least 25% following injury. Many of the proteins are of hepatic origin. A number of them are shown in **Figure 3–13**. The causes of the changes in concentration are incompletely understood, but it can be said that many of the changes make homeostatic sense. Thus, for example, an increase in C-reactive protein activates monocytes and causes further production of cytokines. Other changes that occur in response to injury include somnolence, negative nitrogen balance, and fever.

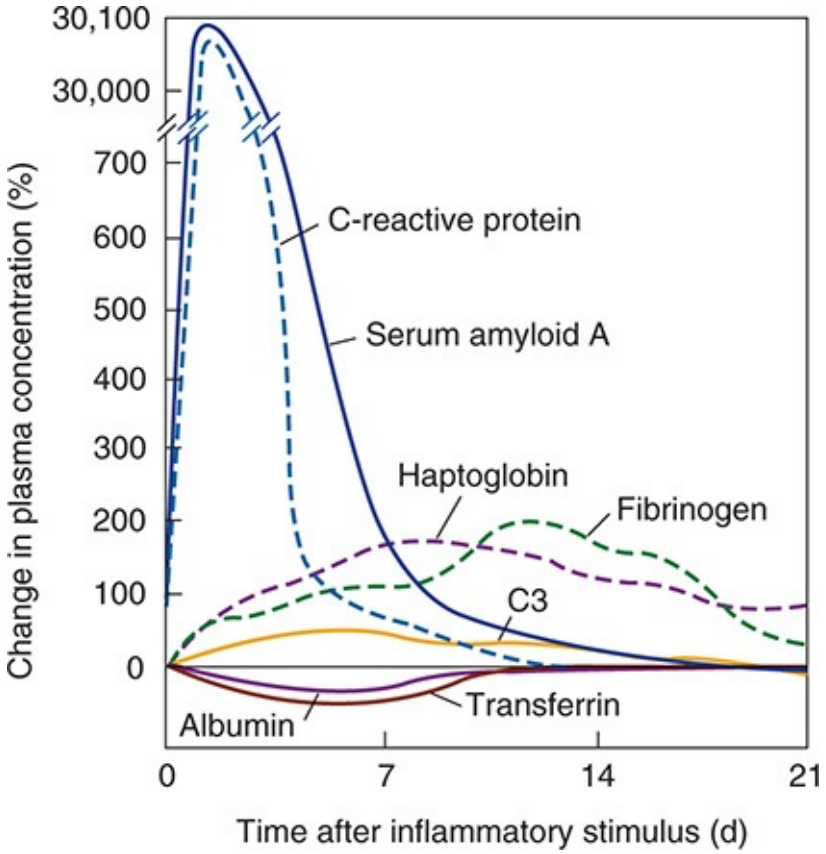


FIGURE 3–13 Time course of changes in some major acute phase proteins. C3, C3 component of complement. (Modified with permission from McAdam KP, Elin RJ, Sipe JD, Wolff SM: Changes in human serum amyloid A and C-reactive protein after etiocholanolone-induced inflammation. J Clin Invest 1978;February;61(2):390-394.)

WOUND HEALING

When tissue is damaged, platelets adhere to exposed matrix via integrins that bind to collagen and laminin (Figure 3–12). Blood coagulation produces thrombin, which promotes platelet aggregation and granule release. The platelet granules generate an inflammatory response. White blood cells are attracted by selectins and bind to integrins on endothelial cells, leading to their extravasation through the blood vessel walls. Cytokines released by the white blood cells and platelets upregulate integrins on macrophages, which migrate to the area of injury, and on fibroblasts and epithelial cells, which mediate wound healing and scar formation. Plasmin aids healing by removing excess fibrin. This aids the migration of keratinocytes into the wound to restore the epithelium under the

scab. Collagen synthesis is upregulated, producing the scar. Wounds gain 20% of their ultimate strength in 3 weeks and later gain more strength, but they never reach more than about 70% of the strength of normal skin.

CHAPTER SUMMARY

- Immunity is the means by which the body is protected from foreign invaders or from abberent host cells, such as tumor cells.
- Innate immunity represents an evolutionarily conserved, primitive response to stereotypical microbial components.
- Acquired immunity is slower to develop than innate immunity, but long-lasting and often more effective due to its greater specificity.
- Genetic rearrangements endow B and T lymphocytes with a vast array of receptors capable of recognizing billions of foreign antigens.
- Self-reactive lymphocytes are normally deleted; a failure of this process leads to autoimmune disease.
- A variety of soluble mediators orchestrate the development of immunologic effector cells, their migration, and their subsequent immune and inflammatory reactions.
- Immune and inflammatory responses are mediated by several additional cell types—granulocytes, monocytes, mast cells, tissue macrophages, and antigen-presenting cells—that arise predominantly from the bone marrow and may circulate or reside in connective tissues.
- Granulocytes mount phagocytic responses that engulf and destroy bacteria. These are accompanied by the release of reactive oxygen species and other mediators into adjacent tissues that may cause tissue injury. Disease can result from abnormal function or development of granulocytes. Deficient immune responses to microbial threats usually result.
- Mast cells and basophils underpin allergic reactions to substances that would be treated as innocuous by nonallergic individuals.
- Inflammatory responses occur in response to infection or injury, and serve to resolve the threat, although they may cause damage to otherwise healthy tissue. A number of chronic diseases reflect excessive inflammatory responses that persist even once the threat is controlled, or are triggered by stimuli that healthy individuals would not respond to.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. Cells responsible for innate immunity are activated most commonly by

   A. glucocorticoids

   B. pollen

   C. carbohydrate sequences in bacterial cell walls

   D. eosinophils

   E. thrombopoietin

2. A biotechnology company is working to design a new therapeutic strategy for cancer that involves triggering an enhanced immune response to cellular proteins that are mutated in the disease. Which of the following immune cells or processes will most likely **not** be required for a successful therapy?

   A. Cytotoxic T cells

   B. Antigen presentation in the context of MHC-II

   C. Proteosomal degradation

   D. Gene rearrangements producing T cell receptors

   E. The immune synapse

3. A six-year-old boy is brought to the pediatrician with complaints of progressive excessive thirst, increased urination, weight loss, and severe fatigue. A glucose tolerance test results in a diagnosis of type 1 diabetes mellitus and the patient is treated effectively with injected insulin. The emergent symptoms in this patient reflect a failure of which immune process?

   A. Antibody synthesis

   B. Neutrophil chemotaxis

   C. Antigen presentation

   D. Tolerance

   E. Complement activation

4. A 20-year-old college student comes to the student health center in April complaining of runny nose and congestion, itchy eyes, and wheezing. She reports that similar symptoms have occurred at the same time each year, and that she obtains some relief from over-the-counter antihistamine drugs, although they make her too drowsy to study. Her symptoms can most likely be attributed to inappropriate synthesis of which of the following antibodies specific for tree pollen?

A. IgA
B. IgD
C. IgE
D. IgG
E. IgM

5. The ability of the blood to phagocytose pathogens and mount a respiratory burst is increased by

A. interleukin-2 (IL-2)
B. granulocyte colony-stimulating factor (G-CSF)
C. erythropoietin
D. interleukin-4 (IL-4)
E. interleukin-5 (IL-5)

6. In an experiment, a scientist treats a group of mice with an antiserum that substantially depletes the number of circulating neutrophils. Compared with untreated control animals, the mice with reduced numbers of neutrophils were found to be significantly more susceptible to death induced by bacterial inoculation. The increased mortality can be ascribed to a relative deficit in which of the following?

A. Acquired immunity
B. Oxidants
C. Platelets
D. Granulocyte/macrophage colony stimulating factor (GM-CSF)
E. Integrins

7. If a nasal biopsy were performed on the patient described in Question 4 while symptomatic, histologic examination of the tissue would most likely reveal degranulation of which of the following cell types?

A. Dendritic cells
B. Lymphocytes
C. Neutrophils
D. Monocytes
E. Mast cells

8. A patient suffering from an acute flare in his rheumatoid arthritis undergoes a procedure where fluid is removed from his swollen and inflamed knee joint. Biochemical analysis of the inflammatory cells recovered from the removed

fluid would most likely reveal a decrease in which of the following proteins?

A. Interleukin-1

B. Tumor necrosis factor-α

C. Nuclear factor-κB

D. IκBα

E. von Willebrand factor

CHAPTER 4

Excitable Tissue: Nerve

OBJECTIVES

After studying this chapter, you should be able to:

- Draw a typical neuron and identify the role played by soma, dendrites, axon, and initial segment in impulse generation and conduction.
- Explain the basis for the resting membrane potential of a neuron and the effect of hyperkalemia and hypokalemia on the resting potential.
- Explain the ionic fluxes that occur during an action potential.
- Compare and contrast how unmyelinated and myelinated neurons propagate impulses.
- Compare the conduction velocity and other properties of different types of sensory and motor nerve fibers.
- Explain the importance of orthograde and retrograde axonal transport.
- Compare the functions of the various types of glia found in the nervous system.
- Identify neuropathologies related to dysfunction of myelin proteins or the loss of myelin.
- Describe the function of neurotrophins.

INTRODUCTION

The basic working unit of the central and peripheral nervous system is the nerve cell or **neuron.** Neurons are identified as excitable cells because they have the ability to be electrically excited resulting in the generation of action potentials. Other examples of excitable cells are skeletal, smooth, and cardiac muscle cells (Chapter 5) and secretory cells of the pancreas. Neurons come in many different shapes and sizes, but they share features that impart in them an ability to receive, process, integrate, and transmit information from external and internal sources to initiate most physiological behaviors. This chapter describes the ionic mechanisms that enable neurons to generate and conduct impulses; Chapter 6 explains how neurons communicate with other neurons and effector organs (synaptic transmission). This chapter also describes the roles played by neuroglia cells and neurotrophins in physiological and pathophysiological processes.

THE NEURON: BASIC WORKING UNIT OF THE NERVOUS SYSTEM

Figure 4–1 shows the basic components of a neuron for the prototypical spinal motor neuron. The cell body **(soma)** contains the nucleus that is the metabolic center of the neuron and stores the hereditary material or DNA. Neurons have several processes called **dendrites** that extend outward from the cell body and arborize extensively to aid their role in receiving incoming signals, processing the information, and then transmitting the information to the soma of the neuron. A typical neuron also has a long fibrous **axon** that originates from a thickened area of the cell body **(axon hillock).** The first portion of the axon is called the **initial segment**. The axon divides into **presynaptic terminals,** each ending in a number of **synaptic knobs** that are also called **terminal buttons** or **boutons.** They contain granules or vesicles in which the synaptic transmitters released by the nerves are stored. Based on the number of processes that emanate from their cell body, neurons can be classified as **unipolar, bipolar, pseudounipolar,** and **multipolar (Figure 4–2)**.

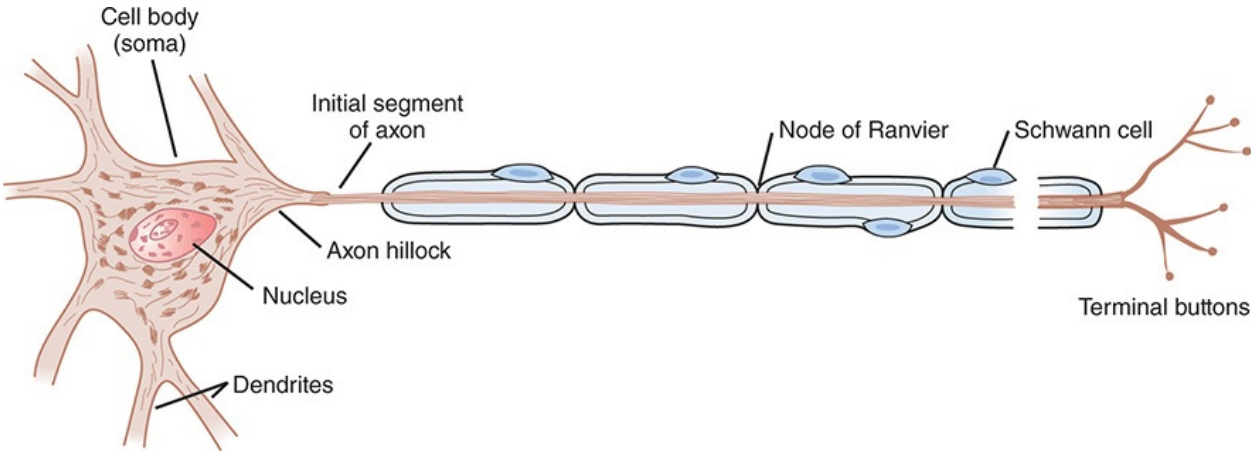


FIGURE 4–1 Motor neuron with a myelinated axon. A motor neuron is composed of a cell body (soma) with a nucleus, several processes called dendrites, and a long fibrous axon that originates from the axon hillock. The first portion of the axon is called the initial segment. A myelin sheath forms from Schwann cells and surrounds the axon except at its ending and at the nodes of Ranvier. Terminal buttons (boutons) are located at the terminal endings.

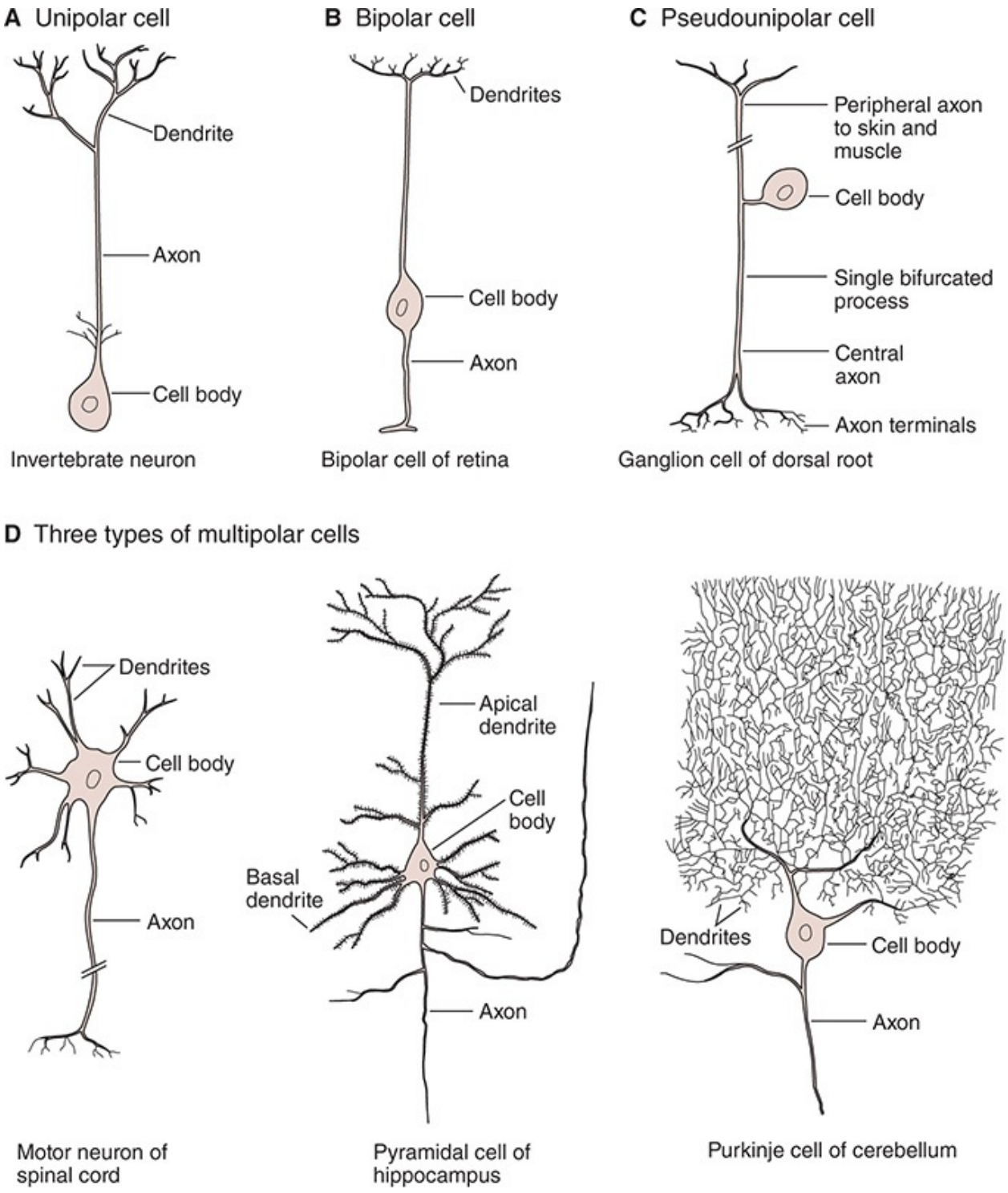


FIGURE 4–2 Some of the types of neurons in the mammalian nervous system. A) Unipolar neurons have one process, with different segments serving as receptive surfaces and releasing terminals. **B)** Bipolar neurons have two specialized processes: a dendrite that carries information to the cell and an axon that transmits information from the cell. **C)** Some sensory neurons are in a

subclass of bipolar cells called pseudounipolar cells. As the cell develops, a single process splits into two, both of which function as axons—one going to skin or muscle and another to the spinal cord. **D)** Multipolar cells have one axon and many dendrites. Examples include motor neurons, hippocampal pyramidal cells with dendrites in the apex and base, and cerebellar Purkinje cells with an extensive dendritic tree in a single plane. (Reproduced with permission from Kandel ER, Schwartz JH, Jessell TM, Siegelbaum SA, Hudspeth AJ (editors): *Principles of Neural Science*, 5th ed. New York, NY: McGraw-Hill; 2013.)

From a functional point of view, neurons generally have four important zones: (1) a dendritic zone where multiple local potential changes generated by synaptic connections are integrated; (2) a site where propagated action potentials are generated (the initial segment in spinal motor neurons, the initial node of Ranvier in cutaneous sensory neurons); (3) an axonal process that transmits propagated impulses to the nerve endings; and (4) the nerve endings, where action potentials cause the release of synaptic transmitters.

The axons of many neurons acquire a **myelin sheath,** a protein–lipid complex that is wrapped around the axon. In the peripheral nervous system, myelin forms when a **Schwann cell** (a type of glia) wraps its membrane around an axon up to 100 times (Figure 4–1). The myelin sheath envelops the axon except at the **nodes of Ranvier**, periodic 1-µm gaps where the axon is unmyelinated (Figure 4–1). The insulating function of myelin and its role in axonal conduction are discussed later in this chapter.

EXCITATION & CONDUCTION

A hallmark of nerve cells is their excitable membrane. Neurons respond to electrical, chemical, or mechanical stimuli by producing local (nonpropagated) or propagated potentials reflecting changes in the conduction of ions across the cell membrane. Depending on their location, the nonpropagated potentials are called **synaptic, generator,** or **electrotonic potentials.** The propagated **action potentials** are the primary electrical responses of neurons; they are the main form of communication within the nervous system. The electrical events in neurons are rapid, measured in milliseconds (ms); and the potential changes are small, measured in millivolts (mV).

The impulse is normally transmitted (conducted) along the axon to its termination. Conduction is an active, self-propagating process, and the impulse moves along the nerve at a constant amplitude and velocity. The process is often

compared to what happens when a match is applied to one end of a trail of gunpowder; by igniting the powder particles immediately in front of it, the flame moves steadily down the trail to its end as it is extinguished in its wake.

RESTING MEMBRANE POTENTIAL

The voltage difference across the cell membrane of a neuron is called a **membrane potential;** it is the difference between the electrical potential in the cytoplasm of the cell and the electrical potential in the extracellular space. The membrane potential results from the separation of positive and negative charges across the cell membrane **(Figure 4–3)**. In order for a potential difference to be present across a membrane lipid bilayer, two conditions must be met. First, there must be an unequal distribution of ions of one or more type across the membrane (ie, a **concentration gradient**). Second, the membrane must be permeable to these ions. The permeability is provided by the existence of channels or pores in the bilayer; these channels are usually permeable to a single type of ion.

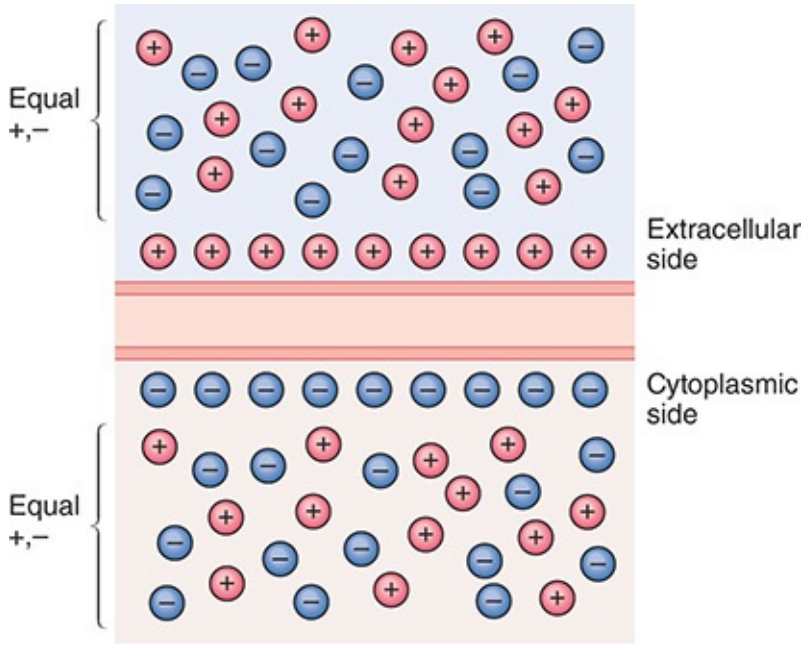


FIGURE 4–3 A membrane potential results from separation of positive and negative charges across the cell membrane. The excess of positive charges (red circles) outside the cell and negative charges (blue circles) inside the cell at rest represents a small fraction of the total number of ions present. (Reproduced with permission from Kandel ER, Schwartz JH, Jessell TM, Siegelbaum SA, Hudspeth AJ (editors): *Principles of Neural Science*, 5th ed. New York, NY:

McGraw-Hill; 2013.)

The **resting membrane potential** represents an equilibrium situation at which the driving force for the membrane-permeant ions down their concentration gradients across the membrane is equal and opposite to the driving force for these ions down their **electrical gradients.** In neurons, the concentration of K^+ is much higher inside than outside the cell, while the reverse is the case for Na^+. This concentration difference is established by **Na, K ATPase**. The outward K^+ concentration gradient results in passive movement of K^+ out of the cell when K^+-selective channels are open. Similarly, the inward Na^+ concentration gradient results in passive movement of Na^+ into the cell when Na^+-selective channels are open.

The resting membrane potential of neurons is usually about –70 mV (step 1 in **Figure 4–4**). Because there are more open K^+ channels than Na^+ channels at rest, the membrane permeability to K^+ is greater. Consequently, the intracellular and extracellular K^+ concentrations are the prime determinants of the resting membrane potential, which is therefore close to the equilibrium potential for K^+. Steady ion leaks cannot continue forever without eventually dissipating the ion gradients. Na, K ATPase prevents this from occurring by actively moving Na^+ and K^+ against their electrochemical gradients.

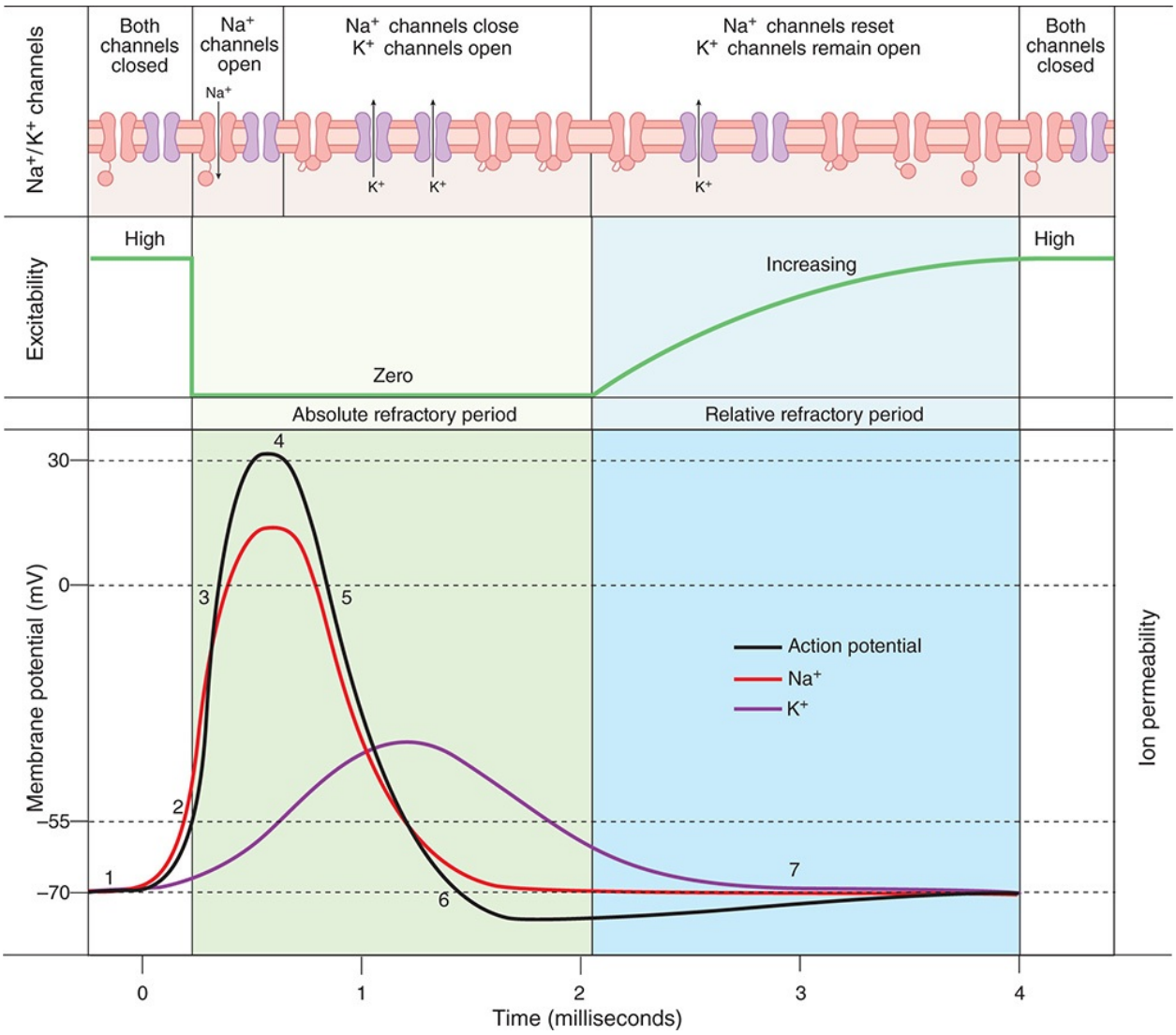


FIGURE 4–4 Changes in membrane potential and relative membrane permeability to Na^+ and K^+ during an action potential. Steps 1 through 7 are detailed in the text. These changes in threshold for activation (excitability) are correlated with the phases of the action potential. (Modified with permission from Silverthorn DU: *Human Physiology: An Integrated Approach*, 5th ed. Pearson, 2010.)

IONIC FLUXES DURING THE ACTION POTENTIAL

Neuronal cell membranes contain many types of ion channels, including both ligand-gated and voltage-gated ion channels. **Ligand-gated ion channels** open

when a ligand (eg, neurotransmitter) binds to them, and **voltage-gated ion channels** open when there is a change in the voltage gradient across the membrane. The behavior of these channels, particularly Na^+ and K^+ channels, explains the electrical events in neurons.

The changes in **membrane conductance** of Na^+ and K^+ that occur during an action potential are shown by steps 1 through 7 in Figure 4–4. The conductance of an ion is the reciprocal of its electrical resistance in the membrane and is a measure of the membrane permeability to that ion. In response to a depolarizing stimulus, some of the voltage-gated Na^+ channels open and Na^+ enters the cell and the membrane is brought to its **threshold potential** (step 2) and the voltage-gated Na^+ channels overwhelm the K^+ and other channels. The entry of Na^+ causes the opening of more voltage-gated Na^+ channels and further depolarization, setting up a **positive feedback loop.** The rapid upstroke in the membrane potential ensues (step 3). The membrane potential moves toward the equilibrium potential for Na^+ (+60 mV) but does not reach it during the action potential (step 4), primarily because the increase in Na^+ conductance is short-lived. The Na^+ channels rapidly enter a closed state called the **inactivated state** and remain in this state for a few milliseconds before returning to the resting state, when they again can be activated. The direction of the electrical gradient for Na^+ is reversed during the **overshoot** because the membrane potential is reversed which limits Na^+ influx; also the voltage-gated K^+ channels open. These factors contribute to **repolarization.** The opening of voltage-gated K^+ channels is slower and more prolonged than the opening of the Na^+ channels; consequently, much of the increase in K^+ conductance comes after the increase in Na^+ conductance (step 5). The net movement of positive charge out of the cell due to K^+ efflux at this time helps complete the process of repolarization. The slow return of the K^+ channels to the closed state also explains the **after-hyperpolarization** (step 6), followed by a return to the resting membrane potential (step 7). Thus, voltage-gated K^+ channels bring the action potential to an end and cause closure of their gates through a **negative feedback process.** **Figure 4–5** shows the sequential feedback control in voltage-gated K^+ and Na^+ channels during the action potential.

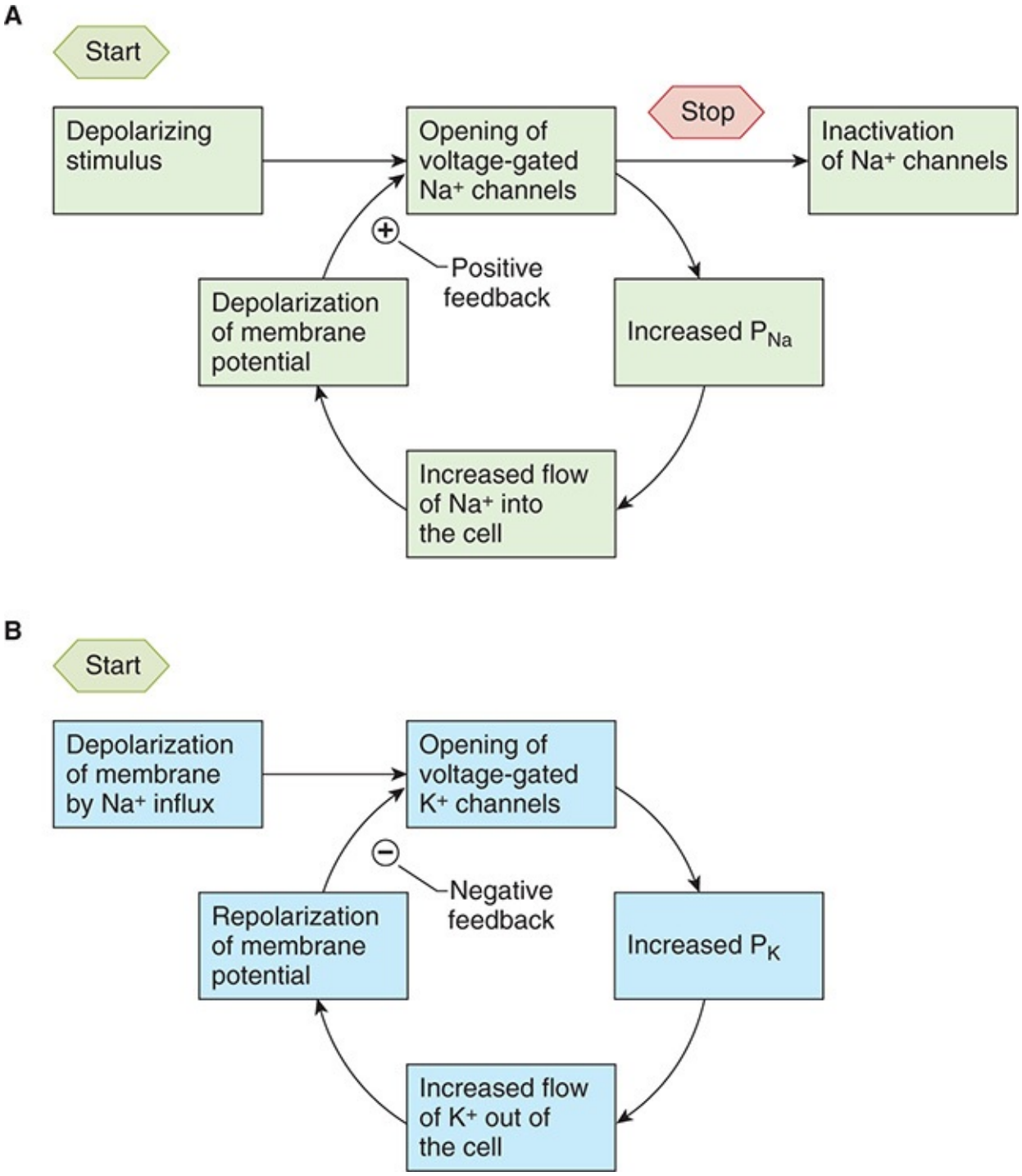


FIGURE 4–5 Feedback control in voltage-gated ion channels in the membrane. A) Na^+ channels exert positive feedback. **B)** K^+ channels exert negative feedback. P_{Na}, P_K is permeability to Na^+ and K^+, respectively. (Reproduced with permission from Widmaier EP, Raff H, Strang KT: *Vander's Human Physiology*. New York, NY: McGraw-Hill; 2008.)

Decreasing the external Na^+ concentration reduces the size of the action potential but has little effect on the resting membrane potential. The lack of much effect on the resting membrane potential is predicted because the permeability of the membrane to Na^+ at rest is relatively low. In contrast, since the resting membrane potential is close to the equilibrium potential for K^+, changes in the external concentration of this ion can have major effects on the resting membrane potential. If the extracellular level of K^+ is increased **(hyperkalemia),** the resting potential moves closer to the threshold for eliciting an action potential and the neuron becomes more excitable. If the extracellular level of K^+ is decreased **(hypokalemia),** the membrane potential is reduced and the neuron is hyperpolarized. **Clinical Box 4–1** describes some of the common causes, signs, and treatments for the deviation of plasma levels of K^+ away from the normal value of 3.5–5.0 mEq/L on excitable cells (eg, neurons, heart, skeletal muscle, smooth muscle).

Other ions, notably Ca^{2+}, can affect the membrane potential through both channel movement and membrane interactions. A decrease in extracellular Ca^{2+} concentration increases the excitability of nerve and muscle cells by decreasing the amount of depolarization needed to initiate the changes in the Na^+ and K^+ conductance that produce the action potential. Conversely, an increase in extracellular Ca^{2+} concentration can stabilize the membrane by reducing excitability.

ALL-OR-NONE ACTION POTENTIALS

The minimal intensity of stimulating current needed to produce an action potential is called the **threshold intensity**; it varies with the stimulus duration. A weak stimulus needs a long duration, and a strong stimulus is sufficient at a short duration. The relation between the strength and the duration of a threshold stimulus is called the **strength–duration curve.** Slowly rising currents fail to induce an action potential in the nerve because the nerve undergoes **adaptation.** The action potential is **all-or-none** in character. That is, no action potential occurs if the stimulus is subthreshold in magnitude, and the action potential has a constant amplitude and form at any stimulus strength above the threshold intensity.

CLINICAL BOX 4–1

Hyperkalemia and Hypokalemia

Potassium homeostasis is critical for the normal functioning of nerves, skeletal muscle, smooth muscle, and the heart. A primary cause of **hyperkalemia** is impairment in the ability of the kidney to excrete K^+ due to advanced renal failure, adrenal insufficiency, distal renal tubular acidosis, type 1 diabetes, and dehydration. Drug-induced hyperkalemia can result from the use of angiotensin II receptor blockers, angiotensin-converting enzyme (ACE) inhibitors, nonsteroidal anti-inflammatory drugs (NSAIDs), and potassium sparing diuretics. Symptoms of hyperkalemia include muscle pain and weakness, numbness, cardiac arrhythmias, and nausea. Extremely high levels of plasma K^+ can lead to cardiac arrest and death. **Hyperkalemic periodic paralysis** is a rare inherited disorder (prevalence of 1 per 100,000 individuals) in which patients experience transient episodes of muscle paralysis due to hyperkalemia. The resting membrane potential of skeletal muscle in affected individuals shifts from a normal value of −90 mV to a value of −60 mV which inactivates Na^+ channels and prevents action potential generation.

The most common cause of **hypokalemia** is increased excretion of K^+, but it can also occur if there is a shift of K^+ from the extracellular to the intracellular space. Hypokalemia can be a side effect of rare genetic disorders of the kidney (**Bartter syndrome** [see Chapter 37] and **Gitelman syndrome), Cushing syndrome,** the use of K^+-wasting diuretics, diabetic **ketoacidosis,** renal tubular acidosis, and familial hypokalemia. Symptoms are somewhat nonspecific but can include weakness and fatigue, constipation, muscle cramping, palpitations, and psychological symptoms such as depression or psychosis. Severe hypokalemia (below 2.5 mEq/L) mainly leads to problems with cardiac rhythmicity and can manifest as bradycardia, tachycardia, premature beats, and atrial or ventricular fibrillation.

THERAPEUTIC HIGHLIGHTS

Treatment of hyperkalemia typically includes treatment of the underlying cause. It may also include a low-potassium diet, intravenous administration of calcium to protect the heart and muscles, or administration of sodium bicarbonate to promote movement of K^+ from the extracellular space back into the cells. Treatment of hypokalemia focuses on ways to reduce K^+ loss (eg, discontinue the use of a diuretic or use a K^+-sparing diuretic), replenish K^+ stores (oral or

intravenous administration of K^+), and determining and treating the cause of the hypokalemia.

ELECTROTONIC POTENTIALS, LOCAL RESPONSE, & FIRING LEVEL

Although subthreshold stimuli do not produce a propagating action potential, they do have an effect on the membrane potential. Applying subthreshold stimuli of fixed duration leads to a localized depolarizing potential change that rises sharply and decays exponentially with time **(Figure 4–6)**. The magnitude of this response drops off rapidly as the distance between the stimulating and recording electrode is increased. Conversely, an anodal current produces a hyperpolarizing potential change of similar duration. These potential changes are called **electrotonic potentials.** As the strength of the current is increased, the response is greater due to the increasing addition of a local response of the membrane. Finally, at 7–15 mV of depolarization (potential of –55 mV), the threshold potential is reached and an action potential occurs.

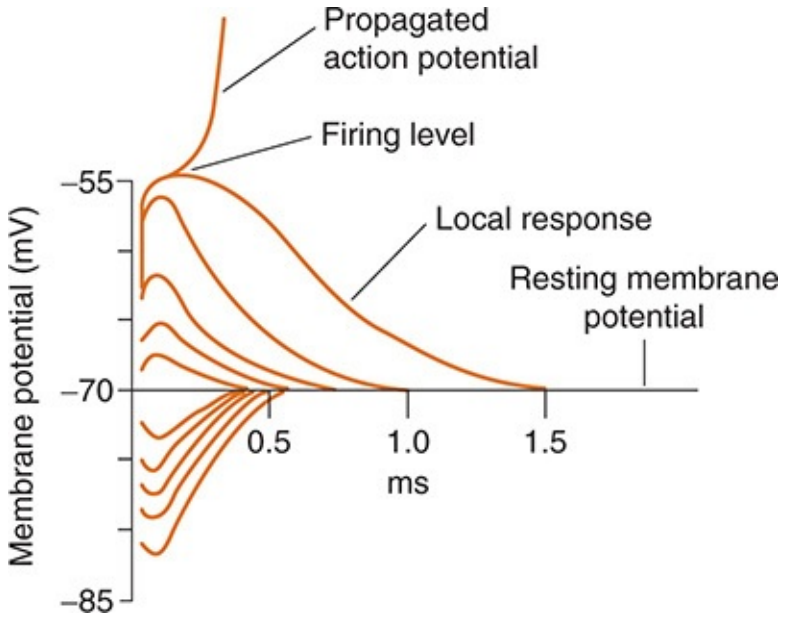


FIGURE 4–6 Electrotonic potentials and local response. The changes in the membrane potential of a neuron following application of stimuli of 0.2, 0.4, 0.6, 0.8, and 1.0 times threshold intensity are shown superimposed on the same time scale. The responses below the horizontal line are those recorded near the anode,

and the responses above the line are those recorded near the cathode. The stimulus of threshold intensity was repeated twice. Once it caused a propagated action potential (top line), and once it did not.

REFRACTORY PERIODS

During the action potential, as well as during electrotonic potentials and the local response, the threshold of the neuron to stimulation changes (Figure 4–4). Hyperpolarizing responses elevate the threshold, and depolarizing potentials lower it as they move the membrane potential closer to the threshold potential. During the local response, the threshold is lowered, but during the rising and much of the falling phases of the spike potential, the neuron is refractory to stimulation. The **refractory period** is divided into an **absolute refractory period,** corresponding to the period from the time the firing level is reached until repolarization is about one-third complete, and a **relative refractory period,** lasting from this point to the start of after-depolarization. During the absolute refractory period, no stimulus, no matter how strong, will excite the nerve. However, during the relative refractory period, stronger than normal stimuli can cause excitation. These changes in threshold are correlated with the phases of the action potential in Figure 4–4.

CONDUCTION OF THE ACTION POTENTIAL

The nerve cell membrane is polarized at rest, with positive charges lined up along the outside of the membrane and negative charges along the inside. During the action potential, this polarity is abolished and for a brief period is actually reversed **(Figure 4–7)**. Positive charges from the membrane ahead of and behind the action potential flow into the area of negativity represented by the action potential (“current sink”). By drawing off positive charges, this flow decreases the polarity of the membrane ahead of the action potential. Such electrotonic depolarization initiates a local response, and when the firing level is reached, a propagated response occurs that in turn electrotonically depolarizes the membrane in front of it.

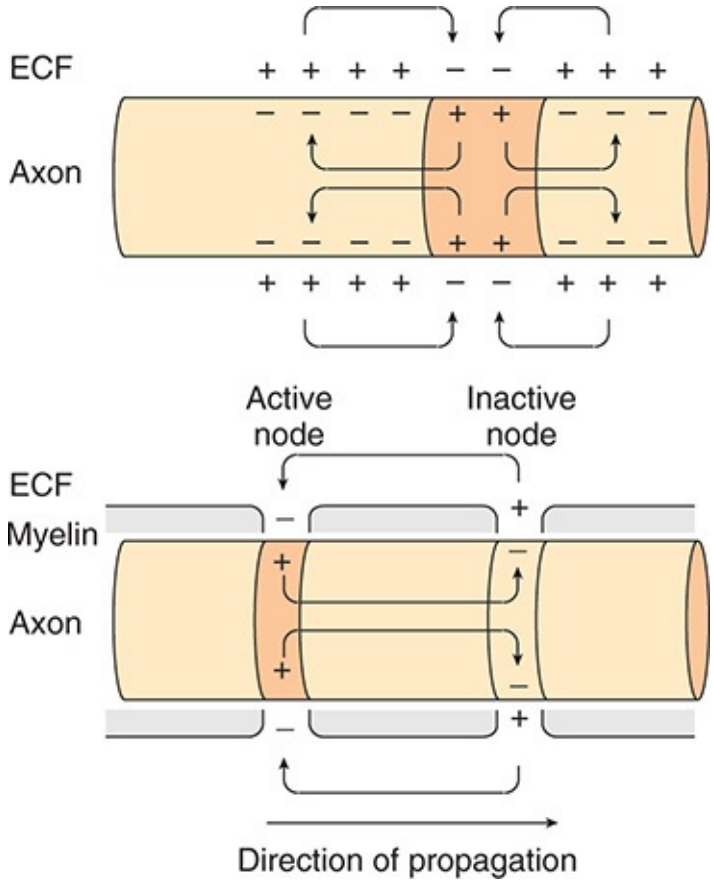


FIGURE 4–7 Local current flow (movement of positive charges) around an impulse in an axon. Top: Unmyelinated axon. **Bottom:** Myelinated axon. Positive charges from the membrane ahead of and behind the action potential flow into the area of negativity represented by the action potential ("current sink"). In myelinated axons, depolarization appears to "jump" from one node of Ranvier to the next (saltatory conduction).

The spatial distribution of ion channels along the axon plays a key role in the initiation and regulation of the action potential. Voltage-gated Na^+ channels are highly concentrated in the nodes of Ranvier and the initial segment in myelinated neurons. The number of Na^+ channels per square micrometer of membrane in myelinated mammalian neurons is 50–75 in the cell body, 350–500 in the initial segment, less than 25 on the surface of the myelin, 2000–12,000 at the nodes of Ranvier, and 20–75 at the axon terminals. Along the axons of unmyelinated neurons, the number is about 110. In many myelinated neurons, the Na^+ channels are flanked by K^+ channels that are involved in repolarization.

Conduction in myelinated axons depends on a similar pattern of circular current flow as just described. However, myelin is an effective insulator, and

current flow through it is negligible. Instead, depolarization in myelinated axons travels from one node of Ranvier to the next, with the current sink at the active node serving to electrotonically depolarize the node ahead of the action potential to the firing level (Figure 4–7). This "jumping" of depolarization from node to node is called **saltatory conduction.** It is a rapid process that allows myelinated axons to conduct up to 50 times faster than the fastest unmyelinated fibers.

NERVE FIBER TYPES & FUNCTION

Mammalian nerve fibers are divided into A, B, and C groups, and the A group can be subdivided into α, β, γ, and δ fibers. In **Table 4–1**, the various fiber types are listed with their diameters, electrical characteristics, and functions. After a stimulus is applied to a nerve, there is a latent period before the start of the action potential. This interval corresponds to the time it takes the impulse to travel along the axon from the site of stimulation to the recording electrodes. Its duration is proportionate to the distance between the stimulating and recording electrodes and inversely proportionate to the speed of conduction. If the duration of the latent period and the distance between the stimulating and recording electrodes are known, **axonal conduction velocity** can be calculated. In general, the greater the diameter of a given nerve fiber, the greater its speed of conduction. The large axons are concerned primarily with proprioceptive sensation, somatic motor function, conscious touch, and pressure, while the smaller axons subserve pain and temperature sensations and autonomic function.

TABLE 4–1 Types of mammalian nerve fibers.

Fiber Type	Function	Fiber Diameter (μm)	Conduction Velocity (m/s)	Spike Duration (ms)	Absolute Refractory Period (ms)
Aα	Proprioception; somatic motor	12–20	70–120		
Aβ	Touch, pressure	5–12	30–70	0.4–0.5	0.4–1
Aγ	Motor to muscle spindles	3–6	15–30		
Aδ	Pain, temperature	2–5	12–30		
B	Preganglionic autonomic	< 3	3–15	1.2	1.2
C, Dorsal root	Pain, temperature	0.4–1.2	0.5–2	2	2
C, Sympathetic	Postganglionic sympathetic	0.3–1.3	0.7–2.3	2	2

Although the letter classification is commonly used to describe motor fibers, a numerical system (Ia, Ib, II, III, and IV) is often used to classify sensory fibers based on their axonal diameter and conduction velocity. **Table 4–2** shows the corresponding classification of the number system and the letter system.

TABLE 4–2 Numerical classification of sensory nerve fibers.

Number	Origin	Fiber Type
Ia	Muscle spindle, annulo-spiral ending	Aα
Ib	Golgi tendon organ	Aα
II	Muscle spindle, flower-spray ending; touch, pressure	Aβ
III	Pain and cold receptors; some touch receptors	Aδ
IV	Pain, temperature, and other receptors	C

In addition to variations in speed of conduction and fiber diameter, the various classes of fibers in peripheral nerves differ in their sensitivity to hypoxia and anesthetics **(Table 4–3)**. This fact has clinical as well as physiologic significance. Local anesthetics depress transmission in the unmyelinated group C fibers before they affect the myelinated group A fibers **(Clinical Box 4–2)**. Conversely, pressure on a nerve can cause loss of conduction in large-diameter motor, touch, and pressure fibers while pain sensation remains relatively intact. Patterns of this type are sometimes seen in individuals who sleep with their arms under their heads for long periods, causing compression of the nerves in the arms. Because of the association of deep sleep with alcoholic intoxication, the syndrome is most common on weekends and has acquired the interesting name Saturday night or Sunday morning paralysis.

TABLE 4–3 Relative susceptibility of mammalian A, B, and C nerve fibers to conduction block produced by various agents.

Agent	Most Susceptible	Intermediate	Least Susceptible
Hypoxia	B	A	C
Pressure	A	B	C
Local anesthetics	C	B	A

AXONAL TRANSPORT

The apparatus for protein synthesis in neurons is located primarily in the soma, with transport of proteins and polypeptides to the axonal ending by **axoplasmic flow.** Thus, the cell body maintains the functional and anatomic integrity of the axon. **Orthograde transport** occurs along microtubules located along the length of the axon; it requires two molecular motors, dynein and kinesin **(Figure 4–8)**. Orthograde transport moves from the cell body toward the axon terminals. It has both fast and slow components, **fast axonal transport** occurs at about 400 mm/day, and **slow axonal transport** occurs at 0.5–10 mm/day. **Retrograde transport** from the nerve ending to the cell body occurs at about 200 mm/day. Synaptic vesicles recycle in the membrane, but some used vesicles are carried back to the cell body and deposited in lysosomes. Some materials taken up at the ending by endocytosis, including **nerve growth factor (NGF)** and some viruses, are also transported back to the cell body.

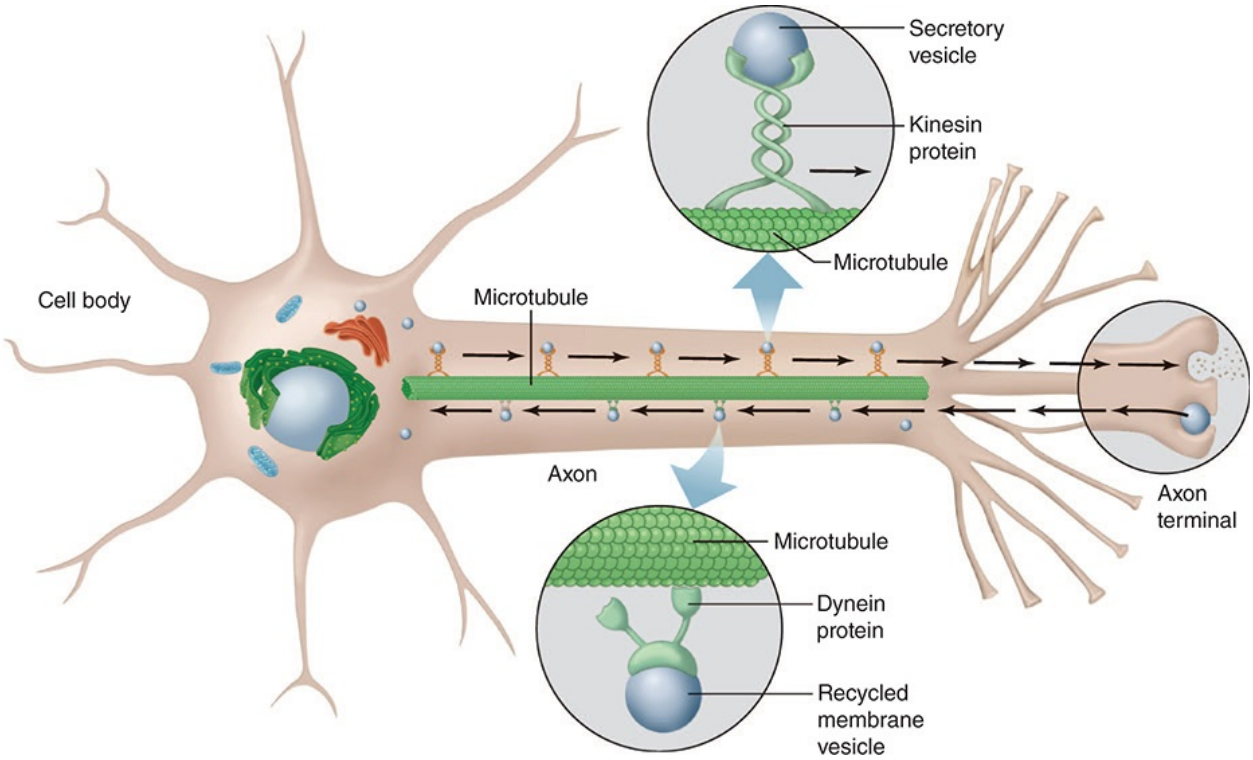


FIGURE 4–8 Axonal transport along microtubules by dynein and kinesin. Fast (400 mm/day) and slow (0.5–10 mm/day) axonal orthograde transport occurs along microtubules that run along the length of the axon from the cell body to the terminal. Retrograde transport (200 mm/day) occurs from the terminal to the cell body. (Reproduced with permission from Widmaier EP, Raff

H, Strang KT: *Vander's Human Physiology*. New York, NY: McGraw-Hill; 2008.)

GLIA

The word *glia* is Greek for *glue*. For many years following their discovery, neuroglia cells were viewed as connective tissue. Today these cells are recognized for their role in communication within the central nervous system (CNS) in partnership with neurons. Unlike most neurons, glia continue to undergo cell division in adulthood and their ability to proliferate is particularly noticeable after brain injury (eg, stroke).

Glia are classified as **microglia** and **macroglia** depending on their size. Microglia are part of the immune system; they are scavenger cells that resemble tissue macrophages and remove debris resulting from injury, infection, and disease (eg, multiple sclerosis [MS], AIDS-related dementia, Parkinson disease, and Alzheimer disease). Microglia arise from macrophages outside of the nervous system and are physiologically and embryologically unrelated to other neural cell types.

There are three types of macroglia: oligodendrocytes, Schwann cells, and astrocytes **(Figure 4–9)**. **Oligodendrocytes** and **Schwann cells** are involved in myelin formation around axons in the CNS and peripheral nervous system, respectively. Unlike the Schwann cell, which forms the myelin on a single axon (Figure 4–1), oligodendrocytes emit multiple processes that form myelin on many neighboring axons. In MS, patchy destruction of myelin occurs in the CNS **(Clinical Box 4–3)**. The loss of myelin is associated with delayed or blocked conduction in the demyelinated axons. Myelin protein zero (P0) and a hydrophobic protein PMP22 are components of the myelin sheath in the peripheral nervous system. Autoimmune reactions to these proteins cause **Guillain–Barré syndrome**, a peripheral demyelinating neuropathy. Mutations in myelin protein genes cause peripheral neuropathies that disrupt myelin and cause axonal degeneration (eg, **Charcot-Marie-Tooth disease**).

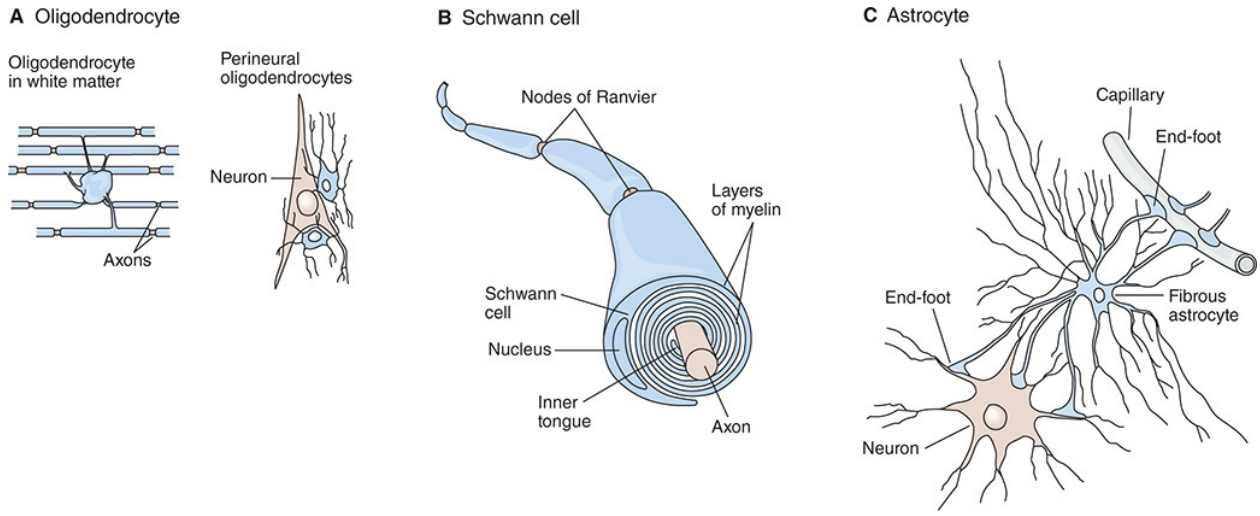


FIGURE 4–9 The principal types of macroglia in the nervous system. A) Oligodendrocytes are small with relatively few processes. Those in the white matter provide myelin, and those in the gray matter support neurons. **B)** Schwann cells provide myelin to the peripheral nervous system. Each cell forms a segment of myelin sheath about 1 mm long; the sheath assumes its form as the inner tongue of the Schwann cell turns around the axon several times, wrapping in concentric layers. Intervals between segments of myelin are the nodes of Ranvier. **C)** Astrocytes are the most common glia in the CNS and are characterized by their starlike shape. They contact both capillaries and neurons and are thought to have a nutritive function. They are also involved in forming the blood-brain barrier. (Reproduced with permission from Kandel ER, Schwartz JH, Jessell TM, Siegelbaum SA, Hudspeth AJ (editors): *Principles of Neural Science*, 5th ed. New York, NY: McGraw-Hill; 2013.)

Astrocytes are found throughout the brain and are subdivided into two groups. **Fibrous astrocytes** are found primarily in white matter and contain many intermediate filaments; **protoplasmic astrocytes** are found in gray matter and have a granular cytoplasm. Both types of astrocytes send processes to blood vessels, where they induce capillaries to form the tight junctions making up the **blood-brain barrier.** They also send processes that envelop synapses and the surface of nerve cells. Protoplasmic astrocytes have a membrane potential that varies with the external K^+ concentration but do not generate propagated potentials. They produce substances that are tropic to neurons, and they help maintain the appropriate concentration of ions and neurotransmitters by taking up K^+ and the neurotransmitters glutamate and γ-aminobutyrate (GABA).

CLINICAL BOX 4–2

Local Anesthesia

Local or regional anesthesia is used to block the conduction of action potentials in sensory and motor nerve fibers. This usually occurs as a result of blockade of voltage-gated Na^+ channels on the nerve cell membrane. This causes a gradual increase in the threshold for electrical excitability of the nerve, a reduction in the rate of rise of the action potential, and a slowing of axonal conduction velocity. There are two major categories of local anesthetics: **ester-linked** (eg, **cocaine, procaine, tetracaine**) or **amide-linked** (eg, **lidocaine, bupivacaine**). In addition to either the ester or amide, all local anesthetics contain an aromatic and an amine group. The structure of the aromatic group determines the drug's hydrophobic characteristics, and the amine group determines its latency to onset of action and its potency. Application of these drugs into the vicinity of a central (eg, **epidural, spinal anesthesia**) or peripheral nerve can lead to rapid, temporary, and near complete interruption of neural traffic to allow a surgical or other potentially noxious procedure to be done without eliciting pain. Cocaine (from the coca shrub, *Erythroxylan coca*) was the first chemical to be identified as having local anesthetic properties and remains the only naturally occurring local anesthetic. Its addictive and toxic properties prompted the development of other local anesthetics. Nociceptive fibers (unmyelinated C fibers) are the most sensitive to the blocking effect of local anesthetics. This is followed by sequential loss of sensitivity to temperature, touch, and deep pressure. Motor nerve fibers are the most resistant to the actions of local anesthetics.

NEUROTROPHINS: THEIR FUNCTION & RECEPTORS

Neurotrophins are necessary for survival and growth of neurons. Some of them are products of muscles or other structures that the neurons innervate, but many in the CNS are produced by astrocytes. These proteins bind to receptors at the endings of a neuron. They are internalized and then transported by retrograde transport to the neuronal cell body where they foster the production of proteins associated with neuronal development, growth, and survival. Other

neurotrophins are produced in soma and transported to the nerve ending where they maintain the integrity of the postsynaptic neuron.

NGF, the first neurotrophin identified, is a protein growth factor that is needed for the growth and maintenance of sympathetic neurons and some sensory neurons. NGF is made up of two *α*, two β, and two γ subunits. The β subunits, each of which has a molecular mass of 13,200 Da, have all the nerve growth-promoting activity, the α subunits have trypsin-like activity, and the γ subunits are serine proteases. The function of the proteases is unknown. The structure of the β subunit of NGF resembles that of insulin.

NGF is picked up by neurons and transported in a retrograde fashion from the nerve endings to the cell body. NGF may be responsible for the growth and maintenance of cholinergic neurons in the basal forebrain and the striatum. NGF-mediated survival of neurons is due to suppression of apoptosis rather than promotion of cell metabolism.

In addition to NGF, there are other neurotrophins, including **brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3),** and **NT-4/5.** They each maintain a different pattern of neurons, although there is some overlap. NT-3 is important for proprioceptor neurons that innervate the muscle spindle and mechanoreceptors in the skin; NT-4/5 is important for neurons that innervate the hair follicle; NGF is important for skin nociceptive neurons. Sympathetic neurons depend on both NGF and NT-3. BDNF acts rapidly and can actually depolarize neurons. BDNF-deficient mice lose peripheral sensory neurons and have severe degenerative changes in their vestibular ganglia and blunted long-term potentiation.

These four established neurotrophins and their three high-affinity **tyrosine kinase associated (Trk) receptors** are listed in **Table 4–4**. Each of these Trk receptors dimerizes and initiates phosphorylation in the cytoplasmic tyrosine kinase domains of the receptors. An additional low-affinity NGF receptor that is a 75-kDa protein is called the **p75 receptor.** This receptor binds all four of the listed neurotrophins with equal affinity. Interestingly, if a p75 receptor becomes activated in the absence of exposure to a neurotrophin, it causes apoptosis or cell death, an effect opposite to the usual growth-promoting and nurturing effects of neurotrophins. Research is ongoing to characterize the distinct roles of p75 and Trk receptors and factors that influence their expression in neurons.

TABLE 4–4 Neurotrophins.

Neurotrophin	Receptor
Nerve growth factor (NGF)	Trk A
Brain-derived neurotrophic factor (BDNF)	Trk B
Neurotrophin 3 (NT-3)	Trk C, Trk B
Neurotrophin 4/5 (NT-4/5)	Trk B

CLINICAL BOX 4–3

Demyelinating Diseases

Normal conduction of action potentials relies on the insulating properties of myelin. Thus, defects in myelin can have major adverse neurologic consequences. One example is **MS,** an autoimmune disease that affects over 3 million people worldwide, usually striking between the ages of 20 and 50 and affecting women about twice as often as men. The causes of MS include both genetic and environmental factors. It is most common among whites living in countries with temperate climates including Europe, southern Canada, northern United States, and southeastern Australia. Environmental triggers include early exposure to viruses such as Epstein-Barr virus and those that cause measles, herpes, chickenpox, or influenza. In MS, antibodies and white blood cells in the immune system attack myelin, causing inflammation and injury to the sheath and eventually the nerves that it surrounds. Loss of myelin leads to leakage of K^+ through voltage-gated channels, hyperpolarization, and failure to conduct action potentials. Initial presentation commonly includes reports of **paraparesis** (weakness in lower extremities) that may be accompanied by mild spasticity and hyperreflexia; **paresthesia;** numbness; urinary incontinence; and heat intolerance. Clinical assessment often reports **optic neuritis,** characterized by blurred vision, a change in color perception, visual field defect (**central scotoma**), and pain with eye movements; **dysarthria;** and **dysphagia.** Symptoms are often exacerbated by increased body temperature or increased ambient temperature. Progression of the disease is variable. In the most common form called **relapsing-remitting MS,** transient episodes appear suddenly, last a few weeks or months, and then gradually disappear. Subsequent episodes can appear years later, and eventually full recovery does not occur. A steadily worsening course with

only minor periods of remission (**secondary-progressive MS**) develops later in many individuals. Others have a progressive form of the disease in which there are no periods of remission (**primary-progressive MS**). Diagnosing MS is very difficult and generally is delayed until multiple episodes occur with deficits separated in time and space. **Nerve conduction tests** can detect slowed conduction in motor and sensory pathways. Cerebral spinal fluid analysis can detect the presence of **oligoclonal** bands indicative of an abnormal immune reaction against myelin. The most definitive assessment is **magnetic resonance imaging (MRI)** to visualize multiple scarred (sclerotic) areas or plaques in the brain. These plaques often appear in the periventricular regions of the cerebral hemispheres.

THERAPEUTIC HIGHLIGHTS

Although there is no cure for MS, **corticosteroids** (eg, **prednisone**) are the most common treatment used to reduce the inflammation that is accentuated during a relapse. Some drug treatments are designed to modify the course of the disease. For example, daily injections of **β-interferons** suppress the immune response to reduce the severity and slow the progression of the disease. **Glatiramer acetate** may block the immune system's attack on the myelin. **Natalizumab** interferes with the ability of potentially damaging immune cells to move from the bloodstream to the CNS. A clinical trial using B cell-depleting therapy with **rituximab,** an anti-CD20 monoclonal antibody, showed that the progression of the disease was slowed in patients younger than 51 years in whom the primary-progressive form of MS was diagnosed. Another clinical trial has shown that oral administration of **fingolimod** slowed the progression of the relapsing- remitting form of MS. This immunosuppressive drug acts by sequestering lymphocytes in the lymph nodes, thereby limiting their access to the CNS. In 2017, the Food and Drug Administration approved the use of **ocrelizumab** for the treatment of primary-progressive MS; it is an immunosuppressive agent that targets the CD20 marker on B lymphocytes.

CLINICAL BOX 4–4

Axonal Regeneration

Peripheral nerve damage is often reversible. Although the axon will

degenerate distal to the damage, connective elements of the so-called **distal stump** often survive. **Axonal sprouting** occurs from the proximal stump, growing toward the nerve ending. This results from **growth-promoting factors** secreted by **Schwann cells** that attract axons toward the distal stump. Adhesion molecules of the immunoglobulin superfamily (eg, the neuron-glia cell adhesion molecule or NgCAM/L1) promote axon growth along cell membranes and extracellular matrices. Inhibitory molecules in the perineurium ensure that the regenerating axons grow in a correct trajectory. Denervated distal stumps are able to upregulate production of **neurotrophins** that promote growth. Once the regenerated axon reaches its target, a new functional connection (eg, neuromuscular junction) is formed, allowing for considerable, although not full, recovery. For example, fine motor control may be permanently impaired because some motor neurons are guided to an inappropriate motor fiber. Nonetheless, recovery of peripheral nerves from damage far surpasses that of central nerve pathways. The proximal stump of a damaged axon in the CNS will form short sprouts, but distant stump recovery is rare, and the damaged axons are unlikely to form new synapses. This is in part because CNS neurons do not have the growth-promoting chemicals needed for regeneration. In fact, CNS myelin is a potent inhibitor of axonal growth. In addition, after a CNS injury, **astrocytic proliferation, activation of microglia, scar formation, inflammation,** and **invasion of immune cells** create an inappropriate environment for regeneration. Thus, treatment of brain and spinal cord injuries focuses on rehabilitation rather than reversing the nerve damage. New research is aiming to identify ways to initiate and maintain axonal growth, to direct regenerating axons to reconnect with their target neurons, and to reconstitute original neuronal circuitry.

THERAPEUTIC HIGHLIGHTS

There is evidence showing that the use of NSAIDs such as ibuprofen can overcome the factors that inhibit axonal growth following injury. This effect is thought to be mediated by the ability of NSAIDs to inhibit RhoA, a small GTPase protein that normally prevents repair of neural pathways and axons. Growth cone collapse in response to myelin-associated inhibitors after nerve injury is prevented by drugs (such as **pertussis toxin**) that interfere with signal transduction via trimeric G-protein. Experimental drugs that inhibit the **phosphoinositide 3-kinase (PI3) pathway** or the **inositol triphosphate (IP3) receptor** have also been shown to promote regeneration after nerve injury.

OTHER FACTORS AFFECTING NEURONAL GROWTH

The regulation of neuronal growth is a complex process. Schwann cells and astrocytes produce **ciliary neurotrophic factor (CNTF).** This factor promotes the survival of damaged and embryonic spinal cord neurons and may prove to be of value in treating human diseases in which motor neurons degenerate. **Glial cell line-derived neurotrophic factor (GDNF)** maintains the survival of midbrain dopaminergic neurons and prevents the apoptosis of spinal motor neurons. Another factor that enhances the growth of neurons is **leukemia inhibitory factor (LIF).** In addition, neurons as well as other cells respond to **insulin-like growth factor I (IGF-I)** and the various forms of **transforming growth factor (TGF), fibroblast growth factor (FGF),** and **platelet-derived growth factor (PDGF).**

Clinical Box 4–4 compares the ability to regenerate neurons after central and peripheral nerve injury.

CHAPTER SUMMARY

- Neurons are composed of a cell body (soma) that is the metabolic center of the neuron, dendrites that extend arborize extensively and are a common zone for receiving input to the neuron, the initial segment of the axon where the action potential is initiated, and a long axon that conducts the action potentials.
- The resting membrane potential of neurons is about –70 mV, which is close to the equilibrium potential for K^+. During hyperkalemia, the resting potential moves closer to the threshold for eliciting an action potential, thus the neuron becomes more excitable. During hypokalemia, the resting membrane potential is reduced and the neuron is hyperpolarized.
- In response to a depolarizing stimulus, voltage-gated Na^+ channels are activated; when the threshold potential is reached, an action potential results. The membrane potential moves toward the equilibrium potential for Na^+. The Na^+ channels are rapidly inactivated before returning to the resting state.
- The direction of the electrical gradient for Na^+ is reversed during the overshoot because the membrane potential is reversed; this limits Na^+

influx. Voltage-gated K^+ channels open to complete the process of repolarization. The slow return of the K^+ channels to the closed state explains after-hyperpolarization, followed by a return to the resting membrane potential.

- The axons of many neurons are wrapped in myelin, a protein-lipid complex that is an effective insulator; depolarization of myelinated axons travels from one node of Ranvier to the next, with the current sink at the active node serving to electrotonically depolarize to the firing level the node ahead of the action potential.
- Nerve fibers are divided into different categories (A, B, and C) based on axonal diameter, conduction velocity, and function. A numerical classification (Ia, Ib, II, III, and IV) is also used for sensory afferent fibers. These various classes differ in their sensitivity to hypoxia and anesthetics.
- Orthograde transport occurs along microtubules that run the length of the axon and requires two molecular motors: dynein and kinesin. It moves from the cell body toward the axon terminals and has both fast (400 mm/day) and slow (0.5–10 mm/day) components. Retrograde transport goes in the opposite direction (from nerve ending to cell body) at a rate of about 200 mm/day.
- There are two main types of glia: microglia and macroglia. Microglia are scavenger cells. Macroglia include oligodendrocytes, Schwann cells, and astrocytes. Oligodendrocytes and Schwann cells are involved in myelin formation; astrocytes produce substances that are tropic to neurons and help maintain the appropriate concentration of ions and neurotransmitters.
- Patchy destruction of myelin within the CNS associated with multiple sclerosis delays the conduction in axons. Autoimmune reactions to the myelin proteins P0 and PMP22 cause Guillain–Barré syndrome, a peripheral demyelinating neuropathy. Mutations in myelin protein genes cause peripheral neuropathies (eg, Charcot-Marie-Tooth disease).
- Neurotrophins (eg, NGF) are carried by retrograde transport to the neuronal cell body where they produce proteins associated with neuronal development, growth, and survival and suppress neuronal apoptosis.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. Glia are critical for the development of the nervous system and have important roles in some neurodegenerative disorder. Which of the following statements correctly describe a property of a type of glia?

   A. Microglia arise from macrophages outside of the nervous system and are physiologically and embryologically similar to other neural cell types.

   B. Fibrous astrocytes are found primarily in the gray matter and induce capillaries to form tight junctions to form the blood-brain barrier.

   C. Protoplasmic astrocytes produce substances that are tropic to neurons to maintain the appropriate concentration of ions and neurotransmitters by taking up K^+ and the neurotransmitters glutamate and GABA.

   D. Oligodendrocytes and Schwann cells are involved in myelin formation around axons in the peripheral nervous system and central nervous system, respectively.

   E. Macroglia are scavenger cells that resemble tissue macrophages and remove debris resulting from injury, infection, and disease.

2. Primary erythromelalgia due to a peripheral nerve sodium channelopathy was diagnosed in a 13-year-old girl who was experiencing frequent episodes of red, painful, warm extremities. Which part of a neuron has the highest concentration of Na^+ channels per square micrometer of cell membrane?

   A. dendrites

   B. cell body near dendrites

   C. initial segment

   D. axonal membrane under myelin

   E. node of Ranvier

3. A 45-year-old woman who works in an office had been experiencing tingling in her index and middle fingers and thumb of her right hand. Recently, her wrist and hand had become weak. Her physician ordered a nerve conduction test to evaluate her for carpal tunnel syndrome. Which one of the following nerves has the slowest conduction velocity?

   A. Aα fibers

   B. Aβ fibers

   C. Aγ fibers

   D. B fibers

   E. C fibers

4. Axoplasmic flow is a cellular process responsible for movement of proteins

and polypeptides within a neuron. Which of the following statements correctly describes a property of orthograde or retrograde axonal transport?

A. Synaptic transmission: Antidromic conduction

B. Molecular motors for orthograde and retrograde transport are dynein and kinesin, respectively.

C. The rate of retrograde fast axonal transport is ∼400 mm/day

D. The rate of orthograde slow axonal transport is ∼200 mm/day

E. Some viruses use retrograde transport to move from the nerve terminal to the soma.

5. For a summer research project, a second-year medical student is introduced to the patch clamp technique in a neurophysiology laboratory. As part of her training, she learns to monitor both membrane potential and individual channel function. Which of the following ionic changes is correctly matched with a component of the action potential?

A. Opening of voltage-gated K^+ channels: After-hyperpolarization

B. A decrease in extracellular Ca^{2+}: Repolarization

C. Opening of voltage-gated Na^+ channels: Depolarization

D. Rapid closure of voltage-gated Na^+ channels: Resting membrane potential

E. Rapid closure of voltage-gated K^+ channels: Relative refractory period

6. A man falls into a deep sleep with one arm under his head. This arm is paralyzed when he awakens, but it tingles, and pain sensation in it is still intact. The reason for the loss of motor function without loss of pain sensation is:

A. A fibers are more susceptible to hypoxia than B fibers.

B. A fibers are more sensitive to pressure than C fibers.

C. C fibers are more sensitive to pressure than A fibers.

D. Motor nerves are more affected by sleep than sensory nerves.

E. Sensory nerves are nearer the bone than motor nerves and hence are less affected by pressure.

7. Neurotrophins foster the production of proteins associated with neuronal development, growth, and survival. Which of the following statements about nerve growth factor (NGF) is true?

A. NGF is made up of one α, two β, and one γ polypeptide subunits.

B. NGF is responsible for the growth and maintenance of adrenergic neurons

in the basal forebrain and the striatum.

C. NGF uses orthograde transport to move from the soma to the nerve terminal of a neuron.

D. NGF is important for the growth of sensory neurons that innervate the muscle spindle.

E. NGF binds to both p75 receptor and Trk A receptors.

8. A 20-year-old female student awakens one morning with severe pain and blurry vision in her left eye; the symptoms abate over several days. About 6 months later, on a morning after playing volleyball with friends, she notices weakness but not pain in her right leg; the symptoms intensify while taking a hot shower. Which of the following is most likely to be the case?

A. The two episodes described are not likely to be related.

B. She may have primary-progressive multiple sclerosis.

C. She may have relapsing-remitting multiple sclerosis.

D. She may have a lumbar disk rupture.

E. She may have Guillain–Barré syndrome.

9. A medical student was working in a neurophysiology lab and was learning factors that determine the resting membrane potential of a neuron. Which of the following statements correctly explains how a change in concentration of an ion inside or outside of the neuron would change its resting membrane potential?

A. A decrease in extracellular Ca^{2+} concentration would stabilize the membrane and reduce its excitability.

B. A decrease in the extracellular Na^{+} concentration would reduce the size of the resting membrane potential.

C. An increase in the extracellular K^{+} concentration would move the resting membrane potential from a normal value of −90 mV to −70 mV.

D. A decrease in the extracellular K^{+} concentration increases the gradient for K^{+} to leak out of the neuron, making the cell more hyperpolarized.

E. A decrease in intracellular Na^{+} concentration would make the resting membrane potential more negative.

10. A precocious 6-year-old girl asked her aunt who was a neurologist at a local medical school to explain how a neuron works. Her aunt explained how different parts of the neuron have a specific role in generating and transmitting an action potential. She identified the part of the neuron where

the action potential is initiated, a part that receives input from other neurons, and a part that conducts the action potential. These would have been as follows:

A. Soma, dendrite, axon

B. Axon hillock, soma, myelin sheath

C. Cell membrane, soma, dendrite

D. Initial segment, dendrite, axon

E. Axon hillock, soma, myelin sheath

CHAPTER 5

Excitable Tissue: Muscle

OBJECTIVES

After studying this chapter, you should be able to:

- Differentiate the major classes of muscle in the body.
- Describe the molecular and electrical makeup of muscle cell excitation–contraction coupling.
- Define elements of the sarcomere that underlie striated muscle contraction.
- Differentiate the role(s) for Ca^{2+} in skeletal, cardiac, and smooth muscle contraction.
- Appreciate muscle cell diversity and function.

INTRODUCTION

Muscle cells, like neurons, can be excited chemically, electrically, and mechanically to produce an action potential that is transmitted along their cell membranes. Unlike neurons, they respond to stimuli by activating a contractile mechanism. The contractile protein myosin and the cytoskeletal protein actin are abundant in muscle, where they are the primary structural components that bring about contraction.

Muscle is generally divided into three types: **skeletal muscle, cardiac muscle,** and **smooth muscle,** although smooth muscle is not a homogeneous single category. Skeletal muscle makes up the great mass of the somatic musculature. It has well-developed cross-striations, does not normally contract in the absence of nervous stimulation, lacks anatomic and functional connections between individual muscle fibers, and is generally under voluntary control. Cardiac muscle also has cross-striations, but it is functionally syncytial and, although it can be modulated via the autonomic nervous system, it can contract rhythmically in the absence of external innervation owing to the presence in the myocardium of pacemaker cells that discharge spontaneously (see Chapter 29). Smooth muscle lacks cross-striations and can be further subdivided into two broad types: unitary (or visceral) smooth muscle and multiunit smooth muscle. The type found in most hollow viscera is functionally syncytial and contains pacemakers that discharge irregularly. The multiunit type found in the eye and in some other locations is not spontaneously active and resembles skeletal muscle in graded contractile ability. Smooth muscle is also classified on the basis of its function or activity pattern to rhythmic/phasic smooth muscle and continuous/tonic smooth muscle.

SKELETAL MUSCLE MORPHOLOGY

ORGANIZATION

Skeletal muscle is made up of individual muscle fibers that are the “building blocks” of the muscular system in the same sense that the neurons are the building blocks of the nervous system. Most skeletal muscles begin and end in tendons, and the muscle fibers are arranged in parallel between the tendinous ends, so that the force of contraction of the units is additive. Each muscle fiber is a single cell that is multinucleated, long, cylindrical, and surrounded by a cell membrane, the **sarcolemma** **(Figure 5–1)**. There are no syncytial bridges between cells. The muscle fibers are made up of myofibrils, which are divisible into individual filaments. These myofilaments contain several proteins that together make up the contractile machinery of the skeletal muscle.

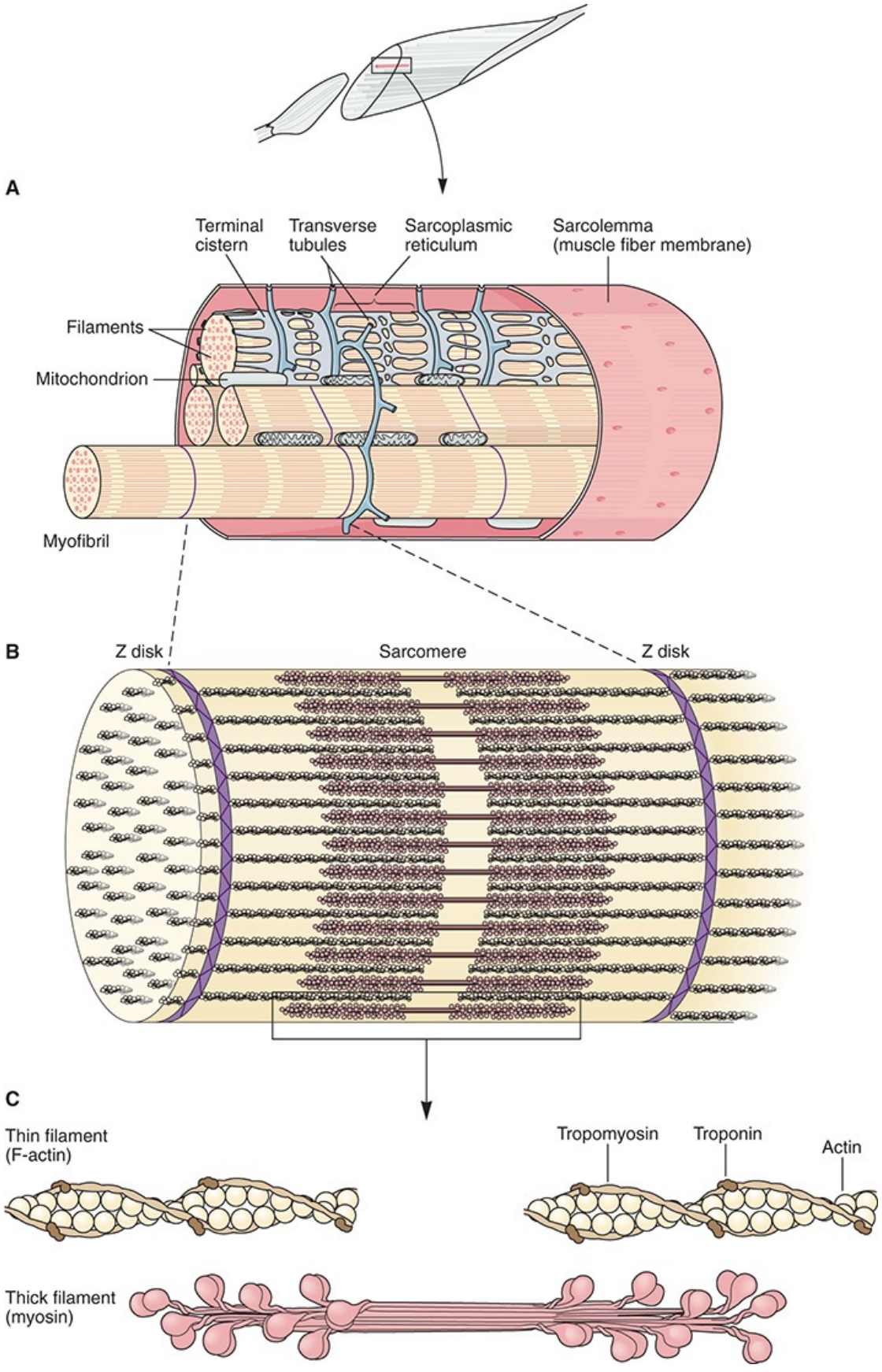

A
Terminal cistern
Transverse tubules
Sarcoplasmic reticulum
Sarcolemma (muscle fiber membrane)
Filaments
Mitochondrion
Myofibril
B
Z disk
Sarcomere
Z disk
C
Thin filament (F-actin)
Tropomyosin
Troponin
Actin
Thick filament (myosin)

FIGURE 5–1 Mammalian skeletal muscle. A) A single muscle fiber surrounded by its sarcolemma has been cut away to show individual myofibrils. The cut surface of the myofibrils shows the arrays of thick and thin filaments. The sarcoplasmic reticulum with its transverse (T) tubules and terminal cisterns surrounds each myofibril. The T tubules invaginate from the sarcolemma and contact the myofibrils twice in every sarcomere. Mitochondria are found between the myofibrils and a basal lamina surrounds the sarcolemma. **B** and **C)** Structural elements of myofibril shown in detail (see also Figure 5–2).

The contractile mechanism in skeletal muscle largely depends on the proteins **myosin-II, actin, tropomyosin,** and **troponin.** Troponin is made up of three subunits: **troponin I, troponin T,** and **troponin C.** Other important proteins in muscle are involved in maintaining the proteins that participate in contraction in appropriate structural relation to one another and to the extracellular matrix.

STRIATIONS

Differences in the refractive indexes of the various parts of the muscle fiber are responsible for the characteristic cross-striations seen in skeletal muscle when viewed under the microscope. The parts of the cross-striations are frequently identified by letters A, H, I, M, and Z **(Figure 5–2)**. The light I band is divided by the dark Z-line, and the dark A band has the lighter H band in its center. A transverse M-line is seen in the middle of the H band, and this line plus the narrow light areas on either side of it are sometimes called the pseudo-H zone. The area between two adjacent Z-lines is called a **sarcomere.** The orderly arrangement of actin, myosin, and related proteins that produces this pattern can also be seen in Figures 5–1 and 5–2. The thick filaments, which are about twice the diameter of the thin filaments, are made up of myosin; the thin filaments are made up of actin, tropomyosin, and troponin. The thick filaments are lined up to form the A bands, whereas the array of thin filaments extends out of the A band and into the less dense staining I bands. The lighter H bands in the center of the A bands are the regions where, when the muscle is relaxed, the thin filaments do not overlap the thick filaments. The Z-lines allow for anchoring of the thin filaments. If a transverse section through the A band is examined under the electron microscope, each thick filament is seen to be surrounded by six thin filaments in a regular hexagonal pattern.

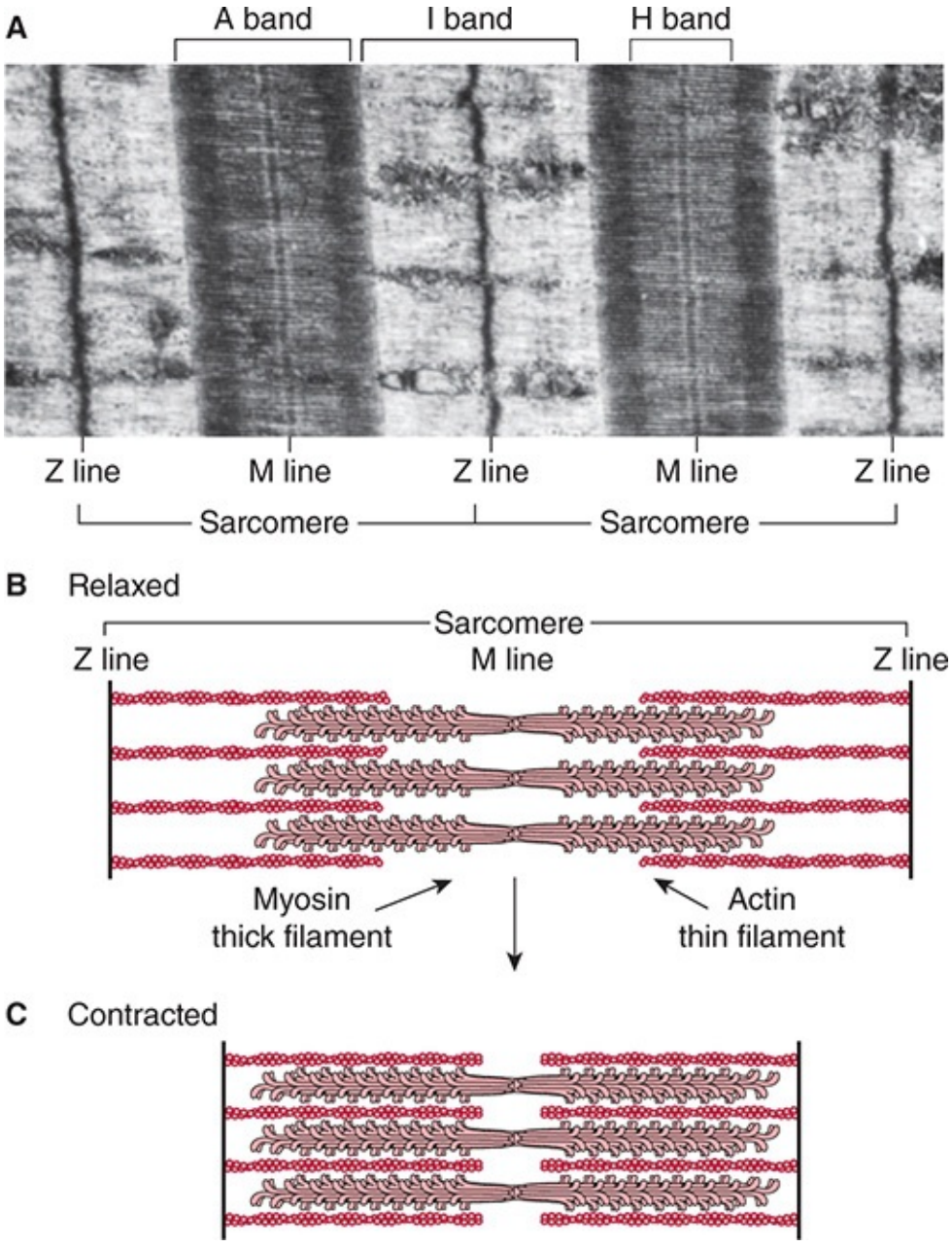


FIGURE 5–2 Skeletal muscle sarcomere. A) Electron micrograph of human gastrocnemius muscle (× 13,500). The sarcomere, named bands, and lines are shown. (Used with permission from GM Walker and GR Schrodt.) **B)** Arrangement of thin (actin) and thick (myosin) filaments and the Z-line in a relaxed skeletal muscle. **C)** Arrangement of thin and thick filaments and the Z-line in a contracted skeletal muscle. Note that the Z-lines come together as the thick and thin filaments slide next to each other during contraction. The thick and thin filaments do not change in size.

The form of myosin found in muscle is myosin-II, with two globular heads and a long tail. The heads of the myosin molecules form cross-bridges with actin. Myosin contains heavy chains and light chains, and its heads are made up of the light chains and the amino terminal portions of the heavy chains. These heads contain an actin-binding site and a catalytic site that hydrolyzes adenosine triphosphate (ATP). The myosin molecules are arranged symmetrically on either side of the center of the sarcomere, and it is this arrangement that creates the light areas in the pseudo-H zone. The M-line is the site of the reversal of polarity of the myosin molecules in each of the thick filaments. At these points, there are slender cross-connections that hold the thick filaments in proper array. Each thick filament contains several hundred myosin molecules.

The thin filaments are polymers made up of two chains of actin that form a long double helix. Tropomyosin molecules are long filaments located in the groove between the two chains in the actin. Each thin filament contains 300–400 actin molecules and 40–60 tropomyosin molecules. Troponin molecules are small globular units located at intervals along the tropomyosin molecules. Each of the three troponin subunits has a unique function: Troponin T binds the troponin components to tropomyosin, troponin I inhibits the interaction of myosin with actin, and troponin C contains the binding sites for the Ca^{2+} that initiates contraction.

Some additional structural proteins that are important in skeletal muscle function include **actinin, titin,** and **desmin.** Actinin binds actin to the Z-lines. Titin, the largest known protein (with a molecular mass near 3,000,000 Da), connects the Z-lines to the M-lines and provides scaffolding for the sarcomere. It contains two kinds of folded domains that provide muscle with its elasticity. At first when the muscle is stretched, there is relatively little resistance as the domains unfold, but with further stretch, there is a rapid increase in resistance that protects the structure of the sarcomere. Desmin adds structure to the Z-lines in part by binding the Z-lines to the plasma membrane. Some muscle disorders associated with these structural components are described in **Clinical Box 5–1**. It should be noted that although these proteins are important in muscle structure/function, by no means do they represent an exhaustive list.

CLINICAL BOX 5–1

Structural & Metabolic Disorders in Muscle Disease

The term **muscular dystrophy** is applied to diseases that cause progressive

weakness of skeletal muscle. About 50 such diseases have been described, some of which include cardiac as well as skeletal muscle. They range from mild to severe and some are eventually fatal. They have multiple causes, but mutations in the genes for the various components of the dystrophin–glycoprotein complex are a prominent cause. The dystrophin gene is one of the largest in the body, and mutations can occur at many different sites in it. **Duchenne muscular dystrophy** is a serious form of dystrophy in which the dystrophin protein is absent from muscle. It is X-linked and usually fatal by the age of 30. In a milder form of the disease, **Becker muscular dystrophy,** dystrophin is present but altered or reduced in amount. Limb-girdle muscular dystrophies of various types are associated with mutations of the genes coding for the sarcoglycans or other components of the dystrophin–glycoprotein complex.

Due to its enormous size and structural role in the sarcomere, **titin** is a prominent target for mutations that give rise to muscle disease. Mutations that encode for shorter titin structure have been associated with dilated cardiomyopathy, while other mutations have been associated with hypertrophic cardiomyopathy. The skeletal muscle-associated tibialis muscular dystrophy is a genetic muscle disease of titin that is predicted to destabilize the folded state of the protein. Interestingly, many of the titin mutations identified thus far are in regions of titin that are expressed in all striated muscles, yet not all muscles are affected in the same way. Such muscle type-specific phenotypes underscore the need to study titin's multiple functions in different muscles, under both normal and pathologic conditions.

Desmin-related myopathies are a very rare heterogeneous group of muscle disorders that typically result in cellular aggregates of desmin. Common symptoms of these diseases are failing and wasting in the distal muscles of the lower limbs that can later be identified in other body areas. Studies in desmin knockout mice have revealed defects in skeletal, smooth, and cardiac muscle, notably in the diaphragm and heart.

Metabolic Myopathies

Mutations in genes that code for enzymes involved in the metabolism of carbohydrates, fats, and proteins to CO_2 and H_2O in muscle and the production of ATP can cause **metabolic myopathies** (eg, McArdle syndrome). Metabolic myopathies all have in common exercise intolerance and the possibility of muscle breakdown due to accumulation of toxic metabolites.

THERAPEUTIC HIGHLIGHTS

Although acute muscle pain and soreness can be treated with anti-inflammatory drugs and rest, the genetic dysfunctions described above are not as easily addressed. The overall goals are to slow muscle function/structure loss and, when possible relieve symptoms associated with the disease. Extensive monitoring, physical therapy, and appropriate drugs including corticosteroids can aid to slow disease progression. Assistive devices and surgery are not uncommon as the diseases progress.

SARCOTUBULAR SYSTEM

The muscle fibrils are surrounded by structures made up of membranes that appear in electron micrographs as vesicles and tubules. These structures form the **sarcotubular system,** which is made up of a **T system** and a **sarcoplasmic reticulum.** The T system of transverse tubules, which is continuous with the sarcolemma of the muscle fiber, forms a grid perforated by the individual muscle fibrils (Figure 5–1). The space between the two layers of the T system is an extension of the extracellular space. The sarcoplasmic reticulum, which forms an irregular curtain around each of the fibrils, has enlarged **terminal cisterns** in close contact with the T system at the junctions between the A and I bands. At these points of contact, the arrangement of the central T system with a cistern of the sarcoplasmic reticulum on either side has led to the use of the term **triads** to describe the system. The T system, which is continuous with the sarcolemma, provides a path for the rapid transmission of the action potential from the cell plasma membrane to all the fibrils in the muscle. The sarcoplasmic reticulum is an important store of Ca^{2+} and also participates in muscle metabolism.

DYSTROPHIN–GLYCOPROTEIN COMPLEX

The large **dystrophin** protein (molecular mass 427,000 Da) forms a rod that connects the thin actin filaments to the transmembrane protein **β-dystroglycan** in the sarcolemma by smaller proteins in the cytoplasm, **syntrophins.** β-Dystroglycan is connected to **merosin** (merosin refers to laminins that contain the α_2 subunit in their trimeric makeup) in the extracellular matrix by **α-dystroglycan** **(Figure 5–3)**. The dystroglycans are in turn associated with a

complex of four transmembrane glycoproteins: α-, β-, γ-, and δ-**sarcoglycan.** This **dystrophin–glycoprotein complex** adds strength to the muscle by providing a scaffolding for the fibrils and connecting them to the extracellular environment. Disruption of these important structural features can result in several different muscular dystrophies (see Clinical Box 5–1).

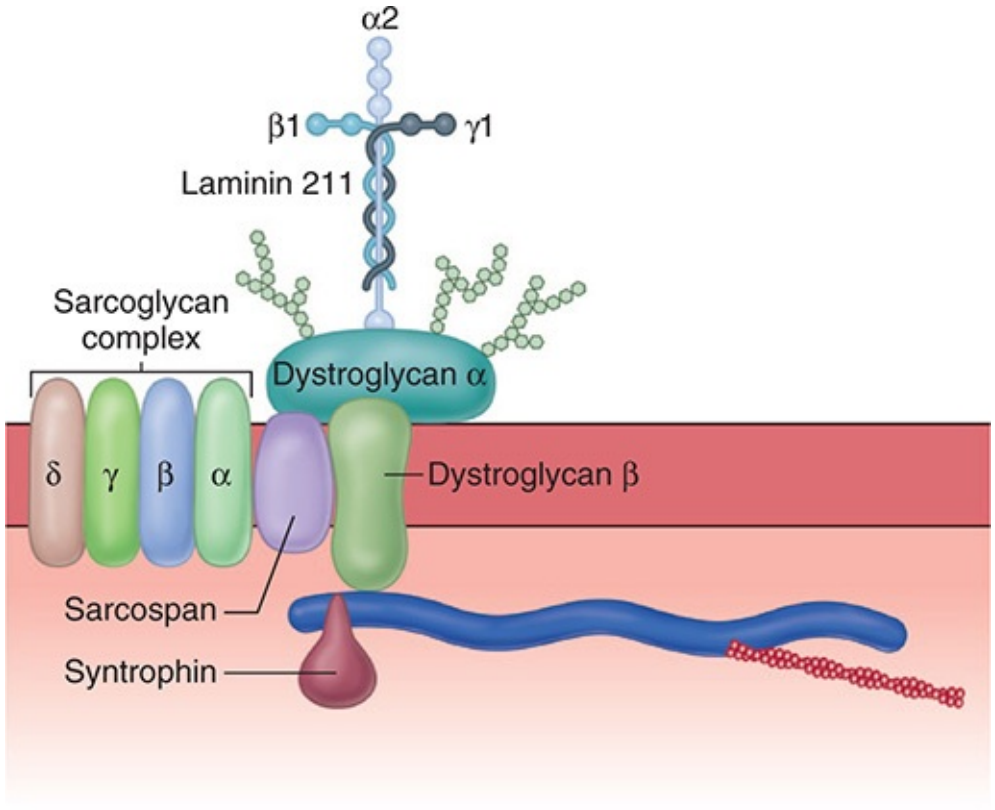


FIGURE 5–3 The dystrophin–glycoprotein complex. Dystrophin connects F-actin to the two members of the dystroglycan (DG) complex, α and β-dystroglycan, and these in turn connect to the merosin subunit of laminin 211 in the extracellular matrix. The sarcoglycan complex of four glycoproteins, α-, β-, γ-, and δ-sarcoglycan, sarcospan, and syntropins are all associated with the dystroglycan complex. There are muscle disorders associated with loss, abnormalities, or both of the sarcoglycans and merosin. (Used with permission of Justin Fallon and Kevin Campbell.)

ELECTRICAL PHENOMENA & IONIC FLUXES

ELECTRICAL CHARACTERISTICS OF

SKELETAL MUSCLE

The electrical events in skeletal muscle and the ionic fluxes that underlie them share distinct similarities to those in nerve, with quantitative differences in timing and magnitude. The resting membrane potential of skeletal muscle is about −90 mV. The action potential lasts 2–4 ms and is conducted along the muscle fiber at about 5 m/s. The absolute refractory period is 1–3 ms long, and the after-polarizations, with their related changes in threshold to electrical stimulation, are relatively prolonged. The initiation of impulses at the myoneural junction is discussed in Chapter 6.

ION DISTRIBUTION & FLUXES

The distribution of ions across the muscle fiber membrane is similar to that across the nerve cell membrane. Approximate values for the various ions and their equilibrium potentials are shown in **Table 5–1**. As in nerves, membrane depolarization is largely a manifestation of Na^+ influx, and repolarization is largely a manifestation of K^+ efflux.

TABLE 5–1 Steady-state distribution of ions in the intracellular and extracellular compartments of mammalian skeletal muscle, and the equilibrium potentials for these ions.

	Concentration (mmol/L)		
Ion[a]	Intracellular Fluid	Extracellular Fluid	Equilibrium Potential (mV)
Na^+	12	145	+65
K^+	155	4	–95
H^+	13×10^{-5}	3.8×10^{-5}	–32
Cl^-	3.8	120	–90
HCO_3^-	8	27	–32
A^-	155	0	…
Membrane potential = –90 mV			

[a] A^- represents organic anions. The value for intracellular Cl^- is calculated from the membrane potential, using the Nernst equation.

CONTRACTILE RESPONSES

It is important to distinguish between the electrical and mechanical events in skeletal muscle. Although one response does not normally occur without the other, their physiological bases and characteristics are different. Muscle fiber membrane depolarization normally starts at the motor endplate, the specialized structure under the motor nerve ending. The action potential is transmitted along the muscle fiber and initiates the contractile response by inducing Ca^{2+} influx and release.

THE MUSCLE TWITCH

A single action potential causes a brief contraction followed by relaxation. This response is called a **muscle twitch.** In **Figure 5–4**, the action potential and the twitch are plotted on the same time scale. The twitch starts about 2 ms after the start of membrane depolarization, before repolarization is complete. The duration of the twitch varies with the type of muscle being tested. “Fast” muscle fibers, primarily those concerned with fine, rapid, precise movement, have twitch durations as short as 7.5 ms. “Slow” muscle fibers, principally those involved in strong, gross, sustained movements, have twitch durations up to 100 ms.

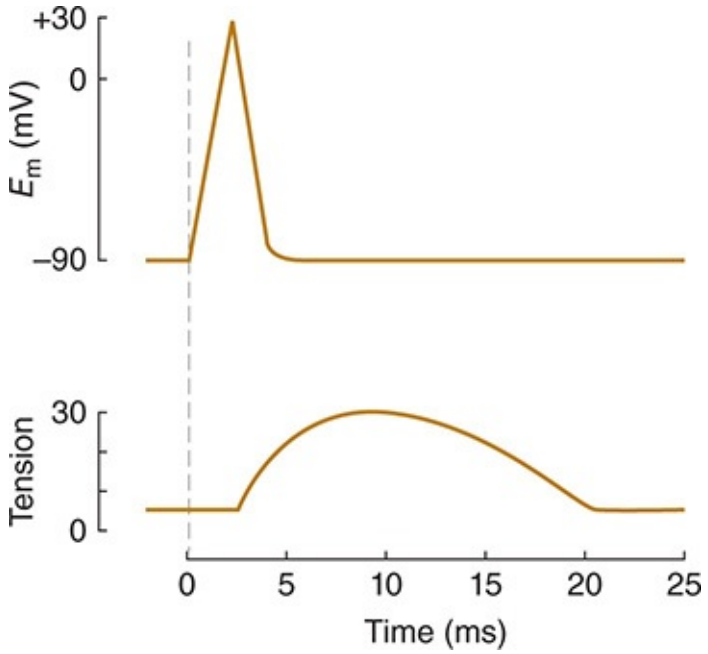


FIGURE 5–4 The electrical and mechanical responses of a mammalian

skeletal muscle fiber to a single maximal stimulus. The electrical response or change in membrane potential (E_m) in mV and the mechanical response or tension (T) in in arbitrary units are plotted on the same abscissa (time in ms). The mechanical response is relatively long-lived compared to the electrical response that initiates contraction.

MOLECULAR BASIS OF CONTRACTION

The process by which the contraction of muscle is brought about is a sliding of the thin filaments over the thick filaments. Note that this shortening is not due to changes in the actual lengths of the thick and thin filaments, rather, due to their increased overlap within the muscle cell. The width of the A bands is constant, whereas the Z-lines move closer together when the muscle contracts and farther apart when it relaxes (Figure 5–2).

The sliding during muscle contraction occurs when the myosin heads bind firmly to actin, bend at the junction of the head with the neck, and then detach. This "power stroke" depends on the simultaneous hydrolysis of ATP. Myosin-II molecules are dimers that have two heads, but only one attaches to actin at any given time. The probable sequence of events of the power stroke is outlined in **Figure 5–5**. In resting muscle, troponin I is bound to actin and tropomyosin and covers the sites where myosin heads interact with actin. Also at rest, the myosin head contains tightly bound adenosine phosphate (ADP) and inorganic phosphate (Pi). Following an action potential, cytosolic Ca^{2+} is increased (due to Ca^{2+} release from the sarcoplasmic reticulum and Ca^{2+} influx through voltage-gated Ca^{2+} channels) and free Ca^{2+} binds to troponin C. This binding results in a weakening of the troponin I interaction with actin and exposes the actin binding site for myosin to allow for formation of myosin/actin cross-bridges. Upon formation of the cross-bridge, ADP is released, causing a conformational change in the myosin head that moves the thin filament relative to the thick filament, comprising the cross-bridge "power stroke." ATP quickly binds to the free site on the myosin, which leads to a detachment of the myosin head from the thin filament. ATP is hydrolyzed and P_i released, causing a "re-cocking" of the myosin head and completing the cycle. As long as cytosolic Ca^{2+} remains elevated and sufficient ATP is available, this cycle repeats. Many myosin heads cycle at or near the same time, and they cycle repeatedly, producing gross muscle contraction. Each power stroke shortens the sarcomere about 10 nm. Each thick filament has about 500 myosin heads, and each head cycles about

five times per second during a rapid contraction.

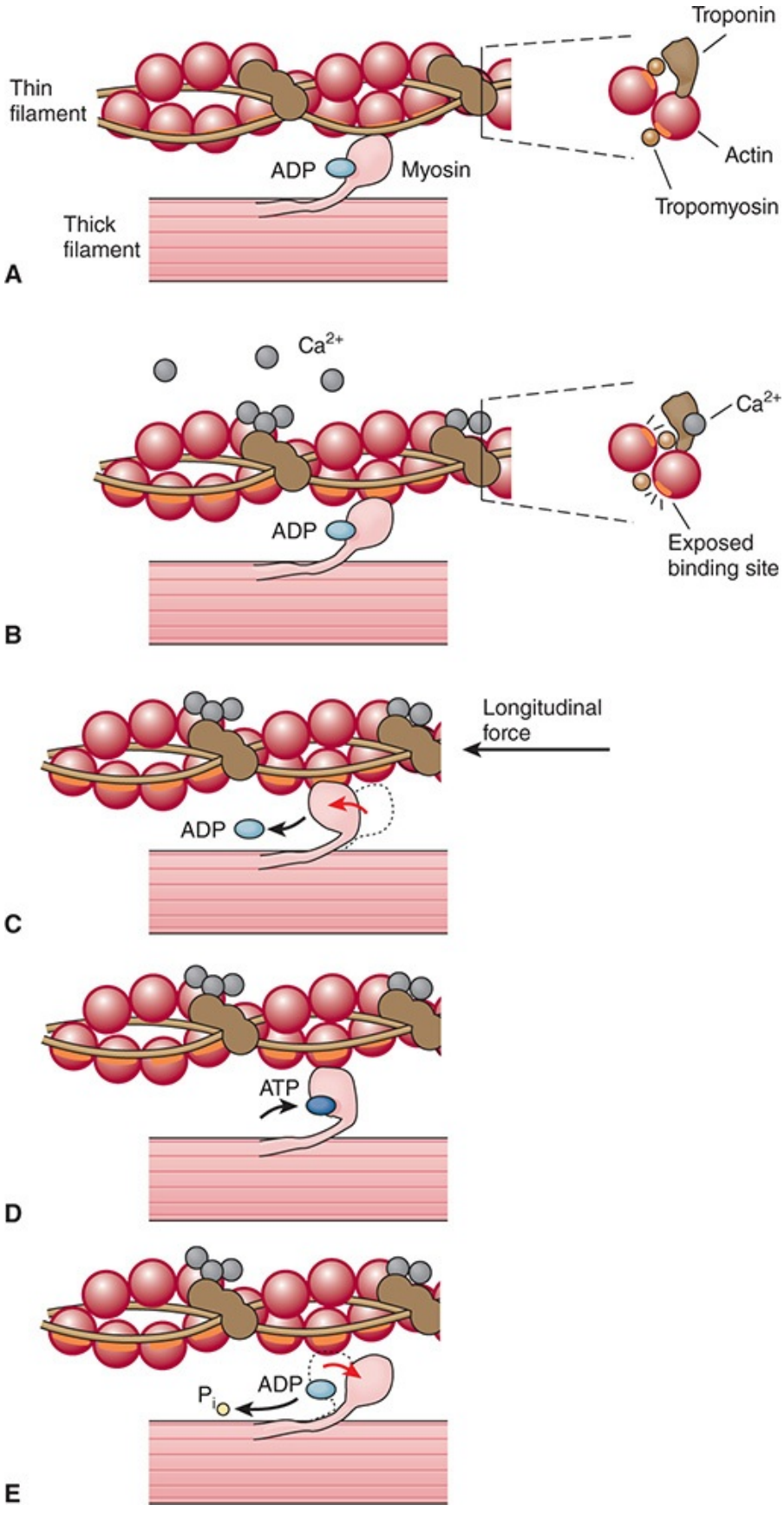
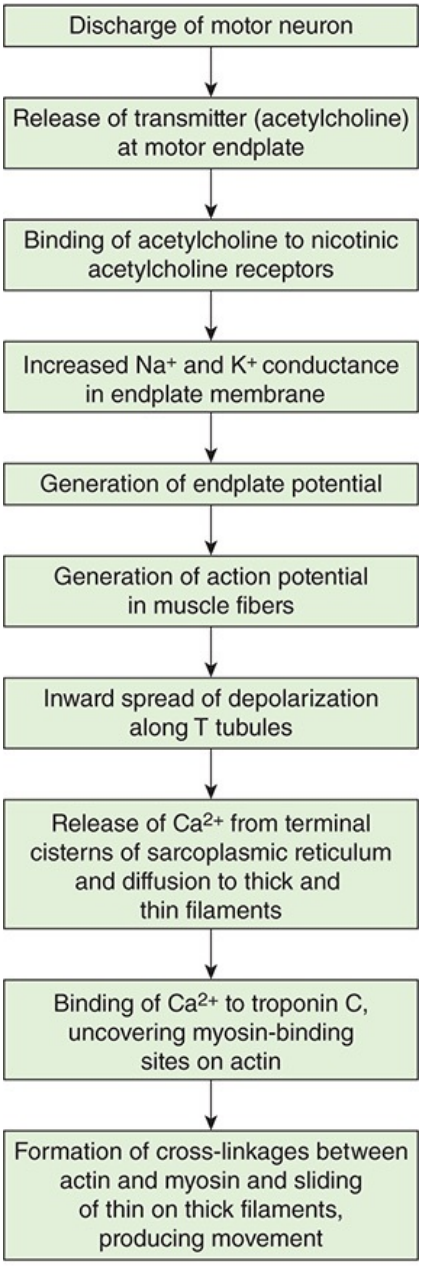

Troponin
Thin filament
Actin
ADP
Myosin
Tropomyosin
Thick filament
A
Ca^{2+}
Ca^{2+}
ADP
Exposed binding site
B
Longitudinal force
ADP
C
ATP
D
P_i
ADP
E

FIGURE 5–5 Power stroke of myosin in skeletal muscle. A) At rest, myosin heads are bound to adenosine diphosphate and are said to be in a "cocked" position in relation to the thin filament, which does not have Ca^{2+} bound to the troponin—tropomyosin complex. **B)** Ca^{2+} bound to the troponin—tropomyosin complex induces a conformational change in the thin filament that allows for myosin heads to cross-bridge with thin filament actin. **C)** Myosin heads rotate, move the attached actin, and shorten the muscle fiber, forming the power stroke. **D)** At the end of the power stroke, ATP binds to a now exposed site and causes a detachment from the actin filament. **E)** ATP is hydrolyzed into ADP and inorganic phosphate (P_i) and this chemical energy is used to "re-cock" the myosin head. (Data from Huxley AF, Simmons RM: Proposed mechanism of force generation in striated muscle. Nature Oct 22;233(5321):533–538, 1971 and Squire JM: Molecular mechanisms in muscular contraction. Trends Neurosci 6:409–413, 1983.)

The process by which membrane depolarization of the muscle fiber initiates contraction is called **excitation–contraction coupling.** The action potential is transmitted to all the fibrils in the fiber via the T system **(Figure 5–6)**. It triggers the release of Ca^{2+} from the terminal cisterns, the lateral sacs of the sarcoplasmic reticulum next to the T system. Depolarization of the T tubule membrane activates the sarcoplasmic reticulum via **dihydropyridine receptors (DHPRs),** named for the drug dihydropyridine, which blocks them **(Figure 5–7)**. DHPRs are voltage-gated Ca^{2+} channels in the T tubule membrane. In cardiac muscle, influx of Ca^{2+} via these channels triggers the release of Ca^{2+} stored in the sarcoplasmic reticulum (ie, Ca^{2+}-induced Ca^{2+} release) by activating the **ryanodine receptor (RyR).** The RyR is named after the plant alkaloid ryanodine that was used in its discovery. The RyR is a ligand-gated Ca^{2+} channel with Ca^{2+} as its natural ligand. In skeletal muscle, Ca^{2+} entry from the extracellular fluid (ECF) by this route is not required for Ca^{2+} release. Instead, the DHPR that serves as the voltage sensor triggers release of Ca^{2+} from the nearby sarcoplasmic reticulum via physical interaction with the RyR. The released Ca^{2+} is quickly amplified through Ca^{2+}-induced Ca^{2+} release. Cytosolic Ca^{2+} concentration is reduced in the muscle cell by the sarcoplasmic or endoplasmic reticulum Ca^{2+} ATPase (SERCA). The SERCA pump uses energy from ATP hydrolysis to remove Ca^{2+} from the cytosol back into the terminal cisterns against its concentration gradient, where it is stored until released by the next

action potential. Once cytosolic Ca^{2+} concentration, or the Ca^{2+} concentration outside the sarcoplasmic reticulum, has been lowered sufficiently, chemical interaction between myosin and actin ceases and the muscle relaxes. Note that ATP provides the energy for both contraction (at the myosin head) and relaxation (via SERCA). If transport or uptake of Ca^{2+} into the sarcoplasmic reticulum is inhibited, relaxation does not occur even though there are no more action potentials; the resulting sustained contraction is called a **contracture.** Alterations in the excitable response in muscle underscore many different pathologic conditions **(Clinical Box 5–2)**.

Steps in contraction



Steps in relaxation

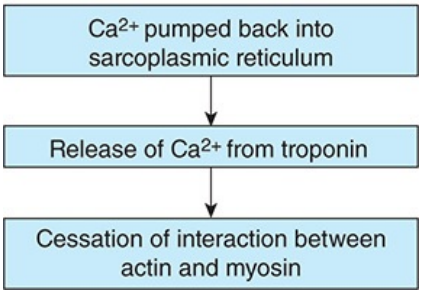


FIGURE 5–6 Flow of information that leads to muscle contraction.

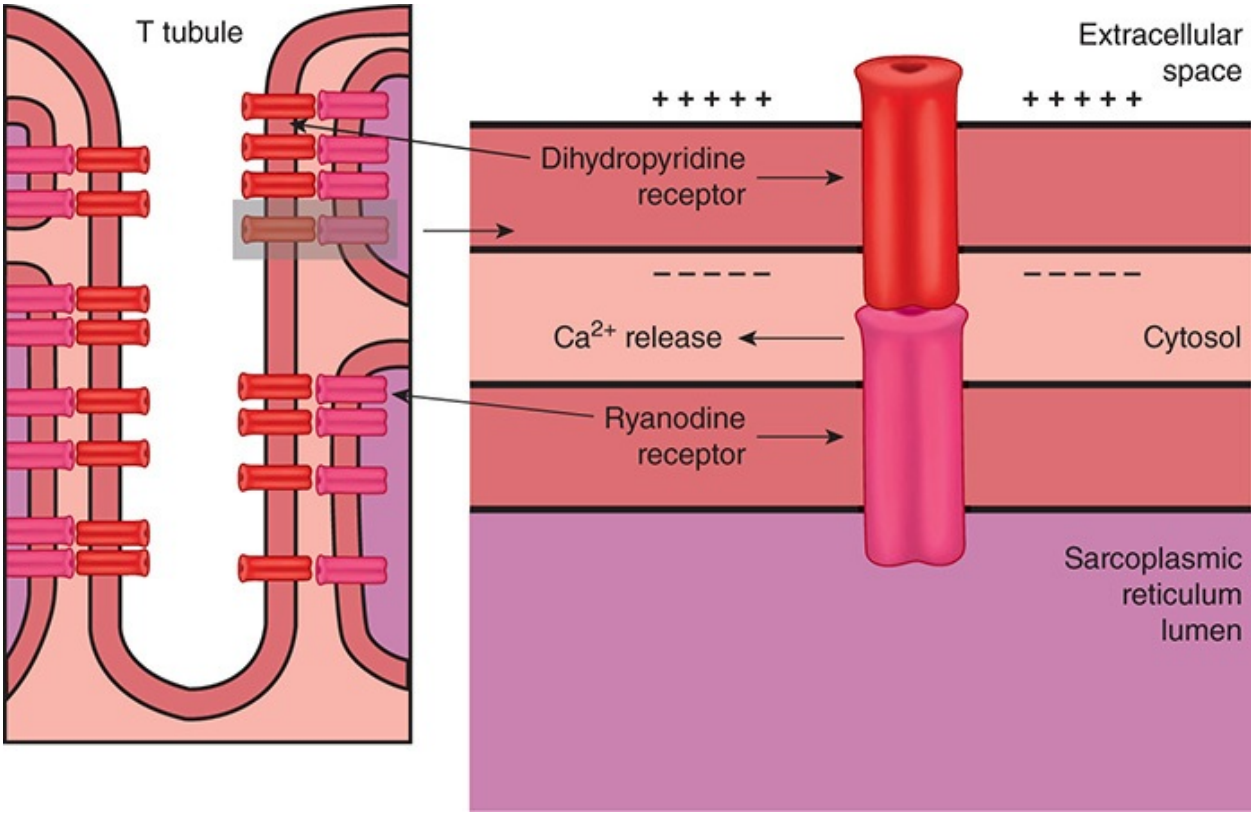


FIGURE 5–7 Relation of the T tubule to the sarcoplasmic reticulum in Ca^{2+} transport. In skeletal muscle, the voltage-gated dihydropyridine receptor in the T tubule triggers Ca^{2+} release from the sarcoplasmic reticulum (SR) via the ryanodine receptor (RyR). Upon sensing a voltage change, there is a physical interaction between the sarcolemmal-bound DHPR and the SR-bound RyR. This interaction gates the RyR and allows for Ca^{2+} release from the SR.

TYPES OF CONTRACTION

Muscular contraction involves shortening of the contractile elements, but because muscles have elastic and viscous elements in series with the contractile mechanism, it is possible for contraction to occur without an appreciable decrease in the length of the whole muscle **(Figure 5–8)**. Such a contraction is called **isometric** (“same measure” or length). Contraction against a constant load with a decrease in muscle length is **isotonic** (“same tension”). Note that because work is the product of force times distance, isotonic contractions do work,

whereas isometric contractions do not. In other situations, muscle can do negative work while lengthening against a constant weight.

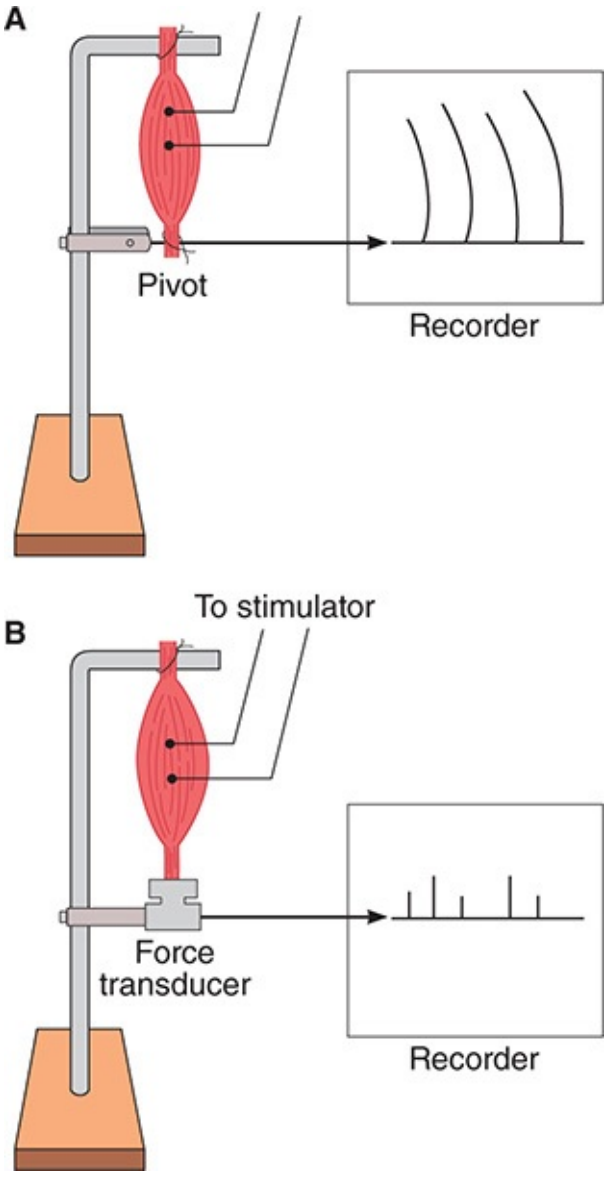


FIGURE 5–8 Isotonic and isometric contractions. A) Muscle preparation arranged for recording isotonic contractions. **B)** Preparation arranged for recording isometric contractions. In **A,** the muscle is fastened to a writing lever that swings on a pivot. In **B,** it is attached to an electronic transducer that measures the force generated without permitting the muscle to shorten.

SUMMATION OF CONTRACTIONS

The electrical response of a muscle fiber to repeated stimulation is like that of nerve. The fiber is electrically refractory only during the rising phase and part of the falling phase of the spike potential. At this time, the contraction initiated by the first stimulus is just beginning. However, because the contractile mechanism does not have a refractory period, repeated stimulation before relaxation has occurred produces additional activation of the contractile elements and a response that is added to the contraction already present. This phenomenon is known as **summation of contractions.** The tension developed during summation is considerably greater than that during the single muscle twitch. With rapidly repeated stimulation, activation of the contractile mechanism occurs repeatedly before any relaxation has occurred, and the individual responses fuse into one continuous contraction. Such a response is called **tetanus (tetanic contraction).** It is a **complete tetanus** when no relaxation occurs between stimuli and an **incomplete tetanus** when periods of incomplete relaxation take place between the summated stimuli. During a complete tetanus, the tension developed is about four times that developed by the individual twitch contractions. The development of an incomplete and a complete tetanus in response to stimuli of increasing frequency is shown in **Figure 5–9**.

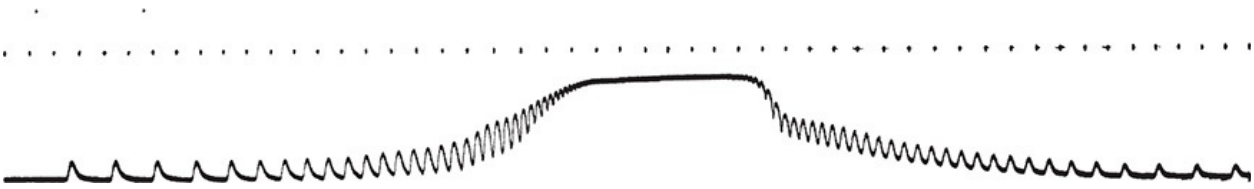


FIGURE 5–9 Tetanus. Isometric tension of a single muscle fiber during continuously increasing and decreasing stimulation frequency. Dots at the top are at intervals of 0.2 s. Note the development of incomplete and then complete tetanus as stimulation is increased, and the return of incomplete tetanus, then full response, as stimulation frequency is decreased.

CLINICAL BOX 5–2

Muscle Channelopathies

Channelopathies are diseases that have as their underlying feature mutations or dysregulation of ion channels. Such diseases are frequently associated with excitable cells, including muscle. In the various forms of clinical **myotonia,** muscle relaxation is prolonged after voluntary contraction. The molecular bases of myotonias are due to dysfunction of channels that shape the action

potential. Myotonia dystrophy is caused by an autosomal dominant mutation that leads to overexpression of a K^+ channel (although the mutation is *not* at the K^+ channel). A variety of myotonias are associated with mutations in Na^+ channels (eg, hyperkalemic periodic paralysis, paramyotonia congenita, or Na^+ channel congenita) or Cl^- channels (eg, dominant or recessive myotonia congenita). **Myasthenia,** defined as abnormal muscle weakness or disease, can also be related to loss of ion channel function in the muscle. In **congenital myasthenia,** the patient has an inheritable disorder of one of ion channels necessary for the transmission of neuronal signaling to muscle response. Mutations in Ca^{2+} channels that allow for neuronal transmitter release or in the acetylcholine receptor nonspecific cation channels, important in recognition of neuronal transmitters, have both been shown to cause congenital myasthenia. Alterations of channel functions can also occur via autoimmune disease, such as that observed in **myasthenia gravis.** In this disease, antibodies to the nicotinic acetylcholine receptor can reduce its functional presence at the muscle membrane by up to 80%, and thus limit muscle response to neuronal transmitter release.

Channelopathies can also occur in the Ca^{2+} release channels in muscle (eg, ryanodine receptors) that amplify the Ca^{2+} response within the cell. Such mutations can cause **malignant hyperthermia.** Patients with this condition display normal muscle function under normal conditions. However, certain anesthetic agents, or in rare cases exposure to high environmental heat or strenuous exercise, can trigger abnormal release of Ca^{2+} from the sarcoplasmic reticulum in the muscle cell, resulting in sustained muscle contraction and heat production. In severe cases, fatality can occur.

THERAPEUTIC HIGHLIGHTS

Although the symptoms associated with each individual channelopathy may be similar, treatments for the individual diseases include a wide variety of drugs that are targeted to the defect in the individual ion channel (or proteins associated with ion channel). Appropriate drug therapy helps improve symptoms and maintain acceptable muscle function. Further interventions related to individual diseases are to avoid muscle movements that exacerbate the disease.

The stimulation frequency at which summation of contractions occurs is

determined by the twitch duration of the particular muscle being studied. For example, if the twitch duration is 10 ms, frequencies less than 1/10 ms (100/s) cause discrete responses interrupted by complete relaxation, and frequencies greater than 100/s cause summation.

RELATION BETWEEN MUSCLE LENGTH, TENSION, & VELOCITY OF CONTRACTION

Both the tension that a muscle develops when stimulated to contract isometrically (the **total tension)** and the **passive tension** exerted by the unstimulated muscle vary with the length of the muscle fiber. This relationship can be studied in a whole skeletal muscle preparation such as that shown in Figure 5–8. The length of the muscle can be varied by changing the distance between its two attachments. At each length, the passive tension is measured, the muscle is then stimulated electrically, and the total tension is measured. The difference between the two values at any length is the amount of tension actually generated by the contractile process, the **active tension.** The records obtained by plotting passive tension and total tension against muscle length are shown in **Figure 5–10**. Similar curves are obtained when single muscle fibers are studied. The length of the muscle at which the active tension is maximal is usually called its optimal **resting length.** The term comes originally from experiments demonstrating that the length of many of the muscles in the body at rest is the length at which they develop maximal tension.

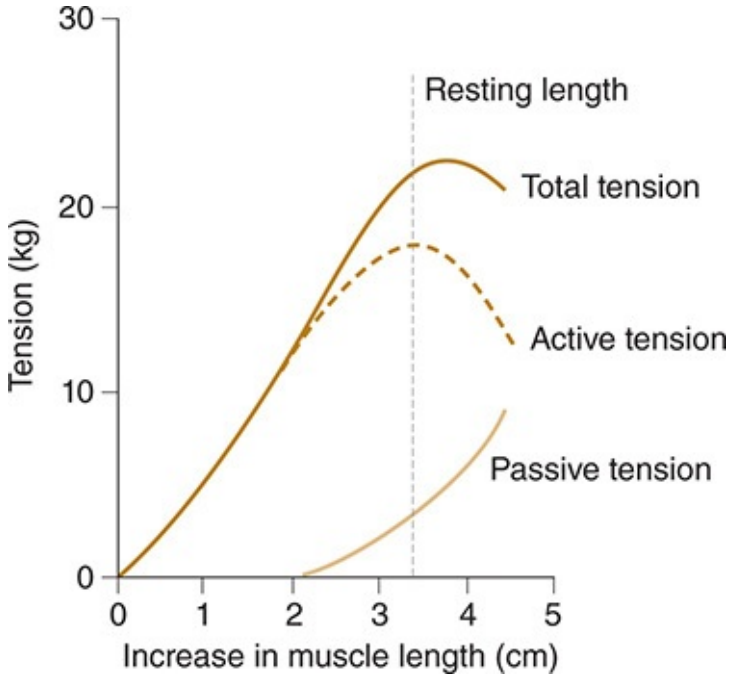


FIGURE 5–10 Length–tension relationship for the human triceps muscle. The passive tension curve measures the tension exerted by this skeletal muscle at each length when it is not stimulated. The total tension curve represents the tension developed when the muscle contracts isometrically in response to a maximal stimulus. The active tension is the difference between the two.

The observed length—tension relation in skeletal muscle can be explained by the sliding filament mechanism of muscle contraction. When the muscle fiber contracts isometrically, the tension developed is proportional to the number of cross-bridges between the actin and the myosin molecules. When muscle is stretched, the overlap between actin and myosin is reduced and the number of cross-linkages is therefore reduced. Conversely, when the muscle is appreciably shorter than resting length, the distance the thin filaments can move is reduced.

The velocity of muscle contraction varies inversely with the load on the muscle. At a given load, the velocity is maximal at the resting length and declines if the muscle is shorter or longer than this length.

FIBER TYPES

Although skeletal muscle fibers resemble one another in a general way, skeletal muscle is a heterogeneous tissue made up of fibers that vary in myosin ATPase activity, contractile speed, and other properties. Muscles are frequently classified

into two types: “slow” and “fast.” These muscles can contain a mixture of three fiber types: type I (for slow-oxidative or SO), type IIA (for fast-oxidative-glycolytic or FOG), or type IIB (for fast glycolytic or FG). Some of the properties associated with types I, IIA, and IIB fibers are summarized in **Table 5–2**. Although these classification schemes are valid for muscles across many mammalian species, there are significant variations of fibers within and between muscles. For example, type I fibers in a given muscle can be larger than type IIA fibers from a different muscle in the same animal. Many of the differences in the fibers that make up muscles stem from differences in the proteins within them. Most of these are encoded by multigene families. Ten different **isoforms** of the myosin heavy chains (MHCs) have been characterized. Each of the two types of light chains also has isoforms. It appears that there is only one form of actin, but multiple isoforms of tropomyosin and all three components of troponin.

TABLE 5–2 Classification of fiber types in skeletal muscles.

	Type I	Type IIA	Type IIB
Other names	Slow, Oxidative (SO)	Fast, Oxidative, Glycolytic (FOG)	Fast, Glycolytic (FG)
Color	Red	Red	White
Myosin ATPase activity	Slow	Fast	Fast
Ca^{2+}-pumping capacity of sarcoplasmic reticulum	Moderate	High	High
Diameter	Small	Large	Large
Glycolytic capacity	Moderate	High	High
Oxidative capacity	High	Moderate	Low
Associated Motor Unit Type	Slow (S)	Fast Resistant to Fatigue (FR)	Fast Fatigable (FF)
Membrane potential = –90 mV			

ENERGY SOURCES & METABOLISM

Muscle contraction requires energy, and muscle has been called “a machine for converting chemical energy into mechanical work.” The immediate source of this energy is ATP, and this is formed by the metabolism of carbohydrates and lipids (see Chapters 1 and 2).

PHOSPHORYLCREATINE

ATP is resynthesized from ADP by the addition of a phosphate group. Some of the energy for this endothermic reaction is supplied by the breakdown of glucose to CO_2 and H_2O, but there also exists in muscle another energy-rich phosphate compound that can supply this energy for short periods. This compound is **phosphorylcreatine,** which is hydrolyzed to creatine and phosphate groups with the release of considerable energy **(Figure 5–11)**. At rest, some ATP in the mitochondria transfers its phosphate to creatine, so that a phosphorylcreatine store is built up. During exercise, the phosphorylcreatine is hydrolyzed at the junction between the myosin heads and actin, forming ATP from ADP and thus permitting contraction to continue.

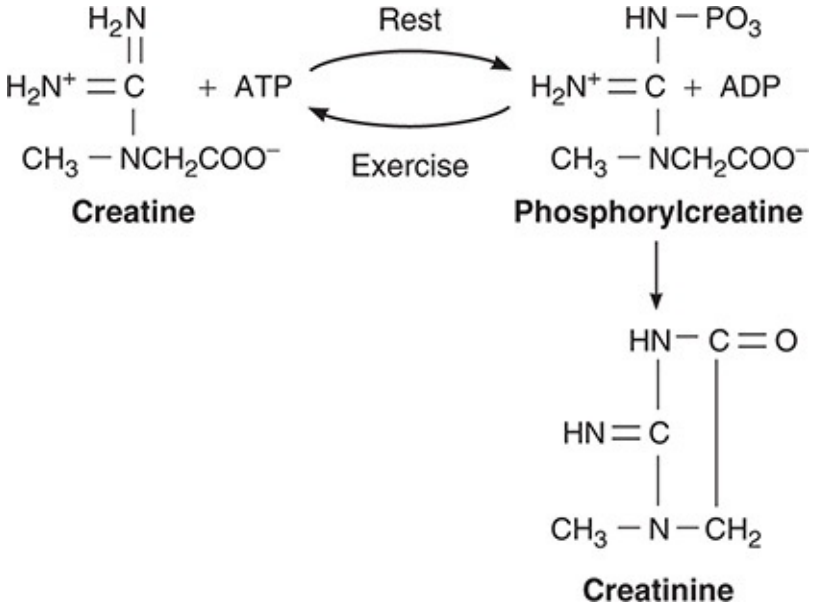


FIGURE 5–11 Creatine, phosphorylcreatine, and creatinine cycling in muscle. During periods of high activity, cycling of phosphorylcreatine allows for quick release of ATP to sustain muscle activity.

CARBOHYDRATE & LIPID BREAKDOWN

At rest and during light exercise, muscles utilize lipids in the form of free fatty acids as their energy source. As the intensity of exercise increases, lipids alone cannot supply energy fast enough and so use of carbohydrate becomes the predominant component in the muscle fuel mixture. Thus, during exercise, much of the energy for phosphorylcreatine and ATP resynthesis comes from the breakdown of glucose to CO_2 and H_2O. Glucose in the bloodstream enters cells, where it is degraded through a series of chemical reactions to pyruvate. Another source of intracellular glucose, and consequently of pyruvate, is glycogen, the carbohydrate polymer that is especially abundant in liver and skeletal muscle. When adequate O_2 is present, pyruvate enters the citric acid cycle and is metabolized—through this cycle and the so-called respiratory enzyme pathway —to CO_2 and H_2O. This process is called **aerobic glycolysis.** The metabolism of glucose or glycogen to CO_2 and H_2O forms large quantities of ATP from ADP. If O_2 supplies are insufficient, the pyruvate formed from glucose does not enter the tricarboxylic acid cycle but is reduced to lactate. This process of **anaerobic glycolysis** is associated with the net production of much smaller quantities of energy-rich phosphate bonds, but it does not require the presence of O_2. A brief overview of the various reactions involved in supplying energy to skeletal muscle is shown in **Figure 5–12**.

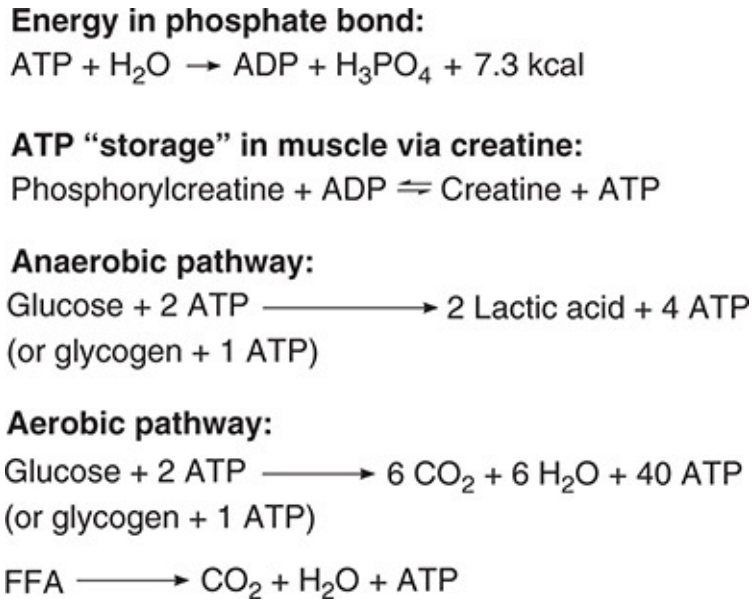


FIGURE 5–12 ATP turnover in muscle cells. Energy released by hydrolysis of

1 mol of ATP and reactions responsible for resynthesis of ATP. The amount of ATP formed per mole of free fatty acid (FFA) oxidized is large but varies with the size of the FFA. For example, complete oxidation of 1 mol of palmitic acid generates 140 mol of ATP.

THE OXYGEN DEBT MECHANISM

During exercise, the muscle blood vessels dilate and blood flow is increased so that the available O_2 supply is increased. Up to a point, the increase in O_2 consumption is proportional to the energy expended, and all the energy needs are met by aerobic processes. However, when muscular exertion is very great, aerobic resynthesis of energy stores cannot keep pace with their utilization. Under these conditions, phosphorylcreatine is still used to resynthesize ATP. In addition, some ATP synthesis is accomplished by using the energy released by the anaerobic breakdown of glucose to lactate. Use of the anaerobic pathway is self-limiting because in spite of rapid diffusion of lactate into the bloodstream, enough accumulates in the muscles to eventually exceed the capacity of the tissue buffers and produce an enzyme-inhibiting decline in pH. However, for short periods, the presence of an anaerobic pathway for glucose breakdown permits muscular exertion of a far greater magnitude than would be possible without it. For example, in a 100-m dash that takes 10 seconds, 85% of the energy consumed is derived anaerobically; in a 2-mile race that takes 10 minutes, 20% of the energy is derived anaerobically; and in a long-distance race that takes 60 minutes, only 5% of the energy comes from anaerobic metabolism.

After a period of exertion is over, extra O_2 is consumed to remove the excess lactate, replenish the ATP and phosphorylcreatine stores, and replace the small amounts of O_2 that were released by myoglobin. Without replenishment of ATP, muscles enter a state of rigor **(Clinical Box 5–3)**. The amount of extra O_2 consumed is proportional to the extent to which the energy demands during exertion exceeded the capacity for the aerobic synthesis of energy stores, that is, the extent to which an **oxygen debt** was incurred. The O_2 debt is measured experimentally by determining O_2 consumption after exercise until a constant, basal consumption is reached and subtracting the basal consumption from the total. The amount of this debt may be six times the basal O_2 consumption, which indicates that the subject is capable of six times the exertion that would have been possible without it.

HEAT PRODUCTION IN MUSCLE

Thermodynamically, the energy supplied to a muscle must equal its energy output. The energy output appears in work done by the muscle, in energy-rich phosphate bonds formed for later use, and in heat. The overall mechanical efficiency of skeletal muscle (work done/total energy expenditure) ranges up to 50% while lifting a weight during isotonic contraction and is essentially 0% during isometric contraction. Energy storage in phosphate bonds is a small factor. Consequently, heat production is considerable. The heat produced in muscle can be measured accurately with suitable thermocouples.

CLINICAL BOX 5–3

Muscle Rigor

When muscle fibers are completely depleted of ATP and phosphorylcreatine, they develop a state of rigidity called rigor. When this occurs after death, the condition is called **rigor mortis.** In rigor, almost all of the myosin heads attach to actin but in an abnormal, fixed, and resistant way. The muscles effectively are locked into place and become quite stiff to the touch.

Resting heat, the heat given off at rest, is the external manifestation of basal metabolic processes. The heat produced in excess of resting heat during contraction is called the **initial heat.** This is made up of **activation heat,** the heat that muscle produces whenever it is contracting, and **shortening heat,** which is proportional in amount to the distance the muscle shortens. Shortening heat is apparently due to some change in the structure of the muscle during shortening.

Following contraction, heat production in excess of resting heat continues for as long as 30 min. This **recovery heat** is the heat liberated by the metabolic processes that restore the muscle to its precontraction state. The recovery heat of muscle is approximately equal to the initial heat; that is, the heat produced during recovery is equal to the heat produced during contraction.

If a muscle that has contracted isotonically is restored to its previous length, extra heat in addition to recovery heat is produced **(relaxation heat).** External work must be done on the muscle to return it to its previous length, and relaxation heat is mainly a manifestation of this work.

PROPERTIES OF SKELETAL MUSCLES IN THE INTACT ORGANISM

THE MOTOR UNIT

Innervation of muscle fibers is critical to muscle function **(Clinical Box 5–4)**. Because the axons of the spinal motor neurons supplying skeletal muscle each branch to innervate several muscle fibers, the smallest possible amount of muscle that can contract in response to the excitation of a single motor neuron is not one muscle fiber but all the fibers supplied by the neuron. Each single motor neuron and the muscle fibers it innervates constitute a **motor unit.** The number of muscle fibers in a motor unit varies. In muscles such as those of the hand and those concerned with motion of the eye (ie, muscles concerned with fine, graded, precise movement), each motor unit innervates very few (on the order of three to six) muscle fibers. On the other hand, values of 600 muscle fibers per motor unit can occur in human leg muscles. The group of muscle fibers that contribute to a motor unit can be intermixed within a muscle. That is, although they contract as a unit, they are not necessarily "neighboring" fibers within the muscle.

Each spinal motor neuron innervates only one kind of muscle fiber, so that all the muscle fibers in a motor unit are of the same type. On the basis of the type of muscle fiber they innervate, and thus on the basis of the duration of their twitch contraction, motor units are divided into S (slow), FR (fast, resistant to fatigue), and FF (fast, fatigable) units. Interestingly, there is also a gradation of innervation of these fibers, with S fibers tending to have a low innervation ratio (ie, small units) and FF fibers tending to have a high innervation ratio (ie, large units). The recruitment of motor units during muscle contraction is not random; rather it follows a general scheme, the **size principle.** In general, a specific muscle action is developed first by the recruitment of S muscle units that contract relatively slowly to produce controlled contraction. Next, FR muscle units are recruited, resulting in more powerful response over a shorter period of time. Lastly, FF muscle units are recruited for the most demanding tasks. For example, in muscles of the leg, the small, slow units are first recruited for standing. As walking motion is initiated, their recruitment of FR units increases. As this motion turns to running or jumping, the FF units are recruited. Of course, there is overlap in recruitment, but, in general, this principle holds true.

CLINICAL BOX 5–4

Denervation of Muscle

In the intact animal healthy skeletal muscle does not contract except in response to stimulation of its motor nerve supply. Destruction of this nerve supply causes muscle atrophy. It also leads to abnormal excitability of the muscle and increases its sensitivity to circulating acetylcholine (**denervation hypersensitivity;** see Chapter 6). Fine, irregular contraction of individual fibers (**fibrillations**) appears. This is the classic picture of a **lower motor neuron lesion.** If the motor nerve regenerates, the fibrillations disappear. Usually, the contractions are not visible grossly, and they should not be confused with **fasciculations,** which are jerky, visible contractions of groups of muscle fibers that occur as a result of pathologic discharge of spinal motor neurons.

The differences between types of muscle units are not inherent but are determined by, among other things, their activity. When the nerve to a slow muscle is cut and the nerve to a fast muscle is spliced to the cut end, the fast nerve grows and innervates the previously slow muscle. However, the muscle becomes fast and corresponding changes take place in its muscle protein isoforms and myosin ATPase activity. This change is due to changes in the pattern of activity of the muscle; in stimulation experiments, changes in the expression of *MHC* genes and consequently of MHC isoforms can be produced by changes in the pattern of electrical activity used to stimulate the muscle. More commonly, muscle fibers can be altered by a change in activity initiated through exercise (or lack thereof). Increased activity can lead to muscle cell hypertrophy, which allows for increase in contractile strength. Types IIA and IIB fibers are most susceptible to these changes. Alternatively, inactivity can lead to muscle cell atrophy and a loss of contractile strength. Type I fibers—that is, the ones used most often—are most susceptible to these changes.

ELECTROMYOGRAPHY

Activation of motor units can be studied by electromyography, the process of recording the electrical activity of muscle. This may be done in unanaesthetized humans by using small metal disks on the skin overlying the muscle as the pick-up electrodes or by using needle or fine wire electrodes inserted into the muscle. The record obtained with such electrodes is the **electromyogram (EMG).** With

needle or fine wire electrodes, it is usually possible to pick up the activity of single muscle fibers. The measured EMG depicts the potential difference between the two electrodes, which is altered by the activation of muscles in between the electrodes. A typical EMG is shown in **Figure 5–13**.

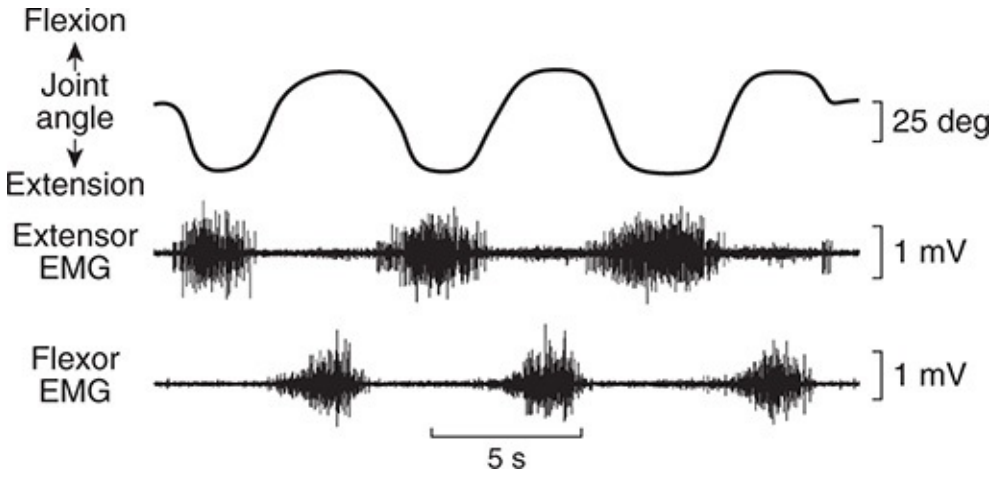


FIGURE 5–13 Relative joint angle and electromyographic tracings from human extensor pollicis longus and flexor pollicis longus during alternate flexion and extension of the distal joint of the thumb. The extensor pollicis longus and flexor pollicis longus extend and flex the distal joint of the thumb, respectively. The distal thumb joint angle (top) is superimposed over the extensor pollicis longus (middle) and flexor pollicus longus (bottom) EMGs. Note the alternate activation and rest patterns as one muscle is used for extension and the other for flexion. (Used with permission of Andrew J. Fuglevand.)

It has been shown by electromyography that little if any spontaneous activity occurs in the skeletal muscles of normal individuals at rest. With minimal voluntary activity a few motor units discharge, and with increasing voluntary effort, more and more are brought into play to monitor the **recruitment of motor units.** Gradation of muscle response is therefore in part a function of the number of motor units activated. In addition, the frequency of discharge in the individual nerve fibers plays a role, the tension developed during a tetanic contraction being greater than that during individual twitches. The length of the muscle is also a factor. Finally, the motor units fire asynchronously, that is, out of phase with one another. This asynchronous firing causes the individual muscle fiber responses to merge into a smooth contraction of the whole muscle. In summary, EMGs can be used to quickly (and roughly) monitor abnormal electrical activity associated with muscle responses.

THE STRENGTH OF SKELETAL MUSCLES

Human skeletal muscle can exert 3–4 kg of tension per square centimeter of cross-sectional area. This figure is about the same as that obtained in a variety of experimental animals and seems to be constant for mammalian species. Because many of the muscles in humans have a relatively large cross-sectional area, the tension they can develop is quite large. The gastrocnemius, for example, not only supports the weight of the whole body during climbing but resists a force several times this great when the foot hits the ground during running or jumping. An even more striking example is the gluteus maximus, which can exert a tension of 1200 kg. The total tension that could be developed if all muscles in the body of an adult man pulled together is approximately 22,000 kg (nearly 25 tons).

BODY MECHANICS

Body movements are generally organized in such a way that they take maximal advantage of the physiological principles outlined above. For example, the attachments of the muscles in the body are such that many of them are normally at or near their resting length when they start to contract. In muscles that extend over more than one joint, movement at one joint may compensate for movement at another in such a way that relatively little shortening of the muscle occurs during contraction. Nearly isometric contractions of this type permit development of maximal tension per contraction. The hamstring muscles extend from the pelvis over the hip joint and the knee joint to the tibia and fibula. Hamstring contraction produces flexion of the leg on the thigh. If the thigh is flexed on the pelvis at the same time, the lengthening of the hamstrings across the hip joint tends to compensate for the shortening across the knee joint. In the course of various activities, the body moves in a way that takes advantage of this. Such factors as momentum and balance are integrated into body movement in ways that make possible maximal motion with minimal muscular exertion. One net effect is that the stress put on tendons and bones rarely exceeds 50% of their failure strength, protecting them from damage.

In walking, each limb passes rhythmically through a support or stance phase when the foot is on the ground and a swing phase when the foot is off the ground. The support phases of the two legs overlap, so that two periods of double support occur during each cycle. There is a brief burst of activity in the leg flexors at the start of each step, and then the leg is swung forward with little more active muscular contraction. Therefore, the muscles are active for only a

fraction of each step, and walking for long periods causes relatively little fatigue.

A young adult walking at a comfortable pace moves at a velocity of about 80 m/min and generates a power output of 150–175 W per step. A group of young adults asked to walk at their most comfortable rate selected a velocity close to 80 m/min, and it was found that they had selected the velocity at which their energy output was minimal. Walking more rapidly or more slowly took more energy.

CARDIAC MUSCLE MORPHOLOGY

The striations in cardiac muscle are similar to those in skeletal muscle, and Z-lines are present. Large numbers of elongated mitochondria are in close contact with the muscle fibrils. The muscle fibers branch and interdigitate, but each is a complete unit surrounded by a cell membrane. Where the end of one muscle fiber abuts on another, the membranes of both fibers parallel each other through an extensive series of folds. These areas, which always occur at Z-lines, are called **intercalated disks** **(Figure 5–14)**. They provide a strong union between fibers, maintaining cell-to-cell cohesion, so that the pull of one contractile cell can be transmitted along its axis to the next. Along the sides of the muscle fibers next to the disks, the cell membranes of adjacent fibers fuse for considerable distances, forming gap junctions. These junctions provide low-resistance bridges for the spread of excitation from one fiber to another. They permit cardiac muscle to function as if it were a syncytium, even though no protoplasmic bridges are present between cells. The T system in cardiac muscle is located at the Z-lines rather than at the A–I junction, where it is located in mammalian skeletal muscle.

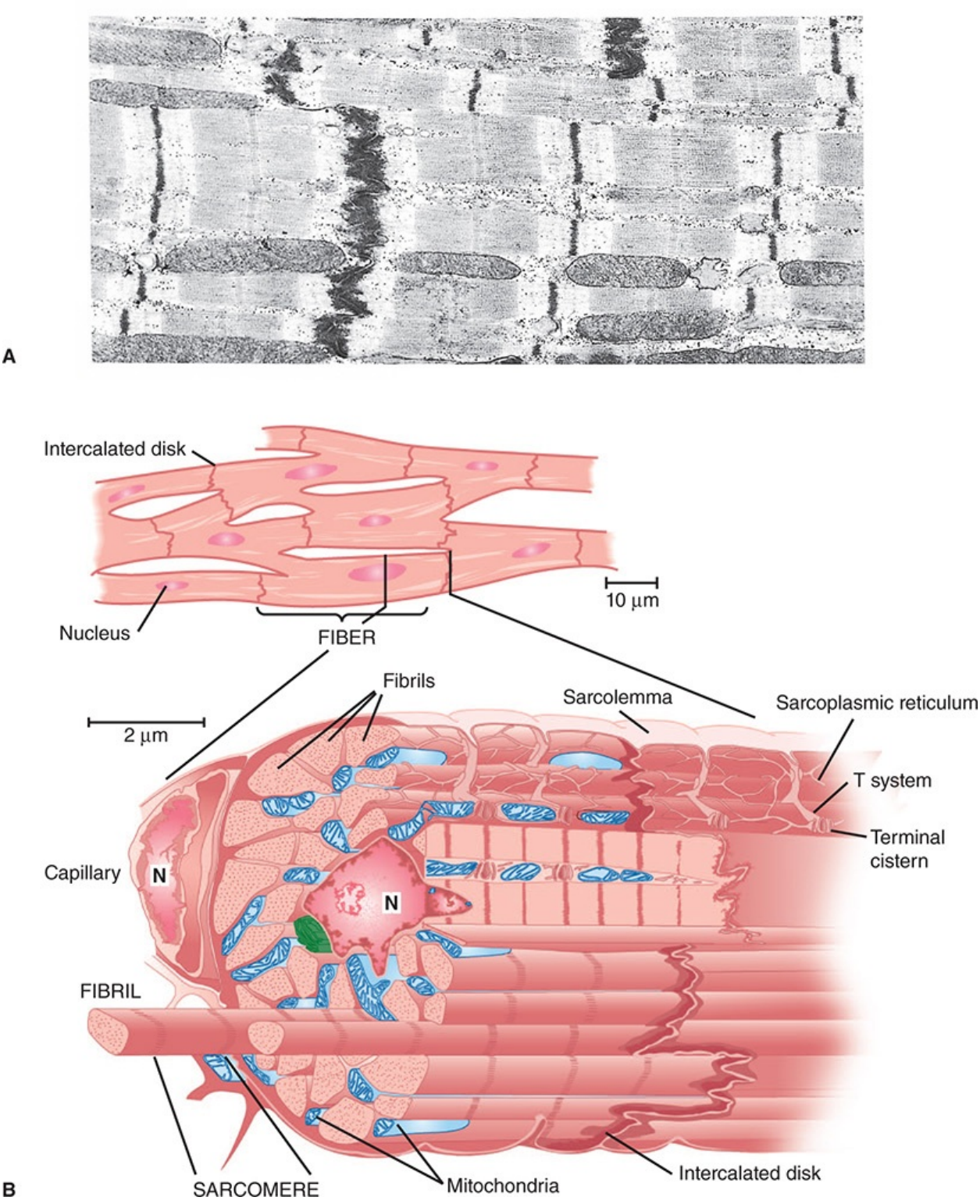


FIGURE 5–14 Cardiac muscle. A) Electron micrograph of cardiac muscle. Note the similarity of the A-I regions seen in the skeletal muscle EM of Figure 5–2. The fuzzy thick lines are intercalated disks and function similarly to the Z-

lines but occur at cell membranes (× 12,000). (Reproduced with permission from Bloom W, Fawcett DW: *A Textbook of Histology*, 10th ed. Saunders, 1975.) **B)** Artist interpretation of cardiac muscle as seen under the light microscope (top) and the electron microscope (bottom). Again, note the similarity to skeletal muscle structure. N, nucleus. (Reproduced with permission from Braunwald E, Ross J, Sonnenblick EH: Mechanisms of contraction of the normal and failing heart. N Engl J Med 1967 October 12;277(15):794–800.)

ELECTRICAL PROPERTIES

RESTING MEMBRANE & ACTION POTENTIALS

The resting membrane potential of individual mammalian cardiac muscle cells is about −90 mV. Stimulation produces a propagated action potential that is responsible for initiating contraction. Although action potentials vary among the cardiomyocytes in different regions of the heart (discussed in Chapter 29), the action potential of a typical ventricular cardiomyocyte can be used as an example **(Figure 5–15)**. Depolarization proceeds rapidly and an overshoot of the zero potential is present, as in skeletal muscle and nerve, but this is followed by a plateau before the membrane potential returns to the baseline. In mammalian hearts, depolarization lasts about 2 ms, but the plateau phase and repolarization last 200 ms or more. Repolarization is therefore not complete until the contraction is half over.

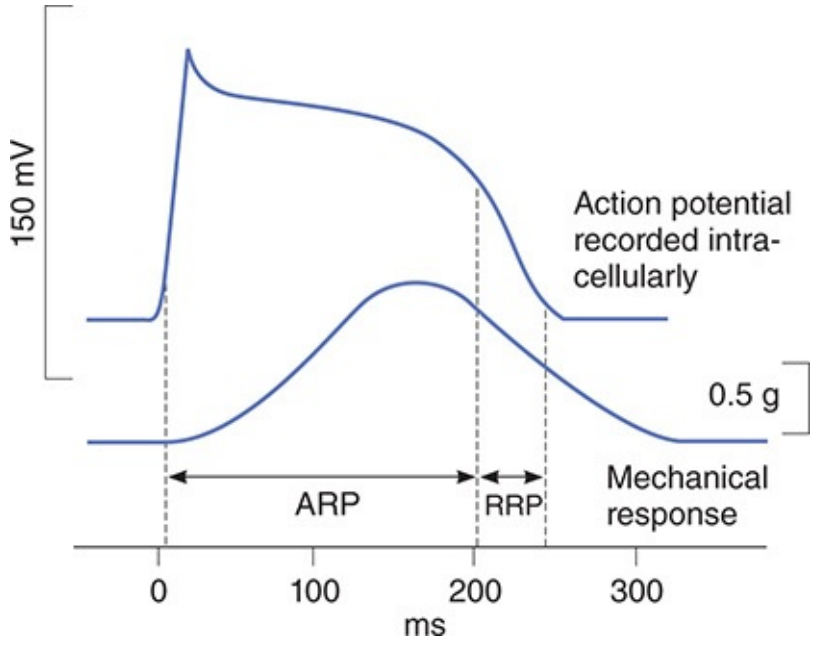


FIGURE 5–15 Comparison of action potentials and contractile response of a mammalian cardiac muscle fiber in a typical ventricular cell. In the top trace, the intracellular recording of the action potential shows the quick depolarization and extended recovery. In the bottom trace, the mechanical response is matched to the extracellular and intracellular electrical activities. Note that in the absolute refractory period (ARP), the cardiac myocyte cannot be excited, whereas in the relative refractory period (RRP) minimal excitation can occur.

As in other excitable tissues, changes in the external K^+ concentration affect the resting membrane potential of cardiac muscle, whereas changes in the external Na^+ concentration affect the magnitude of the action potential. The initial rapid depolarization and the overshoot (phase 0) are due to opening of voltage-gated Na^+ channels similar to that occurring in nerve and skeletal muscle **(Figure 5–16)**. The initial rapid repolarization (phase 1) is due to closure of Na^+ channels and opening of one type of K^+ channel (ie, Kv4.2/KCND2 and/or Kv4.3/CCND3, and KV1.4/KCNA4). The subsequent prolonged plateau (phase 2) is due to a slower but prolonged opening of voltage-gated Ca^{2+} channels. Final repolarization (phase 3) to the resting membrane potential (phase 4) is due to closure of the Ca^{2+} channels and a slow, delayed increase of K^+ efflux through various types of K^+ channels. Cardiac myocytes contain at least two types of voltage-gated Ca^{2+} channels (T- and L-types), but the Ca^{2+} current is mostly due to opening of the slower L-type Ca^{2+} channels. Mutations or dysfunctions in any of these channels lead to serious pathologies of the heart **(eg, Clinical Box 5–5**).

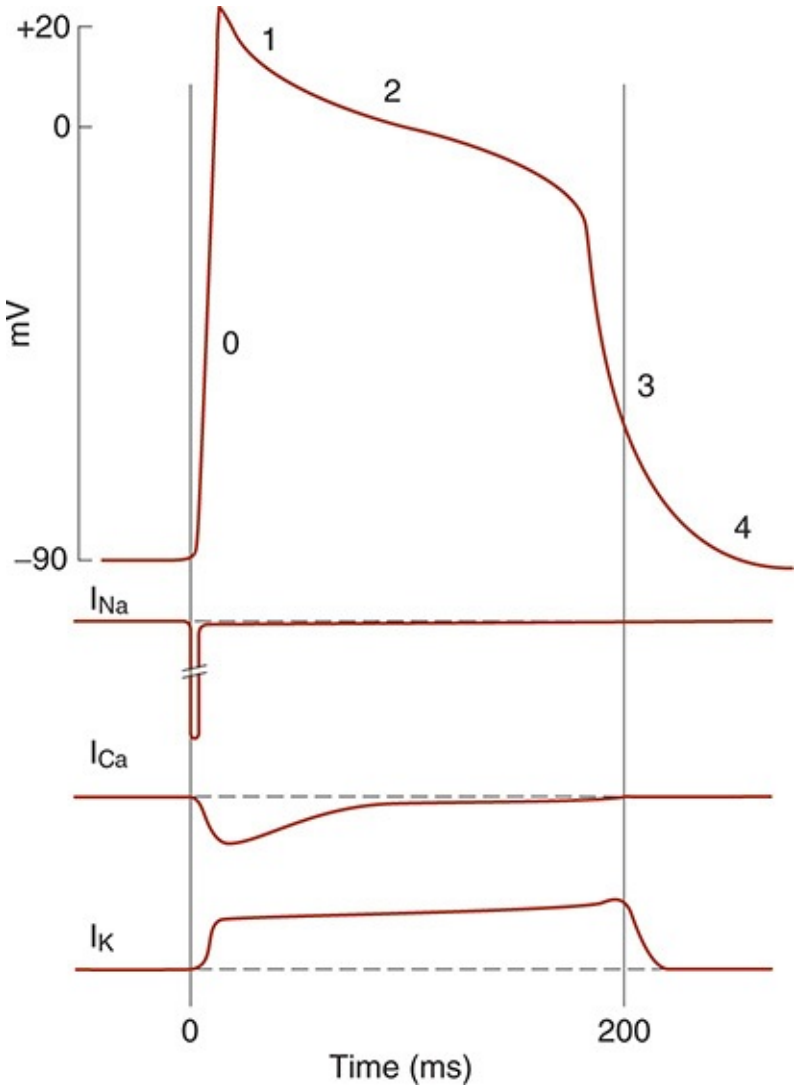


FIGURE 5–16 Dissection of the cardiac action potential. Top: The action potential of a cardiac muscle fiber can be broken down into several phases: 0, depolarization; 1, initial rapid repolarization; 2, plateau phase; 3, late rapid repolarization; 4, baseline. **Bottom:** Diagrammatic summary of Na^+, Ca^{2+}, and cumulative K+ currents during the action potential. As is convention, inward currents are downward, and outward currents are upward.

MECHANICAL PROPERTIES

CONTRACTILE RESPONSE

The contractile response of cardiac muscle begins just after the start of depolarization and lasts about 1.5 times as long as the action potential (Figure 5–

15). The role of Ca^{2+} in excitation–contraction coupling is similar to its role in skeletal muscle (see above). However, it is the influx of extracellular Ca^{2+} through the voltage-sensitive DHPR or voltage-gated Ca^{2+} channels in the T system that triggers Ca^{2+}-induced Ca^{2+} release through the RyR at the sarcoplasmic reticulum. Because there is a net influx of Ca^{2+} during activation, there is also a more prominent role for plasma membrane Ca^{2+} ATPases and the Na^{+}/Ca^{2+} exchanger in recovery of cytosolic Ca^{2+} concentrations. Specific effects of drugs that indirectly alter intracellular Ca^{2+} concentrations are discussed in **Clinical Box 5–6**.

CLINICAL BOX 5–5

Long QT Syndrome

Long QT syndrome (LQTS) is defined as a prolongation of the QT interval observed on an electrocardiogram. LQTS can lead to irregular heartbeats and subsequent fainting, seizure, cardiac arrest, or even death. Although certain medications can lead to LQTS, it is more frequently associated with genetic mutations in a variety of cardiac-expressed ion channels. Mutations in cardiac-expressed voltage-gated K^{+} channel genes (*KCNQ1* or *KCNH2*) account for most of the mutation-based cases of LQTS (~90%). Mutations in cardiac-expressed voltage-gated Na^{+} channel genes (eg, *SCN5A*) or cardiac-expressed Ca^{2+} channel genes (eg, *CACNA1C*) have also been associated with the disease. The fact that mutations in diverse channels all can result in the prolongation of the QT interval and subsequent pathophysiological changes underlies the intricate interplay of these channels in shaping the heart's electrical response.

THERAPEUTIC HIGHLIGHTS

Patients with LQTS should avoid drugs that prolong the QT interval or reduce their serum K^{+} or Mg^{2+} levels; any K^{+} or Mg^{2+} deficiencies should be corrected. Drug interventions in asymptomatic patients remain somewhat controversial, although patients with congenital defects that lead to LQTS are considered candidates for intervention independent of symptoms. In general, β-blockers have been used for LQTS to reduce the risk of cardiac arrhythmias. More specific and effective treatments can be introduced once the underlying cause of LQTS is identified.

During phases 0 to 2 and about half of phase 3 (until the membrane potential reaches approximately −50 mV during repolarization), cardiac muscle cannot be excited again; that is, it is in its **absolute refractory period.** It remains relatively refractory until phase 4. Therefore, tetanus of the type seen in skeletal muscle cannot occur. Of course, tetanization of cardiac muscle for any length of time would have lethal consequences, and in this sense, the fact that cardiac muscle cannot be tetanized is a safety feature.

CLINICAL BOX 5–6

Glycosidic Drugs & Cardiac Contraction

Ouabain and other digitalis glycosides are commonly used to treat failing hearts. These drugs have the effect of increasing the strength of cardiac contraction. Although there is discussion as to full mechanisms, a working hypothesis is based on the ability of these drugs to inhibit the Na, K ATPase in cell membranes of the cardiomyocytes. The block of the Na, K ATPase in cardiomyocytes would result in an increased intracellular Na^+ concentration. Such an increase would result in a decreased inward Na^+ transportation and hence outward Ca^{2+} transportation via the Na^+-Ca^{2+} exchange antiport during the Ca^{2+} recovery period. The resulting increase in intracellular Ca^{2+} concentration in turn increases the strength of contraction of the cardiac muscle. With this mechanism in mind, these drugs can also be quite toxic. Over inhibition of the Na, K ATPase would result in a depolarized cell that could slow conduction, or even spontaneously activate contraction. Alternatively, an overly increased Ca^{2+} concentration could also have ill effects on cardiomyocyte function.

ISOFORMS

Cardiac muscle is generally slow and has relatively low ATPase activity. Its fibers are dependent on oxidative metabolism and hence on a continuous supply of O_2. The human heart contains both the α and the β isoforms of the myosin heavy chain (α MHC and β MHC). β MHC has lower myosin ATPase activity

than α MHC. Both are present in the atria, with the α isoform predominating, whereas the β isoform predominates in the ventricle. The spatial differences in expression contribute to the well-coordinated contraction of the heart.

CORRELATION BETWEEN MUSCLE FIBER LENGTH & TENSION

The relation between initial fiber length and total tension in cardiac muscle is similar to that in skeletal muscle; there is an optimal resting length at which the tension developed on stimulation is maximal. In the body, the initial length of the fibers is determined by the degree of diastolic filling of the heart, and the pressure developed in the ventricle is proportional to the volume of the ventricle at the end of the filling phase **(Starling law of the heart).** The developed tension **(Figure 5–17)** increases as the diastolic volume increases until it reaches a maximum, then tends to decrease. However, unlike skeletal muscle, the decrease in developed tension at high degrees of stretch is not due to a decrease in the number of cross-bridges between actin and myosin, because even severely dilated hearts are not stretched to this degree. The decrease is instead due to beginning disruption of the myocardial fibers.

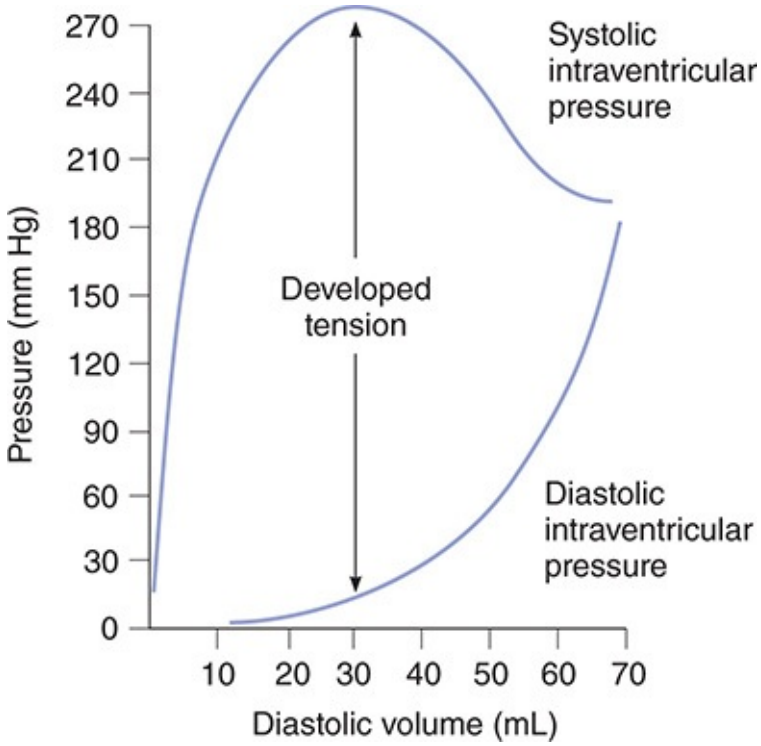


FIGURE 5–17 Length–tension relationship for cardiac muscle. Comparison of the systolic intraventricular pressure (top trace) and diastolic intraventricular pressure (bottom trace) display the developed tension in the cardiomyocyte. Values shown are for canine heart.

The force of contraction of cardiac muscle can be also increased by catecholamines, and this increase occurs without a change in muscle length. This positive ionotropic effect of catecholamines is mediated via innervated β_1-adrenergic receptors, cyclic adenosine monophosphate (AMP), and their effects on Ca^{2+} homeostasis. The heart also contains noninnervated β_2-adrenergic receptors, which also act via cyclic AMP, but their ionotropic effect is smaller and is maximal in the atria. Cyclic AMP activates protein kinase A, and this leads to phosphorylation of the voltage-gated Ca^{2+} channels, causing them to spend more time in the open state. Cyclic AMP also increases the active transport of Ca^{2+} to the sarcoplasmic reticulum, thus accelerating relaxation and consequently shortening systole. This is important when the cardiac rate is increased because it permits adequate diastolic filling (see Chapter 30).

METABOLISM

Mammalian hearts have an abundant blood supply, numerous mitochondria, and a high content of myoglobin, a muscle pigment that can function as an O_2 storage mechanism. Normally, less than 1% of the total energy liberated is provided by anaerobic metabolism. During hypoxia, this figure may increase to nearly 10%, but under totally anaerobic conditions, the energy liberated is inadequate to sustain ventricular contractions. Under basal conditions, 35% of the caloric needs of the human heart are provided by carbohydrate, 5% by ketones and amino acids, and 60% by fat. However, the proportions of substrates utilized vary greatly with the nutritional state. After ingestion of large amounts of glucose, more lactate and pyruvate are used; during prolonged starvation, more fat is used. Circulating free fatty acids normally account for almost 50% of the lipid utilized. In untreated diabetics, the carbohydrate utilization of cardiac muscle is reduced and that of fat is increased.

SMOOTH MUSCLE MORPHOLOGY

Smooth muscle is distinguished anatomically from skeletal and cardiac muscle

because it lacks visible cross-striations. Actin and myosin-II are present, and they slide on each other to produce contraction. However, they are not arranged in regular arrays, as in skeletal and cardiac muscle, and so the striations are absent. Instead of Z-lines, there are **dense bodies** in the cytoplasm and attached to the cell membrane, and these are bound by α-actinin to actin filaments. Smooth muscle also contains tropomyosin, but troponin appears to be absent. The isoforms of actin and myosin differ from those in skeletal muscle. A sarcoplasmic reticulum is present, but it is less extensive than those observed in skeletal or cardiac muscle. In general, smooth muscles contain few mitochondria and depend, to a large extent, on glycolysis for their metabolic needs.

TYPES

There is considerable variation in the structure and function of smooth muscle in different parts of the body. In general, smooth muscle can be divided into **unitary** (or **visceral**) **smooth muscle** and **multiunit smooth muscle.** Unitary smooth muscle occurs in large sheets, has many low-resistance gap junctional connections between individual muscle cells, and functions in a syncytial fashion. Unitary smooth muscle is found primarily in the walls of hollow viscera. The musculature of the intestine, the uterus, and the ureters are examples. Multiunit smooth muscle is made up of individual units with few (or no) gap junctional bridges. It is found in structures such as the iris of the eye, in which fine, graded contractions occur. It is not under voluntary control, but it has many functional similarities to skeletal muscle. Each multiunit smooth muscle cell has en passant endings of nerve fibers, but in unitary smooth muscle there are en passant junctions on fewer cells, with excitation spreading to other cells by gap junctions. In addition, these cells respond to hormones and other circulating substances. Blood vessels have both unitary and multiunit smooth muscle in their walls.

ELECTRICAL & MECHANICAL ACTIVITY

Unitary smooth muscle is characterized by the instability of its membrane potential and by the fact that it shows continuous, irregular contractions that are independent of its nerve supply. This maintained state of partial contraction is called **tonus,** or **tone.** The membrane potential has no true “resting” value, being relatively low (or less negative) when the tissue is active and higher (or more negative) when it is inhibited, but in periods of relative quiescence values for

resting potential are on the order of −20 to −65 mV. Smooth muscle cells can display divergent electrical activity **(eg, Figure 5–18).** There are slow sine wave-like fluctuations a few millivolts in magnitude and spikes that sometimes overshoot the zero potential line and sometimes do not. In many tissues, the spikes have a duration of about 50 ms, whereas in some tissues the action potentials have a prolonged plateau during repolarization, like the action potentials in cardiac muscle. As in the other muscle types, there are significant contributions of K^+, Na^+, and Ca^{2+} channels and Na, K ATPase to this electrical activity. However, discussion of contributions to individual smooth muscle types is beyond the scope of this text.

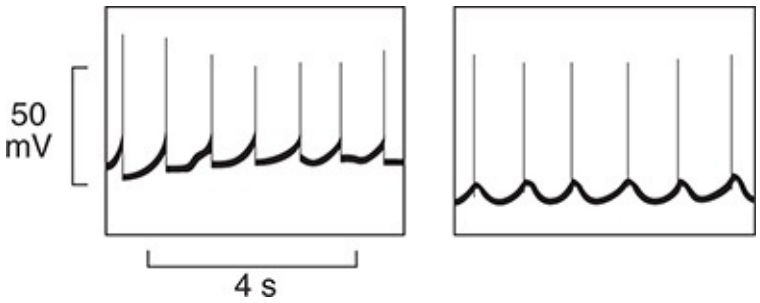


FIGURE 5–18 Electrical activity of individual smooth muscle cells in the guinea pig taenia coli. Left: Pacemaker-like activity with spikes firing at each peak. **Right:** Sinusoidal fluctuation of membrane potential with firing on the rising phase of each wave. In other fibers, spikes can occur on the falling phase of sinusoidal fluctuations and there can be mixtures of sinusoidal and pacemaker potentials in the same fiber.

Because of the continuous activity, it is difficult to study the relation between the electrical and mechanical events in unitary smooth muscle, but in some relatively inactive preparations, a single spike can be generated. In such preparations, the excitation–contraction coupling in unitary smooth muscle can occur with as much as a 500-ms delay. Thus, it is a very slow process compared with that in skeletal and cardiac muscle, in which the time from initial depolarization to initiation of contraction is less than 10 ms. Unlike unitary smooth muscle, multiunit smooth muscle is nonsyncytial and contractions do not spread widely through it. Because of this, the contractions of multiunit smooth muscle are more discrete, fine, and localized than those of unitary smooth muscle.

MOLECULAR BASIS OF CONTRACTION

As in skeletal and cardiac muscle, an increase in cytosolic Ca^{2+} plays a prominent role in the initiation of contraction of smooth muscle. However, the source of Ca^{2+} increase can be quite different in unitary smooth muscle. Depending on the activating stimulus, cytosolic Ca^{2+} increase can be due to Ca^{2+} influx through voltage- or ligand-gated channels in the plasma membrane, Ca^{2+} release or mobilization from intracellular stores (eg, sarcoplasmic reticulum) through the RyR and the **inositol trisphosphate receptor (IP3R)**, Ca^{2+} release channels in the sarcoplasmic reticulum. In addition, the lack of troponin in smooth muscle prevents Ca^{2+} activation via troponin binding. Rather, myosin in smooth muscle must be phosphorylated for activation of the myosin ATPase. Phosphorylation and dephosphorylation of myosin also occur in skeletal muscle, but phosphorylation is not necessary for activation of the ATPase. In smooth muscle, Ca^{2+} binds to calmodulin, and the resulting complex activates **calmodulin-dependent myosin light chain kinase.** This enzyme catalyzes the phosphorylation of the myosin light chain on serine at position 19, increasing its ATPase activity.

Myosin is dephosphorylated by **myosin light chain phosphatase** in the cell. However, dephosphorylation of myosin light chain kinase does not necessarily lead to relaxation of the smooth muscle. Various mechanisms are involved. One appears to be a latch bridge mechanism by which myosin cross-bridges remain attached to actin for some time after the cytoplasmic Ca^{2+} concentration falls. This produces sustained contraction with little expenditure of energy, which is especially important in vascular smooth muscle. Relaxation of the muscle presumably occurs when the Ca^{2+}–calmodulin complex finally dissociates or when some other mechanism comes into play. The events leading to contraction and relaxation of unitary smooth muscle are summarized in **Figure 5–19**. The events in multiunit smooth muscle are generally similar.

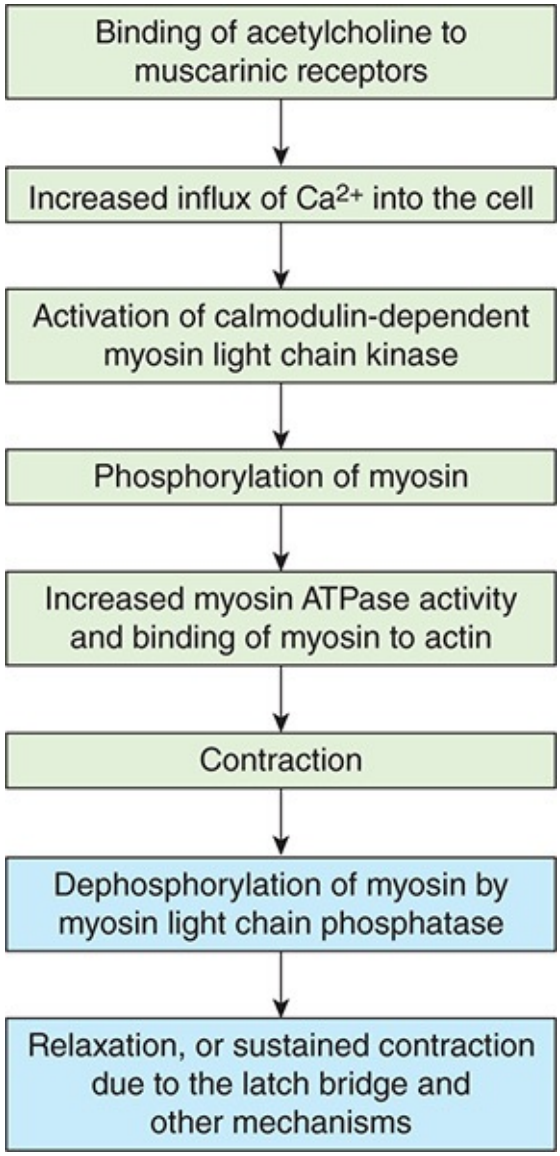


FIGURE 5–19 Sequence of events in contraction and relaxation of smooth muscle. Flow chart illustrates many of the molecular changes that occur from the initiation of contraction to its relaxation. Note the distinct differences from skeletal and cardiac muscle excitation.

Unitary smooth muscle is unique in that, unlike other types of muscle, it contracts when stretched in the absence of any extrinsic innervation. Stretch is followed by a decline (depolarization) in membrane potential, an increase in the frequency of spikes, and a general increase in tone.

If epinephrine or norepinephrine is added to a preparation of intestinal smooth muscle arranged for recording of intracellular potentials in vitro, the membrane potential usually becomes larger (more negative or hyperpolarization), the spikes

decrease in frequency, and the muscle relaxes **(Figure 5–20)**. Norepinephrine is the chemical mediator released at noradrenergic nerve endings, and stimulation of the noradrenergic nerves to the preparation produces inhibitory potentials. Acetylcholine has an effect opposite to that of norepinephrine on the membrane potential and contractile activity of intestinal smooth muscle. If acetylcholine is added to the fluid bathing a smooth muscle preparation in vitro, the membrane potential decreases (less negative or depolarization) and the spikes become more frequent. The muscle becomes more active, with an increase in tonic tension and the number of rhythmic contractions. The effect is mediated by phospholipase C, which produces IP_3 and allows for Ca^{2+} release through IP_3 receptors. In the intact animal, stimulation of cholinergic nerves causes release of acetylcholine, excitatory potentials, and increased intestinal contractions.

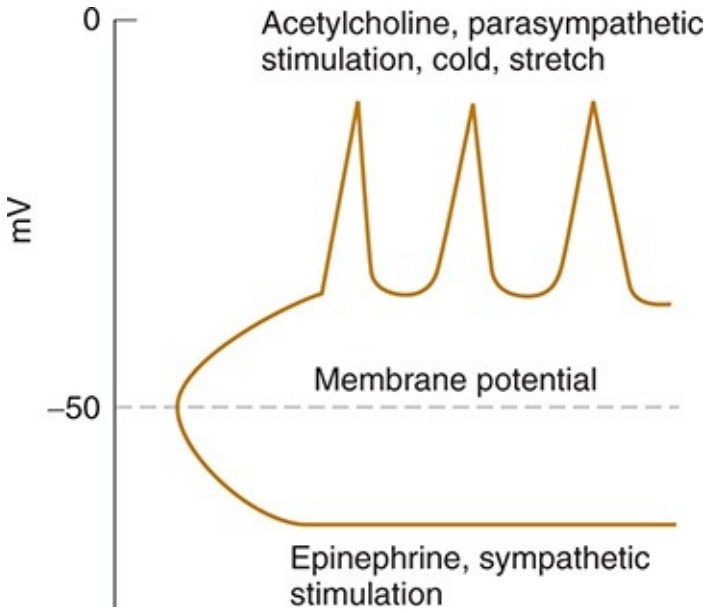


FIGURE 5–20 Effects of various agents on the membrane potential of intestinal smooth muscle. Drugs and hormones can alter firing of smooth muscle action potentials by raising (top trace) or lowering (bottom trace) resting membrane potential.

Like unitary smooth muscle, multiunit smooth muscle is very sensitive to circulating chemical substances and is normally activated by chemical mediators (acetylcholine and norepinephrine) released at the endings of its motor nerves. Norepinephrine in particular tends to persist in the muscle and to cause repeated firing of the muscle after a single stimulus rather than a single action potential. Therefore, the contractile response produced is usually an irregular tetanus rather

than a single twitch. When a single twitch response is obtained, it resembles the twitch contraction of skeletal muscle except that its duration is 10 times long.

RELAXATION

In addition to cellular mechanisms that increase contraction of smooth muscle, there are cellular mechanisms that lead to its relaxation **(Clinical Box 5–7)**. This is especially important in smooth muscle that forms the blood vessels or arteries to increase blood flow. The arterial wall consists of three layers: the intima containing mainly the endothelium or endothelial cells, the media containing mainly smooth muscle cells, and the externa or adventitia containing mainly connective tissues, extracellular matrix and fibroblasts. It was long known that endothelial cells that line the inside (the endothelium) of blood vessels could release a substance that relaxed smooth muscle **(endothelium-derived relaxing factor, EDRF).** EDRF was later identified as the gaseous second messenger molecule, **nitric oxide (NO).** NO produced in endothelial cells is free to diffuse into the smooth muscle for its effects. Once in muscle, NO directly activates a soluble guanylyl cyclase to produce another second messenger molecule, **cyclic guanosine monophosphate (cGMP).** This molecule can activate cGMP-specific protein kinases that can affect ion channels, Ca^{2+} homeostasis, or phosphatases, or all of those mentioned, leading to smooth muscle relaxation (see Chapter 32).

CLINICAL BOX 5–7

Common Drugs That Act on Smooth Muscle

Overexcitation of smooth muscle in the airways, such as that observed during an asthma attack, can lead to bronchoconstriction or bronchospasm. Inhalers that deliver drugs to the conducting airway are commonly used to offset this bronchial smooth muscle contraction, as well as other symptoms in the asthmatic airways. The rapid effects of drugs in inhalers are related to smooth muscle relaxation. Rapid response inhaler drugs (eg, ventolin, albuterol, sambuterol) target β-adrenergic receptors in the airway smooth muscle to elicit a relaxation. Although these β-adrenergic receptor agonists targeting the smooth muscle do not treat all symptoms associated with asthma (eg, inflammation and increased mucus), they act rapidly and frequently allow for sufficient opening of the conducting airway to restore airflow, and thus allow for other treatments to reduce airway obstruction.

Smooth muscle is also a target for drugs developed to increase blood flow and decrease blood pressure. As discussed in the text, NO is a natural signaling molecule that relaxes smooth muscle by raising cyclic guanosine monophosphate (cGMP). This signaling pathway is naturally downregulated by the action of **phosphodiesterase (PDE),** which transforms cGMP into a nonsignaling form, GMP. The drugs sildenafil, tadalafil, and vardenafil are all specific inhibitors of PDE V, an isoform found mainly in the smooth muscle in the corpus cavernosum of the penis (see Chapters 25 and 32) and the pulmonary vasculature. Thus, oral administration of these drugs can block the action of PDE V, increasing blood flow in a very limited region in the body and offsetting erectile dysfunction. These drugs are also used to treat pulmonary arterial hypertension.

FUNCTION OF THE NERVE SUPPLY TO SMOOTH MUSCLE

The effects of acetylcholine and norepinephrine on unitary smooth muscle serve to emphasize two of its important properties: (1) its spontaneous activity in the absence of nervous stimulation and (2) its sensitivity to chemical agents released from nerves locally or brought to it in the circulation. In mammals, unitary muscle usually has a dual nerve supply from the two divisions of the autonomic nervous system. The function of the nerve supply is not to initiate activity in the muscle but rather to modify it. Stimulation of one division of the autonomic nervous system usually increases smooth muscle activity, whereas stimulation of the other decreases it. In some organs, noradrenergic stimulation increases and cholinergic stimulation decreases smooth muscle activity; in others, the reverse is true.

FORCE GENERATION & PLASTICITY OF SMOOTH MUSCLE

Smooth muscle displays a unique economy when compared to skeletal muscle. Despite approximately 20% of the myosin content and a 100-fold difference in ATP use when compared with skeletal muscle, they can generate similar force per cross-sectional area. One of the tradeoffs of obtaining force under these conditions is the noticeably slower contractions when compared to skeletal

muscle. There are several known reasons for these noticeable changes, including unique isoforms of myosin and contractile-related proteins expressed in smooth muscle and their distinct regulation. The unique architecture of the smooth cell and its coordinated units also likely contribute to these changes.

Another special characteristic of smooth muscle is the variability of the tension it exerts at any given length. If a unitary smooth muscle is stretched, it first exerts increased tension. However, if the muscle is held at the greater length after stretching, the tension gradually decreases. Sometimes the tension falls to or below the level exerted before the muscle was stretched. It is consequently impossible to correlate length and developed tension accurately, and no resting length can be assigned. In some ways, therefore, smooth muscle behaves more like a viscous mass than a rigidly structured tissue, and it is this property that is referred to as the **plasticity** of smooth muscle.

The consequences of plasticity can be demonstrated in humans. For example, the tension exerted by the smooth muscle of the bladder wall can be measured at different degrees of distension as fluid is infused into the bladder via a catheter. Initially, tension increases relatively little as volume is increased because of the plasticity of the bladder wall. However, a point is eventually reached at which the bladder contracts forcefully (see Chapter 37).

CHAPTER SUMMARY

- There are three main types of muscle cells: skeletal, cardiac, and smooth.
- Skeletal muscle is a true syncytium under voluntary control. Skeletal muscles receive electrical stimuli from nerve cells to elicit contraction: "excitation–contraction coupling." Action potentials in muscle cells are developed largely through coordination of Na^+ and K^+ channels. Contraction in skeletal muscle cells is coordinated through Ca^{2+} regulation of the actomyosin system that gives the muscle its classic striated pattern under the microscope.
- There are several different types of skeletal muscle fibers (I, IIA, IIB) that have distinct properties in terms of protein makeup and force generation. Skeletal muscle fibers are arranged into motor units of like fibers within a muscle. Skeletal motor units are recruited in a specific pattern as the need for more force is increased.
- Cardiac muscle is a collection of individual cells (cardiomyocytes) that are linked as a syncytium by gap junctional communication. Cardiac muscle

cells also undergo excitation–contraction coupling. Pacemaker cells in the heart can initiate propagated action potentials. Cardiac muscle cells also have a striated, actomyosin system that underlies contraction.

- Smooth muscle exists as individual cells and are frequently under control of the autonomic nervous system.
- There are two broad categories of smooth muscle cells: unitary and multiunit. Unitary smooth muscle contraction is synchronized by gap junctional communication to coordinate contraction among many cells. Multiunit smooth muscle contraction is coordinated by motor units, functionally similar to skeletal muscle.
- Smooth muscle cells contract through an actomyosin system, but do not have well-organized striations. Unlike skeletal and cardiac muscle, Ca^{2+} regulation of contraction is primarily through phosphorylation–dephosphorylation reactions.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. The action potential of skeletal muscle
   A. has a prolonged plateau phase.
   B. spreads inward to all parts of the muscle via the T tubules.
   C. causes the immediate uptake of Ca^{2+} into the lateral sacs of the sarcoplasmic reticulum.
   D. is longer than the action potential of cardiac muscle.
   E. is not essential for contraction.
2. The functions of tropomyosin in skeletal muscle include
   A. sliding on actin to produce shortening.
   B. releasing Ca^{2+} after initiation of contraction.
   C. binding to myosin during contraction.
   D. acting as a “relaxing protein” at rest by covering up the sites where myosin binds to actin.
   E. generating ATP, which it passes to the contractile mechanism.
3. The cross-bridges of the sarcomere in skeletal muscle are made up of
   A. actin.

B. myosin.
C. troponin.
D. tropomyosin.
E. myelin.

4. The contractile response in skeletal muscle
A. starts after the action potential is over.
B. does not last as long as the action potential.
C. produces more tension when the muscle contracts isometrically than when the muscle contracts isotonically.
D. produces more work when the muscle contracts isometrically than when the muscle contracts isotonically.
E. decreases in magnitude with repeated stimulation.

5. Gap junctions
A. are absent in cardiac muscle.
B. are present but of little functional importance in cardiac muscle.
C. are present and provide the pathway for rapid spread of excitation from one cardiac muscle fiber to another.
D. are absent in smooth muscle.
E. connect the sarcotubular system to individual skeletal muscle cells.

CHAPTER 6

Synaptic & Junctional Transmission

OBJECTIVES

After studying this chapter, you should be able to:

- Describe the major location and functional components of a neuron-to-neuron synapse.
- Contrast the ionic fluxes responsible for the fast and slow excitatory and inhibitory postsynaptic potentials.
- Compare and contrast the terms temporal summation and spatial summation and their role in action potential generation in a postsynaptic neuron.
- Explain postsynaptic inhibition, presynaptic inhibition, and presynaptic facilitation.
- Identify the components of the neuromuscular junction and the sequence of events that leads to a propagated action potential in the skeletal muscle fiber.
- Explain how autonomic neurons communicate with their effector organs at a neuroeffector junction.
- Define denervation hypersensitivity.
- Describe some pathologies associated with dysfunction at the neuromuscular junction.

INTRODUCTION

The “all-or-none” type of conduction seen in axons and skeletal muscle has been discussed in Chapters 4 and 5. Impulses are transmitted from one neuron to another at a **synapse.** This is the region where the axon or some other portion of one neuron (**presynaptic neuron**) terminates on the dendrites, soma, or axon of another neuron (**postsynaptic neuron**). Neuron-to-neuron communication occurs across either a **chemical** or an **electrical synapse.** Because most synaptic transmission is chemical, consideration in this chapter is primarily related to chemical neurotransmission.

When a neuron terminates on a muscle, the connection is properly called a **neuromuscular junction** rather than a synapse. Transmission from a nerve to a muscle resembles chemical synaptic transmission from one neuron to another. The contacts between autonomic neurons and smooth and cardiac muscle or glands are less specialized than those between a neuron and skeletal muscle, and transmission in these locations is a more diffuse process. The region where the neuron communicates with the effector organ is called the **neuroeffector junction**. These forms of transmission are also considered in this chapter.

SYNAPTIC TRANSMISSION: FUNCTIONAL ANATOMY

The anatomic structure of synapses varies considerably in the different parts of the mammalian nervous system. The ends of the presynaptic fibers are generally enlarged to form **terminal boutons** or **synaptic knobs** **(Figure 6–1)**. In the cerebral and cerebellar cortex, endings are commonly located on dendrites **(axodendritic synapse)** and frequently on **dendritic spines,** which are small knobs projecting from dendrites **(Figure 6–2)**. Some presynaptic nerves terminate on the soma **(axosomatic synapse)** or axons **(axoaxonal synapses)** of postsynaptic neurons. On average, each neuron divides to form over 2000 synaptic endings; thus, communication between neurons is very complex. Synapses are dynamic structures, increasing and decreasing in complexity and number with use and experience.

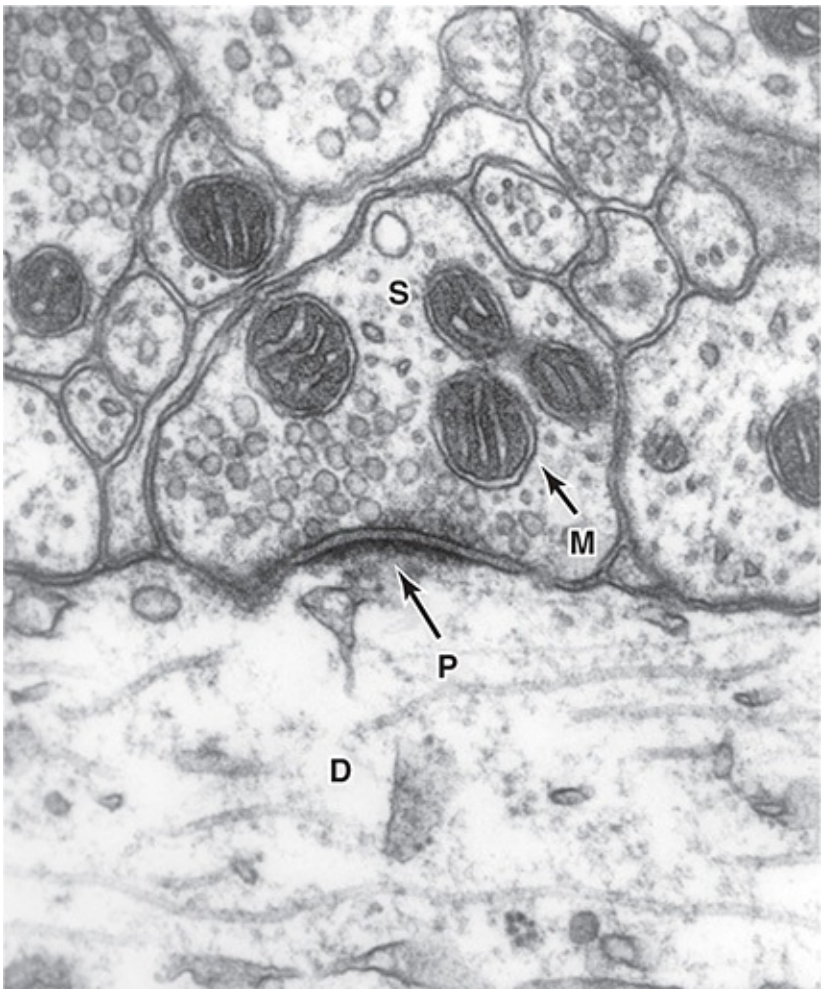
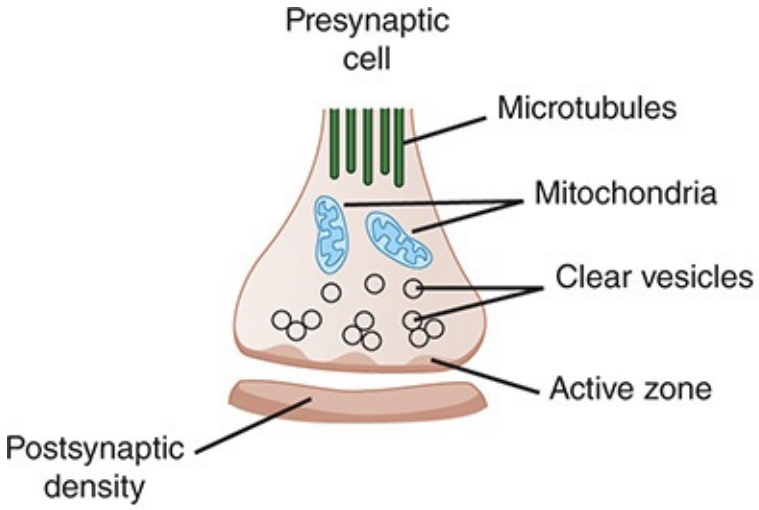


FIGURE 6–1 Electron micrograph of synaptic knob (S) ending on the shaft of a dendrite (D) in the central nervous system. P, postsynaptic density; M, mitochondrion (×56,000).


Presynaptic cell
Microtubules
Mitochondria
Clear vesicles
Active zone
Postsynaptic density

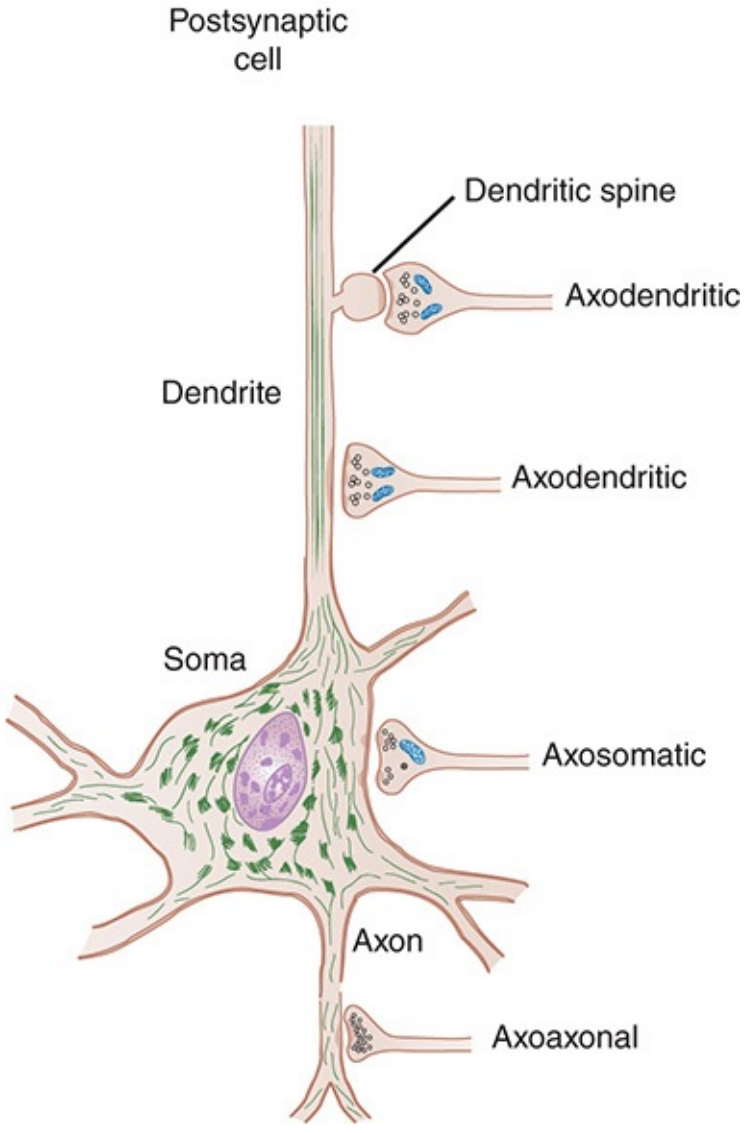

Postsynaptic cell
Dendritic spine
Axodendritic
Dendrite
Axodendritic
Soma
Axosomatic
Axon
Axoaxonal

FIGURE 6–2 Axodendritic, axoaxonal, and axosomatic synapses. Many presynaptic neurons terminate on dendritic spines, as shown at the top, but some also end directly on the shafts of dendrites. Note the presence of clear and granulated synaptic vesicles in endings and clustering of clear vesicles at active zones.

FUNCTIONS OF SYNAPTIC ELEMENTS

The dendrites function as a major site that receives electrical signals from other neurons, processes these signals, and transfers the information to the soma of the neuron. When the dendritic tree of a neuron is extensive and has multiple presynaptic knobs ending on it, there is room for a great interplay of inhibitory and excitatory activity. Dendritic spines can appear, change, and even disappear over a time scale of minutes and hours, not days and months. Also, although protein synthesis occurs mainly in the soma with its nucleus, strands of mRNA migrate into the dendrites. There, each can become associated with a single ribosome in a dendritic spine and produce proteins to alter the effects of input from individual synapses on the spine. These changes in dendritic spines have been implicated in motivation, learning, and long-term memory (see Chapter 15).

Each presynaptic terminal of a chemical synapse is separated from the postsynaptic structure by a **synaptic cleft** that is 20–40 nm wide. Across the synaptic cleft are many neurotransmitter receptors in the postsynaptic membrane, and usually a postsynaptic thickening called the **postsynaptic density** (Figure 6–2). The postsynaptic density is an ordered complex of specific receptors, binding proteins, and enzymes induced by postsynaptic effects.

Inside the presynaptic terminal are many mitochondria, as well as many membrane-enclosed vesicles, which contain neurotransmitters (see Chapter 7). There are three kinds of **synaptic vesicles:** small, clear synaptic vesicles contain acetylcholine, glycine, GABA, or glutamate; small dense-core vesicles contain catecholamines; and large dense-core vesicles contain neuropeptides. The vesicles and the proteins contained within them are synthesized in the neuronal cell body and transported along the axon to the endings by **fast axoplasmic transport**. The neuropeptides in the large dense-core vesicles must also be produced by the protein-synthesizing machinery in the cell body. However, the small clear vesicles and the small dense-core vesicles recycle in the nerve ending **(Figure 6–3)**. These vesicles fuse with the cell membrane and release transmitters through **exocytosis** and are then recovered by endocytosis to be

refilled locally. In some instances, they enter endosomes and are budded off the endosome and refilled, starting the cycle over again. More commonly, however, the synaptic vesicle discharges its contents through a small hole in the cell membrane, then the opening reseals rapidly and the main vesicle stays inside the cell **(kiss-and-run discharge).** In this way, the full endocytotic process is short-circuited.

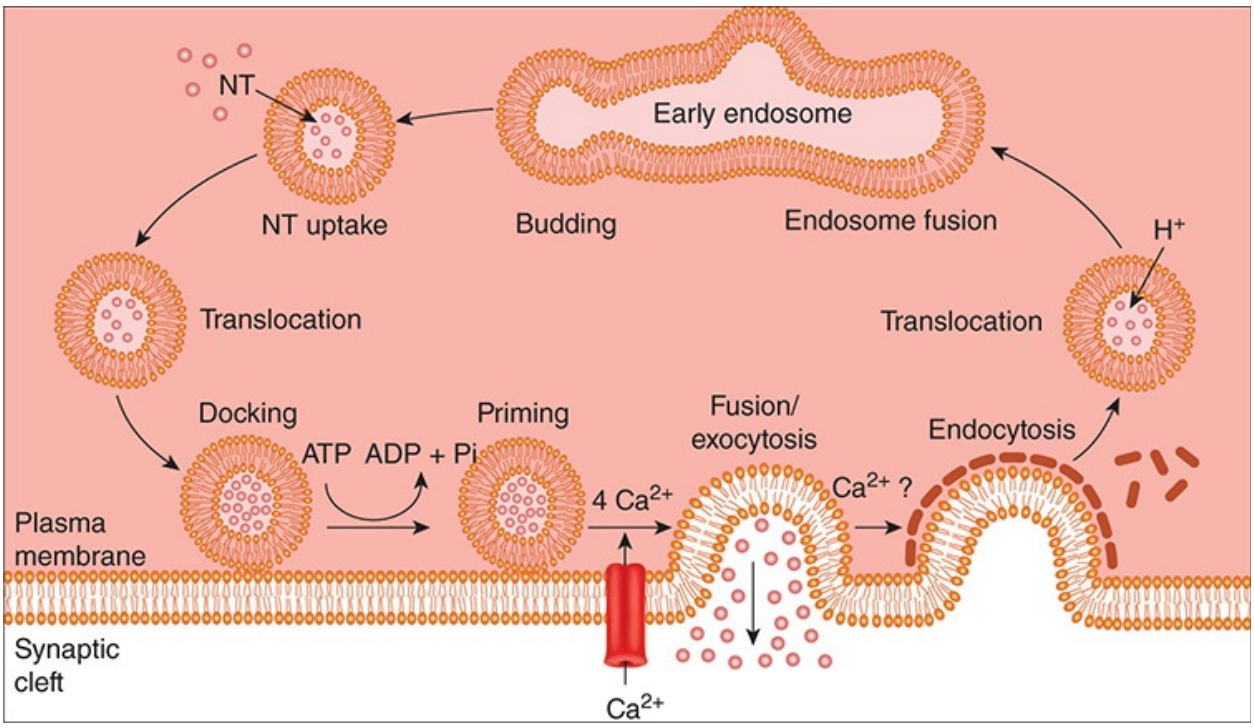


FIGURE 6–3 Small synaptic vesicle cycle in presynaptic nerve terminals. Vesicles bud off the early endosome and then are filled with a neurotransmitter (NT). The vesicles then translocate toward release sites on the plasma membrane where they dock, and then become primed. Following arrival of an action potential at the nerve ending, Ca^{2+} influx triggers fusion and exocytosis of the vesicular contents into the synaptic cleft. The vesicle wall then is coated with clathrin, which likely facilitates endocytosis. Once back inside the cytoplasm, the vesicle becomes uncoated and fuses with the early endosome to start the cycle again.

The large dense-core vesicles are found throughout some presynaptic nerve terminals and release their neuropeptide contents by exocytosis from all parts of the terminal. On the other hand, the small vesicles are located near the synaptic cleft and fuse to the membrane, discharging their contents very rapidly into the cleft at areas of membrane thickening called **active zones** (Figure 6–2). Voltage-

gated Ca^{2+} channels are very close to the release sites at the active zones.

The Ca^{2+} that triggers exocytosis of transmitters enters the presynaptic neurons, and transmitter release starts within 200 μs. In addition, the transmitter must be released close to the postsynaptic receptors to be effective on the postsynaptic neuron. This orderly organization of the synapse depends in part on **neurexins**, presynaptic cell-adhesion molecules that bind to **neuroligins** on the membrane of postsynaptic neurons. Neurexin–neuroligin interactions not only hold synapses together, but they also provide a mechanism for the production of synaptic specificity.

As noted in Chapter 2, vesicle budding, fusion, and discharge of contents with subsequent retrieval of vesicle membrane are fundamental processes occurring in most, if not all, cells. Thus, neurotransmitter secretion at synapses and the accompanying membrane retrieval are specialized forms of the general processes of exocytosis and endocytosis. The fusion of synaptic vesicles with the cell membrane involves the action of several proteins, including the N-ethylmaleimide–sensitive fusion protein (NSF), SNAPs (soluble NSF attachment proteins), and SNAREs (SNAP receptors; **Figure 6–4**). **Synaptobrevin** in the vesicle membrane locks with **syntaxin** and **SNAPs** in the cell membrane; GTPases regulate a multiprotein complex that includes Rab and Sec1/Munc18-like proteins as part of the fusion process. The one-way gate at the synapses is necessary for orderly neural function.

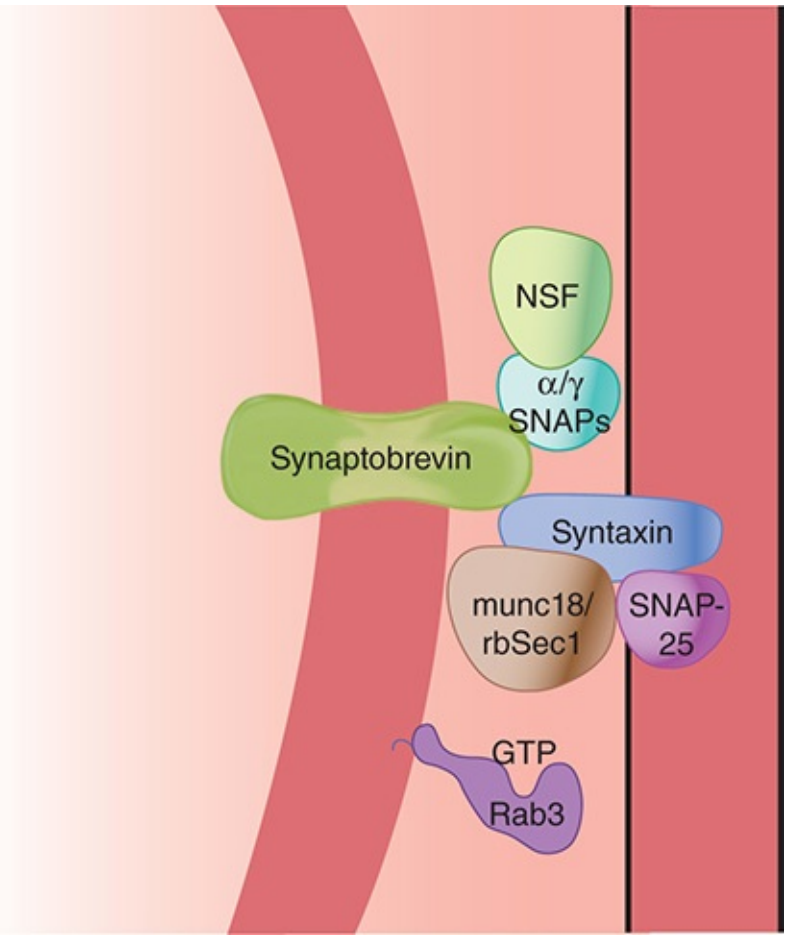


FIGURE 6–4 Main proteins that interact to produce synaptic vesicle docking and fusion in nerve endings. Several proteins are involved in synaptic vesicle docking and fusion, including the N-ethylmaleimide–sensitive fusion protein (NSF), SNAPs (soluble NSF attachment proteins), and SNAREs (SNAP receptors). Synaptobrevin in the vesicle membrane links with syntaxin and SNAPs in the cell membrane; GTPases regulate a multiprotein complex that includes Rab and Sec1/Munc18-like proteins.

Several deadly toxins that block neurotransmitter release are zinc endopeptidases that cleave and hence inactivate proteins in the fusion-exocytosis complex. **Clinical Box 6–1** describes how neurotoxins from bacteria called *Clostridium tetani* and *Clostridium botulinum* can disrupt neurotransmitter release in either the central nervous system (CNS) or at the neuromuscular junction.

ELECTRICAL EVENTS IN POSTSYNAPTIC

NEURONS

EXCITATORY & INHIBITORY POSTSYNAPTIC POTENTIALS

Stimulation of a dorsal root afferent (sensory neuron) can be used to study both excitatory and inhibitory events in α-motor neurons **(Figure 6–5)**. Once an impulse reaches the presynaptic terminals of the sensory neuron, a response can be obtained in the postsynaptic neuron after a **synaptic delay.** The delay is due to the time it takes for the synaptic mediator to be released and to act on the receptors on the membrane of the postsynaptic neuron. Because of it, conduction along a chain of neurons is slower if there are many synapses compared to if there are only a few synapses. Because the minimum time for transmission across one synapse is 0.5 ms, it is possible to determine whether a given reflex pathway is **monosynaptic** or **polysynaptic** (contains more than one synapse) by measuring the synaptic delay.

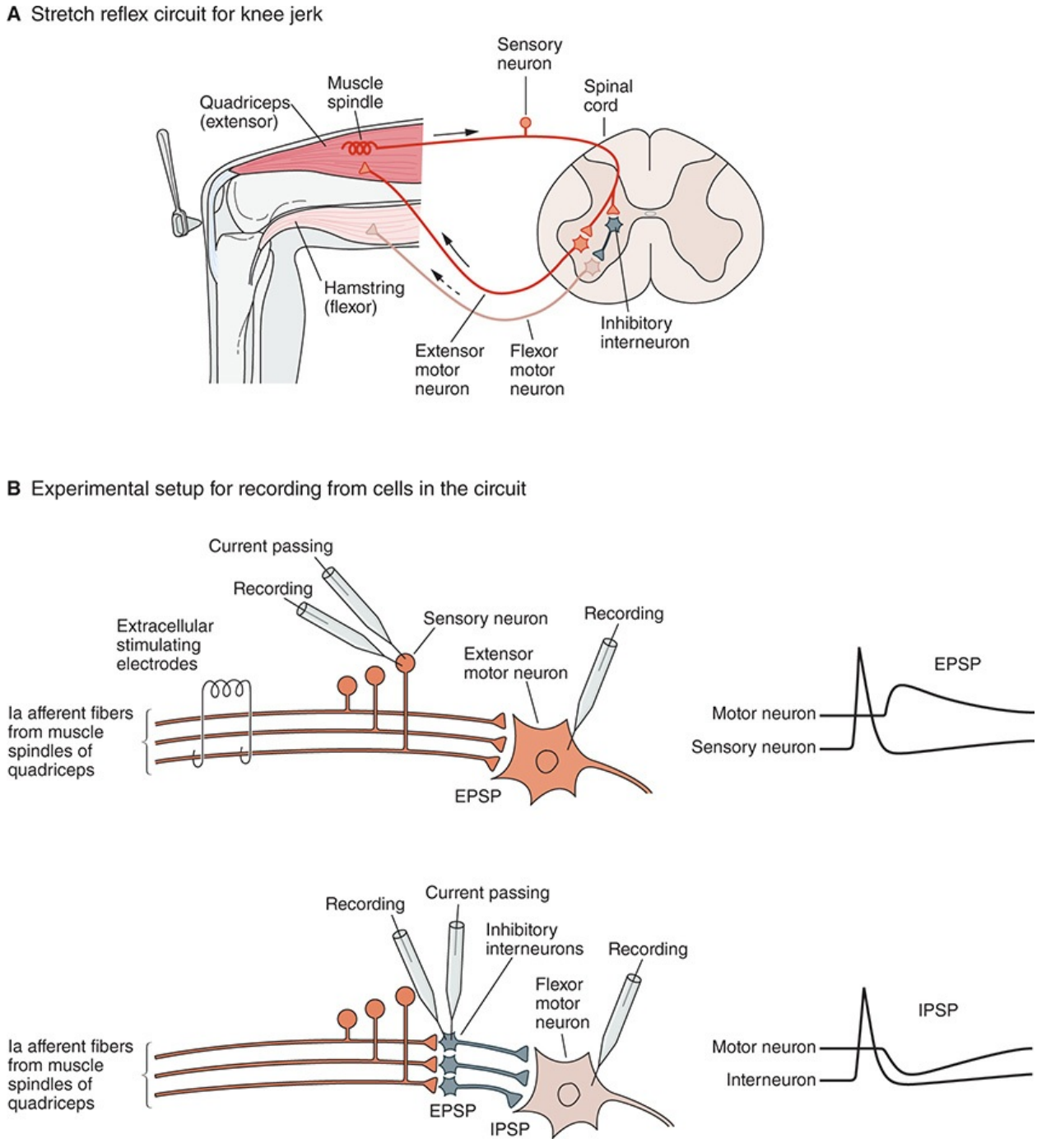


FIGURE 6–5 Excitatory and inhibitory synaptic connections mediating the stretch reflex provide an example of typical circuits within the CNS. A) The stretch receptor sensory neuron of the quadriceps muscle makes an excitatory connection with the extensor motor neuron of the same muscle and an inhibitory interneuron projecting to flexor motor neurons supplying the antagonistic hamstring muscle. **B)** Experimental setup to study excitation and inhibition of the extensor motor neuron. Top panel shows two approaches to elicit an

excitatory (depolarizing) postsynaptic potential or EPSP in the extensor motor neuron–electrical stimulation of the whole Ia afferent nerve using extracellular electrodes and intracellular current passing through an electrode inserted into the cell body of a sensory neuron. Bottom panel shows that current passing through an inhibitory interneuron elicits an inhibitory (hyperpolarizing) postsynaptic potential or IPSP in the flexor motor neuron. EPSP, excitatory postsynaptic potential; IPSP, inhibitory postsynaptic potential. (Reproduced with permission from Kandel ER, Schwartz JH, Jessell TM [editors]: *Principles of Neural Science*, 4th ed. New York, NY: McGraw-Hill; 2000.)

CLINICAL BOX 6–1

Botulinum and Tetanus Toxins

Clostridia are gram-positive bacteria. Two varieties, *Clostridium tetani* and *Clostridium botulinum*, produce some of the most potent biologic toxins **(tetanus toxin** and **botulinum toxin)** known to affect humans. These neurotoxins act by preventing the release of neurotransmitters in the CNS and at the neuromuscular junction. Tetanus toxin binds irreversibly to the presynaptic membrane of the neuromuscular junction and uses retrograde axonal transport to travel to the cell body of the motor neuron in the spinal cord. From there it is picked up by the terminals of presynaptic inhibitory interneurons. The toxin attaches to **gangliosides** in these terminals and blocks the release of glycine and GABA. As a result, the activity of motor neurons is markedly increased. Clinically, tetanus toxin causes spastic paralysis; the characteristic symptom of “lockjaw” is spasms of the masseter muscle. Botulism can result from ingestion of contaminated food, colonization of the gastrointestinal tract in an infant, or wound infection. Botulinum toxins are a family of seven neurotoxins, but it is mainly botulinum toxins A, B, and E that are toxic to humans. Botulinum toxins A and E cleave synaptosome-associated protein-25 **(SNAP-25).** This is a presynaptic membrane protein needed for fusion of synaptic vesicles containing acetylcholine to the terminal membrane, an important step in transmitter release. Botulinum toxin B cleaves **synaptobrevin,** a vesicle-associated membrane protein **(VAMP).** By blocking acetylcholine release at the neuromuscular junction, these toxins cause flaccid paralysis. Symptoms can include ptosis (drooping eyelid), diplopia (double vision), dysarthria (slurred speech), dysphonia (difficulty speaking), and dysphagia (difficulty swallowing).

THERAPEUTIC HIGHLIGHTS

Tetanus can be prevented by treatment with **tetanus toxoid vaccine.** The widespread use of this vaccine in the United States beginning in the mid-1940s led to a marked decline in the incidence of tetanus toxicity. The incidence of botulinum toxicity is also low (about 100 cases per year in the United States), but in those individuals who are affected, the fatality rate is 5–10%. An antitoxin is available for treatment, and those who are at risk for respiratory failure are placed on a ventilator. On the positive side, local injection of small doses of botulinum toxin (**botox**) has proven to be effective in the treatment of a wide variety of conditions characterized by muscle hyperactivity. Examples include injection into the lower esophageal sphincter to relieve achalasia, injection into facial muscles to remove wrinkles, and injection into leg muscles to reduce spasticity in individuals with cerebral palsy.

A single stimulus applied to the sensory nerves characteristically does not lead to the formation of a propagated action potential in the postsynaptic neuron. Instead, the stimulation produces either a transient partial depolarization or a transient hyperpolarization. The initial depolarizing response produced by a single stimulus to the proper input begins about 0.5 ms after the afferent impulse enters the spinal cord. It reaches its peak 1–1.5 ms later and then declines exponentially (Figure 6–5). During this potential, the excitability of the neuron to other stimuli is increased; thus, it is called a fast **excitatory postsynaptic potential (EPSP)**.

The fast EPSP is produced by depolarization of the postsynaptic cell membrane immediately under the presynaptic ending. The excitatory transmitter opens Na^+ or Ca^{2+} channels in the postsynaptic membrane, producing an inward current. The area of current flow thus created is so small that it does not drain off enough positive charge to depolarize the whole membrane. Instead, an EPSP is induced. The EPSP due to activity in one synaptic knob is small, but the depolarizations produced by each of the active knobs summate.

EPSPs are produced by stimulation of some inputs, but stimulation of other inputs produces hyperpolarizing responses. Like the EPSPs, they peak 1–1.5 ms after the stimulus and decrease exponentially (Figure 6–5). During this potential, the excitability of the neuron to other stimuli is decreased; thus, it is called a fast **inhibitory postsynaptic potential (IPSP)**.

A fast IPSP can be produced by a localized increase in Cl^- transport. When an

inhibitory synaptic knob becomes active, the released neurotransmitter triggers the opening of Cl^- channels in the area of the postsynaptic cell membrane under the knob. Cl^- moves down its concentration gradient. The net effect is the transfer of negative charge into the cell, increasing the membrane potential.

The decreased excitability of the nerve cell during the IPSP is due to movement of the membrane potential away from the firing level. Consequently, more excitatory (depolarizing) activity is necessary to reach the firing level. The fact that an IPSP is mediated by Cl^- can be demonstrated by repeating the stimulus while varying the resting membrane potential of the postsynaptic cell. When the membrane potential is at the equilibrium potential for chloride (E_{Cl}), the postsynaptic potential disappears **(Figure 6–6)**, and at more negative membrane potentials, it becomes positive **(reversal potential).**

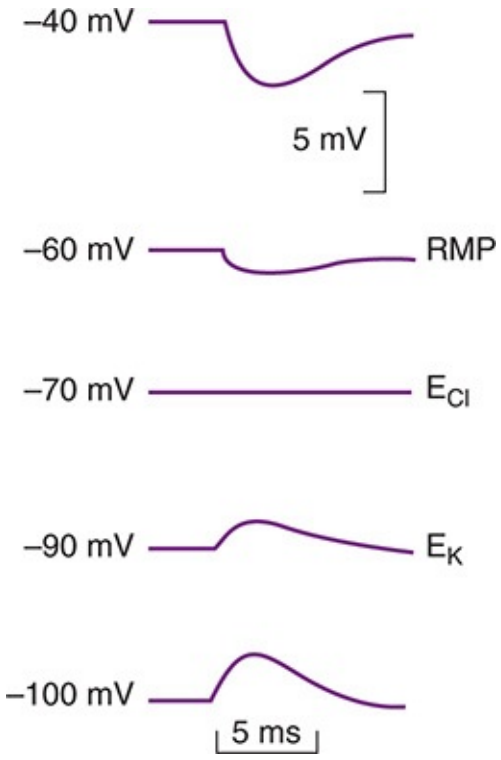


FIGURE 6–6 IPSP is due to increased Cl^- influx during stimulation. This can be demonstrated by repeating the stimulus while varying the resting membrane potential (RMP) of the postsynaptic cell. When the membrane potential is at E_{Cl}, the potential disappears, and at more negative membrane potentials (eg, E_K and below), it becomes positive (reversal potential).

Because IPSPs are net hyperpolarizations, they can be produced by alterations

in other ion channels in the neuron. For example, they can be produced by opening K^+ channels, with movement of K^+ out of the postsynaptic cell, or by closure of Na^+ or Ca^{2+} channels.

SLOW POSTSYNAPTIC POTENTIALS

In addition to the fast EPSPs and IPSPs, slow EPSPs and IPSPs occur in autonomic ganglia, cardiac and smooth muscle, and cortical neurons. These postsynaptic potentials have a latency of 100–500 ms and last several seconds. The slow EPSPs are generally due to decreases in K^+ conductance, and the slow IPSPs are due to increases in K^+ conductance.

ELECTRICAL TRANSMISSION

In electrical synapses, the membranes of the presynaptic and postsynaptic neurons come close together, and gap junctions are formed between the cells (see Chapter 2). Like the intercellular junctions in other tissues, these junctions form low-resistance bridges through which ions can pass with relative ease. At these junctions the impulse reaching the presynaptic terminal generates an EPSP in the postsynaptic cell that, because of the low-resistance bridge between the two, has a much shorter latency than the EPSP at a synapse where transmission is chemical.

GENERATION OF AN ACTION POTENTIAL IN THE POSTSYNAPTIC NEURON

The constant interplay of excitatory and inhibitory activity on the postsynaptic neuron produces a fluctuating membrane potential that is the algebraic summation of the hyperpolarizing and depolarizing potentials. The soma of the neuron thus acts as an integrator. When the level of depolarization reaches the threshold voltage, a propagated action potential will occur. However, the discharge of the neuron is slightly more complicated than this. In motor neurons, the portion of the cell with the lowest threshold for the production of an action potential is the initial segment, the portion of the axon at and just beyond the axon hillock (see Chapter 4). This unmyelinated segment is depolarized or hyperpolarized electrotonically by the current sinks and sources under the

excitatory and inhibitory synaptic knobs. It is the first part of the neuron to fire, and its discharge is propagated in two directions: down the axon and back into the soma. Retrograde firing of the soma in this fashion probably has value in wiping the slate clean for subsequent renewal of the interplay of excitatory and inhibitory activity on the cell.

TEMPORAL & SPATIAL SUMMATION OF POSTSYNAPTIC POTENTIALS

Two passive membrane properties of a neuron affect the ability of postsynaptic potentials to summate to elicit an action potential **(Figure 6–7)**. The **time constant** of a neuron determines the time course of the synaptic potential, and the **length constant** of a neuron determines the degree to which a depolarizing current is reduced as it spreads passively. Figure 6–7 also shows how the time constant of the postsynaptic neuron can affect the amplitude of the depolarization caused by consecutive EPSPs produced by a single presynaptic neuron. The longer the time constant, the greater is the chance for two potentials to summate to induce an action potential. If a second EPSP is elicited before the first EPSP decays, the two potentials summate and, as in this example, their additive effects are sufficient to induce an action potential in the postsynaptic neuron **(temporal summation).** Figure 6–7 also shows how the length constant of a postsynaptic neuron can affect the amplitude of two EPSPs produced by different presynaptic neurons in a process called **spatial summation.** If a neuron has a long length constant, the membrane depolarization induced by input arriving at two points on the neuron can spread to the trigger zone of the neuron with minimal decrement. The two potentials can summate and induce an action potential.

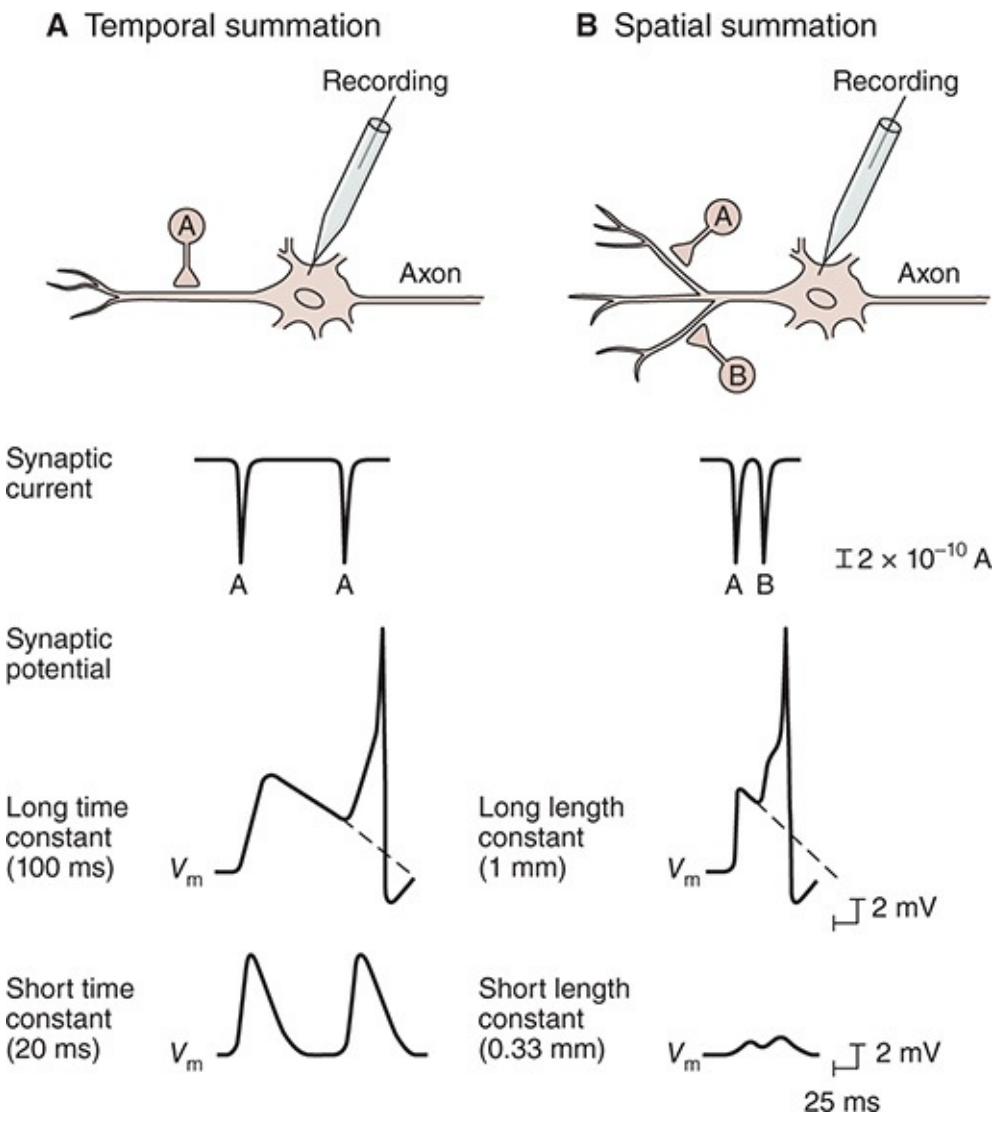


FIGURE 6–7 Central neurons integrate a variety of synaptic inputs through temporal and spatial summation. A) The time constant of the postsynaptic neuron affects the amplitude of the depolarization caused by consecutive EPSPs produced by a single presynaptic neuron. In cases of a long time constant, if a second EPSP is elicited before the first EPSP decays, the two potentials summate to induce an action potential. **B)** The length constant of a postsynaptic cell affects the amplitude of two EPSPs produced by two presynaptic neurons, A andB. If the length constant is long, the depolarization induced at two points on the neuron can spread to the trigger zone with minimal decrement so that the two potentials summate and an action potential is elicited. EPSP, excitatory postsynaptic potential. (Reproduced with permission from Kandel ER, Schwartz JH, Jessell TM [editors]: *Principles of Neural Science*, 4th ed. New York, NY: McGraw-Hill; 2000.)

INHIBITION & FACILITATION AT SYNAPSES

Inhibition within the CNS can be either postsynaptic or presynaptic. Examples of the neuronal connections that can mediate **presynaptic inhibition** and **postsynaptic inhibition** are compared in **Figure 6–8**. Postsynaptic inhibition during the course of an IPSP is called **direct inhibition** because it is not a consequence of previous discharges of the postsynaptic neuron. There are various forms of **indirect inhibition** that is due to the effects of previous postsynaptic neuron discharge. For example, the postsynaptic cell can be refractory to excitation because it has just fired and is in its refractory period. During after-hyperpolarization it is also less excitable. In spinal neurons, especially after repeated firing, this after- hyperpolarization may be large and prolonged.

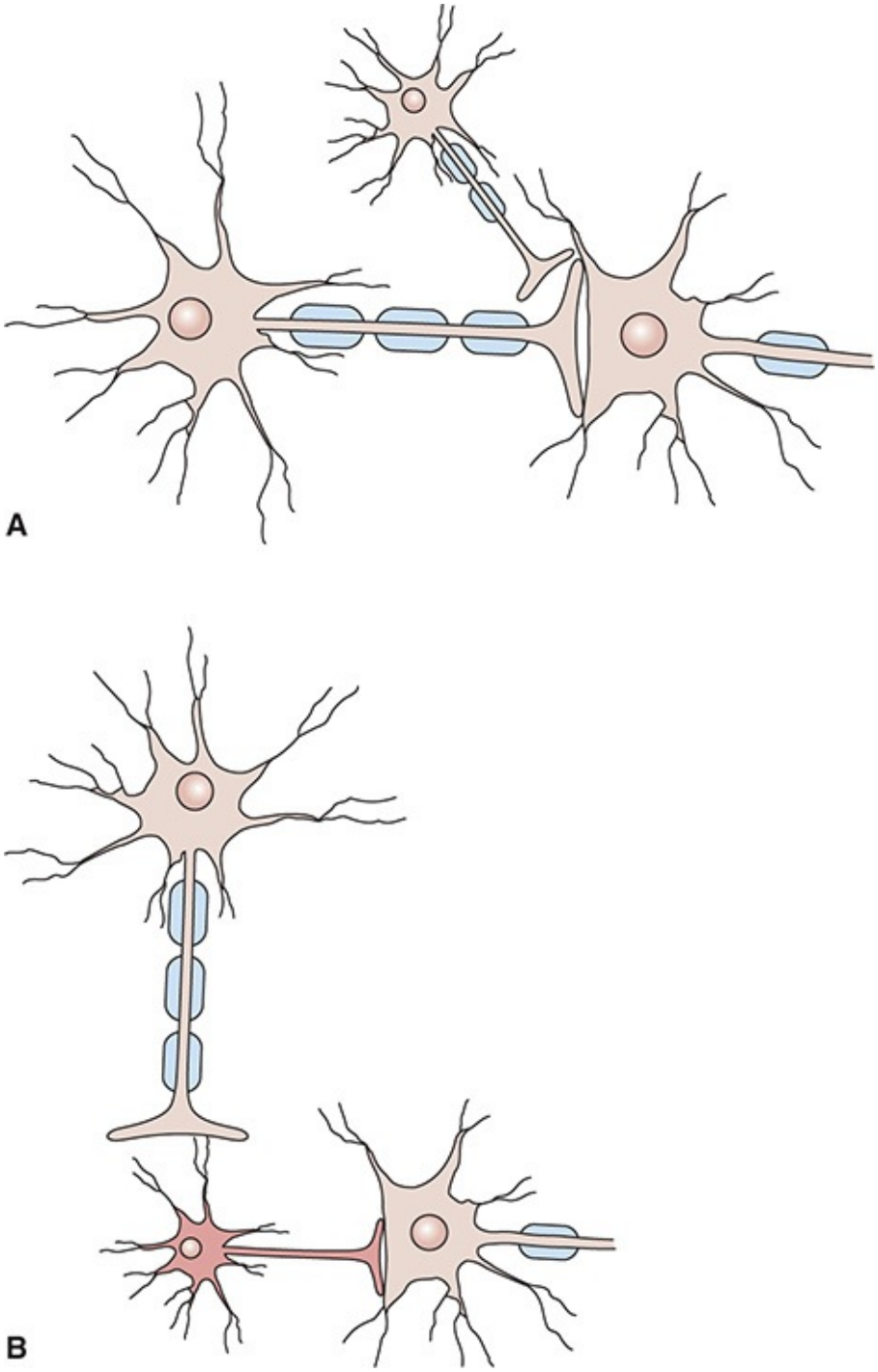


FIGURE 6–8 Comparison of neuronal connections that can produce presynaptic and postsynaptic inhibition. A) Presynaptic inhibition is a process mediated by neurons whose terminals are on excitatory nerve endings, forming axoaxonal synapses and reducing transmitter release from the excitatory neuron. **B)** Postsynaptic inhibition occurs when an inhibitory transmitter such as GABA is released from the nerve terminals of an inhibitory interneuron (dark) that

synapses on a postsynaptic neuron.

POSTSYNAPTIC INHIBITION

Postsynaptic inhibition occurs when an inhibitory transmitter such as glycine or GABA is released from a presynaptic nerve terminal onto the postsynaptic neuron to induce an IPSP in the postsynaptic neuron (Figure 6–8B). Various pathways in the nervous system mediate postsynaptic inhibition, and one illustrative example is presented in Figure 6–5. Afferent fibers from the muscle spindles (stretch receptors) in skeletal muscle project directly to the spinal motor neurons supplying the same muscle. Impulses in this afferent fiber cause an EPSP and, with summation, propagated action potentials in the postsynaptic motor neuron. At the same time, an IPSP is produced in motor neurons supplying the antagonistic muscle, which has an inhibitory interneuron interposed between the afferent fiber and the motor neuron. Therefore, activity in the afferent fibers from the muscle spindles excites the motor neurons supplying the muscle from which the impulses come and inhibits the motor neurons supplying its antagonists **(reciprocal innervation).** These reflexes are considered in more detail in Chapter 12.

PRESYNAPTIC INHIBITION & FACILITATION

Actions at the presynaptic nerve terminal can either reduce (presynaptic inhibition) or enhance (presynaptic facilitation) neurotransmitter release as a mechanism to fine-tune the strength of synaptic transmission. Presynaptic inhibition is a process mediated by neurons whose terminals are on excitatory endings, forming axoaxonal synapses (Figure 6–2 and 6–8A). Activation of the presynaptic receptors increases Cl^- conductance, and this decreases the size of the action potential reaching the excitatory ending **(Figure 6–9)**. This in turn reduces Ca^{2+} entry and consequently the amount of excitatory transmitter released. Voltage-gated K^+ channels are also opened, and the resulting K^+ efflux causes a decrease in Ca^{2+} influx.

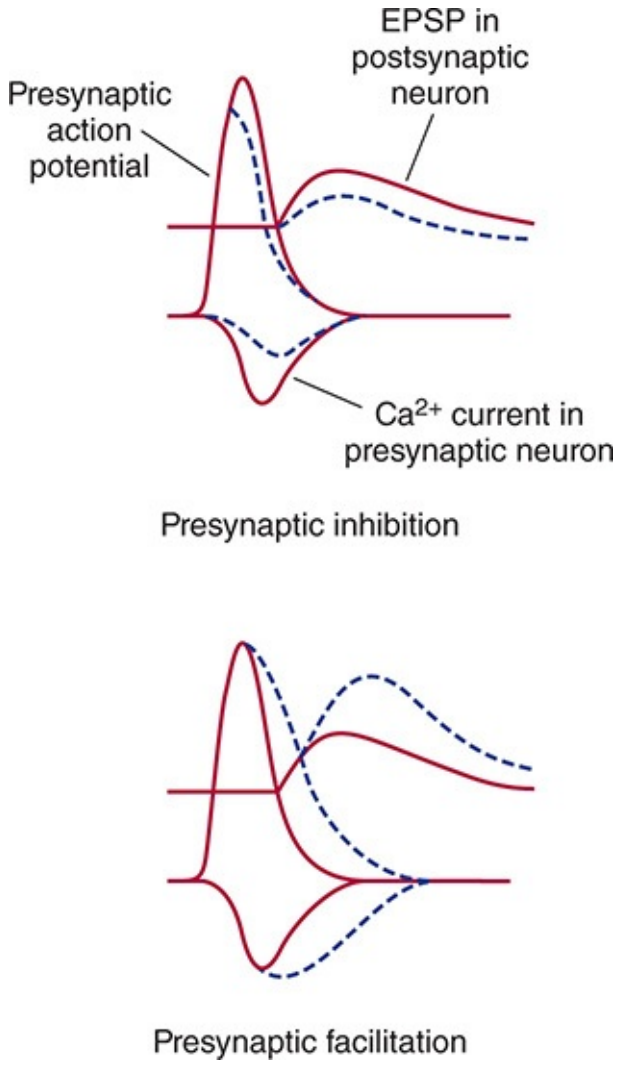


FIGURE 6–9 Effects of presynaptic inhibition and facilitation on the action potential and the Ca^{2+} current in the presynaptic neuron and the EPSP in the postsynaptic neuron. In each case, the solid lines are the controls and the dashed lines the records obtained during inhibition or facilitation. Presynaptic inhibition occurs when activation of presynaptic receptors increases Cl^- conductance, which decreases the size of the action potential. This reduces Ca^{2+} entry and thus the amount of excitatory transmitter released. Presynaptic facilitation is produced when the action potential is prolonged and the Ca^{2+} channels are open for a longer duration. EPSP, excitatory postsynaptic potential. (Reproduced with permission from Kandel ER, Schwartz JH, Jessell TM [editors]: *Principles of Neural Science*, 4th ed. New York, NY: McGraw-Hill; 2000.)

The first transmitter shown to produce presynaptic inhibition was GABA.

Acting via $GABA_A$ receptors, GABA increases Cl^- conductance. $GABA_B$ receptors are also present in the spinal cord and mediate presynaptic inhibition via a G-protein that produces an increase in K^+ conductance. Baclofen, a $GABA_B$ agonist, is effective in the treatment of the spasticity of spinal cord injury and multiple sclerosis, particularly when administered intrathecally via an implanted pump. Other transmitters also mediate presynaptic inhibition by G-protein-mediated effects on Ca^{2+} channels and K^+ channels.

Conversely, **presynaptic facilitation** is produced when the action potential is prolonged (Figure 6–9) and the Ca^{2+} channels are open for a longer period. The molecular events responsible for the production of presynaptic facilitation mediated by serotonin in the sea snail *Aplysia* have been worked out in detail. Serotonin released at an axoaxonal ending increases intraneuronal cyclic adenosine monophosphate (cAMP) levels, and the resulting phosphorylation of one group of K^+ channels closes the channels, slowing repolarization and prolonging the action potential.

ORGANIZATION OF INHIBITORY SYSTEMS

Presynaptic inhibition and postsynaptic inhibition are usually produced by stimulation of certain systems converging on a given postsynaptic neuron. Neurons may also inhibit themselves in a negative feedback manner (negative feedback inhibition). For instance, a spinal motor neuron emits a recurrent collateral that synapses on an inhibitory interneuron **(Renshaw cell)** that terminates on the cell body of spinal motor neuron **(Figure 6–10)**. The Renshaw cell releases **glycine** to reduce the activity of the motor neuron. Similar inhibition via recurrent collaterals is seen in the cerebral cortex and limbic system. Presynaptic inhibition due to descending pathways that terminate on afferent pathways in the dorsal horn may be involved in the gating of pain transmission.

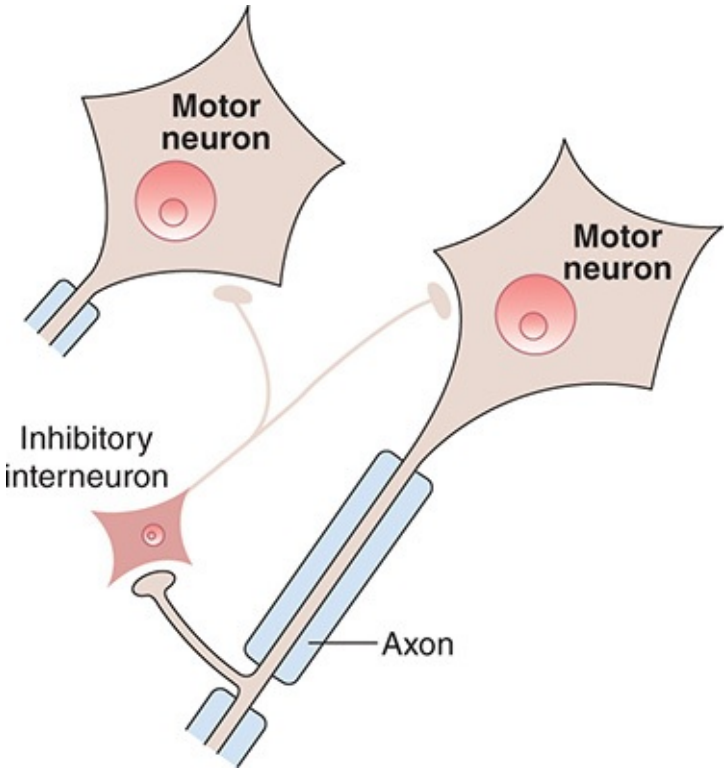


FIGURE 6–10 Negative feedback inhibition of a spinal motor neuron via an inhibitory interneuron. The axon of a spinal motor neuron has a recurrent collateral that synapses on an inhibitory interneuron that terminates on the cell body of the same and other motor neurons. The inhibitory interneuron is called a Renshaw cell and its neurotransmitter is glycine.

Another example of inhibition is that resulting from stimulation of cerebellar basket cells that produces IPSPs in the Purkinje cells. The basket cells and Purkinje cells are both excited by the same parallel-fiber input (see Chapter 12). This arrangement is called **feed-forward inhibition** and may limit the duration of the excitation produced by any given afferent volley.

NEUROMUSCULAR TRANSMISSION

NEUROMUSCULAR JUNCTION

Figure 6–11 shows the components of the neuromuscular junction, the specialized area where a motor nerve terminates on a skeletal muscle fiber. As the axon supplying a skeletal muscle fiber approaches its termination, it loses its myelin sheath and divides into a number of terminal boutons. The terminal

contains many small, clear vesicles that contain acetylcholine, the transmitter at these junctions. The endings fit into **junctional folds** or depressions in the **motor endplate,** the thickened portion of the muscle membrane at this junction. Each endplate receives input from a single nerve fiber. The space between the nerve and the thickened muscle membrane is comparable to the synaptic cleft at a neuron-to-neuron synapse. The whole structure is known as the **neuromuscular junction.**

A

B

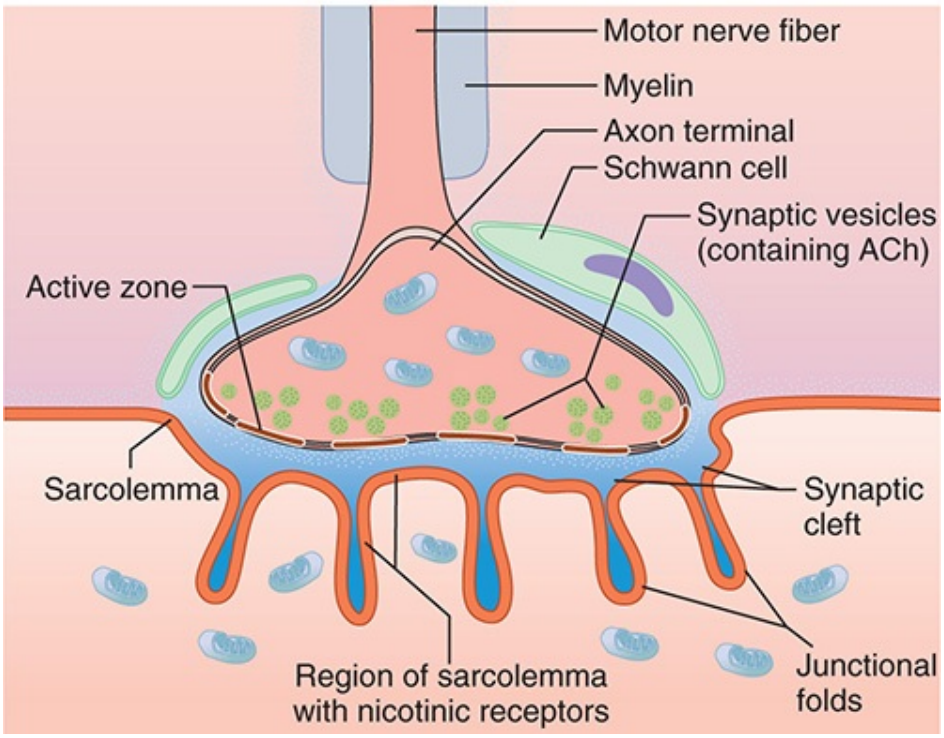

Motor nerve fiber
Myelin
Axon terminal
Schwann cell
Synaptic vesicles (containing ACh)
Active zone
Sarcolemma
Synaptic cleft
Region of sarcolemma with nicotinic receptors
Junctional folds

FIGURE 6–11 The neuromuscular junction. A) Scanning electron micrograph showing branching of motor axons with terminals embedded in grooves in the muscle fiber's surface. **B)** Structure of a neuromuscular junction. ACh, acetylcholine. (Modified with permission from Widmaier EP, Raff H, Strang KT: *Vanders Human Physiology*. New York, NY: McGraw-Hill; 2008.)

SEQUENCE OF EVENTS DURING TRANSMISSION

The events occurring during transmission of impulses from the motor nerve to the muscle are somewhat similar to those occurring at neuron-to-neuron synapses **(Figure 6–12)**. The impulse arriving in the terminal of the motor neuron increases its permeability to Ca^{2+}. Ca^{2+} enters the nerve endings and triggers a marked increase in exocytosis of the acetylcholine-containing synaptic vesicles. The acetylcholine diffuses to nicotinic cholinergic (N_M) receptors that are concentrated at the tops of the junctional folds of the membrane of the motor endplate. Binding of acetylcholine to these receptors increases the Na^+ and K^+ conductance, and the resultant influx of Na^+ produces a depolarizing potential, the **endplate potential.** The current sink created by this local potential depolarizes the adjacent muscle membrane to its firing level. Action potentials are generated on either side of the endplate and are conducted away from the endplate in both directions along the muscle fiber. The muscle action potential, in turn, initiates muscle contraction (see Chapter 5). Acetylcholine is then removed from the synaptic cleft by acetylcholinesterase, which is present in high concentration at the neuromuscular junction.

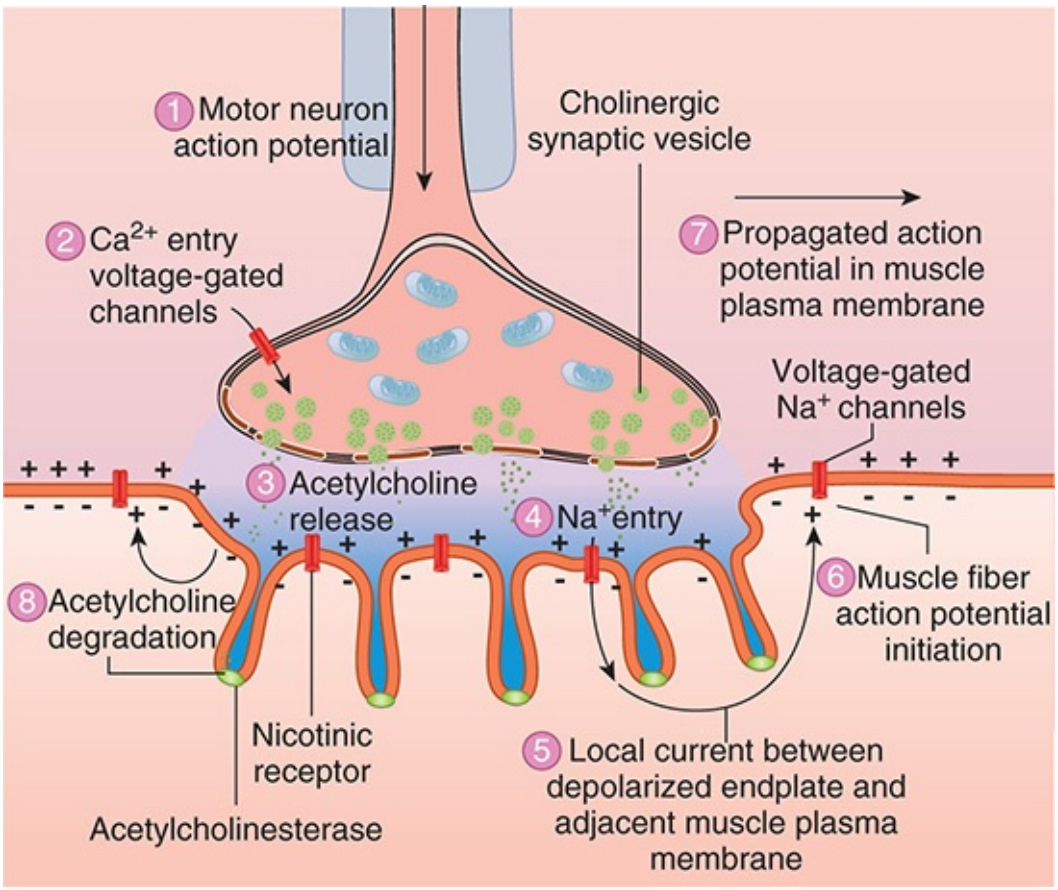


FIGURE 6–12 Events at the neuromuscular junction that lead to an action potential in the muscle fiber plasma membrane. The impulse arriving in the end of the motor neuron increases the permeability of its endings to Ca^{2+}, which enters the endings and triggers exocytosis of the acetylcholine (ACh)-containing synaptic vesicles. ACh diffuses and binds to nicotinic cholinergic (N_M) receptors in the motor endplate, which increases Na^+ and K^+ conductance. The resultant influx of Na^+ produces the endplate potential. The current sink created by this local potential depolarizes the adjacent muscle membrane to its firing level. Action potentials are generated on either side of the endplate and are conducted away from the endplate in both directions along the muscle fiber and the muscle contracts. ACh is then removed from the synaptic cleft by acetylcholinesterase. (Modified with permission from Widmaier EP, Raff H, Strang KT: *Vanders Human Physiology*. New York, NY: McGraw-Hill; 2008.)

QUANTAL RELEASE OF TRANSMITTER

Each nerve impulse releases acetylcholine from about 60 synaptic vesicles, and

each vesicle contains about 10,000 molecules of the neurotransmitter. Small quanta (packets) of acetylcholine are released randomly from the nerve cell membrane at rest. Each produces a minute depolarizing spike called a **miniature endplate potential** that is about 0.5 mV in amplitude. The size of the quanta of acetylcholine released in this way varies directly with the Ca^{2+} concentration and inversely with the Mg^{2+} concentration at the endplate. When a nerve impulse reaches the ending, the number of quanta released increases by several orders of magnitude, and the result is the large endplate potential that exceeds the firing threshold of the muscle fiber. Quantal release of a neurotransmitter also occurs at noradrenergic, glutamatergic, and other synaptic junctions. Two diseases of the neuromuscular junction, myasthenia gravis and Lambert-Eaton myasthenic syndrome, are described in **Clinical Box 6–2** and **Clinical Box 6–3**, respectively.

NERVE ENDINGS IN SMOOTH & CARDIAC MUSCLE

The postganglionic autonomic nerve fibers supplying smooth muscle in various organs branch extensively and come in close contact with the muscle cells **(Figure 6–13)**. Some of these nerve fibers have clear vesicles containing acetylcholine; others have dense-core vesicles containing norepinephrine. There are no recognizable endplates or other postsynaptic specializations at these **neuroeffector junctions**. The nerve fibers run along the membranes of the muscle cells and sometimes groove their surfaces. The nerve branches are beaded with enlargements **(varicosities)** that contain synaptic vesicles. In noradrenergic neurons, the varicosities are about 5 µm apart, with up to 20,000 varicosities per neuron. Neurotransmitter can be released at each of the varicosities along the axon; this arrangement permits one neuron to innervate many effector cells. The type of contact in which a neuron forms a synapse on the surface of a smooth muscle cell and then passes on to make similar contacts with other cells is called a **synapse en passant.**

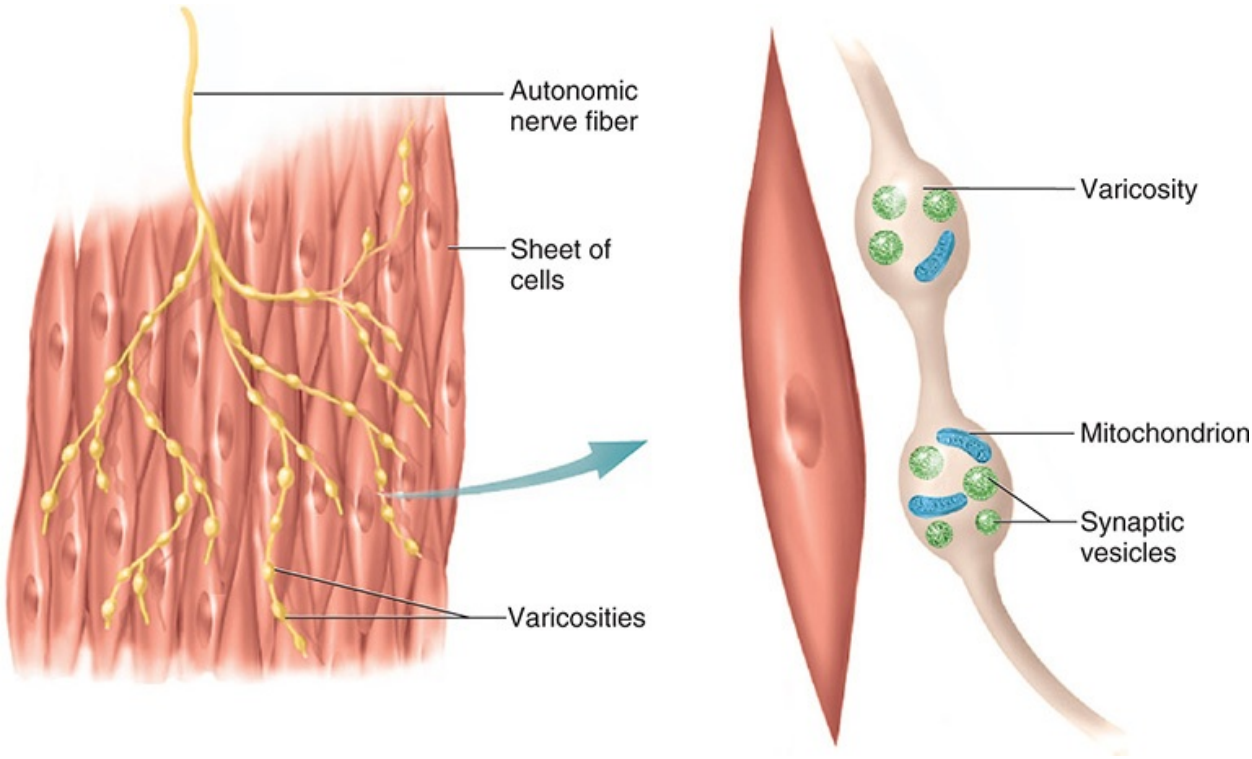


FIGURE 6–13 Endings of postganglionic autonomic neurons on smooth muscle. The nerve fibers run along the membranes of the smooth muscle cells and sometimes groove their surfaces. The multiple branches of postganglionic neurons are beaded with enlargements (varicosities) and contain synaptic vesicles. Neurotransmitter is released from the varicosities and diffuses to receptors on smooth muscle cell plasma membranes. (Reproduced with permission from Widmaier EP, Raff H, Strang KT: *Vanders Human Physiology*. New York, NY: McGraw-Hill; 2008.)

In the heart, cholinergic and noradrenergic nerve fibers end on the sinoatrial node, the atrioventricular node, and the bundle of His (see Chapter 29). Noradrenergic fibers also innervate the ventricular muscle. The exact nature of the endings on nodal tissue is not known. In the ventricle, the contacts between the noradrenergic fibers and the cardiac muscle fibers resemble those found in smooth muscle.

CLINICAL BOX 6–2

Myasthenia Gravis

Myasthenia gravis is a serious and sometimes fatal disease in which skeletal

muscles are weak and tire easily. It occurs in 25 to 125 of every 1 million people worldwide and can occur at any age but seems to have a bimodal distribution, with peak occurrences in individuals in their 20s (mainly women) and 60s (mainly men). It is caused by the formation of circulating antibodies to the skeletal muscle type of **nicotinic cholinergic receptors.** These antibodies destroy some of the receptors and bind others to neighboring receptors, triggering their removal by endocytosis. Normally, the number of quanta released from the motor nerve terminal declines with successive repetitive stimuli. In myasthenia gravis, neuromuscular transmission fails at these low levels of quantal release. This leads to the major clinical feature of the disease, muscle fatigue with sustained or repeated activity. There are two major forms of the disease. In one form, the extraocular muscles are primarily affected. In the second form, there is a generalized skeletal muscle weakness. In severe cases, all muscles, including the diaphragm, can weaken and respiratory failure and death can ensue. The major structural abnormality in myasthenia gravis is the appearance of sparse, shallow, and abnormally wide or absent synaptic clefts in the motor endplate. The postsynaptic membrane has a reduced response to acetylcholine, and there is a 70–90% decrease in the number of receptors per endplate in affected muscles. Patients with myasthenia gravis have a greater than normal tendency to also have rheumatoid arthritis, systemic lupus erythematosus, and polymyositis. About 30% of patients with myasthenia gravis have a maternal relative with an autoimmune disorder. These associations suggest that individuals with myasthenia gravis share a genetic predisposition to autoimmune disease. The thymus may play a role in the pathogenesis of the disease by supplying helper T cells sensitized against thymic proteins that cross-react with acetylcholine receptors. In most patients, the thymus is hyperplastic; and 10–15% have a thymoma.

THERAPEUTIC HIGHLIGHTS

Muscle weakness due to myasthenia gravis improves after a period of rest or after administration of an **acetylcholinesterase inhibitor** such as **neostigmine** or **pyridostigmine.** Cholinesterase inhibitors prevent metabolism of acetylcholine and can thus compensate for the normal decline in released neurotransmitters during repeated stimulation. **Immunosuppressive drugs** (eg, **prednisone, azathioprine,** or **cyclosporine**) can suppress antibody production and have been shown to improve muscle strength in some patients with myasthenia gravis. **Thymectomy** is indicated especially if a thymoma is

suspected in the development of myasthenia gravis. Even in those without thymoma, thymectomy induces remission in 35% and improves symptoms in another 45% of patients.

JUNCTIONAL POTENTIALS

In smooth muscles in which noradrenergic activity is excitatory, stimulation of the noradrenergic nerves produces discrete partial depolarizations that look like small endplate potentials and are called **excitatory junction potentials (EJPs).** These potentials summate with repeated stimuli. Similar EJPs are seen in tissues excited by cholinergic discharges. In tissues inhibited by noradrenergic stimuli, hyperpolarizing **inhibitory junction potentials (IJPs)** are produced by stimulation of the noradrenergic nerves. Junctional potentials spread in an electrotonic fashion.

AXONAL INJURY & DENERVATION SUPERSENSITIVITY

Figure 6–14 shows some of the reactions triggered by injury or section of an axon. Orthograde degeneration **(Wallerian degeneration)** occurs from the point of injury to the nerve terminal, interrupting neural transmission. Distal to the injury, the membrane breaks down and the myelin sheath degenerates. The proximal axon also is affected and may undergo retrograde degeneration and die. The cell body of the injured neuron swells, the nucleus moves to an eccentric position, and the rough endoplasmic reticulum gets fragmented **(chromatolytic reaction).**

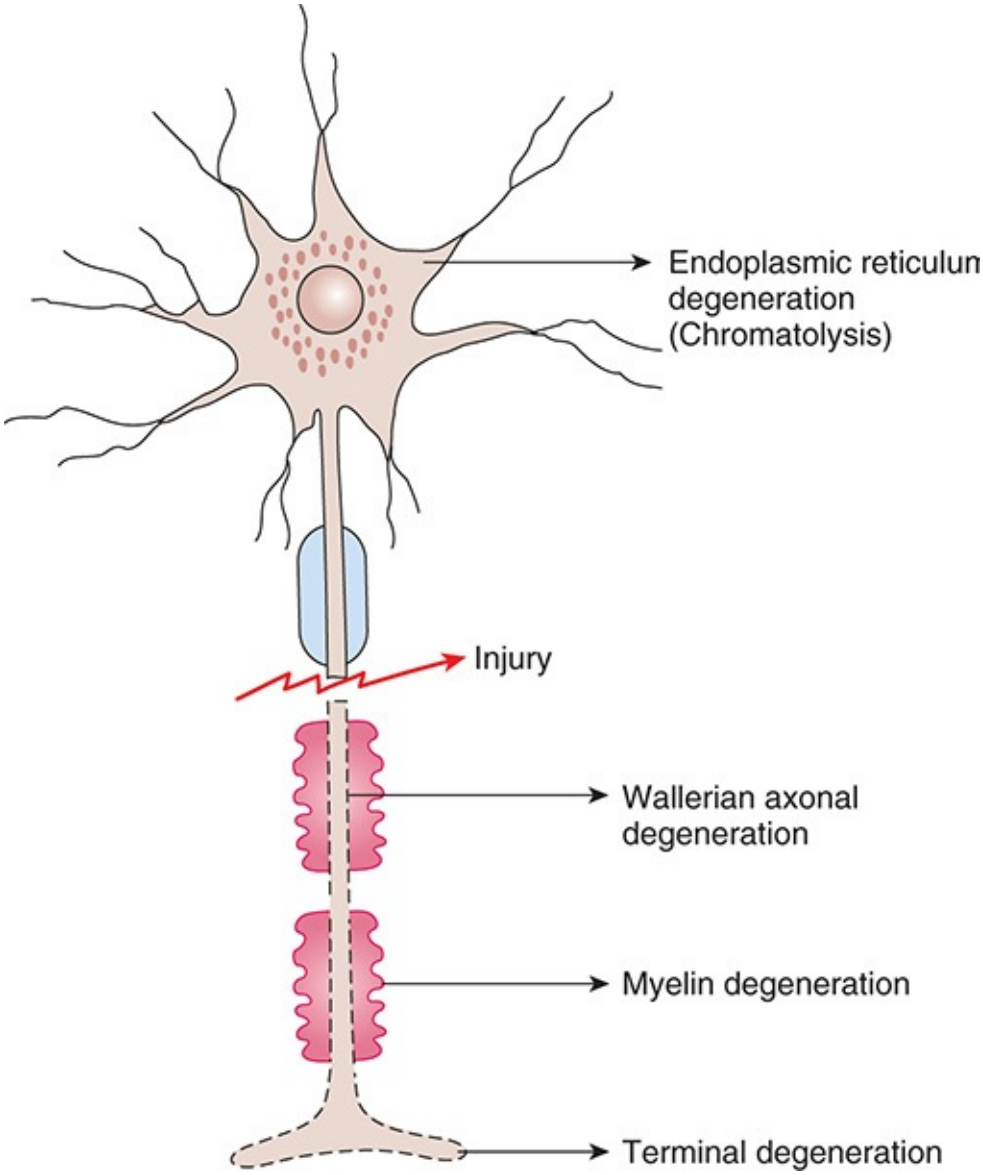


FIGURE 6–14 Changes occurring in a neuron when its axon is crushed or injured. The distal axon stump separates from the cell body, orthograde (Wallerian) degeneration occurs from the point of damage to the terminal, and the myelin sheath degenerates. The cell body of the injured neuron swells and the endoplasmic reticulum is fragmented as part of the chromatolytic reaction.

The nerve then starts to regrow, with multiple small branches projecting along the path the axon previously followed **(regenerative sprouting).** Axons sometimes grow back to their original targets, especially in locations like the neuromuscular junction. However, nerve regeneration is generally limited because axons often become entangled in the area of tissue damage at the site where they were disrupted. This difficulty has been reduced by administration of

neurotrophins (see Chapter 4).

When the motor nerve to skeletal muscle is cut and allowed to degenerate, the muscle gradually becomes extremely sensitive to acetylcholine. This is called **denervation hypersensitivity** or **supersensitivity.** Normally nicotinic receptors are located only in the vicinity of the motor endplate where the axon of the motor nerve terminates. When the motor nerve is severed, there is a marked proliferation of nicotinic receptors over a wide region of the neuromuscular junction. Denervation supersensitivity also occurs at autonomic junctions. Smooth muscle, unlike skeletal muscle, does not atrophy when denervated, but it becomes hyperresponsive to the chemical mediator that normally activates it.

CLINICAL BOX 6–3

Lambert-Eaton Syndrome

In a relatively rare condition called **Lambert-Eaton myasthenic syndrome (LEMS),** muscle weakness is caused by an autoimmune attack against one of the voltage-gated Ca^{2+} channels in the nerve endings at the neuromuscular junction. This decreases the normal Ca^{2+} influx that causes acetylcholine release. The incidence of LEMS in the United States is about 1 case per 100,000 people; it is usually an adult-onset disease that has a similar occurrence in men and women. Proximal muscles of the lower extremities are primarily affected, producing a waddling gait and difficulty raising the arms. Repetitive stimulation of the motor nerve facilitates accumulation of Ca^{2+} in the nerve terminal and increases acetylcholine release, leading to an increase in muscle strength. This is in contrast to myasthenia gravis in which symptoms are exacerbated by repetitive stimulation. About 40% of patients with LEMS also have cancer, especially small cell cancer of the lung. One theory is that antibodies that have been produced to attack the cancer cells may also attack Ca^{2+} channels, leading to LEMS. LEMS has also been associated with lymphosarcoma; malignant thymoma; and cancer of the breast, stomach, colon, prostate, bladder, kidney, or gallbladder. Clinical signs usually precede the diagnosis of cancer. A syndrome similar to LEMS can occur after the use of **aminoglycoside antibiotics,** which also impair Ca^{2+} channel function.

THERAPEUTIC HIGHLIGHTS

Since there is a high comorbidity with small cell lung cancer, the first treatment strategy is to determine if the individual also has cancer and, if so, to treat that appropriately. In patients without cancer, **immunotherapy** is initiated. **Prednisone** administration, **plasmapheresis,** and **intravenous immunoglobulin** are some examples of effective therapies for LEMS. The use of **aminopyridines** facilitates the release of acetylcholine in the neuromuscular junction and can improve muscle strength in LEMS patients. This class of drugs causes blockade of presynaptic K^+ channels and promotes activation of voltage-gated Ca^{2+} channels. Acetylcholinesterase inhibitors can be used but often do not ameliorate the symptoms of LEMS.

Denervation hypersensitivity has multiple causes. As noted in Chapter 2, a deficiency of a given chemical messenger generally produces an upregulation of its receptors. Another factor is a lack of reuptake of secreted neurotransmitters.

CHAPTER SUMMARY

- Most synapses occur on dendrites (axodendritic), but some presynaptic nerves terminate on the soma (axosomatic) or axon (axoaxonic) of a postsynaptic neuron. At chemical synapses, an impulse in the presynaptic axon causes release of a neurotransmitter from synaptic vesicles that diffuses across a synaptic cleft and binds to receptors on the postsynaptic density, triggering events that open or close ion channels in the membrane of the postsynaptic neuron. At electrical synapses, gap junctions between the presynaptic and postsynaptic neurons form low-resistance bridges through which ions pass with relative ease from one neuron to the next.
- An excitatory stimulus opens Na^+ or Ca^{2+} ion channels to produce an EPSP (inward current) in a postsynaptic neuron after a latency of 0.5 ms. An IPSP is produced by a localized increase in Cl^- transport. Slow EPSPs and IPSPs are due to decreases and increases in K^+ conductance, respectively. They occur after a latency of 100–500 ms in autonomic ganglia, cardiac and smooth muscle, and cortical neurons.
- The time constant of a neuron determines the time course of the synaptic potential; the longer the time constant, the greater is the chance for two potentials to summate to induce an action potential (temporal summation).

The length constant of a neuron determines the degree to which a depolarizing current is reduced as it spreads passively; if a neuron has a long length constant, the depolarization induced at two points on the neuron can summate and induce an action potential (spatial summation).

- Postsynaptic inhibition occurs when an inhibitory transmitter (glycine, GABA) is released from a presynaptic nerve terminal and induces an IPSP in the postsynaptic neuron. Presynaptic inhibition is mediated at axoaxonal synapses; activation of presynaptic receptors increases Cl^- conductance, decreasing the size of the action potentials reaching the excitatory ending, and reducing Ca^{2+} entry and the amount of excitatory transmitter released. Presynaptic facilitation occurs when the action potential is prolonged and the Ca^{2+} channels are open for a longer period.
- The axon terminal of motor neurons synapses on the motor endplate on the skeletal muscle membrane to form the neuromuscular junction. An impulse arriving in the motor nerve terminal leads to the entry of Ca^{2+} that triggers the exocytosis of the acetylcholine-containing synaptic vesicles. The acetylcholine diffuses and binds to nicotinic cholinergic receptors on the motor endplate, causing an increase in Na^+ and K^+ conductance; the influx of Na^+ induces the endplate potential and depolarization of the adjacent muscle membrane.
- Autonomic nerve terminals in smooth muscle form a neuroeffector junction; nerve branches are beaded with enlargements (varicosities) that contain synaptic vesicles. Release of neurotransmitter mediates EJPs or IJPs that spread electrotonically.
- When a nerve is damaged and then degenerates, the postsynaptic structure gradually becomes extremely sensitive to the transmitter released by the nerve (denervation hypersensitivity).
- Neurotoxins from bacteria can disrupt neurotransmitter release at the neuromuscular junction (botulinum toxin, tetanus toxin). Autoimmune diseases of the neuromuscular junction include myasthenia gravis (antibodies against nicotinic cholinergic receptors) and Lambert-Eaton myasthenic syndrome (antibodies against voltage-gated Ca^{2+} channels).

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. A neurology resident was conducting research in an electrophysiology laboratory and was studying the roles of cation channels in mediating nonpropagated potentials. Which of the following electrophysiologic events is correctly paired with the change in ionic currents causing the event?

   A. Fast inhibitory postsynaptic potentials (IPSPs) and closing of Cl^- channels

   B. Fast excitatory postsynaptic potentials (EPSPs) and an increase in Ca^{2+} conductance

   C. Endplate potential and an increase in Na^+ conductance

   D. Excitatory junctional potentials and closure of voltage-gated K^+ channels

   E. Slow EPSPs and an increase in K^+ conductance

2. A neurology resident was conducting research in an electrophysiology laboratory and was studying the functional anatomy of synaptic transmission. Which of the following physiologic processes is correctly paired with a structure?

   A. Electrical transmission: synaptic cleft

   B. Positive feedback inhibition: Renshaw cell

   C. Synaptic vesicle docking and fusion: presynaptic nerve terminal

   D. Endplate potential: muscarinic cholinergic receptor

   E. Action potential initiation: axon hillock

3. A medical student was studying the passive membrane properties of neurons and their ability to affect the amplitude of an EPSP recorded from the neuron. She found that in one neuron, applying two stimuli separated by 25 ms to one presynaptic input induced two EPSPs of identical amplitude. In a second neuron, the same type of stimulation induced an EPSP followed an action potential. What can she conclude from this experiment?

   A. The second neuron had a longer time constant than the first neuron.

   B. The second neuron had a shorter time constant than the first neuron.

   C. The second neuron had a longer length constant than the first neuron.

   D. The second neuron had a shorter length constant than the first neuron.

4. A medical student was doing research in a laboratory that studies the neuromuscular junction. Which of the following events at the junction are listed in correct sequential order?

   A. Motor neuron action potential, acetylcholine release, Na^+ entry at the endplate

B. Ca^{2+} entry into the motor nerve terminal, Na^{+} entry at the endplate, muscle fiber action potential

C. Na^{+} entry at the endplate, acetylcholine release, muscle fiber action potential

D. Motor neuron action potential, Na^{+} entry at the endplate, formation of an endplate potential

E. Ca^{2+} entry into the motor nerve terminal, acetylcholine release, Na^{+} entry at the endplate

5. Activation of a sensory nerve from the muscle spindle caused contraction of the extensor muscle and relaxation of the flexor muscle. The relaxation of the flexor muscle is an example of

A. negative feedback inhibition.

B. postsynaptic inhibition.

C. Renshaw cell-mediated inhibition.

D. presynaptic inhibition.

E. indirect inhibition.

6. A medical student was working in a laboratory that studied autonomic neurotransmission. He was recording a potential in a vascular smooth muscle from stimulation of the postganglionic sympathetic nerve. What is the name of the structure on the nerve where the neurotransmitter is stored and what is the name of the response in the smooth muscle?

A. Synaptic vesicle and endplate potential

B. Varicosity and inhibitory postsynaptic potential

C. Large dense-core vesicle and inhibitory junctional potential

D. Varicosity and excitatory junctional potential

E. Small dense-core vesicle and excitatory postsynaptic potential

7. A 35-year-old woman sees her physician to report weakness in the extraocular eye muscles and muscles of the extremities. She states that she feels fine when she gets up in the morning, but the weakness begins soon after she becomes active. The weakness is improved by rest. The physician treats her with an anticholinesterase inhibitor, and she notes immediate return of muscle strength. Her physician diagnoses her with

A. Lambert-Eaton syndrome.

B. myasthenia gravis.

C. multiple sclerosis.

D. Parkinson disease.

E. muscular dystrophy.

8. A 55-year-old woman had an autonomic neuropathy that disrupted the sympathetic nerve supply to the pupillary dilator muscle of her right eye. While having her eyes examined, the ophthalmologist placed phenylephrine in her eyes. The right eye became much more dilated than the left eye. This suggests that

A. the sympathetic nerve to the right eye had regenerated.

B. the parasympathetic nerve supply to the right eye remained intact and compensated for the loss of the sympathetic nerve.

C. phenylephrine blocked the pupillary constrictor muscle of the right eye.

D. denervation hypersensitivity had developed.

E. the left eye also had nerve damage and so was not responding as expected.

9. A 47-year-old woman was admitted to the hospital after experiencing nausea and vomiting for about 2 days followed by severe muscle weakness and neurologic symptoms, including ptosis and dysphagia. She indicated she had eaten at a restaurant the evening before the symptoms began. Laboratory tests were positive for *Clostridium botulinum*. The basis for the muscle weakness in this case was most likely because the toxin

A. blocked the reuptake of neurotransmitter into presynaptic terminals.

B. bound irreversibly to the receptor on the postsynaptic membrane at the neuromuscular junction.

C. reached the cell body of the motor neuron by diffusion into the spinal cord.

D. exerted its adverse effects by a direct action on the skeletal muscle.

E. prevented the release of acetylcholine from motor neurons.

CHAPTER 7

Neurotransmitters & Neuromodulators

OBJECTIVES

After studying this chapter, you should be able to:

- List the major types of neurotransmitters and neuromodulators that are broadly characterized as small-molecule transmitters, large-molecule transmitters, and gas transmitters.
- Summarize the five common steps involved in the biosynthesis, release, action, and removal from the synaptic cleft of the major small-molecule and large-molecule neurotransmitters.
- Compare the actions initiated by binding of a neurotransmitter to an ionotropic (ligand-gated) versus metabotropic (G-protein-coupled, GPCR) receptor and identify the second messengers involved in mediating the actions of neurotransmitters that act on GPCRs.
- Recognize the major distribution of the various types of receptors that mediate the functional responses of the common neurotransmitters: amino acids (glutamate and GABA), acetylcholine, monoamines (norepinephrine, epinephrine, dopamine, and serotonin), and opioid peptides.
- List receptor antagonists for each of the common neurotransmitters.
- Describe the role of nitric oxide and carbon monoxide (CO) in modulating synaptic transmission.

- Provide examples of how neurotransmitter dysfunction contributes to some neuropathological disorders.

INTRODUCTION

The dominant form of neuron-to-neuron or neuron-to-effector organ communication within the mammalian nervous system is mediated by the release of a chemical **neurotransmitter** that induces excitation or inhibition of the postsynaptic target. **Neuromodulators** are chemicals released by neurons that have little or no direct effects on their own but can modify the effects of neurotransmitters. This chapter provides a summary of the major properties of some of the most common chemical neurotransmitters, including excitatory and inhibitory amino acids, acetylcholine, monoamines, and neuropeptides. For many of these chemicals, there are some common steps involved in the process of neurotransmission. These steps include uptake of a neurotransmitter precursor into a nerve terminal, biosynthesis of the neurotransmitter, its storage within **synaptic vesicles**, its release into the **synaptic cleft** in response to the arrival of a wave of depolarization into the presynaptic nerve terminal, binding of the neurotransmitter to **receptors** on the membrane of the postsynaptic target, and finally termination of its actions via diffusion away from the synapse, reuptake into the nerve terminal, or enzymatic degradation.

CHEMISTRY OF TRANSMITTERS

Many neurotransmitters and the enzymes involved in their synthesis and catabolism are localized in nerve endings. There are three main classes of chemical substances that serve as neurotransmitters and neuromodulators: small-molecule transmitters, large-molecule transmitters, and gas transmitters. Small-molecule transmitters include amino acids (eg, **glutamate, γ-aminobutyric acid [GABA]**, and **glycine**), **acetylcholine,** and monoamines (eg, **norepinephrine, epinephrine, dopamine,** and **serotonin**). Large-molecule transmitters include neuropeptides such as **substance P, enkephalin,** and **vasopressin**. Neuropeptides are often co-localized with one of the small-molecule neurotransmitters **(Table 7–1)**. Gas transmitters include nitric oxide (NO) and carbon monoxide (CO).

TABLE 7–1 Examples of co-localization of small-molecule transmitters with neuropeptides.

Small-Molecule Transmitter	Neuropeptide
Glutamate	Substance P
GABA	Cholecystokinin, enkephalin, somatostatin, substance P, thyrotropin-releasing hormone
Glycine	Neurotensin
Acetylcholine	Calcitonin gene-related protein, enkephalin, galanin, gonadotropin-releasing hormone, neurotensin, somatostatin, substance P, vasoactive intestinal polypeptide
Dopamine	Cholecystokinin, enkephalin, neurotensin
Norepinephrine	Enkephalin, neuropeptide Y, neurotensin, somatostatin, vasopressin
Epinephrine	Enkephalin, neuropeptide Y, neurotensin, substance P
Serotonin	Cholecystokinin, enkephalin, neuropeptide Y, substance P, vasoactive intestinal polypeptide

Figure 7–1 shows the biosynthesis of some common small-molecule transmitters released by neurons in the central nervous system (CNS) or peripheral nervous system. **Figure 7–2** shows the location of major groups of neurons that contain norepinephrine, serotonin, dopamine, and acetylcholine. These are some of the major central neurotransmitter and neuromodulatory systems.

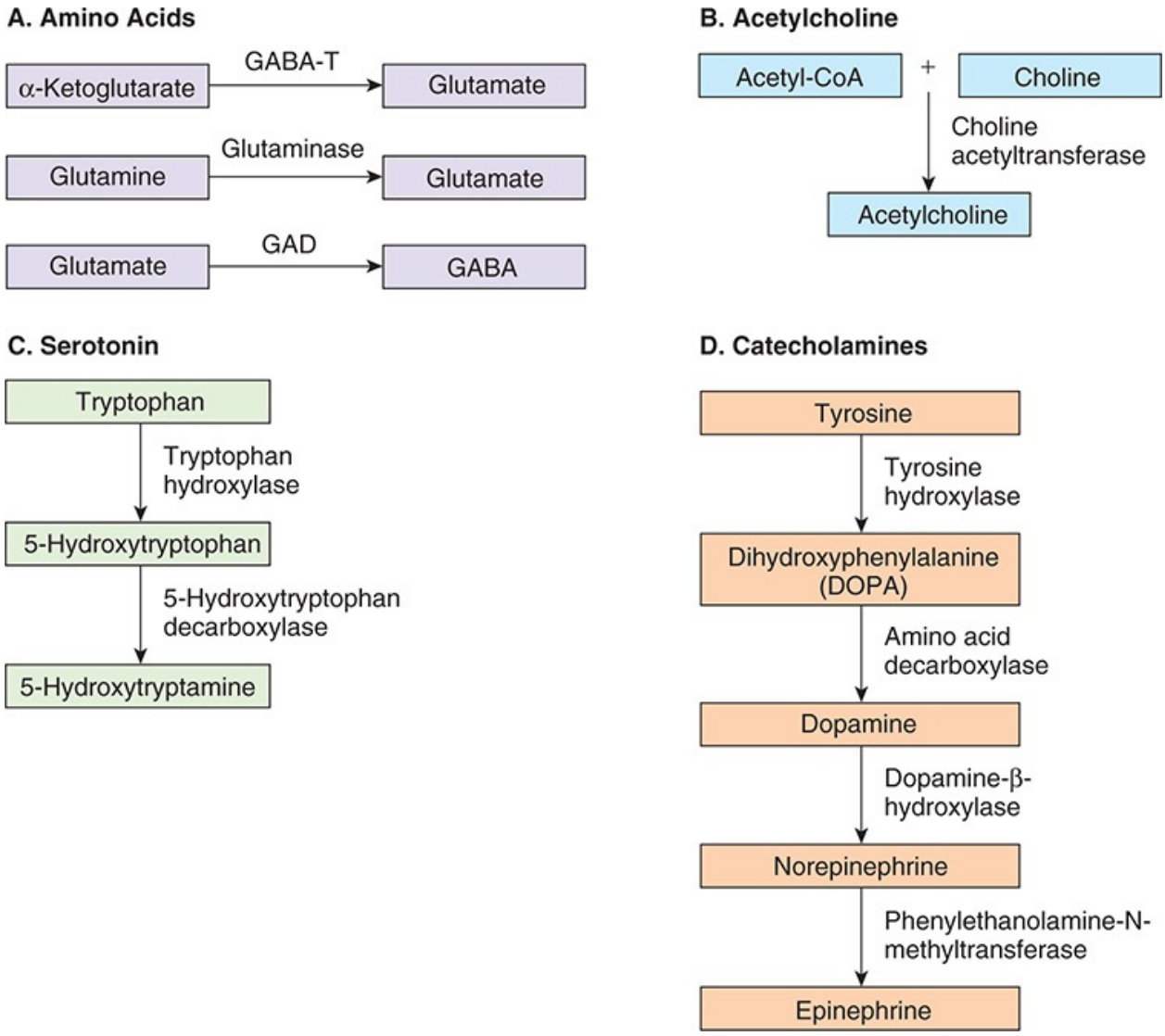


FIGURE 7–1 Biosynthesis of some common small-molecule neurotransmitters. A) Glutamate is synthesized in the Krebs cycle by the conversion of α-ketoglutarate to the amino acid via the enzyme γ-aminobutyric acid transferase (GABA-T) or in nerve terminals by the hydrolysis of glutamine by the enzyme glutaminase. GABA is synthesized by the conversion of glutamate by the enzyme glutamic acid decarboxylase (GAD). **B)** Acetylcholine is synthesized in the cytoplasm of a nerve terminal from acetyl-Co-A and choline by the enzyme choline acetyltransferase. **C)** Serotonin is synthesized from the amino acid tryptophan in a two-step process: the enzymatic hydroxylation of tryptophan to 5-hydroxytryptophan and the enzymatic decarboxylation of this intermediate to form 5-hydroxytryptamine (also called serotonin). **D)** Catecholamines are synthesized from the amino acid tyrosine by a multi-step process. Tyrosine is oxidized to dihydroxyphenylalanine (DOPA) by the enzyme

tyrosine hydroxylase in the cytoplasm of the neuron; DOPA is then decarboxylated to dopamine. In dopaminergic neurons, the process stops there. In noradrenergic neurons, the dopamine is transported into synaptic vesicles where it is converted to norepinephrine by dopamine-β-hydroxylase. In neurons that also contain the enzyme phenylethanolamine-N-methyltransferase, norepinephrine is converted to epinephrine.

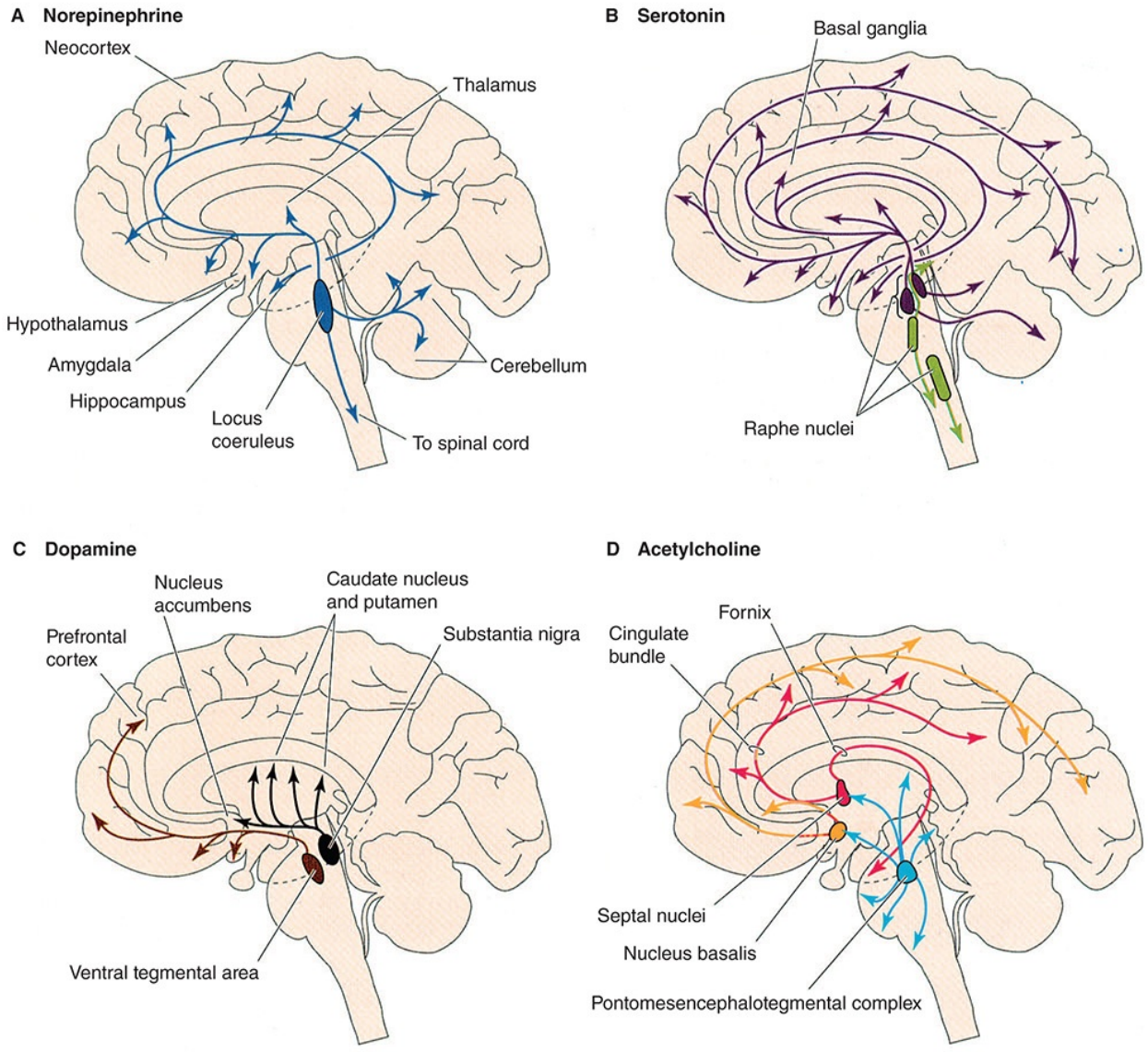


FIGURE 7–2 Four diffusely connected systems of central neuromodulators. **A)** Noradrenergic neurons in the locus coeruleus innervate the spinal cord, cerebellum, several nuclei of the hypothalamus, thalamus, basal telencephalon, and neocortex. **B)** Serotonergic neurons in the raphe nuclei project to the hypothalamus, limbic system, neocortex, cerebellum, and spinal cord. **C)**

Dopaminergic neurons in the substantia nigra project to the striatum and those in the ventral tegmental area of the midbrain project to the prefrontal cortex of the limbic system. **D)** Cholinergic neurons in the basal forebrain complex project to the hippocampus and the neocortex and those in the pontomesencephalotegmental cholinergic complex project to the dorsal thalamus and the forebrain. (Reproduced with permission from Boron WF, Boulpaep EL: *Medical Physiology*. St. Louis, MO: Elsevier; 2005.)

RECEPTORS

The action of a chemical mediator on its target structure is more dependent on the type of receptor on which it acts than on the properties of the mediator per se. The individual receptors, along with their ligands (the molecules that bind to them), are discussed in the following parts of this chapter. However, five common themes about the actions of ligands on receptors have emerged.

First, each chemical mediator has the potential to act on many subtypes of receptors. For example, norepinephrine acts on α_1-, α_2-, β_1-, β_2-, and β_3-adrenergic receptors. This multiplies the possible effects of a given ligand and makes its effects in each cell more selective.

Second, receptors for many neurotransmitters are located on both presynaptic and postsynaptic elements. A presynaptic receptor called an **autoreceptor** often inhibits further release of the transmitter, providing feedback control. For example, norepinephrine acts on α_2-presynaptic receptors to inhibit additional norepinephrine release. A presynaptic **heteroreceptor** is one whose ligand is a chemical other than the transmitter released by the nerve ending on which the receptor is located. For example, norepinephrine acts on a heteroreceptor on a cholinergic nerve terminal to inhibit the release of acetylcholine. In some cases, presynaptic receptors facilitate the release of neurotransmitters.

Third, receptors are grouped into two large families based on structure and function: **ligand-gated channels** (also known as **ionotropic receptors**) and **metabotropic receptors** (also known as **G-protein-coupled receptors [GPCRs]).** In the case of ionotropic receptors, a membrane channel is opened when a ligand binds to the receptor; and activation of the channel usually elicits a brief (few to tens of milliseconds) increase in ionic conductance. Thus, these receptors are important for fast synaptic transmission. Metabotropic receptors are 7-transmembrane GPCRs, and binding of a neurotransmitter to these receptors initiates the production of a second messenger that modulates the

voltage-gated channels on neuronal membranes. The receptors for some neurotransmitters and neuromodulators are listed in **Table 7–2**, along with their principal second messengers and, where established, their net effect on ion channels. This table is an over-simplification. For example, activation of α_2-adrenergic receptors decreases intracellular cyclic adenosine monophosphate (cAMP) concentrations, but there is evidence that the G-protein activated by α_2-adrenergic presynaptic receptors also acts directly on Ca^{2+} channels to inhibit norepinephrine release.

TABLE 7–2 Pharmacology of a selection of receptors for some small-molecule neurotransmitters.

Neurotransmitter	Receptor	Second Messenger	Net Channel Effects	Agonists	Antagonists
Glutamate	AMPA		↑Na^+, K^+	AMPA	CNQX, DNQX
	Kainate		↑Na^+, K^+	Kainate	CNQX, DNQX
	NMDA		↑Na^+, K^+, Ca^{2+}	NMDA	AP5, AP7
	$mGluR_1$	↑cAMP, IP_3, DAG	↓K^+, ↑Ca^{2+}	DHPG	
	$mGluR_5$	↑IP_3, DAG	↓K^+, ↑Ca^{2+}	Quisqualate	
	$mGluR_2$, $mGluR_3$	↓cAMP	↑K^+, ↓Ca^{2+}	DCG-IV	
	$mGluR_4$, $mGluR_{6-7}$	↓cAMP	↓Ca^{2+}	L-AP4	
GABA	$GABA_A$		↑Cl^-	Muscimol	Bicuculline, Gabazine, Picrotoxin
	$GABA_B$	↑IP_3, DAG	↑K^+, ↓Ca^{2+}	Baclofen	Saclofen
Glycine	Glycine		↑Cl^-	Taurine, β-alanine	Strychnine
Acetylcholine	N_M		↑Na^+, K^+	Nicotine	Tubocurarine, Gallamine triethiodide
	N_N		↑Na^+, K^+	Nicotine, lobeline	Trimethaphan
	M_1, M_3, M_5	↑IP_3, DAG	↑Ca^{2+}	Muscarine, Bethanechol, Oxotremorine (M_1)	Atropine, Pirenzipine (M_1)
	M_2, M_4	↓cAMP	↑K^+	Muscarine, Bethanechol (M_2)	Atropine, Tropicamide (M_4)
Norepinephrine	α_1	↑IP_3, DAG	↓K^+	Phenylephrine	Prazosin, Tamsulosin
	α_2	↓cAMP	↑K^+, ↓Ca^{2+}	Clonidine	Yohimbine
	β_1	↑cAMP	↓K^+	Isoproterenol, Dobutamine	Atenolol, Esmolol
	β_2	↑cAMP		Albuterol	Butoxamine
Serotonin	$5HT_{1A}$	↓cAMP	↑K^+	8-OH-DPAT	Metergoline, Spiperone
	$5HT_{1B}$	↓cAMP		Sumatriptan	
	$5HT_{1D}$	↓cAMP	↓K^+	Sumatriptan	
	$5HT_{2A}$	↑IP_3, DAG	↓K^+	Dobutamine	Ketanserin
	$5HT_{2C}$	↑IP_3, DAG		α-Methyl-5-HT	
	$5HT_3$		↑Na^+	α-Methyl-5-HT	Ondansetron
	$5HT_4$	↑cAMP	↓K^+	5-Methoxytryptamine	

8-OH-DPAT, 8-hydroxy-N, N-dipropyl-2-aminotetralin; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate; DAG, diacylglycerol; DCG-IV, 2-(2, 3-dicarboxycyclopropyl) glycine; DHPG, 3-5 dihydroxyphenylglycine; IP_3, inositol triphosphate; L-AP4, 2-amino-4-phosphonobutyrate; NMDA, N-methyl-D-aspartate.

Fourth, receptors are concentrated in clusters on the postsynaptic membrane close to the endings of neurons that secrete the neurotransmitters specific for them. This is generally due to the presence of specific binding proteins for them.

Fifth, in response to prolonged exposure to their ligands, most receptors become unresponsive; that is, they undergo **desensitization.** This can be of two types: **homologous desensitization,** with loss of responsiveness only to the ligand and maintained responsiveness of the cell to other ligands; and **heterologous desensitization,** in which the cell becomes unresponsive to other ligands as well.

REUPTAKE

Neurotransmitters are rapidly transported from the synaptic cleft back into the cytoplasm of the neurons that released them via a process called **reuptake,** which involves a high-affinity, Na^+-dependent membrane transporter. **Figure 7–3** illustrates the reuptake of norepinephrine in a sympathetic postganglionic nerve ending. After release of norepinephrine into the synaptic cleft, it is rapidly routed back into the sympathetic nerve terminal by a **norepinephrine transporter (NET).** A portion of the norepinephrine that re-enters the neuron is sequestered into the synaptic vesicles through the **vesicular monoamine transporter (VMAT).** There are analogous membrane and vesicular transporters for other small-molecule neurotransmitters released at other synapses in the CNS and peripheral nervous system.

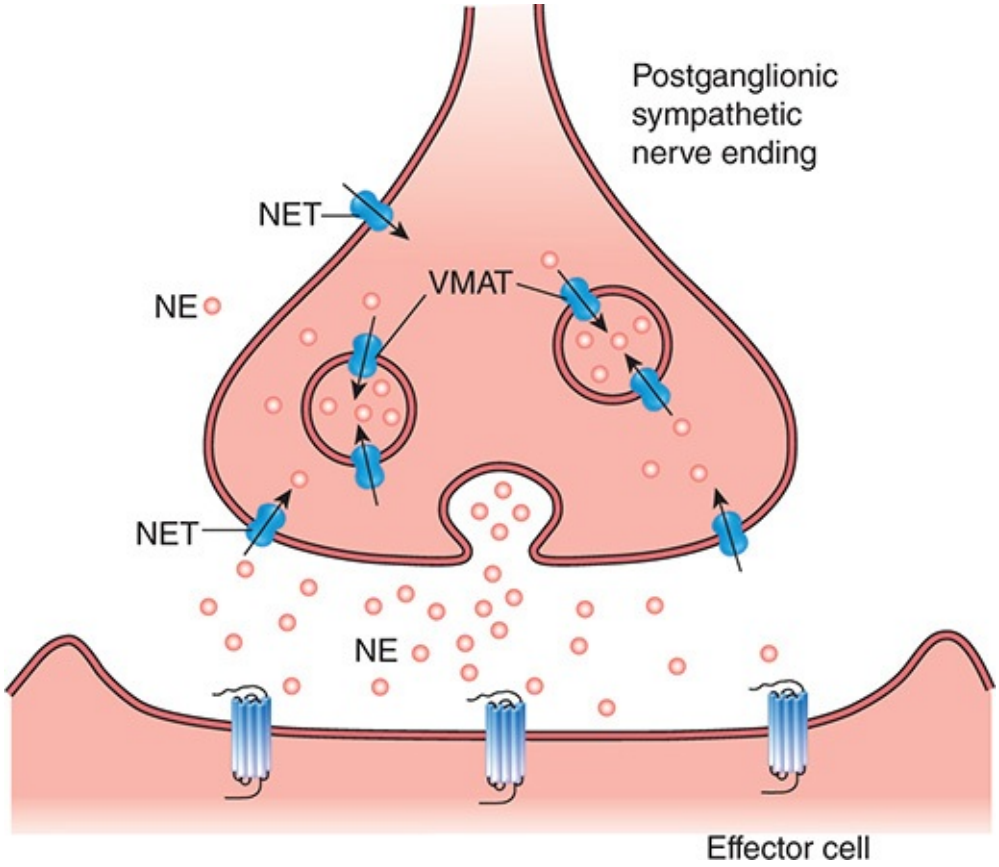


FIGURE 7–3 Fate of monoamines released at synaptic junctions. In each monoamine-secreting neuron, the monoamine is synthesized in the cytoplasm and the secretory granules and its concentration in secretory granules is maintained by the two vesicular monoamine transporters (VMAT). The monoamine is released by exocytosis of the granules, and it acts on G-protein-coupled receptors. In this example, the monoamine is norepinephrine acting on adrenoceptors. Many of these receptors are postsynaptic, but some are presynaptic and some are located on glia. In addition, there is extensive reuptake of the monoamine into the cytoplasm of the presynaptic terminal via a monoamine transporter, in this case the norepinephrine transporter (NET). (Modified with permission from Katzung BG, Masters SB, Trevor AJ: *Basic and Clinical Pharmacology*, 11th ed. New York, NY: McGraw-Hill; 2009.)

Reuptake is a major factor in terminating the action of neurotransmitters, and when it is inhibited, the effects of neurotransmitter release are increased and prolonged. This has clinical consequences. For example, several effective antidepressant drugs are inhibitors of the reuptake of amine transmitters. Glutamate reuptake into neurons and glia is important because glutamate is an excitotoxin that can kill cells by overstimulating them **(see Clinical Box 7–1)**.

During ischemia and anoxia, loss of neurons is increased because glutamate reuptake is inhibited.

SMALL-MOLECULE TRANSMITTERS: EXCITATORY & INHIBITORY AMINO ACIDS, ACETYLCHOLINE, AND MONOAMINES

Glutamate

The amino acid **glutamate** is the main excitatory neurotransmitter in the brain and spinal cord and may be responsible for 75% of the excitatory transmission in the CNS. There are two distinct pathways involved in the synthesis of glutamate (Figure 7–1). In one pathway, **α-ketoglutarate** produced by the Krebs cycle is converted to glutamate by the enzyme **GABA transaminase (GABA-T).** In the second pathway, glutamate is released from the nerve terminal into the synaptic cleft by Ca^{2+}-dependent exocytosis and transported via a **glutamate reuptake transporter** into glia, where it is converted to **glutamine** by the enzyme **glutamine synthetase (Figure 7–4)**. Glutamine then diffuses back into the nerve terminal where it is hydrolyzed back to glutamate by the enzyme **glutaminase.** A membrane transporter also returns glutamate directly into the nerve terminal. Within glutamatergic neurons, glutamate is highly concentrated in synaptic vesicles by a **vesicular glutamate transporter.**

Glutamate Receptors

Glutamate acts on both ionotropic and metabotropic receptors in the CNS (Figure 7–4). There are three subtypes of ionotropic glutamate receptors, each named for its relatively specific agonist. These are the **AMPA** (α-amino-3-hydroxy-5-methylisoxazole-4-propionate), **kainate**, and **NMDA receptors.** Table 7–2 summarizes some of the major properties of these receptors. Ionotropic glutamate receptors are tetramers composed of different subunits whose helical domains span the membranes three times and a short sequence that forms the channel pore. Four AMPA (GluR1–GluR4), five kainate (GluR5–GluR7, KA1, KA2), and six NMDA (NR1, NR2A–NR2D) subunits have been identified, and each is coded by a different gene.

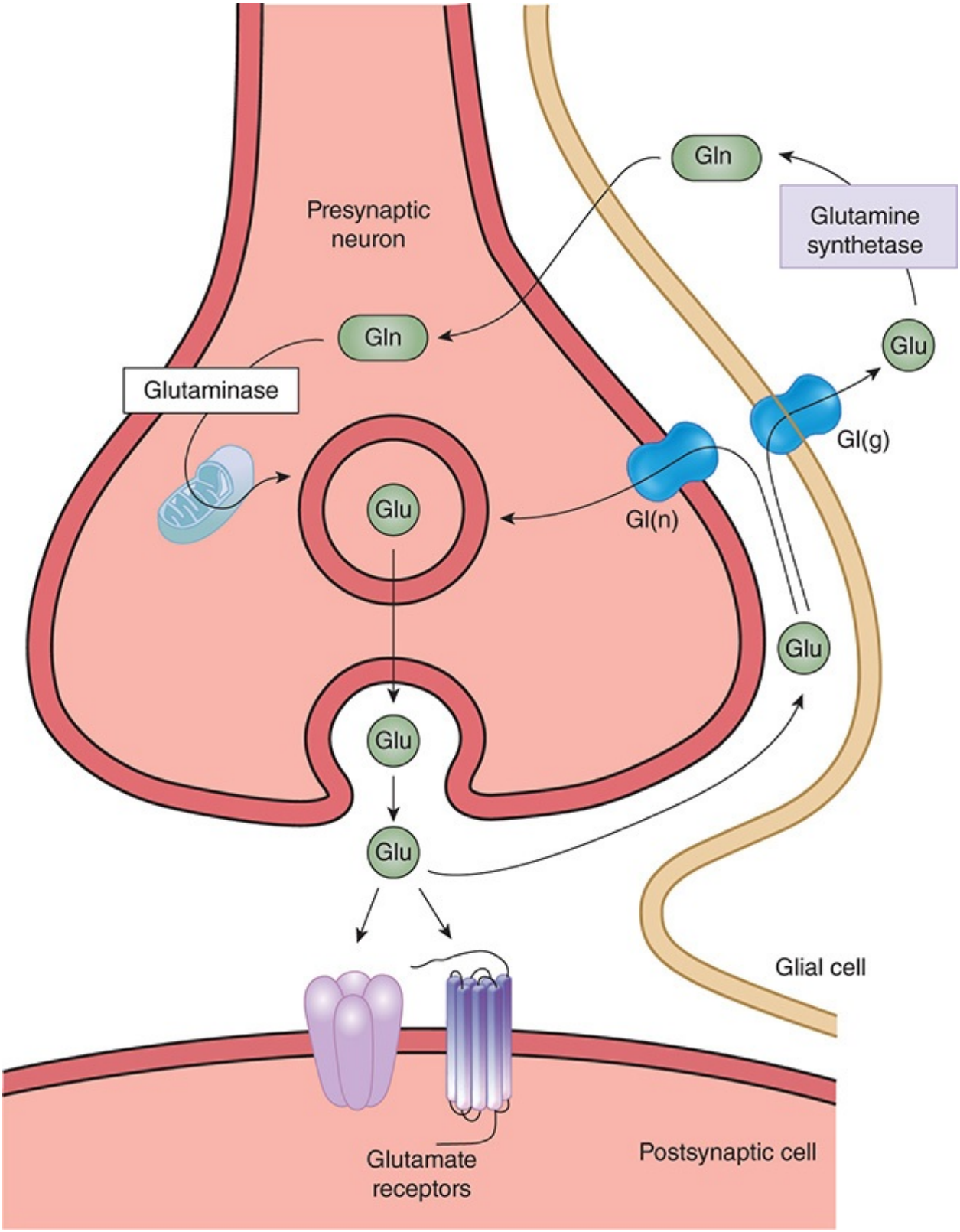


FIGURE 7–4 Biochemical events at a glutamatergic synapse. Glutamate

(Glu) released into the synaptic cleft by Ca^{2+}-dependent exocytosis. Released Glu can act on ionotropic and G-protein-coupled receptors on the postsynaptic neuron. Synaptic transmission is terminated by the active transport of Glu via by a Na^{+}-dependent glutamate transporters located on membranes of the presynaptic terminal [Gl(n)] and glia [Gl(g)]. In glia, Glu is converted to glutamine (Gln) by the enzyme glutamine synthetase; Gln then diffuses into the nerve terminal where it is hydrolyzed back to Glu by the enzyme glutaminase. In the nerve terminal, Glu is highly concentrated in synaptic vesicles by a vesicular glutamate transporter.

The release of glutamate and its binding to AMPA or kainate receptors primarily permits the influx of Na^{+} and the efflux of K^{+}, accounting for a fast-excitatory postsynaptic potential (EPSP). Most AMPA receptors have low Ca^{2+} permeability, but the absence of certain subunits in the receptor complex at some sites allows for the influx of Ca^{2+}, which may contribute to the excitotoxic effect of glutamate (see Clinical Box 7–1).

Activation of the NMDA receptor permits the influx of relatively large amounts of Ca^{2+} along with Na^{+}. When glutamate is in excess in the synaptic cleft, the NMDA receptor-induced influx of Ca^{2+} into neurons is the major basis for the excitotoxic actions of glutamate. The NMDA receptor is unique in several ways **(Figure 7–5)**. First, glycine binding to the NMDA receptor is essential for the receptor to respond to glutamate. Second, when glutamate binds to the NMDA receptor, it opens, but at normal membrane potentials, the channel is blocked by extracellular Mg^{2+}. This block is removed only when the neuron containing the receptor is partially depolarized by the activation of adjacent AMPA and kainate receptors. Third, the EPSP induced by activation of NMDA receptors is slower than that elicited by activation of the AMPA and kainate receptors.

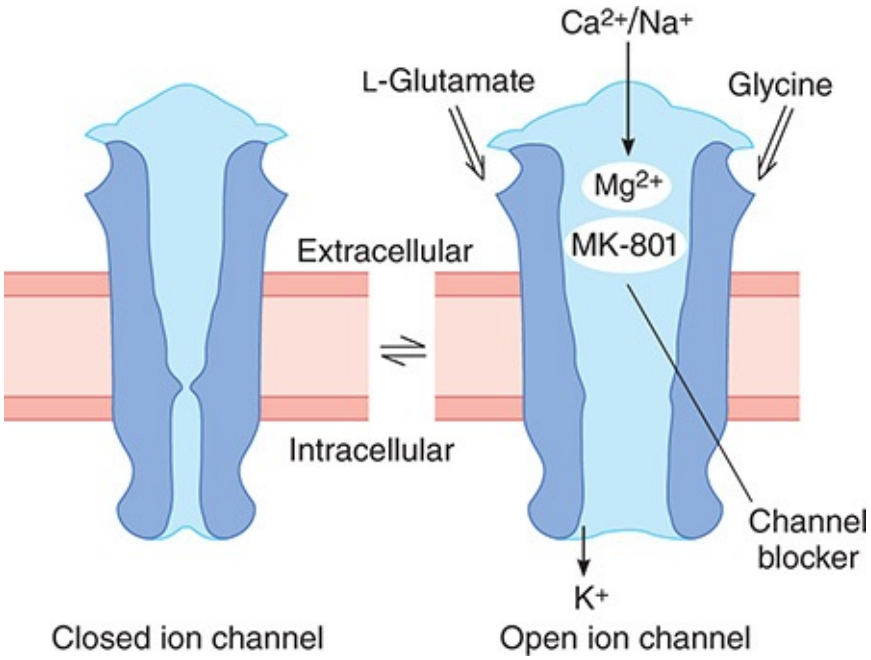


FIGURE 7–5 Diagrammatic representation of the NMDA receptor. When glycine and glutamate bind to the receptor, the closed ion channel (left) opens, but at the resting membrane potential, the channel is blocked by Mg^{2+} (right). This block is removed if partial depolarization is produced by other inputs to the neuron containing the receptor, and Ca^{2+} and Na^{+} enter the neuron. Blockade can also be produced by the drug dizocilpine maleate (MK-801).

Essentially all neurons in the CNS have both AMPA and NMDA receptors. Kainate receptors are located presynaptically on GABA-secreting nerve endings and postsynaptically at various sites, most notably in the hippocampus, cerebellum, and spinal cord. Kainate and AMPA receptors are also found in glia. The concentration of NMDA receptors in the hippocampus is high, and blockade of these receptors prevents **long-term potentiation,** a long-lasting facilitation of transmission in neural pathways following a brief period of high-frequency stimulation. Thus, these receptors may be involved in memory and learning (see Chapter 15).

Activation of the metabotropic glutamate receptors (mGluR) leads to either an increase in intracellular inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) levels or a decrease in intracellular cAMP levels (Table 7–2). There are eight known subtypes of mGluR located at either presynaptic ($mGluR_{2\text{-}4,6\text{-}8}$) or postsynaptic sites ($mGluR_{1,5}$) and are widely distributed in the brain. They may be involved in the production of **synaptic plasticity,** particularly in the

hippocampus and the cerebellum. Activation of presynaptic mGluR autoreceptors on hippocampal neurons limits the release of glutamate from these neurons. Knockout of the gene *mGluR1* causes severe motor incoordination and deficits in spatial learning. Dysregulation of brain levels of the $mGluR_5$ has been linked to neurological disorders including schizophrenia, major depressive disorders, and autism.

CLINICAL BOX 7–1

Excitotoxins

Glutamate is usually cleared from the brain's extracellular fluid by Na^+-dependent uptake systems in neurons and glia, keeping only micromolar levels of the chemical in the extracellular fluid despite millimolar levels inside neurons. However, excessive levels of glutamate occur in response to ischemia, anoxia, hypoglycemia, or trauma. Glutamate and some of its synthetic agonists are unique in that when they act on neuronal cell bodies, they can produce so much Ca^{2+} influx that the neurons die. Excitotoxins play a significant role in the damage done to the brain by a **stroke.** When a cerebral artery is occluded, the cells in the severely ischemic area die. The surrounding partially ischemic cells may survive but lose their ability to maintain the transmembrane Na^+ gradient. The elevated levels of intracellular Na^+ prevent the ability of **astrocytes** to remove glutamate from the brain's extracellular fluid. Therefore, glutamate accumulates to the point that excitotoxic damage and cell death occurs in the **penumbra,** the region around the completely infarcted area. In addition, excessive glutamate receptor activation may contribute to the pathophysiology of some neurodegenerative diseases such as **amyotrophic lateral sclerosis (ALS), Parkinson disease,** and **Alzheimer disease**.

THERAPEUTIC HIGHLIGHTS

Riluzole is a voltage-gated channel blocker that may antagonize N-methyl-D-aspartate (NMDA) receptors. It slows the progression of impairment and modestly improves the life expectancy of patients with ALS. Another NMDA receptor antagonist **memantine** has been used to slow the progressive decline in patients with Alzheimer disease. A third NMDA receptor antagonist, **amantadine,** in conjunction with **levodopa,** improves function in patients with

Parkinson disease.

Pharmacology of Glutamate Synapses

Table 7–2 shows some of the pharmacologic properties of glutamate receptors, examples of the agonists that bind to these receptors, and some of the antagonists that prevent activation of the receptors. The clinical applications of drugs that modulate glutamatergic transmission are still in their infancy. This is because the role of glutamate as a neurotransmitter was discovered much later than that of most other small- molecule transmitters. Intraspinal or extradural administration of NMDA receptor antagonists is used for the treatment of chronic pain. The $mGluR_5$ is a target for development of drugs in the treatment of neuropsychiatric disorders.

GABA

GABA is the major inhibitory mediator in the brain and mediates both presynaptic and postsynaptic inhibition. GABA is formed by decarboxylation of glutamate (Figure 7–1) by the enzyme **glutamate decarboxylase (GAD),** in nerve endings in many parts of the brain. A **vesicular GABA transporter (VGAT)** transports GABA into secretory vesicles. GABA is metabolized primarily by transamination to succinic semialdehyde and then to succinate in the citric acid cycle. **GABA-T** is the enzyme that catalyzes the transamination. After GABA is released from a neuron, a high-affinity GABA transporter allows for its reuptake.

GABA Receptors

Three subtypes of GABA receptors have been identified: $GABA_A$, $GABA_B$, and $GABA_C$ (Table 7–2). The $GABA_A$ and $GABA_B$ receptors **(Figure 7–6)** are widely distributed in the CNS, $GABA_C$ receptors are found almost exclusively in the retina. The $GABA_A$ and $GABA_C$ receptors are ionotropic receptors, activation of which allows the entry of Cl^- into neurons to mediate fast inhibitory postsynaptic potentials (IPSP). The $GABA_B$ receptors are GPCRs that are linked via G-proteins to alter the influx of K^+ and Ca^{2+}; G_i inhibits adenylyl

cyclase to open a K^+ channel, and G_o inhibits or delays Ca^{2+} influx. Activation of $GABA_B$ receptors mediates both presynaptic and slow postsynaptic inhibition.

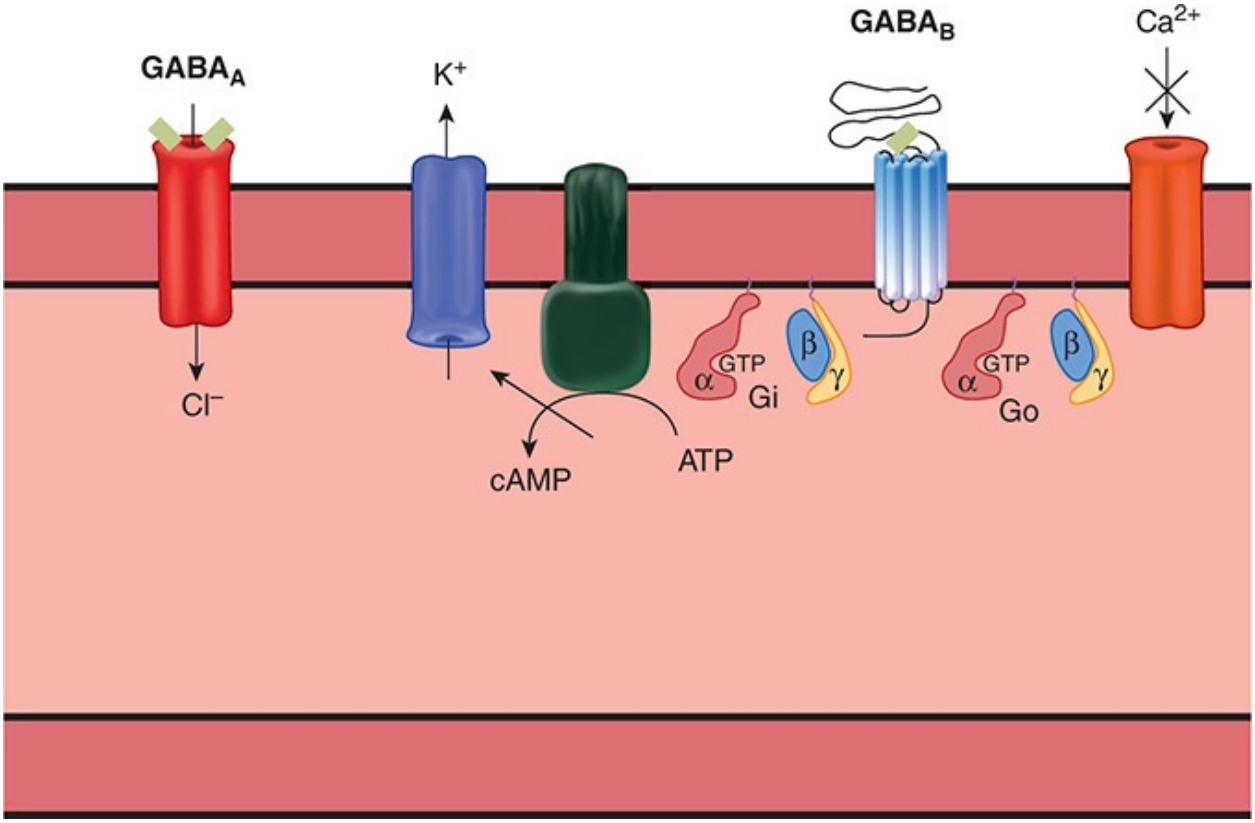


FIGURE 7–6 Diagram of $GABA_A$ and $GABA_B$ receptors, showing their principal actions. Two molecules of GABA (*squares*) bind to the $GABA_A$ receptor to allow an influx of Cl^- to mediate fast inhibitory postsynaptic potentials. One molecule of GABA binds to the $GABA_B$ receptor, which couples to the α-subunit of a G-protein; G_i inhibits adenylyl cyclase to open a K^+ channel and G_o inhibits Ca^{2+} influx.

The $GABA_A$ receptors are pentamers made up of various combinations of six α subunits, four β, four γ, one δ, and one ε. This endows them with considerably different properties from one location to another. However, most synaptic $GABA_A$ receptors have two α, two β, and one γ subunit (Figure 7–6). $GABA_A$ receptors on dendrites, axons, or somas often contain δ and ε subunits in place of the γ subunit. The $GABA_C$ receptors are relatively simple in that they are pentamers of three ρ subunits in various combinations.

Pharmacology of GABA Synapses

Table 7–2 shows some of the pharmacologic properties of GABA receptors, including examples of agonists that bind to receptors and some of the antagonists that prevent activation of these receptors. The increase in Cl^- conductance produced by $GABA_A$ receptors is potentiated by **benzodiazepines** (eg, **diazepam**). Thus, these are examples of neuromodulators. These drugs have marked antianxiety activity and are also effective muscle relaxants, anticonvulsants, and sedatives. Benzodiazepines bind to α subunits of $GABA_A$ receptors. **Barbiturates** such as **phenobarbital** are effective anticonvulsants because they enhance $GABA_A$ receptor-mediated inhibition and suppress AMPA receptor-mediated excitation. The anesthetic actions of barbiturates (**thiopental, pentobarbital,** and **methoxital**) result from their actions as agonists at $GABA_A$ receptors as well as by acting as neuromodulators of GABA transmission. Regional variation in anesthetic actions in the brain parallels the variation in subtypes of $GABA_A$ receptors.

Glycine

Glycine has both excitatory and inhibitory effects in the CNS. When it binds to NMDA receptors, it makes them more sensitive to the actions of glutamate. Glycine may spill over from synaptic junctions into the interstitial fluid and in the spinal cord; for example, it may facilitate pain transmission by NMDA receptors in the dorsal horn. However, glycine mediates direct inhibition in the brainstem and spinal cord. The glycine receptor that mediates inhibition is a Cl^- channel, a pentamer made up of two subunits: the ligand-binding α subunit and the structural β subunit. Like GABA, glycine acts by increasing Cl^- conductance. Its action is antagonized by strychnine. The clinical picture of convulsions and muscular hyperactivity produced by strychnine emphasizes the importance of postsynaptic inhibition in normal neuronal function.

There are three kinds of spinal cord neurons that mediate direct inhibition: neurons that release glycine, neurons that release GABA, and neurons that release both. Neurons that release only glycine have the glycine transporter GLYT2, those that release only GABA have VGAT, and those that release glycine and GABA have both. Glycine and GABA can be found in the same vesicles in these latter neurons.

Acetylcholine

Acetylcholine is the transmitter at the neuromuscular junction, in autonomic ganglia, and in postganglionic parasympathetic nerve-target organ junctions and some postganglionic sympathetic nerve-target junctions (see Chapter 13). In fact, acetylcholine is the transmitter released by all neurons that exit the CNS (cranial nerves, motor neurons, and preganglionic neurons). Acetylcholine is also found in the basal forebrain complex (septal nuclei and nucleus basalis), which projects to the hippocampus and neocortex, and the pontomesencephalic cholinergic complex, which projects to the dorsal thalamus and forebrain (Figure 7–2). These systems may be involved in regulation of sleep-wake states, learning, and memory (see Chapters 14 and 15).

Acetylcholine is found in small, clear synaptic vesicles in high concentration in the terminals of **cholinergic neurons.** It is synthesized in the nerve terminal from choline and acetyl-CoA by the enzyme **choline acetyltransferase (ChAT)** (Figure 7–1 and **Figure 7–7**). Choline used in the synthesis of acetylcholine is transported from the extracellular space into the nerve terminal via a Na^+-dependent **choline transporter (CHT).** Following its synthesis, acetylcholine is transported from the cytoplasm into vesicles by a **vesicle-associated transporter (VAT).** Acetylcholine is released when a nerve impulse triggers the influx of Ca^{2+} into the nerve terminal.

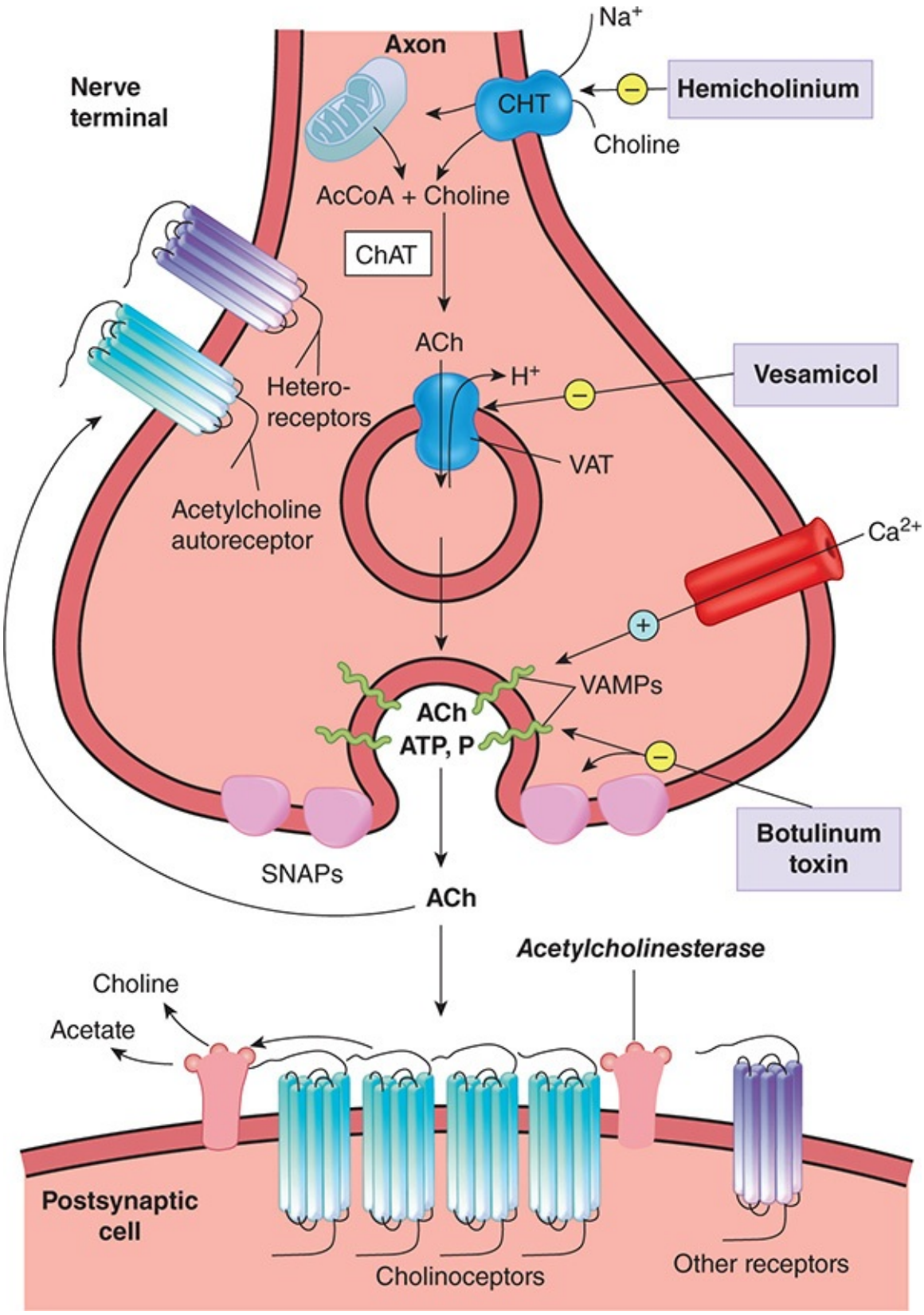


FIGURE 7–7 Biochemical events at a cholinergic synapse. Choline is

transported into the presynaptic nerve terminal by a Na^+-dependent choline transporter (CHT), which can be blocked by the drug hemicholinium. Acetylcholine (ACh) is synthesized from choline and acetyl Co-A (AcCoA) by the enzyme choline acetyltransferase (ChAT) in the cytoplasm. ACh is then transported from the cytoplasm into vesicles by the vesicle-associated transporter (VAT) along with peptides (P) and adenosine triphosphate (ATP). This step can be blocked by the drug vesamicol. ACh is released from the nerve terminal when voltage-sensitive Ca^{2+} channels open, allowing an influx of Ca^{2+}, which leads to fusion of vesicles with the surface membrane and expulsion of ACh and co-transmitters into the synaptic cleft. This process involves synaptosome-associated proteins (SNAPs) and vesicle-associated membrane proteins (VAMPs) and can be prevented by the drug botulinum toxin. The released ACh can act on muscarinic G-protein-coupled receptors on the postsynaptic target (eg, smooth muscle) or on nicotinic ionotropic receptors in autonomic ganglia or the endplate of skeletal muscle (not shown). In the synaptic junction, ACh is readily metabolized by the enzyme acetylcholinesterase. Autoreceptors and heteroreceptors on the presynaptic nerve ending modulate neurotransmitter release.

Acetylcholine must be rapidly removed from the synapse if repolarization is to occur. The removal occurs by way of hydrolysis of acetylcholine to choline and acetate, a reaction catalyzed by the enzyme **acetylcholinesterase** in the synaptic cleft. Acetylcholinesterase molecules are clustered in the postsynaptic membrane of cholinergic synapses. Hydrolysis of acetylcholine by this enzyme is rapid enough to explain the observed changes in Na^+ conductance and electrical activity during synaptic transmission.

Acetylcholine Receptors

Acetylcholine receptors are divided into two main types based on their pharmacologic properties. In sympathetic ganglia and skeletal muscle, nicotine mimics the stimulatory actions of acetylcholine. These actions of acetylcholine are called **nicotinic actions,** and the receptors involved are **nicotinic cholinergic receptors.** Nicotinic receptors are subdivided into those found at the neuromuscular junction (N_M) and those found in the CNS and autonomic ganglia (N_N). Muscarine, the alkaloid responsible for the toxicity of toadstools, mimics the stimulatory action of acetylcholine on smooth muscle and glands. These actions of acetylcholine are called **muscarinic actions,** and the receptors

involved are **muscarinic cholinergic receptors.** Both muscarinic and nicotinic acetylcholine receptors are found in the brain.

The nicotinic acetylcholine receptors are members of a superfamily of ligand-gated ion channels (ionotropic receptors). Each nicotinic cholinergic receptor is made up of five subunits that form a central channel which, when the receptor is activated, permits the passage of Na^+ and other cations. The five subunits are designated as α, β, γ, δ, and ε that are each coded by different genes. The N_M receptor is comprised of two α, one β, one δ, and either one γ or one ε subunit **(Figure 7–8)**. The N_N receptors are comprised of only α and β subunits. Each α subunit has a binding site for acetylcholine, and binding of an acetylcholine molecule to each of them induces a conformational change in the protein so that the channel opens. This increases the conductance of Na^+, and the resulting influx of Na^+ produces a depolarizing potential. A prominent feature of N_N receptors is their high permeability to Ca^{2+}. Many of the N_N receptors in the brain are located presynaptically on glutamate-secreting axon terminals, and they facilitate the release of this neurotransmitter.

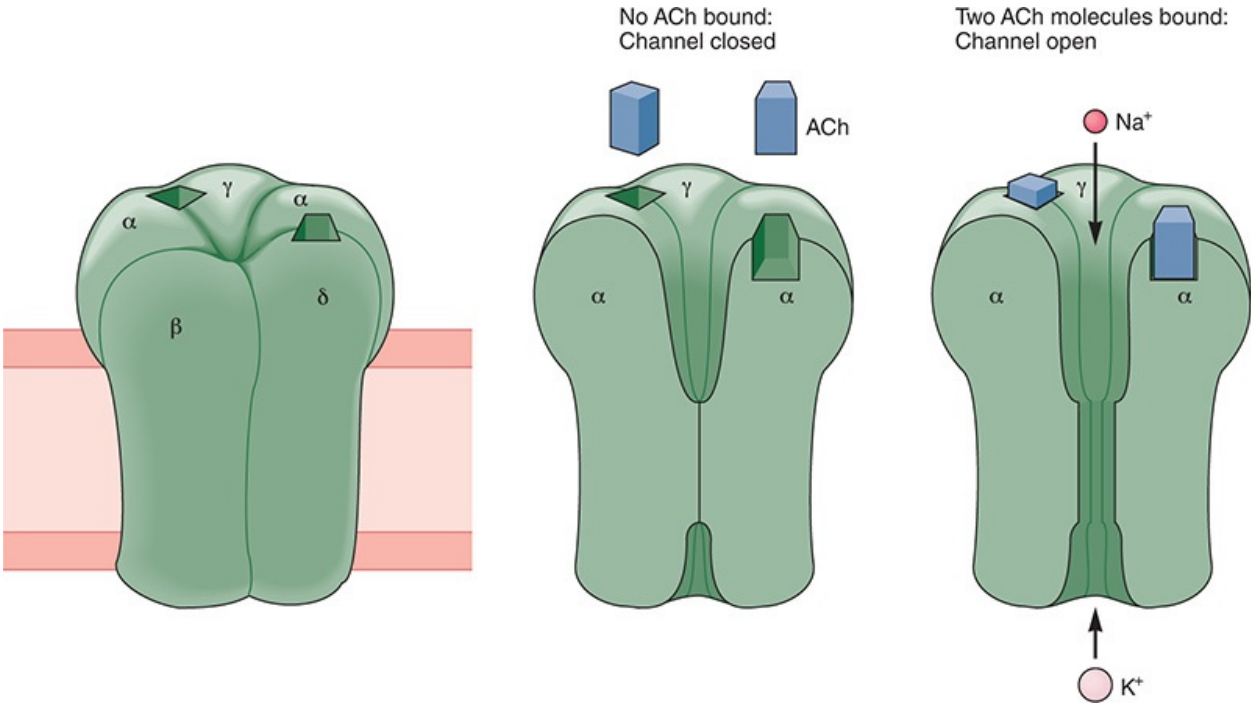


FIGURE 7–8 Three-dimensional model of the nicotinic acetylcholine-gated ion channel. The receptor–channel complex consists of five subunits, all of which contribute to forming the pore. When two molecules of acetylcholine bind to portions of the α-subunits exposed to the membrane surface, the receptor-

channel changes conformation. This opens the pore in the portion of the channel embedded in the lipid bilayer, and both K^+ and Na^+ flow through the open channel down their electrochemical gradient. (Reproduced with permission from Kandel ER, Schwartz JH, Jessell TM [editors]: *Principles of Neural Science*, 4th ed. New York, NY: McGraw-Hill; 2000.)

There are five types of muscarinic cholinergic receptors (M_1–M_5), which are encoded by five separate genes. These are metabotropic receptors that are coupled via G-proteins to adenylyl cyclase, K^+ channels, and/or phospholipase C (Table 7–2). M_1, M_4, and M_5 receptors are located in the CNS; M_2 receptors are in the heart; M_3 receptors are on glands and smooth muscle. M_1 receptors are also located on autonomic ganglia where they can modulate neurotransmission.

Pharmacology of Cholinergic Synapses

Table 7–2 shows some of the major agonists that bind to cholinergic receptors as well as some of the cholinergic receptor antagonists. Figure 7–7 also shows the site of action of various drugs that alter cholinergic transmission. For example, **hemicholinium** blocks the CHT that moves choline into the nerve terminal, and **vesamicol** blocks the VAT that moves acetylcholine into the synaptic vesicle. Also, **botulinum toxin** prevents the release of acetylcholine from the nerve terminal. Chapter 13 includes more information regarding the pharmacology of acetylcholine as related to its role in autonomic neurotransmission.

Norepinephrine & Epinephrine

Norepinephrine is the transmitter released from most sympathetic postganglionic endings where it is found in characteristic small vesicles that have a dense core (granulated vesicles). Norepinephrine and its methyl derivative, epinephrine, are also released by the adrenal medulla (see Chapter 20), but epinephrine is not a mediator at postganglionic sympathetic endings. As discussed in Chapter 6, each sympathetic postganglionic neuron has multiple varicosities along its course, and each of these varicosities is a site at which norepinephrine is released.

Norepinephrine and epinephrine are also released from neurons in the brain. Norepinephrine-containing neurons are properly called **noradrenergic neurons,** although the term **adrenergic neurons** is often used. However, the latter term should be reserved for epinephrine-containing neurons. The cell bodies of the

norepinephrine-containing neurons are found in the locus coeruleus and other medullary and pontine nuclei (Figure 7–2). From the locus coeruleus, the axons of the noradrenergic neurons descend into the spinal cord, enter the cerebellum, and ascend to innervate the paraventricular, supraoptic, and periventricular nuclei of the hypothalamus, the thalamus, the basal telencephalon, and the entire neocortex. The action of norepinephrine in these regions is primarily to modulate neurotransmission.

Biosynthesis & Release of Catecholamines

The principal **catecholamines** found in the body (norepinephrine, epinephrine, and dopamine) are formed by hydroxylation and decarboxylation of the amino acid tyrosine (Figure 7–1 and **Figure 7–9**). Some of the tyrosine is formed from phenylalanine, but most is of dietary origin. **Phenylalanine hydroxylase** is found primarily in the liver **(see Clinical Box 7–2)**. Tyrosine is transported into catecholamine-secreting neurons via a Na^+-dependent carrier. It is converted to dihydroxy-phenylalanine (dopa) and then to dopamine in the cytoplasm of the cells by **tyrosine hydroxylase** and **dopa decarboxylase,** respectively. The decarboxylase is also called **amino acid decarboxylase.** The rate-limiting step in synthesis of catecholamines is the conversion of tyrosine to dopa. Tyrosine hydroxylase is subject to feedback inhibition by dopamine and norepinephrine, thus providing internal control of the synthetic process.

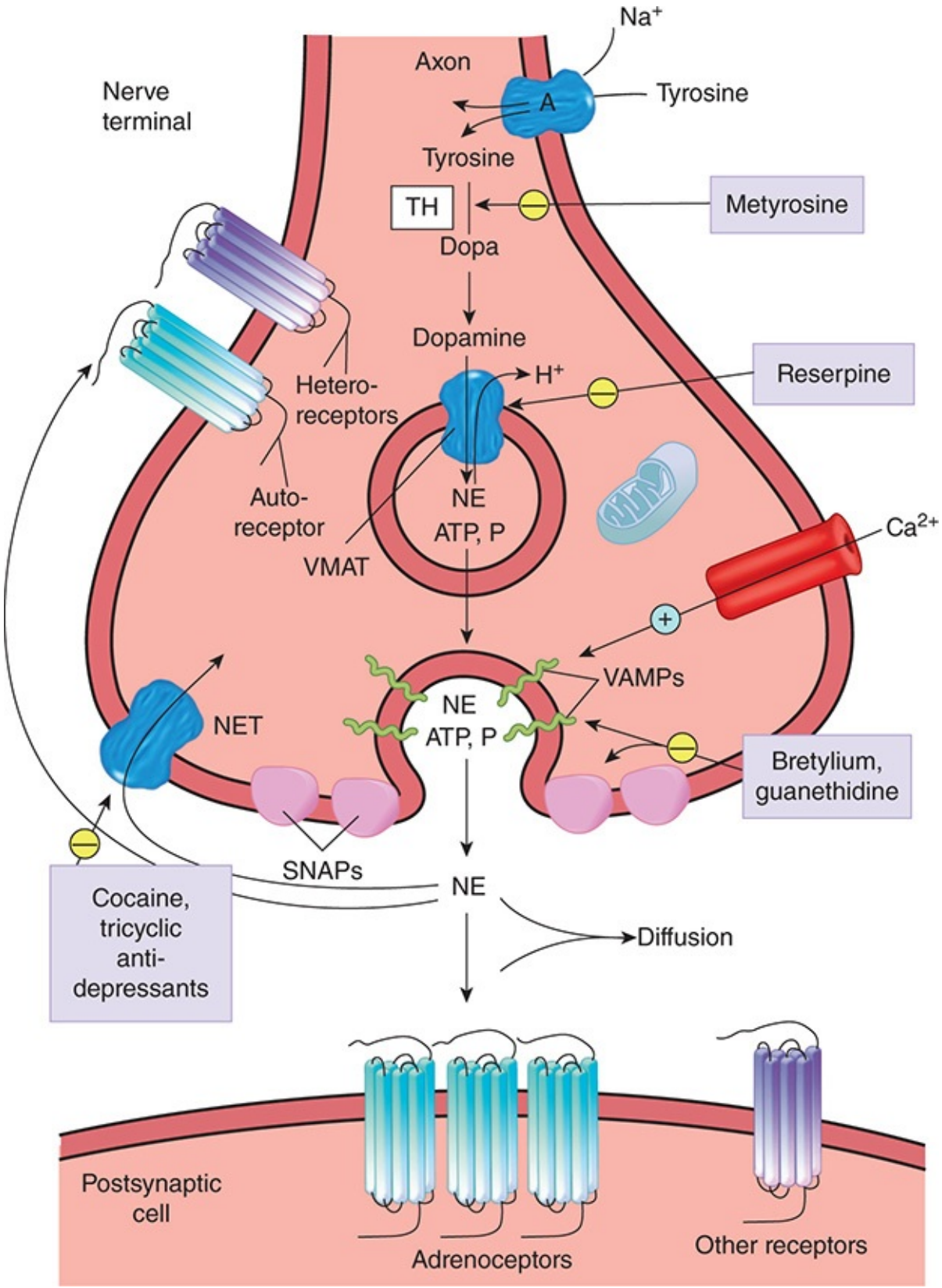


FIGURE 7–9 Biochemical events at a noradrenergic synapse. Tyrosine (Tyr)

is transported into the noradrenergic nerve terminal by a Na^+-dependent carrier (A). The steps involved in the conversion of Tyr to dopamine and dopamine to norepinephrine (NE) are described in Figure 7–1. Metyrosine blocks the action of tyrosine hydroxylase (TH), the rate-limiting step in the production of catecholamines. Dopamine is transported from the cytoplasm into the vesicle by the vesicular monoamine transporter (VMAT), which can be blocked by the drug reserpine. NE and other amines can also be carried by VMAT. Dopamine is converted to NE in the vesicle. An action potential opens voltage-sensitive Ca^{2+} channels to allow an influx of Ca^{2+}, and the vesicles then fuse with the surface membrane to trigger expulsion of NE along with peptides (P) and adenosine triphosphate (ATP). This process involves synaptosome-associated proteins (SNAPs) and vesicle-associated membrane proteins (VAMPs); it can be blocked by drugs such as guanethidine and bretylium. NE released into the nerve terminal can act on G-protein-coupled receptors on the postsynaptic neuron or neuroeffector organ (eg, blood vessels). NE can also diffuse out of the cleft or be transported back into the nerve terminal by the norepinephrine transporter (NET). NET can be blocked by cocaine and tricyclic antidepressants. Autoreceptors and heteroreceptors on the presynaptic nerve ending modulate neurotransmitter release.

Once dopamine is synthesized, it is transported into the vesicle by the **VMAT.** Here the dopamine is converted to norepinephrine by **dopamine β-hydroxylase.** Norepinephrine is the only small-molecule transmitter that is synthesized in synaptic vesicles instead of being transported into the vesicle after its synthesis in the cytoplasm.

Some neurons in the CNS and adrenal medullary cells also contain the cytoplasmic enzyme **phenylethanolamine-N-methyltransferase,** which catalyzes the conversion of norepinephrine to epinephrine. In these cells, norepinephrine leaves the vesicles, is converted to epinephrine in the cytoplasm, and then enters other vesicles for storage until it is released by exocytosis.

Catabolism of Catecholamines

Norepinephrine, like other amine and amino acid transmitters, is removed from the synaptic cleft by binding to postsynaptic receptors, binding to presynaptic receptors, reuptake into the presynaptic neurons, or catabolism. Reuptake via a **NET** is a major mechanism to terminate the actions of norepinephrine (Figure 7–3), and the hypersensitivity of sympathetically denervated structures is explained

in part on this basis. After the noradrenergic neurons are cut, their endings degenerate with loss of NET to remove norepinephrine from the synaptic cleft. Consequently, more norepinephrine from other sources is available to stimulate the receptors on the autonomic effectors.

Epinephrine and norepinephrine are metabolized to biologically inactive products by oxidation and methylation. The former reaction is catalyzed by **monoamine oxidase (MAO)** and the latter by **catechol-O-methyltransferase (COMT).** MAO is located on the outer surface of the mitochondria. MAO is widely distributed and is plentiful in the nerve endings from which catecholamines are released. COMT is also widely distributed, particularly in the liver, kidneys, and smooth muscles. In the brain, it is present in glial cells, and small amounts are found in postsynaptic neurons, but none is found in presynaptic noradrenergic neurons.

Extracellular epinephrine and norepinephrine are for the most part O-methylated, and measurement of the concentrations of the O-methylated derivatives normetanephrine and metanephrine in the urine is a good index of their rate of release. The O-methylated derivatives that are not excreted are largely oxidized, and vanillylmandelic acid is the most plentiful catecholamine metabolite in the urine.

CLINICAL BOX 7–2

Phenylketonuria

Phenylketonuria (PKU) is an example of an inborn error of metabolism. PKU is characterized by severe mental deficiency and the accumulation in the blood, tissues, and urine of large amounts of **phenylalanine** and its keto acid derivatives. It is usually due to decreased function resulting from mutation of the gene for **phenylalanine hydroxylase.** This gene is located on the long arm of chromosome 12. Catecholamines are still formed from tyrosine, and the cognitive impairment is largely due to accumulation of phenylalanine and its derivatives in the blood. The condition can also be caused by **tetrahydrobiopterin (BH4) deficiency.** Because BH4 is a cofactor for tyrosine hydroxylase and tryptophan hydroxylase, as well as phenylalanine hydroxylase, PKU cases due to BH4 deficiency have catecholamine and serotonin deficiencies in addition to hyperphenylalaninemia. These cause hypotonia, inactivity, and developmental problems. BH4 is also essential for the synthesis of NO by NO synthase. Severe BH4 deficiency can lead to

impairment of NO formation, and the CNS may be subjected to increased oxidative stress. Blood phenylalanine levels are usually determined in newborns in North America, Australia, and Europe; if PKU is diagnosed, dietary interventions should be started before the age of 3 weeks to prevent the development of mental retardation.

THERAPEUTIC HIGHLIGHTS

PKU can usually be treated successfully by markedly reducing the amount of phenylalanine in the diet. This means restricting the intake of high-protein foods such as milk, eggs, cheese, meats, and nuts. In individuals with a BH4 deficiency, treatment can include BH4, **levodopa,** and **5-hydroxytryptophan** in addition to a low- phenylalanine diet. The US Food and Drug Administration approved the drug **sapropterin,** a synthetic BH4, for the treatment of some people with PKU.

In the noradrenergic nerve terminals, some of the norepinephrine is constantly being converted by intracellular MAO to the physiologically inactive deaminated derivatives, 3,4- dihydroxymandelic acid and its corresponding glycol. These are then converted to their corresponding O-methyl derivatives, vanillylmandelic acid and 3-methoxy-4-hydroxyphenylglycol.

α- & β-Adrenoceptors

Epinephrine and norepinephrine both act on α- and β-adrenergic receptors **(adrenoceptors),** with norepinephrine having a greater affinity for α-adrenoceptors and epinephrine for β-adrenoceptors. These receptors are GPCRs, and each has multiple subtypes (α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, $\alpha_{2C,}$ and $\beta_{1\text{-}3}$). Most α_1-adrenoceptors are coupled via G_q proteins to phospholipase C, leading to the formation of IP_3 and DAG, which mobilizes intracellular Ca^{2+} stores and activates protein kinase C, respectively. Thus, at many synapses, activation of α_1-adrenoceptors is excitatory to the postsynaptic target. In contrast, α_2-adrenoceptors activate G_i inhibitory proteins to inhibit adenylyl cyclase and decrease cAMP. Other actions of α_2-adrenoceptors are to activate G-protein-coupled inward rectifier K^+ channels to cause membrane hyperpolarization and to inhibit neuronal Ca^{2+} channels. Thus, at many synapses, activation of α_2-

adrenoceptors inhibits the postsynaptic target. Presynaptic α_2-adrenoceptors are autoreceptors which, when activated, inhibit further release of norepinephrine from postganglionic sympathetic nerve terminals. β-Adrenoceptors activate a stimulatory G_S protein to activate adenylyl cyclase to increase cAMP.

α_1-Adrenoceptors are located on smooth muscle and the heart, and α_2-adrenoceptors are located in the CNS, pancreatic islets cells, and nerve terminals. β_1-Adrenoceptors are found in the heart and renal juxtaglomerular cells, β_2-adrenoceptors are located in bronchial smooth muscle and some vascular smooth muscle, and β_3-adrenoceptors are located in adipose tissue.

Pharmacology of Noradrenergic Synapses

Table 7–2 shows some of the common agonists that bind to adrenoceptors as well as some of the common adrenoceptor antagonists. Figure 7–9 also shows the site of action of various drugs that alter noradrenergic transmission. For example, **metyrosine** blocks the action of tyrosine hydroxylase, the rate-limiting step in the synthetic pathway for catecholamine production in the nerve terminal. **Reserpine** blocks the VMAT that moves dopamine into the synaptic vesicle. Also, **bretylium** and **guanethidine** prevent the release of norepinephrine from the nerve terminal. **Cocaine** and **tricyclic antidepressants** block the NET. In addition to the agonists listed in Table 7–2, some drugs mimic the actions of norepinephrine by releasing stored transmitter from the noradrenergic endings. These are called **sympathomimetics** and include **amphetamines** and **ephedrine**. Chapter 13 includes more information regarding the pharmacology of norepinephrine as related to its role in autonomic neurotransmission.

Dopamine

In some parts of the brain, catecholamine synthesis stops at dopamine (Figure 7–1), which can then be released into the synaptic cleft. Active reuptake of dopamine occurs via a Na^+- and Cl^--dependent **dopamine transporter.** Dopamine is metabolized to inactive compounds by MAO and COMT in a manner analogous to the inactivation of norepinephrine. 3,4-Dihydroxyphenylacetic acid and homovanillic acid are conjugated, primarily to sulfate.

Dopaminergic neurons are found in several brain regions (Figure 7–2). One region is the **nigrostriatal system,** which projects from the midbrain substantia

nigra to the striatum in the basal ganglia and is involved in motor control. Another dopaminergic system is the **mesocortical system;** it arises primarily in the ventral tegmental area, which projects to the nucleus accumbens and limbic subcortical areas. The mesocortical system is involved in reward behavior and addiction and in psychiatric disorders such as schizophrenia **(see Clinical Box 7–3)**. Studies using **positive emission tomography (PET)** scanning in healthy humans show that a steady loss of dopamine receptors occurs in the basal ganglia with age.

Dopamine Receptors

Five dopamine receptors have been cloned, but they fall into two major categories: D_1-like (D_1 and D_5) and D_2-like (D_2, D_3, and D_4). All dopamine receptors are GPCRs. Activation of D_1-type receptors leads to an increase in cAMP, whereas activation of D_2-like receptors reduces cAMP levels. Overstimulation of D_2 receptors may contribute to the pathophysiology of schizophrenia (Clinical Box 7–3). D_3 receptors are highly localized, especially to the nucleus accumbens (Figure 7–2). D_4 receptors have a greater affinity than the other dopamine receptors for the "atypical" antipsychotic drug **clozapine,** which is used primarily to treat schizophrenia in individuals who do not respond to other therapies.

Serotonin

Serotonin (5-hydroxytryptamine; 5-HT) is present in highest concentration in blood platelets and in the gastrointestinal tract, where it is found in the enterochromaffin cells and the myenteric plexus. It is also found within the brainstem in the midline raphe nuclei, which project to a wide portion of the CNS including the hypothalamus, limbic system, neocortex, cerebellum, and spinal cord (Figure 7–2).

Serotonin is synthesized from the essential amino acid **tryptophan** (Figure 7–1 and **Figure 7–10**). The rate-limiting step is the conversion of the amino acid to **5-hydroxytryptophan** by **tryptophan hydroxylase.** This is then converted to serotonin by the **aromatic L-amino acid decarboxylase.** Serotonin is transported into the vesicles by the VMAT. After release from serotonergic neurons, serotonin is recaptured by the relatively selective **serotonin transporter (SERT).** Once serotonin is returned to the nerve terminal, it is

either taken back into the vesicles or is inactivated by MAO to form 5-hydroxyindoleacetic acid (5-HIAA). This substance is the principal urinary metabolite of serotonin, and urinary output of 5-HIAA is used as an index of the rate of serotonin metabolism.

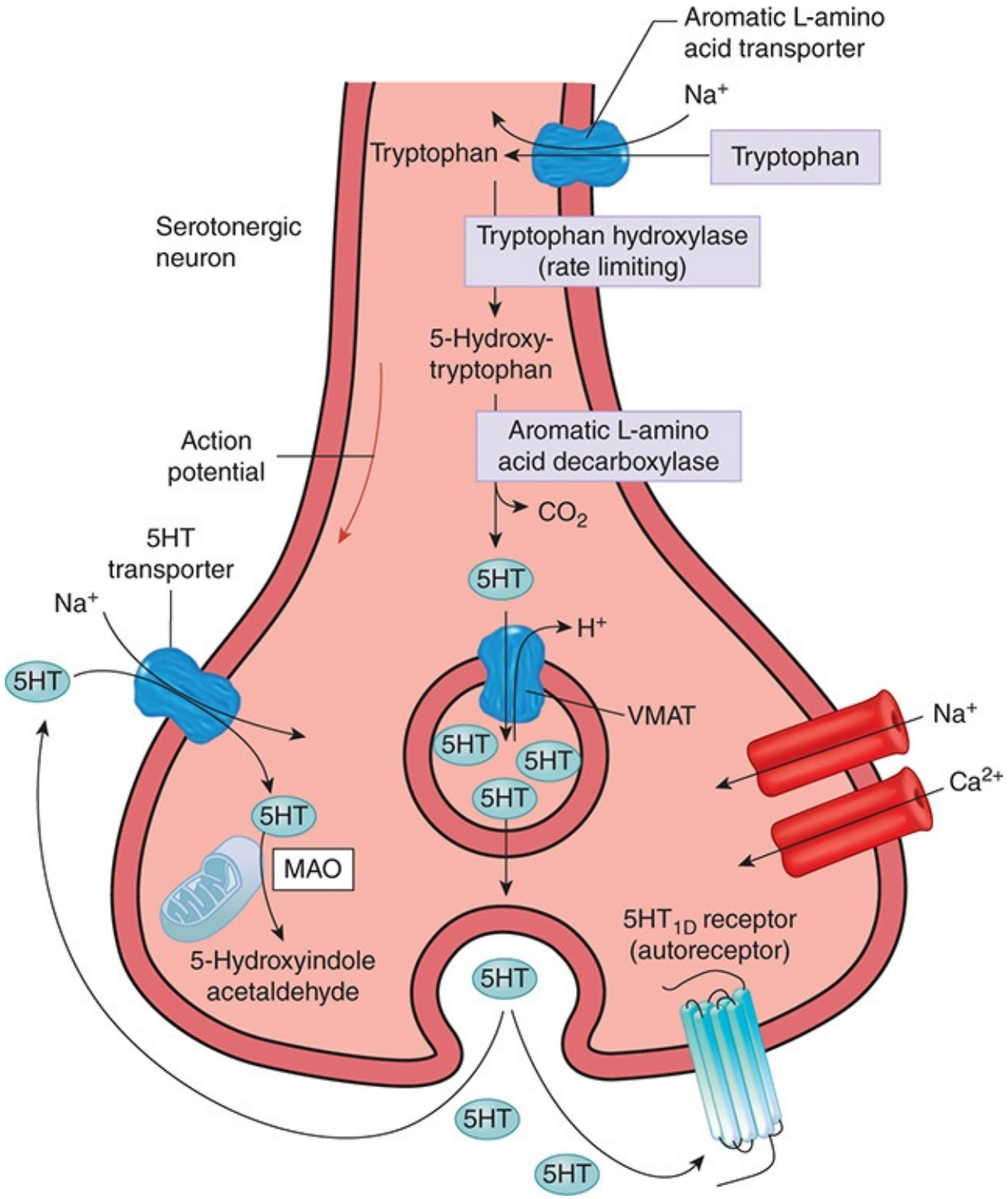


FIGURE 7–10 Biochemical events at a serotonergic synapse. Tryptophan is transported into the serotonergic nerve terminal by a Na^+-dependent aromatic L-amino acid transporter. The steps involved in the conversion of tryptophan to serotonin (5-hydroxytryptamine, 5-HT) are described in Figure 7–1. 5-HT is transported from the cytoplasm into vesicles by the vesicular monoamine transporter (VMAT). 5-HT release occurs when an action potential opens voltage-sensitive Ca^{2+} channels to allow an influx of Ca^{2+} and fusion of vesicles with the surface membrane. 5-HT released into the nerve terminal can act on G-protein-coupled receptors on the postsynaptic neuron (not shown). 5-HT can also diffuse out of the cleft or be transported back into the nerve terminal by the 5-HT transporter. 5-HT can act on presynaptic autoreceptors to inhibit further neurotransmitter release. Cytoplasmic 5-HT is either sequestered in vesicles as described or metabolized to 5-hydroxyindole acetaldehyde by mitochondrial monoamine oxidase (MAO).

CLINICAL BOX 7–3

Schizophrenia

Schizophrenia is an illness involving deficits of multiple brain systems that alter an individual's inner thoughts as well as their interactions with others. Individuals with schizophrenia suffer from hallucinations, delusions, and racing thoughts (positive symptoms); and they experience apathy, difficulty dealing with novel situations, and little spontaneity or motivation (negative symptoms). Worldwide, about 1–2% of the population lives with schizophrenia. A combination of genetic, biologic, cultural, and psychologic factors contributes to the illness. A defect in the **mesocortical system** is responsible for the development of at least some of the symptoms of schizophrenia. Attention was initially focused on overstimulation of limbic **D_2 dopamine receptors. Amphetamine,** which causes release of dopamine and norepinephrine in the brain, causes a schizophrenia-like psychosis; brain levels of D_2 receptors are elevated in individuals; and there is a positive correlation between the anti-schizophrenic activity of many drugs and their ability to block D_2 receptors. However, several recently developed drugs are effective antipsychotic agents but bind to D_2 receptors to a limited degree. Instead, they bind to D_4 receptors, and there is ongoing research into the possibility that these receptors are abnormal in individuals with

schizophrenia.

THERAPEUTIC HIGHLIGHTS

Since the mid-1950s numerous antipsychotic drugs (eg, **chlorpromazine, haloperidol, perphenazine,** and **fluphenazine**) have been used to treat schizophrenia. In the 1990s, new "atypical" antipsychotics were developed. These include **clozapine,** which reduces psychotic symptoms, hallucinations, suicidal behavior, and breaks with reality. However, a potential adverse side effect is agranulocytosis (a loss of the white blood cells), which impairs the ability to fight infections. Other atypical antipsychotics do not cause agranulocytosis, including **risperidone, olanzapine, quetiapine, ziprasidone, aripiprazole,** and **paliperidone.**

Serotonergic Receptors

There are seven classes of 5-HT receptors, and all except one (5-HT_3) are GPCRs and affect adenylyl cyclase or phospholipase C (Table 7–2). Within the 5-HT_1 group are the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F} subtypes. Within the 5-HT_2 group there are 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} subtypes. There are two 5-HT_5 subtypes: 5-HT_{5A} and 5-HT_{5B}. Some of the serotonin receptors are presynaptic and others are postsynaptic.

5-HT_{2A} receptors mediate platelet aggregation and smooth muscle contraction. 5-HT_3 receptors are present in the gastrointestinal tract and the area postrema and are related to vomiting. 5-HT_4 receptors are also present in the gastrointestinal tract, where they facilitate secretion and peristalsis, and in the brain. 5-HT_6 and 5-HT_7 receptors are distributed throughout the limbic system, and the 5-HT_6 receptors have a high affinity for antidepressant drugs.

Pharmacology of Serotonergic Synapses

Table 7–2 shows some of the common agonists that bind to 5-HT receptors as well as some of the common 5-HT receptor antagonists. In addition, tricyclic antidepressants inhibit the uptake of serotonin by blockade of SERT. **Selective serotonin uptake inhibitors (SSRIs)** such as **fluoxetine** are widely used in the

treatment of depression **(see Clinical Box 7–4)**.

CLINICAL BOX 7–4

Major Depression

According to the National Institutes of Mental Health, nearly 21 million Americans over the age of 18 have a mood disorder that includes **major depressive disorder**, **dysthymia**, and **bipolar disease.** The largest group is those with major depression. Major depression has a median age of onset of 32 years and is more prevalent in women than in men. Symptoms of major depression include depressed mood, anhedonia, loss of appetite, insomnia or hypersomnia, restlessness, fatigue, feelings of worthlessness, diminished ability to think or concentrate, and recurrent thoughts of suicide. **Typical depression** is characterized by feelings of sadness, early-morning awakenings, decreased appetite, restlessness, and anhedonia. Symptoms of **atypical depression** include pleasure-seeking behavior and hypersomnia.

The precise cause of depression is unknown, but genetic factors likely contribute. There is strong evidence for a role of central monoamines, including norepinephrine, serotonin, and dopamine. The hallucinogenic agent **lysergic acid diethylamide (LSD)** is a central 5-HT_2 receptor agonist. The transient hallucinations produced by this drug were discovered when the chemist who synthesized it inhaled some by accident. Its discovery drew attention to the correlation between behavior and variations in brain serotonin content. **2,5-Dimethoxy-4-methyl-amphetamine** and **mescaline** and other true hallucinogens are phenylethylamines. However, each of these may exert its effect by binding to 5-HT_2 receptors. **3,4-Methylenedioxymethamphetamine** (MDMA or **Ecstasy**) is a popular drug of abuse that produces euphoria followed by difficulty in concentrating and depression. The drug causes release of serotonin followed by serotonin depletion; the euphoria may be due to the release and the later symptoms to the depletion.

THERAPEUTIC HIGHLIGHTS

In cases of typical depression, drugs such as **fluoxetine (Prozac),** which are selective **serotonin reuptake inhibitors (SSRIs),** are effective as antidepressants. SSRIs are also used to treat **anxiety disorders.** In atypical

depression, SSRIs are often ineffective. Instead, **monoamine oxidase inhibitors (MAOIs)** such as **phenelzine** and **selegiline** have been shown to be effective as antidepressants. However, they have adverse consequences including hypertensive crisis if the patient ingests large quantities of products high in **tyramine,** which include aged cheese, processed meats, avocados, dried fruits, and red wines (especially Chianti). Based on evidence that atypical depression may result from a decrease in both serotonin and dopamine, drugs acting more generally on monoamines have been developed. These drugs, called **atypical antidepressants,** include **bupropion,** which resembles amphetamine and increases both serotonin and dopamine levels in the brain. Bupropion is also used as **smoking cessation therapy.**

LARGE-MOLECULE TRANSMITTERS: NEUROPEPTIDES

Substance P

Substance P is a polypeptide containing 11 amino acid residues that is found in the intestine, various peripheral nerves, and many parts of the CNS. It is one of a family of polypeptides called tachykinins that differ at the amino terminal end but have in common the carboxyl terminal sequence of Phe-X-Gly-LeuMet-NH_2, where X is Val, His, Lys, or Phe. Other members of the family include **neurokinin A** and **neurokinin B.**

There are three **neurokinin receptors (**$\mathbf{NK_1}$**–**$\mathbf{NK_3}$**)**, which are GPCRs. Substance P is the preferred ligand for NK_1 receptors in the CNS, and activation of this receptor leads to increased formation of IP_3 and DAG.

Substance P is found in high concentrations in the endings of primary afferent neurons in the spinal cord, and it is a mediator at the first synapse in pain transmission pathway in the dorsal horn. It is also found in high concentrations in the nigrostriatal system, where its concentration is proportional to that of dopamine, and in the hypothalamus, where it may play a role in neuroendocrine regulation. In the intestine, it is involved in peristalsis. Several recently developed centrally active NK-1 receptor antagonists have been shown to have antidepressant activity. They have also been used as antiemetics in patients undergoing chemotherapy.

Opioid Peptides

The brain and the gastrointestinal tract contain receptors that bind morphine. The search for endogenous ligands for these receptors led to the discovery of two closely related pentapeptides (**enkephalins**) that bind to these opioid receptors: **met-enkephalin** and **leu-enkephalin.** These and other peptides that bind to opioid receptors are called **opioid peptides.** The enkephalins are found in nerve endings in the gastrointestinal tract and many different parts of the brain, and they function as synaptic transmitters. They are found in the substantia gelatinosa and have analgesic activity when injected into the brainstem. They also decrease intestinal motility. Enkephalins are metabolized primarily by two peptidases: enkephalinase A, which splits the Gly-Phe bond, and enkephalinase B, which splits the Gly-Gly bond. Aminopeptidase, which splits the Tyr-Gly bond, also contributes to their metabolism.

Like other small peptides, the endogenous opioid peptides are synthesized as part of larger precursor molecules. More than 20 active opioid peptides have been identified. Each opioid peptide has a prepro form and a pro form from which the signal peptide has been cleaved. **Proenkephalin** is the precursor for met-enkephalin and leu-enkephalin in the brain and adrenal medulla. Each proenkephalin molecule contains four met-enkephalins, one leuenkephalin, one octapeptide, and one heptapeptide. **Proopiomelanocortin,** a large precursor molecule found in the anterior and intermediate lobes of the pituitary gland and the brain, contains **β-endorphin,** a polypeptide of 31 amino acid residues that has metenkephalin at its amino terminal. There are separate enkephalin-secreting and β-endorphin–secreting systems of neurons in the brain. β-Endorphin is also secreted into the bloodstream by the pituitary gland. A third precursor molecule is **prodynorphin,** a protein that contains three leuenkephalin residues associated with dynorphin and neoendorphin. Different types of dynorphins are found in the duodenum and the posterior pituitary and hypothalamus; β-neoendorphins are also found in the hypothalamus.

There are three classes of **opioid receptors:** μ, κ, and δ with various subtypes of each of these. As shown in **Table 7–3**, they differ in physiologic effects and affinity for various opioid peptides. All three are GPCRs, and all inhibit adenylyl cyclase. Activation of μ receptors increases K^+ conductance, hyperpolarizing central neurons and primary afferents. Activation of κ receptors and δ receptors closes Ca^{2+} channels.

TABLE 7–3 Physiologic effects produced by stimulation of opioid receptors.

Receptor	Endogenous Opioid Peptide Affinity	Effect
μ	Endorphins > Enkephalins > Dynorphins	Supraspinal and spinal analgesia Respiratory depression Constipation Euphoria Sedation Increased secretion of growth hormone and prolactin Miosis
κ	Enkephalins > Endorphins and Dynorphins	Supraspinal and spinal analgesia Diuresis Sedation Miosis Dysphoria
δ	Dynorphins > > Endorphins and Enkephalins	Supraspinal and spinal analgesia

Other Polypeptides

Somatostatin is found in various parts of the brain, where it may function as a neurotransmitter with effects on sensory input, locomotor activity, and cognitive function. In the hypothalamus, this growth hormone-inhibiting hormone is secreted into the portal hypophysial vessels; in the endocrine pancreas, it inhibits insulin secretion and the secretion of other pancreatic hormones; and in the gastrointestinal tract, it is an important inhibitory gastrointestinal regulator. A family of five somatostatin receptors have been identified (SSTR1 through SSTR5). All are GPCRs that inhibit adenylyl cyclase and exert various other effects on intracellular messenger systems. SSTR2 mediates cognitive effects and inhibition of growth hormone secretion, whereas SSTR5 mediates the inhibition of insulin secretion.

Vasopressin and **oxytocin** are not only secreted as hormones but also are

released by neurons that project to the brainstem and spinal cord. **Bradykinin, angiotensin II,** and **endothelin** are also released by neurons in the brain. The gastrointestinal hormones, including **vasoactive intestinal polypeptide (VIP), cholecystokinin (CCK-4** and **CCK-8),** are also found in the brain. There are two kinds of CCK receptors in the brain, CCK-A and CCK-B. CCK-8 acts at both binding sites, whereas CCK-4 acts at the CCK-B sites. **Gastrin, neurotensin, galanin,** and **gastrin-releasing peptide** are also found in the gastrointestinal tract and brain. Neurotensin, VIP, and CCK receptors are GPCRs. The hypothalamus contains both gastrin 17 and gastrin 34. VIP produces vasodilation and is found in vasomotor nerve fibers. The functions of these peptides in the nervous system are unknown, although some of the peptides also expressed in the gastrointestinal system have been implicated in satiety (see Chapter 26).

Calcitonin gene-related peptide (CGRP) is present in the CNS and peripheral nervous system, gastrointestinal tract, cardiovascular system, and urogenital system. CGRP is colocalized with either substance P or acetylcholine. CGRP-like immunoreactivity is present in the circulation, and injection of CGRP causes vasodilation. CGRP and the calcium-lowering hormone calcitonin are both products of the calcitonin gene. CGRP has little effect on Ca^{2+} metabolism, and calcitonin is only a weak vasodilator. Release of CGRP from trigeminal afferent fibers may contribute to the pathophysiology of migraine. Actions of this peptide are mediated by two types of metabotropic CGRP receptors.

Neuropeptide Y is a polypeptide that is very abundant throughout the brain and the autonomic nervous system, and it acts on eight receptors: Y_1–Y_8; except for Y_3, these are GPCRs. Activation of these receptors mobilizes Ca^{2+} and inhibits adenylyl cyclase. It acts within the CNS to increase food intake, and Y_1 and Y_5 receptor antagonists may be used to treat obesity. It also acts in the periphery to cause vasoconstriction. It acts on heteroreceptors on postganglionic sympathetic nerve terminals to reduce release of norepinephrine.

GAS TRANSMITTERS

NO, a compound released by the endothelium of blood vessels as endothelium-derived relaxing factor, is also produced in the brain and the enteric nervous system. It is synthesized from arginine, a reaction catalyzed in the brain by one of the three forms of NO synthase. It activates guanylyl cyclase and, unlike most other neurotransmitters, it is a gas, which crosses cell membranes with ease and

binds directly to guanylyl cyclase. NO is not stored in vesicles like other classic neurotransmitters; it is synthesized on demand at postsynaptic sites and diffuses to adjacent sites on the neuron. Synthesis may be triggered by activation of NMDA receptors, which leads to an influx of Ca^{2+} and activation of **neuronal nitric oxide synthase.** It may be the signal by which postsynaptic neurons communicate with presynaptic endings to enhance release of glutamate. It may also play a role in synaptic plasticity and thus in memory and learning.

CO is another diffusible gas that is endogenously formed in CNS neurons and enteric nervous system neurons via the enzymatic degradation of heme by heme oxygenase-2. Like NO it can act as an intracellular messenger to stimulate soluble guanylyl cyclase. It can modulate neurotransmission and has been implicated in having a role in olfaction, pain, and long-term potentiation. Endogenously formed CO may also act centrally to attenuate endotoxin-induced arginine vasopressin release.

OTHER CHEMICAL TRANSMITTERS

Two types of **endocannabinoids** have been identified as neurotransmitters: **2-arachidonyl glycerol** and **anandamide.** They are rapidly synthesized in response to Ca^{2+} influx after a neuron is depolarized. Both act on a cannabinoid receptor (CB_1) with a high affinity for Δ^9-tetrahydrocannabinol, the psychoactive ingredient in marijuana. These receptors are primarily located on presynaptic nerve terminals. The CB_1 receptor triggers a G-protein-mediated decrease in intracellular cAMP levels and is common in central pain pathways as well as in parts of the cerebellum, hippocampus, and cerebral cortex. In addition to inducing euphoria, CB_1 receptor agonists have an anti-nociceptive effect, and CB_1 receptor antagonists enhance nociception. Endocannabinoids also act as retrograde synaptic messengers; they travel back across a synapse after release and bind to presynaptic CB_1 receptors to inhibit further transmitter release. A CB_2 receptor, which also couples to G-proteins, is located primarily in the periphery. Agonists of this class of receptor do not induce the euphoric effects of activation of CB_1 receptors, and they may have potential for use in the treatment of chronic pain.

CHAPTER SUMMARY

- Neurotransmitters and neuromodulators can be divided into three categories: small-molecule transmitters, large-molecule transmitters (neuropeptides), and gas transmitters. Usually neuropeptides are colocalized with one of the small-molecule neurotransmitters.
- For many major neurotransmitters, the common steps involved in the process of neurotransmission include uptake of a neurotransmitter precursor into a nerve terminal; biosynthesis of the neurotransmitter; its storage in synaptic vesicles; its release into the synaptic cleft in response to depolarization of the nerve terminal; binding of the neurotransmitter to receptors on the membrane of the postsynaptic target; and rapid termination of its actions via diffusion away from the synapse, reuptake into the nerve terminal, or enzymatic degradation.
- When a neurotransmitter binds to an ionotropic receptor, the membrane channel is opened and activation of the channel usually elicits a brief increase in ionic conductance. When a neurotransmitter binds to a GPCR, it initiates the production of a second messenger (eg, cAMP, IP_3, and DAG) that modulates the voltage-gated channels on neuronal membranes.
- Major neurotransmitters and neuromodulators include amino acids (glutamate, GABA, and glycine), acetylcholine, monoamines (norepinephrine, epinephrine, dopamine, and serotonin), neuropeptides (substance P and opioids), and gasses (nitric oxide and carbon monoxide).
- Glutamate is the main excitatory neurotransmitter in the CNS. There are two major types of glutamate receptors: metabotropic (GPCR) and ionotropic (ligand-gated ion channels receptors, including kainate, AMPA, and NMDA). Riluzole and memantine are examples of clinically relevant NMDA receptor antagonists.
- GABA is the major inhibitory neurotransmitter in the brain. There are three subtypes of GABA receptors: $GABA_A$ and $GABA_C$ (ligand-gated ion channel) and $GABA_B$ (GPCR). The $GABA_A$ and $GABA_B$ receptors are widely distributed in the CNS. Bicuculline and gabazine are examples of clinically relevant $GABA_A$ receptor antagonists.
- Acetylcholine is found at the neuromuscular junction, in autonomic ganglia, and in postganglionic parasympathetic nerve-target organ junctions and a few postganglionic sympathetic nerve-target junctions. It is also found in the basal forebrain complex and pontomesencephalic cholinergic complex. There are two major types of cholinergic receptors: muscarinic (GPCR) and

nicotinic (ligand-gated ion channel receptors). Atropine is an example of a clinically relevant muscarinic receptor antagonist.

- Norepinephrine-containing neurons are found in the locus coeruleus and other medullary and pontine nuclei. Some neurons also contain phenylethanolamine-N-methyltransferase, which catalyzes the conversion of norepinephrine to epinephrine. Epinephrine and norepinephrine act on α- and β-adrenoceptors, with norepinephrine having a greater affinity for α-adrenoceptors and epinephrine for β-adrenoceptors. They are GPCR, and each has multiple subtypes. Prazosin and atenolol are examples of clinically relevant α- and β-adrenoceptor antagonists, respectively.
- Serotonin (5-HT) is found within the brainstem in the midline raphe nuclei, which project to portions of the hypothalamus, the limbic system, the neocortex, the cerebellum, and the spinal cord. There are at least seven types of 5-HT receptors, and many of these have subtypes. Most are GPCRs.
- The three types of opioid receptors (μ, κ, and δ with various subtypes of each) are all GPCRs that differ in physiologic effects, distribution in the brain and elsewhere, and affinity for various opioid peptides.
- Nitric oxide is synthesized on demand in the brain from arginine, a reaction catalyzed by nitric oxide synthase; it readily diffuses across cell membranes and binds to guanylyl cyclase. It may be the signal by which postsynaptic neurons communicate with presynaptic endings to enhance release of glutamate. Carbon monoxide is endogenously formed in neurons via the enzymatic degradation of heme; it also stimulates soluble guanylyl cyclase to modulate neurotransmission.
- Examples of neuropathologies linked to neurotransmitter dysfunction include ALS (excessive glutamate release), schizophrenia (abnormal activation of dopamine receptors), and major depression (abnormal central actions of monoamines).

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. Which of the following statements about neurotransmitters is true?

A. All neurotransmitters are derived from amino acid precursors.

B. Small-molecule neurotransmitters include dopamine, glycine, acetylcholine, enkephalin, and norepinephrine.

C. Large-molecule transmitters include GABA, endocannabinoids, substance P, and vasopressin.

D. Norepinephrine can act as a neurotransmitter in the periphery and a neuromodulator in the CNS.

E. Nitrous oxide is a neurotransmitter in the CNS.

2. Which of the following statements correctly describes the processes involved in the synthesis, storage, release, binding to a receptor, and termination of action of a common neurotransmitter?

A. Glutamate is synthesized in glia by the enzymatic conversion from glutamine and then diffuses into the neuronal terminal where it is sequestered into vesicles until released by an influx of Ca^{2+} into the cytoplasm after an action potential reaches the nerve terminal, it binds exclusively to ligand-gated ion channel receptors, and is inactivated by reuptake into the nerve terminal.

B. Serotonin is synthesized from trytophan, stored in synaptic vesicles until its release into the synaptic cleft; it then acts primarily on GPCRs and its actions are terminated primarily by reuptake into the presynaptic nerve terminal.

C. Norepinephrine is the only small-molecule transmitter that is synthesized in synaptic vesicles instead of being transported into the vesicle after its synthesis from the amino acid phenylalanine. After its release in response to depolarization, it binds to ligand-gated ion channels or GPCRs and its action is terminated by reuptake into the nerve terminal.

D. Acetylcholine is synthesized from acetlyene, transported from the cytoplasm into vesicles by a vesicle-associated membrane protein, released into the synaptic cleft in response to neuronal depolarization, acts on GPCRs, and its actions are terminated primarily by enzymatic degradation.

3. Which of the following receptors is correctly identified as an ionotropic or a GPCR and correctly paired with the ionic changes and/or second messenger induced by the binding of an agonist?

A. $5\text{-}HT_{1A}$ receptors are GPCRs whose activation increases IP_3 and DAG and increases K^+ conduction.

B. Nicotinic receptors are ionotropic receptors whose activation decreases Na^+ and K^+ conduction.

C. $GABA_A$ receptors are GPCRs whose activation increases cAMP and decreases K^+ conduction.

D. NMDA receptors are ionotropic receptors whose activation increases Na^+, K^+, and Ca^{2+} conductance.

E. Glycine receptors are GPCRs whose activation increases IP_3 and DAG and increases K^+ conductance.

4. A medical student is studying transmission through autonomic ganglia. She studied the effects of two drugs on the activity of a postganglionic neuron. Drug A induced an EPSP in the postganglionic neuron, and drug B blocked the EPSP produced by electrical stimulation of a preganglionic nerve. Drugs A and B might be the following drugs, respectively.

A. Glutamate and glycine

B. Nicotine and atropine

C. Strychnine and atenolol

D. Nicotine and trimethaphan

E. Acetylcholine and phenylephrine

5. A 38-year-old woman was referred to a psychiatrist after telling her primary care physician that she had difficulty sleeping (awakening at 4 AM frequently for the past few months) and a lack of appetite causing a weight loss of over 20 lb. She also said she no longer enjoyed going out with her friends or doing volunteer service for underprivileged children. What type of drug is her doctor most likely to suggest as an initial step in her therapy?

A. A serotonergic receptor antagonist

B. A selective serotonin reuptake inhibitor

C. An inhibitor of monoamine oxidase

D. An amphetamine-like drug

E. A drug that causes an increase in both serotonin and dopamine levels

6. A 55-year-old woman had been receiving long-term treatment with phenelzine for her depression. After she consumed Chianti wine, aged cheddar cheese, processed meats, and dried fruits one night at a party, the following symptoms developed: a severe headache, chest pain, rapid heartbeat, enlarged pupils, increased sensitivity to light, and nausea. What is the most likely cause of these symptoms?

A. The foods were contaminated with botulinum toxin.

B. She had a myocardial infarction.

C. She experienced a migraine headache.

D. She had an unexpected adverse reaction to the mixture of alcohol with her

antidepressant.

E. She had a hypertensive crisis from eating foods high in tyramine while taking a monoamine oxidase inhibitor for her depression.

7. A 27-year-old man was brought to the emergency department by a friend who suspected he had overdosed on a drug. Upon arrival at the emergency department, he had depressed respiration, miosis, and reduced consciousness. Based on these symptoms, what type of drug did the individual likely take and what is its mechanism of action?

A. A drug acting as a D_2 receptor agonist

B. A 5-HT_2 receptor antagonist

C. A δ- and κ-opioid receptor agonist

D. A serotonin reuptake inhibitor

E. A μ-opioid receptor agonist

8. Which of the following statements correctly describes properties of a gas neurotransmitter?

A. Nitric oxide is synthesized by enzymatic degradation of arginine, actively transported across cell membranes, stored in vesicles, released by neuronal depolarization, and binds to nitric oxide receptors.

B. Nitric oxide is synthesized from arginine by nitric oxide synthase, diffuses across cell membranes, is released by neuronal depolarization, and acts on presynaptic nitric oxide receptors to enhance the release of glutamate.

C. Nitric oxide is synthesized from arginine by nitric oxide synthase, diffuses across cell membranes, activates guanylyl cyclase, and may enhance release of glutamate.

D. Carbon monoxide is endogenously formed in neurons by the action of carbon monoxide synthase, stimulates soluble guanylyl cyclase to enhance endotoxin-induced arginine vasopressin release.

E. Carbon monoxide is synthesized in the periphery by enzymatic degradation of heme by heme oxygenase-2, transported to the brain where it activates guanylyl cyclase, and has a role in long-term potentiation.

9. A full-term infant boy is delivered without complications and has normal APGAR scores. Routine newborn screening tests reveal elevated levels of phenylalanine in his blood, leading to a diagnosis of phenylketonuria (PKU). What is the likely outcome if a dietary intervention with restricted intake of high-protein foods is not initiated by the age of 3 weeks?

A. Development of cholestasis
B. Development of neonatal seizures
C. Malformation of the enteric nervous system
D. Autism
E. Profound intellectual disability

SECTION II

Central & Peripheral Neurophysiology

INTRODUCTION TO NEUROPHYSIOLOGY

The central nervous system (CNS) can be likened to a computer processor that is the command center for most if not all of the functions of the body. The peripheral nervous system is like a set of cables that transfers critical data from the CNS to the body and then feeds back information from the body to the CNS. This “computer system” is very sophisticated and is designed to continually make appropriate adjustments to its inputs and outputs in order to allow one to react and adapt to changes in the external and internal environment (sensory systems), to maintain posture, permit locomotion, and use the fine motor control in our hands to create pieces of art (somatomotor system), to maintain homeostasis (autonomic nervous system), to regulate the transitions between sleep and wakefulness (consciousness), and to allow us to recall past events and to communicate with the outside world (higher cortical functions). This section on neurophysiology will describe the fundamental properties and integrative capabilities of neural systems that allow for the exquisite control of this vast array of physiologic functions. Medical fields such as neurology, neurosurgery, and clinical psychology build on the foundation of neurophysiology.

This next series of chapters on neurophysiology contains information that is relevant to many clinical problems. One of the most common reasons that an individual seeks medical advice is because they are in pain. Severe chronic pain involves the rewiring of neural circuits that can result in an unpleasant sensation from even simple touch of the skin. Chronic pain is a devastating health problem that affects nearly 1 in 10 Americans (more than 25 million people). Within the past decade or so there have been considerable advancements in understanding

how pain alters neural activity and in identifying receptors types that are unique to nociceptive pathways. These findings have led to an expanding research effort to develop novel therapies that specifically target synaptic transmission in central nociceptive pathways and in peripheral sensory transduction. This is welcomed by the many individuals who do not get pain relief from nonsteroidal anti-inflammatory drugs or even opioids. These kinds of research breakthroughs depend on a thorough understanding of how the brain and body communicate with each other.

In addition to chronic pain, there are over 600 known neurologic disorders. Nearly 50 million people in the United States alone and an estimated 1 billion people worldwide suffer from the effects of damage to the central or peripheral nervous system. Each year, nearly 7 million people die of a neurologic disorder or its complications. Neurologic disorders include genetic disorders (eg, Huntington disease), demyelinating diseases (eg, multiple sclerosis), developmental disorders (eg, cerebral palsy), degenerative diseases that target specific types of neurons (eg, Parkinson disease and Alzheimer disease), an imbalance of neurotransmitters (eg, depression, anxiety, and eating disorders), trauma (eg, spinal cord and head injury), and convulsive disorders (eg, epilepsy). In addition, there are neurologic complications associated with cerebrovascular problems (eg, stroke) and exposure to neurotoxic chemicals (eg, nerve gases, mushroom poisoning, and pesticides).

Identifying the pathophysiologic basis for neurologic disorders has benefitted from advances in stem cell biology and brain imaging techniques, a greater understanding of the basis for synaptic plasticity of the brain, knowledge about the regulation of receptors and the release of neurotransmitters, and the detection of genetic and molecular defects that lead to neurologic problems. They have also set the stage for identifying better therapies to prevent, reverse, or stabilize the physiologic deficits that more than 600 neurologic disorders cause.

CHAPTER 8

Somatosensory Neurotransmission: Touch, Pain, & Temperature

OBJECTIVES

After studying this chapter, you should be able to:

- Describe the location, type, and function of receptors that mediate the sensations of touch, temperature, and pain.
- Describe the steps involved in sensory transduction and action potential generation in cutaneous mechanoreceptors and nociceptors.
- Explain the basic elements of sensory coding including modality, location, intensity, and duration and how these properties relate to receptor specificity, receptive field, receptor sensitivity, and receptor adaptation.
- Explain the differences between pain and nociception, first and second pain, acute and chronic pain, and hyperalgesia and allodynia.
- Describe and explain the basis for visceral and referred pain.
- Compare the pathway that mediates sensory input from touch, proprioceptive, and vibratory senses to that mediating information from nociceptors and thermoreceptors.
- Describe the deficits caused by lesions of ascending sensory pathways that mediate touch, pain, and temperature.

- Describe processes involved in modulation of transmission in pain pathways.
- Identify drugs used for relief of pain and give the rationale for their use and their clinical effectiveness.

INTRODUCTION

Table 8–1 provides a list of the principle sensory modalities. Sensory receptors convert specific forms of energy into action potentials in sensory neurons. Cutaneous **mechanoreceptors** mediate responses to touch and pressure. **Proprioceptors** in muscles, tendons, and joints relay information about muscle length and tension. **Thermoreceptors** detect the sensations of warmth and cold. **Nociceptors** respond to potentially harmful stimuli such as pain, extreme heat, and extreme cold. **Chemoreceptors** are stimulated by a change in the chemical composition of the local environment. These include receptors for taste and smell as well as visceral receptors that are sensitive to changes in the plasma level of O_2, pH, and osmolality. **Photoreceptors** in the rods and cones in the retina respond to light.

TABLE 8–1 Principle sensory modalities.

Sensory System	Modality	Stimulus	Receptor Class	Receptor Cell Type
Somatosensory	Touch	Tap, flutter 5–40 Hz	Cutaneous mechanoreceptor	Meissner corpuscle
Somatosensory	Touch	Motion	Cutaneous mechanoreceptor	Hair follicle receptor
Somatosensory	Touch	Vibration 60–500 Hz; deep pressure	Cutaneous mechanoreceptor	Pacinian corpuscle
Somatosensory	Touch	Touch, sustained pressure	Cutaneous mechanoreceptor	Merkel cell
Somatosensory	Touch	Skin stretch, vibration	Cutaneous mechanoreceptor	Ruffini corpuscle
Somatosensory	Proprioception	Stretch	Mechanoreceptor	Muscle spindle
Somatosensory	Proprioception	Tension	Mechanoreceptor	Golgi tendon organ
Somatosensory	Temperature	Thermal	Thermoreceptor	Cold and warm receptors
Somatosensory	Pain	Chemical, thermal, and mechanical	Chemoreceptor, thermoreceptor, and mechanoreceptor	Polymodal receptors or chemical, thermal, and mechanical nociceptors
Somatosensory	Itch	Chemical	Chemoreceptor	Chemical nociceptor
Visual	Vision	Light	Photoreceptor	Rods, cones
Auditory	Hearing	Sound	Mechanoreceptor	Hair cells (cochlea)
Vestibular	Balance	Angular acceleration	Mechanoreceptor	Hair cells (semicircular canals)
Vestibular	Balance	Linear acceleration, gravity	Mechanoreceptor	Hair cells (otolith organs)
Olfactory	Smell	Chemical	Chemoreceptor	Olfactory sensory neuron
Gustatory	Taste	Chemical	Chemoreceptor	Taste buds

This chapter describes primarily the characteristics of cutaneous receptors that mediate the sensory modalities of touch, pain, and temperature; the way they generate impulses in afferent neurons; and the central pathways that mediate or modulate information from these receptors. Since pain is one of the main reasons an individual seeks the advice of a clinician, this topic gets considerable attention in this chapter. Receptors involved in the somatosensory modality of proprioception are described in Chapter 12 as they play key roles in the control of balance, posture, and limb movement.

SENSORY RECEPTORS

CUTANEOUS MECHANORECEPTORS

Touch and pressure are sensed by four types of mechanoreceptors **(Figure 8–1).** **Meissner corpuscles** are dendrites encapsulated in connective tissue beneath the epidermis of glabrous (non-hairy) skin and respond to slow vibration. **Merkel cells** are expanded dendritic endings in epidermis of glabrous skin that respond to sustained pressure and touch. **Ruffini corpuscles** are enlarged dendritic endings with elongated capsules in the dermis of glabrous and hairy skin; they respond to stretch and fluttering vibration. **Pacinian corpuscles** are the largest cutaneous mechanoreceptor, 2 mm long and about 1 mm in diameter, in the dermis of glabrous and hairy skin. They are comprised of a nerve ending encapsulated by concentric layers of connective tissue that give it an onion-like appearance. These receptors respond to fast vibration and deep pressure. The sensory nerves from cutaneous mechanoreceptors are myelinated Aα and Aβ fibers whose conduction velocities range from ~70–120 to ~40–75 m/s, respectively.

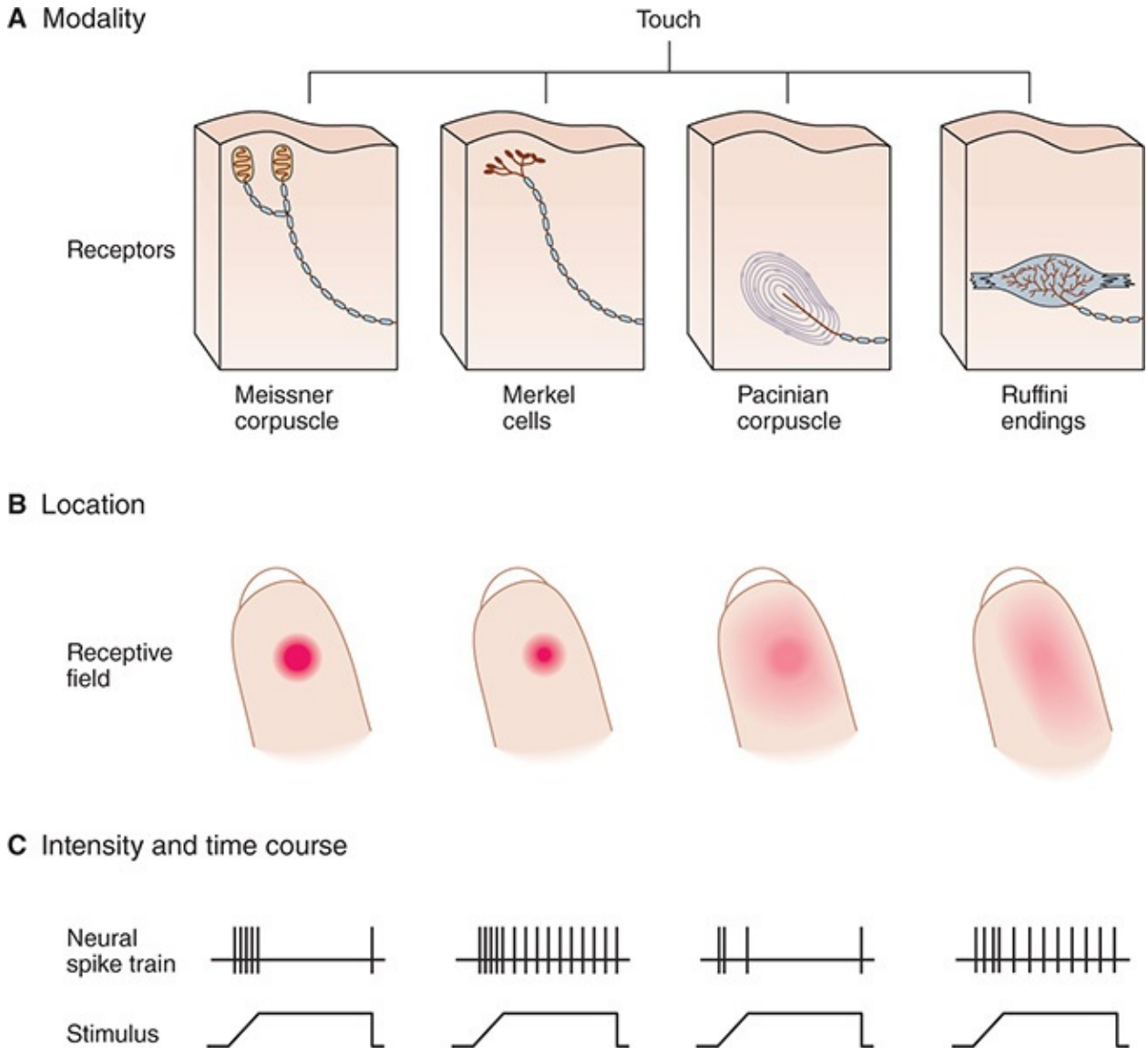


FIGURE 8–1 Sensory systems encode four elementary attributes of stimuli: modality, location, intensity, and duration. A) The human hand has four types of mechanoreceptors; their combined activation produces the sensation of contact with an object. Selective activation of Merkel cells and Ruffini endings causes sensation of steady pressure; selective activation of Meissner and Pacinian corpuscles causes tingling and vibratory sensation. **B)** Location of a stimulus is encoded by spatial distribution of the population of receptors activated. A receptor fires only when the skin close to its sensory terminals is touched. The receptive fields of mechanoreceptors (shown as red areas on fingertips) differ in size and response to touch. Merkel cells and Meissner corpuscles provide the most precise localization as they have the smallest receptive fields and are most sensitive to pressure applied by a small probe. **C)**

Stimulus intensity is signaled by firing rates of individual receptors; duration of stimulus is signaled by time course of firing. The spike trains indicate action potentials elicited by pressure from a small probe at the center of each receptive field. Meissner and Pacinian corpuscles adapt rapidly, the others adapt slowly. (Reproduced with permission from Kandel ER, Schwartz JH, Jessell TM [editors]: *Principles of Neural Science*, 4th ed. New York, NY: McGraw-Hill; 2000.)

NOCICEPTORS

Pain and temperature sensations arise from receptors located on unmyelinated dendrites of sensory neurons located throughout the glabrous and hairy skin as well as deep tissue. **Mechanical nociceptors** respond to strong pressure (eg, from a sharp object). **Thermal nociceptors** are activated by skin temperatures above 45°C or by severe cold (<20°C). **Chemically sensitive nociceptors** respond to chemicals such as bradykinin, histamine, high acidity, and environmental irritants. **Polymodal nociceptors** respond to combinations of these stimuli.

Impulses from nociceptors are transmitted via thinly myelinated Aδ fibers (2–5 µm in diameter) that conduct at rates of ∼12–35 m/s and unmyelinated C fibers (0.4–1.2 µm in diameter) that conduct at low rates of ∼0.5–2 m/s. Activation of Aδ fibers, which release **glutamate,** is responsible for **first pain** (also called **fast pain**), which is a rapid response and mediates the discriminative aspect of pain or the ability to localize the site and intensity of the noxious stimulus. Activation of C fibers, which release a combination of glutamate and **substance P,** is responsible for the delayed **second pain** (also called **slow pain**), which is the dull, intense, diffuse, and unpleasant feeling associated with a noxious stimulus. **Itch** is also related to pain sensation **(see Clinical Box 8–1)**.

A variety of receptors on the endings of nociceptive sensory nerves respond to noxious thermal, mechanical, or chemical stimuli **(Figure 8–2)**. Many of these are part of a family of nonselective cation channels called **transient receptor potential** (TRP) channels. This includes **TRPV1** receptors (the V refers to a group of chemicals called **vanilloids**) that are activated by intense heat, acids, and chemicals such as **capsaicin** (the active ingredient in hot peppers and an example of a vanilloid). TRPV1 receptors can also be activated indirectly by initial activation of TRPV3 receptors in keratinocytes in the skin. Noxious mechanical, cold, and chemical stimuli may activate **TRPA1** receptors (A, for **ankyrin**) on sensory nerve terminals. Sensory nerve endings also have **acid-**

sensing ion channel (ASIC) receptors that are activated by pH changes within a physiologic range and may be the dominant receptors mediating acid-induced pain. In addition to direct activation of receptors on nerve endings, some nociceptive stimuli release intermediate molecules that then activate receptors on the nerve ending. For example, nociceptive mechanical stimuli cause the release of adenosine triphosphate (ATP) that acts on **purinergic receptors** (eg, **P2X**, an ionotropic receptor, and **P2Y**, a G-protein-coupled receptor). **Tyrosine receptor kinase A (TrkA)** is activated by **nerve growth factor (NGF)** that is released due to tissue damage.

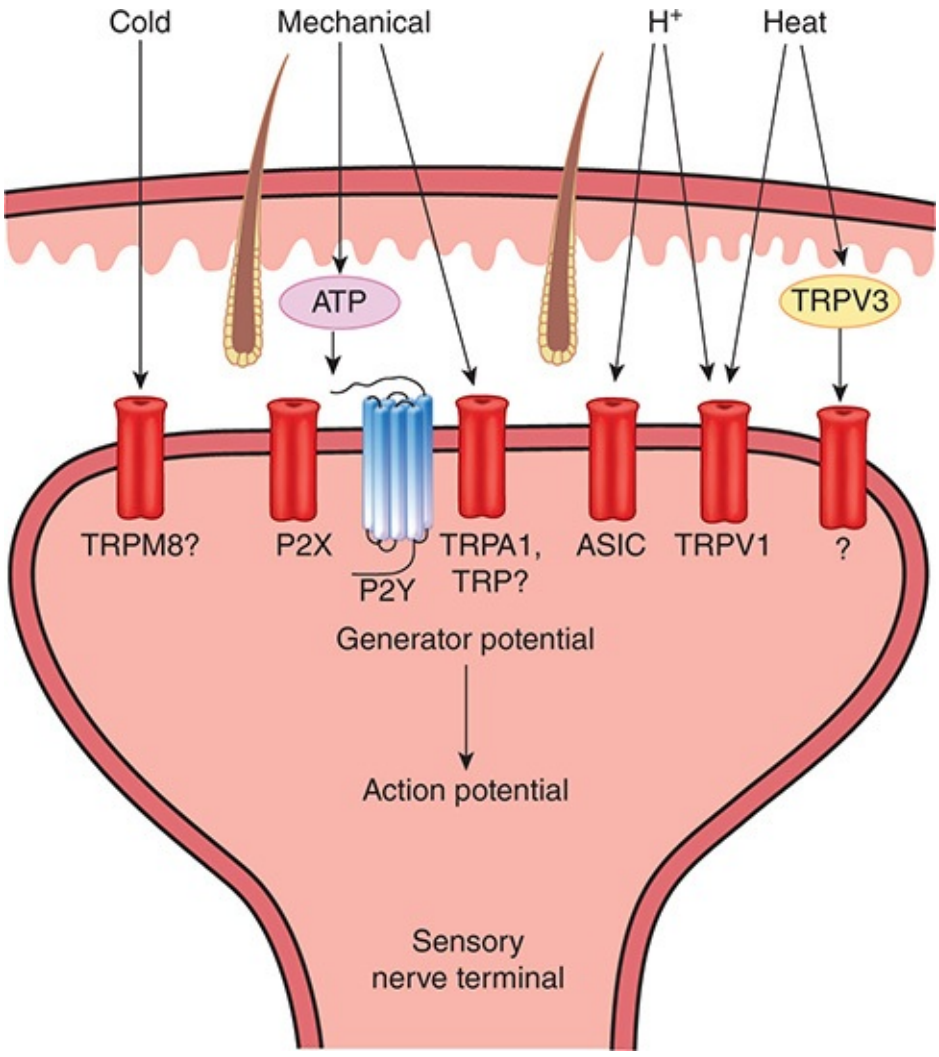


FIGURE 8–2 Receptors on nociceptive unmyelinated nerve terminals in the skin. Nociceptive stimuli (eg, heat) can activate some receptors directly due to transduction of the stimulus energy by receptors (eg, transient receptor potential (TRP) channel TRPV1) or indirectly by activation of TRP channels on

keratinocytes (eg, TRPV3). Nociceptors (eg, mechanoreceptors) can also be activated by the release of intermediate molecules (eg, ATP). ASIC, acid-sensitive ion channel; P2X, ionotropic purinoceptor; P2Y, G-protein-coupled purinergic receptor.

CLINICAL BOX 8–1

Itch

Itching (**pruritus**) is not a major problem for healthy individuals, but severe itching that is difficult to treat occurs in diseases such as chronic kidney disease, some forms of liver disease, atopic dermatitis, and HIV infection. There may be an itch- specific sensory pathway since itch spots have been mapped on the skin and itch-specific fibers have been identified in the ventrolateral spinothalamic tract. Itching can be produced not only by repeated local mechanical stimulation of the skin but also by a variety of chemical agents, including **histamine** and kinins such as **bradykinin**, that are released in the skin in response to tissue damage. Kinins exert their effects by activation of two types of G-protein-coupled receptors, B_1 and B_2. Activation of bradykinin B_2 receptors is a downstream event in **protease-activated receptor-2 (PAR-2)** activation, which induces both a nociceptive and a pruritogenic response.

THERAPEUTIC HIGHLIGHTS

Simple scratching relieves itching because it activates large, fast-conducting afferents that gate transmission in the dorsal horn in a manner analogous to the inhibition of pain by stimulation of similar afferents. **Antihistamines** are primarily effective in reducing pruritus associated with an allergic reaction. In a mouse model exhibiting scratching behavior in response to activation of PAR-2, treatment with a B_2 receptor antagonist reduced the scratching behavior. B_2 receptor antagonists may be a useful therapy for treating pruritic conditions.

Nerve endings also have a variety of receptors that respond to immune mediators that are released in response to tissue injury and mediate inflammatory pain (see Chapter 3). These include B_1 and B_2 receptors (bradykinin), prostanoid receptors (prostaglandins), and cytokine receptors (interleukins).

THERMORECEPTORS

Innocuous **cold receptors** are on dendritic endings of Aδ and C fibers; innocuous **warm receptors** are on C fibers. Mapping experiments show that the skin has discrete cold and heat-sensitive spots. There are 4–10 times as many cold-sensitive as heat-sensitive spots.

TRPM8 receptors are activated by moderate cold; the M refers to **menthol,** the ingredient in mint that gives it its "cool" taste. Cold receptors are inactive at temperatures of 40°C, but then steadily increase their firing rate as skin temperature falls to about 24°C. As skin temperature further decreases, the firing rate of cold receptors decreases until the temperature reaches 10°C. Below that temperature, they are inactive and cold, which becomes an effective local anesthetic.

TRPV3 and **TRPV4** receptors on sensory nerve endings are activated when skin temperatures reach 33–39°C and 25–34°C, respectively. The firing rate of warm receptors can further increase as the skin temperature reaches about 45°C; they become silent if skin temperature is further increased to induce a sensation of pain.

GENERATION OF IMPULSES IN CUTANEOUS RECEPTORS

The way that activation of a sensory receptors leads to an action potential in the nerve that innervates them varies based on the complexity of the sensory receptor. **Figure 8–3** shows how this occurs in the Pacinian corpuscle. A myelinated portion of the sensory nerve along with the first node of Ranvier of the sensory nerve is inside the receptor; the second node of Ranvier is usually near the point at which the nerve fiber leaves the corpuscle.

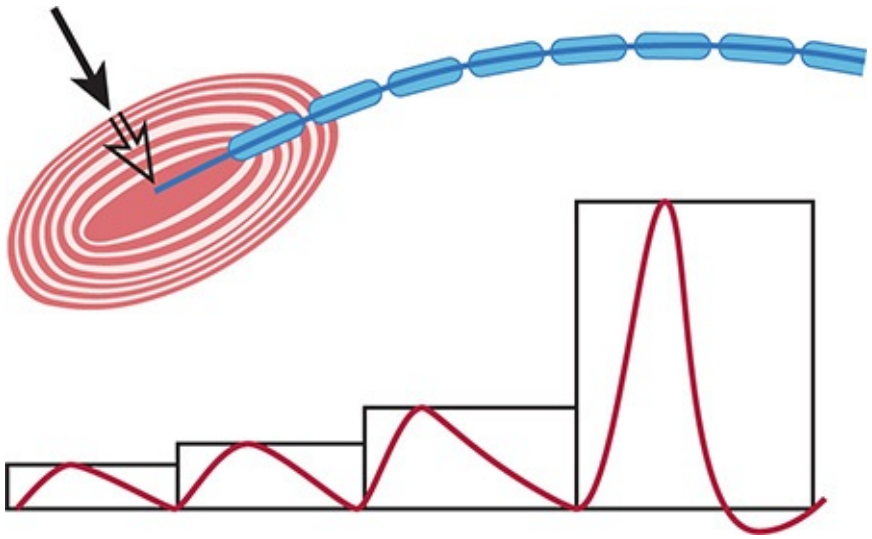


FIGURE 8–3 Demonstration that the generator potential in a Pacinian corpuscle originates in the unmyelinated nerve terminal. The electrical responses to pressures (black arrow) of 1, 2×, 3×, and 4× are shown. The strongest stimulus produced an action potential in the sensory nerve, originating in the center of the corpuscle. (Reproduced with permission from Waxman SG: *Clinical Neuroanatomy*, 26th ed. New York, NY: McGraw-Hill; 2010.)

When a small amount of pressure is applied to a Pacinian corpuscle, a nonpropagated depolarizing potential resembling an excitatory postsynaptic potential is induced (Figure 8–3). The receptor converts mechanical energy into an electrical response, the magnitude of which is proportional to the intensity of the stimulus **(graded receptor potential).** When the magnitude of the generator potential reaches about 10 mV, an action potential is produced at the first node of Ranvier. The nerve then repolarizes. If the generator potential is great enough, the neuron fires again as soon as it repolarizes, and it continues to fire if the generator potential is large enough to bring the membrane potential of the node to the firing level. Thus, the node converts the graded response of the receptor into action potentials, the frequency of which is proportional to the magnitude of the applied stimulus.

SENSORY CODING

Converting a receptor stimulus to a recognizable sensation is termed **sensory coding.** All sensory systems code for four elementary attributes of a stimulus: modality, location, intensity, and duration. **Modality** is the type of energy transmitted by the stimulus. **Location** is the site on the body or space where the

stimulus originated. **Intensity** is signaled by the response amplitude or frequency of action potential generation. **Duration** refers to the time from start to end of a response in the receptor. These attributes of sensory coding are shown for the modality of touch in Figure 8–1.

MODALITY

A sensory receptor is specialized to respond to either mechanical, chemical, thermal, or electromagnetic stimuli **(receptor specificity).** The form of energy to which a receptor is most sensitive is called its **adequate stimulus.** The adequate stimulus for the rods and cones in the eye, for example, is light. Sensory receptors can respond to forms of energy other than their adequate stimuli, but the threshold for these nonspecific responses is much higher. Pressure on the eyeball will stimulate the rods and cones, for example, but the threshold of these receptors to pressure is much higher than the threshold of the pressure receptors in the skin.

LOCATION

A **sensory unit** is a single sensory axon and all its peripheral branches. The **receptive field** of a sensory unit is the spatial distribution from which a stimulus produces a response in the unit (Figure 8–1). Representation of the senses in the skin is punctate. If the skin is carefully mapped, millimeter by millimeter, with a fine hair, a sensation of touch is evoked from spots overlying touch receptors. None is evoked from the intervening areas. Similarly, temperature sensations and pain are produced by stimulation of the skin only over the spots where the receptors for these modalities are located.

One of the most important mechanisms that enable localization of a stimulus site is **lateral inhibition.** Information from sensory neurons whose receptors are at the peripheral edge of the stimulus is inhibited compared to information from the sensory neurons at the center of the stimulus. Thus, lateral inhibition enhances the contrast between the center and periphery of a stimulated area and increases the ability of the brain to localize a sensory input. Lateral inhibition underlies **two-point discrimination** **(see Clinical Box 8–2)**.

CLINICAL BOX 8–2

Neurologic Exam

The **two-point threshold test** is used to measure the size of the receptive fields for light touch. In this procedure, the two points on a pair of calipers are simultaneously positioned on the skin and one determines the minimum distance between the two caliper points that can be perceived as separate points of stimulation **(two-point discrimination threshold).** If the distance is very small, each caliper point is touching the receptive field of only one sensory neuron. If the distance between stimulation points is less than this threshold, only one point of stimulation can be felt. Thus, the two-point discrimination threshold is a measure of **tactile acuity.** The magnitude of two-point discrimination thresholds varies from place to place on the body and is smallest where touch receptors are most abundant. Stimulus points on the back, for instance, must be separated by at least 65 mm before they can be distinguished as separate, whereas on the fingertips two stimuli are recognized if they are separated by as little as 2 mm. Blind individuals benefit from the tactile acuity of fingertips to facilitate the ability to read Braille; the dots forming Braille symbols are separated by 2.5 mm. Two-point discrimination is used to test the integrity of the **dorsal column (medial lemniscus) system,** the central pathway for touch and proprioception.

Vibratory sensibility is tested by applying a vibrating (128-Hz) tuning fork to the skin on the fingertip, tip of the toe, or bony prominences of the toes. The normal response is a “buzzing” sensation that is most marked over bones. **Pallesthesia** is the ability to feel mechanical vibrations. The receptors involved are those for touch, especially **Pacinian corpuscles,** but a time factor is also necessary. A pattern of rhythmic pressure stimuli is interpreted as vibration. The impulses responsible for the vibrating sensation are carried in the dorsal columns. Degeneration of this part of the spinal cord occurs in poorly controlled diabetes, pernicious anemia, vitamin B_{12} deficiencies, or early tabes dorsalis. An increased threshold for vibratory stimuli is an early symptom of this degeneration. Vibratory sensation and proprioception are closely related; when one is diminished, so is the other.

Stereognosis is the perception of the form and nature of an object without looking at it. Healthy persons can readily identify objects such as keys and coins of various denominations. This ability depends on intact touch and pressure sensation and is compromised if the dorsal columns are damaged. **Tactile agnosia** is the inability to identify an object by touch. Impaired stereognosis can be an early sign of damage to the cerebral cortex and sometimes occurs in the absence of any detectable defect in touch and

pressure sensation when there is a lesion in the primary sensory cortex. Stereognosia can also be expressed by the failure to identify an object by sight **(visual agnosia),** the inability to identify sounds or words **(auditory agnosia)** or color **(color agnosia),** or the inability to identify the position of an extremity **(position agnosia).**

INTENSITY

The intensity of sensation is determined by the amplitude of the stimulus applied to the receptor **(Figure 8–4)**. As a greater pressure is applied to the skin, the receptor potential in the mechanoreceptor increases (not shown), and the frequency of the action potentials in a single axon transmitting information to the CNS is increased. Weak stimuli activate the receptors with the lowest thresholds, and stronger stimuli activate those with higher thresholds **(receptor sensitivity).** As the stimulus strength is increased, it also spreads over a large area to "recruit" fibers in the surrounding area. Some of the receptors activated are part of the same sensory unit, and impulse frequency in the unit therefore increases. Because of overlap and interdigitation of one unit with another, receptors of other units are also stimulated and more sensory fibers fire. In this way, more afferent pathways are activated, which is interpreted in the brain as an increase in intensity of the sensation.

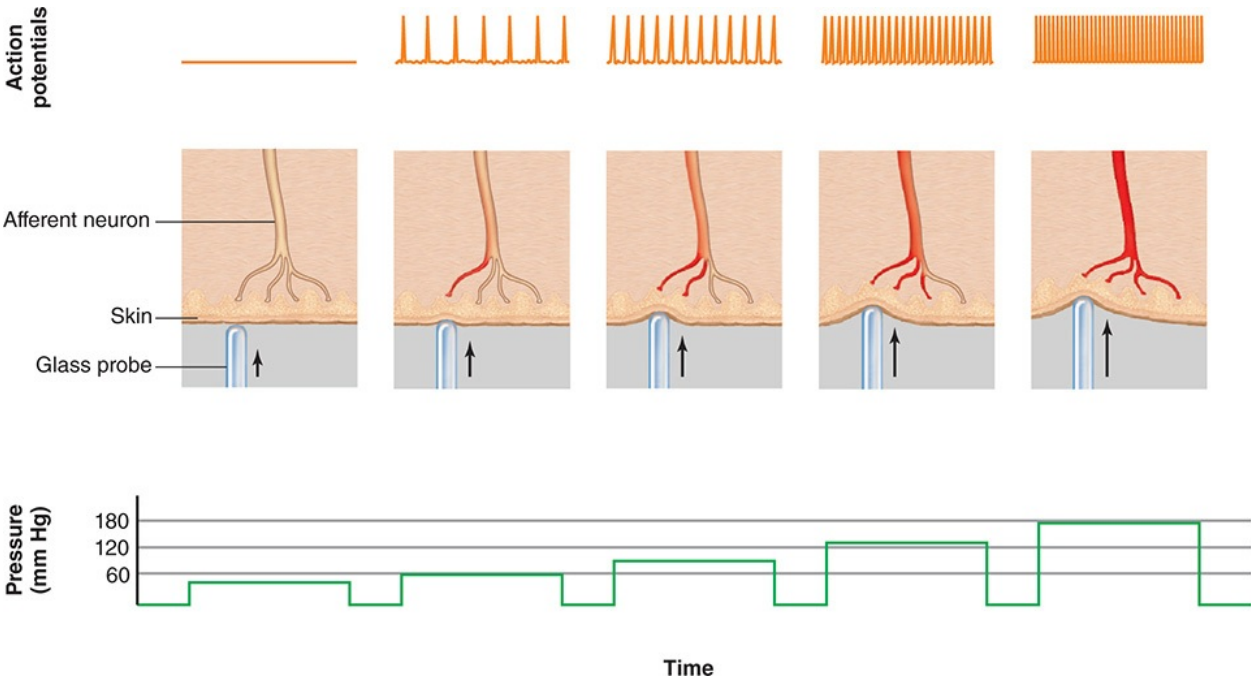


FIGURE 8–4 Relationship between stimulus and impulse frequency in an afferent fiber. Action potentials in an afferent fiber from a mechanoreceptor of a single sensory unit increase in frequency as branches of the afferent neuron are stimulated by pressure of increasing magnitude. (Reproduced with permission from Widmaier EP, Raff H, Strang KT: *Vanders Human Physiology*. 11th ed. New York, NY: McGraw-Hill; 2008.)

DURATION

If a stimulus of constant strength is maintained on a sensory receptor, the frequency of the action potentials in its sensory nerve declines over time. This phenomenon is known as **receptor adaptation.** The degree to which adaptation occurs varies from one sensation to another. Receptors can be classified as **rapidly adapting (phasic) receptors** or **slowly adapting (tonic) receptors.** This is illustrated for the four types of touch receptors in Figure 8–1; Meissner and Pacinian corpuscles are rapidly adapting receptors, and Merkel cells and Ruffini endings are slowly adapting receptors. Muscle spindles and nociceptors are also slowly adapting receptors. Different types of sensory adaptation have some value to the individual. Light touch would be distracting if it were persistent; and, conversely, slow adaptation of spindle input is needed to maintain posture. Similarly, input from nociceptors provides a warning that would be lost if the receptor adapted rapidly.

NEUROLOGIC EXAM

The sensory component of a neurologic exam includes an assessment of sensory modalities including touch, proprioception, vibratory sense, and pain. Cortical sensory function can be tested by placing familiar objects in a patient's hands and asking him or her to identify it with the eyes closed. Clinical Box 8–2 describes some of the common assessments made in a neurologic exam.

PAIN

One of the most common reasons an individual seeks the advice of a clinician is because he or she is in pain. Painful stimuli generally initiate potent withdrawal and avoidance responses. Pain differs from other sensations in that it warns that something is wrong, preempts other signals, and is associated with an unpleasant

affect. Pain is complex because when tissue is damaged, central nociceptive pathways are sensitized and reorganized, which leads to persistent or **chronic pain** **(see Clinical Box 8–3)**.

CLINICAL BOX 8–3

Chronic Pain

A 2009 report in *Scientific American* indicated that 10–20% of the US and European populations experience **chronic pain;** 59% of these individuals are women. Based on a survey of primary care clinicians, only 15% indicated that they felt comfortable treating patients with chronic pain; and 41% said they waited until patients specifically requested opioid pain killers before prescribing them. Nearly 20% of adults with chronic pain indicated that they have visited an alternative medicine therapist. Risk factors for chronic neck and back pain include aging, being female, anxiety, repetitive work, obesity, depression, heavy lifting, and nicotine use. One example of chronic pain is **neuropathic pain** that occurs when nerve fibers are injured. Nerve damage can cause an inflammatory response due to activation of microglia in the spinal cord. Neuropathic pain is excruciating and a difficult condition to treat. The resulting pain lasts much longer than the injury itself. For example, in **causalgia,** a spontaneous burning pain occurs long after seemingly trivial injuries. The pain is often accompanied by **hyperalgesia** and **allodynia. Reflex sympathetic dystrophy** is often present as well. In this condition, the skin in the affected area is thin and shiny, and there is increased hair growth. This results from sprouting and eventual overgrowth of sympathetic nerve fibers into the dorsal root ganglia of the sensory nerves from the injured area. Sympathetic discharge then brings on pain. Thus, it appears that the periphery has been short-circuited and that the relevant altered fibers are being stimulated by norepinephrine at the dorsal root ganglion level.

THERAPEUTIC HIGHLIGHTS

Chronic pain is often refractory to most conventional therapies such as **nonsteroidal anti-inflammatory drugs** and **opioids.** In new efforts to treat chronic pain, some therapies focus on synaptic transmission in central nociceptive pathways and peripheral sensory transduction mechanisms. **TRPV1,** a capsaicin receptor, is activated by noxious stimuli such as heat, protons, and products of inflammation. **Capsaicin transdermal patches** or

creams reduce pain by exhausting the supply of substance P in nerves. Nav1.8 (a tetrodotoxin-resistant voltage-gated sodium channel) is uniquely associated with nociceptive neurons in dorsal root ganglia. **Lidocaine** and **mexiletine** are useful in some cases of chronic pain and may act by blocking this channel. **Ziconotide,** a voltage-gated N-type Ca^{2+} channel blocker, has been approved for intrathecal analgesia in patients with refractory chronic pain. **Gabapentin** is an anticonvulsant drug that is an analog of GABA; it has been shown to be effective in treatment of neuropathic and **inflammatory pain** by acting on voltage-gated Ca^{2+} channels. Two other anticonvulsant drugs, **topiramate** and **valproate (valproic acid),** block voltage-gated Na^{+} channels and are used to treat **migraine** headaches. **NMDA (N-methyl-D-aspartate) receptor antagonists** can be co-administered with an opioid to reduce tolerance to an opioid. Endogenous **cannabinoids** have analgesic actions in addition to their euphoric effects. G-protein-coupled receptor (CB_2) receptor agonists that are devoid of euphoric effects are under development for the treatment of inflammatory and neuropathic pain.

CLASSIFICATION OF PAIN

For scientific and clinical purposes, **pain** is defined by the International Association for the Study of Pain (IASP) as, “an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage.” This is to be distinguished from the term **nociception** that the IASP defines as the unconscious activity induced by a harmful stimulus applied to sense receptors.

Pain is frequently classified as **physiologic** or **acute pain** and **pathologic** or **chronic pain.** Acute pain typically has a sudden onset and recedes during the healing process; it can be regarded as “good pain” as it serves an important protective mechanism. The withdrawal reflex (Chapter 12) is an example of the expression of this protective role of pain. Chronic pain can be considered “bad pain” because it persists long after recovery from an injury and is often refractory to common analgesic agents, including **nonsteroidal anti-inflammatory drugs (NSAIDs**) and **opioids.** Chronic pain can result from inflammation **(inflammatory pain)** or nerve injury (**neuropathic pain**) including diabetic neuropathy, toxin-induced nerve damage, and ischemia (see Clinical Box 8–3).

HYPERALGESIA & ALLODYNIA

Pain is often accompanied by **hyperalgesia** and **allodynia.** Hyperalgesia is an exaggerated response to a noxious stimulus, and allodynia is a sensation of pain in response to a normally innocuous stimulus. An example of the latter is the painful sensation from a warm shower when the skin is damaged by sunburn.

Hyperalgesia and allodynia signify increased sensitivity of nociceptive afferent fibers. **Figure 8–5** shows how chemicals released at the site of injury can further directly activate receptors on sensory nerve endings leading to inflammatory pain. Injured tissues also release chemicals such as K^+ that directly depolarize nerve terminals, making nociceptors more responsive **(sensitization).** Injured tissues also release bradykinin and substance P to further sensitize nociceptive terminals. Histamine is released from mast cells, serotonin (5-HT) from platelets, and prostaglandins from cell membranes, all contributing to the inflammatory process and they activate or sensitize the nociceptors. Some released substances act by releasing another one (eg, bradykinin activates both Aδ and C nerve endings and increases synthesis and release of prostaglandins). Prostaglandin E_2 (a cyclooxygenase metabolite of arachidonic acid) is released from damaged cells and produces hyperalgesia. This explains why aspirin and other NSAIDs (nonselective inhibitors of cyclooxygenase) alleviate pain.

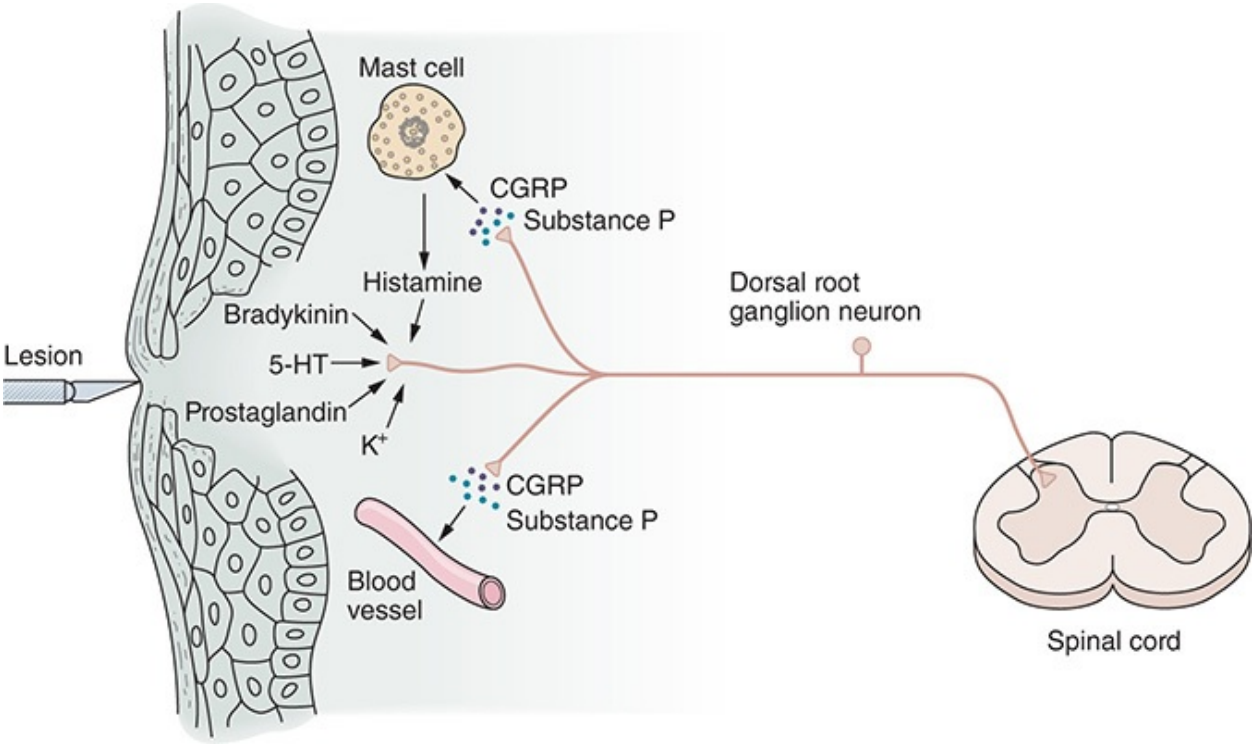


FIGURE 8–5 Chemical mediators are released in response to tissue damage and can sensitize or directly activate nociceptors. These factors contribute to hyperalgesia and allodynia. Tissue injury releases bradykinin and prostaglandins that sensitize or activate nociceptors, which in turn release substance P and calcitonin gene-related peptide (CGRP). Substance P acts on mast cells to cause degranulation and release histamine, which activates nociceptors. Substance P causes plasma extravasation and CGRP dilates blood vessels; the resulting edema causes additional release of bradykinin. Serotonin (5-HT) is released from platelets and activates nociceptors. (Reproduced with permission from Lembeck F: CIBA Foundation Symposium. Summit, NJ: Pitman Medical; 1981.)

In addition to sensitization of nerve endings by chemical mediators, several other changes occur within the periphery and the CNS that can contribute to chronic pain. The NGF released by tissue damage is picked up by nerve terminals and transported retrogradely to cell bodies in dorsal root ganglia where it can alter gene expression. Transport may be facilitated by the activation of TrkA receptors on the nerve endings. In the dorsal root ganglia, the NGF increases production of substance P and converts non-nociceptive neurons to nociceptive neurons (a phenotypic change). The NGF also influences expression of a tetrodotoxin-resistant voltage-gated sodium channel (**Nav1.8**) on dorsal root ganglia cells, further increasing activity.

Damaged nerve fibers undergo sprouting; fibers from touch receptors synapse on spinal dorsal horn neurons that normally receive only nociceptive input (see below). This can explain why innocuous stimuli can induce pain after injury. The combined release of substance P and glutamate from nociceptive afferents in the spinal cord causes excessive activation of **NMDA receptors** on spinal neurons, a phenomenon called “wind-up” that leads to increased activity in pain transmitting pathways. Another change in the spinal cord is due to the activation of **microglia** near afferent nerve terminals in the spinal cord by the release of transmitters from sensory afferents. This, in turn, leads to the release of pro-inflammatory cytokines and chemokines that modulate pain processing by affecting presynaptic release of neurotransmitters and postsynaptic excitability. There are P2X receptors on microglia; antagonists of these receptors may be a useful therapy for treatment of chronic pain.

DEEP & VISCERAL PAIN

The main difference between superficial (cutaneous) and deep or visceral pain is

the nature of the pain evoked by noxious stimuli. This may be due to a relative deficiency of Aδ nerve fibers in deep structures, so there is little rapid, sharp pain. Also, deep pain and visceral pain are poorly localized, nauseating, and frequently accompanied by sweating and changes in blood pressure. Muscle spasms can result from injuries to bones, tendons, and joints. The steadily contracting muscles become ischemic, and ischemia stimulates the pain receptors in the muscles. The pain in turn initiates more spasms, setting up a vicious cycle.

Nociceptors are present in visceral organs, but they are more sparsely distributed than in somatic structures. Afferent fibers from visceral structures reach the CNS via sympathetic and parasympathetic nerves. Their cell bodies are in the dorsal root ganglia and cranial nerve ganglia. Specifically, there are visceral afferents in the facial, glossopharyngeal, and vagus nerves and in the thoracic, upper lumbar, and sacral dorsal roots.

Visceral pain can be severe. The receptors in the walls of the viscera are especially sensitive to distension of these organs. **Intestinal colic** is a pain that waxes and wanes due to muscle spasms that occur after an intestinal obstruction. When a visceral organ is inflamed, or hyperemic, relatively minor stimuli cause severe pain, a form of hyperalgesia.

REFERRED PAIN

Visceral pain often radiates or is referred to other areas. Irritation of a visceral organ frequently produces pain that is felt not at that site but in a somatic structure that may be some distance away **(referred pain).** Knowledge of the common sites of pain referral from each of the visceral organs is of importance to a clinician. One of the best-known examples is referral of cardiac pain to the inner aspect of the left arm. Other examples include pain in the tip of the shoulder caused by irritation of the central portion of the diaphragm and pain in the testicle due to distension of the ureter. Sites of reference are not stereotyped, and unusual reference sites occur with considerable frequency. Ischemic cardiac pain, for instance, may be referred to the right arm, the abdominal region, or even the back, neck, or jaw.

When pain is referred, it is usually to a structure that developed from the same embryonic segment or dermatome as the structure in which the pain originates. For example, the heart and the arm have the same segmental origin, and the testicle migrated with its nerve supply from the primitive urogenital ridge from which the kidney and ureter also developed.

The basis for referred pain may be convergence of somatic and visceral pain fibers on the same second-order neurons in the dorsal horn that project to the thalamus and then to the somatosensory cortex **(Figure 8–6)**. This is called the **convergence-projection theory.** Somatic and visceral neurons converge in the ipsilateral dorsal horn. When the visceral stimulus is prolonged, facilitation of activity from the somatic fiber endings occurs. They now stimulate the second-order neurons, and of course the brain cannot determine whether the stimulus came from the viscera or from the area of referral.

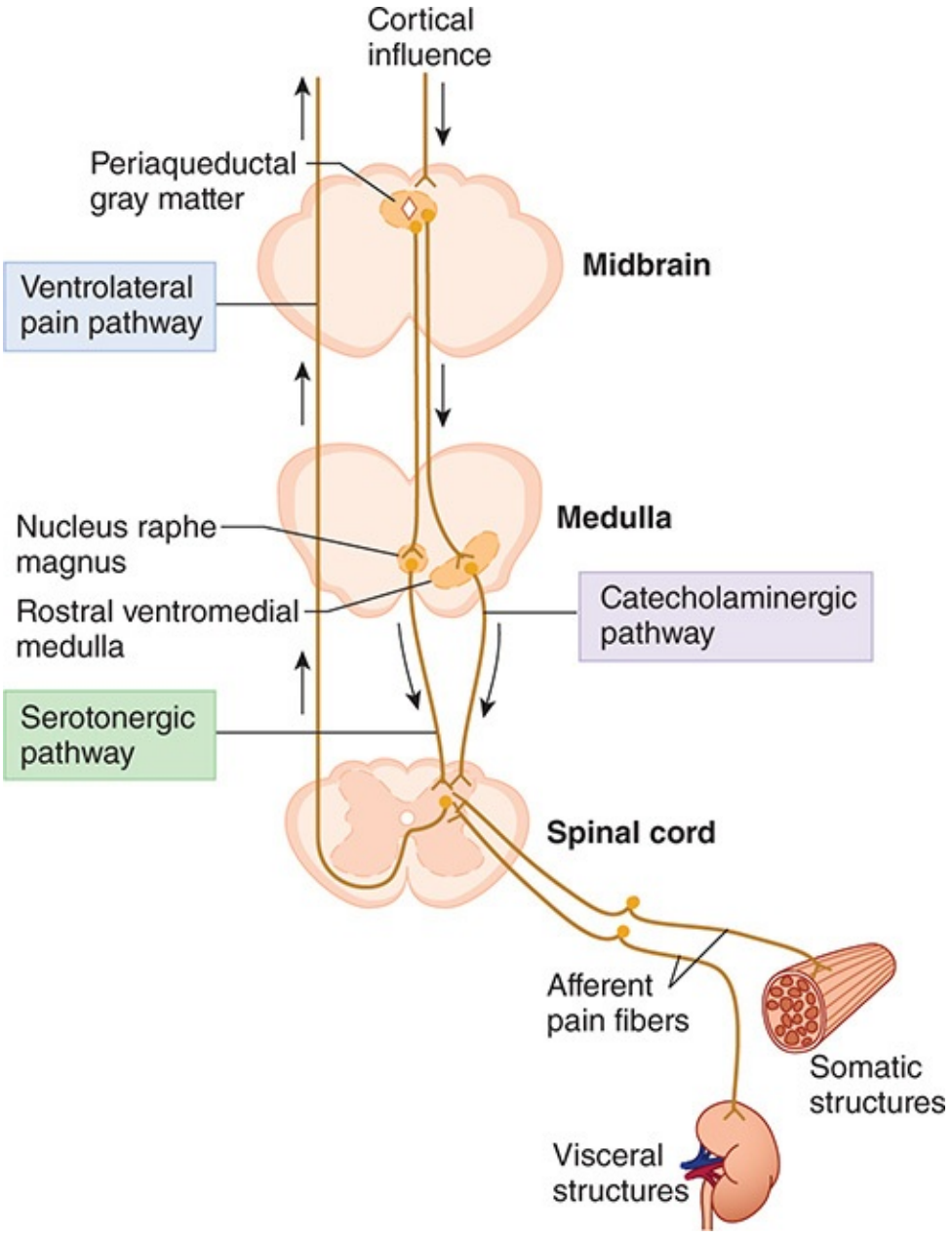


FIGURE 8–6 Schematic illustration of the convergence-projection theory

for referred pain and descending pathways involved in pain control. The basis for referred pain may be convergence of somatic and visceral pain fibers on the same second-order neurons in the dorsal horn of the spinal cord that project higher brain regions. The periaqueductal gray is a part of a descending pathway that includes serotonergic neurons in the nucleus raphe magnus and catecholaminergic neurons in the rostral ventromedial medulla to modulate pain transmission by inhibition of primary afferent transmission in the dorsal horn.

SOMATOSENSORY PATHWAYS

The sensation evoked by impulses generated in a sensory receptor depends in part on the specific part of the brain they ultimately activate. Below is a comparison of the ascending sensory pathway that mediates touch, vibratory sense, and proprioception **(dorsal column** or **medial lemniscal pathway)** and that which mediates pain and temperature **(ventrolateral spinothalamic pathway).**

DORSAL COLUMN PATHWAY

The principal pathways to the cerebral cortex for touch, vibratory sense, and proprioception are shown in **Figure 8–7**. Fibers mediating these sensations ascend ipsilaterally in the dorsal columns of the spinal cord to the medulla, where they synapse in the **gracilus** and **cuneate nuclei.** The second-order neurons from these nuclei cross the midline and ascend in the **medial lemniscus** to end in the contralateral **ventral posterior lateral (VPL) nucleus**. This ascending system is called the dorsal column or medial lemniscal system. The fibers within the dorsal column pathway are joined in the brainstem by fibers mediating sensation from the head. Touch and proprioception from the head are relayed mostly via the main sensory and mesencephalic nuclei of the trigeminal nerve.

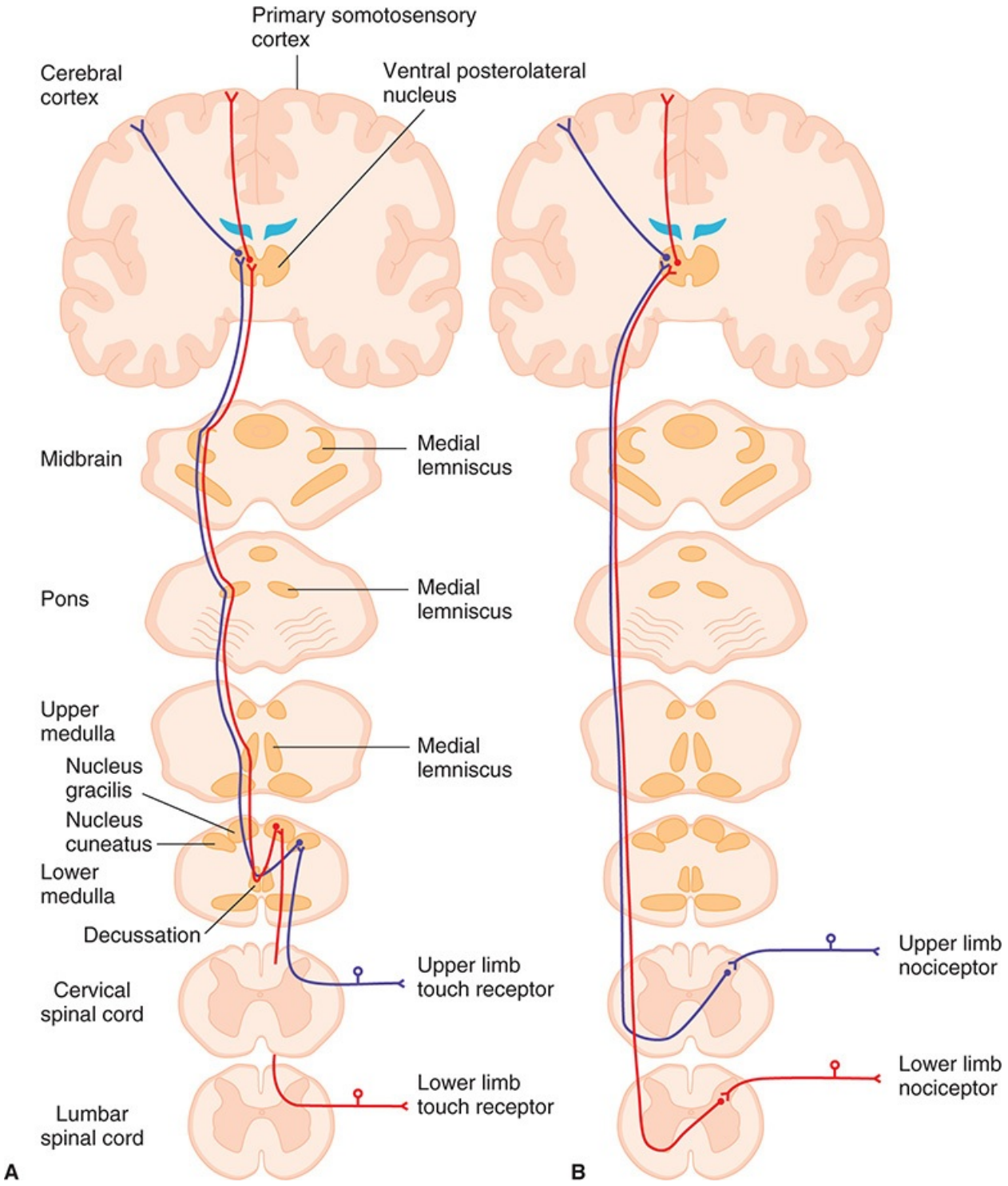


FIGURE 8–7 Ascending tracts carrying sensory information from peripheral receptors to the cerebral cortex. A) Dorsal column pathway mediates touch, vibratory sense, and proprioception. Sensory fibers ascend ipsilaterally via the spinal dorsal columns to medullary gracilus and cuneate nuclei; from there the fibers cross the midline and ascend in the medial

lemniscus to the contralateral thalamic ventral posterior lateral (VPL) and then to the primary somatosensory cortex. **B)** Ventrolateral spinothalamic tract mediates pain and temperature. These sensory fibers terminate in the dorsal horn and projections from there cross the midline and ascend in the ventrolateral quadrant of the spinal cord to the VPL and then to the primary somatosensory cortex.

Somatotopic Organization

Within the dorsal columns, fibers arising from different levels of the cord are somatotopically organized (Figure 8–7). Specifically, fibers from the sacral cord are positioned most medially and those from the cervical cord are positioned most laterally. This arrangement continues in the medulla with lower body (eg, foot) representation in the gracilus nucleus and upper body (eg, finger) representation in cuneate nucleus. The medial lemniscus is organized dorsal to ventral representing from neck to foot.

Somatotopic organization continues through the thalamus and cortex. The VPL thalamic neurons carrying sensory information project in a highly specific way to the **primary somatosensory cortex** in the **postcentral gyrus** of the **parietal lobe** **(Figure 8–8)**. The arrangement of projections to this region is such that the parts of the body are represented in order along the postcentral gyrus, with the legs on top and the head at the foot of the gyrus. Not only is there detailed localization of the fibers from the various parts of the body in the postcentral gyrus but also the size of the cortical receiving area for impulses from a part of the body is proportional to the use of the part. The relative sizes of the cortical receiving areas are shown dramatically in **Figure 8–9**, in which the proportions of the **homunculus** have been distorted to correspond to the size of the cortical receiving areas for each. Note that the cortical areas for sensation from the trunk and back are small, whereas very large areas are concerned with impulses from the hand and the parts of the mouth concerned with speech.

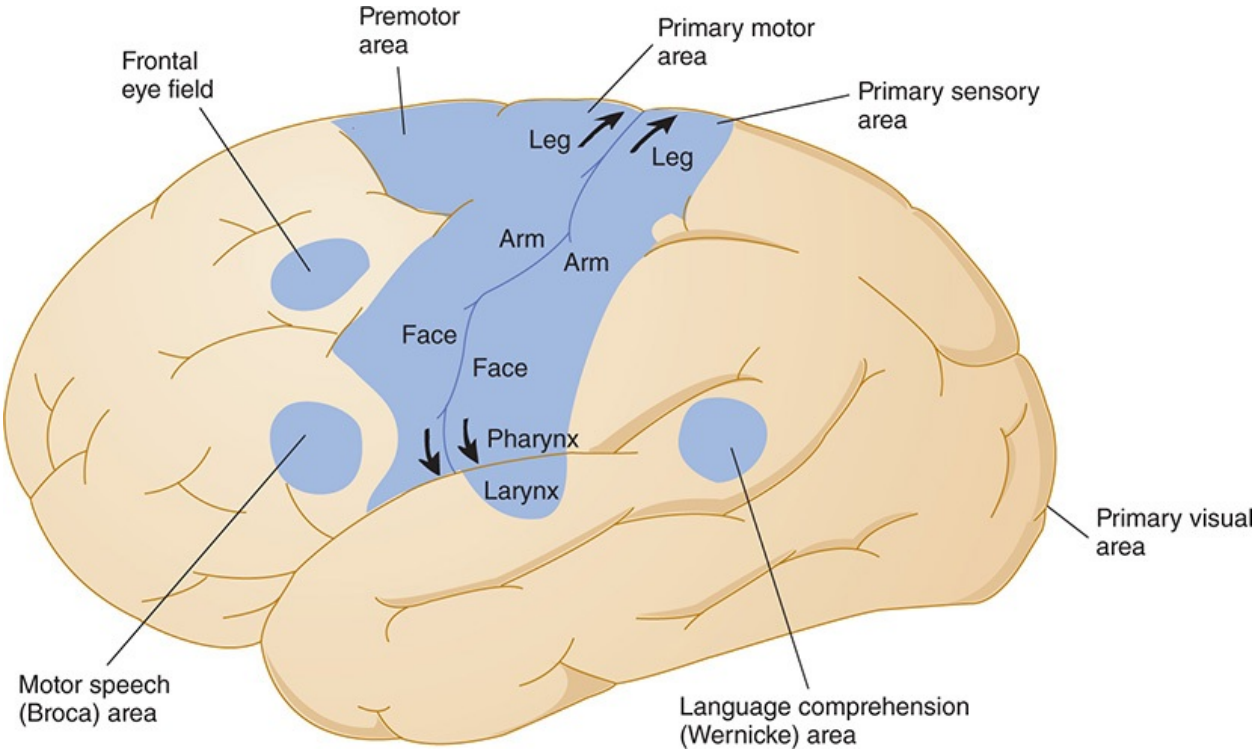


FIGURE 8–8 A lateral view of the left hemisphere showing some principal cortical areas and their functional correlates in the human brain. The primary somatosensory area is in the postcentral gyrus of the parietal lobe, and the primary motor cortex is in the precentral gyrus. (Reproduced with permission from Waxman SG: *Clinical Neuroanatomy*, 26th ed. New York, NY: McGraw-Hill; 2010.)

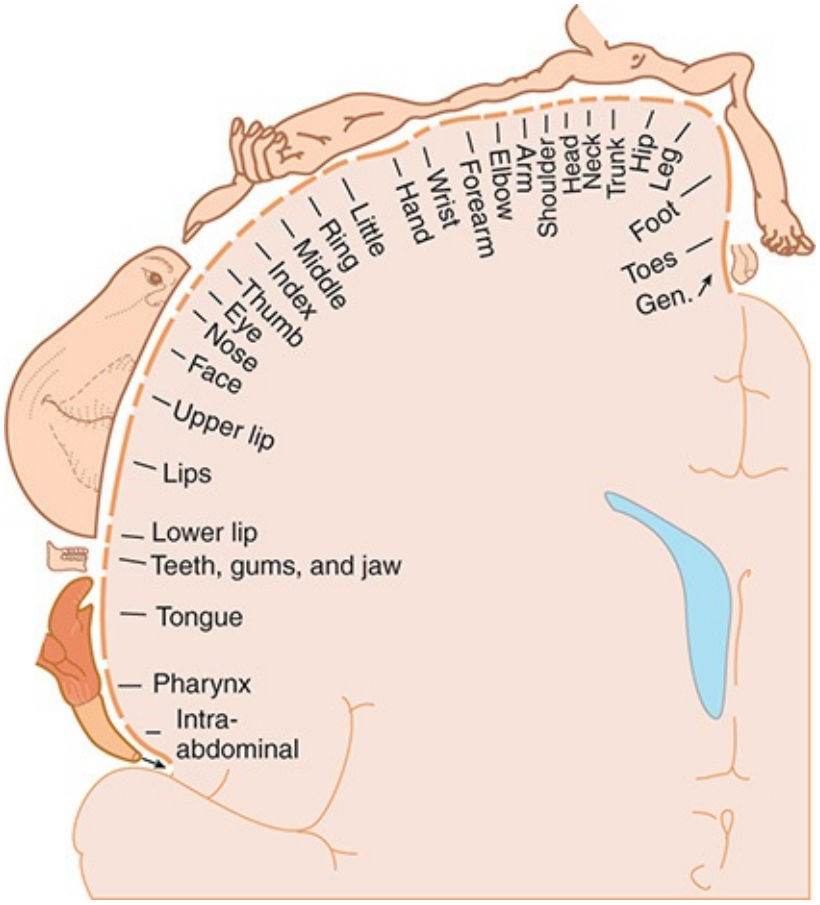


FIGURE 8–9 Sensory homunculus, drawn overlying a coronal section through the postcentral gyrus. The parts of the body are represented in order along the postcentral gyrus, with the legs on top and the head at the foot of the gyrus. The size of the cortical receiving area for impulses from a particular part of the body is proportionate to the use of the part. Gen., genitalia.

In addition to the primary somatosensory cortex, there are two other cortical regions that contribute to the integration of sensory information. The **sensory association area** is in the parietal cortex, and the **secondary somatosensory cortex** is located in the wall of the **lateral fissure** (also called **sylvian fissure**) that separates the temporal from the frontal and parietal lobes. These regions receive input from the primary somatosensory cortex.

Conscious awareness of the positions of the various parts of the body in space depends in part on impulses from sensory receptors in and around the joints. Impulses from these receptors, from touch receptors in the skin and other tissues, and from muscle spindles are synthesized in the cortex into a conscious picture of the position of the body in space (proprioception).

VENTROLATERAL SPINOTHALAMIC TRACT

Fibers from nociceptors and thermoreceptors synapse on neurons in the dorsal horn of the spinal cord. The axons from these dorsal horn neurons cross the midline and ascend in the ventrolateral quadrant of the spinal cord, where they form the ventrolateral spinothalamic pathway (Figure 8–7). Fibers within this tract synapse in the VPL. Some dorsal horn neurons that receive nociceptive input synapse in the reticular formation of the brainstem **(spinoreticular pathway)** and then project to the centrolateral nucleus of the thalamus.

Positron emission tomographic (PET) and functional magnetic resonance imaging (fMRI) studies in healthy humans indicate that pain activates the primary and secondary somatosensory cortex and the cingulate gyrus on the side opposite the stimulus. In addition, the amygdala, frontal lobe, and the insular cortex are activated. These technologies were important in distinguishing two components of pain pathways. The pathway to the primary somatosensory cortex is responsible for the discriminative aspect of pain (pain localization). In contrast, the pathway that includes synapses in the brainstem reticular formation and centrolateral thalamic nucleus projects to the frontal lobe, limbic system, and insular cortex. This pathway mediates the motivational-affective component of pain.

Visceral sensation travels along the same central pathways as somatic sensation in the spinothalamic tracts and thalamic radiations, and the cortical receiving areas for visceral sensation are intermixed with the somatic receiving areas.

CORTICAL PLASTICITY

The extensive neuronal connections described above are not innate and immutable but can be changed relatively rapidly by experience to reflect the use of the represented area. **Clinical Box 8–4** describes remarkable changes in cortical and thalamic organization that occur in response to limb amputation to lead to the phenomenon of **phantom limb pain.**

CLINICAL BOX 8–4

Phantom Limb Pain

In 1551, Ambroise Pare, a military surgeon, wrote “…the patients, long after

the amputation is made, say they still feel pain in the amputated part. Of this they complain strongly, a thing worthy of wonder and almost incredible to people who have not experienced this." This is perhaps the earliest description of **phantom limb pain.** Between 50% and 80% of amputees experience phantom sensations, usually pain, in the region of their amputated limb. Phantom sensations may also occur after the removal of body parts other than the limbs, for example, after amputation of the breast, extraction of a tooth **(phantom tooth pain),** or removal of an eye **(phantom eye syndrome).** Numerous theories have been evoked to explain this phenomenon. The current theory is based on evidence that the brain can reorganize if sensory input is cutoff. The **ventral posterior thalamic nucleus** is one example where this change can occur. In patients who have had their leg amputated, single neuron recordings show that the thalamic region that once received input from the leg and foot now respond to stimulation of the stump (thigh). Others have demonstrated remapping of the somatosensory cortex. For example, in some individuals who have had an arm amputated, stroking different parts of the face can lead to the feeling of being touched in the area of the missing limb.

THERAPEUTIC HIGHLIGHTS

The use of **epidural anesthesia** during the amputation surgery may prevent the acute pain associated with the surgery, thereby reducing the need for opioid therapy in the immediate postoperative period. This anesthetic procedure resulted in a reduced incidence of phantom pain. **Spinal cord stimulation** has been shown to be an effective therapy for phantom pain. Electric current is passed through an electrode that is placed next to the spinal cord to stimulate spinal pathways. This interferes with the impulses ascending to the brain and lessens the pain felt in the phantom limb. Instead, amputees feel a tingling sensation in the phantom limb.

Cortical structures are capable of dramatic reorganization **(plasticity)** after loss of a body part such as a digit; the cortical representation of the neighboring digits spreads into the cortical area that was formerly occupied by the representation of the amputated digit. Cortical connections of sensory units to the cortex have extensive convergence and divergence, with connections that can become weak with disuse and strong with use.

PET scanning in humans also documents plastic changes, sometimes from

one sensory modality to another. Thus, for example, tactile and auditory stimuli increase metabolic activity in the visual cortex in blind individuals. Conversely, deaf individuals respond faster and more accurately than normal individuals to moving stimuli in the visual periphery. Plasticity also occurs in the motor cortex.

EFFECTS OF CNS LESIONS

Clinical Box 8–2 describes some of the deficits noted after damage within the somatosensory pathways. **Clinical Box 8–5** describes the characteristic changes in sensory and motor functions that occur in response to spinal cord hemisection.

Damage to the dorsal columns leads to ipsilateral loss of the ability to detect light touch, vibration, and proprioception from body structures represented caudal to the level of damage. Damage to the ventrolateral spinothalamic pathway leads to contralateral loss of pain and temperature sensation below the level of the lesion. Such spinal damage could occur with a penetrating wound or a tumor.

CLINICAL BOX 8–5

Brown-Séquard Syndrome

A functional hemisection of the spinal cord causes a characteristic and easily recognized clinical picture that reflects damage to ascending sensory (dorsal column pathway, ventrolateral spinothalamic tract) and descending motor (corticospinal tract) pathways, which is called the **Brown-Séquard syndrome.** The lesion to fasciculus gracilus or fasciculus cuneatus leads to ipsilateral loss of discriminative touch, vibration, and proprioception below the level of the lesion. The loss of the spinothalamic tract leads to contralateral loss of pain and temperature sensation beginning one or two segments below the lesion. Damage to the corticospinal tract produces weakness and spasticity in certain muscle groups on the same side of the body. Although a precise spinal hemisection is rare, the syndrome is common because it can be caused by a spinal cord tumor, spinal cord trauma, degenerative disk disease, and ischemia.

THERAPEUTIC HIGHLIGHTS

Spinal or vertebral stabilization is needed when spinal trauma is indicated. Drug

treatments for Brown-Séquard syndrome are based on the etiology and time since onset. High doses of **corticosteroids** have been shown to be of value particularly if administered soon after the onset of such a spinal cord injury. Corticosteroids decrease the inflammation by suppressing polymorphonuclear leukocytes and reverse the increase in capillary permeability. Drugs may be needed to treat spasticity, pain, or other possible complications of the spinal cord injury. Physical therapy is important to maintain strength and joint mobility and improving respiratory function.

Damage to the primary somatosensory cortex does not abolish somatic sensation. Irritation of this region causes **paresthesia** or an abnormal sensation of numbness and tingling on the contralateral side of the body. Destructive lesions impair the ability to localize noxious stimuli in time, space, and intensity. Damage to the cingulate cortex impairs the recognition of the aversive nature of a noxious stimulus.

An infarct in the thalamus can lead to a loss of sensation. **Thalamic pain syndrome** can occur during recovery from a thalamic infarct. The syndrome is characterized by chronic pain on the side of the body contralateral to the stroke.

MODULATION OF PAIN TRANSMISSION

PROCESSING INFORMATION IN THE DORSAL HORN

Transmission in nociceptive pathways can be interrupted by actions within the dorsal horn of the spinal cord at the site of sensory afferent termination. Many people have learned from practical experience that rubbing or shaking an injured area decreases the pain due to the injury. The relief may be due to the simultaneous activation of innocuous cutaneous mechanoreceptors whose afferents emit collaterals that terminate in the dorsal horn. The activity of these cutaneous mechanosensitive afferents may reduce the responsiveness of dorsal horn neurons to their input from nociceptive afferent terminals. This is called the **gate-control mechanism** of pain modulation and it serves as the rationale behind the use of **transcutaneous electrical nerve stimulation (TENS)** for pain relief. This method uses electrodes to activate Aα and Aβ fibers near the injury.

Opioids are a commonly used analgesic that can exert their effects at various

places in the CNS, including in the spinal cord and dorsal root ganglia. **Figure 8–10** shows some of the various modes of action of opioids to decrease nociceptive transmission. There are interneurons in the superficial regions of the dorsal horn that contain endogenous opioid peptides (**enkephalin** and **dynorphin**). These interneurons terminate in the region of the dorsal horn where nociceptive afferents terminate. Opioid receptors are located on the terminals of nociceptive fibers and on dendrites of dorsal horn neurons, allowing for both presynaptic and postsynaptic sites of actions for opioids. Activation of the postsynaptic opioid receptors hyperpolarizes the dorsal horn interneuron by causing an increase in K^+ conductance. Activation of the presynaptic opioid receptors leads to a decrease in Ca^{2+} influx, resulting in a decrease in release of glutamate and substance P. Together these actions reduce the duration of the EPSP in the dorsal horn neuron. Activation of opioid receptors on dorsal root ganglia cell bodies also contributes to reduced transmission from nociceptive afferents.

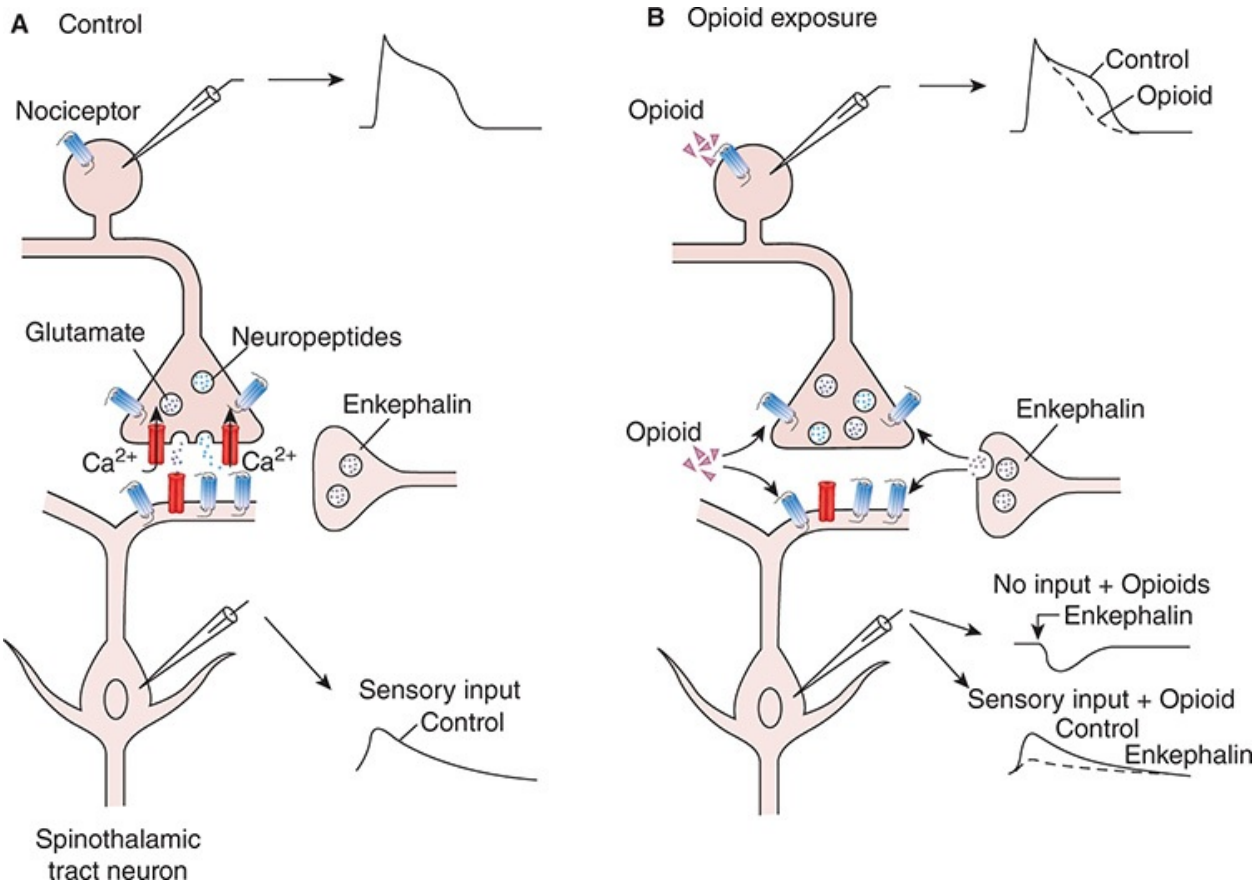


FIGURE 8–10 Actions of opioids to reduce sensory transmission in pain pathways at the level of the dorsal root ganglion (DRG) and spinal cord

dorsal horn region. A) Activation of a nociceptor leads to the release of glutamate and neuropeptides from its nerve terminals that synapse on spinothalamic tract projection neurons. This leads to the depolarization (activation) of spinothalamic tract projection neurons. Enkephalin (ENK)-containing interneurons mediate their effects via opioid receptors on the terminals of nociceptive afferent fibers and on dendrites of dorsal horn neurons to exert both presynaptic and postsynaptic inhibition. **B)** The action of an opioid (eg, morphine) within the DRG is to decrease Ca^{2+} influx leading to a decrease in the duration of the invoked action potential in the nociceptive neuron and a reduction in transmitter release from the nociceptive neuron onto a neuron in the dorsal horn. Opioids also hyperpolarize the membrane of dorsal horn neuron by activation of a K^{+} conductance; opioids also decrease the amplitude of the excitatory postsynaptic potential (EPSP) produced by stimulation of nociceptors.

CLINICAL BOX 8–6

Motivation & Addiction

Neurons in the forebrain **ventral tegmental area** and **nucleus accumbens** are involved in motivated behaviors such as reward, laughter, pleasure, addiction, and fear. These areas are referred to as the brain's **reward center** or **pleasure center.** The **mesocortical dopaminergic neurons** that project from the midbrain to the **nucleus accumbens** and the frontal cortex are also involved. **Addiction,** defined as the repeated compulsive use of a substance despite negative health consequences, can be produced by many different drugs. According to the World Health Organization, over 76 million people worldwide suffer from alcohol abuse, and over 15 million suffer from drug abuse. Not surprisingly, alcohol and drug addiction are associated with the reward system. The best studied addictive drugs are opioids (eg, morphine and heroin); others include cocaine, amphetamine, alcohol, cannabinoids, and nicotine. These drugs affect the brain in different ways, but all have in common the fact that they increase the amount of dopamine available to act on $\mathbf{D_3}$ **receptors** in the nucleus accumbens. Thus, acutely they stimulate the reward system of the brain. Long-term addiction involves the development of **tolerance,** which is the need for increasing amounts of a drug to produce a high. Also, **withdrawal** produces psychological and physical symptoms. One of the characteristics of addiction is the tendency of addicts to relapse after treatment. For opioid addicts, the relapse rate in the first year is about 80%.

Relapse often occurs on exposure to sights, sounds, and situations that were previously associated with drug use. Even a single dose of an addictive drug facilitates release of excitatory neurotransmitters in brain areas concerned with memory. The medial frontal cortex, hippocampus, and amygdala are concerned with memory, and they project via excitatory glutamatergic pathways to the nucleus accumbens. Relatively little is known about the specific brain mechanisms that cause tolerance and dependence. However, the two can be separated. Absence of **β-arrestin-2** blocks tolerance but has no effect on dependence. β-Arrestin-2 is a member of a family of proteins that inhibit heterotrimeric G-proteins by phosphorylating them.

THERAPEUTIC HIGHLIGHTS

Withdrawal symptoms and cravings associated with addiction to opioids can be reversed by treatment with drugs such as **methadone** and **buprenorphine** that act on the same CNS receptors as morphine and heroin. The US Federal Drug Administration has approved the use of three drugs for treatment of alcohol abuse: **naltrexone, acamprosate,** and **disulfiram.** Naltrexone is an opioid receptor antagonist that blocks the reward system and the craving for alcohol. Acamprosate may reduce the withdrawal effects associated with alcohol abuse. Disulfiram causes an accumulation of acetaldehyde by preventing the full degradation of alcohol. This leads to an unpleasant reaction to alcohol ingestion (eg, flushing, nausea, and palpitations). **Topiramate,** a Na^+ channel blocker, is showing promise in clinical trials of alcohol addiction. This is the same drug that is effective in treatment of migraine headaches.

Patients who receive long-term pain management with morphine may become resistant to the drug, requiring progressively higher doses for pain relief. This **acquired tolerance** is different from **addiction,** which refers to a psychological craving. Psychological addiction rarely occurs when morphine is used to treat chronic pain, provided the patient does not have a history of drug abuse. **Clinical Box 8–6** describes mechanisms involved in motivation and addiction.

ROLES OF PERIAQUEDUCTAL GRAY & BRAINSTEM

Another site of action for morphine and endogenous opioid peptides is the

midbrain **periaqueductal gray (PAG).** An injection of opioids into the PAG induces analgesia. The PAG is part of a descending pathway that modulates pain transmission by inhibiting primary afferent transmission in the dorsal horn (Figure 8–6). These PAG neurons project directly to and activate two groups of neurons in the brainstem: serotonergic neurons in the **nucleus raphe magnus** and catecholaminergic neurons in the **rostral ventromedial medulla.** Neurons in these regions project to the dorsal horn of the spinal cord where they release serotonin and norepinephrine, respectively, to inhibit the activity of dorsal horn neurons that receive input from nociceptive afferent fibers (Figure 8–6). This inhibition occurs, at least in part, due to the activation of the dorsal horn enkephalin-containing interneurons. A group of pontine catecholaminergic neurons in the **locus coeruleus** are also elements of this descending pain modulating pathway. These neurons also exert their analgesic effect by the release of norepinephrine in the dorsal horn.

The analgesic effect of **electroacupuncture** may involve the release of endogenous opioids and activation of this descending pain modulatory pathway. Electroacupuncture activates ascending sensory pathways that emit collaterals in the PAG and in the brainstem serotonergic and catecholaminergic regions. The analgesic effect of electroacupuncture is prevented by administration of naloxone, an opioid receptor antagonist.

STRESS-INDUCED ANALGESIA

It is well known that soldiers wounded in the heat of battle often feel no pain until the battle is over. This is an example of **stress-induced analgesia** that can also be exemplified by reduced pain sensitivity when being attacked by a predator or other stressful events. Release of norepinephrine, perhaps from brainstem catecholaminergic neurons, in the amygdala may contribute to this phenomenon. As noted above, the amygdala is a part of the limbic system that is involved in mediating the motivational-affective responses to pain.

The release of endogenous **cannabinoids** such as 2-arachidonoylglycerol (2AG) and anandamide may contribute to stress-induced analgesia. These chemicals can act on two types of G-protein-coupled receptors (CB_1 and CB_2). CB_1 receptors are found in many brain regions, and activation of these receptors accounts for the euphoric actions of cannabinoids. CB_2 receptors are expressed in activated microglia under pathologies that are associated with chronic neuropathic pain (see Clinical Box 8–3). Binding of an agonist to CB_2 receptors

on microglia reduces the inflammatory response and has an analgesic effect. Work is underway to develop selective CB_2 agonists for therapeutic treatment of neuropathic pain.

CHAPTER SUMMARY

- Touch and pressure are sensed by four types of mechanoreceptors that are innervated by myelinated Aα and Aβ sensory afferents. They are rapidly adapting Meissner corpuscles (respond to tapping and slow vibrations), slowly adapting Merkel cells (respond to sustained pressure and touch), slowly adapting Ruffini corpuscles (respond to skin stretch and vibration), and rapidly adapting Pacinian corpuscles (respond to fast vibration and deep pressure).
- Nociceptors and thermoreceptors are located on the free nerve endings of unmyelinated C fibers or lightly myelinated Aδ fibers in hairy and glaborous skin and deep tissues. These nerve endings have various types of receptors that are activated by noxious chemical (eg, TRPV1 and ASIC), mechanical (eg, P2X, P2Y and TRPA1), and thermal (eg, TRPV1) stimuli. In addition, chemical mediators (eg, bradykinin, prostaglandin, serotonin, and histamine) released in response to tissue injury directly activate or sensitize nociceptors.
- The receptor potential is the nonpropagated depolarizing potential recorded in a sensory receptor after an adequate stimulus is applied. As the stimulus intensity is increased, the size of the receptor potential is increased. When it reaches a critical threshold, an action potential is generated in the sensory nerve.
- Converting a receptor stimulus to a recognizable sensation is termed “sensory coding.” All sensory systems code for four elementary attributes of a stimulus: modality (receptor selectivity), location (receptive field), intensity (receptor sensitivity), and duration (receptor adaptation).
- Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage, whereas nociception is the unconscious activity induced by a harmful stimulus applied to sense receptors. First pain is mediated by Aδ fibers and causes a sharp, localized sensation. Second pain is mediated by C fibers and causes a dull, intense, diffuse, and unpleasant feeling. Acute pain has a sudden onset, recedes during the healing process, and serves as an important

protective mechanism. Chronic pain is persistent and caused by nerve damage or prolonged inflammation; it is often associated with hyperalgesia (an exaggerated response to a noxious stimulus) and allodynia (a sensation of pain in response to an innocuous stimulus).

- Visceral pain is poorly localized, unpleasant, and associated with nausea and autonomic symptoms. It often radiates (or is referred) to other somatic structures perhaps due to convergence of somatic and visceral nociceptive afferent fibers on the same second-order neurons in the spinal dorsal horn that project to the thalamus and then to the primary somatosensory cortex.
- Discriminative touch, proprioception, and vibratory sensations are relayed via the dorsal column (medial lemniscus) pathway to the VPL in the thalamus and then to the primary somatosensory cortex. Pain and temperature sensations are mediated via the ventrolateral spinothalamic tract, which projects to the VPL and then to cortex. The discriminative aspect of pain results from activation of the primary somatosensory cortex; the motivational-affective component of pain is from activation of the frontal lobe, limbic system, and insular cortex.
- Damage to the dorsal columns leads to ipsilateral loss of the ability to detect light touch, vibration, and proprioception from body structures represented caudal to the level of damage. Damage to the ventrolateral spinothalamic pathway leads to contralateral loss of pain and temperature sensation below the level of the lesion. Damage to the primary somatosensory impairs the ability to localize noxious stimuli in time, space, and intensity. Damage to the cingulate cortex impairs the recognition of the aversive nature of a noxious stimulus.
- Transmission in pain pathways is modulated by endogenous opioids that can act in the PAG, brainstem, spinal cord, and dorsal root ganglia. Descending pain modulating pathways include neurons in the PAG, nucleus raphe magnus, rostral ventromedial medulla, and locus coeruleus.
- New pain therapies focus on synaptic transmission in nociception and peripheral sensory transduction. Capsaicin transdermal patches or creams reduce pain by exhausting the supply of substance P in nerves and by acting on TRPV1 receptors in the skin. Lidocaine and mexiletine are useful in some cases of chronic pain and act by blocking Nav1.8, which is uniquely associated with nociceptive neurons in dorsal root ganglia. Ziconotide, a voltage-gated N-type Ca^{2+} channel blocker, is used for intrathecal analgesia in patients with refractory chronic pain. Gabapentin, an anticonvulsant drug,

is effective in treatment of neuropathic and inflammatory pain by acting on voltage-gated Ca^{2+} channels. Topiramate, a Na^{+} channel blocker, is another anticonvulsant drug that can be used to treat migraines. NMDA receptor antagonists can be co-administered with an opioid to reduce tolerance to an opioid.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. A 28-year-old man was seen by a neurologist because he had experienced prolonged episodes of tingling and numbness in his right arm. He underwent a neurologic exam to evaluate his sensory nervous system. Which of the following cutaneous mechanoreceptors is correctly paired with the type of stimulus to which it is most apt to respond?

   A. Pacinian corpuscle and rapid vibration

   B. Meissner corpuscle and skin stretch

   C. Merkel cells and slow vibration

   D. Ruffini corpuscles and sustained pressure

2. An MD/PhD student was recording responses in different cutaneous receptors and noted the following. One receptor was inactive until the skin temperature was increased to 33°C and then its firing rate continued to increase as the skin temperature was gradually raised to 45°C. A second receptor was inactive until the skin temperature reached 46°C. A third receptor was inactive at skin temperatures of 40°C, but then steadily increases its firing rate as skin temperature was lowered to 24°C. For each of these cases, classify the type of receptor and the nonselective cation channel that was possibly activated.

   A. Receptor one is a thermal nociceptor and the channel activated was TRPV1; receptor two is a thermal nociceptor and the channel activated was TRPA1; receptor three was an innocuous cold receptor and the channel activated was TRPV4.

   B. Receptor one is an innocuous warm receptor and the channel activated was TRPV1; receptor two is a thermal nociceptor and the channel activated was TRPM8; receptor three was an innocuous cold receptor and the channel activated was TRPV3.

   C. Receptor one is an innocuous warm receptor and the channel activated was TRPV3; receptor two is a thermal nociceptor and the channel activated was

TRPV1; receptor three was an innocuous cold receptor and the channel activated was TRPM8.

D. Receptor one is a thermal nociceptor and the channel activated was TRPA1; receptor two is a thermal nociceptor and the channel activated was TRPV1; receptor three was an innocuous cold receptor and the channel activated was TRPM8.

3. List the steps involved in the generation of an action potential in a sensory nerve fiber beginning with the stimulation of a Pacinian corpuscle.

A. Light touch is applied to the Pacinian corpuscle, and a receptor potential is generated; as the pressure is increased, the size of the receptor potential is increased; when it reaches 30 mV, an action potential is produced at a point of the sensory nerve within the corpuscle.

B. Light touch is applied to the Pacinian corpuscle, and a receptor potential is generated; as more receptors are brought into the receptive field, the size of the receptor potential increases; when it reaches 30 mV, an action potential is produced at the first node of Ranvier.

C. Sustained pressure is applied to the Pacinian corpuscle, and a receptor potential is generated; as more receptors are activated, the size of the receptor potential increases; when it reaches 10 mV, an action potential is produced at the first node of Ranvier.

D. Rapid vibration is applied to the Pacinian corpuscle, and a graded receptor potential is generated; when the receptor potential reaches 10 mV, an action potential is produced at the first node of Ranvier.

E. Rapid vibration is applied to the Pacinian corpuscle, and a receptor potential is generated; when the receptor potential reaches 10 mV, an action potential is produced in the unmyelinated portion of the sensory fiber.

4. A medical student was doing research in a sensory neurophysiology laboratory. In preparation for his research, the principal investigator of the laboratory asked him to compare the four basic attributes of a stimulus to sensory receptors. The four attributes of sensory coding are

A. modality, adequate threshold, sensitivity, and location.

B. adequate threshold, receptive field, adaptation, and projection.

C. specific energy, adequate threshold, sensation, and duration.

D. sensitization, discrimination, energy, and projection.

E. modality, location, intensity, and duration.

5. A 23-year-old woman fell asleep on the beach while sunbathing. She awoke a

few hours later to find that she had a very bad sunburn. That evening while taking a shower, the lukewarm water (40°C) touching her back caused her to feel pain. What types of receptors were activated by the lukewarm water and why did she experience pain?

A. Thermal nociceptors and nociceptive pain

B. Thermal nociceptors and allodynia

C. Thermal nociceptors and hyperalgesia

D. Innocuous thermal receptors and hyperalgesia

E. Innocuous thermal receptors and allodynia

6. A 32-year-old woman experienced the sudden onset of a severe cramping pain in the abdominal region. She also became nauseated. List some of the common features of visceral pain.

A. It results from activation of nociceptors in the viscera that are innervated by the same fibers as innervate skin, induces rapid shard pain, causes spasms of the visceral muscle, and shows relatively rapid adaptation.

B. It is mediated by Aδ and C fibers in the ventral roots of spinal nerves, radiates to a nearby or distant somatic structure, is accompanied by sweating, and is relayed to the cortex by the spinothalamic tract.

C. It is poorly localized, is accompanied by sweating, radiates to a somatic structure that may be some distance away, and is relayed to the somatosensory cortex via the spinothalamic tract.

D. It requires simultaneous activation of nociceptors within and outside of the viscera, causes spasms of the visceral and skeletal muscle, and is relayed to the cortex by the dorsal column pathway.

E. It is well localized, is accompanied by sweating, radiates to a somatic structure that may be some distance away, and causes spasms of visceral muscle.

7. A ventrolateral cordotomy is performed that produces relief of pain in the right leg. It is effective because it interrupts the

A. left dorsal column.

B. left ventrolateral spinothalamic tract.

C. right ventrolateral spinothalamic tract.

D. right medial lemniscal pathway.

E. a direct projection to the primary somatosensory cortex.

8. A medical student is studying neurons that are part of a descending pain

modulating pathway. What brain region is correctly paired with the neurotransmitters it releases and the location where the neurotransmitter is released?

A. Periaqueductal gray neurons release endorphins in the spinal dorsal horn.

B. Nucleus raphe magnus releases serotonin in the dorsal root ganglion.

C. Locus coeruleus neurons release serotonin in the nucleus raphe magnus.

D. Locus coeruleus neurons release norepinephrine in the spinal dorsal horn.

E. Periaqueductal gray releases dynorphin in the rostral ventromedial medulla.

9. A 47-year-old woman experienced a migraine headache that was not relieved by her current pain medications. The doctor opted to prescribe a drug that targets receptors or ion channels within the pain pathway. Which of the following drugs is used to treat migraines and which receptor or ion channel does it affect?

A. Topiramate and voltage-gated Na^+ channel

B. Ziconotide and voltage-gated N-type Ca^{2+} channel

C. Valproate and TRPV1 receptors

D. Carbamazepine and voltage-gated Na^+ channels

E. Gabapentin and Nav1.8

10. A 40-year-old man loses his right hand in a farm accident. Four years later, he has episodes of severe pain in the missing hand (phantom limb pain). A detailed PET scan study of his cerebral cortex might be expected to show

A. expansion of the right hand area in his right primary somatosensory cortex.

B. expansion of the right hand area in his left primary somatosensory cortex.

C. a metabolically inactive spot where his hand area in his left primary somatosensory cortex would normally be.

D. projection of fibers from neighboring sensory areas into the right hand area of his right primary somatosensory cortex.

E. projection of fibers from neighboring sensory areas into the right hand area of his left primary somatosensory cortex.

11. A 50-year-old woman undergoes a neurologic exam that indicates loss of pain and temperature sensitivity, vibratory sense, and proprioception in the left leg. These symptoms could be explained by

A. a tumor on the right medial lemniscal pathway in the sacral spinal cord.

B. a peripheral neuropathy.

C. a tumor on the left medial lemniscal pathway in the sacral spinal cord.

D. a tumor affecting the right posterior paracentral gyrus.
E. a large tumor in the right lumbar ventrolateral spinal cord.

CHAPTER 9

Smell & Taste

OBJECTIVES

After studying this chapter, you should be able to:

- Describe the structure and function of the neural elements in the olfactory epithelium and olfactory bulb.
- Identify the significance of the family of olfactory receptor genes.
- Explain how odorant receptors are activated and the mechanism by which signal transduction occurs in these receptors.
- Label the components of the pathway by which impulses generated in the olfactory epithelium reach five regions of the olfactory cortex.
- Describe the location and cellular composition of taste buds.
- Name the five major taste modalities and compare the signal transduction mechanisms in the receptors mediating these different taste modalities.
- Label the components of the pathways by which impulses generated in taste receptors reach the gustatory region of the insular cortex.
- Name and discuss abnormalities in odor and taste sensations.

INTRODUCTION

Smell (**olfaction**) and taste (**gustation**) are examples of visceral senses because of their close association with gastrointestinal function. Physiologically, they are related to each other as the flavor of food is a combination of its taste and smell. This is why food may taste “different” if one has a cold that depresses the sense of smell. Smell and taste receptors are **chemoreceptors** that are stimulated by chemical molecules in solution in mucus in the nose (**odorants**) and saliva in the mouth (**tastants**). The sensations of smell and taste likely evolved as protective mechanisms to avoid the intake of potentially harmful substances.

SMELL

OLFACTORY EPITHELIUM

The yellowish pigmented **olfactory epithelium** is a specialized portion of the nasal mucosa that covers an area of 10 cm^2 in the roof of the nasal cavity near the septum in humans **(Figure 9–1)**. The olfactory epithelium is the place in the body where the nervous system is closest to the external world. It contains three types of neurons that are important for olfaction: **olfactory sensory neurons, supporting cells, and basal stem cells**.

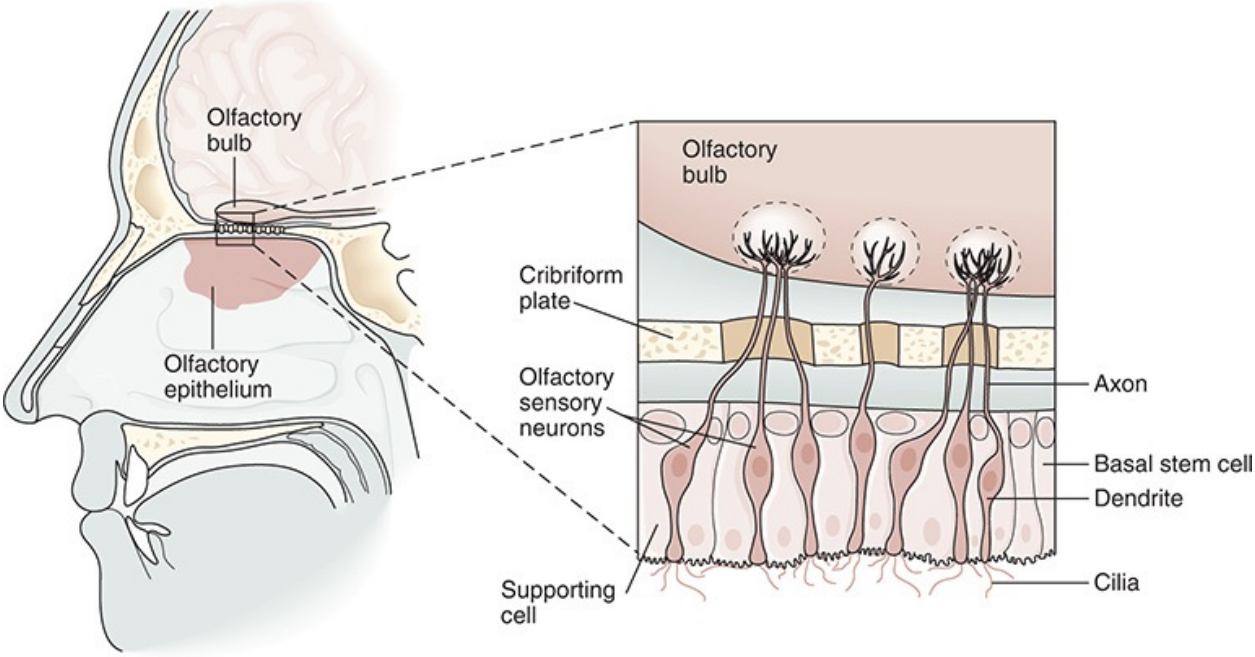


FIGURE 9–1 Structure of the olfactory epithelium. There are three cell types: olfactory sensory neurons, supporting (sustentacular) cells, and basal stem cells

at the base of the epithelium. Each olfactory sensory neuron has a dendrite that projects to the epithelial surface. Numerous cilia protrude into the mucus layer lining the nasal lumen. Odorants bind to specific odorant receptors on the cilia and initiate a cascade of events leading to generation of action potentials in the sensory axon. Each olfactory sensory neuron has a single axon that projects to the olfactory bulb, a small ovoid structure that rests on the cribriform plate of the ethmoid bone. (Reproduced with permission from Kandel ER, Schwartz JH, Jessell TM [editors]: *Principles of Neural Science*, 4th ed. New York, NY: McGraw-Hill; 2000.)

The bipolar olfactory sensory neurons (also called olfactory receptor cells) are responsible for olfactory transduction. They have a short, thick dendrite that projects into the nasal cavity where it terminates in a knob containing 6–12 **cilia** that protrude into the thin layer of mucus overlying the epithelium (Figure 9–1). The axons of the olfactory sensory neurons (ie, **olfactory nerve**) pass through the **cribriform plate** of the ethmoid bone to enter the **olfactory bulbs.**

The supporting cells secrete the mucus that provides the appropriate molecular and ionic environment for odor detection in the olfactory epithelium. Odor-producing molecules (odorants) dissolve in the mucus and bind to **odorant receptors** on the cilia of the olfactory sensory neurons. **Odorant- binding proteins** in the mucus may facilitate the diffusion of odorants to and from the odorant receptor. Basal stem cells undergo mitosis to generate new olfactory sensory neurons as needed to replace those damaged by exposure to the environment; olfactory sensory neurons generally survive for only 1–2 months.

ODORANT RECEPTORS & SIGNAL TRANSDUCTION

The olfactory system can discriminate perhaps more than 1 million distinct odors due in part to the existence of many different functional odorant receptors. There are about 1000 olfactory genes in humans, accounting for 3% of the human genome; approximately 400 of these genes function as odorant receptors. The amino acid sequences of odorant receptors are very diverse, but all are **G-protein-coupled receptors (GPCRs)**. When an odorant molecule binds to its receptor, the G-protein subunits (α, β, γ) dissociate **(Figure 9–2)**. The α-subunit activates adenylyl cyclase to catalyze the production of cAMP which acts as a second messenger to open cation channels, increasing the membrane permeability to Na^+, K^-, and Ca^{2+}. The net effect is an inward-directed Ca^{2+}

current which produces the **graded receptor potential.** This then opens Ca^{2+}-activated Cl^- channels, further depolarizing the cell due to the high intracellular Cl^- levels in olfactory sensory neurons. If the stimulus is sufficient for the receptor potential to exceed its threshold, an action potential in the olfactory nerve (1st cranial nerve) is triggered.

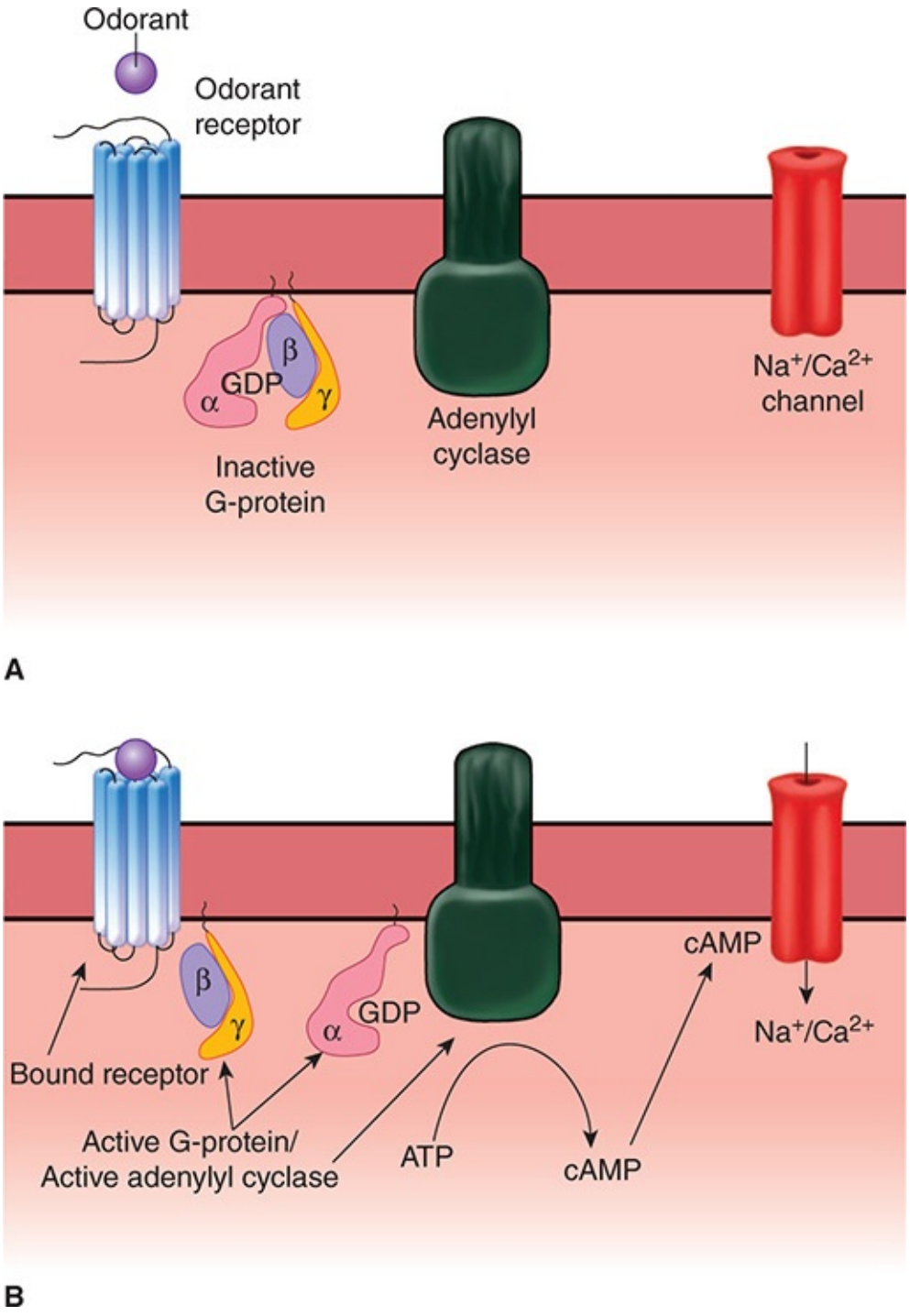


FIGURE 9–2 Signal transduction in an odorant receptor. A) Olfactory receptors are examples of G-protein-coupled receptors; ie, they are associated with three G-protein subunits (α, β, γ). **B)** When an odorant binds to the receptors, the subunits dissociate. The α-subunit of G-proteins activates adenylyl cyclase to catalyze production of cAMP. cAMP acts as a second messenger to open cation channels. Inward diffusion of Na^+ and Ca^{2+} produces depolarization.

OLFACTORY SENSORY PATHWAY

In the olfactory bulb, the axons of the olfactory sensory neurons synapse on the primary dendrites of the **mitral cells** and **tufted cells** **(Figure 9–3)** to form **olfactory glomeruli.** Each olfactory sensory neuron expresses only one of the 400 functional olfactory genes, but each odorant can bind to a large pool of odorant receptors. Each olfactory sensory neuron projects to only one or two glomeruli. This provides a distinct two-dimensional map in the olfactory bulb that is unique to the odorant. The mitral cells with their glomeruli project to different parts of the olfactory cortex. The central olfactory system is able to decode the pattern of receptor-cell activity that signals the identity of the odorant.

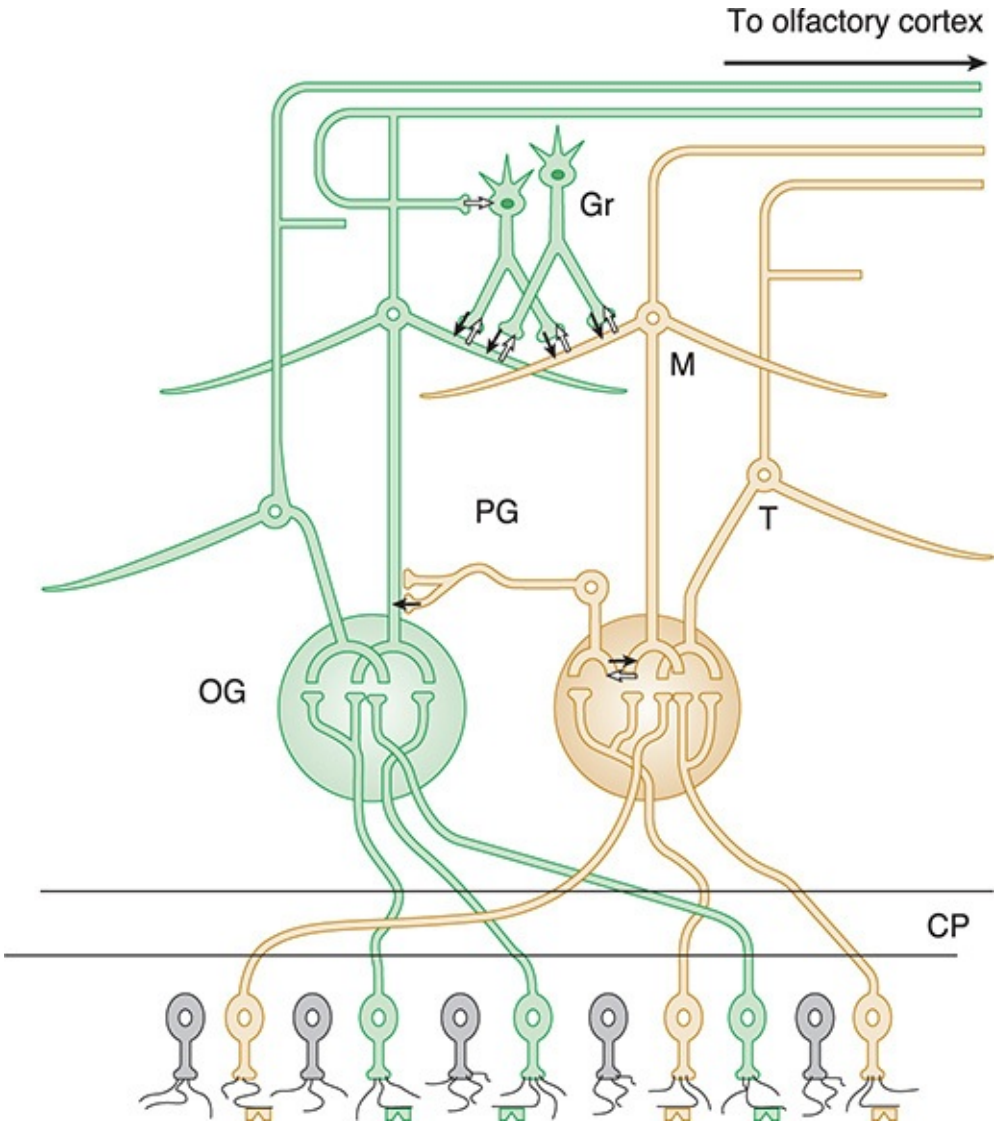


FIGURE 9–3 Basic neural circuits in the olfactory bulb. Olfactory receptor cells with one type of odorant receptor project to one olfactory glomerulus (OG) and olfactory receptor cells with another type of receptor project to a different OG. Solid black arrows signify inhibition via GABA release, and white arrows signify excitatory connections via glutamate release. CP, cribriform plate; Gr, granule cell; M, mitral cell; PG, periglomerular cell; T, tufted cell. (Adapted with permission from Mori K, et al: The olfactory bulb: coding and processing of odor molecular information, Science 1999 Oct 22; 286(5440): 711–715.)

The olfactory bulbs also contain **periglomerular cells,** which are inhibitory neurons connecting one glomerulus to another, and **granule cells,** which have no axons and make reciprocal synapses with the lateral dendrites of the mitral and tufted cells. At these synapses, the mitral or tufted cells excite the granule cell by

releasing **glutamate,** and the granule cells in turn inhibit the mitral or tufted cell by releasing **GABA.** The lateral inhibition mediated by periglomerular and granule cells functions to sharpen and focus olfactory signals.

The axons of the mitral and tufted cells pass posteriorly through the **lateral olfactory stria** to terminate on apical dendrites of pyramidal cells in five regions of the **olfactory cortex: anterior olfactory nucleus, olfactory tubercle, piriform cortex, amygdala,** and **entorhinal cortex** **(Figure 9–4)**. From these regions, information travels directly to the frontal cortex or via the thalamus to the orbitofrontal cortex. Conscious discrimination of odors is dependent on the pathway to the orbitofrontal cortex. The pathway to the amygdala mediates the emotional responses to olfactory stimuli, and the pathway to the entorhinal cortex is concerned with olfactory memories.

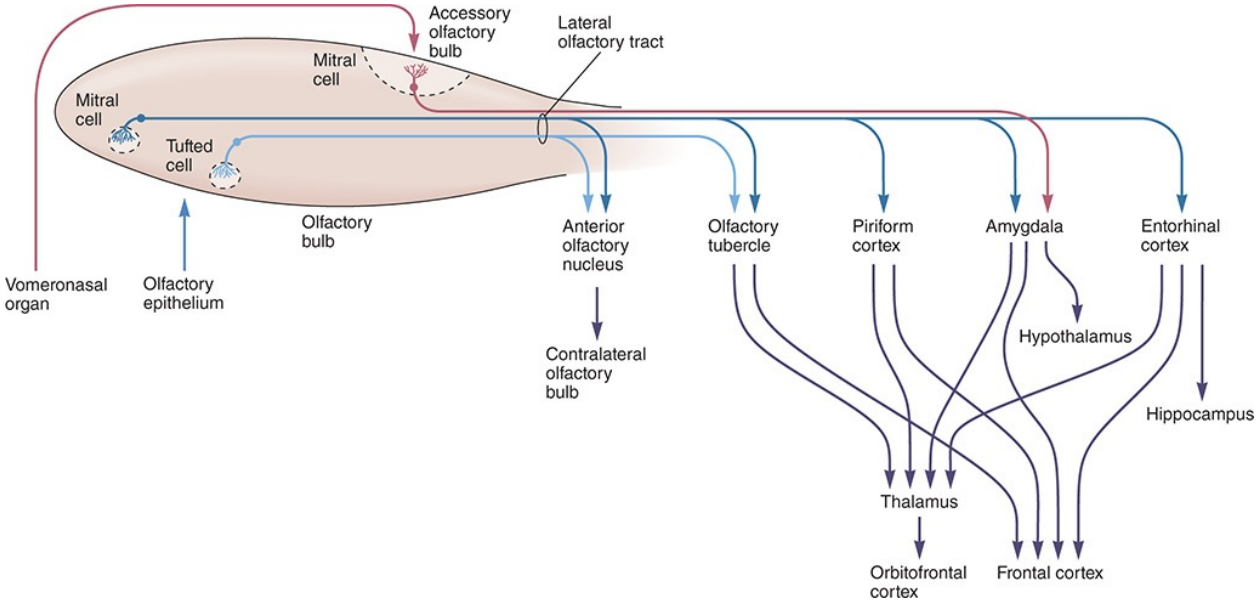


FIGURE 9–4 Diagram of the olfactory pathway. Information is transmitted from the olfactory bulb by axons of mitral and tufted relay neurons in the lateral olfactory tract. Mitral cells project to five regions of the olfactory cortex: anterior olfactory nucleus, olfactory tubercle, piriform cortex, and parts of the amygdala and entorhinal cortex. Tufted cells project to anterior olfactory nucleus and olfactory tubercle; mitral cells in the accessory olfactory bulb project only to the amygdala. Conscious discrimination of odor depends on the neocortex (orbitofrontal and frontal cortices). Emotive aspects of olfaction derive from limbic projections (amygdala and hypothalamus). (Reproduced with permission from Kandel ER, Schwartz JH, Jessell TM [editors]: *Principles of Neural Science*, 4th ed. New York, NY: McGraw-Hill; 2000.)

In some mammals, the nasal cavity contains another patch of olfactory epithelium located along the nasal septum in the **vomeronasal organ** that is concerned with the perception of **pheromones.** Vomeronasal sensory neurons project to the **accessory olfactory bulb** (Figure 9–4) and from there to regions of the amygdala and hypothalamus that are concerned with reproduction and ingestive behavior. The vomeronasal organ has about 100 G-protein-coupled odorant receptors that differ in structure from those in the rest of the olfactory epithelium.

ODOR DETECTION THRESHOLD

Odorants are generally small, containing between 3 and 20 carbon atoms; molecules with the same number of carbon atoms but different structural configurations have different odors. Relatively high water and lipid solubility is characteristic of substances with strong odors. Some common abnormalities in odor detection are described in **Clinical Box 9–1**.

The **odor detection threshold** is the lowest concentration of a chemical that can be detected. Examples of substances detected at very low concentrations include hydrogen sulfide (0.0005 parts per million, ppm), acetic acid (0.016 ppm), kerosene (0.1 ppm), and gasoline (0.3 ppm). Some toxic substances are essentially odorless; they have odor detection thresholds higher than lethal concentrations. For example, carbon dioxide is detected at 74,000 ppm but is lethal at 50,000 ppm. The odor detection threshold for a given odorant is not the same in all individuals.

The sense of smell is said to be more acute in women than in men, and in women it is most acute at the time of ovulation. Although olfactory discrimination is remarkable, determination of differences in the intensity of any given odor is poor. The concentration of an odor-producing substance must be changed by about 30% before a difference can be detected. The comparable visual discrimination threshold is a 1% change in light intensity.

CLINICAL BOX 9–1

Abnormalities in Odor Detection

Anosmia (inability to smell) and **hyposmia** or **hypesthesia** (diminished olfactory sensitivity) can result from simple nasal congestion, nasal polyps, or prolonged use of nasal decongestants. It may also be a sign of a more serious

problem such as damage to the olfactory nerves due to fractures of the cribriform plate or head trauma, tumors (eg, neuroblastomas or meningiomas), and respiratory tract infections. **Congenital anosmia** is a rare disorder in which an individual is born without the ability to smell. Olfactory dysfunction is often one of the earliest clinical symptoms of Alzheimer disease. According to the National Institutes of Health, 1–2% of the North American population under the age of 65 experiences a significant degree of loss of smell. However, 50% of individuals between the ages of 65 and 80 and >75% of those over the age of 80 have an impaired ability to identify smells. Because of the close relationship between taste and smell, anosmia is associated with a reduction in taste sensitivity **(hypogeusia).** Anosmia is generally permanent in cases in which the olfactory nerve or other neural elements in the olfactory neural pathway are damaged. In addition to not being able to experience the enjoyment of pleasant aromas and a full spectrum of tastes, individuals with anosmia are at risk because they are not able to detect the odor from dangers such as gas leaks, fire, and spoiled food. **Hyperosmia** (enhanced olfactory sensitivity) is less common than loss of smell, but pregnant women commonly become oversensitive to smell. **Dysosmia** (distorted sense of smell) can be caused by several disorders including sinus infections, partial damage to the olfactory nerves, and poor dental hygiene. An aura of a disagreeable odor (eg, burning rubber) can occur when an individual experiences an **uncinate seizure** that originates in the medial temporal lobe.

THERAPEUTIC HIGHLIGHTS

Quite often anosmia is a temporary condition due to sinus infection or a common cold, but it can be permanent if caused by nasal polyps or trauma. **Antibiotics** can be prescribed to reduce the inflammation caused by polyps and improve the ability to smell. In some cases, surgery is performed to remove the nasal polyps. Topical **corticosteroids** have also been shown to be effective in reversing the loss of smell due to nasal and sinus diseases.

TASTE

TASTE BUDS

The specialized sense organ for taste (gustation) consists of about 5000 **taste buds** located primarily on the **papillae** of the dorsal surface of the tongue in humans **(Figure 9–5)**. The **fungiform papillae** are rounded structures most numerous near the tip of the tongue; the **circumvallate papillae** are prominent structures arranged in a V on the back of the tongue; the **foliate papillae** are on the posterior edge of the tongue. Each fungiform papilla has up to five taste buds, mostly located at the top of the papilla, while each circumvallate and foliate papilla contain up to 100 taste buds, mostly located along the sides of the papillae. Taste buds are also located in the soft palate, epiglottis, and pharynx.

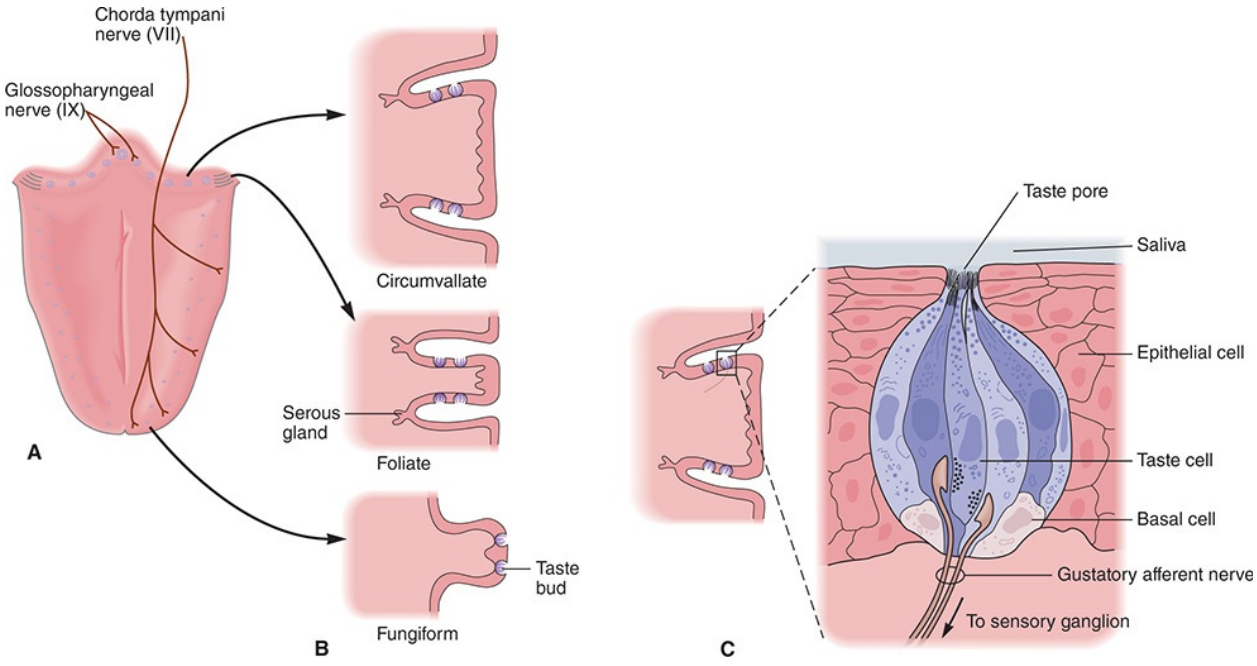


FIGURE 9–5 Taste buds located in papillae of the human tongue. A) Taste buds on the anterior two-thirds of the tongue are innervated by the chorda tympani branch of the facial nerve; those on the posterior one-third of the tongue are innervated by the lingual branch of the glossopharyngeal nerve. **B)** The three major types of papillae (circumvallate, foliate, and fungiform) are located on specific parts of the tongue. **C)** Taste buds are composed of basal stem cells and three types of taste cells (dark, light, and intermediate). Taste cells extend from the base of the taste bud to the taste pore, where microvilli contact tastants dissolved in saliva and mucus. (Modified with permission from Kandel ER, Schwartz JH, Jessell TM [editors]: *Principles of Neural Science*, 4th ed. New York, NY: McGraw-Hill; 2000.)

Each taste bud contains 50–100 **taste receptor cells** and numerous **basal cells**

and **support cells** (Figure 9–5). The taste receptor cells are modified epithelial cells that respond to chemical stimuli or tastants. The apical ends of taste cells have **microvilli** that project into the **taste pore**, a small opening on the dorsal surface of the tongue where tastes cells are exposed to the oral contents. Saliva in the oral cavity acts as a solvent for tastants; after dissolving, the chemical diffuses to the taste receptor sites. Saliva may also function to cleanse the mouth to prepare the taste receptors for a new stimulant.

Each taste bud is innervated by about 50 nerve fibers, and conversely, each nerve fiber receives input from an average of five taste buds. The basal cells arise from the epithelial cells surrounding the taste bud. They differentiate into new taste cells as taste cells survive for only about 10 days. If the sensory nerve is cut, the taste buds it innervates degenerate and eventually disappear.

TASTE PATHWAYS

The sensory nerve fibers from the taste buds on the anterior two-thirds of the tongue travel in the **chorda tympani branch of the facial nerve,** and those from the posterior third of the tongue reach the brainstem via the **glossopharyngeal nerve (Figure 9–6)**. The fibers from areas other than the tongue (eg, pharynx) reach the brain stem via the **vagus nerve.** On each side, the myelinated but relatively slowly conducting taste fibers in these three nerves unite in the gustatory portion of the **nucleus of the tractus solitarius (NTS)** in the medulla oblongata (Figure 9–6). From there, axons of second-order neurons ascend in the ipsilateral medial lemniscus and project directly to the ventral **posteromedial nucleus of the thalamus.** From the thalamus, the axons of the third-order neurons pass to neurons in the **anterior insula** and the frontal operculum in the ipsilateral cerebral cortex. This region is rostral to the face area of the postcentral gyrus, which is probably the area that mediates conscious perception of taste and taste discrimination.

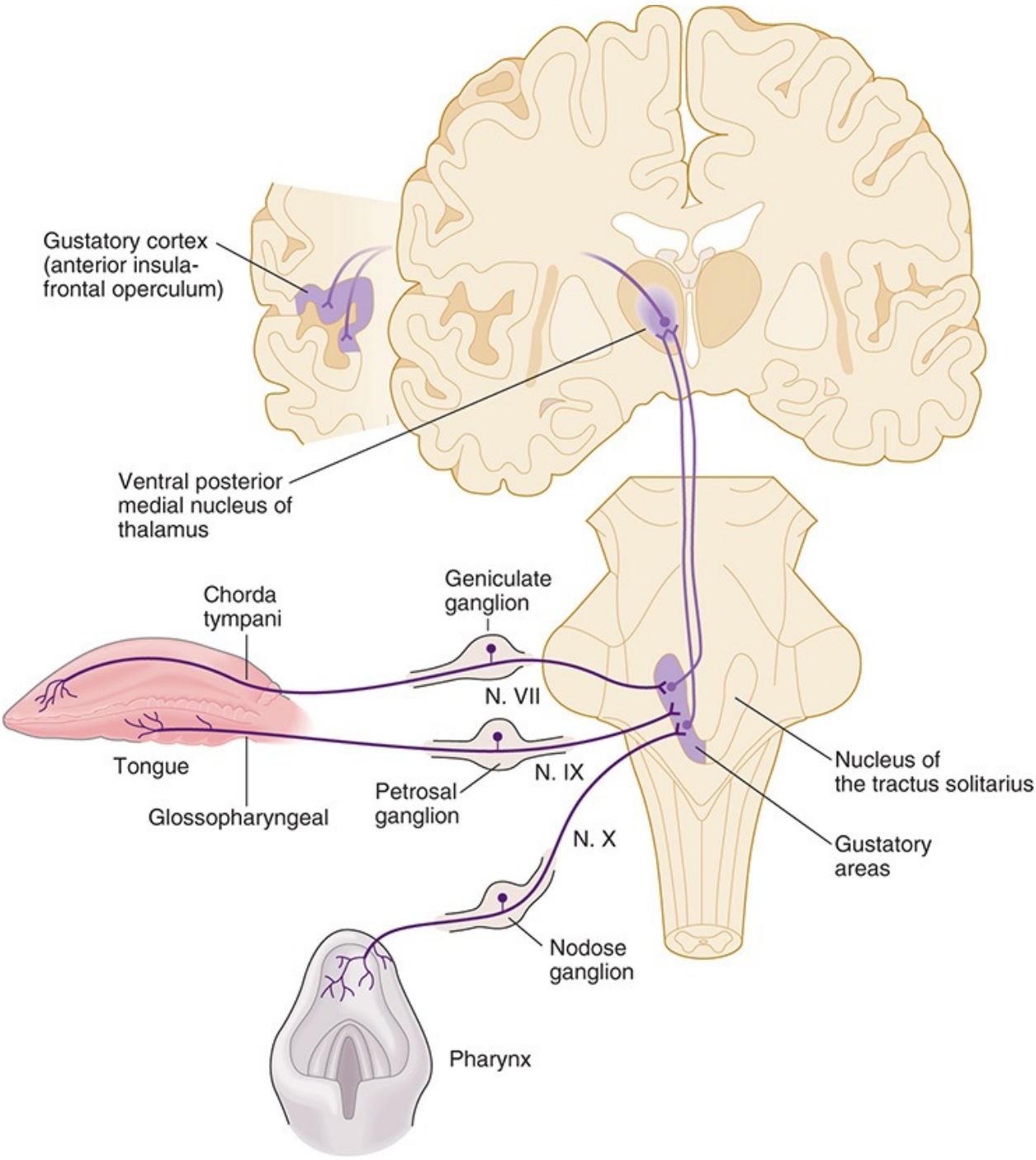


FIGURE 9–6 Diagram of taste pathways. Signals from the taste buds travel via different nerves to gustatory areas of the nucleus of the tractus solitarius, which relays information to the thalamus; the thalamus projects to the gustatory cortex. (Modified with permission from Kandel ER, Schwartz JH, Jessell TM [editors]: *Principles of Neural Science*, 4th ed. New York, NY: McGraw-Hill; 2000.)

Sensory fibers in the trigeminal (5th cranial) nerve also innervate the tongue and contribute to the burning sensation experienced when we eat foods containing capsaicin. Taste buds are surrounded by TRPV1 receptors on trigeminal nociceptive fibers that are activated in response to eating spicy foods.

TASTE MODALITIES, RECEPTORS, & TRANSDUCTION

Humans have five basic taste modalities: **salt, sweet, sour, bitter,** and **umami.** Common stimuli for these sensory modalities are sodium chloride, sucrose, hydrochloric acid, quinine, and monosodium glutamate, respectively. All tastants are sensed from all parts of the tongue and adjacent structures. Afferent nerves to the NTS contain fibers from all types of taste receptors, without any clear localization of types. An individual taste receptor cell may respond to more than one type of tastant. The central nervous system can distinguish the various tastes from one another because each type of taste receptor cell connects to a particular gustatory axon.

Figure 9–7 shows the putative receptors for the five modalities of taste. They include the two major types of receptors: **ligand-gated channels** (ionotropic receptors) and GPCRs (metabotropic receptors). Salt and sour tastes are triggered by activation of ionotropic receptors; sour, bitter, and umami tastes are triggered by activation of metabotropic receptors. Many GPCRs in the human genome are taste receptors (T1Rs, T2Rs families).

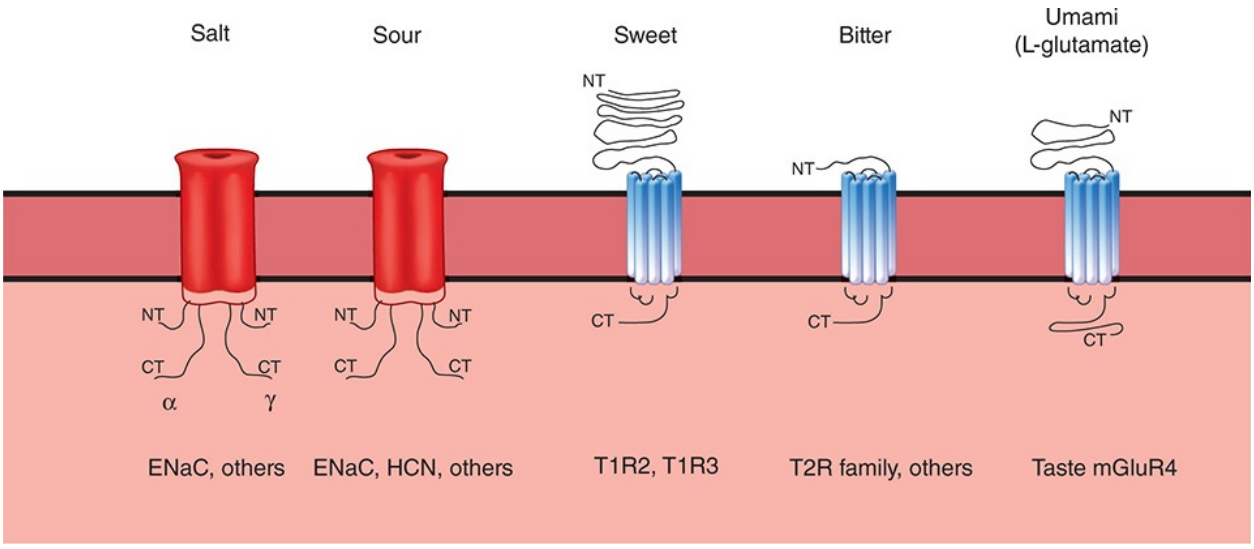


FIGURE 9–7 Signal transduction in taste receptors. Salt and sour tastes are

mediated via the epithelial sodium channel (ENaC). This receptor has two subunits (α and γ), each crossing the membrane twice, resulting in intracellular N and C termini (NT, CT). Salt is sensed following Na^+ movement; sour is mediated by movement of H^+. Sweet, bitter, and umami tastes are sensed via G-protein-coupled receptors that span the membrane seven times and have varying lengths of CT and NT (represented as ribbon structures). Sweet tastes are detected by the T1R2 and T1R3 families; bitter and umami tastes are detected by the T2R family and mGluR4, respectively.

Salt-sensitive taste is mediated by an **epithelial sodium channel (ENaC).** The entry of Na^+ into the salt receptor depolarizes the membrane, generating a receptor potential. The sour taste is triggered by protons (H^+ ions). ENaCs permit the entry of protons and may contribute to the sensation of sour taste. The H^+ ions can also bind to and block a K^+-sensitive channel. The fall in K^+ permeability can depolarize the membrane. Also, a hyperpolarization-activated cyclic nucleotide-gated cation channel (HCN) and other mechanisms may contribute to sour transduction.

Substances that taste sweet are detected by at least two types of GPCRs, T1R2 and T1R3. Sugars taste sweet, but so do compounds such as saccharin that have an entirely different structure. Natural sugars such as sucrose and synthetic sweeteners may act on gustducin via different receptors. Sweet-responsive receptors act via cyclic nucleotides and inositol phosphate metabolism.

Bitter taste is produced by a variety of unrelated compounds. Many of these are poisons, and bitterness serves as a warning to avoid them. Some bitter compounds (eg, quinine) are membrane permeable and bind to and block K^+-selective channels. Many bitter tastants (eg, strychnine) bind to GPCRs (T2R family) that couple to the heterotrimeric G-protein, **gustducin**. Gustducin lowers cAMP and increases the formation of inositol phosphates (IP_3), which releases Ca^{2+} to trigger depolarization.

Umami tastants activate a receptor comprised of T1R1 and T1R3. Umami taste may also involve the activation of a truncated metabotropic glutamate receptor, **mGluR4,** in the taste buds.

TASTE THRESHOLDS & INTENSITY DISCRIMINATION

The ability of humans to discriminate differences in the intensity of tastes is

relatively crude. A 30% change in the concentration of the tastant is necessary before a difference can be detected. **Taste threshold** refers to the minimum concentration at which a substance can be perceived. Table 9–1 shows the threshold concentration of various substances needed for a taste bud to respond. Bitter substances tend to have the lowest threshold. Some toxic substances such as strychnine have a bitter taste at very low concentrations, preventing accidental ingestion of this chemical, which causes fatal convulsions. Some common abnormalities in taste detection are described in Clinical Box 9–2.

TABLE 9–1 Some taste thresholds.

Substance	Taste	Threshold Concentration (μmol/L)
Hydrochloric acid	Sour	100
Sodium chloride	Salt	2000
Strychnine hydrochloride	Bitter	1.6
Glucose	Sweet	80,000
Sucrose	Sweet	10,000
Saccharin	Sweet	23

CLINICAL BOX 9–2

Abnormalities in Taste Detection

Ageusia (absence of the sense of taste) and **hypogeusia** (diminished taste sensitivity) can be caused by damage to the lingual or glossopharyngeal nerve. Neurological disorders such as vestibular schwannoma, Bell palsy, familial dysautonomia, multiple sclerosis, certain infections (eg, primary ameboid meningoencephalopathy), and poor oral hygiene can also cause problems with taste sensitivity. Ageusia can be an adverse side effect of various drugs, including cisplatin and captopril, or vitamin B_3 or zinc deficiencies. Aging and tobacco abuse also contribute to diminished taste. **Dysgeusia** or **parageusia** (unpleasant perception of taste) causes a metallic, salty, foul, or rancid taste. In many cases, dysgeusia is a temporary problem.

Factors contributing to ageusia or hypogeusia can also lead to abnormal taste sensitivity. Taste disturbances can also occur under conditions in which **serotonin (5-HT)** and **norepinephrine (NE)** levels are altered (eg, during anxiety or depression). This implies that these neuromodulators contribute to the determination of **taste thresholds.** Administration of a **5-HT reuptake inhibitor** reduces sensitivity to sucrose (sweet taste) and quinine (bitter taste). In contrast, administration of an **NE reuptake inhibitor** reduces bitter taste and sour thresholds. About 25% of the population has a heightened sensitivity to taste, in particular to bitterness. These individuals are called **supertasters;** this may be due to the presence of an increased number of fungiform papillae on their tongue.

THERAPEUTIC HIGHLIGHTS

Improved oral hygiene and adding zinc supplements to one's diet can correct the inability to taste in some individuals.

CHAPTER SUMMARY

- The olfactory epithelium in the upper portion of the nasal cavity contains three types of cells involved in olfaction: olfactory sensory neurons that are responsible for olfactory transduction, supporting cells that secrete the mucus that provides the appropriate molecular and ionic environment for odor detection, and basal stem cells that generate new olfactory sensory neurons to replace those damaged by exposure to the environment.
- Processing of olfactory information occurs in the olfactory bulb where the axons of olfactory sensory neurons synapse on mitral cells and tufted cells to form olfactory glomeruli. The olfactory bulb also contains inhibitory periglomerular cells and granule cells which make reciprocal synapses with mitral and tufted cells.
- The olfactory system can discriminate perhaps more than 1 million distinct odors due in part to the existence of 400 functional odorant genes (receptors). Each olfactory sensory neuron expresses only one of the 400 functional olfactory genes, but each odorant can bind to a large pool of odorant receptors.
- Odorant receptors are part of a large family of GPCRs. When an odorant

binds to a receptor, the G-protein subunits dissociate and the α-subunit activates adenylyl cyclase to increase production of cAMP which opens cation channels to increase membrane permeability to Na^+, K^-, and Ca^{2+}. Cl^- channels then open to further depolarize olfactory sensory neurons.

- Projections from mitral and tufted cells travel via the lateral olfactory stria directly to five regions of the olfactory cortex: anterior olfactory nucleus, olfactory tubercle, piriform cortex, amygdala, and entorhinal cortex.
- Taste buds are the specialized sense organs for taste and are composed of epithelial taste cells and basal stem cells. Taste buds are located primarily in the mucosa of the walls of papillae of the tongue, but also in the epiglottis, palate, and pharynx.
- The five major taste modalities are salt, sour, bitter, sweet, and umami. Signal transduction mechanisms include passage through ion channels (ENaC for salt and sour), binding to and blocking ion channels (sour), and binding to GPCRs requiring second messenger systems (T1R2, T1R3 for sweet; T2R for bitter; and mGluR4 for umami).
- The afferents from taste buds in the anterior two-thirds of the tongue travel via the facial nerve, those from the posterior one-third of the tongue travel via the glossopharyngeal nerve, and those located elsewhere travel via the vagus nerve. All of the sensory fibers from taste buds synapse in the NTS. From there, axons ascend via the ipsilateral medial lemniscus to the ventral posteromedial nucleus of the thalamus, and onto the anterior insula and frontal operculum in the ipsilateral cerebral cortex.
- Disorders of olfaction include anosmia (inability to smell), hyposmia (diminished olfactory sensitivity), hyperosmia (enhanced olfactory sensitivity), and dysosmia (distorted sense of smell). Causes include damage to the olfactory nerve, tumors, respiratory tract infections, and poor dental hygiene. Disorders of taste include ageusia (absence of the sense of taste), hypogeusia (diminished taste sensitivity), and dysgeusia (unpleasant perception of taste). Causes include damage to the facial or glossopharyngeal nerve, neurological disorders, drugs, vitamin deficiencies, and poor oral hygiene.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. A young boy was diagnosed with congenital anosmia, a rare disorder in which an individual is born without the ability to smell. Which parts of the nervous system might be defective in an individual with congenital anosmia to account for the inability to detect odors?

   A. Glossopharyngeal nerve, olfactory bulb, ventral posterior medial nucleus of the thalamus, and anterior insula-frontal operculum

   B. Olfactory sensory neurons, olfactory glomeruli, nucleus of the tractus solitarius, and ventral posterior lateral nucleus of the thalamus

   C. Olfactory nerve, olfactory bulb, medial olfactory tract, and anterior insula-frontal operculum

   D. Olfactory sensory neuron, 1st cranial nerve, olfactory glomeruli, and frontal cortex

   E. Trigeminal nerve, olfactory glomeruli, lateral olfactory tubercle, and entorhinal cortex

2. While working in a laboratory studying the olfactory system, a medical student was intrigued by the fact that a simple sense organ like the human olfactory epithelium can discriminate perhaps more than 1 million distinct odors. What factors may contribute to this phenomenon?

   A. There are 500 types of odorant receptors and over 1000 types of odorant-binding proteins that sequester odorants to enhance sensory discrimination.

   B. Each olfactory sensory neuron expresses a single odorant receptor gene and projects to a particular subset of mitral cells that connect to distinct parts of the olfactory cortex.

   C. Odorants bind to a mixture of GPCR and ion channel receptors on olfactory sensory neurons and the axons of these sensory neurons form anatomically discrete synaptic units called olfactory glomeruli.

   D. Lateral inhibition within olfactory glomeruli sharpen and focus olfactory signals and granule cells within the olfactory glomerulus make specific projections to the postcentral gyrus in the somatosensory cortex.

   E. There are about 5000 types of odorant receptors and each odorant binds to only one of these.

3. As part of a research experience, a medical student was reviewing reports on the effects of exposure to various neurotoxins on odor detection in humans. Which cells in the olfactory system are responsible for the ability to retain the sense of smell despite the fact that toxins can damage elements of the nasal mucosa?

A. Basal cells in the olfactory bulb undergo mitosis to generate new olfactory sensory neurons.

B. Surviving olfactory sensory neurons undergo neuroplasticity and make connections with the mitral and tufted cells that were originally connected to the destroyed sensory neurons.

C. Supporting cells in the olfactory epithelium release neurotrophic factors that stimulate the genesis of new olfactory sensory neurons.

D. Basal cells in the olfactory epithelium regenerate the neurons comprising the 1st cranial nerve.

E. Olfactory sensory neurons in the olfactory bulb are repaired because the surrounding environment contains neurotrophic factors.

4. A 9-year-old boy had frequent episodes of uncontrollable nose bleeds. At the advice of his clinician, he underwent surgery to correct a problem in his nasal septum. A few days after the surgery, he told his mother he could not smell the cinnamon rolls she was baking in the oven. Which of the following sequence of events occurs when an odorant binds to an odorant receptor?

A. Binding of the odorant to a ligand-gated ion channel promotes the influx of Na^+ ions in the olfactory sensory neuron which leads to the induction of an action potential in the olfactory nerve.

B. Binding of the odorant promotes dissociation of G-protein subunits and the γ-subunit activates adenylyl cyclase to increase cGMP which then selectively opens Na^+ channels on the nerve membrane to induce an action potential in the olfactory nerve.

C. When the odorant binds to a combination of ligand-gated ion channels and GPCRs, there is an influx of Ca^{2+} that triggers an influx of Na^+ to induce an action potential in the olfactory sensory neuron.

D. Binding of the odorant to a GPCR on the cilia of the olfactory sensory neurons causes dissociation of the G-protein subunits, the α-subunit activates adenylyl cyclase to increase cAMP which opens cation channels, leading to increased permeability to Na^+, K^-, and Ca^{2+} on the nerve membrane and depolarization of the olfactory nerve.

5. After watching the movie *Christmas Story*, a 10-year-old boy wanted to see if his tongue would really stick to a frozen pole. Much to his surprise, it did. When he pulled his tongue from the pole, he injured the anterior one-third of his tongue. What sensory nerve arises from this portion of the tongue, where are the cell bodies of these sensory neurons, and where does the nerve

terminate?

A. Facial nerve, geniculate ganglion, and gustatory area of the nucleus of the tractus solitarius

B. Vagus nerve, nodose ganglion, and gustatory area of the nucleus ambiguus

C. Chorda tympani branch of the facial nerve, taste buds, and gustatory area of nucleus of the tractus solitarius

D. Glossopharyngeal nerve, petrosal ganglion, and gustatory area of the nucleus ambiguus

E. Glossopharyngeal nerve, taste buds, and caudal portion of the nucleus of the tractus solitarius

6. A 37-year-old woman was diagnosed with multiple sclerosis. One of the potential consequences of this disorder is diminished taste and smell sensitivity. What is the relationship between the sensations of taste and smell?

A. Odorant receptors and taste receptors are both innervated by trigeminal nerves.

B. The afferent fibers from odorant receptors and taste receptors terminate on the same second-order neurons in the brainstem.

C. Odors from food enter our nasal passages at the same time that the taste receptors in our mouth are stimulated by the food, and the two chemosensory systems interact to establish the flavor of what we ingest.

D. Olfaction is closely related to gustation because odorant and taste receptors send signals to adjacent regions of the postcentral gyrus of the cortex which are connected via axon collaterals.

7. A medical student was doing research on gustatory function with a focus on the anatomy and physiology of taste buds. What are the locations and cellular composition of taste buds?

A. Taste buds are located on the sensory endings of the 7th, 9th, and 10th cranial nerves and contain taste receptors, salivary glands, and basal stem cells.

B. Taste buds are located on the dendrites of the 7th, 9th, and 10th cranial nerves and contain papillae, salivary glands, and taste cells.

C. Taste buds are located in the papillae of the tongue and epiglottis and contain tastants, salivary glands, and basal cells.

D. Taste buds are located in the papillae of the tongue and contain tastants, neuroepithelial cells, and the axons of the 7th, 9th, and 10th cranial nerves.

E. Taste buds are located in the papillae of the tongue and contain taste

receptor cells, support cells, and basal cells.

8. A 31-year-old woman is a smoker who has had poor oral hygiene for most of her life. In the past few years she has noticed a reduced sensitivity to the taste of foods which she used to enjoy eating. What types of taste receptors may be damaged if she has difficulty sensing sweet and bitter substances?

   A. Epithelial sodium channel (sweet) and hyperpolarization-activated cyclic nucleotide-gated cation channel (bitter)

   B. Hyperpolarization-activated cyclic nucleotide-gated cation channel (sweet) and epithelial sodium channel (bitter)

   C. T2R family of GPCRs (sweet) and metabotropic glutamate receptor (mGluR4, bitter)

   D. T1R2 and T1R3 family of GPCRs (sweet) and T2R family of GPCRs (bitter)

   E. Metabotropic glutamate receptor (mGluR4, sweet) and epithelial sodium channel (bitter)

CHAPTER 10
Vision

OBJECTIVES

After studying this chapter, you should be able to:

- Identify the function of the various parts of the eye.
- Explain how light rays in the environment are brought to a focus on the retina and the role of accommodation and the pupil light reflex in this process.
- Explain the refractive deficits responsible for hyperopia, myopia, presbyopia, and astigmatism.
- Describe the functional organization of the retina.
- List the sequence of events involved in phototransduction.
- Describe the electrical responses produced by bipolar cells, horizontal cells, amacrine cells, and ganglion cells.
- Define dark adaptation, visual acuity, and age-related macular degeneration.
- Trace the neural pathways that transmit visual information from photoreceptors to the visual cortex.
- Describe the neural pathways involved in color vision and the types of color blindness.
- Predict the visual field deficits that would occur after lesions within specific

parts of the visual pathway.

- Identify the muscles involved in the four types of eye movements and the function of these movements.

INTRODUCTION

The eyes are complex sense organs that have evolved from primitive light-sensitive spots on the surface of invertebrates. They gather information about the environment; the brain interprets this information to form an image of what appears within the field of vision. Within its protective casing, each eye has a layer of photoreceptors that respond to light, a lens system that focuses the light on these receptors, and a system of nerves that conducts impulses from the receptors to the brain. A great deal of work has been done on the neurophysiology of vision; in fact, it may be the most studied and best understood sensory system. This chapter describes the way the components of the visual system operate to set up conscious visual images.

ANATOMY OF THE EYE

The principal structures of the eye are shown in **Figure 10–1**. The outer white protective layer of the eyeball is the **sclera** through which no light can pass. It is modified anteriorly to form the transparent **cornea,** through which light rays enter the eye. The lateral margin of the cornea is contiguous with the **conjunctiva,** a clear mucous membrane that covers the sclera. Just inside the sclera is the **choroid,** a vascular layer that provides oxygen and nutrients to the structures in the eye. The **retina,** the neural tissue containing the **photoreceptors,** lines the posterior two-thirds of the choroid.

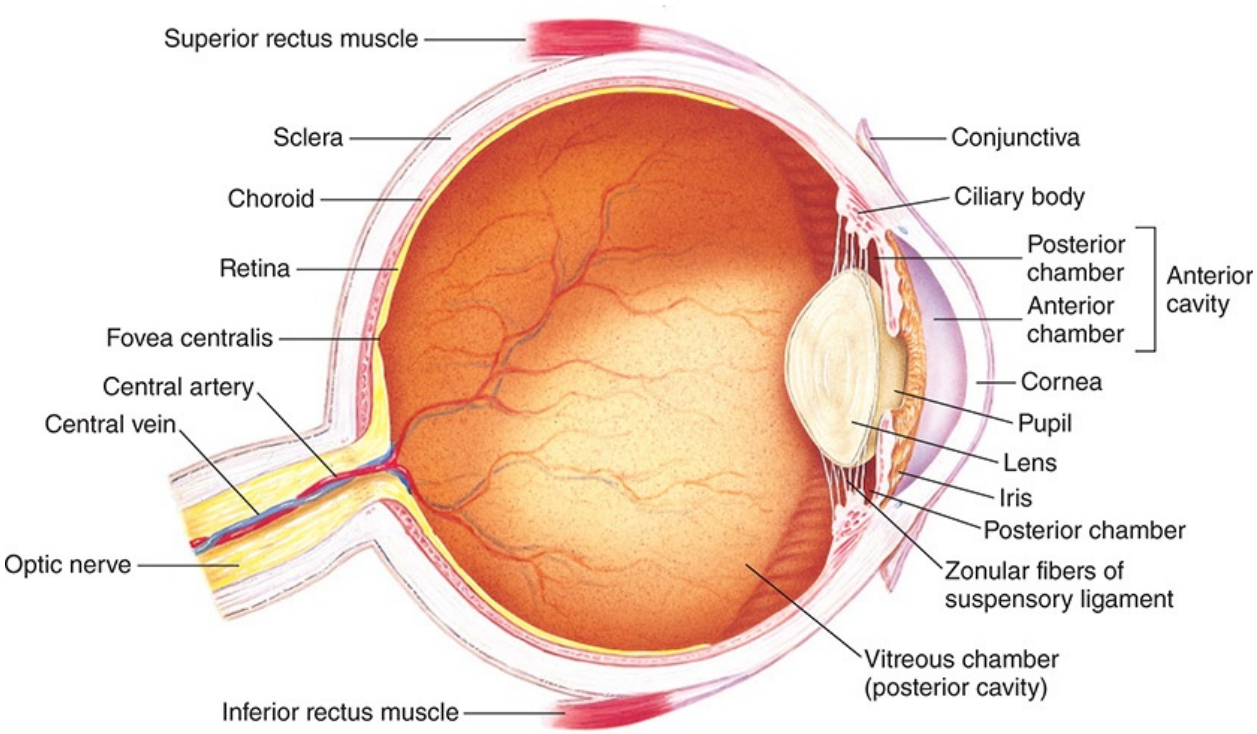


FIGURE 10–1 A schematic of the anatomy of the eye. (Reproduced with permission from Fox SI: *Human Physiology*. New York, NY: McGraw-Hill; 2008.)

The **crystalline lens** is a transparent structure held in place by a circular **lens suspensory ligament (zonule).** The zonule is attached to the **ciliary body** that contains circular muscle fibers and longitudinal muscle fibers that attach near the corneoscleral junction. The pigmented and opaque **iris,** the colored portion of the eye, is in front of the lens. The iris, ciliary body, and choroid are collectively called the **uvea.** The iris contains sphincter muscles that constrict **(miosis)** and radial muscles that dilate **(mydriasis)** the **pupil** that are under the control of parasympathetic and sympathetic nerves, respectively (see Chapter 13). Variations in the diameter of the pupil can produce up to a 16-fold change in the amount of light reaching the retina.

The **aqueous humor** is a clear protein-free liquid that nourishes the cornea and iris; it is produced in the ciliary body by diffusion and active transport from plasma. It flows through the pupil and fills the **anterior chamber** of the eye. It is normally reabsorbed through a network of trabeculae into the **canal of Schlemm,** a venous channel at the junction between the iris and the cornea **(filtration angle).** Obstruction of this outlet leads to increased **intraocular pressure (IOP),** a critical risk factor for glaucoma **(Clinical Box 10–1)**.

The **posterior chamber** is a narrow aqueous-containing space between the iris, zonule, and the lens. The **vitreous chamber** is the space between the lens and the retina that is filled primarily with a clear gelatinous material called the **vitreous (vitreous humor).**

The eye is well protected from injury by the bony walls of the orbit. The cornea is moistened and kept clear by tears that course from the **lacrimal gland** in the upper portion of each orbit across the surface of the eye to empty via the **lacrimal duct** into the nose. Blinking helps keep the cornea moist.

THE IMAGE-FORMING MECHANISM

The eyes convert energy in the visible spectrum into action potentials in the optic nerve. The wavelength of visible light ranges from 397 to 723 nm. The images of objects in the environment are focused on the retina. The light rays striking the retina generate potentials in the photoreceptors. Impulses initiated in the retina are conducted to the cerebral cortex, where they produce the sensation of vision.

PRINCIPLES OF OPTICS

Light rays are bent when they pass from a medium of one density into a medium of a different density, except when they strike perpendicular to the interface **(Figure 10–2)**. The bending of light rays is called **refraction** and is the mechanism that allows one to focus an accurate image onto the retina. Parallel light rays striking a biconvex lens are refracted to a point **(focal point** or **principal focus)** behind the lens. The focal point is on a line passing through the centers of curvature of the lens, the **principal axis.** The distance between the lens and the principal focus is the **focal length.** Light rays from an object that strike a lens more than 6 m (20 ft) away are, for practical purposes, parallel. The rays from an object closer than 6 m are diverging and are therefore brought to a focus farther back on the principal axis than the principal focus. Biconcave lenses cause light rays to diverge.

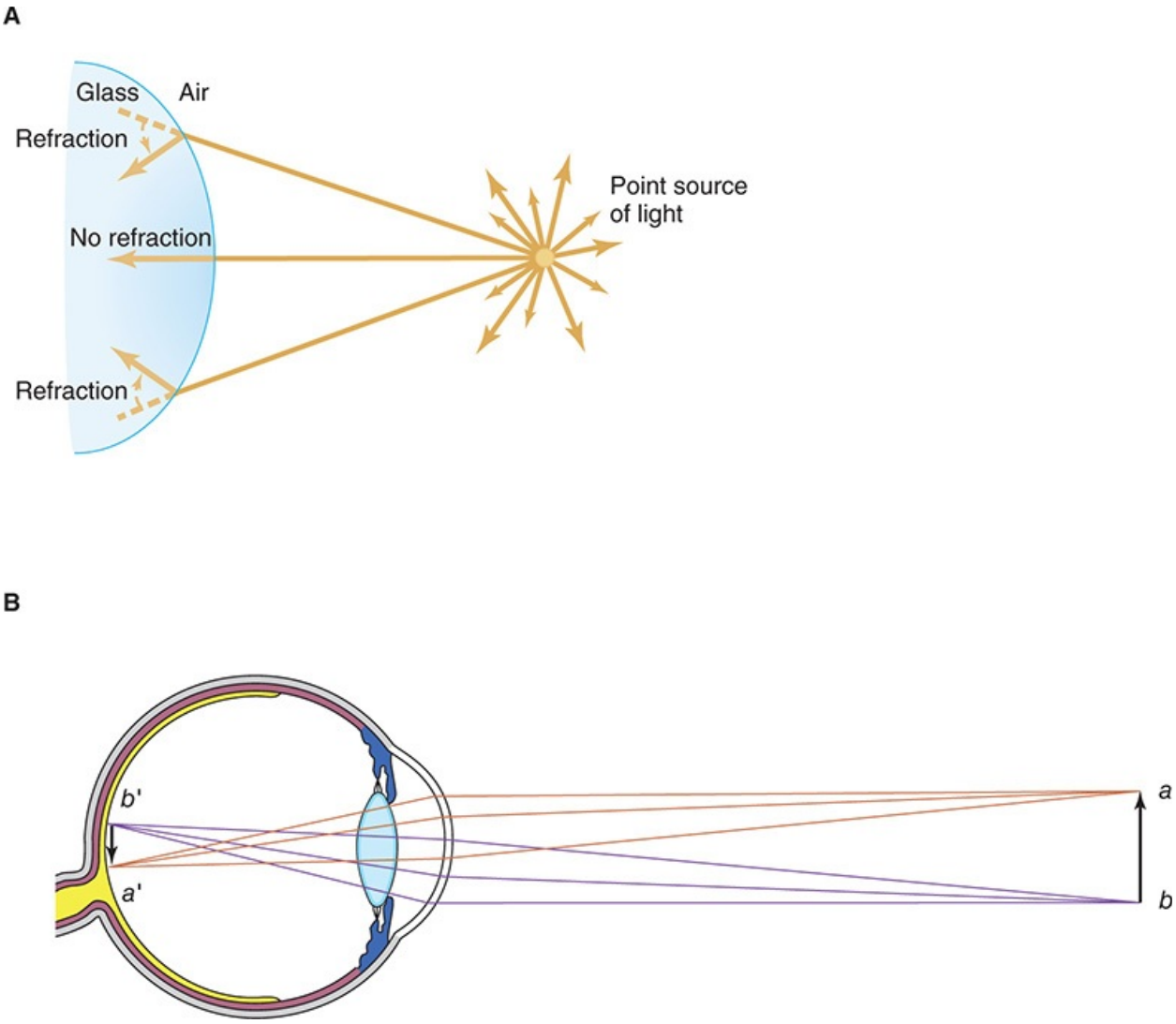


FIGURE 10–2 Focusing point sources of light. A) When diverging light rays enter a dense medium at an angle to its convex surface, refraction bends them inward. **B)** Refraction of light by the lens system. For simplicity, refraction is shown only at the corneal surface (site of greatest refraction) although it also occurs in the lens and elsewhere. Incoming light from *a* (above) and *b* (below) is bent in opposite directions, resulting in *b*′ being above *a*′ on the retina. (Reproduced with permission from Widmaier EP, Raff H, Strang KT: *Vander's Human Physiology*, 11th ed. New York, NY: McGraw-Hill; 2008.)

Refractive power is greatest when the curvature of a lens is greatest. The refractive power of a lens is measured in **diopters,** the number of diopters being the reciprocal of the principal focal distance in meters. For example, a lens with a principal focal distance of 0.25 m has a refractive power of 1/0.25, or 4

diopters. The human eye has a refractive power of 60 diopters at rest.

CLINICAL BOX 10–1

Glaucoma

IOP is not the only cause of **glaucoma,** a degenerative disease in which there is loss of retinal ganglia cells; however, it is a critical risk factor. In 20–50% of the patients with glaucoma, IOP is normal (10–20 mm Hg); however, increased IOP worsens glaucoma, and treatment is aimed at lowering the pressure. Indeed, elevations in IOP due to injury or surgery can cause glaucoma. Glaucoma is caused by poor drainage of the aqueous humor through the filtration angle formed between the iris and the cornea. **Open-angle glaucoma,** a chronic disease, is caused by decreased permeability through the trabeculae into the canal of Schlemm, which leads to an increase in IOP. In some cases, this type of glaucoma is due to a genetic defect. **Closed-angle glaucoma** results from a forward ballooning of the iris so that it reaches the back of the cornea and obliterates the filtration angle, thus reducing the outflow of aqueous humor. If left untreated, glaucoma can lead to blindness.

THERAPEUTIC HIGHLIGHTS

Glaucoma can be treated with agents that decrease the secretion or production of aqueous humor or with drugs that increase outflow of the aqueous humor. β-Adrenoceptor antagonists such as **timolol** decrease the secretion of aqueous fluid. Carbonic anhydrase inhibitors (eg, **dorzolamide, acetazolamide**) also exert their beneficial effects by decreasing the secretion of aqueous humor. Glaucoma can also be treated with cholinergic agonists (eg, **pilocarpine, carbachol, physostigmine**) that increase aqueous outflow by causing ciliary muscle contraction. Aqueous outflow is also increased by **prostaglandins.** Prolonged use of **corticosteroids** can lead to glaucoma and increase the risk of occurrence of ocular infections due to fungi or viruses. A common treatment is a combination of a β-blocker to reduce secretion and a prostaglandin to increase outflow.

In the eye, light is refracted at the anterior surface of the cornea and at the

anterior and posterior surfaces of the lens. The process of refraction can be represented diagrammatically, however, without introducing any appreciable error, by drawing the rays of light as if all refraction occurs at the anterior surface of the cornea (Figure 10–2). Note that the retinal image is inverted. The connections of the retinal receptors are such that from birth any inverted image on the retina is viewed right side up and projected to the visual field on the side opposite to the retinal area stimulated. This perception is present in infants and is innate. If retinal images are turned right side up by means of special lenses, the objects viewed look as if they are upside down.

COMMON DEFECTS OF THE IMAGE-FORMING MECHANISM

Table 10–1 shows some of the common tests included in a comprehensive ophthalmology exam in an adult. In some individuals, the eyeball is shorter than normal, and the parallel rays of light are brought to a focus behind the retina. This abnormality is called **hyperopia** or farsightedness **(Figure 10–3)**. Sustained accommodation, even when viewing distant objects, can partially compensate for the defect, but the prolonged muscular effort is tiring and may cause headaches and blurred vision. The prolonged convergence of the visual axes associated with the accommodation may lead eventually to **strabismus** **(Clinical Box 10–2)**. The defect can be corrected by using glasses with convex lenses, which aid the refractive power of the eye in shortening the focal distance.

TABLE 10–1 Components of a Comprehensive Ophthalmology Exam.

Assessment	Method	Result
Visual acuity	Snellen chart	Nearsightedness, farsightedness, astigmatism
Refraction	Phoropter; retinoscope	Lens power needed for vision correction
Color blindness	Ishihara chart	Protanopia, deuteranopia, or tritanopia
Pupillary light reflex	Penlight	Test integrity of sensory and motor innervation of the eye; aniscoria (uneven pupil size)
Intraocular pressure	Goldmann applanation tonometry	Risk factor for glaucoma
Optic nerve, macula, vessel integrity	Fundoscope, opthalmascope	Macular degeneration, retinal tear
Eyelids, cornea, conjunctiva, sclera, iris	Slit lamp	Cataract, corneal injury, dry eye syndrome, retinal detachment, macular degeneration, retinal vessel occlusion, diabetic retinopathy
Peripheral vision	Perimeter, penlight	Map of peripheral portions of the visual fields
Central vision	Tangent screen	Map of central visual field; identify objective scotoma (blind spots)
Extraocular muscle motility/alignment	Check six cardinal positions of gaze	Heterotropia

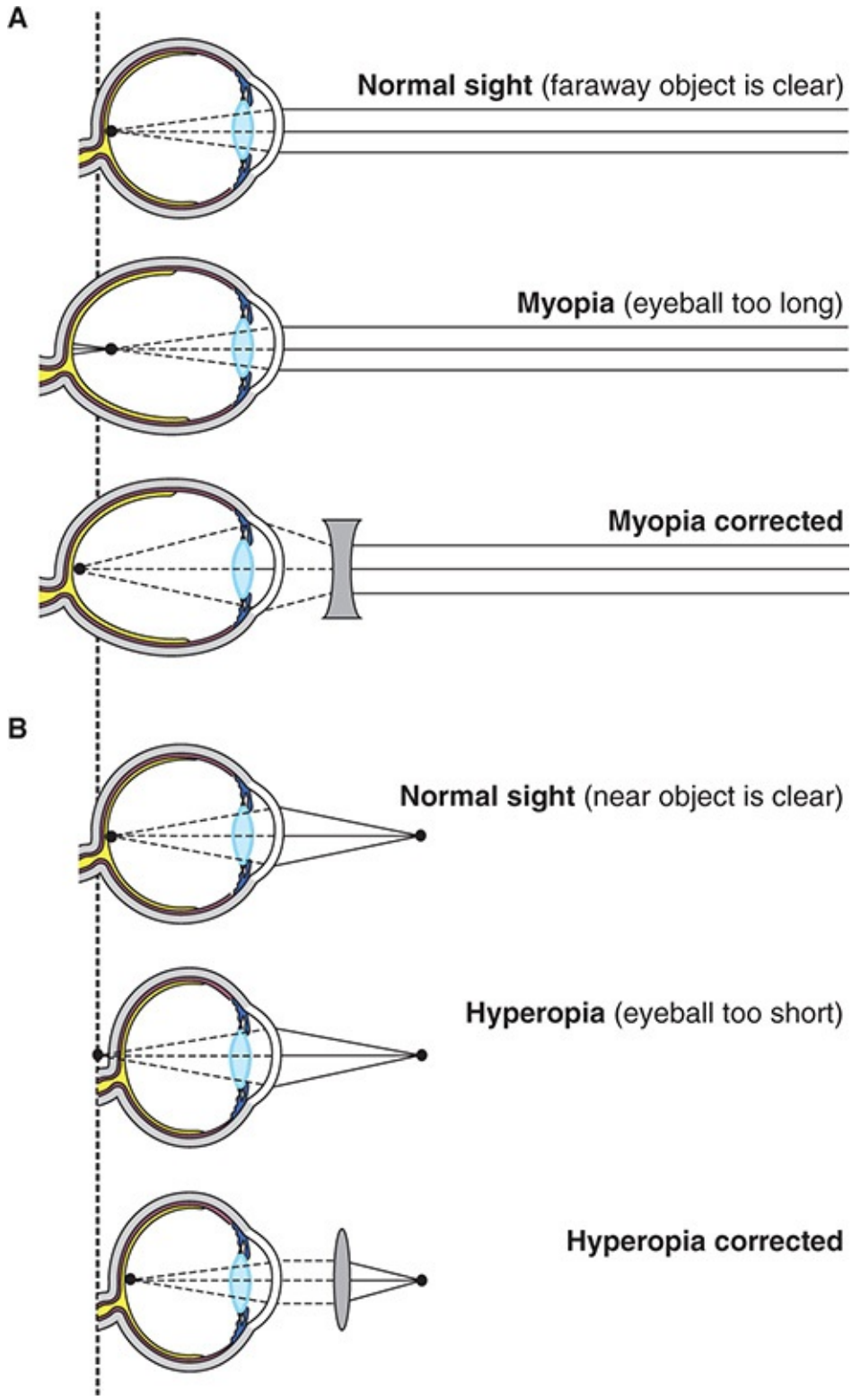


FIGURE 10–3 Common defects of the optical system of the eye. A) In myopia (nearsightedness), the eyeball is too long and light rays focus in front of the retina. Placing a biconcave lens in front of the eye causes the light rays to

diverge slightly before striking the eye, so that they are brought to a focus on the retina. **B)** In hyperopia (farsightedness), the eyeball is too short and light rays come to a focus behind the retina. A biconvex lens corrects this by adding to the refractive power of the lens of the eye. (Reproduced with permission from Widmaier EP, Raff H, Strang KT: *Vander's Human Physiology*, 11th ed. New York, NY: McGraw-Hill; 2008.)

In **myopia** (nearsightedness), the anteroposterior diameter of the eyeball is too long (Figure 10–3). Myopia may be genetic in origin; however, there is a positive correlation between sleeping in a lighted room before the age of 2 and the subsequent development of myopia. Thus, the shape of the eye appears to be determined in part by the refraction presented to it. In young adults, the extensive close work involved in activities such as studying accelerates the development of myopia. This defect can be corrected by glasses with biconcave lenses, which make parallel light rays diverge slightly before they strike the eye.

Astigmatism is a common condition in which the curvature of the cornea is not uniform. When the curvature in one meridian is different from that in others, light rays in that meridian are refracted to a different focus, so that part of the retinal image is blurred. A similar defect may be produced if the lens is pushed out of alignment or the curvature of the lens is not uniform, but these conditions are rare. Astigmatism can usually be corrected with cylindric lenses placed in such a way that they equalize the refraction in all meridians.

ACCOMMODATION

When the **ciliary muscle** is relaxed, parallel light rays striking the optically normal **(emmetropic)** eye are brought to a focus on the retina. As long as this relaxation is maintained, rays from objects closer than 6 m from the observer are brought to a focus behind the retina, and consequently the objects appear blurred. The problem of bringing diverging rays from close objects to a focus on the retina can be solved by increasing the distance between the lens and the retina or by increasing the curvature or refractive power of the lens.

The process by which the curvature of the lens is increased is called **accommodation.** At rest, the lens is held under tension by the lens ligaments. Because the lens substance is malleable and the lens capsule has considerable elasticity, the lens is pulled into a flattened shape. If the gaze is directed at a near object, the ciliary muscle contracts. This decreases the distance between the edges of the ciliary body and relaxes the lens ligaments, so that the lens springs

into a more convex shape **(Figure 10–4)**. The change is greatest in the anterior surface of the lens. In young individuals, the change in shape may add as many as 12 diopters to the refractive power of the eye. The relaxation of the lens ligaments produced by contraction of the ciliary muscle is due partly to the sphincter-like action of the circular muscle fibers in the ciliary body and partly to the contraction of longitudinal muscle fibers that attach anteriorly, near the corneoscleral junction. As these fibers contract, they pull the whole ciliary body forward and inward. This motion brings the edges of the ciliary body closer together. The nearest point to the eye at which an object can be brought into clear focus by accommodation is called the **near point of vision**. The near point recedes throughout life, slowly at first and then rapidly with advancing age, from approximately 9 cm at age 10 to approximately 83 cm at age 60. This recession is due principally to increasing hardness of the lens, with a resulting loss of accommodation due to the steady decrease in the degree to which the curvature of the lens can be increased. By the time a healthy individual reaches age 40–45 years, the loss of accommodation is usually sufficient to make reading and close work difficult. This condition, which is known as **presbyopia**, can be corrected by wearing glasses with convex lenses. In addition to accommodation, the visual axes converge, and the pupil constricts when an individual looks at a near object. This three-part response is called the **near response.**

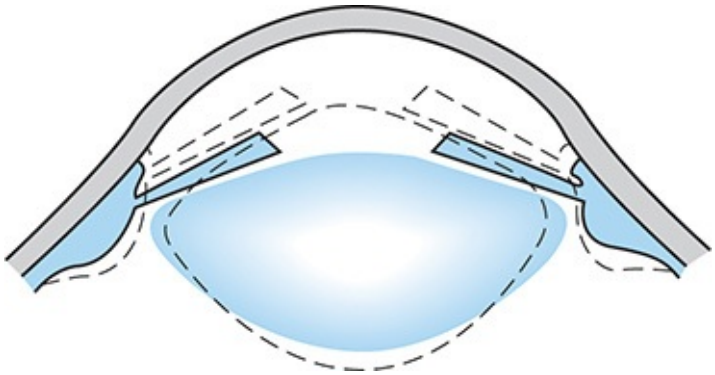


FIGURE 10–4 Accommodation. The solid lines represent the shape of the lens, iris, and ciliary body at rest, and the dashed lines represent the shape during accommodation. When gaze is directed at a near object, ciliary muscles contract. This decreases the distance between the edges of the ciliary body and relaxes the lens ligaments, and the lens becomes more convex.

CLINICAL BOX 10–2

Strabismus & Amblyopia

Strabismus is a misalignment of the eyes and one of the most common eye problems in children, affecting 4% of children under 6 years of age. It is characterized by one or both eyes turning inward (**esotropia**), outward (**exotropia**), upward, or downward. In some cases, more than one of these conditions is present. Strabismus is commonly called “wandering eye” or “crossed-eyes.” It results in visual images that do not fall on corresponding retinal points. When visual images chronically fall on noncorresponding points in the two retinas in young children, one is eventually suppressed **(suppression scotoma).** This suppression is a cortical phenomenon, and rarely develops in adults. It is important to institute treatment before age 6 in affected children, because if the suppression persists, the loss of visual acuity in the eye generating the suppressed image is permanent. A similar suppression with subsequent permanent loss of visual acuity can occur in children in whom vision in one eye is blurred or distorted due to a refractive error. The loss of vision in these cases is called **amblyopia ex anopsia,** a term that refers to uncorrectable loss of visual acuity that is not directly due to organic disease of the eye. Typically, an affected child has one weak eye with poor vision and one strong eye with normal vision. It affects about 3% of the general population. Amblyopia is also referred to as “lazy eye,” and it often coexists with strabismus.

THERAPEUTIC HIGHLIGHTS

Atropine (a cholinergic muscarinic receptor antagonist) and **miotics** such as **echothiophate iodide** can be administered in the eye to correct strabismus and ambylopia. Atropine will blur the vision in the good eye to force the individual to use the weaker eye. Eye muscle training through **optometric vision therapy** has also been proven to be useful, even in patients older than 17 years. Some types of strabismus can be corrected by surgical shortening of some of the eye muscles, by eye muscle training exercises or by using glasses with prisms that bend the light rays sufficiently to compensate for the abnormal position of the eyeball. However, subtle defects in **depth perception** persist. Congenital abnormalities of the visual tracking mechanisms may cause both strabismus and the defective depth perception. In infant monkeys, covering one eye with a patch for 3 months causes a loss of ocular dominance columns; input from the remaining eye spreads to take over all the cortical cells and the patched eye becomes functionally blind. Comparable changes may occur in children with strabismus.

PUPILLARY LIGHT REFLEXES

Light entering one eye constricts the pupil of that eye **(direct light reflex)** as well as the pupil of the other eye **(consensual light reflex).** The sensory fibers initiating this **pupillary light reflex** are in the **optic nerve** (second cranial nerve). The efferent pathway of the reflex is the ipsilateral and contralateral **Edinger-Westphal nuclei** that contain preganglionic parasympathetic neurons within the **oculomotor nerve** (third cranial nerve) that innervate postganglionic neurons in the ciliary ganglion. The reflex not only regulates the amount of light that enters the eye but it also affects the quality of the retinal image (a smaller pupil diameter gives a greater depth of focus).

PHOTO-TRANSDUCTION PROCESS

RETINA

The retina is organized into layers containing different types of cells and neural processes **(Figure 10–5)**. The **outer nuclear layer** contains the photoreceptors **(rods** and **cones).** The **inner nuclear layer** contains the cell bodies of the excitatory and inhibitory interneurons including **bipolar cells, horizontal cells,** and **amacrine cells.** The **ganglion cell layer** contains various types of **ganglion cells** that are the only output neurons of the retina; their axons form the optic nerve. The **outer plexiform layer** is interposed between the outer and inner nuclear layers; the **inner plexiform layer** is interposed between the inner nuclear and ganglion cell layers.

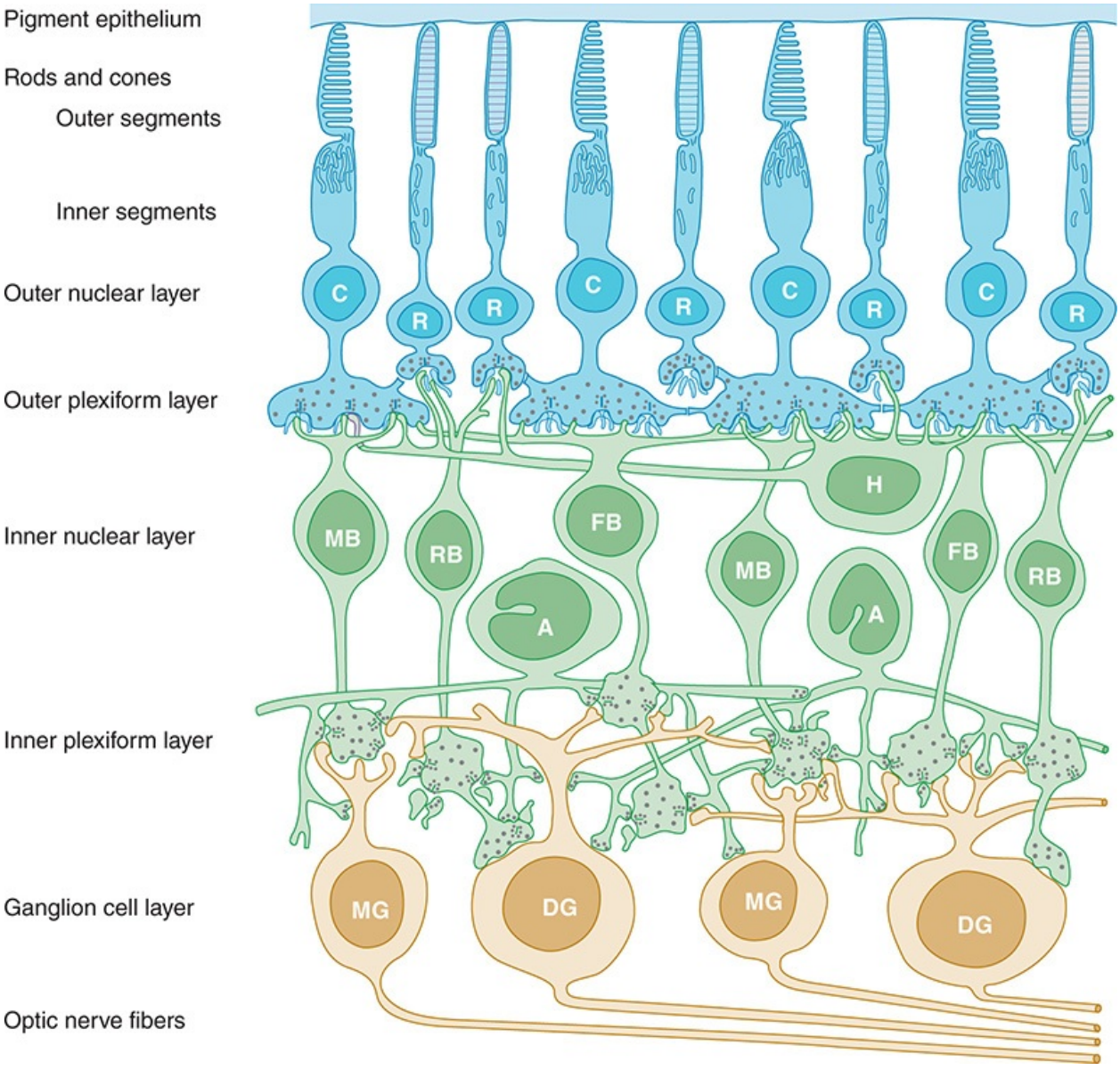


FIGURE 10–5 Neural components of the extrafoveal portion of the retina. C, cone; R, rod; MB, RB, and FB, midget, rod, and flat bipolar cells; DG and MG, diffuse and midget ganglion cells; H, horizontal cells; A, amacrine cells. (Modified with permission from Dowling JE, Boycott BB: Organization of the primate retina: Electron microscopy. Proc R Soc Lond Ser B [Biol] 1966 Nov 15;166(1002):80–111.)

The rods and cones, which are next to the choroid, synapse with bipolar cells; the bipolar cells synapse with ganglion cells. There are various types of bipolar cells that differ in terms of morphology and function. Horizontal cells connect photoreceptor cells to other photoreceptor cells in the outer plexiform layer.

Amacrine cells connect ganglion cells to one another in the inner plexiform layer via processes of varying length and patterns. Amacrine cells also connect with the terminals of bipolar cells. Retinal neurons are also connected via gap junctions.

Because the receptor layer of the retina rests on the **pigment epithelium** next to the choroid, light rays must pass through the ganglion cell and bipolar cell layers to reach the rods and cones. The pigment epithelium absorbs light rays, preventing the reflection of rays back through the retina that would otherwise produce blurring of the visual images.

PHOTORECEPTORS

Each rod and cone photoreceptor is divided into an outer segment, an inner segment that includes a nuclear region, and a synaptic terminal zone **(Figure 10–6)**. The outer segments are modified **cilia** composed of regular stacks of flattened **saccules** or membranous **disks.** The saccules and disks contain the photosensitive compounds that react to light, initiating action potentials in the visual pathways. The inner segments are rich in mitochondria; this is the region that synthesizes the photosensitive compounds. The inner and outer segments are connected by a ciliary stalk through which the photosensitive compounds travel from the inner segment to the outer segment of the rods and cones.

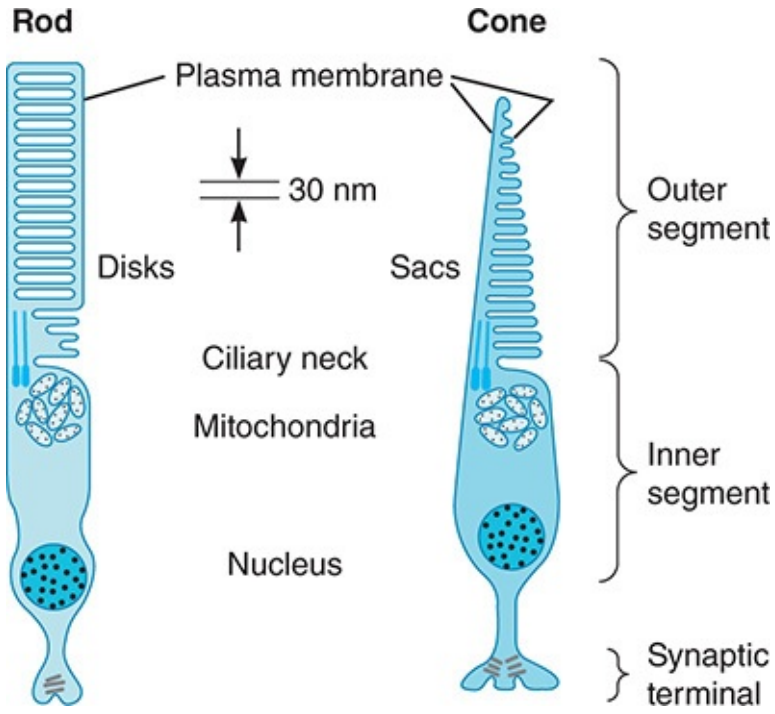


FIGURE 10–6 Schematic diagram of a rod and a cone. Each rod and cone is divided into an outer segment, an inner segment with a nuclear region, and a synaptic zone. The saccules and disks in the outer segment contain photosensitive compounds that react to light to initiate action potentials in the visual pathways. (Reproduced with permission from Lamb TD: Electrical responses of photoreceptors. In: *Recent Advances in Physiology*. No. 10. Baker PF [editor]. Churchill Livingstone, 1984.)

The rods are named for the thin, rodlike appearance of their outer segments. Each rod contains a stack of disk membranes that are flattened membrane-bound intracellular organelles that have detached from the outer membrane and are thus free floating. Cones generally have thick inner segments and conical outer segments, although their morphology varies throughout the retina. The saccules of the cones are formed by infolding of the membrane of the outer segment. Rod outer segments are being constantly renewed by the formation of new disks at the inner edge of the segment and phagocytosis of old disks from the outer tip by cells of the pigment epithelium. Cone renewal is a more diffused process and may occur at multiple sites in the outer segments.

In the extrafoveal portions of the retina, rods predominate **(Figure 10–7)**, and there is a good deal of convergence. *Flat* bipolar cells (Figure 10–5) make synaptic contact with several cones, and *rod* bipolar cells make synaptic contact with several rods. Because there are nearly 6 million cones and 120 million rods in each human eye but only 1.2 million nerve fibers in each optic nerve, the overall convergence of receptors through bipolar cells on ganglion cells is about 105:1. However, there is divergence from this point on. For example, in the visual cortex the number of neurons concerned with vision is 1000 times the number of fibers in the optic nerves.

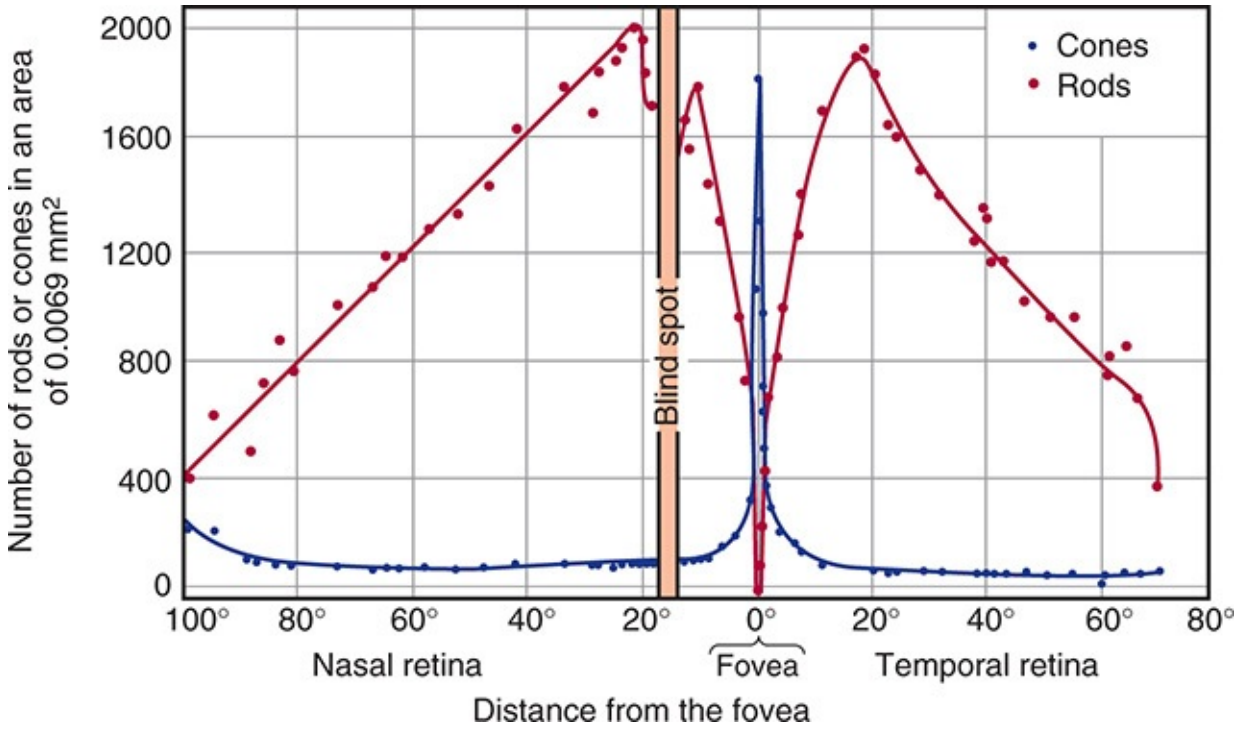


FIGURE 10–7 Rod and cone density along the horizontal meridian through the human retina. A plot of the relative acuity of vision in the various parts of the light-adapted eye would parallel the cone density curve; a similar plot of relative acuity of the dark-adapted eye would parallel the rod density curve.

One of the most important characteristics of the visual system is its ability to function over a wide range of light intensity. When one goes from near darkness to bright sunlight, light intensity increases by 10 log units or a factor of 10 billion. Adjustments in the diameter of the pupil can reduce the fluctuation in intensity; when the diameter is reduced from 8 to 2 mm, its area decreases by a factor of 16 and light intensity at the retina is reduced by more than 1 log unit.

The two types of photoreceptors contribute to our ability to react to fluctuations in intensity. The rods are extremely sensitive to light and are the receptors for night vision **(scotopic vision).** The scotopic visual apparatus resolves the details and boundaries of objects. The cones have a much higher threshold, but the cone system has a much greater acuity and is the system responsible for vision in bright light **(photopic vision)** and for color vision.

THE PHOTORECEPTOR MECHANISM

The potential changes that initiate action potentials in the retina are generated by

the action of light on photosensitive compounds in the rods and cones. When light is absorbed by these substances, their structure changes, and this triggers a sequence of events that initiates neural activity.

The eye is unique in that the receptor potentials of the photoreceptors and the electrical responses of most of the other neural elements in the retina are local, graded potentials, and it is only in the ganglion cells that all-or-none action potentials are generated. The responses of the rods, cones, and horizontal cells are hyperpolarizing, and the responses of the bipolar cells are either hyperpolarizing or depolarizing. Amacrine cells produce depolarizing potentials and spikes that may act as generator potentials for the propagated spikes produced in the ganglion cells.

The cone receptor potential has a sharp onset and offset, whereas the rod receptor potential has a sharp onset and slow offset. The curves relating the amplitude of receptor potentials to stimulus intensity have similar shapes in rods and cones, but the rods are much more sensitive. Therefore, rod responses are proportional to stimulus intensity at levels of illumination that are below the threshold for cones. On the other hand, cone responses are proportional to stimulus intensity at high levels of illumination when the rod responses are maximal and cannot change. Therefore, cones generate good responses to changes in light intensity above background but do not represent absolute illumination well, whereas rods detect absolute illumination.

IONIC BASIS OF PHOTORECEPTOR POTENTIALS

The cGMP-gated cation channels in the outer segments of the rods and cones are open in the dark, so current flows from the inner to the outer segment and to the synaptic ending of the photoreceptor **(Figure 10–8)**. The Na^+, K^+ ATPase in the inner segment maintains ionic equilibrium. Release of synaptic transmitter (glutamate) is steady in the dark. When light strikes the outer segment, the reactions that are initiated close some of the cGMP-gated cation channels to induce a hyperpolarizing receptor potential. The hyperpolarization reduces glutamate release and generates a signal in the bipolar cells that ultimately leads to action potentials in ganglion cells that are transmitted to the brain.

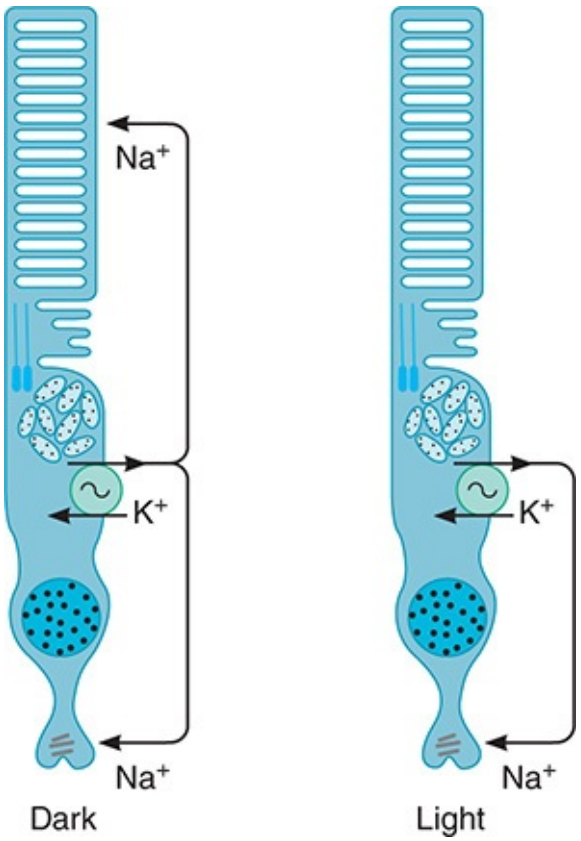


FIGURE 10–8 Effect of light on current flow in visual receptors. In the dark, cGMP-gated cation channels in the outer segment are held open by cGMP. Light leads to increased conversion of cGMP to 5′-GMP, and some of the channels close. This produces hyperpolarization of the synaptic terminal of the photoreceptor.

RHODOPSIN

Rhodopsin (visual purple) is the photosensitive pigment in the rods and is composed of **retinal,** an aldehyde of vitamin A, and the protein **opsin.** Vitamin A is needed for the synthesis of retinal, so a deficiency in this vitamin produces visual abnormalities **(Clinical Box 10–3)**.

Opsin has a molecular weight of 41 kDa and is found in the membranes of the rod disks; it makes up 90% of the total protein in these membranes. Opsin is part of the large family of G-protein-coupled receptors (GPCR). Retinal is parallel to the surface of the membrane **(Figure 10–9)** and is attached to a lysine residue at position 296 in the seventh transmembrane domain.

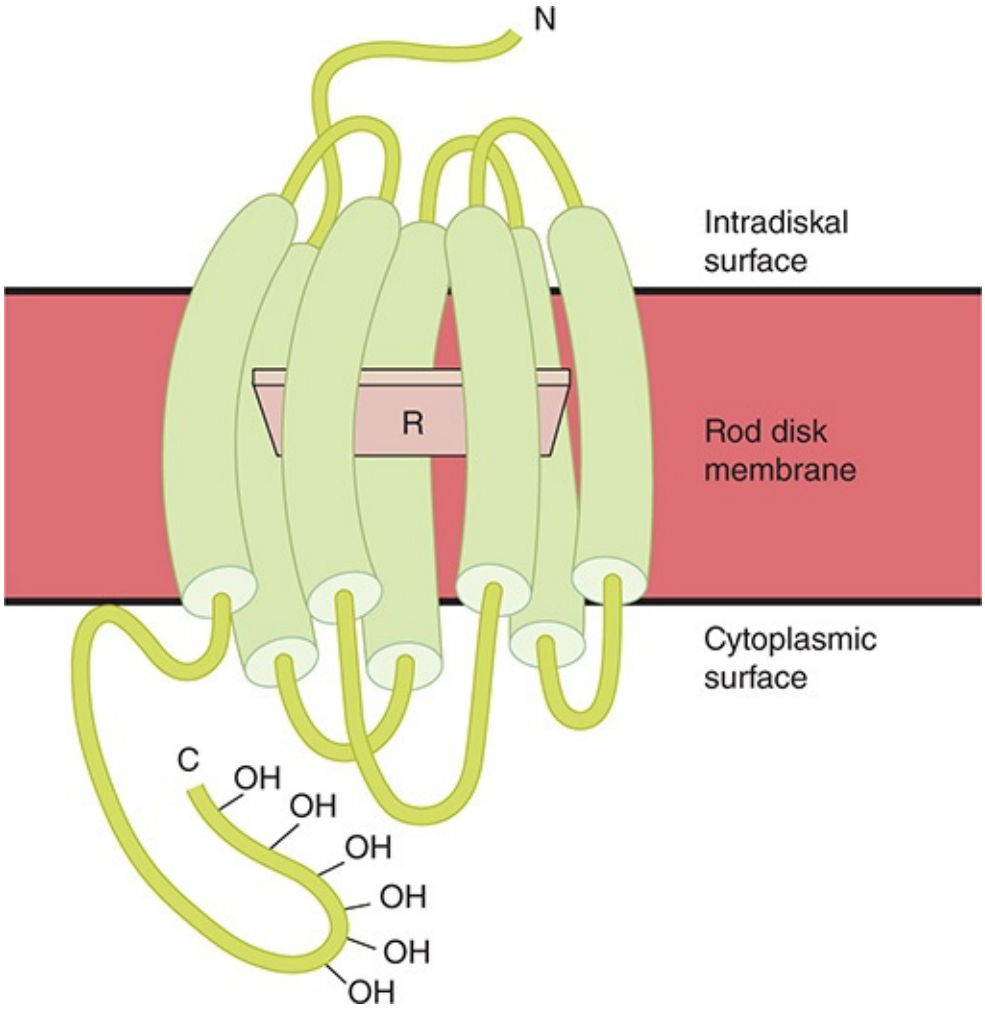


FIGURE 10–9 Diagrammatic representation of the structure of rhodopsin in the rod disk membrane. Rhodopsin is formed when retinal (R) is attached to opsin via a lysine residue at position 296 in the 7th transmembrane domain of the GPCR. GPCR, G-protein-coupled receptor.

CLINICAL BOX 10–3

Vitamin A Deficiency

Vitamin A was the first fat-soluble vitamin identified and is composed of a family of compounds called retinoids. Deficiency is rare in the United States, but it is still a major public health problem in the developing world. Annually, about 80,000 individuals worldwide (mostly children in underdeveloped countries) lose their sight from severe vitamin A deficiency. Vitamin A deficiency is due to inadequate intake of foods high in vitamin A (liver,

kidney, whole eggs, milk, cream, and cheese) or **β-carotene,** a precursor of vitamin A, found in dark green leafy vegetables and yellow or orange fruits and vegetables. One of the earliest visual defects to appear with vitamin A deficiency is night blindness **(nyctalopia).** Vitamin A deficiency also contributes to blindness by causing the eye to become very dry, damaging the cornea **(xerophthalmia)** and retina. Vitamin A first alters rod function, but concomitant cone degeneration occurs as vitamin A deficiency develops. Prolonged deficiency is associated with anatomic changes in the rods and cones followed by degeneration of the neural layers of the retina.

THERAPEUTIC HIGHLIGHTS

Treatment with vitamin A can restore retinal function if given before the receptors are destroyed. Vitamin A–rich foods include liver, chicken, beef, eggs, whole milk, yams, carrots, spinach, kale, and other green vegetables. Other vitamins, especially those of the B complex, are also necessary for the normal functioning of the retina and other neural tissues.

Figure 10–10 summarizes the sequence of events in photoreceptors by which incident light leads to production of a signal in the next succeeding neural unit in the retina. In the dark, the retinal in rhodopsin is in the 11-*cis* configuration. The only action of light is to change the shape of the retinal, converting it to the all-*trans* isomer. This, in turn, alters the configuration of the opsin, and the opsin change activates its associated heterotrimeric G-protein **(transducin)** that has several subunits (Tα, Gβ1, and Gγ1). After 11-*cis* retinal is converted to the all-*trans* configuration, it separates from the opsin in a process called bleaching. This changes the color from the rosy red of rhodopsin to the pale yellow of opsin.

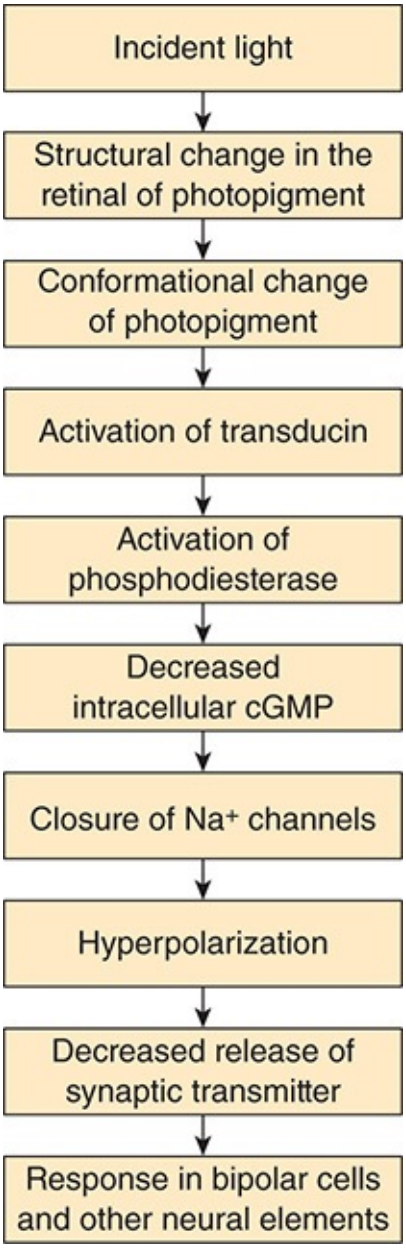


FIGURE 10–10 Sequence of events involved in phototransduction in rods and cones.

Some of the all-*trans* retinal is converted back to the 11-*cis* retinal by retinal isomerase, and then again associates with scotopsin to replenish the rhodopsin supply. Some 11-*cis* retinal is also synthesized from vitaminA. All of these reactions, except the formation of the all-*trans* isomer of retinal, are independent of the light intensity, proceeding equally well in light or darkness. The amount of rhodopsin in the receptors therefore varies inversely with the incident light level.

The G-protein transducin exchanges GDP for GTP, and the α subunit

separates. This subunit remains active until its intrinsic GTPase activity hydrolyzes the GTP. Termination of the activity of transducin is accelerated by its binding of β-arrestin. The α subunit activates cGMP phosphodiesterase to convert cGMP to 5′-GMP.

In darkness, when phosphodiesterase activity is low, cGMP-gated Na^+ channels are maintained in an open state so both Na^+ and Ca^{2+} enter the photoreceptor; the cell is depolarized, and glutamate is released. The light-induced decline in the cytoplasmic cGMP concentration causes some cGMP-gated Na^+ channels to close, reducing the entry of Na^+ and Ca^{2+} and producing the hyperpolarizing potential. This cascade of reactions occurs very rapidly and amplifies the light signal. The amplification helps explain the remarkable sensitivity of rod photoreceptors; these receptors can produce a detectable response to as little as one photon of light.

Calcium ions exert a negative feedback effect on the phototransduction process. In darkness, the relatively high intracellular Ca^{2+} concentration inhibits guanylyl cyclase activity, decreases the activity of the cGMP-gated Na^+ channels, and increases the activity of rhodopsin. The light-induced decrease in Ca^{2+} concentration influences these components of the phototransduction cascade.

PROCESSING OF VISUAL INFORMATION IN THE RETINA

A characteristic of the retinal bipolar and retinal ganglion cells (as well as the lateral geniculate neurons and the neurons in layer 4 of the visual cortex) is that they respond best to a small, circular stimulus and that, within their receptive field, an annulus of light around the center (surround illumination) antagonizes the response to the central spot **(Figure 10–11)**. The center can be excitatory with an inhibitory surround (an **on-center/off-surround cell**) or inhibitory with an excitatory surround (an **off-center/on-surround cell**). The inhibition of the center response by the surround is probably due to inhibitory feedback from one photoreceptor to another mediated via horizontal cells. Thus, activation of nearby photoreceptors by addition of the annulus triggers horizontal cell hyperpolarization, which in turn inhibits the response of the centrally activated photoreceptors. The inhibition of the response to central illumination by an increase in surrounding illumination is an example of **lateral inhibition**—that form of inhibition in which activation of a neural unit is associated with

inhibition of the activity of nearby units. It is a general phenomenon in mammalian sensory systems and helps sharpen the edges of a stimulus and improve discrimination.

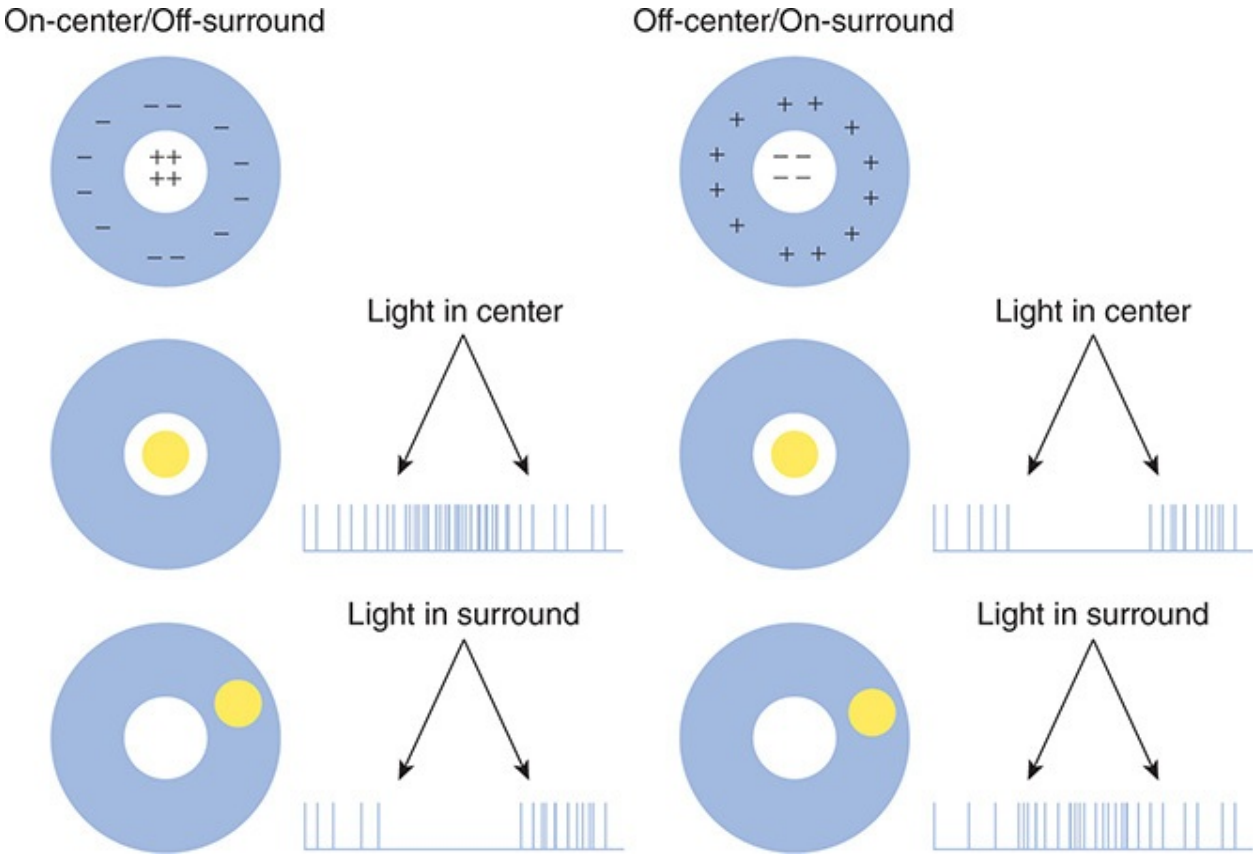


FIGURE 10–11 Responses of two types of retinal ganglion cells to light (yellow circle) focused on a portion of their receptive field. Left side: An *on-center/off-surround* cell responds with an increase in firing rate when the light is placed in the center of the receptive field and with a decrease in firing rate when the light is placed in the surround portion of the receptive field. **Right side:** An *off-center/on-surround* cell responds with a decrease in firing rate when the light is placed in the center and with an increase in firing rate when the light is placed in the surround.

DARK ADAPTATION

If a person spends a considerable length of time in brightly lighted surroundings and then moves to a dimly lighted environment, the retinas slowly become more sensitive to light as the individual becomes “accustomed to the dark.” This

decline in visual threshold is known as **dark adaptation.** It is nearly maximal in about 20 min, although some further decline occurs over longer periods. The time required for dark adaptation depends in part on the time needed to build up the rhodopsin stores that are continuously being broken down in bright light. When one passes suddenly from a dim to a brightly lighted environment, the light seems intensely and even uncomfortably bright until the eyes adapt to the increased illumination and the visual threshold rises. This adaptation occurs over a period of about 5 min and is called **light adaptation,** although it is merely the disappearance of dark adaptation.

The dark adaptation response has two components. The first drop in visual threshold, rapid but small in magnitude, is due to dark adaptation of the cones because when only the foveal, rod-free portion of the retina is tested, the decline proceeds no further. In the peripheral portions of the retina, a further drop occurs because of adaptation of the rods. The total change in threshold between the light-adapted and the fully dark-adapted eye is very great.

Radiologists, aircraft pilots, and others who need maximal visual sensitivity in dim light can avoid having to wait 20 min in the dark to become dark-adapted if they wear red goggles when in bright light. Light wavelengths in the red end of the spectrum stimulate the rods to only a slight degree while permitting the cones to function reasonably well. Therefore, a person wearing red glasses can see in bright light during the time it takes for the rods to become dark-adapted.

VISUAL ACUITY

The optic nerve leaves the eye at a point 3 mm medial to and slightly above the posterior pole of the globe. This region is visible through the ophthalmoscope as the **optic disk** **(Figure 10–12)**. Since there are no visual receptors over the disk, this area of the retina does not respond to light and is known as the **blind spot.** Near the posterior pole of the eye, there is a yellowish pigmented spot called the **macula.** The **fovea** is in the center of the macula; it is a thinned-out, rod-free portion of the retina in which cones are densely packed. Each cone synapses on a single bipolar cell, which, in turn, synapses on a single ganglion cell, providing a direct pathway to the brain. There are very few overlying cells and no blood vessels; thus, the fovea is the point where **visual acuity** is greatest. When attention is attracted to or fixed on an object, the eyes are normally moved so that light rays coming from the object fall on the fovea. **Age-related macular degeneration (AMD)** is a disease in which sharp, central vision is gradually destroyed **(Clinical Box 10–4)**.

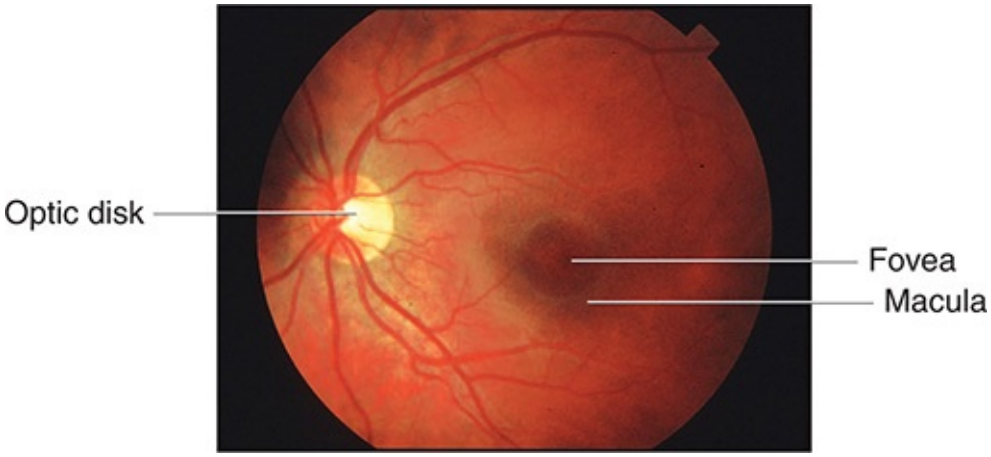


FIGURE 10–12 The fundus of the eye in a healthy human as seen through the ophthalmoscope. The fundus of the eye refers to the interior surface of the eye, opposite the lens, and includes the retina, optic disk, macula and fovea, and posterior pole. Optic nerve fibers leave the eyeball at the optic disk to form the optic nerve. The arteries, arterioles, and veins in the superficial layers of the retina near its vitreous surface can be seen through the ophthalmoscope. (Used with permission of Dr AJ Weber, Michigan State University.)

An ophthalmoscope is used to view the **fundus** which is the interior surface of the eye, opposite to the lens; it includes the retina, optic disk, macula and fovea, and posterior pole (Figure 10–12). The arteries, arterioles, and veins in the superficial layers of the retina near its vitreous surface can be examined. The retinal vessels supply the bipolar and ganglion cells, but the receptors are nourished primarily by the capillary plexus in the choroid. This is why **retinal detachment** is so damaging to the receptor cells. Because this is the one place in the body where arterioles are readily visible, an ophthalmoscope examination is of value in the diagnosis and evaluation of diabetes mellitus, hypertension, and other diseases that affect blood vessels **(Clinical Box 10–5)**.

Glaucoma (Clinical Box 10–1) causes changes in the appearance of the fundus of the eye as seen through an ophthalmoscope **(Figure 10–13)**. The photograph on the left is from a primate with a normal eye and shows an optic disk with a uniform “pinkish” color. The blood vessels appear relatively flat as they cross the margin of the disk. This is because there are a normal number of ganglion cell fibers, and the blood vessels have intact support tissue around them. The photograph on the right is from a primate with glaucoma that was experimentally induced by causing a chronic elevation in intraocular pressure. As is characteristic of glaucomatous optic neuropathy, the disk is pale, especially in the center. The retinal blood vessels are distorted, especially at the disk

margin, due to a lack of support tissue; and there is increased “cupping” of the disk.

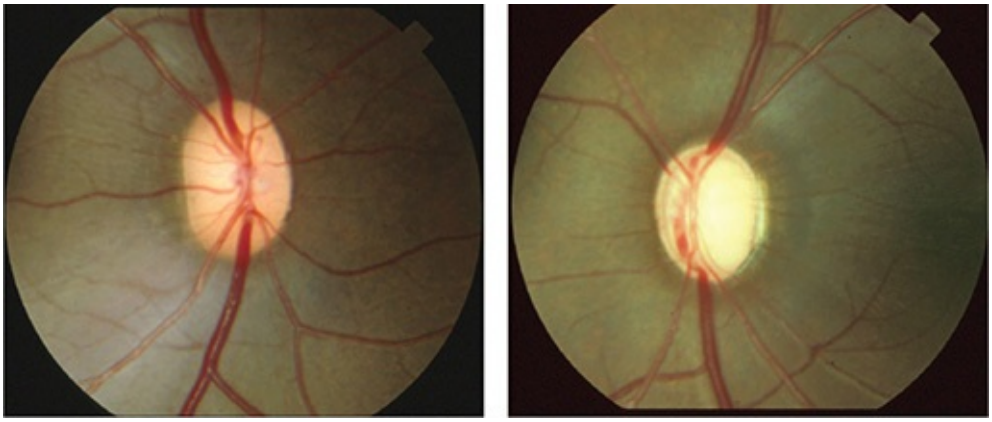


FIGURE 10–13 The fundus of the eye in a normal primate (left) and in a primate with experimentally induced glaucoma (right) as seen through an ophthalmoscope. Normal: uniform “pinkish” color vessels appear relatively flat crossing the margin of disk due to a normal number of ganglion cell fibers and since they have intact support tissue around them. Glaucomatous: disk is pale, especially in center, vessels are distorted, especially at the disk margin due to lack of support tissue and increased “cupping” of the disk. (Used with permission of Dr AJ Weber, Michigan State University.)

CLINICAL BOX 10–4

Visual Acuity and Age-Related Macular Degeneration

Visual acuity is the degree to which the details and contours of objects are perceived, and it is usually defined in terms of the shortest distance by which two lines can be separated and still be perceived as two lines. Visual acuity is often determined by using the **Snellen letter charts** viewed from a distance of 20 ft (6 m). The results are expressed as a fraction; the numerator is 20 (distance from which letters are read) and the denominator is the greatest distance from the chart at which an individual with normal visual acuity can read the line. Normal visual acuity is 20/20; a subject with 20/15 visual acuity has better than normal vision (not farsightedness); and one with 20/100 visual acuity has subnormal vision. Visual acuity is a complex phenomenon and is influenced by many factors, including optical factors (eg, the state of the image-forming mechanisms of the eye), retinal factors (eg, the state of the

cones), and stimulus factors (eg, illumination, brightness of the stimulus, contrast between the stimulus and the background, length of time the subject is exposed to the stimulus). Many drugs can also have adverse side effects on visual acuity. Patients treated with the antiarrhythmic drug **amiodarone** often report corneal changes (**kerotopathy**) including complaints of blurred vision, glare and halos around lights, or light sensitivity. **Aspirin** and other anticoagulants can cause conjunctival or retinal hemorrhaging that can impair vision. **Maculopathy** is a risk factor for those treated with **tamoxifen** for breast cancer. Antipsychotic therapies such as **thioridazine** can cause pigmentary changes that can affect visual acuity, color vision, and dark adaptation.

There are over 20 million individuals in the United States and Europe with age-related macular degeneration or **AMD**, a deterioration of central visual acuity. Nearly 30% of those aged 75 or older have this disorder, and it is the most common cause of visual loss in those over 50 years of age. Women are at greater risk than men for AMD; whites have a greater risk than blacks. There are two types: wet and dry. Wet AMD occurs when fragile blood vessels begin to form under the macula. Blood and fluid leak from these vessels and rapidly damage the macula. Vascular endothelial growth factors (VEGFs) may contribute to the growth of these blood vessels. Dry AMD occurs when the cones in the macula slowly break down, causing a gradual loss of central vision.

THERAPEUTIC HIGHLIGHTS

The US Food and Drug Administration has approved three VEGF inhibitors for the treatment of wet AMD: **ranibizumab** (Lucentis), **bevacizumab** (Avastin), and **aflibercept** (Eylea). **Photodynamic therapy** uses injection of **verteporfin** into the vein in an arm; this drug is activated by a laser light that produces a chemical reaction to destroy abnormal blood vessels. **Laser surgery** can be done to repair damaged blood vessels if they are at a distance from the fovea. However, new vessels may form after the surgery, and vision loss may progress.

CLINICAL BOX 10–5

Eyes Are a Window to Your Health

A thorough exam of the eye (Table 10–1) can reveal a lot about your overall health. In addition to determining your visual acuity and evidence for macular degeneration, a detached retina, or a cataract; an ophthalmologist is able to identify signs of diseases in other organs. **Exophthalmos** (bulging of the eye out of the orbit) can be indicative of **Graves disease** due to an overactive thyroid or an **orbital tumor**. The presence of a gray ring around the cornea **(arcus senilis)** often is linked to high cholesterol and hyperlipidemia. **Ptosis** (a droopy lid) can be evidence of **myasthenia gravis** (neuromuscular disease); a combination of ptosis, **aniscoria** (uneven pupil size), and **facial anhidrosis** (lack of sweating) is indicative of **Horner syndrome** (interruption of the sympathetic innervation of the eye). See Chapter 13 for more information on Horner's syndrome. **Hypertension** can be detected during a retinal exam because high blood pressure causes twisting and kinking of tiny retinal blood vessels. A **fundoscopic exam** can detect **diabetic retinopathy**, a leading cause of blindness, that is associated with **macular edema, microaneurysms** (microscopic bulges protruding from the arterial wall), retinal vessel dilation, **neovascularization** (development of new blood vessels), and **cotton wool spots** (fluffy white patches on the retina due to nerve damage). See Chapter 24 for more information on the adverse consequences of diabetes.

COLOR VISION

Colors have three attributes: **hue, intensity,** and **saturation** (degree of freedom from dilution with white). For any color there is a **complementary color** that, when properly mixed with it, produces a sensation of white. Black is the sensation produced by the absence of light, but it is probably a positive sensation because the blind eye does not "see black"; rather, it "sees nothing."

Another observation of basic importance is the demonstration that the sensation of white, any spectral color, and even the extraspectral color, purple, can be produced by mixing various proportions of red light (wavelength 723–647 nm), green light (575–492 nm), and blue light (492–450 nm). Red, green, and blue are therefore called the **primary colors.** A third important point is that the color perceived depends in part on the color of other objects in the visual field. Thus, for example, a red object is seen as red if the field is illuminated with green or blue light, but as pale pink or white if the field is illuminated with red light. **Clinical Box 10–6** describes color blindness.

RETINAL MECHANISMS

The **Young–Helmholtz theory** of color vision is based on the existence of three kinds of cones, each containing a different photopigment that is maximally sensitive to one of the three primary colors. The sensation of any given color is determined by the relative frequency of the impulses from each of these cone systems **(Figure 10–14)**. One pigment (the blue-sensitive, or short-wave, pigment) absorbs light maximally in the blue-violet portion of the spectrum. Another (the green-sensitive, or middle-wave, pigment) absorbs maximally in the green portion. The third (the red-sensitive, or long-wave, pigment) absorbs maximally in the yellow portion. Blue, green, and red are the primary colors, but the cones with their maximal sensitivity in the yellow portion of the spectrum are sensitive enough in the red portion to respond to red light at a lower threshold than green.

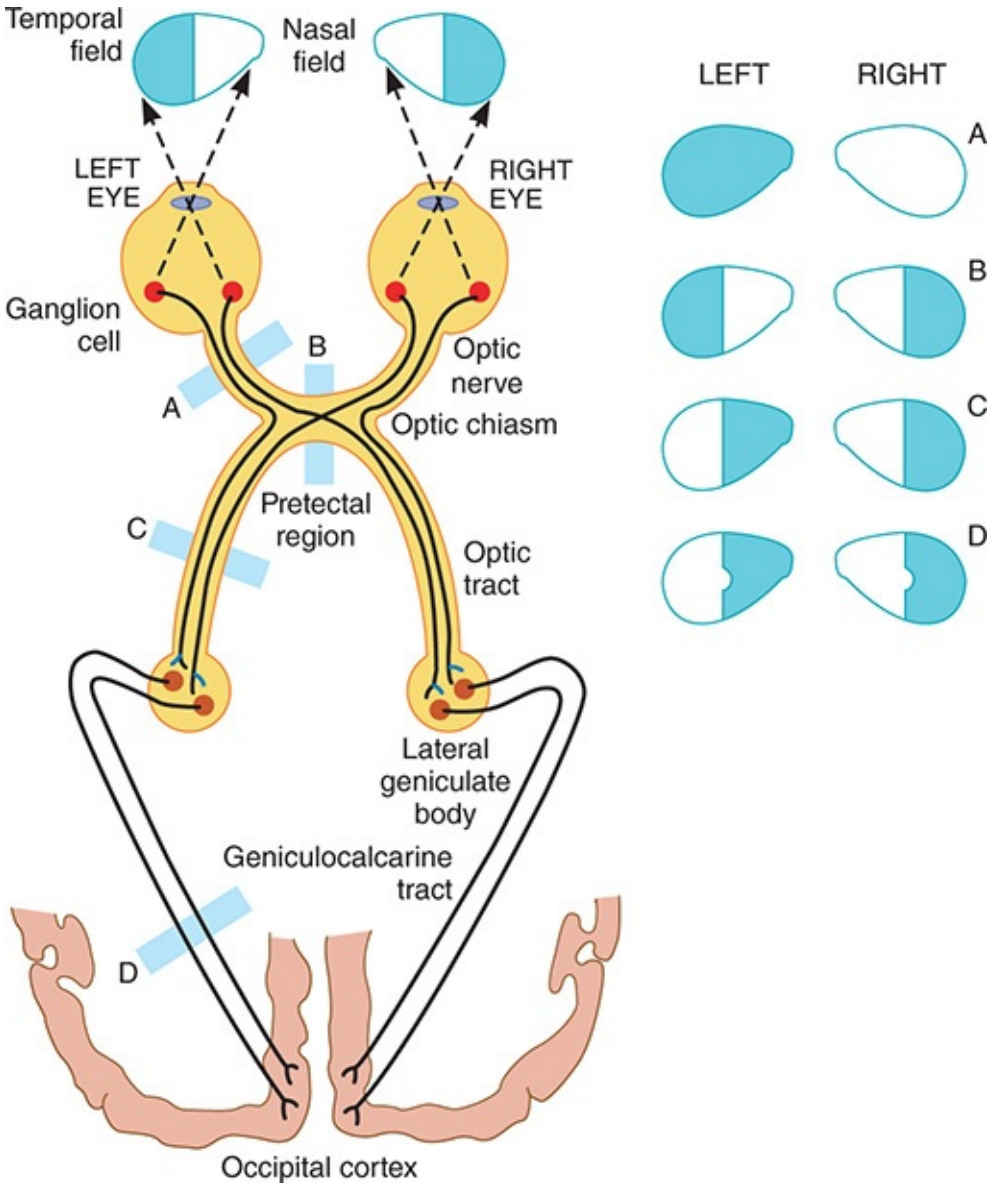


FIGURE 10–14 Visual pathways. Transection of the pathways at the locations indicated by the letters causes the visual field defects shown in the diagrams on the right. The fibers from the nasal half of each retina decussate in the optic chiasm, so that the fibers in the optic tracts are those from the temporal half of one retina and the nasal half of the other. A lesion that interrupts one optic nerve causes blindness in that eye (A). A lesion in one optic tract causes blindness in half of the visual field (C) and is called homonymous (same side of both visual fields) hemianopia (half-blindness). Lesions affecting the optic chiasm destroy fibers from both nasal hemiretinas and produce a heteronymous (opposite sides of the visual fields) hemianopia (B). Occipital lesions may spare the fibers from the macula (as in D) because of the separation in the brain of these fibers from

the others subserving vision.

CLINICAL BOX 10–6

Color Blindness

The most common test for **color blindness** uses the **Ishihara charts,** which are plates containing figures made up of colored spots on a background of similarly shaped colored spots. The figures are intentionally made up of colors that are liable to look the same as the background to an individual who is color blind. Some color-blind individuals are unable to distinguish certain colors, whereas others have only a color weakness. The prefixes “prot-,” “deuter-,” and “trit-” refer to defects of the red, green, and blue cone systems, respectively. Individuals with normal color vision are called **trichromats. Dichromats** are individuals with only two cone systems; they may have protanopia, deuteranopia, or tritanopia. **Monochromats** have only one cone system. Dichromats can match their color spectrum by mixing only two primary colors; monochromats match their color spectrum by varying the intensity of only one. Abnormal color vision is an inherited abnormality in 8% of white males and 0.4% of white females. Tritanopia is rare and shows no sexual selectivity. However, about 2% of color-blind males are dichromats who have protanopia or deuteranopia, and about 6% are anomalous trichromats in whom the red- sensitive or the green-sensitive pigment is shifted in its spectral sensitivity. These abnormalities are inherited as recessive and X-linked characteristics. Color blindness occurs in males if the X chromosome has the abnormal gene. Females show a defect only when both X chromosomes contain the abnormal gene. However, female children of a man with X-linked color blindness are carriers of color blindness and pass the defect on to half of their sons. Therefore, X-linked color blindness skips generations and appears in males of every second generation. Color blindness can also occur in individuals with lesions of area V8 of the visual cortex since this region is uniquely concerned with color vision in humans. This deficit is called **achromatopsia.** Transient blue-green color weakness occurs as a side effect in individuals taking sildenafil (Viagra) for the treatment of erectile dysfunction because the drug inhibits the retinal as well as the penile form of phosphodiesterase.

The gene for human rhodopsin is on chromosome 3, and the gene for the blue-sensitive S cone pigment is on chromosome 7. The other two cone pigments are encoded by genes arranged in tandem on the q arm of the X chromosome. The green-sensitive M and red-sensitive L pigments are very similar in structure. Their opsins show 96% homology of amino acid sequences, but each of these pigments has only 43% homology with the opsin of blue-sensitive pigment, and all three have about 41% homology with rhodopsin.

RESPONSES IN THE VISUAL PATHWAYS & CORTEX

NEURAL PATHWAYS

The axons of the ganglion cells pass caudally in the optic nerve and **optic tract** to end in the **lateral geniculate body** in the thalamus **(Figure 10–15)**. The fibers from each nasal hemiretina decussate in the **optic chiasm.** In the geniculate body, the fibers from the nasal half of one retina and the temporal half of the other synapse on the cells whose axons form the **geniculocalcarine tract.** This tract passes to the occipital lobe of the cerebral cortex. The effects of lesions in these pathways on visual function are discussed in the next section.

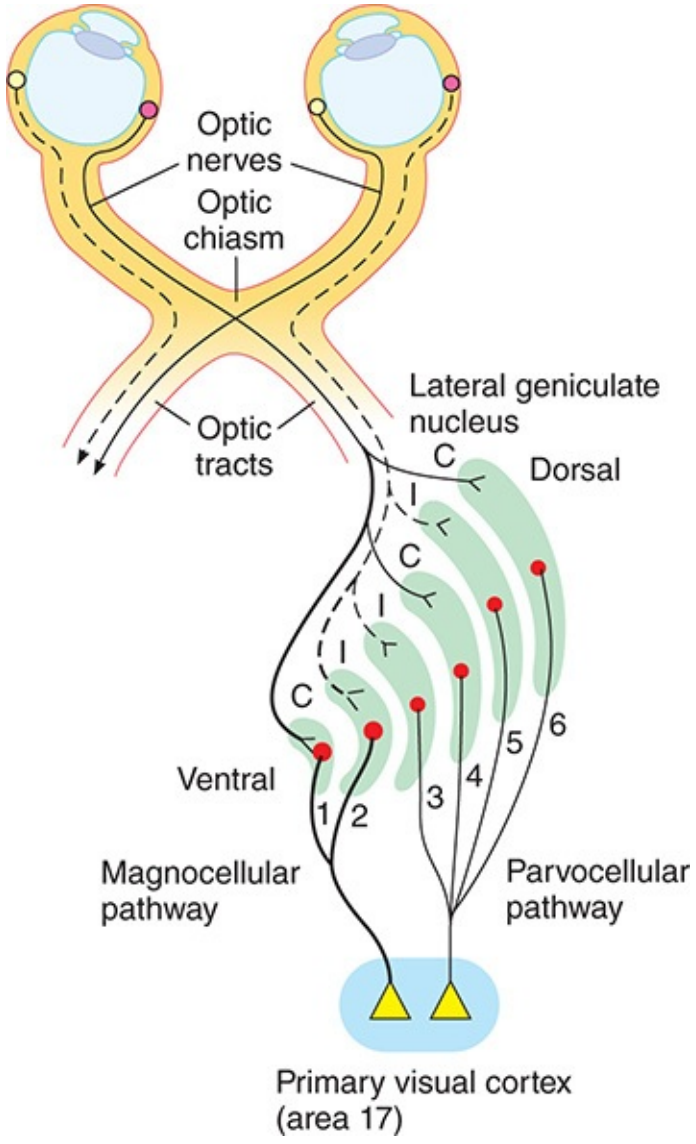


FIGURE 10–15 Ganglion cell projections from the right hemiretina of each eye to the right lateral geniculate body and from this nucleus to the right primary visual cortex. Note the six layers of the geniculate body. P ganglion cells project to layers 3–6, and M ganglion cells project to layers 1 and 2. The ipsilateral (I) and contralateral (C) eyes project to alternate layers. Not shown are the interlaminar area cells, which project via a separate component of the P pathway to blobs in the visual cortex. (Modified with permission from Kandel ER, Schwartz JH, Jessell TM [editors]: *Principles of Neural Science*, 4th ed. New York, NY: McGraw-Hill; 2000.)

The axons of retinal ganglion cells project a detailed spatial representation of the retina on the lateral geniculate body. Each geniculate body contains six well-

defined layers **(Figure 10–16)**. Layers 3–6 have small cells and are called parvocellular, whereas layers 1 and 2 have large cells and are called magnocellular. On each side, layers 1, 4, and 6 receive input from the contralateral eye, whereas layers 2, 3, and 5 receive input from the ipsilateral eye. In each layer, there is a precise point-for-point representation of the retina, and all six layers are in register so that along a line perpendicular to the layers, the receptive fields of the cells in each layer are almost identical. Only 10–20% of the input to the **lateral geniculate nucleus** (LGN) comes from the retina; inputs also occur from the visual cortex and other brain regions. The feedback pathway from the visual cortex is involved in visual processing related to the perception of orientation and motion.

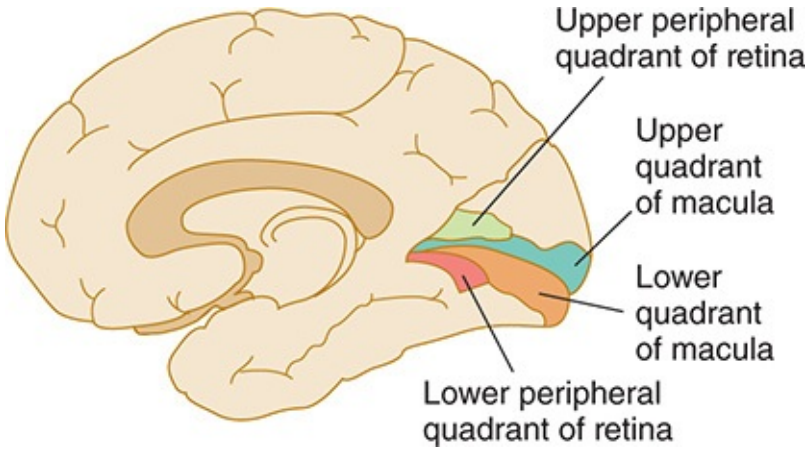


FIGURE 10–16 Medial view of the human right cerebral hemisphere showing projection of the retina on the primary visual cortex in the occipital cortex around the calcarine fissure. The geniculocalcarine fibers from the medial half of the lateral geniculate terminate on the superior lip of the calcarine fissure, and those from the lateral half terminate on the inferior lip. Also, the fibers from the lateral geniculate body that relay macular vision separate from those that relay peripheral vision and end more posteriorly on the lips of the calcarine fissure.

There are several types of retinal ganglion cells. These include large ganglion cells (magno, or M cells), which add responses from different kinds of cones and are concerned with movement and stereopsis. Another type is the small ganglion cells (parvo, or P cells), which subtract input from one type of cone from input from another and are concerned with color, texture, and shape. The M ganglion cells project to the magnocellular portion of the lateral geniculate, whereas the P ganglion cells project to the parvocellular portion. From the LGN, a

magnocellular pathway and a parvocellular pathway project to the visual cortex. The magnocellular pathway, from layers 1 and 2, carries signals for detection of movement, depth, and flicker. The parvocellular pathway, from layers 3–6, carries signals for color vision, texture, shape, and fine detail. The small-field bistratified ganglion cells may be involved in color vision and carry the short (blue) wavelength information to the intralaminar zones of the LGN.

EFFECT OF LESIONS IN THE OPTIC PATHWAYS

Lesions along the visual pathways can be localized with a high degree of accuracy by the effects they produce in the visual fields. The fibers from the nasal half of each retina decussate in the optic chiasm, so that the fibers in the optic tracts are those from the temporal half of one retina and the nasal half of the other. In other words, each optic tract subserves half of the field of vision. Therefore, a lesion that interrupts one optic nerve causes blindness in that eye, but a lesion in one optic tract causes blindness in half of the visual field (Figure 10–15). This defect is classified as a **homonymous** (same side of both visual fields) **hemianopia** (half-blindness).

Lesions affecting the optic chiasm, such as pituitary tumors expanding out of the sella turcica, cause destruction of the fibers from both nasal hemiretinas and produce a **heteronymous** (opposite sides of the visual fields) **hemianopia.** Because the fibers from the macula are located posteriorly in the optic chiasm, hemianopic scotomas developed before vision in the two hemiretinas are completely lost. Selective visual field defects are further classified as bitemporal, binasal, and right or left.

The optic nerve fibers from the upper retinal quadrants subserving vision in the lower half of the visual field terminate in the medial half of the lateral geniculate body, and the fibers from the lower retinal quadrants terminate in the lateral half. The geniculocalcarine fibers from the medial half of the lateral geniculate body terminate on the superior lip of the calcarine fissure, and those from the lateral half terminate on the inferior lip. Furthermore, the fibers from the lateral geniculate body that subserve macular vision separate from those that subserve peripheral vision and end more posteriorly on the lips of the calcarine fissure **(Figure 10–17)**. Because of this anatomic arrangement, occipital lobe lesions may produce discrete quadrantic visual field defects (upper and lower quadrants of each half visual field).

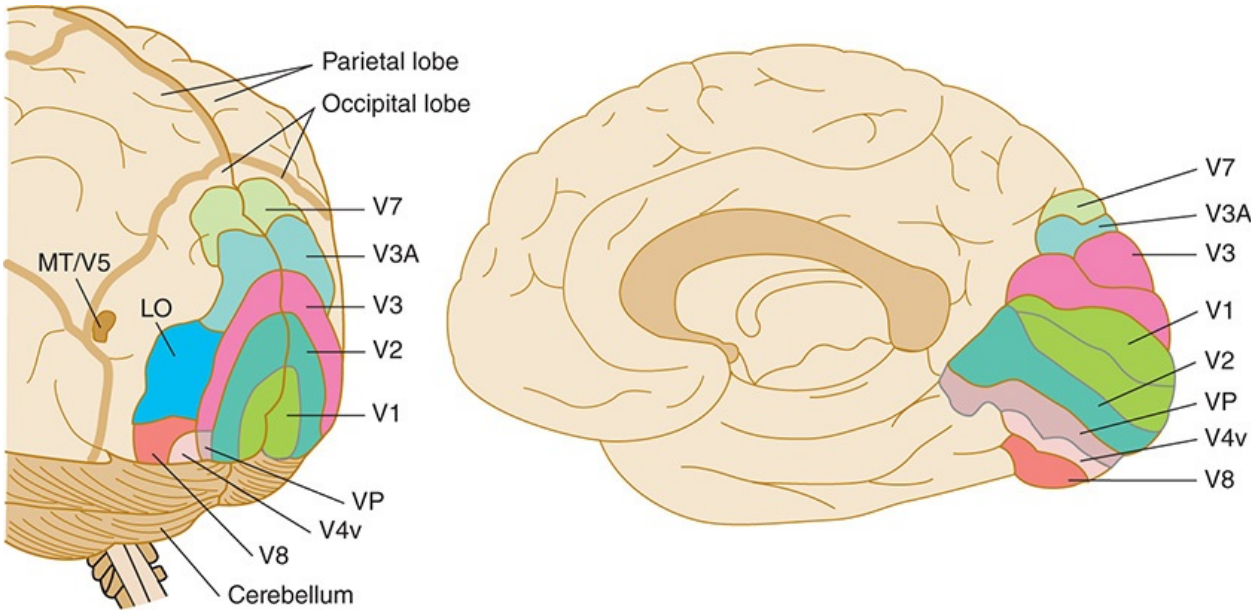


FIGURE 10–17 Some of the main areas to which the primary visual cortex (V1) projects in the human brain. Lateral and medial views. LO, lateral occipital; MT, medial temporal; VP, ventral parietal. See also Table 10–2. (Modified with permission from Logothetis N: Vision: A window on consciousness. Sci Am 1999 Nov;281(5):69–75.)

Macular sparing or loss of peripheral vision with intact macular vision is also common with occipital lesions (Figure 10–15) because the macular representation is separate from that of the peripheral fields and very large relative to that of the peripheral fields. Therefore, occipital lesions must extend considerable distances to destroy both macular and peripheral vision. The fibers to the pretectal region that subserves the pupillary reflex produced by shining a light into the eye leave the optic tracts near the geniculate bodies. Therefore, blindness with preservation of the pupillary light reflex is usually due to bilateral lesions caudal to the optic tract.

PRIMARY VISUAL CORTEX

The primary visual receiving area (**primary visual cortex;** also known as V1) is located on the sides of the calcarine fissure (Figure 10–17). Just as the ganglion cell axons project a detailed spatial representation of the retina on the lateral geniculate body, the lateral geniculate body projects a similar point-for-point representation on the primary visual cortex. In the visual cortex, many nerve cells are associated with each incoming fiber. Like the rest of the neocortex, the

visual cortex has six layers. The axons from the LGN that form the magnocellular pathway end in layer 4, specifically in its deepest part, layer 4C. Many of the axons that form the parvocellular pathway also end in layer 4C. However, the axons from the interlaminar region end in layers 2 and 3.

Layers 2 and 3 of the cortex contain clusters of cells about 0.2 mm in diameter that, unlike the neighboring cells, contain a high concentration of the mitochondrial enzyme cytochrome oxidase. The clusters have been named **blobs.** They are arranged in a mosaic in the visual cortex and are concerned with color vision. However, the parvocellular pathway also carries color opponent data to the deep part of layer 4.

Like the ganglion cells, the lateral geniculate neurons and the neurons in layer 4 of the visual cortex respond to stimuli in their receptive fields with on-centers and inhibitory surrounds or off-centers and excitatory surrounds. A bar of light covering the center is an effective stimulus for them because it stimulates the entire center and relatively little of the surround. However, the bar has no preferred orientation and, as a stimulus, is equally effective at any angle.

The responses of the neurons in other layers of the visual cortex are strikingly different. **Simple cells** respond to bars of light, lines, or edges, but only when they have a specific orientation. When a bar of light is rotated as little as 10° from the preferred orientation, the firing rate of the simple cell is usually decreased; and if the stimulus is rotated much more, the response disappears. **Complex cells** also have a preferred orientation of a linear stimulus but are less dependent on the location of a stimulus in the visual field than the simple cells and the cells in layer 4. They often respond maximally when a linear stimulus is moved laterally without a change in its orientation.

The visual cortex, like the somatosensory cortex, is arranged in vertical columns that are concerned with orientation **(orientation columns).** Each is about 1 mm in diameter. However, the orientation preferences of neighboring columns differ in a systematic way; as one moves from column to column across the cortex, sequential changes occur in orientation preference of 5–10°. Thus, it seems likely that for each ganglion cell receptive field in the visual field, there is a collection of columns in a small area of visual cortex, representing the possible preferred orientations at small intervals throughout the full 360°. The simple and complex cells are called **feature detectors** because they respond to and analyze certain features of the stimulus.

Another feature of the visual cortex is the presence of **ocular dominance columns.** The geniculate cells and the cells in layer 4 receive input from only one eye, and the layer 4 cells alternate with cells receiving input from the other

eye. If a large amount of a radioactive amino acid is injected into one eye, the amino acid is incorporated into protein and transported by axoplasmic flow to the ganglion cell terminals, across the geniculate synapses, and along the geniculocalcarine fibers to the visual cortex. In layer 4, labeled endings from the injected eye alternate with unlabeled endings from the uninjected eye. The result, when viewed from above, is a vivid pattern of stripes that covers much of the visual cortex and is separate from and independent of the grid of orientation columns.

About half of the simple and complex cells receive an input from both eyes. The inputs are identical or nearly so in terms of the portion of the visual field involved and the preferred orientation. However, they differ in strength, so that between the cells to which the input comes totally from the ipsilateral or the contralateral eye, there is a spectrum of cells influenced to different degrees by both eyes.

OTHER CORTICAL AREAS CONCERNED WITH VISION

The primary visual cortex (V1) projects to many other parts of the occipital lobes and other parts of the brain. These are often identified by number (V2, V3, etc) or by letters (LO, MT, etc). The distribution of some of these in the human brain is shown in **Figure 10–18**, and their putative functions are listed in **Table 10–2**. The visual projections from V1 can be divided roughly into a **dorsal** or **parietal pathway,** concerned primarily with motion, and a **ventral** or **temporal pathway,** concerned with shape and recognition of forms and faces. In addition, connections to the sensory areas are important. For example, in the occipital cortex, visual responses to an object are better if the object is felt at the same time. There are many other relevant connections to other systems.

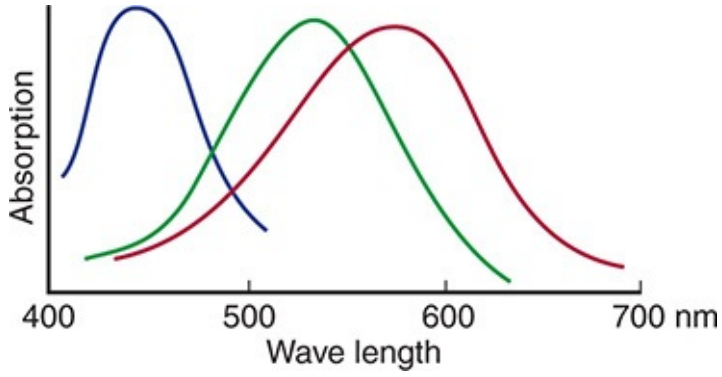


FIGURE 10–18 Absorption spectra of the three cone pigments in the human retina. The S pigment that peaks at 440 nm senses blue, and the M pigment that peaks at 535 nm senses green. The remaining L pigment peaks in the yellow portion of the spectrum, at 565 nm, but its spectrum extends far enough into the long wavelengths to sense red.

TABLE 10–2 Functions of visual projection areas in the human brain.

V1	Primary visual cortex; receives input from lateral geniculate nucleus, begins processing in terms of orientation, edges, etc
V2, V3, VP	Continued processing, larger visual fields
V3A	Motion
V4	Detection of shape, color, and texture of an object
MT/V5	Processes direction of movement
V6	May detect direction and speed of bodily movement
LO	Recognition of shape
V7	Representation of upper and lower quadrants of the visual field
V8	Color vision

LO, lateral occipital; MT, medial temporal; VP, ventral parietal.

NEURAL MECHANISMS OF COLOR VISION

Color is mediated by ganglion cells that subtract or add input from one type of cone to input from another type. Processing in the ganglion cells and the LGN produces impulses that pass along three types of neural pathways that project to V1: a red-green pathway that signals differences between L- and M-cone responses, a blue-yellow pathway that signals differences between S-cone and the sum of L- and M-cone responses, and a luminance pathway that signals the sum of L- and M-cone responses. These pathways project to the blobs and the deep portion of layer 4C of V1. From the blobs and layer 4, color information is projected to V8. However, it is not known how V8 converts color input into the sensation of color.

EYE MOVMENTS

The eye is moved within the orbit by six ocular muscles that are innervated by the oculomotor, trochlear, and abducens nerves. **Figure 10–19** shows the movements produced by the six pairs of muscles. Because the oblique muscles pull medially, their actions vary with the position of the eye. When the eye is turned nasally, the inferior oblique elevates it and the superior oblique depresses it. When it is turned laterally, the superior rectus elevates it and the inferior rectus depresses it. Because much of the visual field is binocular, a very high order of coordination of the movements of the two eyes is necessary if visual images are to fall at all times on corresponding points in the two retinas and diplopia is to be avoided.

There are four types of eye movements, each controlled by a different neural system but sharing the same final common path, the motor neurons that supply the external ocular muscles. **Saccades,** sudden jerky movements, occur as the gaze shifts from one object to another. They bring new objects of interest onto the fovea and reduce adaptation in the visual pathway that would occur if gaze were fixed on a single object for long periods. **Smooth pursuit movements** are tracking movements of the eyes as they follow moving objects. **Vestibular movements,** adjustments that occur in response to stimuli initiated in the semicircular canals, maintain visual fixation as the head moves. **Convergence movements** bring the visual axes toward each other as attention is focused on objects near the observer. Saccadic movements, pursuit movements, and vestibular movements depend on an intact visual cortex. Saccades are programmed in the frontal cortex and the superior colliculi and pursuit movements in the cerebellum.

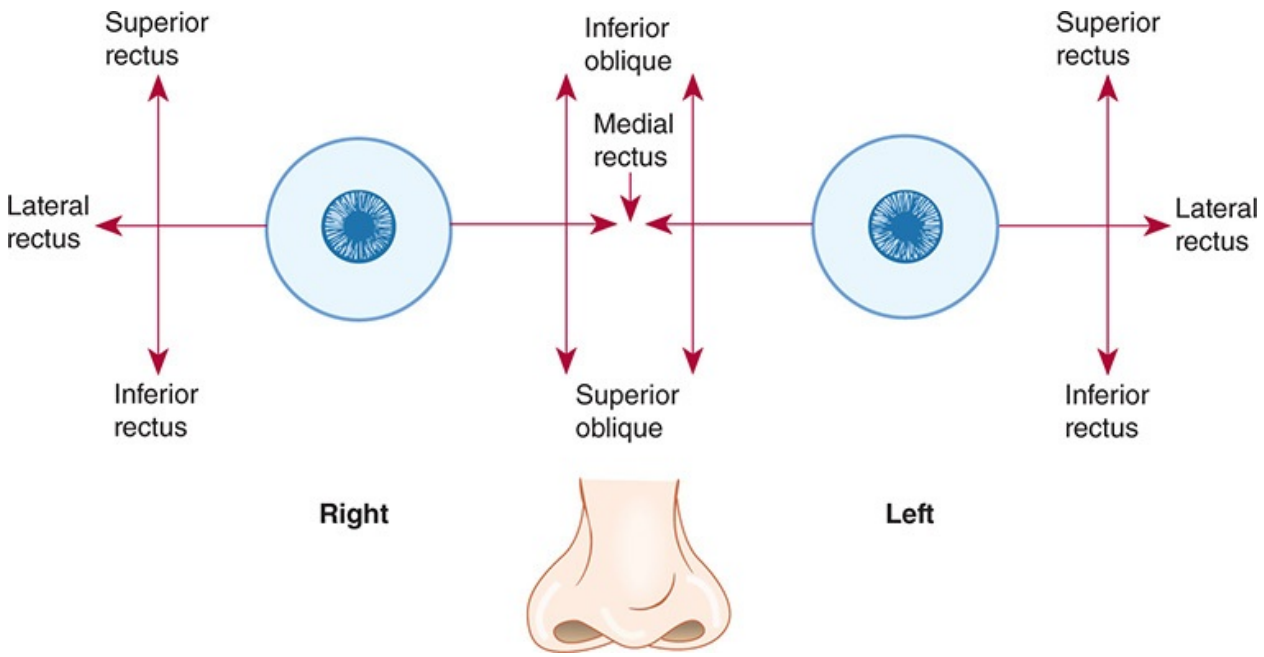


FIGURE 10–19 Diagram of eye muscle actions. The eye is adducted by the medial rectus and abducted by the lateral rectus. The adducted eye is elevated by the inferior oblique and depressed by the superior oblique; the abducted eye is elevated by the superior rectus and depressed by the inferior rectus. (Reproduced with permission from Waxman SG: *Clinical Neuroanatomy*, 26th ed. New York, NY: McGraw-Hill; 2010.)

CHAPTER SUMMARY

- The major parts of the eye are the sclera (protective covering), cornea (transfer light rays), choroid (nourishment), retina (photoreceptor cells), lens, and iris.
- The bending of light rays (refraction) allows one to focus an accurate image onto the retina. Light is refracted at the anterior surface of the cornea and at the anterior and posterior surfaces of the lens.
- To bring diverging rays from close objects to a focus on the retina, the curvature of the lens is increased, a process called accommodation. The pupillary light reflex regulates the amount of light that enters the eye and affects the quality of the retinal image (a smaller pupil diameter gives a greater depth of focus).
- In hyperopia (farsightedness), the eyeball is too short and light rays come to a focus behind the retina. In myopia (nearsightedness), the anteroposterior

diameter of the eyeball is too long. Astigmatism is a common condition in which the curvature of the cornea is not uniform. Presbyopia is the loss of accommodation for near vision.

- The retina is organized into several layers: the outer nuclear layer contains the photoreceptors (rods and cones); the inner nuclear layer contains bipolar cells, horizontal cells, and amacrine cells; and the ganglion cell layer contains the only output neuron of the retina.
- Rhodopsin is the photosensitive pigment in the rods and is composed of retinal and the protein opsin which is a GPCR. Exposure to light causes this sequence of events: structural change in retinal, a conformational change in opsin, activation of transducin, activation of phosphodiesterase, decreased cGMP, closure of cGMP-gated cation channels, hyperpolarization, decrease in neurotransmitter release, and responses in neural elements of the retina.
- In response to light, horizontal cells are hyperpolarized; bipolar cells are either hyperpolarized or depolarized; and amacrine cells are depolarized and develop spikes that may act as generator potentials for the propagated spikes produced in the ganglion cells.
- The decline in visual threshold after spending long periods of time in a dimly lit room is called dark adaptation. The fovea in the center of the retina is the point where visual acuity is greatest. Age-related macular degeneration is a disease in which sharp, central visual acuity is gradually destroyed.
- The visual pathway is from the rods and cones to bipolar cells to ganglion cells then via the optic tract to the thalamic lateral geniculate body to the occipital lobe of the cerebral cortex. The fibers from each nasal hemiretina decussate in the optic chiasm; the fibers from the nasal half of one retina and the temporal half of the other synapse on the cells whose axons form the geniculocalcarine tract.
- Neurons in layer 4 of the visual cortex respond to stimuli in their receptive fields with on-centers and inhibitory surrounds or off-centers and excitatory surrounds. Simple cells respond to bars of light, lines, or edges, but only when they have a particular orientation. Complex cells also have a preferred orientation of a linear stimulus but are less dependent on the location of a stimulus in the visual field. Projections from area V1 can be divided into a dorsal or parietal pathway (concerned primarily with motion) and a ventral or temporal pathway (concerned with shape and recognition of forms and faces).
- The Young–Helmholtz theory of color vision is based on the existence of

three kinds of cones, each containing a different photopigment that is maximally sensitive to one of the three primary colors, with the sensation of any given color being determined by the relative frequency of the impulses from each of these cone systems.

- The fibers from the nasal half of each retina decussate in the optic chiasm, so that the fibers in the optic tracts are those from the temporal half of one retina and the nasal half of the other. A lesion of one optic nerve causes blindness in that eye; a lesion in one optic tract causes blindness in half of the visual field (homonymous hemianopia). A lesion of the optic chiasm destroys fibers from both nasal hemiretinas (heteronymous hemianopia). Occipital lesions may spare the fibers from the macular region.
- Eye movement is controlled by six ocular muscles innervated by the oculomotor, trochlear, and abducens nerves. The inferior oblique muscle turns the eye upward and outward; the superior oblique turns it downward and outward. The superior rectus muscle turns the eye upward and inward; the inferior rectus turns it downward and inward. The medial rectus muscle turns the eye inward; the lateral rectus turns it outward.
- Saccades (sudden jerky movements) occur as the gaze shifts from one object to another, and they reduce adaptation in the visual pathway that would occur if gaze were fixed on a single object for long periods. Smooth pursuit movements are tracking movements of the eyes as they follow moving objects. Vestibular movements occur in response to stimuli in the semicircular canals to maintain visual fixation as the head moves. Convergence movements bring the visual axes toward each other as attention is focused on objects near the observer.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. A visual exam in an 80-year-old man shows he has a reduced ability to see objects in the upper and lower quadrants of the left visual fields of both eyes but some vision remains in the central regions of the visual field. The diagnosis is

   A. central scotoma.

   B. heteronymous hemianopia with macular sparing.

   C. lesion of the optic chiasm.

D. homonymous hemianopia with macular sparing.

E. retinopathy.

2. A 45-year-old woman who had never needed to wear glasses had trouble reading a menu in a dimly-lit restaurant. She then recalled that as of late she needed to have the newspaper closer to her eyes to read it. Her ophthalmologist told her she was experiencing age-related loss of accommodation for near vision (presbyopis) that is due to

A. the inability to increase the tension on the lens ligaments.

B. the inability to increase the curvature of the lens.

C. relaxation of the sphincter muscle of the iris.

D. contraction of the ciliary muscle.

E. increased softness of the lens.

3. A 28-year-old man with severe myopia made an appointment to see his ophthalmologist when he began to notice flashing lights and floaters in his visual field. He was diagnosed with a retinal detachment. The inner nuclear layer of the retina is comprised of

A. the inner segments of the photoreceptors (rods and cones).

B. various types of ganglion cells.

C. bipolar cells, horizontal cells, and amacrine cells.

D. glial cells that generate new rods and cones.

E. cell bodies of the optic nerve.

4. A 65-year-old woman was diagnosed with dry age-related macular degeneration with a foveal-sparing scotoma. The fovea of the eye

A. has the lowest light threshold.

B. is the region of highest visual acuity.

C. contains only red and green cones.

D. contains only rods.

E. is situated over the head of the optic nerve.

5. A 62-year-old man went to his ophthalmologist for his routine eye exam. It included ophthalmoscopy to visualize the interior surface of his eye, opposite to the lens. This portion of the eye is called

A. the optic disk.

B. the macula.

C. the sclera.

D. the conjunctiva.

E. the fundus.

6. Which of the following parts of the eye has the greatest concentration of rods?

A. Ciliary body

B. Iris

C. Optic disk

D. Fovea

E. Parafoveal region

7. The correct sequence of events involved in phototransduction in rods and cones in response to light is:

A. activation of transducin, decreased release of glutamate, structural changes in rhodopsin, closure of cGMP-gated cation channels, and decrease in intracellular cGMP.

B. decreased release of glutamate, activation of transducin, closure of cGMP-gated cation channels, decrease in intracellular cGMP, and structural changes in rhodopsin.

C. structural changes in rhodopsin, decrease in intracellular cGMP, decreased release of glutamate, closure of cGMP-gated cation channels, and activation of transducin.

D. structural changes in rhodopsin, activation of transducin, decrease in intracellular cGMP, closure of cGMP-gated cation channels, and decreased release of glutamate.

E. activation of transducin, structural changes in rhodopsin, closure of cGMP-gated cation channels, decrease in intracellular cGMP, and decreased release of glutamate.

8. A 25-year-old medical student spent a summer volunteering in the sub-Saharan region of Africa. There he noted a high incidence of people reporting difficulty with night vision due to a lack of vitamin A in their diet. Vitamin A is a precursor for the synthesis of

A. rods and cones.

B. retinal.

C. rod transducin.

D. opsin.

E. cone transducin.

9. An 11-year-old boy was having difficulty reading the graphs that his teacher

was showing at the front of classroom. His teacher recommended he be seen by an ophthalmologist. Not only was he asked to look at a Snellen letter chart for visual acuity but he was also asked to identify numbers in an Ishihara chart. He responded that he merely saw a bunch of dots. Abnormal color vision is 20 times more common in males than females because most cases are caused by an abnormal

A. dominant gene on the Y chromosome.

B. recessive gene on the Y chromosome.

C. dominant gene on the X chromosome.

D. recessive gene on the X chromosome.

E. recessive gene on chromosome 22.

10. A 32-year-old man was brought to the emergency department after being found comatose by his wife. The resident in the emergency department assessed his pupillary light reflex as a useful gauge of his brainstem function. He found that when the light was shone into his left eye, neither pupil constricted; but when the light was shone in his right eye, both pupils constricted. The physician determined that damage was within

A. the left optic nerve.

B. the left oculomotor nerve.

C. the right optic nerve.

D. the right oculomotor nerve.

E. the sphincter muscle of the left eye.

11. A tumor was diagnosed near the base of the skull in a 56-year-old woman, impinging on her right optic tract. Which parts of the visual field of each eye are relayed through the right optic tract?

A. The temporal half of left retina and the nasal half of the right retina

B. The nasal half of left retina and the temporal half of the right retina

C. The temporal half of right retina and the nasal half of the left retina

D. The nasal half of right retina and the temporal half of the left retina

E. The temporal and nasal halves of the left retina

12. A 50-year-old man began having difficulty moving his left eye downward and laterally. His primary care physician did testing that revealed he had damage to a cranial nerve that innervated one of the muscles controlling eye movement. Which nerve and muscle allow the eye to move downward and laterally?

A. The oculomotor nerve and the inferior oblique muscle

B. The trochlear nerve and the superior oblique muscle

C. The abducens nerve and the lateral rectus muscle

D. The oculomotor nerve and the superior oblique muscle

E. The trochlear nerve and the inferior rectus muscle

13. A medical student was recording responses of retinal ganglion cells to light focused on a portion of their receptive field. He identified one cell as an off-center/on-surround cell. What might contribute to the reduced firing rate when the light was focused on the center of the receptive field of the ganglion cell?

A. The light caused the release of an inhibitory neurotransmitter released from the terminals of rod cells that synapse on the ganglion cell.

B. The activation of nearby photoreceptors leads to hyperpolarization of a horizontal cell that in turn reduces the activity of the ganglion cell.

C. The activation of nearby photoreceptors triggers an action potential in an amacrine cell that inhibits the ganglion cell.

D. The light caused an action potential in rod and cone receptors within the receptive field and they caused hyperpolarization in the ganglion cell.

CHAPTER 11

Hearing & Equilibrium

OBJECTIVES

After studying this chapter, you should be able to:

- Describe the components and functions of the external, middle, and inner ear.
- Explain the roles of the tympanic membrane, the auditory ossicles (malleus, incus, and stapes), and scala vestibule in sound transmission.
- Describe the way that movements of molecules in the air are converted into impulses generated in hair cells in the cochlea.
- Explain how pitch, loudness, and timbre are coded in the auditory pathways.
- Describe the components of the auditory pathway from the cochlear hair cells to the cerebral cortex.
- Compare the causes of conductive and sensorineural hearing loss and the tests used to distinguish between them.
- Define the following terms: tinnitus, presbycusis, and syndromic and nonsyndromic deafness.
- Explain how cochlear implants and hearing aids function.
- Explain how the receptors in the semicircular canals detect rotational acceleration and how the receptors in the saccule and utricle detect linear acceleration.

- List the major sensory inputs that provide the information that is synthesized in the brain into the sense of position in space.
- Describe the neural mechanisms for vestibular nystagmus and how nystagmus can be used as a diagnostic indicator of the integrity of the vestibular system.
- Describe the cause and clinical signs of the following vestibular disturbances: vertigo, Ménière disease, and motion sickness.

INTRODUCTION

Our ears not only let us detect sounds, but they also help us maintain balance. Receptors for two sensory modalities (hearing and equilibrium) are housed in the ear. The external ear, the middle ear, and the cochlea of the inner ear are involved with hearing. The semicircular canals, the utricle, and the saccule of the inner ear are involved with equilibrium. Both hearing and equilibrium rely on a very specialized type of receptor called a hair cell. There are six groups of hair cells in each inner ear: one in each of the three semicircular canals, one in the utricle, one in the saccule, and one in the cochlea. Receptors in the semicircular canals detect rotational acceleration, those in the utricle detect linear acceleration in the horizontal direction, and the ones in the saccule detect linear acceleration in the vertical direction.

STRUCTURE & FUNCTION OF THE EAR

EXTERNAL & MIDDLE EAR

The external ear is composed of the **auricle** (pinna) that captures sound waves, the **external auditory meatus** (ear canal) through which sound waves travel, and the **tympanic membrane** (eardrum) that moves in and out in response to sound **(Figure 11–1)**. The tympanic membrane marks the beginning of the middle ear.

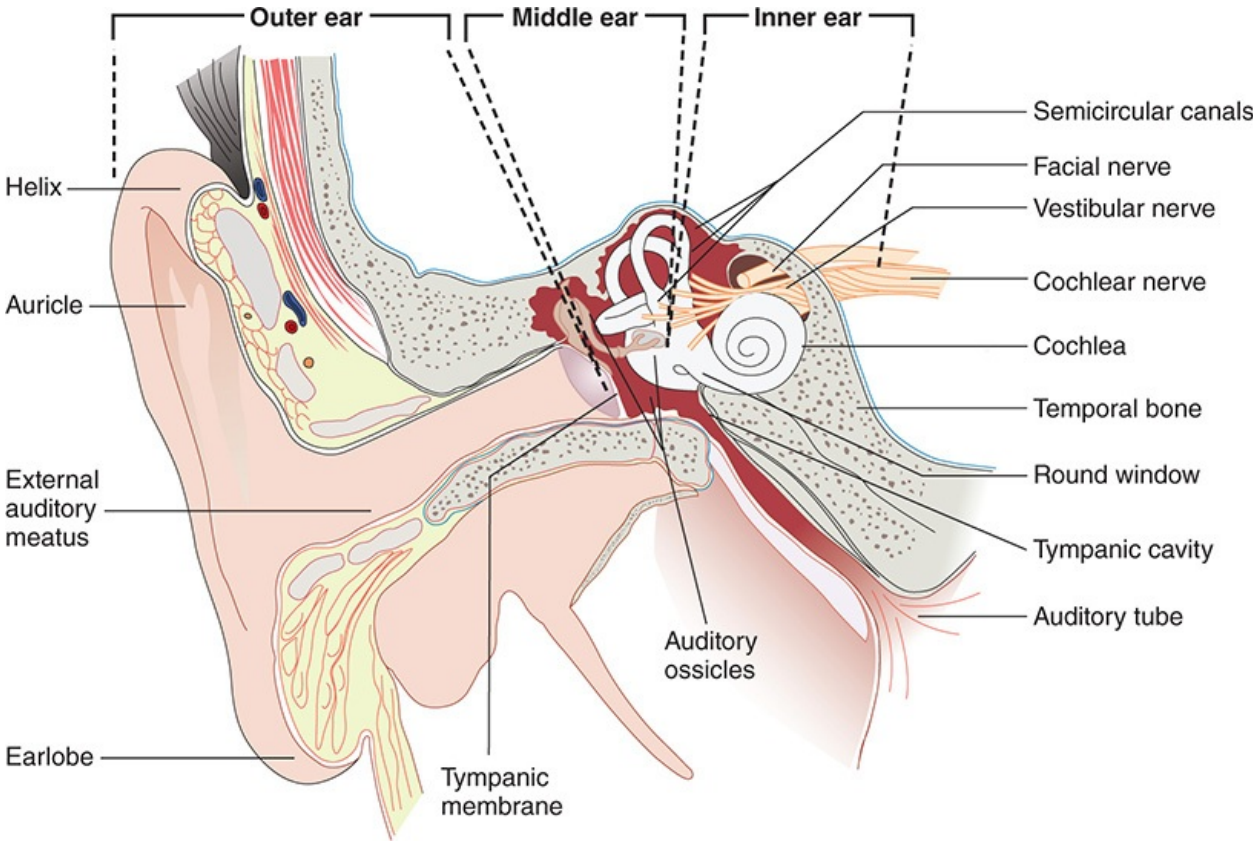


FIGURE 11–1 The structures of the external, middle, and inner portions of the human ear. Sound waves travel from the external ear to the tympanic membrane via the external auditory meatus. The middle ear is an air-filled cavity in the temporal bone; it contains the auditory ossicles. The inner ear is composed of the bony and membranous labyrinths. To make the relationships clear, the cochlea has been turned slightly and the middle ear muscles have been omitted. (Reproduced with permission from Fox SI: *Human Physiology*. New York, NY: McGraw-Hill; 2008.)

The middle ear is an air-filled cavity in the temporal bone that opens via the **eustachian (auditory) tube** into the nasopharynx and through the nasopharynx to the exterior. The tube is usually closed, but during swallowing, chewing, and yawning it opens, keeping the air pressure on the two sides of the eardrum equalized. Figure 11–2 shows the three tiny **auditory ossicles or bones** of the middle ear: **malleus** (hammer), **incus** (anvil), and **stapes** (stirrup). The **manubrium** (handle of the malleus) is attached to the back of the tympanic membrane. Its head is attached to the wall of the middle ear, and its short process is attached to the incus, which in turn articulates with the head of the stapes (stirrup). Its **footplate** is attached by an annular ligament to the walls of the **oval**

window that marks the beginning of the inner ear. Two small skeletal muscles **(tensor tympani** and **stapedius)** are also located in the middle ear. Contraction of the former pulls the manubrium of the malleus medially and decreases the vibrations of the tympanic membrane; contraction of the latter pulls the footplate of the stapes out of the oval window. The functions of the ossicles and muscles are detailed below.

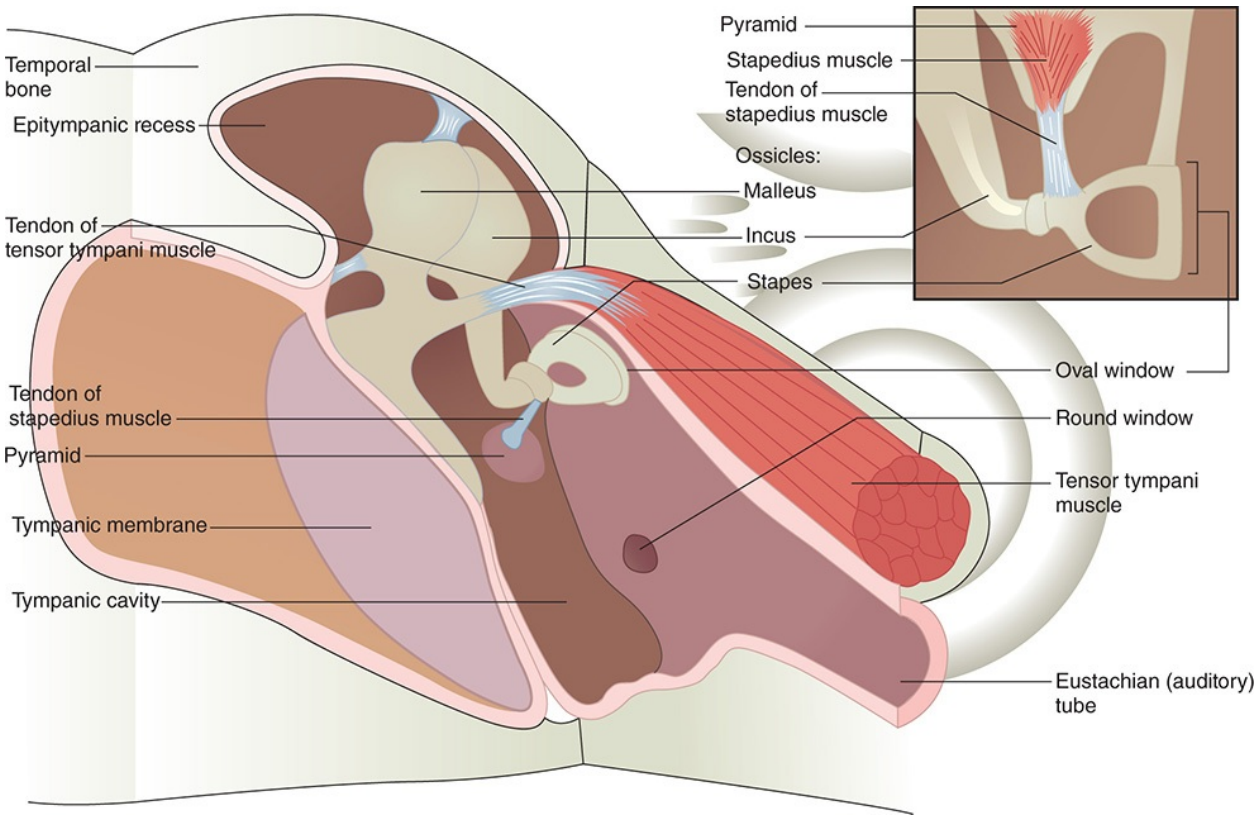


FIGURE 11–2 The medial view of the middle ear containing three auditory ossicles (malleus, incus, and stapes) and two small skeletal muscles (tensor tympani muscle and stapedius). The manubrium (handle of the malleus) is attached to the back of the tympanic membrane. Its head is attached to the wall of the middle ear, and its short process is attached to the incus, which in turn articulates with the head of the stapes. The footplate of the stapes is attached by an annular ligament to the walls of the oval window. Contraction of the tensor tympani muscle pulls the manubrium medially and decreases the vibrations of the tympanic membrane; contraction of the stapedius muscle pulls the footplate of the stapes out of the oval window. (Reproduced with permission from Fox SI: *Human Physiology*. New York, NY: McGraw-Hill; 2008.)

INNER EAR

The inner ear **(labyrinth)** is made up of two parts, one within the other. The **bony labyrinth** is a series of channels in the petrous portion of the **temporal bone** and is filled with a fluid called **perilymph** that has a relatively low concentration of K^+, similar to that of plasma or the cerebrospinal fluid. The **membranous labyrinth** is inside of these bony channels, surrounded by the perilymph; it more or less duplicates the shape of the bony channels and is filled with a K^+-rich fluid called **endolymph.** The labyrinth has three components: the **cochlea** that contains hair cells (receptors) for hearing, **semicircular canals** that contain hair cells that respond to head rotation, and the **otolith organs** that contain hair cells that respond to changes in gravity and head tilt **(Figure 11–3)**.

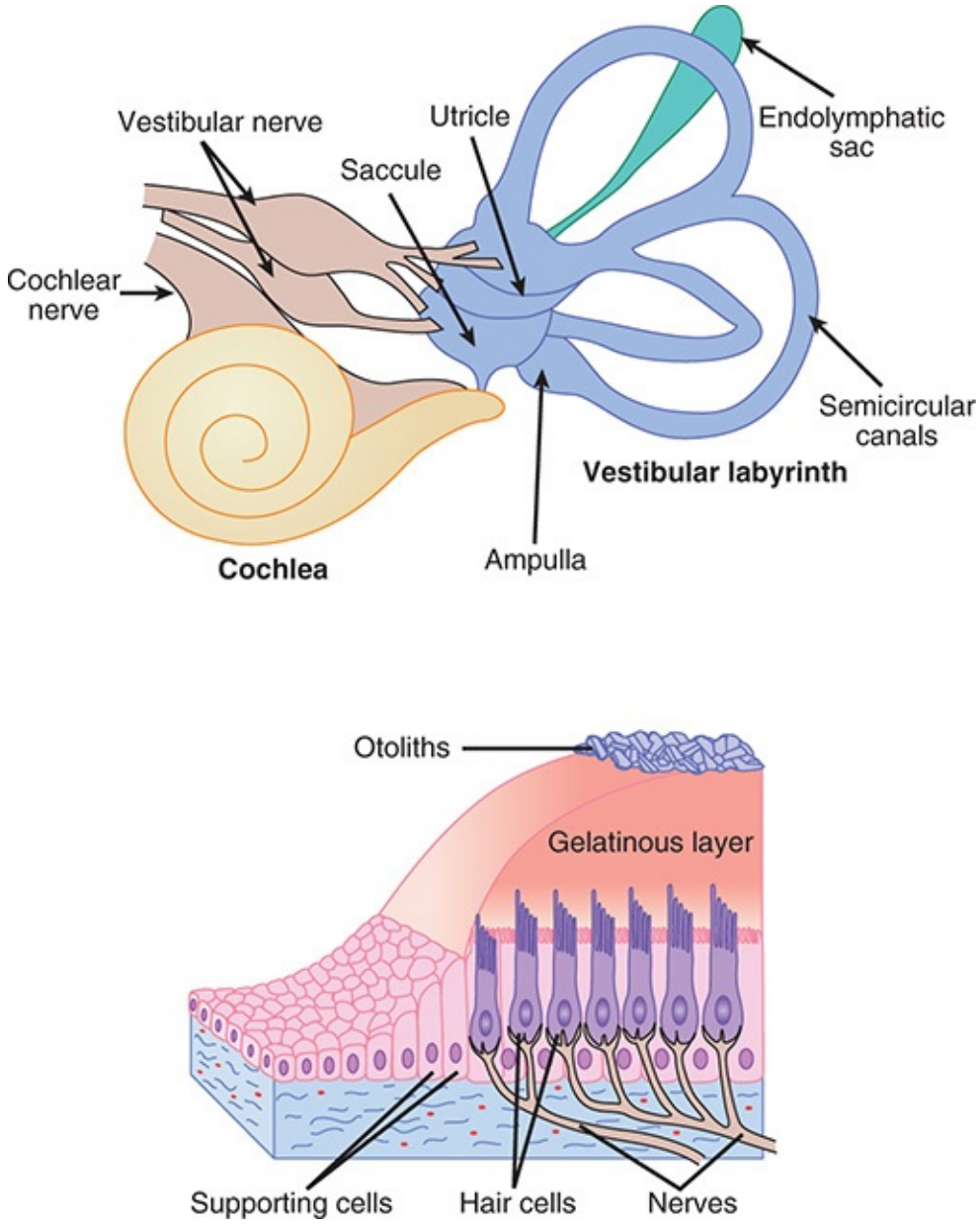


FIGURE 11–3 The membranous labyrinth of the inner ear has three components: semicircular canals, cochlea, and otolith organs. The semicircular canals are sensitive to angular accelerations that deflect the gelatinous cupula and associated hair cells. In the cochlea, hair cells spiral along the basilar membrane within the organ of Corti. Airborne sounds set the eardrum in motion, which is conveyed to the cochlea by bones of the middle ear. This flexes the membrane up and down. Hair cells in the organ of Corti are stimulated by shearing motion. The otolithic organs (saccule and utricle) are sensitive to linear acceleration in vertical and horizontal planes. Hair cells are attached to the otolithic membrane. Information from the cochlear hair cells is carried by the

cochlear division of the auditory (VIII cranial) nerve. Information from the hair cells in the semicircular canals and otolith organs is carried by the vestibular divisions of the auditory nerve.

The cochlea is a 35-mm-long coiled tube that makes two and three quarter turns. The basilar membrane and Reissner membrane divide it into three chambers or **scalae** **(Figure 11–4)**. The upper **scala vestibuli** and lower **scala tympani** contain perilymph and communicate with each other at the apex of the cochlea via a small opening **(helicotrema).** At the base of the cochlea, the scala vestibuli ends at the oval window that is closed by the footplate of the stapes. The scala tympani ends at the **round window,** a foramen on the medial wall of the middle ear that is closed by the flexible **secondary tympanic membrane.** The **scala media,** the middle cochlear chamber, is continuous with the membranous labyrinth and does not communicate with the other two scalae.

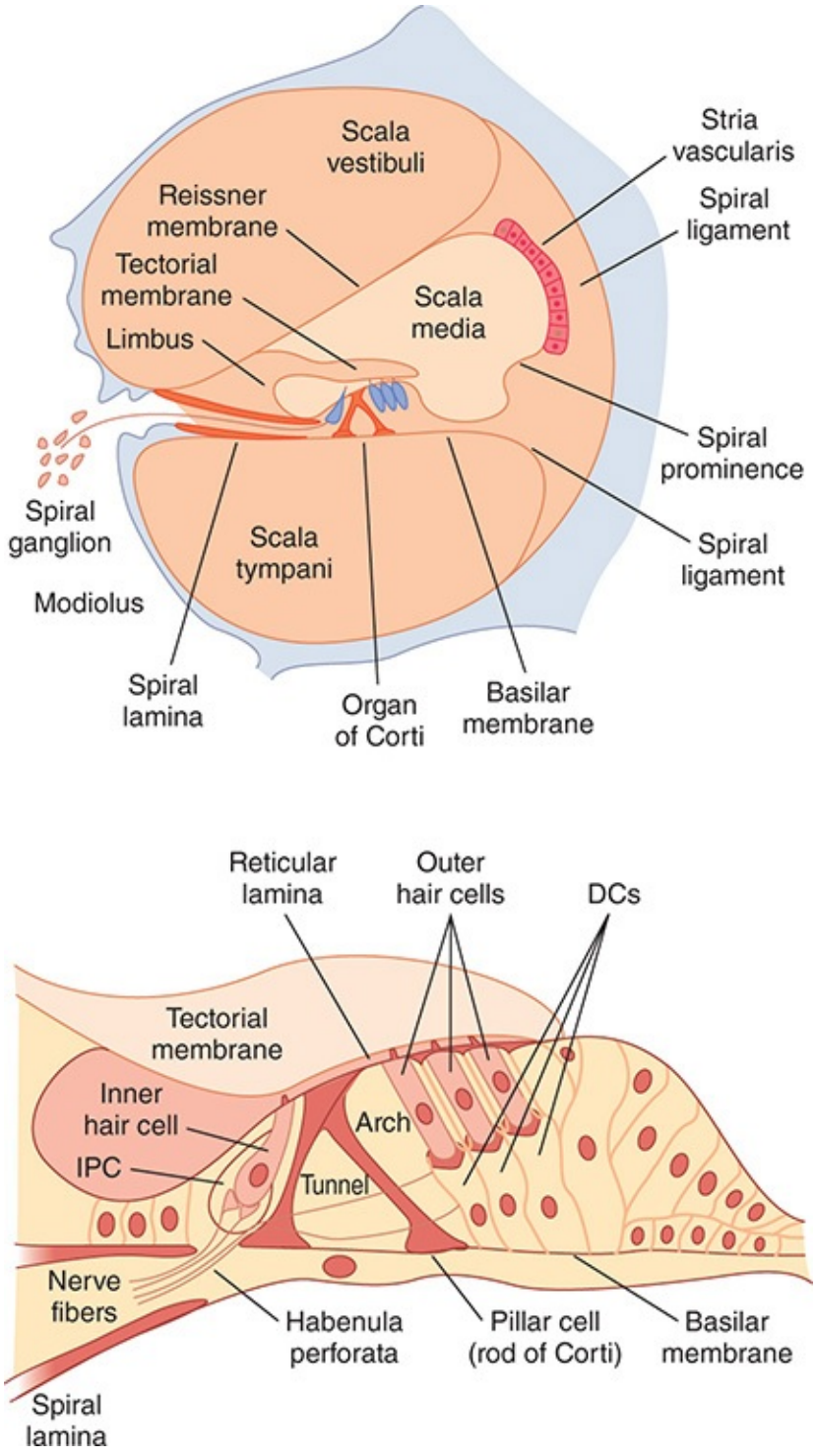


FIGURE 11–4 Schematic of the cochlea and organ of Corti in the membranous labyrinth of the inner ear. Top: The cross section of the cochlea shows the organ of Corti and the three scalae of the cochlea. **Bottom:** This shows the structure of the organ of Corti as it appears in the basal turn of the cochlea. DC, outer phalangeal cells (Deiters cells) supporting outer hair cells;

IPC, inner phalangeal cells supporting inner hair cell. (Reproduced with permission from Pickels JO: *An Introduction to the Physiology of Hearing*, 2nd ed. Academic Press; 1988.)

The spiral-shaped **organ of Corti** on the basilar membrane extends from the apex to the base of the cochlea. It contains the highly specialized auditory receptors (hair cells) whose processes pierce the tough, membrane-like **reticular lamina** that is supported by the **pillar cells** or **rods of Corti** (Figure 11–4). The hair cells are arranged in four rows: three rows of **outer hair cells** lateral to the tunnel formed by the rods of Corti and one row of **inner hair cells** medial to the tunnel. There are 20,000 outer hair cells and 3500 inner hair cells in each human cochlea. Covering the rows of hair cells is a thin, viscous, but elastic **tectorial membrane** in which the tips of the hairs of the outer but not the inner hair cells are embedded. The cell bodies of the sensory neurons that arborize around the bases of the hair cells are located in the **spiral ganglion** within the **modiolus,** the bony core around which the cochlea is wound. Most (90–95%) of these sensory neurons innervate the inner hair cells; only 5–10% innervate the more numerous outer hair cells. By contrast, most of the efferent fibers in the auditory nerve terminate on the outer rather than inner hair cells. The axons of the afferent neurons that innervate the hair cells form the **auditory (cochlear) division** of the eighth cranial nerve.

In the cochlea, gap junctions between the hair cells and the adjacent phalangeal cells prevent endolymph from reaching the base of the cell. However, the basilar membrane is relatively permeable to perilymph in the scala tympani and the tunnel of the organ of Corti. The bases of the hair cells are bathed in perilymph. Because of similar gap junctions, the arrangement is similar for the hair cells in other parts of the inner ear; that is, the processes of the hair cells are bathed in endolymph, whereas their bases are bathed in perilymph.

On each side of the head, the semicircular canals are perpendicular to each other, so that they are oriented in the three planes of space. The **crista ampullaris** (sensory organ of rotation) is located in the expanded end **(ampulla)** of each of the membranous canals (Figure 11–3). Each crista consists of hair cells and supporting cells surmounted by a gelatinous partition **(cupula)** that closes off the ampulla. The processes of the hair cells are embedded in the cupula, and the bases of the hair cells are in close contact with the afferent fibers of the **vestibular division** of the eighth cranial nerve.

A pair of otolith organs, the **saccule** and **utricle,** are located near the center of the membranous labyrinth. The **macula**, the sensory epithelium of these organs, are vertically oriented in the saccule and horizontally located in the utricle when

the head is upright. The maculae contain supporting cells and hair cells, surrounded by an otolithic membrane in which are embedded crystals of calcium carbonate, the **otoliths** (Figure 11–3). The otoliths or **otoconia** range from 3 to 19 µm in length. The processes of the hair cells are embedded in the membrane. The nerve fibers from the hair cells join those from the cristae in the vestibular division of the eighth cranial nerve.

SENSORY RECEPTORS IN THE EAR: HAIR CELLS

The specialized sensory mechanoreceptors in the ear consist of six patches of hair cells in the membranous labyrinth **(Figure 11–5)**. The hair cells in the organ of Corti signal hearing; the hair cells in the utricle signal horizontal acceleration; the hair cells in the saccule signal vertical acceleration; and a patch in each of the three semicircular canals signal rotational acceleration. Each hair cell is embedded in an epithelium made up of supporting cells, with the basal end in close contact with afferent neurons. A hair bundle projects from the apical end. It has one large **kinocilium,** a true but nonmotile cilium, with nine pairs of microtubules around a core and a central pair of microtubules. The kinocilium is lost from the cochlear hair cells in adults. The other 30–150 processes **(stereocilia)** are found in all hair cells; they have cores composed of parallel filaments of actin. There is an orderly structure within the clump of processes on each cell. Along an axis toward the kinocilium, the stereocilia increase progressively in height; along the perpendicular axis, all the stereocilia are of the same height.

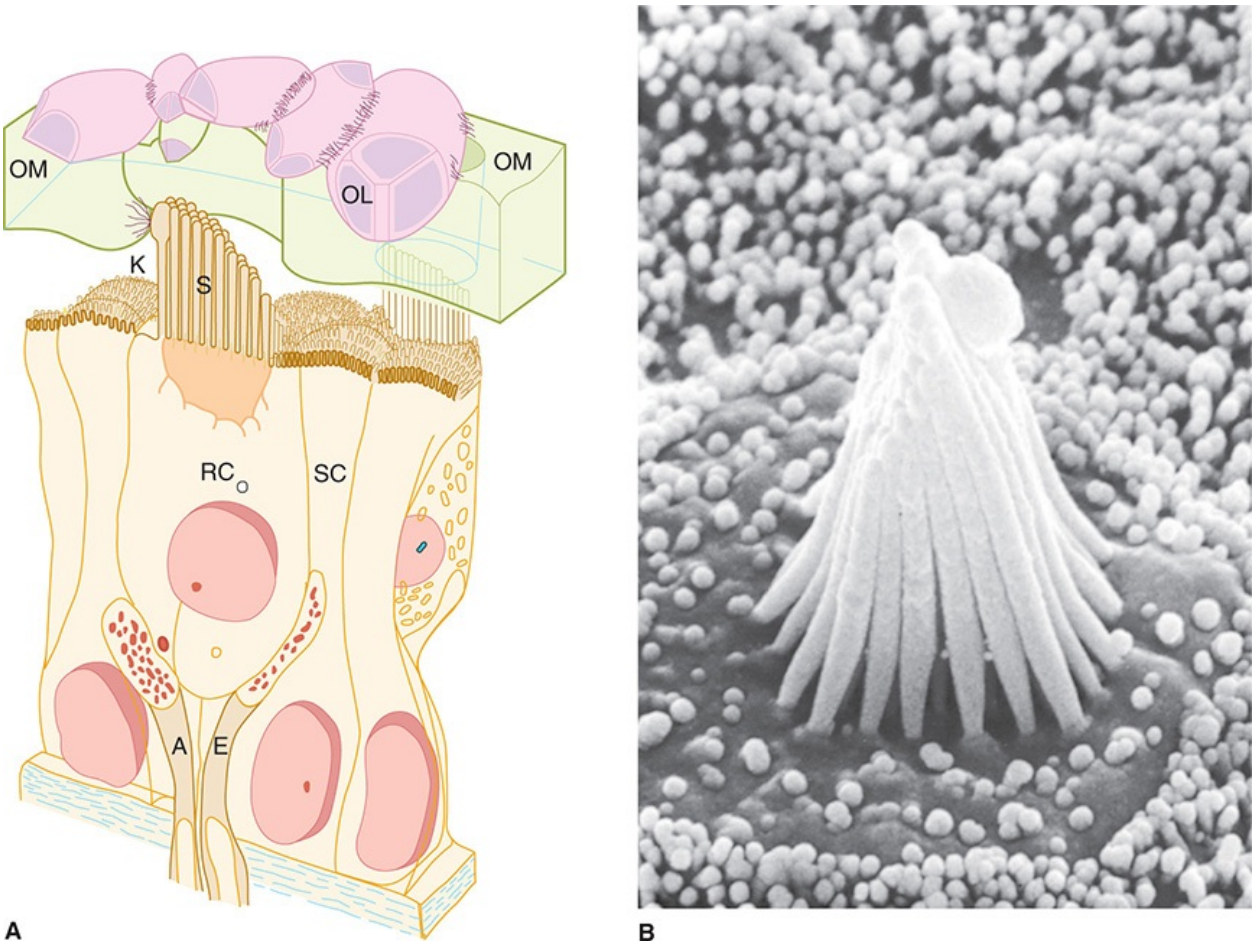


FIGURE 11–5 Structure of hair cell in the saccule. Left: Hair cells in the membranous labyrinth of the ear have a common structure, and each is within an epithelium of supporting cells (SC) surmounted by an otolithic membrane (OM) embedded with crystals of calcium carbonate, the otoliths (OL). Projecting from the apical end are rod-shaped processes, or hair cells (RC), in contact with afferent (A) and efferent (E) nerve fibers. Except in the cochlea, one of these, **kinocilium** (K), is a true but nonmotile cilium with nine pairs of microtubules around its circumference and a central pair of microtubules. The other processes, **stereocilia** (S), are found in all hair cells; they have cores of actin filaments coated with isoforms of myosin. Within the clump of processes on each cell there is an orderly structure. Along an axis toward the kinocilium, the stereocilia increase progressively in height; along the perpendicular axis, all the stereocilia are the same height. (Reproduced with permission from Llinas R, Precht W (eds): *Frog Neurobiology*. Springer; 1976.) **Right:** Scanning electron micrograph of processes on a hair cell in the saccule. The otolithic membrane has been removed. The small projections around the hair cell are microvilli on supporting cells. (Used with permission of AJ Hudspeth.)

ELECTRICAL RESPONSES IN HAIR CELLS

Very fine processes called **tip links** **(Figure 11–6)** tie the tip of each stereocilium to the side of its higher neighbor, and mechanically sensitive cation channels are at the junction in the taller process. When the shorter stereocilia are pushed toward the taller ones, the channel open time is increased. K^+, the most abundant cation in endolymph, and Ca^{2+} enter via the channel and induce depolarization. A myosin-based molecular motor in the taller neighbor then moves the channel toward the base, releasing tension in the tip link. This causes the channel to close and restores the resting state. Depolarization of hair cells causes them to release a neurotransmitter, probably glutamate, which initiates depolarization of neighboring afferent neurons.

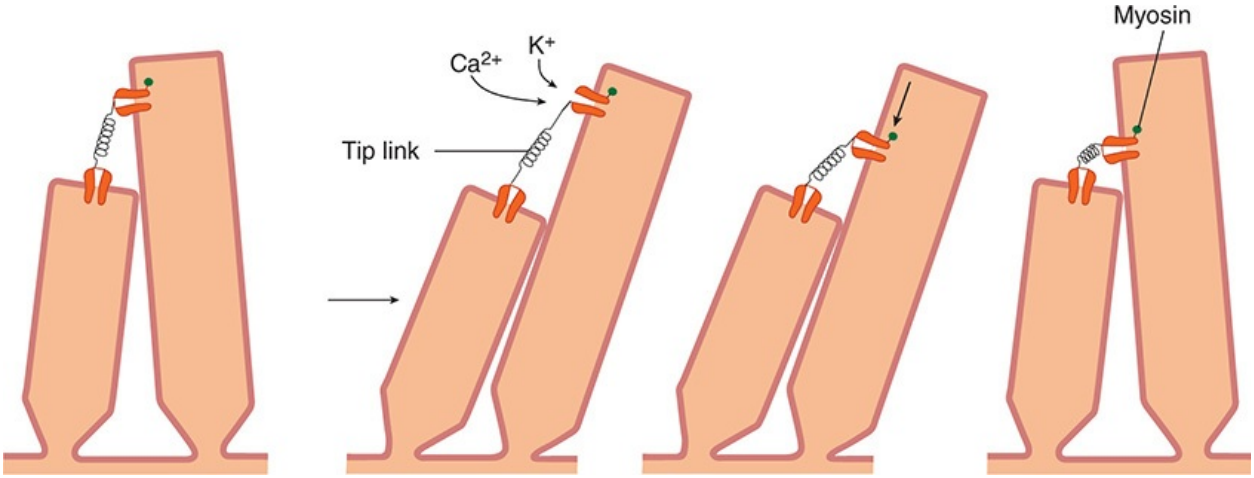


FIGURE 11–6 Schematic representation of the role of tip links in the responses of hair cells. When a stereocilium is pushed toward a taller stereocilium, the tip link is stretched and opens an ion channel in its taller neighbor. The channel next is moved down the taller stereocilium by a molecular motor, so the tension on the tip link is released. When the hairs return to the resting position, the motor moves back up the stereocilium.

The K^+ that enters hair cells via the mechanically sensitive cation channels is recycled **(Figure 11–7)**. It enters supporting cells and then passes on to other supporting cells by way of gap junctions. In the cochlea, it eventually reaches the stria vascularis and is secreted back into the endolymph, completing the cycle.

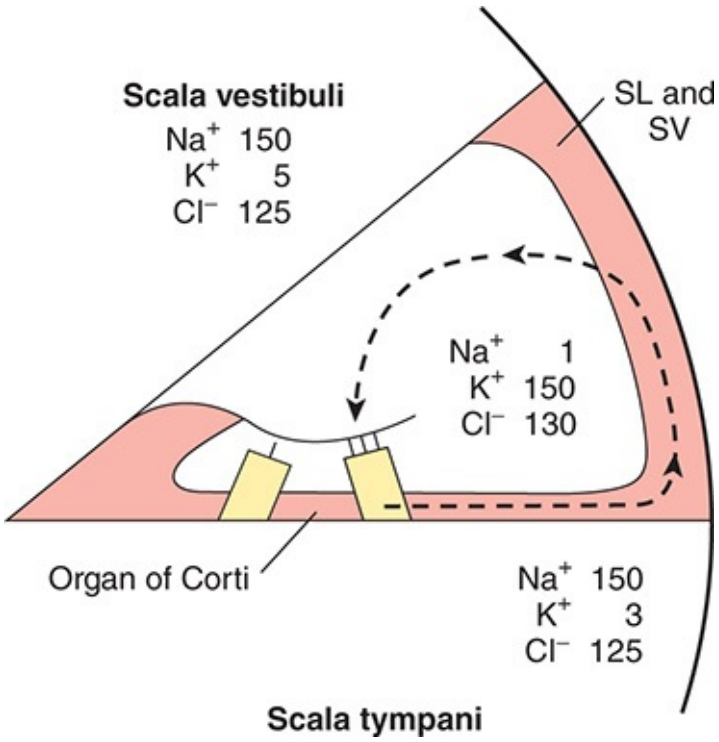


FIGURE 11–7 Ionic composition of perilymph in the scala vestibuli, endolymph in the scala media, and perilymph in the scala tympani. SL, spiral ligament. SV, stria vascularis. The dashed arrow indicates the path by which K^+ recycles from the hair cells to the supporting cells to the spiral ligament and is then secreted back into the endolymph by cells in the stria vascularis.

As described above, the processes of hair cells project into the endolymph and the bases are bathed in perilymph. This arrangement is necessary for the normal production of receptor potentials. The perilymph is formed mainly from plasma. On the other hand, endolymph is formed in the scala media by the stria vascularis and has a high concentration of K^+ and a low concentration of Na^+ (Figure 11–7). Cells in the stria vascularis have a high concentration of Na^+, K^+ ATPase. A unique electrogenic K^+ pump in the stria vascularis may account for the fact that the scala media is electrically positive by 85 mV relative to the scala vestibuli and scala tympani.

The resting membrane potential of hair cells is about −60 mV. When the stereocilia are pushed toward the kinocilium, the membrane potential is decreased to about −50 mV. When the hair bundle is pushed in the opposite direction, the cell is hyperpolarized. Displacing the processes in a direction perpendicular to this axis provides no change in membrane potential, and

displacing the processes in a direction that is intermediate between these two directions induces depolarization or hyperpolarization that is proportional to the degree to which the direction is toward or away from the kinocilium. Thus, the hair processes provide a mechanism for generating changes in membrane potential proportional to the direction and distance the hair moves.

HEARING

SOUND WAVES

Sound is the sensation produced when longitudinal vibrations of molecules in the external environment strike the tympanic membrane. **Figure 11–8** shows a plot of these movements as changes in pressure on the tympanic membrane per unit of time; such movements in the environment are called **sound waves.** The waves travel through air at a speed of 344 m/s (770 mph) at 20°C at sea level; the speed of sound increases with temperature or altitude. Other media can also conduct sound waves, but at a different speed. For example, the speed of sound is 1450 m/s at 20°C in fresh water and is even greater in salt water.

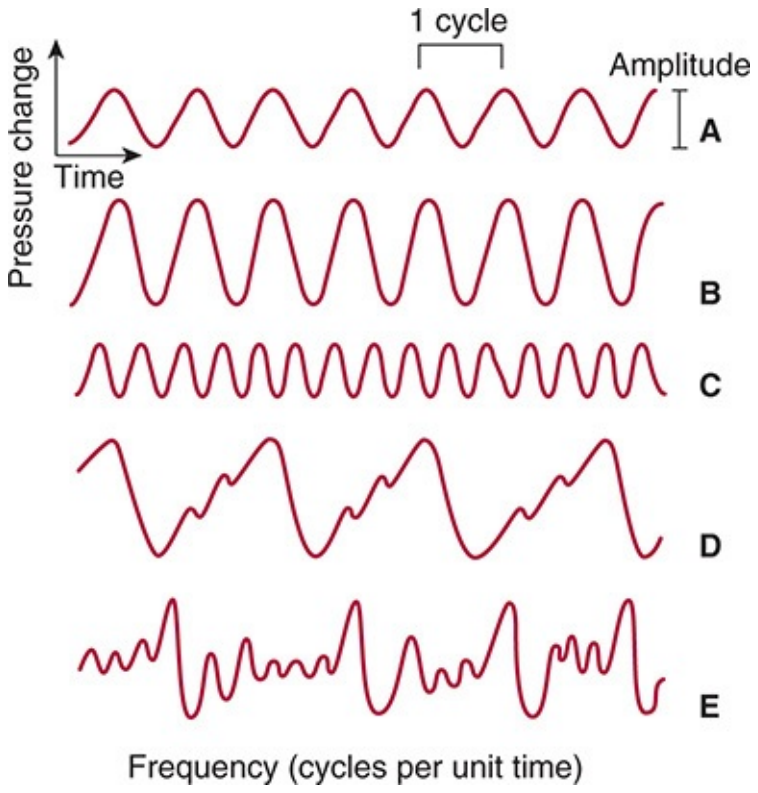


FIGURE 11–8 Characteristics of sound waves. A is the record of a pure tone.

B has a greater amplitude and is louder than **A. C** has the same amplitude as **A** but a greater frequency, and its pitch is higher. **D** is a complex wave form that is regularly repeated. Such patterns are perceived as musical sounds, whereas waves like that shown in **E,** which have no regular pattern, are perceived as noise.

In general, the **loudness** of a sound is directly correlated with the **amplitude** of a sound wave (Figure 11–8). The **pitch** of a sound is directly correlated with the **frequency** (number of waves per unit of time) of the sound wave. Sound waves that have repeating patterns, even though the individual waves are complex, are perceived as musical sounds; aperiodic nonrepeating vibrations cause a sensation of noise. Most musical sounds are made up of a wave with a primary frequency that determines the pitch of the sound plus a number of harmonic vibrations **(overtones)** that give the sound its characteristic **timbre** (quality). Variations in timbre allow us to distinguish the sounds of different musical instruments even though they are playing notes of the same pitch.

Although the pitch of a sound depends primarily on the frequency of the sound wave, loudness also plays a part; low tones (below 500 Hz) seem lower and high tones (above 4000 Hz) seem higher as their loudness increases. Duration also affects pitch to a minor degree. The pitch of a tone cannot be perceived unless it lasts for more than 0.01 s, and pitch rises as duration increases from 0.01 to 0.1 s. Finally, the pitch of complex sounds that include harmonics of a given frequency is still perceived even if the primary frequency is absent.

The amplitude of a sound wave is expressed on a **decibel scale**. The intensity of a sound in **bels** is the logarithm of the ratio of the intensity of that sound and a standard sound. A decibel (dB) is 0.1 bel. The standard sound reference level adopted by the Acoustical Society of America corresponds to 0 dB at a pressure level of 0.000204 × dyne/cm^2, a value that is just at the auditory threshold for the average human. A value of 0 dB does not mean the absence of sound but a sound level of an intensity equal to that of the standard. The 0- to 140-dB range from threshold pressure to a pressure that is potentially damaging to the organ of Corti actually represents a 10^7 (10 million)-fold variation in sound pressure.

A range of 120–160 dB (eg, firearms, jackhammer, and jet plane on takeoff) is classified as painful; 90–110 dB (eg, subway, bass drum, chain saw, and lawn mower) is extremely high; 60–80 dB (eg, alarm clock, busy traffic, dishwasher, and conversation) is very loud; 40–50 dB (eg, moderate rainfall and normal room noise) is moderate; and 30 dB (eg, whisper and library) is faint. Prolonged

or frequent exposure to sounds above 85 dB can cause hearing loss.

The sound frequencies audible to humans range from about 20 to a maximum of 20,000 cycles per second (Hz). The threshold of the human ear varies with the pitch of the sound **(Figure 11–9)**, the greatest sensitivity being in the 1000- to 4000-Hz range. The pitch of the average male voice in conversation is about 120 Hz and that of the average female voice about 250 Hz. The number of pitches that can be distinguished by an average individual is about 2000, but trained musicians can improve on this figure considerably. Pitch discrimination is best in the 1000- to 3000-Hz range and is poor at high and low pitches.

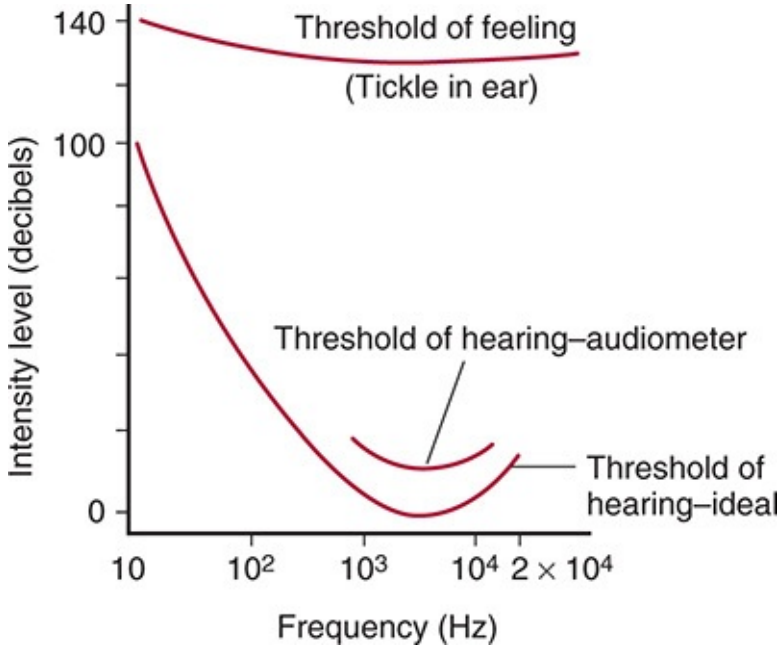


FIGURE 11–9 Human audibility curve. The middle curve is that obtained by audiometry under the usual conditions. The lower curve is that obtained under ideal conditions. At about 140 dB (top curve), sounds are felt as well as heard.

The presence of one sound decreases the ability to hear other sounds, a phenomenon known as **masking.** It is due to the relative or absolute refractoriness of previously stimulated auditory receptors and nerve fibers to other stimuli. The degree to which a given tone masks others is related to its pitch. The masking effect of the background noise in all but the most carefully soundproofed environments raises the auditory threshold by a definite and measurable amount.

SOUND TRANSMISSION

The ear converts sound waves in the external environment into action potentials in the auditory nerves. The waves are transformed by the eardrum and auditory ossicles into movements of the footplate of the stapes. These movements set up waves in the fluid of the inner ear **(Figure 11–10)**. The action of the waves on the organ of Corti generates action potentials in the nerve fibers.

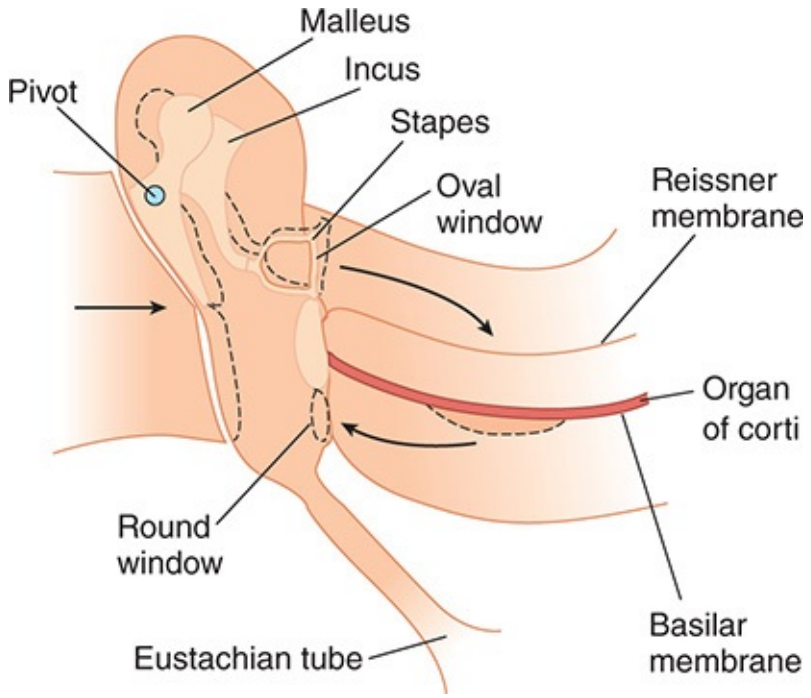


FIGURE 11–10 Schematic representation of the auditory ossicles and the way their movement translates movements of the tympanic membrane into a wave in the fluid of the inner ear. The wave is dissipated at the round window. The movements of the ossicles, the membranous labyrinth, and the round window are indicated by dashed lines. The waves are transformed by the eardrum and auditory ossicles into movements of the footplate of the stapes. These movements set up waves in the fluid of the inner ear. In response to the pressure changes produced by sound waves on its external surface, the tympanic membrane moves in and out to function as a resonator that reproduces the vibrations of the sound source. The motions of the tympanic membrane are imparted to the manubrium of the malleus, which rocks on an axis through the junction of its long and short processes, so that the short process transmits the vibrations of the manubrium to the incus. The incus moves so that the vibrations are transmitted to the head of the stapes. Movements of the head of the stapes swing its footplate.

When sound waves change the pressure on its external surface, the tympanic

membrane moves in and out. The motions of the tympanic membrane are imparted to the manubrium of the malleus. The malleus rocks on an axis through the junction of its long and short processes, so that the short process transmits the vibrations of the manubrium to the incus. The incus moves in such a way that the vibrations are transmitted to the head of the stapes. Movements of the head of the stapes swing its footplate to and fro like a door hinged at the posterior edge of the oval window. The auditory ossicles thus function as a lever system that converts the vibrations of the tympanic membrane into movements of the stapes against the perilymph-filled scala vestibuli of the cochlea (Figure 11–10). This system increases the sound pressure that arrives at the oval window, because the lever action of the malleus and incus multiplies the force 1.3 times and the area of the tympanic membrane is much greater than the area of the footplate of the stapes.

Contraction of the tensor tympani and stapedius muscles of the middle ear cause the manubrium of the malleus to be pulled inward and the footplate of the stapes to be pulled outward to reduce sound transmission (Figure 11–2). Loud sounds initiate the **tympanic reflex** that causes contraction of these muscles. This reflex prevents strong sound waves from causing excessive stimulation of the auditory receptors.

TRAVELING WAVES

The movements of the footplate of the stapes set up a series of traveling waves in the perilymph of the scala vestibuli. A diagram of such a wave is shown in **Figure 11–11**. As the wave moves up the cochlea, its height increases to a maximum and then drops off rapidly. The distance from the stapes to this point of maximum height varies with the frequency of the vibrations initiating the wave. High-pitched sounds generate waves that reach maximum height near the base of the cochlea; low-pitched sounds generate waves that peak near the apex. The bony walls of the scala vestibuli are rigid, but Reissner membrane is flexible. The basilar membrane is not under tension, and it is also readily depressed into the scala tympani by the peaks of waves in the scala vestibuli. Displacements of the fluid in the scala tympani are dissipated into air at the round window. Sound produces distortion of the basilar membrane, and the site at which this distortion is maximal is determined by the sound wave frequency. The tops of the hair cells in the organ of Corti are held rigid by the reticular lamina, and the hairs of the outer hair cells are embedded in the tectorial membrane (Figure 11–4). When the stapes moves, both membranes move in the

same direction, but they are hinged on different axes, so a shearing motion bends the hairs. The hairs of the inner hair cells are not attached to the tectorial membrane, but they are bent by fluid moving between the tectorial membrane and the underlying hair cells.

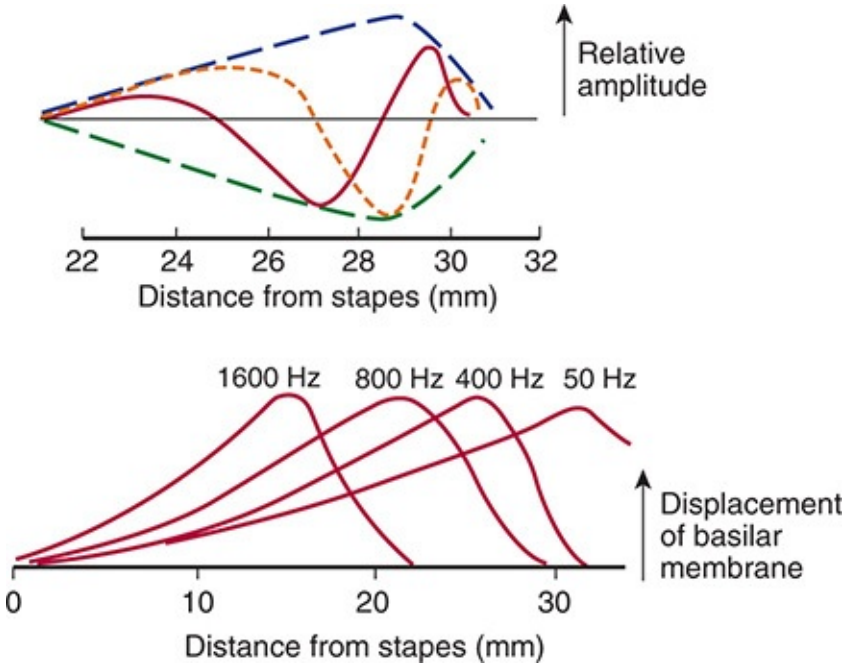


FIGURE 11–11 Traveling waves. Top: The solid and the short-dashed lines represent the wave at two instants of time. The long-dashed line shows the "envelope" of the wave formed by connecting the wave peaks at successive instants. **Bottom:** Displacement of the basilar membrane by the waves generated by stapes vibration of the frequencies shown at the top of each curve.

FUNCTIONS OF THE OUTER HAIR CELLS

The inner hair cells are the primary sensory receptors that generate action potentials in the auditory nerves and are stimulated by the fluid movements. On the other hand, the outer hair cells respond to sound like the inner hair cells, but depolarization makes them shorten and hyperpolarization makes them lengthen. They do this over a very flexible part of the basal membrane, and this action increases the amplitude and clarity of sounds. Thus, outer hair cells amplify sound vibrations entering the inner ear from the middle ear. These changes in outer hair cells occur in parallel with changes in a membrane protein **prestin,** the motor protein of outer hair cells.

The **olivocochlear bundle** is a prominent bundle of efferent fibers in each auditory nerve that arises from both ipsilateral and contralateral superior olivary complexes and ends primarily around the bases of the outer hair cells of the organ of Corti. The activity in this nerve bundle modulates the sensitivity of the hair cells via the release of acetylcholine. The effect is inhibitory, and it may function to block background noise while allowing other sounds to be heard.

ACTION POTENTIALS IN AUDITORY NERVE FIBERS

The frequency of the action potentials in a given auditory nerve fiber determines the loudness of a sound. At low sound intensities, each axon is activated by sounds of only one frequency that depends on the part of the cochlea from which the fiber originates. At higher sound intensities, the individual axons discharge to a wider spectrum of sound frequencies, particularly to frequencies lower than that at which threshold simulation occurs.

The place in the organ of Corti that is maximally stimulated determines the pitch perceived when a sound wave strikes the ear. The traveling wave set up by a tone produces peak depression of the basilar membrane, and consequently maximal receptor stimulation, at one point. As noted above, the distance between this point and the stapes is inversely related to the pitch of the sound, with low tones producing maximal stimulation at the apex of the cochlea and high tones producing maximal stimulation at the base. The pathways from the various parts of the cochlea to the brain are distinct.

CENTRAL AUDITORY PATHWAY

The afferent fibers in the auditory division of the eighth cranial nerve end in **dorsal** and **ventral cochlear nuclei** **(Figure 11–12)**. From there, auditory impulses pass by various routes to the **inferior colliculi,** the centers for auditory reflexes, and via the **medial geniculate body** in the thalamus to the **auditory cortex** located on the superior temporal gyrus of the temporal lobe. Information from both ears converges on each superior olive, and beyond this, most of the neurons respond to inputs from both sides. In humans, low tones are represented anterolaterally and high tones posteromedially in the auditory cortex.

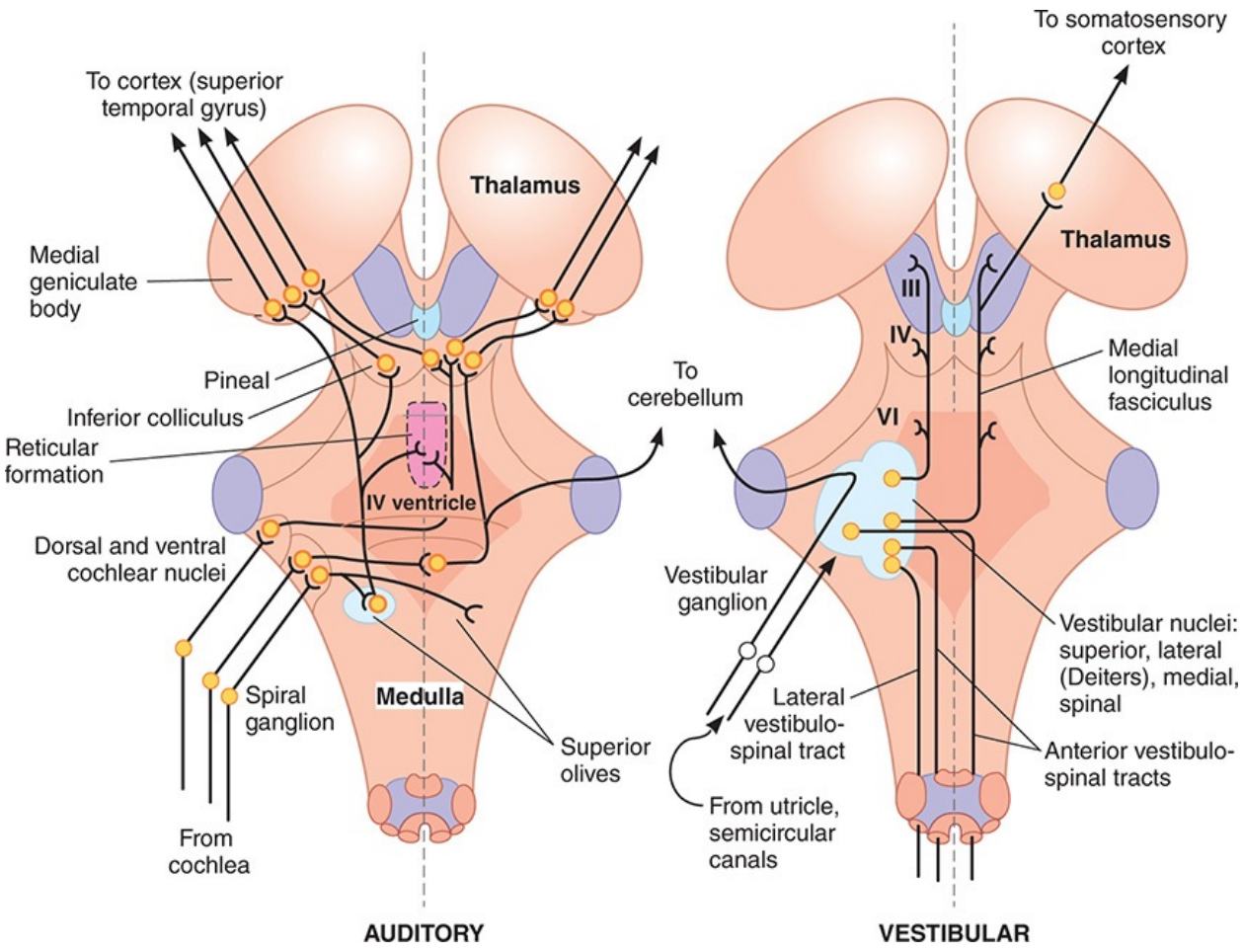


FIGURE 11–12 Simplified diagram of main auditory (left) and vestibular (right) pathways superimposed on a dorsal view of the brainstem. Cerebellum and cerebral cortex have been removed. For the auditory pathway, eighth cranial nerve afferent fibers form the cochlea end in dorsal and ventral cochlear nuclei. From there, most fibers cross the midline and terminate in the contralateral inferior colliculus. From there, fibers project to the medial geniculate body in the thalamus and then to the auditory cortex located on the superior temporal gyrus of the temporal lobe. For the vestibular pathway, the vestibular nerve terminates in the ipsilateral vestibular nucleus. Most fibers from the semicircular canals terminate in the superior and medial divisions of the vestibular nucleus and project to nuclei controlling eye movement. Most fibers from the utricle and saccule terminate in the lateral division, which then projects to the spinal cord. They also terminate on neurons that project to the cerebellum and the reticular formation. The vestibular nuclei also project to the thalamus and from there to the primary somatosensory cortex. The ascending connections to cranial nerve nuclei are concerned with eye movements.

The responses of individual second-order neurons in the cochlear nuclei to sound stimuli are like those of the individual auditory nerve fibers. The frequency at which sounds of the lowest intensity evoke a response varies from unit to unit; with increased sound intensities, the band of frequencies to which a response occurs becomes wider. The major difference between the responses of the first- and second-order neurons is the presence of a sharper "cutoff" on the low-frequency side in the medullary neurons. This greater specificity of the second-order neurons is probably due to an inhibitory process in the brainstem. In the primary auditory cortex, most neurons respond to inputs from both ears, but strips of cells are stimulated by input from the contralateral ear and inhibited by input from the ipsilateral ear.

The increasing availability of positron emission tomography (PET) scanning and functional magnetic resonance imaging (fMRI) has greatly improved the level of knowledge about auditory association areas in humans. The auditory pathways in the cortex resemble the visual pathways in that increasingly complex processing of auditory information takes place along them. An interesting observation is that although the auditory areas look very much the same on the two sides of the brain, there is marked hemispheric specialization. For example, **Wernicke area** (see Figure 8–7) is concerned with the processing of auditory signals related to speech. During language processing, this area is much more active on the left side than on the right side. Wernicke area on the right side is more concerned with melody, pitch, and sound intensity. The auditory pathways are also very plastic, and, like the visual and somatosensory pathways, they are modified by experience. Examples of auditory plasticity in humans include the observation that in individuals who become deaf before language skills are fully developed, sign language activates auditory association areas. Conversely, individuals who become blind early in life are demonstrably better at localizing sound than individuals with normal eyesight.

Musicians provide additional examples of cortical plasticity. In these individuals, the size of the auditory areas activated by musical tones is increased. In addition, violinists have altered somatosensory representation of the areas to which the fingers they use in playing their instruments project. Musicians also have larger cerebellums than nonmusicians, presumably because of learned precise finger movements.

SOUND LOCALIZATION

Determination of the direction from which a sound emanates in the horizontal

plane depends on detecting the difference in time between the arrival of the stimulus in the two ears and the consequent difference in phase of the sound waves on the two sides; it also depends on the fact that the sound is louder on the side closest to the source. The detectable time difference, which can be as little as 20 µs, is the most important factor at frequencies below 3000 Hz; the loudness difference is the most important at frequencies above 3000 Hz. Neurons in the auditory cortex that receive input from both ears respond maximally or minimally when the time of arrival of a stimulus at one ear is delayed by a fixed period relative to the time of arrival at the other ear. This fixed period varies from neuron to neuron.

Sounds coming from directly in front of the individual differ in quality from those coming from behind because each pinna (the visible portion of the exterior ear) is turned slightly forward. Reflections of sound waves from the pinnal surface change as sounds move up or down, and the change in the sound waves is the primary factor in locating sounds in the vertical plane. Sound localization is markedly disrupted by lesions of the auditory cortex.

HEARING LOSS

There are two major categories of hearing loss or deafness: sensorineural and conductive **(Clinical Box 11–1)**. **Sensorineural hearing loss** (deafness) is the most common type of hearing loss; it is usually due to the loss of cochlear hair cells but can be result from damage to the eighth cranial nerve or within central auditory pathways. It often impairs the ability to hear certain pitches while others are unaffected. Aminoglycoside antibiotics such as streptomycin and gentamicin obstruct the mechanosensitive channels in the stereocilia of hair cells (especially outer hair cells) and can cause the cells to degenerate, producing sensorineural hearing loss and abnormal vestibular function. Damage to the hair cells by prolonged exposure to noise is also associated with hearing loss. Other causes include autoimmune disorders, traumatic injuries, acoustic neuromas, tumors of the eighth cranial nerve and cerebellopontine angle, and vascular damage in the medulla.

Conductive hearing loss refers to impaired sound transmission in the external or middle ear and impacts all sound frequencies. Among the causes of conduction hearing loss are plugging of the external auditory canals with wax (cerumen) or foreign bodies, otitis externa (inflammation of the outer ear, “swimmer’s ear”) and otitis media (inflammation of the middle ear) causing fluid accumulation or scarring, or perforation of the eardrum. Severe conductive

deafness can result from **otosclerosis** in which bone is resorbed and replaced with sclerotic bone that grows over the oval window.

Auditory acuity is commonly measured with an **audiometer.** This device presents the subject with pure tones of various frequencies through earphones. At each frequency, the threshold intensity is determined and plotted on a graph as a percentage of normal hearing. This provides an objective measurement of the degree of deafness and a picture of the tonal range most affected.

Conduction and sensorineural deafness can be differentiated by simple tests with a tuning fork. Three of these tests are outlined in Table 11–1. The **Rinne test** compares sound conduction through air and bone. **Bone conduction** is the transmission of vibrations of the bones of the skull to the fluid of the inner ear. The **Weber and Schwabach tests** demonstrate the important masking effect of environmental noise on the auditory threshold.

TABLE 11–1 Common tests with a tuning fork to distinguish between sensorineural and conduction hearing loss.

	Weber	Rinne	Schwabach
Method	Base of vibrating tuning fork placed on vertex of skull	Base of vibrating tuning fork placed on mastoid process until subject no longer hears it, then held in air next to ear	Bone conduction of patient compared with that of healthy subject
Normal	Hears equally on both sides	Hears vibration in air after bone conduction is over	
Conduction deafness (one ear)	Sound louder in diseased ear because masking effect of environmental noise is absent on diseased side	Vibrations in air not heard after bone conduction is over	Bone conduction better than normal (conduction defect excludes masking noise)
Sensorineural deafness (one ear)	Sound louder in normal ear	Vibration heard in air after bone conduction is over, as long as nerve deafness is partial	Bone conduction worse than normal

CLINICAL BOX 11–1

Hearing Loss

Hearing loss is the most common sensory defect in humans. According to the World Health Organization, over 270 million people worldwide have moderate to profound hearing loss, with one-fourth of these cases beginning in childhood. According to the National Institutes of Health, ~15% of Americans between 20 and 69 years of age have high-frequency hearing loss due to exposure to loud sounds or noise at work or in leisure activities (**noise-induced hearing loss**). Both inner and outer hair cells are damaged by excessive noise, but outer hair cells appear to be more vulnerable. The use of

various chemicals (**ototoxins)** also causes hearing loss. These include some antibiotics (streptomycin), loop diuretics (furosemide), and platinum-based chemotherapy agents (cisplatin). These ototoxic agents damage the outer hair cells or the stria vascularis. **Presbycusis,** the gradual hearing loss associated with aging, affects more than one-third of those over age 75 and is probably due to gradual cumulative loss of hair cells and neurons. In most cases, hearing loss is a multifactorial disorder caused by both genetic and environmental factors. Single-gene mutations can cause hearing loss. This type of hearing loss is a monogenic disorder with an autosomal dominant, autosomal recessive, X-linked, or mitochondrial mode of inheritance. Monogenic forms of deafness can be defined as **syndromic** (hearing loss associated with other abnormalities) or **nonsyndromic** (only hearing loss). About 0.1% of newborns have genetic mutations leading to deafness. Nonsyndromic deafness due to genetic mutations can first appear in adults rather than in children and may account for many of the 16% of all adults who have significant hearing impairment. It is estimated that the products of 100 or more genes are essential for normal hearing, and deafness loci have been identified in all but 5 of the 24 human chromosomes. The most common mutation leading to congenital hearing loss is that of the protein connexin 26. This defect prevents the normal recycling of K^+ through the sustentacular cells. Mutations in three nonmuscle myosins also cause deafness. These are myosin-VIIa, associated with the actin in the hair cell processes; myosin-Ib, which is probably part of the "adaptation motor" that adjusts tension on the tip links; and myosin-VI, which is essential in some way for the formation of normal cilia. Deafness is also associated with mutant forms of α-tectin, one of the major proteins in the tectorial membrane. An example of syndromic deafness is **Pendred syndrome,** in which a mutant multifunctional anion exchanger causes deafness and goiter. Another example is one form of the **long QT syndrome** in which one of the K^+ channel proteins, **KVLQT1,** is mutated. In the stria vascularis, the normal form of this protein is essential for maintaining the high K^+ concentration in endolymph, and in the heart it helps maintain a normal QT interval. Individuals who are homozygous for mutant KVLQT1 are deaf and predisposed to the ventricular arrhythmias and sudden death that characterize the long QT syndrome. Mutations of the membrane protein **barttin** can cause deafness as well as the renal manifestations of Bartter syndrome.

THERAPEUTIC HIGHLIGHTS

Cochlear implants are used to treat both children and adults with severe hearing loss. The US Food and Drug Administration reported that, as of December 2012, approximately 324,000 cochlear devices have been implanted worldwide and at least 50,000 are implanted every year. They may be used in children as young as 12 months old. These devices consist of a microphone (picks up environmental sounds), a speech processor (selects and arranges these sounds), a transmitter and receiver/stimulator (converts these sounds into electrical impulses), and an electrode array (sends the impulses to the auditory nerve). Although the implant cannot restore normal hearing, it provides a useful representation of environmental sounds to a deaf person. Those with adult-onset deafness who receive cochlear implants can learn to associate the signals it provides with sounds they remember. Children who receive cochlear implants in conjunction with intensive therapy have been able to acquire speech and language skills. Research is also underway to develop cells that can replace the hair cells in the inner ear. For example, researchers at Stanford University were able to generate cells resembling mechanosensitive hair cells from mouse **embryonic** and **pluripotent stem cells.**

Hearing aids can also be used to treat sensorineural hearing loss in individuals who have significant residual hearing. The microphone component of an analog hearing aid receives sound that converts sound waves (vibrations) to electrical signals; an amplifier then increases the power of the signal and sends it to the ear via a speaker. Digital hearing aids convert sound waves into numerical codes akin to a computer binary code before amplifying them. The code also includes information about pitch or loudness, so it can be programmed to amplify selectively those sound wave frequencies to which the wearer is least sensitive.

Injury to inner ear hair cells can induce random electrical impulses that are then relayed to the auditory cortex, leading to an intermittent or steady, high-pitched ringing in the ear **(tinnitus)**. Tinnitus affects about 50 million Americans and can be a symptom of age-related hearing loss, excessive exposure to loud noises, ear infections, or otosclerosis. In most cases, its cause is unknown. Hypertension and atherosclerosis are risk factors for the development of tinnitus. Also, it can be triggered or worsened by the use of antimalarial drugs, antibiotics, chemotherapeutic drugs, diuretics, or high doses of aspirin.

VESTIBULAR SYSTEM

The vestibular system can be divided into the **vestibular apparatus** and central **vestibular nuclei.** The vestibular apparatus within the inner ear detects head motion and position and transduces this information to a neural signal (Figure 11–3). The vestibular nuclei are primarily concerned with maintaining the position of the head in space. The tracts that descend from these nuclei mediate head-on-neck and head-on-body adjustments.

CENTRAL VESTIBULAR PATHWAY

The cell bodies of the 19,000 neurons supplying the cristae and maculae on each side are located in the vestibular ganglion. Each vestibular nerve terminates in the ipsilateral four-part vestibular nucleus (Figure 11–12) and in the flocculonodular lobe of the cerebellum (not shown in the figure). Fibers from the semicircular canals terminate primarily in the superior and medial divisions of the vestibular nucleus; neurons in this region project mainly to nuclei controlling eye movement (see Chapter 10). Fibers from the utricle and saccule project predominantly to the lateral division (Deiters nucleus) of the vestibular nucleus which then projects to the contralateral spinal cord. The descending vestibular nucleus receives input from the otolith and projects to the cerebellum, reticular formation, and the spinal cord. The ascending connections to cranial nerve nuclei are control eye movements; the vestibular nuclei also ascend to project to thalamocortical neurons.

RESPONSES TO ROTATIONAL ACCELERATION

Rotational acceleration in the plane of a given semicircular canal stimulates its crista. The endolymph, because of its inertia, is displaced in a direction opposite to the direction of rotation. The fluid pushes on the cupula, deforming it. This bends the processes of the hair cells (Figure 11–3). When a constant speed of rotation is reached, the fluid spins at the same rate as the body and the cupula swings back into the upright position. When rotation is stopped, deceleration produces displacement of the endolymph in the direction of the rotation, and the cupula is deformed in a direction opposite to that during acceleration. It returns to mid position in 25–30 s. Movement of the cupula in one direction increases the firing rate of single nerve fibers from the crista; movement in the opposite direction inhibits neural activity **(Figure 11–13)**.

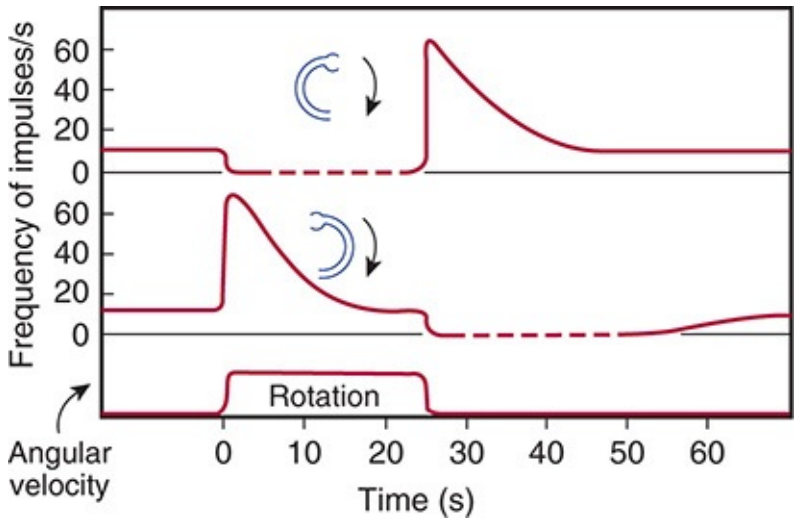


FIGURE 11–13 Ampullary responses to rotation. Average time course of impulse discharge from the ampulla of two semicircular canals during rotational acceleration, steady rotation, and deceleration. Movement of the cupula in one direction increases the firing rate of single nerve fibers from the crista, and movement in the opposite direction inhibits neural activity. (Reproduced with permission from Adrian ED: Discharges from vestibular receptors in the cat. J Physiol 1943; Mar 25; 101(4):389–407.)

Rotation causes maximal stimulation of the semicircular canals most nearly in the plane of rotation. Because the canals on one side of the head are a mirror image of those on the other side, the endolymph is displaced toward the ampulla on one side and away from it on the other. The pattern of stimulation reaching the brain varies with the direction as well as the plane of rotation. Linear acceleration probably fails to displace the cupula and therefore does not stimulate the cristae. However, when one part of the labyrinth is destroyed, other parts take over its functions. **Clinical Box 11–2** describes the characteristic eye movements that occur during a period of rotation.

RESPONSES TO LINEAR ACCELERATION

The utricular and saccular maculae respond to horizontal and vertical acceleration, respectively. The otoliths in the surrounding membrane are denser than the endolymph, and acceleration in any direction causes them to be displaced in the opposite direction, distorting the hair cell processes and generating activity in the vestibular nerve. The maculae also discharge in the absence of head movement due to the pull of gravity on the otoliths.

The impulses generated from these receptors are partly responsible for **labyrinth righting reflexes.** The reflex is initiated by tilting of the head that stimulates the otolithic organs; the response is a compensatory contraction of the neck muscles to keep the head level. A **vestibulo-ocular reflex** stabilizes images on the retina during head movements. Vestibular stimulation during the rotation leads to inhibition of extraocular muscles on one side and activation on the extraocular muscles on the other side.

CLINICAL BOX 11–2

Nystagmus

Nystagmus is the characteristic jerky movement of the eye observed at the start and end of a period of rotation. It is actually a reflex that maintains visual fixation on stationary points while the body rotates. When rotation starts, the eyes move slowly in a direction opposite to the direction of rotation, maintaining visual fixation (**vestibulo-ocular reflex**). When the limit of this movement is reached, the eyes quickly snap back to a new fixation point and then again move slowly in the other direction. The slow component is initiated by impulses from the vestibular labyrinths; the quick component is triggered by a center in the brainstem. Nystagmus is frequently horizontal (ie, the eyes move in the horizontal plane), but it can be vertical (when the head is tipped sideways during rotation) or rotatory (when the head is tipped forward). By convention, the direction of eye movement in nystagmus is identified by the direction of the quick component. The direction of the quick component during rotation is the same as that of the rotation, but the postrotatory nystagmus that occurs due to displacement of the cupula when rotation is stopped is in the opposite direction. When nystagmus is seen at rest, it is a sign of a pathology. Two examples of this are **congenital nystagmus** that is seen at birth and **acquired nystagmus** that occurs later in life. In these clinical cases, nystagmus can persist for hours at rest. Acquired nystagmus can be seen in patients with acute **temporal bone fracture** affecting **semicircular canals** or after damage to the **flocculonodular lobe** or the **fastigial nucleus.** It can also occur as a result of stroke, multiple sclerosis, head injury, and brain tumors. Some drugs (especially antiseizure drugs), alcohol, and sedatives can cause nystagmus.

Nystagmus can be used as a diagnostic indicator of the integrity of the vestibular system. Caloric stimulation can be used to test the function of the

vestibular labyrinth. The semicircular canals are stimulated by instilling warm (40°C) or cold (30°C) water into the external auditory meatus. The temperature difference sets up convection currents in the **endolymph,** with consequent motion of the cupula. In healthy persons, warm water causes nystagmus that bears toward the stimulus, whereas cold water induces nystagmus that bears toward the opposite ear. This test is given the mnemonic **COWS (C**old water nystagmus is **O**pposite side; **W**arm water nystagmus is **S**ame side). In the case of a unilateral lesion in the vestibular pathway, nystagmus is reduced or absent on the side of the lesion. To avoid nystagmus, vertigo, and nausea when irrigating the ear canals in the treatment of ear infections, the fluid used should be at body temperature.

THERAPEUTIC HIGHLIGHTS

There is no cure for acquired nystagmus, and treatment depends on the cause. Correcting the underlying cause (stopping drug usage, surgical removal of a tumor) is often the treatment of choice. Also, rectus muscle surgery has been used successfully to treat some cases of acquired nystagmus. Short-term correction of nystagmus can result from injections of **botulinum toxin** (Botox) to paralyze the ocular muscles.

Although most of the responses to stimulation of the maculae are reflex in nature, vestibular impulses also reach the cerebral cortex. These impulses may mediate conscious perception of motion and supply part of the information necessary for orientation in space. **Vertigo** is the sensation of rotation in the absence of actual rotation and is a prominent symptom when one labyrinth is inflamed.

SPATIAL ORIENTATION

Orientation in space depends in part on input from the vestibular receptors, but visual cues are also important. Spatial orientation also uses information from proprioceptors in joint capsules and from cutaneous touch and pressure receptors. These four inputs are synthesized at a cortical level into a continuous picture of the individual's orientation in space. **Clinical Box 11–3** describes some common vestibular disorders.

CLINICAL BOX 11–3

Vestibular Disorders

Vestibular balance disorders are the ninth most common reason for visits to a primary care clinician. It is one of the most common reasons elderly people seek medical advice. Patients often describe balance problems in terms of vertigo, dizziness, lightheadedness, and motion sickness. Neither lightheadedness nor dizziness is necessarily a symptom of vestibular problems, but **vertigo** is a prominent symptom of a disorder of the inner ear or vestibular system, especially when one labyrinth is inflamed. **Benign paroxysmal positional vertigo** is the most common vestibular disorder and is characterized by episodes of vertigo that occur with particular changes in body position (eg, turning over in bed and bending over). One possible cause is that **otoconia** from the utricle separate from the otolith membrane and become lodged in the canal or cupula of the semicircular canal. This causes abnormal deflections when the head changes position relative to gravity.

Ménière disease is an abnormality of the inner ear causing vertigo or severe dizziness, tinnitus, fluctuating hearing loss, and the sensation of pressure or pain in the affected ear lasting several hours. Symptoms can occur suddenly and recur daily or very rarely. The hearing loss is initially transient but can become permanent. The pathophysiology may involve an immune reaction. An inflammatory response can increase fluid volume within the membranous labyrinth, causing it to rupture and allowing the endolymph and perilymph to mix together. The worldwide prevalence for Ménière disease is ~12 per 1000 individuals. It is diagnosed most often between the ages of 30 and 60, and it affects both sexes similarly.

The nausea, blood pressure changes, sweating, pallor, and vomiting that are the well-known symptoms of **motion sickness** are produced by excessive vestibular stimulation and occur when conflicting information is fed into the vestibular and other sensory systems. **Space motion sickness** (ie, the nausea, vomiting, and vertigo experienced by astronauts) develops when they are first exposed to microgravity and often wears off after a few days of space flight. It can then recur with reentry, as the force of gravity increases again. It is due to mismatches in neural input created by changes in the input from some parts of the vestibular apparatus and other gravity sensors without corresponding changes in the other spatial orientation inputs.

THERAPEUTIC HIGHLIGHTS

Symptoms of benign paroxysmal positional vertigo often subside over weeks or months, but if treatment is needed, one option is a procedure called **canalith repositioning.** This consists of simple and slow maneuvers to position your head to move the otoconia from the semicircular canals back into the vestibule that houses the utricle. There is no cure for Ménière disease, but the symptoms can be controlled by reducing the fluid retention through dietary changes (low-salt or salt-free diet, no caffeine, no alcohol) or medications such as diuretics (eg, **hydrochlorothiazide**). Individuals with Ménière disease often respond to drugs used to alleviate the symptoms of vertigo. **Vestibulosuppressants** such as the **antihistamine meclizine** decrease the excitability of the middle ear labyrinth and block conduction in middle ear vestibular-cerebellar pathway. Motion sickness commonly can be prevented with the use of antihistamines or **scopolamine,** a **cholinergic muscarinic receptor antagonist.**

CHAPTER SUMMARY

- The external ear includes the auricle, external auditory meatus, and tympanic membrane; the external ear captures sound waves and directs them toward the middle ear. The middle ear contains three bones (malleus, incus, and stapes), the Eustachian tube, and the tensor tympani and stapedius muscles. The inner ear contains the cochlea with the receptors (hair cells) for hearing and the semicircular canals with hair cell receptors for balance.
- The pressure changes produced by sound waves cause the tympanic membrane to move in and out; thus, it functions as a resonator to reproduce the vibrations of the sound source. The auditory ossicles serve as a lever system to convert the vibrations of the tympanic membrane into movements of the stapes against the perilymph-filled scala vestibuli of the cochlea.
- Sound is the sensation produced when longitudinal vibrations of air molecules strike the tympanic membrane. The hair cells in the organ of Corti signal hearing. The stereocilia provide a mechanism for generating changes in membrane potential proportional to the direction and distance the hair moves.
- Loudness is correlated with the amplitude of a sound wave, pitch with the frequency, and timbre with harmonic vibrations.

- The activity within the auditory pathway passes from the eighth cranial nerve afferent fibers to the dorsal and ventral cochlear nuclei to the inferior colliculi to the thalamic medial geniculate body and then to the auditory cortex.
- Tinnitus, a high-pitched ringing in the ear, can result from injury to inner ear hair cells. Presbycusis is age-related hearing loss due to the gradual cumulative loss of hair cells. Single-gene mutations can cause hearing loss; these monogenic forms of deafness are defined as either syndromic (hearing loss associated with other abnormalities) or nonsyndromic (only hearing loss).
- Sensorineural hearing loss is usually due to loss of cochlear hair cells but can result from damage to the eighth cranial nerve or central auditory pathway. Conductive hearing loss is due to impaired sound transmission in the external or middle ear and impacts all sound frequencies. Conductive and sensorineural deafness can be differentiated by simple tests (eg, Rinne test) with a tuning fork.
- Cochlear implants consist of a microphone, a speech processor, a transmitter and receiver/stimulator that converts sounds into electrical impulses, and an electrode array that sends the impulses to the auditory nerve. Hearing aids consist of a microphone, an amplifier and a speaker. Analog hearing aids convert sound waves to electrical signals; digital hearing aids convert sound waves into numerical codes that provide information about pitch or loudness.
- Rotational acceleration stimulates the crista in the semicircular canals, displacing the endolymph in a direction opposite to the direction of rotation, deforming the cupula and bending the hair cell. The utricle responds to horizontal acceleration and the saccule to vertical acceleration. Acceleration in any direction displaces the otoliths, distorting the hair cell processes and generating neural activity.
- Spatial orientation is dependent on input from vestibular receptors, visual cues, proprioceptors in joint capsules, and cutaneous touch and pressure receptors.
- Nystagmus is the characteristic jerky movement of the eye at the start and end of a period of rotation; it is actually a reflex that maintains visual fixation on stationary points while the body rotates (vestibulo-ocular reflex). A test that induces nystagmus (COWS) by instilling warm or cold water into the external auditory meatus can be used to evaluate the integrity of the

vestibular system.

- Benign paroxysmal positional vertigo is the most common vestibular disorder and is characterized by episodes of vertigo that occur with particular changes in body. Ménière disease is an abnormality of the inner ear that causes vertigo, tinnitus, hearing loss, and sensation of pressure or pain in the affected ear. Symptoms of motion sickness (eg, nausea, sweating, pallor, and vomiting) occur when conflicting information is fed into the vestibular and other sensory systems.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. A 9-year-old girl complained of ear pain due to an inflammation and build-up of fluids in the middle ear. She was diagnosed with a middle ear infection, acute otitis media of bacterial origin, and she was treated with an antibiotic. The middle ear contains

   A. hair cells that mediate linear acceleration.

   B. the membranous labyrinth containing endolymph.

   C. the bony labyrinth containing perilymph fluid.

   D. cochlear hair cells that mediate hearing.

   E. the auditory ossicles and the tensor tympani and stapedius muscles.

2. A 2-year-old girl was diagnosed with a type of sensorineural deafness. After being evaluated by an audiologist, she was determined to be a good candidate for a cochlear implant. A cochlear implant is comprised of

   A. a microphone, transmitter, and receiver that converts sound into vibrating waves.

   B. artificial hair cells that are able to replace damaged hair cells in the inner ear.

   C. a microphone, amplifier, and speaker that can convert sound waves into electrical signals.

   D. a microphone, speech processor, transmitter, and receiver that converts sound into electrical impulses.

   E. a microphone, transmitter, and receiver that converts sound into numerical codes that provide information about pitch or loudness.

3. After playing the violin for the Boston Symphony Orchestra for 18 years, a

40-year-old man was given the opportunity to follow the dream he had as a child to be a physician like his father. Compared to other students in his medical school class, what distinctive features might be expressed in his auditory system?

A. The pitch of his conversational voice will be about 120 Hz compared to his younger male classmates whose voices are likely to have a pitch of about 250 Hz.

B. When presented with musical tones, a larger area of his auditory cortex will be activated compared to the area activated in the cortex of his classmates who are not musically inclined.

C. As a musician, he will be better able to localize sound than most of his classmates.

D. The Wernicke area on the left side of his brain will be more concerned with melody, pitch, and sound.

E. He will be able to distinguish about 2000 pitches; in contrast, his younger classmates will be able to distinguish only about 1000 pitches.

4. A 40-year-old man, employed as a road construction worker for nearly 20 years, went to his clinician to report that he recently began to notice difficulty hearing during normal conversations. A Weber test showed that sound from a vibrating tuning fork was localized to the right ear. A Schwabach test showed that bone conduction was below normal. A Rinne test showed that both air and bone conductions were abnormal, but air conduction lasted longer than bone conduction. The diagnosis was

A. sensorial hearing loss in both ears.

B. conduction deafness in the right ear.

C. sensorial deafness in the right ear.

D. conduction deafness in the left ear.

E. sensorineural deafness in the left ear.

5. A medical resident was asked to give a lecture to medical students on how sound is transmitted from the environment through the ear. He would have said the following about the roles of the auditory ossicles in this process.

A. The movement of the malleus transmits the vibrations of the manubrium to the incus.

B. The movement of the incus allows the sound wave to go through the oval window.

C. The movement of the stapes transmits the vibrations of the manubrium to

the incus.

D. The movement of the stapes allows the sound wave to go through the round window.

E. The movement of the malleus transmits the vibrations of the tympanic membrane to the stapes.

6. A medical resident was asked to give a lecture to medical students on how the brain synthesizes information to provide the sense of the position of the body in space. He would have said that the following sensory inputs play an important role in spatial orientation.

A. vestibular receptors, cochlear receptors, retinal receptors, and cutaneous pressure receptors

B. cochlear receptors, retinal receptors, proprioceptors, and cutaneous touch and pressure receptors

C. vestibular receptors, visual cues, proprioceptors in joints, and cutaneous touch and pressure receptors

D. middle ear receptors, visual cues, IA and IB sensory fibers, and touch receptors

E. organ of Corti hair cells, membranous labyrinth hair cells, visual clues, and touch receptors

7. The components of the auditory pathway are

A. sensory fibers in the acoustic branch of the eighth cranial nerve, the lateral cochlear nucleus, the superior colliculus, and the auditory cortex.

B. sensory fibers in the auditory branch of the eighth cranial nerve, the lateral cochlear nucleus, the inferior colliculus, lateral geniculate body, and the superior temporal gyrus of the cortex.

C. afferent fibers of the eighth cranial nerve, the dorsal and ventral cochlear nuclei, the inferior colliculi, the lateral geniculate body, and the auditory cortex.

D. sensory fibers in the cochlear branch of the eighth cranial nerve, the dorsal and ventral cochlear nuclei, the inferior colliculus, the medial geniculate body, and the auditory cortex.

E. afferent fibers of the cochlear branch of the eighth cranial nerve, the ventral cochlear nuclei, the superior colliculi, the lateral geniculate body, and the superior temporal gyrus of the cortex.

8. A healthy male medical student volunteered to undergo evaluation of the function of his vestibular system for a class demonstration. The direction of

his nystagmus is expected to be vertical when he is rotated

A. after warm water is put in one of his ears.

B. with his head tipped backward.

C. after cold water is put in both of his ears.

D. with his head tipped sideways.

E. with his head tipped forward.

9. A 65-year old man had an acute injury to the utricle of the inner ear, causing problems with balance. In the utricle, tip links in hair cells are involved in

A. formation of perilymph.

B. depolarization of the stria vascularis.

C. movements of the basement membrane.

D. perception of sound.

E. regulation of distortion-activated ion channels.

10. A 40-year old woman made an appointment with an otolaryngologist due to ringing in her ear that has been interfering with her ability to concentrate. What is a likely diagnosis and a potential cause of this symptom?

A. Pendred syndrome due to inflammation of the middle ear hair cells that induce random electrical impulses in the auditory nerve.

B. Syndromic hearing due to a single gene mutation.

C. Presbycusis that can result from injury to inner ear hair cells that induce random electrical impulses in the eighth cranial nerve.

D. Tinnitus that can be caused by injury to inner ear hair cells that induce random electrical impulses in the auditory nerve.

E. Tinnitus due to an accumulation of perilymph moving over inner ear hair cells.

11. An MD/PhD candidate was doing research on the generation of changes in the membrane potential of cochlear inner hair cells. What steps are involved in this process?

A. When the shorter stereocilia are pushed toward the taller ones, the channel open time is increased, and K^+ and Ca^{2+} enter via the channel and induce hyperpolarization.

B. When the shorter stereocilia are pushed toward the taller ones, the channel open time is increased, and K^+ and Ca^{2+} enter via the channel and induce depolarization.

C. When the taller stereocilia are pushed toward the shorter ones, the channel

open time is increased, and Na^+ and Ca^{2+} exit via the channel and induce hyperpolarization.

D. When the stereocilia move toward the sound source, the channel open time is decreased, and K^+ and Cl^- exit via the channel and induce depolarization.

E. When the stereocilia move toward the sound source, the channel open time is increased, and K^+ and Cl^- enter via the channel and induce depolarization.

12. A 45-year-old woman sought medical advice when she experienced sudden onset of vertigo, tinnitus and hearing loss in her left ear, nausea, and vomiting. She was referred to an otolaryngologist to rule out Ménière disease. Which of the following are possible causes of Ménière disease?

A. Ménière disease is autosomal dominant genetic disorder that weakens the membranous labyrinth of the inner ear.

B. The hair cells of the cochlea are altered to give the sensation of motion even at rest.

C. The otoliths dislodge, enter the semicircular canal, and stimulate the hair cells.

D. An inflammatory response increases fluid volume within the membranous labyrinth, causing it to rupture and allowing the endolymph and perilymph to intermix.

E. The membranous labyrinth on one side has become inflamed.

CHAPTER 12

Reflex & Voluntary Control of Posture & Movement

OBJECTIVES

After studying this chapter, you should be able to:

- Describe the basic elements of a reflex pathway.
- Identify the components, function, and afferent nerve fibers of the muscle spindle.
- Explain the neuronal response initiated by striking the patellar tendon (patellar tendon or knee jerk reflex) that leads to contraction of a muscle.
- Explain how the activity of γ-motor neurons alters the response to muscle stretch.
- Describe the role of Golgi tendon organs in the control of skeletal muscle.
- Define physiologic tremor, clonus, and muscle tone.
- Identify the components and function of the withdrawal reflex pathway.
- Define spinal shock and describe the initial and long-term changes in spinal reflexes that follow spinal cord injury.
- Describe how skilled movements are planned and carried out.
- Contrast the organization of the central pathways involved in the control of axial (posture) and distal (skilled movement, fine motor movements)

muscles.

- Describe the clinical tests and findings that distinguish between upper and lower motor neuron disorders, including the Babinski sign and clonus.
- Identify the pathophysiology and characteristics of cerebral palsy, decerebrate rigidity, and decorticate rigidity.
- Identify the components of the basal ganglia and the pathways that interconnect them, along with the neurotransmitters in each pathway.
- Explain the pathophysiology and symptoms of Parkinson disease, Huntington disease, and other pathologies of the basal ganglia pathways.
- Discuss the functions of the cerebellum and the neurologic abnormalities produced by diseases of this part of the brain.

INTRODUCTION

Somatic motor function depends ultimately on the activity of the spinal motor neurons and homologous neurons in the motor nuclei of the cranial nerves. These neurons, the final common pathways to skeletal muscle, are bombarded by impulses from an immense array of descending pathways, other spinal neurons, and peripheral afferents. Some of these inputs end directly on α-motor neurons, but many exert their effects via interneurons or via γ-motor neurons to the muscle spindles and back through the Ia afferent fibers to the spinal cord. It is the integrated activity of these multiple inputs from spinal, brainstem, midbrain, and cortical levels that regulates the posture of the body and makes coordinated movement possible.

The inputs converging on motor neurons have three major functions: to induce voluntary activity, to adjust body posture, and to make movements smooth and precise. The patterns of voluntary activity are planned within the brain, and the commands are sent to the muscles primarily via the corticospinal and corticobulbar systems. Posture is continually adjusted not only before but also during movement by information carried in descending brainstem pathways and peripheral afferents. Movement is smoothed and coordinated by the medial and intermediate portions of the cerebellum (spinocerebellum) and its connections. The basal ganglia and the lateral portions of the cerebellum (cerebrocerebellum) are part of a feedback circuit to the premotor and motor cortex that is concerned with planning and organizing voluntary movement.

Chapter 11 introduced somatomotor control by describing the role of the

vestibular system in control of balance and equilibrium. This chapter considers two types of motor output: reflex (involuntary) and voluntary. A subdivision of reflex responses includes some rhythmic movements such as swallowing, chewing, scratching, and walking, which are largely involuntary but are subject to voluntary adjustment and control.

GENERAL PROPERTIES OF REFLEXES

The basic unit of integrated reflex activity is the **reflex arc** that consists of a sense organ, an afferent neuron, one or more synapses within a central integrating station, an efferent neuron, and an effector. The afferent neurons enter via the dorsal roots or cranial nerves and have their cell bodies in dorsal root ganglia or homologous cranial nerve ganglia. The efferent fibers leave via the ventral roots or motor cranial nerves.

Activity in the reflex arc starts in a sensory receptor with a **receptor potential** (see Chapter 8) whose magnitude is proportional to the strength of the stimulus **(Figure 12–1)**. This generates all-or-none action potentials in the afferent nerve, the number of action potentials being proportional to the size of the receptor potential. In the central nervous system (CNS), the responses are again graded in terms of excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) at the synaptic junctions (see Chapter 6). When the action potentials in the efferent motor nerve reach the effector, a graded response is induced. If the graded response is adequate to produce action potentials in the muscle, muscle contraction will ensue. The activity in the reflex arc is modified by the multiple inputs converging on the efferent neurons or at any synaptic station within the reflex arc.

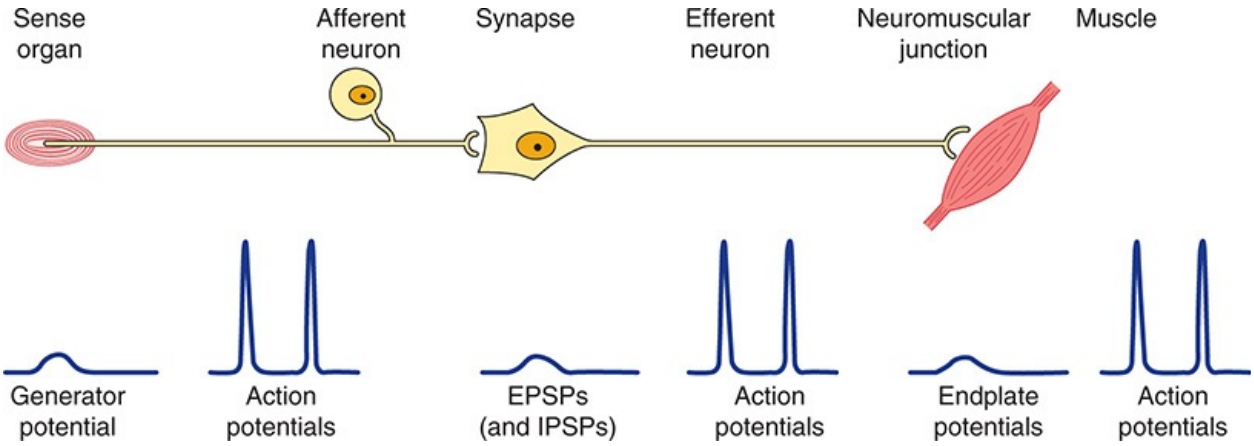


FIGURE 12–1 The reflex arc. Note that at the receptor and in the CNS a

nonpropagated graded response occurs that is proportional to the magnitude of the stimulus. The response at the neuromuscular junction is also graded, though under normal conditions it is always large enough to produce a response in skeletal muscle. On the other hand, in the portions of the arc specialized for transmission (afferent and efferent nerve fibers, muscle membrane), the responses are all-or-none action potentials.

Reflex activity is stereotyped and specific in that a particular stimulus elicits a particular response. The fact that reflex responses are stereotyped does not exclude the possibility of their being modified by experience. Reflexes are adaptable and can be modified to perform motor tasks and maintain balance. Descending inputs from higher brain regions play an important role in modulating and adapting spinal reflexes.

The **α-motor neurons** that supply the extrafusal fibers in skeletal muscles are the efferent side of many reflex arcs. All neural influences affecting muscular contraction ultimately funnel through them to the muscles, thus they are called the **final common pathway.** Numerous inputs converge on α-motor neurons; the surface of an average motor neuron and its dendrites accommodates about 10,000 synaptic knobs. At least five inputs go from the same spinal segment to a typical spinal motor neuron. In addition to these, there are excitatory and inhibitory inputs, generally relayed via interneurons, from other levels of the spinal cord and multiple long-descending tracts from the brain. All of these pathways converge on and determine the activity in the final common pathway.

MONOSYNAPTIC REFLEXES: THE STRETCH REFLEX

The simplest reflex arc is the one with a single synapse between the afferent and efferent neurons (i.e., **monosynaptic reflexes**). Reflex arcs in which interneurons are interposed between the afferent and efferent neurons are called **polysynaptic reflexes.** There can be anywhere from two to hundreds of synapses in a polysynaptic reflex arc.

When a skeletal muscle with an intact nerve supply is stretched, it contracts. This response is called the **stretch reflex** or **myotatic reflex.** The stimulus that initiates this reflex is stretch of the muscle, and the response is contraction of the muscle being stretched. The sense organ is a small encapsulated spindle-like or fusiform-shaped structure called the **muscle spindle,** located within the fleshy part of the muscle. The impulses originating from the spindle are transmitted to

the CNS by fast sensory fibers that pass directly to the motor neurons that supply the same muscle. The neurotransmitter at the central synapse is glutamate. The stretch reflex is the best known and studied monosynaptic reflex and is typified by the **knee jerk reflex** (Clinical Box 12–1).

CLINICAL BOX 12–1

Knee Jerk Reflex

Tapping the patellar tendon elicits the **knee jerk,** a stretch reflex of the quadriceps femoris muscle, because the tap on the tendon stretches the muscle. A similar contraction is observed if the quadriceps is stretched manually. The knee jerk reflex is an example of a **deep tendon reflex (DTR)** in a neurologic exam and is graded on the following scale: 0 (absent), 1+ (hypoactive), 2+ (brisk, normal), 3+ (hyperactive without clonus), 4+ (hyperactive with mild clonus), and 5+ (hyperactive with sustained clonus). Absence of the knee jerk can signify an abnormality anywhere within the reflex arc, including the muscle spindle, the Ia afferent nerve fibers, or the motor neurons to the quadriceps muscle. The most common cause is a peripheral neuropathy from such things as diabetes, alcoholism, and toxins. A hyperactive reflex can signify an interruption of corticospinal and other descending pathways that suppress the activity in the reflex arc. DTR is not specific to an assessment of the knee jerk reflex; stretch reflexes can be elicited from most of the large muscles of the body. Tapping on the tendon of the triceps brachii, for example, causes an extensor response at the elbow as a result of reflex contraction of the triceps. Tapping on the Achilles tendon causes an ankle jerk due to reflex contraction of the gastrocnemius, and tapping on the side of the face causes a stretch reflex in the masseter. The spinal nerve involved in testing various DTRs is as follows: biceps (C5, C6 spinal nerve); triceps (C7 spinal nerve); patellar (L4 spinal nerve); and Achilles tendon (S1 spinal nerve).

STRUCTURE OF MUSCLE SPINDLES

Each muscle spindle has three essential elements: (1) a group of specialized **intrafusal muscle fibers** with contractile polar ends and a noncontractile center, (2) large diameter myelinated afferent nerves (types Ia and II) originating in the

central portion of the intrafusal fibers, and (3) small diameter myelinated efferent nerves supplying the polar contractile regions of the intrafusal fibers **(Figure 12–2A)**. It is important to understand the relationship of these elements to each other and to the muscle itself to appreciate the role of this sense organ in signaling changes in the length of the muscle in which it is located. Changes in muscle length are associated with changes in joint angle; thus, muscle spindles provide information on position (ie, **proprioception**).

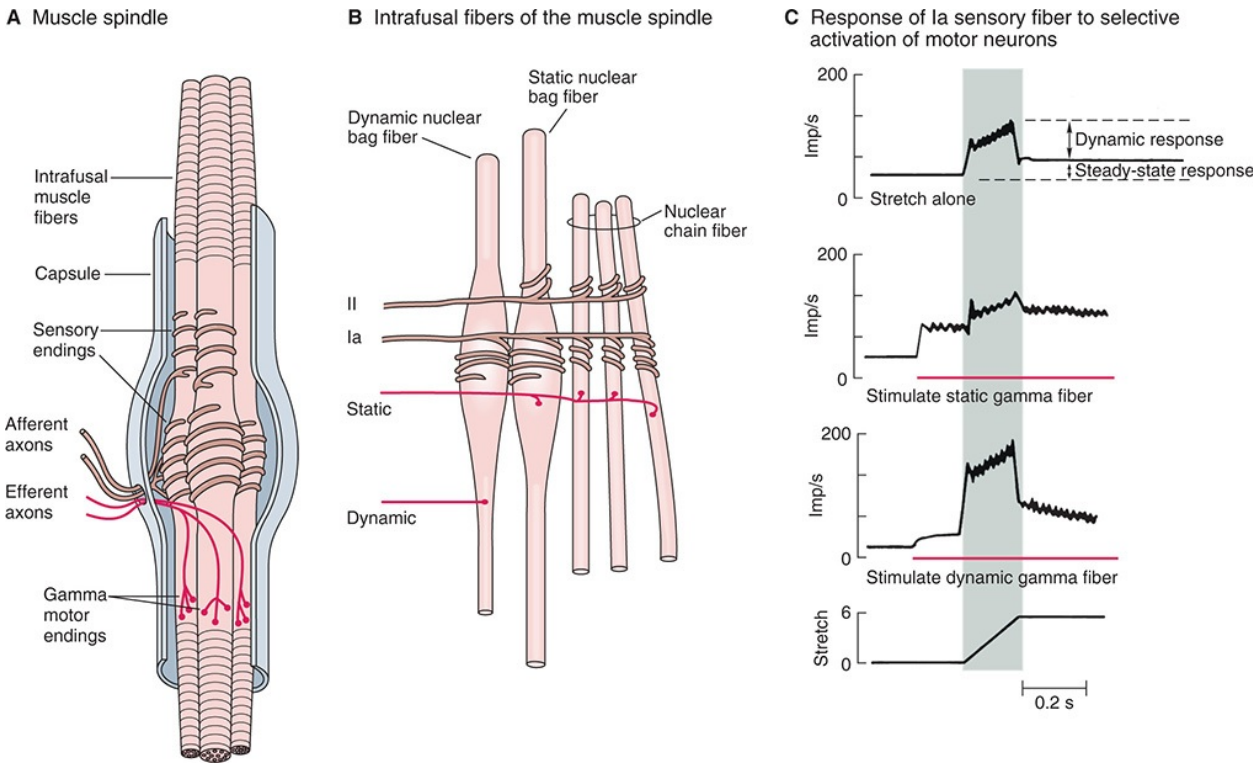


FIGURE 12–2 Mammalian muscle spindle. A) Diagrammatic representation of the main components of mammalian muscle spindle including intrafusal muscle fibers, afferent sensory fiber endings, and efferent motor fibers (γ-motor neurons). **B)** Three types of intrafusal muscle fibers: dynamic nuclear bag, static nuclear bag, and nuclear chain fibers. A single Ia afferent fiber innervates all three types of fibers to form a primary sensory ending. A group II sensory fiber innervates nuclear chain and static bag fibers to form a secondary sensory ending. Dynamic γ-motor neurons innervate dynamic bag fibers; static γ-motor neurons innervate combinations of chain and static bag fibers. **C)** Comparison of discharge pattern of Ia afferent activity during stretch alone and during stimulation of static or dynamic γ-motor neurons. Without γ-stimulation, Ia fibers show a small dynamic response to muscle stretch and a modest increase in steady-state firing. When static γ-motor neurons are activated, the steady-state

response increases and the dynamic response decreases. When dynamic γ-motor neurons are activated, the dynamic response is markedly increased but the steady-state response gradually returns to its original level. (Reproduced with permission from Gray H: *Gray's Anatomy: The Anatomical Basis of Clinical Practice*, 40th ed. St. Louis, MO: Churchill Livingstone/Elsevier; 2009.)

The **intrafusal fibers** are positioned in parallel to the **extrafusal fibers** (the regular contractile units of the muscle) with the ends of the spindle capsule attached to the tendons at either end of the muscle. Intrafusal fibers do not contribute to the overall contractile force of the muscle, but rather serve a pure sensory function. There are two types of intrafusal fibers in mammalian muscle spindles. The first type contains many nuclei in a dilated central area and is called a **nuclear bag fiber** (**Figure 12–2B**). There are two subtypes of nuclear bag fibers: **dynamic** and **static.** The second intrafusal fiber type, the **nuclear chain fiber,** is thinner and shorter and lacks a definite bag. Typically, each muscle spindle contains two or three nuclear bag fibers and about five nuclear chain fibers.

There are two kinds of sensory endings in each spindle, a single **primary (group Ia) ending** and up to eight **secondary (group II) endings** (Figure 12–2B). The Ia afferent fiber wraps around the center of the dynamic and static nuclear bag fibers and nuclear chain fibers. Group II sensory fibers are located adjacent to the centers of the static nuclear bag and nuclear chain fibers; these fibers do not innervate the dynamic nuclear bag fibers. Ia afferents are very sensitive to the velocity of the change in muscle length during a stretch **(dynamic response);** thus, they provide information about the speed of movements and allow for quick corrective movements. The steady-state (tonic) activity of group Ia and II afferents provide information on steady-state length of the muscle **(static response).** The top trace in **Figure 12–2C** shows the dynamic and static components of activity in a Ia afferent during muscle stretch. Note that they discharge most rapidly while the muscle is being stretched (shaded area of graphs) and less rapidly during sustained stretch.

The spindles have a motor nerve supply of their own called **γ-motor neurons;** they are 3–6 µm in diameter and constitute about 30% of the fibers in the ventral roots. There are two types of γ-motor neurons: **dynamic** that supply dynamic nuclear bag fibers and **static** that supply static nuclear bag fibers and nuclear chain fibers. Activation of dynamic γ-motor neurons increases the dynamic sensitivity of the group Ia endings. Activation of the static γ-motor neurons increases the tonic level of activity in both group Ia and II endings, decreases the dynamic sensitivity of group Ia afferents, and can prevent silencing

of Ia afferents during muscle stretch (Figure 12–2C).

CENTRAL CONNECTIONS OF AFFERENT FIBERS

Ia fibers end directly on motor neurons supplying the extrafusal fibers of the same muscle **(Figure 12–3)**. The **reaction time** is the interval between the application of a stimulus and the response. In humans, the reaction time for a stretch reflex is 19–24 ms. Weak stimulation of the sensory nerve from the muscle that stimulates only Ia fibers causes a contractile response with a similar latency. Because the conduction velocities of the afferent and efferent fiber types are known and the distance from the muscle to the spinal cord can be measured, it is possible to calculate how much of the reaction time was taken up by conduction to and from the spinal cord. When this value is subtracted from the reaction time, the remainder (**central delay)** is the time taken for the reflex activity to traverse the spinal cord. The central delay for the knee jerk reflex is 0.6–0.9 ms. Because the minimum synaptic delay is 0.5 ms, only one synapse could have been traversed.

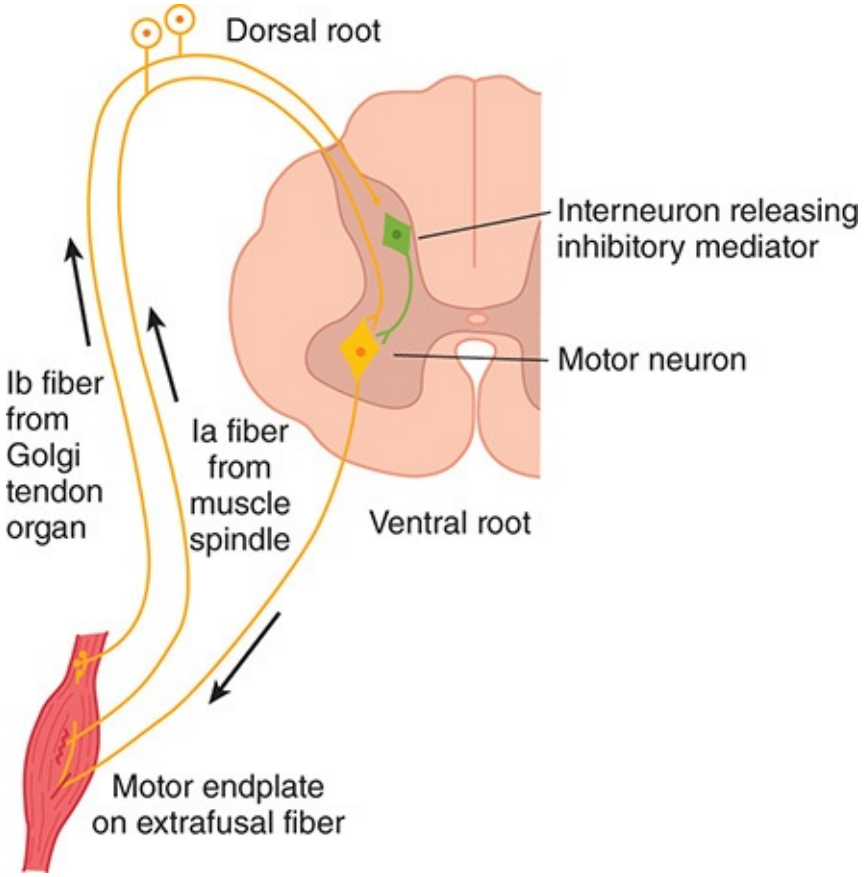


FIGURE 12–3 Diagram illustrating the pathways responsible for the stretch reflex and the inverse stretch reflex. Stretch stimulates the muscle spindle, which activates Ia fibers that excite the motor neuron. Stretch also stimulates the Golgi tendon organ, which activates Ib fibers that excite an interneuron that releases the inhibitory mediator glycine. With strong stretch, the resulting hyperpolarization of the motor neuron is so great that it stops discharging.

FUNCTION OF MUSCLE SPINDLES

When the muscle spindle is stretched, its sensory endings are distorted and receptor potentials are generated. These in turn set up action potentials in the sensory fibers at a frequency proportional to the degree of stretching. Because the spindle is in parallel with the extrafusal fibers, when the muscle is passively stretched, the spindles are also stretched, referred to as **“loading the spindle.”** This initiates reflex contraction of the extrafusal fibers in the muscle. On the other hand, the spindle afferents characteristically stop firing when the muscle is made to contract by electrical stimulation of the α-motor neurons to the extrafusal fibers because the muscle shortens while the spindle is unloaded

(Figure 12–4).

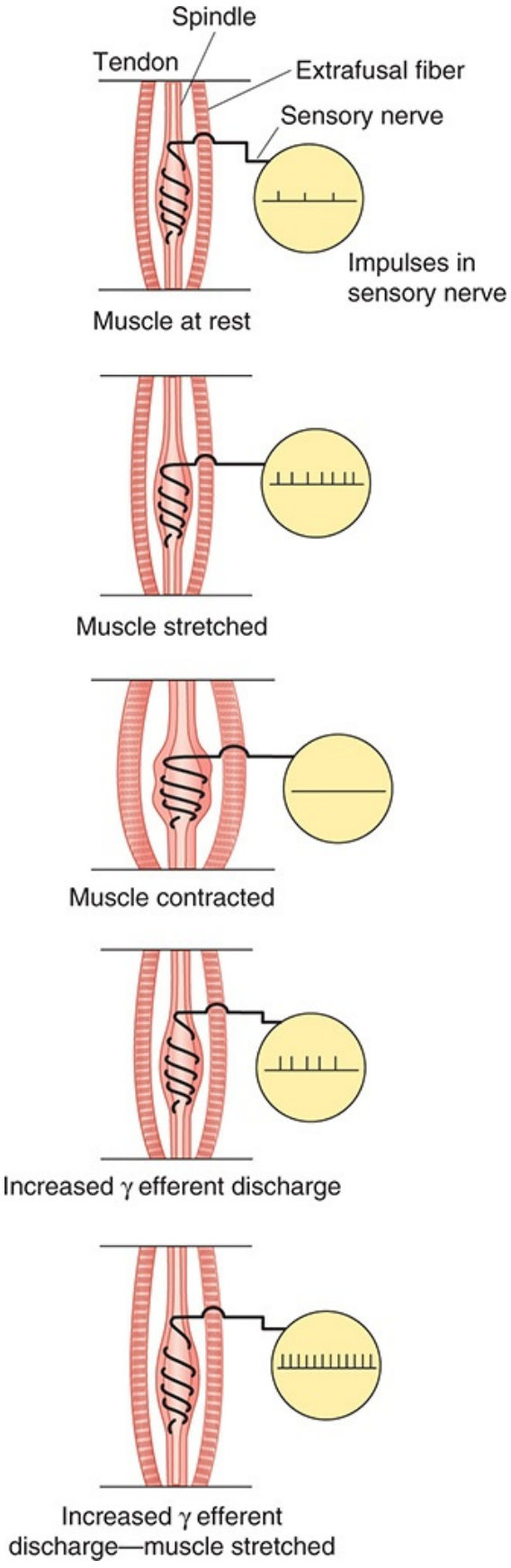

Spindle
Tendon
Extrafusal fiber
Sensory nerve
Impulses in sensory nerve
Muscle at rest
Muscle stretched
Muscle contracted
Increased γ efferent discharge
Increased γ efferent discharge—muscle stretched

FIGURE 12–4 Effect of various conditions on muscle spindle discharge. When the whole muscle is stretched, the muscle spindle is also stretched and its sensory endings are activated at a frequency proportional to the degree of stretching ("loading the spindle"). Spindle afferents stop firing when the muscle contracts ("unloading the spindle"). Stimulation of γ-motor neurons causes the contractile ends of the intrafusal fibers to shorten. This stretches the nuclear bag region, initiating impulses in sensory fibers. If the whole muscle is stretched during stimulation of the γ-motor neurons, the rate of discharge in sensory fibers is further increased.

The muscle spindle and its reflex connections constitute a feedback device that operates to maintain muscle length. If the muscle is stretched, spindle discharge increases and reflex shortening is produced. If the muscle is shortened without a change in γ-motor neuron discharge, spindle afferent activity decreases and the muscle relaxes.

Dynamic and static responses of muscle spindle afferents influence **physiologic tremor.** The response of the Ia sensory fiber endings to the dynamic (phasic) as well as the static events in the muscle is important because the prompt, marked phasic response helps dampen oscillations caused by conduction delays in the feedback loop regulating muscle length. Normally a small oscillation occurs in this feedback loop. This physiologic tremor has low amplitude (barely visible to the naked eye) and a frequency of approximately 10 Hz. Physiologic tremor is a normal phenomenon that affects everyone while maintaining posture or during movements. However, the tremor would be more prominent if it were not for the sensitivity of the spindle to velocity of stretch. It can become exaggerated in some situations such as when we are anxious or tired or because of drug toxicity. Numerous factors contribute to the genesis of physiologic tremor. It is dependent on both central **(inferior olive)** sources and peripheral factors including motor unit firing rates, reflexes, and mechanical resonance.

RECIPROCAL INNERVATION

When a stretch reflex occurs, the opposing muscles relax due to **reciprocal innervation.** Impulses in the Ia fibers from the muscle spindles of the protagonist muscle cause postsynaptic inhibition of the motor neurons to the antagonists. A collateral from each Ia fiber passes in the spinal cord to an

inhibitory interneuron that synapses on a motor neuron supplying the antagonist muscles. This example of postsynaptic inhibition is discussed in Chapter 6, and the pathway is illustrated in Figure 6–5.

EFFECTS OF γ-MOTOR NEURON DISCHARGE

Activation of γ-motor neurons produces a very different picture from that produced by activation of the α-motor neurons. Activation of γ-motor neurons does not lead directly to detectable contraction of the muscles because intrafusal fibers are not strong enough or plentiful enough to cause shortening. However, their activation does cause the contractile ends of the intrafusal fibers to shorten and therefore stretches the nuclear bag portion of the spindles, deforming the endings, and initiating impulses in Ia fibers (Figure 12–4). This in turn can lead to reflex contraction of the muscle. Thus, muscles can be made to contract via activation of the α-motor neurons that innervate the extrafusal fibers or the γ-motor neurons that initiate contraction indirectly via the stretch reflex.

If the whole muscle is stretched during stimulation of the γ-motor neurons, the rate of discharge in the Ia fibers is further increased (Figure 12–4). Increased γ-motor neuron activity thus increases **spindle sensitivity** during stretch.

In response to descending excitatory input to spinal motor circuits, both α- and γ-motor neurons are activated. Because of this **"α-γ coactivation,"** intrafusal and extrafusal fibers shorten together, and spindle afferent activity can occur throughout the period of muscle contraction. In this way, the spindle remains capable of responding to stretch and reflexively adjusting α-motor neuron discharge.

CONTROL OF γ-MOTOR NEURON DISCHARGE

The γ-motor neurons are regulated by descending tracts originating in areas of the brain that also control α-motor neurons (described below). Via these pathways, the sensitivity of the muscle spindles and hence the threshold of the stretch reflexes in various parts of the body can be adjusted and shifted to meet the needs of postural control.

Other factors also influence γ-motor neuron activity. Anxiety causes an increased discharge, a fact that probably explains the hyperactive tendon reflexes sometimes seen in anxious patients. In addition, unexpected movement is associated with a greater efferent discharge. Stimulation of the skin, especially

by noxious agents, increases γ-motor neuron activity to ipsilateral flexor muscle spindles while decreasing that to extensors and produces the opposite pattern in the opposite limb. Trying to pull the hands apart when the flexed fingers are hooked together facilitates the knee jerk reflex **(Jendrassik maneuver),** and this may also be due to increase γ-motor neuron discharge initiated by afferent impulses from the hands.

INVERSE STRETCH REFLEX

Up to a point, the harder a muscle is stretched, the stronger is the reflex contraction. However, when the tension becomes great enough, contraction suddenly ceases and the muscle relaxes. This relaxation in response to strong stretch is called the **inverse stretch reflex.** The receptor for the inverse stretch reflex is in the **Golgi tendon organ** (Figure 12–5) that consists of a netlike collection of knobby nerve endings among the fascicles of a tendon. There are 3–25 muscle fibers per tendon organ. The fibers from the Golgi tendon organs are the Ib group of myelinated, rapidly conducting sensory nerve fibers. Activation of these Ib fibers leads to IPSPs in the motor neurons that supply the muscle from which the fibers arise. The Ib fibers end in the spinal cord on inhibitory interneurons that terminate directly on the motor neurons (Figure 12–3). They also make excitatory connections with motor neurons supplying antagonists to the muscle.

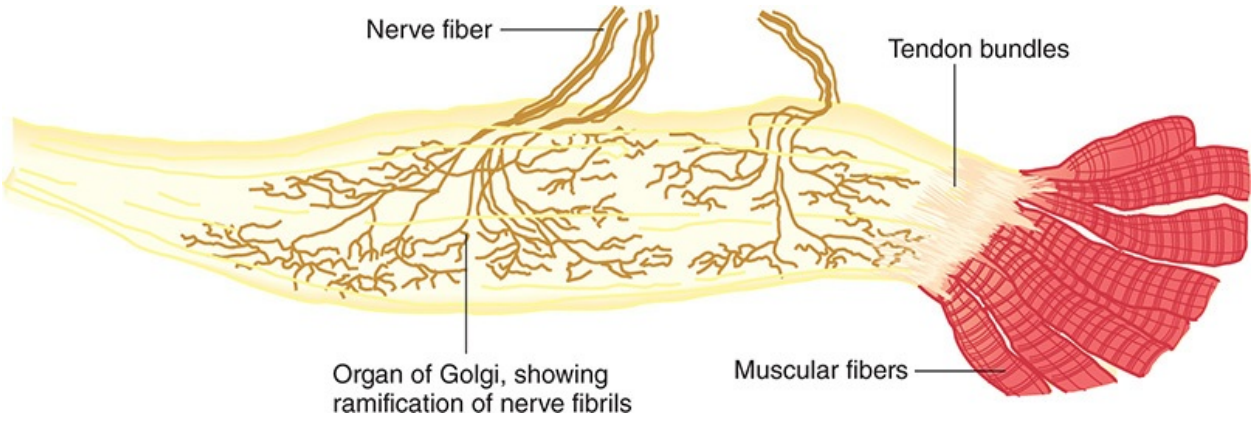


FIGURE 12–5 Golgi tendon organ. This organ is the receptor for the inverse stretch reflex and consists of a netlike collection of knobby nerve endings among the fascicles of a tendon. The innervation is the Ib group of myelinated, rapidly conducting sensory nerve fibers. (Reproduced with permission from Gray H: *Gray's Anatomy: The Anatomical Basis of Clinical Practice,* 40th ed. St. Louis,

MO: Churchill Livingstone/Elsevier; 2009.)

Because the Golgi tendon organs, unlike the spindles, are in series with the muscle fibers, they are stimulated by both passive stretch and active contraction of the muscle. The threshold of the Golgi tendon organs is low. The degree of stimulation by passive stretch is not great because the more elastic muscle fibers take up much of the stretch; therefore, it takes a strong stretch to produce relaxation. However, discharge is regularly produced by contraction of the muscle, and the Golgi tendon organ thus functions as a transducer in a feedback circuit that regulates muscle force in a manner analogous to the spindle feedback circuit that regulates muscle length.

CLINICAL BOX 12–2

Clonus

Clonus is characteristic of states in which there is increased γ-motor neuron activity. This neurologic sign is the occurrence of regular, repetitive, rhythmic contractions of a muscle subjected to sudden, maintained stretch. Only sustained clonus with five or more beats is considered abnormal. Ankle clonus is initiated by brisk, maintained dorsiflexion of the foot; the response is rhythmic plantar flexion at the ankle. The **stretch reflex-inverse stretch reflex sequence** may contribute to this response. However, it can occur on the basis of synchronized motor neuron discharge without Golgi tendon organ activation. The spindles of the tested muscle are hyperactive, and the burst of impulses from them activates all the motor neurons supplying the muscle at once. The consequent muscle contraction stops spindle discharge. However, the stretch has been maintained, and as soon as the muscle relaxes it is again stretched and the spindles stimulated. There are numerous causes of abnormal clonus including traumatic brain injury, brain tumors, strokes, and multiple sclerosis. Clonus may also occur in spinal cord injury that disrupts the descending cortical input to a spinal glycinergic inhibitory interneuron called the **Renshaw cell.** This cell receives excitatory input from α-motor neurons via axon collaterals (and in turn it inhibits the same α-motor neuron). In addition, cortical fibers activating ankle flexors synapse on Renshaw cells (as well as type Ia inhibitory interneurons) that inhibit the antagonistic ankle extensors. This circuitry prevents reflex stimulation of the extensors when flexors are active. Therefore, when the descending cortical fibers are

damaged **(upper motor neuron lesion),** the inhibition of antagonists is absent. The result is repetitive, sequential contraction of ankle flexors and extensors (clonus). Clonus may be seen in patients with amyotrophic lateral sclerosis (ALS), stroke, multiple sclerosis, spinal cord damage, epilepsy, liver or kidney failure, and hepatic encephalopathy.

THERAPEUTIC HIGHLIGHTS

Treatment of clonus often centers on its underlying cause. For some individuals, physical therapy that includes stretching exercises can reduce episodes of clonus. **Immunosuppressants** (eg, **azathioprine** and **corticosteroids**), **anticonvulsants** (eg, **primidone** and **levetiracetam**), and **tranquilizers** (eg, **clonazepam**) are beneficial in the treatment of clonus. **Botulinum toxin** has also been used to block the release of acetylcholine in the muscle, which triggers the rhythmic muscle contractions that are characteristic of clonus.

The primary endings in the spindles and the Golgi tendon organs together regulate the velocity of the muscle contraction, muscle length, and muscle force. The interaction of spindle discharge, tendon organ discharge, and reciprocal innervation determines the firing rate of α-motor neurons **(Clinical Box 12–2)**.

MUSCLE TONE

The resistance of a muscle to stretch is often referred to as its **tone** or **tonus.** If the motor nerve to a muscle is severed, the muscle offers very little resistance and is said to be **flaccid.** A **hypertonic (spastic)** muscle is one in which the resistance to stretch is high because of hyperactive stretch reflexes. Somewhere between the states of flaccidity and spasticity is the ill-defined area of normal tone. The muscles are generally hypotonic when the rate of γ-motor neuron discharge is low and hypertonic when it is high.

When the muscles are hypertonic, the sequence of moderate stretch → muscle contraction, strong stretch → muscle relaxation is clearly seen. Passive flexion of the elbow, for example, meets immediate resistance as a result of the stretch reflex in the triceps muscle. Further stretch activates the inverse stretch reflex. The resistance to flexion suddenly collapses, and the arm flexes. Continued passive flexion stretches the muscle again, and the sequence may be repeated. This sequence of resistance followed by a sudden decrease in resistance when a

limb is moved passively is known as the **clasp-knife effect** because of its resemblance to the closing of a pocket knife.

WITHDRAWAL REFLEX

The **withdrawal reflex** is a typical polysynaptic reflex that occurs in response to a noxious stimulus to the skin or subcutaneous tissues and muscle. The response is flexor muscle contraction and inhibition of extensor muscles, so that the body part stimulated is flexed and withdrawn from the stimulus. When a strong stimulus is applied to a limb, the response includes not only flexion and withdrawal of that limb but also extension of the opposite limb. This **crossed extensor response** is properly part of the withdrawal reflex. Strong stimuli can generate activity in the interneuron pool that spreads to all four extremities.

IMPORTANCE OF THE WITHDRAWAL REFLEX

Flexor responses can be produced by innocuous stimulation of the skin or by stretch of the muscle, but strong flexor responses with withdrawal are initiated only by stimuli that are noxious or at least potentially harmful (ie, **nociceptive stimuli**). The withdrawal reflex serves a protective function as flexion of the stimulated limb gets it away from the source of irritation, and extension of the other limb supports the body.

A weak noxious stimulus to one foot evokes a minimal flexion response; stronger stimuli produce greater and greater flexion as the stimulus irradiates to more and more of the motor neuron pool supplying the muscles of the limb. Stronger stimuli also cause a more prolonged response. A weak stimulus causes one quick flexion movement; a strong stimulus causes prolonged flexion and sometimes a series of flexion movements. This prolonged response is due to prolonged, repeated firing of the motor neurons **(after-discharge)** that is due to continued bombardment of motor neurons by impulses arriving by complicated and circuitous polysynaptic paths.

As the strength of a noxious stimulus is increased, the reaction time is shortened. Spatial and temporal facilitation occur at synapses in the polysynaptic pathway. Stronger stimuli produce more action potentials per second in the active branches and cause more branches to become active; summation of the EPSPs to the threshold level for action potential generation occurs more rapidly.

SPINAL INTEGRATION OF REFLEXES

The responses to spinal cord injury (**SCI**) illustrate the integration of reflexes at the spinal level. The deficits seen after SCI vary, of course, depending on the level of the injury. **Clinical Box 12–3** provides information on long-term problems related to SCI and recent advancements in treatment options.

CLINICAL BOX 12–3

Spinal Cord Injury

It has been estimated that the worldwide annual incidence of sustaining **SCI** is between 10 and 83 per million of the population. Leading causes are vehicular accidents, violence, and sports injuries. The mean age of patients who sustain SCI is 33 years old, and men outnumber women with a nearly 4:1 ratio. Approximately 52% of SCI cases result in quadriplegia and about 42% lead to paraplegia. With quadriplegia, the threshold for the withdrawal reflex is very low; even minor noxious stimuli may cause not only prolonged withdrawal of one extremity but marked flexion-extension patterns in the other three limbs. Stretch reflexes are also hyperactive. Afferent stimuli irradiate from one reflex center to another after SCI. When even a relatively minor noxious stimulus is applied to the skin, it may activate autonomic neurons and produce evacuation of the bladder and rectum, sweating, pallor, and blood pressure swings in addition to the withdrawal response. This distressing **mass reflex** can sometimes be used to give patients with paraplegia a degree of bladder and bowel control. They can be trained to initiate urination and defecation by stroking or pinching their thighs, thus producing an intentional mass reflex. If the cord section is incomplete, the flexor spasms initiated by noxious stimuli can be associated with bursts of pain that are particularly bothersome. They can be treated with considerable success with baclofen, a $GABA_B$ receptor agonist that crosses the blood-brain barrier and facilitates inhibition.

THERAPEUTIC HIGHLIGHTS

Treatment of SCI patients presents complex problems. Administration of corticosteroids such as **methylprednisolone** may have beneficial effects by fostering recovery and minimizing loss of function after SCI. They need to be

given soon after the injury and then discontinued because of the well-established deleterious effects of long-term corticosteroid treatment. Their immediate value is likely due to reduction of the inflammatory response in the damaged tissue. Because SCI patients are immobile, a negative nitrogen balance develops and large amounts of body protein are catabolized. Their body weight compresses the circulation to the skin over bony prominences, causing formation of **pressure ulcers.** The ulcers heal poorly and are prone to infection because of body protein depletion. The tissues that are broken down include the protein matrix of bone and this, plus the immobilization, cause Ca^{2+} to be released in large amounts, leading to **hypercalcemia, hypercalciuria,** and formation of **calcium stones** in the urinary tract. The combination of stones and bladder paralysis cause urinary stasis, which predisposes to **urinary tract infection,** the most common complication of SCI. The search continues for ways to get axons of neurons in the spinal cord to regenerate. Administration of **neurotrophins** shows some promise in experimental animals, and so does implantation of **embryonic stem cells** at the site of injury. Another possibility being explored is bypassing the site of SCI with **brain–computer interface devices.** However, these novel approaches are a long way from routine clinical use.

In all vertebrates, transection of the spinal cord is followed by a period of **spinal shock** during which all spinal reflex responses are profoundly depressed. Subsequently, reflex responses return and become hyperactive. The duration of spinal shock is proportional to the degree of encephalization of motor function in the various species. In frogs and rats it lasts for minutes; in dogs and cats it lasts for 1–2 h; in monkeys it lasts for days; and in humans it usually lasts for a minimum of 2 weeks.

Cessation of tonic bombardment of spinal neurons by excitatory impulses in descending pathways (see below) plays a role in development of spinal shock. In addition, spinal inhibitory interneurons that normally are themselves inhibited may be released from this descending inhibition to become disinhibited. This, in turn, would inhibit motor neurons. The recovery of reflex excitability may be due to the development of denervation hypersensitivity to the mediators released by the remaining spinal excitatory endings. Another contributing factor is sprouting of collaterals from existing neurons, with the formation of additional excitatory endings on interneurons and motor neurons.

The first reflex response to appear as spinal shock wears off in humans is

often a slight contraction of the leg flexors and adductors in response to a noxious stimulus (ie, the withdrawal reflex). In some patients, the knee jerk reflex recovers first. The interval between cord transection and the return of reflex activity is about 2 weeks in the absence of any complications, but if complications are present it is much longer. Once the spinal reflexes begin to reappear after spinal shock, their threshold steadily drops.

Circuits intrinsic to the spinal cord can produce walking movements when stimulated in a suitable manner even after spinal cord transection in cats and dogs. There are two **locomotor pattern generators** in the spinal cord: one in the cervical region and one in the lumbar region. However, this does not mean that spinal animals or humans can walk without stimulation; the pattern generator has to be turned on by tonic discharge of a discrete area in the midbrain, the **mesencephalic locomotor region,** and, of course, this is only possible in patients with incomplete spinal cord transection. Progress is being made in teaching humans with SCI to take a few steps by placing them, with support, on a treadmill.

GENERAL PRINCIPLES OF CENTRAL ORGANIZATION OF MOTOR PATHWAYS

To voluntarily move a limb, the brain must plan a movement, arrange appropriate motion at many different joints at the same time, and adjust the motion by comparing plan with performance. The motor system "learns by doing" and performance improves with repetition. This involves synaptic plasticity. Damage to the cerebral cortex before or during childbirth or during the first 2–3 years of development can lead to cerebral palsy, a disorder that affects muscle tone, movement, and coordination **(Clinical Box 12–4)**.

CLINICAL BOX 12–4

Cerebral Palsy

Cerebral palsy (CP) is a term used to describe any one of several nonprogressive neurologic disorders that occur before or during childbirth or during early childhood. Prenatal factors, including exposure of the developing brain to hypoxia, infections, or toxins, may account for 70–80% of cases of CP. Typical symptoms of the disorder include spasticity, ataxia, deficits in

fine motor control, and abnormal gait (crouched or "scissored gait"). Sensory deficits including loss of vision and hearing as well as learning difficulties and seizures often occur in children with CP. In developed countries, the prevalence of CP is 2–2.5 cases per 1000 live births; however, the incidence of CP in children who are born prematurely is much higher compared with children born at term. Based on differences in the resting tone in muscles and the limbs involved, CP is classified into different groups. The most prevalent type is **spastic CP** that is characterized by **spasticity, hyperreflexia,** clonus, and a **positive Babinski sign.** These are all signs of damage to the corticospinal tract (Clinical Box 12–5). **Dyskinetic CP** is characterized by abnormal involuntary movements (**chorea** and **athetosis**) and may reflect damage to extrapyramidal motor areas. It is not uncommon to have signs of both types of CP. The rarest type is **hypotonic CP** that presents with truncal and extremity hypotonia, hyperreflexia, and persistent primitive reflexes.

THERAPEUTIC HIGHLIGHTS

There is no cure for CP. Treatment often includes physical and occupational therapy. Botulinum toxin injections into affected muscles have been used to reduce muscle spasticity, especially in the gastrocnemius muscle. Other drugs used to treat muscle spasticity in patients with CP include **diazepam** (a benzodiazepine that binds to the $GABA_A$ receptor), **baclofen** (an agonist at presynaptic $GABA_B$ receptors in the spinal cord), and **dantrolene** (a direct muscle relaxant). Various surgeries have been used to treat CP, including **selective dorsal rhizotomy** (section of the dorsal roots) and **tenotomy** (severing the tendon in the gastrocnemius muscles).

Figure 12–6 shows the general motor control scheme with the commands for voluntary movement originating in cortical association areas. The movements are planned in the cortex, the basal ganglia, and the lateral portions of the cerebellar hemispheres, as indicated by increased electrical activity before the movement. The basal ganglia and cerebellum funnel information to the premotor and motor cortex by way of the thalamus. Motor commands from the motor cortex are relayed predominantly via the corticospinal tracts to the spinal cord and the corresponding corticobulbar tracts to motor neurons in the brainstem. However, collaterals from these pathways and a few direct connections from the motor cortex end on brainstem nuclei that project to motor neurons in the

brainstem and spinal cord. These pathways can also mediate voluntary movement. Movement sets up alterations in sensory input from the special senses and from muscles, tendons, joints, and the skin. This feedback information that adjusts and smooths movement is relayed directly to the motor cortex and spinocerebellum. The spinocerebellum then projects to the brainstem. The main brainstem pathways that are concerned with posture and coordination are the rubrospinal, reticulospinal, tectospinal, and vestibulospinal tracts.

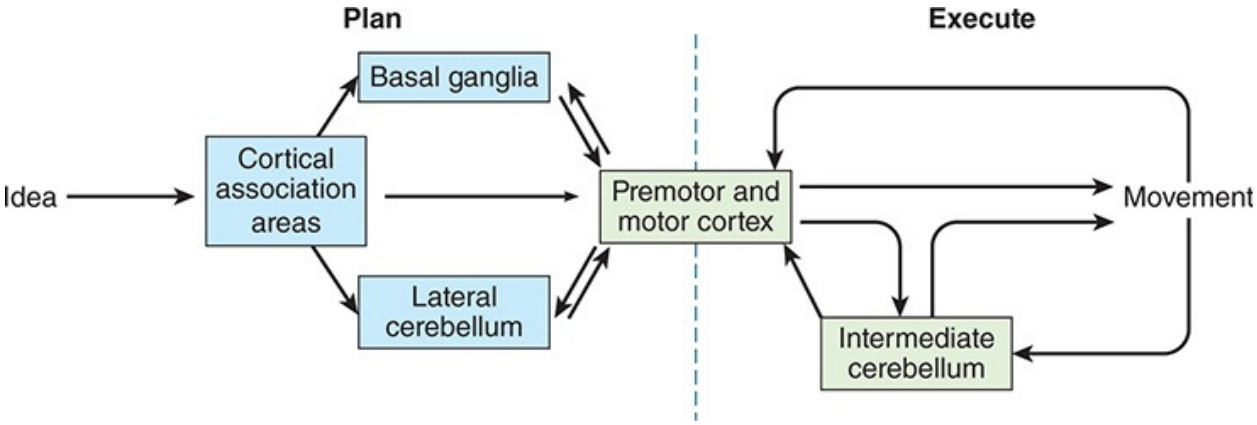


FIGURE 12–6 Control of voluntary movement. Commands for voluntary movement originate in cortical association areas. The cortex, basal ganglia, and cerebellum work cooperatively to plan movements. Movement executed by the cortex is relayed via the corticospinal tracts and corticobulbar tracts to motor neurons. The cerebellum provides feedback to adjust and smoothen movement.

MOTOR CORTEX & VOLUNTARY MOVEMENT

PRIMARY MOTOR CORTEX

The reader can refer to Figure 8–8 for the locations of the major cortical regions involved in motor control. The **primary motor cortex (M1)** is in the precentral gyrus of the frontal lobe, extending into the central sulcus. By means of stimulation experiments in patients undergoing craniotomy under local anesthesia, this region was mapped to show where various parts of the body are represented in the precentral gyrus. **Figure 12–7** shows the **motor homunculus** with the feet at the top of the gyrus and the face at the bottom. The cortical representation of each body part is proportional in size to the skill with which the part is used in fine, voluntary movement. The areas involved in speech and hand movements are especially large in the cortex; use of the pharynx, lips, and

tongue to form words and of the fingers and opposable thumbs to manipulate the environment are activities in which humans are especially skilled.

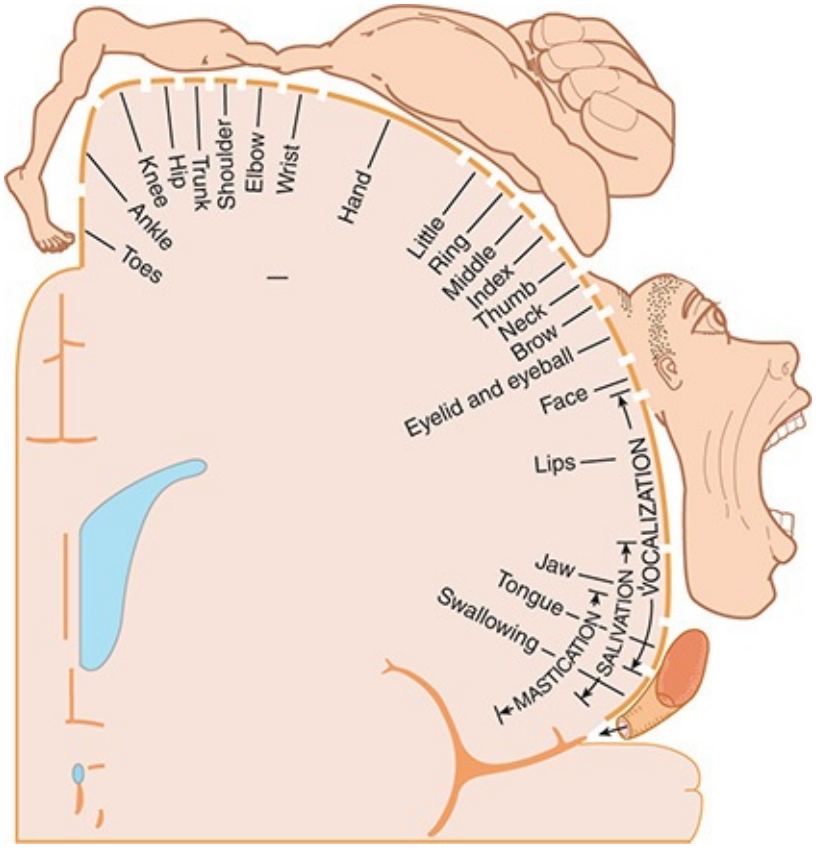


FIGURE 12–7 Motor homunculus. The figure represents, on a coronal section of the precentral gyrus, the location of the cortical representation of the various parts. The size of the various parts is proportional to the cortical area devoted to them. Compare with Figure 8–9 which shows the sensory homunculus. (Reproduced with permission from Penfield W, Rasmussen G: *The Cerebral Cortex of Man*. Macmillan, 1950.)

Modern brain imaging techniques such as **positron emission tomography (PET)** and **functional magnetic resonance imaging (fMRI)** can be used to map the cortex to identify motor areas. **Figure 12–8** shows activation of the hand area of the motor cortex while repetitively squeezing a ball with either the right or left hand.

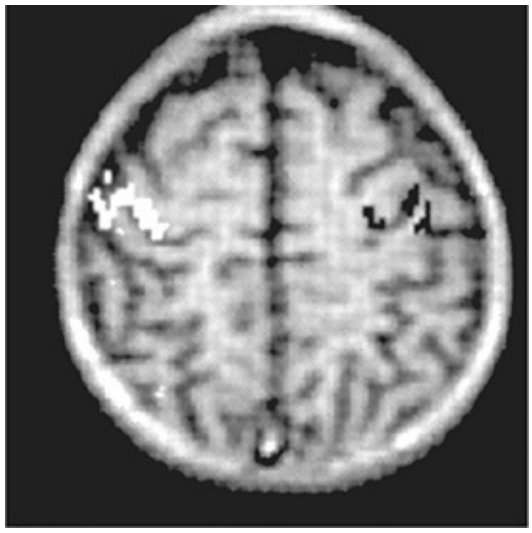


FIGURE 12–8 Hand area of motor cortex demonstrated by functional magnetic resonance imaging (fMRI) in a 7-year-old boy. Changes in signal intensity, measured using a method called echoplanar MRI, result from changes in the flow, volume, and oxygenation of the blood. The child was instructed to repetitively squeeze a foam-rubber ball at the rate of two to four squeezes per second with the right or left hand. Changes in cortical activity with the ball in the right hand are shown in black. Changes in cortical activity with the ball in the left hand are shown in white. (Data from Novotny EJ, et al: Functional magnetic resonance imaging [fMRI] in pediatric epilepsy. Epilepsia 1994;35(Supp 8):36.)

The cells in the cortical motor areas are arranged in a **columnar organization.** M1 neurons represent movements of groups of muscles for different tasks. Neurons in several cortical columns project to the same muscle; moreover, the cells in each column receive sensory input from the peripheral area in which they produce movement, providing the basis for feedback control of movement. Some of this input may be direct and some is relayed from other parts of the cortex.

SUPPLEMENTARY MOTOR AREA

The supplementary motor area is on and above the superior bank of the cingulate sulcus on the medial side of the hemisphere. It projects to M1 and contains a map of the body; but it is less precise than in M1. The supplementary motor area is involved in organizing or planning motor sequences, while M1 executes the movements.

When human subjects count to themselves without speaking, the motor cortex is quiescent, but when they speak the numbers aloud as they count, blood flow increases in M1 and the supplementary motor area. Thus, both regions are involved in voluntary movement when the movements being performed are complex and involve planning.

PREMOTOR CORTEX

The premotor cortex is located anterior to the precentral gyrus, on the lateral and medial cortical surface; it contains a somatotopic map. This region receives input from sensory regions of the parietal cortex and projects to M1, the spinal cord, and the brainstem reticular formation. This region is concerned with setting posture at the start of a planned movement and with getting the individual prepared to move. It is most involved in control of proximal limb muscles needed to orient the body for movement.

POSTERIOR PARIETAL CORTEX

The somatic sensory area and related portions of the posterior parietal lobe project to the premotor cortex. Lesions of the somatic sensory area cause defects in motor performance that are characterized by inability to execute learned sequences of movements such as eating with a knife and fork. Some of the neurons are concerned with aiming the hands toward an object and manipulating it, whereas other neurons are concerned with hand-eye coordination. As described below, neurons in this posterior parietal cortex contribute to the descending pathways involved in motor control.

PLASTICITY

A striking discovery made possible by PET and fMRI is that the motor cortex shows the same kind of plasticity as already described for the sensory cortex in Chapter 8. For example, the finger areas of the contralateral motor cortex enlarge as a pattern of rapid finger movement is learned with the fingers of one hand; this change is detectable at 1 week and maximal at 4 weeks. Cortical areas of output to other muscles also increase in size when motor learning involves these muscles. When a small focal ischemic lesion is produced in the hand area of the motor cortex of monkeys, the hand area may reappear, with return of motor

function, in an adjacent undamaged part of the cortex. Thus, the maps of the motor cortex are not immutable; they change with experience.

CONTROL OF AXIAL & DISTAL MUSCLES

Within the brainstem and spinal cord, pathways and neurons that are concerned with the control of skeletal muscles of the trunk (axial) and proximal portions of the limbs are located medially or ventrally, whereas pathways and neurons that are concerned with the control of skeletal muscles in the distal portions of the limbs are located laterally. The axial muscles are concerned with postural adjustments and gross movements, whereas the distal limb muscles mediate fine, skilled movements. Neurons in the medial portion of the ventral horn innervate proximal limb muscles, particularly the flexors, and lateral ventral horn neurons innervate distal limb muscles. Similarly, the ventral corticospinal tract and medial descending brainstem pathways (tectospinal, reticulospinal, and vestibulospinal tracts) are concerned with adjustments of proximal muscles and posture, and the lateral corticospinal and rubrospinal tracts are concerned with distal limb muscles and, particularly in the case of the lateral corticospinal tract, with skilled voluntary movements.

CORTICOSPINAL & CORTICOBULBAR TRACTS

The somatotopic organization just described for the motor cortex continues throughout the pathways from the cortex to the motor neurons. The axons of neurons from the motor cortex that project to spinal motor neurons form the **corticospinal tracts,** a large bundle of about 1 million fibers. About 80% of these fibers cross the midline in the medullary pyramids to form the **lateral corticospinal tract** **(Figure 12–9)**. The remaining 20% make up the **ventral corticospinal tract,** which does not cross the midline until it reaches the level of the spinal cord at which it terminates. Lateral corticospinal tract neurons make monosynaptic connections to motor neurons, especially those concerned with skilled movements. Many corticospinal tract neurons also synapse on spinal interneurons antecedent to motor neurons; this indirect pathway is important in coordinating groups of muscles.

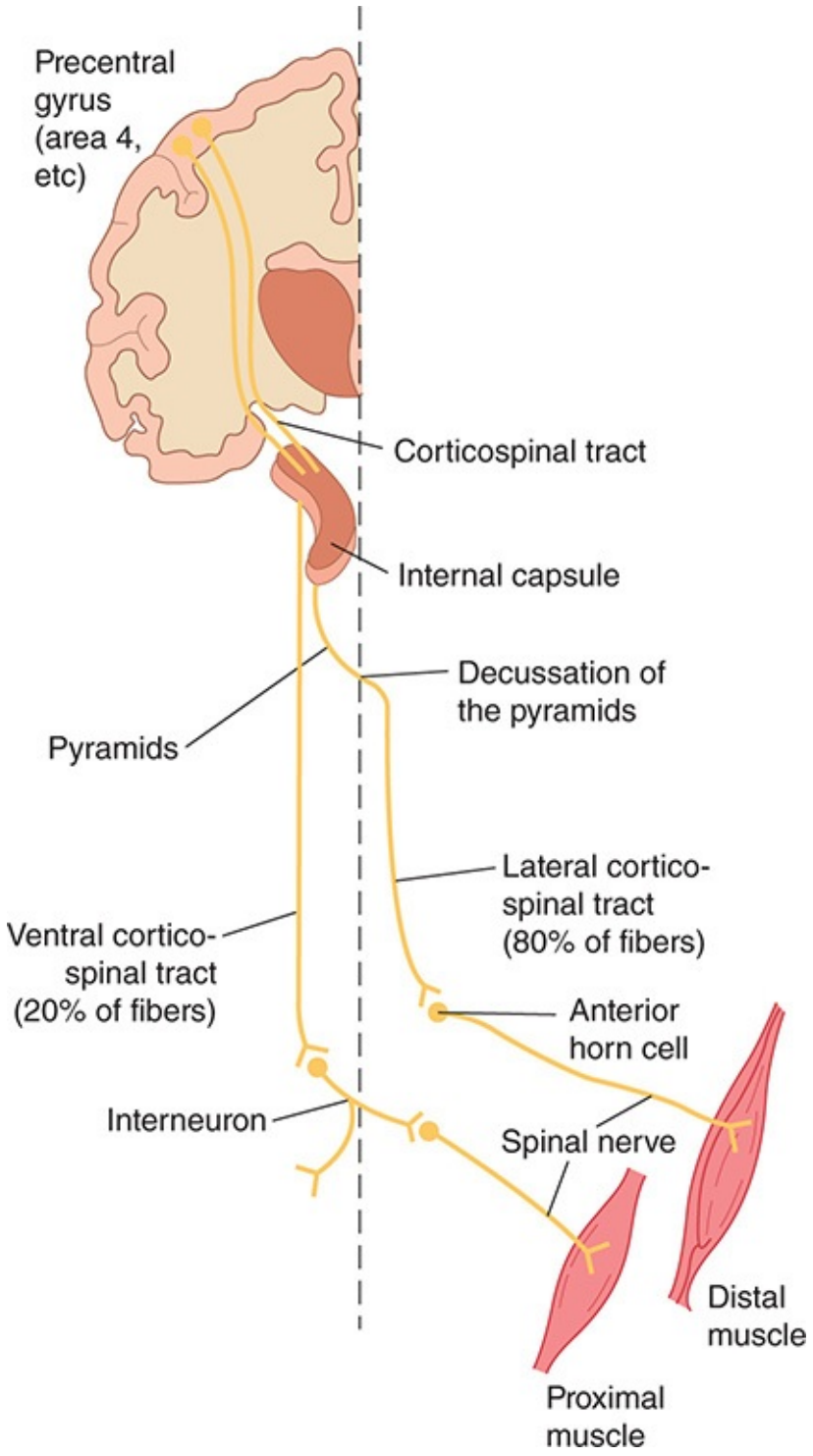


FIGURE 12–9 The corticospinal tracts. This tract originates in the precentral gyrus and passes through the internal capsule. Most fibers decussate in the pyramids and descend in the lateral white matter of the spinal cord to form the lateral division of the tract which can make monosynaptic connections with spinal motor neurons. The ventral division of the tract remains uncrossed until

reaching the spinal cord where axons terminate on spinal interneurons antecedent to motor neurons.

The trajectory from the cortex to the spinal cord passes through the **corona radiata** to the posterior limb of the **internal capsule.** Within the midbrain they traverse the **cerebral peduncle** and the basilar pons until they reach the **medullary pyramids** on their way to the spinal cord.

The **corticobulbar tract** is composed of the fibers that pass from the motor cortex to motor neurons in the trigeminal, facial, and hypoglossal nuclei. Corticobulbar neurons end either directly on the cranial nerve nuclei or on their antecedent interneurons within the brainstem. Their axons traverse through the genu of the internal capsule, the cerebral peduncle (medial to corticospinal tract neurons), to descend with corticospinal tract fibers in the pons and medulla.

The motor system can be divided into lower and upper motor neurons. **Lower motor neurons** refer to the spinal and cranial motor neurons that directly innervate skeletal muscles. **Upper motor neurons** are those in the cortex and brainstem that activate the lower motor neurons. The pathophysiologic responses to damage to lower and upper motor neurons are very distinctive **(Clinical Box 12–5)**.

ORIGINS OF CORTICOSPINAL & CORTICOBULBAR TRACTS

Corticospinal and corticobulbar tract neurons are pyramidal shaped and located in layer V of the cerebral cortex (see Chapter 14). The cortical areas from which these tracts originate were identified on the basis of electrical stimulation that produced prompt discrete movement. About 31% of the corticospinal tract neurons are from M1. The premotor cortex and supplementary motor cortex account for 29% of the corticospinal tract neurons. The other 40% of corticospinal tract neurons originate in the parietal lobe and primary somatosensory area in the postcentral gyrus. The corticospinal and corticobulbar system is the primary pathway for the initiation of skilled voluntary movement.

BRAINSTEM PATHWAYS INVOLVED IN POSTURE & VOLUNTARY MOVEMENT

As mentioned above, spinal motor neurons are organized so that those

innervating the most proximal muscles are located most medially and those innervating the more distal muscles are located more laterally. This organization is also reflected in descending brainstem pathways **(Figure 12–10)**.

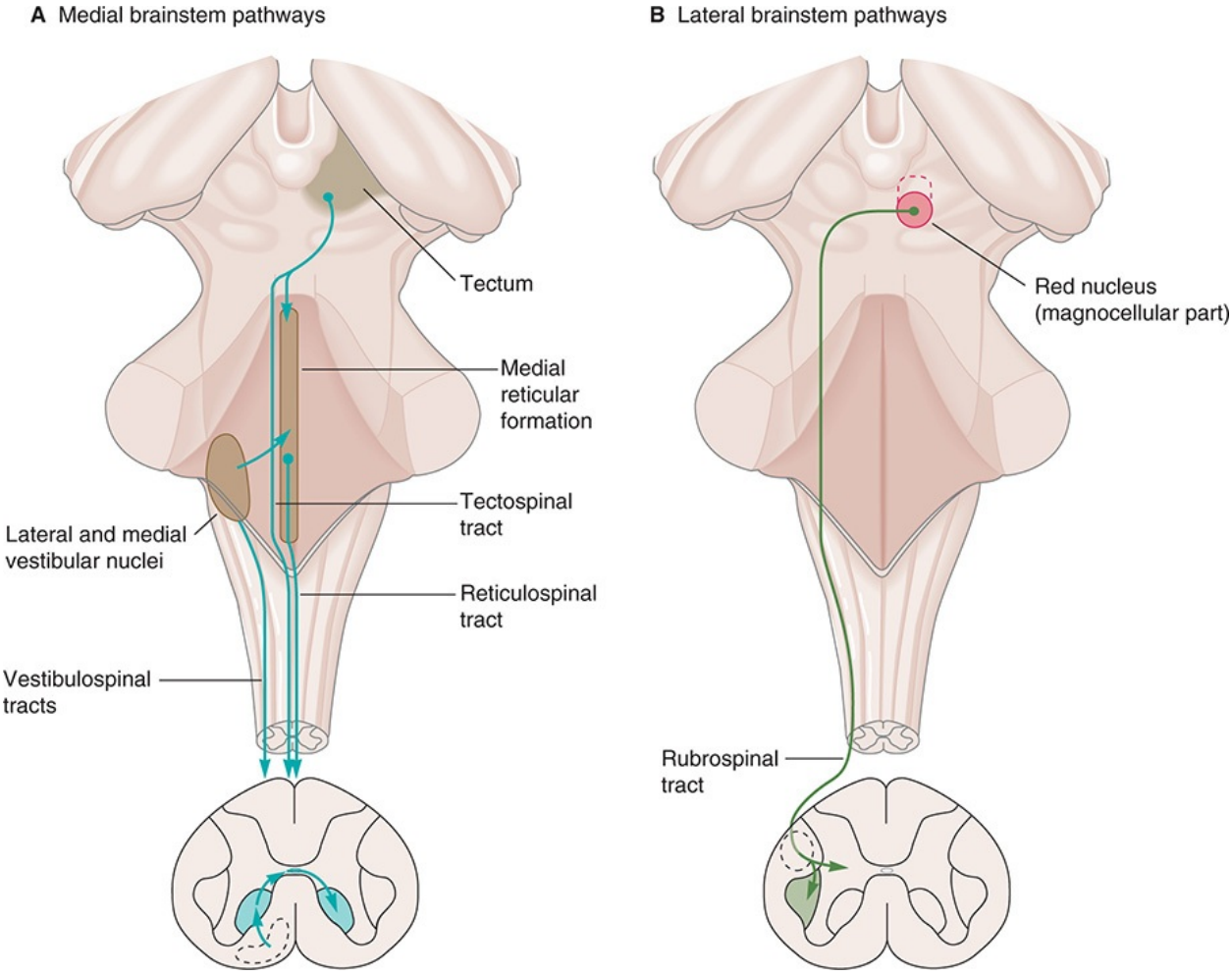


FIGURE 12–10 Medial and lateral descending brainstem pathways involved in motor control. A) Medial pathways (reticulospinal, vestibulospinal, and tectospinal) terminate in ventromedial area of spinal gray matter and control axial and proximal muscles. **B)** Lateral pathway (rubrospinal) terminates in dorsolateral area of spinal gray matter and controls distal muscles. (Reproduced with permission from Kandel ER, Schwartz JH, Jessell TM [editors]: *Principles of Neural Science*, 4th ed. New York, NY: McGraw-Hill; 2000.)

CLINICAL BOX 12–5

Lower versus Upper Motor Neuron Damage

Lower motor neurons are those whose axons terminate on skeletal muscles. Damage to these neurons is associated with **flaccid paralysis, muscular atrophy, fasciculations** (visible muscle twitches that appear as flickers under the skin), **hypotonia** (decreased muscle tone), and **hyporeflexia** or **areflexia.** An example of a disease that leads to lower motor neuron damage is **ALS.** "Amyotrophic" means "no muscle nourishment" and describes the atrophy that muscles undergo because of disuse. "Sclerosis" refers to the hardness felt when a pathologist examines the spinal cord on autopsy; the hardness is due to proliferation of astrocytes and scarring of the lateral columns of the spinal cord. ALS is a selective, progressive degeneration of α-motor neurons. This fatal disease is also known as **Lou Gehrig disease** in remembrance of a famous American baseball player who died of ALS. The worldwide annual incidence of ALS has been estimated to be 0.5–3 cases per 100,000 people. The disease has no racial, socioeconomic, or ethnic boundaries. The life expectancy of ALS patients is usually 3–5 years after diagnosis. ALS is most commonly diagnosed in middle age and affects men more often than women. Most cases of ALS are sporadic in origin; but 5–10% of the cases have a familial link. Possible causes include viruses, neurotoxins, heavy metals, DNA defects (especially in familial ALS), immune system abnormalities, and enzyme abnormalities. About 40% of the familial cases have a mutation in the gene for Cu/Zn superoxide dismutase *(SOD-1)* on chromosome 21. SOD is a free radical scavenger that reduces oxidative stress. A defective *SOD-1* gene permits free radicals to accumulate and kill neurons. An increase in the excitability of deep cerebellar nuclei due to the inhibition of **small-conductance calcium-activated potassium (SK) channels** may contribute to the development of cerebellar ataxia.

Upper motor neurons typically refer to corticospinal tract neurons that innervate spinal motor neurons, but they can also include brainstem neurons that control spinal motor neurons. Damage to these neurons initially causes muscles to become weak and flaccid but eventually leads to spasticity, **hypertonia** (increased resistance to passive movement), hyperactive stretch reflexes, and abnormal plantar extensor reflex (positive Babinski sign). The Babinski sign is dorsiflexion of the great toe and fanning of the other toes when the lateral aspect of the sole of the foot is scratched. In adults, the normal response to this stimulation is plantar flexion in all the toes. The Babinski sign is of value in the localization of disease processes, but its physiologic significance is unknown. In infants whose corticospinal tracts are

not well developed, dorsiflexion of the great toe and fanning of the other toes is the natural response to stimuli applied to the sole of the foot.

THERAPEUTIC HIGHLIGHTS

One of the few drugs shown to modestly slow the progression of ALS is **riluzole,** a drug that opens the SK channels and may be effective in preventing nerve damage caused by excessive release of the excitatory amino acid, glutamate. Spasticity associated with motor neuron disease can be reduced by the muscle relaxant **baclofen** (a derivative of GABA); in some cases, a subarachnoid infusion of baclofen is given via an implanted lumbar pump. Spasticity can also be treated with **tizanidine,** a centrally acting α_2-adrenoceptor agonist; its effectiveness is due to increasing presynaptic inhibition of spinal motor neurons. **Botulinum toxin** is also approved for the treatment of spasticity; this toxin acts by binding to receptors on the cholinergic nerve terminals to decrease the release of acetylcholine, causing neuromuscular blockade.

MEDIAL BRAINSTEM PATHWAYS

The medial brainstem pathways, which work in concert with the ventral corticospinal tract, are the **pontine and medullary reticulospinal, vestibulospinal,** and **tectospinal tracts.** These pathways descend in the ipsilateral ventral columns of the spinal cord and terminate predominantly on interneurons and long propriospinal neurons in the ventromedial part of the ventral horn to control axial and proximal muscles. A few medial pathway neurons synapse directly on motor neurons controlling axial muscles.

The medial and lateral vestibulospinal tracts are involved in vestibular function and are described in Chapter 11. The medial tract originates in the medial and inferior vestibular nuclei and projects bilaterally to cervical spinal motor neurons that control neck musculature. The lateral tract originates in the lateral vestibular nuclei and projects ipsilaterally to neurons at all spinal levels. It activates motor neurons to antigravity muscles (eg, proximal limb extensors) to control posture and balance.

The pontine and medullary reticulospinal tracts project to all spinal levels. They are involved in the maintenance of posture and in modulating muscle tone, especially via an input to γ-motor neurons. Pontine reticulospinal neurons are

primarily excitatory and medullary reticulospinal neurons are primarily inhibitory. The tectospinal tract originates in the superior colliculus of the midbrain and projects to the contralateral cervical spinal cord to control head and eye movements.

LATERAL BRAINSTEM PATHWAY

The main control of distal muscles arises from the lateral corticospinal tract, but neurons within the red nucleus of the midbrain cross the midline and project to interneurons in the dorsolateral part of the spinal ventral horn to also influence motor neurons that control distal limb muscles. This **rubrospinal tract** excites flexor motor neurons and inhibits extensor motor neurons. This pathway is not very prominent in healthy humans, but it may play a role in the posture typical of decorticate rigidity (see below).

POSTURE-REGULATING SYSTEMS

When damage occurs somewhere along the neural axis, the activities integrated below the injury are released from the control of higher brain centers and often are accentuated. Release of this type, long a cardinal principle in neurology, may be due in some situations to disruption of an inhibitory control by higher neural regions.

DECEREBRATION

A complete transection of the brainstem between the superior and inferior colliculi permits the brainstem pathways to function independent of their input from higher brain structures. This **midcollicular decerebration** is diagramed in **Figure 12–11** by the dashed line labeled A. This lesion interrupts all inputs from the cortex (corticospinal and corticobulbar tracts) and red nucleus (rubrospinal tract), primarily to distal muscles of the extremities. The excitatory and inhibitory reticulospinal pathways (primarily to postural extensor muscles) remain intact. The dominance of drive from ascending sensory pathways to the excitatory reticulospinal pathway leads to hyperactivity in extensor muscles in all four extremities **(decerebrate rigidity).** This resembles what ensues after **uncal herniation** due to a supratentorial lesion. Uncal herniation can occur in patients with large tumors or a hemorrhage in the cerebral hemisphere. **Figure**

12–12A shows the posture typical of such a patient. Clinical Box 12–6 describes complications related to uncal herniation.

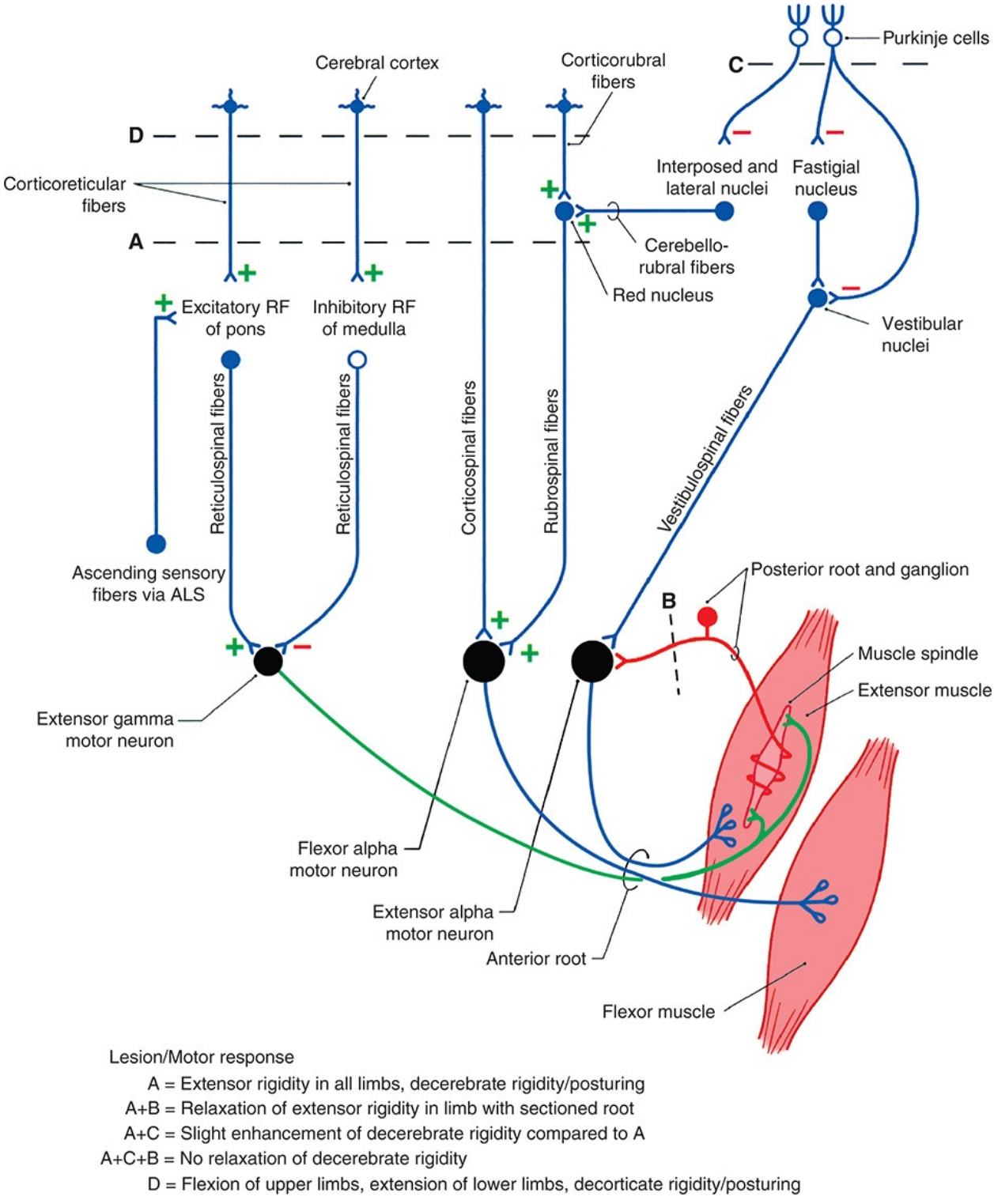


FIGURE 12–11 A circuit drawing representing lesions produced in

experimental animals to replicate decerebrate and decorticate deficits seen in humans. Bilateral transections are indicated by dashed lines A, B, C, and D. Decerebration is at a midcollicular level (A), decortication is rostral to the superior colliculus, dorsal roots sectioned for one extremity (B), and damage to the anterior lobe of cerebellum (C). The objective was to identify anatomic substrates responsible for decerebrate or decorticate rigidity/posturing seen in humans with lesions that either isolate the forebrain from the brainstem or separate rostral from caudal brainstem and spinal cord. (Reproduced with permission from Haines DE (ed): *Fundamental Neuroscience for Basic and Clinical Applications*, 3rd ed. St. Louis, MO: Elsevier; 2006.)

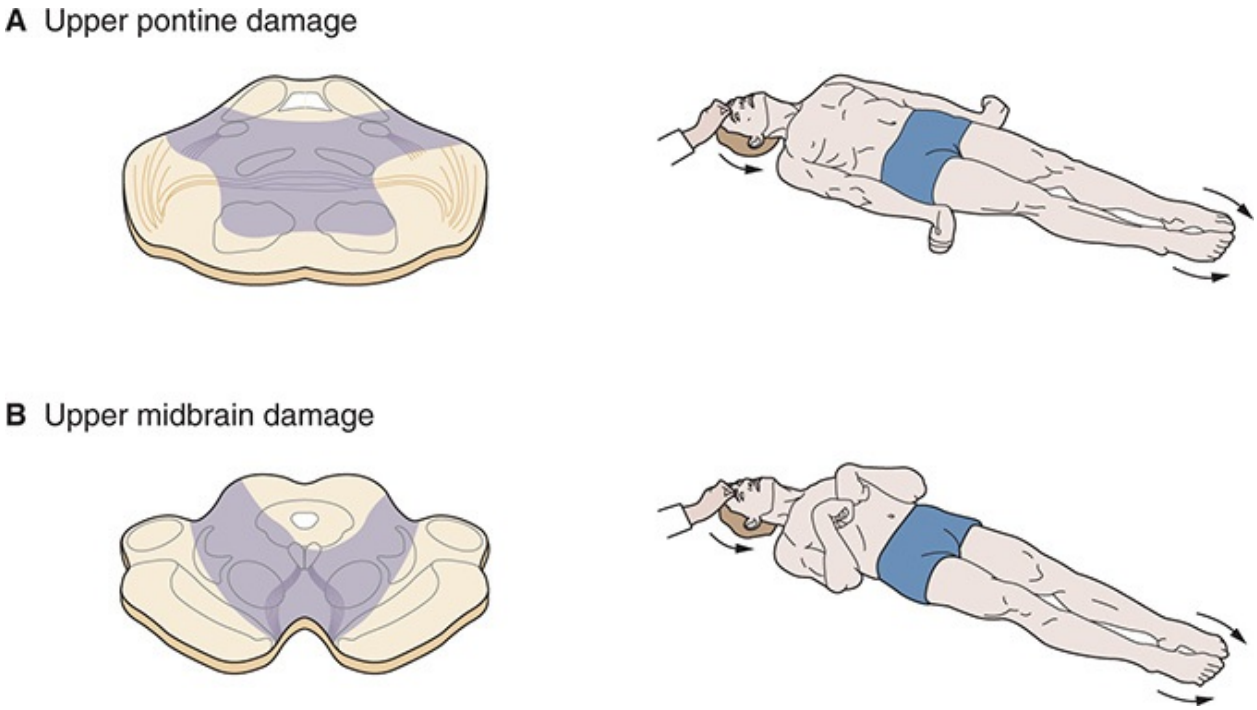


FIGURE 12–12 Decerebrate and decorticate postures. A) Damage to lower midbrain and upper pons causes decerebrate posturing in which lower extremities are extended with toes pointed inward and upper extremities extended with fingers flexed and forearms pronate. Neck and head are extended. **B)** Damage to upper midbrain may cause decorticate posturing in which upper limbs are flexed, lower limbs are extended with toes pointed slightly inward, and head is extended. (Modified with permission from Kandel ER, Schwartz JH, Jessell TM (eds): *Principles of Neural Science*, 4th ed. New York, NY: McGraw-Hill; 2000.)

Decerebrate rigidity is spasticity due to facilitation of the myotatic stretch

reflex. That is, the excitatory input from the reticulospinal pathway activates γ-motor neurons which indirectly activate α-motor neurons (via Ia spindle afferent activity). This is called the **gamma loop.** This was demonstrated by cutting dorsal roots to a limb in midcollicular decerebrate cats (dashed line labeled B in Figure 12–11) that immediately eliminated the hyperactivity of extensor muscles.

Decerebrate rigidity can also lead to direct activation of α-motor neurons. If the anterior lobe of the cerebellum is removed in a decerebrate animal (dashed line labeled C in Figure 12–11), extensor muscle hyperactivity is exaggerated **(decerebellate rigidity).** This lesion eliminates cortical inhibition of the cerebellar fastigial nucleus and secondarily increases excitation to vestibular nuclei. Subsequent dorsal root section does not reverse the rigidity due to activation of α-motor neurons independent of the gamma loop.

DECORTICATION

Injury to the cerebral cortex **(decortication;** dashed line labeled D in Figure 12–11) produces **decorticate rigidity** that is characterized by flexion of the upper extremities at the elbow and extensor hyperactivity in the lower extremities **(Figure 12–12B)**. The flexion is explained by rubrospinal excitation of flexor muscles in the upper extremities; the hyperextension of lower extremities is due to the same changes that occur after midcollicular decerebration.

CLINICAL BOX 12–6

Uncal Herniation

Space-occupying lesions from large tumors, hemorrhages, strokes, or abscesses in the cerebral hemisphere can drive the uncus of the temporal lobe over the edge of the cerebellar tentorium, compressing the ipsilateral cranial nerve III **(uncal herniation).** Before the herniation these patients experience a decreased level of consciousness, lethargy, poorly reactive pupils, deviation of the eye to a “down and out” position, hyperactive reflexes, and a bilateral Babinski sign (due to compression of the ipsilateral corticospinal tract). After the brain herniates, the patients are decerebrate and comatose, have fixed and dilated pupils, and eye movements are absent. Once damage extends to the midbrain, a **Cheyne-Stokes respiratory pattern** develops, characterized by a pattern of waxing-and-waning depth of respiration with interposed periods of

apnea. Eventually, medullary function is lost, breathing ceases, and recovery is unlikely. Hemispheric masses closer to the midline compress the thalamic reticular formation and can cause coma before eye findings develop **(central herniation).** As the mass enlarges, midbrain function is affected, the pupils dilate, and a decerebrate posture ensues. With progressive herniation, pontine vestibular and then medullary respiratory functions are lost.

Decorticate rigidity is seen on the hemiplegic side after hemorrhages or thromboses in the internal capsule. The small arteries in the internal capsule are especially prone to rupture or thrombotic obstruction, so this type of decorticate rigidity is fairly common. Sixty percent of intracerebral hemorrhages occur in the internal capsule, 10% in the cerebral cortex, 10% in the pons, 10% in the thalamus, and 10% in the cerebellum.

BASAL GANGLIA

ORGANIZATION OF THE BASAL GANGLIA

The term **basal ganglia** (or **basal nuclei**) is applied to five interactive structures on each side of the brain **(Figure 12–13)**. These are the **caudate nucleus, putamen,** and **globus pallidus** (three large nuclear masses underlying the cortical mantle), the **subthalamic nucleus,** and **substantia nigra.** The caudate nucleus and putamen collectively form the **striatum;** the putamen and globus pallidus collectively form the **lenticular nucleus.**

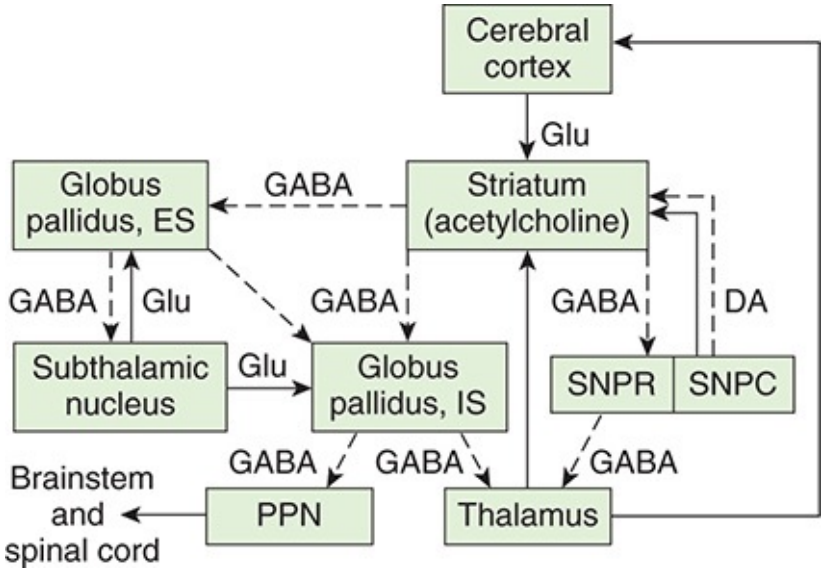


FIGURE 12–13 The basal ganglia. The basal ganglia are composed of the caudate nucleus, putamen, and globus pallidus and the functionally related subthalamic nucleus and substantia nigra. The frontal (coronal) section shows the location of the basal ganglia in relation to surrounding structures.

The globus pallidus is divided into external and internal segments (GPe and GPi); both regions contain inhibitory GABAergic neurons. The substantia nigra is divided into **pars compacta** that uses dopamine as a neurotransmitter and **pars reticulata** that uses GABA as a neurotransmitter. About 95% of striatal neurons are medium spiny neurons that use GABA as a neurotransmitter. The remaining striatal neurons are aspiny interneurons that differ in terms of size and neurotransmitters: large (acetylcholine), medium (somatostatin), and small (GABA).

Figure 12–14 shows the major connections to and from and within the basal ganglia along with the neurotransmitters within these pathways. There are two main inputs to the basal ganglia; they are both excitatory (glutamate), and they both terminate in the striatum. They are from a wide region of the cerebral cortex **(corticostriate pathway)** and from intralaminar nuclei of the thalamus **(thalamostriatal pathway).** The two major outputs of the basal ganglia are from GPi and substantia nigra pars reticulata. Both are inhibitory (GABAergic) and both project to the thalamus. From the thalamus, there is an excitatory (glutamatergic) projection to the prefrontal and premotor cortex. This completes the cortical-basal ganglia-thalamic-cortical loop.

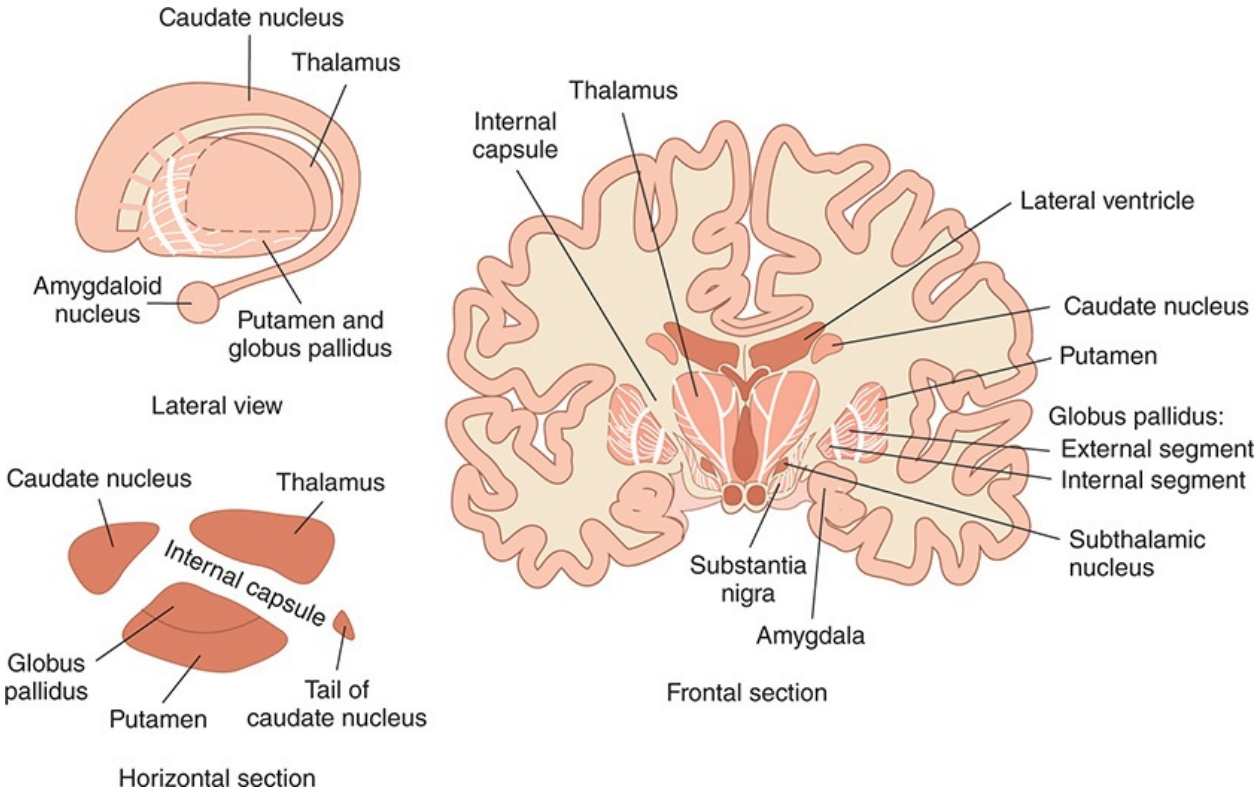


FIGURE 12–14 Diagrammatic representation of the principal connections of the basal ganglia. Solid lines indicate excitatory pathways, dashed lines indicate inhibitory pathways. The transmitters are indicated in the pathways, where they are known. DA, dopamine; Glu, glutamate. Acetylcholine is the transmitter produced by interneurons in the striatum. ES, external segment; IS, internal segment; PPN, pedunculopontine nuclei; SNPC, substantia nigra, pars compacta; SNPR, substantia nigra, pars reticulata. The subthalamic nucleus also projects to the pars compacta of the substantia nigra; this pathway has been omitted for clarity.

The connections within the basal ganglia include a dopaminergic **nigrostriatal projection** from the substantia nigra pars compacta to the striatum and a GABAergic projection from the striatum to substantia nigra pars reticulata. There is an inhibitory projection from the striatum to both GPe and GPi. The subthalamic nucleus receives an inhibitory input from GPe, and in turn the subthalamic nucleus has an excitatory (glutamatergic) projection to both GPe and GPi.

FUNCTION

Neurons in the basal ganglia, like those in the lateral portions of the cerebellar hemispheres, discharge before movements begin. The basal ganglia are involved in the planning and programming of movement or, more broadly, in the processes by which an abstract thought is converted into voluntary action (Figure 12–6). They influence the motor cortex via the thalamus, and the corticospinal pathways provide the final common pathway to motor neurons. In addition, GPi projects to nuclei in the brainstem, and from there to motor neurons in the brainstem and spinal cord. The basal ganglia, particularly the caudate nuclei, also play a role in some cognitive processes. Possibly because of the interconnections of this nucleus with the frontal portions of the neocortex, lesions of the caudate nuclei disrupt performance on tests involving object reversal and delayed alternation. In addition, lesions of the head of the left but not the right caudate nucleus and nearby white matter in humans are associated with a dysarthric form of aphasia that resembles Wernicke aphasia (see Chapter 15).

DISEASES OF THE BASAL GANGLIA IN HUMANS

Three distinct biochemical pathways in the basal ganglia normally operate in a balanced manner: (1) the nigrostriatal dopaminergic system, (2) the intrastriatal cholinergic system, and (3) the GABAergic system that projects from the striatum to the globus pallidus and substantia nigra. When one or more of these pathways become dysfunctional, characteristic motor abnormalities occur. Diseases of the basal ganglia lead to two general types of disorders: **hyperkinetic** and **hypokinetic.** The hyperkinetic conditions are those in which movement is excessive and abnormal, including chorea, athetosis, and ballism. Hypokinetic abnormalities include akinesia and bradykinesia.

Chorea is characterized by rapid, involuntary “dancing” movements. **Athetosis** is characterized by continuous, slow writhing movements. Choreiform and athetotic movements have been likened to the start of voluntary movements occurring in an involuntary, disorganized way. In **ballism,** involuntary flailing, intense, and violent movements occur. **Akinesia** is difficulty in initiating movement and decreased spontaneous movement. **Bradykinesia** is slowness of movement.

In addition to Parkinson disease (described below), there are several other disorders that involve a malfunction within the basal ganglia. A few of these are

described in Clinical Box 12–7. **Huntington disease** is one of an increasing number of human genetic diseases affecting the nervous system that are characterized by **trinucleotide repeat** expansion. Most of these involve cytosine-adenine-guanine (CAG) repeats (Table 12–1), but one involves cytosine-guanine-guanine (CGG) repeats and another involves CTG repeats (T refers to thymine). Increased numbers of a 12-nucleotide repeat are also associated with a rare form of epilepsy.

TABLE 12–1 Examples of trinucleotide repeat diseases.

Disease	Expanded Trinucleotide Repeat	Affected Protein
Huntington disease	CAG	Huntingtin
Spinocerebellar ataxia, types 1, 2, 3, 7	CAG	Ataxin 1, 2, 3, 7
Spinocerebellar ataxia, type 6	CAG	α_{1A} subunit of Ca^{2+} channel
Dentatorubral-pallidoluysian atrophy	CAG	Atrophin
Spinobulbar muscular atrophy	CAG	Androgen receptor
Fragile X syndrome	CGG	FMR-1
Myotonic dystrophy	CTG	DM protein kinase
Friedreich ataxia	GAA	Frataxin

PARKINSON DISEASE

Parkinson disease has both hypokinetic and hyperkinetic features. It was originally described in 1817 by James Parkinson and is named for him. Parkinson disease is the first disease identified as being due to a deficiency in a specific neurotransmitter (Clinical Box 12–8). It results from the degeneration of dopaminergic neurons in the substantia nigra pars compacta. The fibers to the putamen (part of the striatum) are most severely affected.

The hypokinetic features of Parkinson disease are akinesia and bradykinesia, and the hyperkinetic features are **cogwheel rigidity** and **tremor at rest.** The absence of motor activity and the difficulty in initiating voluntary movements are striking. There is a decrease in the normal, unconscious movements such as swinging of the arms during walking, the panorama of facial expressions related to the emotional content of thought and speech, and the multiple "fidgety" actions and gestures that occur in all of us. The rigidity is different from spasticity because motor neuron discharge increases to both the agonist and antagonist muscles. Passive motion of an extremity meets with a plastic, dead-feeling resistance that has been likened to bending a lead pipe and is therefore called **lead pipe rigidity.** Sometimes a series of "catches" takes place during passive motion (cogwheel rigidity), but the sudden loss of resistance seen in a spastic extremity is absent. The tremor, which is present at rest and disappears with activity, is due to regular, alternating 8-Hz contractions of antagonistic muscles.

Figure 12–15 shows the current view of the pathogenesis of the movement disorders in Parkinson disease. In healthy individuals, basal ganglia output is inhibitory via GABAergic nerve fibers. The dopaminergic neurons that project from the substantia nigra to the putamen normally have two effects. They stimulate the D_1 dopamine receptors, which inhibit GPi via direct GABAergic receptors; and they inhibit D_2 receptors, which also inhibit the GPe. In addition, the inhibition reduces the excitatory discharge from the subthalamic nucleus to the GPi. This balance between inhibition and excitation somehow maintains normal motor function. In Parkinson disease, the dopaminergic input to the putamen is lost. This results in decreased inhibition and increased excitation from the subthalamic nucleus to the GPi. The overall increase in inhibitory output to the thalamus and brainstem disorganizes movement.

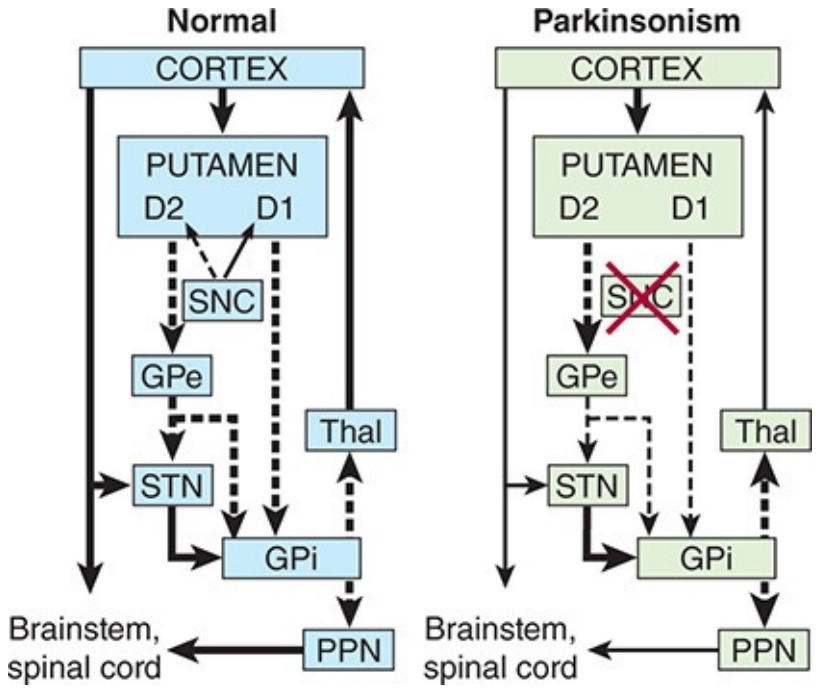


FIGURE 12–15 Probable basal ganglia-thalamocortical circuitry in Parkinson disease. Solid arrows indicate excitatory outputs and dashed arrows inhibitory outputs. The strength of each output is indicated by the width of the arrow. GPe, external segment of the globus pallidus; GPi, internal segment of the globus pallidus; PPN, pedunculopontine nuclei; SNC, pars compacta of the substantia nigra; STN, subthalamic nucleus; Thal, thalamus. See text for details. (Modified with permission from Grafton SC, DeLong M: Tracing the brain circuitry with functional imaging, Nat Med 1997 Jun; 3(6):602–603.)

CLINICAL BOX 12–7

Basal Ganglia Diseases

The initial detectable damage in **Huntington disease** is to medium spiny neurons in the striatum. The loss of this GABAergic pathway to the GPe releases inhibition, permitting the hyperkinetic features of the disease to develop. An early sign is a jerky trajectory of the hand when reaching to touch a spot, especially toward the end of the reach. Later, hyperkinetic **choreiform movements** appear and gradually increase until they incapacitate the patient. Speech becomes slurred and then incomprehensible, and a progressive dementia is followed by death, usually within 10–15 years after the onset of symptoms. Huntington disease affects 5 out of 100,000 people

worldwide. It is inherited as an autosomal dominant disorder, and its onset is usually between the ages of 30 and 50. The abnormal gene responsible for the disease is located near the end of the short arm of chromosome 4. It normally contains 11–34 CAG repeats, each coding for glutamine. In patients with Huntington disease, this number is increased to 42–86 or more copies; and the greater the number of repeats, the earlier the age of onset and the more rapid the progression of the disease. The gene codes for **huntingtin,** a protein that plays a role in axonal trafficking, regulation of gene transcription, and cell survival. Poorly soluble protein aggregates, which are toxic, form in cell nuclei and elsewhere. However, the correlation between aggregates and symptoms is less than perfect. The loss of the function of huntingtin that occurs is proportional to the size of the CAG insert. In animal models of the disease, intrastriatal grafting of fetal striatal tissue improves cognitive performance. In addition, tissue caspase-1 activity is increased in the brains of humans with the disease. Moreover, in mice in which the gene for this apoptosis-regulating enzyme has been knocked out, progression of the disease is slowed.

Wilson disease (or **hepatolenticular degeneration**) is a rare disorder of copper metabolism that has an onset between 6 and 25 years of age, affecting about four times as many females as males. Wilson disease affects about 30,000 people worldwide. It is a genetic autosomal recessive disorder due to a mutation on the long arm of chromosome 13q. It affects the copper-transporting ATPase gene (*ATP7B*) in the liver, leading to an accumulation of copper in the liver and resultant progressive liver damage. About 1% of the population carries a single abnormal copy of this gene but does not develop any symptoms. The disease may develop in a child who inherits the gene from both parents. In affected individuals, copper accumulates in the periphery of the cornea in the eye accounting for the characteristic yellow **Kayser-Fleischer rings.** The dominant neuronal pathology is degeneration of the putamen, a part of the **lenticular nucleus.** Motor disturbances include "wing-beating" tremor or **asterixis, dysarthria,** unsteady gait, and rigidity. **Tardive dyskinesia** is a disease that involves the basal ganglia, but it is caused by medical treatment of another disorder with **neuroleptic drugs** such as phenothiazines or haloperidol. Therefore, tardive dyskinesia is iatrogenic in origin. Long-term use of these drugs may produce biochemical abnormalities in the striatum. The motor disturbances include either temporary or permanent uncontrolled involuntary movements of the face and tongue and cogwheel rigidity. The neuroleptic drugs act via blockade of dopaminergic transmission. Prolonged drug use leads to hypersensitivity of

D_3 dopaminergic receptors and an imbalance in nigrostriatal influences on motor control.

THERAPEUTIC HIGHLIGHTS

Treatment for Huntington disease is directed at treating the symptoms and maintaining quality of life because there is no cure. In general, drugs used to treat the symptoms of this disease have side effects such as fatigue, nausea, and restlessness. In August 2008, the US Food and Drug Administration approved the use of **tetrabenazine** to reduce choreiform movements that characterize the disease. This drug binds reversibly to vesicular monoamine transporters (VMATs) and thus inhibits the uptake of monoamines into synaptic vesicles. It also acts as a dopamine receptor antagonist. Tetrabenazine is the first drug to receive approval for individuals with Huntington disease. It is also used to treat other hyperkinetic movement disorders such as tardive dyskinesia. **Chelating agents** (eg, **penicillamine and trienthine**) are used to reduce the copper in the body in individuals with Wilson disease. Tardive dyskinesia has proven to be difficult to treat. Treatment in patients with psychiatric disorders is often directed at prescribing a neuroleptic with less likelihood of causing the disorder. **Clozapine** is an atypical neuroleptic drug that has been an effective substitute for traditional neuroleptic drugs but with less risk for development of tardive dyskinesia.

Familial cases of Parkinson disease occur, but these are uncommon. The genes for at least five proteins can be mutated. These proteins appear to be involved in ubiquitination. Two of the proteins, **α-synuclein** and **parkin,** interact and are found in **Lewy bodies.** The Lewy bodies are inclusion bodies in neurons that occur in all forms of Parkinson disease.

An important consideration in Parkinson disease is the balance between the excitatory discharge of cholinergic interneurons and the inhibitory dopaminergic input in the striatum. Some improvement is produced by decreasing the cholinergic influence with anticholinergic drugs. More dramatic improvement is produced by administration of L-dopa. Unlike dopamine, this dopamine precursor crosses the blood-brain barrier and helps repair the dopamine deficiency. However, the degeneration of these neurons continues and in 5–7 years the beneficial effects of L-dopa disappear.

CEREBELLUM

The cerebellum sits astride the main sensory and motor systems in the brainstem **(Figure 12–16)**. The medial **vermis** and lateral **cerebellar hemispheres** are more extensively folded and fissured than the cerebral cortex. The cerebellum weighs only 10% as much as the cerebral cortex, but its surface area is about 75% of that of the cerebral cortex. Anatomically, the cerebellum is divided into three parts by two transverse fissures. The posterolateral fissure separates the medial nodulus and the lateral flocculus on either side from the rest of the cerebellum, and the primary fissure divides the remainder into an anterior and a posterior lobe. Lesser fissures divide the vermis into smaller sections, so that it contains 10 primary lobules numbered I–X from superior to inferior.

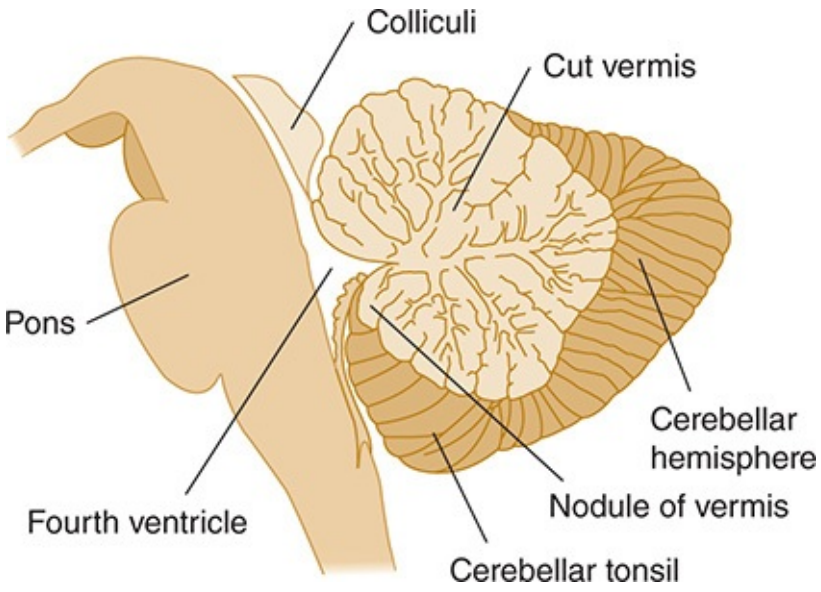


FIGURE 12–16 A midsagittal section through the cerebellum. The medial vermis and lateral cerebellar hemispheres have many narrow, ridge-like folds called folia. Although not shown, the cerebellum is connected to the brainstem by three pairs of peduncles (superior, middle, and inferior). (Reproduced with permission from Waxman SG: *Clinical Neuroanatomy*, 26th ed. New York, NY: McGraw-Hill; 2010.)

The cerebellum is connected to the brainstem by three pairs of peduncles that are located above and around the fourth ventricle. The **superior cerebellar peduncle** includes fibers from deep cerebellar nuclei that project to the brainstem, red nucleus, and thalamus. The **middle cerebellar peduncle** contains only afferent fibers from the contralateral pontine nuclei, and the **inferior**

cerebellar peduncle a mixture of afferent fibers from the brainstem and spinal cord and efferent fibers to the vestibular nuclei.

The cerebellum has an external **cerebellar cortex** separated by white matter from the **deep cerebellar nuclei.** The middle and inferior cerebellar peduncles carry afferent fibers into the cerebellum where they are called **mossy** and **climbing fibers.** These fibers emit collaterals to the deep nuclei and pass to the cortex. There are four deep nuclei: the **dentate,** the **globose,** the **emboliform,** and the **fastigial** nuclei. The globose and the emboliform nuclei are sometimes lumped together as the **interpositus nucleus.**

ORGANIZATION OF THE CEREBELLUM

The cerebellar cortex has three layers: an external molecular layer, a Purkinje cell layer that is only one cell thick, and an internal granular layer. There are five types of neurons in the cortex: Purkinje, granule, basket, stellate, and Golgi cells **(Figure 12–17)**. The **Purkinje cells** are among the largest neurons in the CNS. They have extensive dendritic arbors that extend throughout the molecular layer. Their axons, which are the only output from the cerebellar cortex, project to the deep cerebellar nuclei, especially the dentate nucleus, where they form inhibitory synapses. They also make inhibitory connections with neurons in the vestibular nuclei.

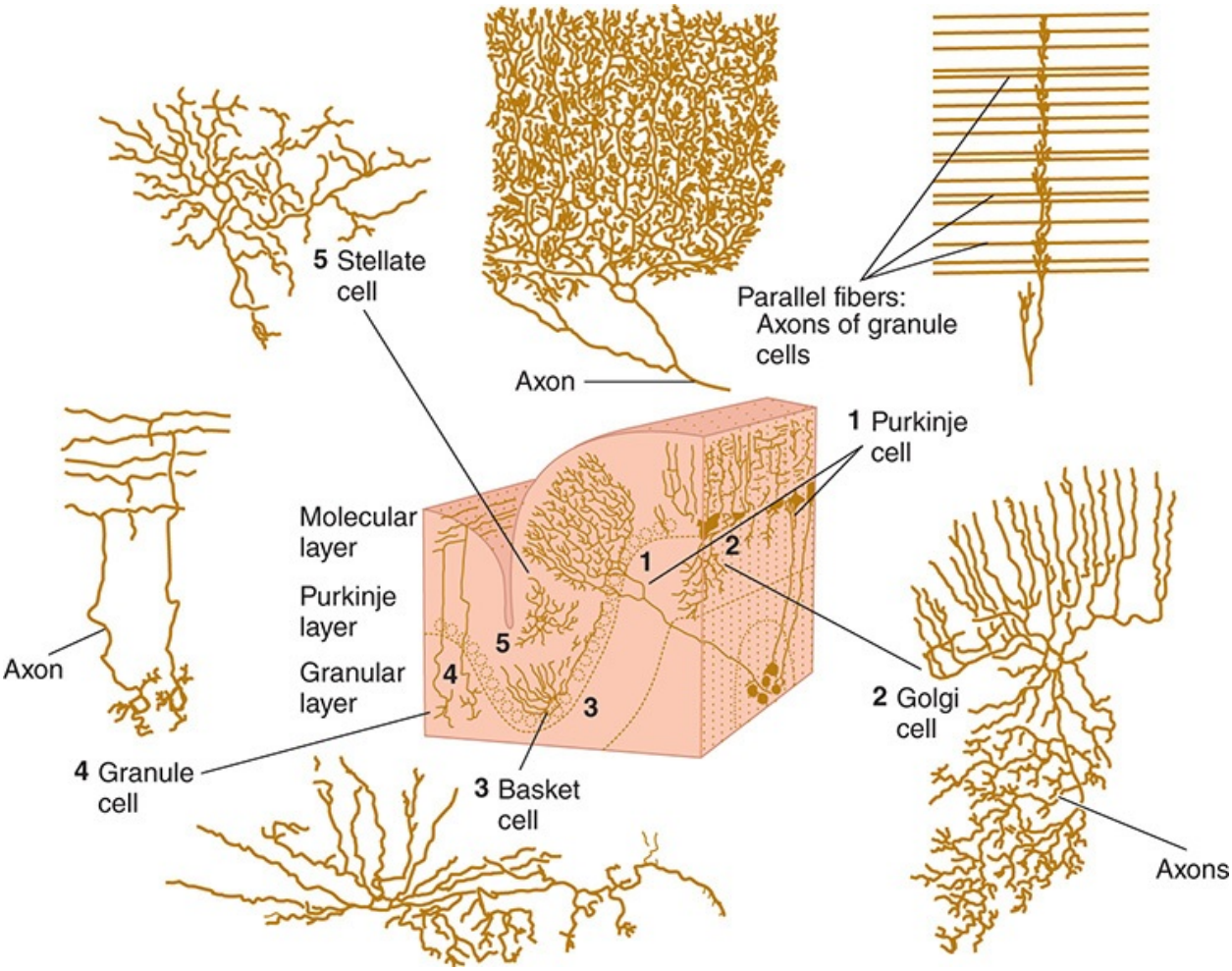


FIGURE 12–17 Location and structure of five neuronal types in the cerebellar cortex. Drawings are based on Golgi-stained preparations. Purkinje cells (1) have processes aligned in one plane; their axons are the only output from the cerebellum. Axons of granule cells (4) traverse and make connections with Purkinje cell processes in molecular layer. Golgi (2), basket (3), and stellate (5) cells have characteristic positions, shapes, branching patterns, and synaptic connections. (For 1 and 2, Reproduced with permission of Ramon y Cajal S: Histologie du Systeme Nerveux II., C.S.I.C. Madrid; For 3–5, Reproduced with permission of Palay SL, Chan-Palay V: *Cerebellar Cortex*. Berlin: Springer-Verlag, 1975.)

The cerebellar **granule cells,** whose cell bodies are in the granular layer, receive excitatory input from the mossy fibers and innervate the Purkinje cells **(Figure 12–18)**. Each sends an axon to the molecular layer, where the axon bifurcates to form a T. The branches of the T are straight and run long distances; thus, they are called **parallel fibers.** The dendritic trees of the Purkinje cells are

markedly flattened and oriented at right angles to the parallel fibers. The parallel fibers form excitatory synapses on the dendrites of many Purkinje cells, and the parallel fibers and Purkinje dendritic trees form a grid of remarkably regular proportions.

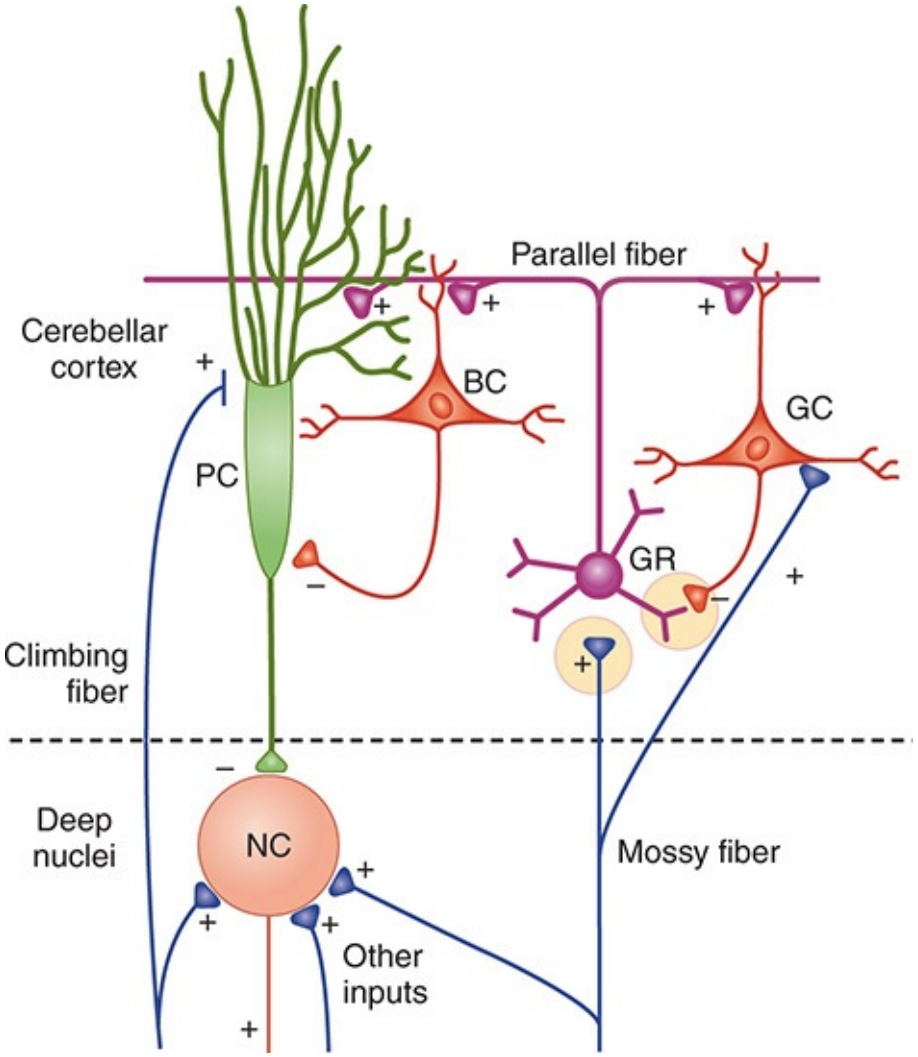


FIGURE 12–18 Diagram of neural connections in the cerebellum. Plus (+) and minus (–) signs indicate whether endings are excitatory or inhibitory. BC, basket cell; GC, Golgi cell; GR, granule cell; NC, cell in deep nucleus; PC, Purkinje cell. Note that PCs and BCs are inhibitory. The connections of the stellate cells, which are not shown, are similar to those of the basket cells, except that they end for the most part on Purkinje cell dendrites.

CLINICAL BOX 12–8

Parkinson Disease

There are 7–10 million people worldwide in whom Parkinson disease has been diagnosed. The disease is 1.5 times more prevalent in men than in women. Each year in the United States, nearly 60,000 new cases are diagnosed. Parkinsonism occurs in sporadic idiopathic form in many middle-aged and elderly individuals and is one of the most common neurodegenerative diseases. It is estimated to occur in 1–2% of individuals over age 65. Dopaminergic neurons and dopamine receptors are steadily lost with age in the basal ganglia in healthy individuals, and an acceleration of these losses apparently precipitates parkinsonism. Symptoms appear when 60–80% of the nigrostriatal dopaminergic neurons degenerate.

Parkinsonism is also seen as a complication of treatment with the phenothiazine group of antipsychotic drugs and other drugs that block dopaminergic D_2 receptors. It can be produced in rapid and dramatic form by injection of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine (MPTP). This effect was discovered by chance when a drug dealer in northern California supplied some of his clients with a homemade preparation of synthetic heroin that contained MPTP. MPTP is a prodrug that is metabolized in astrocytes by the enzyme monoamine oxidase (MOA-B) to produce a potent oxidant, 1-methyl-4-phenylpyridinium (MPP+). MPP+ is taken up by the dopamine transporter into dopaminergic neurons in the substantia nigra, which it destroys without affecting other dopaminergic neurons to any appreciable degree.

THERAPEUTIC HIGHLIGHTS

There is no cure for Parkinson disease, and drug therapies are designed to treat the symptoms. **Sinemet,** a combination of **levodopa** (L-dopa) and **carbidopa,** is the most commonly used drug for the treatment of Parkinson disease. The addition of carbidopa to L-dopa increases its effectiveness and prevents the conversion of L-dopa to dopamine in the periphery and thus reduces some of the adverse side effects of L-dopa (nausea, vomiting, and cardiac rhythm disturbances). Dopamine agonists, including **apomorphine, bromocriptine, pramipexole,** and **ropinirole,** have also proven effective in some patients with Parkinson disease. Taken in combination with L-dopa, cathechol-O-methyltransferase (COMT) inhibitors (eg, **entacapone**) are another class of drugs used to treat this disease. They act by blocking the breakdown of L-dopa, allowing more of it to reach the brain to increase the level of dopamine. MAO-

B inhibitors (eg, **selegiline**) also prevent the breakdown of dopamine. They can be given soon after diagnosis and delay the need for L-dopa.

The US Food and Drug Administration has approved the use of deep brain stimulation (DBS) for the treatment of Parkinson disease. DBS reduces the amount of L-dopa patients need and thus reduces its adverse side effects (eg, involuntary movements called **dyskinesias**). DBS has been associated with reduction of tremors, slowness of movements, and gait problems in some patients. Surgical treatments in general are reserved for those who have exhausted drug therapies or who have not responded favorably to them. Lesions in GPi **(pallidotomy)** or in the subthalamic nucleus **(thalamotomy)** have been performed to help restore the output balance of the basal ganglia toward normal (see Figure 12–15). Another surgical approach is to implant dopamine-secreting tissue in or near the basal ganglia. Transplants of the patient's own adrenal medullary tissue or carotid body works for a while, apparently by functioning as a sort of dopamine minipump, but long-term results have been disappointing. Results with transplantation of fetal striatal tissue have been better, and transplanted cells have been shown to not only survive but also make appropriate connections in the host's basal ganglia. However, some patients with transplants develop dyskinesias due to excessive levels of dopamine.

The other three types of neurons in the cerebellar cortex are inhibitory interneurons. **Basket cells** (Figure 12–17) are located in the molecular layer. They receive excitatory input from the parallel fibers and each projects to many Purkinje cells (Figure 12–18). Their axons form a basket around the cell body and axon hillock of each Purkinje cell they innervate. **Stellate cells** are similar to the basket cells but are located in the more superficial molecular layer. **Golgi cells** are located in the granular layer. Their dendrites project into the molecular layer and receive excitatory input from the parallel fibers (Figure 12–18). Their cell bodies receive excitatory input via collaterals from the incoming mossy fibers. Their axons project to the dendrites of the granule cells where they form an inhibitory synapse.

As already mentioned, the two excitatory main inputs to the cerebellar cortex are climbing fibers and mossy fibers (Figure 12–18). The climbing fibers come from a single source, the inferior olivary nuclei. Each projects to the primary dendrites of a Purkinje cell, around which it entwines like a climbing plant. Proprioceptive input to the inferior olivary nuclei comes from all over the body.

On the other hand, the mossy fibers provide direct proprioceptive input from all parts of the body plus input from the cerebral cortex via the pontine nuclei to the cerebellar cortex. They end on the dendrites of granule cells in complex synaptic groupings called **glomeruli.** The glomeruli also contain the inhibitory endings of the Golgi cells mentioned above.

The fundamental circuits of the cerebellar cortex are relatively simple (Figure 12–18). Climbing fiber inputs exert a strong excitatory effect on single Purkinje cells, whereas mossy fiber inputs exert a weak excitatory effect on many Purkinje cells via the granule cells. The basket and stellate cells are also excited by granule cells via their parallel fibers; and the basket and stellate cells, in turn, inhibit the Purkinje cells **(feed-forward inhibition).** Golgi cells are excited by the mossy fiber collaterals and parallel fibers, and they inhibit transmission from mossy fibers to granule cells. The neurotransmitter released by the stellate, basket, Golgi, and Purkinje cells is GABA, whereas the granule cells release glutamate. GABA acts via $GABA_A$ receptors, but the combinations of subunits in these receptors vary from one cell type to the next. The granule cell is unique in that it appears to be the only type of neuron in the CNS that has a $GABA_A$ receptor containing the α6 subunit.

The output of the Purkinje cells is in turn inhibitory to the deep cerebellar nuclei. As noted above, these nuclei also receive excitatory inputs via collaterals from the mossy and climbing fibers. It is interesting, in view of their inhibitory Purkinje cell input, that the output of the deep cerebellar nuclei to the brainstem and thalamus is always excitatory. Thus, almost all the cerebellar circuitry seems to be concerned solely with modulating or timing the excitatory output of the deep cerebellar nuclei to the brainstem and thalamus. The primary afferent systems that converge to form the mossy fiber or climbing fiber input to the cerebellum are summarized in **Table 12–2**.

TABLE 12–2 Function of principal afferent systems to the cerebellum.[a]

Afferent Tracts	Transmits
Vestibulocerebellar	Vestibular impulses from labyrinths, direct and via vestibular nuclei
Dorsal spinocerebellar	Proprioceptive and exteroceptive impulses from muscle spindles, Golgi tendon organ, and joint receptors of lower limbs and trunk
Ventral spinocerebellar	Proprioceptive and exteroceptive impulses from muscle spindles, Golgi tendon organ, and joint receptors of upper and lower limbs
Cuneocerebellar	Proprioceptive impulses from muscle spindles, Golgi tendon organ, and joint receptors of upper limb and upper thorax
Tectocerebellar	Auditory and visual impulses via inferior and superior colliculi, respectively
Pontocerebellar	Impulses from motor and other parts of cerebral cortex via pontine nuclei
Olivocerebellar	Proprioceptive input from whole body via relay in inferior olive

[a]The olivocerebellar pathway projects to the cerebellar cortex via climbing fibers; the rest of the listed paths project via mossy fibers. Several other pathways transmit impulses from nuclei in the brainstem to the cerebellar cortex and to the deep nuclei, including a serotonergic input from the raphe nuclei to the granular and molecular layers and a noradrenergic input from the locus coeruleus to all three layers.

FUNCTIONAL DIVISIONS

From a functional point of view, the cerebellum is divided into three parts **(Figure 12–19)**. The nodulus in the vermis and the flanking flocculus in the hemisphere on each side form the **vestibulocerebellum** (or **flocculonodular lobe**). This lobe is phylogenetically the oldest part of the cerebellum and has

vestibular connections concerned with equilibrium and eye movements. The rest of the vermis and the adjacent medial portions of the hemispheres form the **spinocerebellum,** the region that receives proprioceptive input from the body as well as a copy of the "motor plan" from the motor cortex. By comparing plan with performance, it smooths and coordinates movements that are ongoing. The vermis projects to the brainstem area concerned with control of axial and proximal limb muscles (medial brainstem pathways, and the hemispheres of the spinocerebellum project to the brainstem areas concerned with control of distal limb muscles (lateral brainstem pathways). The lateral portions of the cerebellar hemispheres **(cerebrocerebellum)** are the newest from a phylogenetic point of view, reaching their greatest development in humans. They interact with the motor cortex in planning and programming movements.

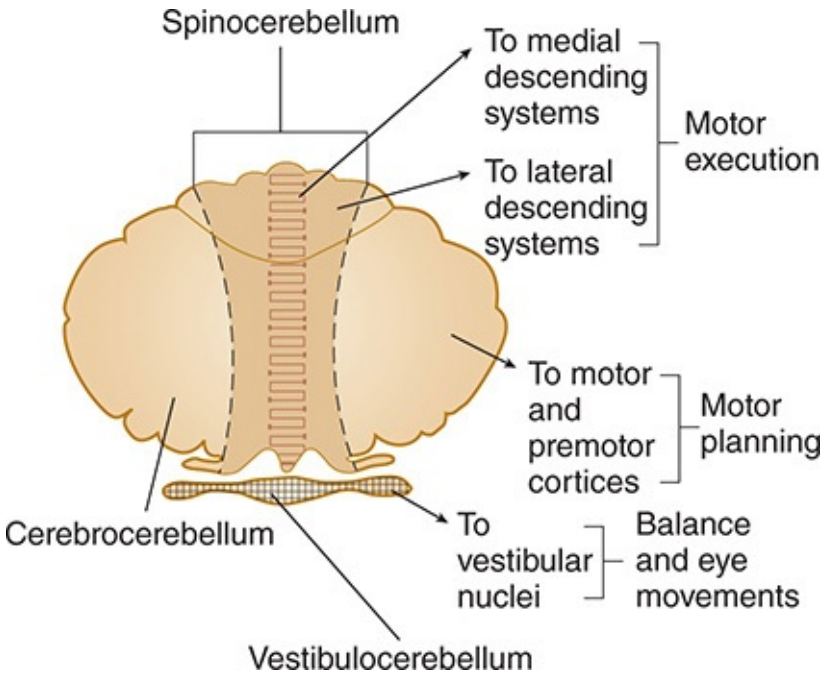


FIGURE 12–19 Three functional divisions of the cerebellum. The nodulus in the vermis and the flanking flocculus in the hemisphere on each side form the vestibulocerebellum which has vestibular connections and is concerned with equilibrium and eye movements. The rest of the vermis and the adjacent medial portions of the hemispheres form the spinocerebellum, the region that receives proprioceptive input from the body as well as a copy of the "motor plan" from the motor cortex. The lateral portions of the cerebellar hemispheres are called the cerebrocerebellum which interacts with the motor cortex in planning and programming movements. (Modified with permission from Kandel ER, Schwartz JH, Jessell TM (eds): *Principles of Neural Science*, 4th ed. New York,

NY: McGraw-Hill; 2000.)

Most of the vestibulocerebellar output passes directly to the brainstem; but the rest of the cerebellar cortex projects to the deep nuclei that then project to the brainstem. The deep nuclei provide the only output for the spinocerebellum and the cerebrocerebellum. The medial portion of the spinocerebellum projects to the fastigial nuclei and from there to the brainstem. The adjacent hemispheric portions of the spinocerebellum project to the emboliform and globose nuclei and from there to the brainstem. The cerebrocerebellum projects to the dentate nucleus and from there either directly or indirectly to the ventrolateral nucleus of the thalamus.

CEREBELLAR DISEASE

Damage to the cerebellum leads to several characteristic abnormalities, including **hypotonia, ataxia,** and **intention tremor.** Motor abnormalities associated with cerebellar damage vary depending on the region involved. **Figure 12–20** illustrates some of these abnormalities. Additional information is provided in **Clinical Box 12–9**.

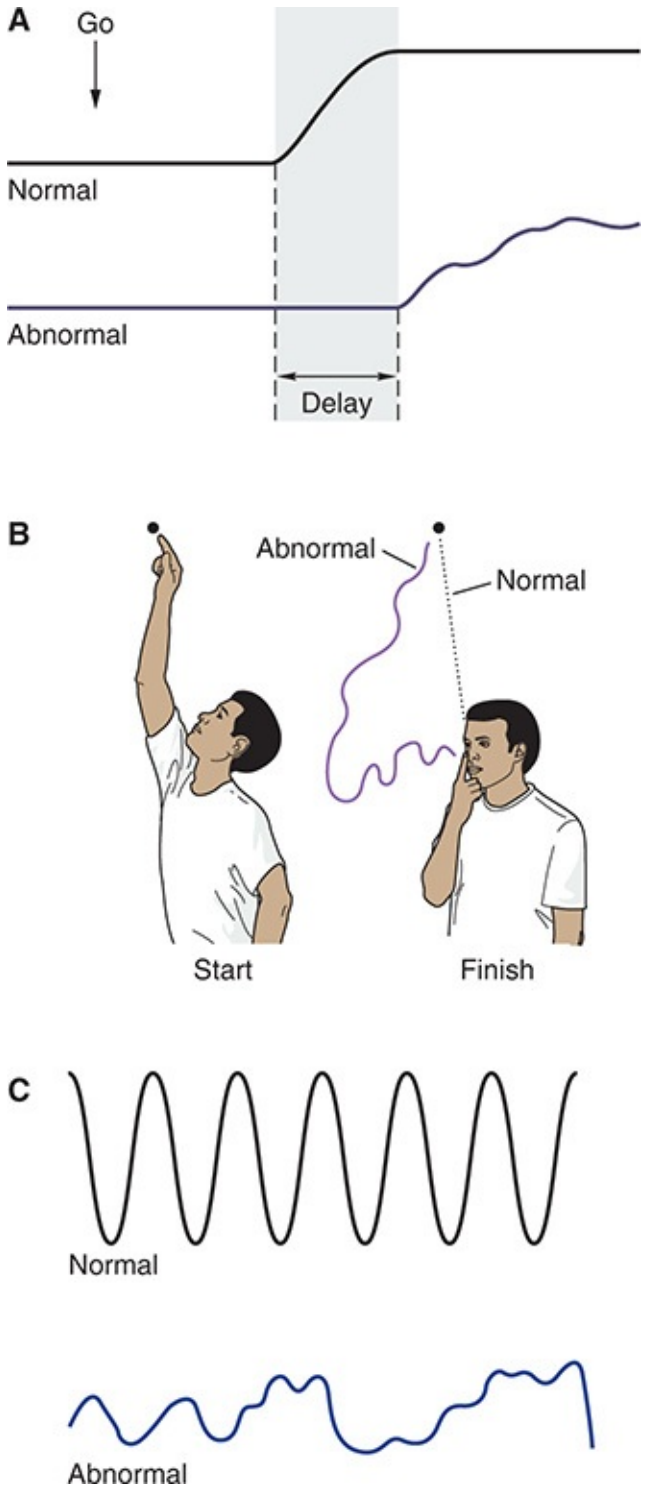


FIGURE 12–20 Typical defects associated with cerebellar disease. A) Lesion of the right cerebellar hemisphere delays initiation of movement. The patient is told to clench both hands simultaneously; right hand clenches later than left (shown by recordings from a pressure bulb transducer squeezed by the patient).

B) Dysmetria and decomposition of movement shown by patient moving his arm from a raised position to his nose. Tremor increases on approaching the nose. **C)** Dysdiadochokinesia occurs in the abnormal position trace of hand and forearm as a cerebellar subject tries alternately to pronate and supinate forearm while flexing and extending elbow as rapidly as possible. (Used with permission from Kandel ER, Schwartz JH, Jessell TM (eds): *Principles of Neural Science*, 4th ed. New York, NY: McGraw-Hill; 2000.)

THE CEREBELLUM & LEARNING

The cerebellum is concerned with learned adjustments that make coordination easier when a given task is performed over and over. As a motor task is learned, activity in the brain shifts from the prefrontal areas to the parietal and motor cortex and the cerebellum. The basis of the learning in the cerebellum is probably the input via the olivary nuclei. The mossy fiber-granule cell-Purkinje cell pathway is highly divergent, allowing an individual Purkinje cell to receive input from many mossy fibers arising from different regions. In contrast, a Purkinje cell receives input from a single climbing fiber but it makes 2000–3000 synapses on it. Climbing fiber activation produces a large, complex spike in the Purkinje cell, and this spike produces long-term modification of the pattern of mossy fiber input to that particular Purkinje cell. Climbing fiber activity is increased when a new movement is being learned, and selective lesions of the olivary complex abolish the ability to produce long-term adjustments in certain motor responses.

CLINICAL BOX 12–9

Cerebellar Disease

Most abnormalities associated with damage to the cerebellum are apparent during movement. The marked **ataxia** is characterized as incoordination due to errors in the rate, range, force, and direction of movement. Ataxia is manifest not only in the wide-based, unsteady, “drunken” gait of patients but also in defects of the skilled movements involved in the production of speech, so that slurred, **scanning speech** results. Many types of ataxia are hereditary, including **Friedreich ataxia** and **Machado-Joseph disease.** There is no cure for hereditary ataxias. Voluntary movements are also highly abnormal when the cerebellum is damaged. For example, attempting to touch an object with a

finger results in overshooting. This **dysmetria** promptly initiates a gross corrective action, but the correction overshoots to the other side, and the finger oscillates back and forth. This oscillation is called an **intention tremor.** Another characteristic of cerebellar disease is the inability to stop movement promptly. Normally, for example, flexion of the forearm against resistance is quickly checked when the resistance force is suddenly broken off. A patient with cerebellar disease cannot stop the movement of the limb, and the forearm flies backward in a wide arc. This abnormal response is known as the **rebound phenomenon** and is one of the reasons these patients show **dysdiadochokinesia,** the inability to perform rapidly alternating opposite movements such as repeated pronation and supination of the hands. Finally, patients with cerebellar disease have difficulty performing actions that involve simultaneous motion at more than one joint. They dissect such movements and carry them out one joint at a time, a phenomenon known as **decomposition of movement.** Other signs of cerebellar deficit in humans point to the importance of the cerebellum in the control of movement. Motor abnormalities associated with cerebellar damage vary depending on the region involved. The major dysfunction seen after damage to the vestibulocerebellum is ataxia, **disequilibrium,** and **nystagmus.** Damage to the vermis and fastigial nucleus (part of the spinocerebellum) leads to scanning speech and disturbances in the control of axial and trunk muscles during attempted antigravity postures. Degeneration of this portion of the cerebellum can result from thiamine deficiency in alcoholics or malnourished individuals. The major dysfunction seen after damage to the cerebrocerebellum is a delay in initiating movements and decomposition of movement.

THERAPEUTIC HIGHLIGHTS

Management of ataxia is primarily supportive and often includes physical, occupational, and speech therapy. Attempts to identify effective drug therapies have met with little success. **DBS** of the ventral intermediate nucleus of the thalamus may reduce cerebellar tremor, but it is less effective in reducing ataxia. A deficiency in **coenzyme Q10** (CoQ10) may contribute to the abnormalities seen in some forms of familial ataxia. If low levels of CoQ10 are detected, treatment to replace the missing CoQ10 has been beneficial.

CHAPTER SUMMARY

- The basic unit of integrated reflex activity is the reflex arc that consists of a sense organ, an afferent neuron, one or more synapses within a central integrating station, an efferent neuron, and an effector.
- A muscle spindle is a group of specialized intrafusal muscle fibers with contractile polar ends and a noncontractile center that is parallel to the extrafusal muscle fibers and innervated by types Ia and II afferent fibers and efferent γ-motor neurons.
- Tapping the patellar tendon causes muscle stretch that activates type Ia fibers that synapse directly on the motor neurons to the extrafusal muscle fibers in the same muscle to cause it to contract (knee jerk reflex).
- Activation of γ-motor neurons causes the contractile ends of the intrafusal fibers to shorten and thus stretches the nuclear bag portion of the spindles, deforming the endings, and initiating impulses in Ia fibers. This can lead to reflex contraction of the muscle. Thus, the γ-motor neurons can initiate contraction indirectly via the stretch reflex.
- A Golgi tendon organ is a netlike collection of knobby nerve endings among the fascicles of a tendon that is in series with extrafusal muscle fibers and innervated by type Ib afferents. They are stimulated by both passive stretch and active contraction of the muscle to relax the muscle (inverse stretch reflex) and function as a transducer to regulate muscle force.
- Physiologic tremor is a rapid (10 Hz), low-amplitude transient tremor in the limbs of healthy individuals; it can become accentuated with anxiety. Clonus is the occurrence of regular, repetitive, rhythmic contractions of a muscle subjected to sudden, maintained stretch; clonus with five or more beats is considered abnormal. Muscle tone is the resistance to muscle stretch. If the muscle offers very little resistance and is said to be flaccid; if resistance to stretch is high, the muscle is spastic. Damage to the cerebral cortex before or during childbirth or during the first 2–3 years of development can lead to cerebral palsy, a disorder that affects muscle tone, movement, and coordination.
- The flexor withdrawal reflex is a polysynaptic reflex that is initiated by nociceptive stimuli; it can serve as a protective mechanism to present further injury.
- Spinal cord injury is followed by a period of spinal shock during which all spinal reflex responses are depressed. In humans, recovery begins about 2

weeks after the injury.

- The supplementary cortex, basal ganglia, and cerebellum participate in the planning of skilled movements. Commands from the primary motor cortex and other cortical regions are relayed via the corticospinal and corticobulbar tracts to spinal and brainstem motor neurons. Cortical areas and motor pathways descending from the cortex are somatotopically organized.
- The ventral corticospinal tract and medial descending brainstem pathways (tectospinal, reticulospinal, and vestibulospinal tracts) regulate proximal muscles and posture. The lateral corticospinal and rubrospinal tracts control distal limb muscles for fine motor control and skilled voluntary movements.
- Lower motor neurons are those whose axons terminate on skeletal muscles; damage to these neurons (eg, ALS) is associated with flaccid paralysis, muscular atrophy, fasciculations, hypotonia, and hyporeflexia or areflexia. Upper motor neurons include corticospinal tract neurons that innervate spinal motor neurons; damage to these neurons initially causes muscles to become weak and flaccid but eventually leads to spasticity, hypertonia, hyperactive stretch reflexes, and a positive Babinski sign.
- Damage to the cerebral cortex before or during childbirth can lead to cerebral palsy, a disorder that affects muscle tone, movement, and coordination. Decerebrate rigidity leads to hyperactivity in extensor muscles in all four extremities; the spasticity is due to facilitation of the stretch reflex. It resembles what occurs with uncal herniation due to a supratentorial lesion. Decorticate rigidity is flexion of the upper extremities at the elbow and extensor hyperactivity in the lower extremities. It occurs on the hemiplegic side after hemorrhage or thrombosis in the internal capsule.
- The basal ganglia include the caudate nucleus, putamen, globus pallidus, subthalamic nucleus, and substantia nigra. The connections between the parts of the basal ganglia include a dopaminergic nigrostriatal projection from the substantia nigra to the striatum and a GABAergic projection from the striatum to substantia nigra.
- Parkinson disease is due to degeneration of the nigrostriatal dopaminergic neurons and is characterized by akinesia, bradykinesia, cogwheel rigidity, and tremor at rest. Huntington disease is characterized by choreiform movements due to the loss of the GABAergic inhibitory pathway to the globus pallidus.
- The cerebellar cortex contains five types of neurons: Purkinje, granule, basket, stellate, and Golgi cells. The two main inputs to the cerebellar cortex

are climbing fibers and mossy fibers. Purkinje cells are the only output from the cerebellar cortex, and they generally project to the deep nuclei. Damage to the cerebellum leads to several characteristic abnormalities, including hypotonia, ataxia, and intention tremor.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. A 48-year-old man was undergoing a thorough neurological exam after falling from a construction platform. The test included an evaluation of his deep tendon reflex. What are the elements of the deep tendon reflex?

   A. Golgi tendon organ, group Ib afferent fibers, spinal inhibitory interneuron, contralateral α-motor neuron, and extrafusal fibers of the skeletal muscle

   B. Golgi tendon organ, group II afferent fibers, spinal inhibitory interneuron, ipsilateral α-motor neuron, and intrafusal fibers of the skeletal muscle

   C. Group Ia sensory fibers originating in the contractile portion of the intrafusal fibers of the muscle spindle, spinal dorsal horn neuron, ipsilateral α-motor neuron, and extrafusal fibers of the skeletal muscle

   D. Group Ia sensory fibers originating in the central portion of the intrafusal fibers of the muscle spindle, ipsilateral α-motor neuron, and extrafusal fibers of the skeletal muscle

   E. Group II sensory fibers originating in the contractile portion of the intrafusal fibers of the muscle spindle, spinal inhibitory interneuron, ipsilateral α-motor neuron, and extrafusal fibers of the skeletal muscle

2. When dynamic γ-motor neurons are activated at the same time as α-motor neurons to muscle,

   A. prompt inhibition of discharge in spindle Ia afferents takes place.

   B. clonus is likely to occur.

   C. the muscle will not contract.

   D. the number of impulses in spindle Ia afferents is smaller than when α discharge alone is increased.

   E. the number of impulses in spindle Ia afferents is greater than when α discharge alone is increased.

3. The inverse stretch reflex

   A. occurs when Ia spindle afferents are inhibited.

B. is a monosynaptic reflex initiated by activation of the Golgi tendon organ.

C. is a disynaptic reflex with a single interneuron inserted between the afferent and efferent limbs.

D. is a polysynaptic reflex with many interneurons inserted between the afferent and efferent limbs.

E. uses type II afferent fibers from the Golgi tendon organ.

4. Withdrawal reflexes are

A. initiated by innocuous stimulation of the skin.

B. can lead to the appearance of clonus.

C. prolonged if the stimulus is strong.

D. an example of a stretch reflex.

E. accompanied by the same response on both sides of the body.

5. While exercising, a 42-year-old woman developed sudden onset of tingling in her right leg and an inability to control movement in that limb. A neurologic exam showed a hyperactive knee jerk reflex and a positive Babinski sign. What is a possible basis for these findings and does it reflect damage to an upper or lower motor neuron?

A. She had a lower thoracic disk rupture that damaged the right side of her spinal cord (upper motor neuron damage).

B. She had a mid-cervical disk rupture that damaged the left side of her spinal cord (upper motor neuron damage).

C. She had a lower lumbar disk rupture that compressed the spinal nerve at that segmental level (lower motor neuron damage).

D. She had a sacral disk rupture that put pressure on the ventral root at that segmental level (lower motor neuron damage).

E. She was experiencing dysfunction of both a lower motor neuron and an upper motor neuron.

6. Increased neural activity before a skilled voluntary movement is *first* seen in the

A. spinal motor neurons.

B. precentral motor cortex.

C. midbrain.

D. cerebellum.

E. cortical association areas.

7. A 58-year-old woman was brought to the emergency department of her local

hospital because of a sudden change of consciousness. All four limbs were extended, suggestive of decerebrate rigidity. A brain CT showed a rostral pontine hemorrhage. What are the underlying neurophysiological changes that lead to the appearance of decerebrate rigidity?

A. Destruction of the rubrospinal tract eliminates inhibition of the cerebellar fastigial nucleus and secondarily increases excitation to vestibular nuclei to activate extensor muscles in the limbs.

B. Loss of the corticospinal pathway disrupts excitatory input to motor neurons controlling flexor muscles, leaving extensor muscles to undergo sustained contraction.

C. Sensory input activates the medullary reticulospinal pathway, which then directly activates motor neurons to extensor muscles in all four extremities.

D. Sensory input activates neurons in the rubrospinal tract that inhibit flexor α-motor neurons and excite extensor α-motor neurons in all four limbs.

E. Sensory input activates the pontine reticulospinal pathway, which then activates primarily γ-motor neurons to extensor muscles in all four extremities.

8. Which of the following correctly describes components of the central pathway responsible for control of posture?

A. The tectospinal pathway originates in the superior colliculus and terminates on neurons in the dorsolateral area of the spinal ventral horn that innervate limb muscles.

B. The medullary reticulospinal pathway terminates on neurons in the ventromedial area of the spinal ventral horn that innervate axial and proximal muscles.

C. The pontine reticulospinal pathway terminates on neurons in the dorsomedial area of the spinal ventral horn that innervate limb muscles.

D. The medial vestibular pathway terminates on neurons in the dorsomedial area of the spinal ventral horn that innervate axial and proximal muscles.

E. The lateral vestibular pathway terminates on neurons in the dorsolateral area of the spinal ventral horn that innervate axial and proximal muscles.

9. Which of the following describes a connection between components of the basal ganglia?

A. The subthalamic nucleus releases glutamate to excite the globus pallidus, internal segment.

B. The substantia nigra pars reticulata releases dopamine to inhibit the

striatum.

C. The substantia nigra pars compacta releases dopamine to excite the globus pallidus, external segment.

D. The striatum releases acetylcholine to excite the substantia nigra pars reticulata.

E. The globus pallidus, external segment releases glutamate to excite the striatum.

10. A 60-year-old man with Parkinson disease, which was diagnosed 15 years ago, has been taking carbidopa and levodopa (Sinemet); until recently, he has been able to continue to work and help with routine jobs around the house. Now his tremor and rigidity interfere with these activities. His clinician has suggested that he undergo deep brain stimulation therapy. The therapeutic effect of L-dopa in patients with Parkinson disease eventually wears off because

A. antibodies to dopamine receptors develop.

B. inhibitory pathways grow into the basal ganglia from the frontal lobe.

C. there is an increase in circulating α-synuclein.

D. the normal action of nerve growth factor (NGF) is disrupted.

E. the dopaminergic neurons in the substantia nigra continue to degenerate.

11. An 8-year-old girl was brought to her pediatrician because her parents noted frequent episodes of gait unsteadiness and speech difficulties. Her mother was concerned because of a family history of Friedreich ataxia. Which of the following is a correct description of connections involving cerebellar neurons?

A. Basket cells release glutamate to activate Purkinje cells.

B. Climbing fiber inputs exert a strong excitatory effect on Purkinje cells, and mossy fiber inputs exert a strong inhibitory effect on Purkinje cells.

C. Granule cells release glutamate to excite basket cells and stellate cells.

D. The axons of Purkinje cells are the sole output of the cerebellar cortex, and they release glutamate to excite the deep cerebellar nuclei.

E. Golgi cells are inhibited by mossy fiber collaterals.

12. After falling down a flight of stairs, a young woman is found to have partial loss of voluntary movement on the right side of her body and loss of pain and temperature sensation on the left side below the midthoracic region. It is probable that she has a lesion

A. damaging the left half of the spinal cord in the lumbar region.

B. damaging the left half of the spinal cord in the upper thoracic region.

C. damaging sensory and motor pathways on the right side of the pons.

D. damaging the right half of the spinal cord in the upper thoracic region.

E. damaging the dorsal half of the spinal cord in the upper thoracic region.

13. At the age of 30, a male postal worker reported weakness in his right leg. Within a year the weakness had spread to his entire right side. A neurologic examination revealed flaccid paralysis, muscular atrophy, fasciculations, hypotonia, and hyporeflexia of muscles in the right arm and leg. Sensory and cognitive function tests were normal. Which of the following diagnosis is likely?

A. A large tumor in the left primary motor cortex

B. A cerebral infarct in the region of the corona radiate

C. A vestibulocerebellar tumor

D. Damage to the basal ganglia

E. Amyotrophic lateral sclerosis

CHAPTER 13

Autonomic Nervous System

OBJECTIVES

After studying this chapter, you should be able to:

- Identify the location of the cell bodies and axonal trajectories of preganglionic and postganglionic sympathetic and parasympathetic neurons.
- Identify the neurotransmitters and receptor types involved in neurotransmission within the autonomic nervous system and its target organs.
- Describe how various drugs alter neurotransmitter synthesis, storage, release, and reuptake and receptor activation and blockade within the autonomic nervous system.
- Describe the ways that the autonomic nervous system contributes to homeostasis.
- Compare the overall functions of the parasympathetic and sympathetic nervous systems.
- Compare and contrast the functions of sympathetic and parasympathetic nerves at targets where they act as functional antagonists, synergistically, and independently.
- Identify the location of forebrain and brainstem neurons and sensory

afferents that are involved in the control of the autonomic nervous system.

- Identify examples of autonomic dysfunction due to primary damage within the autonomic nervous system or as a consequence of other pathologies.
- Describe the composition and functions of the enteric nervous system.

INTRODUCTION

The autonomic nervous system (ANS) is comprised of the **sympathetic nervous system**, the **parasympathetic nervous system**, and the **enteric nervous system**. The ANS is sometimes called the involuntary nervous system because it carries out its functions without requiring a conscious effort. It influences a wide array of physiological processes via its innervation of smooth muscle, cardiac muscle, and pacemaker cells, exocrine and endocrine glands, adipose tissue, liver cells, and lymphatic tissue. In fact, skeletal muscle is the only innervated part of the body that is not under the control of the ANS. The ultimate responsibility of the ANS is to maintain **homeostasis** despite perturbations exerted by the external and internal environments. Although survival may be possible without the ANS, the ability to adapt to environmental stressors and other challenges is severely compromised. The ANS also plays a role in the body's response to an emotional experience. The importance of understanding the functions of the ANS is underscored by the fact that so many prescription and over-the-counter drugs exert their actions on elements of the ANS or its effector targets. Changes in autonomic activity contribute to many diseases (eg, hypertension, heart failure). Also, many neurologic disorders are associated with autonomic dysfunction **(Clinical Box 13–1)**.

CLINICAL BOX 13–1

Multiple System Atrophy & Shy–Drager Syndrome

Multiple system atrophy (MSA) is a neurodegenerative disorder associated with autonomic failure due to loss of preganglionic autonomic neurons in the spinal cord and brainstem. In the absence of an autonomic nervous system, it is difficult to regulate body temperature, fluid and electrolyte balance, and blood pressure. In addition to these autonomic abnormalities, MSA presents with cerebellar, basal ganglia, locus coeruleus, inferior olivary nucleus, and pyramidal tract deficits. MSA is defined as "a sporadic, progressive, adult-

onset disorder characterized by autonomic dysfunction, parkinsonism, and cerebellar ataxia in any combination." **Shy–Drager syndrome** is a subtype of MSA in which autonomic failure dominates. The pathologic hallmark of MSA is cytoplasmic and nuclear inclusions in oligodendrocytes and neurons in central motor and autonomic areas. There is also depletion of monoaminergic, cholinergic, and peptidergic markers in several brain regions and in the cerebrospinal fluid. The cause of MSA remains elusive, but there is some evidence that a neuroinflammatory mechanism causing activation of microglia and production of toxic cytokines may occur in brains of MSA patients. Basal levels of sympathetic activity and plasma norepinephrine levels are normal in MSA patients, but they fail to increase in response to standing or other stimuli and leads to severe **orthostatic hypotension.** In addition to the fall in blood pressure, orthostatic hypotension leads to dizziness, dimness of vision, and even fainting. MSA is also accompanied by parasympathetic dysfunction, including urinary and sexual dysfunction. MSA is most often diagnosed in individuals between 50 and 70 years of age; it affects more men than women. Erectile dysfunction is often the first symptom of the disease. There are also abnormalities in baroreceptor reflex and respiratory control mechanisms. Although autonomic abnormalities are often the first symptoms, 75% of patients with MSA also experience motor disturbances.

THERAPEUTIC HIGHLIGHTS

There is no cure for MSA but various therapies are used to treat specific signs and symptoms of the disease. **Corticosteroids** are often prescribed to retain salt and water to increase blood pressure. In some individuals, parkinsonian-like signs can be alleviated by administration of **levodopa** and **carbidopa (Sinemet).** Various clinical trials are underway to test the effectiveness of using intravenous **immunoglobulins** to counteract the neuroinflammatory process that occurs in MSA; **fluoxetine** (a **serotonin uptake inhibitor**) to prevent orthostatic hypotension, improve mood, and alleviate sleep, pain, and fatigue in MSA patients; and **rasagiline** (a **monoamine oxidase inhibitor**) in MSA patients with parkinsonism.

ANATOMIC ORGANIZATION OF AUTONOMIC

OUTFLOW

GENERAL FEATURES

Figure 13–1 compares some fundamental characteristics of the innervation to skeletal muscles with innervation to smooth muscle, cardiac muscle, and glands. As discussed in Chapter 12, the final common pathway linking the central nervous system (CNS) to skeletal muscles is the α-motor neuron. Similarly, sympathetic and parasympathetic neurons serve as the final common pathway from the CNS to visceral targets. However, in marked contrast to the somatomotor nervous system, the peripheral motor portions of the ANS are made up of two neurons: **preganglionic** and **postganglionic neurons.** The cell bodies of the preganglionic neurons are located in the intermediolateral (IML) column of the spinal cord and in motor nuclei of some cranial nerves. In contrast to the large diameter and rapidly conducting α-motor neurons, preganglionic axons are small-diameter, myelinated, relatively slowly conducting B fibers. The axons of the postganglionic neurons are mostly unmyelinated C fibers and terminate on the visceral effectors.

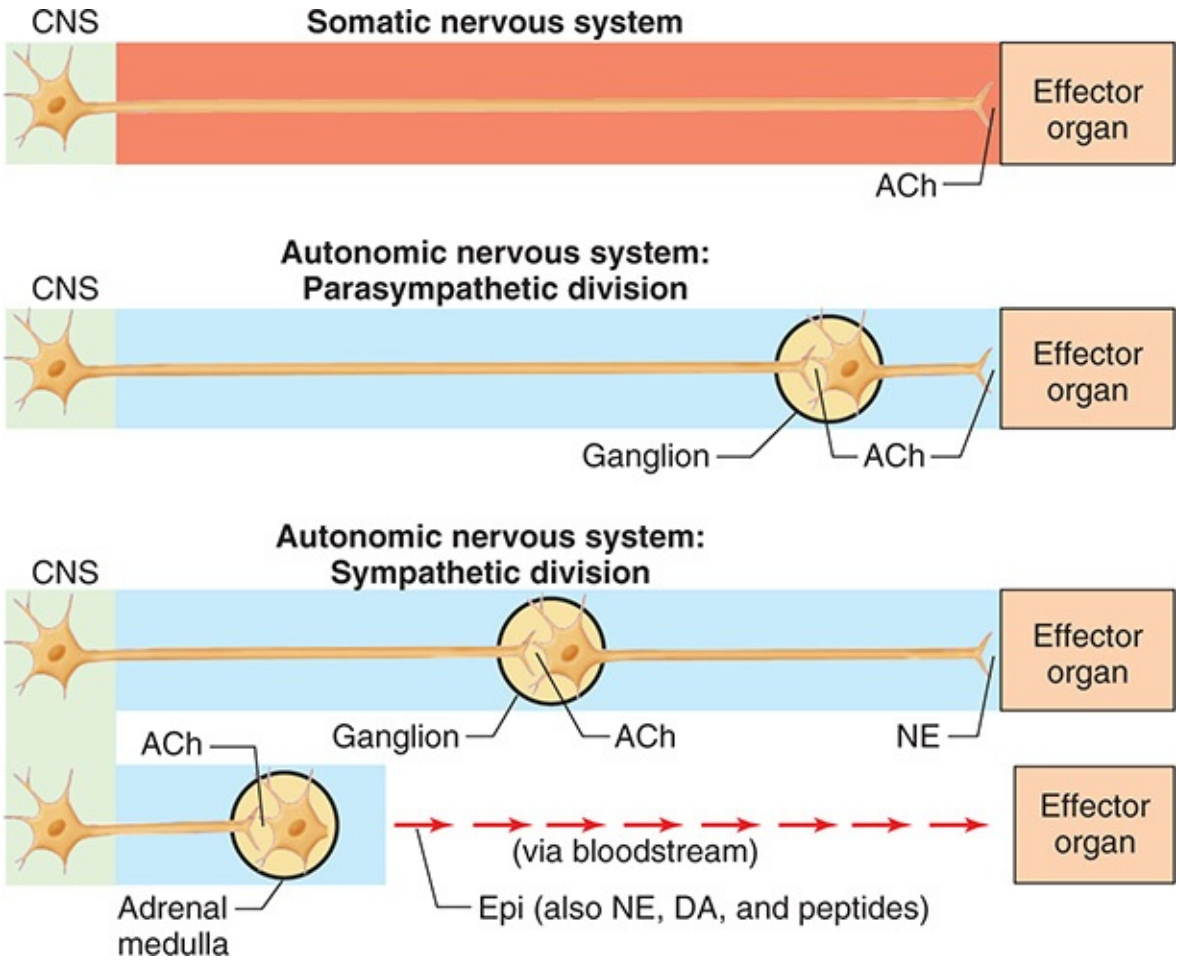


FIGURE 13–1 Comparison of peripheral organization and transmitters released by somatomotor and autonomic nervous systems. In the case of the somatomotor nervous system, the neuron that leaves the spinal cord projects directly to the effector organ. In the case of the autonomic nervous system, there is a synapse between the neuron that leaves the spinal cord and the effector organ (except for neurons that innervate the adrenal medulla). Note that all neurons that leave the central nervous system release acetylcholine (ACh). CNS, central nervous system; DA, dopamine; Epi, epinephrine; NE, norepinephrine. (Used with permission from Widmaier EP, Raff H, Strang KT: *Vander's Human Physiology*. New York, NY: McGraw-Hill; 2008.)

One similar feature of autonomic preganglionic neurons and α-motor neurons is that acetylcholine is released at their nerve terminals (Figure 13–1). This is the neurotransmitter released by all neurons whose axons exit the CNS, including cranial motor neurons, α-motor neurons, γ-motor neurons, preganglionic sympathetic neurons, and preganglionic parasympathetic neurons.

Postganglionic parasympathetic neurons also release acetylcholine; postganglionic sympathetic neurons release either norepinephrine or acetylcholine.

SYMPATHETIC DIVISION OF THE ANS

In contrast to α-motor neurons, which are located at all spinal segments, sympathetic preganglionic neurons are located in the IML of only the first thoracic to the third or fourth lumbar segments. Thus, the sympathetic nervous system is called the thoracolumbar division of the ANS. The axons of the sympathetic preganglionic neurons leave the spinal cord at the level at which their cell bodies are located and exit via the ventral root along with axons of α- and γ-motor neurons **(Figure 13–2)**. They then separate from the ventral root via the **white rami communicans** and project to the adjacent **sympathetic paravertebral ganglion,** where some of them end on the cell bodies of the postganglionic neurons. Paravertebral ganglia are located adjacent to each thoracic and upper lumbar spinal segment; in addition, there are a few ganglia adjacent to the cervical and sacral spinal segments. The ganglia are connected to each other via the axons of preganglionic neurons that travel rostrally or caudally to terminate on postganglionic neurons located at some distance. Together these ganglia and axons form the **sympathetic chain** bilaterally. This arrangement is seen in Figure 13–2 and **Figure 13–3**. Note that, despite the fact that there are no cervical or sacral sympathetic preganglionic neurons, the sympathetic chain includes cervical ganglia that provide innervation to structures in the head (eg, eye and salivary glands) and sacral ganglia that provide innervation of pelvic organs.

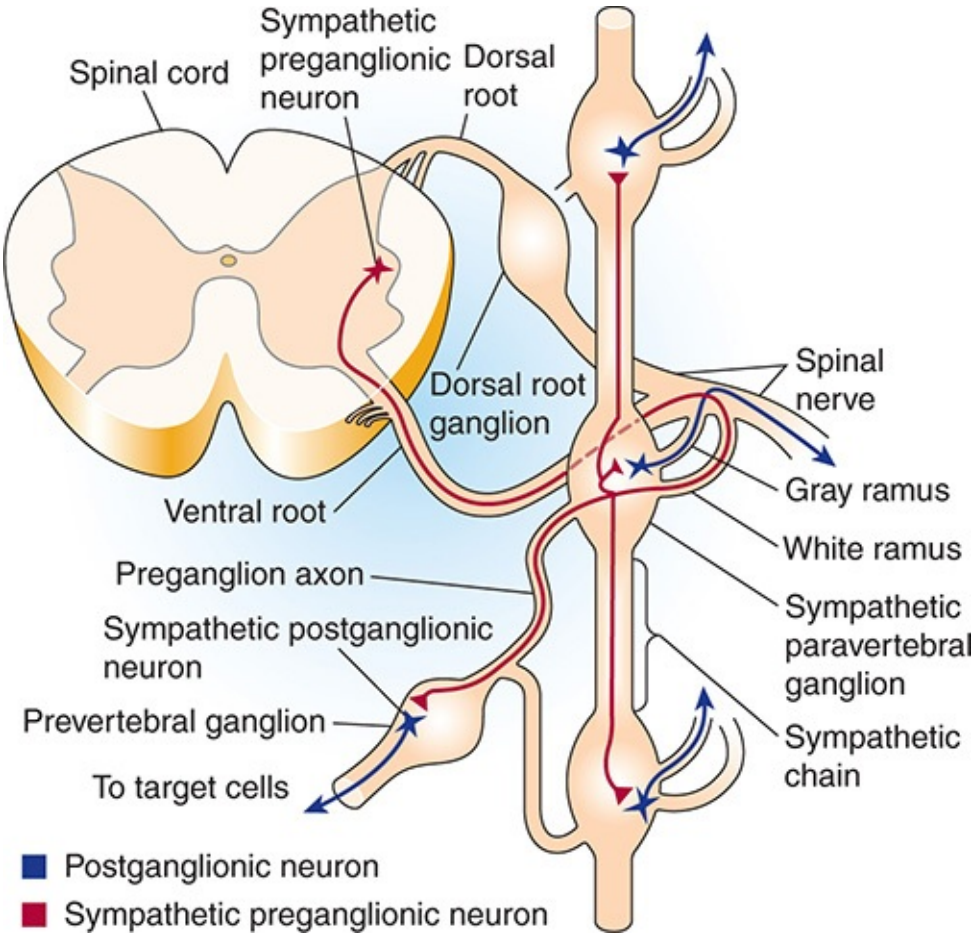


FIGURE 13–2 Projection of sympathetic preganglionic and postganglionic fibers. The drawing shows the thoracic spinal cord, paravertebral, and prevertebral ganglia. Preganglionic neurons are shown in red, and postganglionic neurons in dark blue.

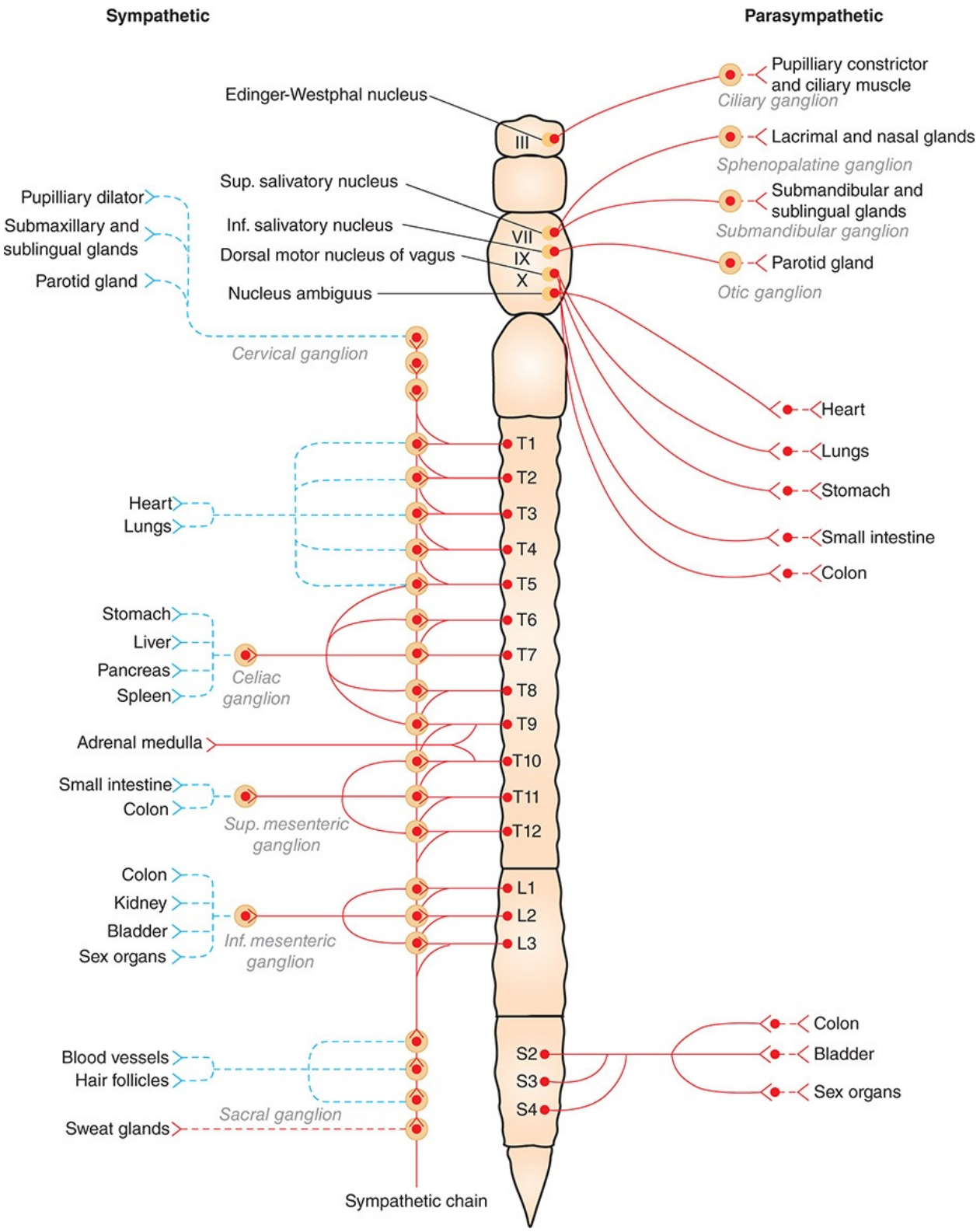


FIGURE 13–3 Organization of sympathetic (left) and parasympathetic (right) nervous systems. Cholinergic nerves are shown in red and noradrenergic

nerves are shown in blue. Preganglionic nerves are solid lines; postganglionic nerves are dashed lines.

Some preganglionic neurons pass through the paravertebral ganglion chain and end on postganglionic neurons located in **prevertebral** (or **collateral**) **ganglia** close to the viscera, including the celiac, superior mesenteric, and inferior mesenteric ganglia (Figure 13–3). There are also preganglionic neurons whose axons terminate directly on the effector organ, the adrenal gland.

The axons of some of the postganglionic neurons leave the chain ganglia and reenter the spinal nerves via the **gray rami communicans** and are distributed to autonomic effectors in the areas supplied by these spinal nerves (Figure 13–2). These postganglionic sympathetic nerves terminate mainly on smooth muscle (eg, blood vessels and hair follicles) and on sweat glands in the limbs. Other postganglionic fibers leave the chain ganglia to enter the thoracic cavity to terminate in visceral organs. Postganglionic fibers from prevertebral ganglia also terminate in visceral targets.

PARASYMPATHETIC DIVISION OF THE ANS

The parasympathetic nervous system is called the **craniosacral division** of the ANS because of the location of its preganglionic neurons within several cranial nerve nuclei (III, VII, IX, and X) and the IML of the sacral spinal cord (Figure 13–3). The cell bodies in the **Edinger-Westphal nucleus** of the oculomotor nerve project to the **ciliary ganglia** to innervate the sphincter (constrictor) muscle of the iris and the ciliary muscle. Neurons in the **superior salivatory nucleus** of the facial nerve project to the **sphenopalatine ganglia** to innervate the lacrimal glands and nasal and palatine mucous membranes and to the **submandibular ganglia** to innervate the submandibular (also called submaxillary) and sublingual glands. The cell bodies in the **inferior salivatory nucleus** of the glossopharyngeal nerve project to the **otic ganglion** to innervate the parotid salivary gland. Vagal preganglionic fibers synapse on ganglia cells clustered within the walls of visceral organs; thus, these parasympathetic postganglionic fibers are very short. Neurons in the **nucleus ambiguus** innervate the sinoatrial (SA) and atrioventricular (AV) nodes in the heart; and neurons in the **dorsal motor vagal nucleus** innervate the esophagus, trachea, lungs, and gastrointestinal tract. The parasympathetic sacral outflow **(pelvic nerve)** supplies the pelvic viscera via branches of the second to fourth sacral spinal nerves.

CHEMICAL TRANSMISSION AT AUTONOMIC JUNCTIONS

ACETYLCHOLINE & NOREPINEPHRINE

Acetylcholine and **norepinephrine** are the principal neurotransmitters synthesized and released by autonomic neurons. The cholinergic autonomic neurons (ie, release acetylcholine) are (1) all preganglionic neurons, (2) all parasympathetic postganglionic neurons, (3) sympathetic postganglionic neurons that innervate sweat glands, and (4) sympathetic postganglionic neurons that end on blood vessels in some skeletal muscles and produce vasodilation when stimulated (sympathetic vasodilator nerves). The remaining sympathetic postganglionic neurons are **noradrenergic** (ie, release norepinephrine). The adrenal medulla is essentially a sympathetic ganglion in which the postganglionic cells have lost their axons and secrete both norepinephrine and epinephrine directly into the bloodstream.

Table 13–1 shows the types of cholinergic and adrenergic receptors at various junctions within the ANS. The junctions in the peripheral autonomic motor pathways are logical sites for pharmacologic manipulation of visceral function. The neurotransmitters are synthesized, stored in the nerve endings, and released near the neurons, muscle cells, or gland cells where they bind to various ion channels or G-protein-coupled receptors (GPCR) to initiate their characteristic actions. The neurotransmitters are then removed from the area by reuptake or metabolism. Each of these steps can be stimulated or inhibited, with predictable consequences. **Table 13–2** lists how various drugs can affect neurotransmission in autonomic neurons and effector sites.

TABLE 13–1 Responses of some effector organs to autonomic nerve activity.

Effector Organs	Parasympathetic Nervous System	Sympathetic Nervous System	
		Receptor Type	Response
Eyes			
Radial muscle of iris	—	α_1	Contraction (mydriasis)
Sphincter muscle of iris	Contraction (miosis)		—
Ciliary muscle	Contraction for near vision		—
Heart			
SA node	Decreased heart rate	β_1	Increased heart rate
Atria and ventricle	Decreased atrial contractility	β_1, β_2	Increased contractility
AV node and Purkinje fibers	Decreased conduction velocity	β_1	Increased conduction velocity
Arterioles			
Skin, splanchnic vessels	—	α_1	Constriction
Skeletal muscle	—	α_1/ β_2, M	Constriction/Dilation
Systemic veins	—	α_1, α_2/ β_2	Constriction/Dilation
Bronchial smooth muscle	Contraction	β_2	Relaxation
Stomach and Intestine			
Motility and tone	Increased	α_1, α_2, β_2	Decreased
Sphincters	Relaxation	α_1	Contraction
Secretion	Stimulation	—	
Gallbladder	Contraction	β_2	Relaxation
Urinary bladder			
Detrusor	Contraction	β_2	Relaxation
Sphincter	Relaxation	α_1	Contraction
Uterus (pregnant)	—	α_1/ β_2	Contraction/Relaxation
Male sex organs	Erection	α_1	Ejaculation
Skin			
Pilomotor muscles	—	α_1	Contraction
Sweat glands	—	M	Secretion
Liver	—	α_1, β_2	Glycogenolysis
Pancreas			
Acini	Increased secretion	α	Decreased secretion
Islet cells	—	α_2/β_2	Decreased/Increased secretion
Salivary glands	Profuse, watery secretion	α_1/β	Thick, viscous secretion/Amylase secretion
Lacrimal glands	Secretion		—
Adipose tissue	—	β_3	Lipolysis

A dash means the target tissue is not innervated by this division of the autonomic nervous system. AV, atrioventricular; SA, sinoatrial. Modified with permission from Brunton LL, Chabner BA, Knollmann BC (eds): *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 12th ed. New York, NY: McGraw-Hill; 2011.

TABLE 13–2 Examples of drugs that affect processes involved in autonomic neurotransmission.

Transmission Process	Drug	Site of Drug Action	Drug Action
Neurotransmitter synthesis	Hemicholinium	Membrane of cholinergic nerve terminals	Blocks choline uptake; slows synthesis
	Metyrosine	Cytoplasm of noradrenergic nerve terminals	Inhibits tyrosine hydroxylase; blocks synthesis
Neurotransmitter storage mechanism	Vesamicol	Vesicles in cholinergic nerve terminals	Prevents storage of acetylcholine
	Reserpine	Vesicles in noradrenergic nerve terminals	Prevents storage of norepinephrine
Neurotransmitter release mechanism	Norepinephrine, dopamine, acetylcholine, prostaglandins	Receptors on cholinergic and adrenergic nerve terminals	Modulates transmitter release
Neurotransmitter reuptake mechanism	Cocaine, tricyclic antidepressants	Adrenergic nerve terminals	Inhibits uptake; prolongs transmitter's action on postsynaptic receptors
Inactivation of neurotransmitter	Edrophonium, neostigmine, physostigmine	Acetylcholinesterase in cholinergic synapses	Inhibits enzyme; prolongs and intensifies actions of acetylcholine
Adrenoceptor activation	α_1: Phenylephrine α_2: Clonidine	Sympathetic postganglionic nerve-effector organ junctions (eg, blood vessels, hair follicles, and radial muscle)	Binds to and activates α-adrenoceptors; ↑ IP_3/DAG cascade (α_1) or ↓ cAMP (α_2)
	β_1: Dobutamine β_2: Albuterol, ritodrine, salmeterol, terbutaline	Sympathetic postganglionic nerve-effector organ junctions (eg, heart, bronchial smooth muscle, and uterine smooth muscle)	Binds to and activates β-adrenoceptors; ↑ cAMP
Adrenoceptor blockade	Nonselective: Phenoxybenzamine α_1: Prazosin, terazosin α_2: Yohimbine	Sympathetic postganglionic nerve-effector organ junctions (eg, blood vessels)	Binds to and blocks α-adrenoceptors
	β_1, β_2: Propranolol $\beta_1 > \beta_2$: Atenolol, esmolol	Sympathetic postganglionic nerve-effector organ junctions (eg, heart and bronchial smooth muscle)	Binds to and blocks β-adrenoceptors
Nicotinic receptor activation	Nicotine	Receptors on autonomic ganglia	Binds to nicotinic receptors; opens Na^+, K^+ channels in postsynaptic membrane
Nicotinic receptor blockade	Hexamethonium, trimethaphan	Receptors on autonomic ganglia	Binds to and blocks nicotinic receptors
Muscarinic receptor activation	Bethanecol	Cholinergic receptors on smooth muscle, cardiac muscle, and glands	Binds to and activates muscarinic receptors; ↑ IP_3/DAG cascade or ↓ cAMP
Muscarinic receptor blockade	Atropine, ipratropium, scopolamine, tropicamide	Cholinergic receptors on smooth muscle, cardiac muscle, and glands	Binds to and blocks muscarinic receptors

Many of these drugs can also act on cholinergic and adrenergic receptors in the central nervous system. For example, the main actions of clonidine and yohimbine to alter blood pressure are by their actions in the brain. cAMP, cyclic adenosine monophosphate; IP_3/DAG, inositol 1,4,5-triphosphate, and diacylglycerol.

CHOLINERGIC NEUROTRANSMISSION

The processes involved in the synthesis and breakdown of acetylcholine were described in Chapter 7. Acetylcholine does not usually circulate in the blood, and the effects of localized cholinergic discharge are generally discrete and of short duration because of the high concentration of acetylcholinesterase at cholinergic nerve endings. This enzyme rapidly breaks down the acetylcholine, terminating its actions.

Transmission in autonomic ganglia is mediated primarily by the actions of acetylcholine on nicotinic cholinergic receptors that are blocked by

hexamethonium **(Figure 13–4)**. These are called N_N receptors to distinguish them from the nicotinic cholinergic receptors (N_M) that are located at the neuromuscular junction and are blocked by D-tubocurarine (curare). Nicotinic receptors are ligand-gated ion channels; binding of an agonist to these receptors opens N^+ and K^+ channels to cause depolarization.

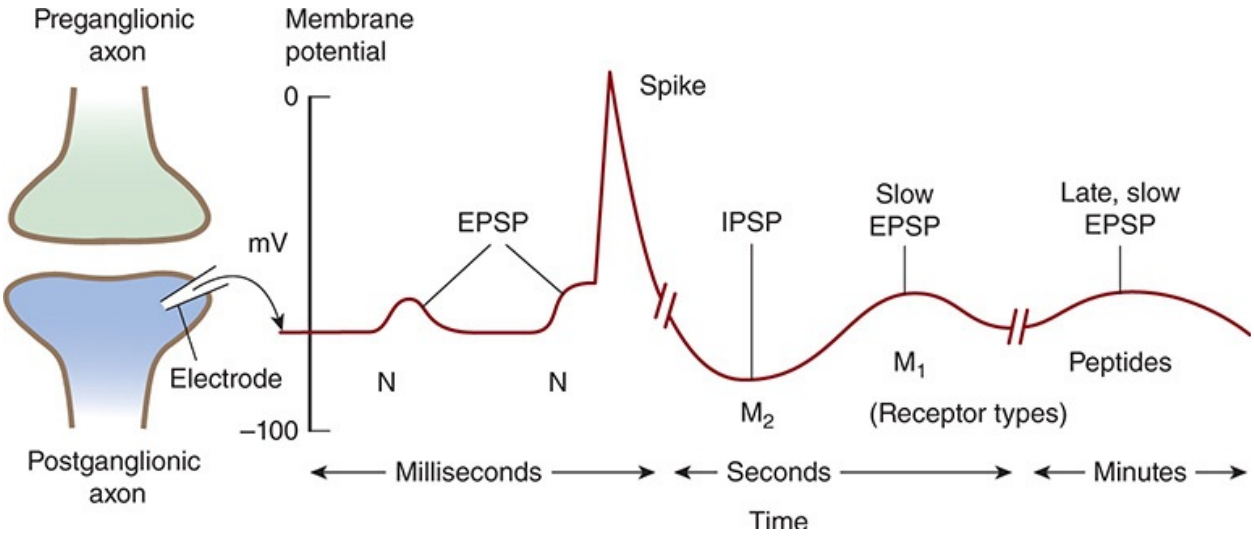


FIGURE 13–4 Schematic of excitatory and inhibitory postsynaptic potentials (EPSP and IPSP) recorded via an electrode in an autonomic ganglion cell. In response to acetylcholine release from the preganglionic neuron, two EPSPs were generated in the postganglionic neuron due to nicotinic (N) receptor activation. The first EPSP was below the threshold for eliciting an action potential, but the second EPSP was suprathreshold and evoked an action potential. This was followed by an IPSP, probably evoked by muscarinic (M_2) receptor activation. The IPSP is then followed by a slower, M_1-dependent EPSP, and this can be followed by an even slower peptide-induced EPSP. (Used with permission from Katzung BG, Maters SB, Trevor AJ: *Basic & Clinical Pharmacology*, 11th ed. New York, NY: McGraw-Hill; 2009.)

The responses produced in postganglionic neurons by stimulation of their preganglionic innervation include both a **fast excitatory postsynaptic potential (EPSP)** that generates action potentials and a prolonged excitatory postsynaptic potential **(slow EPSP).** The slow response may modulate and regulate transmission through the sympathetic ganglia. The initial depolarization is produced by acetylcholine acting on the N_N receptor. The slow EPSP is produced by acetylcholine acting on a muscarinic receptor on the membrane of

the postganglionic neuron.

The release of acetylcholine from postganglionic fibers acts on muscarinic cholinergic receptors that are blocked by atropine. Muscarinic receptors are GPCR and are divided into subtypes M_1–M_5; M_2 and M_3 are the main subtypes found in autonomic target organs. M_2 receptors are found in the heart; binding of an agonist to these receptors opens K^+ channels and inhibits **adenylyl cyclase.** M_3 receptors are located on smooth muscle and glands; binding of an agonist to these receptors leads to the formation of **inositol 1,4,5-triphosphate (IP_3)** and **diacylglycerol (DAG)** and an increase in intracellular Ca^{2+}.

Compounds with muscarinic actions include congeners of acetylcholine and drugs that inhibit acetylcholinesterase. **Clinical Box 13–2** describes some of the signs and therapeutic strategies for the treatment of acute intoxication from **organophosphate cholinesterase inhibitors.** **Clinical Box 13–3** describes an example of **cholinergic poisoning** due to digestion of toxic mushrooms.

NORADRENERGIC NEUROTRANSMISSION

The processes involved in the synthesis, reuptake, and breakdown of norepinephrine were described in Chapter 7. Norepinephrine spreads farther and has a more prolonged action than acetylcholine. Norepinephrine, epinephrine, and dopamine are all found in plasma. The epinephrine and some of the dopamine come from the adrenal medulla, but most of the norepinephrine diffuses into the bloodstream from sympathetic nerve endings. Metabolites of norepinephrine and dopamine also enter the circulation.

The norepinephrine released from sympathetic postganglionic fibers binds to adrenoceptors. These are GPCR and are divided into several subtypes: α_1, α_2, β_1, β_2, and β_3. Table 13–1 shows the locations of these receptor subtypes on smooth muscles, cardiac muscle, and glands. Binding of an agonist to α_1-adrenoceptors activates the G_q-coupling protein that leads to the formation of IP_3 and DAG and an increase in intracellular Ca^{2+}. Binding of an agonist to α_2-adrenoceptors causes dissociation of the inhibitory G-protein G_i to inhibit adenylyl cyclase and decrease **cyclic adenosine monophosphate (cAMP).** Binding of an agonist to β-adrenoceptors activates the G_s-coupling protein to activate adenylyl cyclase and increase cAMP.

There are several diseases or syndromes that result from dysfunction of sympathetic innervation of specific body regions. **Clinical Box 13–4** describes

Horner syndrome that is due to interruption of sympathetic nerves to the face. Clinical Box 13–5 describes a vasospastic condition **(Raynaud phenomenon)** in which blood flow to the fingers and toes is transiently reduced, typically when a sensitive individual is exposed to stress or cold.

CLINICAL BOX 13–2

Organophosphates: Pesticides and Nerve Gases

The World Health Organization estimates that 1–3% of agricultural workers worldwide suffer from **acute pesticide poisoning;** it accounts for significant morbidity and mortality, especially in developing countries. Like **organophosphate** pesticides (eg, **parathion and malathion**), **nerve gases** (eg, **soman** and **sarin**) used in chemical warfare and terrorism inhibit acetylcholinesterase at peripheral and central cholinergic synapses, prolonging the actions of acetylcholine at these synapses. The organophosphate **cholinesterase inhibitors** are readily absorbed by the skin, lung, gut, and conjunctiva, making them very dangerous. They bind to the enzyme and undergo hydrolysis, resulting in a phosphorylated active site on the enzyme. The covalent phosphorous-enzyme bond is very stable and hydrolyzes at a very slow rate. The phosphorylated enzyme complex undergoes a process called **aging** in which one of the oxygen-phosphorous bonds breaks down, which strengthens the phosphorous-enzyme bond. This process occurs within 10 min of exposure to soman. The earliest signs of organophosphate toxicity are indicative of excessive activation of autonomic muscarinic receptors; these include miosis, salivation, sweating, bronchial constriction, vomiting, and diarrhea. CNS signs of toxicity include cognitive disturbances, convulsions, seizures, and even coma; these signs are often accompanied by nicotinic effects such as depolarizing neuromuscular blockade.

THERAPEUTIC HIGHLIGHTS

The muscarinic cholinergic receptor antagonist **atropine** can be given parenterally to control signs of excessive activation of muscarinic cholinergic receptors. Nucleophiles (eg, **pralidoxime)** can break the bond between the organophosphate and the acetylcholinesterase if given soon after exposure to the organophosphate and before aging has occurred. Thus, this drug is called a "cholinesterase regenerator." If **pyridostigmine** is administered in advance of

exposure to a cholinesterase inhibitor, it binds to the enzyme and prevents binding by the toxic organophosphate agent. The protective effects of pyridostigmine dissipate within 3–6 h, but this provides enough time for clearance of the organophosphate from the body. Since the drug cannot cross the blood-brain barrier, protection is limited to peripheral cholinergic synapses. A mixture of pyridostigmine, **carbamate,** and atropine can be administered prophylactically to soldiers and civilians who are at risk for exposure to nerve gases. **Benzodiazepines** can be used to abort the seizures caused by exposure to organophosphates.

CLINICAL BOX 13–3

Mushroom Poisoning

Of more than 5000 species of mushrooms found in the United States, approximately 100 are poisonous and ingestion of about 12 of these can result in death. Estimates are an annual incidence of five cases per 100,000 individuals in the United States. Mushroom poisoning or **mycetism** is divided into rapid-onset (15–30 min after ingestion) and delayed-onset (6–12 h after ingestion) types. In rapid-onset cases caused by mushrooms of the *Inocybe* genus, the symptoms are due to excessive activation of muscarinic cholinergic synapses. The major signs of **muscarinic poisoning** include nausea, vomiting, diarrhea, urinary urgency, vasodilation, sweating, and salivation. Ingestion of mushrooms such as the *Amanita muscaria* exhibit signs of the **antimuscarinic syndrome** rather than muscarinic poisoning because they also contain alkaloids that block muscarinic cholinergic receptors. The classic symptoms of this syndrome are being “red as a beet” (flushed skin), “hot as a hare” (hyperthermia), “dry as a bone” (dry mucous membranes, no sweating), “blind as a bat” (blurred vision and cycloplegia), and “mad as a hatter” (confusion and delirium). The delayed-onset type of mushroom poisoning occurs after ingestion of *Amanita phalloides, Amanita virosa, Galerina autumnalis,* and *Galerina marginata*. These mushrooms cause abdominal cramping, nausea, vomiting, and profuse diarrhea; but the major toxic effects are due to hepatic injury (jaundice and bruising) and associated central effects (confusion, lethargy, and coma). These mushrooms contain **amatoxins** that inhibit RNA polymerase. There is a 60% mortality rate associated with ingestion of these mushrooms.

THERAPEUTIC HIGHLIGHTS

The rapid-onset type muscarinic poisoning can be treated effectively with atropine. Individuals who exhibit the antimuscarinic syndrome can be treated with **physostigmine,** a cholinesterase inhibitor with a 2–4 h duration of action that acts centrally and peripherally. If agitated, these individuals may require sedation with a benzodiazepine or an **antipsychotic agent.** The delayed-onset of toxicity due to ingestion of mushrooms containing amatoxins does not respond to cholinergic drugs. Treatment of amatoxin ingestion includes intravenous administration of fluids and electrolytes to maintain adequate hydration. Administering a combination of a high-dose of **penicillin G** and **silibinin** (a **flavonolignan** found in certain herbs with antioxidant and hepatoprotective properties) can improve survival. If necessary, vomiting can be induced by using activated charcoal to reduce the absorption of the toxin.

CLINICAL BOX 13–4

Horner Syndrome

Horner syndrome is a rare disorder resulting from interruption of preganglionic or postganglionic sympathetic innervation to the face. The problem can result from injury to the nerves, injury to the carotid artery, a stroke or lesion in the brainstem, or a tumor in the lung. In most cases the problem is unilateral, with symptoms occurring only on the side of the damage. The hallmark of Horner syndrome is the triad of **anhidrosis** (reduced sweating), **ptosis** (drooping eyelid), and **miosis** (constricted pupil). Symptoms also include **enophthalmos** (sunken eyeball) and vasodilation.

THERAPEUTIC HIGHLIGHTS

There is no specific pharmacologic treatment for Horner syndrome, but drugs affecting noradrenergic neurotransmission can be used to determine whether the source of the problem is interruption of the preganglionic or postganglionic innervation to the face. Since the iris of the eye responds to topical **sympathomimetic drugs** (ie, direct agonists on adrenoceptors or drugs that increase the release or prevent reuptake of norepinephrine from the nerve terminal), one can easily test the viability of the noradrenergic nerves to the eye.

If the postganglionic sympathetic fibers are damaged, their terminals would degenerate and there would be a loss of stored catecholamines. If the preganglionic fibers are damaged, the postganglionic noradrenergic nerve would remain intact (but be inactive) and would still have stored catecholamines in its terminal. If a drug that causes release of catecholamine stores (eg, **hydroxyamphetamine**) is administered and the constricted pupil does not dilate, one would conclude that the noradrenergic nerve is damaged. If the eye dilates in response to this drug, the catecholamine stores are still able to be released, so the damage must be preganglionic. Administration of **phenylephrine** (α-adrenoceptor agonist) would dilate the pupil regardless of the site of injury as the drug binds to the receptor on the radial muscle of the iris.

CLINICAL BOX 13–5

Raynaud Phenomenon

Approximately 5% of men and 8% of women experience an episodic reduction in blood flow primarily to the fingers, often during exposure to cold or during a stressful situation. Vasospasms in the toes, tip of nose, ears, and penis can also occur. Smoking is associated with an increase in the incidence and severity of the symptoms of Raynaud phenomenon. The symptoms begin to occur between the age of 15 and 25; it is most common in cold climates. The symptoms often include a triphasic change in color of the skin of the digits. Initially, the skin becomes pale or white **(pallor),** cold, and numb. This can be followed by a **cyanotic** period in which the skin turns blue or even purple, during which time the reduced blood flow can cause intense pain. Once the blood flow recovers, the digits often turn deep red **(rubor)** and there can be swelling and tingling. Primary Raynaud phenomenon or **Raynaud disease** refers to the idiopathic appearance of the symptoms in individuals who do not have another underlying disease to account for the symptoms. In such cases, the vasospastic attacks may merely be an exaggeration of a normal response to cold temperature or stress. Secondary Raynaud phenomenon or **Raynaud syndrome** refers to the presence of these symptoms due to another disorder such as **scleroderma, lupus, rheumatoid arthritis, Sjögren syndrome, carpel tunnel syndrome,** and **anorexia.** Although initially thought to reflect an increase in sympathetic activity to the

vasculature of the digits, this is no longer regarded as the mechanism underlying the episodic vasospasms.

THERAPEUTIC HIGHLIGHTS

The first treatment strategy for Raynaud phenomenon is to avoid exposure to the cold, reduce stress, quit smoking, and avoid the use of medications that are vasoconstrictors (eg, β-adrenoceptor antagonists, cold medications, caffeine, and opioids). If the symptoms are severe, drugs may be needed to prevent tissue damage. These include **calcium channel blockers** (eg, **nifedipine**) and **α-adrenoceptor antagonists** (eg, **prazosin**). In individuals who do not respond to pharmacologic treatments, surgical sympathectomy has been done.

NONADRENERGIC & NONCHOLINERGIC TRANSMITTERS

In addition to the "classical neurotransmitters," some autonomic fibers also release neuropeptides. The small granulated vesicles in postganglionic sympathetic neurons contain **adenosine triphosphate (ATP)** and norepinephrine, and the large granulated vesicles contain **neuropeptide Y (NPY).** Low-frequency activation of these nerves promotes release of ATP, and high-frequency stimulation causes release of NPY. Some visceral organs contain purinergic receptors, and ATP is a mediator along with norepinephrine in some autonomic targets.

Many sympathetic fibers innervating the vasculature of viscera, skin, and skeletal muscles release NPY and **galanin** in addition to norepinephrine. **Vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP),** or **substance P** are co-released with acetylcholine by sympathetic nerves to sweat glands **(sudomotor fibers).** VIP is co-localized with acetylcholine in many cranial parasympathetic postganglionic neurons supplying glands. Vagal parasympathetic postganglionic neurons in the gastrointestinal tract contain VIP and the enzymatic machinery to synthesize **nitric oxide (NO).**

RESPONSES OF EFFECTOR ORGANS TO AUTONOMIC NERVE IMPULSES

HOMEOSTASIS

Homeostasis refers to the ability to maintain a stable internal environment despite challenges imposed by shifts in things such as air temperature, atmospheric and blood oxygen and carbon dioxide levels, physical activity, exposure to toxins, disease, drug therapies, fever, and diet. With the wide distribution of autonomic nerves throughout the body, it is not surprising that the ANS works in concert with the endocrine system (see Chapter 16) to maintain homeostatic variables within a physiological range. The ANS plays a fundamental role in the regulation and coordination of many physiologic functions that are critical for homeostasis. These include airflow through the bronchial tree, blood flow, blood gas composition, blood glucose, blood pressure, body temperature, digestion, electrolyte balance, glandular secretions, heart rate, and urination. The failure to maintain homeostasis **(homeostatic imbalance)** can lead to death or a disease. Diseases such as type I diabetes, dehydration, hyperthermia, or hypothermia, heart failure, and hypertension are examples of the consequence of homeostatic imbalance and loss of negative feedback mechanisms to return physiological parameters to normal levels. **Clinical Box 13–6** describes examples of the deleterious consequences of homeostatic imbalance.

CLINICAL BOX 13–6

Homeostatic Imbalance

A failure to maintain homeostasis can cause major dysfunction of organs under the control of the ANS. If we lose our ability to regulate body temperature, one can experience hyperthermia or hypothermia. Failure to maintain energy balance can lead to the development of obesity or diabetes. If your kidney is no longer able to regulate salt and water balance, the body will retain water, salts and metabolic wastes that has many dire consequences (eg, cardiac arrhythmias, hypertension, anemia, and neurological complications). For many physiological systems, the maintenance of homeostasis involves having a sensor (eg, thermoreceptor) to detect the deviation from normal value and a negative feedback mechanism to return the physiological measure (eg, body temperature) back to a normal level (eg, 98.6°F/37°C, normothermia). The body has a complex mechanism to maintain body temperature within a thermal neutral zone of 36–37°C; this is the temperature

range between the low point at which shivering begins and the high point at which sweating begins. When body temperature is elevated by an exposure to high environmental temperatures or physical exertion, thermoreceptors in the skin (see Chapter 8) and the hypothalamus are activated. These receptors send signals to a central control area in the **medial preoptic and anterior hypothalamic nuclei.** Activation of these neurons engages the effector autonomic pathways that promote sweating (activation of sudomotor sympathetic nerves) and cutaneous vasodilation (activation of skin sympathetic nerves). The combination of sweating and cutaneous vasodilation allow for heat dissipation and a return to normothermia. This, in turn, would silence the thermoreceptors and reduce activity emanating from the hypothalamus. If one loses the ability to dissipate heat, hyperthermia or **heat stroke** can ensue. Heat stroke is considered a medical emergency and leads to many indices of homeostatic imbalance: tachycardia, rapid shallow breathing, dizziness, anhidrosis, dry and hot skin, muscle cramping, confusion, and seizures. See Chapter 17 for more details on the mechanisms involved in temperature regulation. Other conditions that can cause anhidrosis and thus the loss of a key homeostatic mechanism to control body temperature include diabetic neuropathy, Parkinson disease, MSA, Sjögren syndrome, Horner syndrome, and alcoholism.

PARASYMPATHETIC AND SYMPATHETIC NERVOUS SYSTEM: PHYSIOLOGICAL ANTAGONISTS, SYNERGISTIC FUNCTIONS, OR INDEPENDENT ACTIONS

The effects of stimulation of the noradrenergic and cholinergic postganglionic nerve fibers are listed in Table 13–1, and they point to another difference between the ANS and the somatomotor nervous system. The release of acetylcholine by α-motor neurons only leads to contraction of skeletal muscles. In contrast, release of acetylcholine onto smooth muscle of some organs leads to contraction (eg, walls of the gastrointestinal tract) while release onto other organs promotes relaxation (eg, sphincters in the gastrointestinal tract). The only way to relax a skeletal muscle is to inhibit the discharges of the α-motor neurons; but for some targets innervated by the ANS, contraction can be shifted to relaxation by switching from activation of the parasympathetic nervous system

to activation of the sympathetic nervous system. This is the case for the many organs that receive dual innervation with antagonistic effects, including the heart, airways, digestive tract, and urinary bladder. For example, stimulation of sympathetic nerves increases heart rate, and stimulation of parasympathetic nerves decreases heart rate.

In other cases, the effects of sympathetic and parasympathetic activation can be considered complementary. An example is the innervation of salivary glands. Parasympathetic activation causes release of watery saliva, while sympathetic activation causes the production of thick, viscous saliva.

The two divisions of the ANS can also act in a synergistic or cooperative manner in the control of some functions. One example is the control of pupil diameter in the eye. Both sympathetic and parasympathetic innervations are excitatory, but the former contracts the radial (or dilator) muscle to cause mydriasis (widening of the pupil) and the latter contracts the sphincter (or constrictor) muscle to cause miosis (narrowing of the pupil). Another example is the synergistic actions of these nerves on sexual function. Activation of parasympathetic nerves to the penis increases penile blood flow and leads to erection while activation of sympathetic nerves to the male reproductive tract causes ejaculation.

There are also several organs that are innervated by only one division of the ANS. In addition to the adrenal gland, most blood vessels, the pilomotor muscles in the skin (hair follicles), and sweat glands are innervated exclusively by sympathetic nerves (sudomotor fibers). The lacrimal muscle (tear gland), ciliary muscle (for accommodation for near vision; see Chapter 10), and the nasopharyngeal gland are innervated exclusively by parasympathetic nerves.

The functions promoted by activity in the parasympathetic nervous system are those concerned with the vegetative aspects of day-to-day living. Parasympathetic action favors digestion and absorption of food by increasing the activity of the intestinal musculature, increasing gastric secretion, and relaxing the pyloric sphincter. For this reason, it is sometimes called the “rest and digest” division of the ANS.

The sympathetic division discharges as a unit in emergency situations and can be called the catabolic nervous system or the “flight or fight” division of the ANS. The effect of this discharge prepares the individual to cope with an emergency. Sympathetic activity dilates the pupils (letting more light into the eyes), accelerates the heartbeat and raises the blood pressure (providing better perfusion of the vital organs and muscles), and constricts the blood vessels of the skin (which limits bleeding from wounds). Noradrenergic discharge also leads to

elevated plasma glucose and free fatty acid levels (supplying more energy). On the basis of effects like these, Walter Cannon called the emergency-induced discharge of the sympathetic nervous system the “preparation for flight or fight.”

The emphasis on mass discharge in stressful situations should not obscure the fact that the sympathetic fibers also subserve other functions and, in fact, there is activity in sympathetic nerves even under resting conditions. For example, tonic sympathetic discharge to the arterioles maintains arterial pressure, and variations in this tonic discharge are the mechanism by which carotid sinus feedback regulation of blood pressure occurs (see Chapter 32). In addition, sympathetic discharge is decreased in fasting animals and increased when fasted animals are again fed. These changes may explain the decrease in blood pressure and metabolic rate produced by fasting and the opposite changes produced by feeding.

DESCENDING INPUTS TO AUTONOMIC PREGANGLIONIC NEURONS

As is the case for α-motor neurons, the activity of autonomic nerves is dependent on both reflexes (eg, baroreceptor and chemoreceptor reflexes) and a balance between descending excitatory and inhibitory inputs from several brain regions. Chapter 32 describes the roles of baroreceptor and chemoreceptor reflexes and medullary neurons in maintaining homeostasis within the cardiovascular system. **Figure 13–5** shows the source of some forebrain and brainstem inputs to sympathetic preganglionic neurons. There are parallel pathways from the hypothalamic paraventricular nucleus, pontine catecholaminergic A5 cell group, rostral ventrolateral medulla, and medullary raphe nuclei. This is analogous to projections from the brainstem and cortex converging on somatomotor neurons in the spinal cord. The rostral ventrolateral medulla is the major source of excitatory input to sympathetic neurons. In addition to these direct pathways to preganglionic neurons, there are many brain regions that feed into these pathways, including the amygdala, mesencephalic periaqueductal gray, caudal ventrolateral medulla, nucleus of the tractus solitarius, and medullary lateral tegmental field. This is analogous to the control of somatomotor function by areas such as the basal ganglia and cerebellum.

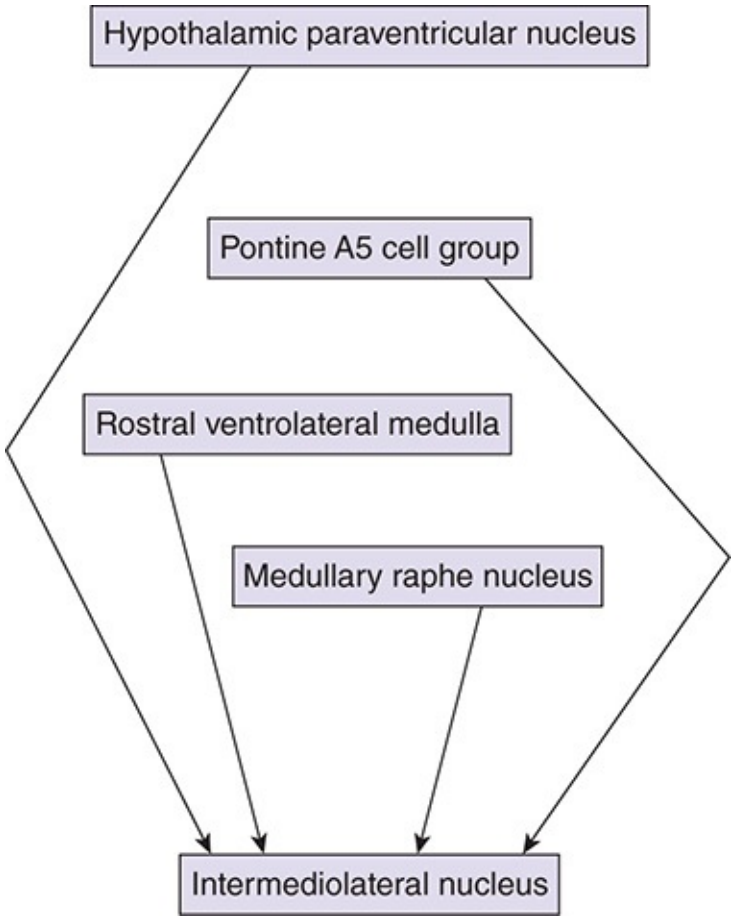


FIGURE 13–5 Pathways that control autonomic responses. Direct projections (solid lines) to autonomic preganglionic neurons include the hypothalamic paraventricular nucleus, pontine A5 cell group, rostral ventrolateral medulla, and medullary raphe.

AUTONOMIC DYSFUNCTION

Drugs, neurodegenerative diseases, trauma, inflammatory processes, and neoplasia are a few examples of factors that can lead to dysfunction of the ANS (Clinical Boxes 13–1 through 13–4). The types of dysfunction can range from complete autonomic failure to autonomic hyperactivity. Among disorders associated with autonomic failure are orthostatic hypotension, neurogenic syncope (vasovagal response), erectile dysfunction, neurogenic bladder, gastrointestinal dysmotility, sudomotor failure, and Horner syndrome. Autonomic hyperactivity can be the basis for neurogenic hypertension, cardiac arrhythmias, neurogenic pulmonary edema, myocardial injury, hyperhidrosis, hyperthermia, and hypothermia.

ENTERIC NERVOUS SYSTEM

The enteric nervous system or third division of the ANS is located within the wall of the digestive tract from the esophagus to the anus. It is composed of two well-organized neural plexuses. The **myenteric plexus** is located between longitudinal and circular layers of muscle and is involved in control of digestive tract motility. The **submucosal plexus** is located between the circular muscle and the luminal mucosa; it senses the environment of the lumen and regulates gastrointestinal blood flow and epithelial cell function.

The enteric nervous system contains all the elements of a nervous system (sensory neurons, interneurons, and motor neurons) leading to it being referred to as a "mini-brain." Sensory neurons innervate receptors in the mucosa that respond to mechanical, thermal, osmotic, and chemical stimuli. Motor neurons control motility, secretion, and absorption by acting on smooth muscle and secretory cells. Interneurons integrate information from sensory neurons and feedback to the enteric motor neurons.

Parasympathetic and sympathetic nerves connect the CNS to the enteric nervous system or directly to the digestive tract. Although the enteric nervous system can function autonomously, normal digestive function often requires communication between the CNS and the enteric nervous system (see Chapter 25).

CHAPTER SUMMARY

- Preganglionic sympathetic neurons are located in the spinal thoracolumbar IML and project to postganglionic neurons in the paravertebral or prevertebral ganglia or the adrenal medulla. Preganglionic parasympathetic neurons are located in motor nuclei of cranial nerves III, VII, IX, and X and the sacral IML and project to ganglia located in or near the effector target. The targets of the ANS include smooth muscle, cardiac muscle and pacemaker cells, exocrine and endocrine glands, adipose tissue, liver cells, and lymphatic tissue.
- Acetylcholine is released at nerve terminals of all preganglionic neurons, postganglionic parasympathetic neurons, and a few postganglionic sympathetic neurons (sweat glands and sympathetic vasodilator fibers). The remaining sympathetic postganglionic neurons release norepinephrine.
- Ganglionic transmission is mediated by activation of nicotinic receptors.

Postganglionic cholinergic transmission is mediated by activation of muscarinic receptors. Postganglionic adrenergic transmission is mediated by activation of α_1-, β-, or β_2-adrenoceptors, depending on the target organ.

- Many commonly used drugs exert their therapeutic actions by serving as agonists (eg, bethanecol, phenylephrine, albuterol) or antagonists (eg, atropine, phenoxybenzamine, atenolol) at autonomic synapses, by blocking neurotransmitter synthesis (eg, metyrosine), or by blocking neurotransmitter release (eg, tricyclic antidepressants).
- The ANS works in concert with the endocrine system to maintain homeostasis or a stable internal environment despite challenges imposed by shifts in things such as air temperature, oxygen and carbon dioxide levels, physical activity, exposure to toxins, disease, drug therapies, fever, and diet.
- Sympathetic activity prepares the individual to cope with an emergency by accelerating the heartbeat, raising blood pressure (perfusion of the vital organs), and constricting the blood vessels of the skin (limits bleeding from wounds). Parasympathetic activity is concerned with the vegetative aspects of day-to-day living and favors digestion and absorption of food by increasing the activity of the intestinal musculature, increasing gastric secretion, and relaxing the pyloric sphincter.
- At many organs that receive dual innervation (eg, heart, airways, digestive tract, and urinary bladder), the two divisions of the ANS act as physiological antagonists. In other cases, the two divisions of the ANS can act in a synergistic manner in the control of some functions (eg, control of pupil diameter). Some organs are innervated by only the sympathetic (eg, blood vessels) or only parasympathetic (eg, ciliary muscle) nervous system.
- Direct projections to sympathetic preganglionic neurons in the IML originate in the hypothalamic paraventricular nucleus, pontine catecholaminergic A5 cell group, rostral ventrolateral medulla, and medullary raphe nuclei.
- The enteric nervous system is located within the wall of the digestive tract and is composed of the myenteric plexus (control of digestive tract motility) and the submucosal plexus (regulates gastrointestinal blood flow and epithelial cell function).

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. Hypertension developed in a 26-year-old man after he began taking amphetamine to boost his energy and to suppress his appetite. Which of the following drugs would be expected to mimic the effects of increased sympathetic discharge on blood vessels?

   A. Phenylephrine

   B. Trimethaphan

   C. Atropine

   D. Reserpine

   E. Albuterol

2. A 35-year-old woman in whom multiple system atrophy was diagnosed had symptoms indicative of failure of sympathetic nerve activity. List expected findings resulting from failure of sympathetic nerve activity to the ventricle of the heart, bronchial smooth muscle, sweat glands, and blood vessels.

   A. Bradycardia, bronchial dilation, reduced sweating, and vasodilation.

   B. Decreased ventricular contractility, bronchial constriction, profuse sweating, and vasodilation.

   C. Tachycardia, bronchial constriction, reduced sweating, and vasoconstriction.

   D. Decreased ventricular contractility, bronchial constriction, reduced sweating, and vasodilation.

   E. Bradycardia, bronchial dilation, profuse sweating, and vasodilation.

3. A 45-year-old man had a meal containing wild mushrooms that he picked in a field earlier in the day. Within a few hours after eating the mushrooms, he developed signs of muscarinic poisoning. List an expected finding resulting from activation of muscarinic receptors on the lacrimal gland, SA node of the heart, sphincter muscle in the urinary tract, and sweat glands.

   A. Increased salivation, bradycardia, relaxation of the urinary tract sphincter, and no change in sweating.

   B. Decreased tear production, tachycardia, contraction of the urinary tract sphincter, and decreased sweating.

   C. Increased tear production, bradycardia, relaxation of the urinary tract sphincter, and increased sweating.

   D. Increased salivation, tachycardia, contraction of the urinary tract sphincter, and no change in sweating.

   E. Increased tear production, bradycardia, contraction of the urinary tract

sphincter, and increased sweating.

4. An MD/PhD candidate was studying control of pupillary diameter by stimulation of the ANS. What is the location of the cell bodies of preganglionic parasympathetic, postganglionic parasympathetic, preganglionic sympathetic, and postganglionic sympathetic nerves controlling pupillary diameter, respectively?

   A. Pupillary nucleus, ciliary ganglion, cervical IML, and cervical paravertebral ganglion.

   B. Second cranial nerve nucleus, pupillary ganglion, cervical IML, and cervical prevertebral ganglion.

   C. Pupillary nucleus, otic ganglion, thoracic IML, and thoracic prevertebral ganglion.

   D. Edinger-Westphal nucleus, pupillary ganglion, thoracic IML, and cervical prevertebral ganglion.

   E. Edinger-Westphal nucleus, ciliary ganglion, thoracic IML, and cervical paravertebral ganglion.

5. An MD/PhD candidate was studying autonomic control of pupillary diameter and accommodation for near vision. How does stimulation of the parasympathetic and sympathetic nerve activity affect these responses?

   A. Parasympathetic nerve activity causes mydriasis by contraction of the sphincter muscle and makes the lens more concave by contraction of the ciliary muscle; sympathetic nerve activity causes miosis by relaxation of the sphincter muscle and makes the lens more convex by contraction of the ciliary muscle.

   B. Parasympathetic nerve activity causes miosis by contraction of the constrictor muscle and causes contraction of the ciliary muscle to make the lens more convex; sympathetic nerve activity causes mydriasis by contraction of the radial muscle and causes relaxation of the ciliary muscle to make the lens more concave.

   C. Parasympathetic nerve activity causes mydriasis by contraction of the sphincter muscle and makes the lens more convex by contraction of the ciliary muscle; sympathetic nerve activity causes miosis by contraction of the dilator muscle and makes the lens more concave by contraction of the ciliary muscle.

   D. Parasympathetic nerve activity causes miosis by contraction of the sphincter muscle and makes the lens more convex by contraction of the ciliary muscle; sympathetic nerve activity causes mydriasis by contraction

of the radial muscle and does not alter the shape of the lens.

E. Parasympathetic nerve activity causes miosis by contraction of the sphincter muscle and makes the lens more convex by contraction of the ciliary muscle; sympathetic nerve activity causes mydriasis by relaxation of the sphincter muscle and does not alter the shape of the lens.

6. A 57-year-old man had severe hypertension that was found to result from a tumor compressing on the surface of the medulla. Which one of the following statements about pathways involved in the control of sympathetic nerve activity is correct?

A. Preganglionic sympathetic nerves receive inhibitory input from the rostral ventrolateral medulla.

B. The major source of excitatory input to preganglionic sympathetic nerves is the paraventricular nucleus of the hypothalamus.

C. The activity of sympathetic preganglionic neurons can be affected by the activity of neurons in the amygdala.

D. Unlike the activity in δ-motor neurons, sympathetic preganglionic neurons are not under any significant reflex control.

E. Under resting conditions, the sympathetic nervous system is not active; it is active only during stress giving rise to the term “flight or fight” response.

7. Diabetic autonomic neuropathy was diagnosed a few years ago in a 53-year-old woman with diabetes. She recently noted abdominal distension and a feeling of being full after eating only a small portion of food, suggesting the neuropathy had extended to her enteric nervous system to cause gastroparesis. What are the components of the enteric nervous system?

A. The enteric nervous system is a specialized subdivision of the parasympathetic nervous system for control of gastrointestinal function and includes specialized preganglionic and postganglionic cholinergic neurons.

B. The enteric nervous system contains the myenteric plexus that regulates gastrointestinal motility and the submucosal plexus that regulates gastrointestinal blood flow and epithelial cell function and includes motor neurons, sensory neurons, and interneurons.

C. The enteric nervous system contains the submucosal plexus that contains motor neurons that control gastric secretions and motility and a myenteric plexus that contains sensory neurons that signal information about the environment, and mucosal interneurons that relay sensory information to the central nervous system.

D. The enteric nervous system contains motor neurons within the circular muscle, sensory neurons within the longitudinal muscle, and interneurons within the mucosa that relay sensory information to the central nervous system.

E. The enteric nervous system the submucosal plexus comprised exclusively of sensory neurons transmit information about the contents of the gastrointestinal tract via interneurons to the myenteric plexus that is comprised exclusively of motor neurons.

8. A respiratory therapist was giving a lecture on how the body responds to changes in arterial blood gases. As part of her lecture, she explained homeostasis as follows.

A. Homeostasis prevents blood gases from deviating from normal values for even brief periods of time.

B. Homeostasis maintains blood gases in a normal range by activation of chemoreceptors that sense the deviation from normal and then engages a positive feedback system to return blood gases to a normal level.

C. Homeostasis maintains blood gases in a normal range by activation of sympathetic and parasympathetic chemoreceptors that then stimulate increased respiratory activity.

D. Homeostasis maintains blood gases in a normal range by activation of chemoreceptors that sense the deviation from normal and then engages a negative feedback system to return blood gases to a normal level.

CHAPTER 14

Electrical Activity of the Brain, Sleep–Wake States, & Circadian Rhythms

OBJECTIVES

After studying this chapter, you should be able to:

- Explain the function of the thalamocortical pathway and ascending arousal system in the control of arousal and consciousness.
- Explain the interplay between brainstem neurons that contain norepinephrine, serotonin, and acetylcholine and diencephalic histaminergic and GABAergic neurons in mediating transitions between sleep and wakefulness.
- Explain the physiological basis and the main clinical uses of the electroencephalogram (EEG).
- Describe possible causes of seizure activity and explain the differences between generalized and partial seizures.
- Identify the primary types of cortical rhythms recorded in an EEG that reflect different states of wakefulness and sleep.
- Summarize the behavioral and EEG characteristics of rapid eye movement (REM) sleep and the four stages of non-REM sleep.
- Describe the pattern of normal nighttime sleep in adults and the variations in

this pattern from birth to old age.

- Describe the symptoms of narcolepsy, sleep apnea, and other sleep disorders.
- Describe the roles of the suprachiasmatic nuclei (SCN) and melatonin in regulation of the circadian rhythm.

INTRODUCTION

Most of the sensory systems introduced in Chapters 8–12 relay impulses from receptors via multiunit pathways to specific sites in the cerebral cortex. The impulses are responsible for perception and localization of individual sensations; however, they must be processed in the awake brain to be perceived. There is a spectrum of behavioral states ranging from deep sleep through alertness with focused attention. Each distinct state is correlated with a discrete pattern of brain electrical activity. Feedback oscillations within the cerebral cortex and between the thalamus and the cortex produce this activity and are determinants of the behavioral state. Arousal can be produced by sensory stimulation and by impulses ascending from the brainstem to the thalamus and then to the cortex. Some of these activities have rhythmic fluctuations that are approximately 24 h in length **(circadian rhythm).**

THALAMOCORTICAL PATHWAYS & ASCENDING AROUSAL SYSTEM

THALAMIC NUCLEI

The **thalamus** within the diencephalon is comprised of groups of nuclei that participate in sensory, motor, and limbic functions. The thalamus is the “gateway to the cerebral cortex” because it processes virtually all information that reaches the cortex. The thalamus also receives input from the cortex.

The thalamus has two major groups of nuclei: those that project diffusely to wide areas of the neocortex **(midline and intralaminar nuclei)** and those that project to discrete regions of the neocortex and limbic system **(specific sensory relay nuclei).** The latter group includes the medial and lateral geniculate bodies that relay auditory and visual impulses to the auditory and visual cortices, respectively, and the ventral posterior lateral (VPL) and ventral posteromedial

nuclei that relay somatosensory information to the postcentral gyrus. The ventral anterior and ventral lateral nuclei receive input from the basal ganglia and the cerebellum and project to the motor cortex. The anterior nuclei receive input from the mammillary bodies and project to the limbic cortex to influence memory and emotion. Most thalamic neurons are excitatory and release glutamate. The thalamus also contains inhibitory neurons in the **thalamic reticular nucleus.** These neurons release **GABA,** and unlike many thalamic neurons, their axons do not project to the cortex. Rather, they are thalamic interneurons and modulate the responses of other thalamic neurons to input coming from the cortex.

CORTICAL ORGANIZATION

The neocortex is arranged in six layers **(Figure 14–1)**. Afferents from the specific nuclei of the thalamus terminate primarily in layer IV; the nonspecific nuclei project to layers I–IV. **Pyramidal neurons,** the most common cell type in the cortex, have extensive vertical dendritic trees that reach toward the cortical surface **(Figure 14–2)**. Their cell bodies are found in all cortical layers except layer I. The axons of pyramidal cells have recurrent collaterals that turn back and synapse on the superficial portions of the dendritic trees. Pyramidal neurons are excitatory neurons that release **glutamate** at their terminals, and they are the only projection neurons of the cortex.

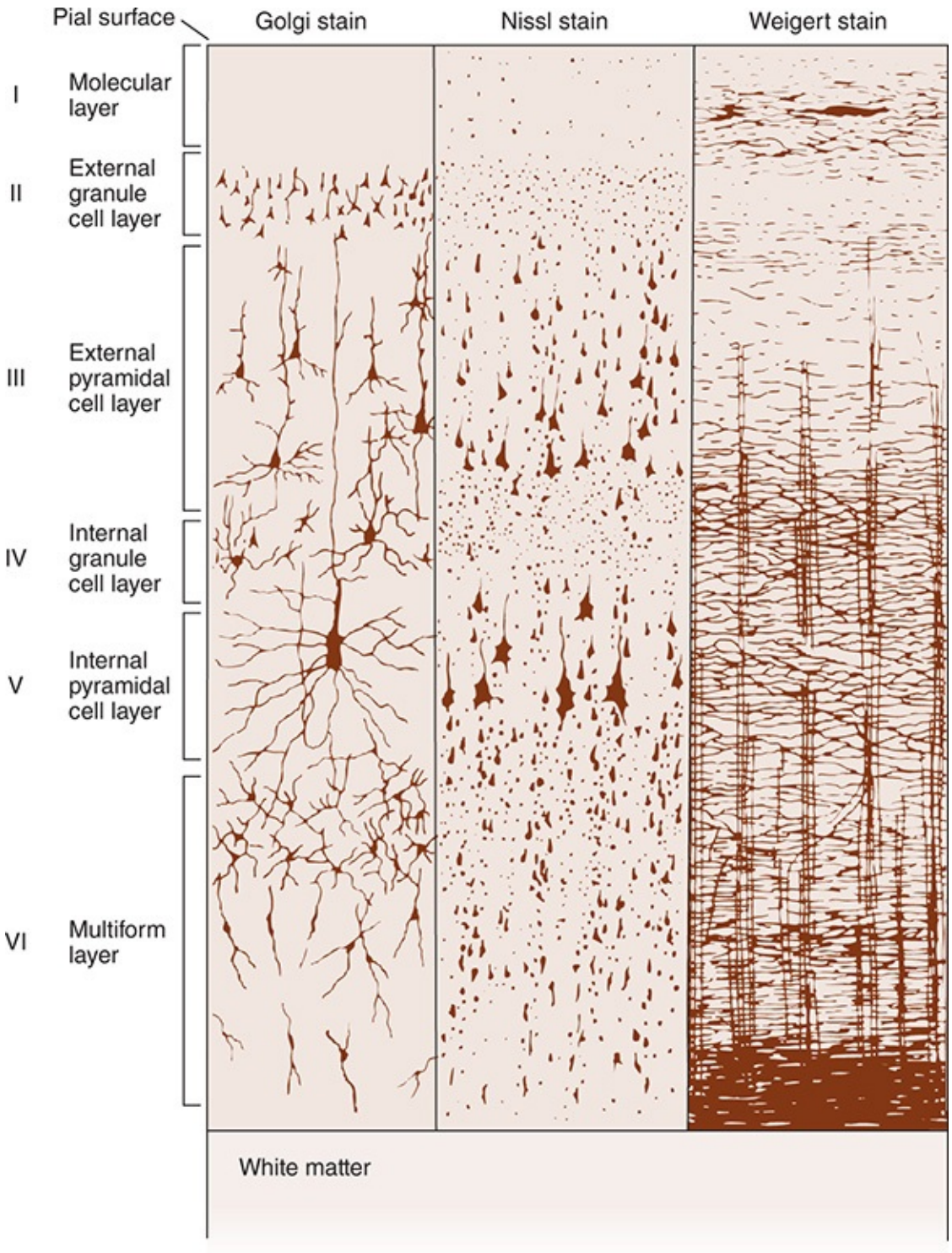


FIGURE 14–1 Structure of the cerebral cortex. The cortical layers are indicated by the numbers. Golgi stain shows neuronal cell bodies and dendrites, Nissl stain shows cell bodies, and Weigert myelin sheath stain shows myelinated nerve fibers. (Modified with permission from Ranson SW, Clark SL: *The*

Anatomy of the Nervous System, 10th ed. St. Louis, MO: Saunders; 1959.)

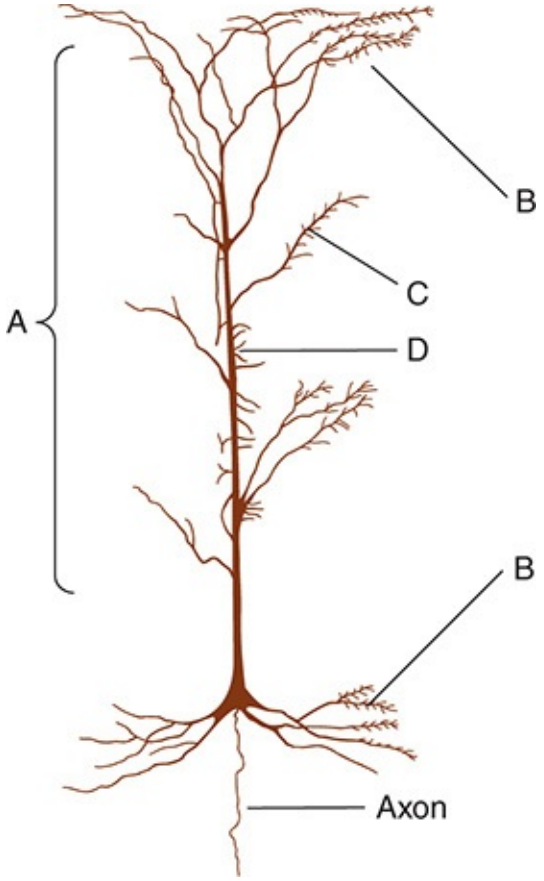


FIGURE 14–2 Neocortical pyramidal cell, showing the distribution of neurons that terminate on it. A denotes nonspecific afferents from the brainstem and the thalamus; **B** denotes recurrent collaterals of pyramidal cell axons; **C** denotes commissural fibers from mirror image sites in the contralateral hemisphere; **D** denotes specific afferents from thalamic sensory relay nuclei. (Based on Scheibel ME, Scheibel AB: Structural organization of nonspecific thalamic nuclei and their projection toward cortex. Brain Res 1967 Sep; 6(1):60–94.)

The other cortical cell types are local circuit interneurons and are classified based on their shape, pattern of projection, and neurotransmitter. Inhibitory interneurons **(basket cells** and **chandelier cells**) release GABA as their neurotransmitter. Basket cells have long axonal endings that surround the soma of pyramidal neurons; they account for most inhibitory synapses on the pyramidal soma and dendrites. Chandelier cells are a powerful source of inhibition of pyramidal neurons because their axonal endings terminate

exclusively on the initial segment of the pyramidal cell axon. Their terminal boutons form short vertical rows that resemble candlesticks, thus accounting for their name. **Spiny stellate cells** are excitatory neurons that release glutamate; these multipolar interneurons are located primarily in layer IV and are a major recipient of sensory information arising from the thalamus.

In addition to being organized into layers, the cerebral cortex is also organized into columns. Neurons within a column have similar response properties, suggesting they comprise a local processing network (eg, orientation and ocular dominance columns in the visual cortex).

Evoked Cortical Potentials

The electrical events that occur in the cortex after stimulation of a sensory receptor can be monitored with a recording electrode. If the electrode is over the primary receiving area for a particular sense, a surface-positive wave appears with a latency of 5–12 ms. This is followed by a small negative wave, and then a larger, more prolonged positive deflection frequently occurs with a latency of 20–80 ms. The first positive-negative wave sequence is the **primary evoked potential;** the second is the **diffuse secondary response.**

The primary evoked potential is highly specific in its location and occurs only where the pathways from a particular sensory system project The positive-negative wave sequence recorded from the surface of the cortex occurs because the superficial cortical layers are positive relative to the initial negativity, then negative relative to the deep hyperpolarization. The surface-positive diffuse secondary response, unlike the primary response, is not highly localized. It appears at the same time over most of the cortex and is due to activity in projections from the midline and intralaminar thalamic nuclei.

ASCENDING AROUSAL SYSTEM

The **ascending arousal system** is a complex polysynaptic pathway comprised of monoaminergic, cholinergic, and histaminergic neurons that project to the intralaminar and reticular nuclei of the thalamus which, in turn, project diffusely to wide regions of the cortex including the frontal, parietal, temporal, and occipital cortices **(Figure 14–3)**. Collaterals funnel into it not only from the long ascending sensory tracts but also from the trigeminal, auditory, visual, and olfactory systems. The complexity of the ascending arousal system and the degree of convergence in it abolish modality specificity, and most neurons are

activated with equal facility by different sensory stimuli. Components of the arousal system include norepinephrine-containing neurons in the pontine locus coeruleus, serotoninergic neurons in the brainstem raphé nuclei, cholinergic neurons in the pontine and midbrain pedunculopontine and laterodorsal tegmental nuclei, and histaminergic neurons in the hypothalamic tuberomammilary nucleus.

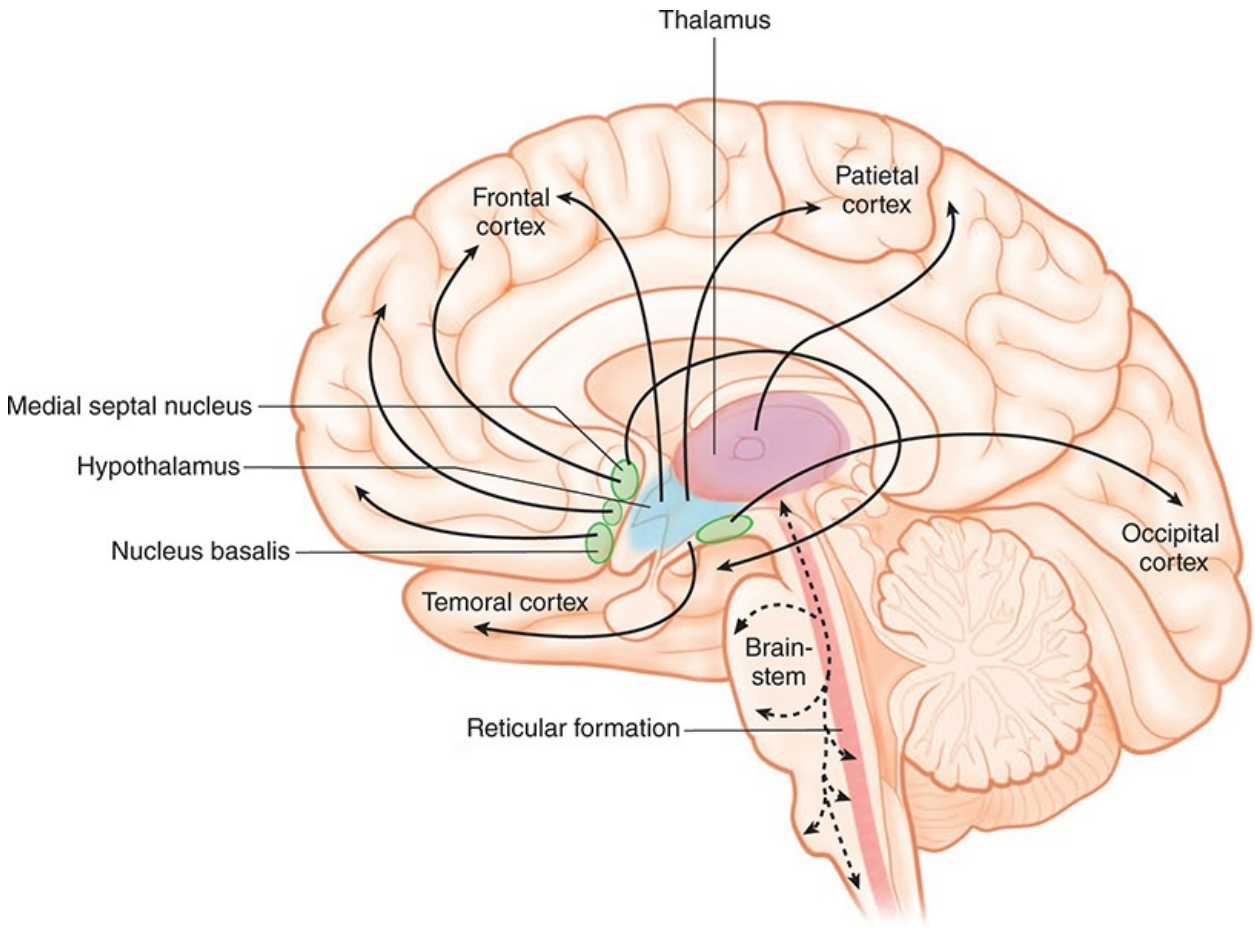


FIGURE 14–3 Cross section through the midline of the human brain showing the ascending arousal system in the brainstem with projections to the intralaminar nuclei of the thalamus and the output from the intralaminar nuclei to many parts of the cerebral cortex. Activation of these areas can be shown by positive emission tomography scans when subjects shift from a relaxed awake state to an attention-demanding task.

NEUROCHEMICAL MECHANISMS PROMOTING SLEEP & AROUSAL

Transitions between sleep and wakefulness manifest a circadian rhythm consisting of an average of 6–8 h of sleep and 16–18 h of wakefulness. Nuclei in both the brainstem and hypothalamus are critical for the transitions between these states of consciousness. As described above, the brainstem ascending arousal system is comprised of several groups of neurons that release norepinephrine, serotonin, acetylcholine, or histamine. The locations and wide projections of these neuronal populations are shown in Figure 7–2. The forebrain is also involved in the control of the sleep–wake cycles via hypothalamic **preoptic neurons** that release **GABA** and **tuberomamilary neurons** that release **histamine.** Also, hypothalamic neurons release **orexin** to play a role in switching between sleep and wakefulness.

One theory regarding the basis for transitions from sleep to wakefulness involves alternating reciprocal activity of different groups of neurons in the ascending arousal system. In this model **(Figure 14–4)**, wakefulness and **rapid eye movement (REM) sleep** are at opposite extremes. When the activity of norepinephrine- and serotonin-containing neurons (locus coeruleus and raphé nuclei) is dominant, activity in acetylcholine-containing pontine neurons is reduced. This pattern of activity contributes to the appearance of the awake state. The reverse of this pattern leads to REM sleep. When there is a more even balance between the activity of the aminergic and cholinergic neurons, **non-REM sleep** occurs. The orexin released from hypothalamic neurons may regulate the changes in activity in these brainstem neurons. An increased release of GABA and reduced release of histamine increase the likelihood of non-REM sleep via deactivation of the thalamus and cortex. Wakefulness occurs when GABA release is reduced and histamine release is increased.

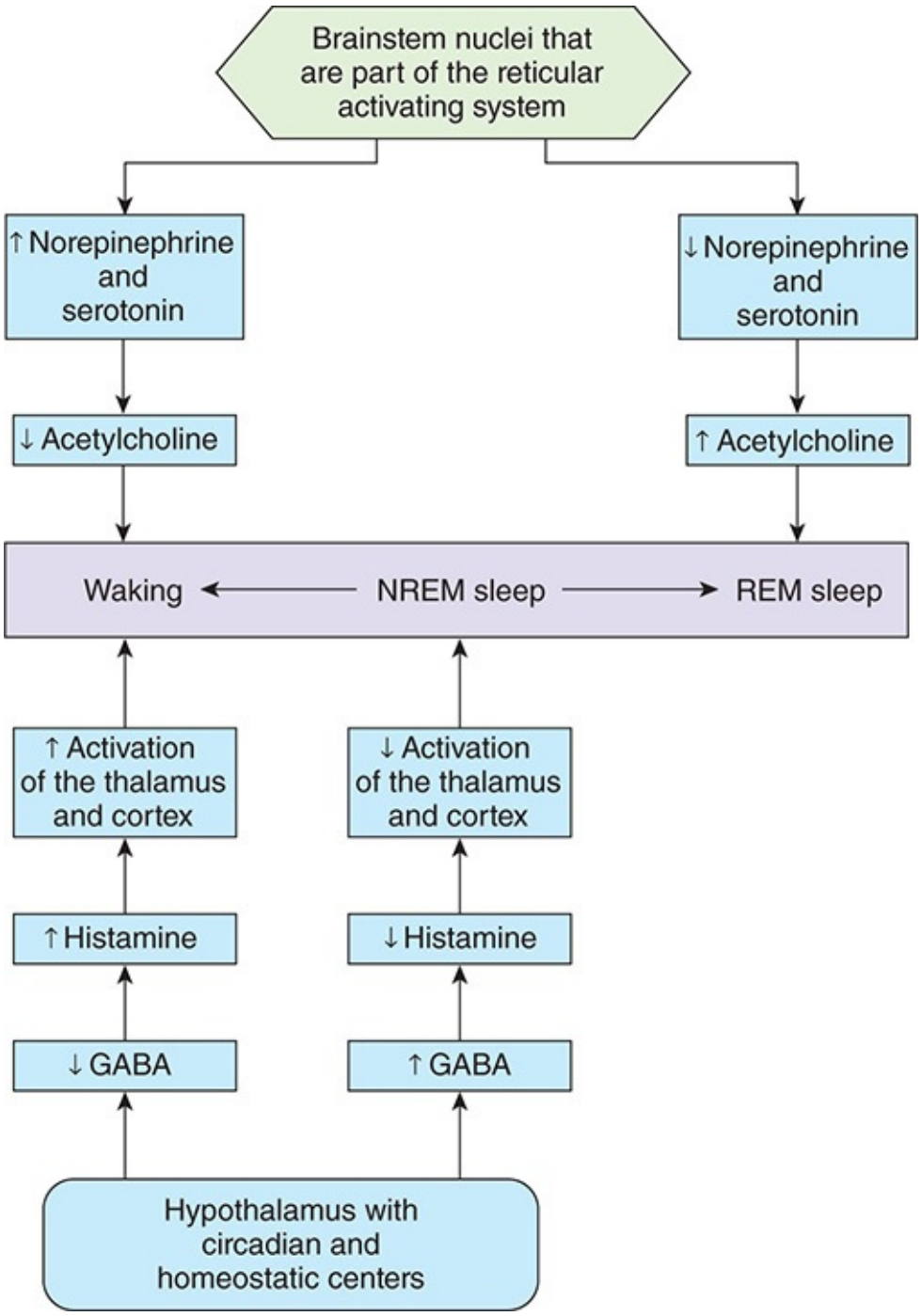


FIGURE 14–4 A model of how alternating activity of brainstem and hypothalamic neurons may influence the different states of consciousness. In this model, wakefulness and REM sleep are at opposite extremes. When the activity of norepinephrine- and serotonin-containing neurons (locus coeruleus and raphe nuclei) is dominant, there is a reduced level of activity in acetylcholine-containing pontine neurons leading to wakefulness. The reverse of

this pattern leads to REM sleep. A more even balance in the activity of these groups of neurons is associated with non-REM (NREM) sleep. Increases in GABA and decreases in histamine promote non-REM sleep via deactivation of the thalamus and cortex. Wakefulness occurs when GABA is reduced and histamine is released. (Used with permission from Widmaier EP, Raff H, Strang KT: *Vander's Human Physiology*, 11th ed. New York, NY: McGraw-Hill; 2008.)

THE ELECTROENCEPHALOGRAM

The term **electroencephalogram (EEG)** refers to a recording that represents the electrical activity of the brain. The EEG can be recorded with scalp electrodes through the unopened skull. The term **electrocorticogram** is used for the recording obtained with electrodes on the pial surface of the cortex.

The EEG recorded from the scalp is a measure of the summation of dendritic postsynaptic potentials rather than action potentials **(Figure 14–5)**. The dendrites of the cortical neurons are a forest of similarly oriented, densely packed units in the superficial layers of the cerebral cortex (Figure 14–1). Propagated potentials can be generated in dendrites. In addition, recurrent axon collaterals end on dendrites in the superficial layers. As excitatory and inhibitory endings on the dendrites of each cell become active, current flows into and out of these current sinks and sources from the rest of the dendritic processes and the cell body. The cell body–dendrite relationship is that of a constantly shifting dipole. Current flow in the dipole produces wavelike potential fluctuations in a volume conductor (Figure 14–5). When the sum of the dendritic activity is negative relative to the cell body, the neuron is depolarized and hyperexcitable; when it is positive, the neuron is hyperpolarized and less excitable.

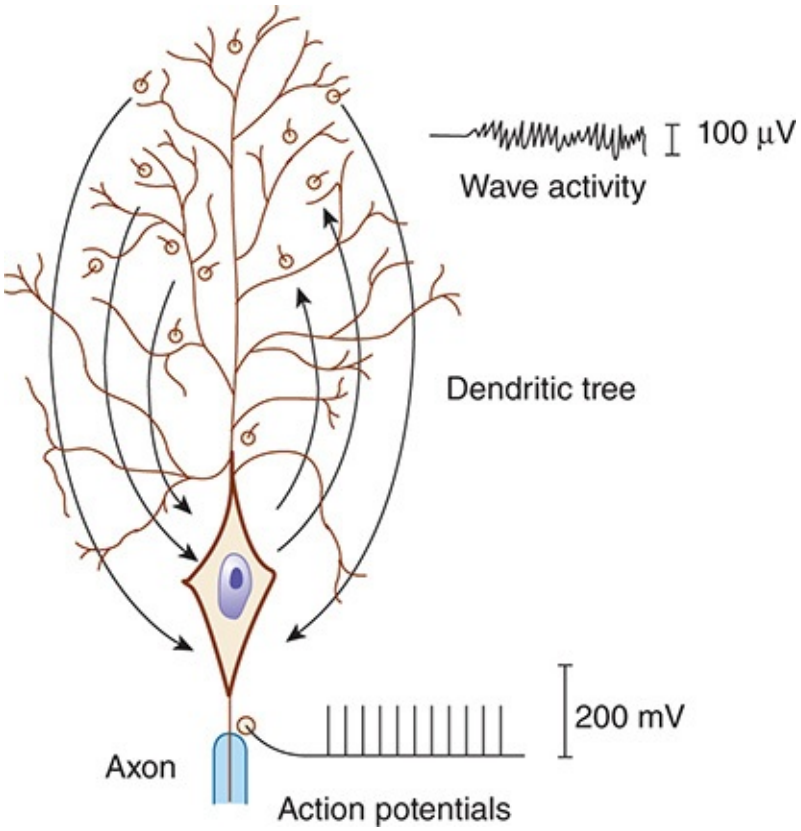


FIGURE 14–5 Diagrammatic comparison of the electrical responses of the axon and the dendrites of a large cortical neuron. Current flow to and from active synaptic knobs on the dendrites produces wave activity, while all-or-none action potentials are transmitted along the axon. When the sum of the dendritic activity is negative relative to the cell body, the neuron is depolarized; when it is positive, the neuron is hyperpolarized. The electroencephalogram recorded from the scalp is a measure of the summation of dendritic postsynaptic potentials rather than action potentials.

CLINICAL USES OF THE EEG

The EEG can be of value in localizing neuropathological processes. When fluid collection overlies a portion of the cortex, activity over this area may be damped. This fact may aid in diagnosing and localizing conditions such as subdural hematomas. Lesions in the cerebral cortex cause local formation of transient disturbances in brain activity, marked by high-voltage abnormal waves that can be recorded with an EEG. Seizure activity can occur because of increased firing of excitatory neurons (eg, release of glutamate) or decreased firing of inhibitory neurons (eg, release GABA).

TYPES OF SEIZURES

Epilepsy is a condition in which there are recurring, unprovoked seizures that may result from damage to the brain. The seizures represent abnormal, highly synchronous neuronal activity. Epilepsy is a syndrome with multiple causes. In some forms, characteristic EEG patterns occur during seizures or between attacks; however, abnormalities are often difficult to demonstrate. Seizures are divided into **partial (focal) seizures** and **generalized seizures.**

Partial seizures originate in a small group of neurons and can result from head injury, brain infection, stroke, or tumor; but often the cause is unknown. Symptoms depend on the seizure focus. They are further subdivided into **simple partial seizures** (without loss of consciousness) and **complex partial seizures** (with altered consciousness). An example of a simple partial seizure is localized jerking movements in one hand progressing to clonic movements of the entire arm lasting about 60–90 s. **Auras** typically precede the onset of a partial seizure and include abnormal sensations. The time after the seizure until normal neurologic function returns is called the **postictal period.**

Generalized seizures are associated with widespread electrical activity and involve both hemispheres simultaneously. They are further subdivided into **convulsive** and **nonconvulsive** categories depending on whether tonic or clonic movements occur. **Absence seizures** (formerly called petit mal seizures) are a form of nonconvulsive generalized seizures characterized by a momentary loss of consciousness. They are associated with 3/s doublets, each consisting of a typical **spike-and-wave** pattern of activity that lasts for about 10 s **(Figure 14–6)**. They are not accompanied by auras or postictal periods. These spike and waves are likely generated by low threshold T-type Ca^{2+} channels in thalamic neurons.

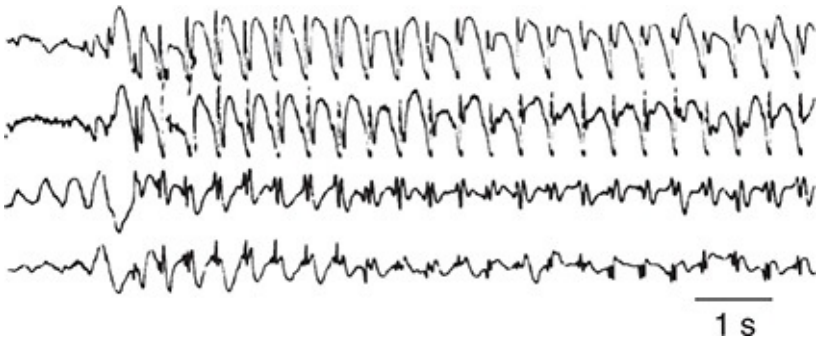


FIGURE 14–6 Absence seizures. This is a recording of four cortical EEG leads from a 6-year-old boy who, during the recording, had one of his “blank spells” in

which he was transiently unaware of his surroundings and blinked his eyelids. Absence seizures are associated with 3/s doublets, each consisting of a typical spike-and-wave pattern of activity that lasts for about 10 s. Time is indicated by the horizontal calibration line. EEG, electroencephalogram. (Reproduced with permission from Waxman SG: *Neuroanatomy with Clinical Correlations*, 25th ed. New York, NY: McGraw-Hill; 2003.)

Tonic-clonic seizures (formerly called grand mal seizure) are the most common convulsive generalized seizure. It is associated with sudden onset of contraction of limb muscles **(tonic phase)** lasting about 30 s, followed by a clonic phase with symmetric jerking of the limbs as a result of alternating contraction and relaxation **(clonic phase)** lasting 1–2 min. There is fast EEG activity during the tonic phase. Slow waves, each preceded by a spike, occur at the time of each clonic jerk; slow waves persist for a while after the attack.

The release of glutamate from astrocytes may play a role in in the pathophysiology of epilepsy. Also, there is evidence that reorganization of astrocytes along with dendritic sprouting and new synapse formation is the structural basis for recurrent excitation in the epileptic brain. **Clinical Box 14–1** describes information on the role of genetic mutations in some forms of epilepsy.

CLINICAL BOX 14–1

Genetic Mutations & Epilepsy

Epilepsy has no geographic, racial, sex, or social bias. It can occur at any age, but is most often diagnosed in infancy, childhood, adolescence, and old age. It is the second most common neurologic disorder after stroke. According to the World Health Organization, an estimated 50 million people worldwide (8.2 per 1000 individuals) experience epileptic seizures. The prevalence in developing countries (such as Colombia, Ecuador, India, Liberia, Nigeria, Panama, United Republic of Tanzania, and Venezuela) is more than 10 per 1000. Many affected individuals experience unprovoked seizures, for no apparent reason, and without any other neurologic abnormalities. These are called **idiopathic epilepsies** and are assumed to be genetic in origin. Mutations in voltage-gated potassium, sodium, and chloride channels have been linked to some forms of idiopathic epilepsy. Mutated ion channels can lead to neuronal hyperexcitability via various pathogenic mechanisms. Scientists have recently identified the mutated gene responsible for

development of **childhood absence epilepsy (CAE);** several patients with CAE have mutations in a subunit gene of the GABA receptor called **GABRB3.** Also, **SCN1A** and **SCN1B mutations** have been identified in an inherited form of epilepsy called **generalized epilepsy with febrile seizures.** SCN1A and SCN1B are sodium channel subunit genes that are widely expressed within the central nervous system. SCN1A mutations are suspected in several other forms of epilepsy.

THERAPEUTIC HIGHLIGHTS

Only about two-thirds of those who experience seizure activity respond to drug therapies. Some respond to surgical interventions (eg, those with temporal lobe seizures), and others respond to vagal nerve stimulation (eg, those with partial seizures). Prior to the 1990s, the most common drugs used to treat seizures **(anticonvulsants)** included phenytoin, valproate, and barbiturates. Newer drugs have become available but, as is the case with the older drugs, they are palliative rather than curative. There are three broad mechanisms of action of anticonvulsant drugs: enhancing inhibitory neurotransmission (increased GABA release), reducing excitatory neurotransmission (decreased glutamate release), or altering ionic conductance. **Gabapentin** is a GABA analog that acts by decreasing Ca^{2+} entry into cells and reducing glutamate release; it is used to treat generalized seizures. **Topiramate** blocks voltage-gated Na^{+} channels associated with glutamate receptors and potentiates the inhibitory effect of GABA; it is also used to treat generalized seizures. **Ethosuximide** reduces the low threshold T-type Ca^{2+} currents in thalamic neurons and is particularly effective in treatment of absence seizures. **Valproate** and **phenytoin** block high-frequency firing of neurons by acting on voltage-gated Na^{+} channels to reduce glutamate release.

SLEEP–WAKE CYCLE: VARIATIONS IN EEG RHYTHMS

ALPHA AND BETA RHYTHMS

In adult humans who are awake but at rest with the mind wandering and the eyes closed, the most prominent component of the EEG is a fairly regular pattern of

waves at a frequency of 8–13 Hz and amplitude of 50–100 µV when recorded from the scalp. This pattern is the **alpha rhythm** (Figure 14–7). It is most marked in the parietal and occipital lobes and is associated with decreased levels of attention. The frequency and magnitude of the EEG rhythm can vary with age, with the use of some drugs, and in some pathological conditions (Clinical Box 14–2).

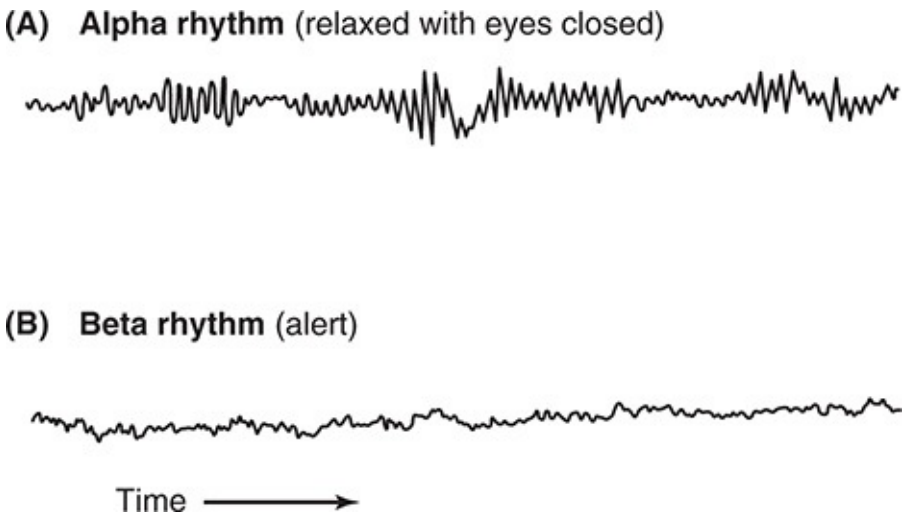


FIGURE 14–7 EEG records showing the alpha and beta rhythms. When attention is focused on something, the 8–13 Hz alpha rhythm is replaced by an irregular 13–30 Hz low-voltage activity, the beta rhythm. This phenomenon is referred to as alpha block, arousal, or the alerting response. (Used with permission from Widmaier EP, Raff H, Strang KT: *Vander's Human Physiology*, 11th ed. New York, NY: McGraw-Hill; 2008.)

When attention is focused on something, the alpha rhythm is replaced by an irregular 13–30 Hz low-voltage activity, the **beta rhythm** (Figure 14–7). This phenomenon is called alpha block and can be produced by any form of sensory stimulation or mental concentration, such as solving arithmetic problems. Another term for this phenomenon is the **arousal** or **alerting response** because it is correlated with the aroused, alert state. It has also been called **desynchronization** because it represents breaking up of the obviously synchronized neural activity necessary to produce regular waves. However, the rapid EEG activity seen in the alert state is also synchronized but at a higher rate. Therefore, the term "desynchronization" is misleading.

SLEEP STAGES: NON-REM & REM SLEEP

Non-REM sleep is divided into four stages **(Figure 14–8)**. Stage 1 non-REM sleep is the transition from wakefulness to sleep, the EEG shows a low-voltage, mixed frequency pattern. A **theta rhythm** (4–7 Hz) can be seen at this stage of sleep. Stage 2 of non-REM sleep is marked by the appearance of sinusoidal waves called **sleep spindles** (7–15 Hz) and occasional high voltage biphasic waves called **K complexes.** Muscle tone is reduced during this time. Stage 3 of non-REM sleep is characterized by the appearance of a high-amplitude **delta rhythm** (0.5–4 Hz) in the EEG, reflecting a further reduction is arousal, and a further reduction in muscle tone. Maximum slowing with large waves is seen in stage 4 of non-REM sleep. Thus, the characteristic of deep sleep is a pattern of rhythmic slow waves, indicative of marked synchronization of cortical and thalamic activity; it is sometimes referred to as slow-wave sleep. While the occurrence of theta and delta rhythms is normal during sleep, their appearance during wakefulness is a sign of brain dysfunction.

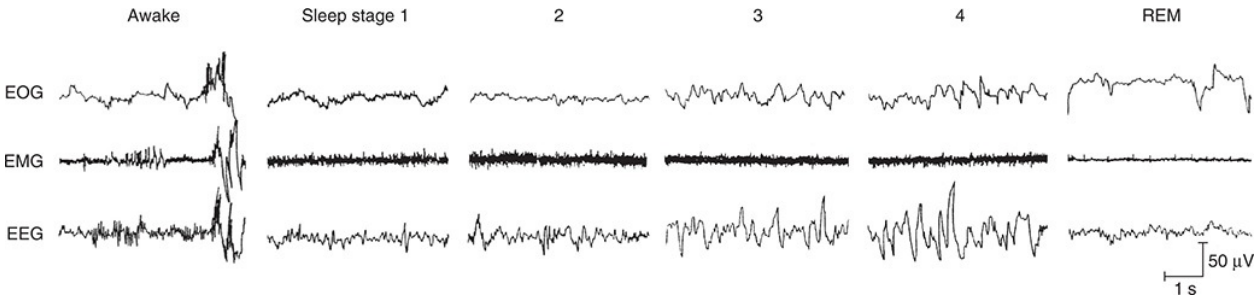


FIGURE 14–8 EEG and muscle activity during various stages of the sleep–wake cycle. Non-REM sleep has four stages. Stage 1 is characterized by a slight slowing of the EEG. Stage 2 has high-amplitude K complexes and spindles. Stages 3 and 4 have slow, high-amplitude delta waves. REM sleep is characterized by eye movements, loss of muscle tone, and a low-amplitude, high-frequency activity pattern. The higher voltage activity in the EOG tracings during stages 2 and 3 reflect high amplitude EEG activity in the prefrontal areas rather than eye movements. EOG, electrooculogram registering eye movements; EMG, electromyogram registering skeletal muscle activity. (Reproduced with permission from Rechtschaffen A, Kales A: *A Manual of Standardized Terminology, Techniques and Scoring System and Sleep Stages of Human Subjects*. Los Angeles: University of California Brain Information Service; 1968.)

CLINICAL BOX 14–2

Variations in the Alpha Rhythm

In humans, the frequency of the dominant EEG rhythm at rest varies with age. In infants, there is fast, beta-like activity, but the occipital rhythm is a slow 0.5–2-Hz pattern. During childhood this latter rhythm speeds up, and the adult alpha pattern gradually appears during adolescence. The frequency of the alpha rhythm is decreased by low blood glucose levels, low body temperature, low levels of adrenal glucocorticoid hormones, and high arterial partial pressure of CO_2 ($PaCO_2$). It is increased by the reverse conditions. Forced over-breathing to lower the $PaCO_2$ is sometimes used clinically to bring out latent EEG abnormalities. The frequency and magnitude of the alpha rhythm is also decreased by metabolic and toxic encephalopathies including those due to hyponatremia and vitamin B_{12} deficiency. The frequency of the alpha rhythm is reduced during acute intoxication with **alcohol, amphetamines, barbiturates, phenytoin,** and **antipsychotics. Propofol,** a hypnotic/sedative drug, can induce a rhythm in the EEG that is analogous to the classic alpha rhythm.

The high-amplitude slow waves seen in the EEG during non-REM sleep are periodically replaced by rapid, low-voltage EEG activity in REM sleep (Figure 14–8). REM sleep gets its name from the characteristic rapid, roving eye movements that occur during this stage of sleep and are recorded as an **electrooculogram (EOG)**. Except for eye movement, there is almost a complete loss of skeletal muscle tone in REM sleep. The threshold for arousal from sleep by sensory stimuli is elevated during this time. Another characteristic of REM sleep is the occurrence of large phasic potentials that originate in the cholinergic neurons in the pons and pass rapidly to the lateral geniculate body and from there to the occipital cortex. They are called **pontogeniculo-occipital (PGO) spikes.**

Positron emission tomography (PET) scans in REM sleep show increased activity in the pontine area, amygdala, and anterior cingulate gyrus, but decreased activity in the prefrontal and parietal cortex. Activity in visual association areas is increased, but activity is decreased in the primary visual cortex. This is consistent with increased emotion and operation of a closed neural system cut off from the areas that relate brain activity to the external world.

DISTRIBUTION OF SLEEP STAGES

In a typical night of sleep, a young adult first enters non-REM sleep, passes through stages 1 and 2, and spends 70–100 min in stages 3 and 4. Sleep then lightens, and a REM period follows. This cycle is repeated at intervals of about 90 min throughout the night **(Figure 14–9)**. The cycles are similar, though there is less stage 3 and 4 sleep and more REM sleep toward morning; thus, four to six REM periods occur per night. REM sleep occupies 80% of total sleep time in premature infants and 50% in full-term neonates. Thereafter, the proportion of REM sleep falls rapidly and plateaus at about 25% until it falls to about 20% in the elderly. Children have more total sleep time (8–10 h) compared to most adults (about 6 h).

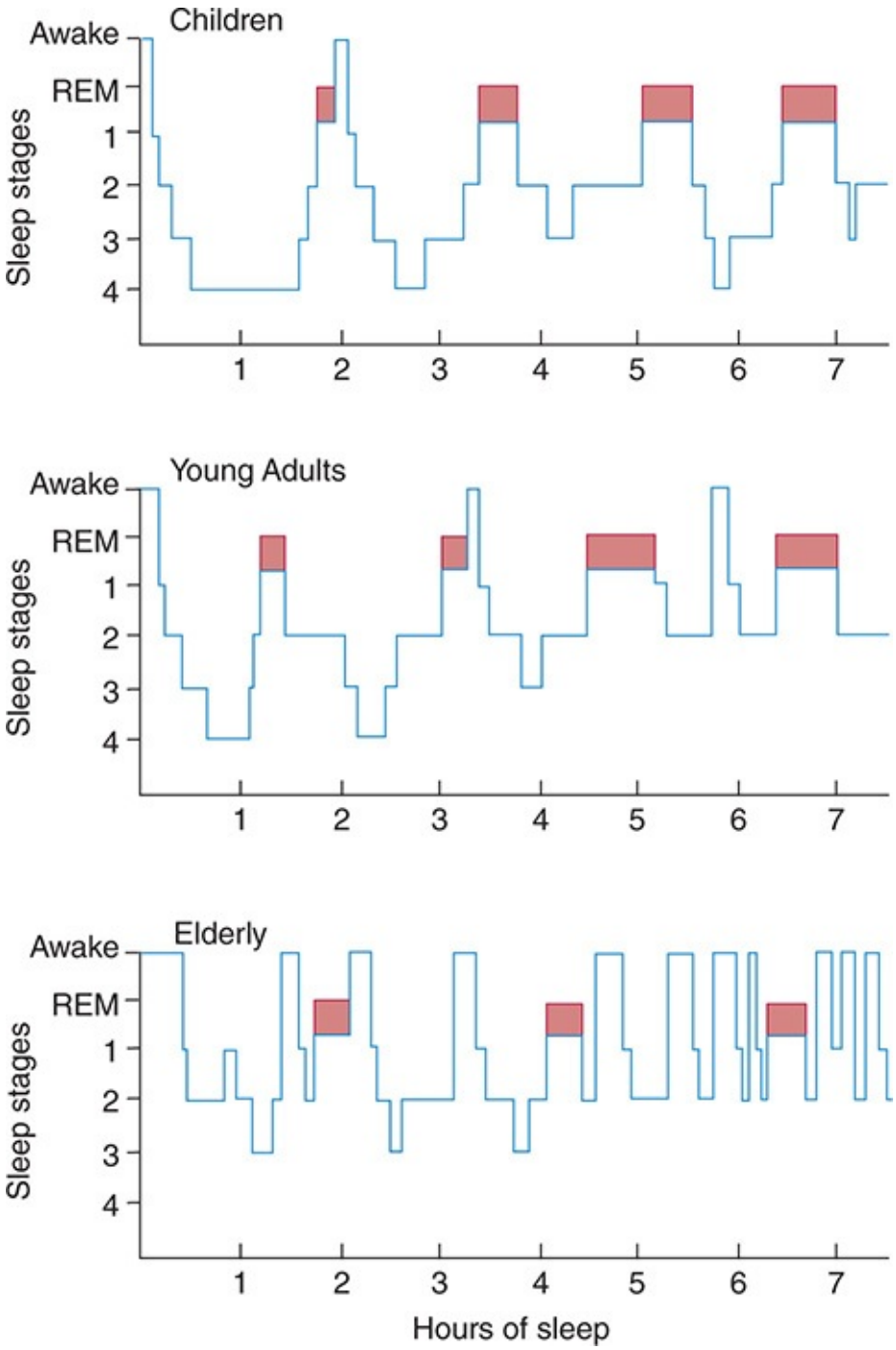


FIGURE 14–9 Normal sleep cycles at various ages. REM sleep is indicated by the darker colored areas. In a typical night of sleep, a young adult first enters non-REM sleep, passes through stages 1 and 2, and spends 70–100 min in stages 3 and 4. Sleep then lightens, and a REM period follows. This cycle is repeated at intervals of about 90 min throughout the night. The cycles are similar, though there is less stage 3 and 4 sleep and more REM sleep toward morning. REM

sleep occupies 50% of total sleep time in neonates; this proportion declines rapidly and plateaus at ~25% until it falls further in the elderly. (Reproduced with permission from Kales AM, Kales JD: Sleep disorders. N Engl J Med 1974; Feb 28; 290(9):487–499.)

Dreaming occurs in both REM and non-REM sleep stages, but their characteristics differ. Dreams that occur during REM sleep tend to be longer and more visual and emotional than those that occur during non-REM sleep.

IMPORTANCE OF SLEEP

Various studies imply that sleep is needed to maintain metabolic-caloric balance, thermal equilibrium, and immune competence. **Clinical Box 14–3** describes several common sleep disorders. If humans are awakened every time they show REM sleep and then permitted to sleep without interruption, they show a great deal more than the normal amount of REM sleep for a few nights. Relatively prolonged REM deprivation does not seem to have adverse psychological effects.

CLINICAL BOX 14–3

Sleep Disorders

Narcolepsy is a chronic neurologic disorder caused by the brain's inability to regulate sleep–wake cycles normally. The affected individual experiences a sudden loss of voluntary muscle tone **(cataplexy),** an eventual irresistible urge to sleep during daytime, and possibly brief episodes of total paralysis at the beginning or end of sleep. Narcolepsy is also characterized by a sudden onset of REM sleep, unlike normal sleep that begins with non-REM, slow-wave sleep. The prevalence of narcolepsy ranges from 1 in 600 in Japan to 1 in 500,000 in Israel, with 1 in 1000 Americans being affected. Narcolepsy has a familial incidence strongly associated with a class II antigen of the major histocompatibility complex on chromosome 6 at the HLA-DR2 or HLA-DQW1 locus, implying a genetic susceptibility to narcolepsy. The HLA complexes are interrelated genes that regulate the immune system (see Chapter 3). Compared to brains from healthy persons, the brains of persons with narcolepsy often contain fewer **hypocretin (orexin)**-producing neurons in the hypothalamus. The HLA complex may increase susceptibility to an

immune attack on these neurons, leading to their degeneration.

Obstructive sleep apnea (OSA) is the most common cause of daytime sleepiness due to fragmented sleep at night; it affects about 24% of middle-aged men and 9% of women in the United States. Breathing ceases for more than 10 s during frequent episodes of obstruction of the upper airway (especially the pharynx) due to a reduction in muscle tone. The apnea causes brief arousals from sleep in order to reestablish upper airway tone. An individual with OSA typically begins to snore soon after falling asleep. The snoring gets progressively louder until it is interrupted by an episode of apnea, which is followed by a loud snort and gasp as the individual tries to breathe. OSA is not associated with a reduction in total sleep time, but individuals with OSA experience a much greater time in stage 1 non-REM sleep (from an average of 10% of total sleep to 30–50%) and a marked reduction in slow-wave sleep (stages 3 and 4 non-REM sleep). The pathophysiology of OSA includes both a reduction in neuromuscular tone at the onset of sleep and a change in the central respiratory drive.

Periodic limb movement disorder (PLMD) is a stereotypical rhythmic extension of the big toe and dorsiflexion of the ankle and knee during sleep lasting for about 0.5–10 s and recurring at intervals of 20–90 s. Movements can actually range from shallow, continual movement of the ankle or toes, to wild and strenuous kicking and flailing of the legs and arms. Electromyograph (EMG) recordings show bursts of activity during the first hours of non-REM sleep associated with brief EEG signs of arousal. The duration of stage 1 non-REM sleep may be increased and that of stages 3 and 4 may be decreased compared to age-matched controls. PLMD is reported to occur in 5% of individuals between the ages of 30 and 50 and increases to 44% of those over the age of 65. PLMD is similar to **restless leg syndrome** or Willis–Ekbom disease in which individuals have an irresistible urge to move their legs while at rest all day long.

Sleepwalking **(somnambulism),** bed-wetting **(nocturnal enuresis),** and **night terrors** are referred to as **parasomnias,** which are sleep disorders associated with arousal from non-REM and REM sleep. Episodes of sleepwalking are more common in children than in adults and occur predominantly in males. They may last several minutes. Somnambulists walk with their eyes open and avoid obstacles, but when awakened they cannot recall the episodes.

THERAPEUTIC HIGHLIGHTS

Excessive daytime sleepiness in patients with narcolepsy can be treated with amphetamine-like stimulants, including **modafinil, methylphenidate (Ritalin),** and **methamphetamine. Gamma hydroxybutyrate (GHB)** is used to reduce the frequency of cataplexy attacks and the incidences of daytime sleepiness. Cataplexy is often treated with antidepressants such as **imipramine** and **desipramine,** but these drugs are not officially approved by the US Federal Drug Administration for such use. The most common treatment for OSA is **continuous positive airflow pressure (CPAP),** a machine that increases airway pressure to prevent airway collapse. Drugs have generally proven to have little or no benefit in treating OSA. **Dopamine agonists,** which are used to treat Parkinson disease, can be used to treat PLMD and restless leg syndrome.

CIRCADIAN RHYTHMS

ROLE OF SUPRACHIASMATIC NUCLEI

Most, if not all, living cells in plants and animals have rhythmic fluctuations in their function on a circadian cycle. Normally they become entrained or synchronized to the day–night light cycle in the environment. If they are not entrained, they become progressively more out of phase with the light–dark cycle because they are longer or shorter than 24 h. In most cases, the **suprachiasmatic nuclei (SCN)** play a major role in the entrainment process **(Figure 14–10)**. The SCN receive information about the light–dark cycle via a special neural pathway, the **retinohypothalamic fibers.** Efferent fibers from the SCN initiate neural and humoral signals that entrain a wide variety of well-known circadian rhythms including the sleep–wake cycle and **melatonin** release from the richly vascularized pineal gland.

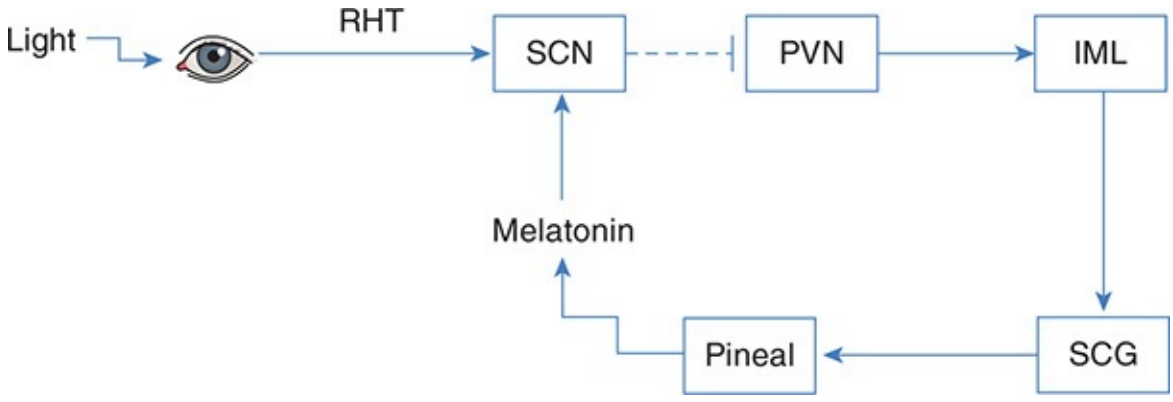


FIGURE 14–10 Secretion of melatonin. The cyclic activity of the suprachiasmatic nucleus (SCN) sets up a circadian rhythm for melatonin release. This rhythm is entrained to light/dark cycles by neurons in the retina. Light signals are relayed via the retinohypothalamic (RHT) fibers to the SCN. GABAergic neurons in the SCN inhibit neurons in the hypothalamic paraventricular nucleus (PVN) which then reduces the activity of sympathetic preganglionic neurons in the spinal intermediolateral nucleus (IML). These sympathetic preganglionic neurons innervate postganglionic neurons in the superior cervical ganglion (SCG) that regulate release of melatonin from the pineal gland.

GABAergic neurons in the SCN inhibit neurons in the hypothalamic paraventricular nucleus. From the hypothalamus, descending pathways converge onto preganglionic sympathetic neurons that innervate the superior cervical ganglion, the site of origin of the postganglionic neurons to the pineal gland.

The SCN have two peaks of circadian activity that may correlate with the observation that exposure to bright light can either advance, delay, or have no effect on the sleep–wake cycle depending on the time of day when it is experienced. During the usual daytime it has no effect, just after dark it delays the onset of the sleep period, and just before dawn it accelerates the onset of the next sleep period. Injections of melatonin have similar effects. **Clinical Box 14–4** describes circadian rhythm disorders that impact the sleep–wake state.

MELATONIN & CIRCADIAN RHYTHMS

Pineal pinealocytes contain melatonin and the enzymes responsible for its synthesis from serotonin by N-acetylation and O-methylation, and they secrete the hormone into the blood and the cerebrospinal fluid **(Figure 14–11)**. Two melatonin G-protein-coupled receptors (MT_1 and MT_2) are found on neurons in the SCN. Activation of MT_1 receptors inhibits adenylyl cyclase and results in sleepiness. Activation of MT_2 receptors stimulates phosphoinositide hydrolysis and may function to synchronize the light–dark cycle.

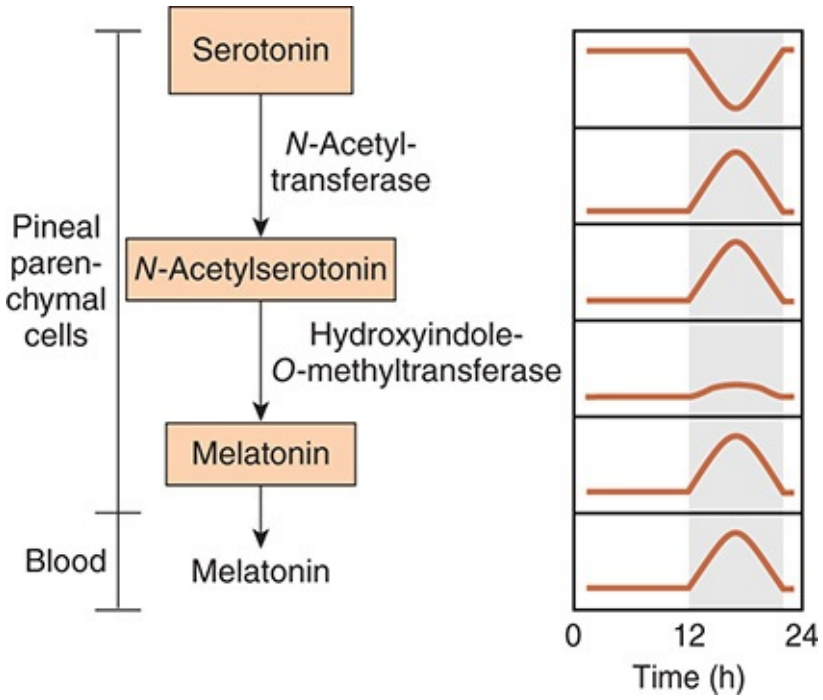


FIGURE 14–11 Diurnal rhythms of compounds involved in melatonin synthesis in the pineal. Melatonin and the enzymes responsible for its synthesis from serotonin are found in pineal pinealocytes; melatonin is secreted into the bloodstream. Melatonin synthesis and secretion are increased during the dark period (shaded area) and maintained at a low level during the light period.

The diurnal change in melatonin secretion may function as a timing signal to coordinate events with the light–dark cycle in the environment. Melatonin synthesis and secretion are increased during the dark period of the day and maintained at a low level during daylight hours (Figure 14–11). This diurnal variation in secretion is due to norepinephrine released from postganglionic sympathetic nerves that innervate the pineal gland (Figure 14–10). Norepinephrine acts via β-adrenoceptors to increase intracellular cAMP, and the cAMP in turn produces a marked increase in *N*-acetyltransferase activity. This results in increased melatonin synthesis and secretion.

CLINICAL BOX 14–4

Insomnia & Circadian Rhythm Disturbances of the Sleep–Wake State

Insomnia is defined as difficulty in initiating and/or maintaining sleep several times a week. Nearly 30% of the adult population experience episodes

of insomnia, and more than 50% of those aged 65 or older have sleep problems. Individuals with persistent episodes of insomnia are more likely to experience accidents, a diminished work experience, and a poorer overall quality of life. Insomnia is often comorbid with **depression,** and both disorders show abnormal regulation of **corticotropin-releasing factor.**

There are two major types of sleep disorders associated with disruption of the circadian rhythm. These are **transient sleep disorders** (jet lag, altered sleep cycle because of shift work, and illness) and **chronic sleep disorders (delayed or advanced sleep phase syndrome).** Those with delayed sleep phase syndrome have the inability to fall asleep in the evenings and awaken in the mornings. However, they have a normal total sleep time. Those with advanced sleep phase syndrome consistently fall asleep in early evening and awaken in early morning. This is seen most often in the elderly and the depressed.

THERAPEUTIC HIGHLIGHTS

Light therapy is an effective treatment in individuals who experience disturbances in their circadian cycle. **Melatonin** can be used to treat jet lag and insomnia in elderly individuals. **Ramelteon** is a MT_1 and MT_2 melatonin receptor agonist that is more effective than melatonin in treating insomnia. **Zolpidem** (Ambien) is an example of a sedative-hypnotic that slows brain activity to promote sleep onset. In addition to treating daytime sleepiness in narcolepsy, **modafinil** has also been used successfully in the treatment of daytime sleepiness due to shift work and to treat delayed sleep disorder syndrome.

CHAPTER SUMMARY

- The thalamus is the gateway to the cortex and includes neurons that project diffusely to wide areas of the neocortex (midline and intralaminar nuclei) and neurons that project to discrete regions of the neocortex (specific sensory relay nuclei).
- The ascending arousal system is comprised of monoaminergic, cholinergic, and histaminergic neurons that project to the intralaminar and reticular nuclei of the thalamus that project diffusely to wide regions of the cortex including the frontal, parietal, temporal, and occipital cortices.

- Wakefulness and REM sleep are at opposite extremes of consciousness. When norepinephrine- and serotonin-containing neurons (locus coeruleus and raphé nuclei) are most active, pontine cholinergic neurons are less active and wakefulness ensues. The reverse of this pattern leads to REM sleep. A more even balance of the activity in these groups of neurons is associated with non-REM sleep. Increases in GABA and decreases in histamine also promote non-REM sleep via deactivation of the thalamus and cortex.
- The EEG reflects the electrical activity (summation of dendritic postsynaptic potentials) of the brain and can be of value in localizing pathologic processes and in characterizing different types of seizures.
- Partial (focal) seizures originate in a small group of neurons and are subdivided into simple (without loss of consciousness) and complex (with altered consciousness). Generalized seizures are associated with widespread electrical activity and are subdivided into convulsive and non-convulsive categories depending on whether tonic or clonic movements occur.
- The major rhythms in the EEG are alpha (8–13 Hz) when awake with eyes closed, beta (13–30 Hz) when alert; theta (4–7 Hz) and delta (0.5–4 Hz) oscillations appear during deep sleep.
- Throughout non-REM sleep, there is some activity of skeletal muscle. A theta rhythm can be seen during stage 1 of sleep. Stage 2 is marked by the appearance of sleep spindles and occasional K complexes. In stage 3, a delta rhythm is dominant. Maximum slowing with large slow waves is seen in stage 4. REM sleep is characterized by low-voltage, high-frequency EEG activity and rapid, roving movements of the eyes.
- A young adult typically passes through stages 1 and 2, and spends 70–100 min in stages 3 and 4. Sleep then lightens, and a REM period follows. This cycle repeats at 90-min intervals throughout the night. REM sleep occupies 50% of total sleep time in full-term neonates; this proportion declines rapidly and plateaus at about 25% until it falls further in old age.
- Narcolepsy is a sudden loss of voluntary muscle tone (cataplexy), an irresistible urge to sleep during daytime, and a sudden onset of REM sleep. OSA is the most common cause of daytime sleepiness; breathing ceases for more than 10 s during frequent episodes of obstruction of the upper airway. Compared to normal sleep patterns, individuals spend more time in stage 1 non-REM sleep and less time in stages 3 and 4 non-REM sleep. PLMD is a stereotypical rhythmic extension of the big toe and dorsiflexion of the ankle

and knee during sleep lasting for about 0.5–10 s and recurring at intervals of 20–90 s.

- The entrainment of biologic processes to the light–dark cycle is regulated by the SCN. The diurnal change in melatonin release from the pineal gland may coordinate events with the light–dark cycle.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. An MD/PhD student was comparing sleep patterns in different age groups. What is expected to be the different about sleep as a function of age?

   A. Non-REM sleep occupies about 50% of total sleep in young adults and falls to about 20% in the elderly.

   B. Young adults experience about 4–6 episodes of REM sleep each night, and infants experience about 5–10 episodes of REM each night.

   C. REM sleep occupies about 80% of total sleep in full-term infants and falls to about 20% in the elderly.

   D. REM sleep occupies about 80% of total sleep in premature infants and falls until it plateaus at about 25% in adults.

   E. In a typical night, a young adult spends 70–100 min in stages 3 and 4 of non-REM sleep, whereas a child spends only about 30 min in these stages of deep sleep.

2. An MD/PhD student was studying the role of thalamocortical pathways in control of arousal. He was preparing his thesis proposal and included the following information on the two major types of thalamic projections to the cortex.

   A. Most thalamic neurons release glutamate throughout the cortex, but thalamic reticular neurons release GABA in the cortex.

   B. Afferents from the specific nuclei of the thalamus terminate in cortical layer I–IV, whereas the nonspecific afferents terminate primarily on spiny stellate cells of layer IV.

   C. Afferents from the specific nuclei of the thalamus terminate primarily on pyramidal neurons in layer IV, whereas the nonspecific afferents terminate primarily on inhibitory basket calls of layer IV.

   D. Thalamic projections to wide regions of the neocortex originate from the midline and intralaminar nuclei; thalamic projections to discrete regions of

the neocortex originate in specific sensory relay nuclei.

3. In a healthy, alert adult sitting with their eyes closed, the dominant EEG rhythm observed with electrodes over the occipital lobes is

   A. delta (0.5–4 Hz).

   B. theta (4–7 Hz).

   C. alpha (8–13 Hz).

   D. beta (18–30 Hz).

   E. fast, irregular low-voltage activity.

4. A 35-year-old man spent the evening in a sleep clinic to determine if he had obstructive sleep apnea. The tests showed that non-REM sleep accounted for over 30% of his total sleep time. Which of the following pattern of changes in central neurotransmitters or neuromodulators are associated with the transition from non-REM to wakefulness?

   A. Decrease in norepinephrine, increase in serotonin, increase in acetylcholine, decrease in histamine, and decrease in GABA.

   B. Decrease in norepinephrine, increase in serotonin, increase in acetylcholine, decrease in histamine, and increase in GABA.

   C. Decrease in norepinephrine, decrease in serotonin, increase in acetylcholine, increase in histamine, and increase in GABA.

   D. Increase in norepinephrine, increase in serotonin, decrease in acetylcholine, increase in histamine, and decrease in GABA.

   E. Increase in norepinephrine, decrease in serotonin, decrease in acetylcholine, increase in histamine, and decrease in GABA.

5. A healthy medical student participated in a research project on the effect of sleep deprivation on their EEG. During the baseline testing phase, no abnormalities were found. The following behavioral and EEG characteristics were likely recorded during stage 2 of non-REM sleep.

   A. Skeletal muscle movements were observed and the EEG showed a mixture of sleep spindles and theta waves.

   B. Skeletal muscle tone was reduced and sleep spindles and K complexes appeared in the EEG.

   C. Skeletal muscle movements were detected and the dominant rhythm in the EEG was delta waves.

   D. There was a complete absence of eye movements and the dominant rhythm in the EEG was theta waves.

E. There was an absence of skeletal muscle and eye movements and the EEG was desynchronized.

6. For the past several months, a 67-year-old woman experienced difficulty initiating and/or maintaining sleep several times a week. A friend suggested that she take melatonin to regulate her sleep–wake cycle. Endogenous melatonin secretion would be increased by

A. reducing the synthesis of serotonin.

B. inhibition of the paraventricular nucleus.

C. stimulation of the superior cervical ganglion.

D. stimulation of the optic nerve.

E. by blockade of hydroxyindole-*O*-methyltransferase.

7. Childhood absence epilepsy was diagnosed in a 10-year-old boy. His EEG showed a bilateral synchronous, symmetric 3-Hz spike-and-wave discharge. Absence seizures are a form of

A. nonconvulsive generalized seizures accompanied by momentary loss of consciousness.

B. complex partial seizures accompanied by momentary loss of consciousness.

C. nonconvulsive generalized seizures without a loss of consciousness.

D. simple partial seizures without a loss of consciousness.

E. convulsive generalized seizures accompanied by momentary loss of consciousness.

8. A child diagnosed with absence epilepsy began treatment with ethosuximide. What is the mechanism of action by which ethosuximide is an effective antiseizure drug?

A. It is a GABA analog that decreases Ca^{2+} entry into cells.

B. It blocks voltage-gated Na^{+} channels associated with glutamate receptors.

C. It potentiates GABA transmission.

D. It is a dopamine receptor agonist.

E. It inhibits T-type Ca^{2+} channels.

9. A 57-year-old professor at a medical school experienced numerous episodes of a sudden loss of muscle tone and an irresistible urge to sleep in the middle of the afternoon. The diagnosis was narcolepsy, which

A. is characterized by a sudden onset of non-REM sleep.

B. has a familial incidence associated with a class II antigen of the major histocompatibility complex.

C. may be due to the presence of an excessive number of orexin-producing neurons in the hypothalamus.

D. is often effectively treated with dopamine receptor agonists.

E. is the most common cause of daytime sleepiness.

CHAPTER 15

Learning, Memory, Language, & Speech

OBJECTIVES

After studying this chapter, you should be able to:

- Describe the role of brain imaging techniques in identifying normal brain function and changes caused by brain damage.
- List the common causes, symptoms, and methods to assess traumatic brain injury (TBI).
- Describe the various forms of memory and identify the parts of the brain involved in memory processing and storage.
- Define synaptic plasticity, long-term potentiation (LTP), long-term depression (LTD), habituation, and sensitization, and explain their roles in learning and memory.
- Identify the abnormalities of brain structure and function that are characteristic of Alzheimer disease.
- Define the terms categorical hemisphere and representational hemisphere and summarize the differences between them.
- Identify the cortical areas important for language and their interconnections.
- Summarize the differences between fluent and nonfluent aphasia and explain each type on the basis of its pathophysiology.

INTRODUCTION

The understanding of brain function in humans has been revolutionized by the development and widespread availability of **positron emission tomographic (PET), functional magnetic resonance imaging (fMRI), computed tomography (CT) scanning,** and other imaging and diagnostic techniques. CT scans provide a high-resolution 3-dimensional image of the brain; it is useful for examining damage to the skull and detecting acute subarachnoid hemorrhage. PET imaging can measure local glucose metabolism, blood flow, and oxygen; fMRI can measure local amounts of oxygenated blood. PET and fMRI provide an index of the level of the activity in various parts of the brain in healthy humans and in those with pathologies or brain injuries **(see Clinical Box 15–1)**. They are used to study not only simple responses but also complex aspects of learning, memory, and perception. Different portions of the cortex are activated when a person is hearing, seeing, speaking, or generating words. **Figure 15–1** shows examples of the use of imaging to compare the functions of the cerebral cortex in processing words in a male versus a female subject.

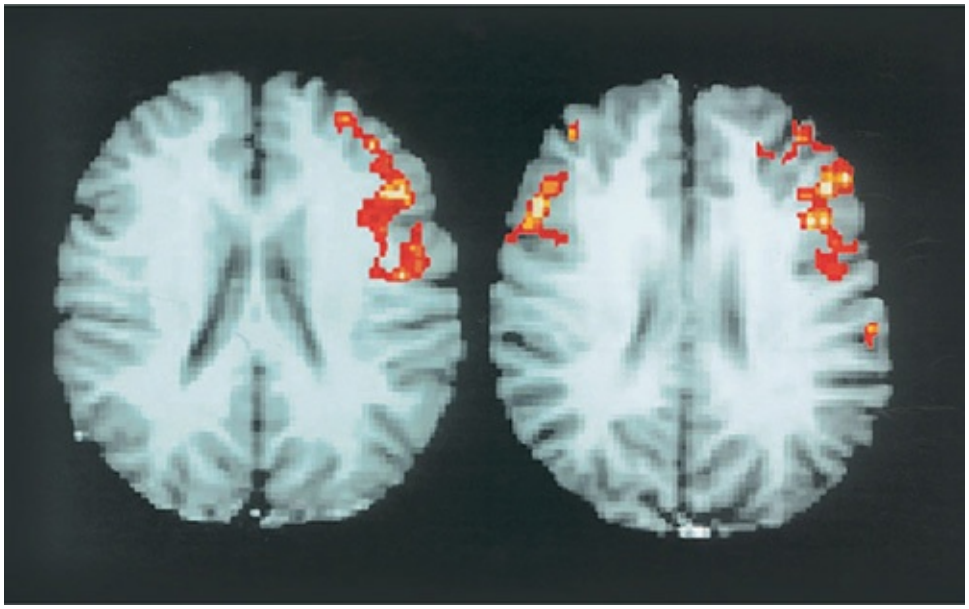


FIGURE 15–1 Comparison of the images of the active areas of the brain in a man (left) and a woman (right) during a language-based activity. Women use both sides of their brain whereas men use only a single side. This difference may reflect different strategies used for language processing. (Used with permission of Shaywitz et al, 1995. NMR Research/Yale Medical School.)

CLINICAL BOX 15–1

Traumatic Brain Injury

Traumatic brain injury (TBI) is defined as a nondegenerative, noncongenital insult to the brain due to an excessive mechanical force or penetrating injury to the head. It can lead to a permanent or temporary impairment of cognitive, physical, emotional, and behavioral functions, and it can be associated with a diminished or altered state of consciousness. TBI is one of the leading causes of death or disability worldwide. According to the Centers for Disease Control and Prevention, each year at least 1.5 million individuals in the United States sustain a TBI. It is most common in children under age 4, in adolescents aged 15–19 years of age, and in adults over the age of 65. In all age groups, TBI occurs twice as often in males compared to females. In about 75% of the cases, the TBI is considered mild and manifests as a concussion. Adults with severe TBI who are treated have a mortality rate of about 30%, but about 50% regain most if not all of their functions with therapy. The leading causes of TBI include falls, motor vehicle accidents, being struck by an object, and assaults. In some cases, areas remote from the actual injury also begin to malfunction, a process called **diaschisis.** TBI is often divided into primary and secondary stages. Primary injury is that caused by the mechanical force (eg, skull fracture and surface contusions) or acceleration–deceleration due to unrestricted movement of the head leading to shear, tensile, and compressive strains. These injuries can cause **intracranial hematoma** (epidural, subdural, or subarachnoid) and **diffuse axonal injury.** Secondary injury is often a delayed response and may be due to impaired cerebral blood flow that can eventually lead to cell death. A **Glasgow Coma Scale** is the most common system used to define the severity of TBI and evaluates motor responses, verbal responses, and eye opening to assess the levels of consciousness and neurologic functioning after an injury. Symptoms of mild TBI include headache, confusion, dizziness, blurred vision, ringing in the ears, a bad taste in the mouth, fatigue, disturbances in sleep, mood changes, and problems with memory, concentration, or thinking. Individuals with moderate or severe TBI show these symptoms as well as vomiting or nausea, convulsions or seizures, an inability to be roused, fixed and dilated pupils, slurred speech, limb weakness, loss of coordination, and increased confusion, restlessness, or agitation. In the most severe cases of TBI, the affected individual may go into a **permanent vegetative state**.

THERAPEUTIC HIGHLIGHTS

The advancements in brain imaging technology have improved the ability of medical personnel to diagnose and evaluate the extent of brain damage. Since little can be done to reverse the brain damage, therapy is initially directed at stabilizing the patient and trying to prevent further (secondary) injury. Medications that can be administered include diuretics (to reduce pressure in the brain), anticonvulsant drugs during the first week post injury (to avoid additional brain damage resulting from a seizure), and coma-inducing drugs (to reduce oxygen demands). This is followed by rehabilitation that includes physical, occupational, and speech/language therapies. Recovery of brain function can be due to several factors: brain regions that were suppressed but not damaged can regain their function, axonal sprouting and redundancy allows other areas of the brain to take over the functions that were lost due to the injury, and behavioral substitution, by learning new strategies to compensate for the deficits.

LEARNING & MEMORY

A characteristic of humans is their ability to alter behavior based on experience. **Learning** is acquisition of the information that makes this possible and **memory** is the retention and storage of that information. The two are obviously closely related and are considered together in this chapter.

FORMS OF MEMORY

From a physiological point of view, memory is divided into explicit and implicit forms **(Figure 15–2)**. **Explicit** or **declarative memory** is associated with consciousness, or at least awareness, and is dependent on the **hippocampus** and other parts of the **medial temporal lobes** of the brain for its retention. **Clinical Box 15–2** describes how tracking a patient with brain damage has led to an awareness of the role of the temporal lobe in declarative memory. **Implicit** or **nondeclarative memory** does not involve awareness, and its retention does not usually involve processing in the hippocampus.

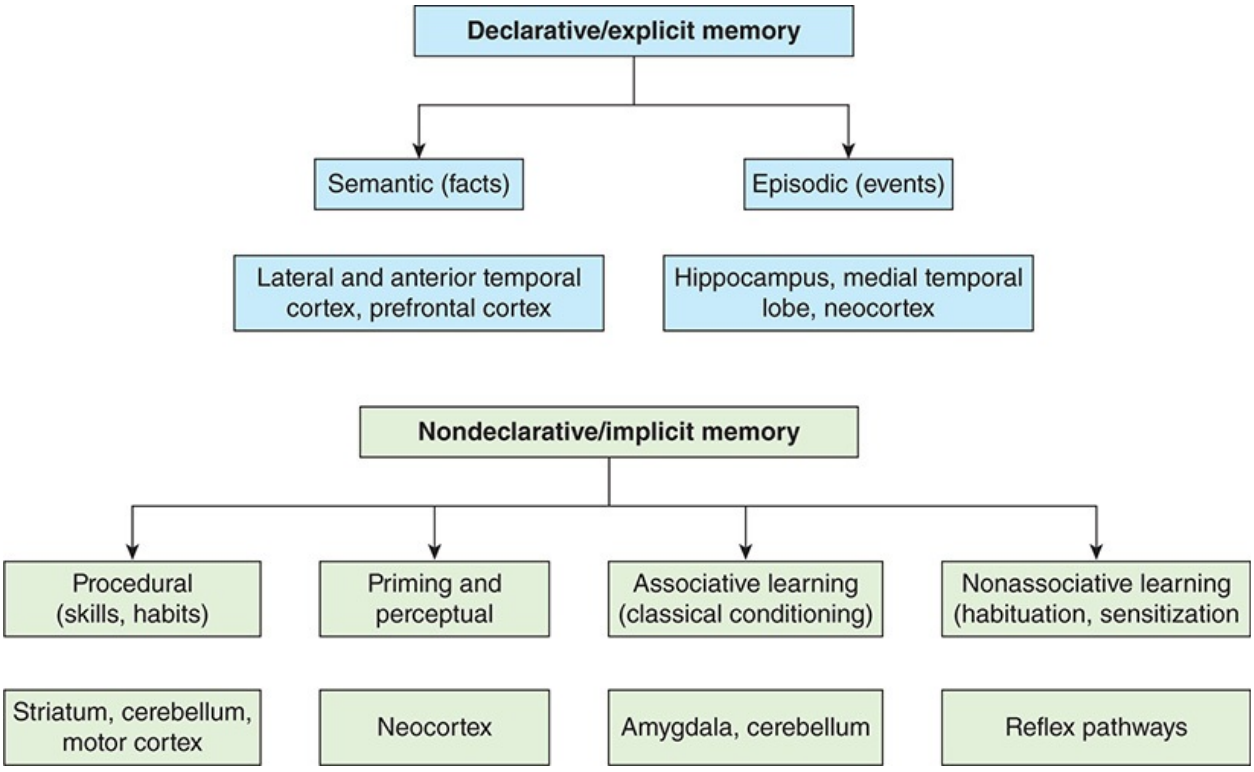


FIGURE 15–2 Forms of memory. Explicit (declarative) memory is associated with consciousness and is dependent on the integrity of the hippocampus, temporal lobes, neocortex, and prefrontal cortex for its retention. Explicit memory is for factual knowledge about people, places, things, and events. Implicit (nondeclarative) memory does not involve awareness, and it does not involve processing in the hippocampus. It requires the integrity of the amygdala, cerebellum, striatum, and neocortex as well as reflex pathways. Implicit memory is important for training reflexive motor or perceptual skills, classical conditioning, and habituation and sensitization. (Modified with permission from Kandel ER, Schwartz JH, Jessell TM [editors]: *Principles of Neural Science*, 4th ed. New York, NY: McGraw-Hill; 2000.)

Explicit memory is for factual knowledge about people, places, and things. It is divided into **semantic memory** for facts (eg, words, rules, and language) and **episodic memory** for events. Explicit memories that are initially required for activities such as riding a bicycle can become implicit once the task is thoroughly learned.

Implicit memory is important for training reflexive motor or perceptual skills and is subdivided into four types. **Priming** is the facilitation of the recognition of words or objects by prior exposure to them and is dependent on the **neocortex.** An example of priming is the improved recall of a word when presented with the

first few letters of it. **Procedural memory** includes skills and habits, which, once acquired, become unconscious and automatic. This type of memory is processed in the **striatum. Associative learning** relates to **classical** and **operant conditioning** in which one learns about the relationship between one stimulus and another. This type of memory is dependent on the **amygdala** for its emotional responses and the **cerebellum** for the motor responses. **Nonassociative** learning includes **habituation** and **sensitization** and is dependent on various reflex pathways.

CLINICAL BOX 15–2

The Case of HM: Defining a Link between Brain Function & Memory

HM was a patient who suffered from bilateral temporal lobe seizures that began following a bicycle accident at age 9. His case has been studied by many scientists and has led to a greater understanding of the link between the **temporal lobe** and **declarative memory.** HM had partial seizures for many years, and then several tonic-clonic seizures by age 16. In 1953, at the age of 27, HM underwent bilateral surgical removal of the amygdala, large portions of the hippocampal formation, and portions of the association area of the temporal cortex. HM's seizures were better controlled after surgery, but removal of the temporal lobes led to devastating memory deficits. He maintained **long-term memory** for events that occurred prior to surgery, but he suffered from **anterograde amnesia.** His **short-term memory** was intact, but he could not commit new events to long-term memory. He had normal procedural memory, and he could learn new puzzles and motor tasks. His case was the first to bring attention to the critical role of temporal lobes in formation of long-term declarative memories and to implicate this region in the conversion of short-term to long-term memories. Later work showed that the **hippocampus** is the primary structure within the temporal lobe involved in this conversion. Because HM retained memories from before surgery, his case also shows that the hippocampus is not involved in the storage of declarative memory. HM died in 2008 and only at that time was his identity released; his full name was Henry Gustav Molaison. An audio-recording and a transcript of the dialog by National Public Radio from the 1990s of HM talking to scientists was released in 2007 and is available at http://www.npr.org/templates/story/story.php?storyId=7584970.

Explicit memory and many forms of implicit memory involve (1) **short-term memory,** which lasts seconds to hours, during which processing in the hippocampus and elsewhere lays down long-term changes in synaptic strength and (2) **long-term memory,** which stores memories for years and sometimes for life. During short-term memory, the memory traces are subject to disruption by trauma and various drugs, whereas long-term memory traces are remarkably resistant to disruption. **Working memory** is a form of short-term memory that keeps information available, usually for very short periods of time, while the individual plans action based on it.

NEURAL BASIS OF MEMORY

The key to memory is alteration in the strength of selected synaptic connections. Second messenger systems contribute to the changes in neural circuitry required for learning and memory. Alterations in cellular membrane channels are often correlated to learning and memory. In all but the simplest of cases, the alteration involves the synthesis of proteins and the activation of genes. This occurs during the change from short-term working memory to long-term memory.

Acquisition of long-term learned responses can be prevented in some cases. For example, there is a loss of memory for the events immediately preceding a brain concussion or electroshock therapy **(retrograde amnesia).** This amnesia can actually encompass many days preceding the event that triggered it; remote memories remain intact.

SYNAPTIC PLASTICITY & LEARNING

Short- and long-term changes in synaptic function can occur because of the history of discharge at a synapse; that is, synaptic conduction can be strengthened or weakened on the basis of past experience. These changes are of great interest because they represent forms of learning and memory. They can be presynaptic or postsynaptic in location.

One form of plastic change is **posttetanic potentiation,** the production of enhanced postsynaptic potentials in response to stimulation. This enhancement lasts up to 60 s and occurs after a brief tetanizing train of stimuli in the presynaptic neuron. The tetanizing stimulation causes Ca^{2+} to accumulate in the presynaptic neuron to such a degree that the intracellular binding sites that keep cytoplasmic Ca^{2+} low are overwhelmed.

Habituation is a simple form of learning in which a neutral stimulus is repeated many times. The first time it is applied it is novel and evokes a reaction (the orienting reflex or "what is it?" response). However, it evokes less and less electrical response as it is repeated. Eventually, the subject becomes habituated to the stimulus and ignores it. This is associated with decreased release of neurotransmitter from the presynaptic terminal because of decreased intracellular Ca^{2+}. The decrease in intracellular Ca^{2+} is due to a gradual inactivation of Ca^{2+} channels. It can be short term, or it can be prolonged if exposure to the benign stimulus is repeated many times. Habituation is a classic example of nonassociative learning.

Sensitization is in a sense the opposite of habituation. Sensitization is the prolonged occurrence of augmented postsynaptic responses after a stimulus to which one has become habituated is paired once or several times with a noxious stimulus. The sensitization is due to presynaptic facilitation. Sensitization may occur as a transient response, or if it is reinforced by additional pairings of the noxious stimulus and the initial stimulus, it can exhibit features of short-term or long-term memory. The short-term prolongation of sensitization is due to a Ca^{2+}-mediated change in adenylyl cyclase that leads to a greater production of cAMP. The **long-term potentiation (LTP)** also involves protein synthesis and growth of the presynaptic and postsynaptic neurons and their connections.

LTP is a rapidly developing persistent enhancement of the postsynaptic potential response to presynaptic stimulation after a brief period of rapidly repeated stimulation of the presynaptic neuron. It resembles posttetanic potentiation but is much more prolonged and can last for days. There are multiple mechanisms by which LTP can occur, some are dependent on changes in the **N-methyl-D-aspartate (NMDA) receptor** and some are independent of the NMDA receptor. LTP is initiated by an increase in intracellular Ca^{2+} in either the presynaptic or postsynaptic neuron.

LTP occurs in many parts of the nervous system but has been studied in greatest detail in a synapse within the hippocampus, specifically the connection of a pyramidal cell in the CA3 region and a pyramidal cell in the CA1 region via the **Schaffer collateral.** This is an example of an NMDA receptor-dependent form of LTP involving an increase in Ca^{2+} in the postsynaptic neuron. Recall that NMDA receptors are permeable to Ca^{2+} as well as to Na^{+} and K^{+}. **Figure 15–3** summarizes the hypothetical basis of the Schaffer collateral LTP. At the resting membrane potential, glutamate release from a presynaptic neuron binds to both NMDA and non-NMDA receptors on the postsynaptic neuron. In the case of the Schaffer collateral, the non-NMDA receptor of interest is the **α-amino-3-**

hydroxy-5-methylisoxazole-4 propionic acid (AMPA) receptor. Na^+ and K^+ can flow only through the AMPA receptor because the presence of Mg^{2+} on the NMDA receptor blocks it. However, the membrane depolarization that occurs in response to high frequency tetanic stimulation of the presynaptic neuron is sufficient to expel the Mg^{2+} from the NMDA receptor, allowing the influx of Ca^{2+} into the postsynaptic neuron. This leads to activation of Ca^{2+}/calmodulin kinase, protein kinase C, and tyrosine kinase which together induce LTP. The Ca^{2+}/calmodulin kinase phosphorylates the AMPA receptors, increasing their conductance, and moves more of these receptors into the synaptic cell membrane from cytoplasmic storage sites. Once LTP is induced, a chemical signal (possibly nitric oxide, NO) is released by the postsynaptic neuron and passes retrogradely to the presynaptic neuron, producing a long-term increase in the quantal release of glutamate.

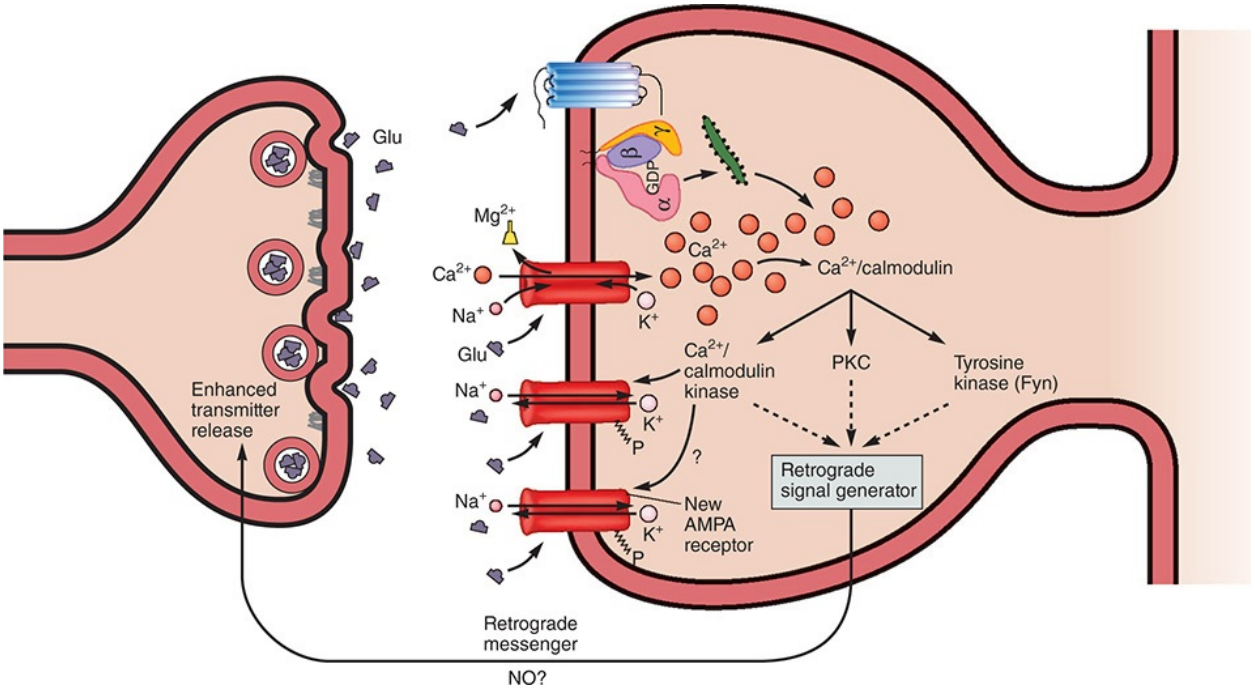


FIGURE 15–3 Production of LTP in Schaffer collaterals in the hippocampus. Glutamate (Glu) released from the presynaptic neuron binds to AMPA and NMDA receptors in the membrane of the postsynaptic neuron. The depolarization triggered by activation of the AMPA receptors relieves the Mg^{2+} block in the NMDA receptor channel, and Ca^{2+} enters the neuron with Na^+. The increase in cytoplasmic Ca^{2+} activates Ca^{2+}/calmodulin kinase, protein kinase C, and tyrosine kinase which together induce LTP. The Ca^{2+}/calmodulin kinase II phosphorylates the AMPA receptors, increasing their conductance, and moves

more AMPA receptors into the synaptic cell membrane from cytoplasmic storage sites. In addition, once LTF is induced, a chemical signal (possibly nitric oxide, NO) is released by the postsynaptic neuron and passes retrogradely to the presynaptic neuron, producing a long-term increase in the quantal release of glutamate. AMPA, α-amino-3-hydroxy-5-methylisoxazole-4 propionic acid; LTP, long-term potentiation; NMDA, N-methyl-D-aspartate.

LTP identified in the mossy fibers of the hippocampus (connecting granule cells in the dentate cortex) is due to an increase in Ca^{2+} in the presynaptic rather than the postsynaptic neuron in response to tetanic stimulation and is independent of NMDA receptors. The influx of Ca^{2+} in the presynaptic neuron is thought to activate Ca^{2+}/calmodulin-dependent adenylyl cyclase to increase cAMP.

Long-term depression (LTD) was first noted in the hippocampus but occurs throughout the brain in the same fibers as LTP. LTD is the opposite of LTP. It resembles LTP in many ways, but it is characterized by a decrease in synaptic strength. It is produced by slower stimulation of presynaptic neurons and is associated with a smaller rise in intracellular Ca^{2+} than occurs in LTP. In the cerebellum, its occurrence requires the phosphorylation of the GluR2 subunit of the AMPA receptors. It may be part of the mechanism by which learning occurs in the cerebellum.

NEUROGENESIS

The traditional view that brain cells are not added after birth is wrong; new neurons form from stem cells throughout life in at least two areas: the olfactory bulb and the hippocampus. This is a process called **neurogenesis.** Experience-dependent growth of new granule cells in the dentate gyrus of the hippocampus may contribute to learning and memory. A reduction in the number of new neurons formed reduces at least one form of hippocampal memory production.

ASSOCIATIVE LEARNING: CONDITIONED REFLEXES

A classic example of associative learning is a **conditioned reflex.** A conditioned reflex is a reflex response to a stimulus that previously elicited little or no response, acquired by repeatedly pairing the stimulus with another stimulus that

normally does produce the response. In Pavlov's classic experiments, the salivation normally induced by placing meat in the mouth of a dog was studied. A bell was rung just before the meat was placed in the dog's mouth, and this was repeated several times until the animal would salivate when the bell was rung even though no meat was placed in its mouth. In this experiment, the meat placed in the mouth was the **unconditioned stimulus (US),** the stimulus that normally produces an innate response. The **conditioned stimulus (CS)** was the bell ringing. After the CS and US had been paired a sufficient number of times, the CS produced the response originally evoked only by the US. The CS had to precede the US. Many somatic, visceral, and other neural changes can be made to occur as conditioned reflex responses. Conditioning of visceral responses is called **biofeedback.**

WORKING MEMORY

As noted above, working memory keeps incoming information available for a short time while deciding what to do with it. It is that form of memory which permits us, for example, to look up a telephone number, and then remember the number while we pick up the telephone and dial the number. It consists of a **central executive** located in the prefrontal cortex, and two "rehearsal systems": a **verbal system** for retaining verbal memories and a parallel **visuospatial system** for retaining visual and spatial aspects of objects. The executive steers information into these rehearsal systems.

HIPPOCAMPUS & MEDIAL TEMPORAL LOBE

Working memory areas are connected to the hippocampus and the adjacent parahippocampal portions of the medial temporal cortex. Output from the hippocampus leaves via the subiculum and the entorhinal cortex and binds together and strengthens circuits in different neocortical areas, forming over time the stable remote memories that can now be triggered by many different cues.

Bilateral destruction of the ventral hippocampus, or Alzheimer disease and similar disease processes that destroy its CA1 neurons, can cause striking defects in short-term memory. Humans with such destruction have intact working memory and remote memory. Their implicit memory processes are generally intact. They perform adequately in terms of conscious memory if they concentrate on what they are doing. However, if they are distracted even briefly,

all memory of what they were doing and what they proposed to do is lost. They are thus capable of new learning and retain old prelesion memories, but they cannot form new long-term memories.

The hippocampus is closely associated with the overlying parahippocampal cortex in the medial frontal lobe. Memory processes can be studied with fMRI and with measurement of evoked potentials (event-related potentials; ERPs) in epileptic patients with implanted electrodes. When subjects recall words, activity in their left frontal lobe and their left parahippocampal cortex increases. In contrast, when they recall pictures or scenes, activity takes place in their right frontal lobe and the parahippocampal cortex on both sides.

The connections of the hippocampus to the diencephalon are also involved in memory. Some people with alcoholism-related brain damage develop impairment of recent memory, and the memory loss correlates well with the presence of pathologic changes in the mamillary bodies that have extensive efferent connections to the hippocampus via the fornix. The mamillary bodies project to the anterior thalamus via the mamillothalamic tract, and lesions of the thalamus cause loss of recent memory. From the thalamus, the fibers concerned with memory project to the prefrontal cortex and from there to the basal forebrain. From the **nucleus basalis of Meynert** in the basal forebrain, a diffuse cholinergic projection goes to the entire neocortex, the amygdala, and the hippocampus. Severe loss of these fibers occurs in Alzheimer disease.

The amygdala is closely associated with the hippocampus and is concerned with encoding and recalling emotionally charged memories. During retrieval of fearful memories, the theta rhythms of the amygdala and the hippocampus become synchronized. In healthy subjects, events associated with strong emotions are remembered better than events without an emotional charge, but in patients with bilateral lesions of the amygdala, this difference is absent.

Individuals with lesions of the ventromedial prefrontal cortex perform poorly on memory tests, but they spontaneously describe events that never occurred. This phenomenon is called confabulation or false memories.

LONG-TERM MEMORY

While the encoding process for short-term explicit memory involves the hippocampus, long-term memories are stored in various parts of the neocortex. Apparently, the various parts of the memories (visual, olfactory, auditory, etc) are localized to the cortical regions concerned with these functions. These pieces are tied together by long-term changes in the strength of transmission at relevant

synaptic junctions so that all the components are brought to consciousness when the memory is recalled.

Once long-term memories have been established, they can be recalled or accessed by many different associations. For example, the memory of a vivid scene can be evoked not only by a similar scene but also by a sound or smell associated with the scene and by words such as “scene,” “vivid,” and “view.” Thus, each stored memory must have multiple routes or keys. Furthermore, many memories have an emotional component or “color,” that is, in simplest terms, memories can be pleasant or unpleasant.

STRANGENESS & FAMILIARITY

Stimulation of some parts of the temporal lobes causes a change in interpretation of one’s surroundings. For example, when the stimulus is applied, the subject may feel strange in a familiar place or may feel that what is happening now has happened before. The occurrence of a sense of familiarity or a sense of strangeness in appropriate situations may help the healthy individual adjust to the environment. In strange surroundings, one is alert and on guard, whereas in familiar surroundings, vigilance is relaxed. An inappropriate feeling of familiarity with new events or in new surroundings is known as the **déjà vu phenomenon** from the French words meaning “already seen.” This occurs occasionally in healthy persons, and it may also occur as an aura (a sensation immediately preceding a seizure) in patients with temporal lobe epilepsy.

ALZHEIMER DISEASE & SENILE DEMENTIA

Alzheimer disease is the most common age-related neurodegenerative disorder. Memory decline initially manifests as a loss of episodic memory, which impedes recollection of recent events. Loss of short-term memory is followed by general loss of cognitive and other brain functions, agitation, depression, the need for constant care, and, eventually, death. **Clinical Box 15–3** describes the etiology and therapeutic strategies for the treatment of Alzheimer disease.

The cytopathologic hallmarks of Alzheimer disease are intracellular **neurofibrillary tangles,** made up in part of hyperphosphorylated forms of the **tau protein** that normally binds to microtubules, and extracellular **amyloid plaques** that have a core of **β-amyloid peptides** surrounded by altered nerve fibers and reactive glial cells **(Figure 15–4)**. The β-amyloid peptides are

products of **amyloid precursor protein (APP),** a transmembrane protein that projects into the extracellular fluid from all nerve cells. This protein is hydrolyzed at three different sites by α-secretase, β-secretase, and γ-secretase, respectively. When APP is hydrolyzed by α-secretase, nontoxic peptide products are produced. However, when it is hydrolyzed by β-secretase and γ-secretase, polypeptides with 40–42 amino acids are produced; the actual length varies because of variation in the site at which γ-secretase cuts the protein chain. These polypeptides are toxic, the most toxic being $A\beta\sigma^{1-42}$. The polypeptides form extracellular aggregates that can stick to AMPA receptors and Ca^{2+} ion channels, increasing Ca^{2+} influx. The polypeptides also initiate an inflammatory response with production of intracellular tangles. The damaged cells eventually die, leading to a third characterization of the brain pathology in individuals with this neurodegenerative disease—atrophy associated with narrowing of the gyri, widening of the sulci, enlargement of the ventricles, and reduction in brain weight.

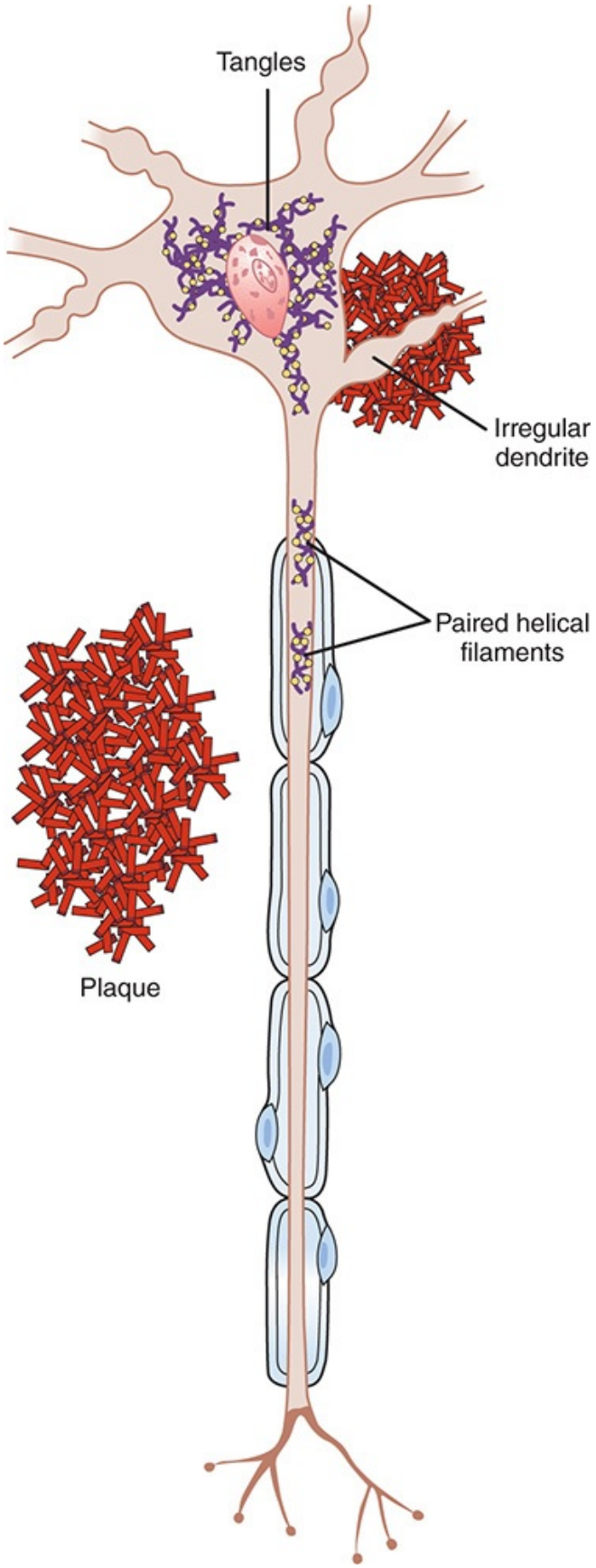

Tangles
Irregular dendrite
Paired helical filaments
Plaque

FIGURE 15–4 Abnormalities in a neuron are associated with Alzheimer disease. The cytopathologic hallmarks are intracellular neurofibrillary tangles and extracellular amyloid plaques that have a core of β-amyloid peptides surrounded by altered nerve fibers and reactive glial cells. A third characterization is brain atrophy. (Used with permission from Kandel ER, Schwartz JH, Jessell TM [editors]: *Principles of Neural Science*, 4th ed. New York, NY: McGraw-Hill; 2000.)

CLINICAL BOX 15–3

Alzheimer Disease

Alzheimer disease was originally characterized in middle-aged people, and similar deterioration in elderly individuals is technically **senile dementia** of the Alzheimer type, though it is frequently just called Alzheimer disease. Both genetic and environmental factors can contribute to the etiology of the disease **(Table 15–1)**. Most cases are sporadic, but a familial form of the disease (accounting for about 5% of the cases) is seen in an early-onset form of the disease. In these cases, the disease is caused by mutations in genes for the amyloid precursor protein on chromosome 21, presenilin 1 on chromosome 14, or presenilin 2 on chromosome 1. It is transmitted in an autosomal dominant mode, so offspring in the same generation have a 50/50 chance of developing familial Alzheimer disease if one of their parents is affected. Each mutation leads to an overproduction of the β-amyloid protein found in neuritic plaques. Senile dementia can be caused by vascular disease and other disorders, but Alzheimer disease is the most common cause, accounting for 50–60% of the cases. The most common risk factor for developing Alzheimer disease is age. This neurodegenerative disease is present in 8–17% of the population over the age of 65, with the incidence nearly doubling every 5 years after reaching the age of 60. In those who are 95 years of age and older, the incidence is 40–50%. It is estimated that by the year 2050, up to 16 million people age 65 and older in the United States will have Alzheimer disease. Although the prevalence of the disease appears to be higher in women, this may be due to their longer life span as the incidence rates are similar for men and women. Alzheimer disease plus the other forms of senile dementia are a major medical problem.

THERAPEUTIC HIGHLIGHTS

Research is aimed at identifying strategies to prevent the occurrence, delay the onset, slow the progression, or alleviate the symptoms of Alzheimer disease. The use of **acetylcholinesterase inhibitors** (eg, **donepezil, galantamine, rivastigmine,** or **tacrine**) in early stages of the disease increases the availability of acetylcholine in the synaptic cleft. This class of drugs has shown some promise in ameliorating global cognitive dysfunction, but not learning and memory impairments in these patients. These drugs also delay the worsening of symptoms for up to 12 months in about 50% of the cases studied. **Memantine** (an NMDA receptor antagonist) prevents glutamate-induced excitotoxicity in the brain and is used to treat moderate to severe Alzheimer disease. It delays but does not prevent worsening of symptoms (eg, loss of memory and confusion) in some patients. Drugs used to block the production of β-amyloid proteins are under development. Also attempts are underway to develop vaccines that would allow the body's immune system to produce antibodies to attack these proteins.

TABLE 15–1 Risk factors associated with the development and progression of Alzheimer disease.

Common	Genes	Environment
Age	ApoE4	Low education level
Family members with dementia	APP (chromosome 21) PS-1 (chromosome 14) PS-2 (chromosome 1) Down syndrome (trisomy 21)	Head trauma Prions Toxins Viruses

APP, amyloid precursor protein; ApoE4, apolipoprotein E4 allele; PS-1, presenilin-1; PS-2, presenilin-2.

An interesting finding that may well have broad physiologic implications is the observation that frequent effortful mental activities, such as doing difficult crossword puzzles and playing board games, slow the onset of cognitive dementia due to Alzheimer disease and vascular disease. The explanation for this "use it or lose it" phenomenon is unknown, but it suggests that the hippocampus and its connections have plasticity like other parts of the brain and skeletal and cardiac muscles.

LANGUAGE & SPEECH

Memory and learning are functions of large parts of the brain, but the centers controlling some of the other "higher functions of the nervous system," particularly the mechanisms related to language, are more or less localized to the neocortex. Speech and other intellectual functions are especially well developed in humans, the animal species in which the neocortical mantle is most highly developed.

COMPLEMENTARY SPECIALIZATION OF THE HEMISPHERES VERSUS "CEREBRAL DOMINANCE"

The term language includes the understanding of the spoken and printed word and expressing ideas in speech and writing. Human language functions depend more on one cerebral hemisphere than on the other. This hemisphere is concerned with categorization and symbolization and is called the **dominant hemisphere.** The **nondominant hemisphere** is specialized in the area of spatiotemporal relations; for example, it is involved in the identification of objects by their form and the recognition of musical themes and in facial recognition. Consequently, the concept of "cerebral dominance" and a dominant and nondominant hemisphere has been replaced by a concept of complementary specialization of the hemispheres, one for sequential-analytic processes (the **categorical hemisphere**) and one for visuospatial relations (the **representational hemisphere**). The categorical hemisphere is concerned with language functions. **Clinical Box 15–4** describes deficits that occur in subjects with representational or categorical hemisphere lesions.

In 96% of right-handed individuals (91% of the human population), the left hemisphere is the dominant or categorical hemisphere; and in the remaining 4%, the right hemisphere is dominant. In 70% of left-handed individuals, the left hemisphere is the dominant hemisphere; in 15% of left-handed persons, the right hemisphere is the categorical hemisphere and in remaining 15%, there is no clear lateralization. Learning disabilities such as **dyslexia** **(see Clinical Box 15–5),** an impaired ability to learn to read, are 12 times as common in left-handers compared to right-handers. The spatial talents of left-handers may be above average; a disproportionately large number of artists, musicians, and mathematicians are left-handed.

CLINICAL BOX 15–4

Lesions of Representational & Categorical Hemispheres

Lesions in the categorical hemisphere produce language disorders, whereas extensive lesions in the representational hemisphere do not. Instead, lesions in the representational hemisphere produce **astereognosis**—the inability to identify objects by feeling them—and other agnosias. **Agnosia** is the general term used for the inability to recognize objects by a particular sensory modality even though the sensory modality itself is intact. Lesions producing these defects are generally in the parietal lobe. Especially when they are in the representational hemisphere, lesions of the inferior parietal lobule, a region in the posterior part of the parietal lobe that is close to the occipital lobe, cause **unilateral inattention** and **neglect.** Individuals with such lesions do not have any apparent primary visual, auditory, or somatesthetic defects, but they ignore stimuli from one side of their bodies or the surrounding space. This leads to failure to care for half their bodies and, in extreme cases, to situations in which individuals shave half their faces, dress half their bodies, or read half of each page. This inability to put together a picture of visual space on one side is due to a shift in visual attention to the side of the brain lesion and can be improved, if not totally corrected, by wearing eyeglasses that contain prisms. Hemispheric specialization extends to other parts of the cortex as well. Patients with lesions in the categorical hemisphere are disturbed about their disability and often depressed, whereas patients with lesions in the representational hemisphere are sometimes unconcerned and even euphoric. Lesions of different parts of the categorical hemisphere produce **fluent, nonfluent,** and **anomic aphasias.** Although aphasias are produced by lesions of the categorical hemisphere, lesions in the representational hemisphere also have effects. For example, they may impair the ability to tell a story or make a joke. They may also impair a subject's ability to get the point of a joke and, more broadly, to comprehend the meaning of differences in inflection and the "color" of speech. This is an example of the way the hemispheres are specialized rather than simply being dominant and nondominant.

THERAPEUTIC HIGHLIGHTS

Treatments for agnosia and aphasia are symptomatic and supportive. Individuals with agnosia can be taught exercises to help them identify objects

that are a necessity for independence. Therapy for individuals with aphasia helps them use remaining language abilities, compensate for language problems, and learn other methods of communicating. Some individuals with aphasia experience recovery but often some disabilities remain. Factors that influence the degree of improvement include the cause and extent of the brain damage, the area of the brain that was damaged, and the age and health of the individual. Computer-assisted therapies can improve retrieval of certain parts of speech as well as allowing an alternative way to communicate.

CLINICAL BOX 15–5

Dyslexia

Dyslexia is characterized by difficulties with learning how to decode at the word level, to spell, and to read accurately and fluently despite having a normal or even higher than normal level of intelligence. It is often due to an inherited abnormality that affects 5% of the population with a similar incidence in boys and girls. Dyslexia is the most common and prevalent of all known learning disabilities. It often coexists with attention deficit disorder. Many individuals with dyslexic symptoms also have problems with short-term memory skills and problems processing spoken language. Acquired dyslexias can occur with brain damage in the left hemisphere's key language areas. Also, in many cases, there is a decreased blood flow in the angular gyrus in the categorical hemisphere. There are several theories to explain the cause of dyslexia. The **phonologic hypothesis** is that dyslexics have a specific impairment in the representation, storage, and/or retrieval of speech sounds. The **rapid auditory processing theory** proposes that the primary deficit is the perception of short or rapidly varying sounds. The **visual theory** is that a defect in the magnocellular portion of the visual system slows processing and also leads to phonemic deficit. More selective speech defects have also been described. For example, lesions limited to the left temporal pole cause inability to retrieve names of places and persons but preserves the ability to retrieve common nouns, verbs, and adjectives.

THERAPEUTIC HIGHLIGHTS

Treatments for children with dyslexia frequently rely on modified teaching

strategies that include the involvement of various senses (hearing, vision, and touch) to improve reading skills. The sooner the diagnosis is made and interventions are applied, the better the prognosis.

PHYSIOLOGY OF LANGUAGE

Language is one of the fundamental bases of human intelligence and a key part of human culture. The primary brain areas concerned with language are arrayed along and near the sylvian fissure (lateral cerebral sulcus) of the categorical hemisphere. A region at the posterior end of the superior temporal gyrus called the **Wernicke area** (Figure 15–5) is concerned with comprehension of auditory and visual information. It projects via the **arcuate fasciculus** to the **Broca area** in the frontal lobe immediately in front of the inferior end of the motor cortex. Broca area processes the information received from Wernicke area into a detailed and coordinated pattern for vocalization and then projects the pattern via a speech articulation area in the insula to the motor cortex. This then initiates the appropriate movements of the lips, tongue, and larynx to produce speech. The probable sequence of events that occurs when a subject names a visual object is shown in Figure 15–6. The angular gyrus behind the Wernicke area appears to process information from words that are read in such a way that they can be converted into the auditory forms of the words in Wernicke area.

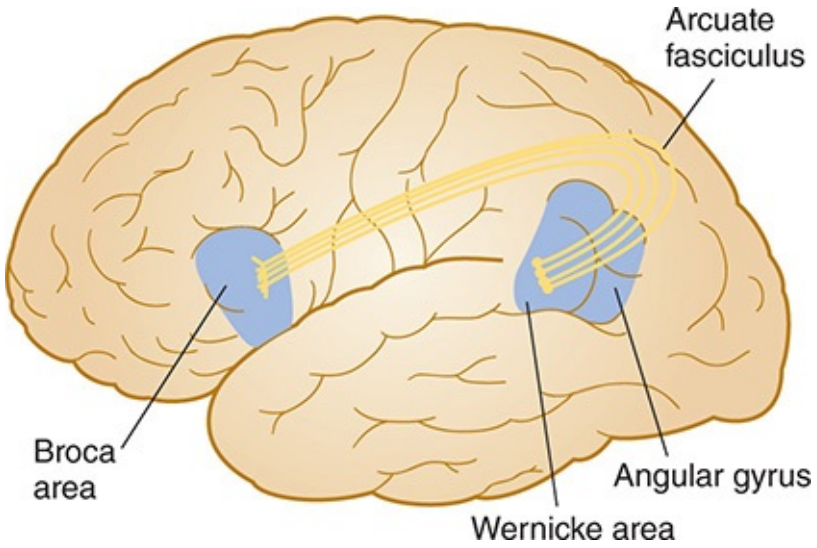


FIGURE 15–5 Location of some of the areas in the categorical hemisphere that are concerned with language functions. Wernicke area is in the posterior

end of the superior temporal gyrus and is concerned with comprehension of auditory and visual information. It projects via the arcuate fasciculus to Broca area in the frontal lobe. Broca area processes information received from Wernicke area into a detailed and coordinated pattern for vocalization and then projects the pattern via a speech articulation area in the insula to the motor cortex, which initiates the appropriate movements of the lips, tongue, and larynx to produce speech.

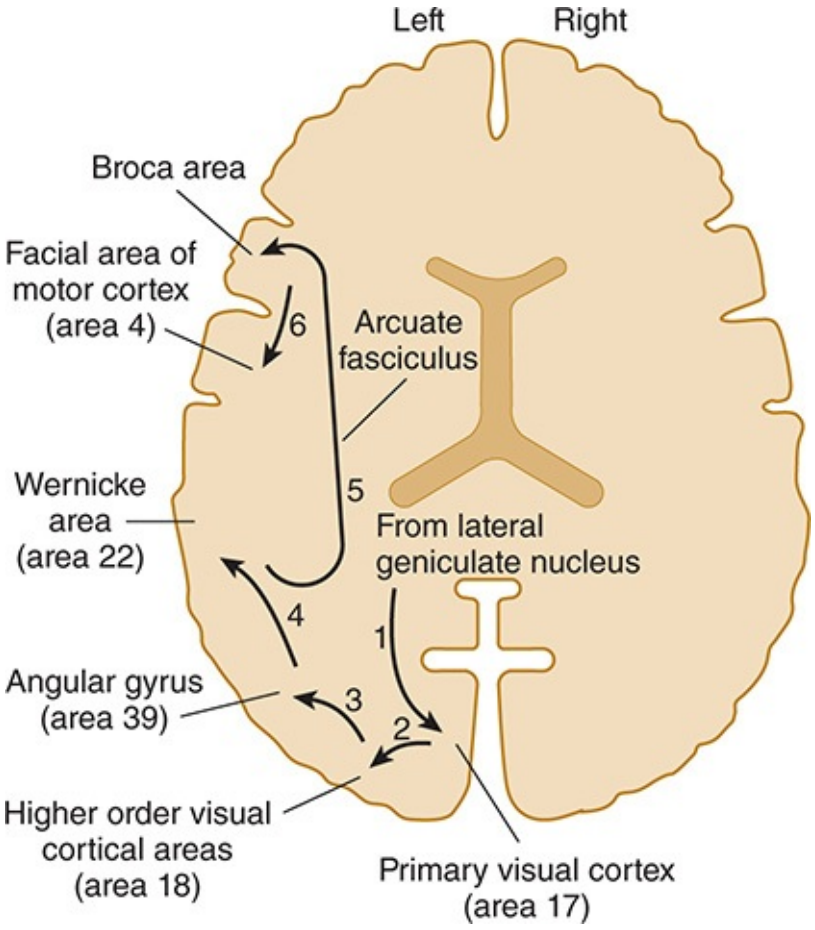


FIGURE 15–6 Path taken by impulses when a subject identifies a visual object, projected on a horizontal section of the human brain. Information travels from the lateral geniculate nucleus in the thalamus to the primary visual cortex, to higher order visual critical areas, and to the angular gyrus. Information then travels from Wernicke area to Broca area via the arcuate fasciculus. Broca area processes the information into a detailed and coordinated pattern for vocalization and then projects the pattern via a speech articulation area in the insula to the motor cortex, which initiates the appropriate movements of the lips, tongue, and larynx to produce speech.

In individuals who learn a second language in adulthood, fMRI reveals that the portion of Broca area concerned with it is adjacent to but separate from the area concerned with the native language. However, in children who learn two languages early in life, only a single area is involved with both. Children acquire fluency in a second language more easily than adults.

LANGUAGE DISORDERS

Aphasias are abnormalities of language functions that are not due to defects of vision or hearing or to motor paralysis. They are caused by lesions in the categorical hemisphere (see Clinical Box 15–4). The most common cause is embolism or thrombosis of a cerebral blood vessel. Aphasias can be classified as **nonfluent, fluent,** or **anomic aphasias.** A lesion of Broca area causes a nonfluent aphasia denoted as an expressive or motor aphasia. Affected individuals have slow speech and difficulty in generating verbal or written words. Patients with severe damage to this area are limited to two or three words to express the whole range of meaning and emotion. Sometimes the words retained are those that were being spoken at the time of the injury or vascular accident that caused the aphasia.

A lesion in Wernicke area produces a type of fluent aphasia in which speech itself is normal but it is full of jargon and neologisms that make little sense. The patient also fails to comprehend the meaning of spoken or written words, so other aspects of the use of language are compromised. Another form of fluent aphasia is a condition in which patients can speak relatively well and have good auditory comprehension but cannot put parts of words together or conjure up words.

When a lesion damages the angular gyrus in the categorical hemisphere without affecting Wernicke or Broca areas, there is no difficulty with speech or the understanding of auditory information; instead there is trouble understanding written language or pictures, because visual information is not processed and transmitted to Wernicke area. The result is a condition called **anomic aphasia.**

The isolated lesions that cause the selective defects described above occur in some patients, but brain destruction is often more general. Consequently, more than one form of aphasia is often present. Frequently, the aphasia is general **(global),** involving both receptive and expressive functions. In this situation, speech is scant as well as nonfluent. Writing is abnormal in all aphasias in which speech is abnormal, but the neural circuits involved are unknown. In addition, deaf persons in whom a lesion develops in the categorical hemisphere lose their

ability to communicate using sign language.

Stuttering is associated with right cerebral dominance and widespread elevated activity in the cerebral cortex and cerebellum, including increased activity in the supplementary motor area. Stimulation of this area can produce **laughter,** with the duration and intensity of the laughter proportional to the intensity of the stimulus.

RECOGNITION OF FACES

An important part of the visual input goes to the inferior temporal lobe, where representations of objects, particularly faces, are stored **(Figure 15–7)**. Faces are particularly important in distinguishing friends from foes and the emotional state of those seen. Storage and recognition of faces is more strongly represented in the right inferior temporal lobe in right-handed individuals, though the left lobe is also active. Damage to this area can cause **prosopagnosia,** the inability to recognize faces. Patients with this abnormality can recognize forms and reproduce them. They can recognize people by their voices, and many of them show autonomic responses when they see familiar as opposed to unfamiliar faces. However, they cannot identify the familiar faces they see. The presence of an autonomic response to a familiar face in the absence of recognition implicates the existence of a separate dorsal pathway for processing information about faces that leads to recognition at only a subconscious level.

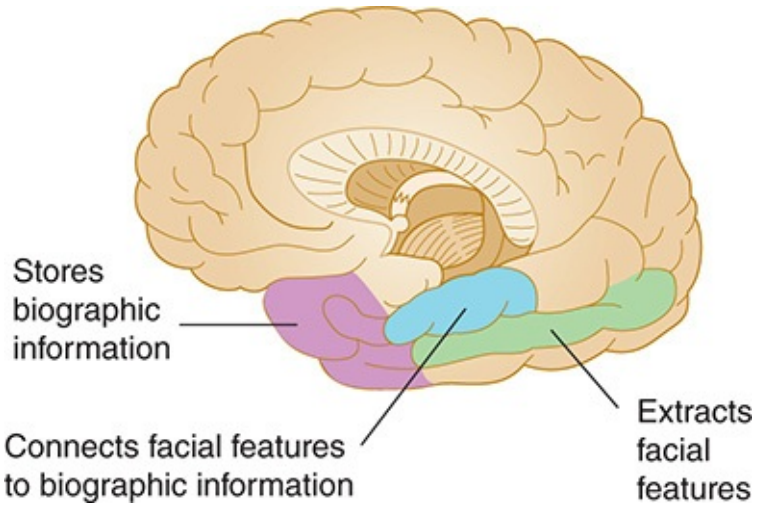


FIGURE 15–7 Areas in the right cerebral hemisphere, in right-handed individuals, that are concerned with recognition of faces. An important part of the visual input goes to the inferior temporal lobe, where representations of

objects, particularly faces, are stored. In humans, storage and recognition of faces is more strongly represented in the right inferior temporal lobe in right-handed individuals, though the left lobe is also active. (Modified with permission from Szpir M: Accustomed to your face. Am Sci 1992; 80:539.)

LOCALIZATION OF OTHER FUNCTIONS

Use of fMRI and PET scanning combined with study of patients with strokes and head injuries has provided insight into the ways serial processing of sensory information produce cognition, reasoning, comprehension, and language. Analysis of the brain regions involved in arithmetic calculations has highlighted two areas. In the inferior portion of the left frontal lobe is an area concerned with number facts and calculations. Frontal lobe lesions can cause **acalculia,** a selective impairment of mathematical ability. There are areas around the intraparietal sulci of both parietal lobes that are concerned with visuospatial representations of numbers.

Two right-sided subcortical structures play a role in accurate navigation. One is the right hippocampus that is concerned with learning the location of places, and the other is the right caudate nucleus that facilitates movement to the places. Men have larger brains than women and are said to have superior spatial skills and ability to navigate.

Other defects seen in patients with localized cortical lesions include, for example, the inability to name animals, though the ability to name other living things is intact. One patient with a left parietal lesion had difficulty with the second half but not the first half of words. Some patients with parietooccipital lesions write only with consonants, omitting vowels. The pattern that emerges from such observations is one of precise sequential processing of information in localized brain areas.

CHAPTER SUMMARY

- CT scans provide a high-resolution 3-dimensional image of the brain or other organ. Both PET imaging and fMRI provide an index of the level of the activity in various parts of the brain in health or disease.
- TBI results from an excessive mechanical force or penetrating injury to the head (eg, falls, motor vehicle accidents, and assaults). It can lead to impaired cognitive, physical, emotional, and behavioral functions and can

be associated with as altered state of consciousness. A Glasgow Coma Scale is used to define the severity of TBI and imaging can identify the extent of the brain damage.

- Memory is divided into explicit (declarative) and implicit (nondeclarative). Explicit memory is further subdivided into semantic and episodic. Implicit memory is further subdivided into priming, procedural, associative learning, and nonassociative learning.
- Declarative memory involves the hippocampus and the medial temporal lobe for retention. Priming is dependent on the neocortex. Procedural memory is processed in the striatum. Associative learning is dependent on the amygdala for its emotional responses and the cerebellum for the motor responses. Nonassociative learning is dependent on various reflex pathways.
- Synaptic plasticity is the ability of neural tissue to change as reflected by LTP (an increased effectiveness of synaptic activity) or LTD (a reduced effectiveness of synaptic activity) after continued use. Habituation is a simple form of learning in which a neutral stimulus is repeated many times. Sensitization is the prolonged occurrence of augmented postsynaptic responses after a stimulus to which one has become habituated is paired once or several times with a noxious stimulus.
- Alzheimer disease is characterized by progressive loss of short-term memory followed by general loss of cognitive function. The cytopathologic hallmarks of Alzheimer disease are intracellular neurofibrillary tangles and extracellular senile plaques.
- Categorical and representational hemispheres are for sequential-analytic processes and visuospatial relations, respectively. Lesions in the categorical hemisphere produce language disorders, whereas lesions in the representational hemisphere produce astereognosis.
- The main cortical regions involved in language are Wernicke area in the upper temporal lobe that projects via the arcuate fasciculus to Broca area in the frontal lobe. Wernicke area is important for comprehension of auditory and visual information; Broca area processes the information from the Wernicke area produces a coordinated pattern for vocalization.
- Aphasias are abnormalities of language functions and are caused by lesions in the categorical hemisphere. They are classified as fluent (Wernicke area), nonfluent (Broca area), and anomic (angular gyrus) based on the location of brain lesions.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. A 23-year-old medical student suffered a traumatic brain injury as a result of a motorcycle accident. He was unconscious and was rushed to the emergency department of the local hospital. A CT scan was performed and appropriate interventions were taken. What kind of information is obtained from a CT scan?

   A. Blood flow in both superficial and deep parts of the brain

   B. Changes in regional brain activity

   C. Functional rather than detailed anatomic information

   D. A high-resolution 3-dimensional image of the brain

   E. Local glucose metabolism, blood flow, and oxygen

2. A 15-year-old boy who was not wearing a helmet was in a bicycle accident. He was rushed to the emergency department of the local hospital where it was diagnosed with mild traumatic brain injury based on the Glasgow Coma Scale. Which of the following symptoms are consistent with this level of traumatic brain injury?

   A. Nausea and vomiting, slurred speech, and blurred vision

   B. Fatigue, sleep disturbances, and mood changes

   C. Limb weakness, confusion, and agitation

   D. Mood changes, restlessness, and nausea

   E. Slurred speech, limb weakness, and fatigue

3. A 52-year-old man had a closed-head injury that caused damage to the prefrontal cortex. As a result, this individual might be expected to experience the following memory disturbance.

   A. Disappearance of remote memories.

   B. Loss of working memory.

   C. Loss of the ability to encode events of the recent past into long-term memory.

   D. Loss of the ability to recall faces and forms.

   E. Production of inappropriate emotional responses when recalling events of the recent past.

4. A 70-year-old woman fell down a flight of stairs, hitting her head on the concrete sidewalk. The trauma caused a severe intracranial hemorrhage

resulting in brain damage. Following this injury, she seemed to ignore her left side; for example, she would only wash the right side of her body or only put a shoe on her right foot. What area of the brain was most likely damaged as a result of this fall down the stairs?

A. Inferior parietal lobe of the representational hemisphere

B. Nucleus basalis of Meynert and related areas of the forebrain

C. Mammillary bodies

D. Angular gyrus in the categorical hemisphere

E. Parietal lobe of the categorical hemisphere

5. An MD/PhD candidate was studying the role of the neocortex in language development. What information is known about the interconnection of cortical areas important for language?

A. Broca area projects to Wernicke area via the arcuate fasciculus.

B. Temporal lobe projects to the frontal lobe via the corpus callosum

C. Broca area projects to Wernicke area via the planum temporale.

D. Temporal lobe projects to Wernicke area via the corpus callosum.

E. Wernicke area projects to Broca area via the arcuate fasciculus.

6. A 67-year-old woman suffered a stroke that damaged the posterior end of the superior temporal gyrus. A lesion of Wernicke area in the categorical hemisphere causes her to

A. lose her short-term memory.

B. experience nonfluent aphasia in which she speaks in a slow, halting voice.

C. experience déjà vu.

D. talk rapidly but make little sense, which is characteristic of fluent aphasia.

E. lose the ability to recognize faces, which is called prosopagnosia.

7. Which of the following is most likely involved in production of LTP?

A. NO release, activation of NMDA receptors, and membrane hyperpolarization

B. Decreased Ca^{2+} in presynaptic or postsynaptic neurons, activation of NMDA receptors, and membrane depolarization

C. Activation of NMDA receptors, NO-induced reduction in glutamate release in a presynaptic neuron, and membrane depolarization

D. Increased Ca^{2+} in presynaptic or postsynaptic neurons, activation of NMDA receptors, and membrane depolarization

E. NO-induced increase in glutamate release in a presynaptic neuron, activation of non-NMDA receptors, membrane hyperpolarization

8. A 79-year-old woman has been experiencing difficulty finding her way back home after her morning walks. Her husband has also noted that she takes much longer to do routine chores around the home and often appears to be confused. He is hoping that this is just due to “old age” but fears it may be a sign of Alzheimer disease. Which of the following is the definitive sign of this disease?

A. Loss of short-term memory

B. The presence of intracellular neurofibrillary tangles and extracellular neuritic plaques with a core of β-amyloid peptides

C. A mutation in genes for amyloid precursor protein (APP) on chromosome 21

D. Rapid reversal of symptoms with the use of acetylcholinesterase inhibitors

E. A loss of cholinergic neurons in the nucleus basalis of Meynert

SECTION III

Endocrine & Reproductive Physiology

The key role of the endocrine system is to maintain whole body homeostasis. This is accomplished via the coordination of hormonal signaling pathways that regulate cellular activity in target organs throughout the body. Endocrine mechanisms are also concerned with the ability of humans to reproduce, and the sexual maturation required for this function. Classic **endocrine glands** are scattered throughout the body and secrete **hormones** into the circulatory system, usually via ductless secretion into the interstitial fluid. **Target organs** express receptors that bind the specific hormone to initiate a cellular response. The endocrine system can be contrasted with the neural regulation of physiologic function that was the focus of the previous section. Endocrine effectors typically provide “broadcast” regulation of multiple tissues and organs simultaneously, with specificity provided for by the expression of relevant receptors. A change in environmental conditions, for example, often calls for an integrated response across many organ systems. Neural regulation, on the other hand, is often exquisitely spatially delimited, such as the ability to contract just a single muscle. Nevertheless, both systems must work collaboratively to allow for minute-to-minute as well as longer term stability of the body’s interior milieu.

Hormones are the soluble messengers of the endocrine system and are classified into steroids, peptides, and amines (see Chapters 1 and 2). Steroid hormones can cross the lipid-containing plasma membrane of cells and usually bind to intracellular receptors. Peptide and amine hormones bind to cell surface receptors. Steroid hormones are produced by the adrenal cortex (Chapter 19), the ovaries (Chapter 22) and the gonads, testes (Chapter 23), in addition to steroid hormones that are made by the placenta during pregnancy (Chapter 22). Amine hormones are derivatives of the amino acid tyrosine and are made by the thyroid

(Chapter 20) and the adrenal medulla (Chapter 19). Interestingly, the tyrosine-derived thyroid hormone behaves more like a steroid than a peptide hormone by binding to an intracellular receptor. The majority of hormones, however, are peptides and they are usually synthesized as preprohormones before being cleaved first to prohormones in the endoplasmic reticulum and then to the active hormone in secretory vesicles.

Diseases of the endocrine system are numerous. Indeed, endocrine and metabolic disorders are among the most common afflictions in developed countries, particularly when nutrition and access to health care provisions are generous and high-risk individuals are identified by regular screening. At least 11 endocrine and metabolic disorders are present in 5% or more of the adult US population, including diabetes mellitus, osteopenia, dyslipidemia, metabolic syndrome, and thyroiditis. For example, type 2 diabetes mellitus is one of the most prevalent endocrine disorders of the 21st century and involves an inability of the body to respond to insulin. The resulting high blood glucose damages many tissues leading to secondary complications (see Chapter 24). In large part, the high and increasing prevalence of diabetes and other metabolic disorders rests on the substantial prevalence of obesity in developed countries, with as many as one-third of the US adult population now considered to be obese, and two-thirds overweight. Indeed, based on a 2009 report, obesity also affects 28% of US children aged 12–17, and while the current prevalence of type 2 diabetes in children is quite low, this prevalence is accordingly expected to rise. Further, a number of endocrine disorders are more prevalent in specific ethnic groups, or in a particular gender. Overall, the burden of endocrine and metabolic disorders, with their protean manifestations and complications, is a serious public health crisis and even highlights an apparent national shortage of trained endocrinologists. Many endocrine disorders must be managed by primary care clinicians as a result.

CHAPTER 16

Basic Concepts of Endocrine Regulation

OBJECTIVES

After studying this chapter, you should be able to:

- Describe hormones and their contribution to whole body homeostatic mechanisms.
- Understand the chemical nature of different classes of hormones and how this determines their mechanism of action on target cells. Contrast membrane bound and intracellular hormone receptors and understand the principles of desensitization, down-regulation, and inactivation.
- Define how hormones are synthesized and secreted by cells of endocrine glands, including how peptide hormones are cleaved from longer precursors.
- Understand the effects of plasma hormone binding proteins (thyroid and steroid hormones) on the availability of hormones to their sites of action, and on the mechanisms that regulate hormone secretion.
- Explain the principles of feedback control (negative and positive) of hormone secretion.
- Understand the principles governing disease states that result from over- or under-production of key hormones.

INTRODUCTION

This section of the text deals with the various endocrine glands that control the function of multiple organ systems of the body. In general, endocrine physiology is concerned with the maintenance of various aspects of **homeostasis.** The mediators of such control mechanisms are soluble factors known as **hormones.** The word hormone was derived from the Greek *horman*, meaning to set in motion. In preparation for specific discussions of the various endocrine systems and their hormones, this chapter will address some concepts of endocrine regulation that are common among all systems.

Another feature of endocrine physiology to keep in mind is that, unlike other physiologic systems that are considered in this text, the endocrine system cannot be cleanly defined along anatomic lines. Rather, the endocrine system is a distributed system of glands and circulating messengers that is often stimulated by the central nervous system or the autonomic nervous system, or both.

HORMONAL SPECIFICITY OF ACTION ON TARGET CELLS

As noted in the introduction to this section, hormones comprise steroids, amines, and peptides. Peptide hormones are by far the most numerous. Many hormones can be grouped into families reflecting their structural similarities as well as the similarities of the receptors they activate.

Steroids and thyroid hormones are distinguished by their predominantly intracellular sites of action, since they can diffuse freely through the cell membrane. They bind to a family of largely cytoplasmic proteins known as nuclear receptors. Upon ligand binding, the receptor–ligand complex translocates to the nucleus where it either homodimerizes, or associates with a distinct liganded nuclear receptor to form a heterodimer. In either case, the dimer binds to DNA to either increase or decrease gene transcription in the target tissue. Individual members of the nuclear receptor family have a considerable degree of homology, and share many functional domains, such as the zinc fingers that permit DNA binding. However, sequence variations allow for ligand specificity as well as binding to specific DNA motifs. In this way, the transcription of distinct genes is regulated by individual hormones.

HORMONE SECRETION

SYNTHESIS & PROCESSING

The regulation of hormone synthesis, of course, depends on their chemical nature. For peptide hormones as well as hormone receptors, synthesis is controlled predominantly at the level of transcription. For amine and steroid hormones, synthesis is controlled indirectly by regulating the production of key synthetic enzymes as well as by substrate availability.

Interestingly, the majority of peptide hormones are synthesized initially as much larger polypeptide chains, and then processed intracellularly by specific proteases to yield the final hormone molecule. In some cases, multiple hormones may be derived from the same initial precursor, depending on the specific processing steps present in a given cell type. Presumably this provides for a level of genetic "economy." It is also notable that the hormone precursors themselves are typically inactive. This may be a mechanism that provides for an additional measure of regulatory control, or, in the case of thyroid hormones, may dictate the site of highest hormone availability.

The synthesis of all of the proteins/peptides discussed above is subject to the normal mechanisms of transcriptional control in the cell (see Chapter 2). In addition, there is provision for exquisitely specific regulation by other hormones, since the regulatory regions of many peptide hormone genes contain binding motifs for the nuclear receptors discussed above. For example, thyroid hormone directly suppresses TSH expression via the thyroid hormone receptor. These specific mechanisms to regulate hormone transcription are essential to the function of feedback loops, as will be addressed in greater detail below. In some cases, the abundance of selected hormones may also be regulated via effects on translation. For example, elevated levels of circulating glucose stimulate the translation of insulin mRNA. These effects are mediated by the ability of glucose to increase the interaction of the insulin mRNA with specific RNA-binding proteins, which increase its stability and enhance its translation. The net effect is a more precise and timely regulation of insulin levels, and thus energy metabolism, than could be accomplished with transcriptional regulation alone.

The precursors for peptide hormones are processed through the cellular machinery that handles proteins destined for export, including trafficking through specific vesicles where the propeptide form can be cleaved to the final active hormones. Mature hormones are also subjected to a variety of posttranslational processing steps, such as glycosylation, which can influence their ultimate biologic activity and/or stability in the circulation. Ultimately, all hormones enter either the constitutive or regulated secretory pathway (see

Chapter 2).

SECRETION

The secretion of many hormones is via a process of exocytosis of stored granules, as discussed in Chapter 2. The exocytotic machinery is activated when the cell type that synthesizes and stores the hormone in question is activated by a specific signal, such as a neurotransmitter or peptide releasing factor. One should, however, contrast the secretion of stored hormones with that of those that are continually released by diffusion (eg, steroids). Control of the secretion of the latter molecules occurs via kinetic influences on the synthetic enzymes or carrier proteins involved in hormone production. For example, the steroidogenic acute regulatory protein (StAR) is a labile protein whose expression, activation, and deactivation are regulated by intracellular signaling cascades and their effectors, including a variety of protein kinases and phosphatases. StAR traffics cholesterol from the outer to the inner membrane leaflet of the mitochondrion. Because this is a rate-limiting first step in the synthesis of the steroid precursor, pregnenolone, this arrangement permits changes in the rate of steroid synthesis, and thus secretion, in response to homeostatic cues such as trophic hormones, cytokines, and stress **(Figure 16–1)**.

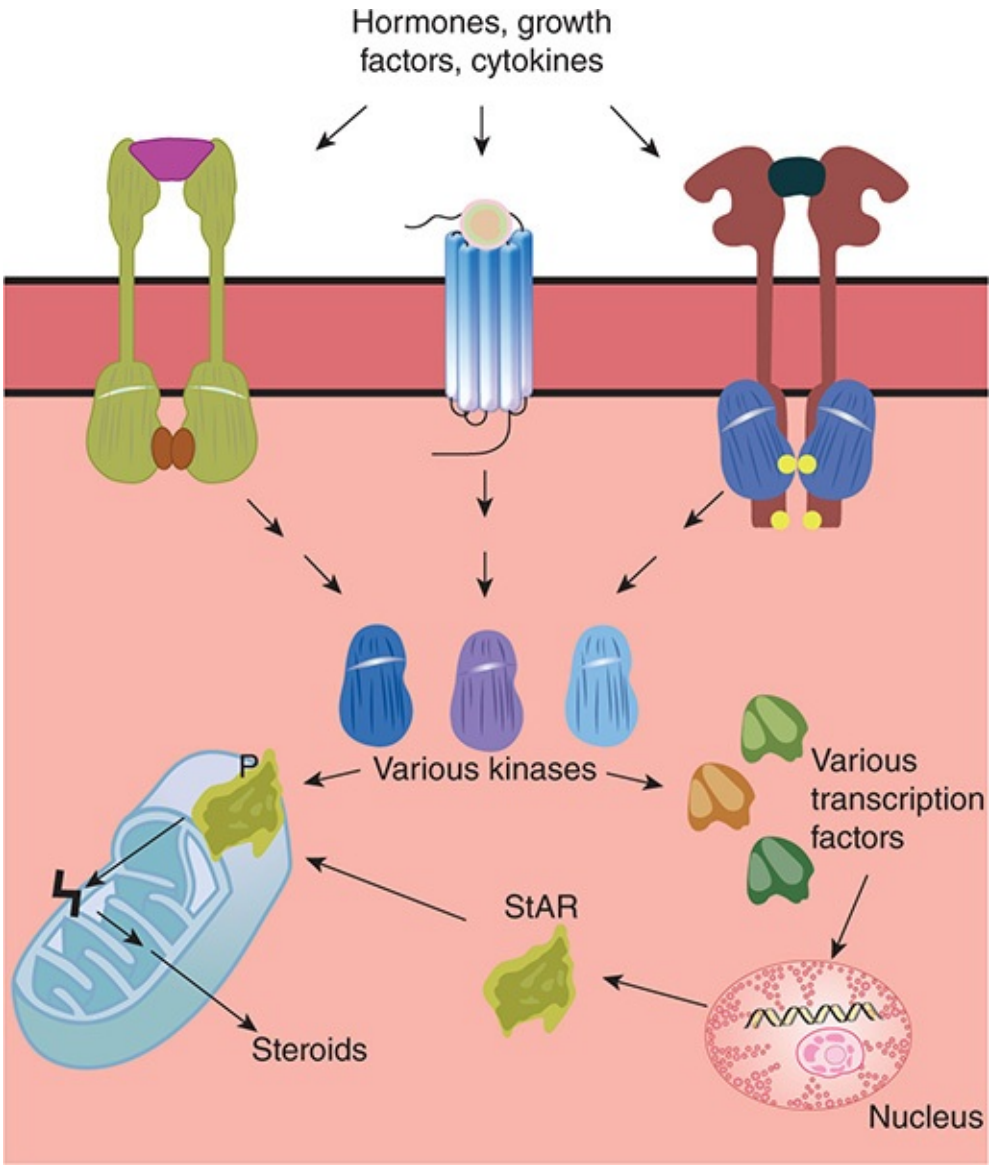


FIGURE 16–1 Regulation of steroid biosynthesis by the steroidogenic acute regulatory protein (StAR). Extracellular signals activate intracellular kinases that, in turn, phosphorylate transcription factors that upregulate StAR expression. StAR is activated by phosphorylation, and facilitates transfer of cholesterol from the outer to inner mitochondrial membrane leaflet. This then allows conversion of cholesterol into pregnenolone, which is the first intermediate in the steroid biosynthetic pathway.

An additional complexity related to hormone secretion relates to the fact that some hormones are secreted in a pulsatile manner. Secretion rates may peak and ebb relative to circadian rhythms, in response to the timing of meals, or as regulated by other pattern generators whose periodicity may range from

milliseconds to years. Pulsatile secretion is often related to the activity of oscillators in the hypothalamus that regulate the membrane potential of neurons, in turn secreting bursts of hormone releasing factors into the hypophysial blood flow that then cause the release of pituitary and other downstream hormones in a similar pulsatile manner (see Chapters 17 and 18). There is evidence that these hormone pulses convey different information to the target tissues that they act upon compared to a steady exposure to a single concentration of the hormone. Therapeutically, pulsatile secretion may pose challenges if, due to deficiency, it proves necessary to replace a particular hormone that is normally secreted in this way.

HORMONE TRANSPORT IN THE BLOOD

In addition to the rate of secretion and its nature (steady vs. pulsatile), a number of factors influence the circulating levels of hormones. These include the rates of hormone degradation and/or uptake, receptor binding and availability of receptors, and the affinity of a given hormone for plasma carriers **(Figure 16–2)**. Stability influences the circulating half-life of a given hormone and has therapeutic implications for hormone replacement therapy, in addition to those posed by pulsatile secretion as discussed above.

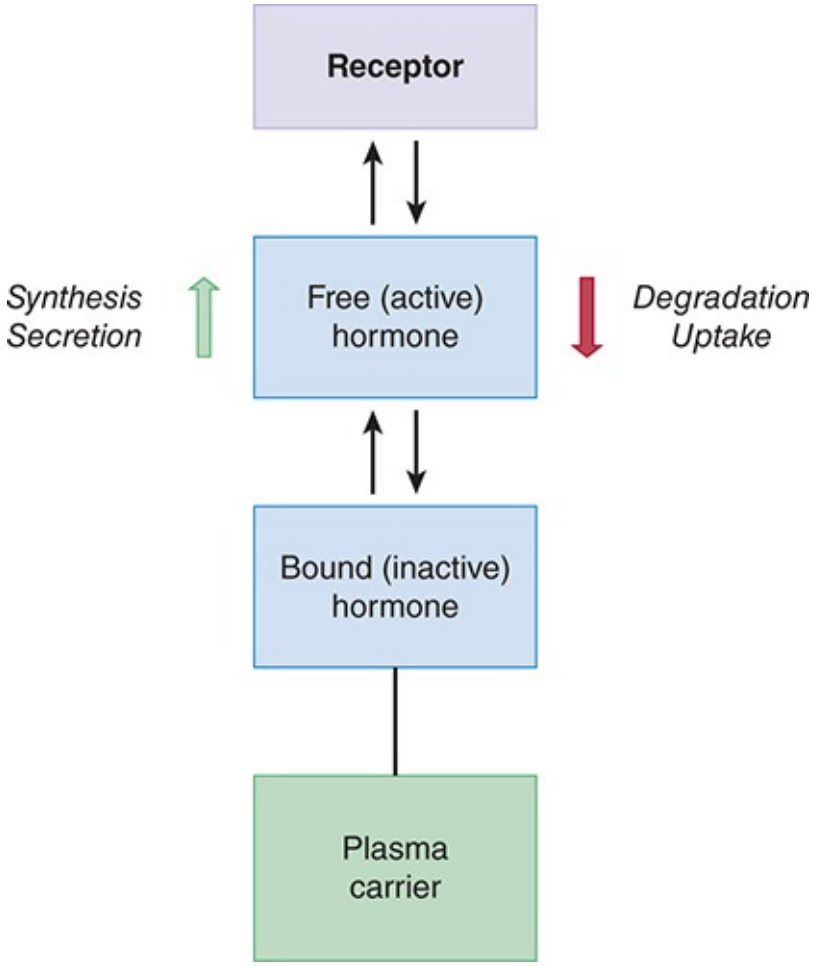


FIGURE 16–2 Summary of factors that determine the level of free hormones circulating in the bloodstream. Factors that increase (green upward arrow) or decrease (red downward arrow) hormone levels are shown. Free hormones also equilibrate with the forms bound to either receptors or plasma carrier proteins.

Plasma carriers for specific hormones have a number of important physiologic functions. First, they serve as a reservoir of inactive hormone and thus provide a hormonal reserve. Bound hormones are typically prevented from degradation or uptake. Thus, the bound hormone reservoir can allow fluctuations in hormonal levels to be smoothed over time. Plasma carriers also restrict the access of the hormone to some sites. Ultimately, plasma carriers may be vital in modulating levels of the free hormone in question. Typically, it is only the free hormone that is biologically active in target tissues or can mediate feedback regulation (see below) since it is the only form able to access the extravascular compartment.

Catecholamine and most peptide hormones are soluble in plasma and are transported as such. In contrast, steroid hormones are hydrophobic and are mostly bound to large proteins called **steroid binding proteins** (SBP), which are synthesized in the liver. As a result, only small amounts of the free hormone are dissolved in the plasma. Specifically, **sex hormone–binding globulin** (SHBG) is a glycoprotein that binds to the sex hormones, testosterone and 17β-estradiol. Progesterone, cortisol, and other corticosteroids are bound by transcortin.

The SBP–hormone complex and the free hormone are in equilibrium in the plasma, and only the free hormone is able to diffuse across cell membranes. SBP have three main functions: they increase the solubility of lipid-based hormones in the blood; they reduce the rate of hormone loss in the urine by preventing the hormones from being filtered in the kidney; and as mentioned above, they provide a source of hormone in the bloodstream that can release free hormone as the equilibrium changes. It follows that an additional way to regulate the availability of hormones that bind to carrier proteins, such as steroids, is to regulate the expression and secretion of the carrier proteins themselves. This is a critical mechanism that regulates the bioavailability of thyroid hormones, for example (see Chapter 20).

In a pathophysiologic setting, some medications can alter levels of binding proteins or displace hormones that are bound to them. In addition, some binding proteins are promiscuous and bind multiple hormones (eg, SHBG). These observations may have clinical implications for endocrine homeostasis, since free hormones are needed to feedback and control their rates of synthesis and secretion (see below).

Finally, the anatomic relationship of sites of release and action of hormones may play a key role in their regulation. For example, a number of hormones are destroyed by passage through the pulmonary circulation or the liver. This may markedly curtail the temporal window within which a given hormone can act.

HORMONE ACTION

Hormones exert a wide range of distinctive actions on a huge number of target cells to effect changes in metabolism, release of other hormones and regulatory substances, changes in ion channel activity, and cell growth, among others **(Clinical Box 16–1)**. Ultimately, the concerted action of the hormones of the body ensures the maintenance of homeostasis. Indeed, all hormones affect homeostasis to some degree. However, a subset of the hormones, including thyroid hormone, cortisol, parathyroid hormone, vasopressin, the

mineralocorticoids, and insulin, are the key contributors to homeostasis **(Table 16–1)**. Detailed information on the precise biologic effects of these molecules can be found in subsequent chapters.

TABLE 16–1 Major hormonal contributors to homeostasis.

Hormone	Source	Action
Thyroid hormone	Thyroid	Controls basal metabolism in most tissues
Cortisol	Adrenal cortex	Energy metabolism; permissive action for other hormones
Mineralocorticoids	Adrenal cortex	Regulate plasma volume via effects on serum electrolytes
Vasopressin	Posterior pituitary	Regulates plasma osmolality via effects on water excretion
Parathyroid hormone	Parathyroids	Regulates calcium and phosphorus levels
Insulin	Pancreas	Regulates plasma glucose concentration

Hydrophilic hormones, including peptides and catecholamines, exert their acute effects by binding to cell surface receptors. Most of these are from the GPCR family. Hydrophobic hormones, on the other hand, predominantly exert their actions via nuclear receptors. There are two classes of nuclear receptors that are important in endocrine physiology. The first class provides direct stimulation of transcription via induction of the binding of a transcriptional co-activator when the hormonal ligand is bound. In the second class, hormone binding triggers simultaneous dislodging of a transcriptional co-repressor and recruitment of a co-activator. The latter class of receptor allows for a wider dynamic range of regulation of the genes targeted by the hormone in question.

In recent years, it has become apparent that a number of receptors for steroid and other hydrophobic hormones are extranuclear, and some may even be present on the cell surface. The characterization of such receptors at a molecular

level, their associated signaling pathways, and indeed proof of their existence has been complicated by the ability of hydrophobic hormones to diffuse relatively freely into all cellular compartments. These **extranuclear receptors,** some of which may be structurally related or even identical to the more classic nuclear receptors, are proposed to mediate rapid responses to steroids and other hormones that do not require alterations in gene transcription. The physiologic effects at these receptors may therefore be distinct from those classically associated with a given hormone. Evidence is accumulating, for example, that plasma membrane receptors for estrogen can mediate acute arterial vasodilation as well as reducing cardiac hypertrophy in pathophysiologic settings. Functions such as these may account for differences in the prevalence of cardiovascular disease in premenopausal and postmenopausal women. In any event, this active area of biomedical investigation is likely to broaden our horizons of the full spectrum of action of steroid hormones.

CLINICAL BOX 16–1

Breast Cancer

Breast cancer is the most common malignancy of women, with about 1 million new cases diagnosed each year worldwide. The proliferation of more than two-thirds of breast tumors are driven by the ovarian hormone, estrogen, by virtue of the fact that the tumor cells express high levels of posttranslationally modified estrogen receptors (ERs). The clinical significance of these molecular findings has been known for more than 100 years, since the Scottish surgeon, Sir Thomas Beatson, reported delayed disease progression in patients with advanced breast cancer following removal of their ovaries. In modern times, determination of whether a given breast cancer is, or is not, **ER-positive** is a critical diagnostic test that guides treatment decisions, as well as an important prognosticator. ER-positive tumors are typically of lower grade, and patients with such tumors have improved survival (although the latter is likely due, at least in part, to the availability of excellent treatment options for ER-positive tumors compared with those that are ER- negative—see below).

THERAPEUTIC HIGHLIGHTS

Estrogen-responsive breast tumors are dependent on the presence of the hormone for growth. In modern times, cells can be deprived of the effects of

estrogen pharmacologically, rather than resorting to oophorectomy. **Tamoxifen** and related agents specifically inhibit the receptor and may also hasten its degradation. In postmenopausal women, where estrogen is derived from the metabolism of testosterone in extragonadal tissues rather than from the ovaries, **aromatase inhibitors** inhibit the conversion of androgens to estrogen, and thereby deprive tumor cells of their critical signal for continued proliferation.

PRINCIPLES OF FEEDBACK CONTROL

A principle that is critical for endocrine physiology is that of **feedback regulation.** This holds that the responsiveness of target cells to hormonal action subsequently "feeds back" to control the inciting endocrine organ. Feedback can regulate the further release of the hormone in either a negative feedback or (more rarely) a positive feedback loop. Positive feedback relates to the enhancement or continued stimulation of the original release mechanism/stimulus. Such mechanisms are really only seen in settings that need to gather momentum to an eventual outcome, such as parturition. Negative feedback is a far more common control mechanism and involves the inhibition or dampening of the initial hormone release mechanism/stimulus. A general scheme for feedback inhibition of endocrine axes is depicted in **Figure 16–3**.

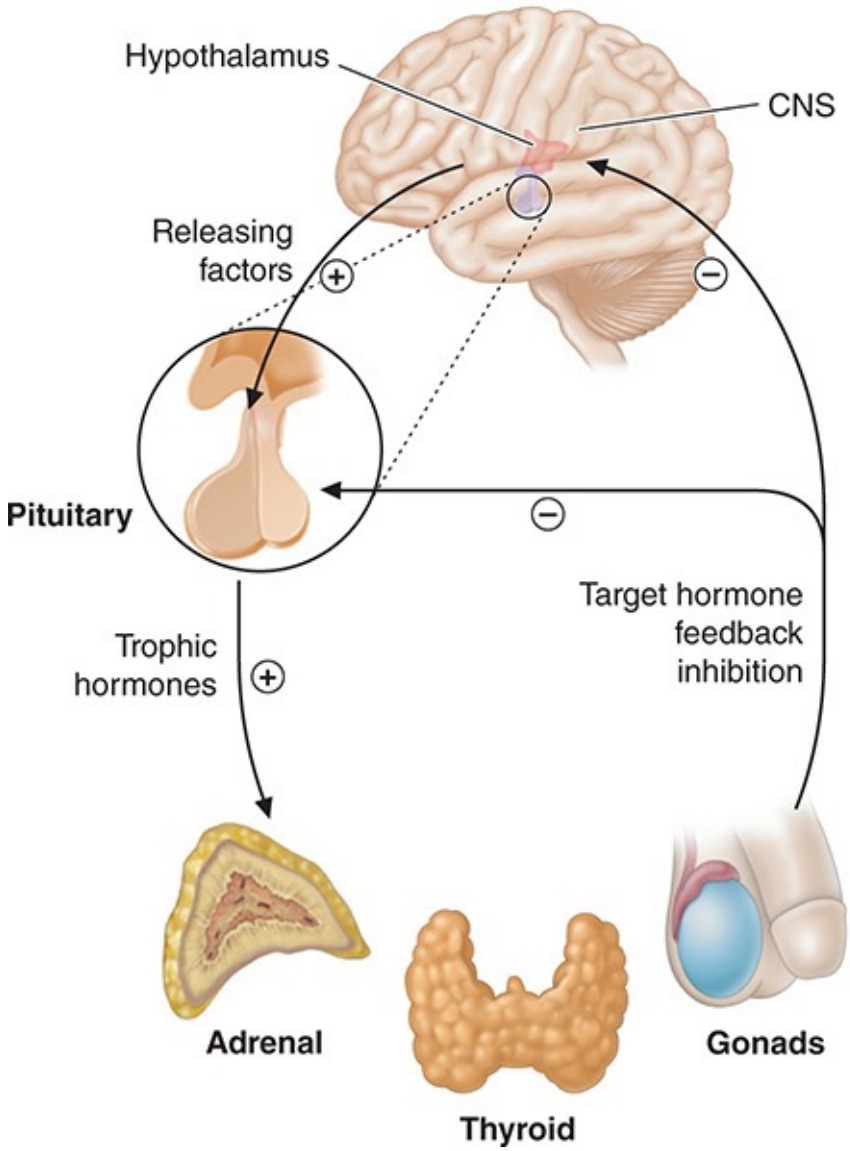


FIGURE 16–3 Summary of feedback loops regulating endocrine axes. CNS, central nervous system. (Reproduced with permission from Jameson JL (ed): *Harrison's Endocrinology*, 2nd ed. New York, NY: McGraw Hill; 2010.)

In general, the endocrine system uses a network of feedback responses to maintain a steady state. Steady state can be explained using blood osmolality as an example **(Figure 16–4)**. Blood osmolality in humans must be maintained within a physiologic range of 275–299 mOsm, and to maintain homeostasis this variable should not exceed that range. To ensure that osmolality does not change in the context of an open system, processes are in place that will add or remove water from the system to ensure a constant osmolality. The osmolality of blood will increase with dehydration and decrease with overhydration. If blood

osmolality increases outside the ideal range (by 10 mOsm or more), osmoreceptors are activated. These signal release of the peptide hormone, vasopressin, into the circulation (from the pituitary). Vasopressin acts on the renal collecting duct, and increases the permeability of the apical (lumenal) plasma membrane to water via the insertion of a protein called an aquaporin. Water is then moved from the urine into the circulation via transcellular transport. The reabsorption of water from the urine to the blood resets the osmolality of the blood to within the physiologic range. The decrease in blood osmolality then exerts a negative feedback on the cells of the hypothalamus and the pituitary and vasopressin release is inhibited, meaning that water reabsorption from the urine is reduced. Further details of this collaboration between the kidneys, hypothalamus, and pituitary are found in Chapter 38.

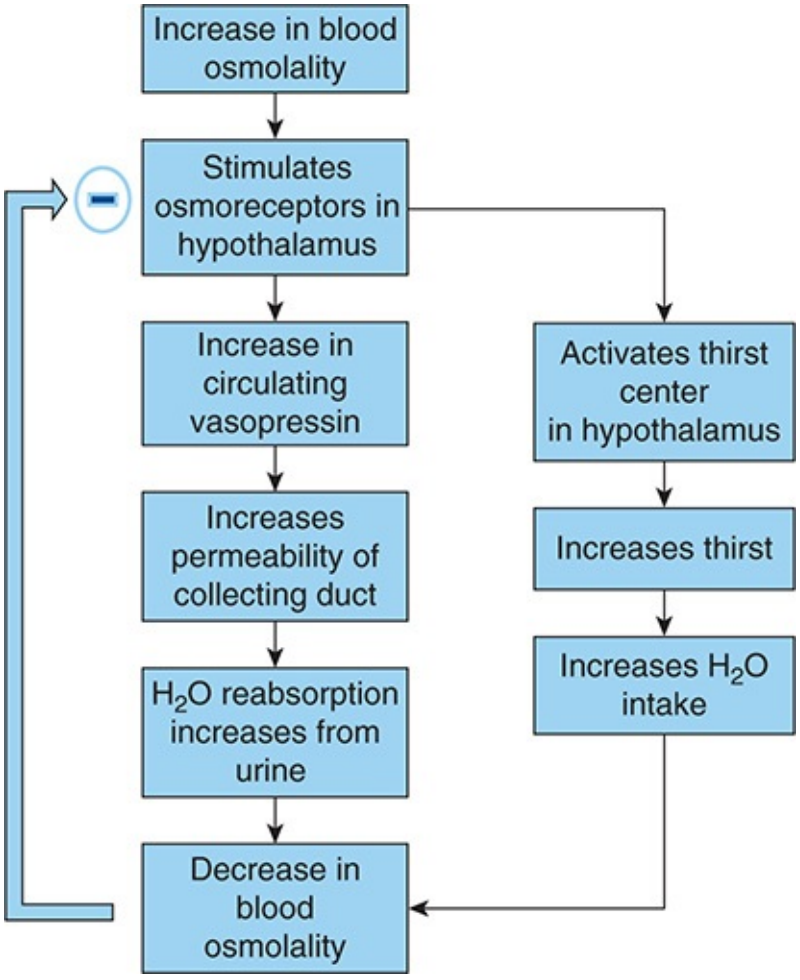


FIGURE 16–4 Feedback loop that ensures homeostasis of blood osmolality. An increase in blood osmolality triggers the thirst mechanism as well as renal conservation of water via the release of vasopressin from the hypothalamus.

Both outcomes decrease blood osmolality back toward the normal range, which feeds back to terminate hypothalamic signaling.

Negative feedback control systems such as those described are the most common feedback/homeostatic systems in the body. Other examples include temperature regulation (see Chapter 17) and the regulation of blood glucose concentrations (see Chapter 24). Feedback control loops also provide for diagnostic strategies in evaluating patients with suspected endocrine disorders. For example, in a patient being evaluated for hypothyroidism, normal levels of TSH (see Chapter 20) tend to rule out a primary defect at the level of the thyroid gland itself, and rather suggest that a defect at the level of the anterior pituitary should be sought. Conversely, if TSH is elevated, it suggests that the normal ability of circulating thyroid hormone to suppress TSH synthesis has been lost, likely due to a reduction in the ability of the thyroid gland to synthesize the hormone **(Clinical Box 16–2)**.

TYPES OF ENDOCRINE DISORDERS

It is pertinent also to discuss briefly the types of disease states where endocrine physiology can become deranged. Additional details of these disease states can be found in ensuing chapters.

HORMONE DEFICIENCY

Deficiencies of particular hormones are most commonly seen in the setting where there is destruction of the glandular structure responsible for their production, often as a result of inappropriate autoimmune attack. For example, in type 1 diabetes mellitus, pancreatic β cells are destroyed leading to an inability to synthesize insulin, often from a very young age. Similarly, hormonal deficiencies arise when there are inherited mutations in the factors responsible for their release or in the receptors for these releasing factors. Defects in the enzymatic machinery needed for hormone production, or a lack of appropriate precursors (eg, iodine deficiency leads to hypothyroidism), will also reduce the amount of the relevant hormone available for bodily requirements.

HORMONE RESISTANCE

Many of the consequences of hormone deficiency can be reproduced in disease states where adequate levels of a given hormone are synthesized and released, but the target tissues become resistant to the hormone's effects. Indeed, there is often overproduction of the implicated hormone in these conditions because the feedback loops that normally serve to shut off hormone synthesis and/or secretion are similarly desensitized. Mutations in hormone receptors (especially nuclear receptors) may result in heritable syndromes of hormone resistance. These syndromes, while relatively rare, usually exhibit severe outcomes, and have provided insights into the basic cell biology of hormone signaling. Functional hormone resistance that develops over time is also seen. Resistance arises from a relative failure of receptor signaling to couple efficiently to downstream intracellular effector pathways that normally mediate the effects of the hormone. The most common example of this is seen in type 2 diabetes mellitus. Target tissues for insulin gradually become more and more resistant to its actions, secondary to reduced activation of phosphatidylinositol 3-kinase and other intracellular signaling pathways. A key factor precipitating this outcome is obesity. In addition, because of excessive insulin secretion, pancreatic β cells become "exhausted" and may eventually fail, necessitating treatment with exogenous insulin. An important therapeutic goal, therefore, is to minimize progression to β cell exhaustion before irreversible insulin resistance sets in, with diet, exercise, and treatment with so-called **insulin sensitizers** (such as metformin and rosiglitazone).

CLINICAL BOX 16–2

Approach to the Patient with Suspected Endocrine Disease

Unlike many of the disorders of individual organ systems considered elsewhere in this volume, the symptoms of endocrine disease may be protean because of the number of body systems that are impacted by hormonal action. Further, many endocrine glands are relatively inaccessible to direct physical examination. Endocrine disorders must therefore be diagnosed on the basis of the symptoms they produce in concert with appropriate biochemical testing. Radioimmunoassays for specific hormones remain the mainstay of diagnostic endocrinolology and can be used to establish steady state concentrations as well as dynamic changes of the hormone in question (the latter requiring repeated blood sampling over time). In addition, the principles of feedback regulation of hormone synthesis and release may allow the clinician to

pinpoint the likely locus of any defect by comparing the levels of hormones in the same axis. For example, if testosterone levels are low but those of luteinizing hormone (LH) are high, this suggests that the testes are unable to respond to LH. Conversely, if both testosterone and LH are low, the problem is more likely to be at the level of the pituitary. Synthetic hormones can also be administered exogenously to test whether increased basal levels of a given hormone can be suppressed, or abnormally low levels can be stimulated by a relevant upstream agent. An example of applying this type of reasoning to the evaluation of suspected hypothyroidism is provided in **Figure 16–5**.

THERAPEUTIC HIGHLIGHTS

The appropriate treatment of endocrine disorders depends on their underlying basis. For example, if a particular hormone or its releasing factor is deficient, hormone replacement therapy is often indicated to ameliorate symptoms as well as long-term negative outcomes (Figure 16–5).

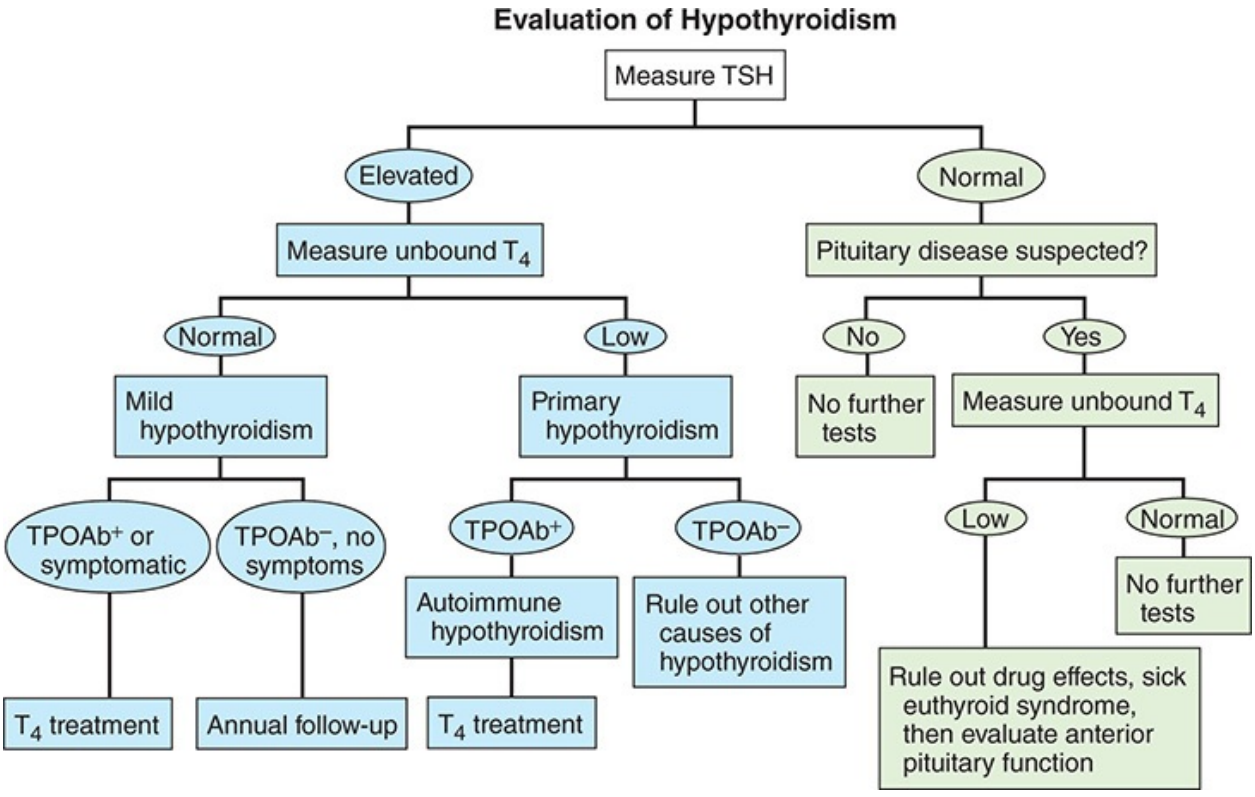


FIGURE 16–5 Summary of a strategy for the laboratory evaluation of hypothyroidism. TSH, thyroid-stimulating hormone; T_4, thyroid hormone;

TPOAb^{+}, positive for autoantibodies to thyroid peroxidase; TPOAb^{-}, antiperoxidase antibodies not present. (Reproduced with permission from Jameson JL [editor]: *Harrison's Endocrinology*, 2nd ed. New York, NY: McGraw Hill; 2010.)

HORMONE EXCESS

The converse of disorders of hormone deficiency or resistance is seen in diseases associated with hormone excess or overstimulation of hormone receptors (or both). A wide variety of endocrine tumors may produce hormones in an excessive and uncontrolled manner. Note that the secretion of hormones from tumor cells may not be subject to the same types of feedback regulation that are seen for the normal source of that hormone. In the setting of an endocrine tumor, exaggerated effects of the hormone are seen. For example, acromegaly, or gigantism, occurs in patients afflicted with an adenoma derived from pituitary somatotropes that secretes excessive quantities of growth hormone (see Chapter 18). In addition, other endocrine tumors may secrete hormones other than those characteristic of the cell type or tissue from which they are originally derived. When hormone production is increased in all of these cases, there usually will also be downregulation of upstream releasing factors due to the triggering of negative feedback loops.

Disorders of hormone excess can also be mimicked by antibodies that bind to, and activate, the receptor for the hormone. A classic example of such a condition is Graves disease, where susceptible individuals generate thyroid-stimulating immunoglobulins (TSIs) that bind to the receptor for TSH. This causes a conformational change that elicits receptor activation, and thus secretion of thyroid hormone in the absence of a physiologic trigger for this event. Diseases associated with hormone excess can also occur in a heritable manner secondary to activating mutations of hormone releasing factor receptors or their downstream targets. As seen for endocrine tumors, these pathophysiologic triggers of excessive hormone release are of course not subject to dampening by negative feedback loops.

CHAPTER SUMMARY

- The endocrine system consists of a distributed set of glands and the chemical messengers that they produce, referred to as hormones. Hormones play a

critical role in ensuring homeostasis (ie, the relative stability of body systems).

- Hormones can be grouped into peptide/protein, amine, and steroid categories. Water-soluble hormones (peptides and catecholamines) bind to cell surface receptors; hydrophobic hormones diffuse into the cell and activate nuclear receptors to regulate gene transcription.
- Hormone availability is dictated by the rate of synthesis, the presence of releasing factors, and rates of degradation or uptake. Free hydrophobic hormones are also in equilibrium with a form bound to plasma protein carriers, the latter representing a hormone reservoir as well as an additional mechanism to regulate hormone availability.
- The synthesis and release of many hormones is subject to regulation by negative feedback loops.
- Disease states can arise in the setting of both hormone deficiency and excess. Hormone deficiencies may be mimicked by inherited defects in their receptors or downstream signaling pathways; hormone excess may be mimicked by autoantibodies that bind to and activate hormone receptors, or by activating mutations of these receptors.

CHAPTER 17

Hypothalamic Regulation of Hormonal Functions

OBJECTIVES

After reading this chapter, you should be able to:

- Describe the anatomic connections between the hypothalamus and the pituitary gland and the functional significance of each connection.
- Define the role of the hypothalamus in producing and secreting hormones of the posterior pituitary.
- Discuss the effects of vasopressin, the receptors on which it acts, and how its secretion is regulated.
- Discuss the effects of oxytocin, the receptors on which it acts, and how its secretion is regulated.
- Outline the hypophysiotropic hormones, and the effect each has on anterior pituitary function.
- List the temperature-regulating mechanisms, and describe the way in which they are integrated under hypothalamic control to maintain normal body temperature in both cold and heat stress.
- Explain how the hypothalamus regulates water intake, and outline how thirst is regulated

- Discuss the pathophysiology of fever.

INTRODUCTION

The **hypothalamus** regulates complex autonomic mechanisms that maintain the chemical constancy of the internal environment, and regulates metabolic endocrine processes to control body temperature and satiety. It synthesizes and secretes **hypothalamic** hormones, and these in turn stimulate or inhibit the secretion of pituitary hormones. The hypothalamus also functions with the limbic system as a unit that regulates emotional and instinctual behavior.

HYPOTHALAMUS: ANATOMIC CONSIDERATIONS

The hypothalamus **(Figure 17–1)** is located in the lower part of the brain above the pituitary gland (Chapter 16, Figure 16–3) and releases hormones directly into the hypophysial portal system, which carries them directly to the pituitary gland. The hypothalamus is the portion of the anterior end of the diencephalon that lies below the hypothalamic sulcus and in front of the interpeduncular nuclei. It is divided into a variety of nuclei and nuclear areas.

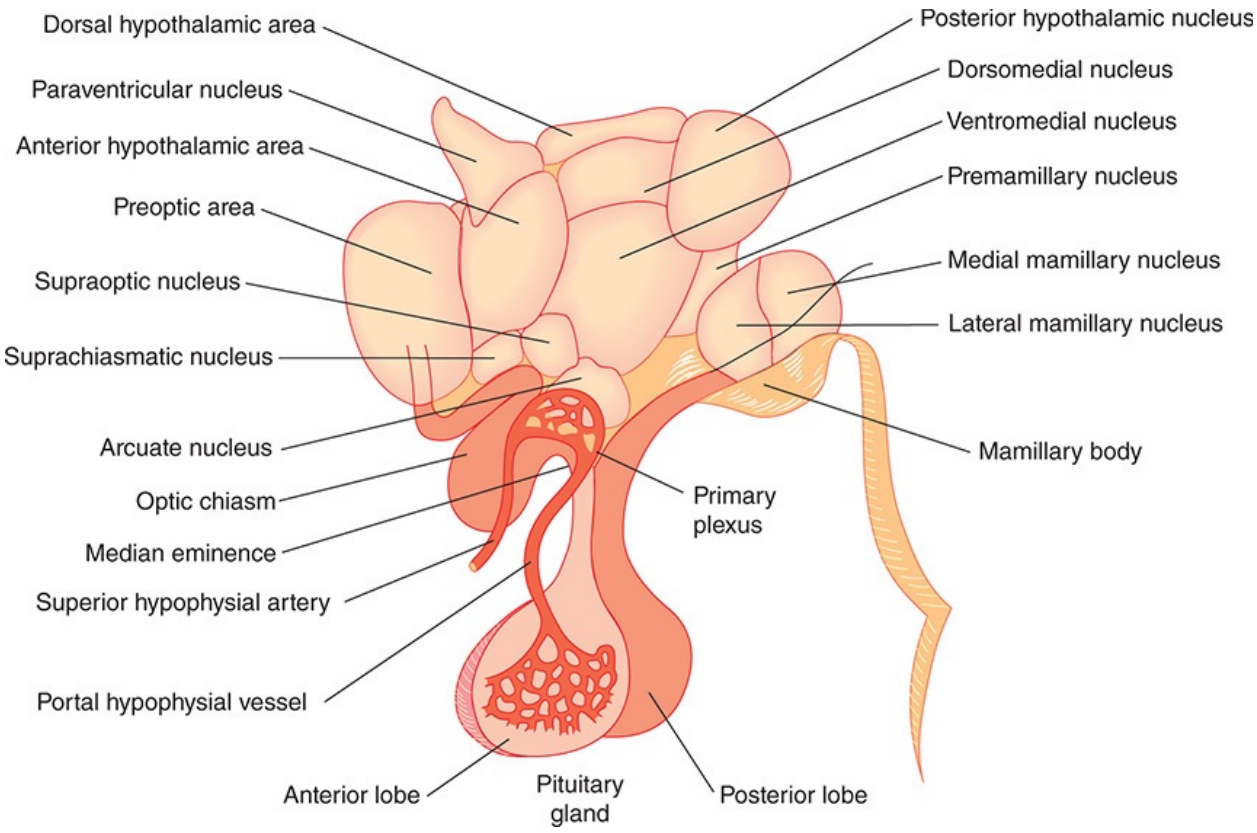


FIGURE 17–1 Human hypothalamus, with a superimposed diagrammatic representation of the portal hypophysial vessels.

AFFERENT & EFFERENT CONNECTIONS OF THE HYPOTHALAMUS

The principal afferent and efferent neural pathways to and from the hypothalamus are mostly unmyelinated. Many connect the hypothalamus to the limbic system. Important connections also exist between the hypothalamus and nuclei in the midbrain tegmentum, pons, and hindbrain.

Norepinephrine-secreting neurons with their cell bodies in the hindbrain end in many different parts of the hypothalamus (see Figure 7–2). Paraventricular neurons that secrete oxytocin and vasopressin project in turn to the hindbrain and the spinal cord. Neurons that secrete epinephrine have their cell bodies in the hindbrain and end in the ventral hypothalamus.

An intrahypothalamic system is composed of dopamine-secreting neurons that have their cell bodies in the arcuate nucleus and end on or near the capillaries that form the portal vessels in the median eminence. Serotonin-

secreting neurons project to the hypothalamus from the raphe nuclei.

RELATION TO THE PITUITARY GLAND

There are neural connections between the hypothalamus and the posterior lobe of the pituitary gland and vascular connections between the hypothalamus and the anterior lobe. Embryologically, the posterior pituitary arises as an evagination of the floor of the third ventricle. It is made up in large part of the endings of axons that arise from cell bodies in the supraoptic and paraventricular nuclei and pass to the posterior pituitary **(Figure 17–2)** via the **hypothalamohypophysial tract**. Most of the supraoptic fibers end in the posterior lobe itself, whereas some of the paraventricular fibers end in the median eminence. The anterior and intermediate lobes of the pituitary arise in the embryo from the rathke pouch, an evagination from the roof of the pharynx (see Figure 18–1). **Sympathetic** nerve fibers reach the anterior lobe from its capsule, and parasympathetic fibers reach it from the petrosal nerves, but few if any nerve fibers pass to it from the hypothalamus. **However**, the **portal hypophysial vessels** form a direct vascular link between the hypothalamus and the anterior pituitary. Arterial twigs from the carotid arteries and circle of willis form a network of fenestrated capillaries called the **primary plexus** on the ventral surface of the hypothalamus (Figure 17–1). Capillary loops also penetrate the median eminence. The capillaries drain into the sinusoidal portal hypophysial vessels that carry blood down the pituitary stalk to the capillaries of the anterior pituitary. This system begins and ends in capillaries without going through the heart and is therefore a true portal system. In birds and some mammals, including humans, there is no other anterior hypophysial arterial supply other than capsular vessels and anastomotic connections from the capillaries of the posterior pituitary. The **median eminence** is generally defined as the portion of the ventral hypothalamus from which the portal vessels arise. This region is outside the blood-brain barrier (see Chapter 33).

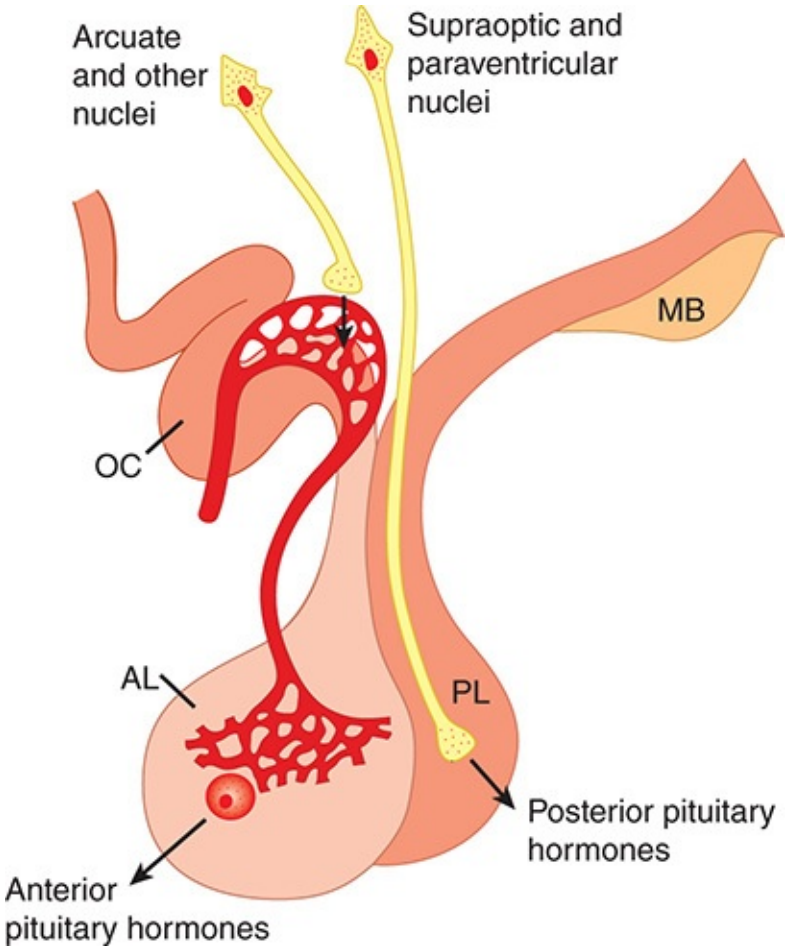


FIGURE 17–2 Secretion of hypothalamic hormones. The hormones of the posterior lobe (PL) are released into the general circulation from the endings of supraoptic and paraventricular neurons, whereas hypophysiotropic hormones are secreted into the portal hypophysial circulation from the endings of arcuate and other hypothalamic neurons. AL, anterior lobe; MB, mamillary bodies; OC, optic chiasm.

HYPOTHALAMIC FUNCTION

The major functions of the hypothalamus are summarized in **Table 17–1**. Some are fairly clear-cut visceral reflexes, and others include complex behavioral and emotional reactions; however, all involve a specific response to a specific stimulus. It is important to keep this in mind in considering hypothalamic function.

TABLE 17–1 Summary of principal hypothalamic regulatory mechanisms.

Function	Afferents from	Integrating Areas
Temperature regulation	Temperature receptors in the skin, deep tissues, spinal cord, hypothalamus, and other parts of the brain	Anterior hypothalamus, response to heat; posterior hypothalamus, response to cold
Neuroendocrine control of:		
Catecholamines	Limbic areas concerned with emotion	Dorsal and posterior hypothalamus
Vasopressin	Osmoreceptors, "volume receptors," others	Supraoptic and paraventricular nuclei
Oxytocin	Touch receptors in breast, uterus, genitalia	Supraoptic and paraventricular nuclei
Thyroid-stimulating hormone (thyrotropin, TSH) via TRH	Temperature receptors in infants, perhaps others	Paraventricular nuclei and neighboring areas
Adrenocorticotropic hormone (ACTH) and β-lipotropin (β-LPH) via CRH	Limbic system (emotional stimuli); reticular formation ("systemic" stimuli); hypothalamic and anterior pituitary cells sensitive to circulating blood cortisol level; suprachiasmatic nuclei (diurnal rhythm)	Paraventricular nuclei
Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) via GnRH	Hypothalamic cells sensitive to estrogens, eyes, touch receptors in skin and genitalia of reflex ovulating species	Preoptic area; other areas
Prolactin via PIH and PRH	Touch receptors in breasts, other unknown receptors	Arcuate nucleus; other areas (hypothalamus inhibits secretion)
Growth hormone via somatostatin and GRH	Unknown receptors	Periventricular nucleus, arcuate nucleus
"Appetitive" behavior:		
Thirst	Osmoreceptors, probably located in the organum vasculosum of the lamina terminalis; angiotensin II uptake in the subfornical organ	Lateral superior hypothalamus
Hunger	Glucostat cells sensitive to rate of glucose utilization; leptin receptors; receptors for other polypeptides	Ventromedial, arcuate, and paraventricular nuclei; lateral hypothalamus
Sexual behavior	Cells sensitive to circulating estrogen and androgen, others	Anterior ventral hypothalamus plus, in the male, piriform cortex
Defensive reactions (fear, rage)	Sense organs and neocortex, paths unknown	Diffuse, in limbic system and hypothalamus
Control of body rhythms	Retina via retinohypothalamic fibers	Suprachiasmatic nuclei

RELATION TO AUTONOMIC FUNCTION

Many years ago, Sherrington called the hypothalamus "the head ganglion of the autonomic system." Stimulation of the hypothalamus produces autonomic responses, but the hypothalamus does not seem to be concerned with the regulation of visceral function per se. Rather, the autonomic responses triggered in the hypothalamus are part of more complex phenomena such as eating, and emotions such as rage. For example, stimulation of various parts of the hypothalamus, especially the lateral areas, produces diffuse sympathetic discharge and increased adrenal medullary secretion—the mass sympathetic discharge seen in animals exposed to stress (the flight or fight reaction; see Chapter 13).

It has been claimed that separate hypothalamic areas control epinephrine and norepinephrine secretion. Differential secretion of one or the other of these adrenal medullary catecholamines does occur in certain situations (see Chapter 20), but the selective increases are small.

Body weight depends on the balance between caloric intake and utilization of calories. Obesity results when the former exceeds the latter. The hypothalamus and related parts of the brain play a key role in the regulation of food intake. Obesity is considered in detail in Chapter 26, and the relation of obesity to diabetes mellitus is discussed in Chapter 24.

Hypothalamic regulation of sleep and circadian rhythms are discussed in Chapter 14.

THIRST

Another appetitive mechanism under hypothalamic control is thirst. Drinking is regulated by plasma osmolality and extracellular fluid (ECF) volume in much the same fashion as vasopressin secretion (see Chapter 38). Water intake is increased by increased effective osmotic pressure of the plasma **(Figure 17–3)**, by decreases in ECF volume, and by psychological and other factors. Osmolality acts via **osmoreceptors,** receptors that sense the osmolality of the body fluids. These osmoreceptors are located in the anterior hypothalamus.

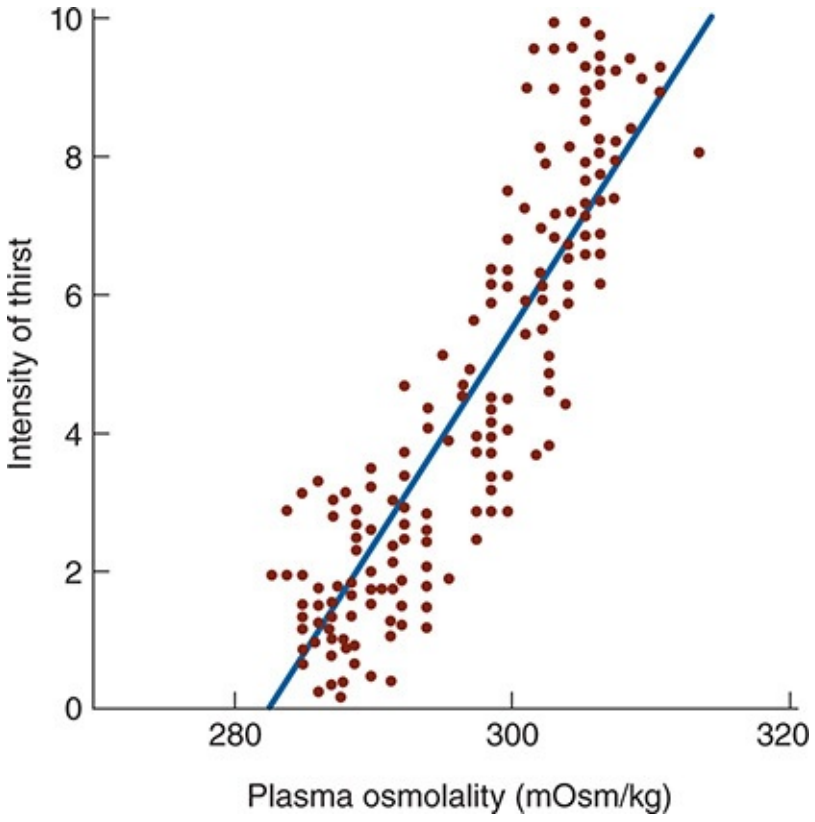


FIGURE 17–3 Relation of plasma osmolality to thirst in healthy adult

humans during infusion of hypertonic saline. The intensity of thirst is measured on a special analog scale. (Reproduced with permission from Thompson CJ et al: The osmotic thresholds for thirst and vasopressin release are similar in healthy humans. Clin Sci Lond 1986; Dec; 71(6): 651–656.)

Decreases in ECF volume also stimulate thirst by a pathway independent of that mediating thirst in response to increased plasma osmolality **(Figure 17–4)**. Thus, hemorrhage causes increased drinking even if there is no change in the osmolality of the plasma. The effect of ECF volume depletion on thirst is mediated in part via the renin–angiotensin system (see Chapter 38). Renin secretion is increased by hypovolemia and results in an increase in circulating angiotensin II. The angiotensin II acts on the **subfornical organ,** a specialized receptor area in the diencephalon (see Figure 33–7), to stimulate the neural areas concerned with thirst. Some evidence suggests that it acts on the **organum vasculosum of the lamina terminalis (OVLT)** as well. These areas are highly permeable and are two of the circumventricular organs located outside the blood-brain barrier (see Chapter 33). However, drugs that block the action of angiotensin II do not completely block the thirst response to hypovolemia, and it appears that the baroreceptors in the heart and blood vessels are also involved.

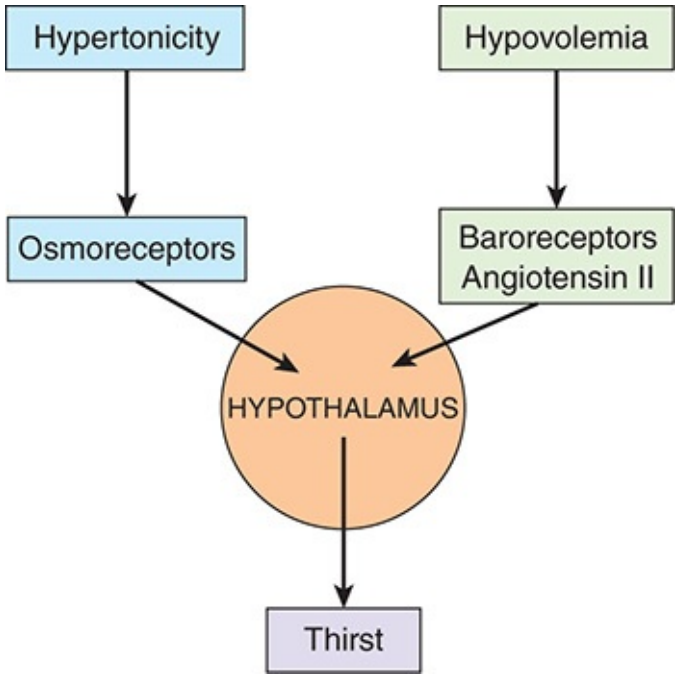


FIGURE 17–4 Diagrammatic representation of the way in which changes in plasma osmolality and changes in ECF volume affect thirst by separate pathways. ECF, extracellular fluid.

The intake of liquids is increased during eating **(prandial drinking).** The increase has been called a learned or habit response, but it has not been investigated in detail. One factor is an increase in plasma osmolality that occurs as food is absorbed. Another may be an action of one or more gastrointestinal hormones on the hypothalamus.

When the sensation of thirst is obtunded, either by direct damage to the diencephalon or by depressed or altered states of consciousness, patients stop drinking adequate amounts of fluid. Dehydration results if appropriate measures are not instituted to maintain water balance. If the protein intake is high, the products of protein metabolism cause an osmotic diuresis (see Chapter 38), and the amounts of water required to maintain hydration are large. Most cases of **hypernatremia** are actually due to simple dehydration in patients with psychoses or hypothalamic disease who do not or cannot increase their water intake when their thirst mechanism is stimulated. Lesions of the anterior communicating artery can also obtund thirst because branches of this artery supply the hypothalamic areas concerned with thirst.

OTHER FACTORS REGULATING WATER INTAKE

A number of other well-established factors contribute to the regulation of water intake. Psychological and social factors are important. Dryness of the pharyngeal mucous membrane causes a sensation of thirst. Patients in whom fluid intake must be restricted sometimes get appreciable relief of thirst by sucking ice chips or a wet cloth.

Dehydrated dogs, cats, camels, and some other animals rapidly drink just enough water to make up their water deficit. They stop drinking before the water is absorbed (while their plasma is still hypertonic), so some kind of pharyngeal gastrointestinal “metering” must be involved. Some evidence suggests that humans have a similar metering ability, though it is not well developed.

CONTROL OF POSTERIOR PITUITARY SECRETION

VASOPRESSIN & OXYTOCIN

In most mammals, the hormones secreted by the posterior pituitary gland are

arginine vasopressin (AVP) and **oxytocin.** In hippopotami and most pigs, arginine in the vasopressin molecule is replaced by lysine to form **lysine vasopressin.** The posterior pituitaries of some species of pigs and marsupials contain a mixture of arginine and lysine vasopressin. The posterior lobe hormones are nanopeptides with a disulfide ring at one end **(Figure 17–5)**.

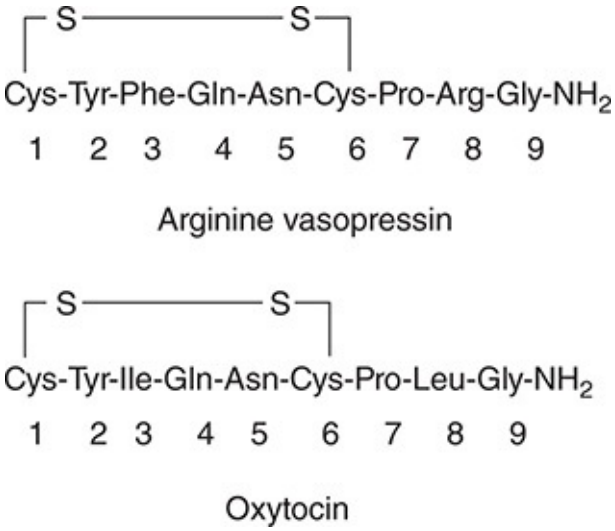


FIGURE 17–5 Arginine vasopressin and oxytocin.

BIOSYNTHESIS, INTRANEURONAL TRANSPORT, & SECRETION

The hormones of the posterior pituitary gland are synthesized in the cell bodies of the magnocellular neurons in the supraoptic and paraventricular nuclei and transported down the axons of these neurons to their endings in the posterior lobe, where they are secreted in response to electrical activity in the endings. Some of the neurons make oxytocin and others make vasopressin, and oxytocin-containing and vasopressin-containing cells are found in both nuclei.

Oxytocin and vasopressin are typical **neural hormones,** that is, hormones secreted into the circulation by nerve cells. This type of neural regulation is compared with other types in **Figure 17–6**. The term **neurosecretion** was originally coined to describe the secretion of hormones by neurons, but the term is somewhat misleading because it appears that all neurons secrete chemical messengers (see Chapter 7).

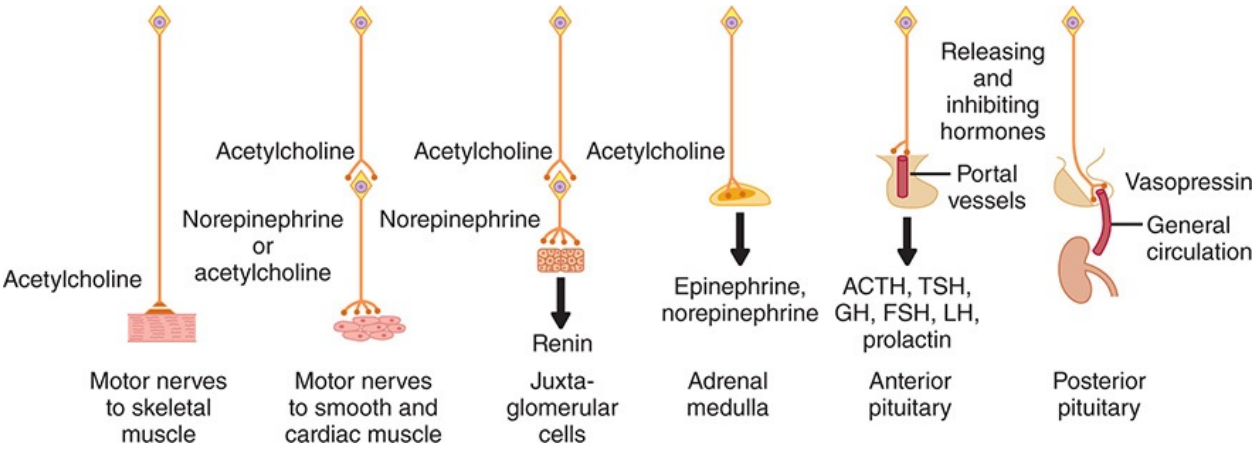


FIGURE 17–6 Neural control mechanisms. In the two situations on the left, neurotransmitters act at nerve endings on muscle; in the two in the middle, neurotransmitters regulate the secretion of endocrine glands; and in the two on the right, neurons secrete hormones into the hypophysial portal or general circulation. ACTH, adrenocorticotropic hormone; FSH, follicle-stimulating hormone; GH, growth hormone; LH, luteinizing hormone; TSH, thyroid-stimulating hormone.

Like other peptide hormones, the posterior lobe hormones are synthesized as part of larger precursor molecules. Vasopressin and oxytocin each have a characteristic **neurophysin** associated with them in the granules in the neurons that secrete them—neurophysin I in the case of oxytocin and neurophysin II in the case of vasopressin. The neurophysins were originally thought to be binding polypeptides, but it now appears that they are simply parts of the precursor molecules. The precursor for AVP, **prepropressophysin,** contains a 19-amino-acid residue leader sequence followed by AVP, neurophysin II, and a glycopeptide **(Figure 17–7)**. **Prepro-oxyphysin,** the precursor for oxytocin, is a similar but smaller molecule that lacks the glycopeptide.

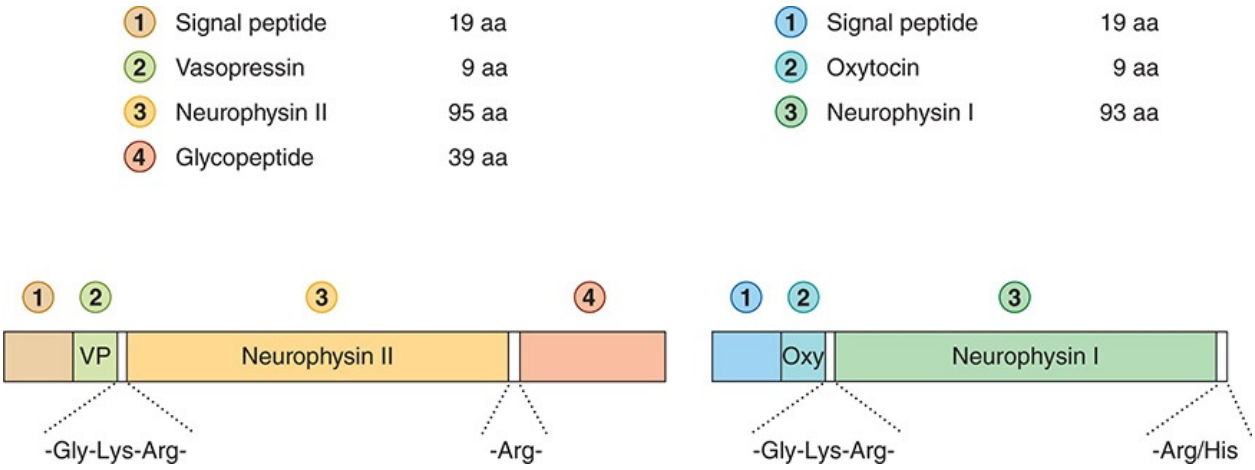


FIGURE 17–7 Structure of bovine prepropressophysin (left) and prepro-oxyphysin (right). Gly in the 10 position of both peptides is necessary for amidation of the Gly residue in position 9. aa, amino acid residues. (Reproduced with permission from Richter D: Molecular events in expression of vasopressin and oxytocin and their cognate receptors. Am J Physiol 1988; Aug; 255(2 Pt 2):F207–F219.)

The precursor molecules are synthesized in the ribosomes of the cell bodies of the neurons. They have their leader sequences removed in the endoplasmic reticulum, are packaged into secretory granules in the Golgi apparatus, and are transported down the axons by axoplasmic flow to the endings in the posterior pituitary. The secretory granules, called **Herring bodies,** are easy to stain in tissue sections, and they have been extensively studied. Cleavage of the precursor molecules occurs as they are being transported, and the storage granules in the endings contain free vasopressin or oxytocin and the corresponding neurophysin. In the case of vasopressin, the glycopeptide is also present. All these products are secreted, but the functions of the components other than the established posterior pituitary hormones are unknown. Physiologic control of vasopressin secretion is described in detail in Chapter 38.

ELECTRICAL ACTIVITY OF MAGNOCELLULAR NEURONS

The oxytocin-secreting and vasopressin-secreting neurons also generate and conduct action potentials, and action potentials reaching their endings trigger the release of hormones by Ca^{2+}-dependent exocytosis. At least in anesthetized rats,

these neurons are silent at rest or discharge at low, irregular rates (0.1–3 spikes/s). However, their response to stimulation varies **(Figure 17–8)**. Stimulation of the nipples causes a synchronous, high-frequency discharge of the oxytocin neurons after an appreciable latency. This discharge causes release of a pulse of oxytocin and consequent milk ejection in postpartum females. On the other hand, stimulation of vasopressin-secreting neurons by a stimulus such as an increase in blood osmolality during dehydration, or loss of blood volume due to hemorrhage, causes an initial steady increase in firing rate followed by a prolonged pattern of phasic discharge in which periods of high-frequency discharge alternate with periods of electrical quiescence **(phasic bursting).** These phasic bursts are generally not synchronous in different vasopressin-secreting neurons. They are well-suited to maintain a prolonged increase in the output of vasopressin, as opposed to the synchronous, relatively short, high-frequency discharge of oxytocin-secreting neurons in response to stimulation of the nipples.

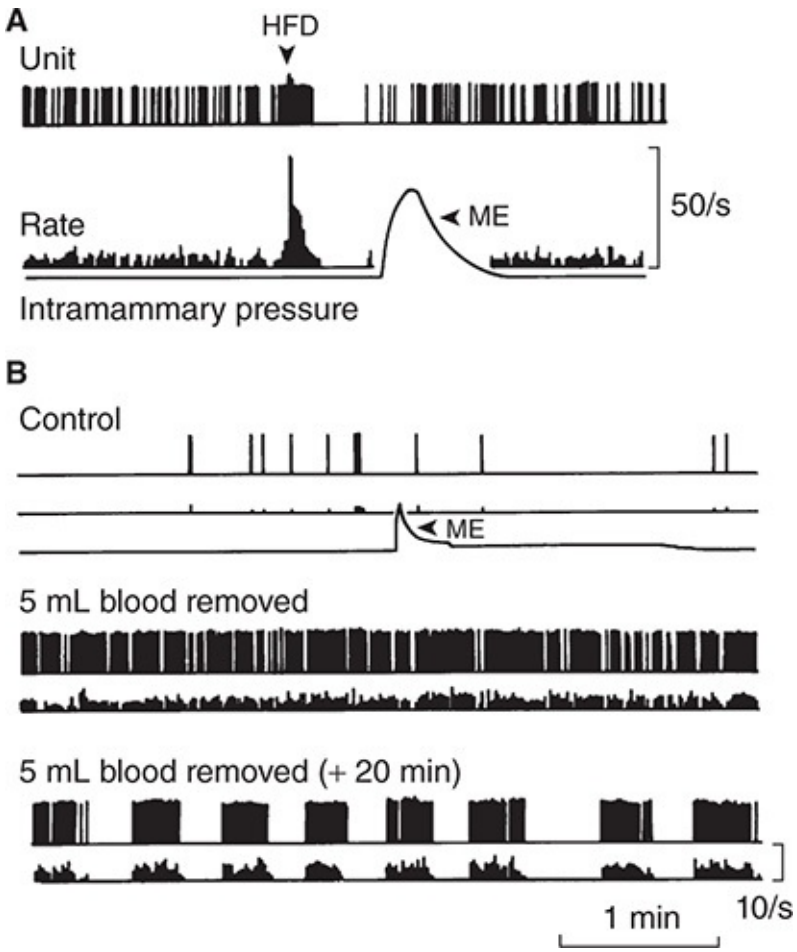


FIGURE 17–8 Responses of magnocellular neurons to stimulation. The

tracings show individual extracellularly recorded action potentials, discharge rates, and intramammary duct pressure. **A)** Response of an oxytocin-secreting neuron. HFD, high-frequency discharge; ME, milk ejection. Stimulation of nipples started before the onset of recording. **B)** Responses of a vasopressin-secreting neuron, showing no change in the slow firing rate in response to stimulation of nipples and a prompt increase in the firing rate when 5 mL of blood was drawn, followed by typical phasic discharge. (Modified with permission from Wakerly JB: Hypothalamic neurosecretory function: Insights from electrophysiological studies of the magno-cellular nuclei. IBRO News 1985; 4:15.)

VASOPRESSIN & OXYTOCIN IN OTHER LOCATIONS

Vasopressin-secreting neurons are found in the suprachiasmatic nuclei, and vasopressin and oxytocin are also found in the endings of neurons that project from the paraventricular nuclei to the brainstem and spinal cord. These neurons appear to be involved in cardiovascular control. In addition, vasopressin and oxytocin are synthesized in the gonads and the adrenal cortex, and oxytocin is present in the thymus. The functions of the peptides in these organs are unsettled.

Vasopressin Receptors

There are at least three kinds of vasopressin receptors: V_{1A}, V_{1B}, and V_2. All are G-protein-coupled. The V_{1A} and V_{1B} receptors act through phosphatidylinositol hydrolysis to increase intracellular Ca^{2+} concentrations. The V_2 receptors act through G_s to increase cyclic adenosine monophosphate levels.

Effects of Vasopressin

Because one of its principal physiologic effects is the retention of water by the kidney, vasopressin is often called the **antidiuretic hormone (ADH).** It increases the permeability of the collecting ducts of the kidney so that water enters the hypertonic interstitium of the renal pyramids (see Chapter 37). The urine becomes concentrated and its volume decreases. The overall effect is

therefore retention of water in excess of solute; consequently, the effective osmotic pressure of the body fluids is decreased. In the absence of vasopressin, the urine is hypotonic to plasma, urine volume is increased, and there is a net water loss. Consequently, the osmolality of the body fluid rises.

Effects of Oxytocin

In humans, oxytocin acts primarily on the breasts and uterus, though it appears to be involved in luteolysis as well (see Chapter 22). A G-protein-coupled oxytocin receptor has been identified in human myometrium, and a similar or identical receptor is found in mammary tissue and the ovary. It triggers increases in intracellular Ca^{2+} levels.

The Milk Ejection Reflex

Oxytocin causes contraction of the **myoepithelial cells** that line the ducts of the breast. This squeezes the milk out of the alveoli of the lactating breast into the large ducts (sinuses) and thence out of the nipple **(milk ejection).** Many hormones acting in concert are responsible for breast growth and the secretion of milk into the ducts (see Chapter 22), but milk ejection in most species requires oxytocin.

Milk ejection is normally initiated by a neuroendocrine reflex. The receptors involved are touch receptors, which are plentiful in the breast—especially around the nipple. Impulses generated in these receptors are relayed from the somatic touch pathways to the supraoptic and paraventricular nuclei. Discharge of the oxytocin-containing neurons causes secretion of oxytocin from the posterior pituitary (Figure 17–8). The suckling of an infant at the breast stimulates the touch receptors, the nuclei are stimulated, oxytocin is released, and the milk is expressed into the sinuses, ready to flow into the mouth of the waiting infant. In lactating women, genital stimulation and emotional stimuli also produce oxytocin secretion, sometimes causing milk to spurt from the breasts.

Other Actions of Oxytocin

Oxytocin causes contraction of the smooth muscle of the uterus. The sensitivity of the uterine musculature to oxytocin is enhanced by estrogen and inhibited by progesterone. The inhibitory effect of progesterone is due to a direct action of

the steroid on uterine oxytocin receptors. In late pregnancy, the uterus becomes very sensitive to oxytocin coincident with a marked increase in the number of oxytocin receptors and oxytocin receptor mRNA (see Chapter 22). Oxytocin secretion is then increased during labor. After dilation of the cervix, descent of the fetus down the birth canal initiates impulses in the afferent nerves that are relayed to the supraoptic and paraventricular nuclei, causing secretion of sufficient oxytocin to enhance labor (Figure 22–22). The amount of oxytocin in plasma is normal at the onset of labor. It is possible that the marked increase in oxytocin receptors at this time allows normal oxytocin levels to initiate contractions, setting up a positive feedback. However, the amount of oxytocin in the uterus is also increased, and locally produced oxytocin may also play a role.

Oxytocin may also act on the nonpregnant uterus to facilitate sperm transport. The passage of sperm up the female genital tract to the uterine tubes, where fertilization normally takes place, depends not only on the motile powers of the sperm but also, at least in some species, on uterine contractions. The genital stimulation involved in coitus releases oxytocin, but whether oxytocin initiates the rather specialized uterine contractions that transport the sperm is as yet unproven. The secretion of oxytocin is also increased by stressful stimuli and, like that of vasopressin, is inhibited by alcohol.

Circulating oxytocin increases at the time of ejaculation in males, and it is possible that this causes increased contraction of the smooth muscle of the vas deferens, propelling sperm toward the urethra.

CONTROL OF ANTERIOR PITUITARY SECRETION

ANTERIOR PITUITARY HORMONES

The anterior pituitary secretes six hormones: **adrenocorticotropic hormone (corticotropin, ACTH), thyroid-stimulating hormone (thyrotropin, TSH), growth hormone, follicle-stimulating hormone (FSH), luteinizing hormone (LH),** and **prolactin (PRL).** An additional polypeptide, β-lipotropin (β-LPH), is secreted with ACTH, but its physiologic role is unknown. The actions of the anterior pituitary hormones are summarized in **Figure 17–9**. The hormones are discussed in detail in subsequent chapters. The hypothalamus plays an important stimulatory role in regulating the secretion of ACTH, β-LPH, TSH, growth hormone, FSH, and LH. It also regulates prolactin secretion, but its effect is predominantly inhibitory rather than stimulatory.

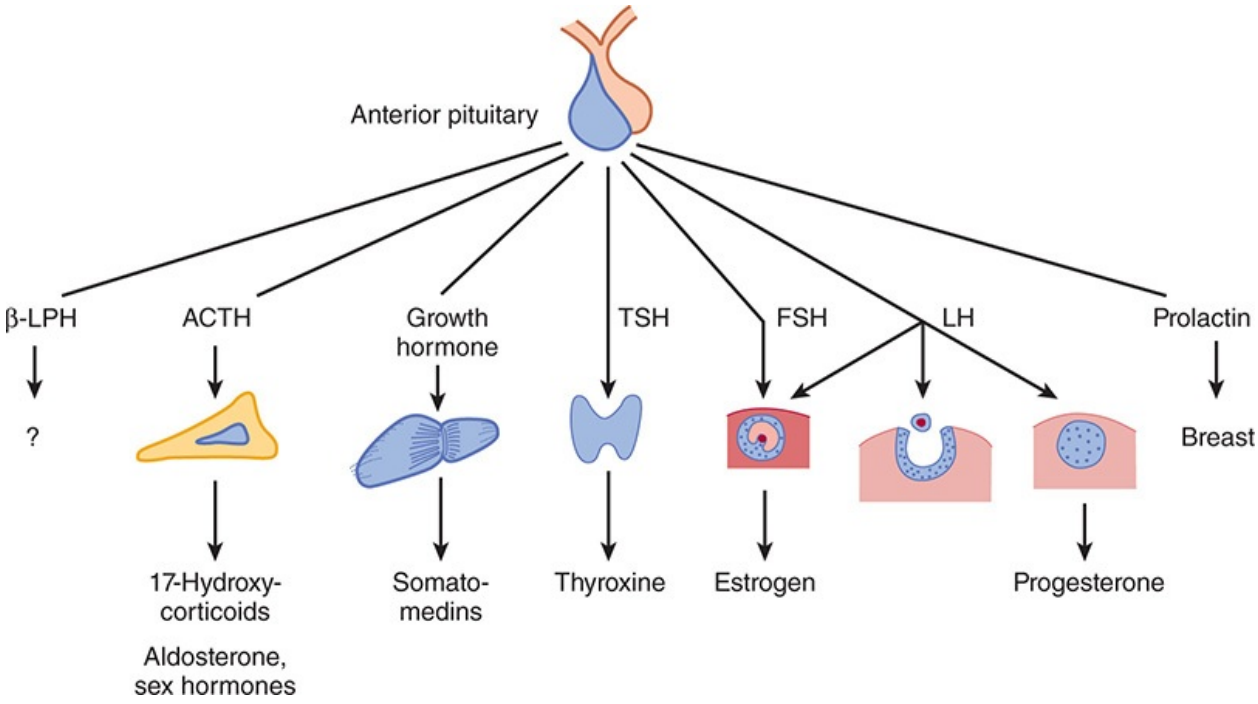


FIGURE 17–9 Anterior pituitary hormones. In women, FSH and LH act in sequence on the ovary to produce growth of the ovarian follicle, ovulation, and formation and maintenance of the corpus luteum. Prolactin stimulates lactation. In men, FSH and LH control the functions of the testes. ACTH, adrenocorticotropic hormone; β-LPH, β-lipotropin; FSH, follicle-stimulating hormone; LH, luteinizing hormone; TSH, thyroid-stimulating hormone.

NATURE OF HYPOTHALAMIC CONTROL

Anterior pituitary secretion is controlled by chemical agents carried in the portal hypophysial vessels from the hypothalamus to the pituitary. These substances used to be called releasing and inhibiting factors, but now they are commonly called **hypophysiotropic hormones.** The latter term seems appropriate since they are secreted into the bloodstream and act at a distance from their site of origin. Small amounts escape into the general circulation, but they are at their highest concentration in portal hypophysial blood.

HYPOPHYSIOTROPIC HORMONES

There are six established hypothalamic releasing and inhibiting hormones **(Figure 17–10)**: **corticotropin-releasing hormone (CRH); thyrotropin-**

releasing hormone (TRH); growth hormone–releasing hormone (GRH); growth hormone–inhibiting hormone (GIH, now generally called **somatostatin); luteinizing hormone–releasing hormone (LHRH,** now generally known as **gonadotropin-releasing hormone [GnRH]);** and **prolactin-inhibiting hormone (PIH).** In addition, hypothalamic extracts contain prolactin-releasing activity, and a **prolactin-releasing hormone (PRH)** has been postulated to exist. TRH, VIP, and several other polypeptides found in the hypothalamus stimulate prolactin secretion, but it is uncertain whether one or more of these peptides is the physiologic PRH. Recently, an orphan receptor was isolated from the anterior pituitary, and the search for its ligand led to the isolation of a 31-amino-acid polypeptide from the human hypothalamus. This polypeptide stimulated prolactin secretion by an action on the anterior pituitary receptor, but additional research is needed to determine whether it is the physiologic PRH. GnRH stimulates the secretion of FSH as well as that of LH, and it seems unlikely that a separate FSH-releasing hormone exists.

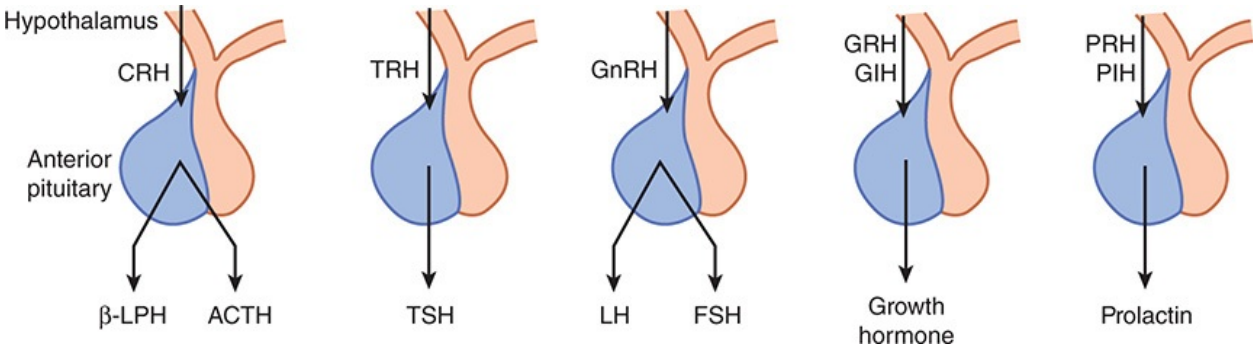


FIGURE 17–10 Effects of hypophysiotropic hormones on the secretion of anterior pituitary hormones. ACTH, adrenocorticotropic hormone; β-LPH, β-lipotropin; CRH, corticotropin-releasing hormone; FSH, follicle-stimulating hormone; GIH, growth hormone–inhibiting hormone; GnRH, gonadotropin-releasing hormone; GRH, growth hormone–releasing hormone; LH, luteinizing hormone; PIH, prolactin-inhibiting hormone; PRH, prolactin-releasing hormone; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone.

The structures of the genes and preprohormones for TRH, GnRH, somatostatin, CRH, and GRH are known. PreproTRH contains six copies of TRH. Several other preprohormones may contain other hormonally active peptides in addition to the hypophysiotropic hormones.

The area from which the hypothalamic releasing and inhibiting hormones are secreted is the median eminence of the hypothalamus. This region contains few

nerve cell bodies, but many nerve endings are in close proximity to the capillary loops from which the portal vessels originate.

The locations of the cell bodies of the neurons that project to the external layer of the median eminence and secrete the hypophysiotropic hormones are shown in **Figure 17–11**, which also shows the location of the neurons secreting oxytocin and vasopressin. The GnRH-secreting neurons are primarily in the medial preoptic area, the somatostatin-secreting neurons are in the periventricular nuclei, the TRH-secreting and CRH-secreting neurons are in the medial parts of the paraventricular nuclei, and the GRH-secreting (and dopamine-secreting) neurons are in the arcuate nuclei.

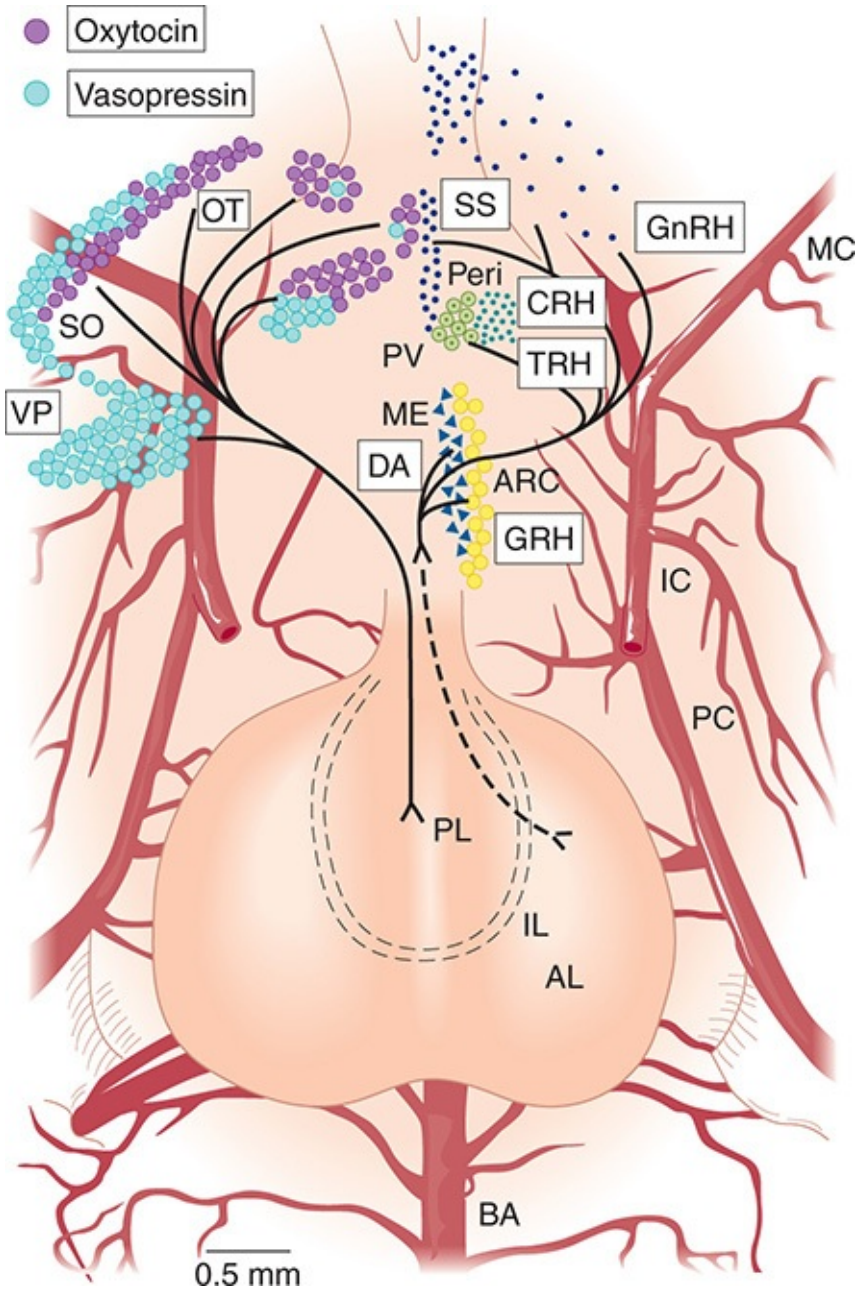


FIGURE 17–11 Location of cell bodies of hypophysiotropic hormone-secreting neurons projected on a ventral view of the hypothalamus and pituitary of the rat. AL, anterior lobe; ARC, arcuate nucleus; BA, basilar artery; CRH, corticotropin-releasing hormone; DA, dopamine; GRH, growth hormone-releasing hormone; GnRH, gonodotropin-releasing hormone; IC, internal carotid artery; IL, intermediate lobe; MC, middle cerebral artery; ME, median eminence; PC, posterior cerebral artery; Peri, periventricular nucleus; PL, posterior lobe; PV, paraventricular nucleus; SO, supraoptic nucleus; TRH, thyroid-releasing hormone. The names of the hormones are enclosed in boxes.

(Used with permission of LW Swanson and ET Cunningham Jr.)

Most, if not all, of the hypophysiotropic hormones affect the secretion of more than one anterior pituitary hormone (Figure 17–10). The FSH-stimulating activity of GnRH has been mentioned previously. TRH stimulates the secretion of prolactin as well as TSH. Somatostatin inhibits the secretion of TSH as well as growth hormone. It does not normally inhibit the secretion of the other anterior pituitary hormones, but it inhibits the abnormally elevated secretion of ACTH in patients with Nelson syndrome. CRH stimulates the secretion of ACTH and β-LPH.

Hypophysiotropic hormones function as neurotransmitters in other parts of the brain, the retina, and the autonomic nervous system (see Chapter 7). In addition, somatostatin is found in the pancreatic islets (see Chapter 24), GRH is secreted by pancreatic tumors, and somatostatin and TRH are found in the gastrointestinal tract (see Chapter 25).

Receptors for most of the hypophysiotropic hormones are coupled to G-proteins. There are two human CRH receptors: hCRH-RI and hCRH-RII. The physiologic role of hCRH-RII is unsettled, though it is found in many parts of the brain. In addition, a **CRH-binding protein** in the peripheral circulation inactivates CRH. It is also found in the cytoplasm of corticotropes in the anterior pituitary, and in this location it might play a role in receptor internalization. However, the exact physiologic role of this protein is unknown. Other hypophysiotropic hormones do not have known binding proteins.

SIGNIFICANCE & CLINICAL IMPLICATIONS

Research delineating the multiple neuroendocrine regulatory functions of the hypothalamus is important because it helps explain how endocrine secretion is matched to the demands of a changing environment. The nervous system receives information about changes in the internal and external environment from the sense organs. It brings about adjustments to these changes through effector mechanisms that include not only somatic movement but also changes in the rate at which hormones are secreted.

The manifestations of hypothalamic disease are neurologic defects, endocrine changes, and metabolic abnormalities such as hyperphagia and hyperthermia. The relative frequencies of the signs and symptoms of hypothalamic disease in one large series of cases are shown in **Table 17–2**. The possibility of hypothalamic pathology should be kept in mind in evaluating all patients with

pituitary dysfunction, especially those with isolated deficiencies of single pituitary tropic hormones.

TABLE 17–2 Symptoms and signs in 60 patients with hypothalamic disease.

Symptoms and Signs	Percentage of Cases
Endocrine and metabolic findings	
Precocious puberty	40
Hypogonadism	32
Diabetes insipidus	35
Obesity	25
Abnormalities of temperature regulation	22
Emaciation	18
Bulimia	8
Anorexia	7
Neurologic findings	
Eye signs	78
Pyramidal and sensory deficits	75
Headache	65
Extrapyramidal signs	62
Vomiting	40
Psychic disturbances, rage attacks, etc	35
Somnolence	30
Convulsions	15

Data from Bauer HG: Endocrine and other clinical manifestations of hypothalamic disease. J Clin Endocrinol 1954;14:13. See also Kahana L, et al: Endocrine manifestations of intracranial extrasellar lesions. J Clin Endocrinol 1962;22:304.

A condition of considerable interest in this context is **Kallmann syndrome,** the combination of hypogonadism due to low levels of circulating gonadotropins **(hypogonadotropic hypogonadism)** with partial or complete loss of the sense of smell (**hyposmia** or **anosmia**). Embryologically, GnRH neurons develop in the nose and migrate up the olfactory nerves and then through the brain to the hypothalamus. If this migration is prevented by congenital abnormalities in the olfactory pathways, the GnRH neurons do not reach the hypothalamus and pubertal maturation of the gonads does not occur. The syndrome is most common in men, and the cause in many cases is mutation of the *KALIG1* gene, a gene on the X chromosome that codes for an adhesion molecule necessary for

the normal development of the olfactory nerve. However, the condition also occurs in women and can be due to other genetic abnormalities.

TEMPERATURE REGULATION

In the body, heat is produced by muscular exercise, assimilation of food, and all the vital processes that contribute to the basal metabolic rate. It is lost from the body by radiation, conduction, and vaporization of water in the respiratory passages and on the skin. Small amounts of heat are also removed in the urine and feces. The balance between heat production and heat loss determines the body temperature. Because the speed of chemical reactions varies with temperature and because the enzyme systems of the body have narrow temperature ranges in which their function is optimal, normal body function depends on a relatively constant body temperature.

Invertebrates generally cannot adjust their body temperatures and so are at the mercy of the environment. Vertebrates have evolved mechanisms for maintaining body temperature by adjusting heat production and heat loss. In reptiles, amphibians, and fish, the adjusting mechanisms are relatively rudimentary, and these species are called “cold-blooded” **(poikilothermic)** because their body temperature fluctuates over a considerable range. In birds and mammals, the “warm-blooded” **(homeothermic)** animals, a group of reflex responses that are primarily integrated in the hypothalamus operate to maintain body temperature within a narrow range in spite of wide fluctuations in environmental temperature. The hibernating mammals are a partial exception. While awake they are homeothermic, but during hibernation their body temperature falls.

NORMAL BODY TEMPERATURE

In homeothermic animals, the actual temperature at which the body is maintained varies from species to species and, to a lesser degree, from individual to individual. In humans, the traditional normal value for the oral temperature is 37°C (98.6°F), but in one large series of normal young adults, the morning oral temperature averaged 36.7°C, with a standard deviation of 0.2°C. Therefore, 95% of all young adults would be expected to have a morning oral temperature of 36.3–37.1°C (97.3–98.8°F; mean ± 1.96 standard deviations). Various parts of the body are at different temperatures, and the magnitude of the temperature difference between the parts varies with the environmental temperature. The

extremities are generally cooler than the rest of the body. The temperature of the scrotum is carefully regulated at 32°C. The rectal temperature is representative of the temperature at the core of the body and varies least with changes in environmental temperature. The oral temperature is normally 0.5°C lower than the rectal temperature, but it is affected by many factors, including ingestion of hot or cold fluids, gum chewing, smoking, and mouth breathing.

The normal human core temperature undergoes a regular circadian fluctuation of 0.5–0.7°C. In individuals who sleep at night and are awake during the day (even when hospitalized at bed rest), it is lowest at about 6:00 AM and highest in the evenings **(Figure 17–12)**. It is lowest during sleep, is slightly higher in the awake but relaxed state, and rises with activity. In women, an additional monthly cycle of temperature variation is characterized by a rise in basal temperature at the time of ovulation (Figure 22–14). Temperature regulation is less precise in young children and they may normally have a temperature that is 0.5°C or so above the established norm for adults.

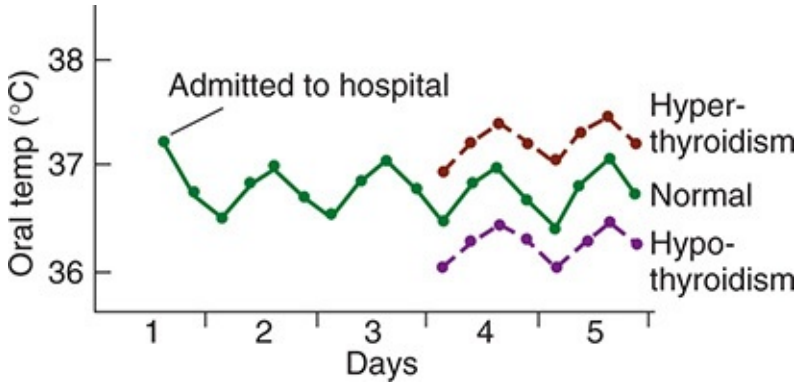


FIGURE 17–12 Typical temperature chart of a hospitalized patient who does not have a febrile disease. Note the slight rise in temperature, due to excitement and apprehension, at the time of admission to the hospital, and the regular circadian temperature cycle.

During exercise, the heat produced by muscular contraction accumulates in the body and the rectal temperature normally rises as high as 40°C (104°F). This rise is due in part to the inability of the heat-dissipating mechanisms to handle the greatly increased amount of heat produced, but evidence suggests that during exercise in addition there is an elevation of the body temperature at which the heat-dissipating mechanisms are activated. Body temperature also rises slightly during emotional excitement, probably owing to unconscious tensing of the muscles. It is chronically elevated by as much as 0.5°C when the metabolic rate

is high, as in hyperthyroidism, and lowered when the metabolic rate is low, as in hypothyroidism (Figure 17–12). Some apparently normal adults chronically have a temperature above the normal range (constitutional hyperthermia).

HEAT PRODUCTION

A variety of basic chemical reactions contribute to body heat production at all times. Ingestion of food increases heat production, but the major source of heat is the contraction of skeletal muscle **(Table 17–3)**. Heat production can be varied by endocrine mechanisms in the absence of food intake or muscular exertion. Epinephrine and norepinephrine produce a rapid but short-lived increase in heat production; thyroid hormones produce a slowly developing but prolonged increase. Furthermore, sympathetic discharge decreases during fasting and is increased by feeding.

TABLE 17–3 Body heat production and heat loss.

Body heat is produced by:	
Basic metabolic processes	
Food intake (specific dynamic action)	
Muscular activity	
Body heat is lost by:	**Percentage of heat lost at 21°C**
Radiation and conduction	70
Vaporization of sweat	27
Respiration	2
Urination and defecation	1

A source of considerable heat, particularly in infants, is **brown adipose tissue (BAT).** This fat has a high rate of metabolism (cells contain mitochondria), and its thermogenic function has been likened to that of an electric blanket. Cold-induced BAT activity is associated with enhanced thermogenesis and energy expenditure in humans, leading to beneficial effects on glucose metabolism and fat mass.

HEAT LOSS

The processes by which heat is lost from the body when the environmental

temperature is below body temperature are listed in Table 17–3. **Conduction** is heat exchange between objects or substances at different temperatures that are in contact with one another. A basic characteristic of matter is that its molecules are in motion, with the amount of motion proportional to the temperature. These molecules collide with the molecules in cooler objects, transferring thermal energy to them. The amount of heat transferred is proportional to the temperature difference between the objects in contact **(thermal gradient).** Conduction is aided by **convection,** the movement of molecules away from the area of contact. Thus, for example, an object in contact with air at a different temperature changes the specific gravity of the air, and because warm air rises and cool air falls, a new supply of air is brought into contact with the object. Of course, convection is greatly aided if the object moves about in the medium or the medium moves past the object, for example, if a subject swims through water or a fan blows air through a room. **Radiation** is the transfer of heat by infrared electromagnetic radiation from one object to another at a different temperature with which it is not in contact. When an individual is in a cold environment, heat is lost by conduction to the surrounding air and by radiation to cool objects in the vicinity. Conversely, of course, heat is transferred to an individual and the heat load is increased by these processes when the environmental temperature is above body temperature. Note that because of radiation, an individual can feel chilly in a room with cold walls even though the room is relatively warm. On a cold but sunny day, the heat of the sun reflected off bright objects exerts an appreciable warming effect. It is the heat reflected from the snow, for example, that in part makes it possible to ski in fairly light clothes even though the air temperature is below freezing.

Because conduction occurs from the surface of one object to the surface of another, the temperature of the skin determines to a large extent the degree to which body heat is lost or gained. The amount of heat reaching the skin from the deep tissues can be varied by changing the blood flow to the skin. When the cutaneous vessels are dilated, warm blood wells into the skin, whereas in the maximally vasoconstricted state, heat is held centrally in the body. The rate at which heat is transferred from the deep tissues to the skin is called the **tissue conductance.** Further, birds have a layer of feathers next to the skin, and most mammals have a significant layer of hair or fur. Heat is conducted from the skin to the air trapped in this layer and from the trapped air to the exterior. When the thickness of the trapped layer is increased by fluffing the feathers or erection of the hairs **(horripilation),** heat transfer across the layer is reduced and heat losses (or, in a hot environment, heat gains) are decreased. “Goose pimples” are the result of horripilation in humans; they are the visible manifestation of cold-

induced contraction of the piloerector muscles attached to the rather meager hair supply. Humans usually supplement this layer of hair with one or more layers of clothes. Heat is conducted from the skin to the layer of air trapped by the clothes, from the inside of the clothes to the outside, and from the outside of the clothes to the exterior. The magnitude of the heat transfer across the clothing, a function of its texture and thickness, is the most important determinant of how warm or cool the clothes feel, but other factors, especially the size of the trapped layer of warm air, are also important. Dark clothes absorb radiated heat and light-colored clothes reflect it back to the exterior.

The other major process transferring heat from the body in humans and other animals that sweat is vaporization of water on the skin and mucous membranes of the mouth and respiratory passages. Vaporization of 1 g of water removes about 0.6 kcal of heat. A certain amount of water is vaporized at all times. This **insensible water loss** amounts to 50 mL/h in humans. When sweat secretion is increased, the degree to which the sweat vaporizes depends on the humidity of the environment. It is common knowledge that one feels hotter on a humid day. This is due in part to the decreased vaporization of sweat, but even under conditions in which vaporization of sweat is complete, an individual in a humid environment feels warmer than an individual in a dry environment. The reason for this difference is unknown, but it seems related to the fact that in the humid environment sweat spreads over a greater area of skin before it evaporates. During muscular exertion in a hot environment, sweat secretion reaches values as high as 1600 mL/h, and in a dry atmosphere, most of this sweat is vaporized. Heat loss by vaporization of water therefore varies from 30 to over 900 kcal/h.

Some mammals lose heat by **panting.** This rapid, shallow breathing greatly increases the amount of water vaporization in the mouth and respiratory passages and therefore the amount of heat lost. Because the breathing is shallow, it produces relatively little change in the composition of alveolar air (see Chapter 34).

The relative contribution of each of the processes that transfer heat away from the body (Table 17–3) varies with the environmental temperature. At 21°C, vaporization is a minor component in humans at rest. As the environmental temperature approaches body temperature, radiation losses decline and vaporization losses increase.

TEMPERATURE-REGULATING MECHANISMS

The reflex and semireflex thermoregulatory responses in humans are listed in

Table 17–4. They include autonomic, somatic, endocrine, and behavioral changes. One group of responses increases heat loss and decreases heat production; the other decreases heat loss and increases heat production. In general, exposure to heat stimulates the former group of responses and inhibits the latter, whereas exposure to cold does the opposite.

TABLE 17–4 Temperature-regulating mechanisms.

Mechanisms activated by cold
Shivering
Hunger
Increased voluntary activity
Increased secretion of norepinephrine and epinephrine
Decreased heat loss
Cutaneous vasoconstriction
Curling up
Horripilation
Mechanisms activated by heat
Increased heat loss
Cutaneous vasodilation
Sweating
Increased respiration
Decreased heat production
Anorexia
Apathy and inertia

Curling up “in a ball” is a common reaction to cold in animals and has a counterpart in the position some people assume on climbing into a cold bed. Curling up decreases the body surface exposed to the environment. Shivering is an involuntary response of the skeletal muscles, but cold also causes a semiconscious general increase in motor activity. Examples include foot stamping and dancing up and down on a cold day. Increased catecholamine secretion is an important endocrine response to cold. Mice unable to make norepinephrine and epinephrine because their dopamine β-hydroxylase gene is

knocked out do not tolerate cold; they have deficient vasoconstriction and are unable to increase thermogenesis in BAT through UCP 1. TSH secretion is increased by cold and decreased by heat in laboratory animals, but the change in TSH secretion produced by cold in adult humans is small and of questionable significance. It is common knowledge that activity is decreased in hot weather—the "it's too hot to move" reaction.

Thermoregulatory adjustments involve local responses as well as more general reflex responses. When cutaneous blood vessels are cooled they become more sensitive to catecholamines and the arterioles and venules constrict. This local effect of cold directs blood away from the skin. Another heat-conserving mechanism that is important in animals living in cold water is heat transfer from arterial to venous blood in the limbs. The deep veins **(venae comitantes)** run alongside the arteries supplying the limbs and heat is transferred from the warm arterial blood going to the limbs to the cold venous blood coming from the extremities (**countercurrent exchange;** see Chapter 37). This limits the ability to maintain heat in the tips of the extremities but conserves body heat.

The reflex responses activated by cold are controlled from the posterior hypothalamus. Those activated by warmth are controlled primarily from the anterior hypothalamus, although some thermoregulation against heat still occurs after decerebration at the level of the rostral midbrain. Stimulation of the anterior hypothalamus causes cutaneous vasodilation and sweating, and lesions in this region cause hyperthermia, with rectal temperatures sometimes reaching 43°C (109.4°F). Posterior hypothalamic stimulation causes shivering, and the body temperature of animals with posterior hypothalamic lesions falls toward that of the environment.

AFFERENTS

The hypothalamus is said to integrate body temperature information from sensory receptors (primarily cold receptors) in the skin, deep tissues, spinal cord, extrahypothalamic portions of the brain, and the hypothalamus itself. Each of these five inputs contributes about 20% of the information that is integrated. There are threshold core temperatures for each of the main temperature-regulating responses and when the threshold is reached the response begins. The threshold is 37°C for sweating and vasodilation, 36.8°C for vasoconstriction, 36°C for nonshivering thermogenesis, and 35.5°C for shivering.

FEVER

Fever is perhaps the oldest and most universally known hallmark of disease. It occurs not only in mammals but also in birds, reptiles, amphibia, and fish. When it occurs in homeothermic animals, the thermoregulatory mechanisms behave as if they were adjusted to maintain body temperature at a higher than normal level, that is, “as if the thermostat had been reset” to a new point above 37°C. The temperature receptors then signal that the actual temperature is below the new set point, and the temperature-raising mechanisms are activated. This usually produces chilly sensations due to cutaneous vasoconstriction and occasionally enough shivering to produce a shaking chill. However, the nature of the response depends on the ambient temperature. The temperature rise in experimental animals injected with a pyrogen is due mostly to increased heat production if they are in a cold environment and mostly to decreased heat loss if they are in a warm environment.

The pathogenesis of fever is summarized in **Figure 17–13**. Toxins from bacteria, such as endotoxin, act on monocytes, macrophages, and Kupffer cells to produce cytokines that act as **endogenous pyrogens (EPs).** There is good evidence that IL-1β, IL-6, IFN-β, IFN-γ, and TNF-α (see Chapter 3) can act independently to produce fever. These circulating cytokines are polypeptides and it is unlikely that they penetrate the brain. Instead, evidence suggests that they act on the OVLT, one of the circumventricular organs (see Chapter 33). This in turn activates the preoptic area of the hypothalamus. Cytokines are also produced by cells in the central nervous system (CNS) when these are stimulated by infection, and these may act directly on the thermoregulatory centers.

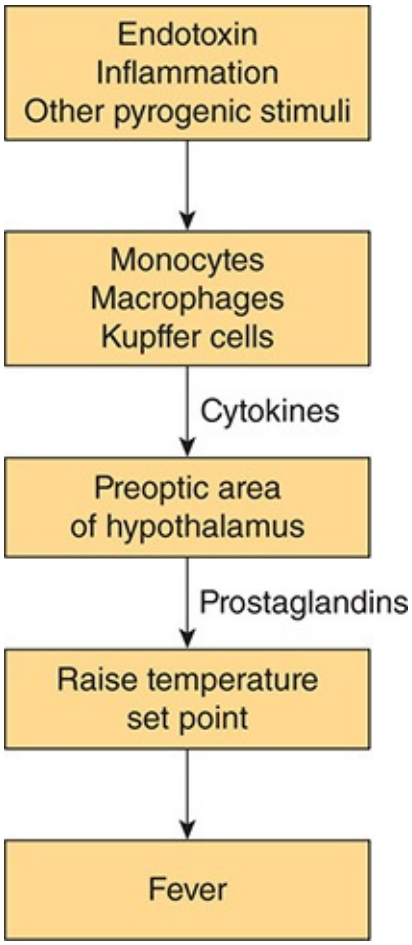


FIGURE 17–13 Pathogenesis of fever.

The fever produced by cytokines is probably due to local release of prostaglandins in the hypothalamus. Intrahypothalamic injection of prostaglandins produces fever. In addition, the antipyretic effect of aspirin is exerted directly on the hypothalamus, and aspirin inhibits prostaglandin synthesis. PGE_2 is one of the prostaglandins that causes fever. It acts on four subtypes of prostaglandin receptors—EP_1, EP_2, EP_3, and EP_4—and knockout of the EP_3 receptor impairs the febrile response to PGE_2, IL-1β, and endotoxin, or bacterial lipopolysaccharide (LPS).

The benefit of fever to the organism is uncertain. A beneficial effect is assumed because fever has evolved and persisted as a response to infections and other diseases. Many microorganisms grow best within a relatively narrow temperature range and a rise in temperature inhibits their growth. In addition, antibody production is increased when body temperature is elevated. Before the advent of antibiotics, fevers were artificially induced for the treatment of

neurosyphilis and proved to be beneficial. Hyperthermia also benefits individuals infected with anthrax; pneumococcal pneumonia; leprosy; and various fungal, rickettsial, and viral diseases. Hyperthermia also slows the growth of some tumors. However, very high temperatures are harmful. A rectal temperature over 41°C (106°F) for prolonged periods results in some permanent brain damage. When the temperature is over 43°C, heat stroke develops and death is common.

In **malignant hyperthermia,** various mutations of the gene coding for the ryanodine receptor (see Chapter 5) lead to excess Ca^{2+} release during muscle contraction triggered by stress. This in turn leads to contractures of the muscles, increased muscle metabolism, and a great increase in heat production in muscle. The increased heat production causes a marked rise in body temperature that is fatal if not treated.

Periodic fevers also occur in humans with mutations in the gene for **pyrin,** a protein found in neutrophils; the gene for mevalonate kinase, an enzyme involved in cholesterol synthesis; and the gene for the type 1 TNF receptor, which is involved in inflammatory responses. However, how any of these three mutant gene products cause fever is unknown.

HYPOTHERMIA

In hibernating mammals, body temperature drops to low levels without causing any demonstrable ill effects on subsequent arousal. This observation led to experiments on induced hypothermia. When the skin or the blood is cooled enough to lower the body temperature in nonhibernating animals or in humans, metabolic and physiologic processes slow down. Respiration and heart rate are very slow, blood pressure is low, and consciousness is lost. At rectal temperatures of about 28°C, the ability to spontaneously return the temperature to normal is lost, but the individual continues to survive and, if rewarmed with external heat, returns to a normal state. If care is taken to prevent the formation of ice crystals in the tissues, the body temperature of experimental animals can be lowered to subfreezing levels without producing any detectable damage after subsequent rewarming.

Humans tolerate body temperatures of 21–24°C (70–75°F) without permanent ill effects, and induced hypothermia has been used in surgery. On the other hand, accidental hypothermia due to prolonged exposure to cold air or cold water is a serious condition and requires careful monitoring and prompt rewarming.

CHAPTER SUMMARY

- Neural connections run between the hypothalamus and the posterior lobe of the pituitary gland, and vascular connections between the hypothalamus and the anterior lobe of the pituitary.
- In most mammals, the hormones secreted by the posterior pituitary gland are vasopressin and oxytocin. Vasopressin increases the permeability of the collecting ducts of the kidney to water, thus concentrating the urine. Oxytocin acts on the breasts (lactation) and the uterus (contraction).
- The anterior pituitary secretes six hormones: adrenocorticotropic hormone (corticotropin, ACTH), thyroid-stimulating hormone (thyrotropin, TSH), growth hormone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and prolactin (PRL).
- Other complex autonomic mechanisms that maintain the chemical constancy and temperature of the internal environment, such as thirst and temperature regulation, are integrated in the hypothalamus.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. Thirst is stimulated by

   A. increases in plasma osmolality and volume.

   B. an increase in plasma osmolality and a decrease in plasma volume.

   C. a decrease in plasma osmolality and an increase in plasma volume.

   D. decreases in plasma osmolality and volume.

   E. injection of vasopressin into the hypothalamus.

2. When an individual is naked in a room in which the air temperature is 21°C (69.8°F) and the humidity 80%, the greatest amount of heat is lost from the body by

   A. elevated metabolism.

   B. respiration.

   C. urination.

   D. vaporization of sweat.

   E. radiation and conduction.

In questions 3–8, select A if the item is associated with (a), B if the item is associated with (b), C if the item is associated with both (a) and (b), and D if the item is associated with neither (a) nor (b).

(a) V_{1A} vasopressin receptors

(b) V_2 vasopressin receptors

3. Activation of G_s
4. Vasoconstriction
5. Increase in intracellular inositol triphosphate
6. Movement of aquaporin
7. Proteinuria
8. Milk ejection

CHAPTER 18

The Pituitary Gland

OBJECTIVES

After studying this chapter, you should be able to:

- Describe the development and structure of the pituitary gland and its relationship to the hypothalamus.
- Identify the hormones secreted by the anterior and posterior lobes of the pituitary and their target organs, and how the numbers of the various cell types in the anterior pituitary are controlled in response to physiologic demands.
- Understand the function of hormones derived from proopiomelanocortin (POMC) and how they are involved in regulating skin coloration.
- Describe how growth hormone is secreted from the anterior pituitary and circulates and activates its receptors, and the stimuli that regulate growth hormone secretion with their underlying mechanisms.
- Understand the role of growth hormone in growth and metabolic function, and how somatomedins (such as insulin-like growth factors) may mediate some of its actions in the periphery.
- Define the normal timeline of growth in humans and identify factors in addition to growth hormone that contribute to its regulation.
- Understand pituitary secretion of gonadotropins and prolactin, how these are

regulated, and the actions of these hormones on reproductive tissues.

- Understand the basis of conditions where pituitary function is abnormal, and how they can be treated.

INTRODUCTION

The pituitary gland, or hypophysis, lies in a pocket of the sphenoid bone at the base of the brain and is closely related to the hypothalamus (see Figure 17–2). It is a coordinating center for control of many downstream endocrine glands, some of which are discussed in subsequent chapters. In many ways, it can be considered to consist of two separate endocrine organs that contain a plethora of hormonally active substances. The anterior lobe of the pituitary secretes **thyroid-stimulating hormone (TSH, thyrotropin), adrenocorticotropic hormone (ACTH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin,** and **growth hormone** (see Figure 17–9), and receives almost all of its blood supply from the portal hypophysial vessels that pass initially through the median eminence, a structure immediately below the hypothalamus. This vascular arrangement positions the cells of the anterior pituitary to respond efficiently to regulatory factors released from the hypothalamus. Of the listed hormones, prolactin acts on the breast. The remaining five are, at least in part, **tropic hormones;** that is, they stimulate secretion of hormonally active substances by other endocrine glands or, in the case of growth hormone, the liver and other tissues (see below). The tropic hormones for some endocrine glands are discussed in the chapter on that gland: TSH in Chapter 19 and ACTH in Chapter 20. However, the gonadotropins FSH and LH, along with prolactin, are covered here. In some species, there is a well-developed intermediate lobe of the pituitary, which contains hormonally active derivatives of the proopiomelanocortin (POMC) molecule that regulate skin pigmentation, among other functions (see below). In humans, the intermediate lobe is rudimentary, and the cells that secrete derivatives of POMC are present in the anterior pituitary.

The posterior lobe of the pituitary consists predominantly of nerves that have their cell bodies in the hypothalamus, and stores **oxytocin** and **vasopressin** in the termini of these neurons, to be released into the bloodstream. The secretion of these hormones, as well as a discussion of the overall role of the hypothalamus and median eminence in regulating both the anterior and posterior pituitary, was covered in Chapter 17.

To avoid redundancy, this chapter will focus predominantly on growth

hormone and its role in growth and facilitating the activity of other hormones, along with a number of general considerations about the pituitary. The melanocyte-stimulating hormones (MSHs) of the intermediate lobe of the pituitary, α-MSH and β-MSH, will also be touched upon.

DEVELOPMENT, STRUCTURE, & CELL TYPES OF THE PITUITARY

GROSS ANATOMY

The anatomy of the pituitary gland is summarized in **Figure 18–1** and discussed in detail in Chapter 17. The posterior pituitary is made up largely of the endings of axons from the supraoptic and paraventricular nuclei of the hypothalamus and arises initially as an extension of this structure. The anterior pituitary, on the other hand, contains endocrine cells that store its characteristic hormones and arises embryologically as an invagination of the pharynx **(Rathke pouch).** In species where it is well developed, the intermediate lobe is formed in the embryo from the dorsal half of Rathke pouch, but is closely adherent to the posterior lobe in the adult. It is separated from the anterior lobe by the remains of the cavity in Rathke pouch, the **residual cleft.**

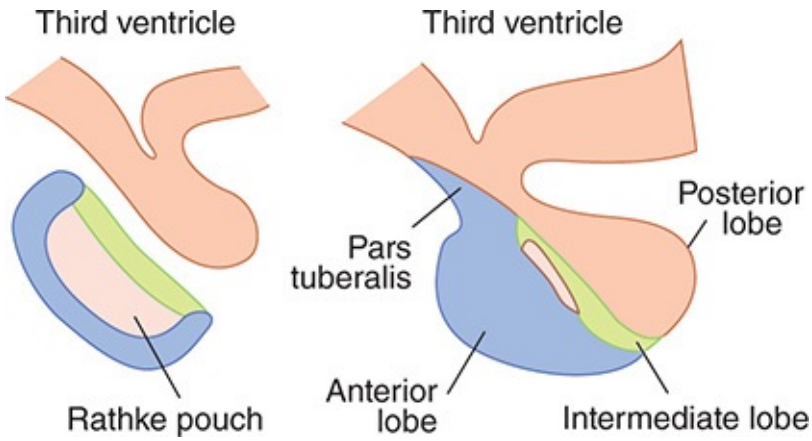


FIGURE 18–1 Diagrammatic outline of the formation of the pituitary (left) and the various parts of the organ in the adult (right).

HISTOLOGY

In the posterior lobe, the endings of the supraoptic and paraventricular axons can

be observed in close relation to blood vessels. **Pituicytes,** stellate cells that are modified astrocytes, are also present.

As noted above, the intermediate lobe is rudimentary in humans—most of its cells are incorporated in the anterior lobe. The anterior pituitary is made up of interlacing cell cords and an extensive network of sinusoidal capillaries. The endothelium of the capillaries is fenestrated, like that in other endocrine organs. The cells contain granules of stored hormone that are extruded from the cells by exocytosis. Their constituents then enter the capillaries to be conveyed to target tissues.

CELL TYPES IN THE ANTERIOR PITUITARY

Five types of secretory cells have been identified in the anterior pituitary by immunocytochemistry and electron microscopy. The cell types are the somatotropes, which secrete growth hormone; the lactotropes (also called mammotropes), which secrete prolactin; the corticotropes, which secrete ACTH; the thyrotropes, which secrete TSH; and the gonadotropes, which secrete FSH and LH. The characteristics of these cells are summarized in **Table 18–1**. Some cells may contain two or more hormones. It is also notable that the three pituitary glycoprotein hormones, FSH, LH, and TSH, while being made up of two subunits, all share a common α subunit that is the product of a single gene and has the same amino acid composition in each hormone, although their carbohydrate residues vary. The α subunit must be combined with a β subunit characteristic of each hormone for maximal physiologic activity. The β subunits, which are produced by separate genes and differ in structure, confer hormonal specificity (see Chapter 16). The α subunits are remarkably interchangeable and hybrid molecules can be created.

TABLE 18–1 Hormone-secreting cells of the human anterior pituitary gland.

Cell Type	Hormones Secreted	Percentage of Total Secretory Cells
Somatotrope	Growth hormone	50
Lactotrope	Prolactin	10–30
Corticotrope	ACTH	10
Thyrotrope	TSH	5
Gonadotrope	FSH, LH	20

ACTH, adrenocorticotropic hormone; FSH, follicle-stimulating hormone; LH, luteinizing hormone; TSH, thyroid-stimulating hormone.

The anterior pituitary also contains folliculostellate cells that send processes between the granulated secretory cells. These cells produce paracrine factors that regulate the growth and function of the secretory cells discussed above. Indeed, the anterior pituitary can adjust the relative proportion of secretory cell types to meet varying requirements for different hormones at different life stages. This plasticity has recently been ascribed to the presence of a small number of pluripotent stem cells that persist in the adult gland.

FORMATION AND FUNCTION OF PROOPIOMELANOCORTIN & DERIVATIVES

BIOSYNTHESIS

Corticotropes of the anterior lobe (or intermediate lobe cells, when present) synthesize a large precursor protein that is cleaved to form a family of hormones. Removal of the signal peptide results in the formation of the prohormone POMC. This molecule is also synthesized in the hypothalamus, the lungs, the gastrointestinal tract, and the placenta. The structure of POMC, as well as its derivatives, is shown in **Figure 18–2**. In corticotropes, it is hydrolyzed to ACTH and β-lipotropin (β-LPH), plus a small amount of β-endorphin, and these substances are secreted. Predominantly in intermediate lobe cells, POMC is further hydrolyzed to corticotropin-like intermediate-lobe peptide (CLIP), γ-LPH, and appreciable quantities of β-endorphin. The functions, if any, of CLIP and γ-LPH are unknown, whereas β-endorphin is an opioid peptide (see Chapter

7) that has the five amino acid residues of met-enkephalin at its amino terminal end. The **melanotropins** α- and β-MSH are also formed but apparently are not secreted in adult humans. In some species, however, these melanotropins have important physiologic functions, as discussed below.

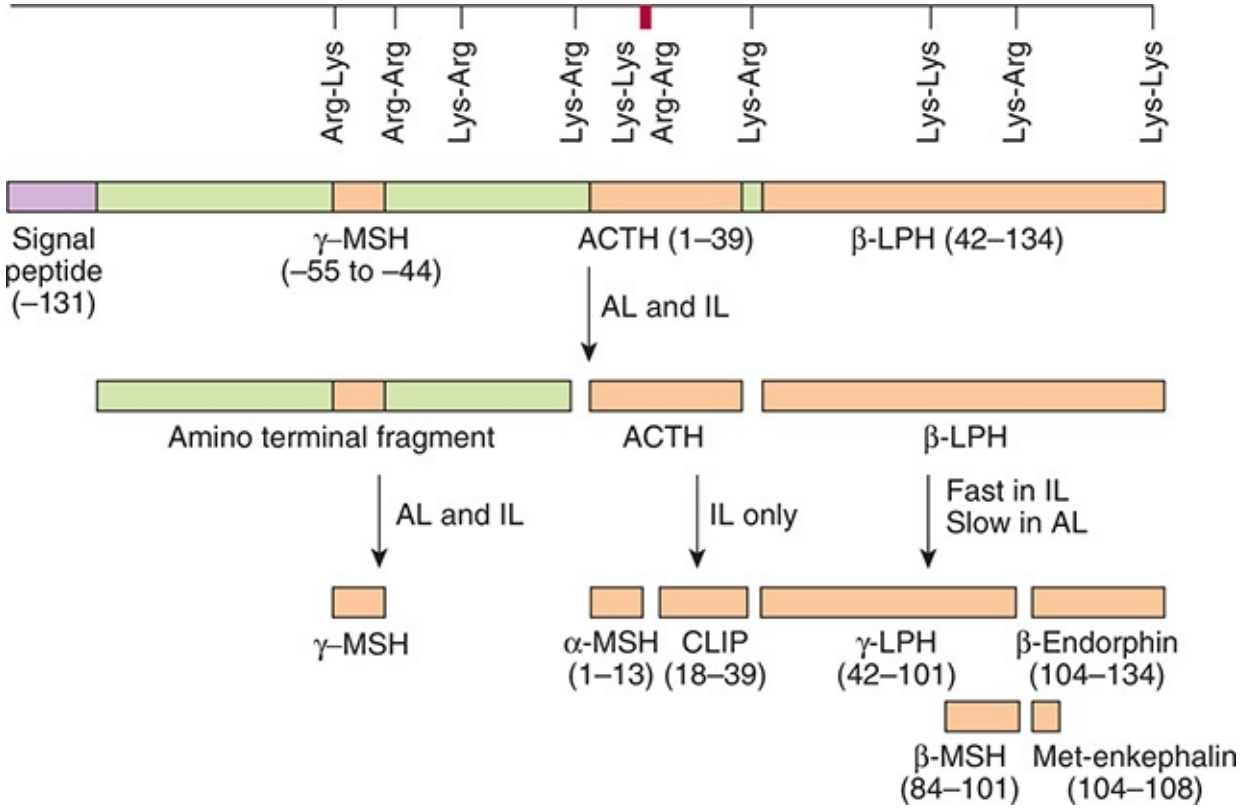


FIGURE 18–2 Schematic representation of the preproopiomelanocortin molecule formed in pituitary cells, neurons, and other tissues. The numbers in parentheses identify the amino acid sequences in each of the polypeptide fragments. The locations of Lys–Arg and other pairs of basic amino acids residues are also indicated; these are the sites of proteolytic cleavage in the formation of the smaller fragments of the parent molecule. ACTH, adrenocorticotropic hormone; AL, anterior lobe; CLIP, corticotropin-like intermediate-lobe peptide; IL, intermediate lobe; LPH, lipotropin; MSH, melanocyte-stimulating hormone.

CONTROL OF SKIN COLORATION & PIGMENT ABNORMALITIES

Fish, reptiles, and amphibia change the color of their skin for thermoregulation,

camouflage, and behavioral displays. They do this in part by moving black or brown granules into or out of the periphery of pigment cells called **melanophores.** The granules are made up of **melanins,** which are synthesized from dopamine (see Chapter 7) and dopaquinone. The movement of these granules is controlled by a variety of hormones and neurotransmitters, including α- and β-MSH, melanin-concentrating hormone, melatonin, and catecholamines.

Mammals have no melanophores containing pigment granules that disperse and aggregate, but they do have **melanocytes,** which have multiple processes containing melanin granules. Melanocytes express **melanotropin-1** receptors. Treatment with MSHs accelerates melanin synthesis, causing readily detectable darkening of the skin in humans in 24 h. As noted above, α- and β-MSH do not circulate in adult humans, and their function is unknown. However, ACTH binds to melanotropin-1 receptors. Indeed, pigmentary changes in several human endocrine diseases are due to changes in circulating ACTH. For example, abnormal pallor is a hallmark of hypopituitarism. Hyperpigmentation occurs in patients with adrenal insufficiency due to primary adrenal disease. Indeed, the presence of hyperpigmentation in association with adrenal insufficiency rules out the possibility that the insufficiency is secondary to pituitary or hypothalamic disease because in these conditions, plasma ACTH is not increased (see Chapter 20). Other disorders of pigmentation result from peripheral mechanisms. Thus, **albinos** have a congenital inability to synthesize melanin resulting from a variety of different genetic defects in the pathways for melanin synthesis. **Piebaldism** is characterized by patches of skin that lack melanin as a result of congenital defects in the migration of pigment cell precursors from the neural crest during embryonic development. Not only the condition but also the precise pattern of the loss is passed from one generation to the next. **Vitiligo** involves a similar patchy loss of melanin, but the loss develops progressively after birth secondary to an autoimmune process that targets melanocytes.

GROWTH HORMONE SECRETION

BIOSYNTHESIS & CHEMISTRY

The long arm of human chromosome 17 contains the growth hormone-hCS cluster that contains five genes: one, *hGH-N*, codes for the most abundant ("normal") form of growth hormone; a second, *hGH-V*, codes for the variant form of growth hormone; two code for human chorionic somatomammotropin (hCS) (see Chapter 22); and the fifth is probably an hCS pseudogene. Only

hGH-N is secreted by the pituitary; hGH-V and hCS are primarily products of the placenta, and as a consequence are only found in appreciable quantities in the circulation during pregnancy (see Chapter 22).

The structure of growth hormone varies considerably from one species to another. Porcine and simian growth hormones have only a transient effect in the guinea pig. In monkeys and humans, bovine and porcine growth hormones do not even have a transient effect on growth, although monkey and human growth hormones are fully active in both monkeys and humans. These facts are relevant to public health discussions surrounding the presence of bovine growth hormones (used to increase milk production) in dairy products, as well as the popularity of growth hormone supplements, marketed via the Internet, with body builders. Controversially, recombinant human growth hormone has also been given to children who are short in stature, but otherwise healthy (ie, without growth hormone deficiency), with apparently limited results.

PLASMA LEVELS, BINDING, & METABOLISM

A portion of circulating growth hormone is bound to a plasma protein that is a large fragment of the extracellular domain of the growth hormone receptor (see below). It appears to be produced by cleavage of receptors in humans, and its concentration is an index of the number of growth hormone receptors in the tissues. Approximately 50% of the circulating pool of growth hormone activity is in the bound form, providing a reservoir of the hormone to compensate for the wide fluctuations that occur in secretion (see below).

The basal plasma growth hormone level measured by radioimmunoassay in adult humans is normally less than 3 ng/mL. This represents both the protein-bound and free forms. Growth hormone is metabolized rapidly, at least in part in the liver. The half-life of circulating growth hormone in humans is 6–20 min, and the daily growth hormone output has been calculated to be 0.2–1.0 mg/d in adults.

HYPOTHALAMIC & PERIPHERAL CONTROL OF GROWTH HORMONE SECRETION

The secretion of growth hormone is not stable over time. Adolescents have the highest circulating levels of growth hormone, followed by children and finally adults. Levels decline in old age, and there has been considerable interest in

injecting growth hormone to counterbalance the effects of aging. There are also diurnal variations in growth hormone secretion superimposed on these developmental stages. Growth hormone is found at relatively low levels during the day, unless specific triggers for its release are present (see below). During sleep, on the other hand, large pulsatile bursts of growth hormone secretion occur. Therefore, it is not surprising that the secretion of growth hormone is under hypothalamic control. The hypothalamus controls growth hormone production by secreting **growth hormone–releasing hormone** (GHRH) as well as somatostatin, which inhibits growth hormone release (see Chapter 17). Thus, the balance between the effects of these hypothalamic factors on the pituitary will determine the level of growth hormone release. The stimuli of growth hormone secretion can therefore act by increasing hypothalamic secretion of GHRH, decreasing secretion of somatostatin, or both. A third regulator of growth hormone secretion is **ghrelin**. The main site of ghrelin synthesis and secretion is the stomach, but it is also produced in the hypothalamus and has marked growth hormone–stimulating activity. In addition, it appears to be involved in the regulation of food intake (see Chapter 26).

Growth hormone secretion is under feedback control (see Chapter 16), like the secretion of other anterior pituitary hormones. It acts on the hypothalamus to antagonize GHRH release. Growth hormone also increases circulating IGF-I, and IGF-I in turn exerts a direct inhibitory action on growth hormone secretion from the pituitary. It also stimulates somatostatin secretion **(Figure 18–3)**.

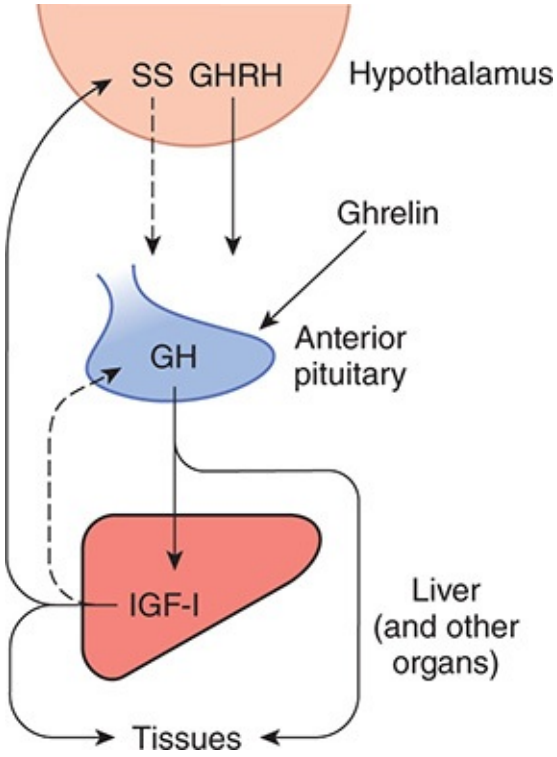


FIGURE 18–3 Feedback control of growth hormone secretion. Solid arrows represent positive effects and dashed arrows represent inhibition. GH, growth hormone; GHRH, growth hormone–releasing hormone; IGF-I, insulin-like growth factor-I; SS, somatostatin.

Other Stimuli Affecting Growth Hormone Secretion

The basal plasma growth hormone concentration ranges from 0 to 3 ng/mL in normal adults. However, secretory rates cannot be estimated from single values because of their irregular nature. Thus, average values over 24 h (see below) and peak values may be more meaningful, albeit difficult to assess. The stimuli that increase and decrease growth hormone secretion are summarized in **Table 18–2**. The stimuli that increase secretion fall into three general categories: (1) conditions such as hypoglycemia and/or fasting in which there is an actual or threatened decrease in the substrate for energy production in cells, (2) conditions in which the amounts of certain amino acids are increased in the plasma, and (3) stressful stimuli. Growth hormone secretion is also increased in persons deprived of rapid eye movement (REM) sleep (see Chapter 14) and inhibited during normal REM sleep.

TABLE 18–2 Stimuli that affect growth hormone secretion in humans.

Stimuli that increase secretion
Hypoglycemia
2-Deoxyglucose
Exercise
Fasting
Increase in circulating levels of certain amino acids
Protein meal
Infusion of arginine and some other amino acids
Glucagon
Lysine vasopressin
Going to sleep
L-dopa and α-adrenergic agonists that penetrate the brain
Apomorphine and other dopamine receptor agonists
Estrogens and androgens
Stressful stimuli (including various psychological stresses)
Pyrogen
Stimuli that decrease secretion
REM sleep
Glucose
Cortisol
FFA
Medroxyprogesterone
Growth hormone and IGF-I

FFA, free fatty acid; IGF, insulin-like growth factor; REM, rapid eye movement.

Glucose infusions lower plasma growth hormone levels and inhibit the response to exercise. The increase produced by 2-deoxyglucose is presumably due to intracellular glucose deficiency, since this compound blocks the catabolism of glucose-6-phosphate. Sex hormones induce growth hormone secretion, increase growth hormone responses to provocative stimuli such as arginine and insulin, and also serve as permissive factors for the action of growth

hormone in the periphery. This likely contributes to the relatively high levels of circulating growth hormone and associated growth spurt in puberty. Growth hormone secretion is also induced by thyroid hormones. Growth hormone secretion is inhibited, on the other hand, by cortisol, free fatty acids (FFA), and medroxyprogesterone.

Growth hormone secretion is increased by L-dopa, which increases the release of dopamine and norepinephrine in the brain, and by the dopamine receptor agonist apomorphine.

GROWTH HORMONE RECEPTORS

The receptor that mediates the cellular effects of growth hormone has a large extracellular portion, a transmembrane domain, and a large cytoplasmic portion. It is a member of the cytokine receptor superfamily, which is discussed in Chapter 3. Growth hormone has two domains that can bind to its receptor, and when it binds to one receptor, the second binding site attracts another, producing a homodimer **(Figure 18–4)**. Dimerization is essential for receptor activation.

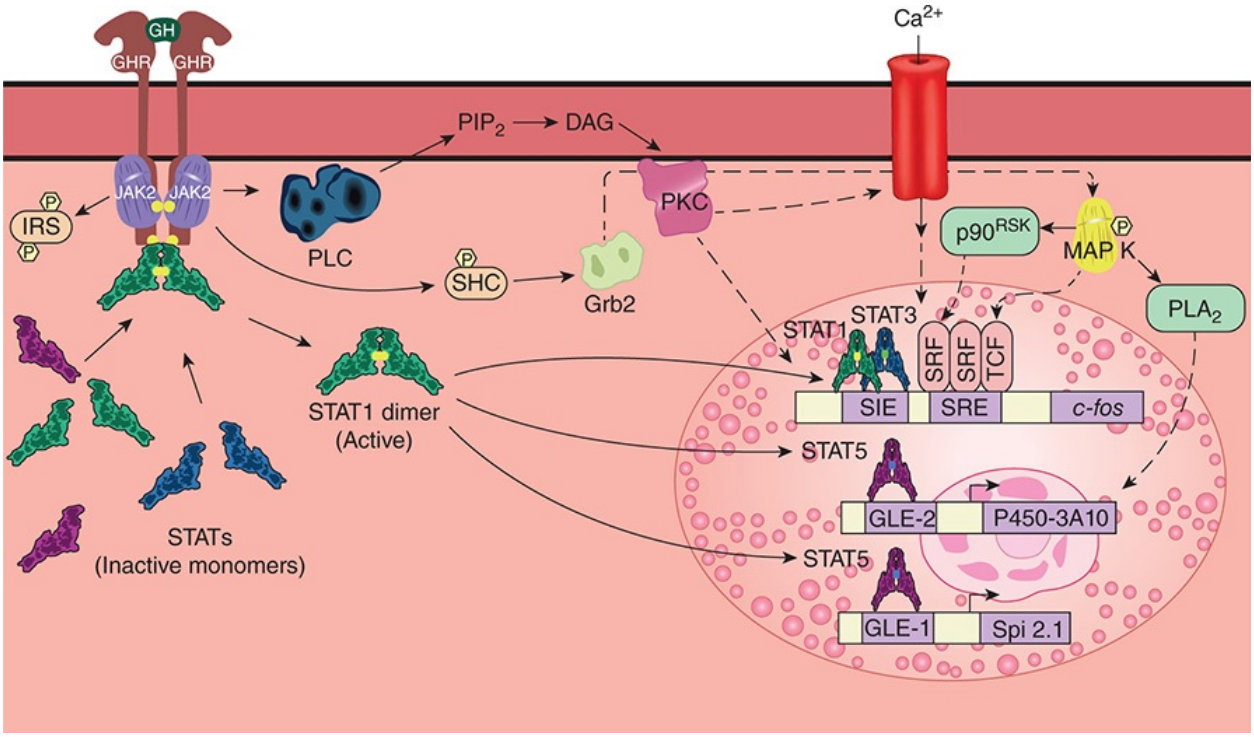


FIGURE 18–4 Some of the principal signaling pathways activated by the dimerized growth hormone receptor (GHR). Solid arrows indicate established pathways; dashed arrows indicate probable pathways. The details of the PLC

pathway and the pathway from Grb2 to MAP K are discussed in Chapter 2. The small uppercase letter Ps in yellow hexagons represent phosphorylation of the factor indicated. GLE-1 and GLE-2, interferon γ-activated response elements; IRS, insulin receptor substrate; $p90^{RSK}$, an S6 kinase; PLA_2, phospholipase A_2; SIE, Sis-induced element; SRE, serum response element; SRF, serum response factor; TCF, ternary complex factor.

Growth hormone has widespread effects in the body (see below), so even though it is not yet possible precisely to correlate intracellular and whole body effects, it is not surprising that, like insulin, growth hormone activates many different intracellular signaling cascades (Figure 18–4). Of particular note is its activation of the JAK2–STAT pathway. JAK2 is a member of the Janus family of cytoplasmic tyrosine kinases. STATs (for signal transducers and activators of transcription) are a family of cytoplasmic transcription factors that, upon phosphorylation by JAK kinases, migrate to the nucleus where they activate various genes. JAK–STAT pathways are known also to mediate the effects of prolactin and various other growth factors.

EFFECTS OF GROWTH HORMONE ON GROWTH

In young animals in which the epiphyses have not yet fused to the long bones (see Chapter 21), growth is inhibited by hypophysectomy and stimulated by growth hormone. Chondrogenesis is accelerated, and as the cartilaginous epiphysial plates widen, they lay down more bone matrix at the ends of long bones. In this way, stature is increased. Prolonged treatment of animals with growth hormone leads to gigantism.

When the epiphyses are closed, linear growth is no longer possible. In this case, an overabundance of growth hormone produces the pattern of bone and soft tissue deformities known in humans as **acromegaly.** The sizes of most of the viscera are increased. The protein content of the body is increased, and the fat content is decreased **(Clinical Box 18–1)**.

EFFECTS FOR GROWTH HORMONE ON PROTEIN & ELECTROLYTE HOMEOSTASIS

Growth hormone is a protein anabolic hormone and produces a positive nitrogen and phosphorus balance, a rise in plasma phosphorus, and a fall in blood urea nitrogen and amino acid levels. In adults with growth hormone deficiency, recombinant human growth hormone produces an increase in lean body mass and a decrease in body fat, along with an increase in metabolic rate and a fall in plasma cholesterol. Gastrointestinal absorption of Ca^{2+} is increased. Na^{+} and K^{+} excretion is reduced by an action independent of the adrenal glands, probably because these electrolytes are diverted from the kidneys to the growing tissues. On the other hand, excretion of the amino acid 4-hydroxyproline is increased during this growth, reflective of the ability of growth hormone to stimulate the synthesis of soluble collagen.

CLINICAL BOX 18–1

Gigantism & Acromegaly

Tumors of the somatotropes of the anterior pituitary (pituitary adenomas) secrete large amounts of growth hormone, leading to **gigantism** in children and to **acromegaly** in adults. If the tumor arises before puberty, the individual may grow to an extraordinary height. After linear growth is no longer possible, on the other hand, the characteristic features of acromegaly arise, including greatly enlarged hands and feet, vertebral changes attributable to osteoarthritis, soft tissue swelling, hirsutism, and protrusion of the brow and jaw. Abnormal growth of internal organs may eventually impair their function such that the condition, which has an insidious onset, can prove fatal if left untreated. Hypersecretion of growth hormone is accompanied by hypersecretion of prolactin in 20–40% of patients with acromegaly. About 25% of patients have abnormal glucose tolerance tests, and 4% develop lactation in the absence of pregnancy. Acromegaly can be caused by extra-pituitary as well as intrapituitary growth hormone–secreting tumors and by hypothalamic tumors that secrete GHRH, but the latter are rare.

THERAPEUTIC HIGHLIGHTS

The mainstay of therapy for acromegaly remains the use of somatostatin analogues that inhibit the secretion of growth hormone. A growth hormone receptor antagonist has become available and has been found to produce clinical improvement in cases of acromegaly that do not respond to other treatments. Surgical removal of the pituitary tumor is also helpful in both

acromegaly and gigantism, but sometimes challenging to perform due to the tumor's often invasive nature. In any case, adjuvant pharmacologic therapy must often be continued after surgery to control ongoing symptoms.

EFFECTS OF GROWTH HORMONE ON CARBOHYDRATE & FAT METABOLISM

The actions of growth hormone on carbohydrate metabolism are discussed in Chapter 24. At least some forms of growth hormone are diabetogenic because they increase hepatic glucose output and exert an anti-insulin effect in muscle. Growth hormone is also ketogenic and increases circulating FFA levels. The increase in plasma FFA, which takes several hours to develop, provides a ready source of energy for the tissues during hypoglycemia, fasting, and stressful stimuli. Growth hormone does not stimulate β cells of the pancreas directly, but it increases the ability of the pancreas to respond to insulinogenic stimuli such as arginine and glucose. This is an additional way growth hormone promotes growth, since insulin has a protein anabolic effect (see Chapter 24).

ROLE OF SOMATOMEDINS IN RESPONSE TO GROWTH HORMONE

The effects of growth hormone on growth, cartilage, and protein metabolism depend on an interaction between growth hormone and **somatomedins,** which are polypeptide growth factors secreted by the liver and other tissues. The first of these factors isolated was called sulfation factor because it stimulated the incorporation of sulfate into cartilage. However, it also stimulated collagen formation, and its name was changed to somatomedin. It then became clear that there are a variety of different somatomedins and that they are members of an increasingly large family of growth factors that affect many different tissues and organs.

In humans probably the only circulating somatomedins are **insulin-like growth factor I (IGF-I, somatomedin C)** and **IGF-II.** These factors are closely related to insulin, except that their C chains are not separated **(Figure 18–5)** and they have an extension of the A chain called the D domain. The hormone relaxin (see Chapter 22) is also a member of this family. Humans have two related

relaxin isoforms, and both resemble IGF-II.

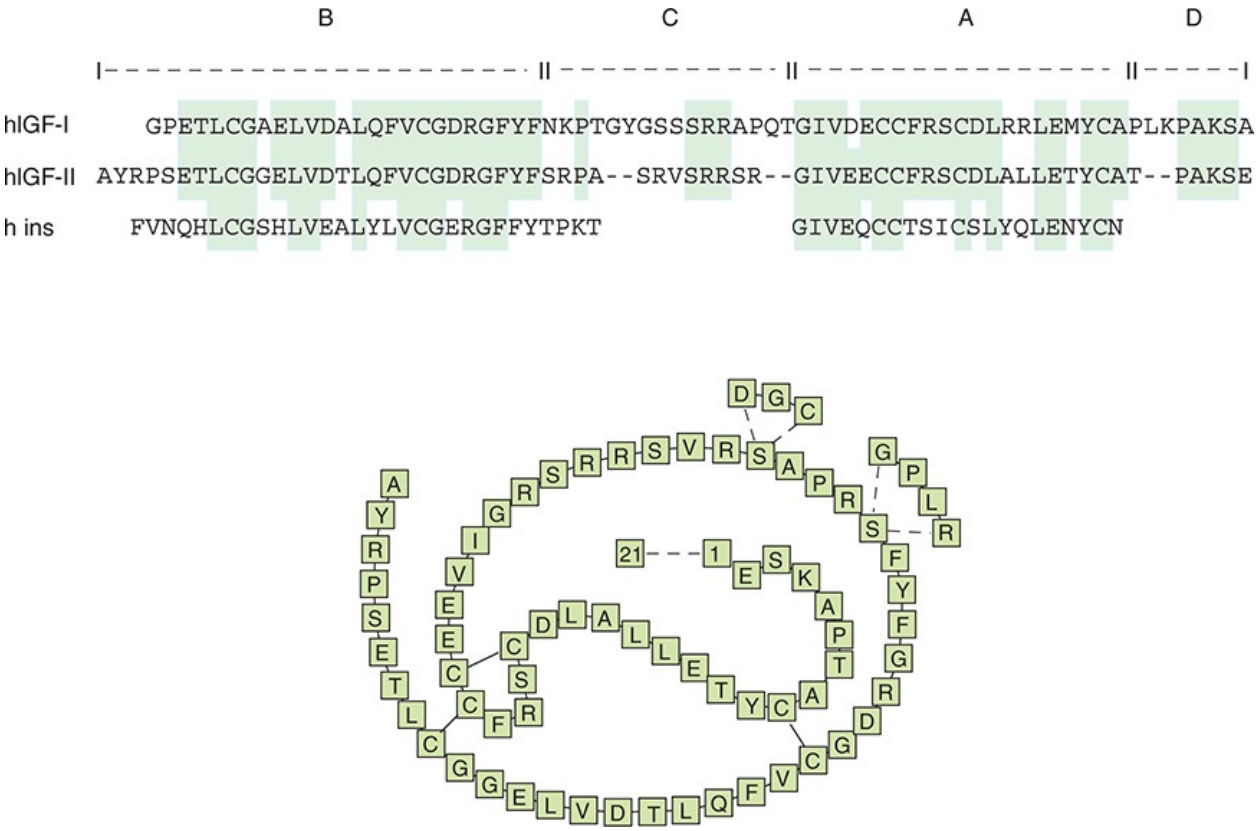


FIGURE 18–5 Structure of human IGF-I, IGF-II, and insulin (ins) (top). The lower panel shows the structure of human IGF-II with its disulfide bonds, as well as three variant structures: a 21-aa extension of the C-terminus, a tetrapeptide substitution at Ser-29, and a tripeptide substitution of Ser-33.

The properties of insulin, IGF-I, and IGF-II are compared in **Table 18–3**. Both IGF-I and IGF-II are tightly bound to proteins in the plasma, prolonging their half-life in the circulation. The contribution of the IGFs to the insulin-like activity in blood is discussed in Chapter 24. The IGF-I receptor is very similar to the insulin receptor and probably uses similar or identical intracellular signaling pathways. The IGF-II receptor has a distinct structure (see Figure 24–5) and is involved in the targeting of acid hydrolases and other proteins to intracellular organelles. Secretion of IGF-I is independent of growth hormone before birth but is stimulated by growth hormone after birth, and it has pronounced growth-stimulating activity. Its concentration in plasma rises during childhood and peaks at the time of puberty, then declines to low levels in old age. IGF-II is largely independent of growth hormone and plays a role in the growth of the fetus before birth. In human fetuses in which it is overexpressed, several organs,

especially the tongue, other muscles, kidneys, heart, and liver, develop out of proportion to the rest of the body. In adults, the gene for IGF-II is expressed only in the choroid plexus and meninges.

TABLE 18–3 Comparison of insulin and the insulin-like growth factors (IGFs).

	Insulin	IGF-I	IGF-II
Other names	—	Somatomedin C	Multiplication-stimulating activity (MSA)
Number of amino acids	51	70	67
Source	Pancreatic β cells	Liver and other tissues	Diverse tissues
Level regulated by	Glucose	Growth hormone after birth, nutritional status	Unknown
Plasma levels	0.3–2 ng/mL	10–700 ng/mL; peaks at puberty	300–800 ng/mL
Plasma-binding proteins	No	Yes	Yes
Major physiologic role	Control of metabolism	Skeletal and cartilage growth	Growth during fetal development

DIRECT & INDIRECT ACTIONS OF GROWTH HORMONE

The understanding of the mechanism of action of growth hormone has evolved. It was originally thought to produce growth by a direct action on tissues, and then later was believed to act solely through its ability to induce somatomedins. However, if growth hormone is injected into one proximal tibial epiphysis, a

unilateral increase in cartilage width is produced, and cartilage, like other tissues, makes IGF-I. A current hypothesis to explain these results holds that growth hormone acts on cartilage to convert stem cells into cells that respond to IGF-I. Locally produced as well as circulating IGF-I then makes the cartilage grow. However, the independent role of circulating IGF-I remains important, since infusion of IGF-I in hypophysectomized rats restores bone and body growth. Overall, it seems that growth hormone and somatomedins can act both in cooperation and independently to stimulate pathways that lead to growth.

Figure 18–6 is a summary of current views of the actions of growth hormone and IGF-I. However, growth hormone probably combines with circulating and locally produced IGF-I in various proportions to produce at least some of the latter effects.

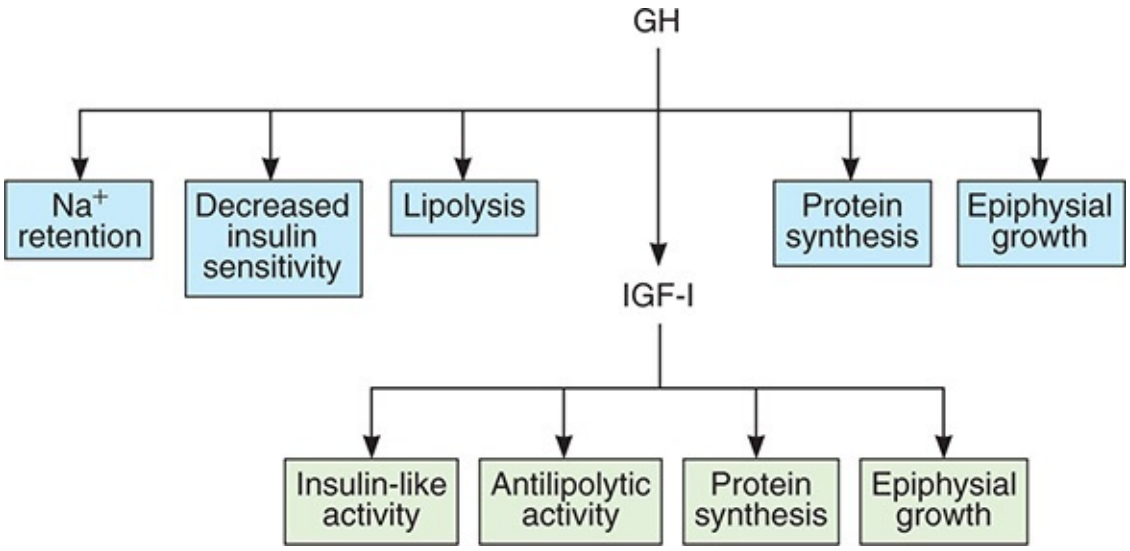


FIGURE 18–6 Direct and indirect actions of growth hormone (GH). The latter are mediated by the ability of GH to induce production of insulin-like growth factor-I (IGF-I). (Used with permission of R. Clark and N. Gesundheit.)

TIMELINE OF GROWTH & OTHER REGULATORS

Growth hormone, while being essentially unimportant for fetal development, is the most important hormone for postnatal growth. However, growth overall is a complex phenomenon that is affected not only by growth hormone and somatomedins but also by thyroid hormones, androgens, estrogens, glucocorticoids, and insulin. It is also affected, of course, by genetic factors, and

it depends on adequate nutrition. It is normally accompanied by an orderly sequence of maturational changes, and it involves accretion of protein and an increase in length and size, not just an increase in weight (which could reflect the formation of fat or retention of salt and water rather than growth per se).

ROLE OF NUTRITION

The food supply is the most important extrinsic factor affecting growth. The diet must be adequate not only in protein content but also in essential vitamins and minerals (see Chapter 26) and in calories, so that ingested protein is not burned for energy. However, the age at which a dietary deficiency occurs appears to be an important consideration. For example, once the pubertal growth spurt has commenced, considerable linear growth continues even if caloric intake is reduced. Injury and disease, on the other hand, stunt growth because they increase protein catabolism.

GROWTH PERIODS

In humans, two periods of rapid growth occur **(Figure 18–7)**: the first in infancy and the second in late puberty just before growth stops. The first period of accelerated growth is partly a continuation of the fetal growth period. The second growth spurt, at the time of puberty, is due to growth hormone, androgens, and estrogens. Because girls mature earlier than boys, this growth spurt appears earlier in girls. Of course, in both sexes the rate of growth of individual tissues varies **(Figure 18–8)**. The eventual cessation of growth is due in large part to closure of the epiphyses in the long bones by estrogens (see Chapter 21). After this time, further increases in height are not possible.

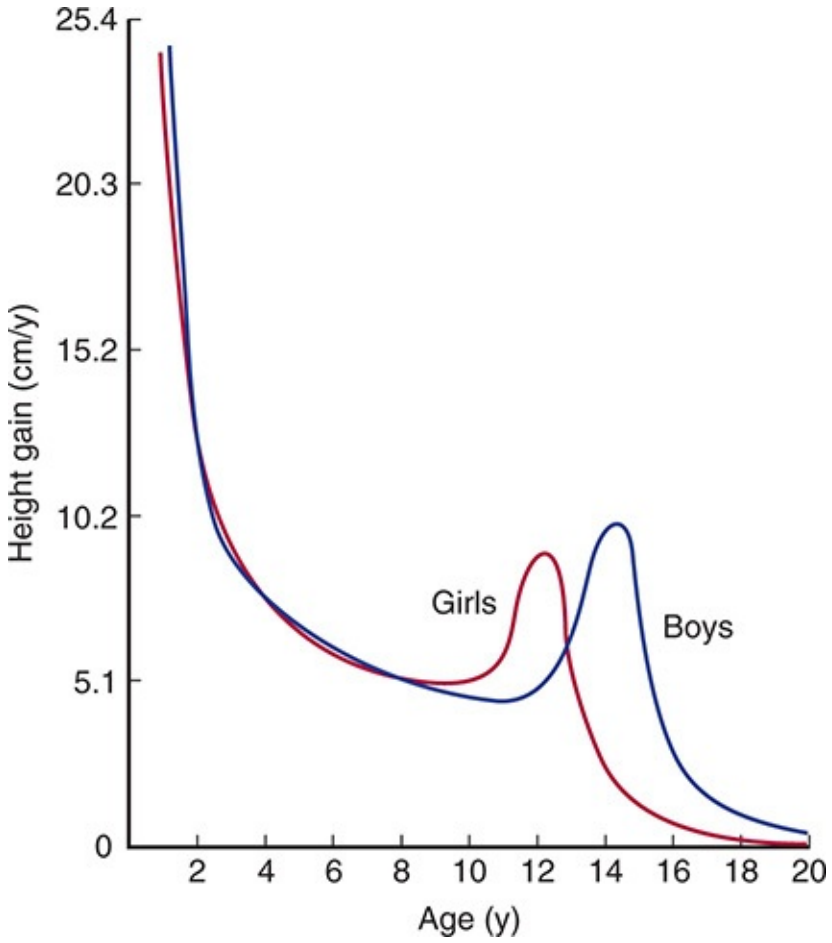


FIGURE 18–7 Rate of growth in boys and girls from birth to age 20.

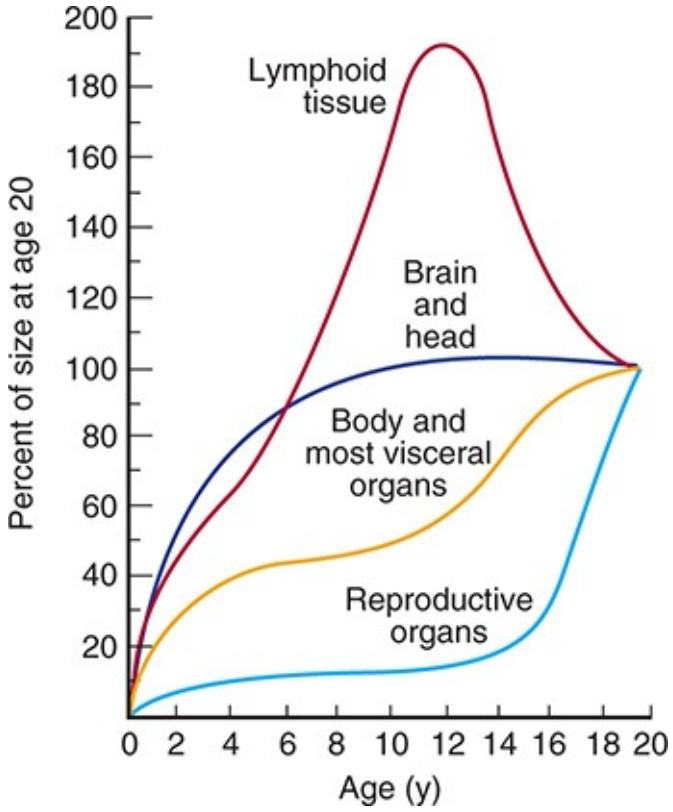


FIGURE 18–8 Growth of different tissues at various ages as a percentage of size at age 20. The curves are composites that include data for both boys and girls.

It is interesting that at least during infancy, growth is not a continuous process but is episodic or saltatory. Increases in the length of human infants of 0.5–2.5 cm in a few days are separated by periods of 2–63 days during which no measurable growth can be detected. The saltatory frequency varies between individuals and summates to final height. This episodic growth apparently reflects discrete times at which chondrocytes are susceptible to differentiation into a hypertrophic phase.

HORMONAL EFFECTS

The contributions of hormones to growth after birth are shown diagrammatically in **Figure 18–9**. Plasma growth hormone is elevated in newborns. Subsequently, average resting levels fall but the spikes of growth hormone secretion are larger, especially during puberty, so the mean plasma level over 24 h is increased; it is 2–4 ng/mL in normal adults, but 5–8 ng/mL in children. Plasma IGF-I levels also

rise during childhood, reaching a peak at 13–17 years of age. In contrast, IGF-II levels are constant throughout postnatal growth.

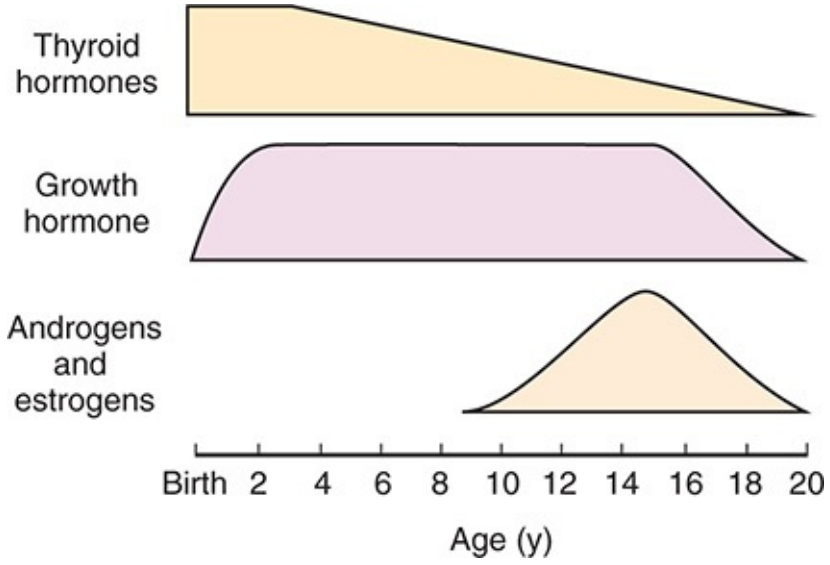


FIGURE 18–9 Relative importance of hormones in human growth at various ages. Thyroid hormones and growth hormones drive the rapid growth rate in the neonatal period and for the first few years of life, with the effect of the thyroid hormones tapering off thereafter. The growth spurt of puberty, on the other hand, involves interactions between the effects of growth hormone and the sex steroids. (Used with permission of D. A. Fisher.)

The growth spurt that occurs at the time of puberty (Figure 18–7) is due in part to the secretion of adrenal androgens at this time in both sexes and the protein anabolic effect of these androgens; however, it is also due to an interaction among sex steroids, growth hormone, and IGF-I. Treatment with estrogens and androgens increases the secretion of growth hormone in response to various stimuli and increases plasma IGF-I secondary to this increase in circulating growth hormone. This, in turn, causes growth.

Although androgens and estrogens initially stimulate growth, estrogens ultimately terminate growth by causing the epiphyses to fuse to the long bones (epiphysial closure). Once the epiphyses have closed, linear growth ceases (see Chapter 21). This is why patients with sexual precocity are apt to be dwarfed. On the other hand, men who were castrated before puberty tend to be tall because their estrogen production is decreased and their epiphyses remain open, allowing some growth to continue past the normal age of puberty.

In hypophysectomized animals, growth hormone increases growth, but this

effect is potentiated by thyroid hormones, which by themselves have no effect on growth. The action of thyroid hormones in this situation is therefore permissive to that of growth hormone, possibly via potentiation of the actions of somatomedins. Thyroid hormones also often appear to be necessary for stimulated growth hormone secretion; basal growth hormone levels are normal in hypothyroidism, but the response to hypoglycemia is frequently blunted. Thyroid hormones have widespread effects on the ossification of cartilage, the growth of teeth, the contours of the face, and the proportions of the body. Hypothyroid dwarfs (also known as **cretins**) therefore have infantile features **(Figure 18–10)**. Patients who are dwarfed because of panhypopituitarism have features consistent with their chronologic age until puberty, but since they do not mature sexually, they have juvenile features in adulthood **(Clinical Box 18–2)**.

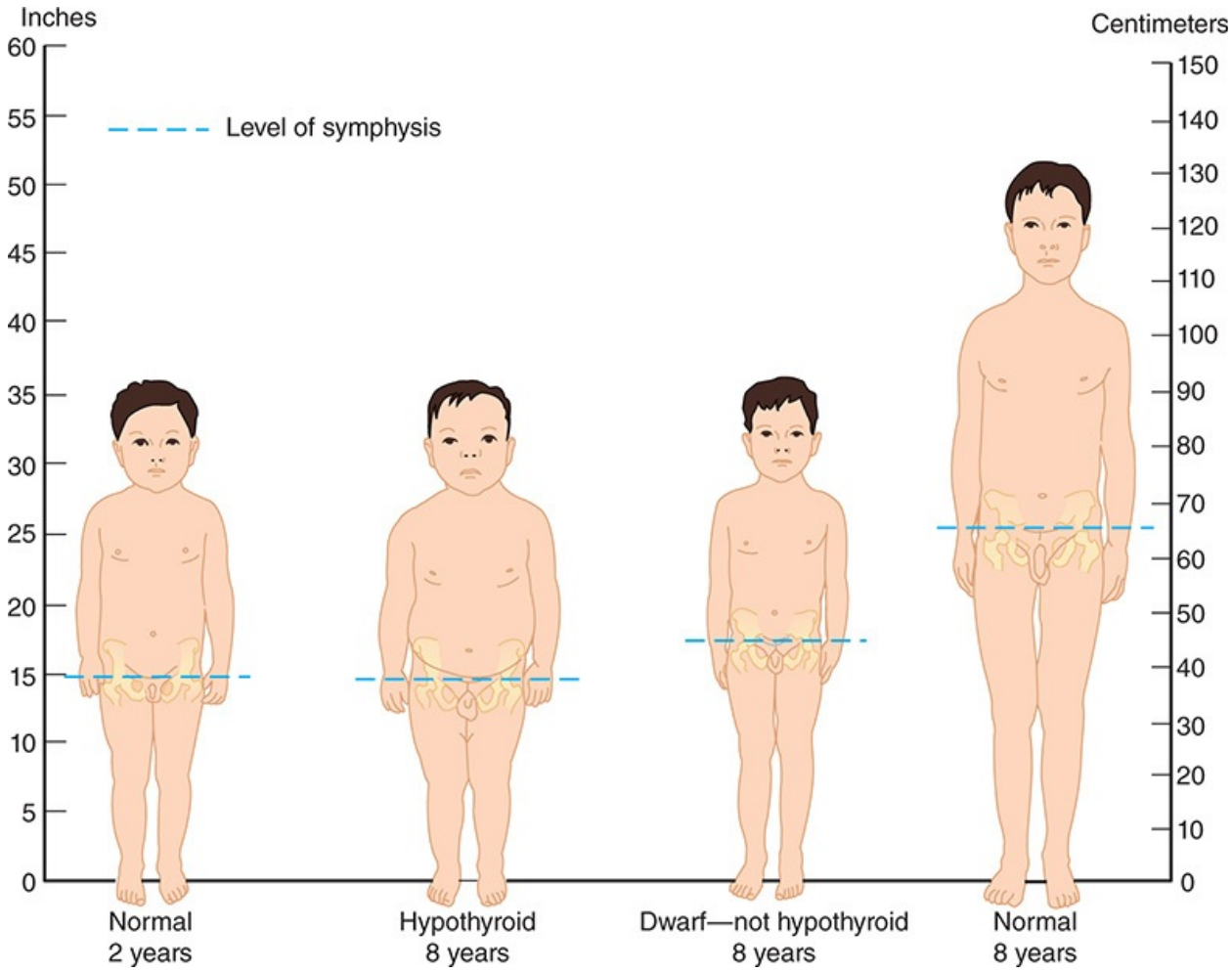


FIGURE 18–10 Normal and abnormal growth. Hypothyroid dwarfs (cretins) retain their infantile proportions, whereas dwarfs of the constitutional type and, to a lesser extent, of the hypopituitary type have proportions characteristic of

their chronologic age. See also Clinical Box 18–2. (Reproduced with permission from Wilkins L: *The Diagnosis and Treatment of Endocrine Disorders in Childhood and Adolescence*, 3rd ed. Thomas; 1966.)

The effect of insulin on growth is discussed in Chapter 24. Diabetic animals fail to grow, and insulin causes growth in hypophysectomized animals. However, the growth is appreciable only when large amounts of carbohydrate and protein are supplied with the insulin.

Adrenocortical hormones other than androgens exert a permissive action on growth in the sense that adrenalectomized animals fail to grow unless their blood pressure and circulations are maintained by replacement therapy. On the other hand, glucocorticoids are potent inhibitors of growth because of their direct action on cells, and treatment of children with pharmacologic doses of corticosteroids slows or stops growth for as long as the treatment is continued.

CATCH-UP GROWTH

Following illness or starvation in children, a period of **catch-up growth** **(Figure 18–11)** takes place during which the growth rate is greater than normal. The accelerated growth usually continues until the previous growth curve is reached, then slows to normal. The mechanisms that bring about and control catch-up growth are poorly understood.

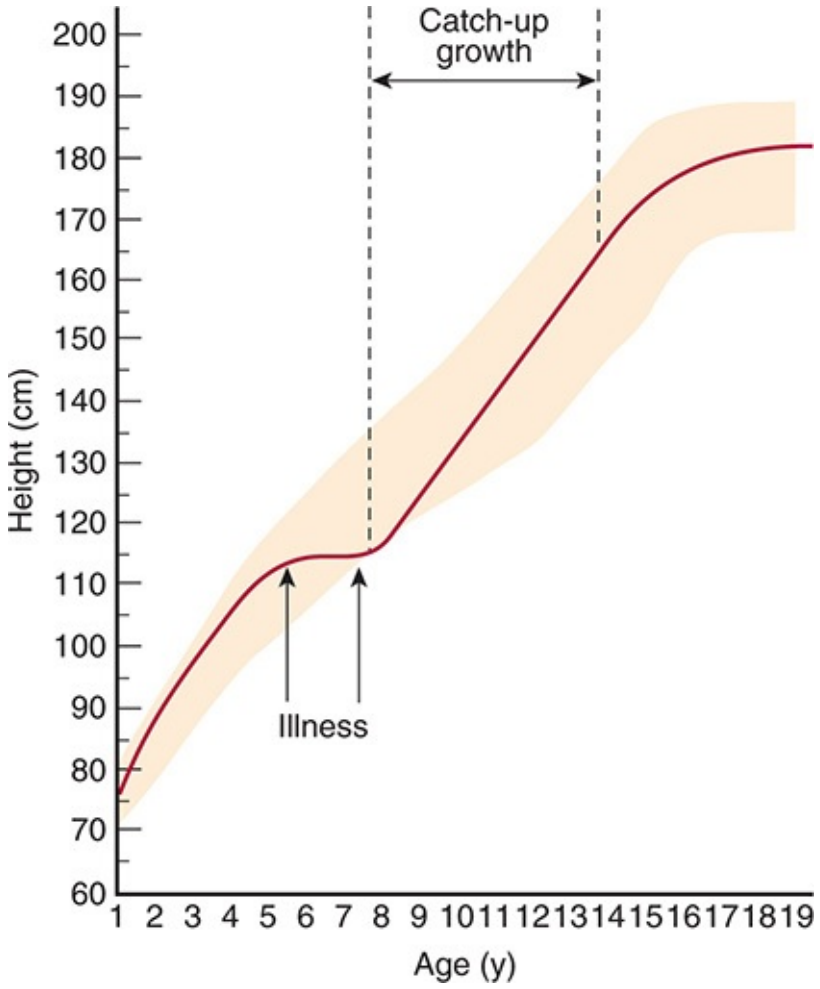


FIGURE 18–11 Growth curve for a normal boy who had an illness beginning at age 5 and ending at age 7. The shaded area shows the range of normal heights for a given age. The red line shows actual growth of the boy studied. Catch-up growth eventually returned his height to his previous normal growth curve. (Modified with permission from Boersma B, Wit JM: Catch-up growth. Endocr Rev 1997 Oct;18(5):646–661.)

PITUITARY GONADOTROPINS & PROLACTIN

CHEMISTRY

FSH and LH are each made up of an α and a β subunit. They are glycoproteins, and their carbohydrate residues increase their potency by markedly slowing their metabolism. The half-life of human FSH is about 170 min; the half-life of LH is about 60 min. Loss-of-function mutations in the FSH receptor cause

hypogonadism. Gain-of-function mutations cause a spontaneous form of **ovarian hyperstimulation syndrome,** a condition in which many follicles are stimulated and cytokines are released from the ovary, causing increased vascular permeability and shock.

CLINICAL BOX 18–2

Dwarfism

The accompanying discussion of growth control should suggest several possible etiologies of short stature. It can be due to GHRH deficiency, growth hormone deficiency, or deficient secretion of IGF-I. Isolated growth hormone deficiency is often due to GHRH deficiency, and in these instances, the growth hormone response to GHRH is normal. However, some patients with isolated growth hormone deficiency have abnormalities of their growth hormone secreting cells. In another group of dwarfed children, the plasma growth hormone concentration is normal or elevated, but their growth hormone receptors are unresponsive as a result of loss-of-function mutations. The resulting condition is known as **growth hormone insensitivity** or **Laron dwarfism.** Plasma IGF-I is markedly reduced, along with its binding protein. African pygmies have normal plasma growth hormone levels and a modest reduction in the plasma level of growth hormone–binding protein. However, their plasma IGF-I concentration fails to increase at the time of puberty and they experience less growth than nonpygmy controls throughout the prepubertal period.

Short stature may also be caused by mechanisms independent of specific defects in the growth hormone axis. It is characteristic of childhood hypothyroidism (cretinism) and occurs in patients with precocious puberty. It is also part of the syndrome of **gonadal dysgenesis** seen in patients who have an XO chromosomal pattern instead of an XX or XY pattern (see Chapter 22). Various bone and metabolic diseases also cause stunted growth, and in many cases there is no known cause (“constitutional delayed growth”). Chronic abuse and neglect can also cause dwarfism in children, independent of malnutrition. This condition is known as **psychosocial dwarfism** or the **Kaspar Hauser syndrome,** named for the patient with the first reported case. Finally, **achondroplasia,** the most common form of dwarfism in humans, is characterized by short limbs with a normal trunk. It is an autosomal dominant condition caused by a mutation in the gene that codes for **fibroblast growth**

factor receptor 3 (FGFR3). This member of the fibroblast growth receptor family is normally expressed in cartilage and the brain.

THERAPEUTIC HIGHLIGHTS

The treatment of dwarfism is dictated by its underlying cause. If treatment to replace the relevant hormone is commenced promptly in appropriate childhood cases, almost normal stature can often be attained. Thus, the availability of recombinant forms of growth hormone and IGF-I has greatly improved treatment in cases where these hormones are deficient.

Human pituitary prolactin has considerable structural similarity to human growth hormone and human chorionic somatomammotropin (hCS). The half-life of prolactin, like that of growth hormone, is about 20 min. Structurally similar prolactins are secreted by the endometrium and by the placenta.

REGULATION OF PROLACTIN SECRETION

The regulatory factors for prolactin secretion by the pituitary overlap, in part, with those causing secretion of growth hormone, but there are important differences, and some stimuli increase prolactin secretion while decreasing that of growth hormone (and vice versa) **(Table 18–4)**. The normal plasma prolactin concentration is approximately 5 ng/mL in men and 8 ng/mL in women. Secretion is tonically inhibited by the hypothalamus, and section of the pituitary stalk leads to an increase in circulating prolactin. Thus, the effect of the hypothalamic prolactin-inhibiting hormone, dopamine, must normally be greater than the effects of the various hypothalamic peptides with prolactin-releasing activity. In humans, prolactin secretion is increased by exercise, surgical and psychological stresses, and stimulation of the nipple (Table 18–4). The plasma prolactin level rises during sleep, the rise starting after the onset of sleep and persisting throughout the sleep period. Secretion is increased during pregnancy, reaching a peak at the time of parturition. After delivery, the plasma concentration falls to nonpregnant levels in about 8 days. Suckling produces a prompt increase in secretion, but the magnitude of this rise gradually declines after a woman has been nursing for more than 3 months. With prolonged lactation, milk secretion occurs with prolactin levels that are in the normal range.

TABLE 18–4 Comparison of factors affecting the secretion of human prolactin and growth hormone.

Factor	Prolactin	Growth Hormone
Sleep	I+	I+
Nursing	I++	N
Breast stimulation in nonlactating women	I	N
Stress	I+	I+
Hypoglycemia	I	I+
Strenuous exercise	I	I
Sexual intercourse in women	I	N
Pregnancy	I++	N
Estrogens	I	I
Hypothyroidism	I	N
TRH	I+	N
Phenothiazines, butyrophenones	I+	N
Opioids	I	I
Glucose	N	D
Somatostatin	N	D+
L-dopa	D+	I+
Apomorphine	D+	I+
Bromocriptine and related ergot derivatives	D+	I

D, moderate decrease; D+, marked decrease; I, moderate increase; I+, marked increase; I++, very marked increase; N, no change; TRH, thyrotropin-releasing hormone.

L-dopa decreases prolactin secretion by increasing the formation of dopamine; bromocriptine and other dopamine agonists inhibit secretion because they stimulate dopamine receptors. Chlorpromazine and related drugs that block dopamine receptors increase prolactin secretion. Thyrotropin-releasing hormone (TRH) stimulates the secretion of prolactin in addition to TSH, and additional

polypeptides with prolactin-releasing activity are present in hypothalamic tissue. Estrogens produce a slowly developing increase in prolactin secretion as a result of a direct action on the lactotropes.

It has now been established that prolactin facilitates the secretion of dopamine in the median eminence. Thus, prolactin acts in the hypothalamus in a negative feedback manner to inhibit its own secretion.

RECEPTORS

The receptors for FSH and LH are G-protein-coupled receptors coupled to adenylyl cyclase through a stimulatory G-protein (G_s; see Chapter 2). In addition, each has an extended, glycosylated extracellular domain.

The human prolactin receptor resembles the growth hormone receptor and is one of the superfamily of receptors that includes the growth hormone receptor and receptors for many cytokines and hematopoietic growth factors (see Chapters 2 and 3). It dimerizes and activates the Janus kinase/signal transducers and activators of transcription (JAK–STAT) pathway and other intracellular enzyme cascades (Figure 18–4).

ACTIONS OF FSH, LH, & PROLACTIN

The testes and ovaries become atrophic when the pituitary is removed or destroyed. The related actions of prolactin and the gonadotropins FSH and LH, as well as those of the gonadotropin secreted by the placenta, are described in detail in Chapters 22 and 23. In brief, FSH helps maintain the spermatogenic epithelium by stimulating Sertoli cells in the male and is responsible for the early growth of ovarian follicles in the female. LH is tropic for the Leydig cells and, in females, is responsible for the final maturation of the ovarian follicles and estrogen secretion from them. It is also responsible for ovulation, the initial formation of the corpus luteum, and secretion of progesterone.

Prolactin causes milk secretion from the breast after estrogen and progesterone priming. Its effect on the breast involves increasing messenger RNA (mRNA) levels and subsequent production of casein and lactalbumin. However, the action of the hormone is not exerted on the cell nucleus and is prevented by inhibitors of microtubules. Prolactin also inhibits the effects of gonadotropins, possibly by an action at the level of the ovary. It thereby prevents ovulation in lactating women. The function of prolactin in normal males is

unsettled, but excess prolactin secreted by tumors causes erectile dysfunction.

EFFECTS OF PITUITARY INSUFFICIENCY

CHANGES IN OTHER ENDOCRINE GLANDS

The widespread changes that develop when the pituitary is removed surgically or destroyed by disease in humans or animals are predictable in terms of the known hormonal functions of the gland. In hypopituitarism, the adrenal cortex atrophies and the secretion of adrenal glucocorticoids and sex hormones falls to low levels. Stress-induced increases in aldosterone secretion are absent, but basal aldosterone secretion and increases induced by salt depletion are normal, at least for some time. Since no mineralocorticoid deficiency is present, salt loss and hypovolemic shock do not develop, but the inability to increase glucocorticoid secretion makes patients with pituitary insufficiency sensitive to stress. The development of salt loss in long-standing hypopituitarism is discussed in Chapter 20. Growth is inhibited (see Clinical Box 18–2). Thyroid function is depressed to low levels, and cold is tolerated poorly. The gonads atrophy, sexual cycles stop, and some of the secondary sex characteristics disappear.

INSULIN SENSITIVITY

Hypophysectomized animals have a tendency to become hypoglycemic, especially when fasted. Hypophysectomy ameliorates diabetes mellitus (see Chapter 24) and markedly increases the hypoglycemic effect of insulin. This is due in part to the deficiency of adrenocortical hormones, but hypophysectomized animals are more sensitive to insulin than adrenalectomized animals because they also lack the anti-insulin effect of growth hormone.

WATER METABOLISM

Although selective destruction of the supraoptic–posterior pituitary causes diabetes insipidus (see Chapter 17), removal of both the anterior and posterior pituitary usually causes no more than a transient polyuria. In the past, there was speculation that the anterior pituitary secreted a “diuretic hormone,” but the amelioration of the diabetes insipidus is actually explained by a decrease in the osmotic load presented for excretion. Osmotically active particles hold water in

the renal tubules (see Chapter 38). Because of the ACTH deficiency, the rate of protein catabolism is decreased in hypophysectomized animals. Because of the TSH deficiency, the metabolic rate is low. Consequently, fewer osmotically active products of catabolism are filtered and urine volume declines, even in the absence of vasopressin. Growth hormone deficiency contributes to the depression of the glomerular filtration rate in hypophysectomized animals, and growth hormone increases the glomerular filtration rate and renal plasma flow in humans. Finally, because of the glucocorticoid deficiency, there is the same defective excretion of a water load that is seen in adrenalectomized animals. The "diuretic" activity of the anterior pituitary can thus be explained in terms of the actions of ACTH, TSH, and growth hormone.

OTHER DEFECTS

When growth hormone deficiency develops in adulthood, it is usually accompanied by deficiencies in other anterior pituitary hormones. The deficiency of ACTH and other pituitary hormones with MSH activity may be responsible for the pallor of the skin in patients with hypopituitarism. There may be some loss of protein in adults, but wasting is not a feature of hypopituitarism in humans, and most patients with pituitary insufficiency are well nourished.

CAUSES OF PITUITARY INSUFFICIENCY IN HUMANS

Tumors of the anterior pituitary cause pituitary insufficiency. Suprasellar cysts, remnants of Rathke pouch that enlarge and compress the pituitary, are another cause of hypopituitarism. In women who have an episode of shock due to postpartum hemorrhage, the pituitary may become infarcted, with the subsequent development of postpartum necrosis **(Sheehan syndrome).** The blood supply to the anterior lobe is vulnerable because it descends on the pituitary stalk through the rigid diaphragma sellae, and during pregnancy the pituitary is enlarged. Pituitary infarction is usually extremely rare in men.

CHAPTER SUMMARY

- The pituitary gland consists of two functional sections in humans: the anterior lobe, which secretes mainly tropic hormones; and the posterior

lobe, which contains nerve endings that project from the hypothalamus and release oxytocin and vasopressin. The anterior lobe receives almost all of its blood supply from the portal hypophysial vessels that carry regulatory factors released by the hypothalamus.

- The pituitary plays a critical role in regulating the function of downstream glands, and also exerts independent endocrine actions on a wide variety of peripheral organs and tissues. Cell types in the anterior lobe include somatotropes (growth hormone), lactotropes (prolactin), corticotropes (ACTH), thyrotropes (TSH), and gonadotropes (FSH, LH), which release the hormones listed in parentheses. The relative proportions of each cell type vary depending on needs at different life stages.
- Corticotropes of the anterior lobe synthesize proopiomelanocortin, which is the precursor of ACTH, endorphins, and melanotropins. ACTH is a primary regulator of skin pigmentation in mammals.
- Growth hormone is synthesized by somatotropes. It is secreted in an episodic manner in response to hypothalamic factors, and secretion is subject to feedback inhibition. A portion of the circulating pool is protein-bound.
- Growth hormone activates growth and influences protein, carbohydrate, and fat metabolism to react to stressful conditions. Many, but not all, of the peripheral actions of growth hormone can be attributed to its ability to stimulate production of IGF-I.
- Growth reflects a complex interplay of growth hormone, IGF-I, and many other hormones as well as extrinsic influences and genetic factors. The consequences of overproduction or underproduction of such influences depends on whether this occurs before or after puberty. Deficiencies in components of the growth hormone pathway in childhood lead to dwarfism; overproduction results in gigantism, acromegaly, or both.
- The pituitary also supplies hormones that regulate reproductive tissues and lactation—FSH, LH, and prolactin. Prolactin, in particular, is regulated by many of the factors that also regulate growth hormone secretion, although specific regulators may have opposing effects.
- Pituitary insufficiency is accompanied by atrophy of the adrenal cortex, sensitivity to stress, growth inhibition, depressed thyroid function, hypoglycemia, pallor, and atrophy of the gonads. Pituitary insufficiency may be caused by tumors or, in women, by infarction following shock due to postpartum hemorrhage.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. A neuroscientist is studying communication between the hypothalamus and pituitary in a rat model. She interrupts blood flow through the median eminence and then measures circulating levels of pituitary hormones following appropriate physiologic stimulation. Secretion of which of the following hormones will be unaffected by the experimental manipulation?

   A. Growth hormone

   B. Prolactin

   C. TSH

   D. FSH

   E. Vasopressin

2. Loss of which of the following pituitary hormones might be expected to increase responses to painful stimuli?

   A. α-Melanocyte–stimulating hormone (α-MSH)

   B. β-MSH

   C. ACTH

   D. Growth hormone

   E. β-Endorphin

3. A 20-year-old African American woman is evaluated for evaluation of patches of skin on her face and hands that have lost pigmentation over a period of weeks. She is otherwise healthy. Blood tests reveal the presence of autoantibodies to melanocytes. The most likely diagnosis is

   A. albinism.

   B. piebaldism.

   C. primary adrenal insufficiency.

   D. vitiligo.

   E. hypopituitarism.

4. A scientist finds that infusion of growth hormone into the median eminence of the hypothalamus in experimental animals inhibits the secretion of growth hormone and concludes that this proves that growth hormone feeds back to inhibit GHRH secretion. Do you accept this conclusion?

   A. No, because growth hormone does not cross the blood-brain barrier.

   B. No, because the infused growth hormone could be stimulating dopamine

secretion.

C. No, because substances placed in the median eminence could be transported to the anterior pituitary.

D. Yes, because systemically administered growth hormone inhibits growth hormone secretion.

E. Yes, because growth hormone binds GHRH, inactivating it.

5. The growth hormone receptor

A. activates G_s.

B. requires dimerization to exert its effects.

C. must be internalized to exert its effects.

D. resembles the IGF-I receptor.

E. resembles the ACTH receptor.

6. A 7-year-old boy is evaluated for short stature. His average circulating growth hormone level is within the normal range for his age, but levels of IGF-I are reduced. His growth failure is most likely due to a defect in

A. GHRH release from the hypothalamus.

B. GHRH receptors.

C. androgen synthesis.

D. estrogen synthesis.

E. growth hormone receptors.

7. A young woman in the first trimester of her first pregnancy comes to the emergency room complaining of lower abdominal pain, nausea, and vomiting that have been resistant to treatment. An ultrasound scan reveals bilateral enlarged ovaries containing multiple cysts and free fluid in the pelvis and abdomen. Her symptoms worsen over time and progress to hemoconcentration and ascites before spontaneously resolving at 18 weeks of gestation. She reports that her mother had developed similar symptoms with each of her own pregnancies. DNA sequencing would be expected to reveal which of the following:

A. A loss of function mutation in FSH

B. A loss of function mutation in LH

C. A gain of function mutation in the FSH receptor

D. A gain of function mutation in the LH receptor

E. A loss of function mutation in gonadotropin-releasing hormone

8. During childbirth, a woman suffers a serious hemorrhage and goes into shock. After she recovers, she displays symptoms of hypopituitarism. Which of the following will not be expected in this patient?

A. Cachexia

B. Infertility

C. Pallor

D. Low basal metabolic rate

E. Intolerance to stress

CHAPTER 19

The Adrenal Medulla & Adrenal Cortex

OBJECTIVES

After studying this chapter, you should be able to:

- Name the three catecholamines secreted by the adrenal medulla and summarize their biosynthesis, metabolism, and function.
- List the stimuli that increase adrenal medullary secretion.
- Differentiate between C_{18}, C_{19}, and C_{21} steroids and give examples of each.
- Outline the steps involved in steroid biosynthesis in the adrenal cortex.
- Name the plasma proteins that bind adrenocortical steroids and discuss their physiologic role.
- Name the major site of adrenocortical hormone metabolism and the principal metabolites produced from glucocorticoids, adrenal androgens, and aldosterone.
- Describe the mechanisms by which glucocorticoids and aldosterone produce changes in cellular function.
- Define the physiological and pharmacological effects of glucocorticoids.
- Contrast the physiological and pathological effects of adrenal androgens.
- Describe the mechanisms that regulate secretion of glucocorticoids and adrenal sex hormones.

- Explain the actions of aldosterone and describe the mechanisms that regulate aldosterone secretion.
- Describe the main features of the diseases caused by an excess or deficiency of each of the hormones of the adrenal gland.

INTRODUCTION

The **adrenal glands** are endocrine organs that produce several hormones including **catecholamines** and **steroid hormones**. There are two adrenal glands, one sitting on top of each kidney **(Figure 19–1)**. Each adrenal gland has an outer cortex that secretes the steroid hormones, **mineralocorticoids, glucocorticoids and androgens**, and an inner **medulla** that secretes the catecholamines, **epinephrine, norepinephrine,** and **dopamine**.

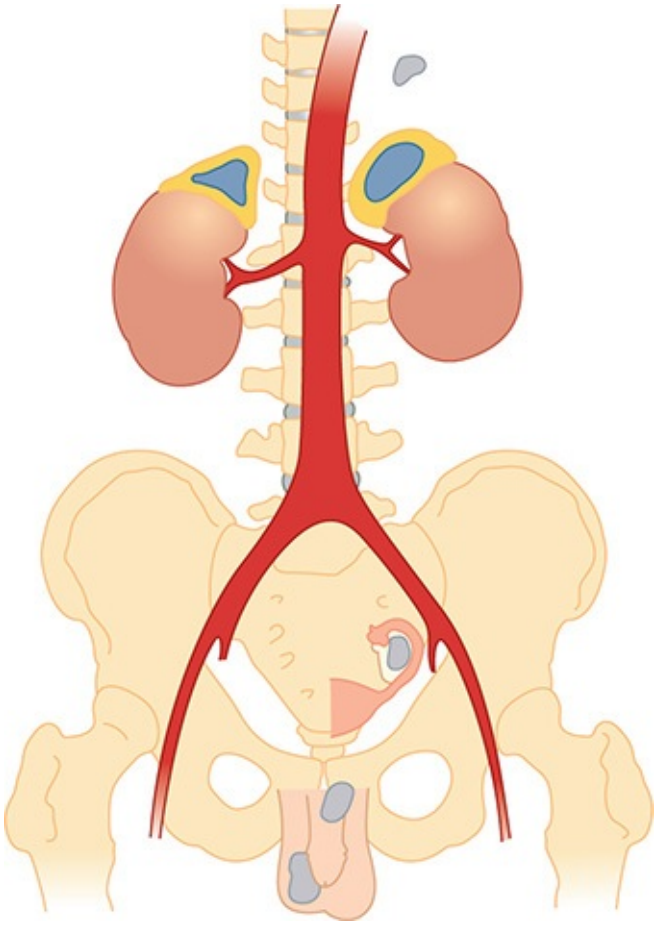


FIGURE 19–1 Human adrenal glands. Adrenocortical tissue is yellow; adrenal medullary tissue is blue. Note the location of the adrenals at the superior

pole of each kidney. Also shown are extra-adrenal sites (gray) at which cortical and medullary tissue is sometimes found. (Reproduced with permission from Williams RH: *Textbook of Endocrinology*, 4th ed. St. Louis, MO: Saunders; 1968.)

The adrenal cortex secretes **glucocorticoids (eg, cortisol)** which are steroids with widespread effects on the metabolism of carbohydrate and protein; and a **mineralocorticoid (aldosterone**) essential to the maintenance of Na^+ balance and extracellular fluid (ECF) volume.Mineralocorticoids and the glucocorticoids are necessary for survival. It is also a secondary site of **androgen** synthesis, secreting sex hormones such as testosterone, which can exert effects on reproductive function. Adrenocortical secretion is controlled primarily by adrenocorticotropic hormone (ACTH), from the anterior pituitary (Chapter 20), but mineralocorticoid secretion is also subject to independent control by circulating factors, of which the most important is **angiotensin II,** a peptide formed in the bloodstream by the action of **renin.**

The adrenal medulla is in effect a sympathetic ganglion in which the postganglionic neurons have lost their axons and become secretory cells. The cells secrete when stimulated by the preganglionic nerve fibers that reach the gland via the splanchnic nerves. Adrenal medullary hormones (epinephrine, norepinephrine, and dopamine) work mostly to prepare the body for emergencies, the so-called “fight-or-flight” responses.

ADRENAL MORPHOLOGY

The adrenal medulla, which constitutes 28% of the mass of the adrenal gland, is made up of interlacing cords of densely innervated granule-containing cells that abut on venous sinuses. Two cell types can be distinguished morphologically: an epinephrine-secreting type that has larger, less dense granules; and a norepinephrine-secreting type in which smaller, very dense granules fail to fill the vesicles in which they are contained. In humans, 90% of the cells are the epinephrine-secreting type and 10% are the norepinephrine-secreting type. The type of cell that secretes dopamine is unknown. **Paraganglia,** small groups of cells resembling those in the adrenal medulla, are found near the thoracic and abdominal sympathetic ganglia (Figure 19–1).

In adult mammals, the adrenal cortex is divided into three zones **(Figure 19–2)**. The outer **zona glomerulosa** is made up of whorls of cells that are continuous with the columns of cells that form the **zona fasciculata.** These

columns are separated by venous sinuses. The inner portion of the zona fasciculata merges into the **zona reticularis,** where the cell columns become interlaced in a network. The zona glomerulosa makes up 15% of the mass of the adrenal gland; the zona fasciculata, 50%; and the zona reticularis, 7%. The adrenocortical cells contain abundant lipid, especially in the outer portion of the zona fasciculata. All three cortical zones secrete **corticosterone,** but the active enzymatic mechanism for aldosterone biosynthesis is limited to the zona glomerulosa, whereas the enzymatic mechanisms for forming cortisol and sex hormones are found in the two inner zones. Furthermore, subspecialization occurs within the inner two zones, with the zona fasciculata secreting mostly glucocorticoids and the zona reticularis secreting mainly sex hormones.

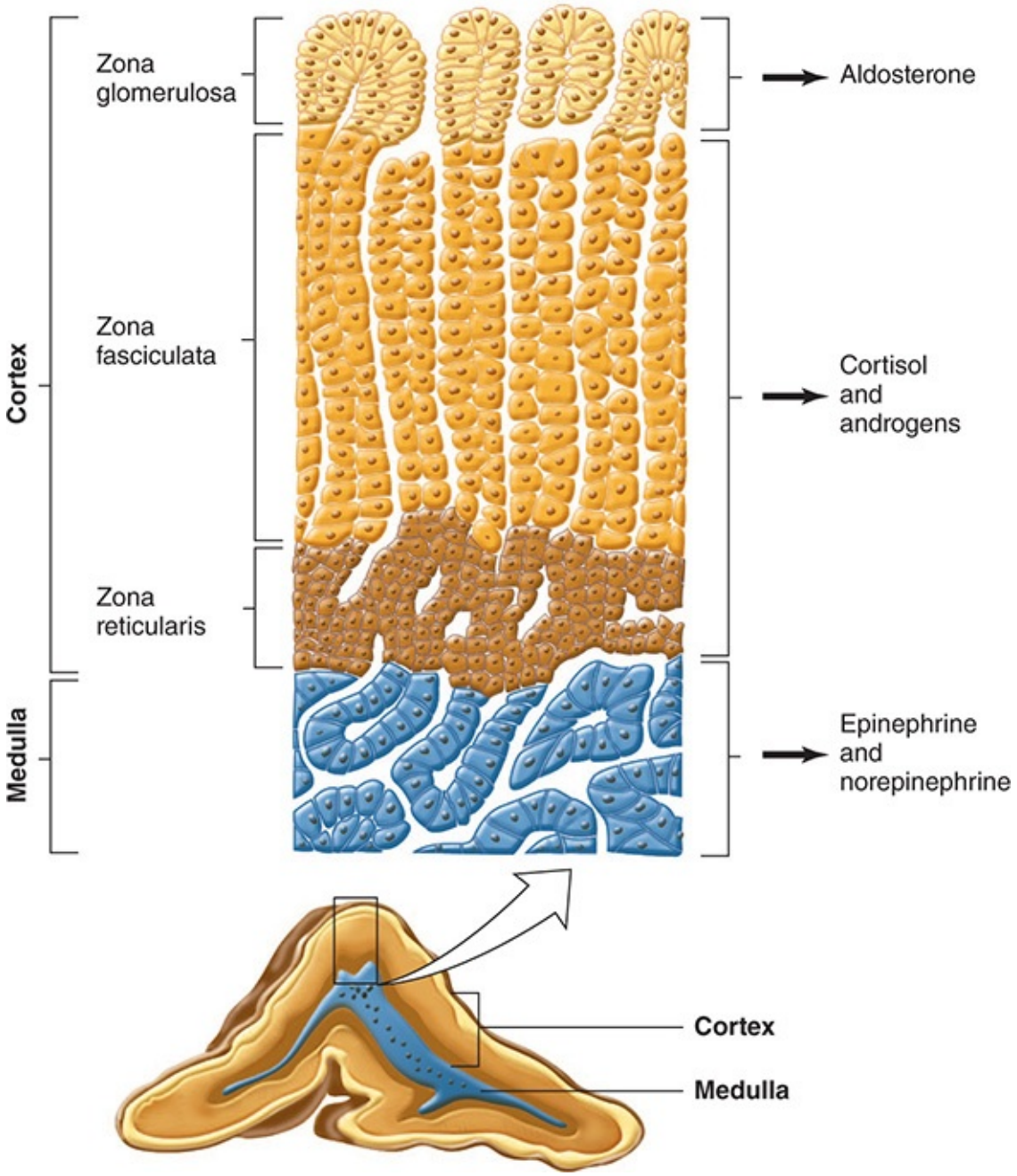


FIGURE 19–2 Section through an adrenal gland showing both the medulla and the zones of the cortex, as well as the hormones they secrete. (Reproduced with permission from Widmaier EP, Raff H, Strang KT: *Vander's Human Physiology: The Mechanisms of Body Function*, 11th ed. New York, NY: McGraw-Hill; 2008.)

Arterial blood reaches the adrenal from many small branches of the phrenic and renal arteries and the aorta. From a plexus in the capsule, blood flows

through the cortex to the sinusoids of the medulla. The medulla is also supplied by a few arterioles that pass directly to it from the capsule. In most species, including humans, blood from the medulla flows into a central adrenal vein. The blood flow through the adrenal is large, as it is in most endocrine glands.

During fetal life, the human adrenal is large and under pituitary control, but the three zones of the permanent cortex represent only 20% of the gland. The remaining 80% is the large **fetal adrenal cortex,** which undergoes rapid degeneration at the time of birth. A major function of this fetal adrenal is synthesis and secretion of sulfate conjugates of androgens that are converted in the placenta to estrogens (see Chapter 22). No structure is comparable to the human fetal adrenal in laboratory animals.

An important function of the zona glomerulosa, in addition to aldosterone synthesis, is the formation of new cortical cells. The adrenal medulla does not regenerate, but when the inner two zones of the cortex are removed, a new zona fasciculata and zona reticularis regenerate from glomerular cells attached to the capsule. Small capsular remnants regrow large pieces of adrenocortical tissue. Immediately after hypophysectomy, the zona fasciculata and zona reticularis begin to atrophy, whereas the zona glomerulosa is unchanged because of the action of angiotensin II on this zone. The ability to secrete aldosterone and conserve Na^+ is normal for some time after hypophysectomy, but in long-standing hypopituitarism, aldosterone deficiency may develop, apparently because of the absence of a pituitary factor that maintains the responsiveness of the zona glomerulosa. Injections of ACTH and stimuli that cause endogenous ACTH secretion produce hypertrophy of the zona fasciculata and zona reticularis but actually decrease, rather than increase, the size of the zona glomerulosa.

The cells of the adrenal cortex contain large amounts of smooth endoplasmic reticulum, which is involved in the steroid-forming process. Other steps in steroid biosynthesis occur in the mitochondria. The structure of steroid-secreting cells is very similar throughout the body. The typical features of such cells are shown in **Figure 19–3**.

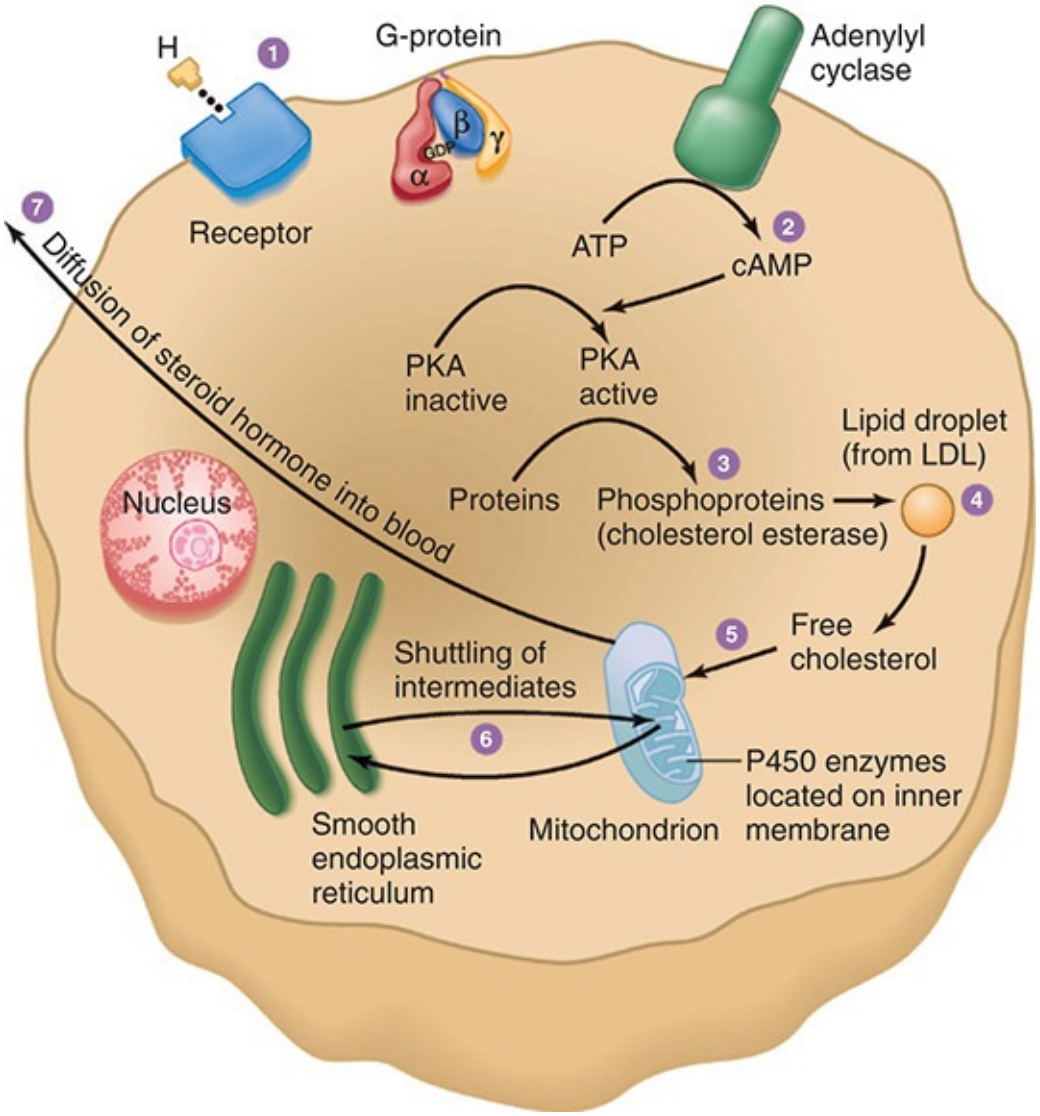


FIGURE 19–3 Schematic overview of the structures of steroid-secreting cells and the intracellular pathway of steroid synthesis. LDL, low-density lipoprotein; PKA, protein kinase A. (Reproduced with permission from Widmaier EP, Raff H, Strang KT: *Vander's Human Physiology: The Mechanisms of Body Function*, 11th ed. New York, NY: McGraw-Hill; 2008.)

ADRENAL MEDULLA: STRUCTURE & FUNCTION OF MEDULLARY HORMONES

CATECHOLAMINES

Norepinephrine, epinephrine, and small amounts of dopamine are synthesized by

the adrenal medulla. Cats and some other species secrete mainly norepinephrine, but in dogs and humans, most of the catecholamine output in the adrenal vein is epinephrine. Norepinephrine also enters the circulation from noradrenergic nerve endings.

The structures of norepinephrine, epinephrine, and dopamine and the pathways for their biosynthesis and metabolism are discussed in Chapter 7. Norepinephrine is formed by hydroxylation and decarboxylation of tyrosine, and epinephrine by methylation of norepinephrine. Phenylethanolamine- N-methyltransferase (PNMT), the enzyme that catalyzes the formation of epinephrine from norepinephrine, is found in appreciable quantities only in the brain and the adrenal medulla. Adrenal medullary PNMT is induced by glucocorticoids. Although relatively large amounts are required, the glucocorticoid concentration is high in the blood draining from the cortex to the medulla. After hypophysectomy, the glucocorticoid concentration of this blood falls and epinephrine synthesis is decreased. In addition, glucocorticoids are apparently necessary for the normal development of the adrenal medulla; in 21β-hydroxylase deficiency, glucocorticoid secretion is reduced during fetal life and the adrenal medulla is dysplastic. In untreated 21β-hydroxylase deficiency, circulating catecholamines are low after birth.

In plasma, about 95% of the dopamine and 70% of the norepinephrine and epinephrine are conjugated to sulfate. Sulfate conjugates are inactive and their function is unsettled. In recumbent humans, the normal plasma level of free norepinephrine is about 300 pg/mL (1.8 nmol/L). On standing, the level increases 50–100% **(Figure 19–4)**. The plasma norepinephrine level is generally unchanged after adrenalectomy, but the free epinephrine level, which is normally about 30 pg/mL (0.16 nmol/L), falls to essentially zero. The epinephrine found in tissues other than the adrenal medulla and the brain is for the most part absorbed from the bloodstream rather than synthesized in situ. Interestingly, low levels of epinephrine reappear in the blood some time after bilateral adrenalectomy, and these levels are regulated like those secreted by the adrenal medulla. They may come from cells such as the intrinsic cardiac adrenergic (ICA) cells (see Chapter 13), but their exact source is unknown.

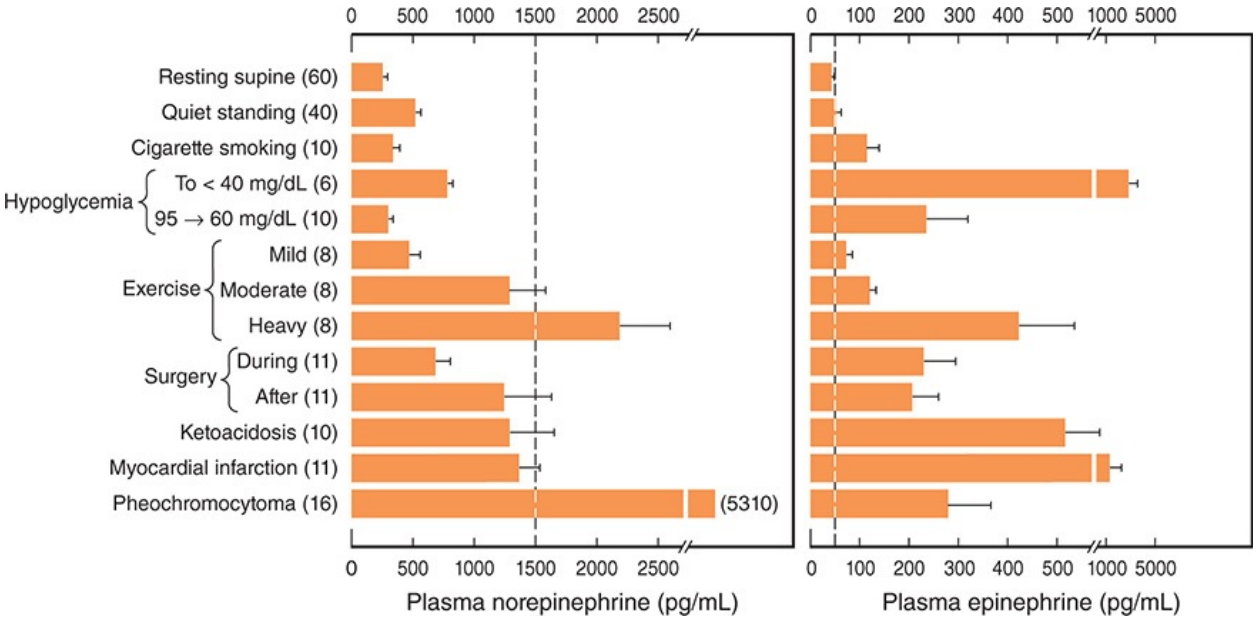


FIGURE 19–4 Norepinephrine and epinephrine levels in human venous blood in various physiologic and pathologic states. Note that the horizontal scales are different. The numbers to the left in parentheses are the numbers of subjects tested. In each case, the vertical dashed line identifies the threshold plasma concentration at which detectable physiologic changes are observed. (Modified and reproduced with permission from Cryer PE: Physiology and pathophysiology of the human sympathoadrenal neuroendocrine system. N Engl J Med 1980; Aug 21; 303(8):436–444.)

Plasma dopamine levels are normally very low, about 0.13 nmol/L. Most plasma dopamine is thought to be derived from sympathetic noradrenergic ganglia.

The catecholamines have a half-life of about 2 min in the circulation. For the most part, they are methoxylated and then oxidized to 3-methoxy-4-hydroxymandelic acid (vanillylmandelic acid [VMA]; see Chapter 7). About 50% of the secreted catecholamines appear in the urine as free or conjugated metanephrine and normetanephrine, and 35% as VMA. Only small amounts of free norepinephrine and epinephrine are excreted. In normal humans, about 30 µg of norepinephrine, 6 µg of epinephrine, and 700 µg of VMA are excreted per day.

OTHER SUBSTANCES SECRETED BY THE ADRENAL MEDULLA

In the medulla, norepinephrine and epinephrine are stored in granules with ATP. The granules also contain chromogranin A (see Chapter 7). Secretion is initiated by acetylcholine released from the preganglionic neurons that innervate the secretory cells. Acetylcholine activates cation channels allowing Ca^{2+} to enter the cells from the ECF and trigger the exocytosis of the granules. In this fashion, catecholamines, ATP, and proteins from the granules are all released into the blood together.

Epinephrine-containing cells of the medulla also contain and secrete opioid peptides (see Chapter 7). The precursor molecule is preproenkephalin. Most of the circulating metenkephalin comes from the adrenal medulla. The circulating opioid peptides do not cross the blood-brain barrier.

Adrenomedullin, a vasodepressor polypeptide found in the adrenal medulla, is discussed in Chapter 32.

EFFECTS OF EPINEPHRINE & NOREPINEPHRINE

In addition to mimicking the effects of noradrenergic nervous discharge, norepinephrine and epinephrine exert metabolic effects that include glycogenolysis in liver and skeletal muscle, mobilization of free fatty acids (FFA), increased plasma lactate, and stimulation of the metabolic rate. The effects of norepinephrine and epinephrine are brought about by actions on two classes of receptors: α- and β-adrenergic receptors. α-Receptors are subdivided into two groups, α_1 and α_2 receptors, and β-receptors into three groups, β_1, β_2, and β_3 receptors (see Chapter 7). There are three subtypes of α_1-receptors and three subtypes of α_2-receptors (see Table 7–2).

Norepinephrine and epinephrine both increase the force and rate of contraction of the isolated heart. These responses are mediated by β_1-receptors. The catecholamines also increase myocardial excitability, causing extrasystoles and, occasionally, more serious cardiac arrhythmias. Norepinephrine produces vasoconstriction in most if not all organs via α_1-receptors, but epinephrine dilates the blood vessels in skeletal muscle and the liver via β_2-receptors. This usually overbalances the vasoconstriction produced by epinephrine elsewhere, and the total peripheral resistance drops. When norepinephrine is infused slowly in normal animals or humans, the systolic and diastolic blood pressures rise. The **hypertension** stimulates the carotid and aortic baroreceptors, producing reflex

bradycardia that overrides the direct cardioacceleratory effect of norepinephrine. Consequently, cardiac output per minute falls. Epinephrine causes a widening of the pulse pressure but because baroreceptor stimulation is insufficient to obscure the direct effect of the hormone on the heart, cardiac rate and output increase. These changes are summarized in **Figure 19–5**.

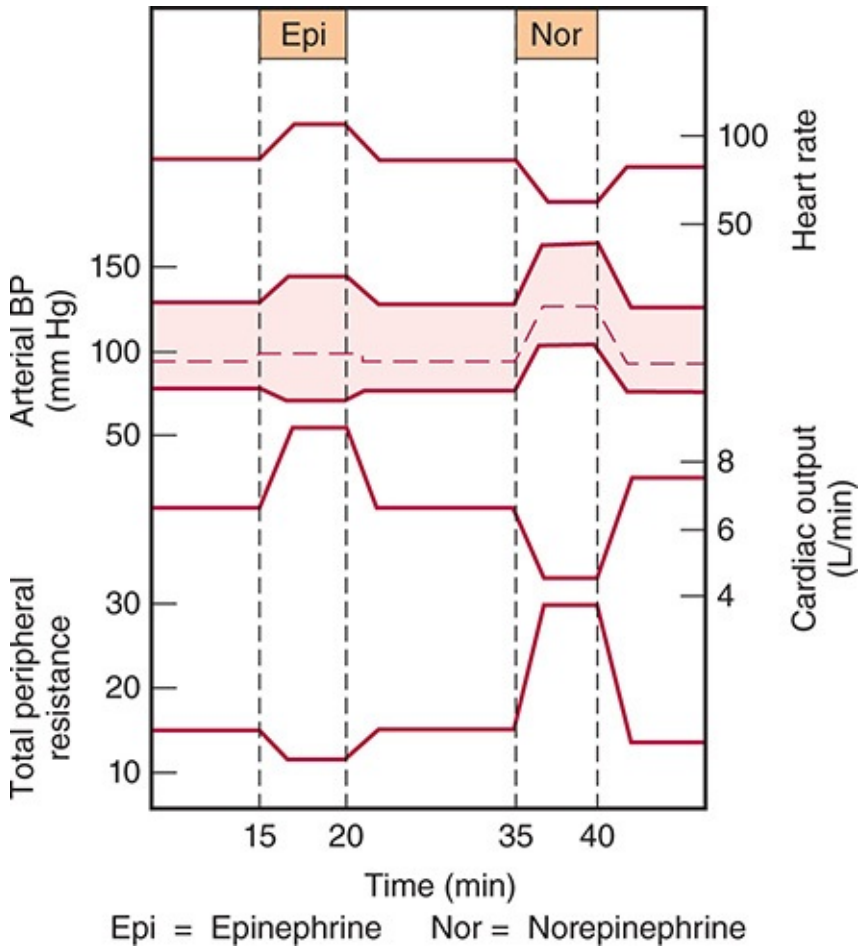


FIGURE 19–5 Circulatory changes produced in humans by the slow intravenous infusion of epinephrine and norepinephrine.

Catecholamines increase alertness (see Chapter 14). Epinephrine and norepinephrine are equally potent in this regard, although in humans epinephrine usually evokes more anxiety and fear.

The catecholamines have several different actions that affect blood glucose. Epinephrine and norepinephrine both cause glycogenolysis. They produce this effect via β-adrenergic receptors that increase cyclic adenosine monophosphate (cAMP), with activation of phosphorylase, and via α-adrenergic receptors that increase intracellular Ca^{2+} (see Chapter 7). In addition, the catecholamines

increase the secretion of insulin and glucagon via β-adrenergic mechanisms and inhibit the secretion of these hormones via α-adrenergic mechanisms.

Norepinephrine and epinephrine also produce a prompt rise in the metabolic rate that is independent of the liver and a smaller, delayed rise that is abolished by hepatectomy and coincides with the rise in blood lactate concentration. The initial rise in metabolic rate may be due to cutaneous vasoconstriction, which decreases heat loss and leads to a rise in body temperature, or to increased muscular activity, or both. The second rise is probably due to oxidation of lactate in the liver. Mice unable to make norepinephrine or epinephrine because their dopamine β-hydroxylase gene is knocked out are intolerant of cold, but surprisingly, their basal metabolic rate is elevated. The cause of this elevation is unknown.

When injected, epinephrine and norepinephrine cause an initial rise in plasma K^+ because of release of K^+ from the liver and then a prolonged fall in plasma K^+ because of an increased entry of K^+ into skeletal muscle that is mediated by β_2-adrenergic receptors. Some evidence suggests that activation of α-receptors opposes this effect.

The increases in plasma norepinephrine and epinephrine that are needed to produce the various effects listed above have been determined by infusion of catecholamines in resting humans. In general, the threshold for the cardiovascular and the metabolic effects of norepinephrine is about 1500 pg/mL, that is, about five times the resting value (Figure 19–4). Epinephrine, on the other hand, produces tachycardia when the plasma level is about 50 pg/mL, that is, about twice the resting value. The threshold for increased systolic blood pressure and lipolysis is about 75 pg/mL; the threshold for hyperglycemia, increased plasma lactate, and decreased diastolic blood pressure is about 150 pg/mL; and the threshold for the α-mediated decrease in insulin secretion is about 400 pg/mL. Plasma epinephrine often exceeds these thresholds. On the other hand, plasma norepinephrine rarely exceeds the threshold for its cardiovascular and metabolic effects, and most of its effects are due to its local release from postganglionic sympathetic neurons. Most adrenal medullary tumors **(pheochromocytomas)** secrete norepinephrine or epinephrine or both and produce sustained hypertension. However, 15% of epinephrine-secreting tumors secrete this catecholamine episodically, producing intermittent bouts of palpitations, headache, glycosuria, and extreme systolic hypertension. These same symptoms are produced by intravenous injection of a large dose of epinephrine.

EFFECTS OF DOPAMINE

The physiologic function of the dopamine in the circulation is unknown. However, injected dopamine produces renal vasodilation, probably by acting on a specific dopaminergic receptor. It also produces vasodilation in the mesentery. Elsewhere, it produces vasoconstriction, probably by releasing norepinephrine, and it has a positive inotropic effect on the heart by an action on β_1-adrenergic receptors. The net effect of moderate doses of dopamine is an increase in systolic pressure and no change in diastolic pressure. Because of these actions, dopamine is useful in the treatment of traumatic and cardiogenic shock (see Chapter 32).

Dopamine is made in the renal cortex. It causes natriuresis and may exert this effect by inhibiting renal Na, K, ATPase.

REGULATION OF ADRENAL MEDULLARY SECRETION

NEURAL CONTROL

Certain drugs act directly on the adrenal medulla, but physiologic stimuli affect medullary secretion through the nervous system. Catecholamine secretion is low in basal states, but the secretion of epinephrine and, to a lesser extent, that of norepinephrine is reduced even further during sleep.

Increased adrenal medullary secretion is part of the diffuse sympathetic discharge provoked in emergency situations, which Cannon called the "emergency function of the sympathoadrenal system." The ways in which this discharge prepares the individual for flight or fight are described in Chapter 13, and the increases in plasma catecholamines under various conditions are shown in Figure 19–4.

The metabolic effects of circulating catecholamines are probably important, especially in certain situations. The calorigenic action of catecholamines in animals exposed to cold is an example, and so is the glycogenolytic effect (see Chapter 24) in combating hypoglycemia.

SELECTIVE SECRETION

When adrenal medullary secretion is increased, the ratio of norepinephrine to epinephrine in the adrenal effluent is generally unchanged. However,

norepinephrine secretion tends to be selectively increased by emotional stresses with which the individual is familiar, whereas epinephrine secretion rises selectively in situations in which the individual does not know what to expect.

ADRENAL CORTEX: STRUCTURE & BIOSYNTHESIS OF ADRENOCORTICAL HORMONES

CLASSIFICATION & STRUCTURE

The hormones of the adrenal cortex are derivatives of cholesterol. Like cholesterol, bile acids, vitamin D, and ovarian and testicular steroids, they contain the **cyclopentanoperhydrophenanthrene nucleus** **(Figure 19–6)**. Gonadal and adrenocortical steroids are of three types: C_{21} steroids, which have a two-carbon side chain at position 17; C_{19} steroids, which have a keto or hydroxyl group at position 17; and C_{18} steroids, which, in addition to a 17-keto or hydroxyl group, have no angular methyl group attached to position 10. The adrenal cortex secretes primarily C_{21} and C_{19} steroids. Most of the C_{19} steroids have a keto group at position 17 and are therefore called **17-ketosteroids.** The C_{21} steroids that have a hydroxyl group at the 17 position in addition to the side chain are often called 17-hydroxycorticoids or 17-hydroxycorticosteroids.

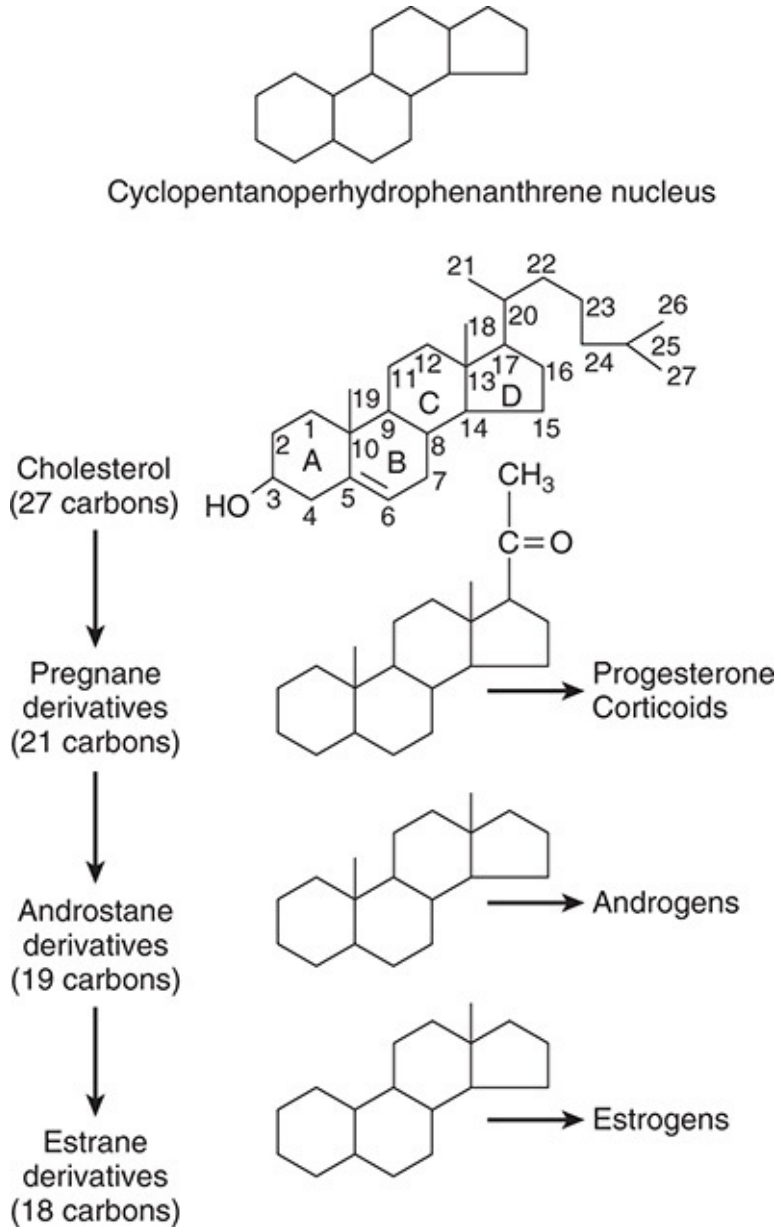


FIGURE 19–6 Basic structure of adrenocortical and gonadal steroids. The letters in the formula for cholesterol identify the four basic rings, and the numbers identify the positions in the molecule. As shown here, the angular methyl groups (positions 18 and 19) are usually indicated simply by straight lines.

The C_{19} steroids have androgenic activity. The C_{21} steroids are classified, using Selye's terminology, as mineralocorticoids or glucocorticoids. All secreted C_{21} steroids have both mineralocorticoid and glucocorticoid activity; **mineralocorticoids** are those in which effects on Na^+ and K^+ excretion

predominate and **glucocorticoids** are those in which effects on glucose and protein metabolism predominate.

The details of steroid nomenclature and isomerism can be found elsewhere. However, it is pertinent to mention that the Greek letter Δ indicates a double bond and that the groups that lie above the plane of each of the steroid rings are indicated by the Greek letter β and a solid line (—OH), whereas those that lie below the plane are indicated by α and a dashed line (- - -OH). Thus, the C_{21} steroids secreted by the adrenal have a Δ^4-3-keto configuration in the A ring. In most naturally occurring adrenal steroids, 17-hydroxy groups are in the α configuration, whereas 3-, 11-, and 21-hydroxy groups are in the β configuration. The 18-aldehyde configuration of naturally occurring aldosterone is the D form. L-aldosterone is physiologically inactive.

SECRETED STEROIDS

Innumerable steroids have been isolated from adrenal tissue, but the only steroids normally secreted in physiologically significant amounts are the mineralocorticoid **aldosterone,** the glucocorticoids **cortisol** and **corticosterone,** and the androgens **dehydroepiandrosterone (DHEA)** and **androstenedione.** The structures of these steroids are shown in **Figure 19–7** and **Figure 19–8**. **Deoxycorticosterone** is a mineralocorticoid that is normally secreted in about the same amount as aldosterone **(Table 19–1)** but has only 3% of the mineralocorticoid activity of aldosterone. Its effect on mineral metabolism is usually negligible, but in diseases in which its secretion is increased, its effect can be appreciable. Most of the estrogens that are not formed in the ovaries are produced in the circulation from adrenal androstenedione. Almost all the dehydroepiandrosterone is secreted conjugated with sulfate, although most if not all of the other steroids are secreted in the free, unconjugated form **(Clinical Box 19–1)**.

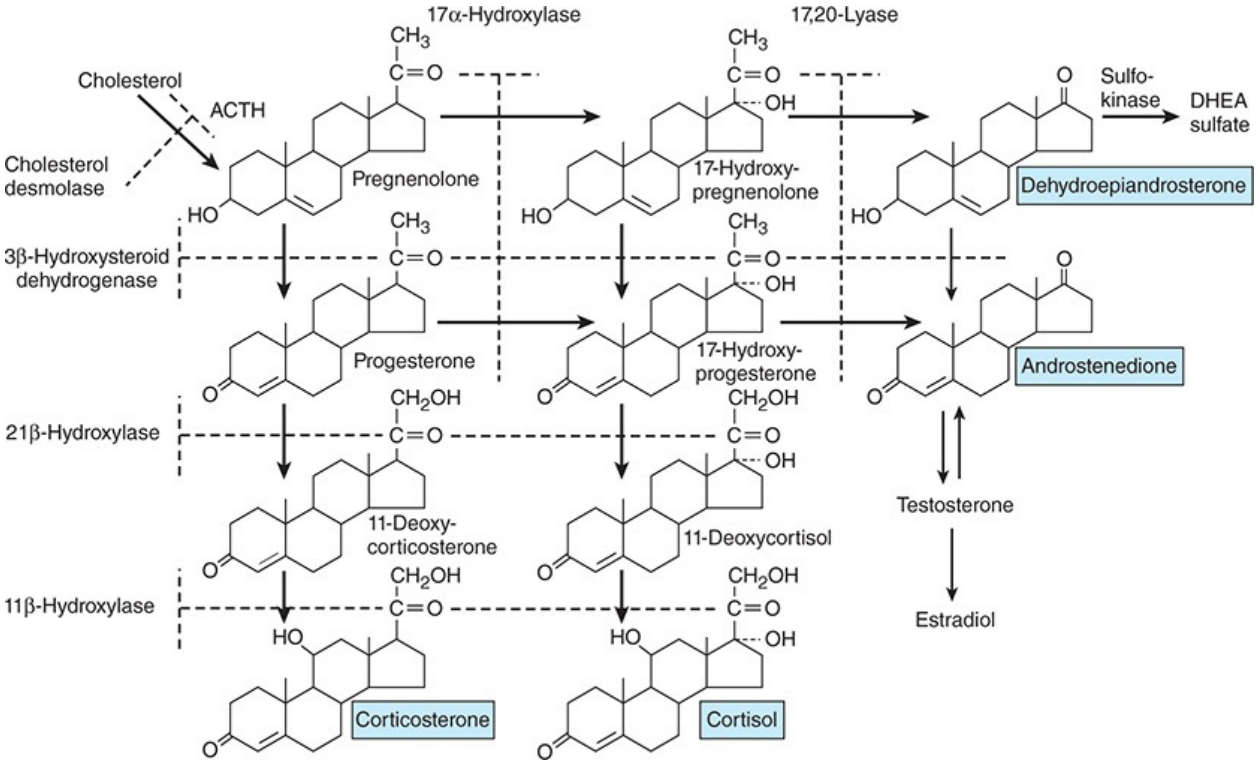


FIGURE 19–7 Outline of hormone biosynthesis in the zona fasciculata and zona reticularis of the adrenal cortex. The major secretory products are underlined. The enzymes for the reactions are shown on the left and at the top of the chart. When a particular enzyme is deficient, hormone production is blocked at the points indicated by the broken lines. ACTH, adrenocorticotropic hormone; DHEA, dehydroepiandrosterone.

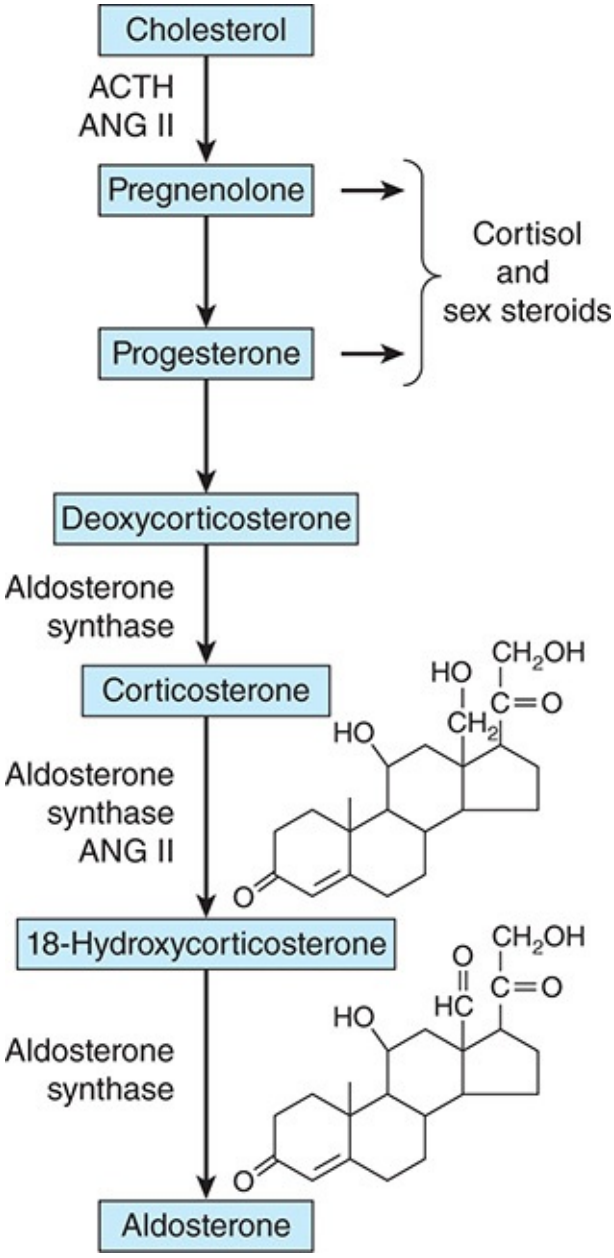


FIGURE 19–8 Hormone synthesis in the zona glomerulosa. The zona glomerulosa lacks 17α-hydroxylase activity, and only the zona glomerulosa can convert corticosterone to aldosterone because it is the only zone that normally contains aldosterone synthase. ACTH, adrenocorticotropic hormone; ANG II, angiotensin II.

TABLE 19–1 Principal adrenocortical hormones in adult humans.[a]

Name	Synonyms	Average Plasma Concentration (Free and Bound)[a] µg/dL)	Average Amount Secreted (mg/24 h)
Cortisol	Compound F, hydrocortisone	13.9	10
Corticosterone	Compound B	0.4	3
Aldosterone		0.0006	0.15
Deoxycorticosterone	DOC	0.0006	0.20
Dehydroepiandrosterone sulfate	DHEAS	175.0	20

[a]All plasma concentration values except DHEAS are fasting morning values after overnight recumbency.

CLINICAL BOX 19–1

Synthetic Steroids

As with many other naturally occurring substances, the activity of adrenocortical steroids can be increased by altering their structure. A number of synthetic steroids are available that have many times the activity of cortisol. The relative glucocorticoid and mineralocorticoid potencies of the natural steroids are compared with those of the synthetic steroids 9α-fluorocortisol, prednisolone, and dexamethasone in **Table 19–2**. The potency of dexamethasone is due to its high affinity for glucocorticoid receptors and its long half-life. Prednisolone also has a long half-life.

TABLE 19–2 Relative potencies of corticosteroids compared with cortisol.[a]

Steroid	Glucocorticoid Activity	Mineralocorticoid Activity
Cortisol	1.0	1.0
Corticosterone	0.3	15
Aldosterone	0.3	3000
Deoxycorticosterone	0.2	100
Costisone	0.7	0.8
Prednisolone	4	0.8
9α-Fluorocortisol	10	125
Dexamethasone	25	–0

[a]Values are approximations based on liver glycogen deposition or anti-inflammatory assays for glucocorticoid activity, and effect on urinary Na^+/K^+ or maintenance of adrenalectomized animals for mineralocorticoid activity. The last three steroids listed are synthetic compounds that do not occur naturally.

SPECIES DIFFERENCES

In all species from amphibia to humans, the major C_{21} steroid hormones secreted by adrenocortical tissue appear to be aldosterone, cortisol, and corticosterone, although the ratio of cortisol to corticosterone varies. Birds, mice, and rats secrete corticosterone almost exclusively; dogs secrete approximately equal amounts of the two glucocorticoids; and cats, sheep, monkeys, and humans secrete predominantly cortisol. In humans, the ratio of secreted cortisol to corticosterone is approximately 7:1.

STEROID BIOSYNTHESIS

The major paths by which the naturally occurring adrenocortical hormones are synthesized in the body are summarized in Figure 19–7 and 19–8. The precursor of all steroids is cholesterol. Some of the cholesterol is synthesized from acetate, but most of it is taken up from LDL in the circulation. LDL receptors are especially abundant in adrenocortical cells. The cholesterol is esterified and

stored in lipid droplets. **Cholesterol ester hydrolase** catalyzes the formation of free cholesterol in the lipid droplets **(Figure 19–9)**. The cholesterol is transported to mitochondria by a sterol carrier protein. In the mitochondria, it is converted to pregnenolone in a reaction catalyzed by an enzyme known as **cholesterol desmolase** or **side-chain cleavage enzyme.** This enzyme, like most of the enzymes involved in steroid biosynthesis, is a member of the cytochrome P450 superfamily and is also known as **P450scc** or **CYP11A1.** For convenience, the various names of the enzymes involved in adrenocortical steroid biosynthesis are summarized in **Table 19–3**.

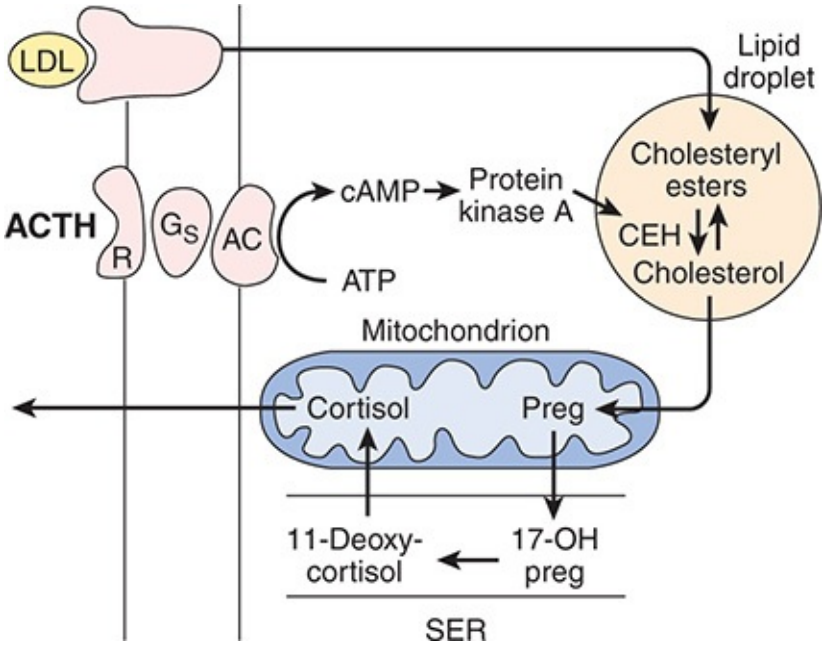


FIGURE 19–9 Mechanism of action of ACTH on cortisol-secreting cells in the inner two zones of the adrenal cortex. When ACTH binds to its receptor (R), adenylyl cyclase (AC) is activated via Gs. The resulting increase in cAMP activates protein kinase A, and the kinase phosphorylates cholesteryl ester hydrolase (CEH), increasing its activity. Consequently, more free cholesterol is formed and converted to pregnenolone. Note that in the subsequent steps in steroid biosynthesis, products are shuttled between the mitochondria and the smooth endoplasmic reticulum (SER). Corticosterone is also synthesized and secreted. ACTH, adrenocorticotropic hormone; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate.

TABLE 19–3 Nomenclature for adrenal steroidogenic enzymes and their location in adrenal cells.

Trivial Name	P450	CYP	Location
Cholesterol desmolase; side-chain cleavage enzyme	P450scc	CYP11A1	Mitochondria
3β-Hydroxysteroid dehydrogenase	. . .	. . .	SER
17α-Hydroxylase, 17,20-lyase	P450c17	CYP17	Mitochondria
21β-Hydroxylase	P450c21	CYP21A2	SER
11β-Hydroxylase	P450c11	CYP11B1	Mitochondria
Aldosterone synthase	P450c11AS	CYP11B2	Mitochondria

SER, smooth endoplasmic reticulum.

Pregnenolone moves to the smooth endoplasmic reticulum, where some of it is dehydrogenated to form progesterone in a reaction catalyzed by **3β-hydroxysteroid dehydrogenase.** This enzyme has a molecular weight of 46,000 and is not a cytochrome P450. It also catalyzes the conversion of 17α-hydroxypregnenolone to 17α-hydroxyprogesterone, and dehydroepiandrosterone to androstenedione (Figure 22–7) in the smooth endoplasmic reticulum. The 17α-hydroxypregnenolone and the 17α-hydroxyprogesterone are formed from pregnenolone and progesterone, respectively (Figure 19–7) by the action of **17α-hydroxylase.** This is another mitochondrial P450, and it is also known as **P450c17** or **CYP17.** Located in another part of the same enzyme is **17,20-lyase** activity that breaks the 17,20 bond, converting 17α-pregnenolone and 17α-progesterone to the C_{19} steroids dehydroepiandrosterone and androstenedione.

Hydroxylation of progesterone to 11-deoxycorticosterone and of 17α-hydroxyprogesterone to 11-deoxycortisol occurs in the smooth endoplasmic reticulum. These reactions are catalyzed by 21β-hydroxylase, a cytochrome P450 that is also known as **P450c21** or **CYP21A2.**

11-Deoxycorticosterone and the 11-deoxycortisol move back to the mitochondria, where they are 11-hydroxylated to form corticosterone and cortisol. These reactions occur in the zona fasciculata and zona reticularis and are catalyzed by 11β-hydroxylase, a cytochrome P450 also known as **P450c11** or

CYP11B1.

In the zona glomerulosa there is no 11β-hydroxylase, but a closely related enzyme called **aldosterone synthase** is present. This cytochrome P450 is 95% identical to 11β-hydroxylase and is also known as **P450c11AS** or **CYP11B2.** The genes that code CYP11B1 and CYP11B2 are both located on chromosome 8. However, aldosterone synthase is normally found only in the zona glomerulosa. The zona glomerulosa also lacks 17α-hydroxylase. This is why the zona glomerulosa makes aldosterone but fails to make cortisol or sex hormones.

Furthermore, subspecialization occurs within the inner two zones. The zona fasciculata has more 3β-hydroxysteroid dehydrogenase activity than the zona reticularis, and the zona reticularis has more of the cofactors required for the 17,20-lyase activity of 17α-hydroxylase. Therefore, the zona fasciculata makes more cortisol and corticosterone, and the zona reticularis makes more androgens. Most of the dehydroepiandrosterone that is formed is converted to dehydroepiandrosterone sulfate (DHEAS) by **adrenal sulfokinase,** and this enzyme is localized in the zona reticularis as well.

ACTION OF ACTH

ACTH binds to high-affinity receptors on the plasma membrane of adrenocortical cells. This activates adenylyl cyclase via G_s. The resulting reactions (Figure 19–9) lead to a prompt increase in the formation of pregnenolone and its derivatives, with secretion of the latter. Over longer periods, ACTH also increases the synthesis of the P450s involved in the synthesis of glucocorticoids.

ACTIONS OF ANGIOTENSIN II

Angiotensin II binds to AT_1 receptors (see Chapter 38) in the zona glomerulosa that act via a G-protein to activate phospholipase C. The resulting increase in protein kinase C fosters the conversion of cholesterol to pregnenolone (Figure 19–8) and facilitates the action of aldosterone synthase, resulting in increased secretion of aldosterone.

ENZYME DEFICIENCIES

The consequences of inhibiting any of the enzyme systems involved in steroid

biosynthesis can be predicted from Figures 19–7 and 19–8. Congenital defects in the enzymes lead to deficient cortisol secretion and the syndrome of **congenital adrenal hyperplasia.** The hyperplasia is due to increased ACTH secretion. Cholesterol desmolase deficiency is fatal in utero because it prevents the placenta from making the progesterone necessary for pregnancy to continue. A cause of severe congenital adrenal hyperplasia in newborns is a loss of function mutation of the gene for the **steroidogenic acute regulatory (StAR) protein.** This protein is essential in the adrenals and gonads but not in the placenta for the normal movement of cholesterol into the mitochondria to reach cholesterol desmolase, which is located on the matrix space side of the internal mitochondrial membrane (see Chapter 16, Figure 16-1). In its absence, only small amounts of steroids are formed. The degree of ACTH stimulation is marked, resulting eventually in accumulation of large numbers of lipoid droplets in the adrenal. For this reason, the condition is called **congenital lipoid adrenal hyperplasia.** Because androgens are not formed, female genitalia develop regardless of genetic sex (see Chapter 22). In 3β hydroxysteroid dehydrogenase deficiency, another rare condition, DHEA secretion is increased. This steroid is a weak androgen that can cause some masculinization in females with the disease, but it is not adequate to produce full masculinization of the genitalia in genetic males. Consequently, **hypospadias,** a condition where the opening of the urethra is on the underside of the penis rather than its tip, is common. In fully developed 17α-hydroxylase deficiency, a third rare condition due to a mutated gene for **CYP17,** no sex hormones are produced, so female external genitalia are present. However, the pathway leading to corticosterone and aldosterone is intact, and elevated levels of 11-deoxycorticosterone and other mineralocorticoids produce hypertension and hypokalemia. Cortisol is deficient, but this is partially compensated by the glucocorticoid activity of corticosterone.

Unlike the defects discussed in the preceding paragraph, 21β-hydroxylase deficiency is common, accounting for 90% or more of the enzyme deficiency cases. The 21β-hydroxylase gene, which is in the HLA complex of genes on the short arm of chromosome 6 (see Chapter 3) is one of the most polymorphic in the human genome. Mutations occur at many different sites in the gene, and the abnormalities that are produced therefore range from mild to severe. Production of cortisol and aldosterone are generally reduced, so ACTH secretion and consequently production of precursor steroids are increased. These steroids are converted to androgens, producing **virilization.** The characteristic pattern that develops in females in the absence of treatment is the **adrenogenital syndrome.** Masculization may not be marked until later in life and mild cases can be detected only by laboratory tests. In 75% of the cases, aldosterone deficiency

causes appreciable loss of Na^+ (**salt-losing form** of adrenal hyperplasia). The resulting hypovolemia can be severe.

In 11β-hydroxylase deficiency, virilization plus excess secretion of 11-deoxycortisol and 11-deoxycorticosterone take place. Because the former is an active mineralocorticoid, patients with this condition also have salt and water retention and, in two-thirds of the cases, hypertension (**hypertensive form** of congenital adrenal hyperplasia).

Glucocorticoid treatment is indicated in all of the virilizing forms of congenital adrenal hyperplasia because it repairs the glucocorticoid deficit and inhibits ACTH secretion, reducing the abnormal secretion of androgens and other steroids.

Expression of the cytochrome P450 enzymes responsible for steroid hormone biosynthesis depends on **steroid factor-1 (SF-1),** an orphan nuclear receptor. If *Ft2-F1*, the gene for SF-1, is knocked out, the gonads as well as adrenals fail to develop and additional abnormalities are present at the pituitary and hypothalamic level.

TRANSPORT, METABOLISM, & EXCRETION OF ADRENOCORTICAL HORMONES

GLUCOCORTICOID BINDING

Cortisol is bound in the circulation to an α globulin called **transcortin** or **corticosteroid-binding globulin (CBG).** A minor degree of binding to albumin also takes place. Corticosterone is similarly bound but to a lesser degree. The half-life of cortisol in the circulation is therefore longer (about 60–90 min) than that of corticosterone (50 min). Bound steroids are physiologically inactive (see Chapter 16). In addition, relatively little free cortisol and corticosterone are found in the urine because of protein binding.

The equilibrium between cortisol and its binding protein and the implications of binding in terms of tissue supplies and ACTH secretion are summarized in **Figure 19–10**. The bound cortisol functions as a circulating reservoir of hormone that keeps a supply of free cortisol available to the tissues. The relationship is similar to that of T_4 and its binding protein (see Chapter 20). At normal levels of total plasma cortisol (13.5 µg/dL or 375 nmol/L), very little free cortisol is present in the plasma, but the binding sites on CBG become saturated when the total plasma cortisol exceeds 20 µg/dL. At higher plasma levels,

binding to albumin increases, but the main increase is in the unbound fraction.

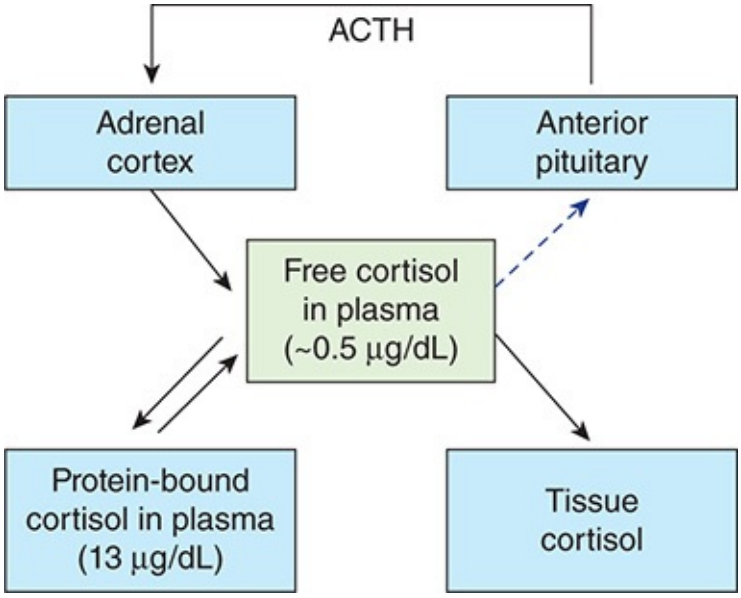


FIGURE 19–10 The interrelationships of free and bound cortisol. The dashed arrow indicates that cortisol inhibits ACTH secretion. The value for free cortisol is an approximation; in most studies, it is calculated by subtracting the protein-bound cortisol from the total plasma cortisol. ACTH, adrenocorticotropic hormone.

CBG is synthesized in the liver and its production is increased by estrogen. CBG levels are elevated during pregnancy and depressed in cirrhosis, nephrosis, and multiple myeloma. When the CBG level rises, more cortisol is bound, and initially the free cortisol level drops. This stimulates ACTH secretion, and more cortisol is secreted until a new equilibrium is reached at which the bound cortisol is elevated but the free cortisol is normal. Changes in the opposite direction occur when the CBG level falls. This explains why pregnant women have high total plasma cortisol levels without symptoms of glucocorticoid excess and, conversely, why some patients with nephrosis have low total plasma cortisol without symptoms of glucocorticoid deficiency.

METABOLISM & EXCRETION OF GLUCOCORTICOIDS

Cortisol is metabolized in the liver, which is the principal site of glucocorticoid catabolism. Most of the cortisol is reduced to dihydrocortisol and then to

tetrahydrocortisol, which is conjugated to glucuronic acid **(Figure 19–11)**. The glucuronyl transferase system responsible for this conversion also catalyzes the formation of the glucuronides of bilirubin (see Chapter 28) and a number of hormones and drugs. Competitive inhibition takes place between these substrates for the enzyme system.

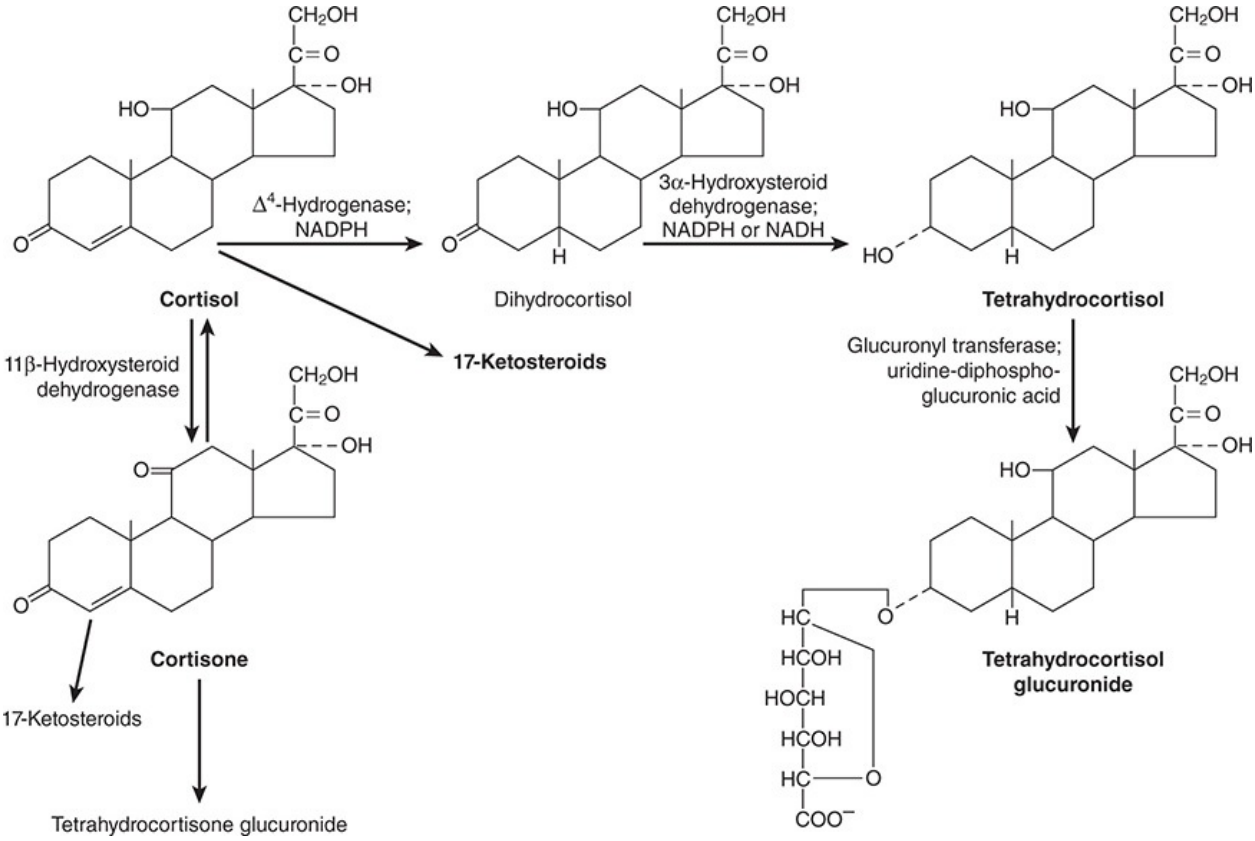


FIGURE 19–11 Outline of hepatic metabolism of cortisol.

CLINICAL BOX 19–2

Variations in the Rate of Hepatic Metabolism

The rate of hepatic inactivation of glucocorticoids is depressed in liver disease and, interestingly, during surgery and other stresses. Thus, in stressed humans, the plasma-free cortisol level rises higher than it does with maximal ACTH stimulation in the absence of stress.

The liver and other tissues contain the enzyme 11β hydroxysteroid

dehydrogenase. There are at least two forms of this enzyme. Type 1 catalyzes the conversion of cortisol to cortisone and the reverse reaction, though it functions primarily as a reductase, forming cortisol from corticosterone. Type 2 catalyzes almost exclusively the one-way conversion of cortisol to cortisone. Cortisone is an active glucocorticoid because it is converted to cortisol, and it is well known because of its extensive use in medicine. It is not secreted in appreciable quantities by the adrenal glands. Little, if any, of the cortisone formed in the liver enters the circulation, because it is promptly reduced and conjugated to form tetrahydrocortisone glucuronide. The tetrahydroglucuronide derivatives ("conjugates") of cortisol and corticosterone are freely soluble. They enter the circulation, where they do not become bound to protein. They are rapidly excreted in the urine.

About 10% of the secreted cortisol is converted in the liver to the 17-ketosteroid derivatives of cortisol and cortisone. The ketosteroids are conjugated for the most part to sulfate and then excreted in the urine. Other metabolites, including 20-hydroxy derivatives, are formed. There is an enterohepatic circulation of glucocorticoids and about 15% of the secreted cortisol is excreted in the stool. The metabolism of corticosterone is similar to that of cortisol, except that it does not form a 17-ketosteroid derivative **(see Clinical Box 19–2)**.

ALDOSTERONE

Aldosterone is bound to protein to only a slight extent, and its half-life is short (about 20 min). The amount secreted is small (Table 19–1), and the total plasma aldosterone level in humans is normally about 0.006 µg/dL (0.17 nmol/L), compared with a cortisol level (bound and free) of about 13.5 µg/dL (375 nmol/L). Much of the aldosterone is converted in the liver to the tetrahydroglucuronide derivative, but some is changed in the liver and in the kidneys to an 18-glucuronide. This glucuronide, which is unlike the breakdown products of other steroids, is converted to free aldosterone by hydrolysis at pH 1.0, and it is therefore often referred to as the "acid-labile conjugate." Less than 1% of the secreted aldosterone appears in the urine in the free form. Another 5% is in the form of the acid-labile conjugate, and up to 40% is in the form of the tetrahydroglucuronide.

17-KETOSTEROIDS

The major adrenal androgen is the 17-ketosteroid dehydroepiandrosterone, although androstenedione is also secreted. The 11-hydroxy derivative of androstenedione and the 17-ketosteroids formed from cortisol and cortisone by side chain cleavage in the liver are the only 17-ketosteroids that have an =O or an —OH group in the 11 position (“11-oxy-17-ketosteroids”). Testosterone is also converted to a 17-ketosteroid. Because the daily 17-ketosteroid excretion in normal adults is 15 mg in men and 10 mg in women, about two-thirds of the urinary ketosteroids in men are secreted by the adrenal or formed from cortisol in the liver and about one-third are of testicular origin.

Etiocholanolone, one of the metabolites of the adrenal androgens and testosterone, can cause fever when it is unconjugated (see Chapter 17). Certain individuals have episodic bouts of fever due to periodic accumulation in the blood of unconjugated etiocholanolone (“etiocholanolone fever”).

EFFECTS OF ADRENAL ANDROGENS & ESTROGENS

ANDROGENS

Androgens are the hormones that exert masculinizing effects and they promote protein anabolism and growth (see Chapter 23). Testosterone from the testes is the most active androgen and the adrenal androgens have less than 20% of its activity. Secretion of the adrenal androgens is controlled acutely by ACTH and not by gonadotropins. However, the concentration of DHEAS increases until it peaks at about 225 mg/dL in the early 20s, and then falls to very low values in old age **(Figure 19–12)**. These long-term changes are not due to changes in ACTH secretion and appear to be due instead to a rise and then a gradual fall in the lyase activity of 17α-hydroxylase.

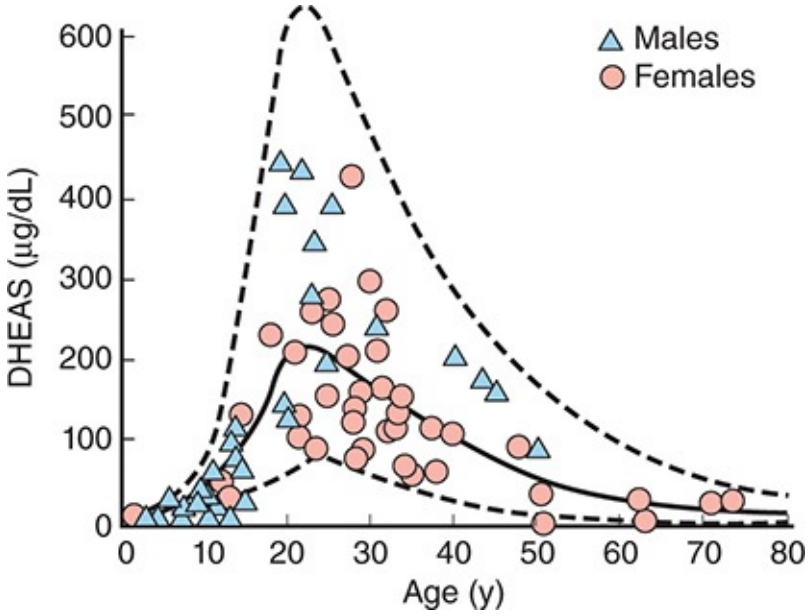


FIGURE 19–12 Change in serum dehydroepiandrosterone sulfate (DHEAS) with age. The middle line is the mean, and the dashed lines identify ±1.96 standard deviations. (Reproduced, with permission, from Smith MR, et al: A radioimmunoassay for the estimation of serum dehydroepiandrosterone sulfate in normal and pathological sera. Clin Chim Acta 1975; Nov 15; 65(1):5–13.)

All but about 0.3% of the circulating DHEA is conjugated to sulfate (DHEAS). The secretion of adrenal androgens is nearly as great in castrated males and females as it is in normal males, so it is clear that these hormones exert very little masculinizing effect when secreted in normal amounts. However, they can produce appreciable masculinization when secreted in excessive amounts. In adult males, excess adrenal androgens merely accentuate existing characteristics, but in prepubertal boys they can cause precocious development of the secondary sex characteristics without testicular growth **(precocious pseudopuberty).** In females they cause female pseudo-hermaphroditism and the adrenogenital syndrome. Some health practitioners recommend injections of dehydroepiandrosterone to combat the effects of aging (see Chapter 1), but results to date are controversial at best.

ESTROGENS

The adrenal androgen androstenedione is converted to testosterone and to estrogens (aromatized) in fat and other peripheral tissues. This is an important source of estrogens in postmenopausal women and men (see Chapters 22 and

23).

PHYSIOLOGIC EFFECTS OF GLUCOCORTICOIDS

ADRENAL INSUFFICIENCY

In untreated adrenal insufficiency, Na^+ loss and shock occurs due to the lack of mineralocorticoid activity, as well as abnormalities of water, carbohydrate, protein, and fat metabolism due to the lack of glucocorticoids. These metabolic abnormalities are eventually fatal despite mineralocorticoid treatment. Small amounts of glucocorticoids correct the metabolic abnormalities, in part directly and in part by permitting other reactions to occur. It is important to separate these physiologic actions of glucocorticoids from the quite different effects produced by large amounts of the hormones.

MECHANISM OF ACTION

The multiple effects of glucocorticoids are triggered by binding to glucocorticoid receptors, and the steroid–receptor complexes act as transcription factors that promote the transcription of certain segments of DNA (see Chapter 1). This, in turn, leads via the appropriate mRNAs to synthesis of enzymes that alter cell function. In addition, it seems likely that glucocorticoids have nongenomic actions.

EFFECTS ON INTERMEDIARY METABOLISM

The actions of glucocorticoids on the intermediary metabolism of carbohydrate, protein, and fat are discussed in Chapter 24. They include increased protein catabolism and increased hepatic glycogenesis and gluconeogenesis. Glucose- 6-phosphatase activity is increased, and the plasma glucose level rises. Glucocorticoids exert an anti-insulin action in peripheral tissues and make diabetes worse. However, the brain and the heart are spared, so the increase in plasma glucose provides extra glucose to these vital organs. In diabetics, glucocorticoids raise plasma lipid levels and increase ketone body formation, but in normal individuals, the increase in insulin secretion provoked by the rise in plasma glucose obscures these actions. In adrenal insufficiency, the plasma

glucose level is normal as long as an adequate caloric intake is maintained, but fasting causes hypoglycemia that can be fatal. The adrenal cortex is not essential for the ketogenic response to fasting.

PERMISSIVE ACTION

Small amounts of glucocorticoids must be present for a number of metabolic reactions to occur, although the glucocorticoids do not produce the reactions by themselves. This effect is called their **permissive action.** Permissive effects include the requirement for glucocorticoids to be present for glucagon and catecholamines to exert their calorigenic effects (see above and Chapter 24), for catecholamines to exert their lipolytic effects, and for catecholamines to produce pressor responses and bronchodilation.

EFFECTS ON ACTH SECRETION

Glucocorticoids inhibit ACTH secretion, which represents a negative feedback response on the pituitary. ACTH secretion is increased in adrenalectomized animals. The consequences of the negative feedback action of cortisol on ACTH secretion are discussed below in the section on regulation of glucocorticoid secretion.

VASCULAR REACTIVITY

In adrenally insufficient animals, vascular smooth muscle becomes unresponsive to norepinephrine and epinephrine. The capillaries dilate and, terminally, become permeable to colloidal dyes. Failure to respond to the norepinephrine liberated at noradrenergic nerve endings probably impairs vascular compensation for the hypovolemia of adrenal insufficiency and promotes vascular collapse. Glucocorticoids restore vascular reactivity.

EFFECTS ON THE NERVOUS SYSTEM

Changes in the nervous system in adrenal insufficiency that are reversed only by glucocorticoids include the appearance of electroencephalographic waves slower than the normal β rhythm, and personality changes. The latter, which are mild, include irritability, apprehension, and inability to concentrate.

EFFECTS ON WATER METABOLISM

Adrenal insufficiency is characterized by an inability to excrete a water load, causing the possibility of water intoxication. Only glucocorticoids repair this deficit. In patients with adrenal insufficiency who have not received glucocorticoids, glucose infusion may cause high fever (“glucose fever”) followed by collapse and death. Presumably, the glucose is metabolized, the water dilutes the plasma, and the resultant osmotic gradient between the plasma and the cells causes the cells of the thermoregulatory centers in the hypothalamus to swell to such an extent that their function is disrupted.

The cause of defective water excretion in adrenal insufficiency is unsettled. Plasma vasopressin levels are elevated in adrenal insufficiency and reduced by glucocorticoid treatment. The glomerular filtration rate is low, and this probably contributes to the reduction in water excretion. The selective effect of glucocorticoids on the abnormal water excretion is consistent with this possibility, because even though the mineralocorticoids improve filtration by restoring plasma volume, the glucocorticoids raise the glomerular filtration rate to a much greater degree.

EFFECTS ON THE BLOOD CELLS & LYMPHATIC ORGANS

Glucocorticoids decrease the number of circulating eosinophils by increasing their sequestration in the spleen and lungs. Glucocorticoids also lower the number of basophils in the circulation and increase the number of neutrophils, platelets, and red blood cells (**Table 19-4**).

TABLE 19–4 Typical effects of cortisol on the white and red blood cell counts in humans (cells/μL).

Cell	Normal	Cortisol-Treated
White blood cells		
Total	9000	10,000
PMNs	5760	8330
Lymphocytes	2370	1080
Eosinophils	270	20
Basophils	60	30
Monocytes	450	540
Red blood cells	5 million	5.2 million

Glucocorticoids decrease the circulating lymphocyte count and the size of the lymph nodes and thymus by inhibiting lymphocyte mitotic activity. They reduce secretion of cytokines by inhibiting the effect of NF-κB on the nucleus. The reduced secretion of the cytokine IL-2 leads to reduced proliferation of lymphocytes (see Chapter 3), and these cells undergo apoptosis.

RESISTANCE TO STRESS

The term **stress** as used in biology has been defined as any change in the environment that changes or threatens to change an existing optimal steady state. Most, if not all, of these stresses activate processes at the molecular, cellular, or systemic level that tend to restore the previous state, that is, they are homeostatic reactions. Some, but not all, of the stresses stimulate ACTH secretion. The increase in ACTH secretion is essential for survival when the stress is severe. If animals are then hypophysectomized, or adrenalectomized but treated with maintenance doses of glucocorticoids, they die when exposed to the same stress.

The reason an elevated circulating ACTH, and hence glucocorticoid level, is essential for resisting stress remains for the most part unknown. Most of the stressful stimuli that increase ACTH secretion also activate the sympathetic nervous system, and part of the function of circulating glucocorticoids may be maintenance of vascular reactivity to catecholamines. Glucocorticoids are also necessary for the catecholamines to exert their full FFA-mobilizing action, and the FFAs are an important emergency energy supply. However, sympathectomized animals tolerate a variety of stresses with relative impunity. Another theory holds that glucocorticoids prevent other stress-induced changes

from becoming excessive. At present, all that can be said is that stress causes increases in plasma glucocorticoids to high “pharmacologic” levels that in the short run are life-saving.

It should also be noted that the increase in ACTH, which is beneficial in the short term, becomes harmful and disruptive in the long term, causing among other things, the abnormalities of Cushing syndrome.

PHARMACOLOGIC & PATHOLOGIC EFFECTS OF GLUCOCORTICOIDS

CUSHING SYNDROME

The clinical picture produced by prolonged increases in plasma glucocorticoids was described by Harvey Cushing and is called **Cushing syndrome** **(Figure 19–13)**. It may be **ACTH-independent** or **ACTH-dependent.** The causes of ACTH-independent Cushing syndrome include glucocorticoid-secreting adrenal tumors, adrenal hyperplasia, and prolonged administration of exogenous glucocorticoids for diseases such as rheumatoid arthritis. Rare but interesting ACTH-independent cases have been reported in which adrenocortical cells abnormally express receptors for gastric inhibitory polypeptide (GIP) (see Chapter 25), vasopressin (see Chapter 38), β-adrenergic agonists, IL-1, or gonadotropin-releasing hormone (GnRH; see Chapter 22), causing these peptides to increase glucocorticoid secretion. The causes of ACTH-dependent Cushing syndrome include ACTH-secreting tumors of the anterior pituitary gland and tumors of other organs, usually the lungs, that secrete ACTH (ectopic ACTH syndrome) or corticotropin releasing hormone (CRH). Cushing syndrome due to anterior pituitary tumors is often called **Cushing disease** because these tumors were the cause of the cases described by Cushing. However, it is confusing to speak of Cushing disease as a subtype of Cushing syndrome, and the distinction seems to be of little more than historical value.

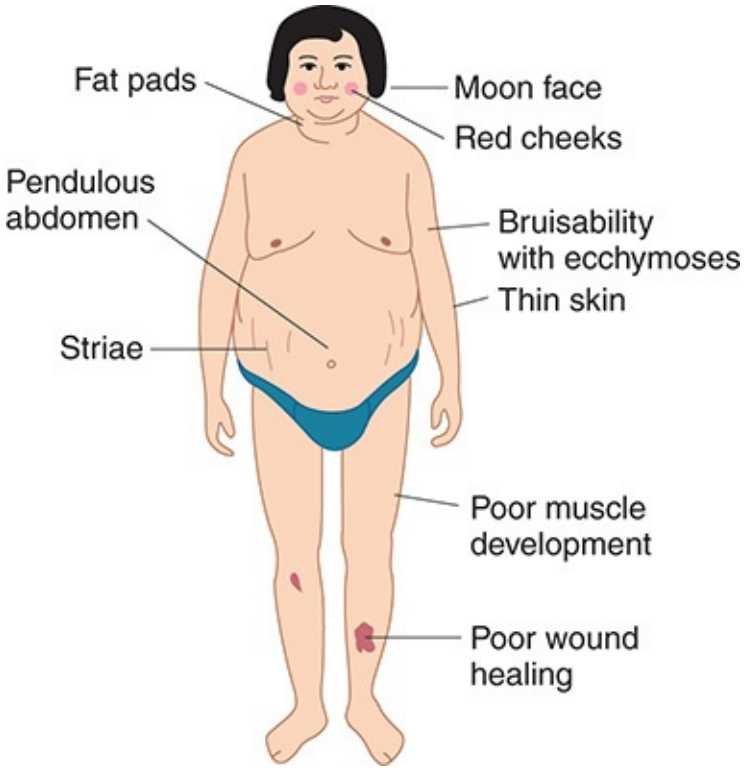


FIGURE 19–13 Typical findings in Cushing syndrome. (Reproduced with permission from Forsham PH, Di Raimondo VC: *Traumatic Medicine and Surgery for the Attorney*. Butterworth; 1960.)

Patients with Cushing syndrome are protein-depleted as a result of excess protein catabolism. The skin and subcutaneous tissues are therefore thin and the muscles are poorly developed. Wounds heal poorly, and minor injuries cause bruises and ecchymoses. The hair is thin and scraggly. Many patients with the disease have some increase in facial hair and acne, but this is caused by the increased secretion of adrenal androgens and often accompanies the increase in glucocorticoid secretion.

Body fat is redistributed in a characteristic way. The extremities are thin, but fat collects in the abdominal wall, face, and upper back, where it produces a "buffalo hump." As the thin skin of the abdomen is stretched by the increased subcutaneous fat depots, the subdermal tissues rupture to form prominent reddish purple **striae.** These scars are seen normally whenever a rapid stretching of skin occurs, but in normal individuals the striae are usually inconspicuous and lack the intense purplish color.

Many of the amino acids liberated from catabolized proteins are converted into glucose in the liver and the resultant hyperglycemia and decreased peripheral utilization of glucose may be sufficient to precipitate insulin-resistant

diabetes mellitus, especially in patients genetically predisposed to diabetes. Hyperlipidemia and ketosis are associated with the diabetes, but acidosis is usually not severe.

The glucocorticoids are present in such large amounts in Cushing syndrome that they may exert a significant mineralocorticoid action. Deoxycorticosterone secretion is also elevated in cases due to ACTH hypersecretion. The salt and water retention plus the facial obesity cause the characteristic plethoric, rounded "moon-faced" appearance, and there may be significant K^+ depletion and weakness. About 85% of patients with Cushing syndrome are hypertensive. The hypertension may be due to increased deoxycorticosterone secretion, increased angiotensinogen secretion, or a direct glucocorticoid effect on blood vessels (see Chapter 32).

Glucocorticoid excess leads to bone dissolution by decreasing bone formation and increasing bone resorption. This leads to **osteoporosis,** a loss of bone mass that leads eventually to collapse of vertebral bodies and other fractures. The mechanisms by which glucocorticoids produce their effects on bone are discussed in Chapter 21.

Glucocorticoids in excess accelerate the basic electroencephalographic rhythms and produce mental aberrations ranging from increased appetite, insomnia, and euphoria to frank toxic psychoses. As noted above, glucocorticoid deficiency is also associated with mental symptoms, but the symptoms produced by glucocorticoid excess are more severe.

ANTI-INFLAMMATORY & ANTI-ALLERGIC EFFECTS OF GLUCOCORTICOIDS

Glucocorticoids inhibit the inflammatory response to tissue injury. The glucocorticoids also suppress manifestations of allergic disease that are due to the release of histamine from mast cells and basophils. Both of these effects require high levels of circulating glucocorticoids and cannot be produced by administering steroids without producing the other manifestations of glucocorticoid excess. Furthermore, large doses of exogenous glucocorticoids inhibit ACTH secretion to the point that severe adrenal insufficiency can be a dangerous problem when therapy is stopped. However, local administration of glucocorticoids, for example, by injection into an inflamed joint or near an irritated nerve, produces a high local concentration of the steroid, often without enough systemic absorption to cause serious side effects.

The actions of glucocorticoids in patients with bacterial infections are dramatic but dangerous. For example, in pneumococcal pneumonia or active tuberculosis, the febrile reaction, the toxicity, and the lung symptoms disappear, but unless antibiotics are given at the same time, the bacteria spread throughout the body. It is important to remember that the symptoms are the warning that disease is present; when these symptoms are masked by treatment with glucocorticoids, there may be serious and even fatal delays in diagnosis and the institution of treatment with antimicrobial drugs.

The role of NF-κB in the anti-inflammatory and anti-allergic effects of glucocorticoids has been mentioned above and is discussed in Chapter 3. An additional action that combats local inflammation is inhibition of phospholipase A_2. This reduces the release of arachidonic acid from tissue phospholipids and consequently reduces the formation of leukotrienes, thromboxanes, prostaglandins, and prostacyclin (see Chapter 32).

OTHER EFFECTS

Large doses of glucocorticoids inhibit growth, decrease growth hormone secretion (see Chapter 18), induce PNMT, and decrease thyroid-stimulating hormone secretion. During fetal life, glucocorticoids accelerate the maturation of surfactant in the lungs (see Chapter 34).

REGULATION OF GLUCOCORTICOID SECRETION

ROLE OF ACTH

Both basal secretion of glucocorticoids and the increased secretion provoked by stress depend on ACTH from the anterior pituitary (Chapter 18). Angiotensin II also stimulates the adrenal cortex, but its effect is mainly on aldosterone secretion. Large doses of a number of other naturally occurring substances, including vasopressin, serotonin, and vasoactive intestinal polypeptide (VIP), are capable of stimulating the adrenal directly, but there is no evidence that these agents play any role in the physiologic regulation of glucocorticoid secretion.

CHEMISTRY & METABOLISM OF ACTH

ACTH is a single-chain polypeptide containing 39 amino acids. Its origin from proopiomelanocortin (POMC) in the pituitary is discussed in Chapter 18. The first 23 amino acids in the chain generally constitute the active "core" of the molecule. Amino acids 24–39 constitute a "tail" that stabilizes the molecule and varies slightly in composition from species to species. The ACTHs that have been isolated are generally active in all species but antigenic in heterologous species.

ACTH is inactivated in blood in vitro more slowly than in vivo; its half-life in the circulation in humans is about 10 min. A large part of an injected dose of ACTH is found in the kidneys, but neither nephrectomy nor evisceration appreciably enhances its in vivo activity, and the site of its inactivation is not known.

EFFECT OF ACTH ON THE ADRENAL

After hypophysectomy, glucocorticoid synthesis and output decline within 1 h to very low levels, although some hormone is still secreted. Within a short time after an injection of ACTH (in dogs, less than 2 min), glucocorticoid output is increased. With low doses of ACTH, the relationship between the log of the dose and the increase in glucocorticoid secretion is linear. However, the maximal rate at which glucocorticoids can be secreted is rapidly reached, and this "ceiling on output" also exists in humans. The effects of ACTH on adrenal morphology and the mechanism by which it increases steroid secretion have been discussed above.

ADRENAL RESPONSIVENESS

ACTH not only produces prompt increases in glucocorticoid secretion but also increases the sensitivity of the adrenal to subsequent doses of ACTH. Conversely, single doses of ACTH do not increase glucocorticoid secretion in chronically hypophysectomized animals and patients with hypopituitarism, and repeated injections or prolonged infusions of ACTH are necessary to restore normal adrenal responses to ACTH. Decreased responsiveness is also produced by doses of glucocorticoids that inhibit ACTH secretion. The decreased adrenal responsiveness to ACTH is detectable within 24 h after hypophysectomy and increases progressively with time **(Figure 19–14)**. It is marked when the adrenal is atrophic but develops before visible changes occur in adrenal size or

morphology.

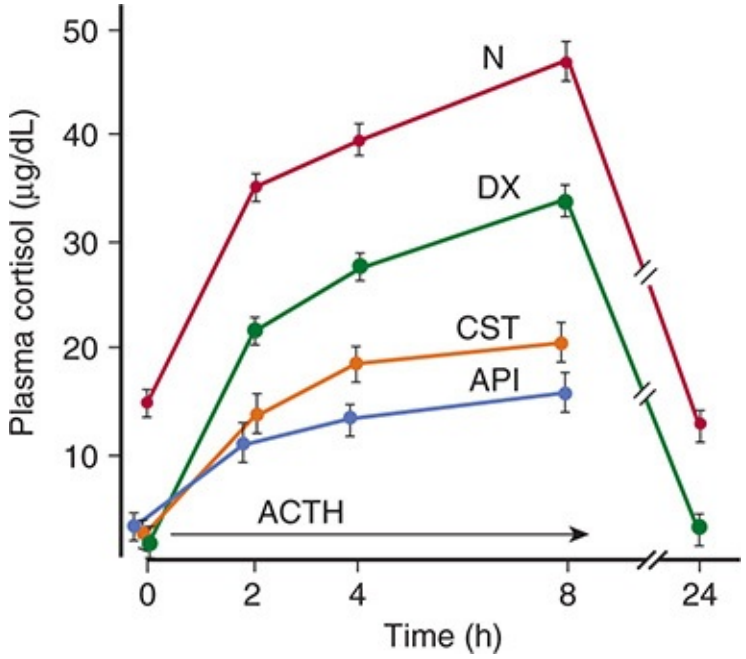


FIGURE 19–14 Loss of ACTH responsiveness when ACTH secretion is decreased in humans. The 1- to 24-amino-acid sequence of ACTH was infused intravenously (IV) in a dose of 250 µg over 8 h. CST, long-term corticosteroid therapy; DX, dexamethasone 0.75 mg every 8 h for 3 days; API, anterior pituitary insufficiency; N, normal subjects. (Reproduced with permission from Kolanowski J, et al: Adrenocortical response upon repeated stimulation with corticotropin in patients lacking endogenous corticotropin secretion. Acta Endocrinol [Kbh] 1977;85:595.)

CIRCADIAN RHYTHM

ACTH is secreted in irregular bursts throughout the day and plasma cortisol tends to rise and fall in response to these bursts **(Figure 19–15)**. In humans, the bursts are most frequent in the early morning, and about 75% of the daily production of cortisol occurs between 4:00 AM and 10:00 AM. The bursts are least frequent in the evening. This **diurnal (circadian) rhythm** in ACTH secretion is present in patients with adrenal insufficiency receiving constant doses of glucocorticoids. It is not due to the stress of getting up in the morning, traumatic as that may be, because the increased ACTH secretion occurs before waking up. If the “day” is lengthened experimentally to more than 24 h, that is, if the individual is isolated and the day’s activities are spread over more than 24

h, the adrenal cycle also lengthens, but the increase in ACTH secretion still occurs during the period of sleep. The biologic clock responsible for the diurnal ACTH rhythm is located in the suprachiasmatic nuclei of the hypothalamus (see Chapter 17).

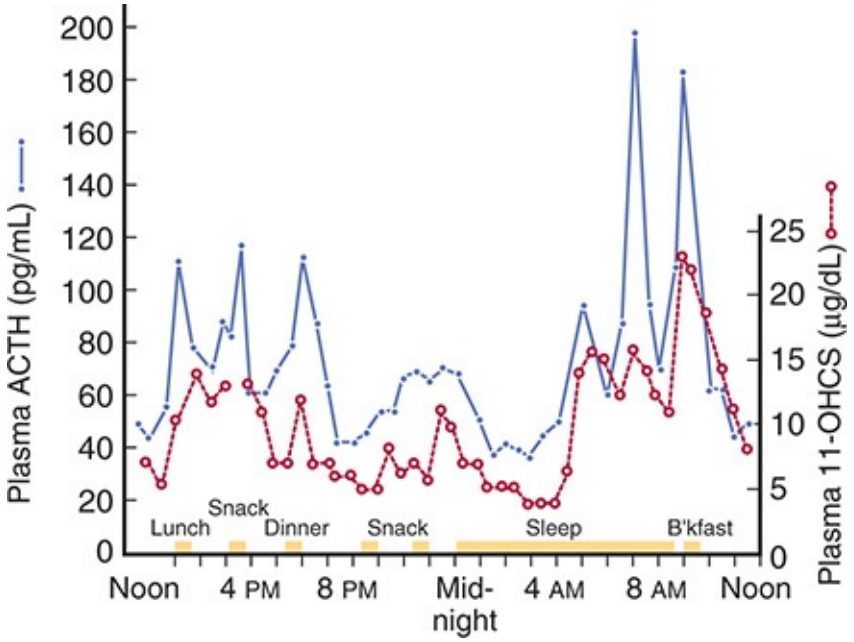


FIGURE 19–15 Fluctuations in plasma ACTH and glucocorticoids throughout the day in a normal girl (age 16). The ACTH was measured by immunoassay and the glucocorticoids as 11-oxysteroids (11-OHCS). Note the greater ACTH and glucocorticoid rises in the morning, before awakening. ACTH, adrenocorticotropic hormone. (Reproduced, with permission, from Krieger DT, et al: Characterization of the normal temporal pattern of plasma corticosteroid levels. J Clin Endocrinol Metab 1971; Feb; 32(2):266–284.)

THE RESPONSE TO STRESS

The morning plasma ACTH concentration in a healthy resting human is about 25 pg/mL (5.5 pmol/L). ACTH and cortisol values in various abnormal conditions are summarized in **Figure 19–16**. During severe stress, the amount of ACTH secreted exceeds the amount necessary to produce maximal glucocorticoid output. However, prolonged exposure to ACTH in conditions such as the ectopic ACTH syndrome increases the adrenal maximum.

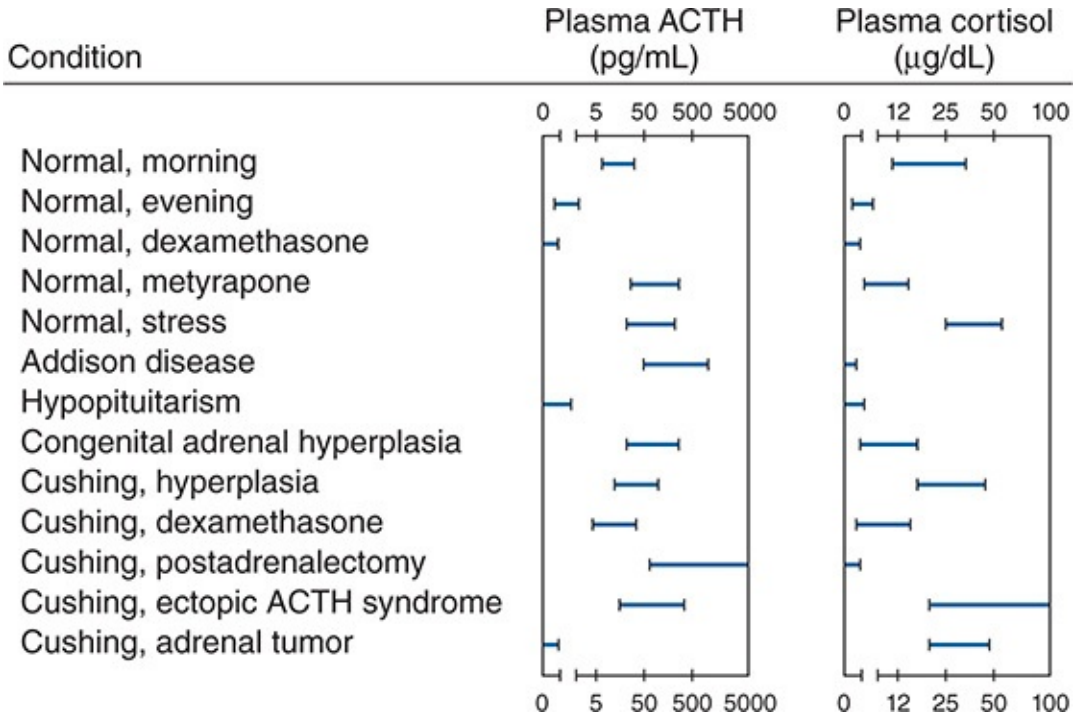


FIGURE 19–16 Plasma concentrations of ACTH and cortisol in various clinical states. ACTH, adrenocorticotropic hormone. (Reproduced with permission from Williams RH [editor]: *Textbook of Endocrinology*, 5th ed. Saunders, 1974.)

Increases in ACTH secretion to meet emergency situations are mediated almost exclusively through the hypothalamus via release of CRH. This polypeptide is produced by neurons in the paraventricular nuclei. It is secreted in the median eminence and transported in the portal-hypophysial vessels to the anterior pituitary, where it stimulates ACTH secretion (see Chapter 18). If the median eminence is destroyed, increased secretion in response to many different stresses is blocked. Afferent nerve pathways from many parts of the brain converge on the paraventricular nuclei. Fibers from the amygdaloid nuclei mediate responses to emotional stresses, and fear, anxiety, and apprehension cause marked increases in ACTH secretion. Input from the suprachiasmatic nuclei provides the drive for the diurnal rhythm. Impulses ascending to the hypothalamus via the nociceptive pathways and the reticular formation trigger increased ACTH secretion in response to injury (Figure 19–16). The baroreceptors exert an inhibitory input via the nucleus of the tractus solitarius.

GLUCOCORTICOID FEEDBACK

Free glucocorticoids inhibit ACTH secretion, and the degree of pituitary inhibition is proportional to the circulating glucocorticoid level. The inhibitory effect is exerted at both the pituitary and the hypothalamic levels. The inhibition is due primarily to an action on DNA, and maximal inhibition takes several hours to develop, although more rapid "fast feedback" also occurs. The ACTH-inhibiting activity of the various steroids parallels their glucocorticoid potency. A drop in resting corticoid levels stimulates ACTH secretion, and in chronic adrenal insufficiency the rate of ACTH synthesis and secretion is markedly increased.

Thus, the rate of ACTH secretion is determined by two opposing forces: the sum of the neural and possibly other stimuli converging through the hypothalamus to increase ACTH secretion, and the magnitude of the braking action of glucocorticoids on ACTH secretion, which is proportional to their level in the circulating blood **(Figure 19–17)**.

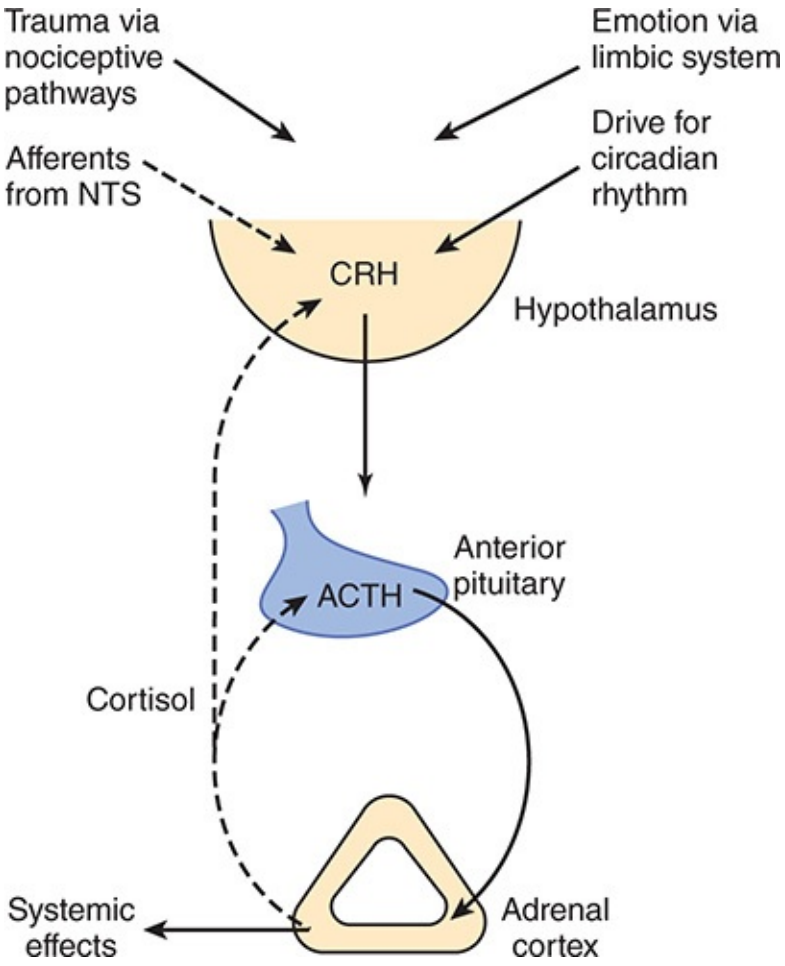


FIGURE 19–17 Feedback control of the secretion of cortisol and other

glucocorticoids via the hypothalamic–pituitary–adrenal axis. The dashed arrows indicate inhibitory effects and the solid arrows indicate stimulating effects. ACTH, adrenocorticotropic hormone; CRH, corticotropin-releasing hormone; NTS, nucleus tractus solitarius.

The dangers involved when prolonged treatment with anti-inflammatory doses of glucocorticoids is stopped deserve emphasis. Not only is the adrenal atrophic and unresponsive after such treatment, but even if its responsiveness is restored by injecting ACTH, the pituitary may be unable to secrete normal amounts of ACTH for as long as a month. The cause of the deficiency is presumably diminished ACTH synthesis. Thereafter, ACTH secretion slowly increases to supranormal levels. These in turn stimulate the adrenal, and glucocorticoid output rises, with feedback inhibition gradually reducing the elevated ACTH levels to normal **(Figure 19–18)**. The complications of sudden cessation of steroid therapy can usually be avoided by slowly decreasing the steroid dose over a long period of time.

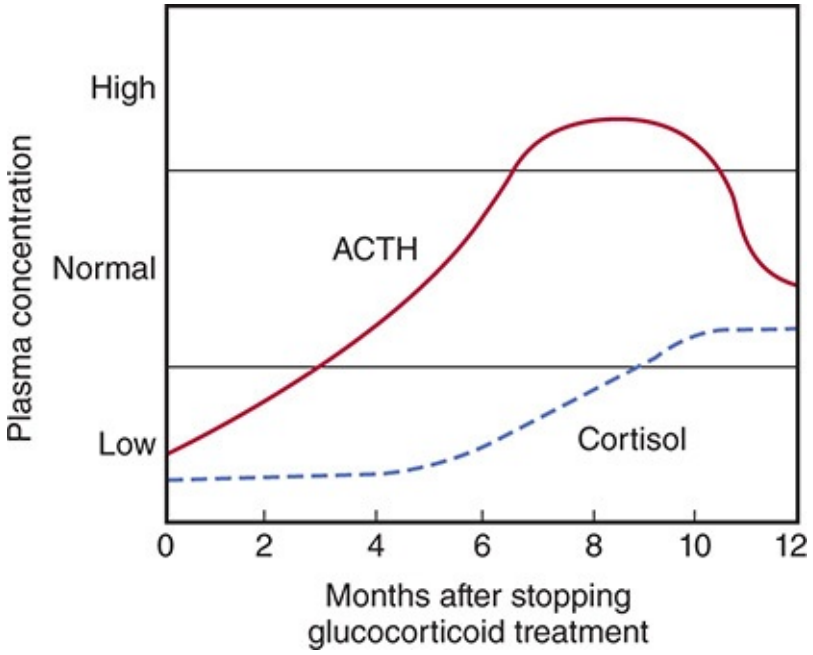


FIGURE 19–18 Pattern of plasma ACTH and cortisol values in patients recovering from prior long-term daily treatment with large doses of glucocorticoids. ACTH, adrenocorticotropic hormone. (Used with permission of R Ney.)

EFFECTS OF MINERALOCORTICOIDS

ACTIONS

Aldosterone and other steroids with mineralocorticoid activity increase the reabsorption of Na^+ from the urine, sweat, saliva, and the contents of the colon. Thus, mineralocorticoids cause retention of Na^+ in the ECF. This expands ECF volume. In the kidneys, they act primarily on the **principal cells (P cells)** of the collecting ducts (see Chapter 37). Under the influence of aldosterone, increased amounts of Na^+ are in effect exchanged for K^+ and H^+ in the renal tubules, producing a K^+ diuresis **(Figure 19–19)** and an increase in urine acidity.

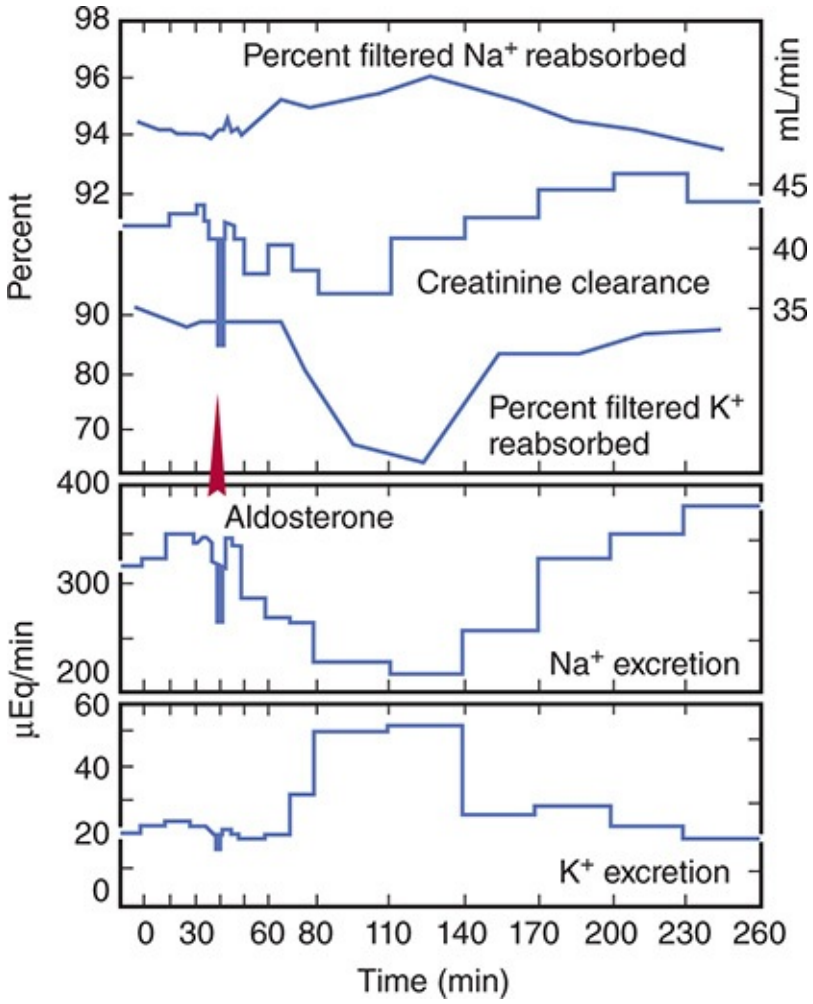


FIGURE 19–19 Effect of aldosterone (5 µg as a single dose injected into the aorta) on electrolyte excretion in an adrenalectomized dog. The scale for creatinine clearance is on the right.

MECHANISM OF ACTION

Like many other steroids, aldosterone binds to a cytoplasmic receptor, and the receptor–hormone complex moves to the nucleus where it alters the transcription of mRNAs. This in turn increases the production of proteins that alter cell function. The aldosterone-stimulated proteins have two effects—a rapid effect, to increase the activity of epithelial sodium channels (ENaCs) by increasing the insertion of these channels into the cell membrane from a cytoplasmic pool; and a slower effect to increase the synthesis of ENaCs. Among the genes activated by aldosterone is the gene for **serum- and glucocorticoid-regulated kinase (sgk),** a serine-threonine protein kinase. The gene for sgk is an early response gene, and sgk increases ENaC activity. Aldosterone also increases the mRNAs for the three subunits that make up ENaCs. The fact that sgk is activated by glucocorticoids as well as aldosterone is not a problem because glucocorticoids are inactivated at mineralocorticoid receptor sites. However, aldosterone activates the genes for other proteins in addition to sgk and ENaCs and inhibits others. Therefore, the exact mechanism by which aldosterone-induced proteins increase Na^+ reabsorption is still unsettled.

Evidence is accumulating that aldosterone also binds to the cell membrane and by a rapid, nongenomic action increases the activity of membrane Na^+–K^+ exchangers. This produces an increase in intracellular Na^+, and the second messenger involved is probably IP_3. In any case, the principal effect of aldosterone on Na^+ transport takes 10–30 min to develop and peaks even later (Figure 19-19), indicating that it depends on the synthesis of new proteins by a genomic mechanism.

RELATION OF MINERALOCORTICOID TO GLUCOCORTICOID RECEPTORS

It is intriguing that in vitro, the mineralocorticoid receptor (MR) has an appreciably higher affinity for glucocorticoids than the glucocorticoid receptor does, and glucocorticoids are present in large amounts in vivo. This raises the question of why glucocorticoids do not bind to the mineralocorticoid receptors in the kidneys and other locations and produce mineralocorticoid effects. At least in part, the answer is that the kidneys and other mineralocorticoid-sensitive tissues also contain the enzyme **11β-hydroxysteroid dehydrogenase type 2.** This enzyme leaves aldosterone untouched, but it converts cortisol to cortisone

(Figure 19–11) and corticosterone to its 11-oxy derivative. These 11-oxy derivatives do not bind to the receptor **(Clinical Box 19–3)**.

CLINICAL BOX 19–3

Apparent Mineralocorticoid Excess

If 11β-hydroxysteroid dehydrogenase type 2 is inhibited or absent, cortisol has marked mineralocorticoid effects. The resulting syndrome is called **apparent mineralocorticoid excess (AME).** Patients with this condition have the clinical picture of hyperaldosteronism because cortisol is acting on their mineralocorticoid receptors, and their plasma aldosterone level as well as their plasma renin activity is low. The condition can be due to congenital absence of the enzyme.

THERAPEUTIC HIGHLIGHTS

Prolonged ingestion of licorice can also cause an increase in blood pressure. Outside of the United States, licorice contains glycyrrhetinic acid, which inhibits 11β-hydroxysteroid dehydrogenase type 2. Individuals who eat large amounts of licorice have an increase in MR-activated sodium absorption via the epithelial sodium channel ENaC in the renal collecting duct, and blood pressure can rise.

OTHER STEROIDS THAT AFFECT NA^+ EXCRETION

Aldosterone is the principal mineralocorticoid secreted by the adrenals, although corticosterone is secreted in sufficient amounts to exert a minor mineralocorticoid effect (Tables 19–1 and 19–2). Deoxycorticosterone, which is secreted in appreciable amounts only in abnormal situations, has about 3% of the activity of aldosterone. Large amounts of progesterone and some other steroids cause natriuresis, but there is little evidence that they play any normal role in the control of Na^+ excretion.

EFFECT OF ADRENALECTOMY

In adrenal insufficiency, Na^+ is lost in the urine; K^+ is retained, and the plasma K^+ rises. When adrenal insufficiency develops rapidly, the amount of Na^+ lost from the ECF exceeds the amount excreted in the urine, indicating that Na^+ also must be entering cells. When the posterior pituitary is intact, salt loss exceeds water loss, and the plasma Na^+ falls **(Table 19–5)**. However, the plasma volume is also reduced, resulting in hypotension, circulatory insufficiency and, eventually, fatal shock. These changes can be prevented to a degree by increasing dietary NaCl intake. Rats survive indefinitely on extra salt alone, but in dogs and most humans, the amount of supplementary salt needed is so large that it is almost impossible to prevent eventual collapse and death unless mineralocorticoid treatment is also instituted **(see Clinical Box 19–4)**.

TABLE 19–5 Typical plasma electrolyte levels in normal humans and patients with adrenocortical diseases.

	Plasma Electolytes (mEq/L)			
State	Na^+	K^+	Cl^-	HCO_3^-
Normal	142	4.5	105	25
Adrenal insufficiency	120	6.7	85	25
Primary hyperaldosteronism	145	2.4	96	41

REGULATION OF ALDOSTERONE SECRETION

STIMULI

The principal conditions that increase aldosterone secretion are summarized in **Table 19–6**. Some of them also increase glucocorticoid secretion; others selectively affect the output of aldosterone. The primary regulatory factors involved are ACTH from the pituitary, renin from the kidney via angiotensin II, and a direct stimulatory effect on the adrenal cortex of a rise in plasma K^+ concentration.

TABLE 19–6 Conditions that increase aldosterone secretion.

Glucocorticoid secretion also increased

Surgery
Anxiety
Physical trauma
Hemorrhage

Glucocorticoid secretion unaffected

High potassium intake
Low sodium intake
Constriction of inferior vena cava in thorax
Standing
Secondary hyperaldosteronism (in some cases of heart failure, cirrhosis, and nephrosis)

CLINICAL BOX 19–4

Secondary Effects of Excess Mineralocorticoids

A prominent feature of prolonged mineralocorticoid excess (Table 20–5) is K^+ depletion due to prolonged K^+ diuresis. H^+ is also lost in the urine. Na^+ is retained initially, but the plasma Na^+ is elevated only slightly if at all, because water is retained with the osmotically active sodium ions. Consequently, ECF volume is expanded and the blood pressure rises. When the ECF expansion passes a certain point, Na^+ excretion is usually increased in spite of the continued action of mineralocorticoids on the renal tubules. This **escape phenomenon (Figure 19–20)** is probably due to increased secretion of atrial natriuretic peptide (ANP) (see Chapter 38). Because of increased excretion of Na^+ when the ECF volume is expanded, mineralocorticoids do not produce edema in normal individuals and patients with hyperaldosteronism. However, escape may not occur in certain disease states, and in these situations, continued expansion of ECF volume leads to edema (see Chapters 37 and 38).

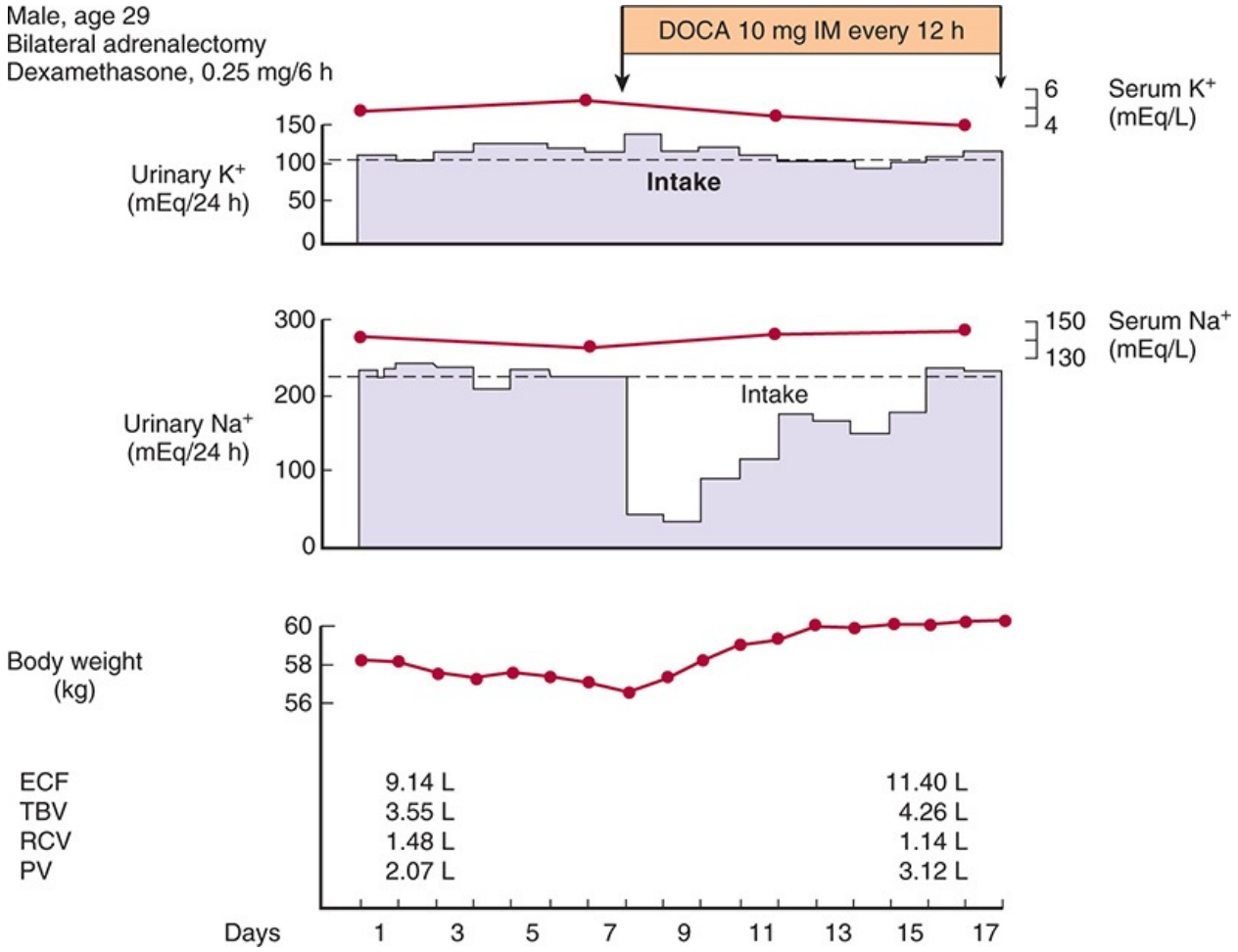


FIGURE 19–20 “Escape” from the sodium-retaining effect of desoxycorticosterone acetate (DOCA) in an adrenalectomized patient. ECF, extracellular fluid volume; PV, plasma volume; RCV, red cell volume; TBV, total blood volume. (Used with permission of EG Biglieri.)

EFFECT OF ACTH

When first administered, ACTH stimulates the output of aldosterone as well as that of glucocorticoids and sex hormones. Although the amount of ACTH required to increase aldosterone output is somewhat greater than the amount that stimulates maximal glucocorticoid secretion **(Figure 19–21)**, it is well within the range of endogenous ACTH secretion. The effect is transient, and even if ACTH secretion remains elevated, aldosterone output declines in 1 or 2 days. On the other hand, the output of the mineralocorticoid deoxycorticosterone remains elevated. The decline in aldosterone output is partly due to decreased renin

secretion secondary to hypervolemia, but it is possible that some other factor also decreases the conversion of corticosterone to aldosterone. After hypophysectomy, the basal rate of aldosterone secretion is normal. The increase normally produced by surgical and other stresses is absent, but the increase produced by dietary salt restriction is unaffected for some time. Later on, atrophy of the zona glomerulosa complicates the picture in long-standing hypopituitarism, and this may lead to salt loss and hypoaldosteronism.

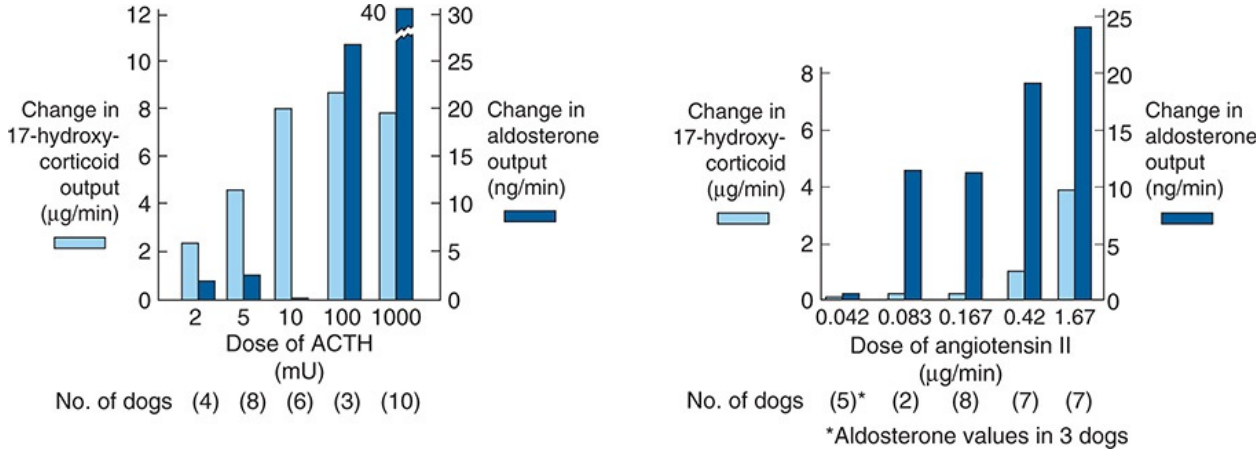


FIGURE 19–21 Changes in adrenal venous output of steroids produced by ACTH in nephrectomized hypophysectomized dogs. ACTH, adrenocorticotropic hormone.

Normally, glucocorticoid treatment does not suppress aldosterone secretion. However, an interesting recently described syndrome is **glucocorticoid-remediable aldosteronism (GRA).** This is an autosomal dominant disorder in which the increase in aldosterone secretion produced by ACTH is no longer transient. The hypersecretion of aldosterone and the accompanying hypertension are remedied when ACTH secretion is suppressed by administering glucocorticoids. The genes encoding aldosterone synthase and 11β-hydroxylase are 95% identical and are close together on chromosome 8. In individuals with GRA, there is unequal crossing over so that the 5′regulatory region of the 11β-hydroxylase gene is fused to the coding region of the aldosterone synthase gene. The product of this hybrid gene is an ACTH-sensitive aldosterone synthase.

EFFECTS OF ANGIOTENSIN II & RENIN

The octapeptide angiotensin II is formed in the body from angiotensin I, which is

liberated by the action of renin on circulating angiotensinogen (see Chapter 38). Injections of angiotensin II stimulate adrenocortical secretion and, in small doses, affect primarily the secretion of aldosterone. The sites of action of angiotensin II are both early and late in the steroid biosynthetic pathway. The early action is on the conversion of cholesterol to pregnenolone, and the late action is on the conversion of corticosterone to aldosterone (Figure 19–8). Angiotensin II does not increase the secretion of deoxycorticosterone, which is controlled by ACTH.

Renin is secreted from the juxtaglomerular cells that surround the renal afferent arterioles as they enter the glomeruli (see Chapter 38). Aldosterone secretion is regulated via the renin–angiotensin system in a feedback manner **(Figure 19–22)**. A drop in ECF volume or intra-arterial vascular volume leads to a reflex increase in renal nerve discharge and decreases renal arterial pressure. Both changes increase renin secretion, and the angiotensin II formed by the action of renin increases the rate of secretion of aldosterone. The aldosterone causes Na^+ and, secondarily, water retention, expanding ECF volume, and shutting off the stimulus that initiated increased renin secretion.

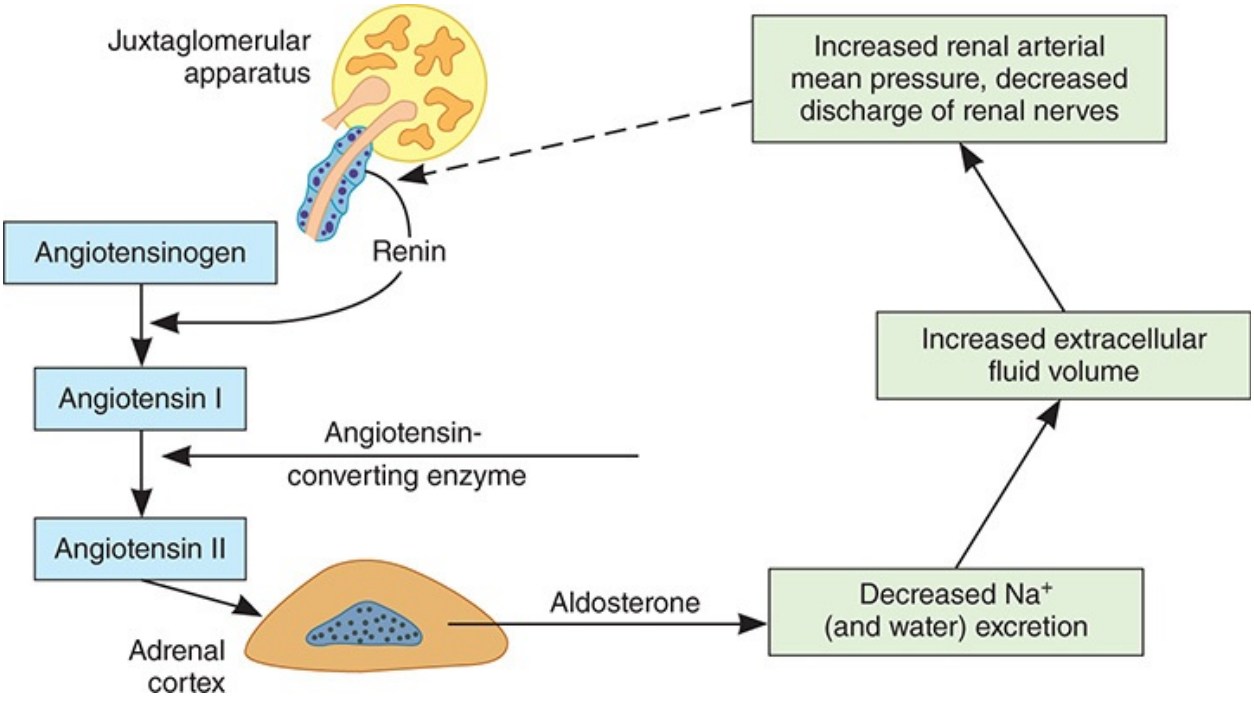


FIGURE 19–22 Feedback mechanism regulating aldosterone secretion. The dashed arrow indicates inhibition.

Hemorrhage stimulates ACTH and renin secretion. Like hemorrhage,

standing and constriction of the thoracic inferior vena cava decrease intrarenal arterial pressure. Dietary sodium restriction also increases aldosterone secretion via the renin–angiotensin system **(Figure 19–23)**. Such restriction reduces ECF volume, but aldosterone and renin secretion are increased before any consistent decrease in blood pressure takes place. Consequently, the initial increase in renin secretion produced by dietary sodium restriction is probably due to a reflex increase in the activity of the renal nerves. The increase in circulating angiotensin II produced by salt depletion upregulates the angiotensin II receptors in the adrenal cortex and hence increases the response to angiotensin II, whereas it down-regulates the angiotensin II receptors in the blood vessels.

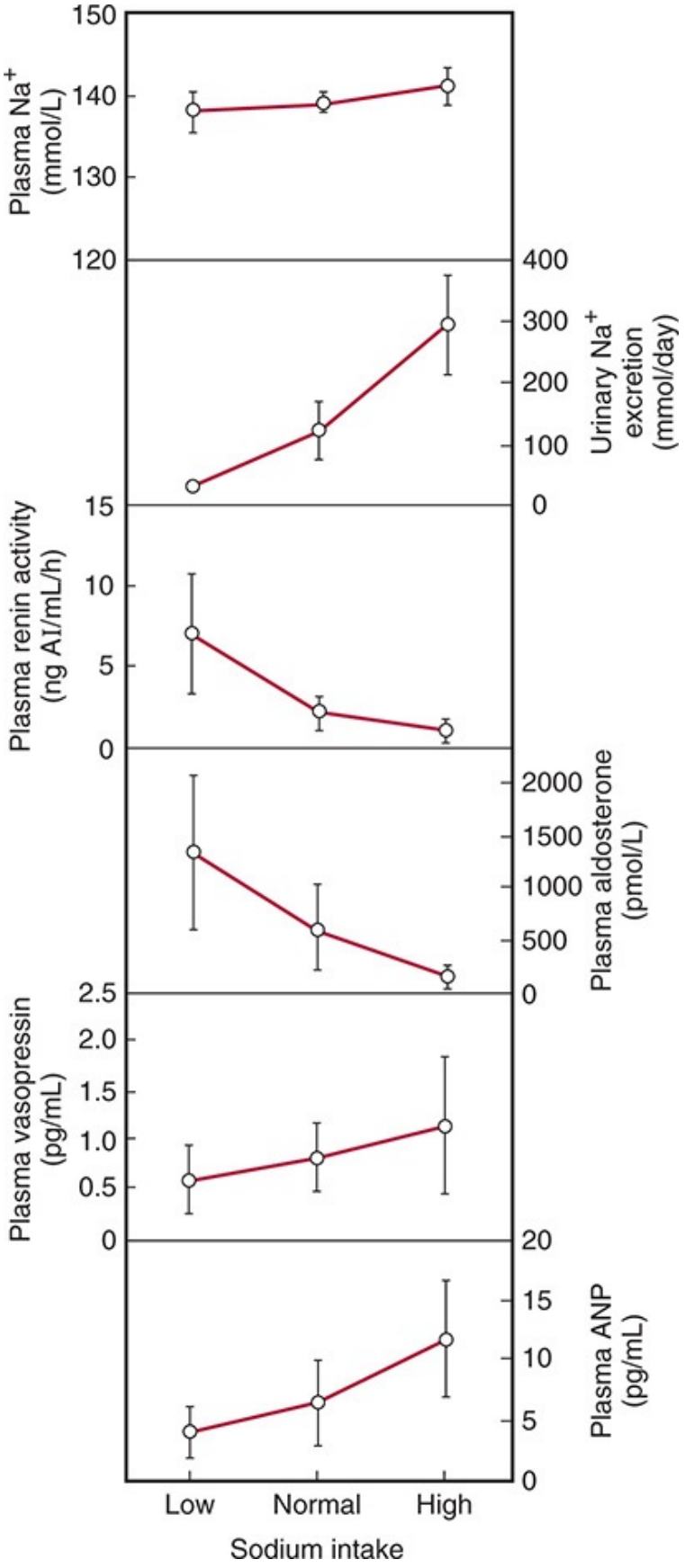

Plasma Na^+ (mmol/L)
150
140
130
120
Urinary Na^+ excretion (mmol/day)
400
300
200
100
0
Plasma renin activity (ng AI/mL/h)
15
10
5
0
Plasma aldosterone (pmol/L)
2000
1500
1000
500
0
Plasma vasopressin (pg/mL)
2.5
2.0
1.5
1.0
0.5
0
Plasma ANP (pg/mL)
20
15
10
5
0
Low
Normal
High
Sodium intake

FIGURE 19–23 Effect of low-, normal-, and high-sodium diets on sodium metabolism and plasma renin activity, aldosterone, vasopressin, and ANP in normal humans. (Data from Sagnella GA, et al: Plasma atrial natriuretic peptide: Its relationship to changes in sodium in-take, plasma renin activity, and aldosterone in man. Clin Sci 1987; 72:25.)

ELECTROLYTES & OTHER FACTORS

An acute decline in plasma Na^+ of about 20 mEq/L stimulates aldosterone secretion, but changes of this magnitude are rare. However, the plasma K^+ level need increase only 1 mEq/L to stimulate aldosterone secretion, and transient increases of this magnitude may occur after a meal, particularly if it is rich in K^+. Like angiotensin II, K^+ stimulates the conversion of cholesterol to pregnenolone and the conversion of deoxycorticosterone to aldosterone. It appears to act by depolarizing the cell, which opens voltage-gated Ca^{2+} channels, increasing intracellular Ca^{2+}. The sensitivity of the zona glomerulosa to angiotensin II and consequently to a low-sodium diet is decreased by a low-potassium diet.

In normal individuals, plasma aldosterone concentrations increase during the portion of the day that the individual is carrying on activities in the upright position. This increase is due to a decrease in the rate of removal of aldosterone from the circulation by the liver and an increase in aldosterone secretion due to a postural increase in renin secretion. Individuals who are confined to bed show a circadian rhythm of aldosterone and renin secretion, with the highest values in the early morning before awakening. Atrial natriuretic peptide (ANP) inhibits renin secretion and decreases the responsiveness of the zona glomerulosa to angiotensin II (see Chapter 38). The mechanisms by which ACTH, angiotensin II, and K^+ stimulate aldosterone secretion are summarized in **Table 19–7**.

TABLE 19–7 Second messengers involved in the regulation of aldosterone secretion.

Secretagogue	Intracellular Mediator
ACTH	Cyclic AMP, protein kinase A
Angiotensin II	Diacylglycerol, protein kinase C
K^+	Ca^{2+} via voltage-gated Ca^{2+} channels

ROLE OF MINERALOCORTICOIDS IN THE REGULATION OF SALT BALANCE

Variations in aldosterone secretion is only one of many factors affecting Na^+ excretion. Other major factors include the glomerular filtration rate, ANP, the presence or absence of osmotic diuresis, and changes in tubular reabsorption of Na^+ independent of aldosterone. It takes some time for aldosterone to act. When one rises from the supine to the standing position, aldosterone secretion increases and Na^+ is retained from the urine. However, the decrease in Na^+ excretion develops too rapidly to be explained solely by increased aldosterone secretion. The primary function of the aldosterone-secreting mechanism is the defense of intravascular volume, but it is only one of the homeostatic mechanisms involved in this regulation.

SUMMARY OF THE EFFECTS OF ADRENOCORTICAL HYPER- & HYPOFUNCTION IN HUMANS

Recapitulating the manifestations of excess and deficiency of the adrenocortical hormones in humans is a convenient way to summarize the multiple and complex actions of these steroids. A characteristic clinical syndrome is associated with excess secretion of each of the types of hormones.

Excess androgen secretion causes masculinization **(adrenogenital syndrome)** and precocious pseudopuberty or female pseudohermaphroditism.

Excess glucocorticoid secretion produces a moon-faced, plethoric appearance, with trunk obesity, purple abdominal striae, hypertension, osteoporosis, protein depletion, mental abnormalities and, frequently, diabetes mellitus **(Cushing syndrome).** Excess mineralocorticoid secretion leads to K^+ depletion and Na^+ retention, usually without edema but with weakness, hypertension, tetany, polyuria, and hypokalemic alkalosis **(hyperaldosteronism).** This condition may be due to primary adrenal disease **(primary hyperaldosteronism; Conn syndrome)** such as an adenoma of the zona glomerulosa, unilateral or bilateral adrenal hyperplasia, adrenal carcinoma, or by GRA. In patients with primary hyperaldosteronism, renin secretion is depressed. **Secondary hyperaldosteronism** with high plasma renin activity is caused by cirrhosis, heart failure, and nephrosis. Increased renin secretion is also found in individuals with

the salt-losing form of the adrenogenital syndrome (see above), because their ECF volume is low. In patients with elevated renin secretion due to renal artery constriction, aldosterone secretion is increased; in those in whom renin secretion is not elevated, aldosterone secretion is normal. The relationship of aldosterone to hypertension is discussed in Chapter 32.

Primary adrenal insufficiency due to disease processes that destroy the adrenal cortex is called **Addison disease.** The condition used to be a relatively common complication of tuberculosis, but now it is usually due to autoimmune inflammation of the adrenal. Patients lose weight, are tired, and become chronically hypotensive. They have small hearts, probably because the hypotension decreases the work of the heart. Eventually, severe hypotension and shock **(addisonian crisis)** develop. This is due not only to mineralocorticoid deficiency but to glucocorticoid deficiency as well. Fasting causes fatal hypoglycemia, and any stress causes collapse. Water is retained, and there is always the danger of water intoxication. Circulating ACTH levels are elevated. The diffuse tanning of the skin and the spotty pigmentation characteristic of chronic glucocorticoid deficiency are due, at least in part, to the melanocyte-stimulating hormone (MSH) activity of the ACTH in the blood. Pigmentation of skin creases on the hands and the gums are common. Minor menstrual abnormalities occur in women, but the deficiency of adrenal sex hormones usually has little effect in the presence of normal testes or ovaries.

Secondary adrenal insufficiency is caused by pituitary diseases that decrease ACTH secretion, and **tertiary adrenal insufficiency** is caused by hypothalamic disorders disrupting CRH secretion. Both are usually milder than primary adrenal insufficiency because electrolyte metabolism is affected to a lesser degree. In addition, there is no pigmentation because in both of these conditions, plasma ACTH is low, not high.

Cases of isolated aldosterone deficiency have also been reported in patients with renal disease and a low circulating renin level **(hyporeninemic hypoaldosteronism).** In addition, **pseudohypoaldosteronism** is produced when there is resistance to the action of aldosterone. Patients with these syndromes have marked hyperkalemia, salt wasting, and hypotension, and they may develop metabolic acidosis.

CHAPTER SUMMARY

- The adrenal gland consists of the adrenal medulla that secretes dopamine and the catecholamines epinephrine and norepinephrine, and the adrenal cortex

that secretes steroid hormones.

- Norepinephrine and epinephrine act on two classes of receptors, α- and β-adrenergic receptors, and exert metabolic effects that include glycogenolysis in liver and skeletal muscle, mobilization of FFA, increased plasma lactate, and stimulation of the metabolic rate.
- The hormones of the adrenal cortex are derivatives of cholesterol and include the mineralocorticoid aldosterone, the glucocorticoids cortisol and corticosterone, and the androgens dehydroepiandrosterone (DHEA) and androstenedione.
- Androgens are the hormones that exert masculinizing effects, and they promote protein anabolism and growth. The adrenal androgen androstenedione is converted to testosterone and to estrogens (aromatized) in fat and other peripheral tissues. This is an important source of estrogens in men and postmenopausal women.
- The mineralocorticoid aldosterone has effects on Na^+ and K^+ excretion and glucocorticoids affect glucose and protein metabolism.
- Glucocorticoid secretion is dependent on ACTH from the anterior pituitary and is increased by stress. Angiotensin II increases the secretion of aldosterone.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. Which of the following is produced only by *large amounts* of glucocorticoids?

   A. Normal responsiveness of fat depots to norepinephrine
   B. Maintenance of normal vascular reactivity
   C. Increased excretion of a water load
   D. Inhibition of the inflammatory response
   E. Inhibition of ACTH secretion

2. Which of the following are *incorrectly* paired?

   A. Gluconeogenesis : Cortisol
   B. Free fatty acid mobilization : Dehydroepiandrosterone
   C. Muscle glycogenolysis : Epinephrine
   D. Kaliuresis : Aldosterone

E. Hepatic glycogenesis : Insulin

3. Which of the following hormones has the shortest plasma half-life?

A. Corticosterone

B. Renin

C. Dehydroepiandrosterone

D. Aldosterone

E. Norepinephrine

4. Mole for mole, which of the following has the greatest effect on Na^+ excretion?

A. Progesterone

B. Cortisol

C. Vasopressin

D. Aldosterone

E. Dehydroepiandrosterone

5. Mole for mole, which of the following has the greatest effect on plasma osmolality?

A. Progesterone

B. Cortisol

C. Vasopressin

D. Aldosterone

E. Dehydroepiandrosterone

6. The secretion of which of the following would be *least* affected by a decrease in extracellular fluid volume?

A. CRH

B. Arginine vasopressin

C. Dehydroepiandrosterone

D. Estrogens

E. Aldosterone

7. A young man presents with a blood pressure of 175/110 mm Hg. He is found to have a high circulating aldosterone but a low circulating cortisol. Glucocorticoid treatment lowers his circulating aldosterone and lowers his blood pressure to 140/85 mm Hg. He probably has an abnormal

A. 17α-hydroxylase.

B. 21β-hydroxylase.

C. 3β-hydroxysteroid dehydrogenase.

D. aldosterone synthase.

E. cholesterol desmolase.

8. A 32-year-old woman presents with a blood pressure of 155/96 mm Hg. In response to questioning, she admits that she loves licorice and eats some at least three times a week. She probably has a low level of

A. type 2 11β-hydroxysteroid dehydrogenase activity.

B. ACTH.

C. 11β-hydroxylase activity.

D. glucuronyl transferase.

E. norepinephrine.

9. In its action in cells, aldosterone

A. increases transport of ENaCs from the cytoplasm to the cell membrane.

B. does not act on the cell membrane.

C. binds to a receptor excluded from the nucleus.

D. may activate a heat shock protein.

E. also binds to glucocorticoid receptors.

CHAPTER 20

The Thyroid Gland

OBJECTIVES

After studying this chapter, you should be able to:

- Describe the development, anatomy, and histology of the thyroid gland, and how these relate to its function, as well as the changes in histology that accompany the activation of secretion with reabsorption of thyroglobulin from the colloid.
- Define the chemical nature of the thyroid hormones and how they are synthesized. Understand the critical role of iodine in the thyroid gland and how its transport is controlled, as well as how it is incorporated into the tyrosine residues of thyroglobulin at the interface between thyrocytes and colloid via the process of organification.
- Describe the relative roles of binding to albumin, transthyretin, and thyroxine-binding globulin in the transport of thyroid hormones, the availability of free hormones, and peripheral metabolism.
- Identify the role of the hypothalamus and pituitary in regulating thyroid function and the feedback loops that establish the level of secretion of thyroid hormones.
- Define the effects of the thyroid hormones in homeostasis, metabolism, and growth.

- Understand the basis of conditions where thyroid function is abnormal and how they can be treated.

INTRODUCTION

The thyroid gland is one of the larger endocrine glands of the body. The gland has two primary functions. The first is to secrete the thyroid hormones, which maintain the level of metabolism in the tissues that is optimal for their normal function. Thyroid hormones stimulate O_2 consumption by most of the cells in the body, help regulate lipid and carbohydrate metabolism, and thereby influence body mass and mentation. Consequences of thyroid gland dysfunction depend on the life stage at which they occur. The thyroid is not essential for life, but its absence or hypofunction during fetal and neonatal life results in severe mental retardation and dwarfism. In adults, hypothyroidism is accompanied by mental and physical slowing and poor resistance to cold. Conversely, excess thyroid secretion leads to body wasting, nervousness, tachycardia, tremor, and excess heat production. Thyroid function is controlled by the thyroid-stimulating hormone (TSH, thyrotropin) that is secreted by the anterior lobe of the pituitary. The secretion of this hormone is in turn increased by thyrotropin-releasing hormone (TRH) from the hypothalamus and is also subject to negative feedback control by high circulating levels of thyroid hormones acting on the anterior pituitary and the hypothalamus.

The second function of the thyroid gland is to secrete calcitonin, a hormone that regulates circulating levels of calcium. This function of the thyroid gland is discussed in Chapter 21 in the broader context of whole body calcium homeostasis.

DEVELOPMENT, STRUCTURE, AND CELL TYPES OF THE THYROID GLAND

GROSS ANATOMY

The thyroid is a butterfly-shaped gland that straddles the trachea in the front of the neck. It develops from an evagination of the floor of the pharynx, and a **thyroglossal duct** marking the path of the thyroid from the tongue to the neck

sometimes persists in the adult. The two lobes of the human thyroid are connected by a bridge of tissue, the **thyroid isthmus,** and there is sometimes a **pyramidal lobe** arising from the isthmus in front of the larynx **(Figure 20–1)**. The gland is well vascularized, and the thyroid has one of the highest rates of blood flow per gram of tissue of any organ in the body.

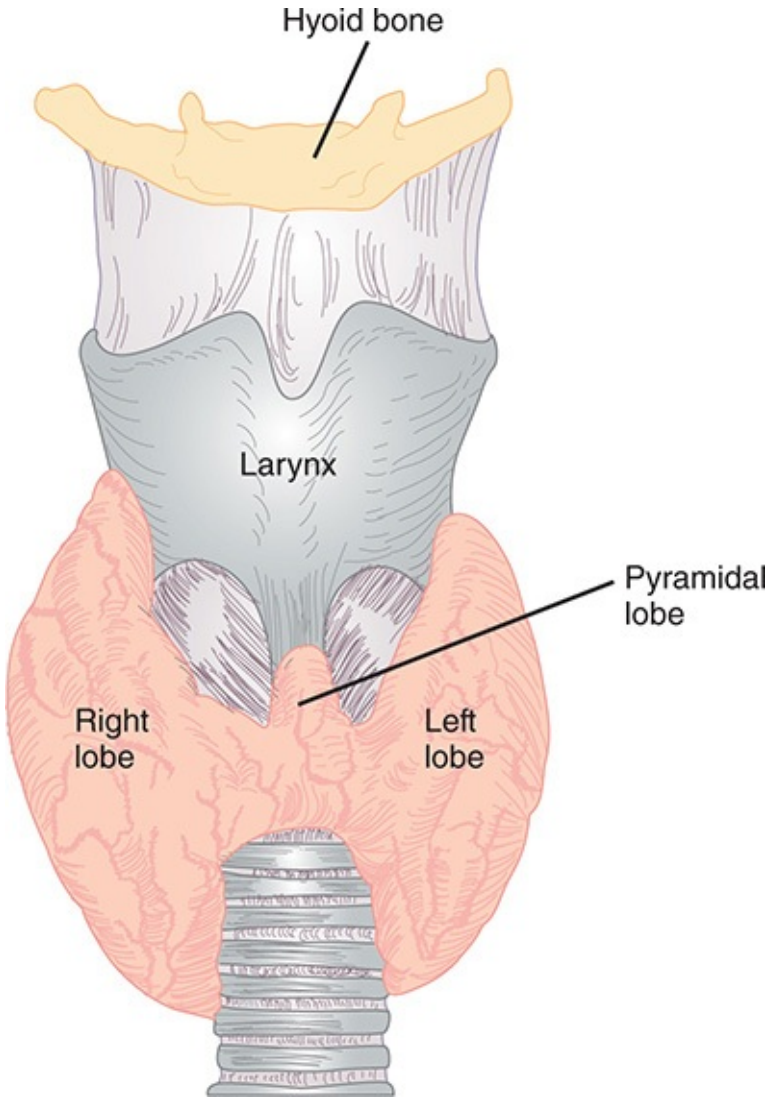


FIGURE 20–1 The human thyroid.

HISTOLOGY

The portion of the thyroid concerned with the production of thyroid hormone consists of multiple **follicles.** Each spherical follicle is surrounded by a single layer of polarized epithelial cells and filled with proteinaceous material called

colloid. Colloid consists predominantly of the glycoprotein, thyroglobulin. When the gland is inactive, the colloid is abundant, the follicles are large, and the cells lining them are flat. When the gland is active, the follicles are small, the cells are cuboid or columnar, and areas where the colloid is being actively reabsorbed into the thyrocytes are visible on histology as "reabsorption lacunae" **(Figure 20–2)**.

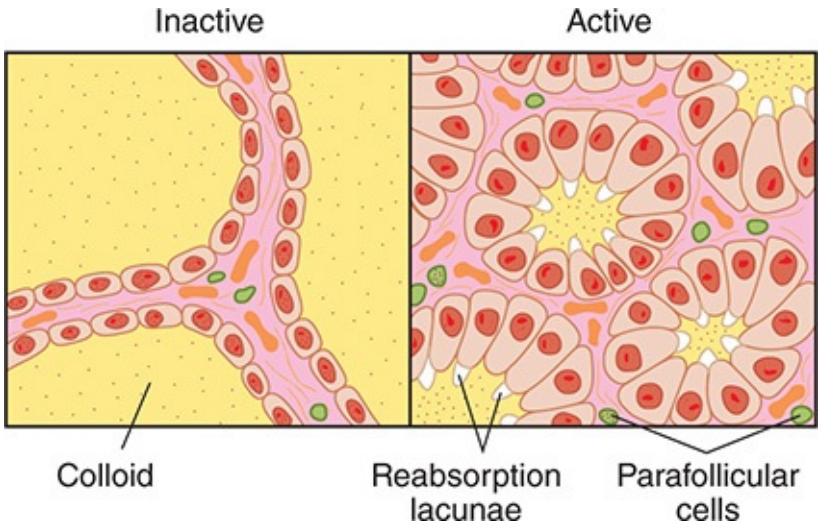


FIGURE 20–2 Thyroid histology. The appearance of the gland when it is inactive (left) and actively secreting (right) is shown. Note the small, punched-out "reabsorption lacunae" in the colloid next to the cells in the active gland.

Microvilli project into the colloid from the apexes of the thyrocytes and canaliculi extend into them. The endoplasmic reticulum is prominent, a feature common to most glandular cells, and secretory granules containing thyroglobulin are seen **(Figure 20–3)**. The individual thyroid cells rest on a basal lamina that separates them from the adjacent capillaries. The capillaries are fenestrated, like those of other endocrine glands (see Chapter 31).

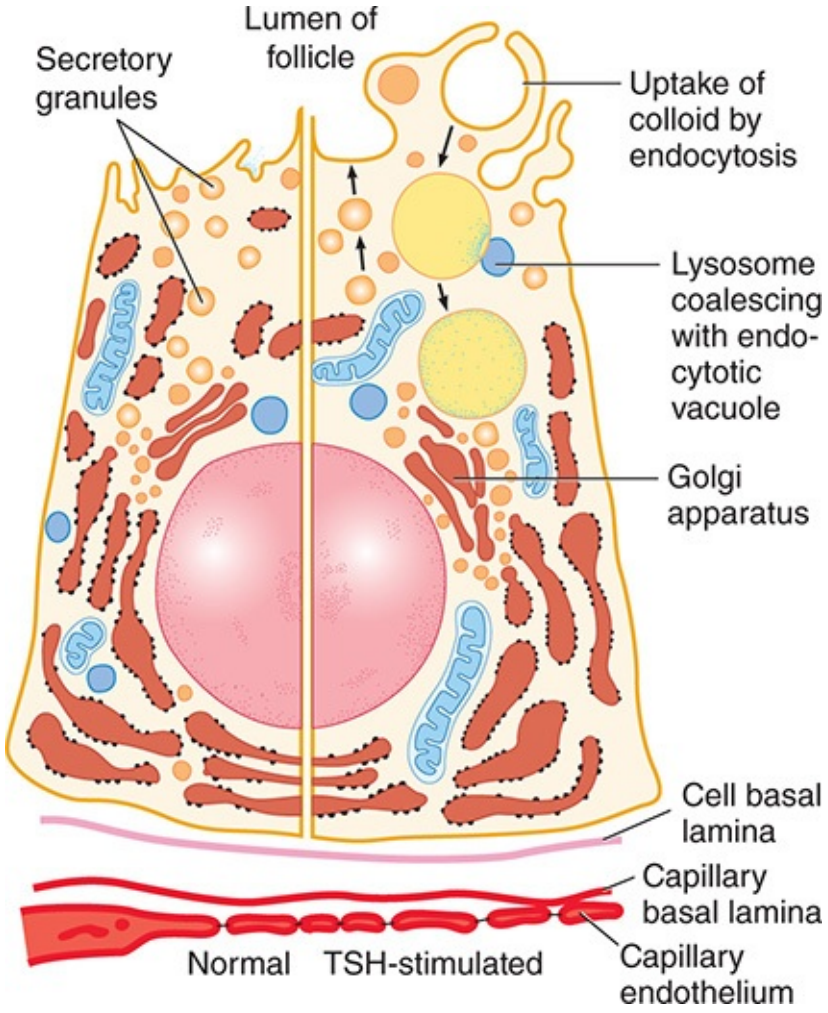


FIGURE 20–3 Thyrocyte. Left: Normal pattern. **Right:** After TSH stimulation. The arrows on the right show the secretion of thyroglobulin into the colloid. On the right, endocytosis of the colloid and merging of a colloid-containing vacuole with a lysosome are also shown. The cell rests on a capillary with gaps (fenestrations) in the endothelial wall.

FORMATION & SECRETION OF THYROID HORMONES

CHEMISTRY

The primary hormone secreted by the thyroid is **thyroxine (T_4),** along with much lesser amounts of **triiodothyronine (T_3).** T_3 has much greater biologic activity than T_4 and is also specifically generated at its site of action in

peripheral tissues by deiodination of T_4 (see below). Both hormones are iodine-containing amino acids **(Figure 20–4)**. Small amounts of reverse triiodothyronine (3,3′,5′-triiodothyronine, RT_3) and other compounds are also found in thyroid venous blood. Whether RT_3 is biologically active remains unclear.

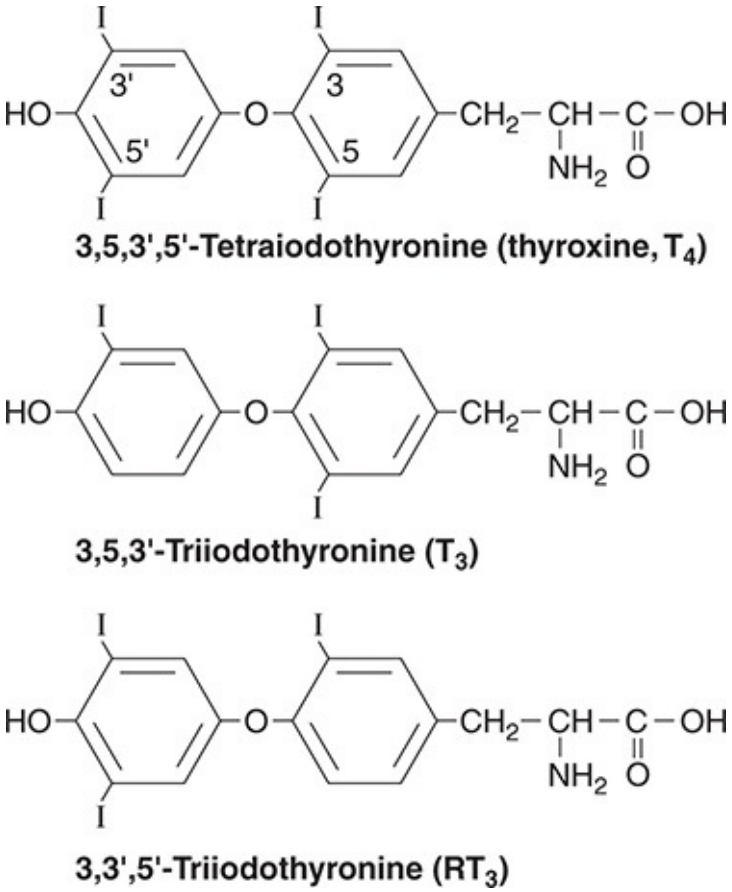


FIGURE 20–4 Thyroid hormones. The numbers in the rings in the T_4 formula indicate the numbers of positions in the molecule. RT_3, reverse triiodothyronine.

IODINE HOMEOSTASIS

Iodine is an essential raw material for thyroid hormone synthesis. Dietary iodide is absorbed by the intestine and enters the circulation; its subsequent fate is summarized in **Figure 20–5**. The minimum daily iodine intake that will maintain normal thyroid function is 150 µg in adults. In most developed countries, supplementation of table salt means that the average dietary intake is

approximately 500 µg/day. The principal organs that take up circulating I^- are the thyroid, which uses it to make thyroid hormones, and the kidneys, which excrete it in the urine. About 120 µg/day enter the thyroid at normal rates of thyroid hormone synthesis and secretion. The thyroid secretes 80 µg/day in the form of T_3 and T_4, while 40 µg/day diffuses back into the extracellular fluid (ECF). Circulating T_3 and T_4 are metabolized in the liver and other tissues, with the release of a further 60 µg of I^- per day into the ECF. Some thyroid hormone derivatives are excreted in the bile, and some of the iodine in them is reabsorbed (enterohepatic circulation), but there is a net loss of I^- in the stool of approximately 20 µg/day. The total amount of I^- entering the ECF is thus 500 + 40 + 60, or 600 µg/day; 20% of this I^- enters the thyroid, whereas 80% is excreted in the urine.

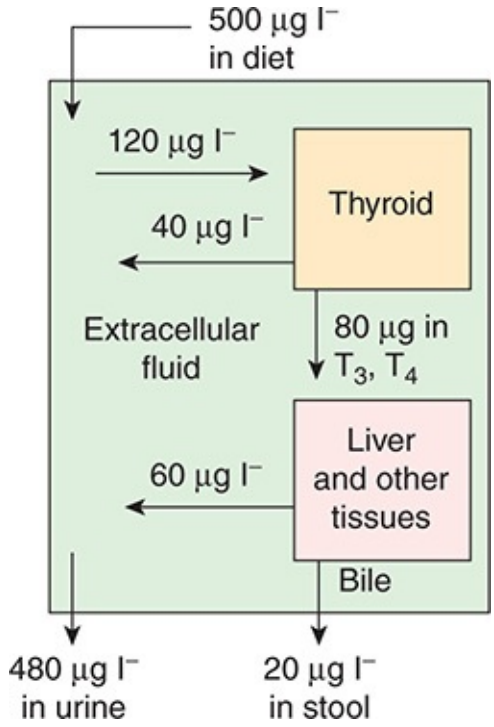


FIGURE 20–5 Iodide metabolism. The figure shows the movement of iodide among various body compartments on a daily basis.

IODIDE TRANSPORT ACROSS THYROCYTES

The basolateral membranes of thyrocytes facing the capillaries contain a **symporter** that transports two Na^+ ions and one I^- ion into the cell with each

cycle, against the electrochemical gradient for I^-. This Na^+/I^- symporter **(NIS)** is capable of producing intracellular I^- concentrations that are 20–40 times as great as the concentration in plasma **(Figure 20–6)**. The process involved is secondary active transport (see Chapter 2), with the energy provided by active transport of Na^+ out of thyroid cells by Na, K ATPase. NIS is regulated both by transcriptional means and by active trafficking into and out of the thyrocyte basolateral membrane; in particular, TSH (see below) induces both NIS expression and the retention of NIS in the basolateral membrane, where it can mediate sustained iodide uptake.

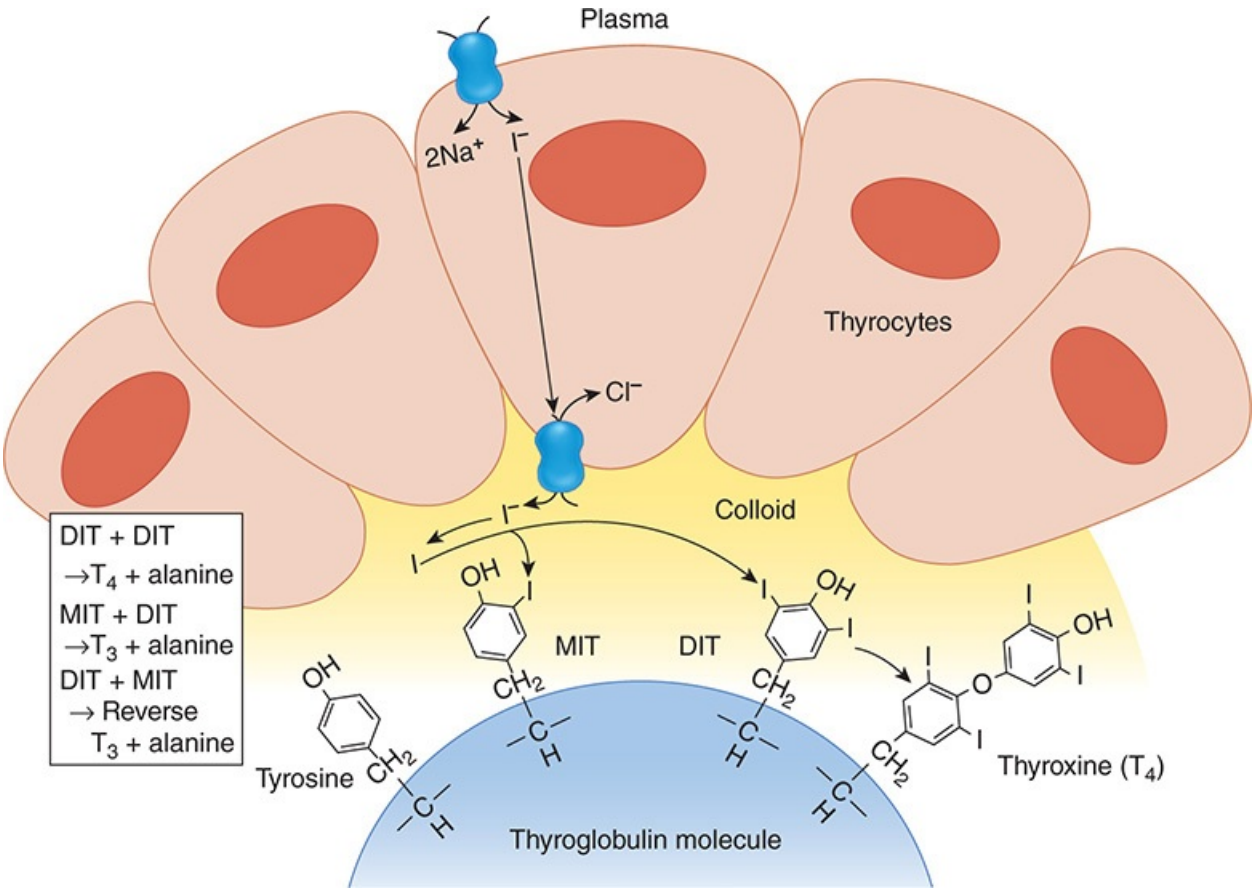


FIGURE 20–6 Outline of thyroid hormone biosynthesis. Iodide (I^-) is transported vectorially from the plasma across the epithelial cells of the thyroid gland by specific transporters. The iodide is converted to iodine, which reacts with tyrosine residues exposed on the surface of thyroglobulin molecules resident in the colloid. Iodination of tyrosine takes place at the apical border of the thyroid cells while the tyrosine moieties remain bound to thyroglobulin via peptide linkages.

Iodide must also exit the thyrocyte across the apical membrane to access the colloid, where the initial steps of thyroid hormone synthesis occur. This transport step is believed to be mediated, at least in part, by a Cl^-/I^- exchanger known as **pendrin** (Figure 20–6). This protein was first identified as the product of the gene responsible for the Pendred syndrome, which causes thyroid dysfunction and deafness. Pendrin (SLC26A4) is one member of the larger family of SLC26 anion exchangers.

The relationship of thyroid function to iodide is unique. Iodide is essential for normal thyroid function, but iodide deficiency and iodide excess both inhibit thyroid function.

The salivary glands, the gastric mucosa, the placenta, the ciliary body of the eye, the choroid plexus, the mammary glands, and certain cancers derived from these tissues also express NIS and can transport iodide against a concentration gradient, but the transporter in these tissues is not affected by TSH. The physiologic significance of all these extrathyroidal iodide-concentrating mechanisms is obscure, but they may provide pathways for radioablation of NIS-expressing cancer cells using iodide radioisotopes. This approach is also useful for the ablation of thyroid cancers.

THYROID HORMONE SYNTHESIS & SECRETION

At the interface between the thyrocyte and the colloid, iodide undergoes a process referred to as organification. First, it is oxidized to iodine, and then incorporated into the carbon 3 position of tyrosine residues that are part of the thyroglobulin molecule in the colloid (Figure 20–6). **Thyroglobulin** is a glycoprotein made up of two subunits. It contains 123 tyrosine residues, but only 4–8 of these are normally incorporated into thyroid hormones. Thyroglobulin is synthesized in the thyrocytes and secreted into the colloid by exocytosis of granules. The oxidation and reaction of iodide with the secreted thyroglobulin is mediated by **thyroid peroxidase,** a membrane-bound enzyme found in the thyrocyte apical membrane. The thyroid hormones so produced remain part of the thyroglobulin molecule until needed. As such, colloid represents a reservoir of thyroid hormones, and humans can ingest a diet completely devoid of iodide for up to 2 months before a decline in circulating thyroid hormone levels is seen. When there is a need for thyroid hormone secretion, colloid is internalized by the thyrocytes by endocytosis, and directed toward lysosomal degradation. Thus, the

peptide bonds of thyroglobulin are hydrolyzed, and free T_4 and T_3 are discharged into cytosol and thence to the capillaries (see below). Thyrocytes thus have four functions: They collect and transport iodine, they synthesize thyroglobulin and secrete it into the colloid, they fix iodine to the thyroglobulin to generate thyroid hormones, and they remove the thyroid hormones from thyroglobulin and secrete them into the circulation.

Thyroid hormone synthesis is a multistep process. Thyroid peroxidase generates reactive iodine species that can attack thyroglobulin. The first product is monoiodotyrosine (MIT). MIT is next iodinated on the carbon 5 position to form diiodotyrosine (DIT). Two DIT molecules then undergo an oxidative condensation to form T_4 with the elimination of the alanine side chain from the molecule that forms the outer ring. There are two theories of how this **coupling reaction** occurs. One holds that the coupling occurs with both DIT molecules attached to thyroglobulin (intramolecular coupling). The other holds that the DIT that forms the outer ring is first detached from thyroglobulin (intermolecular coupling). In either case, thyroid peroxidase is involved in coupling as well as iodination. T_3 is formed by condensation of MIT with DIT. RT_3 is likely formed by condensation of DIT with MIT. In the normal human thyroid, the average distribution of iodinated compounds is 3% MIT, 33% DIT, 35% T_4, and 7% T_3. Only traces of RT_3 and other components are present.

The human thyroid secretes about 80 µg (103 nmol) of T_4, 4 µg (7 nmol) of T_3, and 2 µg (3.5 nmol) of RT_3 per day **(Figure 20–7)**. MIT and DIT are not secreted. These iodinated tyrosines can be deiodinated by a microsomal **iodotyrosine deiodinase.** This represents a mechanism to recover iodine and bound tyrosines and recycle them for additional rounds of hormone synthesis. The iodine liberated by deiodination of MIT and DIT is reutilized in the gland and normally provides about twice as much iodide for hormone synthesis as NIS does. In patients with congenital absence of the iodotyrosine deiodinase, MIT and DIT appear in the urine and there are symptoms of iodine deficiency (see below). Iodinated thyronines are resistant to the activity of iodotyrosine deiodinase, thus allowing T_4 and T_3 to pass into the circulation.

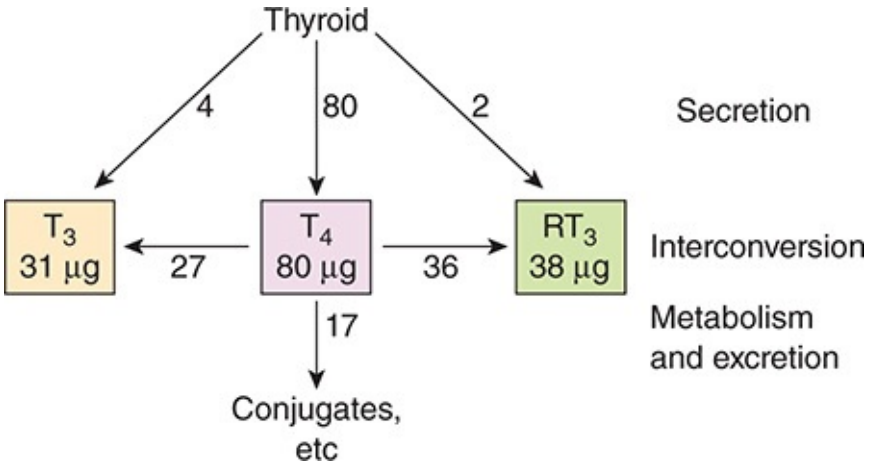


FIGURE 20–7 Secretion and interconversion of thyroid hormones in normal adult humans. Figures are in micrograms per day. Note that most of the T_3 and RT_3 are formed from T_4 deiodination in the tissues and only small amounts are secreted by the thyroid. T_4 is also conjugated for subsequent excretion from the body.

TRANSPORT & METABOLISM OF THYROID HORMONES

PROTEIN BINDING

The normal total **plasma T_4** level in adults is approximately 8 µg/dL (103 nmol/L), and the **plasma T_3** level is approximately 0.15 µg/dL (2.3 nmol/L). T_4 and T_3 are relatively lipophilic; thus, their free forms in plasma are in equilibrium with a much larger pool of protein-bound thyroid hormones in plasma and in tissues. Free thyroid hormones are added to the circulating pool by the thyroid. It is the free thyroid hormones in plasma that are physiologically active and that feed back to inhibit pituitary secretion of TSH **(Figure 20–8)**. The function of protein-binding appears to be the maintenance of a large pool of hormone that can readily be mobilized as needed. In addition, at least for T_3, hormone-binding prevents excess uptake by the first cells encountered and promotes uniform tissue distribution. Total T_4 and T_3 can both be measured by radioimmunoassay. There are also direct assays that specifically measure only the free forms of the hormones. The latter are the more clinically relevant measures given that these are the active forms, and also due to both acquired and

congenital variations in the concentrations of binding proteins between individuals.

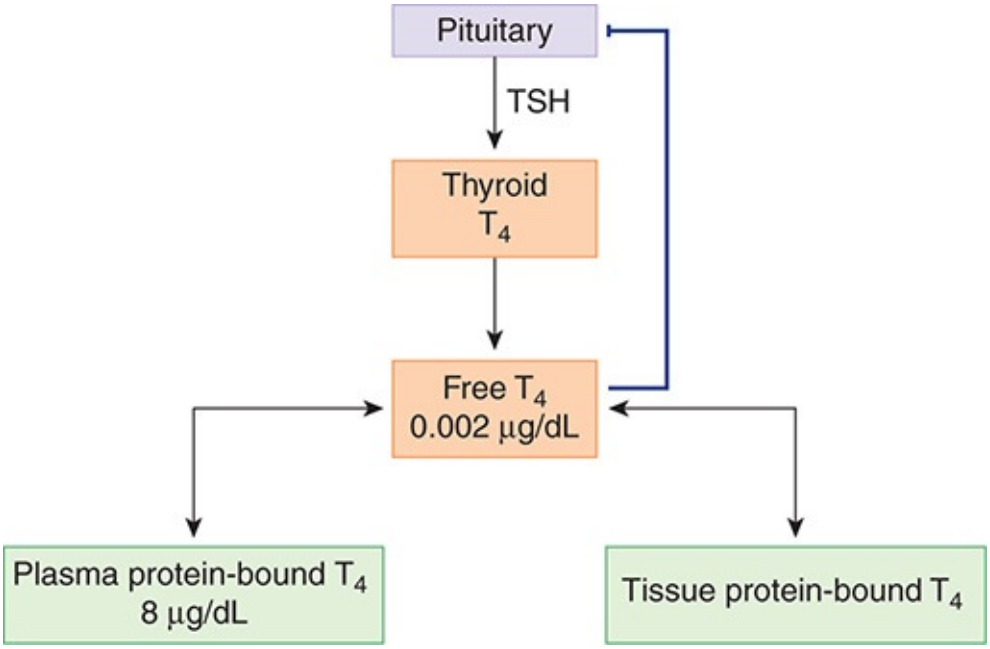


FIGURE 20–8 Regulation of thyroid hormone synthesis. T_4 is secreted by the thyroid in response to TSH. Free T_4 secreted by the thyroid into the circulation is in equilibrium with T_4 bound to both plasma and tissue proteins. Free T_4 also feeds back to inhibit TSH secretion by the pituitary. Not shown, T_3 is also secreted (in small amounts) by the thyroid and produced (in larger amounts) from T_4 in the periphery by deiodination. T_3 also feeds back to reduce secretion of TSH from the pituitary.

The plasma proteins that bind thyroid hormones are **albumin, transthyretin** (formerly called **thyroxine-binding prealbumin),** and **thyroxine-binding globulin (TBG).** Of the three proteins, albumin has the largest **capacity** to bind T_4 (ie, it can bind the most T_4 before becoming saturated) and TBG has the smallest capacity. However, the **affinities** of the proteins for T_4 (ie, the avidity with which they bind T_4 under physiologic conditions) are such that most of the circulating T_4 is bound to TBG **(Table 20–1)**. Smaller amounts of T_4 are bound to transthyretin and albumin.

TABLE 20–1 Binding of thyroid hormones to plasma proteins in normal adult humans.

Protein	Plasma Concentration (mg/dL)	Amounts of Circulating Hormone Bound (%)	
		T_4	T_3
Thyroxine-binding globulin (TBG)	2	67	46
Transthyretin (thyroxine-binding prealbumin, TBPA)	15	20	1
Albumin	3500	13	53

Normally, 99.98% of the T_4 in plasma is bound; the free T_4 level is only about 2 ng/dL. There is very little T_4 in the urine. Its biologic half-life is long (about 6–7 days), and its volume of distribution is less than that of ECF (10 L, or about 15% of body weight). All of these properties are characteristic of a substance that is strongly bound to protein.

T_3 is not bound to quite as great an extent; of the 0.15 µg/dL normally found in plasma, 0.2% (0.3 ng/dL) is free. The remaining 99.8% is protein-bound, 46% to TBG and most of the remainder to albumin, with very little binding to transthyretin (Table 20–1). The lesser binding of T_3 correlates with the facts that T_3 has a shorter half-life than T_4 and that its action on the tissues is much more rapid. RT_3 also binds to TBG.

FLUCTUATIONS IN BINDING

When a sudden, sustained increase in the concentration of thyroid-binding proteins in the plasma takes place, the concentration of free thyroid hormones falls. This change is temporary, however, because the decrease in the concentration of free thyroid hormones in the circulation stimulates TSH secretion, which in turn causes an increase in the production of free thyroid hormones. A new equilibrium is eventually reached at which the total quantity of thyroid hormones in the blood is elevated but the concentration of free hormones, the rate of their metabolism, and the rate of TSH secretion are normal. Corresponding changes in the opposite direction occur when the concentration of thyroid-binding protein is reduced. Consequently, patients with

elevated or decreased concentrations of binding proteins, particularly TBG, are typically neither hyperthyroid nor hypothyroid; that is, they are **euthyroid.**

TBG levels are elevated in estrogen-treated patients and during pregnancy, as well as after treatment with various drugs **(Table 20–2)**. They are depressed by glucocorticoids, androgens, the weak androgen danazol, and the cancer chemotherapeutic agent L-asparaginase. A number of other drugs, including salicylates, the anticonvulsant phenytoin, and the cancer chemotherapeutic agents mitotane (o, p′-DDD) and 5-fluorouracil, inhibit binding of T_4 and T_3 to TBG and consequently produce changes similar to those produced by a decrease in TBG concentration. Changes in total plasma T_4 and T_3 can also be produced by changes in plasma concentrations of albumin and prealbumin.

TABLE 20–2 Effect of variations in the concentrations of thyroid hormone–binding proteins in the plasma on various parameters of thyroid function after equilibrium has been reached.

Condition	Concentrations of Binding Proteins	Total Plasma T_4, T_3, RT_3	Free Plasma T_4, T_3, RT_3	Plasma TSH	Clinical State
Hyperthyroidism	Normal	High	High	Low	Hyperthyroid
Hypothyroidism	Normal	Low	Low	High	Hypothyroid
Estrogens, methadone, heroin, antipsychotic drugs, clofibrate	High	High	Normal	Normal	Euthyroid
Glucocorticoids, androgens, danazol, asparaginase	Low	Low	Normal	Normal	Euthyroid

METABOLISM OF THYROID HORMONES

T_4 and T_3 are deiodinated in the liver, the kidneys, and many other tissues. These deiodination reactions serve not only to catabolize the hormones, but also to provide a local supply specifically of T_3, which is believed to be the primary mediator of the physiologic effects of thyroid secretion. One-third of the circulating T_4 is normally converted to T_3 in adult humans, and 45% is converted to RT_3. As shown in Figure 20–7, only about 13% of the circulating T_3 is secreted by the thyroid while 87% is formed by deiodination of T_4; similarly, only 5% of the circulating RT_3 is secreted by the thyroid and 95% is formed by deiodination of T_4. It should be noted as well that marked differences in the ratio of T_3 to T_4 occur in various tissues. Two tissues that have very high T_3/T_4 ratios are the pituitary and the cerebral cortex, due to the expression of specific deiodinases, as discussed below. In the brain, in particular, high levels of

deiodinase activity ensure an ample supply of active T_3.

Three different deiodinases act on thyroid hormones: D_1, D_2, and D_3. All are unique in that they contain the rare amino acid selenocysteine, with selenium in place of sulfur, which is essential for their enzymatic activity. D_1 is present in high concentrations in the liver, kidneys, thyroid, and pituitary. It appears primarily to be responsible for maintaining the formation of T_3 from T_4 in the periphery. D_2 is present in the brain, pituitary, and brown fat. It also contributes to the formation of T_3. In the brain, it is located in astroglia and provides a supply of T_3 to neurons. D_3 is also present in the brain and in reproductive tissues. It acts only on the 5 position of T_4 and T_3 and is probably the main source of RT_3 in the blood and tissues.

Some of the T_4 and T_3 are further converted to deiodotyrosines by deiodinases. T_4 and T_3 are also conjugated in the liver to form sulfates and glucuronides. These conjugates enter the bile and pass into the intestine. The thyroid conjugates are hydrolyzed, and some are thereafter reabsorbed (enterohepatic circulation), but others are excreted in the stool. In addition, some T_4 and T_3 pass directly from the circulation to the intestinal lumen. The iodide lost by these routes amounts to about 4% of the total daily iodide loss.

FLUCTUATIONS IN DEIODINATION

Much more RT_3 and much less T_3 are formed during fetal life, and the ratio shifts to that of adults about 6 weeks after birth. Various drugs inhibit deiodinases, producing a fall in plasma T_3 levels and a reciprocal rise in RT_3. Selenium deficiency has the same effect. A wide variety of nonthyroidal illnesses also suppress deiodinases. These include burns, trauma, advanced cancer, cirrhosis, chronic kidney disease, myocardial infarction, and febrile states. The low-T_3 state produced by these conditions disappears with recovery.

Diet also has a clear-cut effect on conversion of T_4 to T_3. In fasted individuals, plasma T_3 is reduced by 10–20% within 24 h and by about 50% in 3–7 days, with a corresponding rise in RT_3 **(Figure 20–9)**. Free and bound T_4 levels remain essentially normal. During more prolonged starvation, RT_3 returns to normal but T_3 remains depressed. At the same time, the basal metabolic rate (BMR) falls and urinary nitrogen excretion, an index of protein breakdown, is

decreased. Thus, the decline in T_3 conserves calories and protein. Conversely, overfeeding increases T_3 and reduces RT_3.

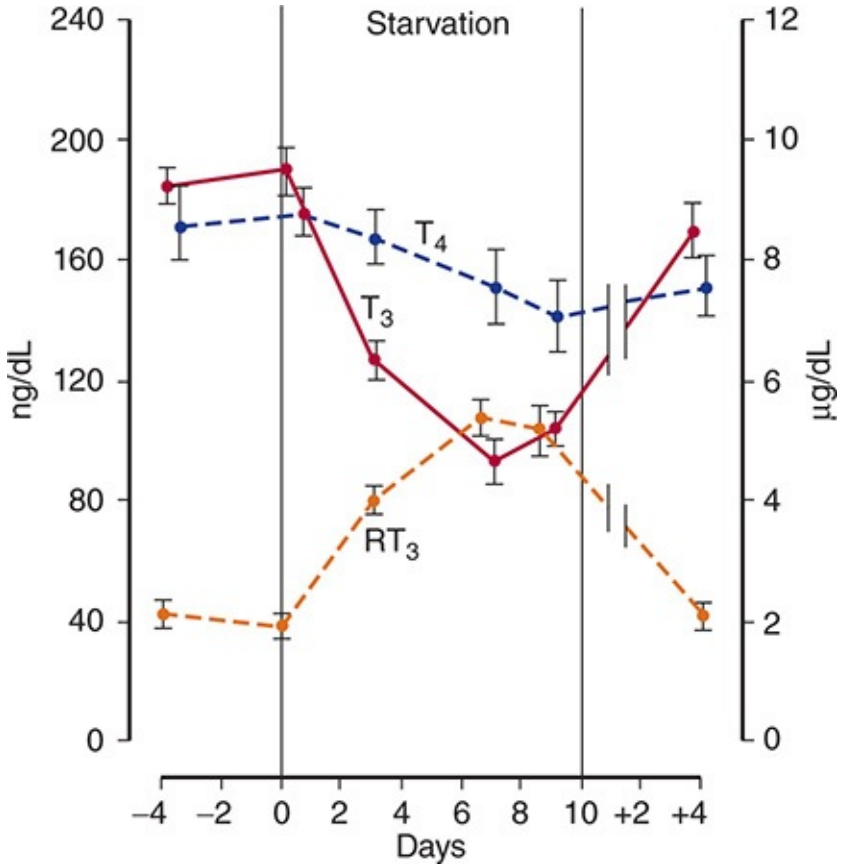


FIGURE 20–9 Effect of starvation on plasma levels of T_4, T_3, and RT_3 in humans. The scale for T_3 and RT_3 is on the left and the scale for T_4 is on the right. The most pronounced effect is a reduction in T_3 levels with a reciprocal rise in RT_3. The changes, which conserve calories by reducing tissue metabolism, are reversed promptly by refeeding. Similar changes occur in wasting diseases. (Reproduced with permission from Burger AG: New aspects of the peripheral action of thyroid hormones. Triangle 1983;22:175. Copyright © 1983 Sandoz Ltd., Basel, Switzerland.)

REGULATION OF THYROID SECRETION

Thyroid function is regulated primarily by variations in the circulating level of pituitary TSH (Figure 20–8). TSH secretion is increased by the hypothalamic hormone TRH (see Chapter 17) and inhibited in a negative feedback manner by

circulating free T_4 and T_3. The effect of T_4 is enhanced by production of T_3 in the cytoplasm of the pituitary cells by the D_2 they contain. TSH secretion is also inhibited by stress, and in experimental animals it is increased by cold and decreased by warmth.

CHEMISTRY & METABOLISM OF TSH

Human TSH is a glycoprotein made up of α and β subunits that become noncovalently linked in the pituitary thyrotropes. TSH-α is identical to the α subunit of luteinizing hormone, follicle-stimulating hormone, and human chorionic gonadotropin (hCG) (see Chapters 18 and 22). The functional specificity of TSH is therefore conferred by the β subunit.

The biologic half-life of human TSH is about 60 min. TSH is degraded for the most part in the kidneys and to a lesser extent in the liver. Secretion is pulsatile, and mean output starts to rise at about 9:00 PM, peaks at midnight, and then declines thereafter. The normal secretion rate is about 110 µg/day. The average plasma level is about 2 µg/mL.

Because the α subunit in hCG is the same as that in TSH, large amounts of hCG can activate TSH receptors nonspecifically. In some patients with benign or malignant tumors of placental origin, plasma hCG levels can rise so high that they produce mild hyperthyroidism.

EFFECTS OF TSH ON THE THYROID

When the pituitary is removed, thyroid function is depressed and the gland atrophies; when TSH is administered, thyroid function is stimulated. TSH acts via its receptor, a typical G-protein-coupled, seven-transmembrane receptor that activates adenylyl cyclase through G_s. It also activates phospholipase C (PLC). Within a few minutes after the injection of TSH, there are increases in iodide binding, synthesis of T_3, T_4, and iodotyrosines, secretion of thyroglobulin into the colloid, and endocytosis of colloid. Iodide trapping is increased in a few hours; blood flow increases; and, with long-term TSH treatment, the thyrocytes hypertrophy and the weight of the gland increases.

Whenever TSH stimulation is prolonged, the thyroid becomes detectably enlarged. Enlargement of the thyroid is called a **goiter.**

OTHER FACTORS AFFECTING THYROID GROWTH

In addition to TSH receptors, thyrocytes express receptors for insulin-like growth factor I (IGF-I) and epidermal growth factor (EGF). IGF-I and EGF promote growth, whereas interferon-γ and tumor necrosis factor-α inhibit growth. The effect of the cytokines implies that thyroid function might be inhibited in the setting of chronic inflammation, which could contribute to cachexia, or weight loss.

CONTROL MECHANISMS

The mechanisms regulating thyroid secretion are summarized in Figure 20–8. The negative feedback effect of thyroid hormones on TSH secretion is exerted in part at the hypothalamic level, but it is also due in large part to an action on the pituitary, since T_4 and T_3 block the increase in TSH secretion produced by TRH. Infusion of either T_4 or T_3 reduces circulating TSH, which declines measurably within 1 h. The day-to-day maintenance of thyroid secretion depends on the feedback interplay of thyroid hormones with TSH and TRH (Figure 20–8). The adjustments that appear to be mediated via TRH include the increased secretion of thyroid hormones produced by cold and, presumably, the decrease produced by heat. It is worth noting that although cold produces clear-cut increases in circulating TSH in human infants, the rise produced by cold in adults is negligible. Consequently, in adults, increased heat production due to increased thyroid hormone secretion **(thyroid hormone thermogenesis)** plays little if any role in the response to cold. Stress has an inhibitory effect on TRH secretion. Glucocorticoids also inhibit TSH secretion.

EFFECTS OF THYROID HORMONES

Some of the widespread effects of thyroid hormones in the body are secondary to stimulation of O_2 consumption **(calorigenic action),** although the hormones also affect growth and development in mammals, help regulate lipid metabolism, and increase the absorption of carbohydrates from the intestine **(Table 20–3)**. They also increase the dissociation of oxygen from hemoglobin by increasing red cell 2,3-diphosphoglycerate (DPG) (see Chapter 35).

TABLE 20–3 Physiologic effects of thyroid hormones.

Target Tissue	Effect	Mechanism
Heart	Chronotropic and inotropic	Increased number of β-adrenergic receptors Enhanced responses to circulating catecholamines Increased proportion of α-myosin heavy chain (with higher ATPase activity)
Adipose tissue	Catabolic	Stimulated lipolysis
Muscle	Catabolic	Increased protein breakdown
Bone	Developmental	Promote normal growth and skeletal development
Nervous system	Developmental	Promote normal brain development
Gut	Metabolic	Increased rate of carbohydrate absorption
Lipoprotein	Metabolic	Formation of LDL receptors
Other	Calorigenic	Stimulated oxygen consumption by metabolically active tissues (exceptions: testes, uterus, lymph nodes, spleen, anterior pituitary) Increased metabolic rate

Modified with permission from McPhee SJ, Lingarra VR, Ganong WF (editors): *Pathophysiology of Disease*, 6th ed. New York, NY: McGraw-Hill; 2010.

MECHANISM OF ACTION

Thyroid hormones enter cells and T_3 binds to thyroid hormone receptors **(TRs),** members of the superfamily of hormone-sensitive nuclear transcription factors. T_4 can also bind, but not as avidly. The hormone–receptor complex then

migrates to the nucleus and binds to DNA via zinc fingers and increases (or in some cases, decreases) the expression of a variety of different genes that code for proteins regulating cell function (see Chapters 1 and 16).

There are two human TR genes: an α receptor gene on chromosome 17 and a β receptor gene on chromosome 3. By alternative splicing, each forms at least two different mRNAs and therefore two different receptor proteins. TRβ2 is found only in the brain, but TRα1, TRα2, and TRβ1 are widely distributed. TRα2 differs from the other three in that it does not bind thyroid hormones due to a unique C-terminus, and may actually antagonize the activity of the other TRs. TRs bind to DNA as monomers, homodimers, and heterodimers with other nuclear receptors, particularly the retinoid X receptor **(RXR).** The TR/RXR heterodimer does not bind to 9-*cis* retinoic acid, the usual ligand for RXR, but TR binding to DNA is greatly enhanced in response to thyroid hormones when the receptor is in the form of this heterodimer. There are also coactivator and corepressor proteins that affect the actions of TRs. Presumably, this complexity underlies the ability of thyroid hormones to produce many different effects in the body.

In most of its actions, T_3 acts more rapidly and is three to five times more potent than T_4 **(Figure 20–10)**. This is because T_3 is less tightly bound to plasma proteins than is T_4, but binds more avidly to thyroid hormone receptors. As previously noted, RT_3 is inert.

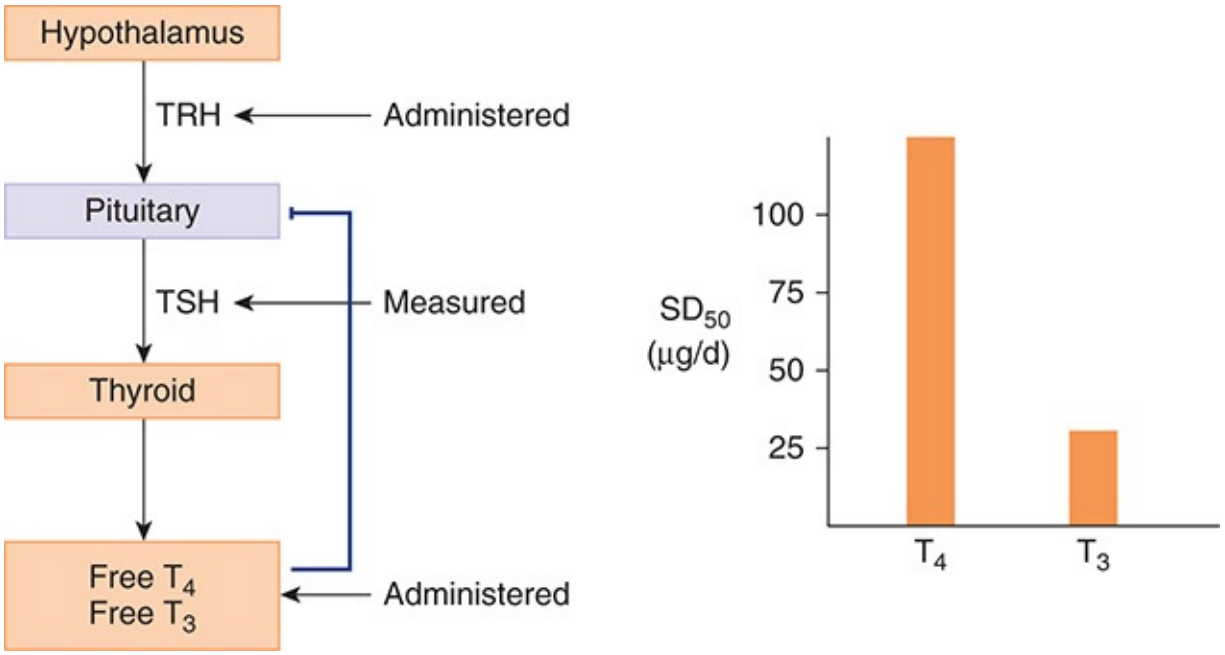


FIGURE 20–10 Experiment demonstrating the relative potency of T_4 and T_3. Healthy male volunteers were given TRH to stimulate TSH production in the absence or presence of various doses of T_3 or T_4. The left hand portion of the figure shows the relationships between the hormones that were administered or measured. On the right, the calculated doses of T_3 and T_4 needed to reduce the TSH response induced by TRH by 50% (SD_{50}) are shown. Note the substantially greater potency of T_3. (Data from Sawin CT, Hershman JM, Chopra IJ: The comparative effect of T_4 and T_3 on the TSH response to TRH in young adult men. J Clin Endo Metab 1977; 44:273–278.)

CALORIGENIC ACTION

T_4 and T_3 increase O_2 consumption by almost all metabolically active tissues. The exceptions are the adult brain, testes, uterus, lymph nodes, spleen, and anterior pituitary. T_4 actually depresses the O_2 consumption of the anterior pituitary, presumably because it inhibits TSH secretion. The increase in metabolic rate produced by a single dose of T_4 becomes measurable after a latent period of several hours and lasts 6 days or more.

Some of the calorigenic effect of thyroid hormones is due to metabolism of the fatty acids they mobilize. In addition, thyroid hormones increase the activity of the membrane-bound Na, K ATPase in many tissues.

EFFECTS SECONDARY TO CALORIGENESIS

When the metabolic rate is increased by T_4 and T_3 in adults, nitrogen excretion is increased; if food intake is not increased, endogenous protein and fat stores are catabolized and weight is lost. In hypothyroid children, small doses of thyroid hormones cause a positive nitrogen balance because they stimulate growth, but large doses cause protein catabolism similar to that produced in the adult. The potassium liberated during protein catabolism appears in the urine, and there is also an increase in urinary hexosamine and uric acid excretion.

When the metabolic rate is increased, the need for all vitamins is increased and vitamin deficiency syndromes may be precipitated. Thyroid hormones are necessary for hepatic conversion of carotene to vitamin A, and the accumulation of carotene in the bloodstream **(carotenemia)** in hypothyroidism is responsible

for the yellowish tint of the skin. Carotenemia can be distinguished from jaundice because in the former condition the sclera are not yellow.

The skin normally contains a variety of proteins combined with polysaccharides, hyaluronic acid, and chondroitin sulfuric acid. In hypothyroidism, these complexes accumulate, promoting water retention and the characteristic puffiness of the skin (myxedema). When thyroid hormones are administered, the proteins are metabolized, and diuresis continues until the myxedema is cleared.

Milk secretion is decreased in hypothyroidism and stimulated by thyroid hormones, a fact sometimes put to practical use in the dairy industry. Thyroid hormones do not stimulate the metabolism of the uterus but are essential for normal menstrual cycles and fertility.

EFFECTS ON THE CARDIOVASCULAR SYSTEM

Large doses of thyroid hormones cause enough extra heat production to lead to a slight rise in body temperature (Chapter 17), which in turn activates heat-dissipating mechanisms. Peripheral resistance decreases because of cutaneous vasodilation, and this increases levels of renal Na^+ and water absorption, expanding blood volume. Cardiac output is increased by the direct action of thyroid hormones, as well as that of catecholamines, on the heart, so that pulse pressure and cardiac rate are increased and circulation time is shortened.

T_3 is not formed from T_4 in cardiac myocytes, but circulating T_3 enters myocytes, combines with its receptors, and enters the nucleus, where the complex promotes the expression of some genes and inhibits the expression of others. Those that are enhanced include the genes for α-myosin heavy chain, sarcoplasmic reticulum Ca^{2+} ATPase, β-adrenergic receptors, G-proteins, Na, K ATPase, and certain K^+ channels. Those that are inhibited include the genes for β-myosin heavy chain, phospholamban, two types of adenylyl cyclase, TRs, and NCX, the Na^+–Ca^{2+} exchanger. The net result is increased heart rate and force of contraction.

The two myosin heavy chain (MHC) isoforms, α-MHC and β-MHC, produced by the heart are encoded by two highly homologous genes located on the short arm of chromosome 17. Each myosin molecule consists of two heavy chains and two pairs of light chains (see Chapter 5). The myosin containing β-MHC has less ATPase activity than the myosin containing α-MHC. α-MHC predominates in the atria in adults, and its level is increased by treatment with

thyroid hormone. This increases the speed of cardiac contraction. Conversely, expression of the α-MHC gene is depressed and that of the β-MHC gene is enhanced in hypothyroidism.

EFFECTS ON THE NERVOUS SYSTEM

In hypothyroidism, mentation is slow and the cerebrospinal fluid (CSF) protein level is elevated. Thyroid hormones reverse these changes, and large doses cause rapid mentation, irritability, and restlessness. Overall, cerebral blood flow and glucose and O_2 consumption by the brain are normal in adult hypothyroidism and hyperthyroidism. However, thyroid hormones enter the brain in adults and are found in gray matter in numerous different locations. In addition, astrocytes in the brain convert T_4 to T_3, and there is a sharp increase in brain D_2 activity after thyroidectomy that is reversed within 4 h by a single intravenous dose of T_3. Some of the effects of thyroid hormones on the brain are probably secondary to increased responsiveness to catecholamines, with consequent increased activation of the reticular activating system (see Chapter 14). In addition, thyroid hormones have marked effects on brain development. The parts of the central nervous system (CNS) most affected are the cerebral cortex and the basal ganglia. In addition, the cochlea is also affected. Consequently, thyroid hormone deficiency during development causes mental retardation, motor rigidity, and deaf–mutism. Deficiencies in thyroid hormone synthesis secondary to a failure of thyrocytes to transport iodide presumably also contribute to deafness in Pendred syndrome, discussed above.

Thyroid hormones also exert effects on reflexes. The reaction time of stretch reflexes (see Chapter 12) is shortened in hyperthyroidism and prolonged in hypothyroidism. Measurement of the reaction time of the ankle jerk (Achilles reflex) once attracted attention as a clinical test for evaluating thyroid function, but this reaction time is also affected by other diseases and thus is not a specific assessment of thyroid activity.

RELATION TO CATECHOLAMINES

The actions of thyroid hormones and the catecholamines norepinephrine and epinephrine are intimately interrelated. Epinephrine increases the metabolic rate, stimulates the nervous system, and produces cardiovascular effects similar to those of thyroid hormones, although the duration of these actions is brief.

Norepinephrine has generally similar actions. The toxicity of the catecholamines is markedly increased in rats treated with T_4. Although plasma catecholamine levels are normal in hyperthyroidism, the cardiovascular effects, tremulousness, and sweating that are seen in the setting of excess thyroid hormones can be reduced or abolished by sympathectomy. They can also be reduced by drugs such as propranolol that block β-adrenergic receptors. Indeed, propranolol and other β-blockers are used extensively in the treatment of thyrotoxicosis and in the treatment of the severe exacerbations of hyperthyroidism called **thyroid storms.** However, even though β-blockers can weakly inhibit extrathyroidal conversion of T_4 to T_3, and consequently may produce a small fall in plasma T_3, they have little effect on the other actions of thyroid hormones. Presumably, the functional synergism observed between catecholamines and thyroid hormones, particularly in pathologic settings, arises from their overlapping biologic functions as well as the ability of thyroid hormones to increase expression of catecholamine receptors and the signaling effectors to which they are linked.

EFFECTS ON SKELETAL MUSCLE

Muscle weakness occurs in most patients with hyperthyroidism **(thyrotoxic myopathy),** and when the hyperthyroidism is severe and prolonged, the myopathy may be severe. The muscle weakness may be due in part to increased protein catabolism. Thyroid hormones affect the expression of the MHC genes in skeletal as well as cardiac muscle (see Chapter 5). However, the effects produced are complex and their relation to the myopathy is not established. Hypothyroidism is also associated with muscle weakness, cramps, and stiffness.

EFFECTS ON CARBOHYDRATE METABOLISM

Thyroid hormones increase the rate of absorption of carbohydrates from the gastrointestinal tract, an action that is probably independent of their calorigenic action. In hyperthyroidism, therefore, the plasma glucose level rises rapidly after a carbohydrate meal, sometimes exceeding the renal threshold. However, it falls again at a rapid rate.

EFFECTS ON CHOLESTEROL METABOLISM

Thyroid hormones lower circulating cholesterol levels. The plasma cholesterol

level drops before the metabolic rate rises, which indicates that this action is independent of the stimulation of O_2 consumption. The decrease in plasma cholesterol concentration is due to increased formation of low-density lipoprotein (LDL) receptors in the liver, resulting in increased hepatic removal of cholesterol from the circulation. Despite considerable effort, however, it has not been possible to produce a clinically useful thyroid hormone analog that lowers plasma cholesterol without increasing metabolism.

EFFECTS ON GROWTH

Thyroid hormones are essential for normal growth and skeletal maturation (see Chapter 21). In hypothyroid children, bone growth is slowed and epiphysial closure delayed. In the absence of thyroid hormones, growth hormone secretion is also depressed. This further impairs growth and development, since thyroid hormones normally potentiate the effect of growth hormone on tissues.

DISORDERS OF THYROID FUNCTION

Based on the foregoing, some of the causes and effects of either under- or overactive thyroid function (hypo- and hyperthyroid conditions, respectively) should be predictable **(Clinical Boxes 20–1** and **20–2)**. Altered regulation of thyroid-responsive physiological systems can also be seen in the setting of thyroid hormone resistance, most commonly attributed to mutations in TRβ **(Clinical Box 20–3)**.

The amount of thyroid hormone required to maintain normal function in thyroidectomized individuals is defined as the amount necessary to return plasma TSH to normal. Indeed, measurement of TSH is regarded as one of the best tests of thyroid function. The amount of T_4 that normalizes plasma TSH in individuals lacking a functional thyroid gland (**athyreotic)** averages 112 µg of T_4 by mouth per day in adults. About 80% of this dose is absorbed from the gastrointestinal tract. It produces a slightly greater than normal level of free T_4 but a normal level of free T_3, indicating that circulating T_3 may be the principal feedback regulator of TSH secretion in humans.

CLINICAL BOX 20–1

Reduced Thyroid Function

The syndrome of adult **hypothyroidism** is generally called **myxedema,** although this term is also used to refer specifically to the skin changes in the syndrome. Hypothyroidism may be the end result of a number of diseases of the thyroid gland, or it may be secondary to pituitary or hypothalamic failure. In the latter two conditions, the thyroid remains able to respond to TSH. Thyroid function may be reduced by a number of conditions **(Table 20–4)**. For example, when the dietary iodine intake falls below 50 µg/day, thyroid hormone synthesis is inadequate and secretion declines. As a result of increased TSH secretion, the thyroid hypertrophies, producing an **iodine deficiency goiter** that may become very large. Such "endemic goiters" have been substantially reduced by the practice of adding iodide to table salt. Drugs may also inhibit thyroid function. Most do so either by interfering with the iodide-trapping mechanism or by blocking the organic binding of iodine. In either case, TSH secretion is stimulated by the decline in circulating thyroid hormones, and a goiter is produced. Paradoxically, another substance that inhibits thyroid function under certain conditions is iodide itself. In normal individuals, large doses of iodide act directly on the thyroid to produce a mild and transient inhibition of organic binding of iodide and hence of hormone synthesis. This inhibition is known as the **Wolff–Chaikoff effect.**

In completely athyreotic adults, the BMR falls to about 40%. The hair is coarse and sparse, the skin is dry and yellowish (carotenemia), and cold is poorly tolerated. Mentation is slow, memory is poor, and in some patients there are severe mental symptoms ("myxedema madness"). Plasma cholesterol is elevated. Children who are hypothyroid from birth or before are called **cretins.** They are dwarfed and mentally retarded. Worldwide, congenital hypothyroidism is one of the most common causes of preventable mental retardation. The main causes are included in Table 20–4. They include not only maternal iodine deficiency and various congenital abnormalities of the fetal hypothalamo–pituitary–thyroid axis, but also maternal antithyroid antibodies that cross the placenta and damage the fetal thyroid. T_4 also crosses the placenta, and unless the mother is hypothyroid, growth and development are normal until birth. If treatment is started at birth, the prognosis for normal growth and development is good, and mental retardation can generally be avoided; for this reason, screening tests for congenital hypothyroidism are becoming routine. When the mother is hypothyroid as well, as in the case of iodine deficiency, the mental deficiency is more severe and less responsive to treatment after birth. It has been estimated that 20

million people in the world now have various degrees of brain damage caused by iodine deficiency in utero.

Uptake of tracer doses of radioactive iodine can be used to assess thyroid function (contrast this with the use of large doses to ablate thyroid tissue in cases of hyperthyroidism (Clinical Box 20–2)).

THERAPEUTIC HIGHLIGHTS

The treatment of hypothyroidism depends on the underlying mechanisms. Iodide deficiency can be addressed by adding it to the diet, as is done routinely in developed countries. In congenital hypothyroidism, levothyroxine—a synthetic form of the thyroid hormone T_4—can be given. It is important that this take place as soon as possible after birth, with levels regularly monitored, to minimize long-term adverse effects.

TABLE 20–4 Causes of congenital hypothyroidism.

Maternal iodine deficiency
Fetal thyroid dysgenesis
Inborn errors of thyroid hormone synthesis
Maternal antithyroid antibodies that cross the placenta
Fetal hypopituitary hypothyroidism

CLINICAL BOX 20–2

Hyperthyroidism

The symptoms of an overactive thyroid gland follow logically from the actions of thyroid hormone discussed in this chapter. Thus, hyperthyroidism is characterized by nervousness; weight loss; hyperphagia; heat intolerance; increased pulse pressure; a fine tremor of the outstretched fingers; warm, soft skin; sweating; and a BMR from +10 to as high as +100. It has various causes (Table 20–5); however, the most common cause is **Graves disease (Graves hyperthyroidism),** which accounts for 60–80% of the cases. This is an autoimmune disease, more common in women, in which antibodies to the TSH receptor stimulate the receptor. This produces marked T_4 and T_3

secretion and enlargement of the thyroid gland (goiter). However, due to the feedback effects of T_4 and T_3, plasma TSH is low, not high. Another hallmark of Graves disease is the occurrence of swelling of tissues in the orbits, producing protrusion of the eyeballs **(exophthalmos).** This occurs in 50% of patients and often precedes the development of obvious hyperthyroidism. Other antithyroid antibodies are present in Graves disease, including antibodies to thyroglobulin and thyroid peroxidase. In Hashimoto thyroiditis, autoimmune antibodies and infiltrating cytotoxic T cells ultimately destroy the thyroid, but during the early stage the inflammation of the gland causes excess thyroid hormone secretion and thyrotoxicosis similar to that seen in Graves disease.

THERAPEUTIC HIGHLIGHTS

Some of the symptoms of hyperthyroidism can be controlled by the **thioureylenes.** These are a group of compounds related to thiourea, which inhibit the iodination of monoiodotyrosine and block the coupling reaction. The two used clinically are propylthiouracil and methimazole. Iodination of tyrosine is inhibited because propylthiouracil and methimazole compete with tyrosine residues for iodine and become iodinated. In addition, propylthiouracil but not methimazole inhibits D_2 deiodinase, reducing the conversion of T_4 to T_3 in many extrathyroidal tissues. In severe cases, hyperthyroidism can also be treated by the infusion of radioactive iodine, which accumulates in the gland and then partially destroys it. Surgery is also considered if the thyroid becomes so large that it affects swallowing and/or breathing.

TABLE 20–5 Causes of hyperthyroidism.

Thyroid overactivity
Graves disease
Solitary toxic adenoma
Toxic multinodular goiter
Early stages of Hashimoto thyroiditis[a]
TSH-secreting pituitary tumor
Mutations causing constitutive activation of TSH receptor
Other rare causes

Extrathyroidal

Administration of T_3 or T_4 (factitious or iatrogenic hyperthyroidism)

Ectopic thyroid tissue

[a]Note that ultimately the thyroid will be destroyed in Hashimoto disease, resulting in hypothyroidism. Many patients only present after they become hypothyroid, and do not recall a transient phase of hyperthyroidism.

CLINICAL BOX 20–3

Thyroid Hormone Resistance

Some mutations in the gene that codes for TRβ are associated with resistance to the effects of T_3 and T_4. Most commonly, there is resistance to thyroid hormones in the peripheral tissues and the anterior pituitary gland. Patients with this abnormality are usually not clinically hypothyroid, because they maintain plasma levels of T_3 and T_4 that are high enough to overcome the resistance, and hTRα is unaffected. However, plasma TSH is inappropriately high relative to the high circulating T_3 and T_4 levels and is difficult to suppress with exogenous thyroid hormone. Some patients have thyroid hormone resistance only in the pituitary. They have hypermetabolism and elevated plasma T_3 and T_4 levels with normal, nonsuppressible levels of TSH. A few patients apparently have peripheral resistance with normal pituitary sensitivity. They have hypometabolism despite normal plasma levels of T_3, T_4, and TSH. An interesting finding is that **attention deficit hyperactivity disorder,** a condition frequently diagnosed in children who are overactive and impulsive, is much more common in individuals with thyroid hormone resistance than in the general population. This suggests that hTRβ may play a special role in brain development.

THERAPEUTIC HIGHLIGHTS

Most patients remain euthyroid in this condition, even in the face of a goiter. It is important to consider thyroid hormone resistance in the differential diagnosis of Graves disease to avoid the inappropriate use of antithyroid medications or

even thyroid ablation. Isolated peripheral resistance to thyroid hormones can be treated by supplying large doses of synthetic T_4 exogenously. These are sufficient to overcome the resistance and increase the metabolic rate.

CHAPTER SUMMARY

- The thyroid gland straddles the trachea in the front of the neck and consists of multiple acini (follicles). Each follicle is surrounded by a single layer of epithelial cells and is filled with colloid, consisting predominantly of thyroglobulin. When the gland is active, areas where the colloid is being reabsorbed are visible (reabsorption lacunae).
- The gland transports and fixes iodide to amino acids present in thyroglobulin to generate the thyroid hormones thyroxine (T_4) and triiodothyronine (T_3). Iodide is transported into the follicle epithelial cells via the Na^+/I^- symporter (NIS) and from there to the colloid via the chloride/iodide exchanger, pendrin. Thyroid peroxidase converts iodide to iodine then incorporates this into the carbon 3 position of tyrosine residues in thyroglobulin in the process of organification.
- Thyroid hormones circulate in the plasma predominantly in protein-bound forms. Only the free hormones are biologically active, and both feed back to reduce secretion of TSH.
- Synthesis and secretion of thyroid hormones is stimulated by thyroid-stimulating hormone (TSH) from the pituitary, which in turn is released in response to thyrotropin-releasing hormone (TRH) from the hypothalamus. These releasing factors are controlled by changes in whole body status (eg, exposure to cold or stress).
- Thyroid hormones exert their effects by entering cells and binding to thyroid receptors. The liganded forms of thyroid receptors are nuclear transcription factors that alter gene expression.
- Thyroid hormones stimulate metabolic rate, calorigenesis, cardiac function, and normal mentation, and interact synergistically with catecholamines. Thyroid hormones also play critical roles in development, particularly of the nervous system, and growth.
- Disease results with both underactivity and overactivity of the thyroid gland. Hypothyroidism is accompanied by mental and physical slowing in adults,

and by mental retardation and dwarfism if it occurs in neonatal life. Overactivity of the thyroid gland, which most commonly is caused by autoantibodies that trigger secretion (Graves disease), results in body wasting, nervousness, and tachycardia.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. A 40-year-old woman comes to her primary care clinician complaining of nervousness and an unexplained weight loss of 20 pounds over the past 3 months despite her impression that she is eating all the time. On physical examination, her eyes are found to be protruding, her skin is moist and warm, and her fingers have a slight tremor. Compared to a healthy individual, a biopsy of her thyroid gland would most likely reveal which of the following:

   A. Decreased numbers of reabsorption lacunae

   B. Decreased evidence of endocytosis

   C. A decrease in the cross-sectional area occupied by colloid

   D. Increased levels of NIS in the basolateral membrane of thyrocytes

   E. Decreased evidence of lysosomal activity

2. Which of the following is *not* essential for normal biosynthesis of thyroid hormones?

   A. Iodine

   B. Ferritin

   C. Thyroglobulin

   D. Protein synthesis

   E. TSH

3. The enzyme primarily responsible for the conversion of T_4 to T_3 in the periphery is

   A. D_1 thyroid deiodinase.

   B. D_2 thyroid deiodinase.

   C. D_3 thyroid deiodinase.

   D. thyroid peroxidase.

   E. None of the above.

4. Increasing intracellular I^- due to the action of NIS is an example of

A. endocytosis.

B. passive diffusion.

C. Na^+ and K^+ cotransport.

D. primary active transport.

E. secondary active transport.

5. The metabolic rate is *least* affected by an increase in the plasma level of

A. TSH.

B. TRH.

C. TBG.

D. Free T_4.

E. Free T_3.

6. In which of the following conditions is it *most* likely that the TSH response to TRH will be reduced?

A. Hypothyroidism due to tissue resistance to thyroid hormone

B. Hypothyroidism due to disease destroying the thyroid gland

C. Hyperthyroidism due to circulating antithyroid antibodies with TSH activity

D. Hyperthyroidism due to diffuse hyperplasia of thyrotropes of the anterior pituitary

E. Iodine deficiency

7. Hypothyroidism due to disease of the thyroid gland is associated with increased plasma levels of

A. cholesterol.

B. albumin.

C. RT_3.

D. iodide.

E. TBG.

8. A young woman has puffy skin and a hoarse voice. Her plasma TSH concentration is low but increases markedly when she is given TRH. She probably has

A. hyperthyroidism due to a thyroid tumor.

B. hypothyroidism due to a primary abnormality in the thyroid gland.

C. hypothyroidism due to a primary abnormality in the pituitary gland.

D. hypothyroidism due to a primary abnormality in the hypothalamus.
E. hyperthyroidism due to a primary abnormality in the hypothalamus.

9. Which of the following would be *least* affected by injections of TSH?
A. Thyroidal uptake of iodine
B. Synthesis of thyroglobulin
C. Cyclic adenosine monophosphate (cAMP) in thyroid cells
D. Cyclic guanosine monophosphate (cGMP) in thyroid cells
E. Size of the thyroid

10. Thyroid hormone receptors bind to DNA in which of the following forms?
A. A heterodimer with the prolactin receptor
B. A heterodimer with the growth hormone receptor
C. A heterodimer with the retinoid X receptor
D. A heterodimer with the insulin receptor
E. A heterodimer with the progesterone receptor

CHAPTER 21

Hormonal Control of Calcium & Phosphate Metabolism & the Physiology of Bone

OBJECTIVES

After studying this chapter, you should be able to:

- Understand the importance of maintaining homeostasis of body calcium and phosphate concentrations.
- Describe the body pools of calcium, their rates of turnover, and the organs that play central roles in regulating movement of calcium between stores.
- Delineate the mechanisms of calcium and phosphate absorption and excretion.
- Identify the major hormones—vitamin D, parathyroid hormone, and calcitonin—and other factors that regulate calcium and phosphate homeostasis, their sites of synthesis, targets of their action, and consequences of dysfunction.
- Define the basic anatomy of bone and understand how linear bone growth is arrested after puberty.
- Delineate the cell types that regulate bone formation and resorption and their mechanism of action, and discuss diseases that result from abnormalities in bone homeostasis.

INTRODUCTION

Calcium is an essential intracellular signaling molecule and also plays a variety of extracellular functions, thus the control of body calcium concentrations is vitally important. The components of the system that maintains calcium homeostasis include cell types that sense changes in extracellular calcium and release calcium-regulating hormones, and the targets of these hormones, including the kidneys, bones, and intestine, that respond with changes in calcium mobilization, excretion, or uptake. Three hormones are primarily concerned with the regulation of calcium homeostasis. **1,25-Dihydroxycholecalciferol** is a steroid hormone formed from vitamin D by successive hydroxylations in the liver and kidneys. Its primary action is to increase calcium absorption from the intestine. **Parathyroid hormone (PTH)** is secreted by the parathyroid glands. Its main action is to mobilize calcium from bone and increase urinary phosphate excretion. **Calcitonin,** a calcium-lowering hormone that in mammals is secreted primarily by cells in the thyroid gland, inhibits bone resorption. Although the role of calcitonin seems to be relatively minor, all three hormones probably operate in concert to maintain the constancy of the calcium level in the body fluids. Phosphate homeostasis is likewise critical to normal body function, particularly given its inclusion in adenosine triphosphate (ATP), its role as a biologic buffer, and its role as a modifier of proteins, thereby altering their functions. Many of the systems that regulate calcium homeostasis also contribute to that of phosphate, albeit sometimes in a reciprocal manner, and thus will also be discussed in this chapter.

CALCIUM & PHOSPHORUS BALANCE IN THE BODY

CALCIUM

The body of a young adult human contains about 1100 g (27.5 moles) of calcium. Ninety-nine percent of the calcium is in the skeleton. Plasma calcium, normally at a concentration of around 10 mg/dL (5 mEq/L, 2.5 mmol/L), is partly bound to protein and partly diffusible **(Table 21–1)**. The distribution of calcium inside the cells and the role of Ca^{2+} as a second messenger molecule are discussed in Chapter 2.

TABLE 21–1 Distribution (mg/dL) of calcium in normal human plasma.

Total diffusible		**5.36**
Ionized (Ca^{2+})	4.72	
Complexed to HCO_3^-, citrate, etc	0.64	
Total nondiffusible (protein-bound)		**4.64**
Bound to albumin	3.68	
Bound to globulin	0.96	
Total plasma calcium		**10.00**

It is the free, ionized calcium (Ca^{2+}) in the body fluids that is a vital second messenger and is necessary for blood coagulation, muscle contraction, and nerve function. A decrease in extracellular Ca^{2+} exerts a net excitatory effect on nerve and muscle cells in vivo (see Chapters 4 and 5). The result is **hypocalcemic tetany,** which is characterized by extensive spasms of skeletal muscle, involving especially the muscles of the extremities and the larynx. Laryngospasm can become so severe that the airway is obstructed and fatal asphyxia is produced. Ca^{2+} also plays an important role in blood clotting (see Chapter 31), but in vivo, fatal tetany would occur before the clotting reaction would be compromised.

Because the extent of Ca^{2+} binding by plasma proteins is proportional to the plasma protein level, it is important to know the plasma protein level when evaluating the total plasma calcium. Other electrolytes and pH also affect the free Ca^{2+} level. Thus, for example, symptoms of tetany can occur at higher total calcium levels if the patient hyperventilates, thereby increasing plasma pH. Plasma proteins are more ionized when the pH is high, providing more protein anions to bind with and sequester free Ca^{2+}.

The calcium in bone is of two types: a readily exchangeable reservoir and a much larger pool of stable calcium that is only slowly exchangeable. Two independent but interacting homeostatic systems affect the calcium in bone. One is the system that regulates plasma Ca^{2+}, providing for the movement of about 500 mmol of Ca^{2+} per day into and out of the readily exchangeable pool in the bone **(Figure 21–1)**. The other system involves bone remodeling by the constant interplay of bone resorption and deposition (see following text). However, the Ca^{2+} interchange between plasma and this stable pool of bone calcium is only about 7.5 mmol/d.

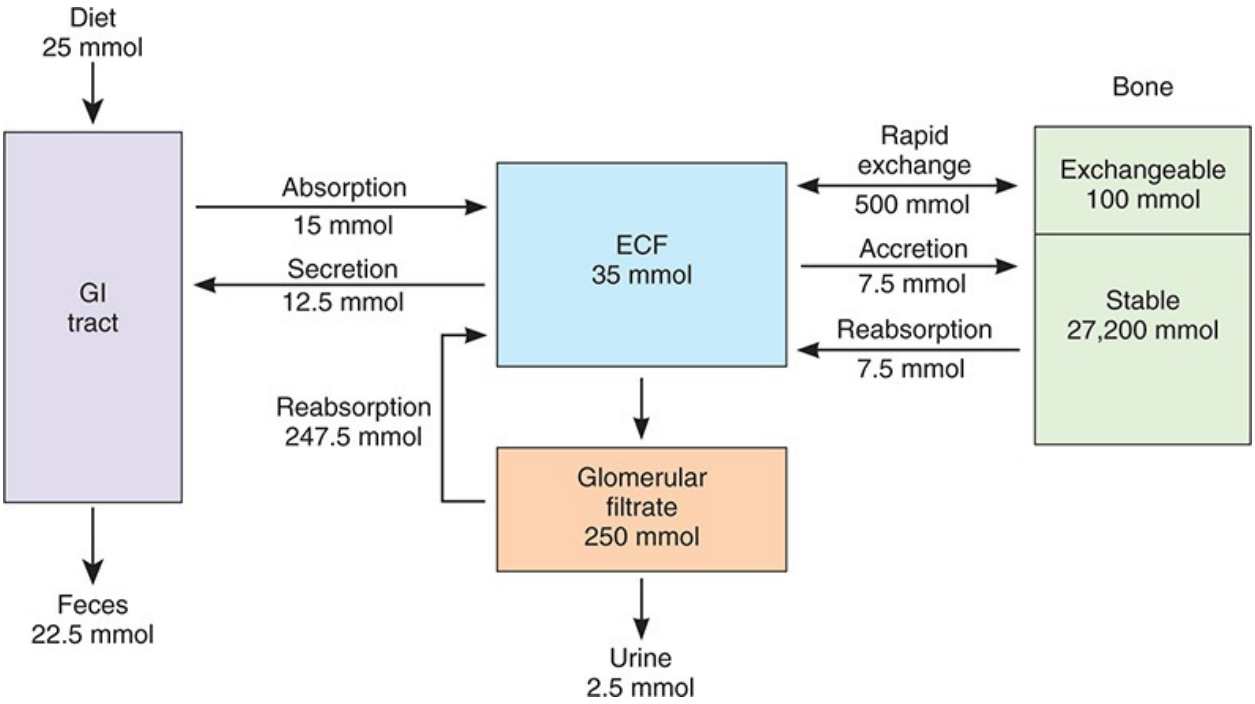


FIGURE 21–1 Calcium metabolism in an adult human. A typical daily dietary intake of 25 mmol Ca^{2+} (1000 mg) moves through many body compartments. Note that the majority of body calcium is in bones, in a pool that is only slowly exchangeable with the extracellular fluid (ECF).

Ca^{2+} is taken up into the body across the brush border of intestinal epithelial cells via channels known as transient receptor potential vanilloid type 6 (TRPV6) and binds to an intracellular protein known as calbindin-D_{9k}. Calbindin-D_{9k} sequesters the absorbed calcium so that it does not disturb epithelial signaling processes that involve calcium. The absorbed Ca^{2+} is thereby delivered to the basolateral membrane of the epithelial cell, from where it can be transported into the bloodstream by either a Na^+/Ca^{2+} exchanger (NCX1) or the Ca^{2+}-Mg^{2+} ATPase, $PMCA_{1b}$ **(Figure 21-2)**. Nevertheless, it should be noted that recent studies indicate that some intestinal Ca^{2+} uptake persists even in the absence of TRPV6 and calbindin-D_{9k}, suggesting that additional pathways are likely also involved in this critical process. The overall transport process is regulated by 1,25-dihydroxycholecalciferol (see below). As Ca^{2+} uptake rises, moreover, 1,25-dihydroxycholecalciferol levels fall in response to increased plasma Ca^{2+}.

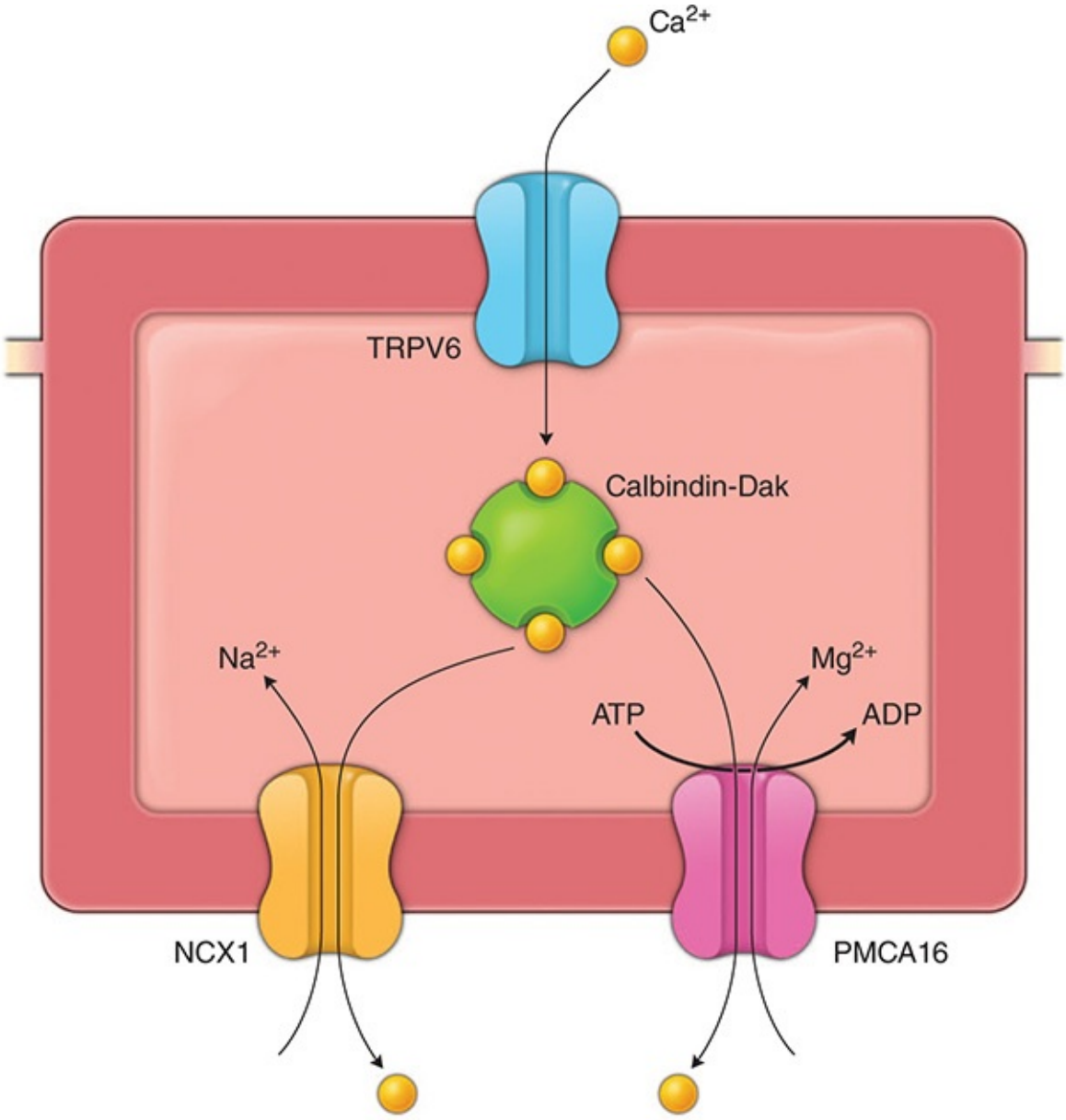


FIGURE 21–2 Intestinal absorption of calcium. Calcium is taken up across the enterocyte apical membrane via TRPV6 calcium channels. It is sequestered in the cytosol by calbindin-D_{9k} and trafficked to the basolateral membrane, from which it is exported to the bloodstream by either NCX1 (taking advantage of the low intracellular sodium concentration to drive calcium efflux) or $PMCA_{1b}$, with expenditure of cellular energy. The expression levels of TRPV6, calbindin-D_{9k}, and $PMCA_{1b}$ are all upregulated by 1,25-dihydroxycholecalciferol.

Plasma Ca^{2+} is filtered in the kidneys, but 98–99% of the filtered Ca^{2+} is reabsorbed. About 60% of the reabsorption occurs in the proximal tubules and

the remainder in the ascending limb of the loop of Henle and the distal tubule. Distal tubular reabsorption depends on the TRPV5 channel, whose expression is regulated by PTH.

PHOSPHORUS

Phosphate is found in ATP, cyclic adenosine monophosphate (cAMP), 2,3-diphosphoglycerate, many proteins, and other vital compounds in the body. Phosphorylation and dephosphorylation of proteins are involved in the regulation of cell function (see Chapter 2). Therefore, it is not surprising that, like calcium, phosphate metabolism is closely regulated. Total body phosphorus is 500–800 g (16.1–25.8 moles), 85–90% of which is in the skeleton. Total plasma phosphorus is about 12 mg/dL, with two-thirds of this total in organic compounds and the remainder as inorganic phosphorus (P_i) (mostly in PO_4^{3-}, HPO_4^{2-}, and $H_2PO_4^-$). The amount of phosphorus normally entering bone is about 3 mg (97 µmol)/kg/d, with an equal amount leaving via resorption.

P_i in the plasma is filtered in the glomeruli, and 85–90% of the filtered P_i is reabsorbed. Active transport in the proximal tubule accounts for most of the reabsorption and involves two related sodium-dependent P_i cotransporters, NaPi-IIa and NaPi-IIc. NaPi-IIa is powerfully inhibited by PTH, which causes its internalization and degradation and thus a reduction in renal P_i reabsorption (see below).

P_i is absorbed in the duodenum and small intestine. Uptake occurs by a transporter related to those in the kidney, NaPi-IIb, that takes advantage of the low intracellular Na^+ concentration established by the Na, K ATPase on the basolateral membrane of intestinal epithelial cells to load P_i against its concentration gradient. However, the pathway by which P_i exits into the bloodstream is not known. Many stimuli that increase Ca^{2+} absorption, including 1,25-dihydroxycholecalciferol, also increase P_i absorption via increased NaPi-IIb expression and/or its insertion into the enterocyte apical membrane.

VITAMIN D & THE HYDROXYCHOLECALCIFEROLS

CHEMISTRY

The active transport of Ca^{2+} and PO_4^{3-} from the intestine is increased by a metabolite of **vitamin D.** The term "vitamin D" is used to refer to a group of closely related sterols produced by the action of ultraviolet light on certain provitamins **(Figure 21–3)**. Vitamin D_3, which is also called cholecalciferol, is produced in the skin of mammals from 7-dehydrocholesterol by the action of sunlight. The reaction involves the rapid formation of previtamin D_3, which is then converted more slowly to vitamin D_3. Vitamin D_3 and its hydroxylated derivatives are transported in the plasma bound to vitamin D-binding protein (DBP). Vitamin D_3 is also ingested in the diet.

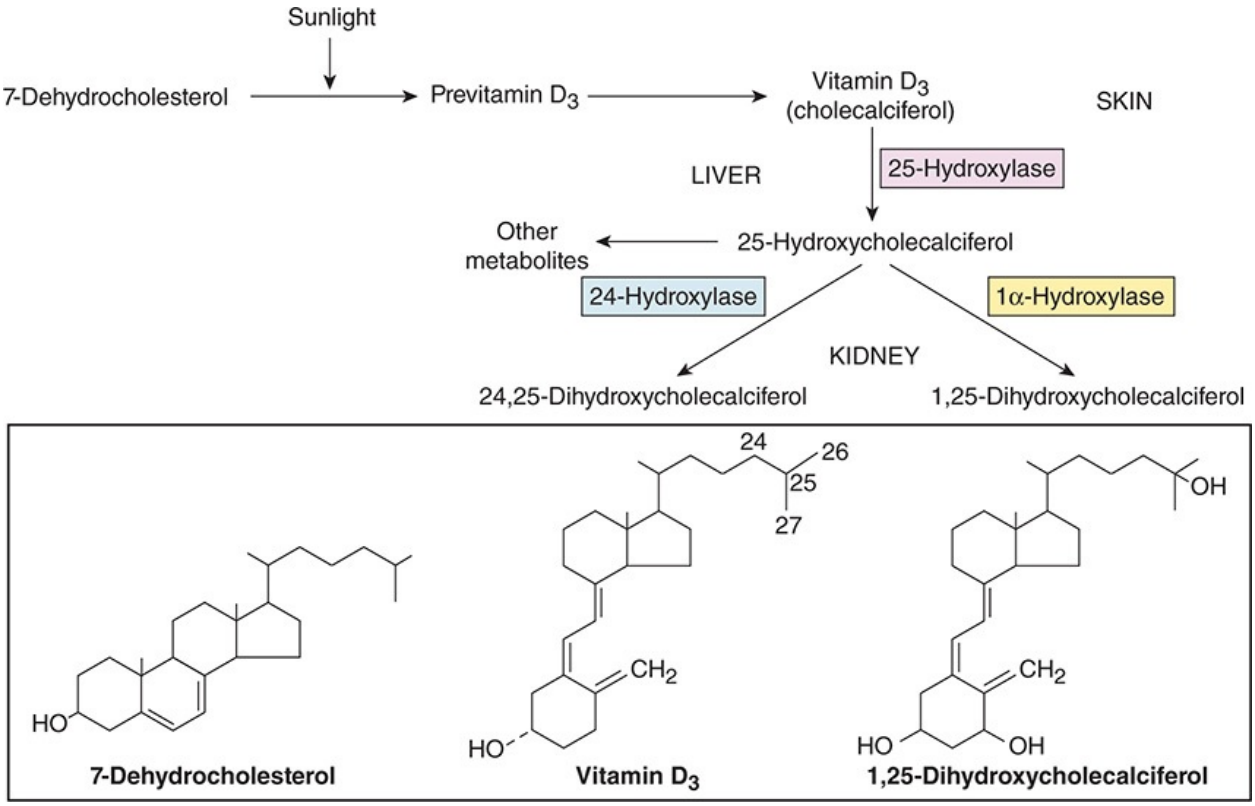


FIGURE 21–3 Formation and hydroxylation of vitamin D_3. 25-Hydroxylation takes place in the liver, and the other hydroxylations occur primarily in the kidneys. The structures of 7-dehydrocholesterol, vitamin D_3, and 1,25-dihydroxycholecalciferol are also shown in the boxed area.

Vitamin D_3 is metabolized by enzymes that are members of the cytochrome P450 (CYP) superfamily (see Chapters 1 and 28). In the liver, vitamin D_3 is converted to **25-hydroxycholecalciferol** (calcidiol, 25-(OH)D_3). The 25-

hydroxycholecalciferol is further converted in the cells of the proximal tubules of the kidneys to the more active metabolite **1,25-dihydroxycholecalciferol,** which is also called calcitriol or 1,25-$(OH)_2D_3$. 1,25-Dihydroxycholecalciferol is also made in the placenta, in keratinocytes in the skin, and in macrophages. The normal plasma level of 25-hydroxycholecalciferol is about 30 ng/mL, and that of 1,25-dihydroxycholecalciferol is about 0.03 ng/mL (approximately 100 pmol/L). The less active metabolite 24,25-dihydroxycholecalciferol is also formed in the kidneys (Figure 21–3).

MECHANISM OF ACTION

1,25-Dihydroxycholecalciferol stimulates the expression of a number of gene products involved in Ca^{2+} transport and handling via its receptor (VDR), which acts as a transcriptional regulator in its ligand-bound form. One target is the family of **calbindin-D** proteins. These are members of the troponin C superfamily of Ca^{2+}-binding proteins that also includes calmodulin (see Chapter 2). Calbindin-Ds are found in human intestine, brain, and kidneys. In the intestinal epithelium and many other tissues, two calbindins are induced: calbindin-D_{9K} and calbindin-D_{28K}, with molecular weights of 9000 and 28,000, respectively. 1,25-Dihydroxycholecalciferol also increases the number of Ca^{2+}–ATPase and TRPV6 molecules in the intestinal cells, and thus, the overall capacity for absorption of dietary calcium.

In addition to increasing Ca^{2+} absorption from the intestine, 1,25-dihydroxycholecalciferol facilitates Ca^{2+} reabsorption in the kidneys via increased TRPV5 expression in the proximal tubules, increases the synthetic activity of osteoblasts, and is necessary for normal calcification of matrix **(Clinical Box 21–1)**. The stimulation of osteoblasts brings about a secondary increase in the activity of osteoclasts (see below).

REGULATION OF SYNTHESIS

The formation of 25-hydroxycholecalciferol does not appear to be stringently regulated. However, the formation of 1,25- dihydroxycholecalciferol in the kidneys, which is catalyzed by the renal 1α-hydroxylase, is regulated in a feedback manner by plasma Ca^{2+} and PO_4^{3+} **(Figure 21–4)**. When the plasma Ca^{2+} level is high, little 1,25-dihydroxycholecalciferol is produced, and the

kidneys produce the relatively inactive metabolite 24,25-dihydroxycholecalciferol instead. This effect of Ca^{2+} on production of 1,25-dihydroxycholecalciferol thereby reduces the signal for Ca^{2+} absorption from the intestine (see previous text). Conversely, when the plasma Ca^{2+} level is low, PTH secretion is increased, and expression of 1α-hydroxylase is stimulated by PTH. The production of 1,25-dihydroxycholecalciferol is also increased by low plasma PO_4^{3-} levels and inhibited by high plasma PO_4^{3-} levels, by a direct inhibitory effect of PO_4^{3-} on the 1α-hydroxylase. Additional control of 1,25-dihydroxycholecalciferol formation results from its direct negative feedback effect on 1α-hydroxylase activity, a positive feedback action on the formation of 24,25-dihydroxycholecalciferol, and a direct action on the parathyroid gland to inhibit PTH expression.

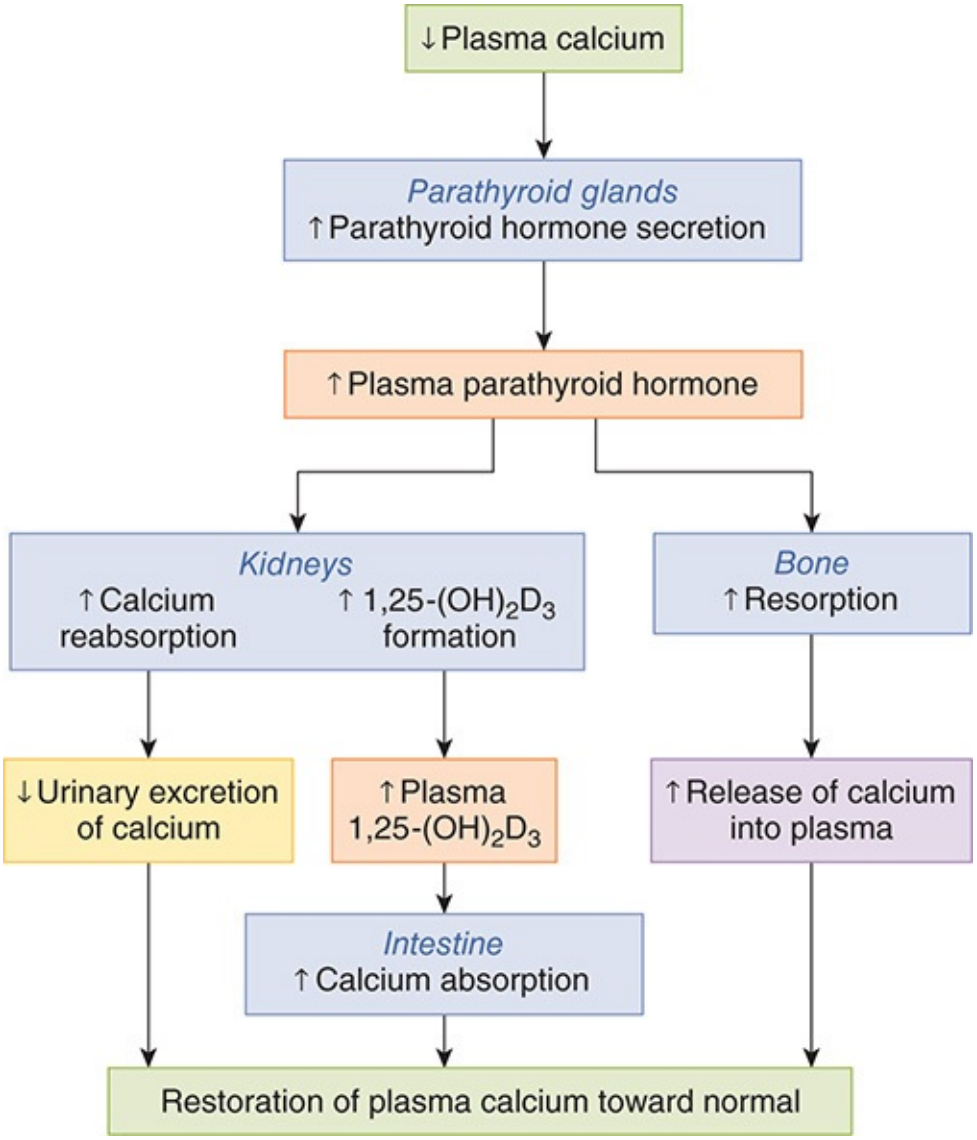


FIGURE 21–4 Effects of PTH and 1,25-dihydroxycholecalciferol on whole body calcium homeostasis. A reduction in plasma calcium stimulates parathyroid hormone secretion. PTH in turn causes calcium conservation and production of 1,25-dihydroxycholecalciferol in the kidneys, the latter of which increases calcium uptake in the intestine. PTH also releases calcium from the readily exchangeable pool in the bone. All of these actions act to restore normal plasma calcium. (Reproduced with permission from Widmaier EP, Raff H, Strang KT: *Vander's Human Physiology*, 10th ed. New York, NY: McGraw-Hill; 2006.)

CLINICAL BOX 21–1

Rickets & Osteomalacia

Vitamin D deficiency causes defective calcification of bone matrix and the disease called **rickets** in children and **osteomalacia** in adults. Even though 1,25-dihydroxycholecalciferol is necessary for normal mineralization of bone matrix, the main defect in this condition is failure to deliver adequate amounts of Ca^{2+} and PO_4^{3-} to the sites of mineralization. The full-blown condition in children is characterized by weakness and bowing of weight-bearing bones, dental defects, and hypocalcemia. In adults, the condition is less obvious. It used to be most commonly due to inadequate exposure to the sun in smoggy cities, but now it is more commonly due to inadequate intake of the provitamins on which the sun acts in the skin. These cases respond to administration of vitamin D. The condition can also be caused by inactivating mutations of the gene for renal 1α-hydroxylase, or in severe kidney or liver diseases, in which case there is no response to vitamin D but a normal response to 1,25- dihydroxycholecalciferol **(type I vitamin D–resistant rickets).** In rare instances, it can be due to inactivating mutations of the gene for the VDR **(type II vitamin D–resistant rickets),** in which case there is a deficient response to both vitamin D and 1,25-dihydroxycholecalciferol.

THERAPEUTIC HIGHLIGHTS

Treatment of these conditions depends on the underlying biochemical basis, as indicated above. Routine supplementation of milk with vitamin D has greatly reduced the occurrence of rickets in Western countries, but the condition remains among the most common childhood diseases in developing countries. Orthopedic surgery may be necessary in severely affected children.

An “anti-aging” protein called α-Klotho has also recently been discovered to play important roles in calcium and phosphate homeostasis, in part by reciprocal effects on 1,25- dihydroxycholecalciferol levels. Mice deficient in α-Klotho displayed accelerated aging, decreased bone mineral density, calcifications, and hypercalcemia and hyperphosphatemia. α-Klotho stabilizes the membrane localization of proteins important in calcium and phosphate (re)absorption, such as TRPV5 and Na, K ATPase. Likewise, it enhances the activity of another factor, fibroblast growth factor 23 (FGF23), at its receptor. FGF23 thereby decreases renal NaPi-IIa and NaPi-IIc expression and inhibits the production of 1α-hydroxylase, reducing levels of 1,25-dihydroxycholecalciferol.

THE PARATHYROID GLANDS

ANATOMY

Humans usually have four parathyroid glands: two embedded in the superior poles of the posterior thyroid and two in its inferior poles **(Figure 21–5)**. Each parathyroid gland is a richly vascularized disk, about 3 × 6 × 2 mm, containing two distinct types of cells **(Figure 21–6)**. The abundant **chief cells,** which contain a prominent Golgi apparatus plus endoplasmic reticulum and secretory granules, synthesize and secrete **PTH.** The less abundant and larger **oxyphil cells** contain oxyphil granules and large numbers of mitochondria in their cytoplasm. In humans, few oxyphil cells are seen before puberty, and thereafter they increase in number with age. Their function is unknown. Consequences of loss of the parathyroid glands are discussed in **Clinical Box 21–2**.

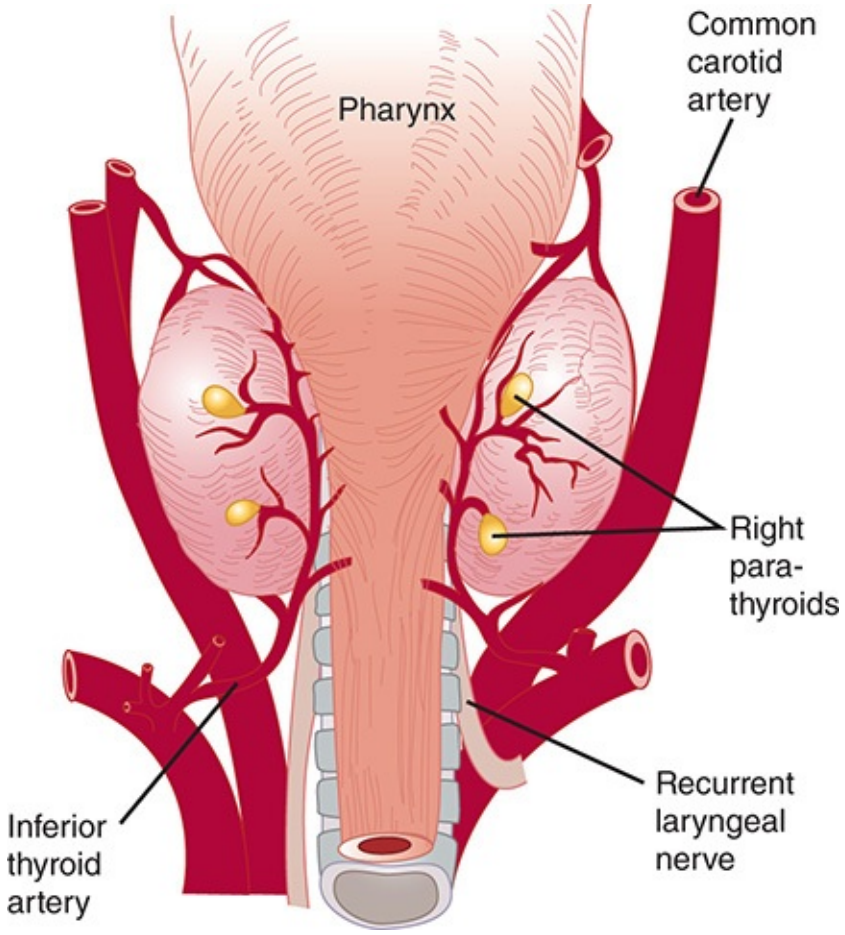


FIGURE 21–5 The human parathyroid glands, viewed from behind. The glands are small structures adherent to the posterior surface of the thyroid gland.

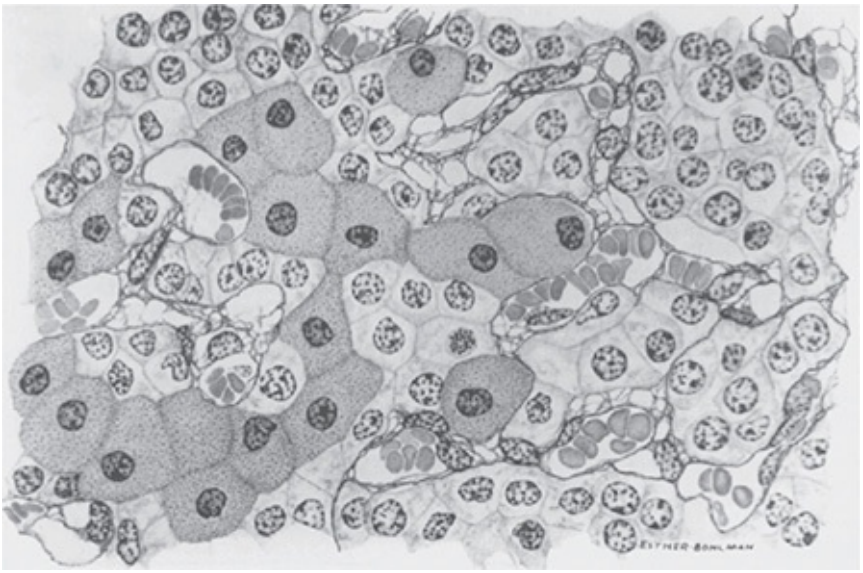


FIGURE 21–6 Section of human parathyroid. (Reduced 50% from ×960.) Small cells are chief cells; large stippled cells (especially prominent in the lower left of picture) are oxyphil cells. (Reproduced with permission from Fawcett DW: *Bloom and Fawcett, A Textbook of Histology*, 11th ed. Saunders, 1986.)

CLINICAL BOX 21–2

Effects of Parathyroidectomy

Occasionally, inadvertent parathyroidectomy occurs in humans during thyroid surgery. This can have serious consequences as PTH is essential for life. After parathyroidectomy, there is a steady decline in the plasma Ca^{2+} level. Signs of neuromuscular hyperexcitability appear, followed by full-blown hypocalcemic tetany (see text). Plasma phosphate levels usually rise as the plasma Ca^{2+} level falls. Symptoms usually develop 2–3 days postoperatively but may not appear for several weeks or more. The signs of tetany in humans include **Chvostek sign,** a quick contraction of the ipsilateral facial muscles elicited by tapping over the facial nerve at the angle of the jaw, and **Trousseau sign,** a spasm of the muscles of the upper extremity that causes flexion of the wrist and thumb with extension of the fingers. In individuals with mild tetany in whom spasm is not yet evident, Trousseau sign can sometimes be produced by occluding the circulation for a few minutes with a blood pressure cuff.

THERAPEUTIC HIGHLIGHTS

Treatment centers around replacing the PTH that would normally be produced by the missing glands. Injections of PTH can be given to correct the chemical abnormalities, and the symptoms then disappear. Injections of Ca^{2+} salts can also give temporary relief.

SYNTHESIS & METABOLISM OF PTH

Human PTH is a linear polypeptide with a molecular weight of 9500 that contains 84 amino acid residues **(Figure 21–7)**. It is synthesized as part of a larger molecule containing 115 amino acid residues **(preproPTH).** On entry of preproPTH into the endoplasmic reticulum, a leader sequence is removed from the amino terminal to form the 90-amino-acid polypeptide **proPTH.** Six additional amino acid residues are removed from the amino terminal of proPTH in the Golgi apparatus, and the 84-amino-acid polypeptide PTH is packaged in secretory granules and released as the main secretory product of the chief cells.

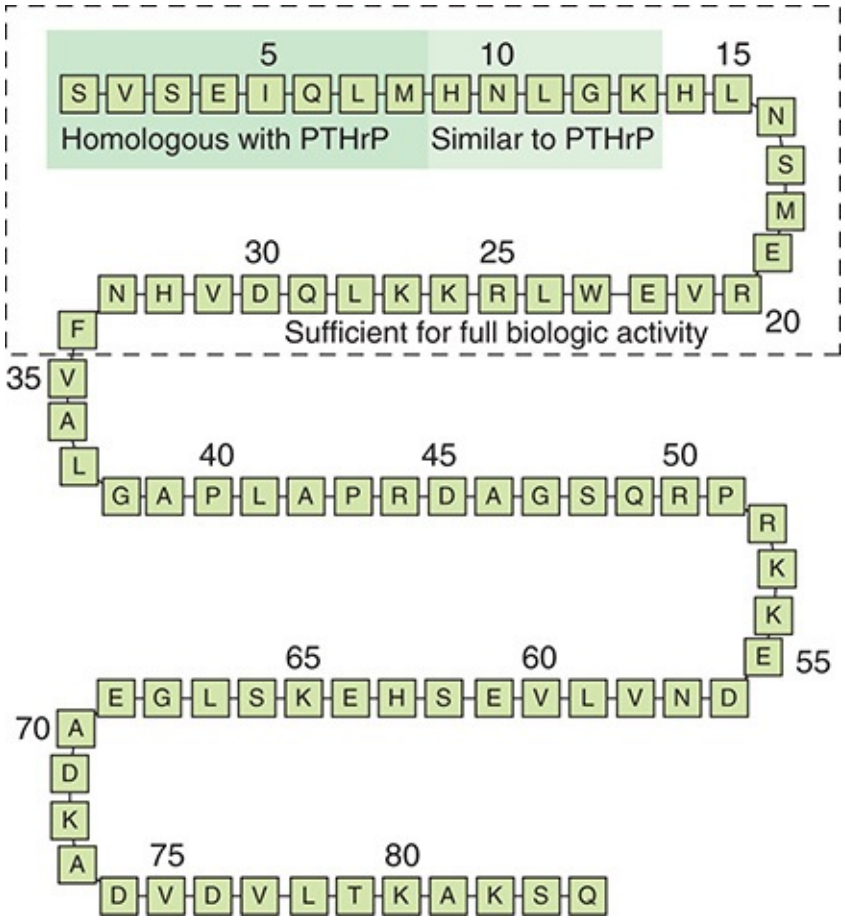


FIGURE 21–7 Parathyroid hormone. The solid boxes show residues that are identical to, or similar to, those at the N-terminus of parathyroid hormone–related peptide (PTHrP). The dashed box indicates that the first 34 amino acids of PTH are sufficient for full biologic activity. Interestingly, this is also the case for PTHrP, although after the first 13 amino acids, its sequence is divergent from that of PTH.

The normal plasma level of intact PTH is 10–55 pg/mL. The half-life of PTH is approximately 10 min, and the secreted polypeptide is rapidly cleaved by Kupffer cells in the liver into inactive fragments. PTH and these fragments are then cleared by the kidneys. Currently used immunoassays for PTH are designed only to measure mature PTH (ie, 84 amino acids) and not its fragments in order to obtain an accurate measure of “active” PTH.

ACTIONS

PTH acts directly on bone to increase bone resorption and mobilize Ca^{2+}. In

addition to increasing plasma Ca^{2+}, PTH increases phosphate excretion in the urine and thereby depresses plasma phosphate levels. This **phosphaturic action** is due to a decrease in reabsorption of phosphate via effects on NaPi-IIa in the proximal tubules, as discussed previously. PTH also increases reabsorption of Ca^{2+} in the distal tubules, although Ca^{2+} excretion in the urine is often increased in hyperparathyroidism because the increase in the load of filtered calcium overwhelms the effect on reabsorption **(Clinical Box 21–3)**. PTH also increases the formation of 1,25- dihydroxycholecalciferol, and this increases Ca^{2+} absorption from the intestine. On a longer time scale, PTH stimulates both osteoblasts and osteoclasts.

MECHANISM OF ACTION

It now appears that there are at least three different PTH receptors. One also binds parathyroid hormone–related protein (PTHrP; see below) and is known as the hPTH/PTHrP receptor. A second receptor, PTH2 (hPTH2-R), does not bind PTHrP and is found in the brain, placenta, and pancreas. In addition, there is evidence for a third receptor, CPTH, which reacts with the carboxyl terminal rather than the amino terminal of PTH. The first two receptors are coupled to G_s, and via this heterotrimeric G-protein they activate adenylyl cyclase, increasing intracellular cAMP. The hPTH/PTHrP receptor also activates PLC via G_q, increasing intracellular Ca^{2+} concentrations and activating protein kinase C **(Figure 21–8)**. However, the way these second messengers affect Ca^{2+} in bone is unsettled.

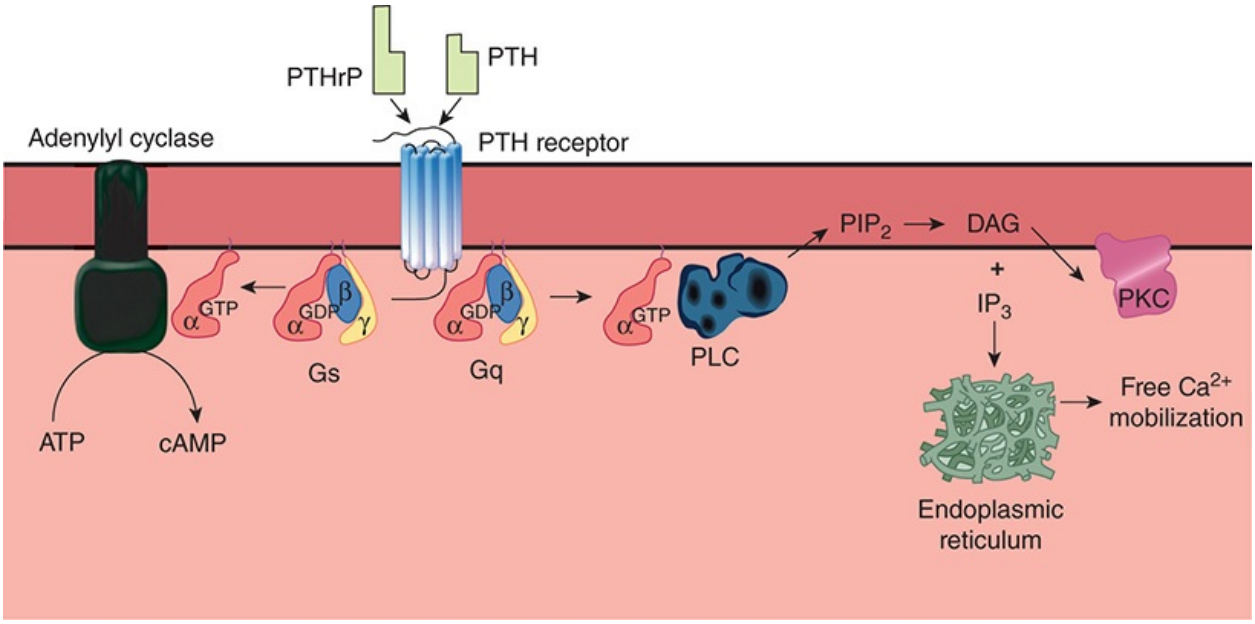


FIGURE 21–8 Signal transduction pathways activated by PTH or PTHrP binding to the hPTH/PTHrP receptor. Intracellular cAMP is increased via G_s and adenylyl cyclase (AC). Diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3) are increased via G_q and phospholipase C (PLC). DAG and IP_3 activate protein kinase C (PKC) and mobilization of calcium from the endoplasmic reticulum.

CLINICAL BOX 21–3

Diseases of Parathyroid Excess

Hyperparathyroidism due to hypersecretion from a functioning parathyroid tumor in humans is characterized by hypercalcemia and hypophosphatemia. Humans with PTH-secreting adenomas are usually asymptomatic, with the condition detected when plasma Ca^{2+} is measured in conjunction with a routine physical examination. However, there may be minor changes in personality, and calcium-containing kidney stones occasionally form. In conditions such as chronic kidney disease and rickets, in which the plasma Ca^{2+} level is chronically low, the associated stimulation of the parathyroid glands causes compensatory parathyroid hypertrophy and secondary hyperparathyroidism. The plasma Ca^{2+} level is low in chronic kidney disease primarily because the diseased kidneys lose the ability to form 1,25-dihydroxycholecalciferol. Finally, mutations in the Ca^{2+}-sensing receptor

gene *CASR* cause predictable long-term changes in plasma Ca^{2+}. Individuals heterozygous for inactivating mutations have familial benign hypocalciuric hypercalcemia, a condition in which there is a chronic moderate elevation in plasma Ca^{2+} because the feedback inhibition of PTH secretion by Ca^{2+} is reduced. Plasma PTH levels are normal or even elevated. However, children who are homozygous for inactivating mutations develop neonatal severe primary hyperparathyroidism. Conversely, individuals with gain-of-function mutations in the *CASR* gene develop familial hypercalciuric hypocalcemia due to increased sensitivity of the parathyroid glands to plasma Ca^{2+}.

THERAPEUTIC HIGHLIGHTS

Subtotal parathyroidectomy is sometimes necessary in patients in whom parathyroid adenoma or hyperplasia with associated hypercalcemia and resulting symptoms develops. However, because parathyroid disease is often benign or only slowly progressing, surgery remains controversial in most patients and is typically reserved for those who have experienced life-threatening complications of hypercalcemia.

In the disease called **pseudohypoparathyroidism,** the signs and symptoms of hypoparathyroidism develop but the circulating level of PTH is normal or even elevated. Because tissues fail to respond to the hormone, this is a receptor disease. There are two forms. In the more common form, a congenital 50% reduction of the activity of G_s occurs and PTH fails to produce a normal increase in cAMP concentration. In a different, less common form, the cAMP response is normal but the phosphaturic action of the hormone is defective.

REGULATION OF SECRETION

Circulating Ca^{2+} acts directly on the parathyroid glands in a negative feedback manner to regulate the secretion of PTH. The key to this regulation is a cell membrane Ca^{2+} sensing receptor, CaSR. Activation of this G-protein-coupled receptor leads to phosphoinositide turnover in many tissues. In the parathyroid, its activation inhibits PTH secretion. In this way, when the plasma Ca^{2+} level is high, PTH secretion is inhibited and Ca^{2+} is deposited in the bones. When it is low, secretion is increased and Ca^{2+} is mobilized from the bones.

1,25-Dihydroxycholecalciferol acts directly on the parathyroid glands to decrease preproPTH mRNA. Increased plasma P_i stimulates PTH secretion by lowering plasma levels of free Ca^{2+} and inhibiting the formation of 1,25-dihydroxycholecalciferol. Magnesium is required to maintain normal parathyroid secretory responses. Impaired PTH release along with diminished target organ responses to PTH account for the hypocalcemia that occasionally occurs in magnesium deficiency (Clinical Boxes 21–2 and 21–3).

PTHrP

PTHrP, another protein with PTH activity, is produced by many different tissues in the body. PTHrP and PTH have marked homology at their amino terminal ends and they both bind to the hPTH/PTHrP receptor, yet their physiologic effects are very different. How is this possible when they bind to the same receptor? For one thing, PTHrP is primarily a paracrine factor, acting close to where it is produced. It may be that circulating PTH cannot reach at least some of these sites. Second, subtle conformational differences may be produced by binding of PTH versus PTHrP to their receptor, despite their structural similarities. Another possibility is action of one or the other hormone on additional, more selective receptors.

PTHrP has a marked effect on the growth and development of cartilage in utero. Mice in which both alleles of the PTHrP gene are knocked out have severe skeletal deformities and die soon after birth. In normal animals, on the other hand, PTHrP-stimulated cartilage cells proliferate and their terminal differentiation is inhibited. PTHrP is also expressed in the brain, where evidence indicates that it inhibits excitotoxic damage to developing neurons. In addition, there is evidence that it is involved in Ca^{2+} transport in the placenta. PTHrP is also found in keratinocytes in the skin, in smooth muscle, and in the teeth, where it is present in the enamel epithelium that caps each tooth. In the absence of PTHrP, teeth cannot erupt.

HYPERCALCEMIA OF MALIGNANCY

Hypercalcemia is a common metabolic complication of cancer. About 20% of hypercalcemic patients have bone metastases that produce hypercalcemia secondary to bone erosion **(local osteolytic hypercalcemia).** Evidence suggests that this erosion is produced by prostaglandins such as prostaglandin E_2 arising

from the tumor. Hypercalcemia in the remaining 80% of the patients is due to elevated circulating levels of PTHrP **(humoral hypercalcemia of malignancy).** The tumors responsible for this hypersecretion include cancers of the breast, kidney, ovary, and skin.

CALCITONIN

ORIGIN

In dogs, perfusion of the thyroparathyroid region with solutions containing high concentrations of Ca^{2+} leads to a fall in peripheral plasma Ca^{2+}, and after damage to this region, Ca^{2+} infusions cause a greater increase in plasma Ca^{2+} than they do in control animals. These and other observations led to the discovery that a Ca^{2+}-lowering as well as a Ca^{2+}-elevating peptide hormone was secreted by structures in the neck. This Ca^{2+}-lowering hormone has been named **calcitonin.** In mammals, calcitonin is produced by the **parafollicular cells** of the thyroid gland, which are also known as the clear or C cells.

SECRETION & METABOLISM

Calcitonin secretion is increased when the thyroid gland is exposed to a plasma calcium level of approximately 9.5 mg/dL. Above this level, plasma calcitonin is directly proportional to plasma calcium. β-Adrenergic agonists, dopamine, and estrogens also stimulate calcitonin secretion. Gastrin, cholecystokinin (CCK), glucagon, and secretin have also been reported to stimulate calcitonin secretion, with gastrin being the most potent stimulus (see Chapter 25). Thus, the plasma calcitonin level is elevated in Zollinger–Ellison syndrome and in pernicious anemia (see Chapter 25). However, the dose of gastrin needed to stimulate calcitonin secretion is supraphysiologic and not seen after eating in normal individuals, so dietary calcium in the intestine probably does not induce secretion of a calcium-lowering hormone prior to the calcium being absorbed. In any event, the actions of calcitonin are short-lived because it has a half-life of less than 10 min in humans.

ACTIONS

Receptors for calcitonin are found in bones and the kidneys. Calcitonin lowers

circulating calcium and phosphate levels. It exerts its calcium-lowering effect by inhibiting bone resorption. This action is direct, and calcitonin also inhibits the activity of osteoclasts in vitro. It also increases Ca^{2+} excretion in the urine.

The exact physiologic role of calcitonin is uncertain. The calcitonin content of the human thyroid is low, and after thyroidectomy, bone density and plasma Ca^{2+} level are normal as long as the parathyroid glands are intact. In addition, after thyroidectomy, there are only transient abnormalities of Ca^{2+} homeostasis when a Ca^{2+} load is injected. This may be explained in part by secretion of calcitonin from tissues other than the thyroid. However, there is general agreement that the hormone has little long-term effect on the plasma Ca^{2+} level in adult animals and humans. Further, unlike PTH and 1,25-dihydroxycholecalciferol, calcitonin does not appear to be involved in phosphate homeostasis. Moreover, patients with medullary carcinoma of the thyroid have a very high circulating calcitonin level but no symptoms directly attributable to the hormone, and their bones are essentially normal. No syndrome due to calcitonin deficiency has been described. More hormone is secreted in young individuals, and it may play a role in skeletal development. In addition, it may protect the bones of the mother from excess calcium loss during pregnancy. Bone formation in the infant and lactation are major drains on Ca^{2+} stores, and plasma concentrations of 1,25-dihydroxycholecalciferol are elevated in pregnancy. They would cause bone loss in the mother if bone resorption were not simultaneously inhibited by an increase in the plasma calcitonin level.

SUMMARY OF CALCIUM HOMEOSTATIC MECHANISMS

The actions of the three principal hormones that regulate the plasma concentration of Ca^{2+} can now be summarized. PTH increases plasma Ca^{2+} by mobilizing this ion from bone. It increases Ca^{2+} reabsorption in the kidney, but this may be offset by the increase in filtered Ca^{2+}. It also increases the formation of 1,25-dihydroxycholecalciferol. 1,25-Dihydroxycholecalciferol increases Ca^{2+} absorption from the intestine and increases Ca^{2+} reabsorption in the kidneys. Calcitonin inhibits bone resorption and increases the amount of Ca^{2+} in the urine.

EFFECTS OF OTHER HORMONES & HUMORAL

AGENTS ON CALCIUM METABOLISM

Calcium metabolism is affected by various hormones in addition to 1,25-dihydroxycholecalciferol, PTH, and calcitonin. **Glucocorticoids** lower plasma Ca^{2+} levels by inhibiting osteoclast formation and activity acutely, but over long periods they cause osteoporosis by decreasing bone formation and increasing bone resorption. They decrease bone formation by inhibiting protein synthesis in osteoblasts. They also decrease the absorption of Ca^{2+} and PO_4^{3-} from the intestine and increase the renal excretion of these ions. The decrease in plasma Ca^{2+} concentration also increases the secretion of PTH, and bone resorption is facilitated. **Growth hormone** increases Ca^{2+} excretion in the urine, but it also increases intestinal absorption of Ca^{2+}, and this effect may be greater than the effect on excretion, with a resultant positive calcium balance. Insulin-like growth factor I (IGF-I) generated by the action of growth hormone stimulates protein synthesis in bone. As noted previously, **thyroid hormones** may cause hypercalcemia, hypercalciuria, and, in some instances, osteoporosis. **Estrogens** prevent osteoporosis by inhibiting the stimulatory effects of certain cytokines on osteoclasts. **Insulin** increases bone formation, and there is significant bone loss in untreated diabetes.

BONE PHYSIOLOGY

Bone is a special form of connective tissue with a collagen framework impregnated with Ca^{2+} and PO_4^{3-} salts, particularly **hydroxyapatites,** which have the general formula $Ca_{10}(PO_4)_6(OH)_2$. Bone is also involved in overall Ca^{2+} and PO_4^{3-} homeostasis. It protects vital organs, and the rigidity it provides permits locomotion and the support of loads against gravity. Old bone is constantly being resorbed and new bone formed, permitting remodeling that allows it to respond to the stresses and strains that are put upon it. It is a living tissue that is well vascularized and has a total blood flow of 200–400 mL/min in adult humans.

STRUCTURE

There are two types of bone: **compact** or **cortical bone,** which makes up the outer layer of most bones **(Figure 21–9)** and accounts for 80% of the bone in the

body; and **trabecular** or **spongy bone** inside the cortical bone, which makes up the remaining 20% of bone in the body. In compact bone, the surface-to-volume ratio is low, and bone cells lie in lacunae. They receive nutrients by way of canaliculi that ramify throughout the compact bone (Figure 21–9). Trabecular bone is made up of spicules or plates, with a high surface to volume ratio and many cells sitting on the surface of the plates. Nutrients diffuse from bone extracellular fluid (ECF) into the trabeculae, but in compact bone, nutrients are provided via **haversian canals** (Figure 21–9), which contain blood vessels. Around each haversian canal, collagen is arranged in concentric layers, forming cylinders called **osteons** or **haversian systems.**

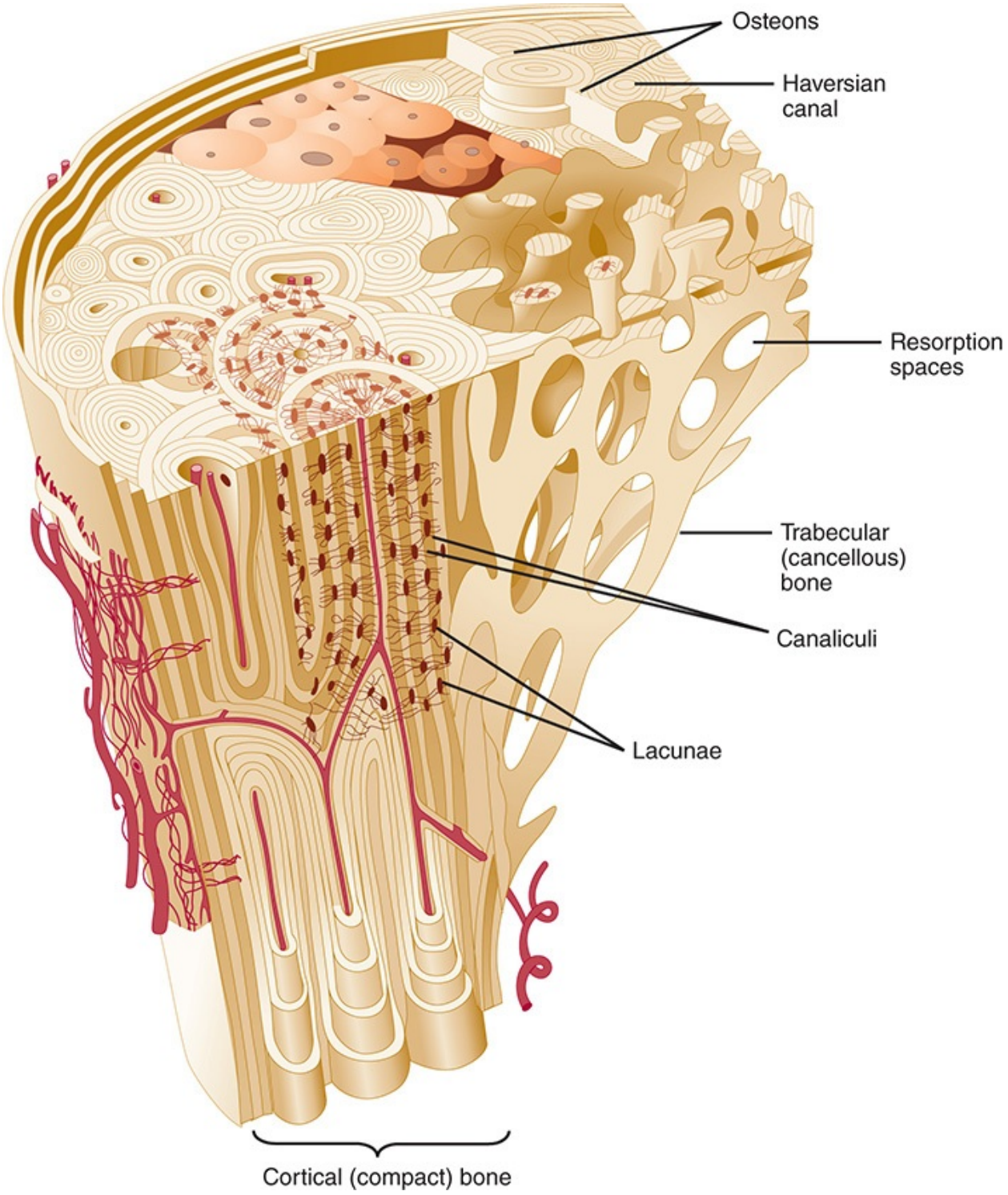


FIGURE 21–9 Structure of compact and trabecular bone. The compact bone is shown in horizontal section (top) and vertical section (bottom). (Reproduced with permission from Williams PL et al (editors): *Gray's Anatomy*, 37th ed. Churchill Livingstone; 1989.)

The protein in bone matrix is over 90% type I collagen, which is also the major structural protein in tendons and skin. This collagen, which weight for weight is as strong as steel, is made up of a triple helix of three polypeptides bound tightly together. Two of these are identical α_1 polypeptides encoded by one gene, and one is an α_2 polypeptide encoded by a different gene. Collagens make up a family of structurally related proteins that maintain the integrity of many different organs.

BONE GROWTH

During fetal development, most bones are modeled in cartilage and then transformed into bone by ossification **(enchondral bone formation).** The exceptions are the clavicles, the mandibles, and certain bones of the skull, in which mesenchymal cells form bone directly **(intramembranous bone formation).**

During growth, specialized areas at the ends of each long bone **(epiphyses)** are separated from the shaft of the bone by a plate of actively proliferating cartilage, the **epiphysial plate** **(Figure 21–10)**. The bone increases in length as this plate lays down new bone on the end of the shaft. The width of the epiphysial plate is proportional to the rate of growth. The width is affected by a number of hormones, but most markedly by growth hormone and IGF-I (see Chapter 18).

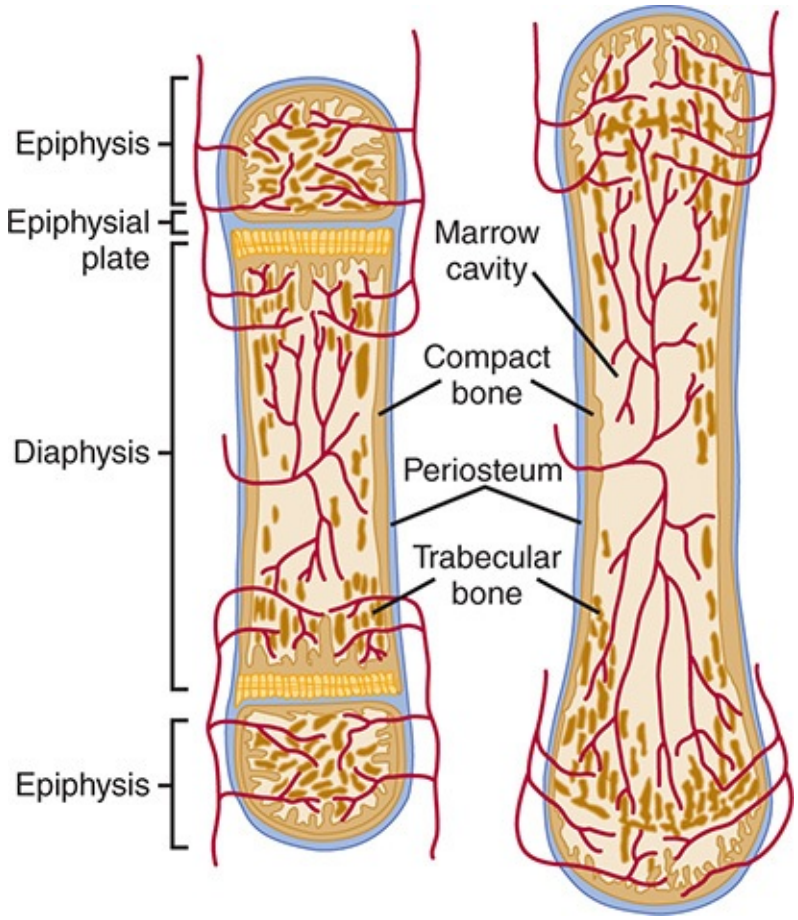


FIGURE 21–10 Structure of a typical long bone before (left) and after (right) epiphysial closure. Note the rearrangement of cells and growth of the bone as the epiphysial plate closes (see text for details).

Linear bone growth can occur as long as the epiphyses are separated from the shaft of the bone, but such growth ceases after the epiphyses unite with the shaft **(epiphysial closure)**. The cartilage cells stop proliferating, become hypertrophic, and secrete vascular endothelial growth factor (VEGF), leading to vascularization and ossification. The epiphyses of the various bones close in an orderly temporal sequence, the last epiphyses closing after puberty. The normal age at which each of the epiphyses closes is known, and the "bone age" of a young individual can be determined by radiographing the skeleton and noting which epiphyses are open and which are closed.

The **periosteum** is a dense fibrous, vascular, and innervated membrane that covers the surface of bones. This layer consists of an outer layer of collagenous tissue and an inner layer of fine elastic fibers containing cells that contribute to bone growth. The periosteum covers all surfaces of the bone except for those

capped with cartilage (eg, at the joints) and serves as a site of attachment of ligaments and tendons. As one ages, the periosteum becomes thinner and loses some of its vasculature. This renders bones more susceptible to injury and disease.

BONE FORMATION & RESORPTION

The cells responsible for bone formation are **osteoblasts** and the cells responsible for bone resorption are **osteoclasts.**

Osteoblasts are modified fibroblasts. Their initial development from the mesenchyme is the same as that of fibroblasts. Later, ossification-specific transcription factors, such as runt-related transcription factor 2 (Runx2; also known as core binding factor subunit alpha-1), contribute to their differentiation. The importance of this transcription factor in bone development is underscored in knockout mice deficient for the *RUNX2* gene. These mice develop to term with their skeletons made exclusively of cartilage; no ossification occurs. Normal osteoblasts, on the other hand, are able to lay down type 1 collagen and form new bone.

Osteoclasts are members of the monocyte family. Stromal cells in the bone marrow, osteoblasts, and T lymphocytes all express receptor activator for nuclear factor kappa beta ligand (RANKL) on their surface. When these cells come in contact with appropriate monocytes expressing RANK (ie, the RANKL receptor) two distinct signaling pathways are initiated: (1) There is a RANKL/RANK interaction between the cell pairs and (2) mononuclear phagocyte colony stimulating factor (M-CSF) is secreted by the nonmonocytic cells and it binds to its corresponding receptor on the monocytes (colony stimulating factor 1 receptor, CSF1R). The combination of these two signaling events leads to differentiation of the monocytes into osteoclasts. The precursor cells also secrete **osteoprotegerin (OPG),** which limits differentiation of the monocytes by competing with RANK for binding of RANKL.

Osteoclasts erode and absorb previously formed bone. They become attached to bone via integrins in a membrane extension called the **sealing zone.** This creates an isolated area (bone-resorbing compartment) between the bone and a portion of the osteoclast. Proton pumps (ie, H^+-dependent ATPases) then move from endosomes into the cell membrane apposed to the isolated area, and they acidify the area to approximately pH 4.0. Similar proton pumps are found in the endosomes and lysosomes of all eukaryotic cells, but in only a few other instances do they move into the cell membrane. Thus, the sealed-off space

formed by the osteoclast resembles a large lysosome. The acidic pH dissolves hydroxyapatite, and acid proteases secreted by the cell break down collagen, forming a shallow depression in the bone **(Figure 21–11)**. The products of bone digestion are then endocytosed and move across the osteoclast by transcytosis (see Chapter 2), with release into the interstitial fluid. The collagen breakdown products have pyridinoline structures, and pyridinolines can be measured in the urine as an index of the rate of bone resorption.

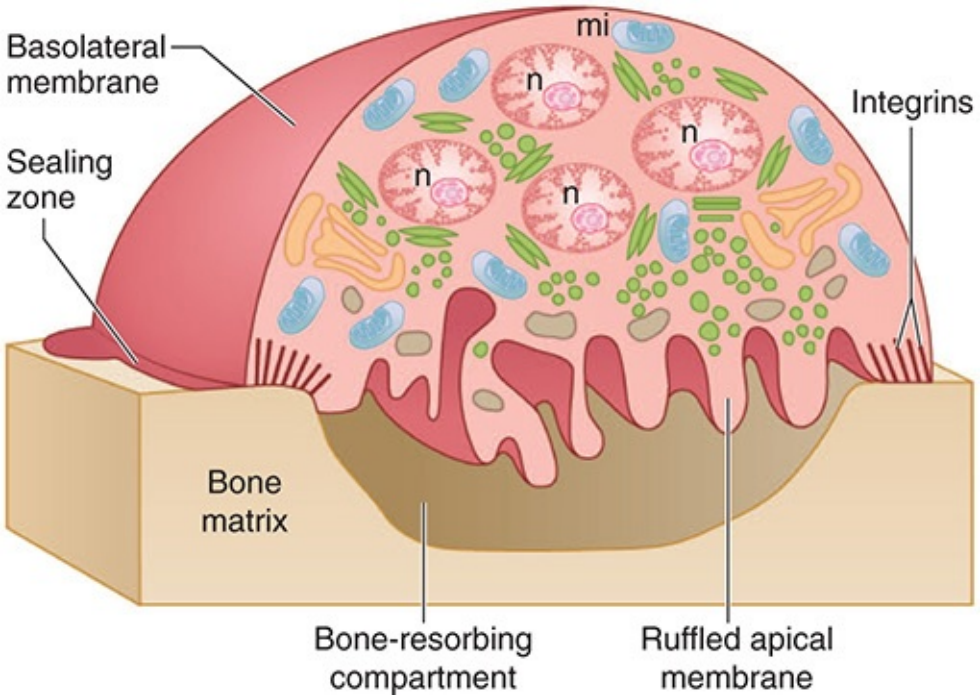


FIGURE 21–11 Osteoclast resorbing bone. The edges of the cell are tightly sealed to bone, permitting secretion of acid from the ruffled apical membrane and consequent erosion of the bone underneath the cell. Note the multiple nuclei (n) and mitochondria (mi). (Used with permission of R. Baron.)

Throughout life, bone is being constantly resorbed and new bone is being formed. The calcium in bone turns over at a rate of 100% per year in infants and 18% per year in adults. Bone remodeling is mainly a local process carried out in small areas by populations of cells called bone-remodeling units. First, osteoclasts resorb bone, and then osteoblasts lay down new bone in the same general area. This cycle takes about 100 days. The shape of bones may also change as bone is resorbed in one location but added in another. Osteoclasts tunnel into cortical bone followed by osteoblasts, whereas trabecular bone remodeling occurs on the surface of the trabeculae. About 5% of the bone mass

is being remodeled by about 2 million bone-remodeling units in the human skeleton at any one time. The renewal rate for bone is about 4% per year for compact bone and 20% per year for trabecular bone. The remodeling is related in part to the stresses and strains imposed on the skeleton by gravity.

At the cellular level, there is some regulation of osteoclast formation by osteoblasts via the RANKL–RANK and the M-CSF–CSF1R mechanism; however, specific feedback mechanisms of osteoclasts on osteoblasts are not well defined. In a broader sense, the bone-remodeling process is primarily under endocrine control. Not surprisingly, hormonal control of bone metabolism can be quite complex, and this can be illustrated by examining effects of the weight-associated hormone leptin on bone metabolism. When administered intracerebroventricularly, leptin decreases bone formation. This is thought to occur via release of various substances from the hypothalamus that can reduce osteoblast function. However, circulating leptin can increase bone mass through osteoblast and pre-osteoblast cell signaling pathways. More generally, PTH accelerates bone resorption, and estrogens slow bone resorption by inhibiting the production of bone-eroding cytokines.

BONE DISEASE

The diseases produced by selective abnormalities of the cells and processes discussed above illustrate the interplay of factors that maintain normal bone function.

In **osteopetrosis,** a rare and often severe disease, the osteoclasts are defective and are unable to resorb bone in their usual manner so the osteoblasts operate unopposed. The result is a steady increase in bone density, neurologic defects due to narrowing and distortion of foramina through which nerves normally pass, and hematologic abnormalities due to crowding out of the marrow cavities. Mice lacking the protein encoded by the immediate-early gene c-*fos* develop osteopetrosis; osteopetrosis also occurs in mice lacking the PU.1 transcription factor. This suggests that all these factors are involved in normal osteoclast development and function.

On the other hand, **osteoporosis** is caused by a relative excess of osteoclastic function. Loss of bone matrix in this condition **(Figure 21–12)** is marked, and the incidence of fractures is increased. Fractures are particularly common in the distal forearm (Colles fracture), vertebral body, and hip. All of these areas have a high content of trabecular bone, and because trabecular bone is more active metabolically, it is lost more rapidly. Fractures of the vertebrae with compression

cause kyphosis, with the production of a typical “widow’s hump” that is common in elderly women with osteoporosis. Fractures of the hip in elderly individuals are associated with a mortality rate of 12–20%, and half of those who survive require prolonged expensive care.

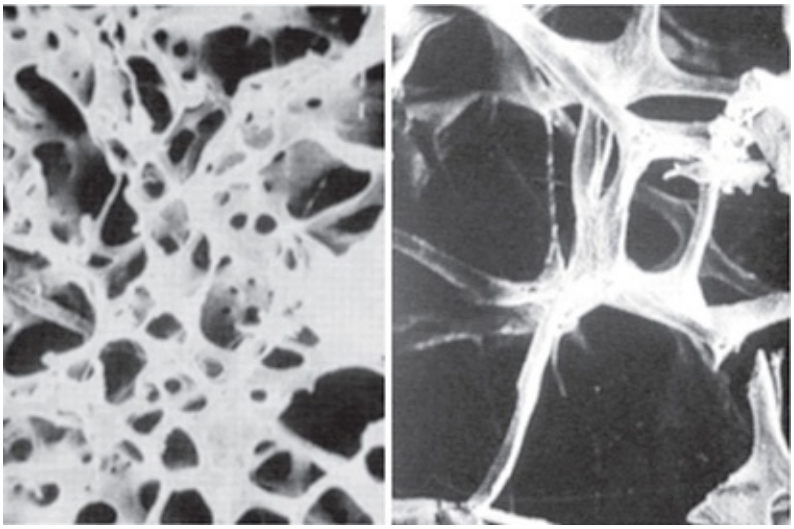


FIGURE 21–12 Normal trabecular bone (left) compared with trabecular bone from a patient with osteoporosis (right). The loss of mass in osteoporosis leaves bones more susceptible to breakage.

Osteoporosis has multiple causes, but by far the most common form is **involutional osteoporosis.** Humans normally gain bone during growth early in life. After a plateau, they begin to lose bone as they grow older **(Figure 21–13)**. When this loss is accelerated or exaggerated, it leads to osteoporosis **(Clinical Box 21–4)**. Increased intake of calcium, particularly from natural sources such as milk, and moderate exercise may help prevent or slow the progress of osteoporosis, although their effects are not very large. Bisphosphonates such as etidronate, which inhibit osteoclastic activity, increase the mineral content of bone and decrease the rate of new vertebral fractures when administered in a cyclical manner. Fluoride stimulates osteoblasts, making bone more dense, but it has proven to be of little value in the treatment of the disease.

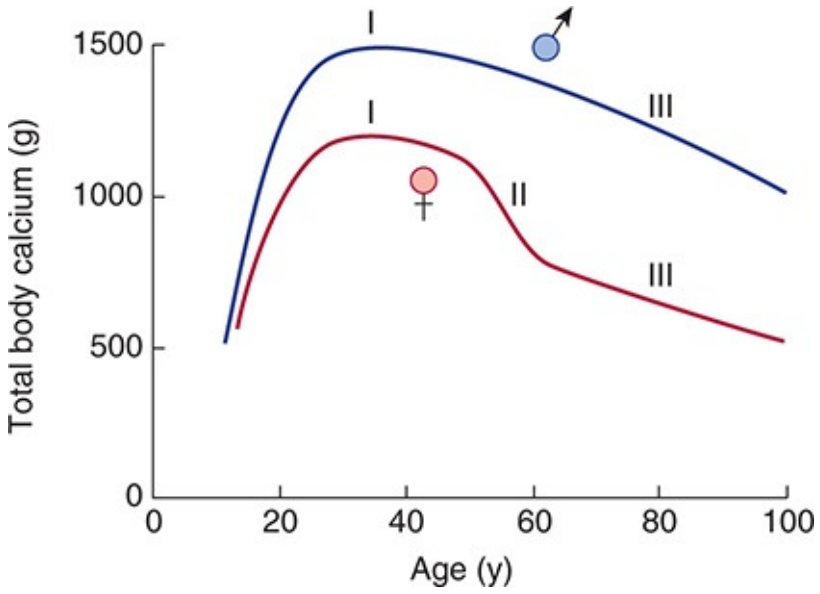


FIGURE 21–13 Total body calcium, an index of bone mass, at various ages in men and women. Note the rapid increase to young adult levels (phase I) followed by the steady loss of bone with advancing age in both sexes (phase III) and the superimposed rapid loss in women after menopause (phase II). (Reproduced with permission from Evans TG, Williams TF (editors): *Oxford Textbook of Geriatric Medicine*. Oxford University Press; 1992.)

CHAPTER SUMMARY

- Calcium and inorganic phosphate ions fulfill physiologic roles in cell signaling, nerve function, muscle contraction, and blood coagulation, among others. The majority of the calcium in the body is stored in the bones but it is the free, ionized calcium in the cells and extracellular fluids that is physiologically relevant. Phosphate is likewise predominantly stored in the bones.
- Calcium and phosphate are absorbed from the diet in a regulated fashion, enter the extracellular fluid, and move into and out of the bones. Circulating calcium and phosphate are filtered by the kidneys, and typically largely reabsorbed unless renal wasting is called for when circulating levels of the minerals are elevated.
- Circulating levels of calcium and phosphate ions are controlled by cells that sense the levels of these electrolytes in the blood and are thereby stimulated to release hormones, and these hormones mobilize the minerals from the bones, increase intestinal absorption, and/or modulate renal wasting.

- The major hormones regulating calcium and phosphate homeostasis are 1,25-dihydroxycholecalciferol (a derivative of vitamin D that is produced cooperatively by the skin, liver and kidneys) and parathyroid hormone, secreted by the parathyroid glands. Calcitonin is also capable of regulating levels of these ions, but its full physiologic contribution is unclear.
- 1,25-Dihydroxycholecalciferol acts to elevate plasma calcium and phosphate by predominantly transcriptional mechanisms, whereas parathyroid hormone elevates calcium but decreases phosphate by increasing the latter's renal excretion. Calcitonin lowers both calcium and phosphate levels. Deficiencies of 1,25-dihydroxycholecalciferol, or mutations in its receptor, also lead to decreases in circulating calcium, defective calcification of the bones, and bone weakness. Disease states also result from either deficiencies or overproduction of parathyroid hormone, with reciprocal effects on calcium and phosphate.
- Bone is a highly structured mass with outer cortical and inner trabecular layers. Regulated bone growth through puberty occurs through epiphysial plates. These plates are located near the end of the bone shaft and fuse with the shaft of the bone to cease linear bone growth.
- Bone is constantly remodeled by osteoclasts, which erode and absorb bone, and osteoblasts, which lay down new bone. An imbalance between the activities of these two cell types can lead to a steady increase in bone density when osteoblasts function unopposed (osteopetrosis) or a loss of bone mass when there is a relative excess of osteoclast activity (osteoporosis).

CLINICAL BOX 21–4

Osteoporosis

Adult women have less bone mass than adult men, and after menopause they initially lose it more rapidly than men of comparable age. Consequently, they are more prone to development of serious osteoporosis. The cause of bone loss after menopause is primarily estrogen deficiency, and estrogen treatment arrests the progress of the disease. Estrogens inhibit secretion of cytokines such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor (TNF-α), cytokines that otherwise foster the development of osteoclasts. Estrogen also stimulates production of transforming growth factor (TGF-β), and this

cytokine increases apoptosis of osteoclasts.

Bone loss can also occur in both men and women as a result of inactivity. In patients who are immobilized for any reason, and during space flight, bone resorption exceeds bone formation and **disuse osteoporosis** develops. The plasma calcium level is not markedly elevated, but plasma concentrations of PTH and 1,25-dihydroxycholecalciferol fall and large amounts of calcium are lost in the urine.

THERAPEUTIC HIGHLIGHTS

Hormone therapy has traditionally been used to offset osteoporosis. **Estrogen replacement therapy** begun shortly after menopause can help maintain bone density. However, it appears that even small doses of estrogens may increase the incidence of uterine and breast cancer, and in carefully controlled studies, estrogens do not protect against cardiovascular disease. Therefore, treatment of a postmenopausal woman with estrogens is no longer used as a primary option. **Raloxifene** is a selective estrogen receptor modulator that can mimic the beneficial effects of estrogen on bone density in postmenopausal women without some of the risks associated with estrogen. However, this too carries risk of side effects (eg, blood clots). Other hormone treatments include the use of **calcitonin** and the PTH analogue **teriparatide.** An alternative to hormone treatments is the **bisphosphonates.** These drugs can inhibit bone breakdown, preserve bone mass, and even increase bone density in the spine and hip to reduce the risk of fractures. Unfortunately, these drugs also can cause mild to serious side effects and require monitoring for patient suitability. In addition to hormones and medications listed above, **physical therapy** to increase appropriate mechanical load and improve balance and muscle strength can significantly improve quality of life.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. A patient with parathyroid deficiency developing 10 days after inadvertent damage to the parathyroid glands during thyroid surgery would probably have

   A. low plasma phosphate and Ca^{2+} levels and tetany.

   B. low plasma phosphate and Ca^{2+} levels and tetanus.

C. a low plasma Ca^{2+} level, increased muscular excitability, and spasm of the muscles of the upper extremity (Trousseau sign).

D. high plasma phosphate and Ca^{2+} levels and bone demineralization.

E. increased muscular excitability, a high plasma Ca^{2+} level, and bone demineralization.

2. In an experiment, a rat is infused with a small volume of a calcium chloride solution, or sodium chloride as a control. Compared to the control condition, which of the following would result from the calcium load?

A. Bone demineralization

B. Increased formation of 1,25-dihydroxycholecalciferol

C. Decreased secretion of calcitonin

D. Decreased blood coagulability

E. Increased formation of 24,25-dihydroxycholecalciferol

3. In the same rat, which of the following would be increased?

A. Intestinal absorption of calcium

B. Expression of TRPV5 in the renal proximal tubule

C. NaPi-IIb abundance in the enterocyte apical membrane

D. Urinary excretion of calcium

E. Expression of calbindins

4. Which of the following is *not* involved in regulating plasma Ca^{2+} levels?

A. Kidneys

B. Skin

C. Liver

D. Lungs

E. Intestine

5. 1,25-Dihydroxycholecalciferol affects intestinal Ca^{2+} absorption through a mechanism that

A. includes alterations in the activity of genes.

B. activates adenylyl cyclase.

C. decreases cell turnover.

D. changes gastric acid secretion.

E. involves degradation of apical calcium channels.

6. Which of the following would you expect to find in a patient whose diet has

been low in calcium for 2 months?

A. Increased formation of 24,25-dihydroxycholecalciferol

B. Decreased expression of TRPV6 in intestinal epithelial cells

C. Increased parathyroid hormone secretion

D. A high plasma calcitonin concentration

E. Increased plasma phosphate

7. The skeleton of a normal male college student would be expected to display which of the following features, relative to that of his 7-year-old brother?

A. Merging of cortical bone and trabecular bone.

B. Differentiation of osteoclasts and osteoblasts.

C. An extended amount of proliferating cartilage that contributes to bone elongation.

D. A meeting of the lacunae with the trabecular bone.

E. Ephyses that are united with the bone shaft.

8. A mouse is engineered to lack a transcription factor necessary for the normal development of osteoclasts. Compared to normal littermate mice, which of the following would be reduced in the knock-out animals?

A. Phosphate deposition in trabecular bone

B. Hydroxyapatite levels in bone

C. Osteoblast proliferation

D. Secretion of acid proteases

E. Bone collagen

CHAPTER 22

Reproductive Development & Function of the Female Reproductive System

OBJECTIVES

After studying this chapter, you should be able to:

- Outline the role of chromosomes, hormones, and related factors in sex determination and development.
- Summarize the hormonal changes that occur at puberty in females.
- Describe oogenesis within the ovarian follicle. Explain the roles of FSH, LH and inhibin in oogenesis and maturation of follicles.
- Describe ovulation and the role of the corpus luteum in the physiologic changes that occur in the female reproductive organs during the menstrual cycle.
- Outline the hormonal changes and their physiologic effects during perimenopause and menopause.
- Describe the hormonal regulation of estrogen and progesterone biosynthesis, transport, metabolism, targets, and actions.
- Describe the roles of the pituitary and the hypothalamus in the regulation of ovarian function, and the role of feedback loops in this process.
- Describe the process of fertilization, implantation and the development of

the placenta

- Discuss the hormonal changes that accompany pregnancy and parturition.
- Outline the processes involved in lactation including neuroendocrine regulation of milk secretion and ejection.

INTRODUCTION

Modern genetics and experimental embryology make it clear that, in most species of mammals, the multiple differences between the male and the female depend primarily on a single chromosome (the Y chromosome) and a single pair of endocrine structures, namely the testes in the male and the ovaries in the female. The differentiation of the primitive gonads into testes or ovaries in utero is genetically determined in humans, but the formation of male genitalia depends on the presence of a functional, secreting testis; in the absence of testicular tissue, development is female. Evidence indicates that male sexual behavior and, in some species, the male pattern of gonadotropin secretion are due to the action of male hormones on the brain in early development. After birth, the gonads remain quiescent until adolescence, when they are activated by gonadotropins from the anterior pituitary. Hormones secreted by the gonads at this time cause the appearance of features typical of the adult male or female and the onset of the sexual cycle in the female. In human females, ovarian function regresses after a number of years and sexual cycles cease (the menopause). In males, gonadal function slowly declines with advancing age, but the ability to produce viable gametes persists.

In both sexes, the gonads have a dual function: the production of germ cells **(gametogenesis)** and the secretion of **sex hormones.** The **androgens** are steroid sex hormones that are masculinizing in their action; the **estrogens** are those that are feminizing. Both types of hormones are normally secreted in both sexes. The ovaries secrete large amounts of estrogens and small amounts of androgens, a pattern that is reversed in males. Androgens are secreted from the adrenal cortex in both sexes, and some of the androgens are converted to estrogens in fat and other extragonadal and extra-adrenal tissues. The ovaries also secrete **progesterone,** a steroid that has special functions in preparing the uterus for pregnancy.

Particularly during pregnancy, the ovaries secrete the polypeptide hormone **relaxin,** which loosens the ligaments of the pubic symphysis and softens the cervix, facilitating delivery of the fetus. In both sexes, the gonads secrete other

polypeptides, including **inhibin B,** a polypeptide that inhibits follicle-stimulating hormone (FSH) secretion.

The secretory and gametogenic functions of the gonads are both dependent on the secretion of the anterior pituitary gonadotropins, FSH, and luteinizing hormone (LH). The sex hormones and inhibin B feed back to inhibit gonadotropin secretion. In males, gonadotropin secretion is noncyclic; but in postpubertal females an orderly, sequential secretion of gonadotropins is necessary for the occurrence of menstruation, pregnancy, and lactation.

SEX DIFFERENTIATION & DEVELOPMENT

CHROMOSOMAL SEX

The Sex Chromosomes

Sex is determined genetically by two chromosomes, called the **sex chromosomes,** to distinguish them from the **somatic chromosomes (autosomes).** In humans and many other mammals, the sex chromosomes are called X and Y. The Y chromosome is necessary and sufficient for the production of testes, and the testis-determining gene product is called SRY (for sex- determining region of the Y chromosome). SRY is a DNA-binding regulatory protein. It bends the DNA and acts as a transcription factor that initiates transcription of a cascade of genes necessary for testicular differentiation, including the gene for **müllerian-inhibiting substance (MIS;** see below). The gene for SRY is located near the tip of the short arm of the human Y chromosome. Diploid male cells contain an X and a Y chromosome (XY pattern), whereas female cells contain two X chromosomes (XX pattern). As a consequence of meiosis during gametogenesis, each normal ovum contains a single X chromosome, but half of the normal sperm contain an X chromosome and half contain a Y chromosome **(Figure 22–1)**. When a sperm containing a Y chromosome fertilizes an ovum, an XY pattern results and the zygote develops into a **genetic male.** When fertilization occurs with an X-containing sperm, an XX pattern and a **genetic female** results. Cell division and the chemical nature of chromosomes are discussed in Chapter 1.

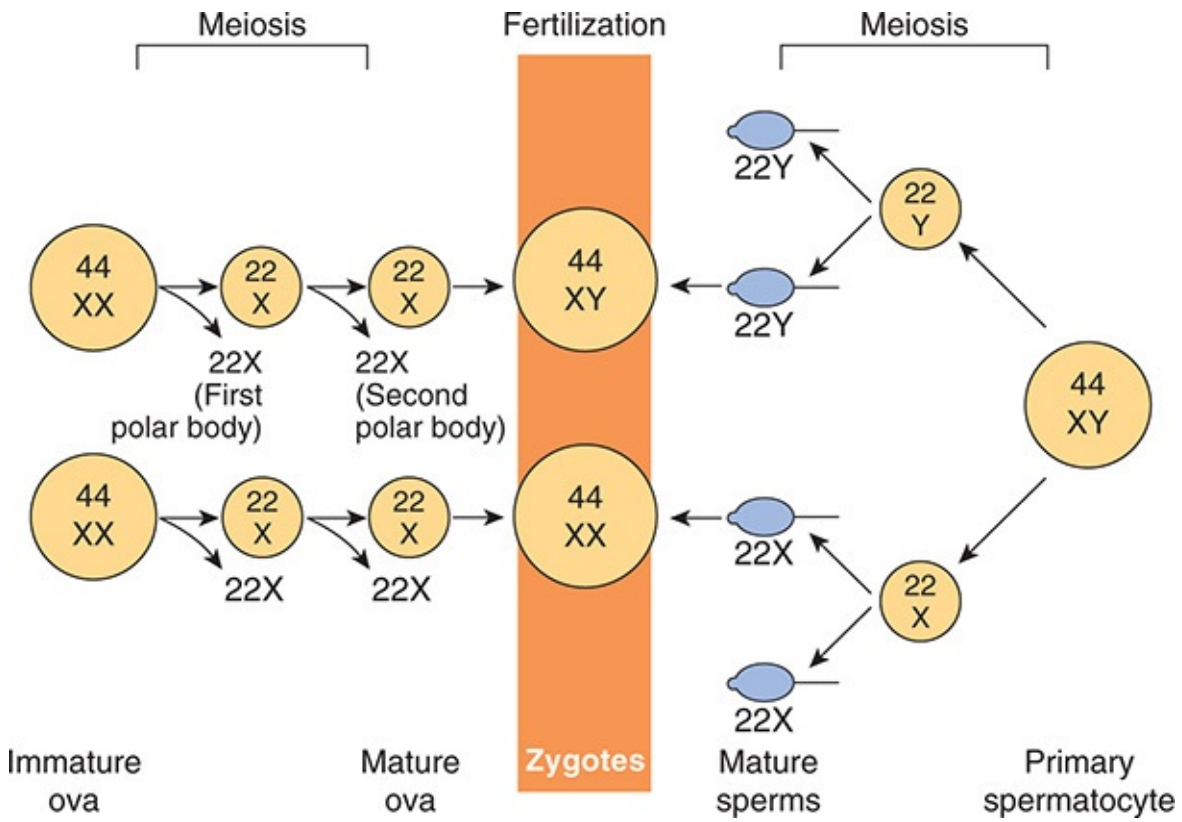


FIGURE 22–1 Basis of genetic sex determination. In the two-stage meiotic division in the female, only one cell survives as the mature ovum. In the male, the meiotic division results in the formation of four sperms, two containing the X and two the Y chromosome. Fertilization thus produces a male zygote with 22 pairs of autosomes plus an X and a Y or a female zygote with 22 pairs of autosomes and two X chromosomes. Note that for clarity, this figure and Figure 22–6 and 22–7 differ from the current international nomenclature for karyotypes, which lists the total number of chromosomes followed by the sex chromosome pattern. Thus, XO is 45, X; XY is 46, XY; XXY is 47, XXY, and so on.

Human Chromosomes

Human chromosomes can be studied in detail. Human cells are grown in tissue culture; treated with the drug colchicine, which arrests mitosis at the metaphase; exposed to a hypotonic solution that makes the chromosomes swell and disperse; and then “squashed” onto slides. Staining techniques make it possible to identify the individual chromosomes **(Figure 22–2)**. There are 46 chromosomes: in males, 22 pairs of autosomes plus an X chromosome and a Y chromosome; in females, 22 pairs of autosomes plus two X chromosomes. The individual

chromosomes are usually arranged in an arbitrary pattern **(karyotype).** The individual autosome pairs are identified by the numbers 1–22 on the basis of their morphologic characteristics.

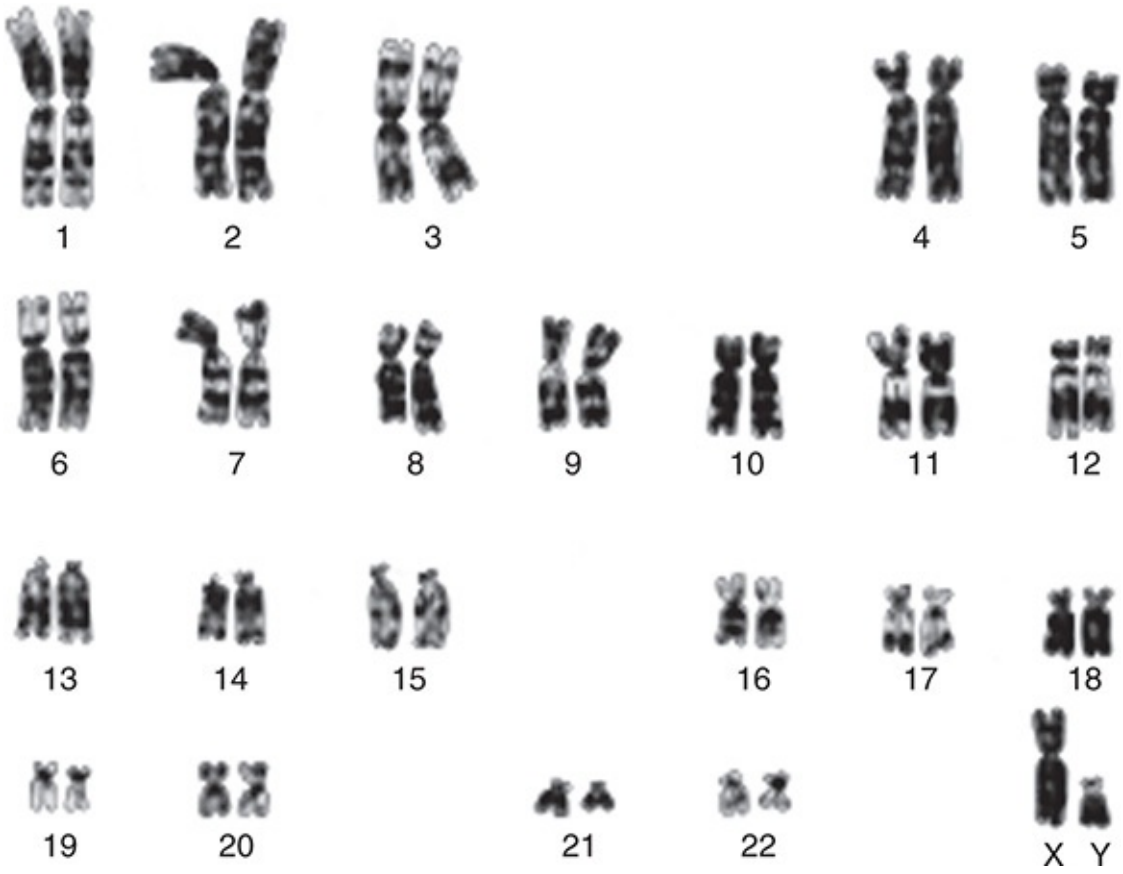


FIGURE 22–2 Karyotype of chromosomes from a normal male. The chromosomes have been stained with Giemsa stain, which produces a characteristic banding pattern. (Reproduced with permission from Lingappa VJ, Farey K: *Physiological Medicine*. New York, NY: McGraw-Hill; 2000.)

Sex Chromatin

Soon after cell division has started during embryonic development, one of the two X chromosomes of the somatic cells in normal females becomes functionally inactive. In abnormal individuals with more than two X chromosomes, only one remains active. The process that is normally responsible for inactivation is initiated in an X-inactivation center in the chromosome, probably via the transactivating factor CTCF (for CCCTC-binding factor), which is also induced during gene imprinting. However, the details of the inactivation

process are still incompletely understood. The choice of which X chromosome remains active is random, so normally one X chromosome remains active in approximately half of the cells and the other X chromosome is active in the other half. The selection persists through subsequent divisions of these cells, and consequently some of the somatic cells in adult females contain an active X chromosome of paternal origin and some contain an active X chromosome of maternal origin.

In normal cells, the inactive X chromosome condenses and can be seen in various types of cells, usually near the nuclear membrane, as the **Barr body,** also called sex chromatin **(Figure 22–3)**. Thus, there is a Barr body for each X chromosome in excess of one in the cell. The inactive X chromosome is also visible as a small "drumstick" of chromatin projecting from the nuclei of 1–15% of the polymorphonuclear leukocytes in females but not in males (Figure 22–3).

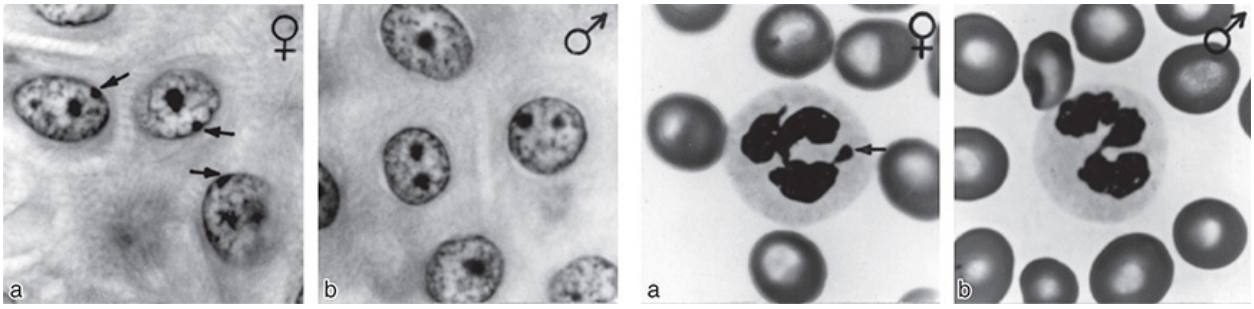


FIGURE 22–3 Left: Barr body (arrows) in the epidermal spinous cell layer. Right: Nuclear appendage ("drumstick") identified by arrow in white blood cells. (Reproduced with permission from Grumbach MM, Barr ML: Cytologic tests of chromosomal sex in relation to sex anomalies in man. Recent Prog Horm Res 1958;14:255–324.)

EMBRYOLOGY OF THE HUMAN REPRODUCTIVE SYSTEM

Development of the Gonads

On each side of the embryo, a primitive gonad arises from the genital ridge, a condensation of tissue near the adrenal gland. The gonad develops a **cortex** and a **medulla.** Until the sixth week of development, these structures are identical in both sexes. In genetic males, the medulla develops during the seventh and eighth weeks into a testis, and the cortex regresses. Leydig and Sertoli cells appear, and

testosterone and MIS are secreted. In genetic females, the cortex develops into an ovary and the medulla regresses. The embryonic ovary does not secrete hormones. Hormonal treatment of the mother has no effect on gonadal (as opposed to ductal and genital) differentiation in humans, although it does in some experimental animals.

Embryology of the Genitalia

The embryology of the gonads is summarized in **Figures 22–4** and **22–5**. In the seventh week of gestation, the embryo has both male and female primordial genital ducts (Figure 22–4). In a normal female fetus, the müllerian duct system then develops into uterine tubes (oviducts) and a uterus. In the normal male fetus, the wolffian duct system on each side develops into the epididymis and vas deferens. The external genitalia are similarly bipotential until the eighth week (Figure 22–5). Thereafter, the urogenital slit disappears and male genitalia form, or, alternatively, it remains open and female genitalia form.

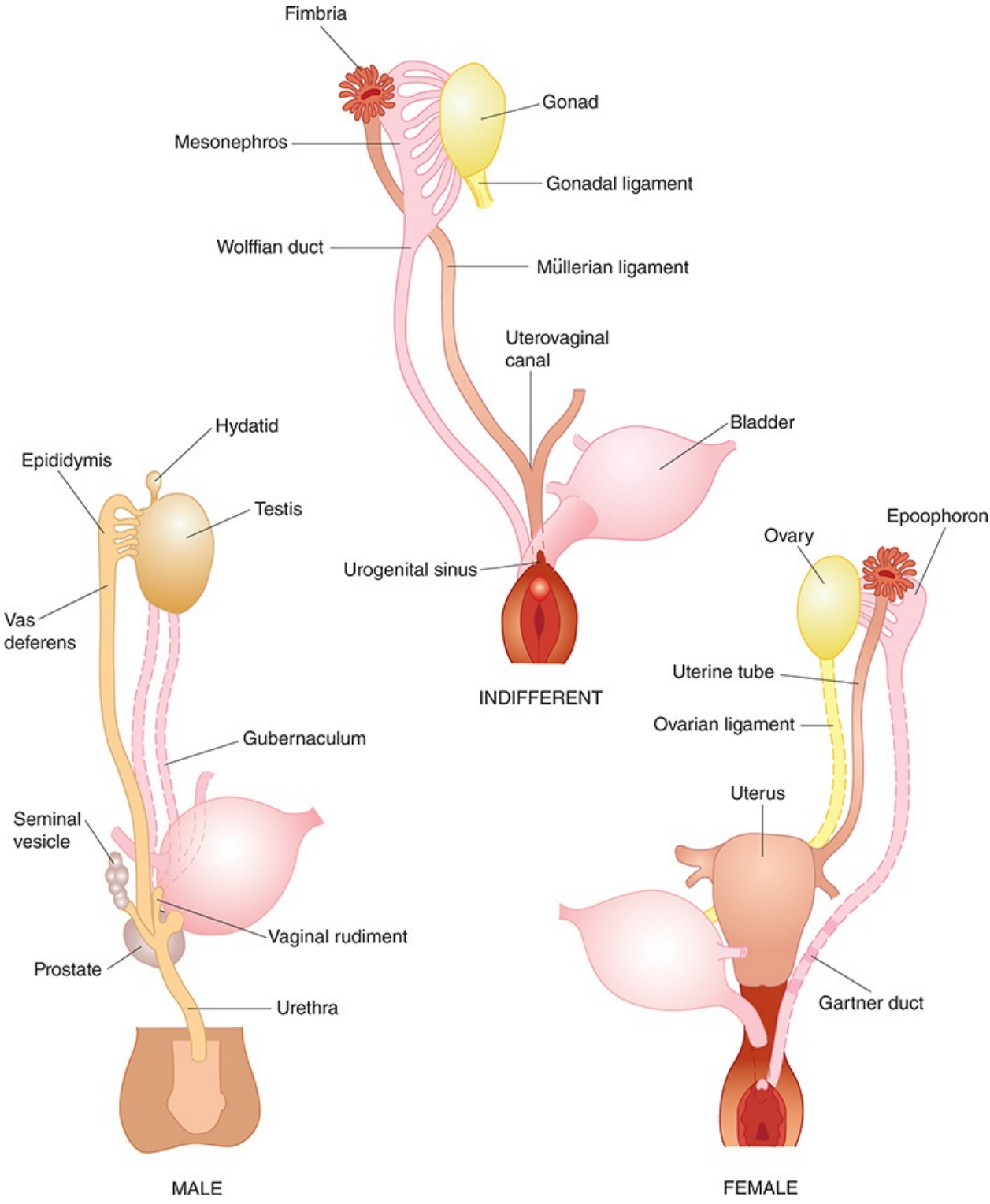


FIGURE 22–4 Embryonic differentiation of male and female internal genitalia (genital ducts) from wolffian (male) and müllerian (female) primordia. (Reproduced with permission from Wilson JD, Foster DW [editors]: *Williams Textbook of Endocrinology*, 7th ed. Saunders; 1985.)

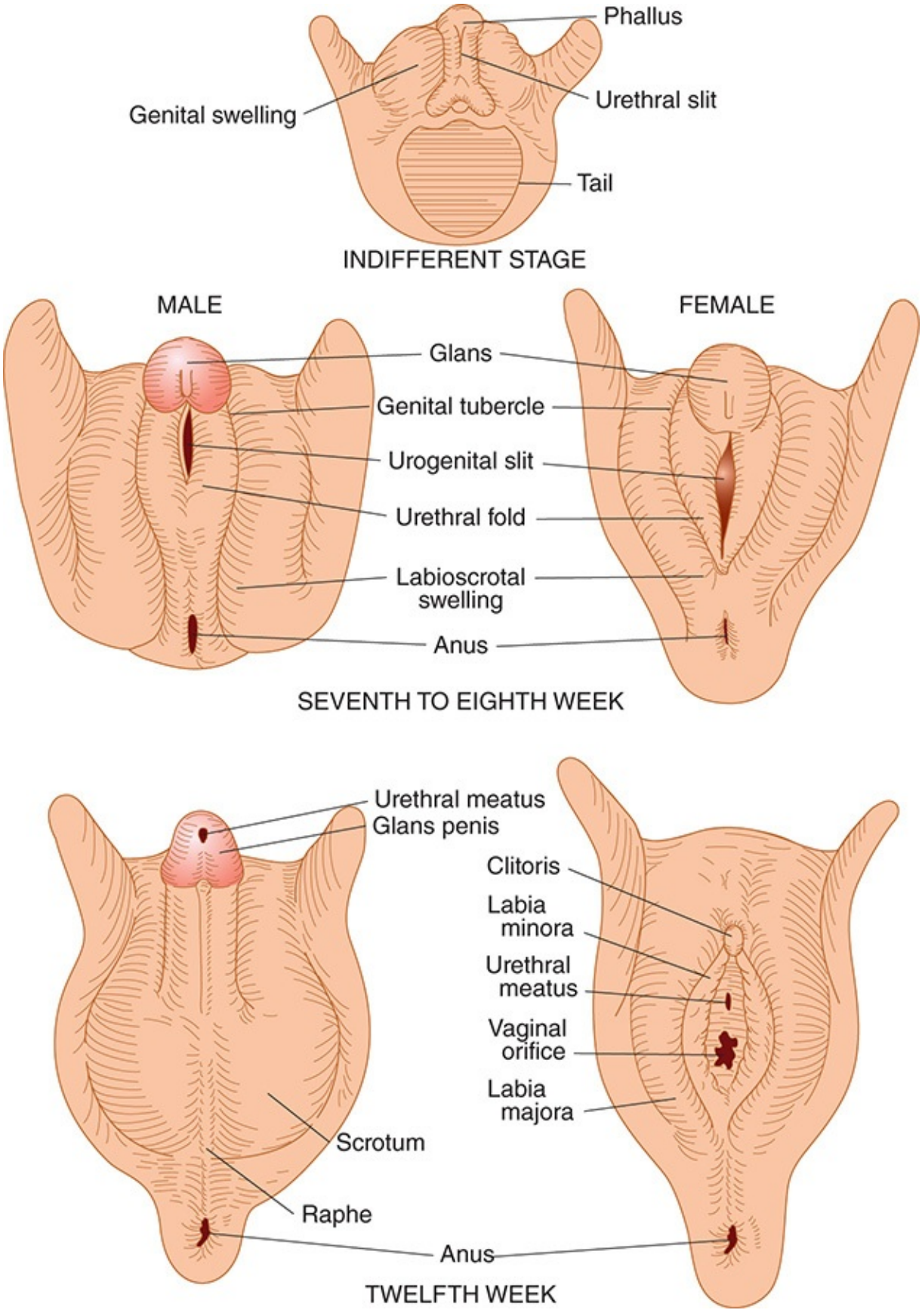

Phallus
Genital swelling
Urethral slit
Tail
INDIFFERENT STAGE
MALE
FEMALE
Glans
Genital tubercle
Urogenital slit
Urethral fold
Labioscrotal swelling
Anus
SEVENTH TO EIGHTH WEEK
Urethral meatus
Glans penis
Clitoris
Labia minora
Urethral meatus
Vaginal orifice
Labia majora
Scrotum
Raphe
Anus
TWELFTH WEEK

FIGURE 22–5 Differentiation of male and female external genitalia from indifferent primordial structures in the embryo.

When the embryo has functional testes, male internal and external genitalia develop. The Leydig cells of the fetal testis secrete testosterone, and the Sertoli cells secrete MIS (also called müllerian regression factor, or MRF). MIS is a 536-amino-acid homodimer that is a member of the transforming growth factor β (TGF-β) super-family of growth factors, which includes inhibins and activins.

In their effects on the internal as opposed to the external genitalia, MIS and testosterone act unilaterally. MIS causes regression of the müllerian ducts by apoptosis on the side on which it is secreted, and testosterone fosters the development of the vas deferens and related structures from the wolffian ducts. The testosterone metabolite dihydrotestosterone induces the formation of male external genitalia and male secondary sex characteristics **(Figure 22–6)**.

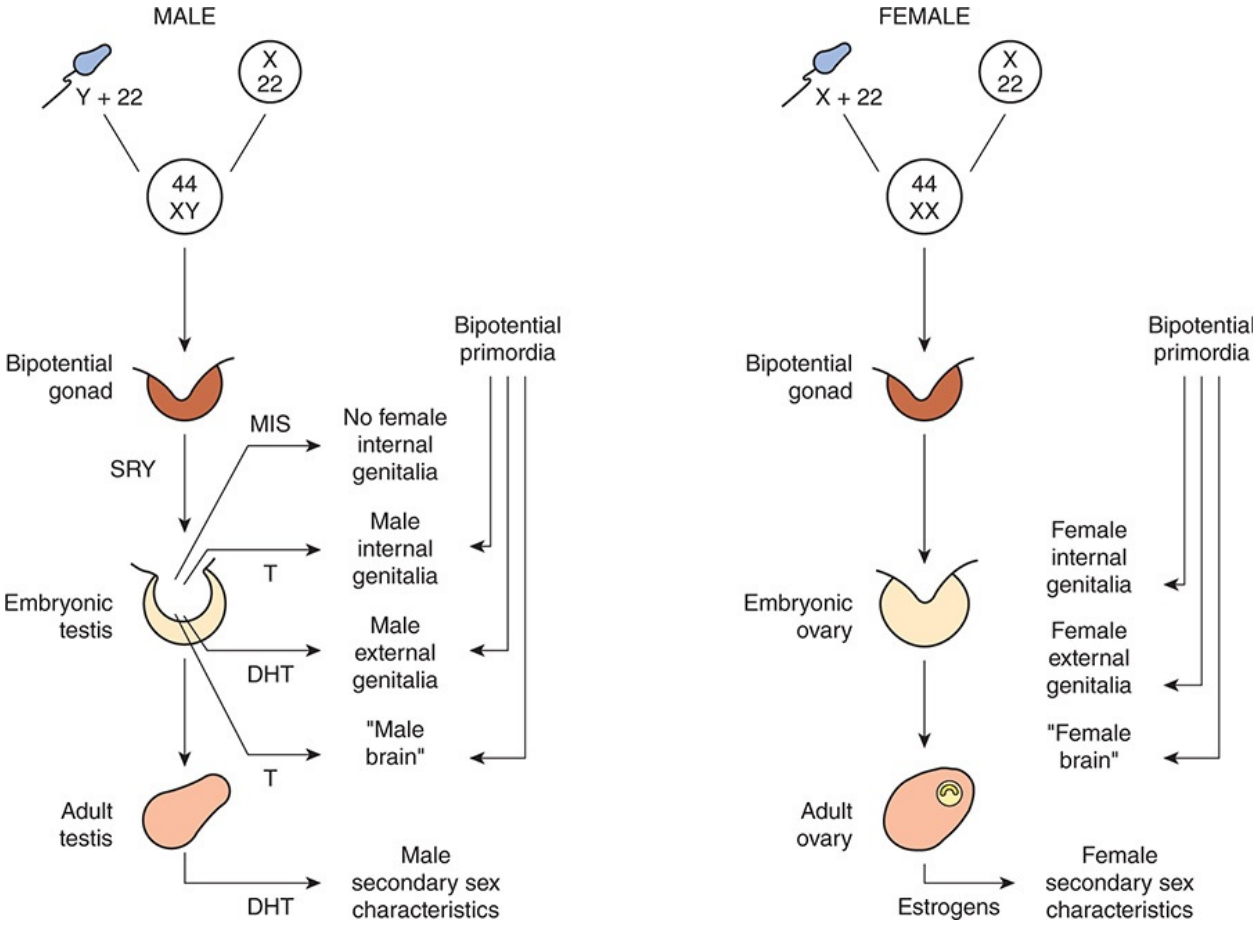


FIGURE 22–6 Diagrammatic summary of normal sex determination,

differentiation, and development in humans. DHT, dihydrotestosterone, MIS, müllerian-inhibiting substance; T, testosterone.

MIS continues to be secreted by the Sertoli cells, and it reaches mean values of 48 ng/mL in plasma in 1- to 2-year-old boys. Thereafter, it declines to low levels by the time of puberty and persists at low but detectable levels throughout life. In girls, MIS is produced by granulosa cells in small follicles in the ovaries, but plasma levels are very low or undetectable until puberty. Thereafter, plasma MIS is about the same as in adult men, that is, about 2 ng/mL. The functions of MIS after early embryonic life are unsettled, but it is probably involved in germ cell maturation in both sexes and in control of testicular descent in boys.

Development of the Brain

At least in some species, the development of the brain as well as the external genitalia is affected by androgens early in life. In rats, a brief exposure to androgens during the first few days of life causes the male pattern of sexual behavior and the male pattern of hypothalamic control of gonadotropin secretion to develop after puberty. In the absence of androgens, female patterns develop (see Chapter 17). In monkeys, similar effects on sexual behavior are produced by exposure to androgens in utero, but the pattern of gonadotropin secretion remains cyclical. Early exposure of female human fetuses to androgens also appears to cause subtle but significant masculinizing effects on behavior. However, women with adrenogenital syndrome due to congenital adrenocortical enzyme deficiency (see Chapter 20) develop normal menstrual cycles when treated with cortisol. Thus, the human, like the monkey, appears to retain the cyclical pattern of gonadotropin secretion despite exposure to androgens in utero.

ABERRANT SEXUAL DIFFERENTIATION

Chromosomal Abnormalities

From the preceding discussion, it might be expected that abnormalities of sexual development could be caused by genetic or hormonal abnormalities as well as by other nonspecific teratogenic influences, and this is indeed the case. The major classes of abnormalities are listed in **Table 22–1**.

TABLE 22–1 Classification of the major disorders of sex differentiation in humans.[a]

Chromosomal disorders
Gonadal dysgenesis (XO and variants)
"Superfemales" (XXX)
Seminiferous tubule dysgenesis (XXY and variants)
True hermaphroditism
Developmental disorders
Female pseudohermaphroditism
Congenital virilizing adrenal hyperplasia of fetus
Maternal androgen excess
Virilizing ovarian tumor
Iatrogenic: Treatment with androgens or certain synthetic progestational drugs
Male pseudohermaphroditism
Androgen resistance
Defective testicular development
Congenital 17α-hydroxylase deficiency
Congenital adrenal hyperplasia due to blockade of pregnenolone formation
Various nonhormonal anomalies

[a]Many of these syndromes can have great variation in degree and, consequently, in manifestations.

Nondisjunction of sex chromosomes during the first division in meiosis results in distinct defects **(see Clinical Box 22–1; Figure 22–7)**. Meiosis is a two-stage process, and although nondisjunction usually occurs during the first meiotic division, it can occur in the second, producing more complex chromosomal abnormalities. In addition, nondisjunction or simple loss of a sex chromosome can occur during the early mitotic divisions after fertilization. The consequence of faulty mitoses in the early zygote is **mosaicism,** in which two or

more populations of cells have different chromosome complements. **True hermaphroditism,** the condition in which the individual has both ovaries and testes, is probably due to XX/XY mosaicism and related mosaic patterns, although other genetic aberrations are possible.

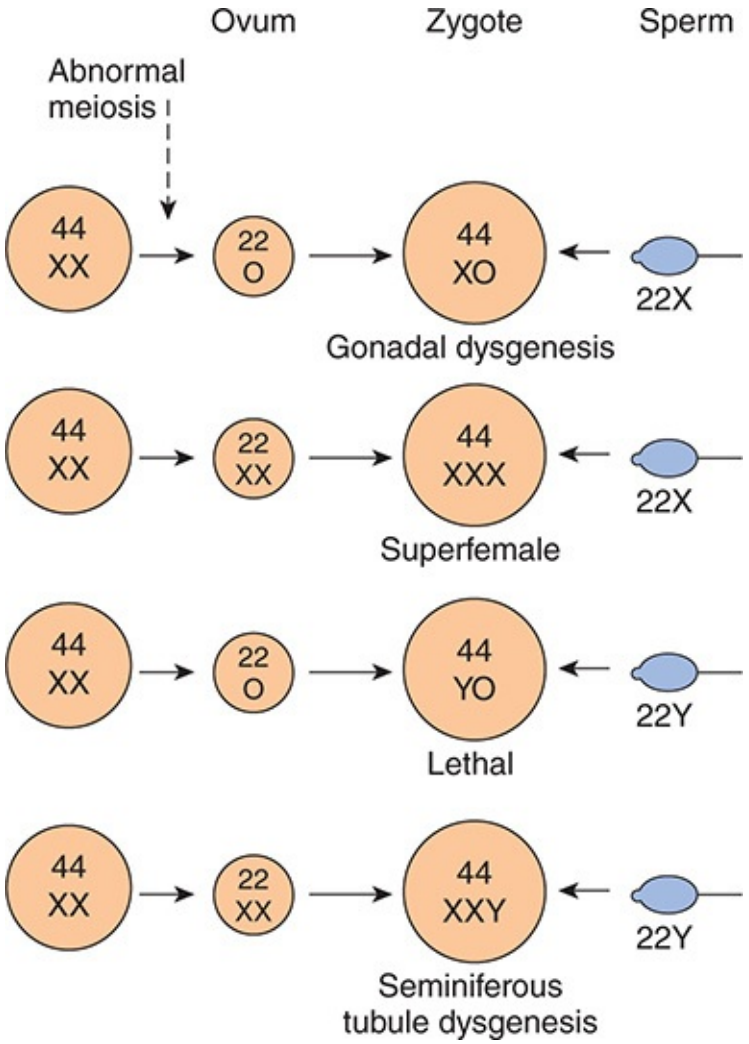


FIGURE 22–7 Summary of four possible defects produced by maternal nondisjunction of the sex chromosomes at the time of meiosis. The YO combination is believed to be lethal, and the fetus dies in utero.

Chromosomal abnormalities also include transposition of parts of chromosomes to other chromosomes. Rarely, genetic males are found to have the XX karyotype because the short arm of their father's Y chromosome was transposed to their father's X chromosome during meiosis and they received that X chromosome along with their mother's. Similarly, deletion of the small portion of the Y chromosome containing SRY produces females with the XY karyotype.

Hormonal Abnormalities

Development of the male external genitalia occurs normally in genetic males in response to androgen secreted by the embryonic testes, but male genital development may also occur in genetic females exposed to androgens from some other source during the 8th to the 13th weeks of gestation. The syndrome that results is **female pseudohermaphroditism.** A pseudohermaphrodite is an individual with the genetic constitution and gonads of one sex and the genitalia of the other. After the 13th week, the genitalia are fully formed, but exposure to androgens can cause hypertrophy of the clitoris. Female pseudohermaphroditism may be due to congenital virilizing adrenal hyperplasia (see Chapter 19), or it may be caused by androgens administered to the mother. Conversely, one cause of the development of female external genitalia in genetic males **(male pseudohermaphroditism)** is defective testicular development. Because the testes also secrete MIS, genetic males with defective testes have female internal genitalia.

Another cause of male pseudohermaphroditism is **androgen resistance,** in which, as a result of various congenital abnormalities, male hormones cannot exert their full effects on the tissues. One form of androgen resistance is a **5α-reductase deficiency,** in which the enzyme responsible for the formation of dihydrotestosterone, the active form of testosterone, is decreased **(Figure 22–8)**. The consequences of this deficiency are discussed in Chapter 23. Other forms of androgen resistance are due to various mutations in the androgen receptor gene, and the resulting defects in receptor function range from minor to severe. Mild defects cause infertility with or without gynecomastia. When the loss of receptor function is complete, the **testicular feminizing syndrome,** now known as **complete androgen resistance syndrome,** results. In this condition, MIS is present and testosterone is secreted at normal or even elevated rates. The external genitalia are female, but the vagina ends blindly because there are no female internal genitalia. At puberty, enlarged breasts develop in persons with this syndrome. The syndrome usually remains undiagnosed until women seek medical advice because of lack of menstruation.

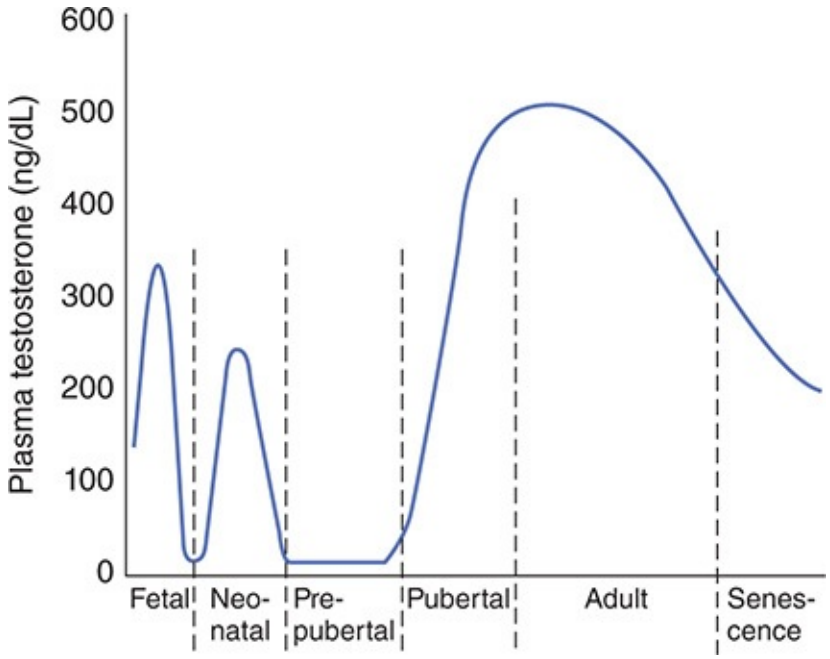


FIGURE 22–8 Plasma testosterone levels at various ages in human males.

CLINICAL BOX 22–1

Chromosomal Abnormalities

An established defect in gametogenesis is **nondisjunction,** a phenomenon in which a pair of chromosomes fail to separate, so that both go to one of the daughter cells during meiosis. Four of the abnormal zygotes that can form as a result of nondisjunction of one of the X chromosomes during oogenesis are shown in Figure 25–7. In individuals with the XO chromosomal pattern, the gonads are rudimentary or absent, so that female external genitalia develop, stature is short, other congenital abnormalities are often present, and no sexual maturation occurs at puberty. This syndrome is called **gonadal dysgenesis** or, alternatively, **ovarian agenesis** or **Turner syndrome.** Individuals with the XXY pattern, the most common sex chromosome disorder, have the genitalia of a normal male. Testosterone secretion at puberty is often great enough for the development of male characteristics; however, the seminiferous tubules are abnormal, and the incidence of mental retardation is higher than normal. This syndrome is known as **seminiferous tubule dysgenesis** or **Klinefelter syndrome.** The XXX (“superfemale”) pattern is second in frequency only to the XXY pattern and may be even more common in the general population, since it does not seem to be associated

with any characteristic abnormalities. The YO combination is probably lethal.

Nondisjunction of chromosome 21 produces **trisomy 21,** the chromosomal abnormality associated with **Down syndrome.** The additional chromosome 21 is normal, so Down syndrome is a pure case of gene excess causing abnormalities.

Many other chromosomal abnormalities occur as well as numerous diseases due to defects in single genes. These conditions are generally diagnosed in utero by analysis of fetal cells in a sample of amniotic fluid collected by inserting a needle through the abdominal wall **(amniocentesis)** or, earlier in pregnancy, by examining fetal cells obtained by a needle biopsy of chorionic villi **(chorionic villus sampling)**.

THERAPEUTIC HIGHLIGHTS

Many of the syndromes mentioned have effects on multiple organ systems, and patients must be carefully monitored with a multidisciplinary approach to avert the consequences of cardiovascular defects, infections secondary to urinary tract and renal malformations, and the psychological impact of reproductive implications. Girls with Turner syndrome and evidence of gonadal failure are also treated with low-dose estrogen to evoke puberty, followed by gradual replacement of mature estrogen levels to permit feminization. Conversely, patients with Klinefelter syndrome are often supplemented with androgens to improve virilization and libido.

It is worth noting that genetic males with congenital blockage of the formation of pregnenolone are pseudohermaphrodites because testicular as well as adrenal androgens are normally formed from pregnenolone. Male pseudohermaphroditism also occurs when there is a congenital deficiency of 17α-hydroxylase (see Chapter 19).

PUBERTY

As noted above, a burst of testosterone secretion occurs in male fetuses before birth (see Chapter 23). In the neonatal period there is another burst, with unknown function, but thereafter the Leydig cells become quiescent. There follows in all mammals a period in which the gonads of both sexes are quiescent until they are activated by gonadotropins from the pituitary to bring about the

final maturation of the reproductive system. This period of final maturation is known as **adolescence.** It is often also called **puberty,** although puberty, strictly defined, is the period when the endocrine and gametogenic functions of the gonads have first developed to the point where reproduction is possible. In girls, the first event is **thelarche,** the development of breasts, followed by **pubarche,** the development of axillary and pubic hair, and then by **menarche,** the first menstrual period. Initial menstrual periods are generally anovulatory, and regular ovulation appears about a year later. In contrast to the situation in adulthood, removal of the gonads during the period from soon after birth to puberty causes only a small increase in gonadotropin secretion, so gonadotropin secretion is not being held in check by the gonadal hormones. In children between the ages of 7 and 10, a slow increase in estrogen and androgen secretion precedes the more rapid rise in the early teens **(Figure 22–9)**.

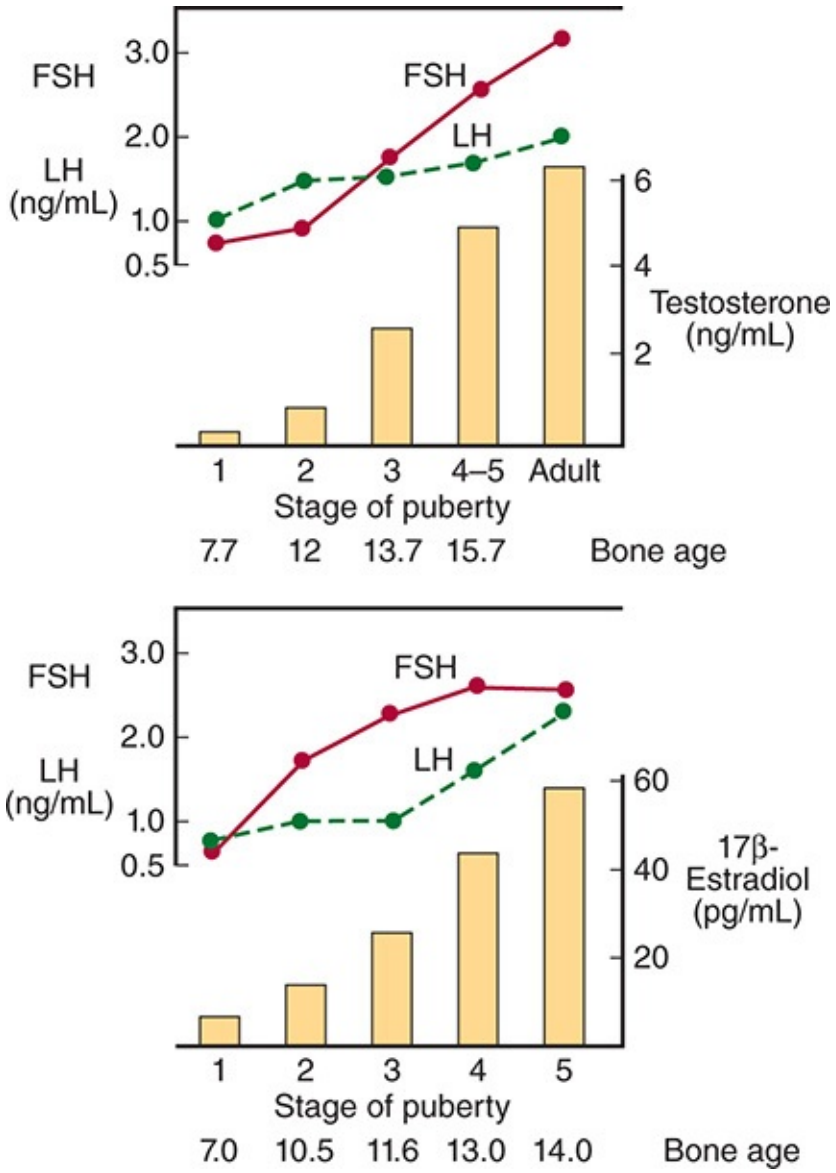


FIGURE 22–9 Changes in plasma hormone concentrations during puberty in boys (top) and girls (bottom). Stage 1 of puberty is preadolescence in both sexes. In boys, stage 2 is characterized by beginning enlargement of the testes, stage 3 by penile enlargement, stage 4 by growth of the glans penis, and stage 5 by adult genitalia. In girls, stage 2 is characterized by breast buds, stage 3 by elevation and enlargement of the breasts, stage 4 by projection of the areolas, and stage 5 by adult breasts. FSH, follicle-stimulating hormone; LH, luteinizing hormone. (Modified and reproduced with permission from Berenberg SR [ed]: *Puberty: Biologic and Psychosocial Components*. HE Stenfoert Kroese BV; 1975.)

The age at the time of puberty is variable. In Europe and the United States, it

has been declining at the rate of 1–3 months per decade for more than 175 years. In the United States in recent years, puberty generally occurs between the ages of 8 and 13 in girls and 9 and 14 in boys.

Another event that occurs in humans at the time of puberty is an increase in the secretion of adrenal androgens (see Figure 19–12). The onset of this increase is called **adrenarche.** It occurs at age 8–10 years in girls and age 10–12 years in boys. Dehydroepiandrosterone (DHEA) values peak at about age 25 in women and slightly later than that in men. They then decline slowly to low values in old age. The rise appears to be due to an increase in the activity of 17α-hydroxylase.

Control of the Onset of Puberty

The gonads of children can be stimulated by gonadotropins; their pituitaries contain gonadotropins and their hypothalami contain gonadotropin-releasing hormone (GnRH) (see Chapter 17). However, their gonadotropins are not secreted. In immature monkeys, normal menstrual cycles can be brought on by pulsatile injection of GnRH, and they persist as long as the pulsatile injection is continued. Thus, it seems clear that pulsatile secretion of GnRH brings on puberty. During the period from birth to puberty, a neural mechanism is operating to prevent the normal pulsatile release of GnRH. The nature of the mechanism inhibiting the GnRH pulse generator is unknown. However, one or more genes produce products that stimulate secretion of GnRH, and inhibition of these genes before puberty is an interesting possibility **(Clinical Box 22–2)**.

PRECOCIOUS & DELAYED PUBERTY

Sexual Precocity

The major causes of precocious sexual development in humans are listed in **Table 22–2**. Early development of secondary sexual characteristics without gametogenesis is caused by abnormal exposure of immature males to androgen or females to estrogen. This syndrome should be called **precocious pseudopuberty** to distinguish it from **true precocious puberty** due to an early but otherwise normal pubertal pattern of gonadotropin secretion from the pituitary.

TABLE 22–2 Classification of the causes of precocious sexual development in humans.

True precocious puberty
Constitutional
Cerebral: Disorders involving posterior hypothalamus
Tumors
Infections
Developmental abnormalities
Gonadotropin-independent precocity
Precocious pseudopuberty (no spermatogenesis or ovarian development)
Adrenal
Congenital virilizing adrenal hyperplasia
Androgen-secreting tumors (in males)
Estrogen-secreting tumors (in females)
Gonadal
Leydig cell tumors of testis
Granulosa cell tumors of ovary
Miscellaneous

CLINICAL BOX 22–2

Leptin

It has been argued for some time that a critical body weight must normally be reached for puberty to occur. Thus, for example, young women who engage in strenuous athletics lose weight and stop menstruating, as do girls with anorexia nervosa. If these girls start to eat and gain weight, they menstruate again, that is, they "go back through puberty." It now appears that leptin, the satiety-producing hormone secreted by fat cells, may be the link between body weight and puberty. Obese ob/ob mice that cannot make leptin are infertile, and their fertility is restored by injections of leptin. Leptin treatment also induces precocious puberty in immature female mice. However, the way that leptin fits into the overall control of puberty remains to be determined.

Constitutional precocious puberty, that is, precocious puberty in which no cause can be determined, is more common in girls than in boys. In both sexes, tumors or infections involving the hypothalamus cause precocious puberty.

Indeed, in one large series of cases, precocious puberty was the most common endocrine symptom of hypothalamic disease. In experimental animals, precocious puberty can be produced by hypothalamic lesions. Apparently the lesions interrupt a pathway that normally holds pulsatile GnRH secretion in check. Pineal tumors are sometimes associated with precocious puberty, but evidence indicates that these tumors are associated with precocity only when there is secondary damage to the hypothalamus.

Precocious gametogenesis and steroidogenesis can occur without the pubertal pattern of gonadotropin secretion (gonadotropin-independent precocity). At least in some cases of this condition, the sensitivity of LH receptors to gonadotropins is increased because of an activating mutation in the G-protein that couples the receptors to adenylyl cyclase.

CLINICAL BOX 22–3

Hyperprolactinemia

Up to 70% of the patients with chromophobe adenomas of the anterior pituitary have elevated plasma prolactin levels. In some instances, the elevation may be due to damage to the pituitary stalk, but in most cases, the tumor cells are actually secreting the hormone. The hyperprolactinemia may cause galactorrhea, but in many individuals no demonstrable endocrine abnormalities are present. Conversely, most women with galactorrhea have normal prolactin levels; definite elevations are found in less than one-third of patients with this condition.

Another interesting observation is that 15–20% of women with secondary amenorrhea have elevated prolactin levels, and when prolactin secretion is reduced, normal menstrual cycles and fertility return. Prolactin may produce amenorrhea by blocking the action of gonadotropins on the ovaries. The hypogonadism produced by prolactinomas is associated with osteoporosis due to estrogen deficiency.

As noted previously, hyperprolactinemia in men is associated with erectile dysfunction and hypogonadism that disappear when prolactin secretion is reduced.

THERAPEUTIC HIGHLIGHTS

Prescription drug use is a common cause of hyperprolactinemia. Prolactin

secretion in the pituitary is suppressed by the brain chemical dopamine. Use of drugs that block the effects of dopamine can cause the pituitary to secrete prolactin. Examples of some prescription drugs that can cause hyperprolactinemia include haloperidol and phenothiazines; most antipsychotic medications; and cisapride, which is used to treat nausea and gastroesophageal reflux in cancer patients. If possible, the drug suspected as causing hyperprolactinemia should be withdrawn, or the dose titrated. Regardless of the etiology, treatment should endeavor to restore normal prolactin levels to avoid suppressive effects on the ovaries and preserve bone density. Dopamine agonists also provide benefit in many cases, including prolactinoma, and can be used in patients for whom an inciting pharmaceutical agent cannot be withdrawn.

Delayed or Absent Puberty

The normal variation in the age at which adolescent changes occur is so wide that puberty cannot be considered to be pathologically delayed until the menarche has failed to occur by the age of 17 or testicular development by the age of 20. Failure of maturation due to panhypopituitarism is associated with dwarfing and evidence of other endocrine abnormalities. Patients with the XO chromosomal pattern and gonadal dysgenesis are also dwarfed. In some individuals, puberty is delayed even though the gonads are present and other endocrine functions are normal. In males, this clinical picture is called **eunuchoidism.** In females, it is called **primary amenorrhea** **(Clinical Box 22–3)**.

THE FEMALE REPRODUCTIVE SYSTEM

THE MENSTRUAL CYCLE

The reproductive system of women **(Figure 22–10)**, unlike that of men, shows regular cyclic changes that teleologically may be regarded as periodic preparations for fertilization and pregnancy. In humans and other primates, the cycle is a **menstrual** cycle, and its most conspicuous feature is the periodic vaginal bleeding that occurs with the shedding of the uterine mucosa **(menstruation).** The length of the cycle is notoriously variable in women, but an average figure is 28 days from the start of one menstrual period to the start of the

next. By common usage, the days of the cycle are identified by number, starting with the first day of menstruation.

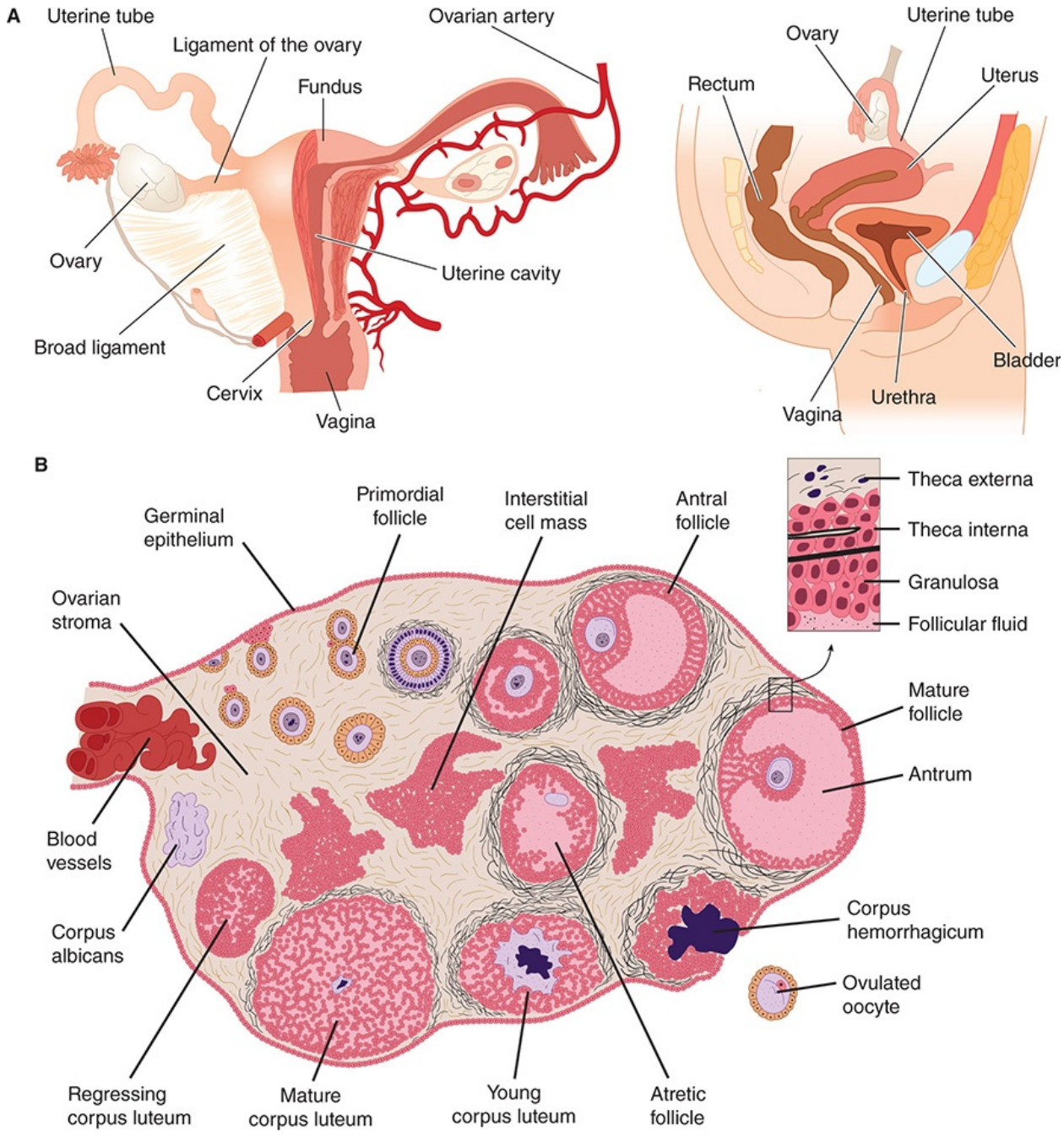


FIGURE 22–10 Functional anatomy of the female reproductive tract. The female reproductive organs include the ovaries, the uterus and the fallopian tubes, and the breast/mammary glands. The sequential development of a follicle, the formation of a corpus luteum and follicular atresia are shown.

Ovarian Cycle

From the time of birth, there are many **primordial follicles** under the ovarian capsule. Each contains an immature ovum (Figure 22–10). At the start of each cycle, several of these follicles enlarge, and a cavity forms around the ovum **(antrum formation).** This cavity is filled with follicular fluid. In humans, usually one of the follicles in one ovary starts to grow rapidly on about the sixth day and becomes the **dominant follicle,** while the others regress, forming **atretic follicles.** The atretic process involves apoptosis. It is uncertain how one follicle is selected to be the dominant follicle in this **follicular phase** of the menstrual cycle, but it seems to be related to the ability of the follicle to secrete the estrogen inside it that is needed for final maturation. When women are given human pituitary gonadotropin preparations by injection, many follicles develop simultaneously.

The structure of a maturing ovarian **(graafian)** follicle is shown in Figure 22–10. The primary source of circulating estrogen is the granulosa cells of the ovaries; however, the cells of the **theca interna** of the follicle are necessary for the production of estrogen as they secrete androgens that are aromatized to estrogen by the granulosa cells.

At about the 14th day of the cycle, the distended follicle ruptures, and the ovum is extruded into the abdominal cavity. This is the process of **ovulation.** The ovum is picked up by the fimbriated ends of the uterine tubes (oviducts). It is transported to the uterus and, unless fertilization occurs, out through the vagina.

The follicle that ruptures at the time of ovulation promptly fills with blood, forming what is sometimes called a **corpus hemorrhagicum.** Minor bleeding from the follicle into the abdominal cavity may cause peritoneal irritation and fleeting lower abdominal pain (“mittelschmerz”). The granulosa and theca cells of the follicle lining promptly begin to proliferate, and the clotted blood is rapidly replaced with yellowish, lipid-rich **luteal cells,** forming the **corpus luteum.** This initiates the **luteal phase** of the menstrual cycle, during which the luteal cells secrete estrogen and progesterone. Growth of the corpus luteum depends on its developing an adequate blood supply, and there is evidence that vascular endothelial growth factor (VEGF) (see Chapter 31) is essential for this process.

If pregnancy occurs, the corpus luteum persists and usually there are no more periods until after delivery. If pregnancy does not occur, the corpus luteum begins to degenerate about 4 days before the next menses (24th day of the cycle)

and is eventually replaced by scar tissue, forming a **corpus albicans.**

The ovarian cycle in other mammals is similar, except that in many species more than one follicle ovulates and multiple births are the rule. Corpora lutea form in some submammalian species but not in others.

In humans, no new ova are formed after birth. During fetal development, the ovaries contain over 7 million primordial follicles. However, many undergo atresia (involution) before birth and others are lost after birth. At the time of birth, there are 2 million ova, but 50% of these are atretic. The million that are normal undergo the first part of the first meiotic division at about this time and enter a stage of arrest in prophase in which those that survive persist until adulthood. Atresia continues during development, and the number of ova in both of the ovaries at the time of puberty is less than 300,000 (Figure 22–14). Only one of these ova per cycle (or about 500 in the course of a normal reproductive life) normally reaches maturity; the remainder degenerate. Just before ovulation, the first meiotic division is completed. One of the daughter cells, the **secondary oocyte,** receives most of the cytoplasm, while the other, the **first polar body,** fragments and disappears. The secondary oocyte immediately begins the second meiotic division, but this division stops at metaphase and is completed only when a sperm penetrates the oocyte. At that time, the **second polar body** is cast off and the fertilized ovum proceeds to form a new individual. The arrest in metaphase is due, at least in some species, to formation in the ovum of the protein **pp39mos**, which is encoded by the **cmos** protooncogene. When fertilization occurs, the pp39mos is destroyed within 30 min by **calpain,** a calcium-dependent cysteine protease.

Uterine Cycle

At the end of menstruation, all but the deep layers of the endometrium have sloughed. A new endometrium then regrows under the influence of estrogens from the developing follicle. The endometrium increases rapidly in thickness from the 5th to the 14th days of the menstrual cycle. As the thickness increases, the uterine glands are drawn out so that they lengthen **(Figure 22–11)**, but they do not become convoluted or secrete to any degree. These endometrial changes are called proliferative, and this part of the menstrual cycle is sometimes called the **proliferative phase.** It is also called the preovulatory or follicular phase of the cycle. After ovulation, the endometrium becomes more highly vascularized and slightly edematous under the influence of estrogen and progesterone from the corpus luteum. The glands become coiled and tortuous and they begin to

secrete a clear fluid. Consequently, this phase of the cycle is called the **secretory** or **luteal phase.** Late in the luteal phase, the endometrium, like the anterior pituitary, produces prolactin, but the function of this endometrial prolactin is unknown.

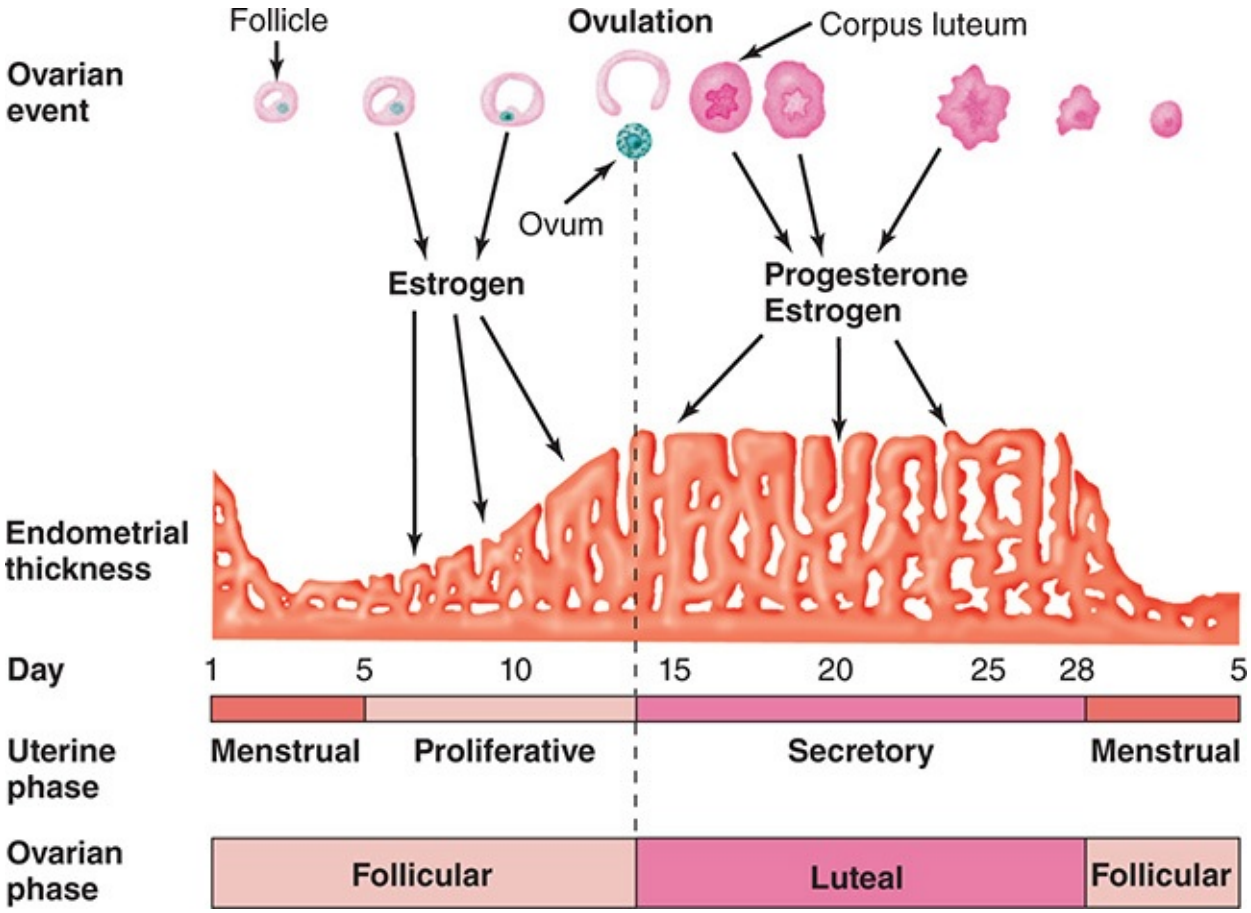


FIGURE 22–11 Relationship between ovarian and uterine changes during the menstrual cycle. (Reproduced with permission from Windmaier EP, Raff H, Strang KT: *Vander's Human Physiology: The Mechanisms of Body Function*, 11th ed. New York, NY: McGraw-Hill; 2008.)

The endometrium is supplied by two types of arteries. The superficial two-thirds of the endometrium that is shed during menstruation, the **stratum functionale,** is supplied by long, coiled **spiral arteries,** whereas the deep layer that is not shed, the **stratum basale,** is supplied by short, straight **basilar arteries.**

When the corpus luteum regresses, hormonal support for the endometrium is withdrawn. The endometrium becomes thinner, which adds to the coiling of the spiral arteries. Foci of necrosis appear in the endometrium, and these coalesce.

In addition, spasm and degeneration of the walls of the spiral arteries take place, leading to spotty hemorrhages that become confluent and produce the menstrual flow.

The vasospasm is probably produced by locally released prostaglandins. Large quantities of prostaglandins are present in the secretory endometrium and in menstrual blood, and infusions of prostagladin $F_{2\alpha}$($PGF_{2\alpha}$) produce endometrial necrosis and bleeding.

From the point of view of endometrial function, the proliferative phase of the menstrual cycle represents restoration of the epithelium from the preceding menstruation, and the secretory phase represents preparation of the uterus for implantation of the fertilized ovum. The length of the secretory phase is remarkably constant at about 14 days, and the variations seen in the length of the menstrual cycle are due for the most part to variations in the length of the proliferative phase. When fertilization fails to occur during the secretory phase, the endometrium is shed and a new cycle starts.

Normal Menstruation

Menstrual blood is predominantly arterial, with only 25% of the blood being of venous origin. It contains tissue debris, prostaglandins, and relatively large amounts of fibrinolysin from endometrial tissue. The fibrinolysin lyses clots, so that menstrual blood does not normally contain clots unless the flow is excessive.

The usual duration of the menstrual flow is 3–5 days, but flows as short as 1 day and as long as 8 days can occur in normal women. The amount of blood lost may range normally from slight spotting to 80 mL; the average amount lost is 30 mL. Loss of more than 80 mL is abnormal. Obviously, the amount of flow can be affected by various factors, including the thickness of the endometrium, medication, and diseases that affect the clotting mechanism.

Anovulatory Cycles

In some instances, ovulation fails to occur during the menstrual cycle. Such anovulatory cycles are common for the first 12–18 months after menarche and again before the onset of the menopause. When ovulation does not occur, no corpus luteum is formed and the effects of progesterone on the endometrium are absent. Estrogens continue to cause growth, however, and the proliferative endometrium becomes thick enough to break down and begins to slough. The

time it takes for bleeding to occur is variable, but it usually occurs in less than 28 days from the last menstrual period. The flow is also variable and ranges from scanty to relatively profuse.

Cyclical Changes in the Uterine Cervix

Although it is continuous with the body of the uterus, the cervix of the uterus is different in a number of ways. The mucosa of the uterine cervix does not undergo cyclical desquamation, but there are regular changes in the cervical mucus. Estrogen makes the mucus thinner and more alkaline, changes that promote the survival and transport of sperm. Progesterone makes it thick, tenacious, and cellular. The mucus is thinnest at the time of ovulation, and its elasticity, or **spinnbarkeit,** increases so that by midcycle, a drop can be stretched into a long, thin thread that may be 8–12 cm or more in length. In addition, it dries in an arborizing, fern-like pattern **(Figure 22–12)** when a thin layer is spread on a slide. After ovulation and during pregnancy, it becomes thick and fails to form the fern pattern.

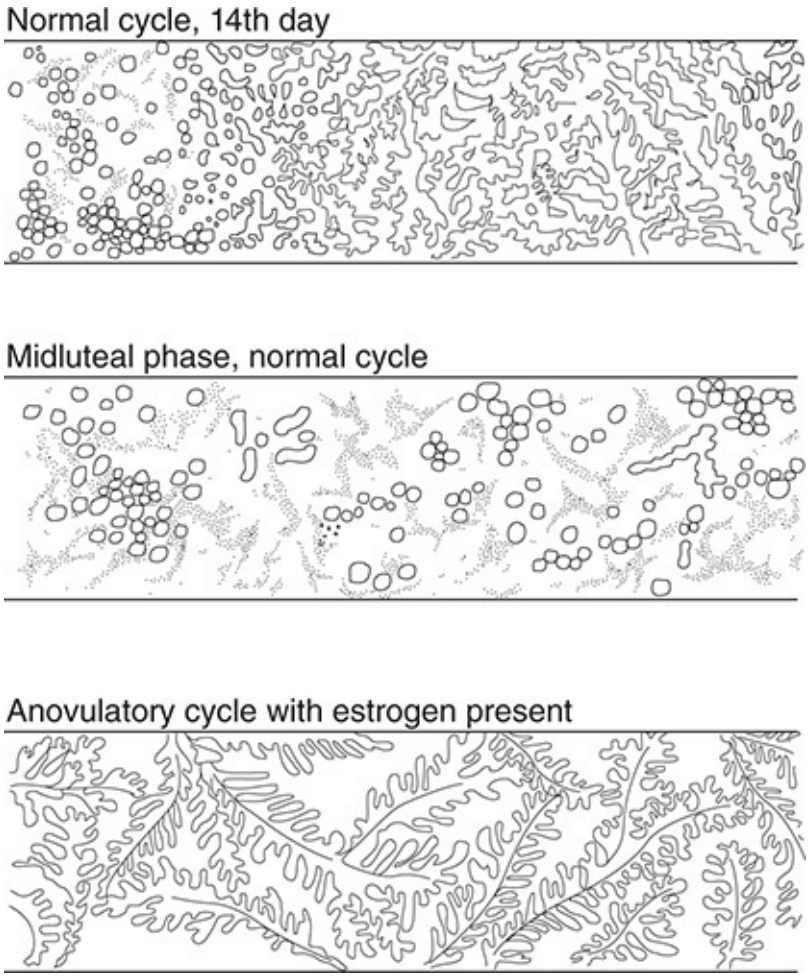


FIGURE 22–12 Patterns formed when cervical mucus is smeared on a slide, permitted to dry, and examined under the microscope. Progesterone makes the mucus thick and cellular. In the smear from a patient who did not ovulate **(bottom),** no progesterone is present to inhibit the estrogen-induced fern pattern.

Vaginal Cycle

Under the influence of estrogens, the vaginal epithelium becomes cornified, and cornified epithelial cells can be identified in the vaginal smear. Under the influence of progesterone, a thick mucus is secreted, and the epithelium proliferates and becomes infiltrated with leukocytes. The cyclical changes in the vaginal smear in rats are relatively marked. The changes in humans and other species are similar but not so clear-cut.

Cyclical Changes in the Breasts

Although lactation normally does not occur until the end of pregnancy, cyclical changes take place in the breasts during the menstrual cycle. Estrogens cause proliferation of mammary ducts, whereas progesterone causes growth of lobules and alveoli. The breast swelling, tenderness, and pain experienced by many women during the 10 days preceding menstruation are probably due to distension of the ducts, hyperemia, and edema of the interstitial tissue of the breast. All these changes regress, along with the symptoms, during menstruation.

Changes During Intercourse

During sexual excitement in women, fluid is secreted onto the vaginal walls, probably because of release of vasoactive intestinal polypeptide (VIP) from vaginal nerves. A lubricating mucus is also secreted by the vestibular glands. The upper part of the vagina is sensitive to stretch, while tactile stimulation from the labia minora and clitoris adds to the sexual excitement. These stimuli are reinforced by tactile stimuli from the breasts and, as in men, by visual, auditory, and olfactory stimuli, which may build to the crescendo known as orgasm. During orgasm, autonomically mediated rhythmic contractions occur in the vaginal walls. Impulses also travel via the pudendal nerves and produce rhythmic contraction of the bulbocavernosus and ischiocavernosus muscles. The vaginal contractions may aid sperm transport but are not essential for it, since fertilization of the ovum is not dependent on female orgasm.

Indicators of Ovulation

Knowing when during the menstrual cycle ovulation occurs is important in increasing fertility or, conversely, in family planning. A convenient and reasonably reliable indicator of the time of ovulation is a change—usually a rise—in the basal body temperature **(Figure 22–13)**. The rise starts 1–2 days after ovulation. Women interested in obtaining an accurate temperature chart should use a digital thermometer and take their temperatures (oral or rectal) in the morning before getting out of bed. The cause of the temperature change at the time of ovulation is probably the increase in progesterone secretion, since progesterone is thermogenic.

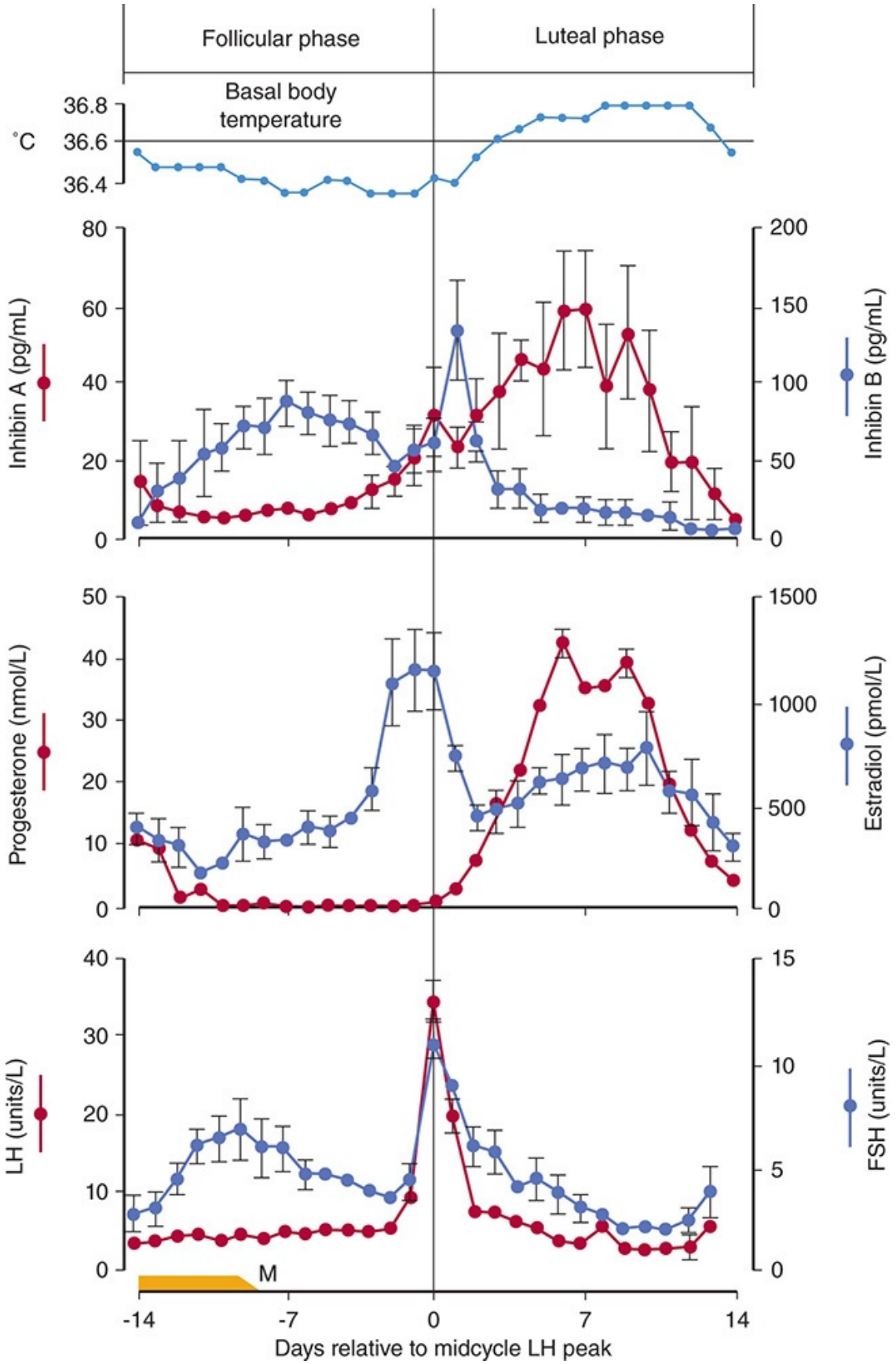

Follicular phase
Luteal phase
Basal body temperature
36.8
36.6
36.4
°C
80
60
40
20
0
Inhibin A (pg/mL)
200
150
100
50
0
Inhibin B (pg/mL)
50
40
30
20
10
0
Progesterone (nmol/L)
1500
1000
500
0
Estradiol (pmol/L)
40
30
20
10
0
LH (units/L)
15
10
5
0
FSH (units/L)
M
-14
-7
0
7
14
Days relative to midcycle LH peak

FIGURE 22–13 Basal body temperature and plasma hormone concentrations (mean ± standard error) during the normal human menstrual cycle. Values are aligned with respect to the day of the midcycle LH peak. FSH, follicle-stimulating hormone; LH, luteinizing hormone; M, menses.

A surge in LH secretion triggers ovulation, and ovulation normally occurs about 9 h after the peak of the LH surge at midcycle (Figure 22–13). The ovum lives for approximately 72 h after it is extruded from the follicle, but it is fertilizable for a much shorter time than this. In a study of the relation of isolated intercourse to pregnancy, 36% of women had a detected pregnancy following intercourse on the day of ovulation, but with intercourse on days after ovulation, the percentage was zero. Isolated intercourse on the first and second day before ovulation also led to pregnancy in about 36% of women. A few pregnancies resulted from isolated intercourse on day 3, 4, or 5 before ovulation, although the percentage was much lower, for example, 8% on day 5 before ovulation. Thus, some sperms can survive in the female genital tract and fertilize the ovum for up to 120 h before ovulation, but the most fertile period is clearly the 48 h before ovulation. However, for those interested in the "rhythm method" of contraception, it should be noted that there are rare but documented cases in the literature of pregnancy resulting from isolated coitus on every day of the cycle.

The Estrous Cycle

Mammals other than primates do not menstruate, and their sexual cycle is called an **estrous cycle.** It is named for the conspicuous period of "heat" **(estrus)** at the time of ovulation, normally the only time during which the sexual interest of the female is aroused. In spontaneously ovulating species with estrous cycles, such as the rat, no episodic vaginal bleeding occurs but the underlying endocrine events are essentially the same as those in the menstrual cycle. In other species, ovulation is induced by copulation (reflex ovulation).

MENOPAUSE

The human ovaries become unresponsive to gonadotropins with advancing age, and their function declines, so that sexual cycles disappear **(menopause).** This unresponsiveness is associated with and probably caused by a decline in the number of primordial follicles, which becomes precipitous at the time of

menopause **(Figure 22–14)**. The ovaries no longer secrete progesterone and 17β-estradiol in appreciable quantities, and estrogen is formed only in small amounts by aromatization of androstenedione in peripheral tissues (see Chapter 20). The uterus and the vagina gradually become atrophic. As the negative feedback effect of estrogens and progesterone is reduced, secretion of FSH is increased, and plasma FSH increases to high levels, LH levels are moderately high. Old female mice and rats have long periods of diestrus and increased levels of gonadotropin secretion. In women, a period called perimenopause precedes menopause and can last up to 10 years. During perimenopause FSH levels will increase before an increase in LH is observed due to a decrease in estrogen, progesterone, and inhibins and the menses become irregular. This usually occurs between the ages of 45 and 55. **The average age at onset of the menopause is 51 years.**

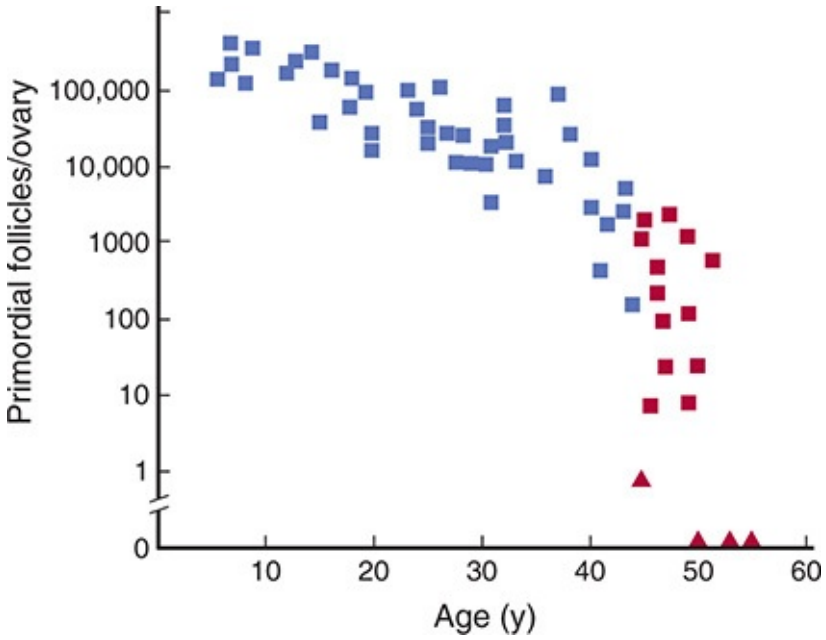


FIGURE 22–14 Number of primordial follicles per ovary in women at various ages. Blue squares, premenopausal women (regular menses); red squares, perimenopausal women (irregular menses for at least 1 year); red triangles, postmenopausal women (no menses for at least 1 year). Note that the vertical scale is a log scale and that the values are from one rather than two ovaries. (Redrawn by PM Wise and reproduced with permission from Richardson SJ, Senikas V, Nelson JF: Follicular depletion during the menopausal transition: Evidence for accelerated loss and ultimate exhaustion. J Clin Endocrinol Metab 1987;65:1231.)

The loss of ovarian function causes many symptoms such as sensations of warmth spreading from the trunk to the face (hot flushes; also called hot flashes) and night sweats. In addition, the onset of menopause increases the risk of many diseases such as osteoporosis, ischemic heart disease, and renal disease.

Hot flushes are said to occur in 75% of menopausal women and may continue intermittently for as long as 40 years. They also occur when early menopause is produced by bilateral ovariectomy, and they are prevented by estrogen treatment. In addition, they occur after castration in men. Their cause is unknown. However, they coincide with surges of LH secretion. LH is secreted in episodic bursts at intervals of 30–60 min or more **(circhoral secretion),** and in the absence of gonadal hormones these bursts are large. Each hot flush begins with the start of a burst. However, LH itself is not responsible for the symptoms, because they can continue after removal of the pituitary. Instead, it appears that some estrogen-sensitive event in the hypothalamus initiates both the release of LH and the episode of flushing.

Although the function of the testes tends to decline slowly with advancing age, the evidence is unclear whether there is a "male menopause" **(andropause)** similar to that occurring in women.

OVARIAN HORMONES

Chemistry, Biosynthesis, & Metabolism of Estrogens

The naturally occurring estrogens are **17β-estradiol, estrone,** and **estriol (Figure 22–15)**. They are C18 steroids that do not have an angular methyl group attached to the 10 position or a Δ4-3-keto configuration in the A ring. They are secreted primarily by the granulosa cells of the ovarian follicles, the corpus luteum, and the placenta. Their biosynthesis depends on the enzyme **aromatase** (CYP19), which converts testosterone to estradiol and androstenedione to estrone (Figure 22–15). The latter reaction also occurs in fat, liver, muscle, and the brain.

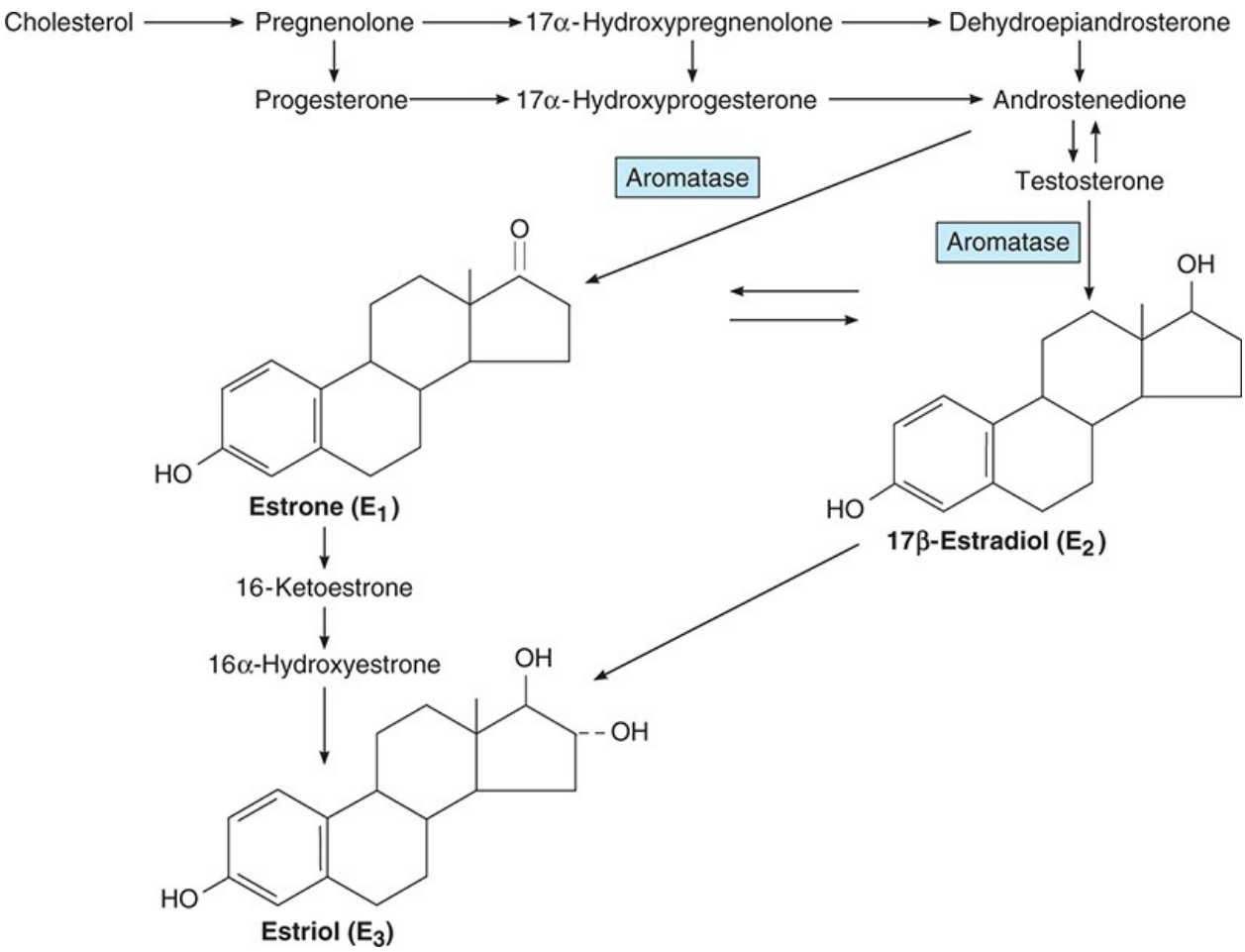


FIGURE 22–15 Biosynthesis and metabolism of estrogens. The formulas of the precursor steroids are shown in Figure 19–7.

Theca interna cells have many LH receptors, and LH acts via cAMP to increase conversion of cholesterol to androstenedione. The theca interna cells supply androstenedione to the granulosa cells. The granulosa cells make estradiol when provided with androgens **(Figure 22–16)**, and it appears that the estradiol they form in primates is secreted into the follicular fluid. Granulosa cells have many FSH receptors, and FSH facilitates their secretion of estradiol by acting via cAMP to increase their aromatase activity. Mature granulosa cells also acquire LH receptors, and LH also stimulates estradiol production.

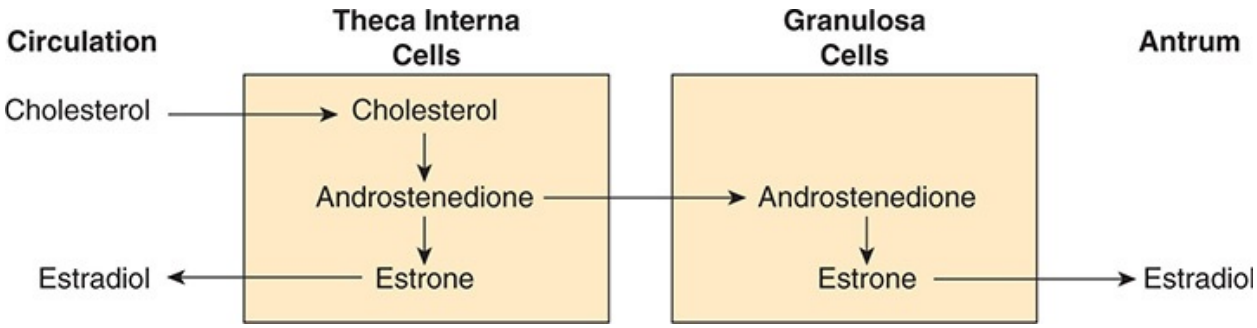


FIGURE 22–16 Interactions between theca and granulosa cells in estradiol synthesis and secretion.

Two percent of the circulating estradiol is free, and the remainder is bound to protein: 60% to albumin and 38% to the same gonadal steroid-binding globulin (GBG) that binds testosterone.

In the liver, estradiol, estrone, and estriol are converted to glucuronide and sulfate conjugates. All these compounds, along with other metabolites, are excreted in the urine. Appreciable amounts are secreted in the bile and reabsorbed into the bloodstream (enterohepatic circulation).

Secretion

The concentration of estradiol in the plasma during the menstrual cycle is shown in Figure 22–14. Almost all of this estrogen comes from the ovary, and two peaks of secretion occur: one just before ovulation and one during the midluteal phase. The estradiol secretion rate is 36 µg/day (133 nmol/day) in the early follicular phase, 380 µg/day just before ovulation, and 250 µg/day during the midluteal phase **(Table 22–3)**. After menopause, estrogen secretion declines to low levels.

TABLE 22–3 Twenty-four-hour production rates of sex steroids in women at different stages of the menstrual cycle.

Sex Steroids	Early Follicular	Preovulatory	Midluteal
Progesterone (mg)	1.0	4.0	25.0
17-Hydroxyprogesterone (mg)	0.5	4.0	4.0
Dehydroepiandrosterone (mg)	7.0	7.0	7.0
Androstenedione (mg)	2.6	4.7	3.4
Testosterone (µg)	144.0	171.0	126.0
Estrone (µg)	50.0	350.0	250.0
Estradiol (µg)	36.0	380.0	250.0

Data from Baird DT, Fraser IS: Blood production and ovarian secretion rates of estradiol-17 beta and estrone in women throughout the menstrual cycle. J Clin Endocrinal Metab 1974 Jun; 38(6):1009-1017.

As noted previously, the estradiol production rate in men is about 50 µg/day (184 nmol/day).

Effects on the Female Genitalia

Estrogens facilitate the growth of the ovarian follicles and increase the motility of the uterine tubes. Their role in the cyclic changes in the endometrium, cervix, and vagina has been discussed previously. They increase uterine blood flow and have important effects on the smooth muscle of the uterus. In immature and castrated females, the uterus is small and the myometrium atrophic and inactive. Estrogens increase the amount of uterine muscle and its content of contractile proteins. Under the influence of estrogens, the muscle becomes more active and excitable, and action potentials in the individual fibers become more frequent. The “estrogen-dominated” uterus is also more sensitive to oxytocin.

Chronic treatment with estrogens causes the endometrium to hypertrophy. When estrogen therapy is discontinued, sloughing takes place with **withdrawal bleeding.** Some “breakthrough” bleeding may occur during treatment when estrogens are given for long periods. Prolonged exposure to estrogen alone (unopposed by progesterone) has been indicated as a risk factor in the development of endometrial cancer.

Effects on Endocrine Organs

Estrogens decrease FSH secretion. Under some circumstances, they inhibit LH secretion (negative feedback); in other circumstances, they increase LH secretion (positive feedback). Women are sometimes given large doses of estrogens for 4–6 days to prevent conception after coitus during the fertile period (postcoital or "morning-after" contraception). However, in this instance, pregnancy is probably prevented by interference with implantation of the ovum rather than changes in gonadotropin secretion.

Estrogens cause increased secretion of angiotensinogen and thyroid-binding globulin. They exert an important protein anabolic effect in chickens and cattle, possibly by stimulating the secretion of androgens from the adrenal, and estrogen treatment has been used commercially to increase the weight of domestic animals. They cause epiphysial closure in humans (see Chapter 21).

Effects on the Central Nervous System

The estrogens are responsible for estrous behavior in animals, and they increase libido in humans. They apparently exert this action by a direct effect on certain neurons in the hypothalamus. Estrogens also increase the proliferation of dendrites on neurons and the number of synaptic knobs in rats.

Effects on the Breasts

Estrogens produce duct growth in the breasts and are largely responsible for breast enlargement at puberty in girls; they have been called the growth hormones of the breast. They are responsible for the pigmentation of the areolas, although pigmentation usually becomes more intense during the first pregnancy than it does at puberty. The role of estrogens in the overall control of breast growth and lactation is discussed below.

Female Secondary Sex Characteristics

The body changes that develop in girls at puberty—in addition to enlargement of breasts, uterus, and vagina—are due in part to estrogens, which are the "feminizing hormones," and in part simply to the absence of testicular androgens. Women have narrow shoulders and broad hips, thighs that converge, and arms that diverge (wide **carrying angle**). This body configuration, plus the

female distribution of fat in the breasts and buttocks, is seen also in castrate males. In women, the larynx retains its prepubertal proportions and the voice stays high-pitched. Women have less body hair and more scalp hair, and the pubic hair generally has a characteristic flat-topped pattern (female escutcheon). However, growth of pubic and axillary hair in both sexes is due primarily to androgens rather than estrogens.

Other Actions

Normal women retain salt and water and gain weight just before menstruation. Estrogens cause some degree of salt and water retention. However, aldosterone secretion is slightly elevated in the luteal phase, and this also contributes to the premenstrual fluid retention.

Estrogens are said to make sebaceous gland secretions more fluid and thus to counter the effect of testosterone and inhibit formation of **comedones** ("black-heads") and acne. The liver palms, spider angiomas, and slight breast enlargement seen in advanced liver disease are due to increased circulating estrogens. The increase appears to be due to decreased hepatic metabolism of androstenedione, making more of this androgen available for conversion to estrogens.

Estrogens have a significant plasma cholesterol-lowering action, and they rapidly produce vasodilation by increasing the local production of NO. Their action on bone is discussed in Chapter 21.

Mechanism of Action

There are two principal types of nuclear estrogen receptors: estrogen receptor α (ERα) encoded by a gene on chromosome 6; and estrogen receptor β (ERβ), encoded by a gene on chromosome 14. Both are members of the nuclear receptor super-family (see Chapter 2). After binding estrogen, they form homodimers and bind to DNA, altering its transcription. Some tissues contain one type or the other, but overlap also occurs, with some tissues containing both ERα and ERβ. ERα is found primarily in the uterus, kidneys, liver, and heart, whereas ERβ is found primarily in the ovaries, prostate, lungs, gastrointestinal tract, hemopoietic system, and central nervous system (CNS). ERα and ERβ can also form heterodimers. Male and female mice in which the gene for ERα has been knocked out are sterile, develop osteoporosis, and continue to grow because their epiphyses do not close. ERβ female knockouts are infertile, but ERβ male

knockouts are fertile even though they have hyperplastic prostates and loss of fat. Both receptors exist in isoforms and, like thyroid receptors, can bind to various activating and stimulating factors. In some situations, ERβ can inhibit ERα transcription. Thus, their actions are complex, multiple, and varied.

Most of the effects of estrogens are genomic, that is, due to actions on the nucleus, but some are so rapid that it is difficult to believe they are mediated via production of mRNAs. These include effects on neuronal discharge in the brain and, possibly, feedback effects on gonadotropin secretion. Evidence is accumulating that these effects are mediated by cell membrane receptors that appear to be structurally related to the nuclear receptors and produce their effects by intracellular mitogen-activated protein kinase pathways. Similar rapid effects of progesterone, testosterone, glucocorticoids, aldosterone, and 1,25-dihydroxycholecalciferol may also be produced by membrane receptors (see Chapter 16).

Synthetic & Environmental Estrogens

The ethinyl derivative of estradiol is a potent estrogen and, unlike the naturally occurring estrogens, is relatively active when given by mouth because it is resistant to hepatic metabolism. The activity of the naturally occurring hormones is low when they are administered by mouth because the portal venous drainage of the intestine carries them to the liver, where they are inactivated before they can reach the general circulation. Some nonsteroidal substances and a few compounds found in plants also have estrogenic activity. The plant estrogens are rarely a problem in human nutrition, but they may cause undesirable effects in farm animals. **Dioxins,** which are found in the environment and are produced by a variety of industrial processes, can activate estrogen response elements on genes. However, they have been reported to have antiestrogenic as well as estrogenic effects, and their role, if any, in the production of human disease remains a matter of disagreement and debate.

Because natural estrogens have undesirable as well as desirable effects (eg, they preserve bone in osteoporosis but can cause uterine and breast cancer), there has been an active search for “tailor-made” estrogens that have selective effects in humans. Two compounds, **tamoxifen** and **raloxifene,** show promise in this regard. Neither combats the symptoms of menopause, but both have the bone-preserving effects of estradiol. In addition, tamoxifen does not stimulate the breast, and raloxifene does not stimulate the breast or uterus. The way the effects of these selective estrogen receptor modulators **(SERMs)** are brought

about is related to the complexity of the estrogen receptors and hence to differences in the way that the receptor–ligand complexes they form bind to DNA.

Chemistry, Biosynthesis, & Metabolism of Progesterone

Progesterone is a C_{21} steroid **(Figure 22–17)** secreted by the corpus luteum, the placenta, and (in small amounts) the follicle. It is an important intermediate in steroid biosynthesis in all tissues that secrete steroid hormones, and small amounts apparently enter the circulation from the testes and adrenal cortex. About 2% of the circulating progesterone is free whereas 80% is bound to albumin and 18% is bound to corticosteroid-binding globulin. Progesterone has a short half-life and is converted in the liver to pregnanediol, which is conjugated to glucuronic acid and excreted in the urine.

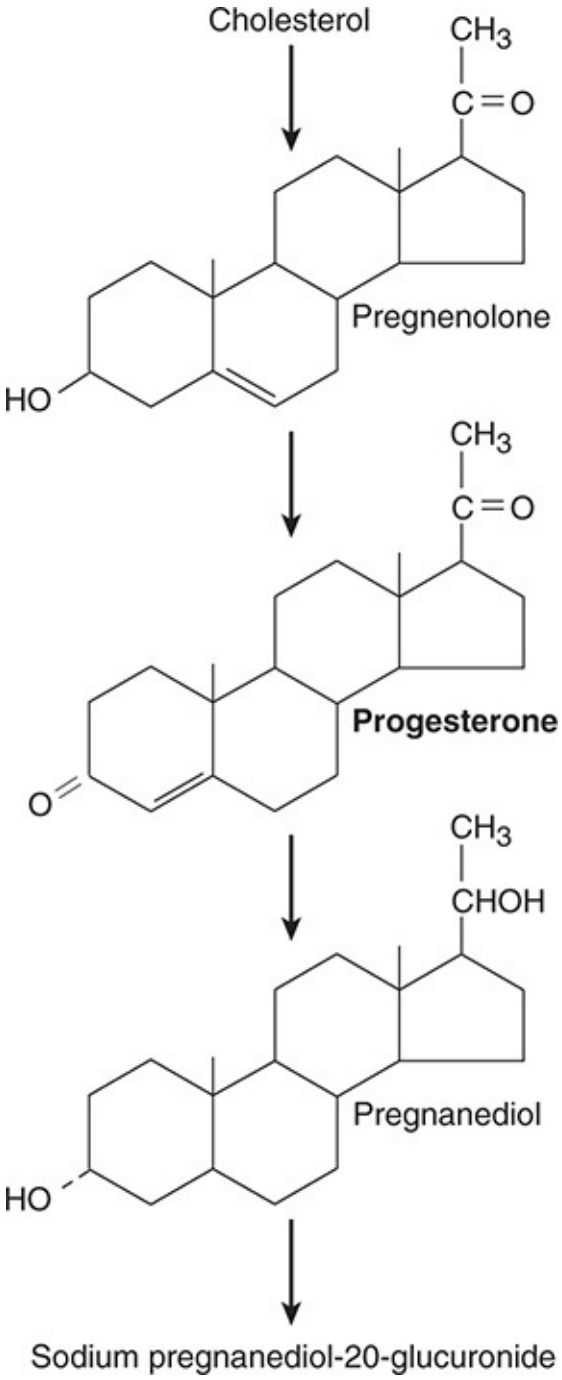


FIGURE 22–17 Biosynthesis of progesterone and major pathway for its metabolism. Other metabolites are also formed.

Secretion

In men, the plasma progesterone level is approximately 0.3 ng/mL (1 nmol/L). In women, the level is approximately 0.9 ng/mL (3 nmol/L) during the follicular

phase of the menstrual cycle (Figure 22–14). The difference is due to secretion of small amounts of progesterone by cells in the ovarian follicles; theca cells provide pregnenolone to the granulosa cells, which convert it to progesterone. Late in the follicular phase, progesterone secretion begins to increase. During the luteal phase, the corpus luteum produces large quantities of progesterone (Table 22–3) and plasma progesterone is markedly increased to a peak value of approximately 18 ng/mL (60 nmol/L).

The stimulating effect of LH on progesterone secretion by the corpus luteum is due to activation of adenylyl cyclase and involves a subsequent step that is dependent on protein synthesis.

Actions

The principal target organs of progesterone are the uterus, the breasts, and the brain. Progesterone is responsible for the progestational changes in the endometrium and the cyclical changes in the cervix and vagina described above. It has an antiestrogenic effect on the myometrial cells, decreasing their excitability, their sensitivity to oxytocin, and their spontaneous electrical activity while increasing their membrane potential. It also decreases the number of estrogen receptors in the endometrium and increases the rate of conversion of 17β-estradiol to less active estrogens.

In the breast, progesterone stimulates the development of lobules and alveoli. It induces differentiation of estrogen-prepared ductal tissue and supports the secretory function of the breast during lactation.

The feedback effects of progesterone are complex and are exerted at both the hypothalamic and pituitary levels. Large doses of progesterone inhibit LH secretion and potentiate the inhibitory effect of estrogens, preventing ovulation.

Progesterone is thermogenic and is probably responsible for the rise in basal body temperature at the time of ovulation. It stimulates respiration, and the alveolar PCO_2 (see Chapter 34) in women during the luteal phase of the menstrual cycle is lower than that in men. In pregnancy, the PCO_2 falls as progesterone secretion rises. However, the physiologic significance of this respiratory response is unknown.

Large doses of progesterone produce natriuresis, probably by blocking the action of aldosterone on the kidney. The hormone does not have a significant anabolic effect.

Mechanism of Action

The effects of progesterone, like those of other steroids, are brought about by an action on DNA to initiate synthesis of new mRNA. The progesterone receptor is bound to a heat shock protein in the absence of the steroid, and progesterone binding releases the heat shock protein, exposing the DNA-binding domain of the receptor. The synthetic steroid **mifepristone (RU 486)** binds to the receptor but does not release the heat shock protein, and it blocks the binding of progesterone. Because the maintenance of early pregnancy depends on the stimulatory effect of progesterone on endometrial growth and its inhibition of uterine contractility, mifepristone combined with a prostaglandin can be used to produce elective abortions.

There are two isoforms of the progesterone receptor—PR_A and PR_B—that are produced by differential processing from a single gene. PR_A is a truncated form, but it is likely that both isoforms mediate unique subsets of progesterone action.

Substances that mimic the action of progesterone are sometimes called **progestational agents, gestagens,** or **progestins.** They are used along with synthetic estrogens as oral contraceptive agents.

Relaxin

Relaxin is a polypeptide hormone that is produced in the corpus luteum, uterus, placenta, and mammary glands in women and in the prostate gland in men. During pregnancy, it relaxes the pubic symphysis and other pelvic joints and softens and dilates the uterine cervix. Thus, it facilitates delivery. It also inhibits uterine contractions and may play a role in the development of the mammary glands. In nonpregnant women, relaxin is found in the corpus luteum and the endometrium during the secretory but not the proliferative phase of the menstrual cycle. Its function in nonpregnant women is unknown. In men, it is found in semen, where it may help maintain sperm motility and aid in sperm penetration of the ovum.

In most species there is only one relaxin gene, but in humans there are two genes on chromosome 9 that code for two structurally different polypeptides that both have relaxin activity. However, only one of these genes is active in the ovary and the prostate.

CONTROL OF OVARIAN FUNCTION

FSH from the pituitary is responsible for the early maturation of the ovarian follicles, and FSH and LH together are responsible for their final maturation. A burst of LH secretion (Figure 22–14) is responsible for ovulation and the initial formation of the corpus luteum. A smaller midcycle burst of FSH secretion also occurs, the significance of which is uncertain. LH stimulates the secretion of estrogen and progesterone from the corpus luteum.

Hypothalamic Components

The hypothalamus occupies a key position in the control of gonadotropin secretion. Hypothalamic control is exerted by GnRH secreted into the portal hypophysial vessels. GnRH stimulates the secretion of FSH as well as LH.

GnRH is normally secreted in episodic bursts, and these bursts produce the circhoral peaks of LH secretion. They are essential for normal secretion of gonadotropins. If GnRH is administered by constant infusion, the GnRH receptors in the anterior pituitary downregulate and LH secretion declines to zero. However, if GnRH is administered episodically at a rate of one pulse per hour, LH secretion is stimulated. This is true even when endogenous GnRH secretion has been prevented by a lesion of the ventral hypothalamus.

It is now clear not only that episodic secretion of GnRH is a general phenomenon but also that fluctuations in the frequency and amplitude of the GnRH bursts are important in generating the other hormonal changes that are responsible for the menstrual cycle. Frequency is increased by estrogens and decreased by progesterone and testosterone. The frequency increases late in the follicular phase of the cycle, culminating in the LH surge. During the secretory phase, the frequency decreases as a result of the action of progesterone **(Figure 22–18)**, but when estrogen and progesterone secretion decrease at the end of the cycle, the frequency once again increases.

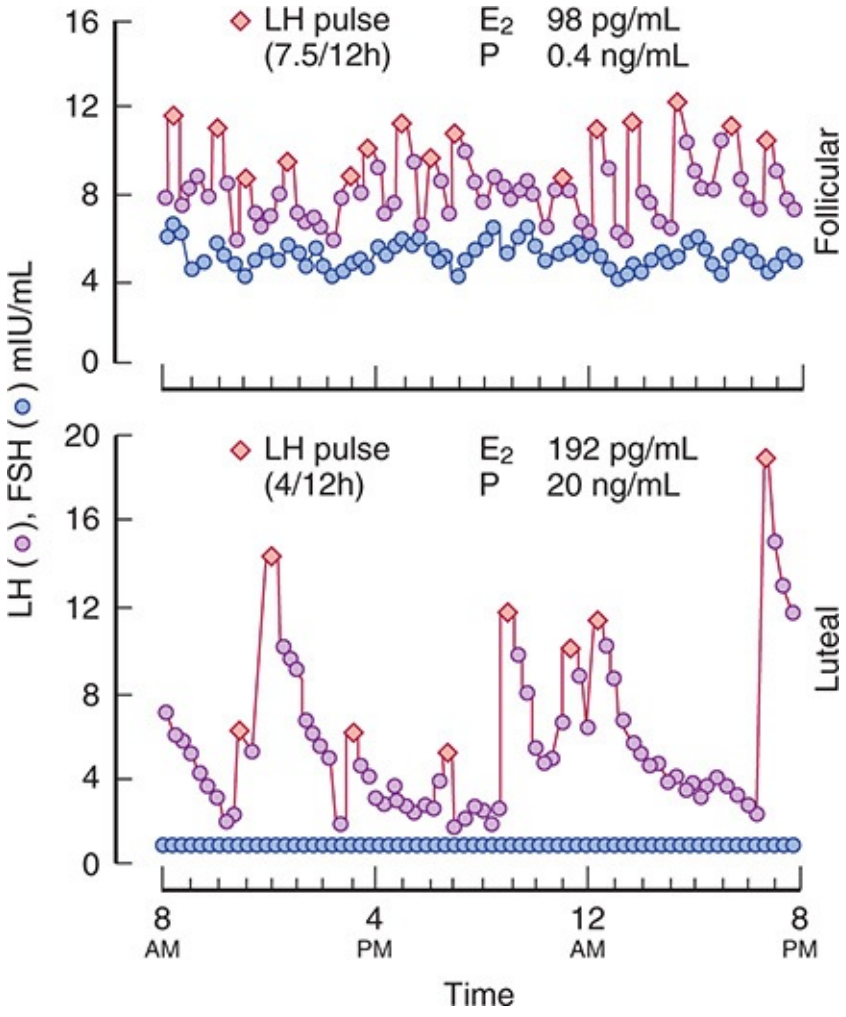


FIGURE 22–18 Episodic secretion of LH (s) and FSH (d) during the follicular stage (top) and the luteal stage (bottom) of the menstrual cycle. The numbers above each graph indicate the numbers of LH pulses per 12 hours and the plasma estradiol (E_2) and progesterone (P) concentrations at these two times of the cycle. (Reproduced with permission from Marshall JC, Kelch RO: Gonadotropin-releasing hormone: Role of pulsatile secretion in the regulation of reproduction. N Engl J Med 1986; Dec 4; 315(23):1459–1468.)

At the time of the midcycle LH surge, the sensitivity of the gonadotropes to GnRH is greatly increased because of their exposure to GnRH pulses at a specific frequency. This self-priming effect of GnRH is important in producing a maximum LH response.

The nature and the exact location of the GnRH pulse generator in the hypothalamus are still unsettled. However, it is known in a general way that norepinephrine and possibly epinephrine in the hypothalamus increase GnRH

pulse frequencies. Conversely, opioid peptides such as the enkephalins and β-endorphin reduce the frequency of GnRH pulses.

The downregulation of pituitary receptors and the consequent decrease in LH secretion produced by constantly elevated levels of GnRH has led to the use of long-acting GnRH analogs to inhibit LH secretion in precocious puberty and in cancer of the prostate.

Feedback Effects

Changes in plasma LH, FSH, sex steroids, and inhibin during the menstrual cycle are shown in Figure 22–13, and their feedback relations are diagrammed in **Figure 22–19**. During the early part of the follicular phase, inhibin B is low and FSH is modestly elevated, fostering follicular growth. LH secretion is held in check by the negative feedback effect of the rising plasma estrogen level. At 36–48 h before ovulation, the estrogen feedback effect becomes positive, and this initiates the burst of LH secretion (LH surge) that produces ovulation. Ovulation occurs about 9 h after the LH peak. FSH secretion also peaks, despite a small rise in inhibin, probably because of the strong stimulation of gonadotropes by GnRH. During the luteal phase, the secretion of LH and FSH is low because of the elevated levels of estrogen, progesterone, and inhibin.

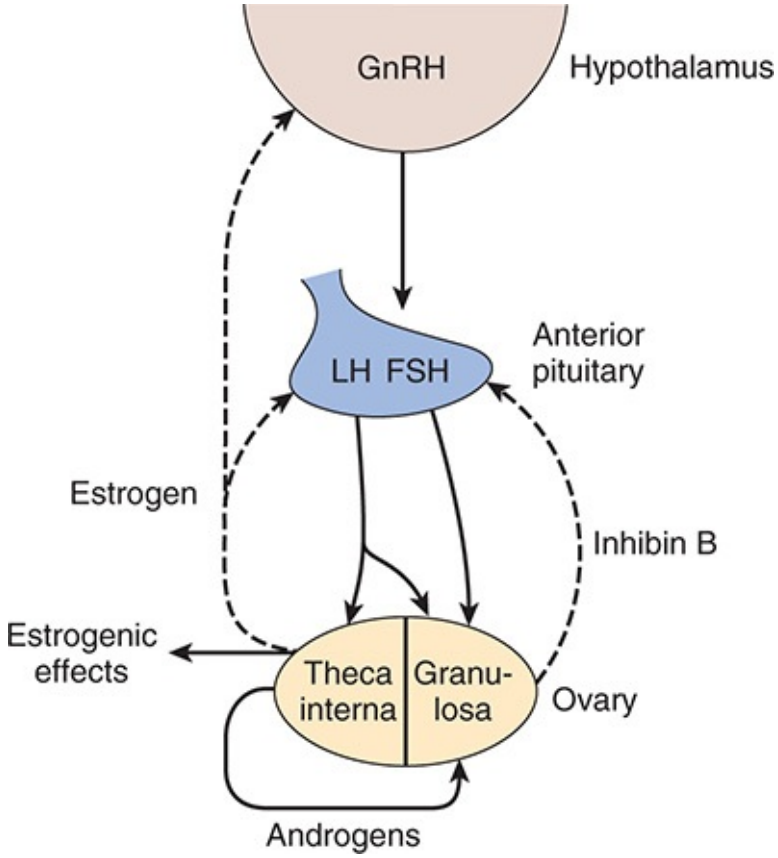


FIGURE 22–19 Feedback regulation of ovarian function. The cells of the theca interna provide androgens to the granulosa cells, and theca cells also produce the circulating estrogens that inhibit the secretion of GnRH, LH, and FSH. Inhibin from the granulosa cells inhibits FSH secretion. LH regulates the thecal cells, whereas the granulosa cells are regulated by both LH and FSH. The dashed arrows indicate inhibitory effects and the solid arrows stimulatory effects. FSH, follicle-stimulating hormone; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone.

It should be emphasized that a moderate, constant level of circulating estrogen exerts a negative feedback effect on LH secretion, whereas during the cycle, an elevated estrogen level exerts a positive feedback effect and stimulates LH secretion. In monkeys, it has been demonstrated that estrogens must also be elevated for a minimum time to produce positive feedback. When circulating estrogen was increased about 300% for 24 h, only negative feedback was seen; but when it was increased about 300% for 36 h or more, a brief decline in secretion was followed by a burst of LH secretion that resembled the midcycle surge. When circulating levels of progesterone were high, the positive feedback effect of estrogen was inhibited. In primates, there is evidence that both the

negative and the positive feedback effects of estrogen are exerted in the hypothalamus and recent studies have identified a role for the peptide **kisspeptin**, which is expressed in neurons in the hypothalamus (in humans, kisspeptin positive neurons are concentrated in the infundibular nucleus). Estrogen and progesterone modulate kisspeptin activity though receptors expressed on kisspeptin neurons.

Control of the Cycle

In an important sense, regression of the corpus luteum **(luteolysis)** starting 3–4 days before menses is the key to the menstrual cycle. $PGF_{2\alpha}$ appears to be a physiologic luteolysin, but this prostaglandin is only active when endothelial cells producing ET-1 (see Chapter 32) are present. Therefore, it appears that at least in some species luteolysis is produced by the combined action of $PGF_{2\alpha}$ and ET-1. In some domestic animals, oxytocin secreted by the corpus luteum appears to exert a local luteolytic effect, possibly by causing the release of prostaglandins. Once luteolysis begins, the estrogen and progesterone levels fall and the secretion of FSH and LH increases. A new crop of follicles develops, and then a single dominant follicle matures as a result of the action of FSH and LH. Near midcycle, estrogen secretion from the follicle rises. This rise augments the responsiveness of the pituitary to GnRH and triggers a burst of LH secretion. The resulting ovulation is followed by formation of a corpus luteum. Estrogen secretion drops, but progesterone and estrogen levels then rise together, along with inhibin B. The elevated levels inhibit FSH and LH secretion for a while, but luteolysis again occurs and a new cycle starts.

Reflex Ovulation

Female cats, rabbits, mink, and some other animals have long periods of estrus, during which they ovulate only after copulation. Such **reflex ovulation** is brought about by afferent impulses from the genitalia and the eyes, ears, and nose that converge on the ventral hypothalamus and provoke an ovulation-inducing release of LH from the pituitary. In species such as rats, monkeys, and humans, ovulation is a spontaneous periodic phenomenon, but neural mechanisms are also involved. Ovulation can be delayed 24 h in rats by administering pentobarbital or various other neurally active drugs 12 h before the expected time of follicle rupture.

Contraception

Methods commonly used to prevent conception are listed in Table 22–4, along with their failure rates. Once conception has occurred, abortion can be produced by progesterone antagonists such as mifepristone.

TABLE 22–4 Relative effectiveness of frequently used contraceptive methods.

Method	Failures per 100 Woman-Years
Vasectomy	0.02
Tubal ligation and similar procedures	0.13
Oral contraceptives	
>50 ug estrogen and progestin	0.32
<50 ug estrogen and progestin	0.27
Progestin only	1.2
IUD	
Copper 7	1.5
Loop D	1.3
Diaphragm	1.9
Condom	3.6
Withdrawal	6.7
Spermicide	11.9
Rhythm	15.5

Data from Vessey M, Lawless M, Yeates D: Efficacy of different contraceptive methods. Lancet 1982;1:841. Reproduced with permission.

Implantation of foreign bodies in the uterus causes changes in the duration of the sexual cycle in a number of mammalian species. In humans, such foreign bodies do not alter the menstrual cycle, but they act as effective contraceptive devices. Intrauterine implantation of pieces of metal or plastic **(intrauterine devices**, **IUDs)** has been used in programs aimed at controlling population growth. Although the mechanism of action of IUDs is still unsettled, they seem in general to prevent sperm from fertilizing ova. Those containing copper appear to exert a spermatocidal effect. IUDs that slowly release progesterone or

synthetic progestins have the additional effect of thickening cervical mucus so that entry of sperm into the uterus is impeded. IUDs can cause intrauterine infections, but these usually occur in the first month after insertion and in women exposed to sexually transmitted diseases.

Women undergoing long-term treatment with relatively large doses of estrogen do not ovulate, probably because they have depressed FSH levels and multiple irregular bursts of LH secretion rather than a single midcycle peak. Women treated with similar doses of estrogen plus a progestational agent do not ovulate because the secretion of both gonadotropins is suppressed. In addition, the progestin makes the cervical mucus thick and unfavorable to sperm migration, and it may also interfere with implantation. For contraception, an orally active estrogen such as ethinyl estradiol is often combined with a synthetic progestin such as norethindrone. The pills are administered for 21 days, then withdrawn for 5–7 days to permit menstrual flow, and started again. Like ethinyl estradiol, norethindrone has an ethinyl group on position 17 of the steroid nucleus, so it is resistant to hepatic metabolism and consequently is effective by mouth. In addition to being a progestin, it is partly metabolized to ethinyl estradiol, and for this reason it also has estrogenic activity. Small as well as large doses of estrogen are effective (Table 22–4).

Implants made up primarily of progestins such as levonorgestrel are now seeing increased use in some parts of the world. These are inserted under the skin and can prevent pregnancy for up to 5 years. They often produce amenorrhea, but otherwise they appear to be effective and well tolerated.

ABNORMALITIES OF OVARIAN FUNCTION

Menstrual Abnormalities

Some women who are infertile have **anovulatory cycles;** they fail to ovulate but have menstrual periods at fairly regular intervals. As noted above, anovulatory cycles are the rule for the first 1–2 years after menarche and again before the menopause. **Amenorrhea** is the absence of menstrual periods. If menstrual bleeding has never occurred, the condition is called **primary amenorrhea.** Some women with primary amenorrhea have small breasts and other signs of failure to mature sexually. Cessation of cycles in a woman with previously normal periods is called **secondary amenorrhea.** The most common cause of secondary amenorrhea is pregnancy, and the old clinical maxim that “secondary amenorrhea should be considered to be due to pregnancy until proved otherwise”

has considerable merit. Other causes of amenorrhea include emotional stimuli and changes in the environment, hypothalamic diseases, pituitary disorders, primary ovarian disorders, and various systemic diseases. Evidence suggests that in some women with hypothalamic amenorrhea, the frequency of GnRH pulses is slowed as a result of excess opioid activity in the hypothalamus. In encouraging preliminary studies, the frequency of GnRH pulses was increased by administration of the orally active opioid blocker naltrexone.

The terms **hypomenorrhea** and **menorrhagia** refer to scanty and abnormally profuse flow, respectively, during regular periods. **Metrorrhagia** is bleeding from the uterus between periods, and **oligomenorrhea** is reduced frequency of periods. **Dysmenorrhea** is painful menstruation. The severe menstrual cramps that are common in young women quite often disappear after the first pregnancy. Most of the symptoms of dysmenorrhea are due to accumulation of prostaglandins in the uterus, and symptomatic relief has been obtained by treatment with inhibitors of prostaglandin synthesis.

Some women develop symptoms such as irritability, bloating, edema, decreased ability to concentrate, depression, headache, and constipation during the last 7–10 days of their menstrual cycles. These symptoms of the **premenstrual syndrome (PMS)** have been attributed to salt and water retention. However, it seems unlikely that this or any of the other hormonal alterations that occur in the late luteal phase are responsible because the time course and severity of the symptoms are not modified if the luteal phase is terminated early and menstruation produced by administration of mifepristone. The antidepressant fluoxetine, which is a serotonin reuptake inhibitor, and the benzodiazepine alprazolam produce symptomatic relief, and so do GnRH-releasing agonists in doses that suppress the pituitary–ovarian axis. How these diverse clinical observations fit together to produce a picture of the pathophysiology of PMS is still unknown **(Clinical Box 22–4)**.

PREGNANCY

Fertilization & Implantation

In humans, **fertilization** of the ovum by the sperm (see Chapter 23) usually occurs in the ampulla of the uterine tube. Fertilization involves (1) chemoattraction of the sperm to the ovum by substances produced by the ovum; (2) adherence to the **zona pellucida,** the membranous structure surrounding the ovum; (3) penetration of the zona pellucida and the acrosome reaction; and (4)

adherence of the sperm head to the cell membrane of the ovum, with breakdown of the area of fusion and release of the sperm nucleus into the cytoplasm of the ovum **(Figure 22–20)**. Millions of sperm are deposited in the vagina during intercourse. Eventually, 50–100 sperm reach the ovum, and many of them contact the zona pellucida. Sperm bind to a receptor in the zona, and this is followed by the **acrosomal reaction,** that is, the breakdown of the acrosome, the lysosome-like organelle on the head of the sperm (Figure 23–4). Various enzymes are released, including the trypsin-like protease **acrosin.** Acrosin facilitates but is not required for the penetration of the sperm through the zona pellucida. When one sperm reaches the membrane of the ovum, fusion to the ovum membrane is mediated by **fertilin,** a protein on the surface of the sperm head that resembles the viral fusion proteins that permit some viruses to attack cells. The fusion provides the signal that initiates development. In addition, the fusion sets off a reduction in the membrane potential of the ovum that prevents polyspermy, the fertilization of the ovum by more than one sperm. This transient potential change is followed by a structural change in the zona pellucida that provides protection against polyspermy on a more long-term basis.

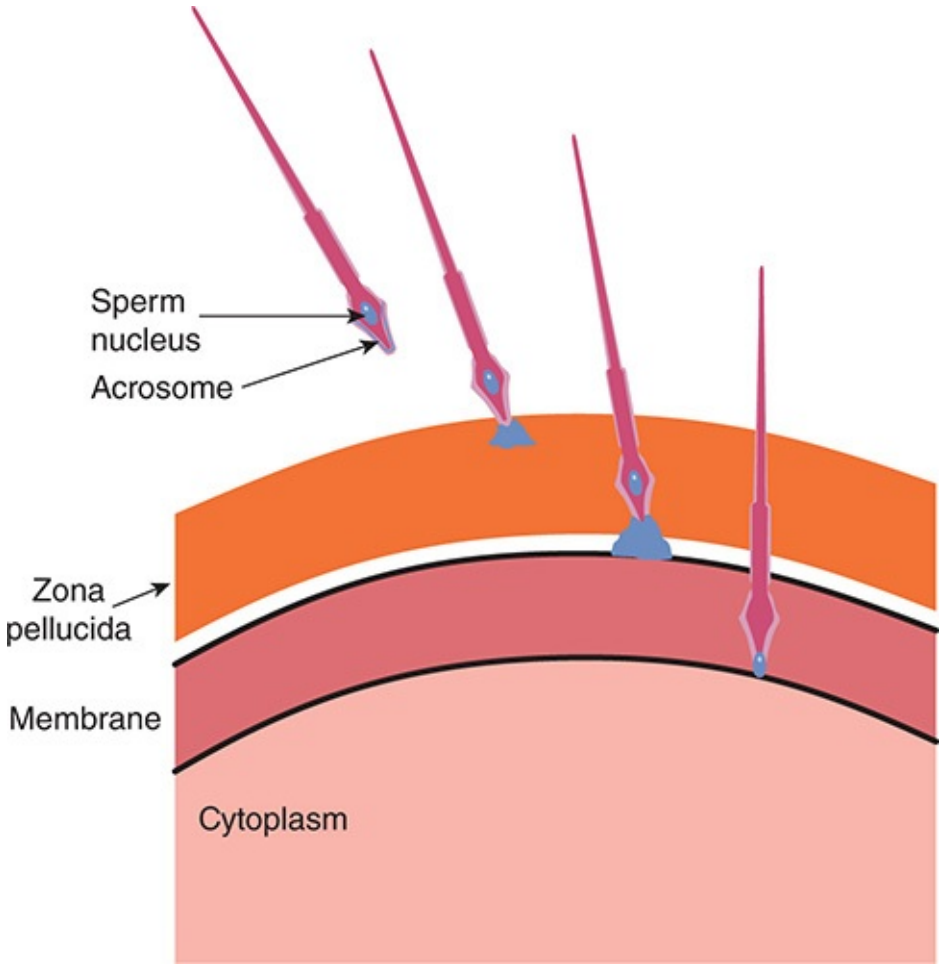


FIGURE 22–20 Sequential events in fertilization in mammals. Sperm are attracted to the ovum, bind to the zona pellucida, release acrosomal enzymes, penetrate the zona pellucida, and fuse with the membrane of the ovum, releasing the sperm nucleus into its cytoplasm. Current evidence indicates that the side, rather than the tip, of the sperm head fuses with the egg cell membrane. (Modified with permission from Vacquier VD: Evolution of gamete recognition proteins. Science 1998; Sep 25; 281(5385):1995–1998.)

CLINICAL BOX 22–4

Genetic Defects Causing Reproductive Abnormalities

A number of single-gene mutations cause reproductive abnormalities when they occur in women. Examples include (1) Kallmann syndrome, which causes hypogonadotropic hypogonadism; (2) GnRH resistance, FSH resistance, and LH resistance, which are due to defects in the GnRH, FSH, or

LH receptors, respectively; and (3) aromatase deficiency, which prevents the formation of estrogens. These are all caused by loss-of-function mutations. An interesting gain-of-function mutation causes the **McCune–Albright syndrome,** in which Gsα becomes constitutively active in certain cells but not others (mosaicism) because a somatic mutation after initial cell division has occurred in the embryo. It is associated with multiple endocrine abnormalities, including precocious puberty and amenorrhea with galactorrhea.

The developing embryo, now called a **blastocyst,** moves down the tube into the uterus. This journey takes about 3 days, during which the blastocyst reaches the 8- or 16-cell stage. Once in contact with the endometrium, the blastocyst becomes surrounded by an outer layer of **syncytiotrophoblast,** a multi-nucleate mass with no discernible cell boundaries, and an inner layer of **cytotrophoblast** made up of individual cells. The syncytiotrophoblast erodes the endometrium, and the blastocyst burrows into it **(implantation).** The implantation site is usually on the dorsal wall of the uterus. A placenta then develops, and the trophoblast remains associated with it.

Failure to Reject the "Fetal Graft"

It should be noted that the fetus and the mother are two genetically distinct individuals, and the fetus is in effect a transplant of foreign tissue in the mother. However, the transplant is tolerated, and the rejection reaction that is characteristically produced when other foreign tissues are transplanted (see Chapter 3) fails to occur. The way the "fetal graft" is protected is unknown. However, one explanation may be that the placental trophoblast, which separates maternal and fetal tissues, does not express the polymorphic class I and class II major histocompatibility complex (MHC) genes and instead expresses *HLA-G*, a nonpolymorphic gene. Therefore, antibodies against the fetal proteins do not develop. In addition, there is Fas ligand on the surface of the placenta, and this binds to T cells, causing them to undergo apoptosis (see Chapter 3).

Infertility

The vexing clinical problem of infertility often requires extensive investigation before a cause is found. In 30% of cases, the problem is in the man; in 45%, the

problem is in the woman; in 20%, both partners have a problem; and in 5%, no cause can be found. **In vitro fertilization,** that is, removing mature ova, fertilizing them with sperm, and implanting one or more of them in the uterus at the four-cell stage is of some value in these cases. It has a 5–10% chance of producing a live birth.

Endocrine Changes

In all mammals, the corpus luteum in the ovary at the time of fertilization fails to regress and instead enlarges in response to stimulation by gonadotropic hormones secreted by the placenta. The placental gonadotropin in humans is called **human chorionic gonadotropin (hCG).** The enlarged **corpus luteum of pregnancy** secretes estrogens, progesterone, and relaxin. Progesterone and relaxin help maintain pregnancy by inhibiting myometrial contractions; progesterone prevents prostaglandin production by the uterus, which stops contractions from occurring. In humans, the placenta produces sufficient estrogen and progesterone from maternal and fetal precursors to take over the function of the corpus luteum after the sixth week of pregnancy. Ovariectomy before the sixth week thus leads to abortion, but ovariectomy thereafter has no effect on the pregnancy. The function of the corpus luteum begins to decline after 8 weeks of pregnancy, but it persists throughout pregnancy. hCG secretion decreases after an initial marked rise, but estrogen and progesterone secretion increase until just before parturition **(Table 22–5)**.

TABLE 22–5 Hormone levels in human maternal blood during normal pregnancy.

Hormone	Approximate Peak Value	Time of Peak Secretion
hCG	5 mg/mL	First trimester
Relaxin	1 ng/mL	First trimester
hCS	15 mg/mL	Term
Estradiol	16 ng/mL	Term
Estriol	14 ng/mL	Term
Progesterone	190 ng/mL	Term
Prolactin	200 ng/mL	Term

Human Chorionic Gonadotropin

hCG is a glycoprotein that contains galactose and hexosamine. It is produced by the syncytiotrophoblast. Like the pituitary glycoprotein hormones, it is made up of α and β subunits. hCG-α is identical to the α subunit of LH, FSH, and TSH. The molecular weight of hCG-α is 18,000, and that of hCG-β is 28,000. hCG is primarily luteinizing and luteotropic and has little FSH activity. It can be measured by radioimmunoassay and detected in the blood as early as 6 days after conception. Its presence in the urine in early pregnancy is the basis of the various laboratory tests for pregnancy, and it can sometimes be detected in the urine as early as 14 days after conception. It appears to act on the same receptor as LH. hCG is not absolutely specific for pregnancy. Small amounts are secreted by a variety of gastrointestinal and other tumors in both sexes, and hCG has been measured in individuals with suspected tumors as a "tumor marker." It also appears that the fetal liver and kidney normally produce small amounts of hCG.

Human Chorionic Somatomammotropin

The syncytiotrophoblast also secretes large amounts of a protein hormone that is lactogenic and has a small amount of growth-stimulating activity. This hormone has been called **chorionic growth hormone-prolactin (CGP)** and **human placental lactogen (hPL),** but it is now generally called **human chorionic somatomammotropin (hCS).** The structure of hCS is very similar to that of human growth hormone (see Chapter 18), and it appears that these two hormones and prolactin evolved from a common progenitor hormone. Large quantities of hCS are found in maternal blood, but very little reaches the fetus. Secretion of growth hormone from the maternal pituitary is not increased during pregnancy and may actually be decreased by hCS. However, hCS has most of the actions of growth hormone and apparently functions as a "maternal growth hormone of pregnancy" to bring about the nitrogen, potassium, and calcium retention, lipolysis, and decreased glucose utilization seen in this state. These latter two actions divert glucose to the fetus. The amount of hCS secreted is proportional to the size of the placenta, which normally weighs about one-sixth as much as the fetus. Low hCS levels are a sign of placental insufficiency.

Other Placental Hormones

In addition to hCG, hCS, progesterone, and estrogens, the placenta secretes other

hormones. Human placental fragments probably produce proopiomelanocortin (POMC). In culture, they release corticotropin-releasing hormone (CRH), β-endorphin, α-melanocyte-stimulating hormone (MSH), and dynorphin A, all of which appear to be identical to their hypothalamic counterparts. They also secrete GnRH and inhibin, and since GnRH stimulates and inhibin inhibits hCG secretion, locally produced GnRH and inhibin may act in a paracrine fashion to regulate hCG secretion. The trophoblast cells and amnion cells also secrete leptin, and moderate amounts of this satiety hormone enter the maternal circulation. Some also enters the amniotic fluid. Its function in pregnancy is unknown. The placenta also secretes prolactin in a number of forms.

Finally, the placenta secretes the α subunits of hCG, and the plasma concentration of free α subunits rises throughout pregnancy. These α subunits acquire a carbohydrate composition that makes them unable to combine with β subunits, and their prominence suggests that they have a function of their own. It is interesting in this regard that the secretion of the prolactin produced by the endometrium also appears to increase throughout pregnancy, and it may be that the circulating α subunits stimulate endometrial prolactin secretion. The cytotrophoblast of the human chorion contains prorenin (see Chapter 38). A large amount of prorenin is also present in amniotic fluid, but its function in this location is unknown.

Fetoplacental Unit

The fetus and the placenta interact in the formation of steroid hormones. The placenta synthesizes pregnenolone and progesterone from cholesterol. Some of the progesterone enters the fetal circulation and provides the substrate for the formation of cortisol and corticosterone in the fetal adrenal glands **(Figure 22–21)**. Some of the pregnenolone enters the fetus and, along with pregnenolone synthesized in the fetal liver, is the substrate for the formation of dehydroepiandrosterone sulfate (DHEAS) and 16-hydroxydehydroepiandrosterone sulfate (16-OHDHEAS) in the fetal adrenal. Some 16- hydroxylation also occurs in the fetal liver. DHEAS and 16-OHDHEAS are transported back to the placenta, where DHEAS forms estradiol and 16-OHDHEAS forms estriol. The principal estrogen formed is estriol, and since fetal 16- OHDHEAS is the principal substrate for the estrogens, the urinary estriol excretion of the mother can be monitored as an index of the state of the fetus.

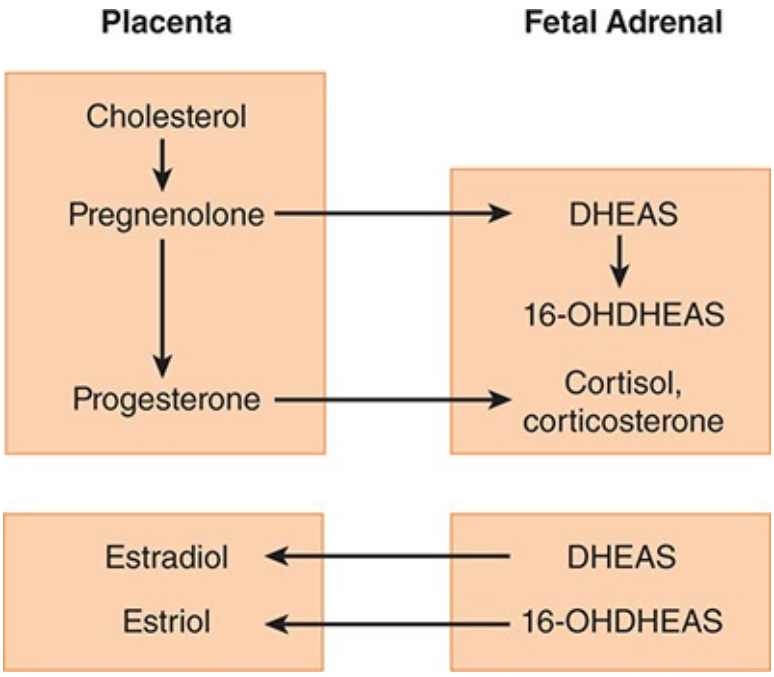


FIGURE 22–21 Interactions between the placenta and the fetal adrenal cortex in the production of steroids.

Parturition

The duration of pregnancy in humans averages 270 days from fertilization (284 days from the first day of the menstrual period preceding conception). Irregular uterine contractions increase in frequency in the last month of pregnancy.

The difference between the body of the uterus and the cervix becomes evident at the time of delivery. The cervix, which is firm in the nonpregnant state and throughout pregnancy until near the time of delivery, softens and dilates, while the body of the uterus contracts and expels the fetus.

There is still considerable uncertainty about the mechanisms responsible for the onset of labor. One factor is the increase in circulating estrogens produced by increased circulating DHEAS. This makes the uterus more excitable, increases the number of gap junctions between myometrial cells, and causes production of more prostaglandins, which in turn cause uterine contractions. In humans, CRH secretion by the fetal hypothalamus increases and is supplemented by increased placental production of CRH. This increases circulating adrenocorticotropic hormone (ACTH) in the fetus, and the resulting increase in cortisol hastens the maturation of the respiratory system. Thus, in a sense, the fetus picks the time to be born by increasing CRH secretion.

The number of oxytocin receptors in the myometrium and the decidua (the endometrium of pregnancy) increases more than 100-fold during pregnancy and reaches a peak during early labor. Estrogens increase the number of oxytocin receptors, and uterine distension late in pregnancy may also increase their formation. In early labor, the oxytocin concentration in maternal plasma is not elevated from the prelabor value of about 25 pg/mL. It is possible that the marked increase in oxytocin receptors causes the uterus to respond to normal plasma oxytocin concentrations. However, at least in rats, the amount of oxytocin mRNA in the uterus increases, reaching a peak at term; this suggests that locally produced oxytocin also participates in the process.

Premature onset of labor is a problem because premature infants have a high mortality rate and often require intensive, expensive care. Intramuscular 17α-hydroxyprogesterone causes a significant decrease in the incidence of premature labor. The mechanism by which it exerts its effect is uncertain, but it may be that the steroid provides a stable level of circulating progesterone. Progesterone relaxes uterine smooth muscle, inhibits the action of oxytocin on the muscle, and reduces the formation of gap junctions between the muscle fibers. All these actions would be expected to inhibit the onset of labor.

Once labor is started, the uterine contractions dilate the cervix, and this dilation in turn sets up signals in afferent nerves that increase oxytocin secretion **(Figure 22–22)**. The plasma oxytocin level rises and more oxytocin becomes available to act on the uterus. Thus, a positive feedback loop is established that aids delivery and terminates on expulsion of the products of conception. Oxytocin increases uterine contractions in two ways: (1) It acts directly on uterine smooth muscle cells to make them contract and (2) it stimulates the formation of prostaglandins in the decidua. The prostaglandins enhance the oxytocin-induced contractions.

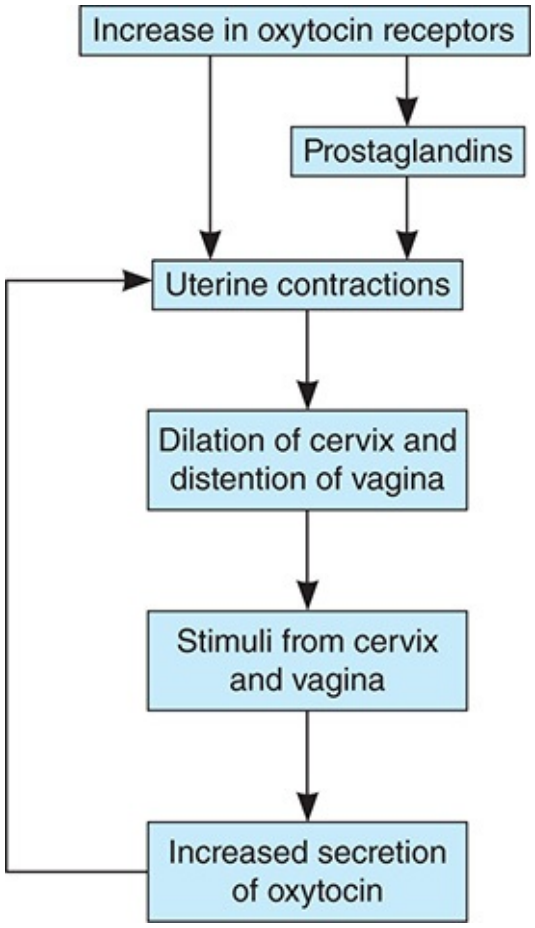


FIGURE 22–22 Role of oxytocin in parturition.

During labor, spinal reflexes and voluntary contractions of the abdominal muscles ("bearing down") also aid in delivery. However, delivery can occur without bearing down and without a reflex increase in secretion of oxytocin from the posterior pituitary gland, since paraplegic women can go into labor and deliver.

LACTATION

Development of the Breasts

Many hormones are necessary for full mammary development. In general, estrogens are primarily responsible for proliferation of the mammary ducts and progesterone for the development of the lobules. In rats, some prolactin is also needed for development of the glands at puberty, but it is not known if prolactin is necessary in humans. During pregnancy, prolactin levels increase steadily until

term, and levels of estrogens and progesterone are elevated as well, producing full lobuloalveolar development.

Secretion & Ejection of Milk

The composition of human and cows' milk is shown in **Table 22–6**. In estrogen- and progesterone-primed rodents, injections of prolactin cause the formation of milk droplets and their secretion into the ducts. Oxytocin causes contraction of the myoepithelial cells lining the duct walls, with consequent ejection of the milk through the nipple.

TABLE 22–6 Composition of colostrum and milk.[a]

Component	Human Colostrum	Human Milk	Cows' Milk
Water (g)	...	88	88
Lactose (g)	5.3	6.8	5.0
Protein (g)	2.7	1.2	3.3
Casein: lactalbumin ratio	...	1:2	3:1
Fat (g)	2.9	3.8	3.7
Linoleic acid	...	8.3% of fat	1.6% of fat
Sodium (mg)	92	15	58
Potassium (mg)	55	55	138
Chloride (mg)	117	43	103
Calcium (mg)	31	33	125
Magnesium (mg)	4	4	12
Phosphorus (mg)	14	15	100
Iron (mg)	0.09[b]	0.15[b]	0.10[b]
Vitamin A (µg)	89	53	34
Vitamin D (µg)	...	0.03[b]	0.06[b]
Thiamine (µg)	15	16	42
Riboflavin (µg)	30	43	157
Nicotinic acid (µg)	75	172	85
Ascorbic acid (mg)	4.4[b]	4.3[b]	1.6[b]

[a]Weights per deciliter.

[b]Poor source.

Reproduced with permission from Findlay ALR: Lactation. Res Reprod (Nov) 1974;6(6).

Initiation of Lactation after Delivery

The breasts enlarge during pregnancy in response to high circulating levels of estrogens, progesterone, prolactin, and possibly hCG. Some milk is secreted into the ducts as early as the fifth month, but the amounts are small compared with the surge of milk secretion that follows delivery. In most animals, milk is secreted within an hour after delivery, but in women it takes 1–3 days for the milk to "come in."

After expulsion of the placenta at parturition, the levels of circulating estrogens and progesterone abruptly decline. The drop in circulating estrogen initiates lactation. Prolactin and estrogen synergize in producing breast growth, but estrogen antagonizes the milk-producing effect of prolactin on the breast. Indeed, in women who do not wish to breastfeed their babies, estrogens may be administered to stop lactation.

Suckling not only evokes reflex oxytocin release and milk ejection, it also maintains and augments the secretion of milk because of the stimulation of prolactin secretion it produces.

Effect of Lactation on Menstrual Cycles

Women who do not breastfeed their infants usually have their first menstrual period 6 weeks after delivery. However, women who breastfeed regularly have amenorrhea for 25–30 weeks. Breastfeeding stimulates prolactin secretion, and evidence suggests that prolactin inhibits GnRH secretion, inhibits the action of GnRH on the pituitary, and antagonizes the action of gonadotropins on the ovaries. Ovulation is inhibited, and the ovaries are inactive, so estrogen and progesterone output falls to low levels. Consequently, only 5–10% of women become pregnant again during the suckling period, and breastfeeding has long been known to be an important, if only partly effective, method of birth control. Furthermore, almost 50% of the cycles in the first 6 months after resumption of menses are anovulatory **(Clinical Box 22–5)**.

Gynecomastia

Breast development in the male is called **gynecomastia.** It may be unilateral but is more commonly bilateral. It is common in newborns because of transplacental passage of maternal estrogens (occurring in about 75%). It also occurs in a mild, transient form in 70% of normal boys at the time of puberty and in many men

over the age of 50. It occurs in androgen resistance. It is a complication of estrogen therapy and is seen in patients with estrogen-secreting tumors. It is found in a wide variety of seemingly unrelated conditions, including eunuchoidism, hyperthyroidism, and cirrhosis of the liver. Digitalis can produce it, apparently because cardiac glycosides are weakly estrogenic. It can also be caused by many other drugs. It has been seen in malnourished prisoners of war, but only after they were liberated and eating an adequate diet. A feature common to many and perhaps all cases of gynecomastia is an increase in the plasma estrogen:androgen ratio due to either increased circulating estrogens or decreased circulating androgens.

CLINICAL BOX 22–5

Chiari–Frommel Syndrome

An interesting, although rare, condition is persistence of lactation **(galactorrhea)** and amenorrhea in women who do not breastfeed after delivery. This condition, called the **Chiari–Frommel syndrome,** may be associated with some genital atrophy and is due to persistent prolactin secretion without the secretion of the FSH and LH necessary to produce maturation of new follicles and ovulation. A similar pattern of galactorrhea and amenorrhea with high circulating prolactin levels is seen in nonpregnant women with chromophobe pituitary tumors and in women in whom the pituitary stalk has been sectioned during treatment of cancer.

HORMONES & CANCER

About 35% of carcinomas of the breast in women of childbearing age are **estrogen-dependent;** their continued growth depends on the presence of estrogens in the circulation. The tumors are not cured by decreasing estrogen secretion, but symptoms are dramatically relieved, and the tumor regresses for months or years before recurring (see Chapter 16). Women with estrogen-dependent tumors often have a remission when their ovaries are removed. Inhibition of the action of estrogens with **tamoxifen** also produces remissions, and inhibition of estrogen formation with drugs that inhibit **aromatase** (Figure 22–15) is even more effective.

CHAPTER SUMMARY

- Differences between males and females depend primarily on a single chromosome (the Y chromosome) and a single pair of endocrine structures (the gonads); testes in the male and ovaries in the female.
- The gonads have a dual function: the production of germ cells (gametogenesis) and the secretion of sex hormones. The testes secrete large amounts of androgens, principally testosterone, but they also secrete small amounts of estrogens. The ovaries secrete large amounts of estrogens and small amounts of androgens.
- The reproductive system of women has regular cyclical changes that can be thought of as periodic preparations for fertilization and pregnancy. In humans and other primates, the cycle is a **menstrual** cycle, and features the periodic vaginal bleeding that occurs with the shedding of the uterine mucosa **(menstruation).**
- Ovaries also secrete progesterone, a steroid that has special functions in preparing the uterus for pregnancy. During pregnancy, the ovaries secrete relaxin, which facilitates the delivery of the fetus. In both sexes, the gonads secrete other polypeptides, including inhibin B, a polypeptide that inhibits FSH secretion.
- In women, a period called perimenopause precedes menopause, and can last up to 10 years; during this time the menstrual cycles become irregular and the level of inhibins decrease.
- Once in menopause, the ovaries no longer secrete progesterone and 17β-estradiol and estrogen is formed only in small amounts by aromatization of androstenedione in peripheral tissues.
- The naturally occurring estrogens are **17β-estradiol, estrone,** and **estriol.** They are secreted primarily by the granulosa cells of the ovarian follicles, the corpus luteum, and the placenta. Their biosynthesis depends on the enzyme **aromatase** (CYP19), which converts testosterone to estradiol and androstenedione to estrone. The latter reaction also occurs in fat, liver, muscle, and the brain.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. If a young woman has high plasma levels of T_3, cortisol, and renin activity but her blood pressure is only slightly elevated and she has no symptoms or signs of thyrotoxicosis or Cushing syndrome, the most likely explanation is that
   A. she has been treated with TSH and ACTH.
   B. she has been treated with T_3 and cortisol.
   C. she is in the third trimester of pregnancy.
   D. she has an adrenocortical tumor.
   E. she has been subjected to chronic stress.
2. In humans, fertilization usually occurs in the
   A. vagina.
   B. cervix.
   C. uterine cavity.
   D. uterine tubes.
   E. abdominal cavity.
3. Which of the following is *not* a steroid?
   A. 17α-hydroxyprogesterone
   B. Estrone
   C. Relaxin
   D. Pregnenolone
   E. Etiocholanolone
4. Which of the following probably triggers the onset of labor?
   A. ACTH in the fetus
   B. ACTH in the mother
   C. Prostaglandins
   D. Oxytocin
   E. Placental renin

CHAPTER 23

Function of the Male Reproductive System

OBJECTIVES

After studying this chapter, you should be able to:

- Name the key hormones secreted by Leydig cells and Sertoli cells of the testes.
- Outline the steps involved in spermatogenesis.
- Outline the mechanisms that produce erection and ejaculation.
- Know the general structure of testosterone and describe its biosynthesis, transport, metabolism, and actions.
- Describe the processes involved in regulation of testosterone secretion.

INTRODUCTION

The role for a functional, secreting testis in the formation of male genitalia, the action of male hormones on the brain in early development, and development of the male reproductive system through adolescence and into adulthood were discussed in the previous chapter. As observed in the female, male gonads have a dual function: the production of germ cells **(gametogenesis)** and the secretion of **sex hormones.** The **androgens** are the steroid sex hormones that are masculinizing in their action. The testes secrete large amounts of androgens,

principally **testosterone,** but they also secrete small amounts of estrogens. Unlike that observed in females, male gonadotropin secretion is noncyclical, and once mature, male gonadal function slowly declines with advancing age, but the ability to produce viable gametes persists. In this chapter, the discussion will be focused on the structure and physiology of the mature male reproductive system.

THE MALE REPRODUCTIVE SYSTEM

STRUCTURE

The testes are made up of loops of convoluted **seminiferous tubules,** in the walls of which the spermatozoa are formed from the primitive germ cells **(spermatogenesis).** Both ends of each loop drain into a network of ducts in the head of the **epididymis.** From there, spermatozoa pass through the tail of the epididymis into the **vas deferens.** They enter through the **ejaculatory ducts** into the urethra in the body of the **prostate** at the time of ejaculation **(Figure 23–1)**. Between the tubules in the testes are nests of cells containing lipid granules, the **interstitial cells of Leydig** (Leydig cells; **Figures 23–2** and **23–3**), which secrete testosterone into the bloodstream. The spermatic arteries to the testes are tortuous, and blood in them runs parallel but in the opposite direction to blood in the pampiniform plexus of spermatic veins. This anatomic arrangement may permit countercurrent exchange of heat and testosterone. The principles of countercurrent exchange are considered in detail in relation to the kidney in Chapter 37.

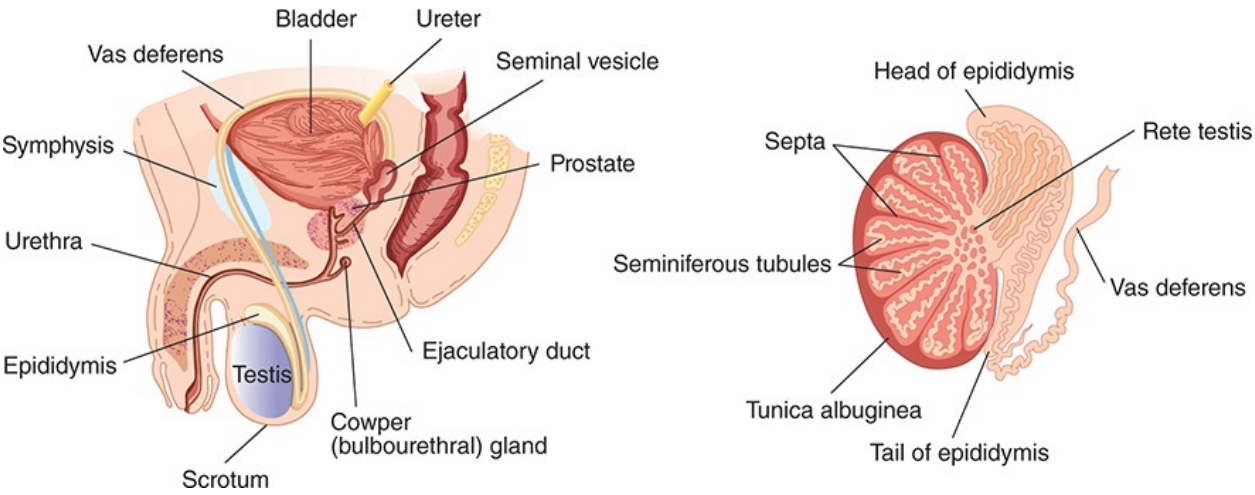


FIGURE 23–1 Male reproductive system. Anatomic features of the male reproductive system in relation to the bladder are shown at left. A cross section

of the duct system within the testis is shown at right.

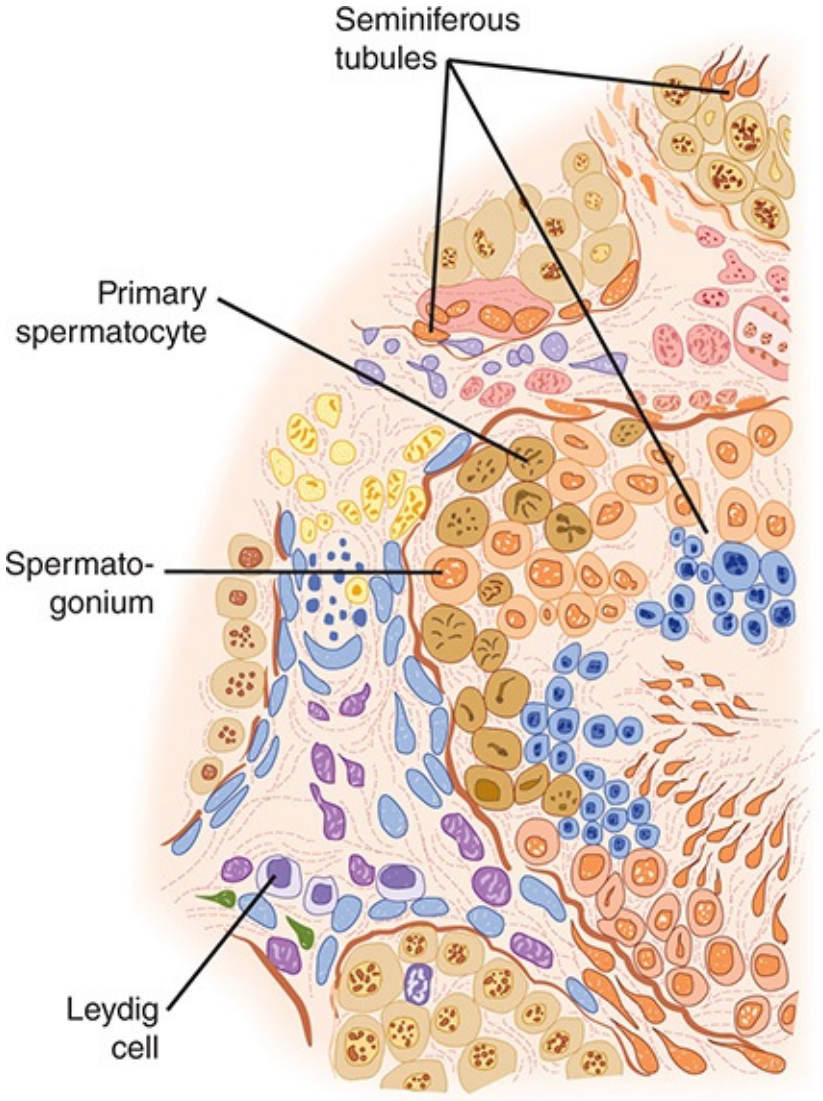


FIGURE 23–2 Section of the human testis. Cross section shows the diversity of cellular anatomy within the testis.

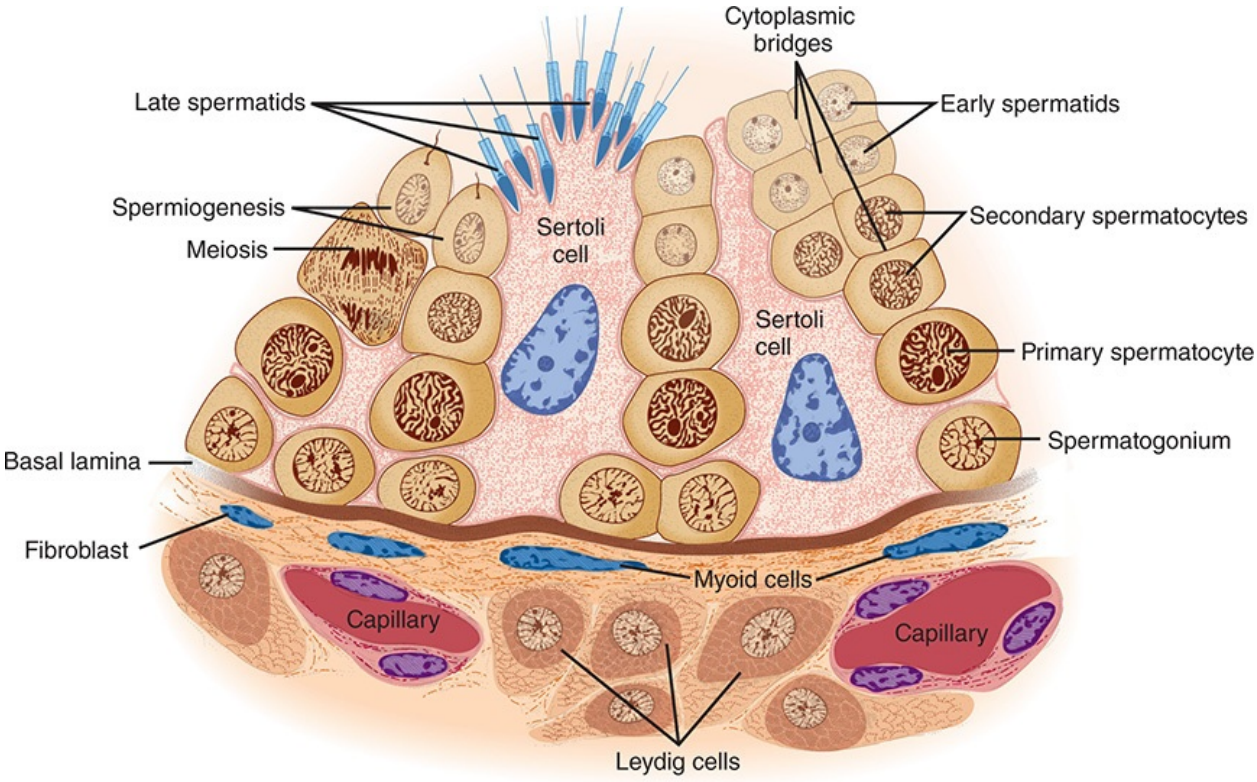


FIGURE 23–3 Seminiferous epithelium. Note that maturing germ cells remain connected by cytoplasmic bridges through the early spermatid stage and that these cells are closely invested by Sertoli cell cytoplasm as they move from the basal lamina to the lumen. (Reproduced with permission from Junqueira LC, Carneiro J: *Basic Histology: Text & Atlas*, 10th ed. New York, NY: McGraw-Hill; 2003.)

GAMETOGENESIS & EJACULATION

Blood-Testis Barrier

The walls of the seminiferous tubules are lined by primitive germ cells and **Sertoli cells,** large, complex glycogen-containing cells that stretch from the basal lamina of the tubule to the lumen (Figure 23–3). Germ cells must stay in contact with Sertoli cells to survive; this contact is maintained by cytoplasmic bridges. Tight junctions between adjacent Sertoli cells near the basal lamina form a **blood-testis barrier** that prevents many large molecules from passing from the interstitial tissue and the part of the tubule near the basal lamina (basal compartment) to the region near the tubular lumen (adluminal compartment) and

the lumen. However, steroids penetrate this barrier with ease, and evidence suggests that some proteins also pass from the Sertoli cells to the Leydig cells, and vice versa, to function in a paracrine manner. In addition, maturing germ cells must pass through the barrier as they move to the lumen. This appears to occur without disruption of the barrier by coordinated breakdown of the tight junctions above the germ cells and formation of new tight junctions below them.

The fluid in the lumen of the seminiferous tubules is quite different from plasma; it contains very little protein and glucose but is rich in androgens, estrogens, K^+, inositol, and glutamic and aspartic acids. Maintenance of its composition depends on the blood-testis barrier. The barrier also protects the germ cells from bloodborne noxious agents, prevents antigenic products of germ cell division and maturation from entering the circulation and generating an autoimmune response, and may help establish an osmotic gradient that facilitates movement of fluid into the tubular lumen.

Spermatogenesis

Spermatogonia, the primitive germ cells next to the basal lamina of the seminiferous tubules, mature into **primary spermatocytes** (Figure 23–3). This process begins during adolescence. The primary spermatocytes undergo meiotic division, reducing the number of chromosomes. In this two-stage process, they divide into **secondary spermatocytes** and then into **spermatids,** which contain the haploid number of 23 chromosomes. The spermatids mature into **spermatozoa (sperm).** As a single spermatogonium divides and matures, its descendants remain tied together by cytoplasmic bridges until the late spermatid stage. This arrangement helps ensure synchrony of the differentiation of each clone of germ cells. The estimated number of spermatids formed from a single spermatogonium is 512. The formation of a mature sperm from a primitive germ cell by spermatogenesis in humans spans approximately 74 days.

Each sperm is an intricate motile cell, rich in DNA, with a head that is made up mostly of chromosomal material **(Figure 23–4)**. Covering the head like a cap is the **acrosome,** a lysosome-like organelle rich in enzymes involved in sperm penetration of the ovum and other events associated with fertilization. The motile tail of the sperm is wrapped in its proximal portion by a sheath holding numerous mitochondria. The membranes of late spermatids and spermatozoa contain a special small form of angiotensin-converting enzyme (ACE) called **germinal angiotensin-converting enzyme (gACE).** Germinal ACE is transcribed from the same gene as the somatic ACE (sACE); however, gACE

displays tissue-specific expression based on alternative transcription initiation sites and alternate splicing patterns. The full function of gACE has yet to be elucidated, although gACE-specific knockout mouse models are sterile.

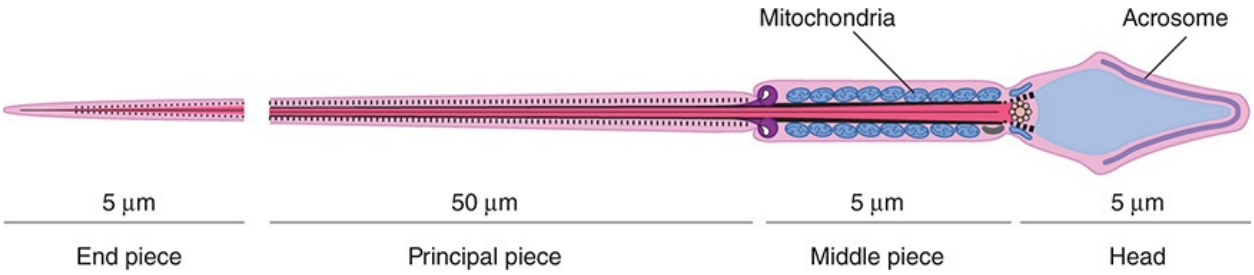


FIGURE 23–4 Human spermatozoon, profile view. Note the acrosome, an organelle that covers half the sperm head inside the plasma membrane of the sperm. (Reproduced with permission from Junqueira LC, Carneiro J: *Basic Histology: Text & Atlas*, 11th ed. New York, NY: McGraw-Hill; 2005.)

Spermatids mature into spermatozoa in deep folds of the cytoplasm of the Sertoli cells (Figure 23–3). Mature spermatozoa are released from the Sertoli cells and become free in the lumens of the tubules. The Sertoli cells secrete **androgen-binding protein (ABP), inhibin,** and **Mullerian inhibiting substance (MIS**). They do not synthesize androgens, but they contain **aromatase (CYP19),** the enzyme responsible for conversion of androgens to estrogens, and they can produce estrogens. ABP probably functions to maintain a high, stable supply of androgen in the tubular fluid. Inhibin inhibits follicle-stimulating hormone (FSH) secretion.

FSH and androgens maintain the gametogenic function of the testis. After hypophysectomy, injection of luteinizing hormone (LH) produces a high local concentration of androgen in the testes, and this maintains spermatogenesis. The stages from spermatogonia to spermatids appear to be androgen-independent. However, the maturation from spermatids to spermatozoa depends on androgen acting on the Sertoli cells in which the developing spermatozoa are embedded. FSH acts on the Sertoli cells to facilitate the last stages of spermatid maturation. In addition, it promotes the production of ABP.

An interesting observation is that the estrogen content of the fluid in the rete testis (Figure 23–1) is high, and the walls of the rete testis contain numerous α estrogen receptors (ERα). In this region, fluid is reabsorbed and the spermatozoa are concentrated. If this does not occur, the sperm entering the epididymis are diluted in a large volume of fluid, resulting in reduced fertility.

Further Development of Spermatozoa

Spermatozoa leaving the testes are not fully mobile. They continue their maturation and acquire motility during their passage through the epididymis. Motility is obviously important in vivo, but fertilization occurs in vitro if an immotile spermatozoon from the head of the epididymis is microinjected directly into an ovum. The ability to move forward **(progressive motility),** which is acquired in the epididymis, involves activation of a unique set of cation channel proteins from the **CatSper** family, which are localized to the principal piece of the sperm tail. CatSpers form an alkaline-sensitive Ca^{2+} channel that becomes more active as the sperm go from the acidic vagina (pH ~5) to the cervical mucus (pH ~8). Sperm from knockout mice that do not express CatSper1-4 have altered motility and are infertile, emphasizing the important role of these channel proteins. In addition, spermatozoa express olfactory receptors, and ovaries produce odorant-like molecules. Recent evidence indicates that these molecules and their receptors interact, fostering movement of the spermatozoa toward the ovary (chemotaxis).

Ejaculation of the spermatozoon involves contractions of the vas deferens mediated in part by P2X purinoreceptors, ligand-gated cation channels that open in response to ATP (see Chapter 7), and fertility is reduced in mice in which these receptors are knocked out.

Once ejaculated into the female, the spermatozoa move up the uterus to the isthmus of the uterine tubes, where they slow down and undergo **capacitation.** This further maturation process involves two components: increasing the motility of the spermatozoa and facilitating their preparation for the acrosome reaction. However, the role of capacitation appears to be facilitatory rather than obligatory, because fertilization is readily produced in vitro. From the isthmuses, the capacitated spermatozoa move rapidly to the tubal ampullas, where fertilization takes place.

Effect of Temperature

Spermatogenesis requires a temperature considerably lower than that of the interior of the body. The testes are normally maintained at a temperature of about 32°C. They are kept cool by air circulating around the scrotum and probably by heat exchange in a countercurrent manner between the spermatic arteries and veins. When the testes are retained in the abdomen or when, in experimental animals, they are held close to the body, degeneration of the tubular walls and

sterility result. Situations that increase heat around the testes in humans (e.g., hot baths [43–45°C for 30 min/d] and insulated athletic supporters) can reduce sperm counts, in some cases by 90%. However, the reductions produced in this manner are not consistent enough to make the procedures reliable forms of male contraception. In addition, evidence suggests a seasonal effect in men, with sperm counts being greater in the winter regardless of the temperature to which the scrotum is exposed.

Semen

The fluid that is ejaculated at the time of orgasm, the **semen,** contains sperm and the secretions of the seminal vesicles, prostate, Cowper glands, and, probably, the urethral glands **(Table 23–1)**. An average volume per ejaculate is 2.5–3.5 mL after several days of abstinence from sexual activity. The volume of semen and the sperm count decrease rapidly with repeated ejaculation. Even though it takes only one sperm to fertilize the ovum, each milliliter of semen normally contains about 100 million sperm. Reduction in sperm production is associated with infertility: 50% of men with counts of 20–40 million/mL and essentially all of those with counts under 20 million/mL are sterile. The presence of many morphologically abnormal or immotile spermatozoa also correlates with infertility. The **prostaglandins** in semen, which come from the seminal vesicles, are at high concentrations, but their function in semen is unknown. The causes of male infertility, as well as the underlying mechanisms of sperm in fertilization, are used as clues in developing male contraception **(Clinical Box 23–1)**.

TABLE 23–1 Composition of human semen.

Color: White, opalescent	
Specific gravity: 1.028	
pH: 7.35–7.50	
Sperm count: Average about 100 million/mL, with < 20% abnormal forms	
Other components: Fructose (1.5–6.5 mg/mL) Phosphorylcholine Ergothioneine Ascorbic acid Flavins Prostaglandins	From seminal vesicles (contributes 60% of total volume)
Spermine Citric acid Cholesterol, phospholipids Fibrinolysin, fibrinogenase Zinc Acid phosphatase	From prostate (contributes 20% of total volume)
Phosphate Bicarbonate	Buffers
Hyaluronidase	From the acrosome of the sperm cells after it has reached the oocyte

CLINICAL BOX 23–1

Male Contraception

Several methods independent of physical intervention (such as hormonal control of sperm development, targeting of cation channel proteins important in fertilization [e.g., CatSpers], and the use of natural compounds that limit sperm function) have been explored as male contraceptives. However, considering the number of sperm and their ability to regenerate, methods that

sufficiently reduce sperm production or limit function with an absence of side effects have been difficult to obtain. In lieu of pharmacological control, the most common male contraception continues to be bilateral ligation of the vas deferens **(vasectomy),** a relatively safe and convenient contraceptive procedure. Interestingly, antibodies against spermatozoa develop in ~50% of the men who have undergone vasectomy; in monkeys, the presence of such antibodies is associated with a higher incidence of infertility after restoration of the patency of the vas. However, the anti-sperm antibodies do not appear to have any other adverse effects. Alternatives to ligation are vas occlusion methods, such as silicone plugs, that aim to block the vas deferens while leaving the tube intact, making reversal easier if desired. Not surprisingly, such methods are not as effective as traditional vasectomy.

THERAPEUTIC HIGHLIGHTS

Vasectomy reversal: Whereas it was once quite difficult to restore the patency of the vas in those wishing to restore fertility, the success rate for such operations has improved steadily. Successful reversal of vasectomy usually results in meaningful sperm counts within months, although delays of a year or more are not unusual. The ultimate success, as measured by pregnancy, is recorded in ~50% of reversals within 2 years.

Human sperm move at a speed of about 3 mm/min through the female genital tract. Sperm reach the uterine tubes 30–60 min after copulation. Contractions of the female organs may facilitate the transport of the sperm to the uterine tubes.

Erection

Erection is initiated by dilation of the arterioles of the penis. As the erectile tissue of the penis fills with blood, the veins are compressed, blocking outflow and adding to the turgor of the organ. The integrating centers in the lumbar segments of the spinal cord are activated by impulses in afferents from the genitalia and descending tracts that mediate erection in response to erotic psychological stimuli. The efferent parasympathetic fibers are in the pelvic splanchnic nerves **(nervi erigentes).** The fibers presumably release acetylcholine and the vasodilator vasoactive intestinal polypeptide (VIP) as cotransmitters (see Chapter 7).

Nonadrenergic noncholinergic fibers are also present in the nervi erigentes, and these contain large amounts of **nitric oxide synthase (NOS),** the enzyme that catalyzes the formation of nitric oxide (NO; see Chapter 32). NO activates soluble guanylyl cyclase, resulting in increased production of cyclic guanosine monophosphate (cGMP), and cGMP is a potent vasodilator. Injection of NOS inhibitors prevents the erection normally produced by stimulation of the pelvic nerve in experimental animals. Thus, it seems clear that NO plays a prominent role in the production of erection. The drugs sildenafil (Viagra), tadalafil (Cialis), and vardenafil (Levitra) all inhibit the breakdown of cGMP by phosphodiesterases and have gained worldwide fame for the treatment of erectile dysfunction. The multiple phosphodiesterases (PDEs) in the body have been divided into 11 isoenzyme families, and these drugs are all most active against PDE-5, the type of phosphodiesterase found in the corpora cavernosa (Clinical Box 5–7). It is worth noting, however, that these drugs can also produce significant inhibition of PDE-6 (and others, if taken at high doses). PDE-6 is found in the retina, and one of the side effects of these drugs is a transient loss of the ability to discriminate between blue and green (see Chapter 9).

Normally, erection is terminated by sympathetic vasoconstrictor impulses to the penile arterioles.

Ejaculation

Ejaculation is a two-part spinal reflex that involves **emission,** the movement of the semen into the urethra; and **ejaculation** proper, the propulsion of the semen out of the urethra at the time of orgasm. The afferent pathways are mostly fibers from touch receptors in the glans penis that reach the spinal cord through the internal pudendal nerves. Emission is a sympathetic response, integrated in the upper lumbar segments of the spinal cord and effected by contraction of the smooth muscle of the vasa deferentia and seminal vesicles in response to stimuli in the hypogastric nerves. The semen is propelled out of the urethra by contraction of the bulbocavernosus muscle, a skeletal muscle. The spinal reflex centers for this part of the reflex are in the upper sacral and lowest lumbar segments of the spinal cord, and the motor pathways traverse the first to third sacral roots and the internal pudendal nerves.

Prostate-Specific Antigen

The prostate produces and secretes into the semen and the bloodstream a 30-kDa

serine protease generally called **prostate-specific antigen (PSA).** The gene for PSA has two androgen response elements. PSA hydrolyzes the sperm motility inhibitor semenogelin in semen, and it has several substrates in plasma, but its precise function in the circulation is unknown. An elevated plasma PSA occurs in prostate cancer and has been widely used as a screening test for this disease. However, PSA is also elevated in benign prostatic hyperplasia and prostatitis, and the effectiveness of PSA screening as a sole tool in diagnosis of prostate cancer has been called into question.

ENDOCRINE FUNCTION OF THE TESTES

Chemistry & Biosynthesis of Testosterone

Testosterone, the principal hormone of the testes, is a C_{19} steroid with a hydroxyl group in the 17 position **(Figure 23–5)**. It is synthesized from cholesterol in the Leydig cells and is also formed from androstenedione secreted by the adrenal cortex. The biosynthetic pathways in all endocrine organs that form steroid hormones are similar, the organs differing only in the enzyme systems they contain. In the Leydig cells, the 11- and 21-hydroxylases found in the adrenal cortex (see Figure 20–7) are absent, but 17α-hydroxylase is present. Pregnenolone is therefore hydroxylated in the 17 position and then subjected to side chain cleavage to form dehydroepiandrosterone. Androstenedione is also formed via progesterone and 17-hydroxyprogesterone, but this pathway is less prominent in humans. Dehydroepiandrosterone and androstenedione are then converted to testosterone.

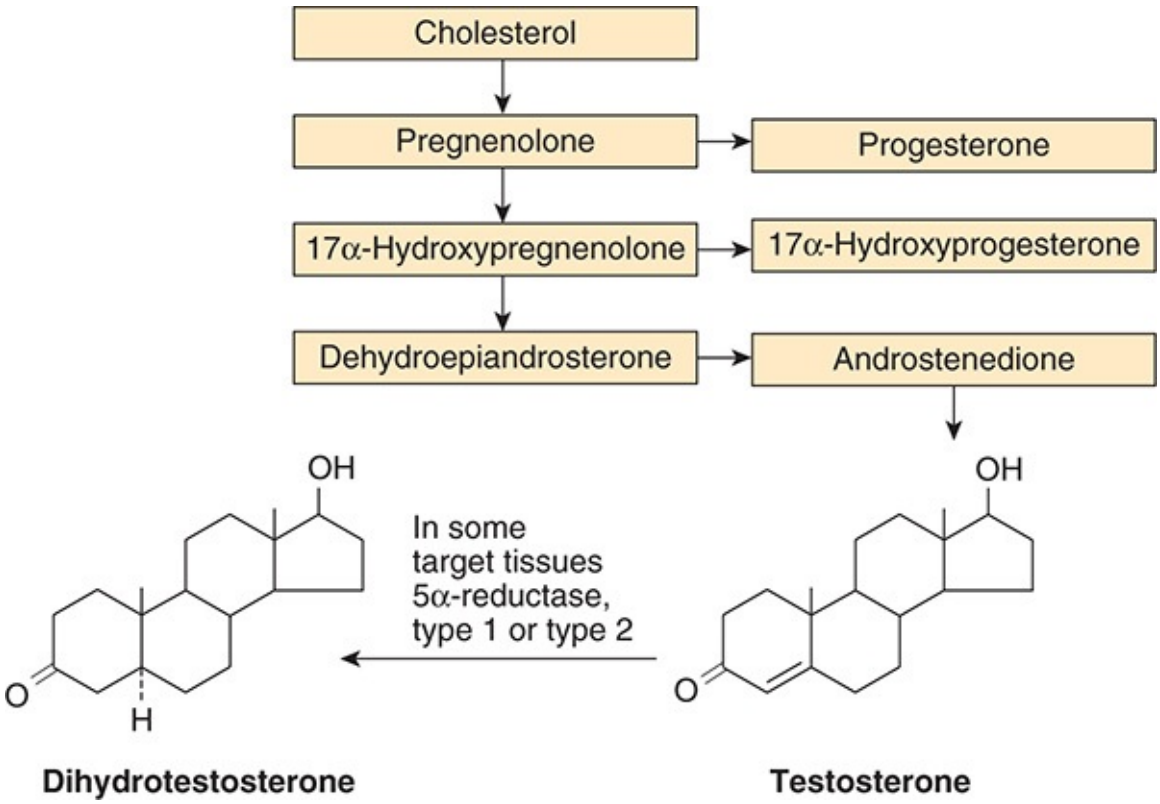


FIGURE 23–5 Biosynthesis of testosterone. The formulas of the precursor steroids are shown in Figure 20–7. Although the main secretory product of the Leydig cells is testosterone, some of the precursors also enter the circulation.

The secretion of testosterone is under the control of luteinizing hormone (LH), and the mechanism by which LH stimulates Leydig cells involves increased formation of cyclic adenosine monophosphate (cAMP) via the G-protein- coupled LH receptor and G_s. Cyclic AMP increases the formation of cholesterol from cholesterol esters and the conversion of cholesterol to pregnenolone via the activation of protein kinase A.

Secretion

The testosterone secretion rate is 4–9 mg/d (13.9–31.33 µmol/d) in normal adult males. Small amounts of testosterone are also secreted in females, with the major source being the ovary, but possibly from the adrenal as well.

Transport & Metabolism

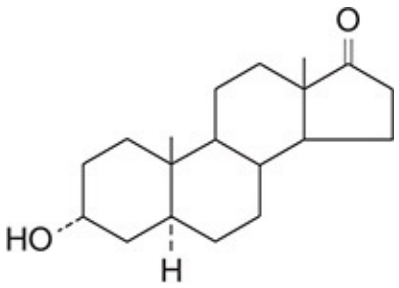
Ninety-eight percent of the testosterone in plasma is bound to protein: 65% is bound to a β-globulin called **gonadal steroid–binding globulin (GBG)** or **sex steroid–binding globulin,** and 33% to albumin **(Table 23–2)**. GBG also binds estradiol. The plasma testosterone level (free and bound) is 300–1000 ng/dL (10.4–34.7 nmol/L) in adult men (Figure 22–8), compared with 30–70 ng/dL (1.04–2.43 nmol/L) in adult women. It declines somewhat with age in men.

TABLE 23–2 Distribution of gonadal steroids and cortisol in plasma.

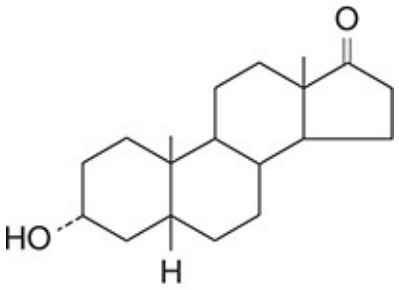
		% Bound to		
Steroid	**% Free**	**CBG**	**GBG**	**Albumin**
Testosterone	2	0	65	33
Androstenedione	7	0	8	85
Estradiol	2	0	38	60
Progesterone	2	18	0	80
Cortisol	4	90	0	6

CBG, corticosteroid-binding globulin; GBG, gonadal steroid–binding globulin. (Used with permission of S Munroe.)

A small amount of circulating testosterone is converted to estradiol, but most of the testosterone is converted to 17-ketosteroids, principally androsterone and its isomer etiocholanolone **(Figure 23–6)**, and excreted in the urine. About two-thirds of the urinary 17-ketosteroids are of adrenal origin, and one-third are of testicular origin. Although most of the 17-ketosteroids are weak androgens (they have 20% or less the potency of testosterone), it is worth emphasizing that not all 17-ketosteroids are androgens and not all androgens are 17-ketosteroids. Etiocholanolone, for example, has no androgenic activity, and testosterone itself is not a 17-ketosteroid.



Androsterone



Etiocholanolone

FIGURE 23–6 Testosterone metabolites. Androsterone and etiocholanolone are two 17-ketosteroid metabolites of testosterone that are secreted in the urine.

Actions

In addition to their actions during development, testosterone and other androgens exert an inhibitory feedback effect on pituitary LH secretion; develop and maintain the male secondary sex characteristics; exert an important protein-anabolic, growth-promoting effect; and, along with FSH, maintain spermatogenesis.

Secondary Sex Characteristics

The widespread changes in hair distribution, body configuration, and genital size that develop in boys at puberty—the male **secondary sex characteristics**—are summarized in Table 23–3. The prostate and seminal vesicles enlarge, and the seminal vesicles begin to secrete fructose. This sugar appears to function as the main nutritional supply for the spermatozoa. The psychological effects of testosterone are difficult to define in humans, but in experimental animals, androgens provoke boisterous and aggressive play. The effects of androgens and

estrogens on sexual behavior are considered in detail in Chapter 22. Although body hair is increased by androgens, scalp hair is decreased **(Figure 23–7)**. Hereditary baldness often fails to develop unless dihydrotestosterone (DHT) is present.

TABLE 23–3 Changes at puberty in boys (male secondary sex characteristics).

External genitalia: Penis increases in length and width. Scrotum becomes pigmented and rugose.
Internal genitalia: Seminal vesicles enlarge and secrete and begin to form fructose. Prostate and bulbourethral glands enlarge and secrete.
Voice: Larynx enlarges, vocal cords increase in length and thickness, and voice becomes deeper.
Hair growth: Beard appears. Hairline on scalp recedes anterolaterally. Pubic hair grows with male (triangle with apex up) pattern. Hair appears in axillas, on chest, and around anus; general body hair increases.
Mental: More aggressive, active attitude. Interest in opposite sex develops.
Body conformation: Shoulders broaden; muscles enlarge.
Skin: Sebaceous gland secretion thickens and increases (predisposing to acne).

FIGURE 23–7 Hairline in children and adults. The hairline of the woman is like that of the child, whereas that of the man is indented in the lateral frontal region.

Anabolic Effects

Androgens increase the synthesis and decrease the breakdown of protein, leading to an increase in the rate of growth. It used to be argued that they cause the epiphyses to fuse to the long bones, thus eventually stopping growth, but it now appears that epiphysial closure is due to estrogens (see Chapter 21). Secondary to their anabolic effects, androgens cause moderate Na^+, K^+, H_2O, Ca^{2+}, SO_4^{2-}, and PO_4^{3-} retention, and they also increase the size of the kidneys. Doses of exogenous testosterone that exert significant anabolic effects are also masculinizing and increase libido, which limits the usefulness of the hormone as an anabolic agent in patients with wasting diseases. Attempts to develop synthetic steroids in which the anabolic action is separated from the androgenic action have not been successful.

Mechanism of Action

Like other steroids, testosterone binds to an intracellular receptor, for example, androgen receptor (or NR3C4), and the receptor/steroid complex then binds to DNA in the nucleus, facilitating transcription of various genes. In addition, testosterone is converted to **DHT** by 5α-reductase in some target cells (Figure 23–5 and **Figure 23–8**), and DHT binds to the same intracellular receptor as testosterone. DHT also circulates, with a plasma level that is about 10% of the testosterone level. Testosterone–receptor complexes are less stable than DHT–receptor complexes in target cells, and they conform less well to the DNA-binding state. Thus, DHT formation is a way of amplifying the action of testosterone in target tissues. Humans have two 5α-reductases that are encoded by different genes. Type 1 5α-reductase is present in skin throughout the body and is the dominant enzyme in the scalp. Type 2 5α-reductase is present in genital skin, the prostate, and other genital tissues.

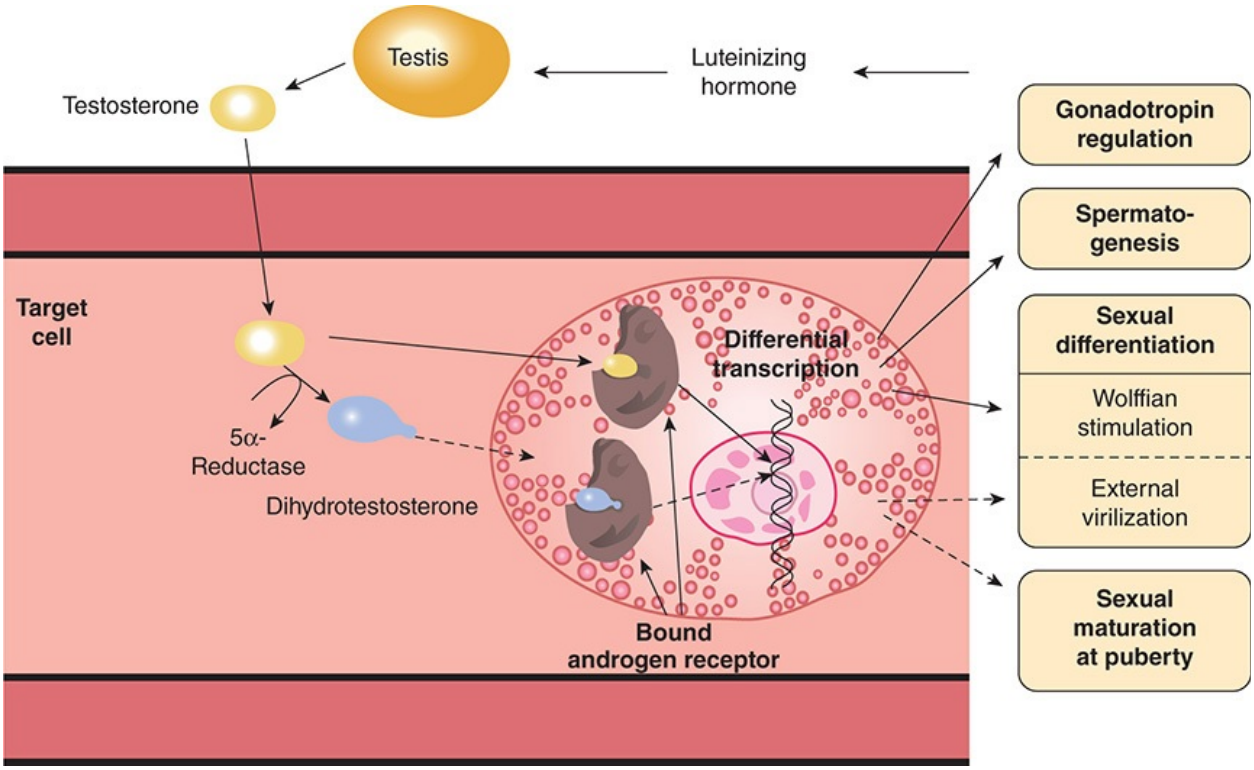


FIGURE 23–8 Schematic diagram of the actions of testosterone (solid arrows) and dihydrotestosterone (dashed arrows). Note that they both bind to the same receptor, but DHT binds more effectively. (Reproduced with permission of Wilson JD, Griffin JE, Russell DW: Steroid 5 alpha-reductase 2 deficiency. Endocr Rev 1993 Oct; 14(5):577–593.)

Testosterone–receptor complexes are responsible for the maturation of Wolffian duct structures and consequently for the formation of male internal genitalia during development, but DHT–receptor complexes are needed to form male external genitalia (Figure 23–8). DHT–receptor complexes are also primarily responsible for enlargement of the prostate and probably of the penis at the time of puberty, as well as for the facial hair, the acne, and the temporal recession of the hairline. On the other hand, the increase in muscle mass and the development of male sex drive and libido depend primarily on testosterone rather than DHT **(see Clinical Box 23–2)**.

Testicular Production of Estrogens

Over 80% of the estradiol and 95% of the estrone in the plasma of adult men are formed by extragonadal and extra-adrenal aromatization of circulating

testosterone and androstenedione. The remainder comes from the testes. Some of the estradiol in testicular venous blood comes from the Leydig cells, but some is also produced by aromatization of androgens in Sertoli cells. In men, the plasma estradiol level is 20–50 pg/mL (73–184 pmol/L) and the total production rate is approximately 50 µg/d (184 nmol/d). In contrast to the situation in women, estrogen production moderately increases with advancing age in men.

CLINICAL BOX 23–2

Congenital 5α-Reductase Deficiency

Congenital 5α-reductase deficiency, in which the gene for type 2 5α-reductase is mutated, is common in certain parts of the Dominican Republic. It produces an interesting form of male pseudohermaphroditism. Individuals with this syndrome are born with male internal genitalia including testes, but they have female external genitalia and are usually raised as girls. However, when they reach puberty, LH secretion and circulating testosterone levels are increased. Consequently, they develop male body contours and male libido. At this point, they usually change their gender identities and "become boys." The clitoris enlarges ("penis-at-12 syndrome") to the point that some of the individuals can have intercourse with women. This enlargement probably occurs because, with the high LH, enough testosterone is produced to overcome the need for DHT amplification in the genitalia.

THERAPEUTIC HIGHLIGHTS

5α-Reductase–inhibiting drugs are being used clinically to treat benign prostatic hyperplasia, and **finasteride,** the most extensively used drug, has its greatest effect on type 2 5α-reductase.

CONTROL OF TESTICULAR FUNCTION

FSH is tropic for Sertoli cells, and FSH and androgens maintain the gametogenic function of the testes. FSH also stimulates the secretion of ABP and inhibin. Inhibin feeds back to inhibit FSH secretion. LH is tropic for Leydig cells and stimulates the secretion of testosterone, which in turn feeds back to inhibit LH secretion. Hypothalamic lesions in animals and hypothalamic disease in humans

lead to atrophy of the testes and loss of their function.

Inhibins

Testosterone reduces plasma LH, but, except in large doses, it has no effect on plasma FSH. Plasma FSH is elevated in patients who have atrophy of the seminiferous tubules but normal levels of testosterone and LH secretion. These observations led to the search for **inhibin,** a factor of testicular origin that inhibits FSH secretion. There are two inhibins in extracts of testes in men and in antral fluid from ovarian follicles in women. They are formed from three polypeptide subunits: a glycosylated α subunit with a molecular weight of 18,000, and two nonglycosylated β subunits, β_A and β_B, each with a molecular weight of 14,000. The subunits are formed from precursor proteins **(Figure 23–9)**. The α subunit combines with β_A to form a heterodimer and with β_B to form another heterodimer, with the subunits linked by disulfide bonds. Both $\alpha\beta_A$ (inhibin A) and $\alpha\beta_B$ (inhibin B) inhibit FSH secretion by a direct action on the pituitary, although it appears that it is inhibin B that is the FSH-regulating inhibin in adult men and women. Inhibins are produced by Sertoli cells in males and granulosa cells in females.

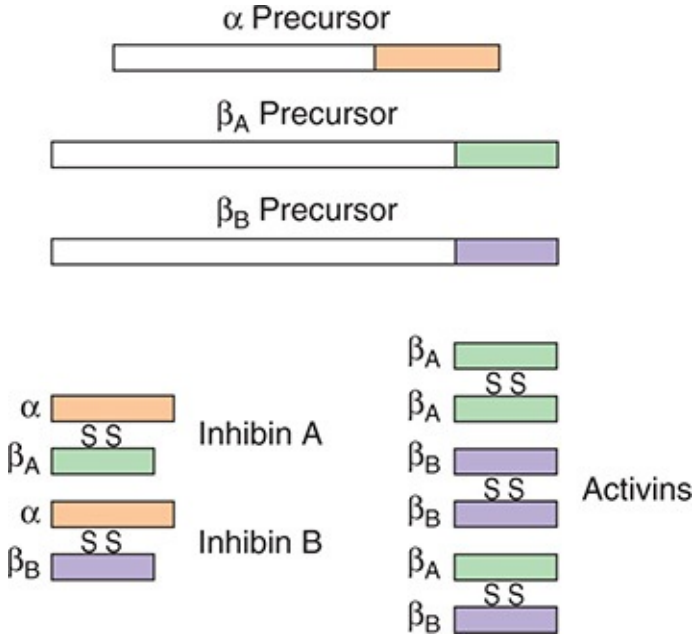


FIGURE 23–9 Inhibin and activin production. From three precursor proteins (α, β_A, and β_B), five separate inhibins and activins are produced. SS, disulfide bonds.

The heterodimer $\beta_A\beta_B$ and the homodimers $\beta_A\beta_A$ and $\beta_B\beta_B$ are also formed. They stimulate rather than inhibit FSH secretion and consequently are called **activins.** Their function in reproduction is unsettled. However, the inhibins and activins are members of the transforming growth factor beta (TGF-β) superfamily of dimeric growth factors that also includes MIS. **Activin receptors** have been identified and belong to the serine/threonine kinase receptor family. Inhibins and activins are found not only in the gonads but also in the brain and many other tissues. In the bone marrow, activins are involved in the development of white blood cells. In embryonic life, activins are involved in the formation of mesoderm. All mice with a targeted deletion of the α-inhibin subunit gene initially exhibit normal growth but then develop gonadal stromal tumors, so the gene is a tumor suppressor gene.

In plasma, α_2-macroglobulin binds activins and inhibins. In tissues, activins bind to a family of four glycoproteins called **follistatins.** Binding of the activins inactivates their biological activity, but the relation of follistatins to inhibin and their physiological function remain unsettled.

Steroid Feedback

A current “working hypothesis” of the way the functions of the testes are regulated by steroids is shown in **Figure 23–10**. Castration is followed by a rise in the pituitary content and secretion of FSH and LH, and hypothalamic lesions prevent this rise. Testosterone inhibits LH secretion by acting directly on the anterior pituitary and by inhibiting the secretion of gonadotropin-releasing hormone (GnRH) from the hypothalamus. Inhibin acts directly on the anterior pituitary to inhibit FSH secretion.

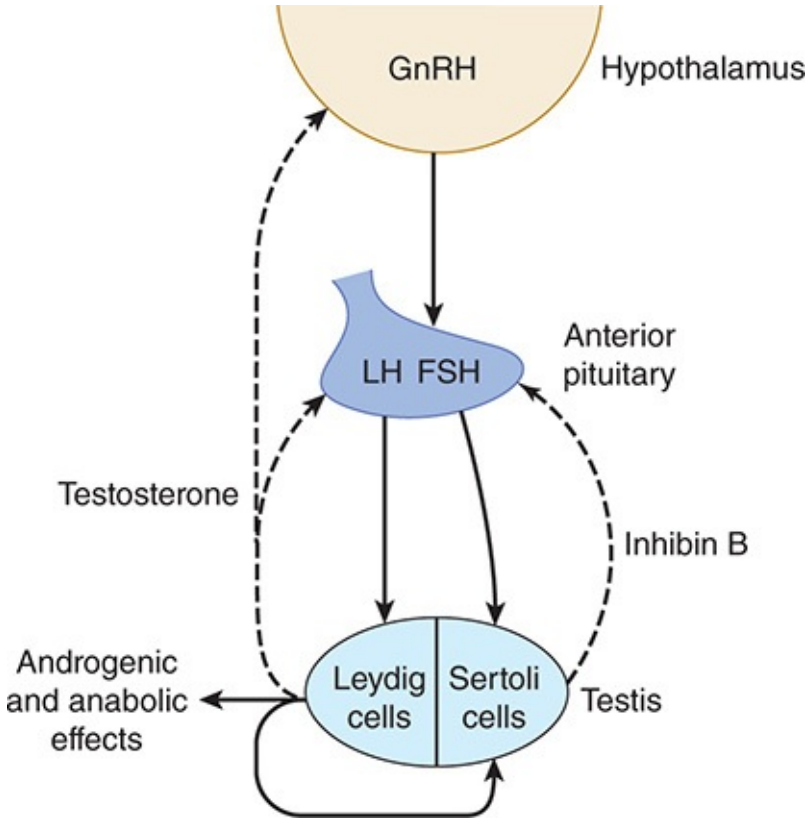


FIGURE 23–10 The hypothalamus, pituitary, and testes interact via signaling molecules. Postulated signaling pathways between the hypothalamus, anterior pituitary, and testis are shown. Solid arrows indicate excitatory effects, whereas dashed arrows indicate inhibitory effects.

In response to LH, some of the testosterone secreted from the Leydig cells bathes the seminiferous epithelium and provides the high local concentration of androgen to the Sertoli cells that is necessary for normal spermatogenesis. Systemically administered testosterone does not raise the androgen level in the testes to as great a degree, and it inhibits LH secretion. Consequently, the net effect of systemically administered testosterone is generally a decrease in sperm count. Testosterone therapy has been suggested as a means of male contraception. However, the dose of testosterone needed to suppress spermatogenesis causes sodium and water retention. The possible use of inhibins as male contraceptives is being explored.

ABNORMALITIES OF TESTICULAR FUNCTION

Cryptorchidism

The testes develop in the abdominal cavity and normally migrate to the scrotum during fetal development. **Testicular descent** to the inguinal region depends on MIS, and descent from the inguinal region to the scrotum depends on other factors. Descent is incomplete on one or, less commonly, both sides in 10% of newborn males, with the testes remaining in the abdominal cavity or inguinal canal. Gonadotropic hormone treatment speeds descent in some cases, or the defect can be corrected surgically. Spontaneous descent of the testes is the rule, and the proportion of boys with undescended testes **(cryptorchidism)** falls to 2% at age 1 year and 0.3% after puberty. However, early treatment is now recommended despite these figures because the incidence of malignant tumors is higher in undescended than in scrotal testes and because after puberty the higher temperature in the abdomen eventually causes irreversible damage to the spermatogenic epithelium.

Male Hypogonadism

The clinical picture of male hypogonadism depends on whether testicular deficiency develops before or after puberty. In adults, if it is due to testicular disease, circulating gonadotropin levels are elevated **(hypergonadotropic hypogonadism);** if it is secondary to disorders of the pituitary or the hypothalamus (eg, Kallmann syndrome), circulating gonadotropin levels are decreased **(hypogonadotropic hypogonadism).** If the endocrine function of the testes is lost in adulthood, the secondary sex characteristics regress slowly because it takes very little androgen to maintain them once they are established. The growth of the larynx during adolescence is permanent, and the voice remains deep. Men castrated in adulthood suffer some loss of libido, although the ability to copulate persists for some time. They occasionally have hot flushes and are generally more irritable, passive, and depressed than men with intact testes. When the Leydig cell deficiency dates from childhood, the clinical picture is that of **eunuchoidism.** Eunuchoid individuals over the age of 20 are characteristically tall, although not as tall as hyperpituitary giants, because their epiphyses remain open and some growth continues past the normal age of puberty. They have narrow shoulders and small muscles, a body configuration resembling that of the adult female. The genitalia are small and the voice high-pitched. Pubic hair and axillary hair are present because of adrenocortical androgen secretion. However, the hair is sparse, and the pubic hair has the female “triangle with the base up” distribution rather than the “triangle with the base down” pattern (male escutcheon) seen in normal males.

Androgen-Secreting Tumors

"Hyperfunction" of the testes in the absence of tumor formation is not a recognized entity. Androgen-secreting Leydig cell tumors are rare and cause detectable endocrine symptoms only in prepubertal boys, in whom precocious pseudopuberty develops (Table 22–2).

Hormones & Cancer

Some carcinomas of the prostate are **androgen-dependent** and regress temporarily after the removal of the testes or treatment with GnRH agonists in doses that are sufficient to produce downregulation of the GnRH receptors on gonadotropes and decrease LH secretion.

CHAPTER SUMMARY

- The gonads have a dual function: the production of germ cells (gametogenesis) and the secretion of sex hormones. The testes secrete large amounts of androgens, principally testosterone, but they also secrete small amounts of estrogens.
- Spermatogonia develop into mature spermatozoa in the seminiferous tubules via a process called spermatogenesis. This is a multistep process that includes maturation of spermatogonia into primary spermatocytes, which undergo meiotic division, resulting in haploid secondary spermatocytes. Several further divisions result in spermatids. Each cell division from a spermatogonium to a spermatid is incomplete, with cells remaining connected via cytoplasmic bridges. Spermatids eventually mature into motile spermatozoa to complete spermatogenesis; this last part of maturation is called spermiogenesis.
- Testosterone is the principal hormone of the testis. It is synthesized from cholesterol in Leydig cells. The secretion of testosterone from Leydig cells is under control of luteinizing hormone at a rate of 4–9 mg/d in adult males. Most testosterone is bound to albumin or to gonadal steroid–binding globulin in the plasma. Testosterone plays an important role in the development and maintenance of male secondary sex characteristics, as well as other defined functions.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. Full development and function of the seminiferous tubules require
   A. somatostatin.
   B. LH.
   C. oxytocin.
   D. FSH.
   E. androgens and FSH.
2. In human males, testosterone is produced mainly by the
   A. Leydig cells.
   B. Sertoli cells.
   C. seminiferous tubules.
   D. epididymis.
   E. vas deferens.
3. NOS contributes to erection by:
   A. raising cAMP levels that relax smooth muscles and increase blood flow.
   B. blocking phosphodiesterases to increase cGMP levels that release smooth muscle and increase blood flow.
   C. activating soluble guanylyl cyclases to increase cGMP levels that relax smooth muscle and increase blood flow.
   D. raising intracellular Ca^{2+} concentrations that relax smooth muscles and increase blood flow.
4. Testosterone is produced
   A. in the testes after reduction of dihydrotestosterone.
   B. in Leydig cells from cholesterol and pregnenolone precursors.
   C. by LH in Leydig cells.
   D. as a precursor for several membrane lipids.

CHAPTER 24

Endocrine Functions of the Pancreas & Regulation of Carbohydrate Metabolism

OBJECTIVES

After studying this chapter, you should be able to:

- List the hormones that affect the plasma glucose concentration and briefly describe the action of each.
- Describe the structure of the pancreatic islets and name the hormones secreted by each of the cell types in the islets.
- Describe the structure of insulin and outline the steps involved in its biosynthesis and release into the bloodstream.
- List the consequences of insulin deficiency and explain how each of these abnormalities is produced.
- Describe insulin receptors, the way they mediate the effects of insulin, and the way they are regulated.
- Describe the types of glucose transporters found in the body and the function of each.
- List the major factors that affect the secretion of insulin.
- Describe the structure of glucagon and other physiologically active peptides produced from its precursor.

- List the physiologically significant effects of glucagon and the factors that regulate glucagon secretion.
- Describe the physiologic effects of somatostatin in the pancreas.
- Outline the mechanisms by which thyroid hormones, adrenal glucocorticoids, catecholamines, and growth hormone affect carbohydrate metabolism.
- Understand the major differences between type 1 and type 2 diabetes.

INTRODUCTION

The pancreas is a glandular organ that contributes to the physiological regulation of the endocrine and digestive system. It is located in the abdominal cavity, below the liver and behind the stomach (Figure 25-11, Chapter 25). The **islets of Langerhans** are the endocrine cells within the pancreas that secrete four polypeptides with regulatory activity. Two of these, **insulin** and **glucagon,** are hormones and have important functions in the regulation of the intermediary metabolism of carbohydrates, proteins, and fats. The third polypeptide, **somatostatin,** plays a role in the regulation of islet cell secretion, and the fourth, **pancreatic polypeptide,** is probably concerned primarily with the regulation of ion transport in the intestine. Glucagon, somatostatin, and possibly pancreatic polypeptide are also secreted by cells in the mucosa of the gastrointestinal tract (Chapter 25).

Insulin is anabolic, increasing the storage of glucose, fatty acids, and amino acids. Glucagon is catabolic, mobilizing glucose, fatty acids, and the amino acids from stores into the bloodstream. The two hormones are thus reciprocal in their overall action and are reciprocally secreted in most circumstances. Insulin excess causes hypoglycemia, which leads to convulsions and coma. Insulin deficiency, either absolute or relative, causes **diabetes mellitus** (chronic elevated blood glucose), a complex and debilitating disease that if untreated is eventually fatal. Glucagon deficiency can cause hypoglycemia, and glucagon excess makes diabetes worse. Excess pancreatic production of somatostatin causes hyperglycemia and other manifestations of diabetes.

A variety of other hormones also have important roles in the regulation of carbohydrate metabolism.

ISLET CELL STRUCTURE

The islets of Langerhans **(Figure 24–1)** are ovoid, 76- × 175-µm collections of cells. The islets are scattered throughout the pancreas, although they are more plentiful in the tail than in the body and head. β-Islets make up about 2% of the volume of the gland, whereas the exocrine portion of the pancreas (see Chapter 25) makes up 80%, and ducts and blood vessels make up the remainder. Humans have 1–2 million islets. Each has a copious blood supply; blood from the islets, like that from the gastrointestinal tract (but unlike that from any other endocrine organs) drains into the hepatic portal vein.

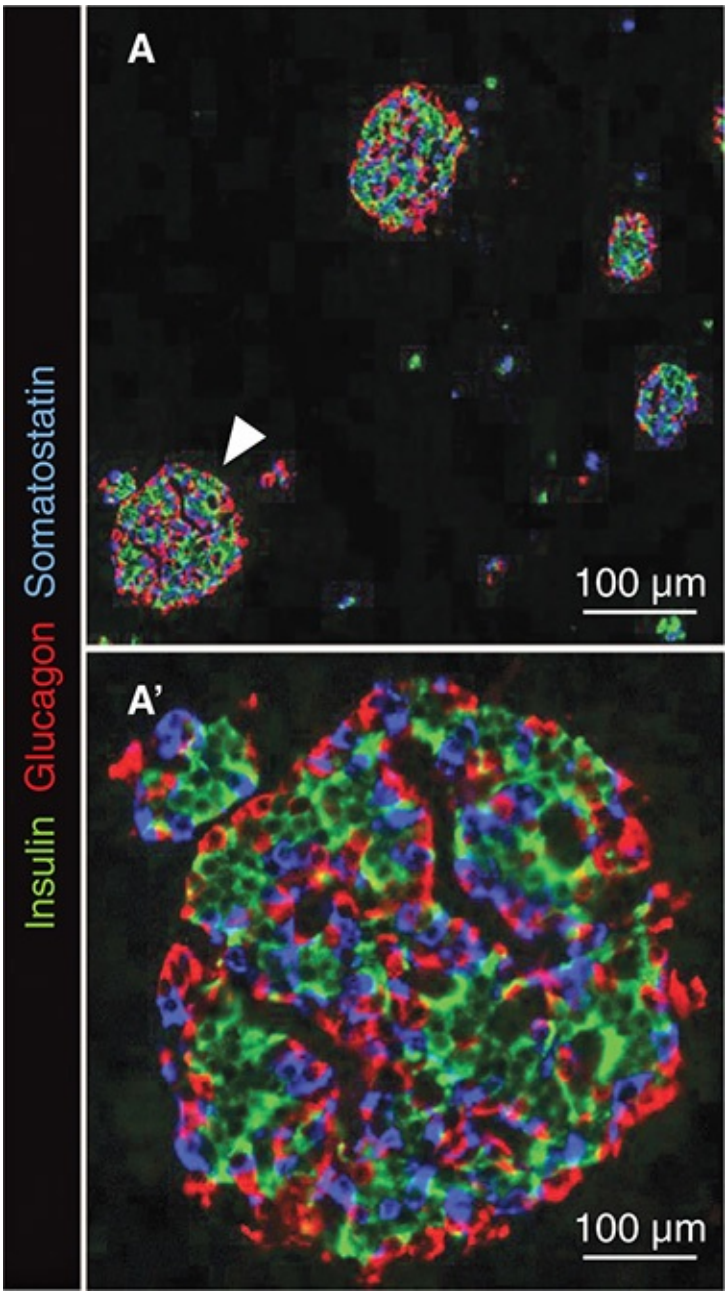


FIGURE 24–1 Islet of Langerhans in the human pancreas. Green fluorescent labeling are insulin containing B cells within the islets, red fluorescent labeling are glucagon containing A cells and blue fluorescent labeling are somatostatin containing D cells. Surrounding dark area is pancreatic acinar tissue. (Used with permission from N. Hart).

The cells in the islets can be divided into types on the basis of their staining properties and morphology. Humans have at least four distinct cell types: A, B, D, and F cells. A, B, and D cells are also called α, β, and δ cells. However, this leads to confusion in view of the use of Greek letters to refer to other structures in the body, particularly adrenergic receptors (see Chapter 7). The A cells secrete glucagon, the B cells secrete insulin, the D cells secrete somatostatin, and the F cells secrete pancreatic polypeptide. The B cells, which are the most common and account for 60–75% of the cells in the islets, are generally located in the center of each islet. They tend to be surrounded by the A cells, which make up 20% of the total, and the less common D and F cells. The islets in the tail, the body, and the anterior and superior part of the head of the human pancreas have many A cells and few if any F cells in the outer rim, whereas in rats and probably in humans, the islets in the posterior part of the head of the pancreas have a relatively large number of F cells and few A cells. The A-cell-rich (glucagon-rich) islets arise embryologically from the dorsal pancreatic bud, and the F-cell-rich (pancreatic polypeptide-rich) islets arise from the ventral pancreatic bud. These buds arise separately from the duodenum.

The B cell granules are packets of insulin in the cell cytoplasm. The shape of the packets varies from species to species; in humans, some are round whereas others are rectangular **(Figure 24–2)**. In the B cells, the insulin molecule forms polymers and also complexes with zinc. The differences in the shape of the packets are probably due to differences in the size of polymers or zinc aggregates of insulin. The A granules, which contain glucagon, are relatively uniform from species to species **(Figure 24–3)**. The D cells also contain large numbers of relatively homogeneous granules.

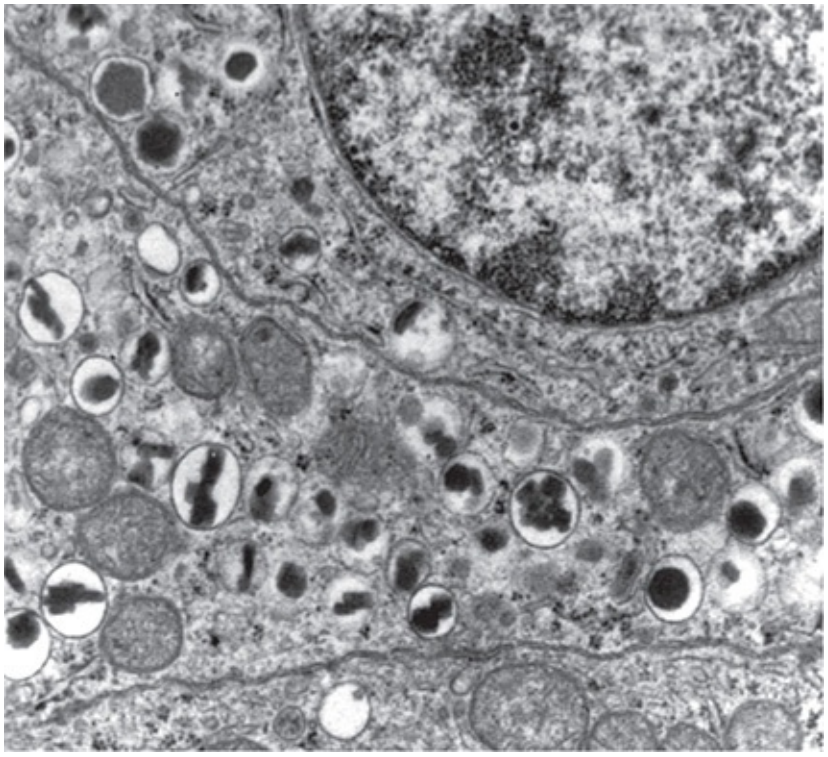


FIGURE 24–2 Electronmicrograph of two adjoining B cells in a human pancreatic islet. The B granules are the crystals in the membrane-lined vesicles. They vary in shape from rhombic to round (× 26,000). (Reproduced with permission from Fawcett DW: *Bloom and Fawcett A Textbook of Histology*, 11th ed. St. Louis, MO: Saunders; 1986.)

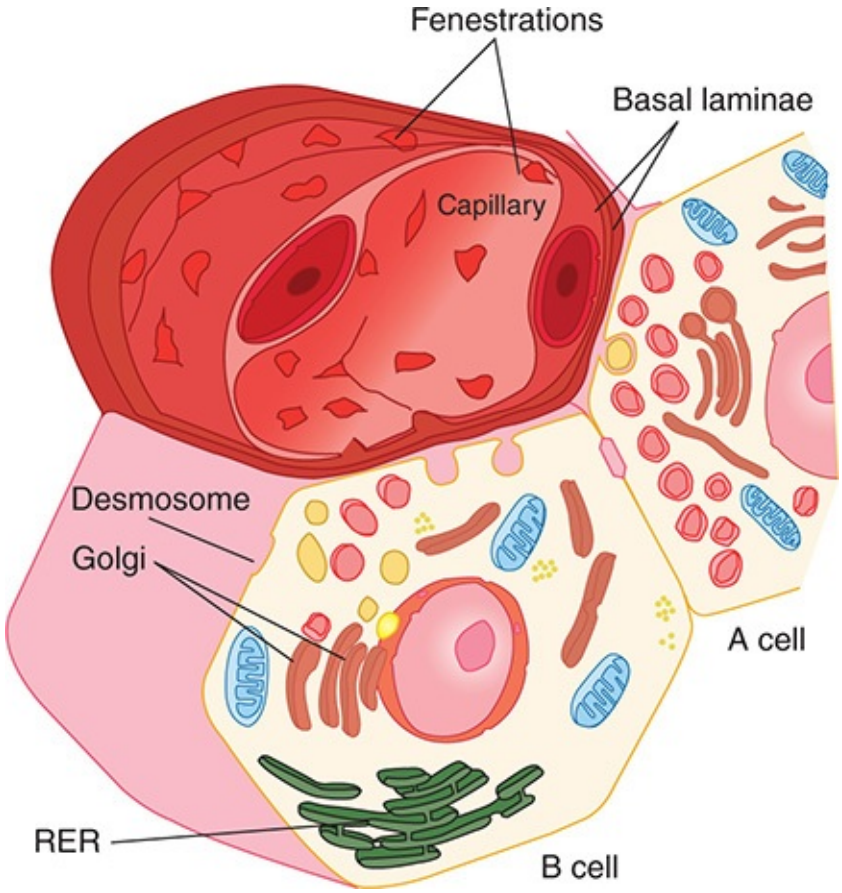


FIGURE 24–3 A and B cells, showing their relation to a blood vessel. Insulin from the B cell and glucagon from the A cell are secreted by exocytosis and cross the basal lamina of the cell and the basal lamina of the capillary before entering the lumen of the fenestrated capillary. RER, rough endoplasmic reticulum. (Reproduced with permission from Junqueira IC, Carneiro J: *Basic Histology: Text and Atlas*, 10th ed. New York, NY: McGraw-Hill; 2003.)

STRUCTURE, BIOSYNTHESIS, & SECRETION OF INSULIN

STRUCTURE & SPECIES SPECIFICITY

Insulin is a polypeptide containing two chains of amino acids linked by disulfide bridges. Minor differences occur in the amino acid composition of the molecule from species to species. The differences are generally not sufficient to affect the biologic activity of a particular insulin in heterologous species but are sufficient to make the insulin antigenic. If insulin of one species is injected for a prolonged

period into another species, the anti-insulin antibodies formed inhibit the injected insulin. Almost all humans who have received commercial bovine insulin for more than 2 months have antibodies against bovine insulin, but the titer is usually low. Porcine insulin differs from human insulin by only one amino acid residue and has low antigenicity. Human insulin produced in bacteria by recombinant DNA technology is now widely used to avoid antibody formation.

BIOSYNTHESIS & SECRETION

Insulin is synthesized in the rough endoplasmic reticulum of the B cells (Figure 24–3). It is then transported to the Golgi apparatus, where it is packaged into membrane-bound granules. These granules move to the plasma membrane by a process involving microtubules, and their contents are expelled by exocytosis (see Chapters 2 and 16). The insulin then crosses the basal lamina of the B cell and a neighboring capillary and the fenestrated endothelium of the capillary to reach the bloodstream. The fenestrations are discussed in detail in Chapter 31.

Like other polypeptide hormones and related proteins that enter the endoplasmic reticulum, insulin is synthesized as part of a larger preprohormone (see Chapter 1). The gene for insulin is located on the short arm of chromosome 11 in humans. It has two introns and three exons. **Preproinsulin** originates from the endoplasmic reticulum. The remainder of the molecule is then folded, and the disulfide bonds are formed to make **proinsulin.** The peptide segment connecting the A and B chains, the **connecting peptide (C peptide),** facilitates the folding and then is detached in the granules before secretion. Two proteases are involved in processing the proinsulin. Normally, 90–97% of the product released from the B cells is insulin along with equimolar amounts of C peptide. The rest is mostly proinsulin. C peptide can be measured by radioimmunoassay, and its level in blood provides an index of B cell function in patients receiving exogenous insulin.

FATE OF SECRETED INSULIN

INSULIN & INSULIN-LIKE ACTIVITY IN BLOOD

Plasma contains a number of substances with insulin-like activity in addition to insulin. The activity that is not suppressed by anti-insulin antibodies has been called **nonsuppressible insulin-like activity (NSILA).** Most, if not all, of this

activity persists after pancreatectomy and is due to the insulin-like growth factors **IGF-I** and **IGF-II** (see Chapter 18). These IGFs are polypeptides. Small amounts are free in the plasma (low-molecular-weight fraction), but large amounts are bound to proteins (high-molecular-weight fraction).

One may well ask why pancreatectomy causes diabetes mellitus when NSILA persists in the plasma. However, the insulin-like activities of IGF-I and IGF-II are weak compared to that of insulin and likely subserve other specific functions.

METABOLISM

The half-life of insulin in the circulation in humans is about 5 min. Insulin binds to insulin receptors, and some is internalized. It is destroyed by proteases in the endosomes formed by the endocytotic process.

EFFECTS OF INSULIN

The physiologic effects of insulin are far-reaching and complex. They are conveniently divided into rapid, intermediate, and delayed actions **(Table 24–1)**. The best known is the hypoglycemic effect, but there are additional effects on amino acid and electrolyte transport, many enzymes, and growth. The net effect of the hormone is storage of carbohydrate, protein, and fat. Therefore, insulin is appropriately called the "hormone of abundance."

TABLE 24–1 Principal actions of insulin.

Rapid (seconds)
Increased transport of glucose, amino acids, and K^+ into insulin-sensitive cells
Intermediate (minutes)
Stimulation of protein synthesis
Inhibition of protein degradation
Activation of glycolytic enzymes and glycogen synthase
Inhibition of phosphorylase and gluconeogenic enzymes
Delayed (hours)
Increase in mRNAs for lipogenic and other enzymes

Courtesy of ID Goldfine.

The actions of insulin on adipose tissue; skeletal, cardiac, and smooth muscle; and the liver are summarized in **Table 24–2**.

TABLE 24–2 Effects of insulin on various tissues.

Adipose tissue
Increased glucose entry
Increased fatty acid synthesis
Increased glycerol phosphate synthesis
Increased triglyceride deposition
Activation of lipoprotein lipase
Inhibition of hormone-sensitive lipase
Increased K^+ uptake
Muscle
Increased glucose entry
Increased glycogen synthesis
Increased amino acid uptake
Increased protein synthesis in ribosomes
Decreased protein catabolism
Decreased release of gluconeogenic amino acids
Increased ketone uptake
Increased K^+ uptake
Liver
Decreased ketogenesis
Increased protein synthesis
Increased lipid synthesis
Decreased glucose output due to decreased gluconeogenesis, increased glycogen synthesis, and increased glycolysis
General
Increased cell growth

GLUCOSE TRANSPORTERS

Glucose enters cells by **facilitated diffusion** (see Chapter 1) or, in the intestine and kidneys, by secondary active transport with Na^+. In muscle, adipose, and

some other tissues, insulin stimulates glucose entry into cells by increasing the number of glucose transporters (GLUTs) in the cell membranes.

The GLUTs that are responsible for facilitated diffusion of glucose across cell membranes are a family of closely related proteins that span the cell membrane 12 times and have their amino and carboxyl terminals inside the cell. They differ from and have no homology with the **Sodium**-dependent **glucose** cotransporters (SGLT-1 and SGLT-2), which are responsible for the secondary active transport of glucose in the intestine (see Chapter 26) and renal tubules (see Chapter 38), although the SGLTs also have 12 transmembrane domains.

Seven different GLUTs, named GLUT 1–7 in order of discovery, have been characterized **(Table 24–3)**. They contain 492–524 amino acid residues and their affinity for glucose varies. Each transporter appears to have evolved for special tasks. GLUT-4 is the transporter in muscle and adipose tissue that is stimulated by insulin. A pool of GLUT-4 molecules is maintained within vesicles in the cytoplasm of insulin-sensitive cells. When the insulin receptors of these cells are activated, the vesicles move rapidly to the cell membrane and fuse with it, inserting the transporters into the cell membrane **(Figure 24–4)**. When insulin action ceases, the transporter-containing patches of membrane are endocytosed and the vesicles are ready for the next exposure to insulin. Activation of the insulin receptor brings about the movement of the vesicles to the cell membrane by activating phosphatidylinositol 3-kinase (Figure 24–4). Most of the other GLUT transporters that are not insulin-sensitive appear to be constitutively expressed in the cell membrane.

TABLE 24–3 Glucose transporters in mammals.

	Function	K_m (mM)[a]	Major Sites of Expression
Secondary active transport (Na^1-glucose cotransport)			
SGLT 1	Absorption of glucose	0.1–1.0	Small intestine, renal tubules
SGLT 2	Absorption of glucose	1.6	Renal tubules
Facilitated diffusion			
GLUT 1	Basal glucose uptake	1–2	Placenta, blood-brain barrier, brain, red cells, kidneys, colon, many other organs
GLUT 2	B-cell glucose sensor; transport out of intestinal and renal epithelial cells	12–20	B cells of islets, liver, epithelial cells of small intestine, kidneys
GLUT 3	Basal glucose uptake	<1	Brain, placenta, kidneys, many other organs
GLUT 4	Insulin-stimulated glucose uptake	5	Skeletal and cardiac muscle, adipose tissue, other tissues
GLUT 5	Fructose transport	1–2	Jejunum, sperm
GLUT 6	Unknown	—	Brain, spleen, and leukocytes
GLUT 7	Glucose 6-phosphate transporter in endoplasmic reticulum	—	Liver

[a]The K_m is the glucose concentration at which transport is half-maximal.

Data from Stephens JM, Pilch PF: The metabolic regulation and vesicular transport of GLUT 4, the major insulin-responsive glucose transporter. Endocr Rev 1995;16:529.

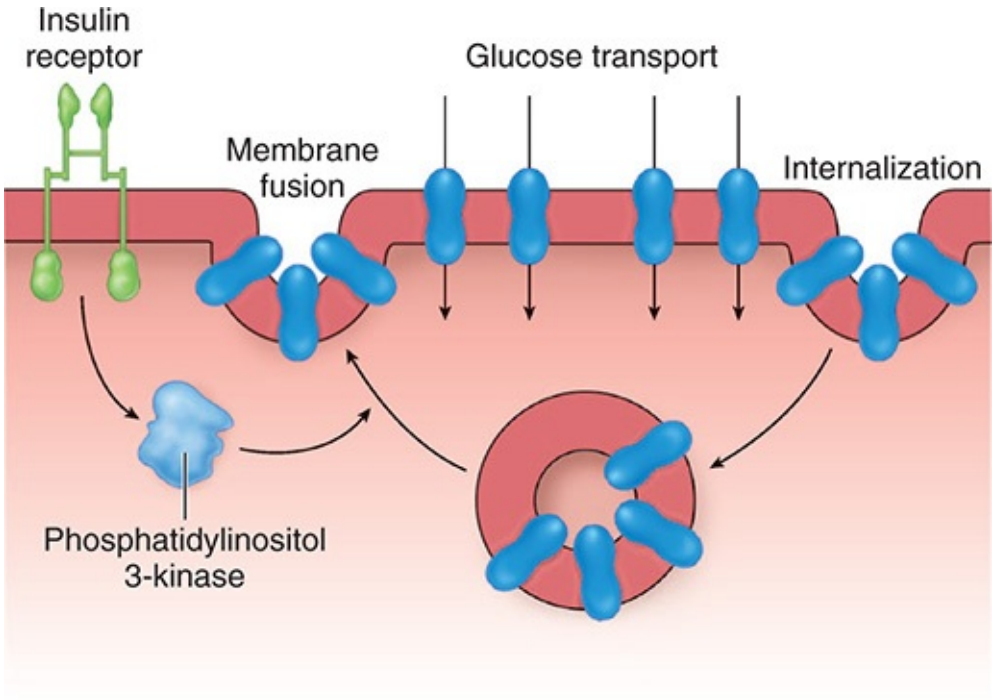


FIGURE 24–4 Cycling of GLUT-4 transporters through endosomes in insulin-sensitive tissues. Activation of the insulin receptor causes activation of phosphatidylinositol 3-kinase, which speeds translocation of the GLUT-4–containing endosomes into the cell membrane. The GLUT-4 transporters then mediate glucose transport into the cell.

In the tissues in which insulin increases the number of GLUTs in cell membranes, the rate of phosphorylation of the glucose, once it has entered the cells, is regulated by other hormones. Growth hormone and cortisol both inhibit phosphorylation in certain tissues. Transport is normally so rapid that it is not a rate-limiting step in glucose metabolism. However, it is rate-limiting in B cells.

Insulin also increases the entry of glucose into liver cells, but it does not exert this effect by increasing the number of GLUT-4 transporters in the cell membranes. Instead, it induces glucokinase, and this increases the phosphorylation of glucose, so that the intracellular free glucose concentration stays low, facilitating the entry of glucose into the cell.

Insulin-sensitive tissues also contain a population of GLUT-4 vesicles that move into the cell membrane in response to exercise, a process that occurs independent of the action of insulin. This is why exercise lowers blood sugar. A 5′-adenosine monophosphate (AMP)–activated kinase may trigger the insertion of these vesicles into the cell membrane.

INSULIN PREPARATIONS

The maximal decline in plasma glucose occurs 30 min after intravenous injection of insulin. After subcutaneous administration, the maximal fall occurs in 2–3 h. A wide variety of insulin preparations are now available commercially. These include insulins that have been complexed with protamine and other polypeptides to delay absorption and degradation, and synthetic insulins in which there have been changes in amino acid residues. In general, they fall into three categories: rapid, intermediate-acting, and long-acting (24–36 h).

Relation To Potassium

Insulin causes K^+ to enter cells, with a resultant lowering of the extracellular K^+ concentration. Infusions of insulin and glucose significantly lower the plasma K^+ level in normal individuals and are very effective for the temporary relief of hyperkalemia in patients with renal failure. **Hypokalemia** often develops when patients with diabetic acidosis are treated with insulin. The reason for the intracellular migration of K^+ is still uncertain. However, insulin increases the activity of Na, K ATPase in cell membranes, so that more K^+ is pumped into cells.

OTHER ACTIONS

The hypoglycemic and other effects of insulin are summarized in temporal terms in Table 24–1, and the net effects on various tissues are summarized in Table 24–2. The action on glycogen synthase fosters glycogen storage, and the actions on glycolytic enzymes favor glucose metabolism to two carbon fragments (see Chapter 1), with resulting promotion of lipogenesis. Stimulation of protein synthesis from amino acids entering the cells and inhibition of protein degradation foster growth.

The anabolic effect of insulin is aided by the protein-sparing action of adequate intracellular glucose supplies. Failure to grow is a symptom of diabetes in children, and insulin stimulates the growth of immature hypophysectomized rats to almost the same degree as growth hormone.

MECHANISM OF ACTION

INSULIN RECEPTORS

Insulin receptors are found on many different cells in the body, including cells in which insulin does not increase glucose uptake.

The insulin receptor, which has a molecular weight of approximately 340,000, is a tetramer made up of two α and two β glycoprotein subunits **(Figure 24–5)**. All these are synthesized on a single mRNA and then proteolytically separated and bound to each other by disulfide bonds. The gene for the insulin receptor has 22 exons and in humans is located on chromosome 19. The α subunits bind insulin and are extracellular, whereas the β subunits span the membrane. The intracellular portions of the β subunits have tyrosine kinase activity. The α and β subunits are both glycosylated, with sugar residues extending into the interstitial fluid.

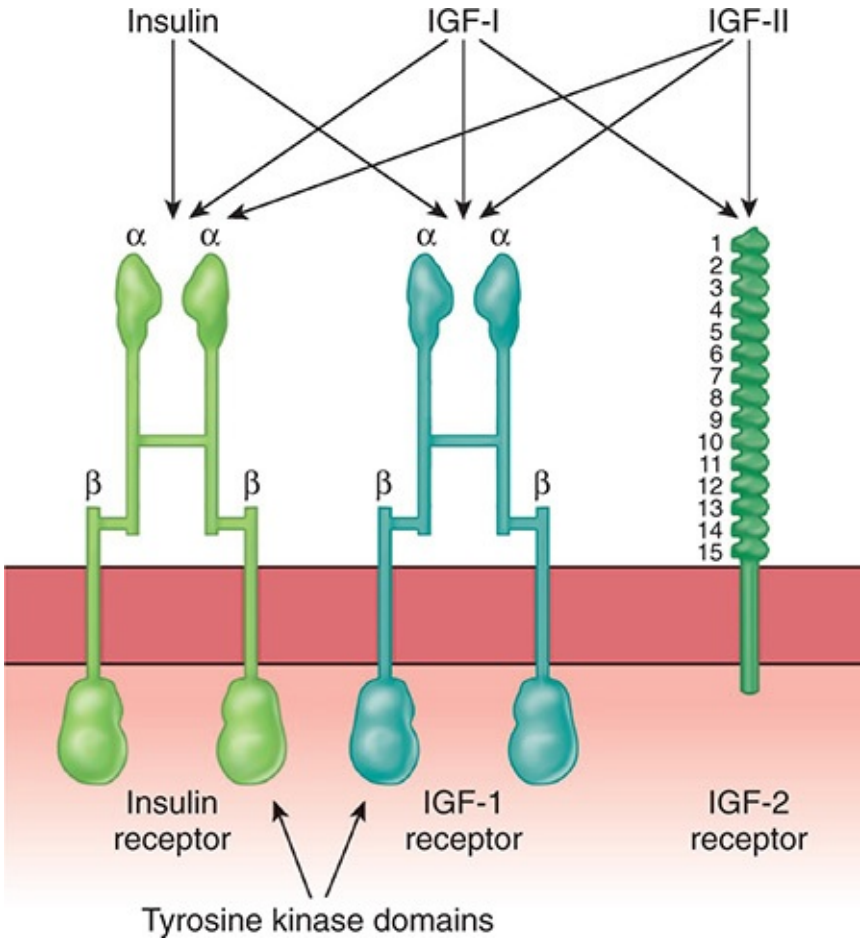


FIGURE 24–5 Insulin, IGF-I, and IGF-II receptors. Each hormone binds primarily to its own receptor, but insulin also binds to the IGF-I receptor, and IGF-I and IGF-II bind to all three. The purple boxes are intracellular tyrosine

kinase domains. Note the marked similarity between the insulin receptor and the IGF-I receptor; also note the 15 repeat sequences in the extracellular portion of the IGF-II receptor.

Binding of insulin triggers the tyrosine kinase activity of the β subunits, producing autophosphorylation of the β subunits on tyrosine residues. The autophosphorylation, which is necessary for insulin to exert its biologic effects, triggers phosphorylation of some cytoplasmic proteins and dephosphorylation of others, mostly on serine and threonine residues. Insulin receptor substrate (IRS-1) mediates some of the effects in humans but there are other effector systems as well **(Figure 24–6)**. For example, mice in which the insulin receptor gene is knocked out show marked growth retardation in utero, have abnormalities of the central nervous system (CNS) and skin, and die at birth of respiratory failure, whereas IRS-1 knockouts show only moderate growth retardation in utero, survive, and are insulin-resistant but otherwise nearly normal.

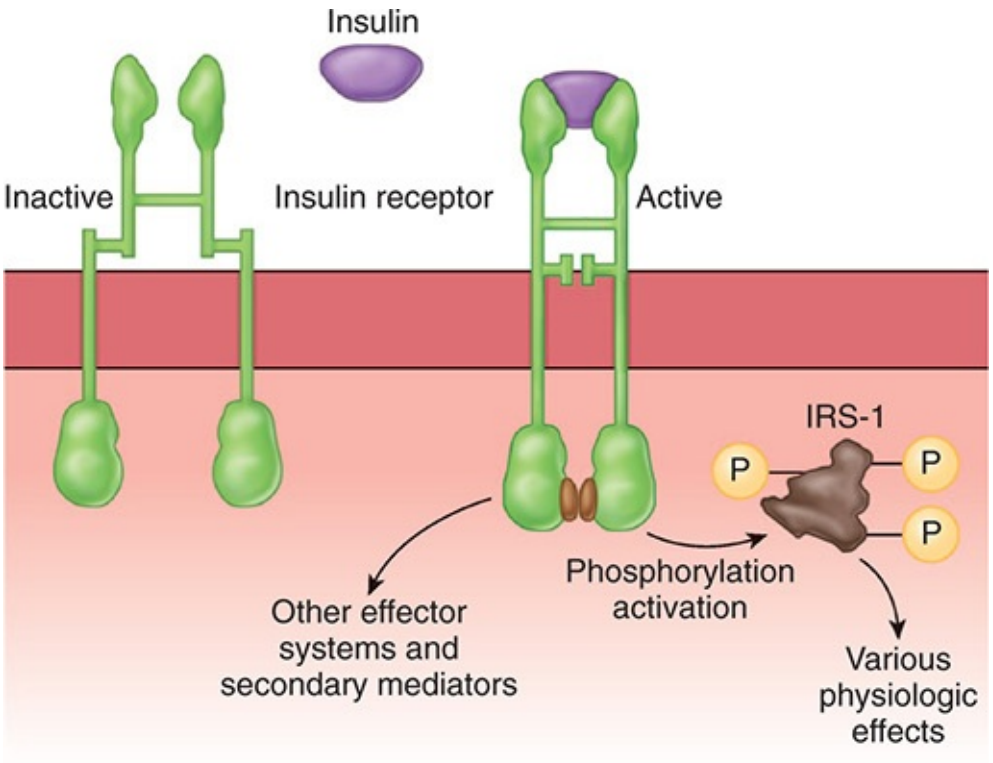


FIGURE 24–6 Intracellular responses triggered by insulin binding to the insulin receptor. Circles labeled P represent phosphate groups. IRS-1, insulin receptor substrate-1.

The growth-promoting protein anabolic effects of insulin are mediated via

phosphatidylinositol 3-kinase (PI3K), and evidence indicates that in invertebrates, this pathway is involved in the growth of nerve cells and axon guidance in the visual system.

It is interesting to compare the insulin receptor with other related receptors. The insulin receptor is very similar to the receptor for IGF-I but different from the receptor for IGF-II (Figure 24–5). Other receptors for growth factors and receptors for various oncogenes also are tyrosine kinases. However, the amino acid composition of these receptors is quite different.

When insulin binds to its receptors, they aggregate in patches and are taken up into the cell by receptor-mediated endocytosis (see Chapter 2). Eventually, the insulin–receptor complexes enter lysosomes, where the receptors are broken down or recycled. The half-life of the insulin receptor is about 7 h.

CONSEQUENCES OF INSULIN DEFICIENCY

The far-reaching physiologic effects of insulin are highlighted by a consideration of the extensive and serious consequences of insulin deficiency **(Clinical Box 24–1)**.

In humans, insulin deficiency is a common pathologic condition. In animals, it can be produced by pancreatectomy; by administration of alloxan, streptozocin, or other toxins that in appropriate doses cause selective destruction of the B cells of the pancreatic islets; by administration of drugs that inhibit insulin secretion; and by administration of anti-insulin antibodies. Strains of mice, rats, hamsters, guinea pigs, miniature swine, and monkeys that have a high incidence of spontaneous diabetes mellitus have also been described.

CLINICAL BOX 24–1

Diabetes Mellitus

The constellation of abnormalities caused by insulin deficiency is called **diabetes mellitus.** Greek and Roman physicians used the term “diabetes” to refer to conditions in which the cardinal finding was a large urine volume, and two types were distinguished: “diabetes mellitus,” in which the urine tasted sweet; and “diabetes insipidus,” in which the urine had little taste. Today, the term “diabetes insipidus” is reserved for conditions in which there is a deficiency of the production or action of vasopressin (see Chapter 38), and the unmodified word “diabetes” is generally used as a synonym for

diabetes mellitus.

The cause of clinical diabetes is always a deficiency of the effects of insulin at the tissue level. **Type 1 diabetes,** or **insulin-dependent diabetes mellitus (IDDM),** is due to insulin deficiency caused by autoimmune destruction of the B cells in the pancreatic islets, and it accounts for 3–5% of cases and usually presents in children. **Type 2 diabetes, or non–insulin-dependent diabetes mellitus (NIDDM),** is characterized by the dysregulation of insulin release from the B cells, along with insulin resistance in peripheral tissues such as skeletal muscle, brain, and liver. Type 2 diabetes historically presented in overweight or obese adults, although it is increasingly being diagnosed in children as childhood obesity increases.

Diabetes is characterized by polyuria (passage of large volumes of urine), polydipsia (excessive drinking), weight loss in spite of polyphagia (increased appetite), hyperglycemia, glycosuria, ketosis, acidosis, and coma. Widespread biochemical abnormalities are present, but the fundamental defects to which most of the abnormalities can be traced are (1) reduced entry of glucose into various "peripheral" tissues and (2) increased liberation of glucose into the circulation from the liver. Therefore, there is an extracellular glucose excess and, in many cells, an intracellular glucose deficiency—a situation that has been called "starvation in the midst of plenty." Also, the entry of amino acids into muscle is decreased and lipolysis is increased.

THERAPEUTIC HIGHLIGHTS

In type 1 diabetes, the mainstay of therapy is provision of exogenous insulin, carefully titrated to dietary intake of glucose. In type 2 diabetes, lifestyle changes such as alterations in the diet or increased exercise can often delay symptoms in early disease, but these are difficult to secure. Insulin-sensitizing drugs represent second-line agents (see Chapter 16).

GLUCOSE TOLERANCE

In diabetes, glucose piles up in the bloodstream, especially after meals. If a glucose load is given to a diabetic, the plasma glucose rises higher and returns to the baseline more slowly than it does in normal individuals. The response to a standard oral test dose of glucose, the **oral glucose tolerance test,** is used in the clinical diagnosis of diabetes **(Figure 24–7)**.

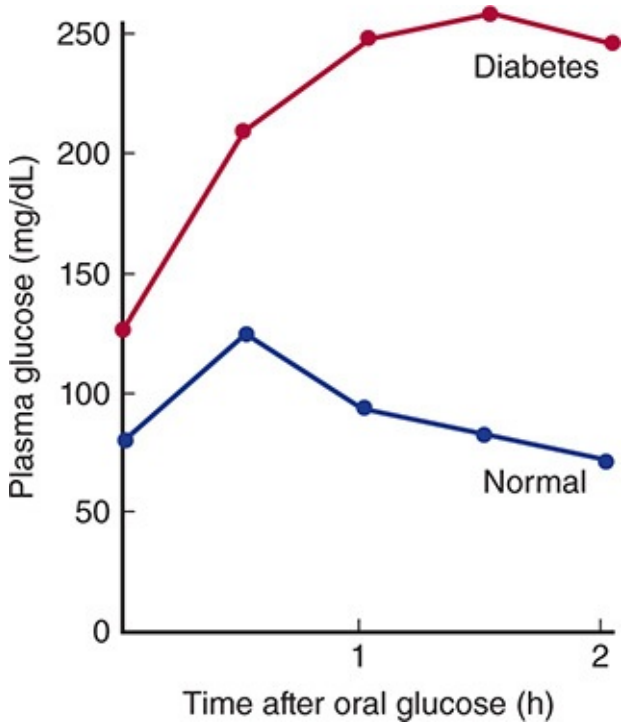


FIGURE 24–7 Oral glucose tolerance test. Adults are given 75 g of glucose in 300 mL of water. In normal individuals, the fasting venous plasma glucose is less than 115 mg/dL, the 2-hour value is less than 140 mg/dL, and no value is greater than 200 mg/dL. Diabetes mellitus is present if the 2-hour value and one other value are greater than 200 mg/dL. Impaired glucose tolerance is diagnosed when the values are above the upper limits of normal but below the values diagnostic of diabetes.

Impaired glucose tolerance in diabetes is due in part to reduced entry of glucose into cells **(decreased peripheral utilization).** In the absence of insulin, the entry of glucose into skeletal, cardiac, and smooth muscle and other tissues is decreased **(Figure 24–8)**. Glucose uptake by the liver is also reduced, but the effect is indirect. Intestinal absorption of glucose is unaffected, as is its reabsorption from the urine by the cells of the proximal tubules of the kidneys. Glucose uptake by most of the brain and the red blood cells is also normal.

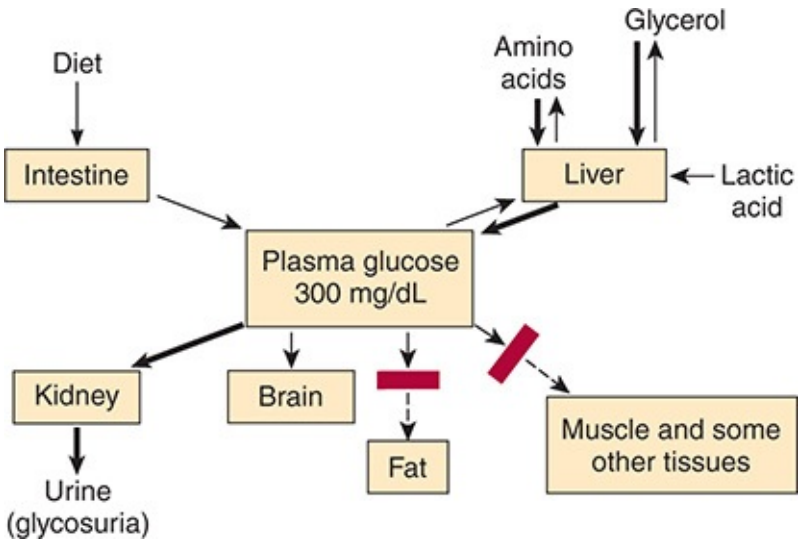


FIGURE 24–8 Disordered plasma glucose homeostasis in insulin deficiency. The heavy arrows indicate reactions that are accentuated. The rectangles across arrows indicate reactions that are blocked.

The second and the major cause of hyperglycemia in diabetes is derangement of the glucostatic function of the liver (see Chapter 28). The liver takes up glucose from the bloodstream and stores it as glycogen, but because the liver contains glucose-6-phosphatase it also discharges glucose into the bloodstream. Insulin facilitates glycogen synthesis and inhibits hepatic glucose output. When the plasma glucose is high, insulin secretion is normally increased and hepatic glucogenesis is decreased. This response does not occur in type 1 diabetes mellitus (as insulin is absent) or in type 2 diabetes mellitus (as tissues are insulin-resistant). Glucagon can contribute to hyperglycemia as it stimulates gluconeogenesis. Glucose output by the liver can be stimulated by catecholamines, cortisol, and growth hormone (ie, during a stress response).

EFFECTS OF HYPERGLYCEMIA

Hyperglycemia by itself can cause symptoms resulting from the hyperosmolality of the blood. In addition, there is glycosuria because the renal capacity for glucose reabsorption is exceeded. Excretion of the osmotically active glucose molecules entails the loss of large amounts of water (osmotic diuresis; see Chapter 38). The resultant dehydration activates the mechanisms regulating water intake, leading to polydipsia. There is an appreciable urinary loss of Na^+ and K^+ as well. For every gram of glucose excreted, 4.1 kcal is lost from the

body. Increasing the oral caloric intake to cover this loss simply raises the plasma glucose further and increases the glycosuria, so mobilization of endogenous protein and fat stores and weight loss are not prevented.

When plasma glucose is episodically elevated over time, small amounts of hemoglobin A are nonenzymatically glycated to form **HbA1c** (see Chapter 31). Careful control of the diabetes with insulin reduces the amount formed and consequently HbA1c concentration is measured clinically as an integrated index of diabetic control for the 4- to 6-week period before the measurement.

The role of chronic hyperglycemia in production of the long-term complications of diabetes is discussed below.

EFFECTS OF INTRACELLULAR GLUCOSE DEFICIENCY

The abundance of glucose outside the cells in diabetes contrasts with the intracellular deficit. Glucose catabolism is normally a major source of energy for cellular processes, and in diabetes energy requirements can be met only by drawing on protein and fat reserves. Mechanisms are activated that greatly increase the catabolism of protein and fat, and one of the consequences of increased fat catabolism is ketosis.

Deficient glucose utilization and deficient hormone sensing (insulin, leptin, CCK) in the cells of the hypothalamus that regulate satiety are the probable causes of hyperphagia in diabetes. The feeding area of the hypothalamus is not inhibited and thus satiety is not sensed so food intake is increased.

Glycogen depletion is a common consequence of intracellular glucose deficit, and the glycogen content of liver and skeletal muscle in diabetic animals is usually reduced.

CHANGES IN PROTEIN METABOLISM

In diabetes, the rate at which amino acids are catabolized to CO_2 and H_2O is increased. In addition, more amino acids are converted to glucose in the liver. The increased gluconeogenesis has many causes. Glucagon stimulates gluconeogenesis, and hyperglucagonemia is generally present in diabetes. Adrenal glucocorticoids also contribute to increased gluconeogenesis when they are elevated in severely ill diabetics. The supply of amino acids is increased for gluconeogenesis because, in the absence of insulin, less protein synthesis occurs

in muscle and hence blood amino acid levels rise. Alanine is particularly easily converted to glucose. In addition, the activity of the enzymes that catalyze the conversion of pyruvate and other two-carbon metabolic fragments to glucose is increased. These include phosphoenolpyruvate carboxykinase, which facilitates the conversion of oxaloacetate to phosphoenolpyruvate (see Chapter 1). They also include fructose 1,6-diphosphatase, which catalyzes the conversion of fructose diphosphate to fructose 6-phosphate, and glucose 6-phosphatase, which controls the entry of glucose into the circulation from the liver. Increased acetyl-CoA increases pyruvate carboxylase activity, and insulin deficiency increases the supply of acetyl-CoA because lipogenesis is decreased. Pyruvate carboxylase catalyzes the conversion of pyruvate to oxaloacetate (see Figure 1–22).

In diabetes, the net effect of accelerated protein conversion to CO_2, H_2O, and glucose, plus diminished protein synthesis, is protein depletion and wasting. Protein depletion from any cause is associated with poor "resistance" to infections.

FAT METABOLISM IN DIABETES

The principal abnormalities of fat metabolism in diabetes are accelerated lipid catabolism, with increased formation of ketone bodies, and decreased synthesis of fatty acids and triglycerides. The manifestations of the disordered lipid metabolism are so prominent that diabetes has been called "more a disease of lipid than of carbohydrate metabolism."

Fifty percent of an ingested glucose load is normally burned to CO_2 and H_2O; 5% is converted to glycogen; and 30–40% is converted to fat in the fat depots. In diabetes, less than 5% of ingested glucose is converted to fat, despite a decrease in the amount burned to CO_2 and H_2O, and no change in the amount converted to glycogen. Therefore, glucose accumulates in the bloodstream and spills over into the urine.

The role of lipoprotein lipase and hormone-sensitive lipase in the regulation of the metabolism of fat depots is discussed in Chapter 1. In diabetes, conversion of glucose to fatty acids in the depots is decreased because of the intracellular glucose deficiency. Insulin inhibits the hormone-sensitive lipase in adipose tissue, and, in the absence of this hormone, the plasma level of **free fatty acids** (NEFA, UFA, FFA) is more than doubled. The increased glucagon also contributes to the mobilization of FFA. Thus, the FFA level parallels the plasma glucose level in diabetes and in some ways is a better indicator of the severity of

the diabetic state. In the liver and other tissues, the fatty acids are catabolized to acetyl-CoA. Some of the acetyl-CoA is burned along with amino acid residues to yield CO_2 and H_2O in the citric acid cycle. However, the supply exceeds the capacity of the tissues to catabolize the acetyl-CoA.

In addition to the previously mentioned increase in gluconeogenesis and marked outpouring of glucose into the circulation, the conversion of acetyl-CoA to malonyl-CoA and thence to fatty acids is markedly impaired. This is due to a deficiency of acetyl-CoA carboxylase, the enzyme that catalyzes the conversion. The excess acetyl-CoA is converted to ketone bodies **(Clinical Box 24–2)**.

In uncontrolled diabetes, the plasma concentration of triglycerides and chylomicrons as well as FFA is increased, and the plasma is often lipemic. The rise in these constituents is mainly due to decreased removal of triglycerides into the fat depots. The decreased activity of lipoprotein lipase contributes to this decreased removal.

CLINICAL BOX 24–2

Ketosis

When excess acetyl-CoA is present in the body, some of it is converted to acetoacetyl-CoA and then, in the liver, to acetoacetate. Acetoacetate and its derivatives, acetone and β-hydroxybutyrate, enter the circulation in large quantities (see Chapter 1).

These circulating ketone bodies are an important source of energy in fasting. Half of the metabolic rate in fasted normal dogs is said to be due to metabolism of ketones. The rate of ketone utilization in diabetics is also appreciable. It has been calculated that the maximal rate at which fat can be catabolized without significant ketosis is 2.5 g/kg body weight/d in diabetic humans. In untreated diabetes, production is much greater than this, and ketone bodies pile up in the bloodstream.

ACIDOSIS

As noted in Chapter 1, acetoacetate and β-hydroxybutyrate are anions of the fairly strong acids acetoacetic acid and β-hydroxybutyric acids. The hydrogen ions from these acids are buffered, but the buffering capacity is soon exceeded if

production is increased. The resulting acidosis stimulates respiration, producing the rapid, deep respiration described by Kussmaul as “air hunger” and named (for him) **Kussmaul breathing.** The urine becomes acidic. However, when the ability of the kidneys to replace the plasma cations accompanying the organic anions with H^+ and NH_4^+ is exceeded, Na^+ and K^+ are lost in the urine. The electrolyte and water losses lead to dehydration, hypovolemia, and hypotension. Finally, the acidosis and dehydration depress consciousness to the point of coma. Diabetic acidosis is a medical emergency. Now that the infections that used to complicate the disease can be controlled with antibiotics, acidosis is the most common cause of early death in persons with clinical diabetes.

In severe acidosis, total body Na^+ is markedly depleted, and when Na^+ loss exceeds water loss, plasma Na^+ may also be low. Total body K^+ is also low, but the plasma K^+ is usually normal, partly because extracellular fluid (ECF) volume is reduced and partly because K^+ moves from cells to ECF when the ECF H^+ concentration is high. Another factor tending to maintain the plasma K^+ is the lack of insulin-induced entry of K^+ into cells.

COMA

Coma in diabetes can be due to acidosis and dehydration. However, the plasma glucose can be elevated to such a degree that independent of plasma pH, the hyperosmolarity of the plasma causes unconsciousness **(hyperosmolar coma).** Accumulation of lactate in the blood **(lactic acidosis)** may also complicate diabetic ketoacidosis if the tissues become hypoxic, and lactic acidosis may itself cause coma. Brain edema occurs in about 1% of children with ketoacidosis, and it can cause coma. Its cause is unsettled, but it is a serious complication, with a mortality rate of about 25%.

CHOLESTEROL METABOLISM

In diabetes, the plasma cholesterol level is usually elevated and this plays a role in the accelerated development of the atherosclerotic vascular disease that is a major long-term complication of diabetes in humans. The rise in plasma cholesterol level is due to an increase in the plasma concentration of very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) (see Chapter 1). These in turn may be due to increased hepatic production of VLDL or decreased removal of VLDL and LDL from the circulation.

SUMMARY

Because of the complexities of the metabolic abnormalities in diabetes, a summary is in order. One of the key features of insulin deficiency **(Figure 24–9)** is decreased entry of glucose into many tissues (decreased peripheral utilization). Also, the net release of glucose from the liver is increased (increased production), due in part to glucagon excess. The resultant hyperglycemia leads to glycosuria and a dehydrating osmotic diuresis. Dehydration leads to polydipsia. In the face of intracellular glucose deficiency, appetite is stimulated, glucose is formed from protein (gluconeogenesis), and energy supplies are maintained by metabolism of proteins and fats. Weight loss, debilitating protein deficiency, and inanition are the result.

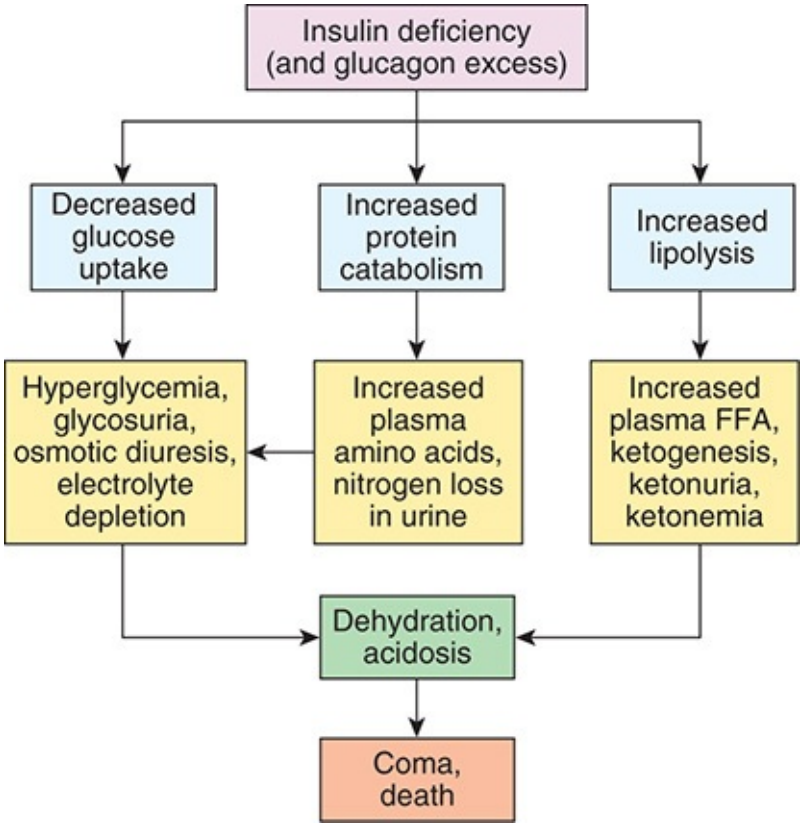


FIGURE 24–9 Effects of insulin deficiency. FFA, free fatty acids. (Used with permission of RJ Havel.)

Fat catabolism is increased and the system is flooded with triglycerides and FFA. Fat synthesis is inhibited and the overloaded catabolic pathways cannot handle the excess acetyl-CoA that is formed. In the liver, the acetyl-CoA is

converted to ketone bodies. Two of these are organic acids, and metabolic acidosis develops as ketones accumulate. Na^+ and K^+ depletion is added to the acidosis because these plasma cations are excreted with the organic anions not covered by the H^+ and NH_4^+ secreted by the kidneys. Finally, the acidotic, hypovolemic, hypotensive, depleted animal or patient becomes comatose because of the toxic effects of acidosis, dehydration, and hyperosmolarity on the nervous system and dies if treatment is not instituted.

All of these abnormalities are corrected by administration of insulin. Although emergency treatment of acidosis also includes administration of alkali to combat the acidosis as well as parenteral water, Na^+, and K^+ to replenish body stores, only insulin repairs the fundamental defects in a way that permits a return to normal.

INSULIN EXCESS

SYMPTOMS

All the known consequences of insulin excess are manifestations, directly or indirectly, of the effects of hypoglycemia on the nervous system. Except in individuals who have been fasting for some time, glucose is the only fuel used in appreciable quantities by the brain. The carbohydrate reserves in neural tissue are very limited and normal function depends on a continuous glucose supply. As the plasma glucose level falls, the first symptoms are palpitations, sweating, and nervousness due to autonomic discharge. These appear at plasma glucose values slightly lower than the value at which autonomic activation first begins, because the threshold for symptoms is slightly above the threshold for initial activation. At lower plasma glucose levels, so-called **neuroglycopenic symptoms** begin to appear. These include hunger as well as confusion and the other cognitive abnormalities. At even lower plasma glucose levels, lethargy, coma, convulsions, and eventually death occur. Obviously, the onset of hypoglycemic symptoms calls for prompt treatment with glucose or glucose-containing drinks such as orange juice. Although a dramatic disappearance of symptoms is the usual response, abnormalities ranging from intellectual dulling to coma may persist if the hypoglycemia was severe or prolonged.

COMPENSATORY MECHANISMS

One important compensation for hypoglycemia is cessation of the secretion of endogenous insulin. Inhibition of insulin secretion is complete at a plasma glucose level of about 80 mg/dL **(Figure 24–10)**. In addition, hypoglycemia triggers increased secretion of at least four counterregulatory hormones: glucagon, epinephrine, growth hormone, and cortisol. The epinephrine response is reduced during sleep. Glucagon and epinephrine increase the hepatic output of glucose by increasing glycogenolysis. Growth hormone decreases the utilization of glucose in various peripheral tissues, and cortisol has a similar action. The keys to counterregulation appear to be epinephrine and glucagon: if the plasma concentration of either increases, the decline in the plasma glucose level is reversed; but if both fail to increase, there is little if any compensatory rise in the plasma glucose level. The actions of the other hormones are supplementary.

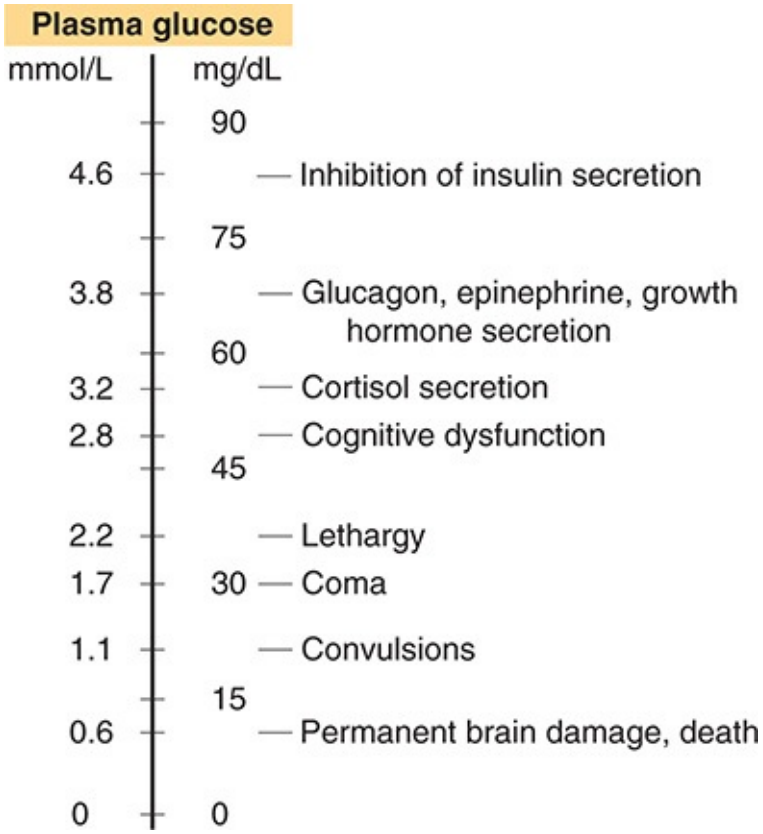


FIGURE 24–10 Plasma glucose levels at which various effects of hypoglycemia appear.

Note that the autonomic discharge and release of counterregulatory hormones normally occur at a higher plasma glucose level than the cognitive deficits and other more serious CNS changes (Figure 24–10). For diabetics treated with

insulin, the symptoms caused by the autonomic discharge serve as a warning to seek glucose replacement. However, particularly in long-term diabetics who have been tightly regulated, the autonomic symptoms may not occur, and the resulting **hypoglycemia unawareness** can be a clinical problem of some magnitude.

REGULATION OF INSULIN SECRETION

The normal concentration of insulin measured by radioimmunoassay in the peripheral venous plasma of fasting normal humans is 0–70 μU/mL (0–502 pmol/L). The amount of insulin secreted in the basal state is about 1 unit/h, with a 5-fold to 10-fold increase following ingestion of food. Therefore, the average amount secreted per day in a normal human is about 40 units (287 nmol).

Factors that stimulate and inhibit insulin secretion are summarized in **Table 24–4**.

TABLE 24–4 Factors affecting insulin secretion.

Stimulators	Inhibitors
Glucose	Somatostatin
Mannose	2-Deoxyglucose
Amino acids (leucine, arginine, others)	Mannoheptulose
Intestinal hormones (GIP, GLP-1 [7–36], gastrin, secretin, CCK; others?)	α-Adrenergic stimulators (norepinephrine, epinephrine)
β-Keto acids	β-Adrenergic blockers (propranolol)
Acetylcholine	
Glucagon	Galanin
Cyclic AMP and various cAMP-generating substances	Diazoxide Thiazide diuretics
β-Adrenergic stimulators	K^+ depletion
Theophylline	Phenytoin
Sulfonylureas	Alloxan Microtubule inhibitors Insulin

EFFECTS OF THE PLASMA GLUCOSE LEVEL

It has been known for many years that glucose acts directly on pancreatic B cells to increase insulin secretion. The response to glucose is biphasic; there is a rapid but short-lived increase in secretion followed by a more slowly developing prolonged increase.

Glucose enters the B cells via GLUT-2 transporters and is phosphorylated by glucokinase then metabolized to pyruvate in the cytoplasm **(Figure 24–11)**. The pyruvate enters the mitochondria and is metabolized to CO_2 and H_2O via the citric acid cycle with the formation of ATP by oxidative phosphorylation. The ATP enters the cytoplasm, where it inhibits ATP-sensitive K^+ channels, reducing K^+ efflux. This depolarizes the B cell, and Ca^{2+} enters the cell via voltage-gated

Ca^{2+} channels. The Ca^{2+} influx causes exocytosis of a readily releasable pool of insulin-containing secretory granules, producing the initial spike of insulin secretion.

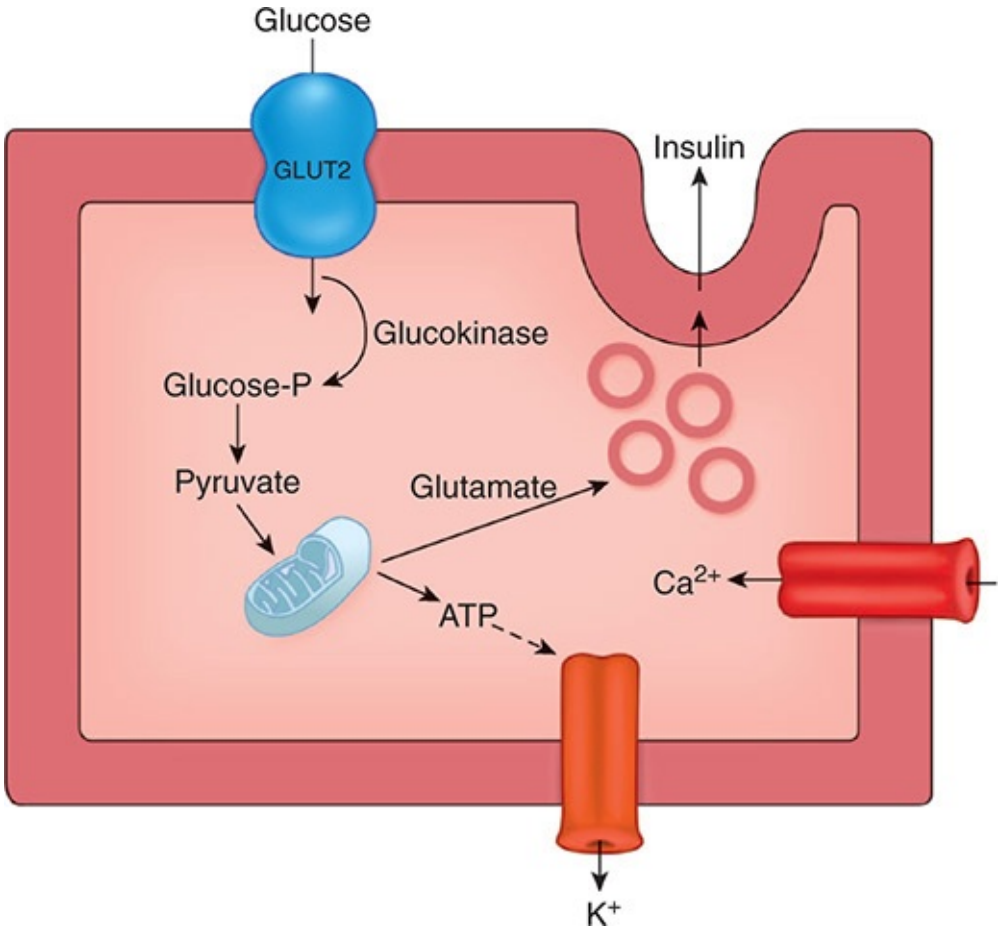


FIGURE 24–11 Insulin secretion. Glucose enters B cells by GLUT-2 transporters. It is phosphorylated and metabolized to pyruvate (Pyr) in the cytoplasm. The Pyr enters the mitochondria and is metabolized via the citric acid cycle. The ATP formed by oxidative phosphorylation inhibits ATP-sensitive K^+ channels, reducing K^+ efflux. This depolarizes the B cell, and Ca^{2+} influx is increased. The Ca^{2+} stimulates release of insulin by exocytosis. Glutamate (Glu) is also formed, and this primes secretory granules, preparing them for exocytosis.

Metabolism of pyruvate via the citric acid cycle also causes an increase in intracellular glutamate. The glutamate appears to act on a second pool of secretory granules, committing them to the releasable form. The action of glutamate may be to decrease the pH in the secretory granules, a necessary step

in their maturation. The release of these granules then produces the prolonged second phase of the insulin response to glucose. Thus, glutamate appears to act as an intracellular second messenger that primes secretory granules for secretion.

The feedback control of plasma glucose on insulin secretion normally operates with great precision so that plasma glucose and insulin levels parallel each other with remarkable consistency.

PROTEIN & FAT DERIVATIVES

Insulin stimulates the incorporation of amino acids into proteins and combats the fat catabolism that produces the β-keto acids. Therefore, it is not surprising that arginine, leucine, and certain other amino acids stimulate insulin secretion, as do β-keto acids such as acetoacetate. Like glucose, these compounds generate ATP when metabolized, and this closes ATP-sensitive K^+ channels in the B cells. In addition, L-arginine is the precursor of NO, and NO stimulates insulin secretion.

ORAL HYPOGLYCEMIC AGENTS

Tolbutamide and other sulfonylurea derivatives such as acetohexamide, tolazamide, glipizide, and glyburide are orally active hypoglycemic agents that lower blood glucose by increasing the secretion of insulin. They only work in patients with some remaining B cells and are ineffective after pancreatectomy or in type 1 diabetes. They bind to the ATP-inhibited K^+ channels in the B cell membranes and inhibit channel activity, depolarizing the B cell membrane and increasing Ca^{2+} influx and hence insulin release, independent of increases in plasma glucose.

Persistent hyperinsulinemic hypoglycemia of infancy is a condition in which plasma insulin is elevated despite the hypoglycemia. The condition is caused by mutations in the genes for various enzymes in B cells that decrease K^+ efflux via the ATP-sensitive K^+ channels. Treatment consists of administration of diazoxide, a drug that increases the activity of the K^+ channels or, in more severe cases, subtotal pancreatectomy.

The biguanide metformin is an oral hypoglycemic agent that acts in the absence of insulin. Metformin acts primarily by reducing gluconeogenesis and therefore decreasing hepatic glucose output. It is sometimes combined with a sulfonylurea in the treatment of type 2 diabetes. Metformin can cause lactic acidosis, but the incidence is usually low.

Troglitazone (Rezulin) and related **thiazolidinediones** are also used in the treatment of diabetes because they increase insulin-mediated peripheral glucose disposal, thus reducing insulin resistance. They bind to and activate peroxisome proliferator-activated receptor γ (PPARγ) in the nucleus of cells. Activation of this receptor, which is a member of the superfamily of hormone-sensitive nuclear transcription factors, has a unique ability to normalize a variety of metabolic functions.

CYCLIC AMP & INSULIN SECRETION

Stimuli that increase cAMP levels in B cells increase insulin secretion, including β-adrenergic agonists, glucagon, and phosphodiesterase inhibitors such as theophylline.

Catecholamines have a dual effect on insulin secretion; they inhibit insulin secretion via α_2-adrenergic receptors and stimulate insulin secretion via β-adrenergic receptors. The net effect of epinephrine and norepinephrine is usually inhibition. However, if catecholamines are infused after administration of α-adrenergic blocking drugs, the inhibition is converted to stimulation.

EFFECT OF AUTONOMIC NERVES

Branches of the right vagus nerve innervate the pancreatic islets, and stimulation of this parasympathetic pathway causes increased insulin secretion via M_3 receptors (see Table 7–2). Atropine blocks the response and acetylcholine stimulates insulin secretion. The effect of acetylcholine, like that of glucose, is due to increased cytoplasmic Ca^{2+}, but acetylcholine activates phospholipase C, with the released IP_3 releasing the Ca^{2+} from the endoplasmic reticulum.

Stimulation of the sympathetic nerves to the pancreas inhibits insulin secretion. The inhibition is produced by released norepinephrine acting on α_2-adrenergic receptors. However, if α-adrenergic receptors are blocked, stimulation of the sympathetic nerves causes increased insulin secretion mediated by β_2-adrenergic receptors. The polypeptide galanin is found in some of the autonomic nerves innervating the islets, and galanin inhibits insulin secretion by activating the K^+ channels that are inhibited by ATP. Thus, although the denervated pancreas responds to glucose, the autonomic innervation of the pancreas is involved in the overall regulation of insulin secretion **(Clinical Box 24–3)**.

INTESTINAL HORMONES

Orally administered glucose exerts a greater insulin-stimulating effect than intravenously administered glucose, and orally administered amino acids also produce a greater insulin response than intravenous amino acids. These observations led to exploration of the possibility that a substance secreted by the gastrointestinal mucosa stimulated insulin secretion. Glucagon, glucagon derivatives, secretin, cholecystokinin (CCK), gastrin, and gastric inhibitory peptide (GIP) all have such an action (see Chapter 25), and CCK potentiates the insulin-stimulating effects of amino acids. However, GIP is the only one of these peptides that produces stimulation when administered in doses that reflect blood GIP levels produced by an oral glucose load.

CLINICAL BOX 24-3

Effects of K^+ Depletion

K^+ depletion decreases insulin secretion, and K^+-depleted patients, for example, patients with primary hyperaldoster-onism (see Chapter 20), develop diabetic glucose tolerance curves. These curves are restored to normal by K^+ repletion.

THERAPEUTIC HIGHLIGHTS

The thiazide diuretics, which cause loss of K^+ as well as Na^+ in the urine (see Chapter 37), decrease glucose tolerance and make diabetes worse. They apparently exert this effect primarily because of their K^+-depleting effects, although some of them also cause pancreatic islet cell damage. Potassium-sparing diuretics, such as amiloride, should be substituted in the diabetic patient who needs such treatment.

Recently, attention has focused on glucagon-like polypeptide 1 (7–36) (GLP-1 [7–36]) as an additional gut factor that stimulates insulin secretion. This polypeptide is a product of preproglucagon. B cells have GLP-1 (7–36) receptors as well as GIP receptors, and GLP-1 (7–36) is a more potent insulinotropic hormone than GIP. GIP and GLP-1 (7–36) both appear to act by increasing Ca^{2+} influx through voltage-gated Ca^{2+} channels.

The possible roles of pancreatic somatostatin and glucagon in the regulation of insulin secretion are discussed below.

LONG-TERM CHANGES IN B CELL RESPONSES

The magnitude of the insulin response to a given stimulus is determined in part by the secretory history of the B cells. Individuals fed a high-carbohydrate diet for several weeks not only have higher fasting plasma insulin levels but also show a greater secretory response to a glucose load than individuals fed an isocaloric low-carbohydrate diet.

Although B cells respond to stimulation with hypertrophy like other endocrine cells, they become exhausted and stop secreting **(B cell exhaustion)** when the stimulation is marked or prolonged. The pancreatic reserve is large and it is difficult to produce B cell exhaustion in normal animals, but if the pancreatic reserve is reduced by partial pancreatectomy, exhaustion of the remaining B cells can be initiated by any procedure that chronically raises the plasma glucose level. For example, diabetes can be produced in animals with limited pancreatic reserves by anterior pituitary extracts, growth hormone, thyroid hormones, or the prolonged continuous infusion of glucose alone. The diabetes precipitated by hormones in animals is at first reversible, but with prolonged treatment it becomes permanent. The transient diabetes is usually named for the agent producing it, for example, “hypophysial diabetes” or “thyroid diabetes.” Permanent diabetes persisting after treatment has been discontinued is indicated by the prefix meta-, for example, **“metahypophysial diabetes”** or **“metathyroid diabetes.”** When insulin is administered along with the diabetogenic hormones, the B cells are protected, probably because the plasma glucose is lowered, and diabetes does not develop.

It is interesting in this regard that genetic factors may be involved in the control of B cell reserve. In mice in which the gene for IRS-1 has been knocked out (see above), a robust compensatory B cell response occurs. However, in IRS-2 knockouts, the compensation is reduced and a more severe diabetic phenotype is produced.

GLUCAGON

CHEMISTRY

Human glucagon, a linear polypeptide with a molecular weight of 3485, is produced by the A cells of the pancreatic islets and the upper gastrointestinal tract. It contains 29 amino acid residues. All mammalian glucagons appear to have the same structure. Human preproglucagon **(Figure 24–12)** is a 179-amino-acid protein that is found in pancreatic A cells, in L cells in the lower gastrointestinal tract, and in the brain. It is the product of a single mRNA, but it is processed differently in different tissues. In A cells, it is processed primarily to glucagon and **major proglucagon fragment (MPGF).** In L cells, it is processed primarily to **glicentin,** a polypeptide that consists of glucagon extended by additional amino acid residues at either end, plus **glucagon-like polypeptides 1 and 2 (GLP-1** and **GLP-2).** Some **oxyntomodulin** is also formed, and in both A and L cells, residual **glicentin-related polypeptide (GRPP)** is left. Glicentin has some glucagon activity. GLP-1 and GLP-2 have no definite biologic activity by themselves. However, GLP-1 is processed further by removal of its amino-terminal amino acid residues and the product, **GLP-1 (7–36),** is a potent stimulator of insulin secretion that also increases glucose utilization (see above). GLP-1 and GLP-2 are also produced in the brain. The function of GLP-1 in this location is uncertain, but GLP-2 appears to be the mediator in a pathway from the nucleus tractus solitarius (NTS) to the dorsomedial nuclei of the hypothalamus, and injection of GLP-2 lowers food intake. Oxyntomodulin inhibits gastric acid secretion, though its physiologic role is unsettled, and GRPP does not have any established physiologic effects.

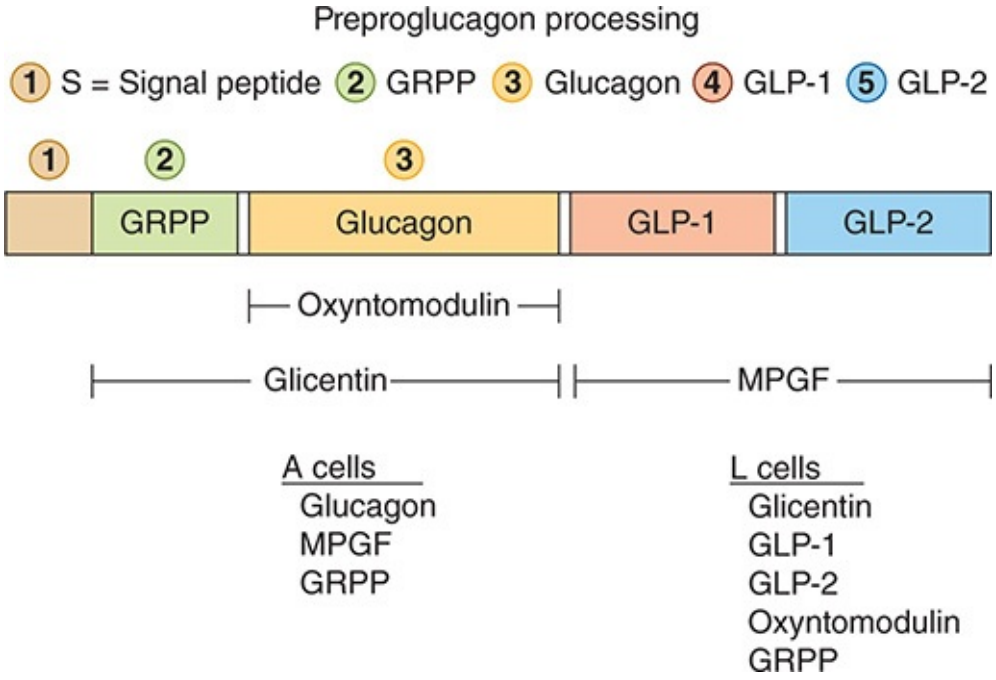


FIGURE 24–12 Posttranslational processing of preproglucagon in A and L cells. S, signal peptide; GRPP, glicentin-related polypeptide; GLP, glucagon-like polypeptide; Oxy, oxyntomodulin; MPGF, major proglucagon fragment. (Modified with permission from Drucker DJ: Glucagon and glucagon-like peptides. Pancreas 1990; July; 5(4):484–488.)

ACTION

Glucagon is glycogenolytic, gluconeogenic, lipolytic, and ketogenic. It acts on G-protein-coupled receptors with a molecular weight of about 190,000. In the liver, it acts via G_s to activate adenylyl cyclase and increase intracellular cAMP. This leads via protein kinase A to activation of phosphorylase and therefore to increased breakdown of glycogen and an increase in plasma glucose. However, glucagon acts on different glucagon receptors located on the same hepatic cells to activate phospholipase C, and the resulting increase in cytoplasmic Ca^{2+} also stimulates glycogenolysis. Protein kinase A also decreases the metabolism of glucose-6-phosphate **(Figure 24–13)** by inhibiting the conversion of phosphoenolpyruvate to pyruvate. It also decreases the concentration of fructose 2,6-diphosphate and this in turn inhibits the conversion of fructose 6-phosphate to fructose 1,6-diphosphate. The resultant buildup of glucose-6-phosphate leads to increased glucose synthesis and release.

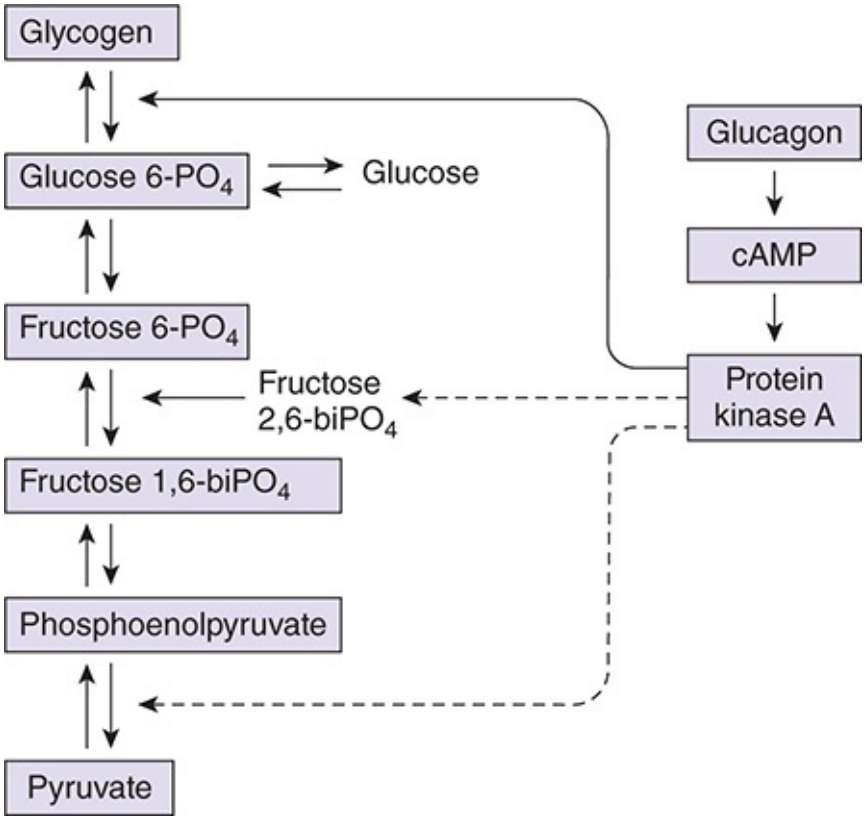


FIGURE 24–13 Mechanisms by which glucagon increases glucose output from the liver. Solid arrows indicate facilitation; dashed arrows indicate inhibition.

Glucagon does not cause glycogenolysis in muscle. It increases gluconeogenesis from available amino acids in the liver and elevates the metabolic rate. It increases ketone body formation by decreasing malonyl-CoA levels in the liver. Its lipolytic activity, which leads in turn to increased ketogenesis, is discussed in Chapter 1. The calorigenic action of glucagon is not due to the hyperglycemia per se but probably to the increased hepatic deamination of amino acids.

Large doses of exogenous glucagon exert a positive inotropic effect on the heart (see Chapter 30) without producing increased myocardial excitability, presumably because they increase myocardial cAMP. Use of this hormone in the treatment of heart disease has been advocated, but there is no evidence for a physiologic role of glucagon in the regulation of cardiac function. Glucagon also stimulates the secretion of growth hormone, insulin, and pancreatic somatostatin.

METABOLISM

Glucagon has a half-life in the circulation of 5–10 min. It is degraded by many tissues but particularly by the liver. Because glucagon is secreted into the portal vein and reaches the liver before it reaches the peripheral circulation, peripheral blood levels are relatively low. The rise in peripheral blood glucagon levels produced by excitatory stimuli is exaggerated in patients with cirrhosis, presumably because of decreased hepatic degradation of the hormone.

REGULATION OF SECRETION

The principal factors known to affect glucagon secretion are summarized in **Table 24–5**. Secretion is increased by hypoglycemia and decreased by a rise in plasma glucose. Pancreatic B cells contain GABA, and evidence suggests that coincident with the increased insulin secretion produced by hyperglycemia, GABA is released and acts on the A cells to inhibit glucagon secretion by activating $GABA_A$ receptors. The $GABA_A$ receptors are Cl^- channels, and the resulting Cl^- influx hyperpolarizes the A cells.

TABLE 24–5 Factors affecting glucagon secretion.

Stimulators	Inhibitors
Amino acids (particularly the glucogenic amino acids: alanine, serine, glycine, cysteine, and threonine)	Glucose
CCK, gastrin	Somatostatin
Cortisol	Secretin
Exercise	FFA
Infections	Ketones
Other stresses	Insulin
β-Adrenergic stimulators	Phenytoin
Theophylline	α-Adrenergic stimulators
Acetylcholine	GABA

Secretion is also increased by stimulation of the sympathetic nerves to the

pancreas, and this sympathetic effect is mediated via β-adrenergic receptors and cAMP. It appears that the A cells are like the B cells in that stimulation of β-adrenergic receptors increases secretion and stimulation of α-adrenergic receptors inhibits secretion. However, the pancreatic response to sympathetic stimulation in the absence of blocking drugs is increased secretion of glucagon, so the effect of β-receptors predominates in the glucagon-secreting cells. The stimulatory effects of various stresses and possibly of exercise and infection are mediated at least in part via the sympathetic nervous system. Vagal stimulation also increases glucagon secretion.

A protein meal and infusion of various amino acids increase glucagon secretion. It seems appropriate that the glucogenic amino acids are particularly potent in this regard, since these are the amino acids that are converted to glucose in the liver under the influence of glucagon. The increase in glucagon secretion following a protein meal is also valuable, since the amino acids stimulate insulin secretion and the secreted glucagon prevents the development of hypoglycemia while the insulin promotes storage of the absorbed carbohydrates and lipids. Glucagon secretion increases during starvation. It reaches a peak on the third day of a fast, at the time of maximal gluconeogenesis. Thereafter, the plasma glucagon level declines as fatty acids and ketones become the major sources of energy.

During exercise, there is an increase in glucose utilization that is balanced by an increase in glucose production caused by an increase in circulating glucagon levels.

The glucagon response to oral administration of amino acids is greater than the response to intravenous infusion of amino acids, suggesting that a glucagon-stimulating factor is secreted from the gastrointestinal mucosa. CCK and gastrin increase glucagon secretion, whereas secretin inhibits it. Because CCK and gastrin secretion are both increased by a protein meal, either hormone could be the gastrointestinal mediator of the glucagon response. The inhibition produced by somatostatin is discussed below.

Glucagon secretion is also inhibited by FFA and ketones. However, this inhibition can be overridden, since plasma glucagon levels are high in diabetic ketoacidosis.

INSULIN–GLUCAGON MOLAR RATIOS

As noted previously, insulin is glycogenic, antigluconeogenetic, antilipolytic, and antiketotic in its actions. It thus favors storage of absorbed nutrients and is a

"hormone of energy storage." Glucagon, on the other hand, is glycogenolytic, gluconeogenetic, lipolytic, and ketogenic. It mobilizes energy stores and is a "hormone of energy release." Because of their opposite effects, the blood levels of both hormones must be considered in any given situation. It is convenient to think in terms of the molar ratios of these hormones.

The insulin–glucagon molar ratios fluctuate markedly because the secretion of glucagon and insulin are both modified by the conditions that preceded the application of any given stimulus **(Table 24–6)**. Thus, for example, the insulin–glucagon molar ratio on a balanced diet is approximately 2.3. An infusion of arginine increases the secretion of both hormones and raises the ratio to 3.0. After 3 days of starvation, the ratio falls to 0.4, and an infusion of arginine in this state lowers the ratio to 0.3. Conversely, the ratio is 25 in individuals receiving a constant infusion of glucose and rises to 170 on ingestion of a protein meal during the infusion (Table 24–6). The rise occurs because insulin secretion rises sharply, while the usual glucagon response to a protein meal is abolished. Thus, when energy is needed during starvation, the insulin–glucagon molar ratio is low, favoring glycogen breakdown and gluconeogenesis; conversely, when the need for energy mobilization is low, the ratio is high, favoring the deposition of glycogen, protein, and fat **(Clinical Box 24–4)**.

TABLE 24–6 Insulin–glucagon molar ratios (I/G) in blood in various conditions.

Condition	Hepatic Glucose Storage (S) or Production (P)[a]	I/G
Glucose availability		
Large carbohydrate meal	4+ (S)	70
Intravenous glucose	2+ (S)	25
Small meal	1+ (S)	7
Glucose need		
Overnight fast	1+ (P)	2.3
Low-carbohydrate diet	2+ (P)	1.8
Starvation	4+ (P)	0.4

[a]1+ to 4+ indicate relative magnitude.

Courtesy of RH Unger.

OTHER ISLET CELL HORMONES

In addition to insulin and glucagon, the pancreatic islets secrete somatostatin and pancreatic polypeptide into the bloodstream. In addition, somatostatin may be involved in regulatory processes within the islets that adjust the pattern of hormones secreted in response to various stimuli.

SOMATOSTATIN

Somatostatin and its receptors are discussed in Chapter 7. Somatostatin 14 (SS 14) and its amino terminal-extended form somatostatin 28 (SS 28) are found in the D cells of pancreatic islets. Both forms inhibit the secretion of insulin, glucagon, and pancreatic polypeptide and act locally within the pancreatic islets in a paracrine fashion. SS 28 is more active than SS 14 in inhibiting insulin secretion, and it apparently acts via the SSTR5 receptor (see Chapter 7). Patients with somatostatin-secreting pancreatic tumors **(somatostatinomas)** develop hyperglycemia and other manifestations of diabetes that disappear when the tumor is removed. Dyspepsia also develops as a result of slow gastric emptying and decreased gastric acid secretion, and gallstones, which are precipitated by decreased gallbladder contraction due to inhibition of CCK secretion. The secretion of pancreatic somatostatin is increased by several of the same stimuli that increase insulin secretion, that is, glucose and amino acids, particularly arginine and leucine. It is also increased by CCK. Somatostatin is released from the pancreas and the gastrointestinal tract into the peripheral blood.

CLINICAL BOX 24–4

Macrosomia & GLUT-1 Deficiency

Infants born to diabetic mothers often have high birth weights and large organs (**macrosomia**). This condition is caused by excess circulating insulin in the fetus, which in turn is caused in part by stimulation of the fetal pancreas by high blood glucose and amino acids from the diabetic mother.

Free insulin in maternal blood is destroyed by proteases in the placenta, but antibody-bound insulin is protected, so it reaches the fetus.

Infants with **GLUT-1 deficiency** have defective transport of glucose across the blood-brain barrier. They have low cerebrospinal fluid glucose in the presence of normal plasma glucose, seizures, and developmental delay.

PANCREATIC POLYPEPTIDE

Human pancreatic polypeptide is a linear polypeptide that contains 36 amino acid residues and is produced by F cells in the islets. It is closely related to two other 36-amino acid polypeptides, **polypeptide YY,** a gastrointestinal peptide (see Chapter 25), and **neuropeptide Y,** which is found in the brain and the autonomic nervous system (see Chapter 7). All end in tyrosine and are amidated at their carboxyl terminal. At least in part, pancreatic polypeptide secretion is under cholinergic control; plasma levels fall after administration of atropine. Its secretion is increased by a meal containing protein and by fasting, exercise, and acute hypoglycemia. Secretion is decreased by somatostatin and intravenous glucose. Infusions of leucine, arginine, and alanine do not affect it, so the stimulatory effect of a protein meal may be mediated indirectly. Pancreatic polypeptide slows the absorption of food in humans, and it may smooth out the peaks and valleys of absorption. However, its exact physiologic function is still uncertain.

ORGANIZATION OF THE PANCREATIC ISLETS

The presence in the pancreatic islets of hormones that affect the secretion of other islet hormones suggests that the islets function as secretory units in the regulation of nutrient homeostasis. Somatostatin inhibits the secretion of insulin, glucagon, and pancreatic polypeptide **(Figure 24–14)**; insulin inhibits the secretion of glucagon; and glucagon stimulates the secretion of insulin and somatostatin. As noted above, A and D cells and pancreatic polypeptide-secreting cells are generally located around the periphery of the islets, with the B cells in the center. There are clearly two types of islets, glucagon-rich islets and pancreatic polypeptide-rich islets, but the functional significance of this separation is not known. The islet cell hormones released into the ECF probably diffuse to other islet cells and influence their function (paracrine communication; see Chapter 25). It has been demonstrated that gap junctions are present between A, B, and D cells and that these permit the passage of ions and other small molecules from one cell to another, which could coordinate their secretory functions.

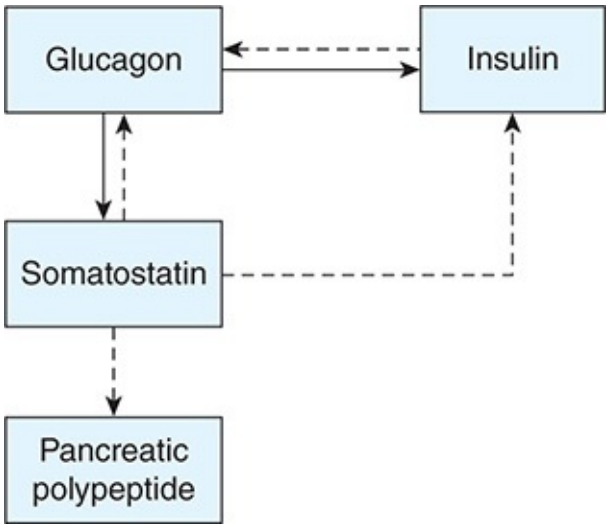


FIGURE 24–14 Effects of islet cell hormones on the secretion of other islet cell hormones. Solid arrows indicate stimulation; dashed arrows indicate inhibition.

EFFECTS OF OTHER HORMONES & EXERCISE ON CARBOHYDRATE METABOLISM

Exercise has direct effects on carbohydrate metabolism. Many hormones in addition to insulin, IGF-I, IGF-II, glucagon, and somatostatin also have important roles in the regulation of carbohydrate metabolism. They include epinephrine, thyroid hormones, glucocorticoids, and growth hormone. The other functions of these hormones are considered elsewhere, but it seems wise to summarize their effects on carbohydrate metabolism in the context of the present chapter.

EXERCISE

The entry of glucose into skeletal muscle is increased during exercise in the absence of insulin by causing an insulin- independent increase in the number of GLUT-4 transporters in muscle cell membranes (see above). This increase in glucose entry persists for several hours after exercise, and regular exercise training can also produce prolonged increases in insulin sensitivity. Exercise can precipitate hypoglycemia in diabetics not only because of the increase in muscle uptake of glucose but also because absorption of injected insulin is more rapid during exercise. Patients with diabetes should take in extra calories or reduce

their insulin dosage when they exercise.

CATECHOLAMINES

The activation of phosphorylase in liver by catecholamines is discussed in Chapter 1. Activation occurs via β-adrenergic receptors, which increase intracellular cAMP, and α-adrenergic receptors, which increase intracellular Ca^{2+}. Hepatic glucose output is increased, producing hyperglycemia. In muscle, the phosphorylase is also activated via cAMP and presumably via Ca^{2+}, but the glucose-6-phosphate formed can be catabolized only to pyruvate because of the absence of glucose-6- phosphatase. For reasons that are not entirely clear, large amounts of pyruvate are converted to lactate, which diffuses from the muscle into the circulation **(Figure 24–15)**. The lactate is oxidized in the liver to pyruvate and converted to glycogen. Therefore, the response to an injection of epinephrine is an initial glycogenolysis followed by a rise in hepatic glycogen content. Lactate oxidation may be responsible for the calorigenic effect of epinephrine (see Chapter 19). Epinephrine and norepinephrine also liberate FFA into the circulation, and epinephrine decreases peripheral utilization of glucose.

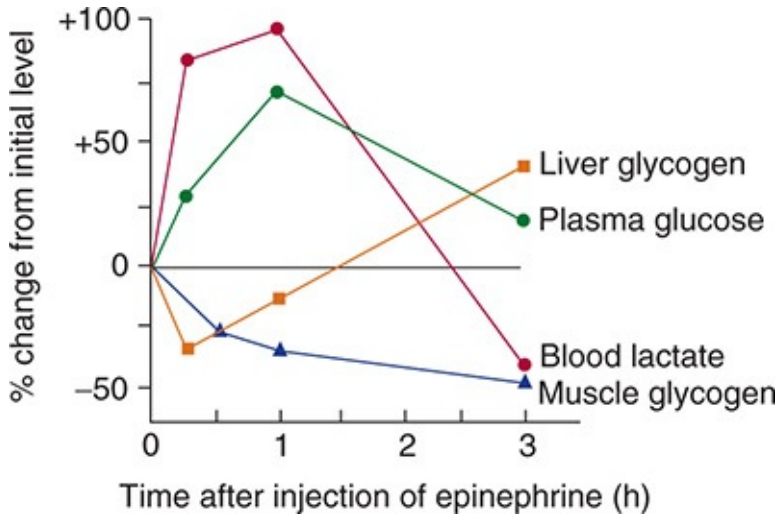


FIGURE 24–15 Effect of epinephrine on tissue glycogen, plasma glucose, and blood lactate levels in fed rats. (Reproduced with permission from Ruch TC, Patton HD [editors]: *Physiology and Biophysics*, 20th ed, St. Louis, MO: Saunders; 1973.)

THYROID HORMONES

Thyroid hormones make experimental diabetes worse; thyrotoxicosis aggravates clinical diabetes; and metathyroid diabetes can be produced in animals with decreased pancreatic reserve. The principal diabetogenic effect of thyroid hormones is to increase absorption of glucose from the intestine, but the hormones also cause (probably by potentiating the effects of catecholamines) some degree of hepatic glycogen depletion. Glycogen-depleted liver cells are easily damaged. When the liver is damaged, the glucose tolerance curve is diabetic because the liver takes up less of the absorbed glucose. Thyroid hormones may also accelerate the degradation of insulin. All these actions have a hyperglycemic effect and, if the pancreatic reserve is low, may lead to B cell exhaustion.

ADRENAL GLUCOCORTICOIDS

Glucocorticoids from the adrenal cortex (see Chapter 19) elevate blood glucose and produce a diabetic type of glucose tolerance curve. In humans, this effect may occur only in individuals with a genetic predisposition to diabetes. Glucose tolerance is reduced in 80% of patients with Cushing syndrome (see Chapter 19), and 20% of these patients have frank diabetes. The glucocorticoids are necessary for glucagon to exert its gluconeogenic action during fasting. They are gluconeogenic themselves, but their role is mainly permissive. In adrenal insufficiency, the blood glucose is normal as long as food intake is maintained, but fasting precipitates hypoglycemia and collapse. The plasma-glucose-lowering effect of insulin is greatly enhanced in patients with adrenal insufficiency. In animals with experimental diabetes, adrenalectomy markedly ameliorates the diabetes. The major diabetogenic effects are an increase in protein catabolism with increased gluconeogenesis in the liver; increased hepatic glycogenesis and ketogenesis; and a decrease in peripheral glucose utilization relative to the blood insulin level that may be due to inhibition of glucose phosphorylation.

GROWTH HORMONE

Human growth hormone makes clinical diabetes worse, and 25% of patients with growth hormone–secreting tumors of the anterior pituitary have diabetes. Hypophysectomy ameliorates diabetes and decreases insulin resistance even more than adrenalectomy, whereas growth hormone treatment increases insulin resistance.

The effects of growth hormone are partly direct and partly mediated via IGF-I (see Chapter 18). Growth hormone mobilizes FFA from adipose tissue, thus favoring ketogenesis. It decreases glucose uptake into some tissues ("anti-insulin action"), increases hepatic glucose output, and may decrease tissue binding of insulin. Indeed, it has been suggested that the ketosis and decreased glucose tolerance produced by starvation are due to hypersecretion of growth hormone. Growth hormone does not stimulate insulin secretion directly, but the hyperglycemia it produces secondarily stimulates the pancreas and may eventually exhaust the B cells.

HYPOGLYCEMIA & DIABETES MELLITUS IN HUMANS

HYPOGLYCEMIA

"Insulin reactions" are common in type 1 diabetics and occasional hypoglycemic episodes are the price of good diabetic control in most diabetics. Glucose uptake by skeletal muscle and absorption of injected insulin both increase during exercise (see above).

Symptomatic hypoglycemia also occurs in nondiabetics, and a review of some of the more important causes serves to emphasize the variables affecting plasma glucose homeostasis. Chronic mild hypoglycemia can cause incoordination and slurred speech, and the condition can be mistaken for drunkenness. Mental aberrations and convulsions in the absence of frank coma also occur. When the level of insulin secretion is chronically elevated by an **insulinoma,** a rare, insulin-secreting tumor of the pancreas, symptoms are most common in the morning. This is because a night of fasting has depleted hepatic glycogen reserves. However, symptoms can develop at any time, and in such patients, the diagnosis may be missed. Some cases of insulinoma have been erroneously diagnosed as epilepsy or psychosis. Hypoglycemia also occurs in some patients with large malignant tumors that do not involve the pancreatic islets, and the hypoglycemia in these cases is apparently due to excess secretion of IGF-II.

As noted above, the autonomic discharge caused by lowered blood glucose that produces shakiness, sweating, anxiety, and hunger normally occurs at plasma glucose levels that are higher than the glucose levels that cause cognitive dysfunction, thereby serving as a warning to ingest sugar. However, in some individuals, these warning symptoms fail to occur before the cognitive

symptoms, due to cerebral dysfunction (desensitization), and this **hypoglycemia unawareness** is potentially dangerous. The condition is prone to develop in patients with insulinomas and in diabetics receiving intensive insulin therapy, so it appears that repeated bouts of hypoglycemia cause the eventual development of hypoglycemia unawareness. If blood sugar rises again for some time, the warning symptoms again appear at a higher plasma glucose level than cognitive abnormalities and coma. The reason why prolonged hypoglycemia causes loss of the warning symptoms is unsettled.

In liver disease, the glucose tolerance curve is diabetic but the fasting plasma glucose level is low **(Figure 24–16)**. In **functional hypoglycemia,** the plasma glucose rise is normal after a test dose of glucose, but the subsequent fall overshoots to hypoglycemic levels, producing symptoms 3–4 h after meals. This pattern is sometimes seen in individuals in whom diabetes develops later. Patients with this syndrome should be distinguished from the more numerous patients with similar symptoms due to psychological or other problems who do not have hypoglycemia when blood is drawn during the symptomatic episode. It has been postulated that the overshoot of the plasma glucose is due to insulin secretion stimulated by impulses in the right vagus, but cholinergic blocking agents do not routinely correct the abnormality. In some thyrotoxic patients and in patients who have had gastrectomies or other operations that speed the passage of food into the intestine, glucose absorption is abnormally rapid. The plasma glucose rises to a high, early peak, but it then falls rapidly to hypoglycemic levels because the wave of hyperglycemia evokes a greater than normal rise in insulin secretion. Symptoms characteristically occur about 2 h after meals.

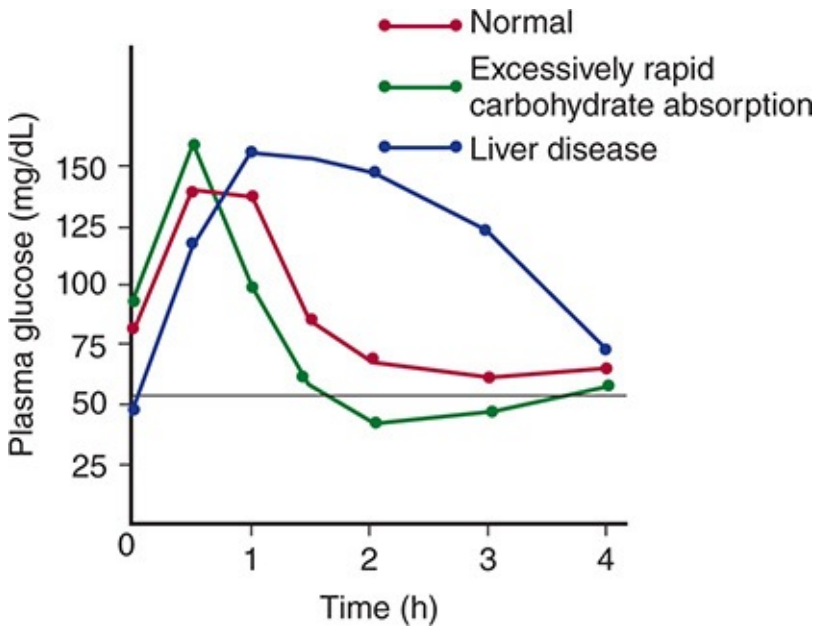


FIGURE 24–16 Typical glucose tolerance curves after an oral glucose load in liver disease and in conditions causing excessively rapid absorption of glucose from the intestine. The horizontal line is the approximate plasma glucose level at which hypoglycemic symptoms may appear.

DIABETES MELLITUS

The incidence of diabetes mellitus in the human population has reached epidemic proportions worldwide and it is increasing at a rapid rate. In 2010 an estimated 285 million people worldwide had diabetes, according to the International Diabetes Federation. The federation predicts as many as 438 million will have diabetes by 2030. Ninety percent of the present cases are type 2 diabetes, and most of the increase will be in type 2, paralleling the increase in the incidence of obesity.

Diabetes is sometimes complicated by acidosis and coma, and in long-standing diabetes, additional complications occur. These include microvascular, macrovascular, and neuropathic disease. The microvascular abnormalities are proliferative scarring of the retina **(diabetic retinopathy)** leading to blindness and renal disease **(diabetic nephropathy)** leading to chronic kidney disease. The macrovascular abnormalities are due to accelerated atherosclerosis, which is secondary to increased plasma LDL. The result is an increased incidence of stroke and myocardial infarction. The neuropathic abnormalities **(diabetic neuropathy)** involve the autonomic nervous system and peripheral nerves. The

neuropathy plus the atherosclerotic circulatory insufficiency in the extremities and reduced resistance to infection can lead to chronic ulceration and gangrene, particularly in the feet.

The ultimate cause of the microvascular and neuropathic complications is chronic hyperglycemia, and tight control of the diabetes reduces their incidence. Intracellular hyperglycemia activates the enzyme aldose reductase. This increases the formation of sorbitol in cells, which in turn reduces cellular Na, K ATPase. In addition, intracellular glucose can be converted to so-called Amadori products, and these in turn can form **advanced glycosylation end products (AGEs),** which cross-link matrix proteins. This damages blood vessels. The AGEs also interfere with leukocyte responses to infection.

TYPES OF DIABETES

The cause of clinical diabetes is always a deficiency of the effects of insulin at the tissue level, but the deficiency may be relative. One of the common forms, **type 1,** or **insulin-dependent diabetes mellitus (IDDM),** is due to insulin deficiency caused by autoimmune destruction of the B cells in the pancreatic islets; the A, D, and F cells remain intact. The second common form, **type 2,** or **noninsulin-dependent diabetes mellitus (NIDDM),** is characterized by insulin resistance.

In addition, some cases of diabetes are due to other diseases or conditions such as chronic pancreatitis, total pancreatectomy, Cushing syndrome (see Chapter 19), and acromegaly (see Chapter 18). These make up 5% of the total cases and are sometimes classified as **secondary diabetes.**

Type 1 diabetes usually develops before the age of 40 and hence is called **juvenile diabetes.** Patients with this disease are not obese and they have a high incidence of ketosis and acidosis. Various anti-B cell antibodies are present in plasma, but the current thinking is that type 1 diabetes is primarily a T lymphocyte–mediated disease. Definite genetic susceptibility is present as well; if the disease develops in one identical twin, the chances are 1 in 3 that it will also develop in the other twin. In other words, the **concordance rate** is about 33%. The main genetic abnormality is in the major histocompatibility complex on chromosome 6, making individuals with certain types of histocompatibility antigens (see Chapter 3) much more prone to the disease. Other genes are also involved.

Immunosuppression with drugs such as cyclosporine ameliorate type 1 diabetes if given early in the disease before all islet B cells are lost. Attempts

have been made to treat type 1 diabetes by transplanting pancreatic tissue or isolated islet cells, but results to date have been poor, largely because B cells are easily damaged and it is difficult to transplant enough of them to normalize glucose responses.

As mentioned above, type 2 is the most common type of diabetes and is usually associated with obesity. It usually develops after age 40 and is not associated with total loss of the ability to secrete insulin. It has an insidious onset, is rarely associated with ketosis, and is usually associated with normal B cell morphology and insulin content if the B cells have not become exhausted. The genetic component in type 2 diabetes is actually stronger than the genetic component in type 1 diabetes; in identical twins, the concordance rate is higher, ranging in some studies to nearly 100%.

In some patients, type 2 diabetes is due to defects in identified genes. Over 60 of these defects have been described. They include defects in glucokinase (about 1% of the cases), the insulin molecule itself (about 0.5% of the cases), the insulin receptor (about 1% of the cases), GLUT-4 (about 1% of the cases), or IRS-1 (about 15% of the cases). In maturity-onset diabetes occurring in young individuals (MODY), which accounts for about 1% of the cases of type 2 diabetes, loss-of-function mutations have been described in six different genes. Five of the genes code for transcription factors affecting the production of enzymes involved in glucose metabolism. The sixth is the gene for glucokinase (Figure 24–11), the enzyme that controls the rate of glucose phosphorylation and hence its metabolism in the B cells. However, the vast majority of cases of type 2 diabetes are almost certainly polygenic in origin, and the actual genes involved are still unknown.

OBESITY, THE METABOLIC SYNDROME, & TYPE 2 DIABETES

Obesity is increasing in incidence and relates to the regulation of food intake and energy balance and overall nutrition. It deserves additional consideration in this chapter because of its special relation to disordered carbohydrate metabolism and diabetes. As body weight increases, insulin resistance increases, that is, there is a decreased ability of insulin to move glucose into fat and muscle and to shut off glucose release from the liver. Weight reduction decreases insulin resistance. Associated with obesity there is hyperinsulinemia, dyslipidemia (characterized by high circulating triglycerides and low high-density lipoprotein [HDL]), and

accelerated development of atherosclerosis. This combination of findings is commonly called the **metabolic syndrome,** or **syndrome X.** Some of the patients with the syndrome are prediabetic, whereas others have frank type 2 diabetes. It has not been proved but it is logical to assume that the hyperinsulinemia is a compensatory response to the increased insulin resistance and that frank diabetes develops in individuals with reduced B cell reserves.

These observations and other data strongly suggest that fat produces a chemical signal or signals that act on muscles and the liver to increase insulin resistance. Evidence for this includes the recent observation that when GLUTs are selectively knocked out in adipose tissue, there is an associated decrease in glucose transport in muscle in vivo, but when the muscles of those animals are tested in vitro their transport is normal.

One possible signal is the circulating level of FFAs, which is elevated in many insulin-resistant states. Other possibilities are peptides and proteins secreted by fat cells. It is now clear that white fat depots are not inert lumps but are actually endocrine tissues that secrete not only leptin but also other hormones that affect fat metabolism. These adipose-derived hormones are commonly termed **adipokines** as they are *cytokines* secreted by *adipose tissue*. Known adipokines are leptin, adiponectin, and resistin.

Some adipokines decrease, rather than increase, insulin resistance. Leptin and adiponectin, for example, decrease insulin resistance, whereas resistin increases insulin resistance. Further complicating the situation, marked insulin resistance is present in the rare metabolic disease **congenital lipodystrophy,** in which fat depots fail to develop. This resistance is reduced by leptin and adiponectin. Finally, a variety of knockouts of intracellular second messengers have been reported to increase insulin resistance. It is unclear how, or indeed if, these findings fit together to provide an explanation of the relation of obesity to insulin tolerance, but the topic is obviously an important one and it is under intensive investigation.

CHAPTER SUMMARY

- Four polypeptides with hormonal activity are secreted by the pancreas: insulin, glucagon, somatostatin, and pancreatic polypeptide.
- Insulin increases the entry of glucose into cells. In skeletal muscle cell it increases the number of GLUT-4 transporters in the cell membranes. In liver it induces glucokinase, which increases the phosphorylation of glucose, facilitating the entry of glucose into the cell.

- Insulin causes K^+ to enter cells, with a resultant lowering of the extracellular K^+ concentration. Insulin increases the activity of Na, K ATPase in cell membranes, so that more K^+ is pumped into cells. Hypokalemia often develops when patients with diabetic acidosis are treated with insulin.
- Insulin receptors are found on many different cells in the body and have two subunits, α and β. Binding of insulin to its receptor triggers a signaling pathway that involves autophosphorylation of the β subunits on tyrosine residues. This triggers phosphorylation of some cytoplasmic proteins and dephosphorylation of others, mostly on serine and threonine residues.
- The constellation of abnormalities caused by insulin deficiency is called diabetes mellitus. Type 1 diabetes is due to insulin deficiency caused by autoimmune destruction of the B cells in the pancreatic islets. Type 2 diabetes is characterized by the dysregulation of insulin release from the B cells, along with insulin resistance in peripheral tissues such as skeletal muscle, brain, and liver.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. Which of the following are *incorrectly* paired?

   A. B cells: insulin

   B. D cells: somatostatin

   C. A cells: glucagons

   D. Pancreatic exocrine cells: chymotrypsinogen

   E. F cells: gastrin

2. Which of the following are *incorrectly* paired?

   A. Epinephrine: increased glycogenolysis in skeletal muscle

   B. Insulin: increased protein synthesis

   C. Glucagon: increased gluconeogenesis

   D. Progesterone: increased plasma glucose level

   E. Growth hormone: increased plasma glucose level

3. Which of the following would be *least* likely to be seen 14 days after a rat is injected with a drug that kills all of its pancreatic B cells?

   A. A rise in the plasma H^+ concentration

B. A rise in the plasma glucagon concentration

C. A fall in the plasma HCO_3^- concentration

D. A fall in the plasma amino acid concentration

E. A rise in plasma osmolality

4. When the plasma glucose concentration falls to low levels, a number of different hormones help combat the hypoglycemia. After intravenous administration of a large dose of insulin, the return of a low blood sugar level to normal is delayed in

A. adrenal medullary insufficiency.

B. glucagon deficiency.

C. combined adrenal medullary insufficiency and glucagon deficiency.

D. thyrotoxicosis.

E. acromegaly.

5. Insulin increases the entry of glucose into

A. all tissues.

B. renal tubular cells.

C. the mucosa of the small intestine.

D. most neurons in the cerebral cortex.

E. skeletal muscle.

6. Glucagon increases glycogenolysis in liver cells but ACTH does not because

A. cortisol increases the plasma glucose level.

B. liver cells have an adenylyl cyclase different from that in adrenocortical cells.

C. ACTH cannot enter the nucleus of liver cells.

D. the membranes of liver cells contain receptors different from those in adrenocortical cells.

E. liver cells contain a protein that inhibits the action of ACTH.

7. A meal rich in proteins containing the amino acids that stimulate insulin secretion but low in carbohydrates does not cause hypoglycemia because

A. the meal causes a compensatory increase in T_4 secretion.

B. cortisol in the circulation prevents glucose from entering muscle.

C. glucagon secretion is also stimulated by the meal.

D. the amino acids in the meal are promptly converted to glucose.

E. insulin does not bind to insulin receptors if the plasma concentration of amino acids is elevated.

SECTION IV

Gastrointestinal Physiology

For unicellular organisms that exist in a sea of nutrients, it is possible to satisfy nutritional requirements simply with the activity of membrane transport proteins that permit the uptake of specific molecules into the cytosol. However, for multicellular organisms, including humans, the challenges of delivering nutrients to appropriate sites in the body are significantly greater, particularly if the organisms are terrestrial. Further, most of the food we eat is in the form of macromolecules, and even when these are digested to their component monomers, most of the end products are water-soluble and do not readily cross cell membranes (a notable exception are the constituents of dietary lipids). Thus, the gastrointestinal system has evolved to permit nutrient acquisition and assimilation into the body, while prohibiting the uptake of undesirable substances (toxins and microbial products, as well as microbes themselves). The latter situation is complicated by the fact that the intestine maintains a lifelong relationship with a rich microbial ecosystem residing in its lumen, a relationship that is largely mutually beneficial if the microbes are excluded from the systemic compartment.

The intestine is a continuous tube that extends from mouth to anus and is formally contiguous with the external environment. A single cell layer of columnar epithelial cells comprises the semipermeable barrier across which controlled uptake of nutrients takes place. Various glandular structures empty into the intestinal lumen at points along its length, providing for digestion of food components, signaling to distal segments, and regulation of the microbiota. There are also important motility functions that move the intestinal contents and resulting waste products along the length of the gut, and a rich innervation that regulates motility, secretion, and nutrient uptake, in many cases in a manner that is independent of the central nervous system (CNS). There are also a large number of endocrine cells that release hormones that work together with

neurotransmitters to coordinate overall regulation of the gastrointestinal system. In general, there is a considerable redundancy of control systems as well as excess capacity for nutrient digestion and uptake. This served humans well in ancient times when food sources were scarce but may now contribute to the modern epidemic of obesity.

The liver, while playing important roles in whole body metabolism, is usually considered a part of the gastrointestinal system for two main reasons. First, it provides for excretion from the body of lipid-soluble waste products that cannot enter the urine. These are secreted into the bile and thence into the intestine to be excreted with the feces. Second, the blood flow draining the intestine is arranged such that substances that are absorbed pass first through the liver, allowing for the removal and metabolism of any toxins that have inadvertently been taken up, as well as clearance of particulates, such as small numbers of enteric bacteria.

In this section, the function of the gastrointestinal system and liver will be considered, and the ways in which the various segments communicate to provide an integrated response to a mixed meal (proteins, carbohydrates, and lipids). The relevance of gastrointestinal physiology for the development of digestive diseases will also be considered. While many are rarely life-threatening (with some notable exceptions, such as specific cancers) digestive diseases represent a substantial burden in terms of morbidity and lost productivity. A 2009 report of the US National Institutes of Diabetes, Digestive and Kidney Diseases found that on an annual basis, for every 100 US residents, there were 35 ambulatory care visits and nearly 5 overnight hospital stays that involved a gastrointestinal diagnosis. Digestive diseases also appear to be increasing in this population (although mortality, principally from cancers, is thankfully in decline). On the other hand, digestive diseases, and in particular infectious diarrhea, remain important causes of mortality in developing countries where clean sources of food and water cannot be assured. In any event, the burden of digestive diseases provides an important impetus for gaining a full understanding of gastrointestinal physiology, since it is a failure of such physiology that most often leads to disease. Conversely, an understanding of specific digestive conditions can often illuminate physiologic principles, as will be stressed in this section.

CHAPTER 25

Overview of Gastrointestinal Function & Regulation

OBJECTIVES

After studying this chapter, you should be able to:

- Understand the functional significance of the gastrointestinal system, and in particular, its roles in nutrient assimilation, excretion, and immunity.
- Describe the structure of the gastrointestinal tract, the glands that drain into it, and its subdivision into functional segments.
- List the major gastrointestinal secretions, their components, and the stimuli that regulate their production.
- Identify the nature and sources of the major hormones, other peptides, and key neurotransmitters of the gastrointestinal system, and their targets and mechanisms of action.
- Describe the special features of the enteric nervous system, the mucosal immune system and the splanchnic circulation.
- Describe water balance in the gastrointestinal tract and explain how the level of luminal fluidity is adjusted to allow for digestion and absorption.

INTRODUCTION

The primary function of the gastrointestinal tract is to serve as a portal whereby nutrients and water can be absorbed into the body. In fulfilling this function, the meal is mixed with a variety of secretions that arise from both the gastrointestinal tract itself and organs that drain into it, such as the pancreas, gallbladder, and salivary glands. Likewise, the intestine displays a variety of motility patterns that serve to mix the meal with digestive secretions and move it along the length of the gastrointestinal tract. Ultimately, residues of the meal that cannot be absorbed, along with cellular debris, are expelled from the body. All of these functions are tightly regulated in concert with the ingestion of meals. Thus, the gastrointestinal system has evolved a large number of regulatory mechanisms that act both locally and over long distances to coordinate the function of the gut and the organs that drain into it. In keeping with its functional continuity with the external environment and its lifelong interaction with a complex microbiota, moreover, the gastrointestinal tract also harbors well-developed innate and adaptive immune systems. Nevertheless, it remains an important portal for infection.

STRUCTURAL CONSIDERATIONS

The parts of the gastrointestinal tract that are encountered by the meal or its residues include, in order, the mouth, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, and anus **(Figure 25–1)**. Throughout the length of the intestine, glandular structures deliver secretions into the lumen, particularly in the stomach and mouth. Also important in the process of digestion are secretions from the pancreas and the biliary system of the liver. The intestine itself also has a very substantial surface area, which is important for its absorptive function. The intestinal tract is functionally divided into segments by means of muscle rings known as **sphincters,** which restrict the flow of intestinal contents to optimize digestion and absorption. These sphincters include the upper and lower esophageal sphincters, the pylorus that retards emptying of the stomach, the ileocecal valve that retains colonic contents (including large numbers of bacteria) in the large intestine, and the inner and outer anal sphincters. After toilet training, the latter permits delaying the elimination of wastes until a time when it is socially convenient.

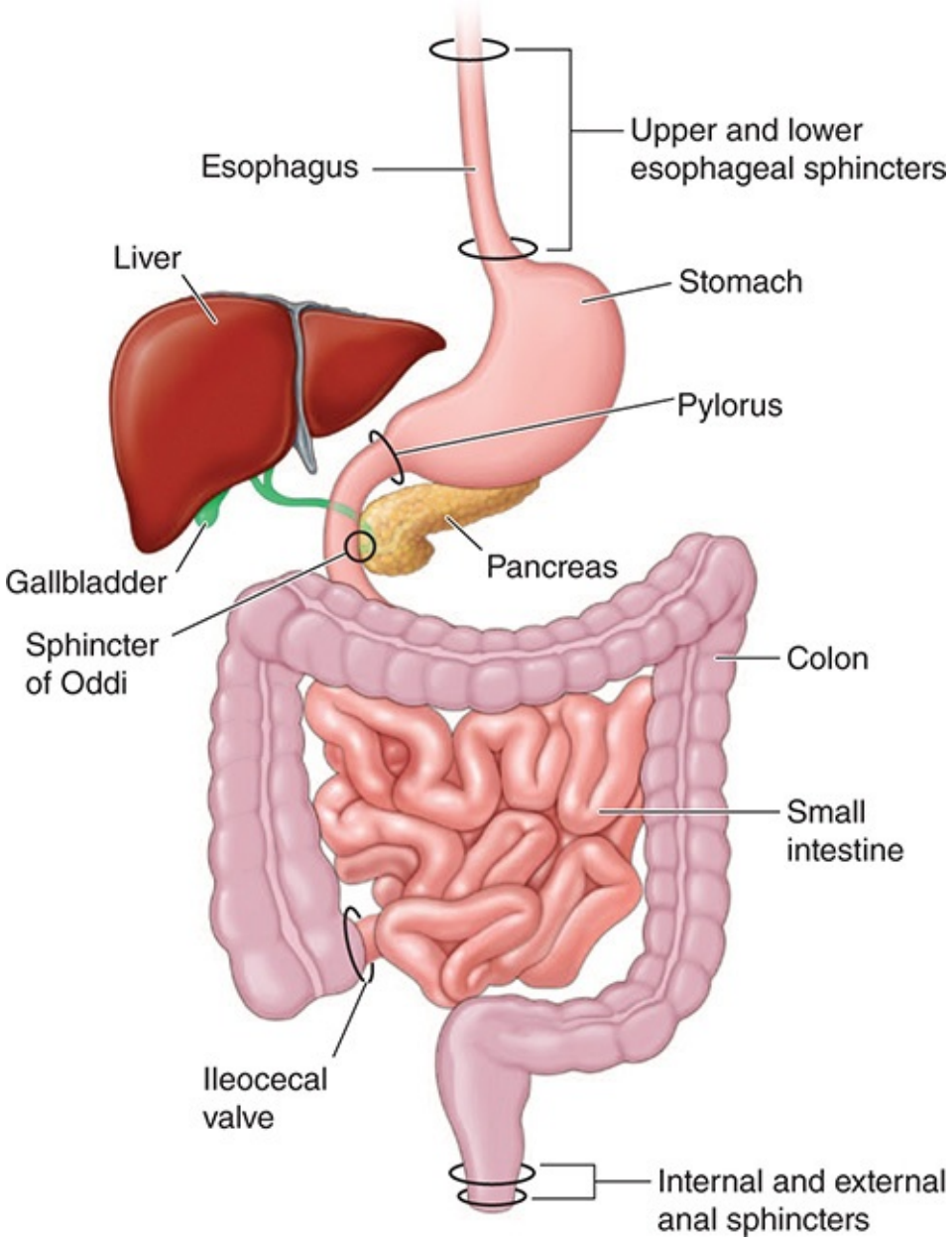


FIGURE 25–1 Overall anatomy of the gastrointestinal system The figure also shows division of the gastrointestinal tract into functional segments by sphincters and valves.

The intestine is composed of functional layers **(Figure 25–2)**. Immediately adjacent to nutrients in the lumen is a single layer of columnar epithelial cells. This represents the barrier that nutrients must traverse to enter the body. Below the epithelium is a layer of loose connective tissue known as the lamina propria, which contains many immune and inflammatory cells even in health and in turn is surrounded by concentric layers of smooth muscle, oriented circumferentially

and then longitudinally to the axis of the gut (the circular and longitudinal muscle layers, respectively). Sandwiched between the two muscle layers, and within the submucosa, are the myenteric and submucosal nerve plexuses of the enteric nervous system, respectively. Sensory nerves project toward the epithelium, and secretomotor nerves innervate both the epithelium and the muscle layers. The intestine is also amply supplied with blood vessels and lymphatics.

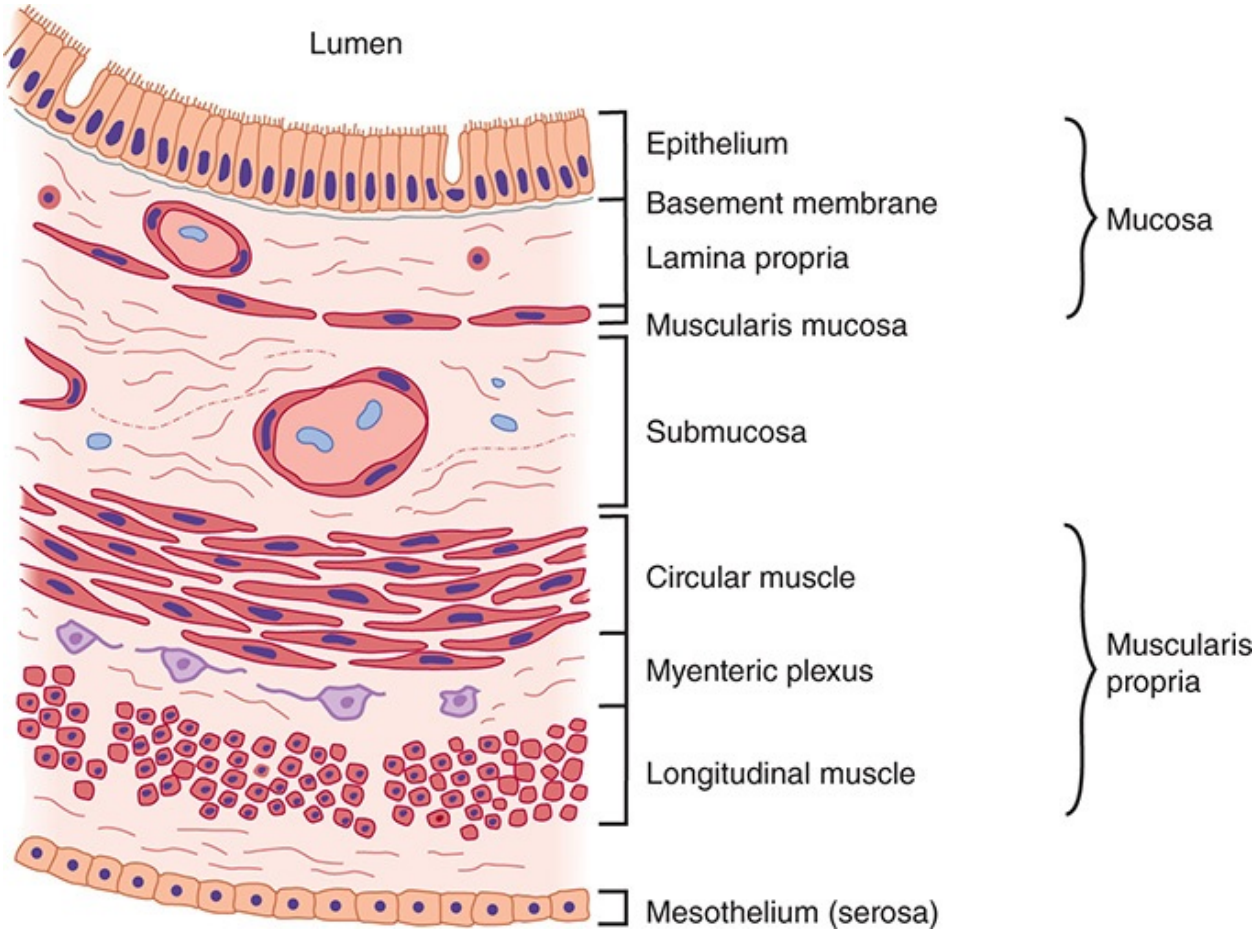


FIGURE 25–2 Organization of the wall of the intestine into functional layers. (Adapted with permission from Yamada T: *Textbook of Gastroenterology*, 4th ed. New York, NY: Lippincott Williams & Wilkins; 2003.)

The epithelium of the intestine is also further specialized in a way that maximizes the surface area available for nutrient absorption. Throughout the small intestine, it is folded up into fingerlike projections called villi **(Figure 25–3)**. Between the villi are infoldings known as crypts. Stem cells that give rise to both crypt and villus epithelial cells reside toward the base of the crypts and are

responsible for completely renewing the epithelium every few days or so. Indeed, the gastrointestinal epithelium is one of the most rapidly dividing tissues in the body. Daughter cells undergo several rounds of cell division in the crypts then migrate out onto the villi, where they are eventually shed and lost in the stool. The villus epithelial cells are also notable for the extensive microvilli that characterize their apical membranes. These microvilli are endowed with a dense glycocalyx (the brush border) that probably protects the cells to some extent from the effects of digestive enzymes. Some digestive enzymes are also actually part of the brush border, being membrane-bound proteins. These so-called “brush border hydrolases” perform the final steps of digestion for specific nutrients.

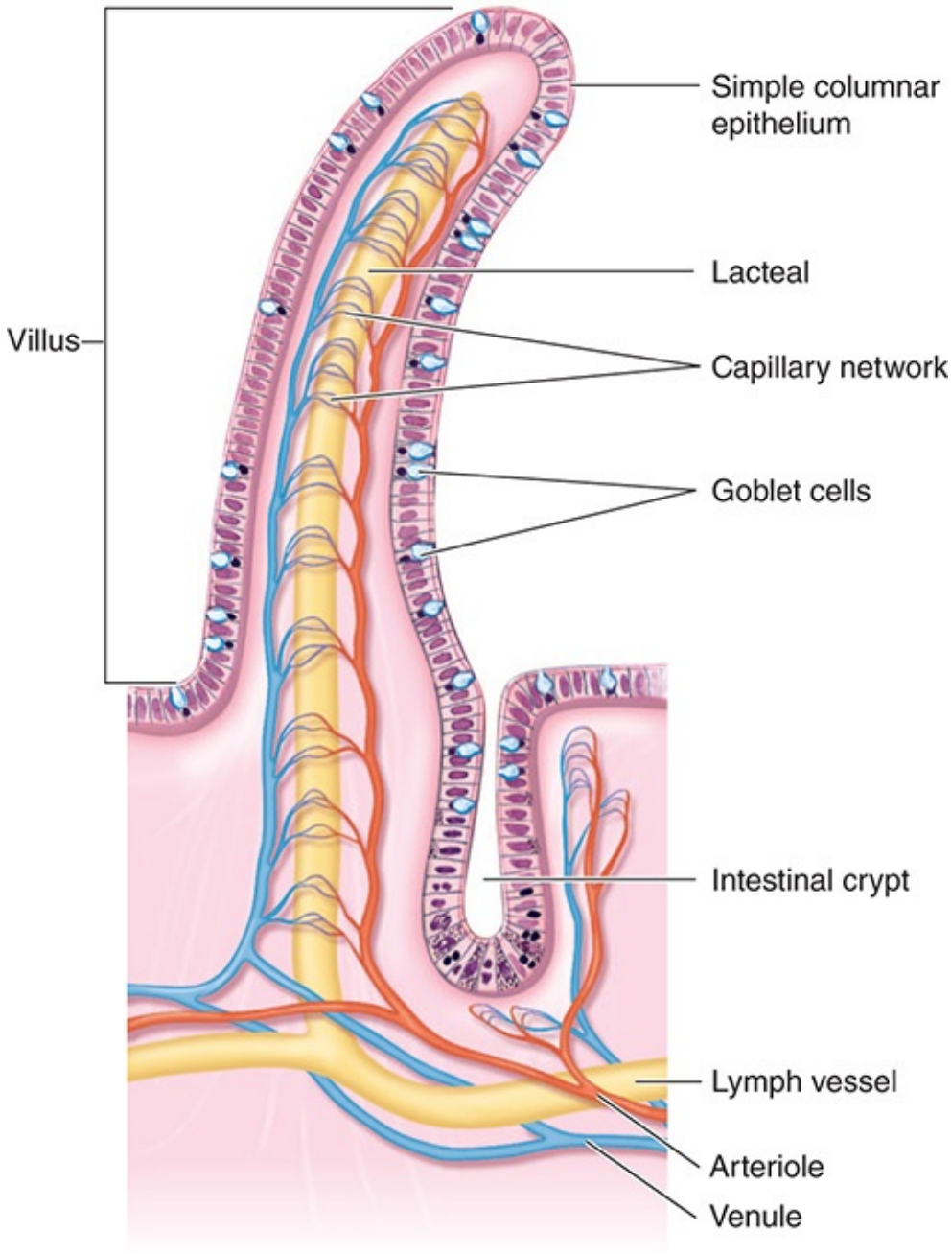


FIGURE 25–3 The structure of intestinal villi and crypts. The epithelial layer also contains scattered endocrine cells and intraepithelial lymphocytes. The crypt base contains Paneth cells, which secrete antimicrobial peptides, as well as the stem cells that provide for continual turnover of the crypt and villus epithelium. The epithelium turns over every 3–5 days in healthy adult humans. (Reproduced with permission from Fox SI: *Human Physiology*, 10th ed. New York, NY: McGraw-Hill; 2008.)

GASTROINTESTINAL SECRETIONS

SALIVARY SECRETION

The ability of the gastrointestinal tract to digest nutrients rests on the sequential exposure of the meal to a series of specific secretions. The first secretion encountered when food is ingested is saliva. Saliva is produced by three pairs of salivary glands (the **parotid, submandibular,** and **sublingual glands**) that drain into the oral cavity. It has a number of organic constituents that serve to initiate digestion (particularly of starch, mediated by amylase) and which also protect the oral cavity from bacteria (such as immunoglobulin A and lysozyme). Saliva also serves to lubricate the food bolus (aided by mucins). Saliva is hypotonic compared with plasma and alkaline; the latter feature is important to neutralize any gastric secretions that reflux into the esophagus.

The salivary glands consist of blind end pieces (acini) that produce the primary secretion containing the organic constituents dissolved in a fluid that is essentially identical in its composition to plasma. The salivary glands are extremely active when maximally stimulated, secreting their own weight in saliva every minute. To accomplish this, they are richly endowed with surrounding blood vessels that dilate when salivary secretion is initiated. The composition of the saliva is then modified as it flows from the acini out into ducts that eventually coalesce and deliver the saliva into the mouth. Na^+ and Cl^- are extracted and K^+ and bicarbonate are added. Because the ducts are relatively impermeable to water, the loss of NaCl renders the saliva hypotonic, particularly at low secretion rates. As the rate of secretion increases, there is less time for NaCl to be extracted and the tonicity of the saliva rises, but it always stays somewhat hypotonic with respect to plasma. Overall, the three pairs of salivary glands that drain into the mouth supply 1000–1500 mL of saliva per day.

Salivary secretion is almost entirely controlled by neural influences, with the parasympathetic branch of the autonomic nervous system playing the most prominent role **(Figure 25–4)**. Sympathetic input slightly modifies the composition of saliva (by increasing proteinaceous content), but has little influence on volume. Secretion is triggered by reflexes that are stimulated by the physical act of chewing, but is actually initiated even before the meal is taken into the mouth as a result of central triggers that are prompted by thinking about, seeing, or smelling food. Indeed, salivary secretion can readily be conditioned, as in the classic experiments of Pavlov where dogs were conditioned to salivate in response to a ringing bell by associating this stimulus with a meal. Salivary

secretion is also prompted by nausea but inhibited by fear or during sleep.

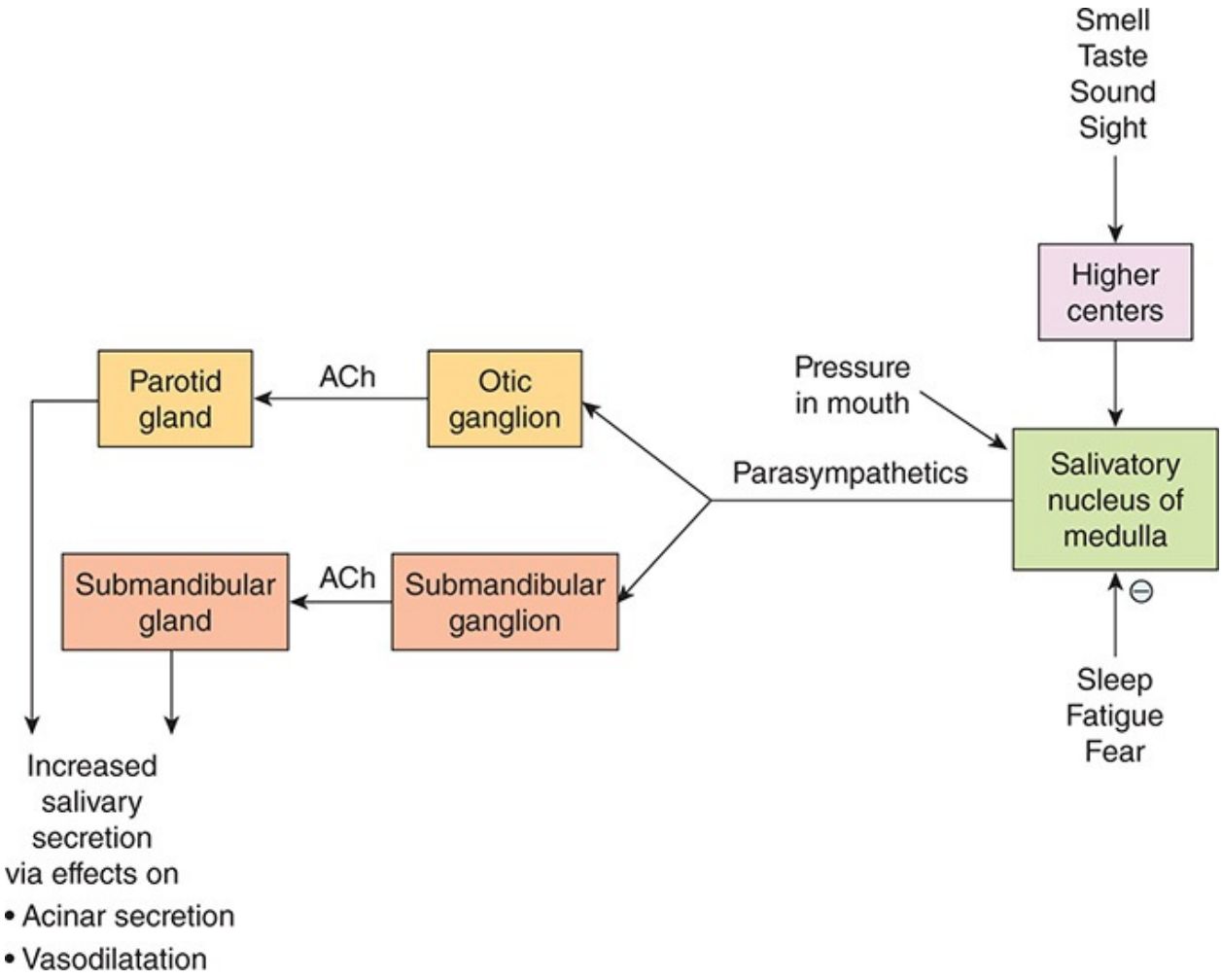


FIGURE 25–4 Regulation of salivary secretion by the parasympathetic nervous system. ACh, acetylcholine. Saliva is also produced by the sublingual glands (not depicted), but these are the minor contributors to both resting and stimulated salivary flows. (Adapted with permission from Barrett KE: *Gastrointestinal Physiology*. New York, NY: McGraw-Hill; 2006.)

Saliva performs a number of important functions: it facilitates swallowing, keeps the mouth moist, serves as a solvent for the molecules that stimulate the taste buds, aids speech by facilitating movements of the lips and tongue, and keeps the mouth and teeth clean. The saliva also has some antibacterial action, and patients with deficient salivation **(xerostomia)** have a higher than normal incidence of dental caries. The buffers in saliva help maintain the oral pH at about 7.0.

GASTRIC SECRETION

Food is stored in the stomach; mixed with acid, mucus, and pepsin; and released at a controlled, steady rate into the duodenum.

ANATOMIC CONSIDERATIONS

The gross anatomy of the stomach is shown in **Figure 25–5**. The gastric mucosa contains many deep glands. In the cardia and the pyloric region, the glands secrete mucus. In the body of the stomach, including the fundus, the glands also contain **parietal cells** and **chief cells** **(Figure 25–6)**. These secretions mix with mucus secreted by the cells in the necks of the glands. Several of the glands open onto a common chamber **(gastric pit)** that opens in turn onto the surface of the mucosa. Mucus is also secreted along with HCO_3^- by mucus cells on the surface of the epithelium between glands.

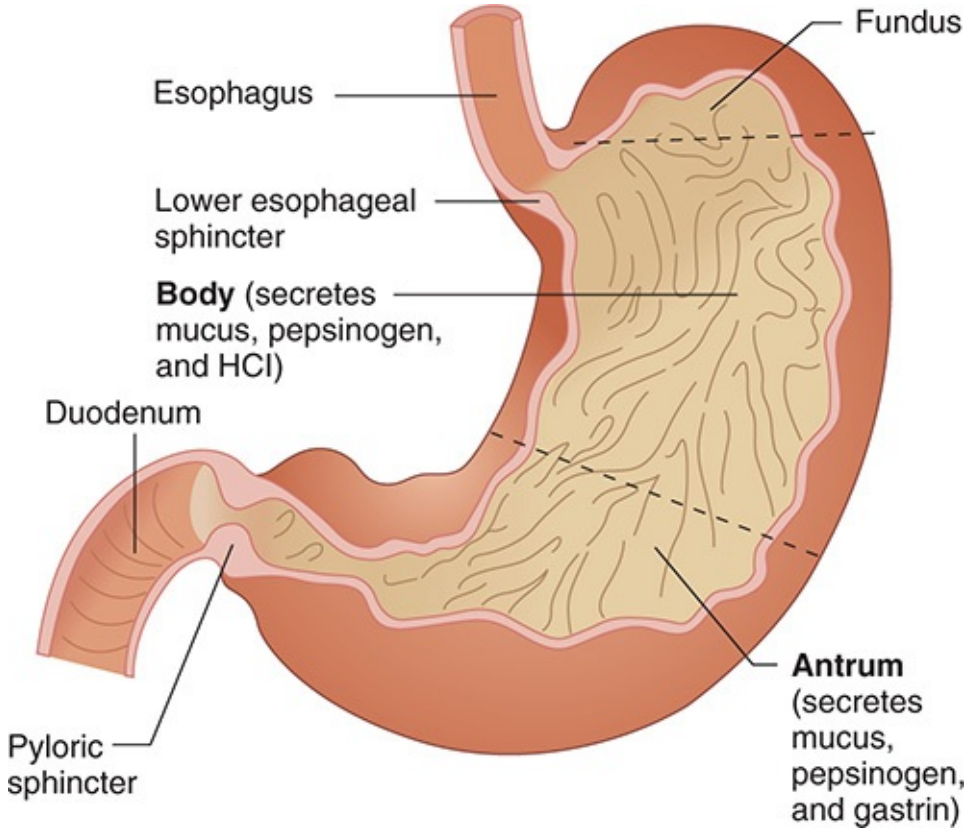


FIGURE 25–5 Anatomy of the stomach. The principal secretions of the body and antrum are listed in parentheses. (Reproduced with permission from

Widmaier EP, Raff H, Strang KT: *Vander's Human Physiology: The Mechanisms of Body Function*, 11th ed. New York, NY: McGraw-Hill; 2008.)

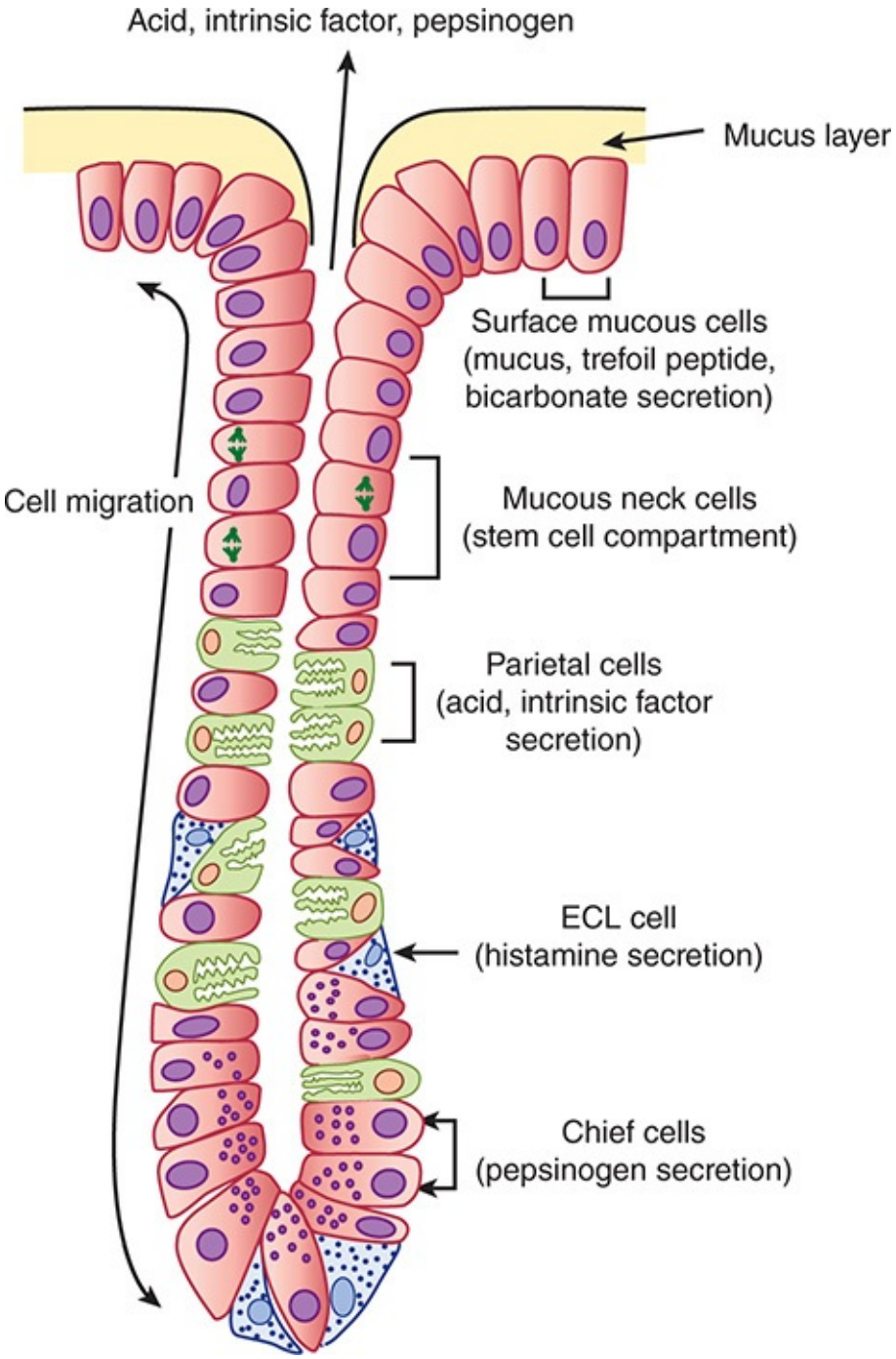


FIGURE 25–6 Structure of a gastric gland from the fundus or body of the stomach. These acid- and pepsinogen-producing glands are referred to as "oxyntic" glands in some sources. Similarly, some sources refer to parietal cells as oxyntic cells. ECL, enterochromaffin-like. (Adapted with permission from

Barrett KE: *Gastrointestinal Physiology*. New York, NY: McGraw-Hill; 2006.)

The stomach has a very rich blood and lymphatic supply. Its parasympathetic nerve supply comes from the vagi and its sympathetic supply from the celiac plexus.

ORIGIN & REGULATION OF GASTRIC SECRETION

The stomach adds a significant volume of digestive juices to the meal. Like salivary secretion, the stomach readies itself to receive the meal before it is actually taken in, during the so-called cephalic phase that can be influenced by food preferences. Subsequently, there is a gastric phase of secretion that is quantitatively the most significant, and finally an intestinal phase once the meal has left the stomach. Each phase is closely regulated by both local and distant triggers.

The gastric secretions **(Table 25–1)** arise from glands in the wall of the stomach that drain into its lumen, and also from the surface cells that secrete primarily mucus and bicarbonate to protect the stomach from digesting itself, as well as substances known as trefoil peptides that stabilize the mucus-bicarbonate layer. The glandular secretions of the stomach differ in different regions of the organ. The most characteristic secretions derive from the glands in the fundus or body of the stomach. These contain the distinctive parietal cells, which secrete hydrochloric acid and intrinsic factor; and chief cells, which produce pepsinogens and gastric lipase (Figure 25–5). The acid secreted by parietal cells serves to sterilize the meal and also to begin the hydrolysis particularly of dietary protein. Intrinsic factor is important for the later absorption of vitamin B_{12}, or cobalamin. Pepsinogen is the precursor of pepsin, which initiates protein digestion. Lipase similarly begins the digestion of dietary fats.

TABLE 25–1 Contents of normal gastric juice (fasting state).

Cations: Na^+, K^+, Mg^{2+}, H^+ (pH approximately 3.0)
Anions: Cl^-, HPO_4^{2-}, SO_4^{2-}
Pepsins
Lipase
Mucus

Intrinsic factor

There are three primary stimuli of gastric secretion, each with a specific role to play in matching the rate of secretion to functional requirements **(Figure 25–7)**. Gastrin is a hormone that is released by G cells in the antrum of the stomach both in response to a specific neurotransmitter released from enteric nerve endings, known as gastrin-releasing peptide (GRP) and also in response to the presence of oligopeptides in the gastric lumen. Gastrin is then carried through the bloodstream to the fundic glands, where it binds to receptors not only on parietal (and likely, chief) cells to activate secretion, but also on so-called enterochromaffin-like cells (ECL cells) that are located in the gland, and release histamine. Histamine is also a trigger of parietal cell secretion, via binding to H_2-receptors. Finally, parietal and chief cells can also be stimulated by acetylcholine, released from enteric nerve endings in the fundus.

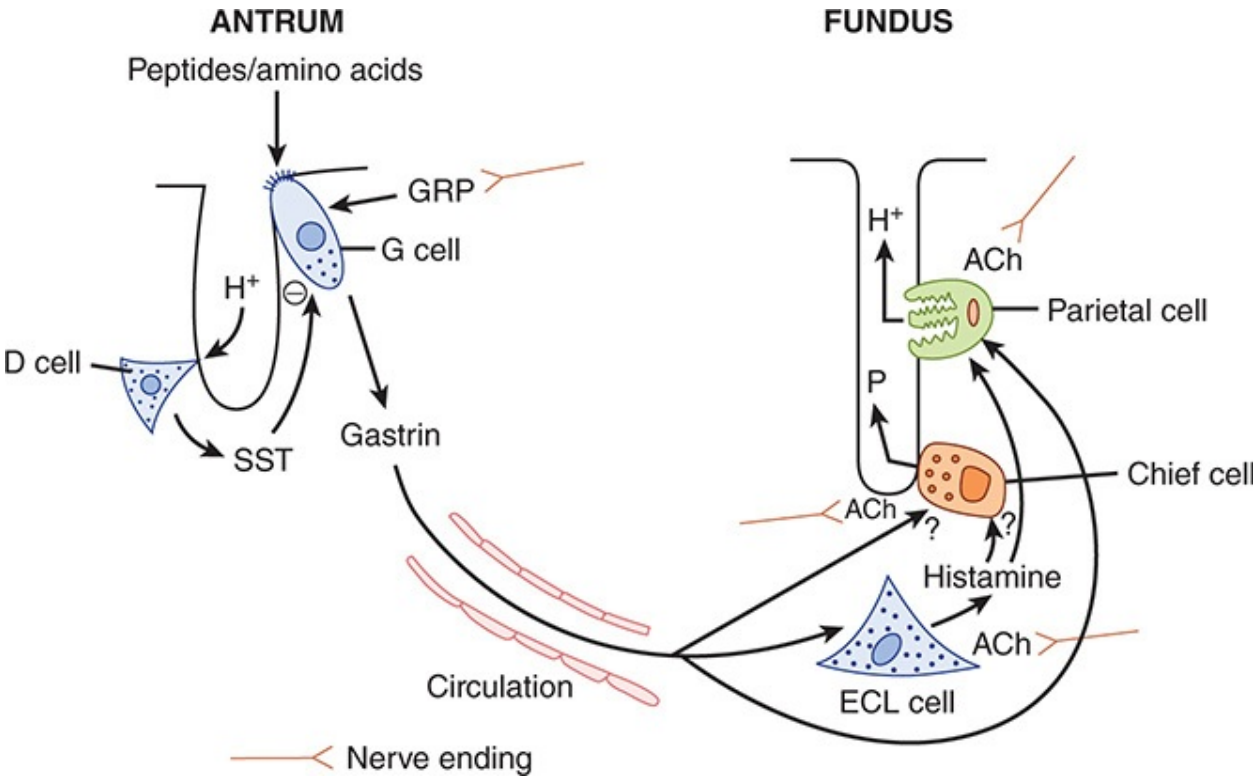


FIGURE 25–7 Regulation of gastric acid and pepsin secretion by soluble mediators and neural input. Gastrin is released from G cells in the antrum in response to gastrin-releasing peptide (GRP) and travels through the circulation to influence the activity of enterochromaffin-like (ECL) cells and parietal cells. ECL cells release histamine, which also acts on parietal cells. Acetylcholine

(ACh), released from nerves, is an agonist for ECL cells, chief cells, and parietal cells. Other specific agonists of the chief cell are not well understood. Gastrin release is negatively regulated by luminal acidity via the release of somatostatin from antral D cells. P, pepsinogen. (Adapted with permission from Barrett KE: *Gastrointestinal Physiology*. New York, NY: McGraw-Hill; 2006.)

Gastric secretion that occurs during the cephalic phase is activated predominantly by vagal input that originates from the brain region known as the dorsal vagal complex, which coordinates input from higher centers. Vagal outflow to the stomach then releases GRP and acetylcholine, thereby initiating secretory function. However, before the meal enters the stomach, there are few additional triggers and thus the amount of secretion is limited. Once the meal is swallowed, on the other hand, meal constituents trigger substantial release of gastrin and the physical presence of the meal also distends the stomach and activates stretch receptors, which provoke a "vago-vagal" as well as local reflexes that further amplify secretion during the gastric phase. The presence of the meal also buffers gastric acidity. Acidity otherwise would serve as a feedback inhibitory signal to shut off secretion, secondary to the release of somatostatin, which inhibits both G and ECL cells as well as secretion by parietal cells themselves (Figure 25–7). This probably represents a key mechanism whereby gastric secretion is terminated after the meal moves from the stomach into the small intestine.

Gastric parietal cells are highly specialized for their unusual task of secreting concentrated acid **(Figure 25–8)**. The cells are packed with mitochondria that supply energy to drive the apical H^+, K^+-ATPase, or proton pump, that moves H^+ ions out of the parietal cell against a concentration gradient of more than a million-fold. At rest, the proton pumps are sequestered within the parietal cell in a series of membrane compartments known as tubulovesicles. When the parietal cell begins to secrete, on the other hand, these vesicles fuse with invaginations of the apical membrane known as canaliculi, thereby substantially amplifying the apical membrane area and positioning the proton pumps to begin acid secretion **(Figure 25–9)**. The apical membrane also contains potassium channels, which supply the K^+ ions to be exchanged for H^+, and Cl^- channels that supply the counterion for HCl secretion **(Figure 25–10)**. The secretion of protons is also accompanied by the release of equivalent numbers of bicarbonate ions into the bloodstream, which are later used to neutralize gastric acidity once its function is complete (Figure 25–10).

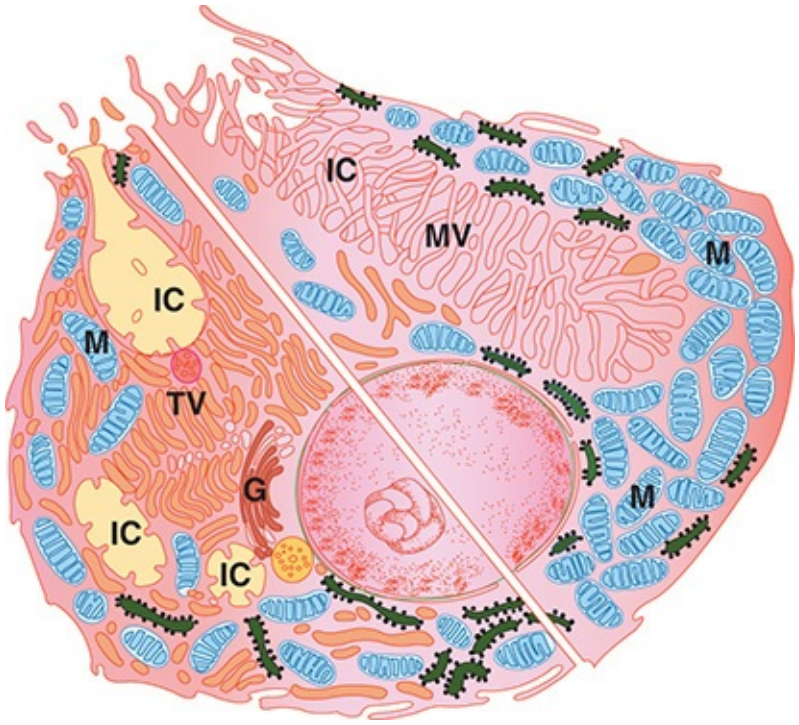


FIGURE 25–8 Composite diagram of a parietal cell, showing the resting state (lower left) and the active state (upper right). The resting cell has intracellular canaliculi (IC), which open on the apical membrane of the cell, and many tubulovesicular structures (TV) in the cytoplasm. When the cell is activated, the TVs fuse with the cell membrane and microvilli (MV) project into the canaliculi, so the area of cell membrane in contact with gastric lumen is greatly increased. M, mitochondrion; G, Golgi apparatus. (Reproduced with pemission of Ito S, Schofield GC: Studies on the depletion and accumulation of microvilli and changes in the tubulovesicular compartment of mouse parietal cells in relation to gastric acid secretion. J Cell Biol 1974; Nov; 63(2 Pt 1):364–382.)

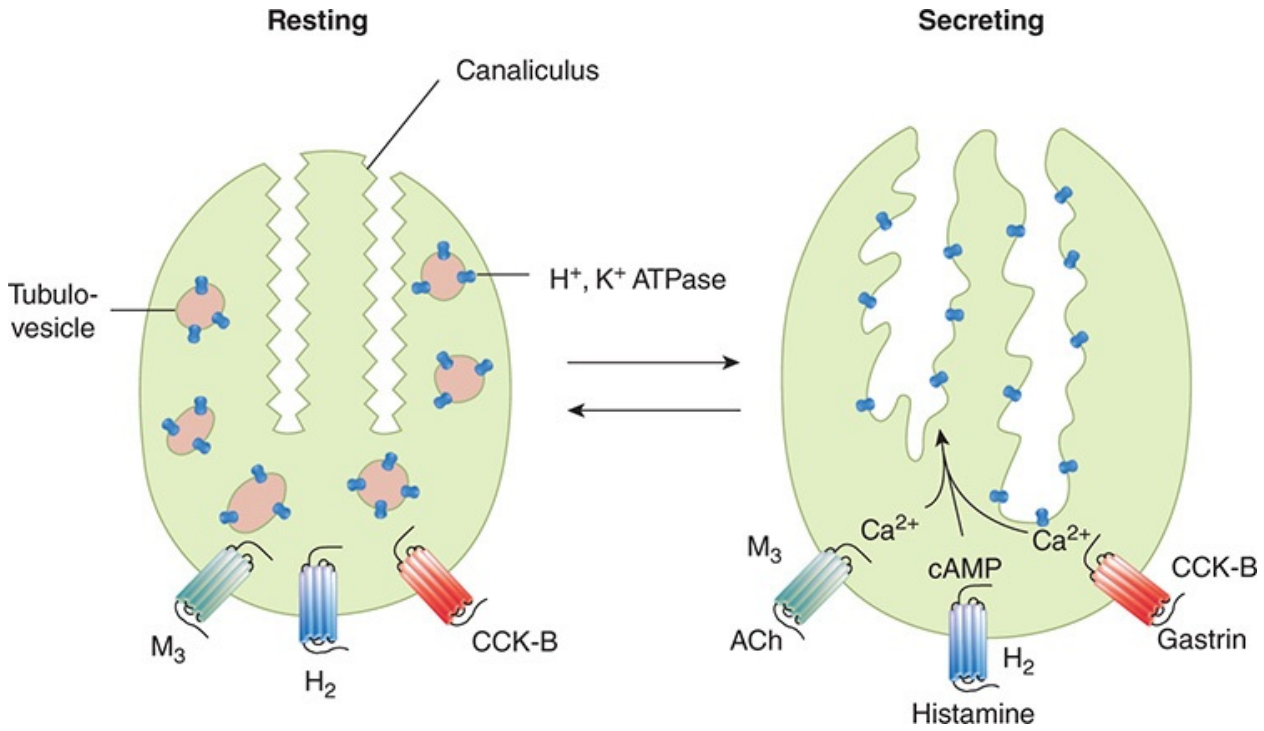


FIGURE 25–9 Parietal cell receptors and schematic representation of the morphologic changes depicted in Figure 25–8. Amplification of the apical surface area is accompanied by an increased density of H^+, K^+–ATPase molecules at this site. Note that acetylcholine (ACh) and gastrin signal via calcium, whereas histamine signals via cAMP. (Adapted with permission from Barrett KE: *Gastrointestinal Physiology*. New York, NY: McGraw-Hill; 2006.)

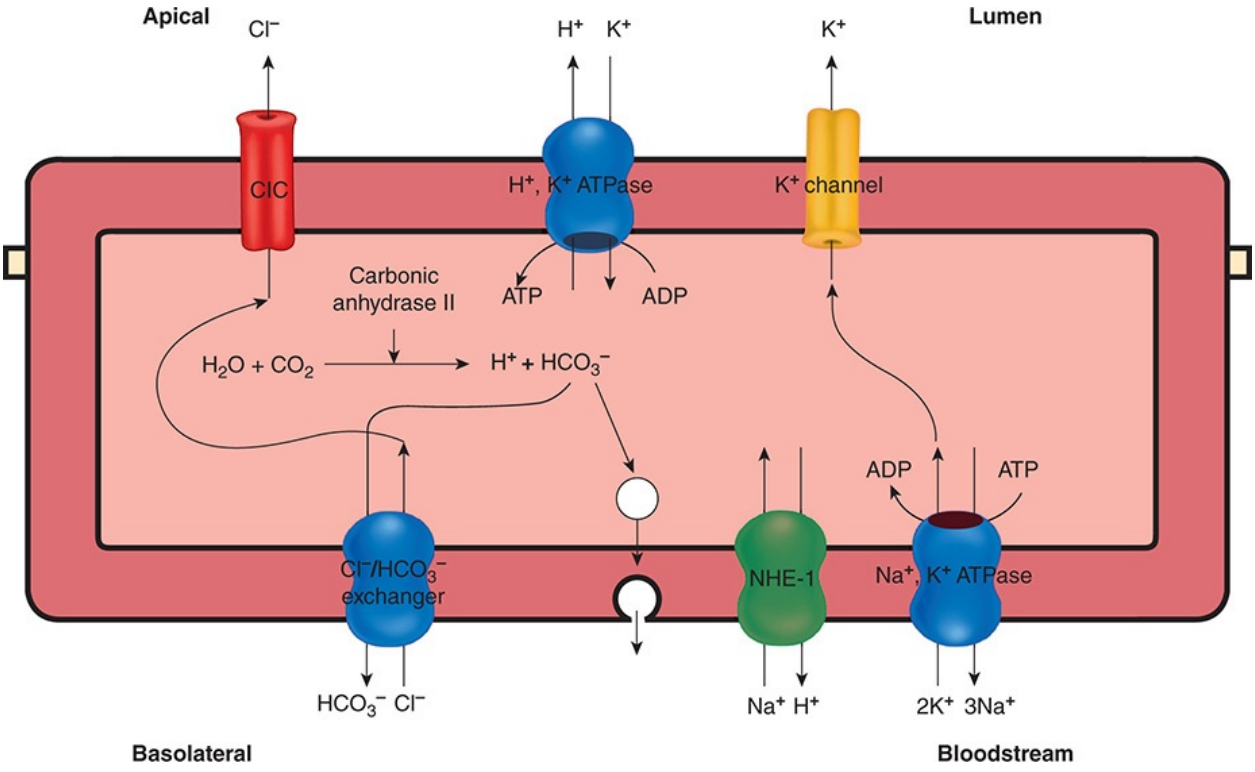


FIGURE 25–10 Ion transport proteins of parietal cells. Protons are generated in the cytoplasm via the action of carbonic anhydrase II. Bicarbonate ions are exported from the basolateral pole of the cell either by vesicular fusion or via a chloride/bicarbonate exchanger. The sodium/hydrogen exchanger, NHE1, on the basolateral membrane is considered a "housekeeping" transporter that maintains intracellular pH in the face of cellular metabolism during the unstimulated state.

The three agonists of the parietal cell—gastrin, histamine, and acetylcholine—each bind to distinct receptors on the basolateral membrane (Figure 25–9). Gastrin and acetylcholine promote secretion by elevating cytosolic free calcium concentrations, whereas histamine increases intracellular cyclic adenosine 3′,5′-monophosphate (cAMP). The net effects of these second messengers are the transport and morphologic changes described above. However, it is important to be aware that the two distinct pathways for activation are synergistic, with a greater than additive effect on secretion rates when histamine plus gastrin or acetylcholine, or all three, are present simultaneously. The physiologic significance of this synergism is that high rates of secretion can be stimulated with relatively small changes in availability of each of the stimuli. Synergism is also therapeutically significant because secretion can be markedly inhibited by blocking the action of only one of the triggers (most commonly that of histamine, via H_2-antagonists that are widely used therapies for adverse effects

of excessive gastric secretion) **(Clinical Box 25–1)**.

Gastric secretion adds about 2.5 L/day to the intestinal contents. However, despite their substantial volume and fine control, gastric secretions are dispensable for the full digestion and absorption of a meal, with the exception of cobalamin absorption. This illustrates an important facet of gastrointestinal physiology, namely that digestive and absorptive capacities are markedly in excess of normal requirements. On the other hand, if gastric secretion is chronically reduced, individuals may display increased susceptibility to infections acquired via the oral route.

PANCREATIC SECRETION

The pancreatic juice contains enzymes that are of major importance in digestion **(see Table 25–2)**. Its secretion is controlled in part by a reflex mechanism and in part by the gastrointestinal hormones secretin and cholecystokinin (CCK).

TABLE 25–2 Principal digestive enzymes.[a]

Source	Enzyme	Activator	Substrate	Catalytic Function or Products
Salivary glands	Salivary α-amylase	Cl^-	Starch	Hydrolyzes 1:4α linkages, producing α-limit dextrins, maltotriose, and maltose
Stomach	Pepsins (pepsinogens)	HCl	Proteins and polypeptides	Cleave peptide bonds adjacent to aromatic amino acids
	Gastric lipase		Triglycerides	Fatty acids and glycerol
Exocrine pancreas	Trypsin (trypsinogen)	Enteropeptidase	Proteins and polypeptides	Cleave peptide bonds on carboxyl side of basic amino acids (arginine or lysine)
	Chymotrypsins (chymotrypsinogens)	Trypsin	Proteins and polypeptides	Cleave peptide bonds on carboxyl side of aromatic amino acids
	Elastase (proelastase)	Trypsin	Elastin, some other proteins	Cleaves bonds on carboxyl side of aliphatic amino acids
	Carboxypeptidase A (procarboxypeptidase A)	Trypsin	Proteins and polypeptides	Cleave carboxyl terminal amino acids that have aromatic or branched aliphatic side chains
	Carboxypeptidase B (procarboxypeptidase B)	Trypsin	Proteins and polypeptides	Cleave carboxyl terminal amino acids that have basic side chains
	Colipase (procolipase)	Trypsin	Fat droplets	Binds pancreatic lipase to oil droplet in the presence of bile acids
	Pancreatic lipase	…	Triglycerides	Monoglycerides and fatty acids
	Cholesteryl ester hydrolase	…	Cholesteryl esters	Cholesterol
	Pancreatic α-amylase	Cl^-	Starch	Same as salivary α-amylase
	Ribonuclease	…	RNA	Nucleotides
	Deoxyribonuclease	…	DNA	Nucleotides
	Phospholipase A_2 (pro-phospholipase A_2)	Trypsin	Phospholipids	Fatty acids, lysophospholipids
Intestinal mucosa	Enteropeptidase	…	Trypsinogen	Trypsin
	Aminopeptidases	…	Polypeptides	Cleave amino terminal amino acid from peptide
	Carboxypeptidases	…	Polypeptides	Cleave carboxyl terminal amino acid from peptide
	Endopeptidases	…	Polypeptides	Cleave between residues in midportion of peptide
	Dipeptidases	…	Dipeptides	Two amino acids
	Maltase	…	Maltose, maltotriose	Glucose
	Lactase	…	Lactose	Galactose and glucose
	Sucrase[b]	…	Sucrose; also maltotriose and maltose	Fructose and glucose
	Isomaltase[b]	…	α-Limit dextrins, maltose Maltotriose	Glucose
	Nuclease and related enzymes	…	Nucleic acids	Pentoses and purine and pyrimidine bases
Cytoplasm of mucosal cells	Various peptidases	…	Di-, tri-, and tetrapeptides	Amino acids

[a]Corresponding proenzymes, where relevant, are shown in parentheses.

[b]Sucrase and isomaltase are separate subunits of a single protein.

ANATOMIC CONSIDERATIONS

The portion of the pancreas that secretes pancreatic juice is a compound alveolar gland resembling the salivary glands. This **exocrine pancreas** should be

distinguished from the **endocrine pancreas** that produces insulin and other hormones from the islets of Langerhans (see Chapter 24), although both are contained within the same organ. Granules containing the digestive enzymes **(zymogen granules)** are formed in the cell and discharged by exocytosis (see Chapter 2) from the apexes of the cells into the lumens of the pancreatic ducts **(Figure 25–11)**. The small duct radicles coalesce into a single duct (pancreatic duct of Wirsung), which joins the bile duct to form the ampulla of the bile duct (also known as the ampulla of Vater) **(Figure 25–12)**. The ampulla opens through the duodenal papilla, and its orifice is encircled by the sphincter of Oddi.

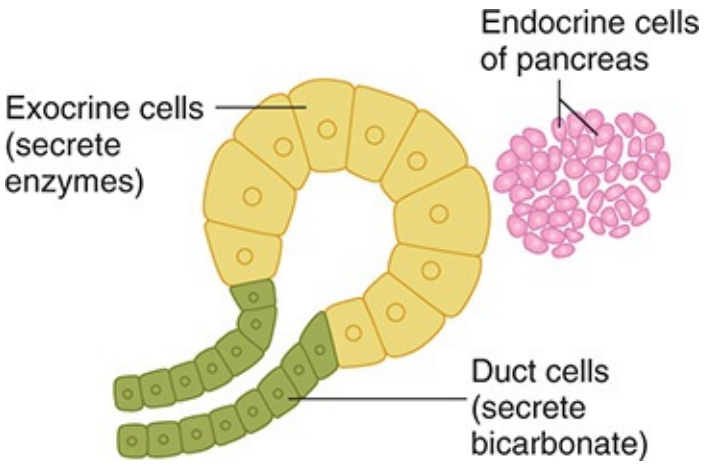


FIGURE 25–11 Structure of the pancreas. (Reproduced with permission from Widmaier EP, Raff H, Strang KT: *Vander's Human Physiology: The Mechanisms of Body Function*, 11th ed. New York, NY: McGraw-Hill; 2008.)

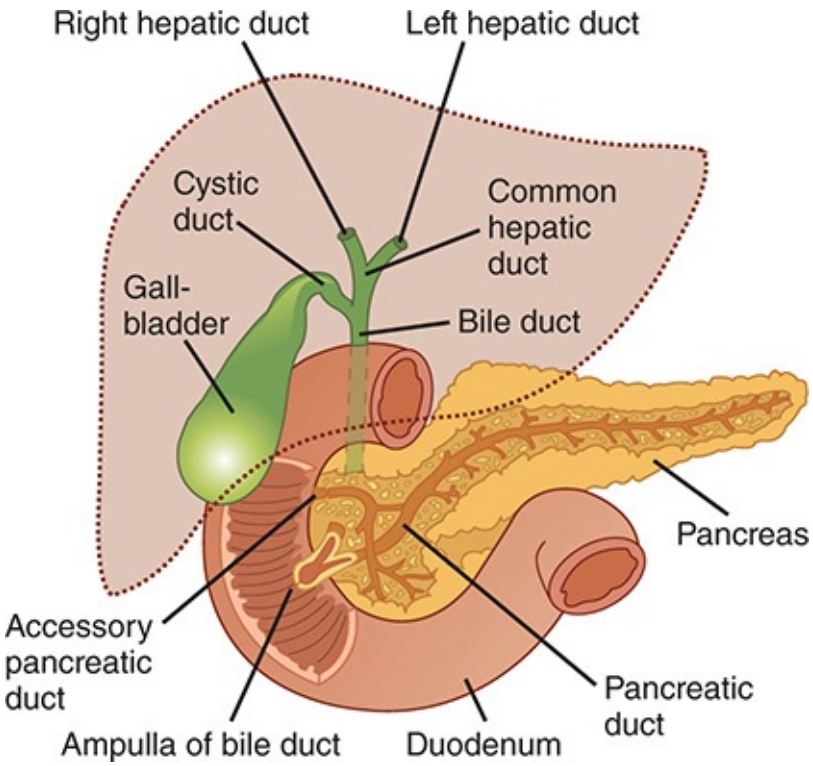


FIGURE 25–12 Connections of the ducts of the gallbladder, liver, and pancreas. (Adapted with permission from Bell GH, Emslie-Smith D, Paterson CR: *Textbook of Physiology and Biochemistry*, 9th ed. Churchill Livingstone, 1976.)

CLINICAL BOX 25–1

Peptic Ulcer Disease

Gastric and duodenal ulceration in humans is related primarily to a breakdown of the barrier that normally prevents irritation and autodigestion of the mucosa by the gastric secretions. Infection with the bacterium *Helicobacter pylori* disrupts this barrier, as do aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit the production of prostaglandins and consequently decrease mucus and HCO_3^- secretion. NSAIDs are widely used to combat pain and treat arthritis. An additional cause of ulceration is prolonged excess secretion of acid. An example of this is the ulcers that occur in the **Zollinger–Ellison syndrome**. This syndrome is seen in patients with gastrinomas. These tumors can occur in the stomach and duodenum, but most of them are found in the pancreas. The gastrin causes

prolonged hypersecretion of acid, and severe ulcers are produced.

THERAPEUTIC HIGHLIGHTS

Gastric and duodenal ulcers can be given a chance to heal by inhibition of acid secretion with drugs such as omeprazole and related drugs that inhibit H^+–K^+ ATPase ("proton pump inhibitors") or with histamine H_2-receptor antagonists. If present, *H. pylori* can be eradicated with antibiotics, and NSAID-induced ulcers can be treated by stopping the NSAID or, when this is not advisable, by treatment with the prostaglandin agonist misoprostol. Gastrinomas can sometimes be removed surgically.

COMPOSITION OF PANCREATIC JUICE

The pancreatic juice is alkaline **(Table 25–3)** and has a high HCO_3^- content (approximately 113 mEq/L vs 24 mEq/L in plasma). About 1500 mL of pancreatic juice is secreted per day. Bile and intestinal juices are also neutral or alkaline, and these three secretions neutralize the gastric acid, raising the pH of the duodenal contents to 6.0–7.0. By the time the chyme reaches the jejunum, its pH is nearly neutral.

TABLE 25–3 Composition of normal human pancreatic juice.

Cations: Na^+, K^+, Ca^{2+}, Mg^{2+} (pH approximately 8.0)
Anions: HCO_3^-, Cl^-, SO_4^{2-}, HPO_4^{2-}
Digestive enzymes (see Table 25–2; 95% of protein in juice)
Other proteins

The pancreatic juice also contains a range of digestive enzymes, but most of these are released in inactive forms and only activated when they reach the intestinal lumen (see Chapter 26). The enzymes are activated following proteolytic cleavage by trypsin, itself a pancreatic protease that is released as an inactive precursor (trypsinogen). The potential danger of the release within the pancreas of a small amount of active trypsin is apparent; the resulting chain reaction would produce active enzymes that could digest the organ. It is therefore not surprising that the pancreas also normally secretes a trypsin

inhibitor.

Another enzyme activated by trypsin is phospholipase A_2. This enzyme splits a fatty acid off phosphatidylcholine (PC), forming lyso-PC. Lyso-PC damages cell membranes. It has been hypothesized that in **acute pancreatitis,** a severe and sometimes fatal disease, phospholipase A_2 is activated prematurely in the pancreatic ducts, with the formation of lyso-PC from the PC that is a normal constituent of bile. This causes disruption of pancreatic tissue and necrosis of surrounding fat.

Small amounts of pancreatic digestive enzymes normally leak into the circulation, but in acute pancreatitis, the circulating levels of the digestive enzymes rise markedly. Measurement of the plasma amylase or lipase concentration is therefore of value in diagnosing the disease.

REGULATION OF THE SECRETION OF PANCREATIC JUICE

Secretion of pancreatic juice is primarily under hormonal control. Secretin acts on the pancreatic ducts to cause copious secretion of a very alkaline pancreatic juice that is rich in HCO_3^- and poor in enzymes. The effect on duct cells is due to an increase in intracellular cAMP. Secretin also stimulates bile secretion. CCK acts on the acinar cells to cause the release of zymogen granules and production of pancreatic juice rich in enzymes but low in volume. Its effect is mediated by phospholipase C (see Chapter 2). Thus, by acting together, CCK and secretin add enzymes to the pancreatic juice and ensure that they are washed into the intestine.

The response to intravenous secretin is shown in **Figure 25–13**. Note that as the volume of pancreatic secretion increases, its Cl^- concentration falls and its HCO_3^- concentration increases. Although HCO_3^- is secreted in the small ducts, it is reabsorbed in the large ducts in exchange for Cl^- **(Figure 25–14)**. The magnitude of the exchange is inversely proportional to the rate of flow.

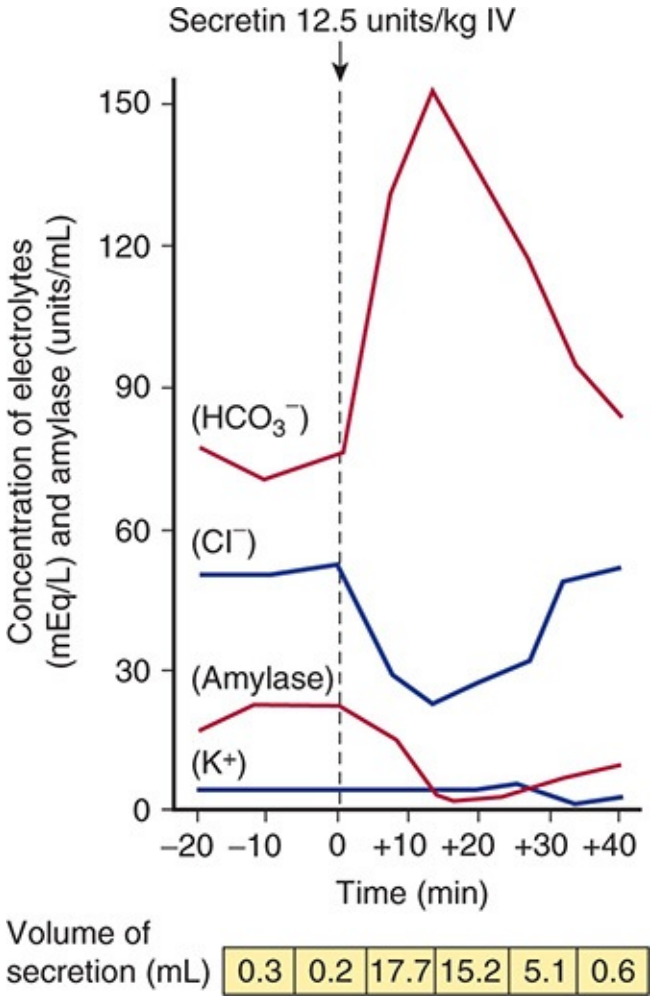


FIGURE 25–13 Effect of a single dose of secretin on the composition and volume of the pancreatic juice in humans. Note the reciprocal changes in the concentrations of chloride and bicarbonate after secretin is infused. The fall in amylase concentration reflects dilution as the volume of pancreatic juice increases.

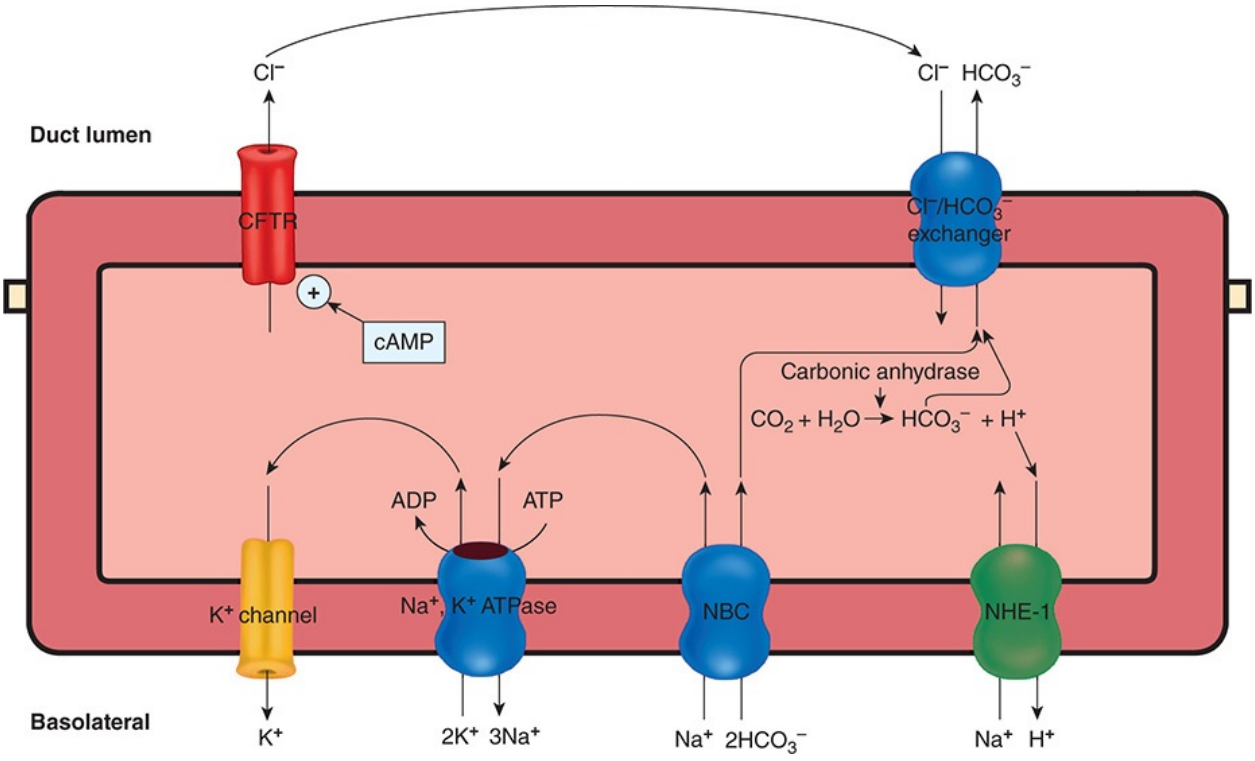


FIGURE 25–14 Ion transport pathways present in pancreatic duct cells. CFTR, cystic fibrosis transmembrane conductance regulator; NHE-1, sodium/hydrogen exchanger-1; NBC, sodium-bicarbonate cotransporter.

Like CCK, acetylcholine acts on acinar cells via phospholipase C to cause discharge of zymogen granules, and stimulation of the vagi causes secretion of a small amount of pancreatic juice rich in enzymes. There is evidence for vagally mediated, conditioned reflex secretion of pancreatic juice in response to the sight or smell of food.

BILIARY SECRETION

An additional secretion important for gastrointestinal function, bile, arises from the liver. The bile acids contained therein are important in the digestion and absorption of fats. In addition, bile serves as a critical excretory fluid by which the body disposes of lipid soluble end products of metabolism as well as lipid soluble xenobiotics. Bile is also the only route by which the body can dispose of cholesterol—either in its native form, or following conversion to bile acids. In this chapter and the next, the role of bile as a digestive fluid will be emphasized. In Chapter 28, a more general consideration of the transport and metabolic

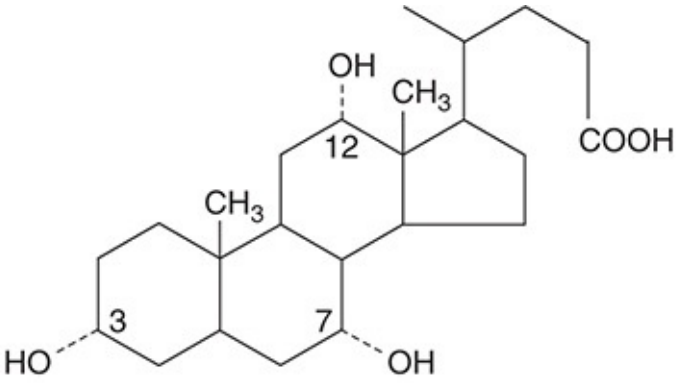
functions of the liver will be presented.

Bile

Bile is made up of the bile acids, bile pigments, and other substances dissolved in an alkaline electrolyte solution that resembles pancreatic juice. About 500 mL is secreted per day. Some of the components of the bile are reabsorbed in the intestine and then excreted again by the liver **(enterohepatic circulation)**.

The glucuronides of the **bile pigments,** bilirubin and biliverdin, are responsible for the golden yellow color of bile. The formation of these breakdown products of hemoglobin is discussed in detail in Chapter 28.

When considering bile as a digestive secretion, it is the **bile acids** that represent the most important components. They are synthesized from cholesterol and secreted into the bile conjugated to glycine or taurine. The four major bile acids found in humans are listed in **Figure 25–15**. In common with vitamin D, cholesterol, and a variety of steroid hormones, bile acids contain the steroid nucleus (see Chapter 19). The two principal (primary) bile acids formed in the liver are cholic acid and chenodeoxycholic acid. In the colon, bacteria convert cholic acid to deoxycholic acid and chenodeoxycholic acid to lithocholic acid. In addition, small quantities of ursodeoxycholic acid are formed from chenodeoxycholic acid. Because they are formed by bacterial action, deoxycholic, lithocholic, and ursodeoxycholic acids are called secondary bile acids.



Cholic acid

	Group at position			Percent in human bile
	3	7	12	
Cholic acid	OH	OH	OH	50
Chenodeoxycholic acid	OH	OH	H	30
Deoxycholic acid	OH	H	OH	15
Lithocholic acid	OH	H	H	5

FIGURE 25–15 Human bile acids. The numbers in the formula for cholic acid refer to the positions in the steroid ring.

The bile acids have a number of important actions: they reduce surface tension and, in conjunction with phospholipids and monoglycerides, are responsible for the emulsification of fat preparatory to its digestion and absorption in the small intestine (see Chapter 26). They are **amphipathic,** that is, they have both hydrophilic and hydrophobic domains; one surface of the molecule is hydrophilic because the polar peptide bond and the carboxyl and hydroxyl groups are on that surface, whereas the other surface is hydrophobic. Therefore, the bile acids tend to form cylindrical disks called **micelles (Figure 25–16)**. Their hydrophilic portions face out and their hydrophobic portions face in. Above a certain concentration, called the **critical micellar concentration,** all bile salts added to a solution form micelles. Ninety to 95% of the bile acids are absorbed from the small intestine. Once they are deconjugated, they can be absorbed by nonionic diffusion, but most are absorbed in their conjugated forms from the terminal ileum **(Figure 25–17)** by an extremely efficient Na^+–bile salt cotransport system (ABST) whose activity is secondarily driven by the low intracellular sodium concentration established by the basolateral Na^+, K^+ ATPase. The remaining 5–10% of the bile salts enter the colon and are converted to the salts of deoxycholic acid and lithocholic acid. Lithocholate is relatively

insoluble and is mostly excreted in the stools; only 1% is absorbed. However, deoxycholate is absorbed.

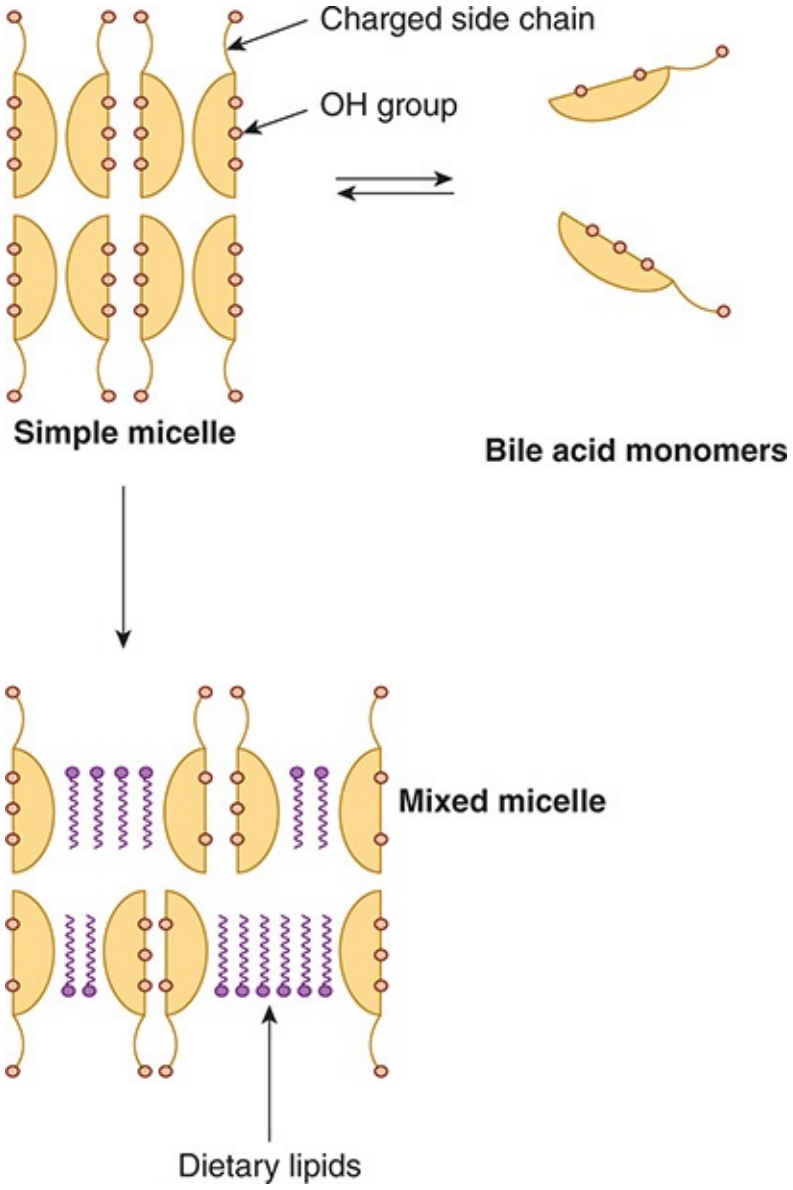


FIGURE 25–16 Physical forms adopted by bile acids in solution. Micelles are shown in cross-section and are actually thought to be cylindrical in shape. Mixed micelles of bile acids present in intestinal contents also incorporate dietary lipids. (Adapted with permission from Barrett KE: *Gastrointestinal Physiology*. New York, NY: McGraw-Hill; 2006.)

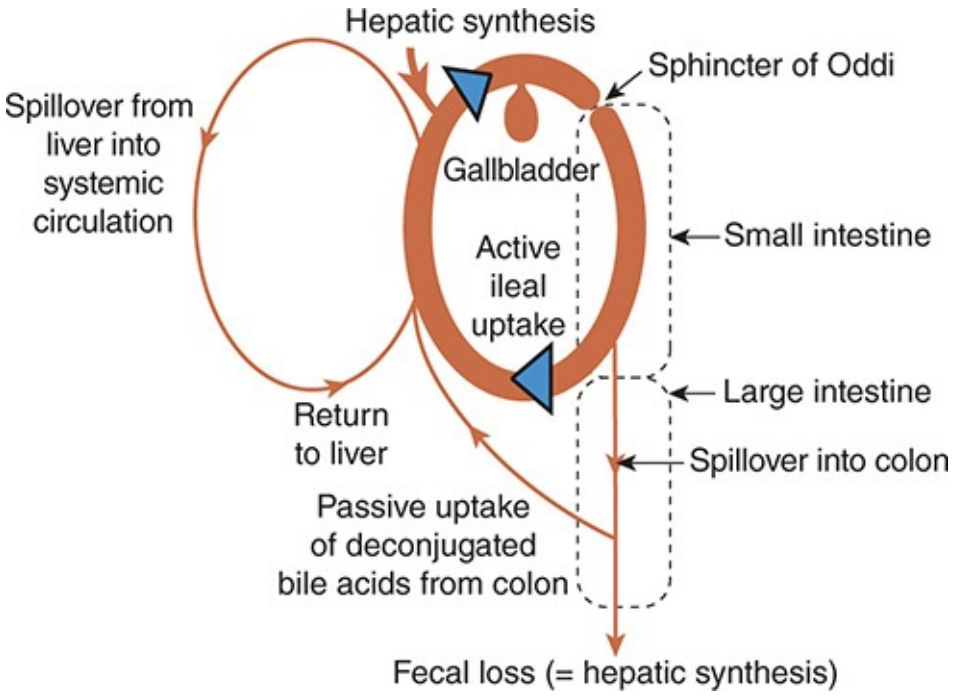


FIGURE 25–17 Quantitative aspects of the circulation of bile acids. The majority of the bile acid pool circulates between the small intestine and liver. A minority of the bile acid pool is in the systemic circulation (due to incomplete hepatocyte uptake from the portal blood) or spills over into the colon and is lost to the stool. Fecal loss must be equivalent to hepatic synthesis of bile acids at steady state. (Adapted with permission from Barrett KE: *Gastrointestinal Physiology*. New York, NY: McGraw-Hill; 2006.)

The absorbed bile acids are transported back to the liver in the portal vein and reexcreted in the bile (enterohepatic circulation) (Figure 25–17). Those lost in the stool are replaced by synthesis in the liver; the normal rate of bile acid synthesis is 0.2–0.4 g/day. The total bile acid pool of approximately 3.5 g recycles repeatedly via the enterohepatic circulation; it has been calculated that the entire pool recycles twice per meal and 6–8 times per day.

GASTROINTESTINAL REGULATION

The various functions of the gastrointestinal tract, including secretion, digestion, and absorption (Chapter 26), and motility (Chapter 27), must be regulated in an integrated way to ensure efficient assimilation of nutrients after a meal. There are three main modalities for gastrointestinal regulation that operate in a complementary fashion to ensure that function is appropriate. First, **endocrine**

regulation is mediated by the release of hormones by triggers associated with the meal. These hormones travel through the bloodstream to change the activity of a distant segment of the gastrointestinal tract, an organ draining into it (eg, the pancreas), or both. Second, some similar mediators are not sufficiently stable to persist in the bloodstream, but instead alter the function of cells in the local area where they are released, in a **paracrine** fashion. Finally, the intestinal system is endowed with extensive neural connections. These include connections to the CNS **(extrinsic innervation),** but also the activity of the largely autonomous **enteric nervous system** that comprises both sensory and secretomotor neurons. The enteric nervous system integrates central input to the gut but can also regulate gut function independently in response to changes in the luminal environment. In some cases, the same substance can mediate regulation by endocrine, paracrine, and neurocrine pathways (eg, CCK, see below).

HORMONES/PARACRINES

Biologically active polypeptides that are secreted by nerve cells and gland cells in the mucosa act in a paracrine fashion, but they also enter the circulation. Measurement of their concentrations in blood after a meal has shed light on the roles these **gastrointestinal hormones** play in the regulation of gastrointestinal secretion and motility.

When large doses of the hormones are given, their actions overlap. However, their physiologic effects appear to be relatively discrete. On the basis of structural similarity and, to a degree, similarity of function, the key hormones fall into one of two families: the gastrin family, the primary members of which are gastrin and CCK; and the secretin family, the primary members of which are secretin, glucagon, vasoactive intestinal peptide (VIP; actually a neurotransmitter, or neurocrine), and gastric inhibitory polypeptide (also known as glucose-dependent insulinotropic peptide, or GIP). There are also other regulatory peptides that do not fall into either of these families.

ENTEROENDOCRINE CELLS

More than 15 types of hormone-secreting **enteroendocrine cells** have been identified in the mucosa of the stomach, small intestine, and colon. Many of these secrete only one hormone and are identified by letters (G cells, S cells, etc). Others manufacture serotonin or histamine and are called **enterochromaffin** or **ECL cells,** respectively.

GASTRIN

Gastrin is produced by cells called G cells in the antral portion of the gastric mucosa **(Figure 25–18)**. G cells are flask-shaped, with a broad base containing many gastrin granules and a narrow apex that reaches the mucosal surface. Microvilli project from the apical end into the lumen. Receptors mediating gastrin responses to changes in gastric contents are present on the microvilli. Other cells in the gastrointestinal tract that secrete hormones have a similar morphology.

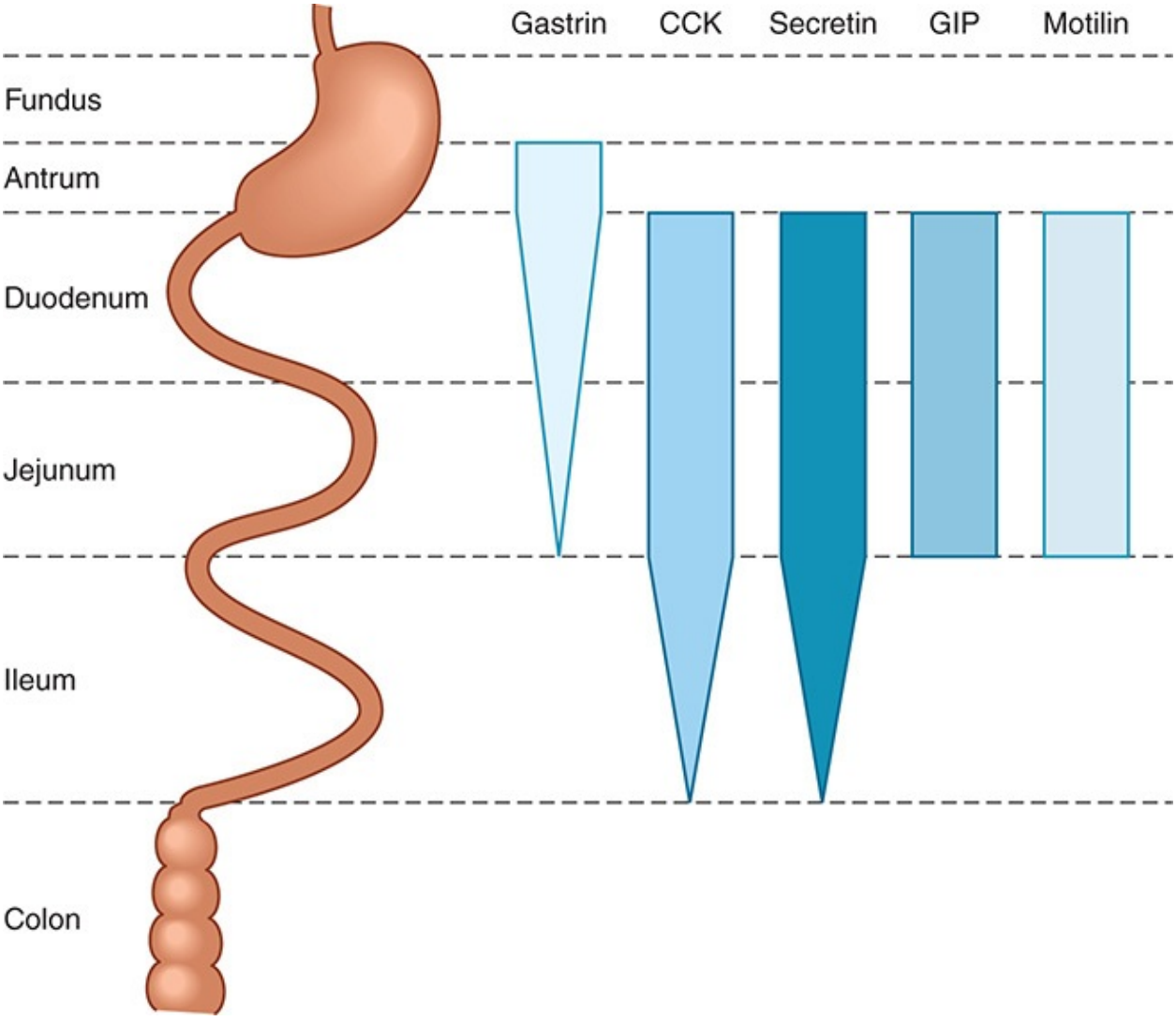


FIGURE 25–18 Sites of production of the five gastrointestinal hormones along the length of the gastrointestinal tract. The width of the bars reflects the relative abundance at each location.

The precursor for gastrin, preprogastrin, is processed into fragments of various sizes. Three main fragments contain 34, 17, and 14 amino acid residues. All have the same carboxyl terminal. These forms are also known as G 34, G 17, and G 14 gastrins, respectively. Gastrins may also be derivatized with sulfation of the tyrosine that is the sixth amino acid residue from the carboxyl terminal. Approximately equal amounts of nonsulfated and sulfated forms are present in blood and tissues, and they are equally active. The carboxyl terminal phenylalanine is also amidated, which likely enhances the peptide's stability in the plasma by rendering it resistant to carboxypeptidases. G 14 and G 17 have half-lives of 2–3 min in the circulation, whereas G 34 has a half-life of 15 min. Gastrins are inactivated primarily in the kidney and small intestine.

The principal physiologic actions of gastrin are stimulation of gastric acid and pepsin secretion and stimulation of the growth of the mucosa of the stomach and small and large intestines **(trophic action)**. Gastrin secretion is affected by the contents of the stomach, the rate of discharge of the vagus nerves, and bloodborne factors **(Table 25–4)**. Gastrin secretion is also increased by the presence of the products of protein digestion in the stomach, particularly amino acids, which act directly on the G cells. Phenylalanine and tryptophan are particularly effective. Gastrin acts via a receptor (CCK-B) that is related to the primary receptor (CCK-A) for cholecystokinin (see below). This likely reflects the structural similarity of the two hormones, and may result in some overlapping actions if excessive quantities of either hormone are present (eg, in the case of a gastrin-secreting tumor, or gastrinoma).

TABLE 25–4 Stimuli that affect gastrin secretion.

Stimuli that increase gastrin secretion
Luminal
Peptides and amino acids
Distention
Neural
Increased vagal discharge via GRP
Bloodborne
Calcium
Epinephrine
Stimuli that inhibit gastrin secretion
Luminal
Acid
Somatostatin
Bloodborne
Secretin, GIP, VIP, glucagon, calcitonin

Acid in the antrum inhibits gastrin secretion, partly by a direct action on G cells and partly by release of somatostatin, a relatively potent inhibitor of gastrin secretion. The effect of acid is the basis of a negative feedback loop regulating gastrin secretion. Increased secretion of the hormone increases acid secretion, but the acid then feeds back to inhibit further gastrin secretion. In conditions such as pernicious anemia in which the acid-secreting cells of the stomach are damaged, gastrin secretion is chronically elevated.

CHOLECYSTOKININ

CCK is secreted by endocrine cells known as I cells in the mucosa of the upper small intestine. It has a plethora of actions in the gastrointestinal system, but the most important appear to be the stimulation of pancreatic enzyme secretion; the contraction of the gallbladder (the action for which it was named); and relaxation of the sphincter of Oddi, which allows both bile and pancreatic juice to flow into the intestinal lumen.

Like gastrin, CCK is produced from a larger precursor. Prepro-CCK is also processed into many fragments, all of which have the same five amino acids at the carboxyl terminal as gastrin. The carboxyl terminal is amidated, and the tyrosine that is the seventh amino acid residue from the carboxyl terminal is

sulfated. Unlike gastrin, the nonsulfated form of CCK has not been found in tissues. The half-life of circulating CCK is about 5 min, but little is known about its metabolism.

In addition to its secretion by I cells, CCK is found in nerves in the distal ileum and colon. It is also found in neurons in the brain, especially the cerebral cortex, and in nerves in many parts of the body (see Chapter 7). In the brain, it may be involved in the regulation of food intake.

In addition to its primary actions, CCK augments the action of secretin in producing secretion of an alkaline pancreatic juice. It also inhibits gastric emptying, exerts a trophic effect on the pancreas, increases the synthesis of enterokinase (see chapter 26), and may enhance the motility of the small intestine and colon. There is some evidence that, along with secretin, it augments the contraction of the pyloric sphincter, thus preventing the reflux of duodenal contents into the stomach. Two CCK receptors have been identified. CCK-A receptors are primarily located in the periphery, whereas both CCK-A and CCK-B (gastrin) receptors are found in the brain. Both activate PLC, and signal via calcium (see Chapter 2).

The secretion of CCK is increased by contact of the intestinal mucosa with the products of digestion, particularly peptides and amino acids, and also by the presence in the duodenum of fatty acids containing more than 10 carbon atoms. There are also two protein-releasing factors that activate CCK secretion, known as CCK-releasing peptide and monitor peptide, which derive from the intestinal mucosa and pancreas, respectively. Because the bile and pancreatic juice that enter the duodenum in response to CCK enhance the digestion of protein and fat, and the products of this digestion stimulate further CCK secretion, a sort of positive feedback operates in the control of CCK secretion. However, the positive feedback is terminated when the products of digestion move on to the lower portions of the gastrointestinal tract, and also because CCK-releasing peptide and monitor peptide are degraded by proteolytic enzymes once these are no longer occupied in digesting dietary proteins.

SECRETIN

Secretin occupies a unique position in the history of physiology. In 1902, Bayliss and Starling first demonstrated that the excitatory effect of duodenal stimulation on pancreatic secretion was due to a bloodborne factor. Their research led to the identification of the first hormone, secretin. Secretin is secreted by S cells that are located deep in the glands of the mucosa of the upper portion of the small

intestine. The structure of secretin is different from that of CCK and gastrin, but very similar to that of GIP, glucagon, and VIP. Only one active form of secretin has been isolated. Its half-life is about 5 min, but little is known about its metabolism.

Secretin increases the secretion of bicarbonate by the duct cells of the pancreas and biliary tract. It thus causes the secretion of a watery, alkaline pancreatic juice. Its action on pancreatic duct cells is mediated via cAMP. It also augments the action of CCK in producing pancreatic secretion of digestive enzymes. It decreases gastric acid secretion and may cause contraction of the pyloric sphincter.

The secretion of secretin is increased by the products of protein digestion and by acid bathing the mucosa of the upper small intestine. The release of secretin by acid is another example of feedback control: Secretin causes alkaline pancreatic juice to flood into the duodenum, neutralizing the acid from the stomach and thus inhibiting further secretion of the hormone.

GIP

GIP contains 42 amino acid residues and is produced by K cells in the mucosa of the duodenum and jejunum. Its secretion is stimulated by glucose and fat in the duodenum, and because in large doses it inhibits gastric secretion and motility, it was named gastric inhibitory peptide. However, it now appears that it does not have significant gastric inhibiting activity when administered in amounts comparable to those seen after a meal. In the meantime, it was found that GIP stimulates insulin secretion at physiological levels. For this reason, it is often called **glucose-dependent insulinotropic peptide**. The glucagon derivative GLP-1 (7–36) (see Chapter 24) also stimulates insulin secretion and may also be a physiologic B cell–stimulating hormone of the gastrointestinal tract.

The integrated action of gastrin, CCK, secretin, and GIP in facilitating digestion and utilization of absorbed nutrients is summarized in **Figure 25–19**.

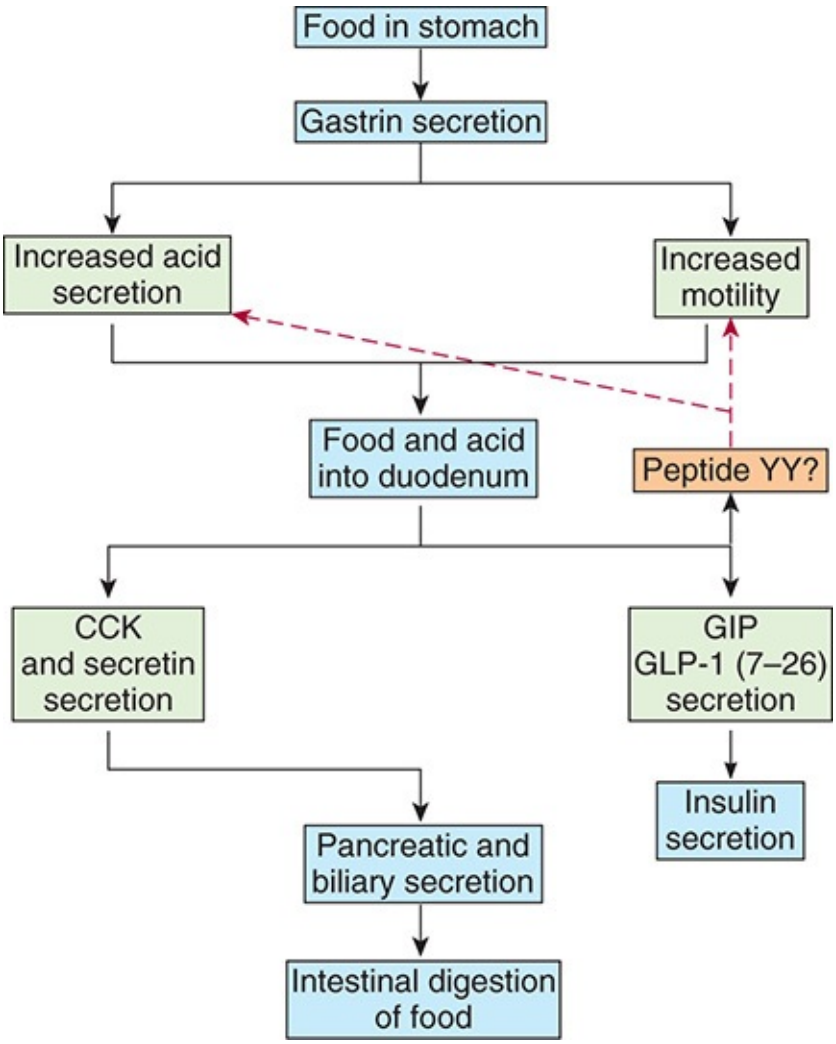


FIGURE 25–19 Integrated action of gastrointestinal hormones in regulating digestion and utilization of absorbed nutrients. The dashed arrows indicate inhibition. The exact identity of the hormonal factor or factors from the intestine that inhibit(s) gastric acid secretion and motility is unsettled, but it may be peptide YY.

VIP

VIP contains 28 amino acid residues. It is found in nerves in the gastrointestinal tract and thus is not itself a hormone, despite its similarities to secretin. VIP is, however, found in blood, in which it has a half-life of about 2 min. In the intestine, it markedly stimulates intestinal secretion of electrolytes and hence of water. Its other actions include relaxation of intestinal smooth muscle, including sphincters; dilation of peripheral blood vessels; and inhibition of gastric acid

secretion. It is also found in the brain and many autonomic nerves (see Chapter 7), where it often occurs in the same neurons as acetylcholine. It potentiates the action of acetylcholine in salivary glands. However, VIP and acetylcholine do not coexist in neurons that innervate other parts of the gastrointestinal tract. VIP-secreting tumors (VIPomas) have been described in patients with severe diarrhea.

MOTILIN

Motilin is a polypeptide containing 22 amino acid residues that is secreted by enterochromaffin cells and Mo cells in the stomach, small intestine, and colon. It acts on G-protein-coupled receptors on enteric neurons in the duodenum and colon and produces contraction of smooth muscle in the stomach and intestines in the period between meals (see Chapter 27).

SOMATOSTATIN

Somatostatin, the growth hormone–inhibiting hormone originally isolated from the hypothalamus, is secreted as a paracrine by D cells in the pancreatic islets (see Chapter 24) and by similar D cells in the gastrointestinal mucosa. It exists in tissues in two forms, somatostatin 14 and somatostatin 28, and both are secreted. Somatostatin inhibits the secretion of gastrin, VIP, GIP, secretin, and motilin. Its secretion is stimulated by acid in the lumen, and it acts in a paracrine fashion to mediate the inhibition of gastrin secretion produced by acid. It also inhibits pancreatic exocrine secretion; gastric acid secretion and motility; gallbladder contraction; and the absorption of glucose, amino acids, and triglycerides.

OTHER GASTROINTESTINAL PEPTIDES

Peptide YY

The structure of peptide YY is discussed in Chapter 24. It inhibits gastric acid secretion and motility and is a good candidate to be the gastric inhibitory peptide (Figure 25–19). Its release from the jejunum is stimulated by fat.

Others

Ghrelin is secreted primarily by the stomach and appears to play an important role in the central control of feeding (see Chapter 26). It also stimulates growth hormone secretion by acting directly on receptors in the pituitary (see Chapter 18). Its levels increase before a meal and exogenous administration of this hormone markedly enhances appetite and food intake. Secretion is markedly reduced in patients who have undergone gastric bypass surgery for the treatment of severe obesity, which may contribute to the efficacy of the treatment.

Substance P is found in endocrine and nerve cells in the gastrointestinal tract and may enter the circulation. It increases the motility of the small intestine. The neurotransmitter **GRP** is present in the vagal nerve endings that terminate on G cells and is the neurotransmitter producing vagally mediated increases in gastrin secretion. **Glucagon** from the gastrointestinal tract may be responsible (at least in part) for the hyperglycemia seen after pancreatectomy.

Guanylin is a gastrointestinal polypeptide that binds to guanylyl cyclase. It is made up of 15 amino acid residues and is secreted by cells of the intestinal mucosa. Stimulation of guanylyl cyclase increases the concentration of intracellular cyclic 3′,5′-guanosine monophosphate (cGMP), and this in turn causes increased secretion of Cl^- into the intestinal lumen. Guanylin appears to act predominantly in a paracrine fashion, and it is produced in cells from the pylorus to the rectum. In an interesting example of molecular mimicry, the heat-stable enterotoxin of certain diarrhea-producing strains of *Escherichia coli* has a structure very similar to guanylin and activates guanylin receptors in the intestine. Guanylin receptors are also found in the kidneys, the liver, and the female reproductive tract, and guanylin may act in an endocrine fashion to regulate fluid movement in these tissues as well, and particularly to integrate the actions of the intestine and kidneys.

THE ENTERIC NERVOUS SYSTEM

Two major networks of nerve fibers are intrinsic to the gastrointestinal tract: the **myenteric plexus** between the outer longitudinal and middle circular muscle layers, and the **submucous plexus** between the middle circular layer and the mucosa (Figure 25–2). Collectively, these neurons constitute the **enteric nervous system.** The system contains about 100 million sensory neurons, interneurons, and motor neurons in humans—as many as are found in the whole spinal cord—and the system is probably best viewed as a displaced part of the CNS that is concerned with the regulation of gastrointestinal function. It is sometimes referred to as the “little brain” for this reason. It is connected to the

CNS by parasympathetic and sympathetic fibers but can function autonomously without these connections (see below). The myenteric plexus innervates the longitudinal and circular smooth muscle layers and is concerned primarily with motor control, whereas the submucous plexus innervates the glandular epithelium, intestinal endocrine cells, and submucosal blood vessels and is primarily involved in the control of intestinal secretion. The neurotransmitters in the system include acetylcholine, the amines norepinephrine and serotonin, the amino acid γ-aminobutyrate (GABA), the purine adenosine triphosphate (ATP), the gases NO and CO, and many different peptides and polypeptides. Some of these peptides also act in a paracrine fashion, and some enter the bloodstream, becoming hormones. Not surprisingly, most of them are also found in the brain.

EXTRINSIC INNERVATION

The intestine receives a dual extrinsic innervation from the autonomic nervous system, with parasympathetic cholinergic activity generally increasing the activity of intestinal smooth muscle and sympathetic noradrenergic activity generally decreasing it while causing sphincters to contract. The preganglionic parasympathetic fibers consist of about 2000 vagal efferents and other efferents in the sacral nerves. They generally end on cholinergic nerve cells of the myenteric and submucous plexuses. The sympathetic fibers are postganglionic, but many of them end on postganglionic cholinergic neurons, where the norepinephrine they secrete inhibits acetylcholine secretion by activating α_2 presynaptic receptors. Other sympathetic fibers appear to end directly on intestinal smooth muscle cells. The electrical properties of intestinal smooth muscle are discussed in Chapter 5. Still other fibers innervate blood vessels, where they produce vasoconstriction. It appears that the intestinal blood vessels have a dual innervation: they have an extrinsic noradrenergic innervation and an intrinsic innervation by fibers of the enteric nervous system. VIP and NO are among the mediators in the intrinsic innervation, which seems, among other things, to be responsible for the increase in local blood flow **(hyperemia)** that accompanies digestion of food. It is unsettled whether the blood vessels have an additional cholinergic innervation.

GASTROINTESTINAL (MUCOSAL) IMMUNE SYSTEM

The mucosal immune system was mentioned in Chapter 3, but it bears repeating here that the continuity of the intestinal lumen with the outside world also makes the gastrointestinal system an important portal for infection. Similarly, the intestine benefits from interactions with a complex community of commensal (ie, nonpathogenic) bacteria that provide beneficial metabolic functions as well as likely increasing resistance to pathogens. In the face of this constant microbial stimulation, it is not surprising that the intestine of mammals has developed a sophisticated set of both innate and adaptive immune mechanisms to distinguish friend from foe. Indeed, the intestinal mucosa contains more lymphocytes than are found in the circulation, as well as large numbers of inflammatory cells that are placed to rapidly defend the mucosa if epithelial defenses are breached. It is likely that immune cells, and their products, also impact the physiologic function of the epithelium, endocrine cells, nerves and smooth muscle, particularly at times of infection and if inappropriate immune responses are perpetuated, such as in inflammatory bowel diseases (see Chapter 3).

A massive amount of information is emerging about the nature and complexity of the microbes that make up the intestinal microbiota, and the potential roles of these microbes in health and disease both within and beyond the gastrointestinal system. The number of microbes increases dramatically as one moves aborally, and is highest in the colon where the microbiota is dominated by strict anaerobes. The microbes supply a large number of metabolites that cannot be produced by mammalian cells, including vitamins, and salvage nutrients that cannot otherwise be digested by pancreatic enzymes. Thus, dietary fiber is digested to yield short chain fatty acids that can subsequently be absorbed across the colonic epithelium. Bacteria also deconjugate bile acids, allowing their unconjugated forms (which are more hydrophobic) to be passively absorbed back into the portal circulation. It is also likely that the collective properties of the microbiota increase resistance to colonization by pathogenic microorganisms, educate the mucosal immune system during early post-natal life, and send signals to the brain that modify behavior. When the balance of the gut microbial community is deranged (known as **dysbiosis**) as a result of disease or the use of broad spectrum antibiotics, there may be an impact on intestinal physiology and/or an opportunity for pathogens to colonize the gut. For example, *Clostridium difficile* is a pathogen that often overgrows in the gut of hospitalized patients who have received antibiotics. It causes significant gastrointestinal symptoms and can be very difficult to eradicate. Recent clinical studies have shown that patients suffering from recurrent *C. difficile* infections can often benefit dramatically from the transplant (via enema) of the fecal microbes from a healthy donor.

GASTROINTESTINAL (SPLANCHNIC) CIRCULATION

A final general point that should be made about the gastrointestinal tract relates to its unusual circulatory features. The blood flow to the stomach, intestines, pancreas, and liver is arranged in a series of parallel circuits, with all the blood from the intestines and pancreas draining via the portal vein to the liver **(Figure 25–20)**. The blood from the intestines, pancreas, and spleen drains via the hepatic portal vein to the liver and from the liver via the hepatic veins to the inferior vena cava. The viscera and the liver receive about 30% of the cardiac output via the celiac, superior mesenteric, and inferior mesenteric arteries. The liver receives about 1300 mL/min from the portal vein and 500 mL/min from the hepatic artery during fasting, and the portal supply increases still further after meals.

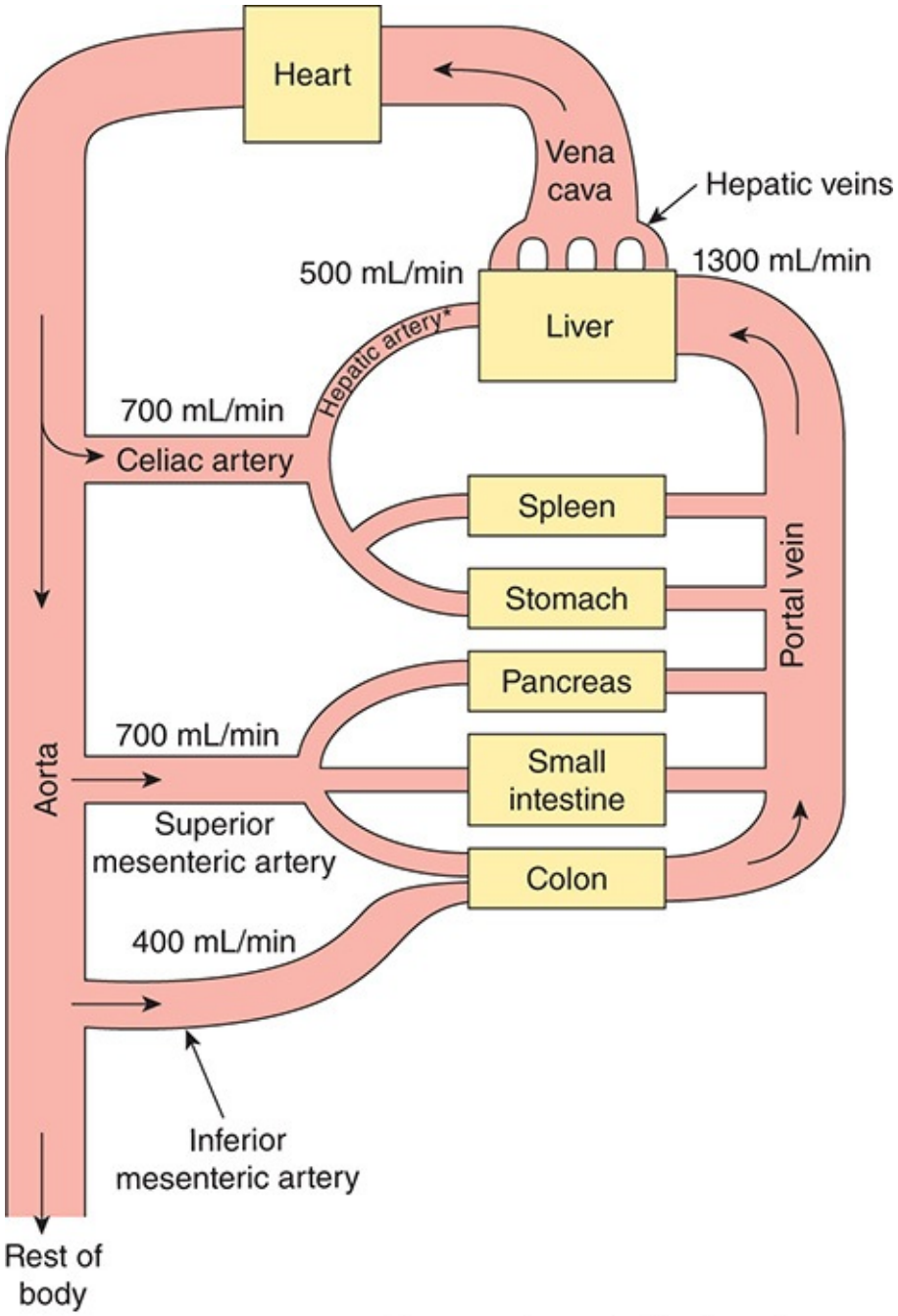


FIGURE 25–20 Schematic of the splanchnic circulation under fasting conditions. Note that even during fasting, the liver receives the majority of its blood supply via the portal vein.

INTESTINAL FLUID & ELECTROLYTE

TRANSPORT

The intestine supplies a fluid environment in which the processes of digestion and absorption can occur. Then, when the meal has been assimilated, fluid used during digestion and absorption is reclaimed by transport back across the epithelium to avoid dehydration. Water moves passively into and out of the gastrointestinal lumen, driven by electrochemical gradients established by the active transport of ions and other solutes. In the period after a meal, much of the fluid reuptake is driven by the coupled transport of nutrients, such as glucose, with sodium ions. In the period between meals, absorptive mechanisms center exclusively around electrolytes. In both cases, secretory fluxes of fluid are largely driven by the active transport of chloride ions into the lumen, although absorption still predominates overall.

Overall water balance in the gastrointestinal tract is summarized in **Table 25–5**. The intestines are presented each day with about 2000 mL of ingested fluid plus 7000 mL of secretions from the mucosa of the gastrointestinal tract and associated glands. Ninety-eight percent of this fluid is reabsorbed, with a daily fluid loss of only 200 mL in the stools.

TABLE 25–5 Daily water turnover (mL) in the gastrointestinal tract.

Ingested		2000
Endogenous secretions		7000
Salivary glands	1500	
Stomach	2500	
Bile	500	
Pancreas	1500	
Intestine	+1000	
	7000	
Total input		9000
Reabsorbed		8800
Jejunum	5500	
Ileum	2000	
Colon	+1300	
	8800	
Balance in stool		200

Data from Moore EW: *Physiology of Intestinal Water and Electrolyte Absorption.* American Gastroenterological Association, 1976.

In the small intestine, secondary active transport of Na^+ is important in bringing about absorption of glucose, some amino acids, and other substances such as bile acids (see above). Conversely, the presence of glucose in the intestinal lumen facilitates the reabsorption of Na^+. In the period between meals, when nutrients are not present, sodium and chloride are absorbed together from the lumen by the coupled activity of a sodium/hydrogen exchanger (NHE) and chloride/bicarbonate exchanger in the apical membrane, in a so-called electroneutral mechanism **(Figure 25–21)**. Water then follows to maintain an osmotic balance. In the colon, moreover, an additional electrogenic mechanism for sodium absorption is expressed, particularly in the distal colon. In this mechanism, sodium enters across the apical membrane via an ENaC (epithelial sodium) channel that is identical to that expressed in the distal tubule of the kidney **(Figure 25–22)**. This underpins the ability of the colon to desiccate the stool and ensure that only a small portion of the fluid load used daily in the digestion and absorption of meals is lost from the body. Following a low-salt diet, increased expression of ENaC in response to aldosterone increases the ability to reclaim sodium from the stool.

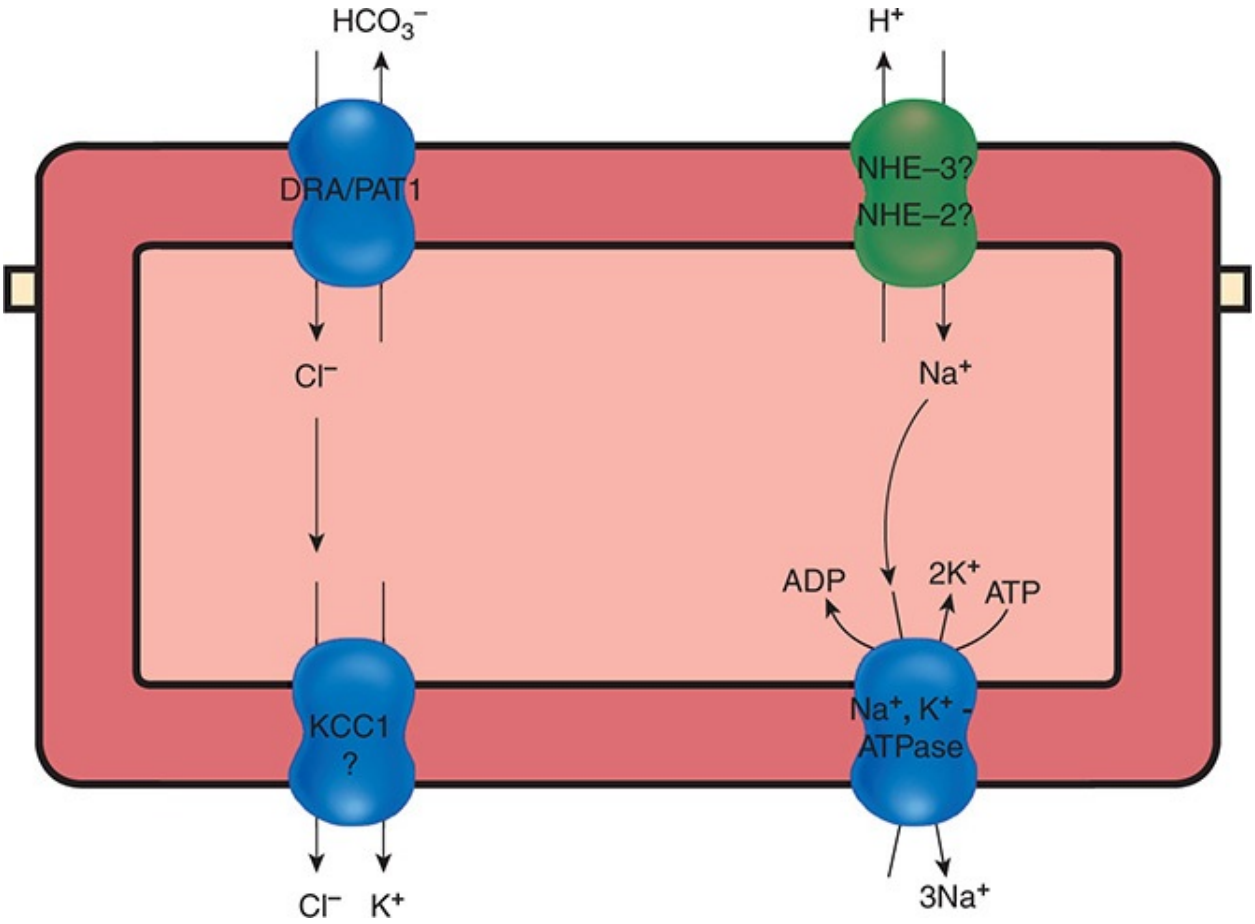


FIGURE 25–21 Electroneutral NaCl absorption in the small intestine and colon. NaCl enters across the apical membrane via the coupled activity of a sodium/hydrogen exchanger (NHE) and a chloride/bicarbonate exchanger (down-regulated in adenoma (DRA), or putative anion transporter-1 (PAT1)). A putative potassium/chloride cotransporter (KCC1) in the basolateral membrane provides for chloride exit, whereas sodium is extruded by the Na^+, K^+ ATPase.

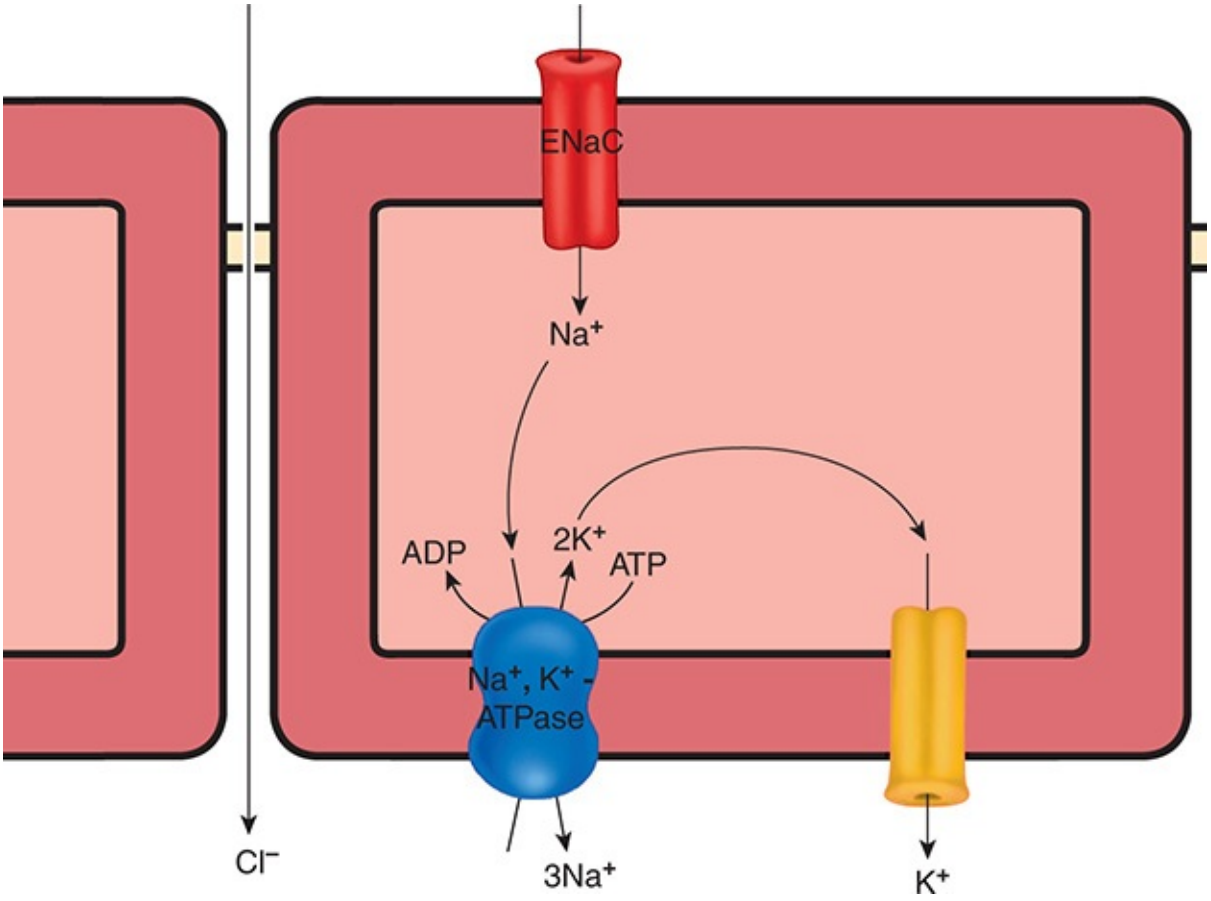


FIGURE 25–22 Electrogenic sodium absorption in the colon. Sodium enters the epithelial cell via apical epithelial sodium channels (ENaC), and exits via the Na^+, K^+ ATPase.

Despite the predominance of absorptive mechanisms, secretion also takes place continuously throughout the small intestine and colon to adjust the local fluidity of the intestinal contents as needed for mixing, diffusion, and movement of the meal and its residues along the length of the gastrointestinal tract. Cl^- normally enters enterocytes from the interstitial fluid via Na^+–K^+–$2Cl^-$ cotransporters in their basolateral membranes **(Figure 25–23)**, and the Cl^- is then secreted into the intestinal lumen via channels that are regulated by various protein kinases. The cystic fibrosis transmembrane conductance regulator (CFTR) channel that is defective in the disease of cystic fibrosis is quantitatively most important, and is activated by protein kinase A and hence by cAMP **(Clinical Box 25–2)**.

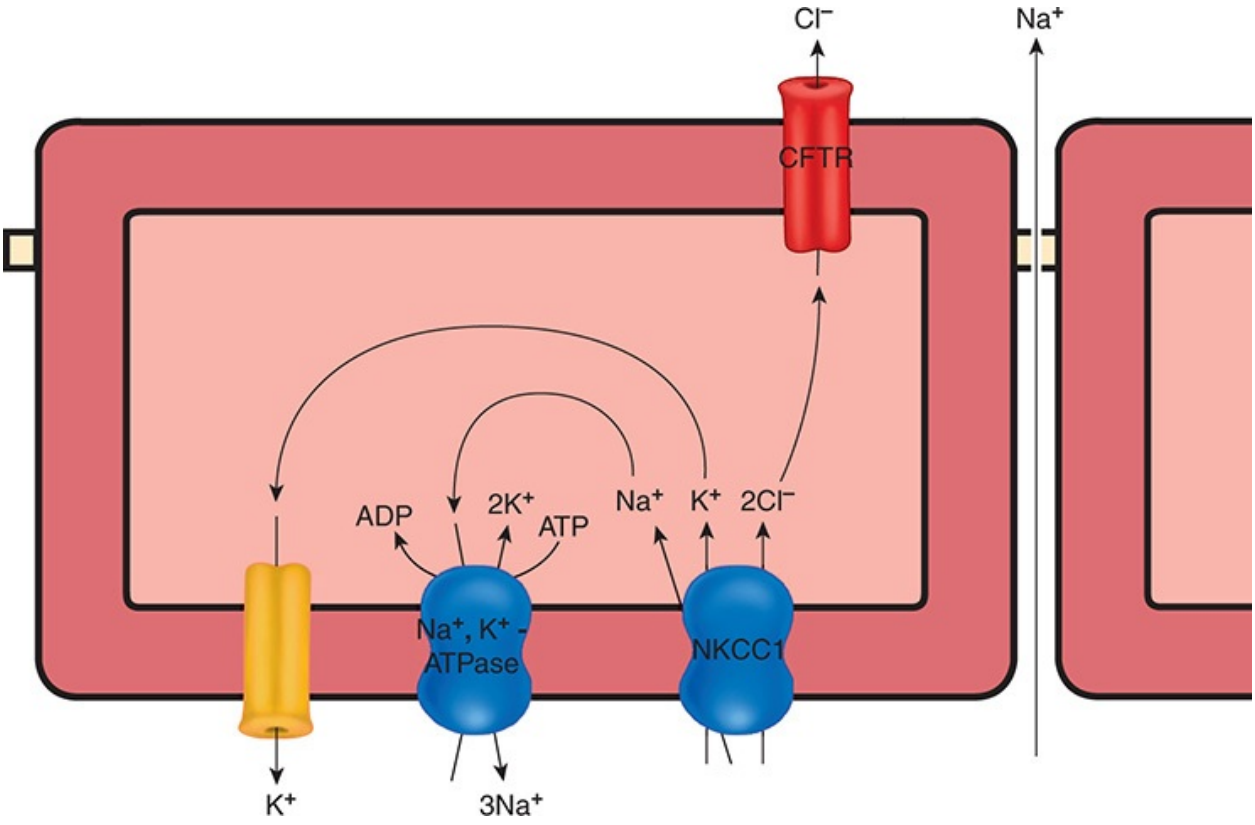


FIGURE 25–23 Chloride secretion in the small intestine and colon. Chloride uptake occurs via the sodium/potassium/2 chloride cotransporter, NKCC1. Chloride exit is via the cystic fibrosis transmembrane conductance regulator (CFTR) as well as perhaps via other chloride channels, not shown.

Water moves into or out of the intestine until the osmotic pressure of the intestinal contents equals that of the plasma. The osmolality of the duodenal contents may be hypertonic or hypotonic, depending on the meal ingested, but by the time the meal enters the jejunum, its osmolality is close to that of plasma. This osmolality is maintained throughout the rest of the small intestine; the osmotically active particles produced by digestion are removed by absorption, and water moves passively out of the gut along the osmotic gradient thus generated. In the colon, Na^+ is absorbed and water moves passively with it, again along the osmotic gradient. **Saline cathartics** such as magnesium sulfate are poorly absorbed salts that retain their osmotic equivalent of water in the intestine, thus increasing intestinal volume and consequently exerting a laxative effect. The amount of fluid in the lumen will also depend on the rate of intestinal motility. When motility is slow, there is more time for absorption. Several antidiarrheal drugs exert their effects by desynchronizing muscle contraction in the gut and slowing propulsive motility (see also Chapter 27).

CLINICAL BOX 25–2

Cholera

Cholera is a severe secretory diarrheal disease that often occurs in epidemics associated with natural disasters where normal sanitary practices break down. Along with other secretory diarrheal illnesses produced by bacteria and viruses, cholera causes a significant amount of morbidity and mortality, particularly among the young and in developing countries. The cAMP concentration in intestinal epithelial cells is increased in cholera. The cholera bacillus stays in the intestinal lumen, but it produces a toxin that binds to receptors on the apical membrane of intestinal epithelial cells, and this permits the A subunit of the toxin to enter the cell. The toxin binds adenosine diphosphate ribose to the α subunit of G_s, inhibiting its GTPase activity (see Chapter 2). Thereafter, the constitutively activated G-protein produces prolonged stimulation of adenylyl cyclase and a marked increase in the intracellular cAMP concentration. In addition to increased Cl^- secretion, the function of NHE3 is reduced, thus reducing NaCl absorption. The resultant increase in electrolyte and water content of the intestinal contents causes the diarrhea. However, Na^+, K^+ ATPase, and the Na^+/glucose cotransporter are unaffected, so coupled reabsorption of glucose and Na^+ bypasses the defect.

THERAPEUTIC HIGHLIGHTS

Treatment for cholera is mostly supportive, since the infection will eventually clear, although antibiotics are sometimes used. The most important therapeutic approach is to ensure that the large volumes of fluid, along with electrolytes, lost to the stool are replaced to avoid dehydration. Stool volumes can approach 20 L per day. When sterile supplies are available, fluids and electrolytes can most conveniently be replaced intravenously. However, this is often not possible in the setting of an epidemic. Instead, the persistent activity of the Na^+/glucose cotransporter provides a physiologic basis for the treatment of Na^+ and water loss by oral administration of solutions containing NaCl and glucose. Oral rehydration solution, a prepackaged mixture of sugar and salt to be dissolved in water, is a simple remedy that has dramatically reduced mortality in epidemics of cholera and other diarrheal diseases in developing countries.

Some K^+ is secreted into the intestinal lumen, especially as a component of mucus. K^+ channels are present in the luminal as well as the basolateral membrane of the enterocytes of the colon, so K^+ is secreted into the colon. In addition, K^+ moves passively down its electrochemical gradient. The accumulation of K^+ in the colon is partially offset by H^+–K^+ ATPase in the luminal membrane of cells in the distal colon, with resulting active transport of K^+ into the cells. Nevertheless, loss of ileal or colonic fluids in chronic diarrhea can lead to severe hypokalemia. When the dietary intake of K^+ is high for a prolonged period, aldosterone secretion is increased and more K^+ enters the colonic lumen. This is due in part to the appearance of more Na^+, K^+ ATPase pumps in the basolateral membranes of the cells, with a consequent increase in intracellular K^+ and K^+ diffusion across the luminal membranes of the cells.

CHAPTER SUMMARY

- The gastrointestinal system evolved as a portal to permit controlled nutrient uptake in multicellular organisms and to excrete the residues of meals, as well as endogenous lipid-soluble metabolic waste products. It is functionally continuous with the outside environment and has a well-developed immune system.
- The gastrointestinal tract is lined by a continuous layer of columnar epithelial cells that are continuously replaced, and folded into structures known as crypts and villi. The lamina propria and submucosa lie below the epithelium and contain immune cells, blood vessels, and lymphatics. Circular and longitudinal muscle layers provide for motility functions.
- Digestive secretions serve to chemically alter the components of meals (particularly macromolecules) such that their constituents can be absorbed across the epithelium. Meal components are acted on sequentially by saliva, gastric juice, pancreatic juice, and bile, which contain enzymes, ions, water, and other specialized components.
- Gastrointestinal functions are regulated in an integrated fashion by endocrine, paracrine, and neurocrine mechanisms. Hormones and paracrine factors are released from enteroendocrine cells in response to signals coincident with the intake of meals.
- The enteric nervous system conveys information from the central nervous system to the gastrointestinal tract, but also often can activate programmed

responses of secretion and motility in an autonomous fashion.

- The intestine harbors an extensive mucosal immune system that regulates responses to the complex microbiota normally resident in the lumen, as well as defending the body against invasion by pathogens.
- The intestine has an unusual circulation, in that the majority of its venous outflow does not return directly to the heart, but rather is directed initially to the liver via the portal vein.
- The intestine and the organs that drain into it secrete about 8 L of fluid per day, which is added to water consumed in food and beverages. Most of this fluid is reabsorbed, leaving only approximately 200 mL to be lost to the stool. Fluid secretion and absorption are both dependent on the active epithelial transport of ions, nutrients, or both.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. A researcher conducts a study of the regulation of salivary secretion in a group of volunteer medical students under various conditions. Which of the following conditions would be expected to be associated with the lowest rates of secretion?

   A. Chewing gum

   B. Undergoing a mock dental exam

   C. Sleep

   D. Exposure to a nauseating odor

   E. Resting control conditions

2. A patient suffering from anemia comes to his physician complaining of frequent bouts of gastroenteritis. A blood test reveals antibodies directed against gastric parietal cells. The anemia in this patient is attributable to hyposecretion of which gastric product?

   A. Histamine

   B. Gastrin

   C. Pepsinogen

   D. Intrinsic factor

   E. Hydrochloric acid

3. A 50-year-old man comes to see his clinician complaining of severe epigastric

pain, frequent heartburn, and unexplained weight loss of 20 pounds over a 6-month period. He claims to have obtained no relief from over-the-counter H_2 antihistamine drugs. He is referred to a gastroenterologist, and upper endoscopy reveals erosions and ulcerations in the proximal duodenum and an increased output of gastric acid in the fasting state. The patient is most likely to have a tumor secreting which of the following hormones?

A. Secretin

B. Somatostatin

C. Motilin

D. Gastrin

E. Cholecystokinin

4. Which of the following has the highest pH?

A. Gastric juice

B. Colonic luminal contents

C. Pancreatic juice

D. Saliva

E. Contents of the intestinal crypts

5. A 60-year-old woman undergoes total pancreatectomy because of the presence of a tumor. Which of the following outcomes would *not* be expected after she recovers from the operation?

A. Steatorrhea

B. Hyperglycemia

C. Metabolic acidosis

D. Weight gain

E. Decreased absorption of amino acids

6. In a germ-free animal, which of the following will not be present?

A. Cholic acid

B. Lithocholic acid

C. Gastrin

D. Cholecystokinin

E. Trypsin

7. A woman suffering from Crohn's disease undergoes a surgical resection of her diseased terminal ileum. Following her recovery from surgery and reintroduction of a normal diet, which of the following will not be increased?

A. Hepatic synthesis of bile acids

B. Levels of conjugated bile acids in the portal vein

C. Stool fat

D. Stool volume

E. Hepatic uptake of unconjugated bile acids

8. Water is absorbed in the jejunum, ileum, and colon and excreted in the feces. Arrange these in order of the amount of water absorbed or excreted from the greatest to the smallest.

A. Colon, jejunum, ileum, feces

B. Feces, colon, ileum, jejunum

C. Jejunum, ileum, colon, feces

D. Colon, ileum, jejunum, feces

E. Feces, jejunum, ileum, colon

9. Following a natural disaster in Haiti, there is an outbreak of cholera among displaced persons living in a tent encampment. The affected individuals display severe diarrheal symptoms because of which of the following changes in intestinal transport?

A. Increased Na^+–K^+ cotransport in the small intestine

B. Increased K^+ secretion into the colon

C. Reduced K^+ absorption in the crypts of Lieberkühn

D. Increased Na^+ absorption in the small intestine

E. Increased Cl^- secretion into the intestinal lumen

CHAPTER 26

Digestion & Absorption of Nutrients

OBJECTIVES

After studying this chapter, you should be able to:

- Understand the basic principles of nutrition, how nutrients are delivered to the body, and the chemical processes needed to convert them to a form suitable for absorption.
- List the major dietary carbohydrates and define the luminal and brush border processes that produce absorbable monosaccharides as well as the transport mechanisms that provide for the uptake of these hydrophilic molecules.
- Understand the process of protein assimilation, and the ways in which it is comparable to, or converges from, that used for carbohydrates.
- Define the stepwise processes of lipid digestion and absorption and the role of bile acids in solubilizing the products of lipolysis.
- Understand the basis of the anatomic reserve for nutrient absorption, the adaptive processes that maintain absorption even when a portion of the bowel is lost, and the basis of the malabsorption syndrome and its impact.
- Identify the source and functions of short-chain fatty acids in the colon.
- Delineate the requirements for, and mechanisms of uptake for vitamins and minerals.

- Explain the processes that regulate overall food intake, as well as the mechanisms and consequences of obesity.

INTRODUCTION

The gastrointestinal system is the portal through which nutritive substances, vitamins, minerals, and fluids enter the body. Proteins, fats, and complex carbohydrates are broken down into absorbable units **(digested),** principally, although not exclusively, in the small intestine. The products of digestion and the vitamins, minerals, and water cross the mucosa and enter the lymph or the blood **(absorption).** The digestive and absorptive processes are the subject of this chapter.

Digestion of the major foodstuffs is an orderly process involving the action of a large number of **digestive enzymes** discussed in the previous chapter. Enzymes from the salivary glands attack carbohydrates (and fats in some species); enzymes from the stomach attack proteins and fats; and enzymes from the exocrine portion of the pancreas attack carbohydrates, proteins, lipids, DNA, and RNA. Other enzymes that complete the digestive process are found in the luminal membranes and the cytoplasm of the cells that line the small intestine. The action of the enzymes is aided by the hydrochloric acid secreted by the stomach and the bile secreted by the liver.

Most substances pass from the intestinal lumen into the enterocytes and then out of the enterocytes to the interstitial fluid. The processes responsible for movement across the luminal cell membrane are often quite different from those responsible for movement across the basal and lateral cell membranes to the interstitial fluid.

NUTRITIONAL PRINCIPLES

Certain substances are essential constituents of any human diet. An optimal diet includes, in addition to sufficient water (see Chapter 37), adequate calories, protein, fat, minerals, and vitamins.

CALORIC INTAKE & DISTRIBUTION

The caloric value of the dietary intake must be approximately equal to the energy

expended if body weight is to be maintained. In addition to the 2000 kcal/d necessary to meet basal needs, 500–2500 kcal/d (or more) are required to meet the energy demands of daily activities.

The distribution of the calories among carbohydrate, protein, and fat is determined partly by physiologic factors and partly by taste and economic considerations. A daily protein intake of 1 g/kg body weight to supply the eight nutritionally essential amino acids **(Table 26–1)** and other amino acids is desirable. The **essential amino acids** are those that cannot be synthesized by the human body, and thus must be obtained from dietary protein. The source of the protein is also important. **Grade I proteins,** the animal proteins of meat, fish, dairy products, and eggs, contain all amino acids, including the essential amino acids, in approximately the proportions required for protein synthesis and other uses. On the other hand, most plant proteins are **grade II** because they supply different proportions of amino acids and some lack one or more of the essential amino acids. The protein needs of vegetarians can be met by ingesting strategic mixtures of grade II proteins, but the intake must often be large because of amino acid wastage.

TABLE 26–1 Amino acids that are essential for human nutrition

Amino Acid Class	Amino Acids
Basic	Lysine, histidine
Aliphatic	Valine, leucine, isoleucine
Aromatic	Phenylalanine, tryptophan
Hydroxyl	Threonine
Sulfur	Methionine

Fat is the most compact form of food, since it supplies 9.3 kcal/g. However, often it is also the most expensive. Indeed, internationally there is a reasonably good positive correlation between fat intake and standard of living. Typically, Western diets have contained large amounts (100 g/d or more). In Central and South American Indian communities where corn (carbohydrate) is the dietary staple, adults live without ill effects for years on a very low fat intake. Therefore, provided that the needs for essential fatty acids (FA) are met, a low-fat intake does not seem to be harmful, and a diet low in saturated fats is desirable.

Carbohydrate is the cheapest source of calories and provides 50% or more of

the calories in most diets. In the average middle-class American diet, approximately 50% of the calories come from carbohydrate, 15% from protein, and 35% from fat. When calculating dietary needs, it is usual to meet the protein requirement first and then split the remaining calories between fat and carbohydrate, depending on taste, income, and other factors. For example, a 65-kg man who is moderately active needs about 2800 kcal/d to maintain his weight. He should eat at least 65 g of protein daily, supplying 267 (65 × 4.1) kcal. A reasonable figure for fat intake is 50–60 g. The rest of the caloric requirement can be met by supplying carbohydrate.

DIGESTION & ABSORPTION: CARBOHYDRATES

DIGESTION

The principal dietary carbohydrates are polysaccharides, disaccharides, and monosaccharides. Starches (glucose polymers) and their derivatives are the only polysaccharides that are digested to any degree in the human gastrointestinal tract by human enzymes. Amylopectin, which typically constitutes around 75% of dietary starch, is a branched molecule, whereas amylose is a straight chain with only α1:4 linkages **(Figure 26–1)**. The disaccharides **lactose** (milk sugar) and **sucrose** (table sugar) are also ingested, along with the monosaccharides fructose and glucose.

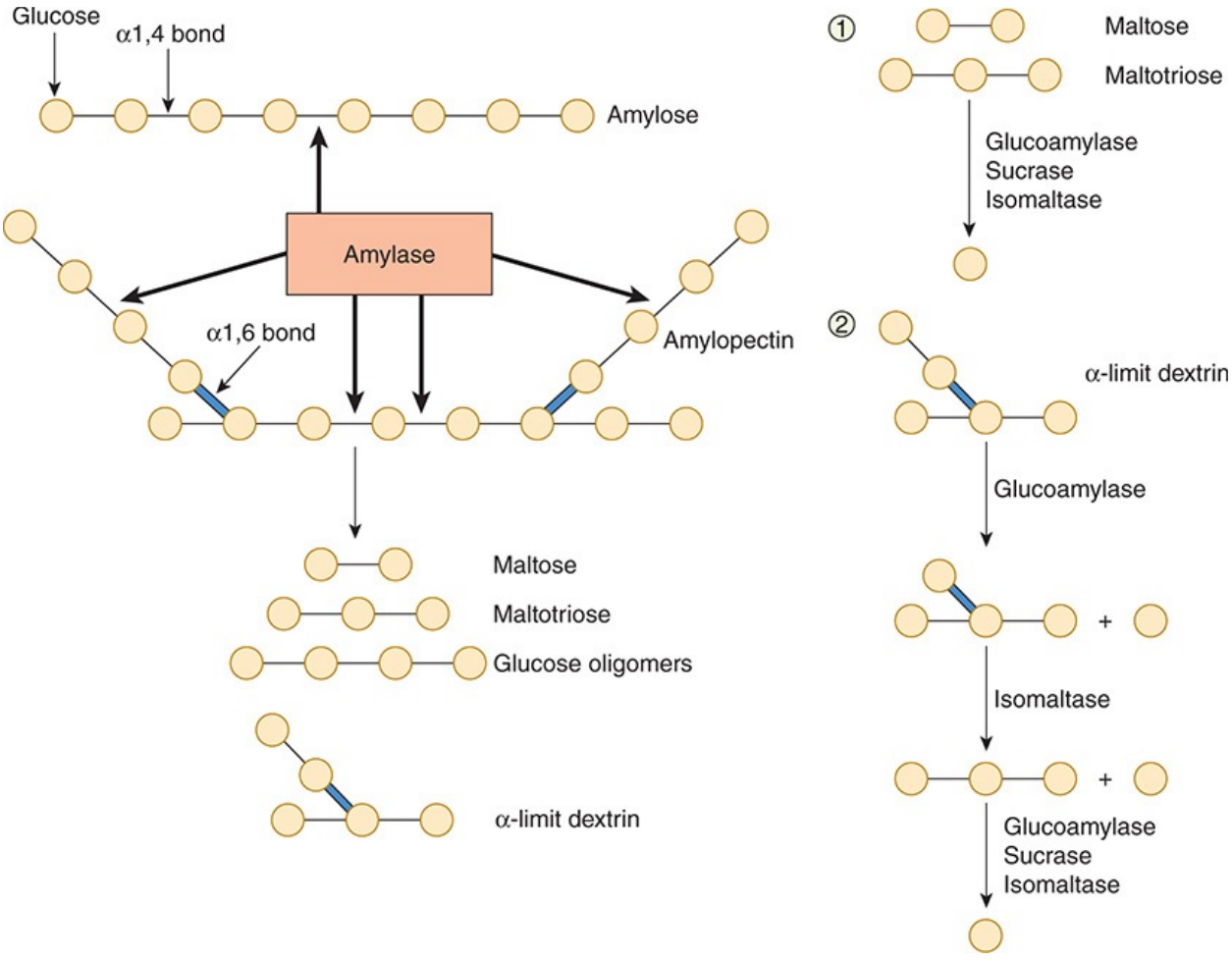


FIGURE 26–1 Left: Structure of amylose and amylopectin, which are polymers of glucose (indicated by circles). These molecules are partially digested by the enzyme amylase, yielding the products shown at the bottom of the figure. **Right:** Brush border hydrolases responsible for the sequential digestion of the products of luminal starch digestion (1, linear oligomers; 2, α-limit dextrins).

In the mouth, starch is attacked by salivary α-amylase. The optimal pH for this enzyme is 6.7. However, it remains partially active even once it moves into the stomach, despite the acidic gastric juice, because the active site is protected in the presence of substrate to some degree. In the small intestine, both the salivary and the pancreatic α-amylase also act on the ingested polysaccharides. Both enzymes hydrolyze internal α1:4 linkages but spare α1:6 linkages and terminal α1:4 linkages. Consequently, the end products of α-amylase digestion are oligosaccharides: the disaccharide **maltose;** the trisaccharide **maltotriose;** and **α-limit dextrins,** branched polymers of glucose containing an average of

about eight glucose molecules with α1:6 linkages (Figure 26–1).

The oligosaccharidases responsible for the further digestion of the starch derivatives are located in the brush border of small intestinal epithelial cells (Figure 26–1). Some of these enzymes have more than one substrate. **Isomaltase** is mainly responsible for hydrolysis of α1:6 linkages. Along with **maltase** and **sucrase,** it also breaks down maltotriose and maltose. Sucrase and isomaltase are initially synthesized as a single glycoprotein chain that is inserted into the brush border membrane. It is then hydrolyzed by pancreatic proteases into sucrase and isomaltase subunits.

Sucrase hydrolyzes sucrose into a molecule of glucose and a molecule of fructose. In addition, **lactase** hydrolyzes lactose to glucose and galactose. Overall, brush border digestion of carbohydrates generates monosaccharides at high concentrations at precisely the location where they will be absorbed. This may serve to sequester them from bacteria and avoid bacterial overgrowth in the small intestine.

Deficiency of one or more of the brush border oligosaccharidases may cause diarrhea, bloating, and flatulence after ingestion of sugar **(Clinical Box 26–1)**. The diarrhea is due to the increased number of osmotically active oligosaccharide molecules that remain in the intestinal lumen, causing the volume of the intestinal contents to increase. In the colon, bacteria break down some of the oligosaccharides, further increasing the number of osmotically active particles. The bloating and flatulence are due to the production of gas (CO_2 and H_2) from disaccharide residues in the lower small intestine and colon.

ABSORPTION

Hexoses are rapidly absorbed across the wall of the small intestine **(Table 26–2)**. Essentially all the hexoses are removed before the remains of a meal reach the terminal part of the ileum. The sugar molecules pass from the mucosal cells to the blood in the capillaries draining into the portal vein.

TABLE 26–2 Normal transport of substances by the intestine and location of maximum absorption or secretion.[a]

Absorption of	Small Intestine			Colon
	Upper[b]	Mid	Lower	
Sugars (glucose, galactose, etc)	++	+++	++	0
Amino acids	++	++	++	0
Water-soluble and fat-soluble vitamins except vitamin B_{12}	+++	++	0	0
Betaine, dimethylglycine, sarcosine	+	++	++	?
Antibodies in newborns	+	++	+++	?
Pyrimidines (thymine and uracil)	+	+	?	?
Long-chain fatty acid absorption and conversion to triglyceride	+++	++	+	0
Bile acids	+	+	+++	+
Vitamin B_{12}	0	+	+++	0
Na^+	+++	++	+++	+++
K^+	+	+	+	Sec
Ca^{2+}	+++	++	+	?
Fe^{2+}	+++	+	+	?
Cl^-	+++	++	+	+
SO_4^{2-}	++	+	0	?

[a]Amount of absorption is graded + to +++. Sec, secreted when luminal K^+ is low.

[b]Upper small intestine refers primarily to jejunum, although the duodenum is similar in most cases studied (with the notable exception that the duodenum secretes HCO_3^- and shows little net absorption or secretion of NaCl).

The transport of glucose and galactose depends on Na^+ in the intestinal lumen; a high concentration of Na^+ on the mucosal surface of the cells facilitates sugar influx into the epithelial cells while a low concentration inhibits sugar influx into the epithelial cells. This is because these sugars and Na^+ share the same **cotransporter,** or **symport,** the **sodium-dependent glucose transporter** (SGLT, Na^+ glucose cotransporter) **(Figure 26–2)**. There are two members of this family of transporters, SGLT-1 and SGLT-2. SGLT-1 is responsible for uptake of dietary glucose from the gut, whereas SGLT-2 is responsible for glucose transport out of the renal tubules (see Chapter 37).

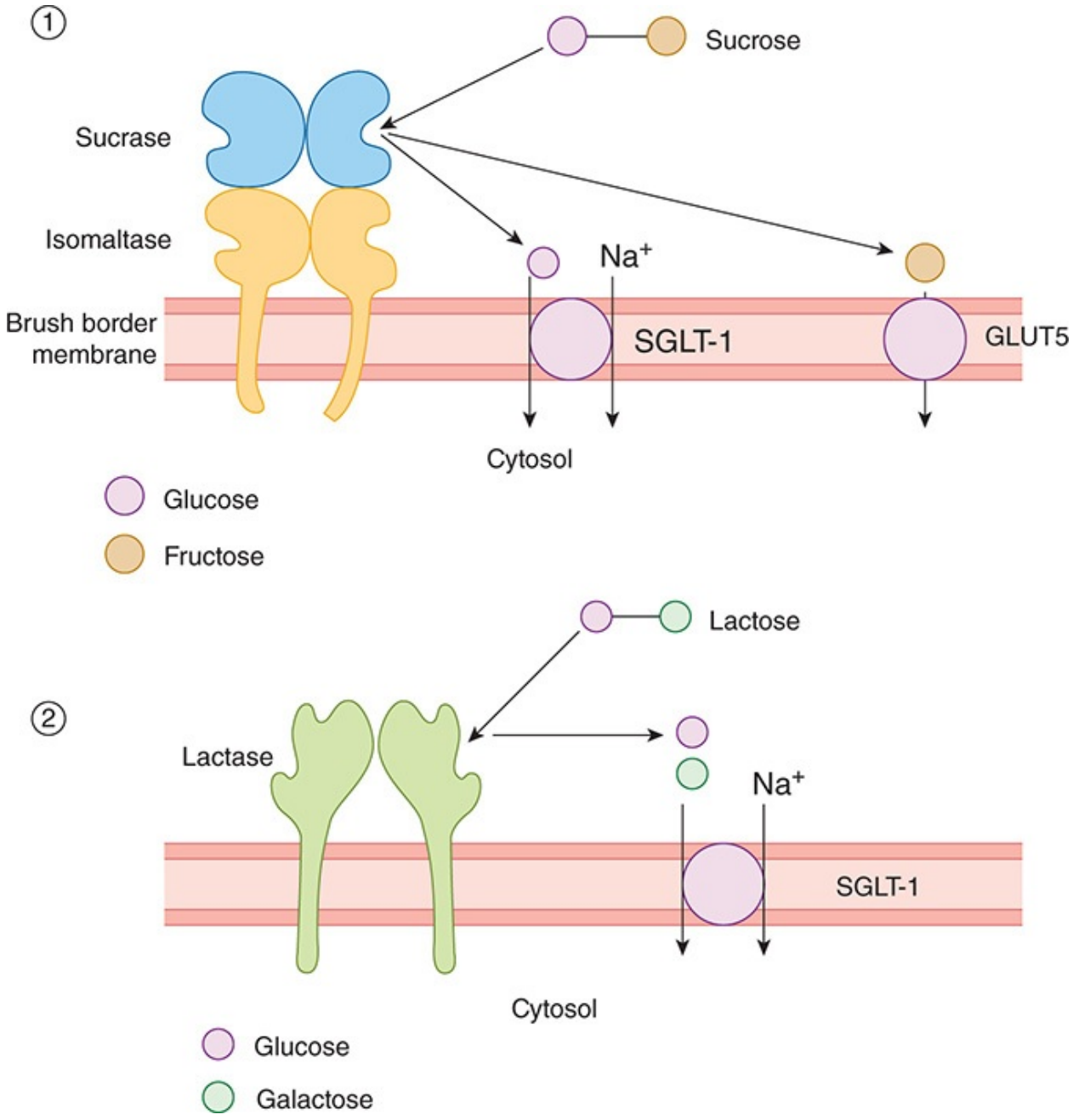


FIGURE 26–2 Brush border digestion and assimilation of the disaccharides sucrose (panel 1) and lactose (panel 2). Uptake of glucose and galactose is driven secondarily by the low intracellular sodium concentration established by the basolateral Na^+, K^+ ATPase (not shown). SGLT-1, sodium-glucose cotransporter-1.

Because the intracellular Na^+ concentration is low in intestinal epithelial cells (as it is in other cells), Na^+ moves into the cell along its concentration gradient.

Glucose moves with the Na^+ and is released in the cell (Figure 26–2). The Na^+ is then exported basolaterally by the Na^+, K^+ ATPase, and any glucose that is not needed for cellular requirements leaves the cell by facilitated diffusion via GLUT2 into the interstitium and thence to the capillaries. Thus, glucose transport is an example of secondary active transport (see Chapter 2); the energy for glucose transport is provided indirectly by the active transport of Na^+ out of the cell. This maintains the concentration gradient across the luminal border of the cell, so that more Na^+ and consequently more glucose enter. When the Na^+/glucose cotransporter is congenitally defective, the resulting **glucose/galactose malabsorption** causes severe diarrhea that is often fatal if glucose and galactose are not promptly removed from the diet. Glucose and its polymers can also be used to retain Na^+ in diarrheal disease, as was discussed in Chapter 25.

CLINICAL BOX 26–1

Lactose Intolerance

In most mammals and in many races of humans, intestinal lactase activity is high at birth, then declines to low levels during childhood and adulthood. The low lactase levels are associated with intolerance to milk **(lactose intolerance).** Most Europeans and their American descendants retain sufficient intestinal lactase activity in adulthood; the incidence of lactase deficiency in northern and western Europeans is only about 15%. However, the incidence in blacks, American Indians, Asians, and Mediterranean populations is 70–100%. When such individuals ingest dairy products, they are unable to digest lactose sufficiently, and so symptoms such as bloating, pain, gas, and diarrhea are produced by the unabsorbed osmoles that are subsequently digested by colonic bacteria.

THERAPEUTIC HIGHLIGHTS

The simplest treatment for lactose intolerance is to avoid dairy products in the diet, but this can sometimes be challenging (or undesirable for the individual who loves ice cream). Symptoms can be ameliorated by administration of commercial lactase preparations, but this is expensive.

As indicated, SGLT-1 also transports galactose, but fructose utilizes a different mechanism. Its absorption is independent of Na^+ or the transport of glucose and galactose; it is transported instead by facilitated diffusion from the intestinal lumen into the enterocytes by GLUT5 and out of the enterocytes into the interstitium by GLUT2. Some fructose is also converted to glucose in mucosal cells.

Insulin has little effect on intestinal transport of sugars. In this respect, intestinal absorption resembles glucose reabsorption in the proximal convoluted tubules of the kidneys (see Chapter 37); neither process requires phosphorylation, and both are essentially normal in diabetes but are depressed by the drug phlorizin. The maximal rate of glucose absorption from the intestine is about 120 g/h.

PROTEINS & NUCLEIC ACIDS

PROTEIN DIGESTION

Protein digestion begins in the stomach, where pepsin cleaves some of the peptide linkages. Like many of the other enzymes concerned with protein digestion, pepsin is secreted in the form of an inactive precursor **(proenzyme)** and activated in the gastrointestinal tract. The pepsin precursor is called pepsinogen and is activated by gastric acid.

Pepsin hydrolyzes the bonds between aromatic amino acids such as phenylalanine or tyrosine and a second amino acid, so the products of peptic digestion are polypeptides of very diverse sizes. Because pepsin has a pH optimum of 1.6–3.2, its action is terminated when the gastric contents are mixed with the alkaline pancreatic juice in the duodenum and jejunum. The pH of the intestinal contents in the duodenal bulb is 3.0–4.0, but rapidly rises; in the rest of the duodenum it is about 6.5.

In the small intestine, the polypeptides formed by digestion in the stomach are further digested by the powerful proteolytic enzymes of the pancreas and intestinal mucosa. Trypsin, chymotrypsin, and elastase act at interior peptide bonds in the peptide molecules and are called **endopeptidases.** These powerful protein-splitting enzymes of the pancreatic juice are secreted as inactive proenzymes. The formation of the active endopeptidases occurs only when they have reached their site of action, secondary to the action of the brush border hydrolase, **enterokinase** (**Figure 26–3**). Thus, trypsinogen is converted to the active enzyme trypsin by **enterokinase** when the pancreatic juice enters the

duodenum. Enterokinase contains 41% polysaccharide, and this high polysaccharide content apparently prevents it from being digested itself before it can exert its effect. Trypsin converts chymotrypsinogen into chymotrypsin and other proenzymes into active enzymes (Figure 26–3). Trypsin can also activate trypsinogen; therefore, once some trypsin is formed, there is an auto-catalytic chain reaction. Enterokinase deficiency occurs as a congenital abnormality and leads to protein malnutrition.

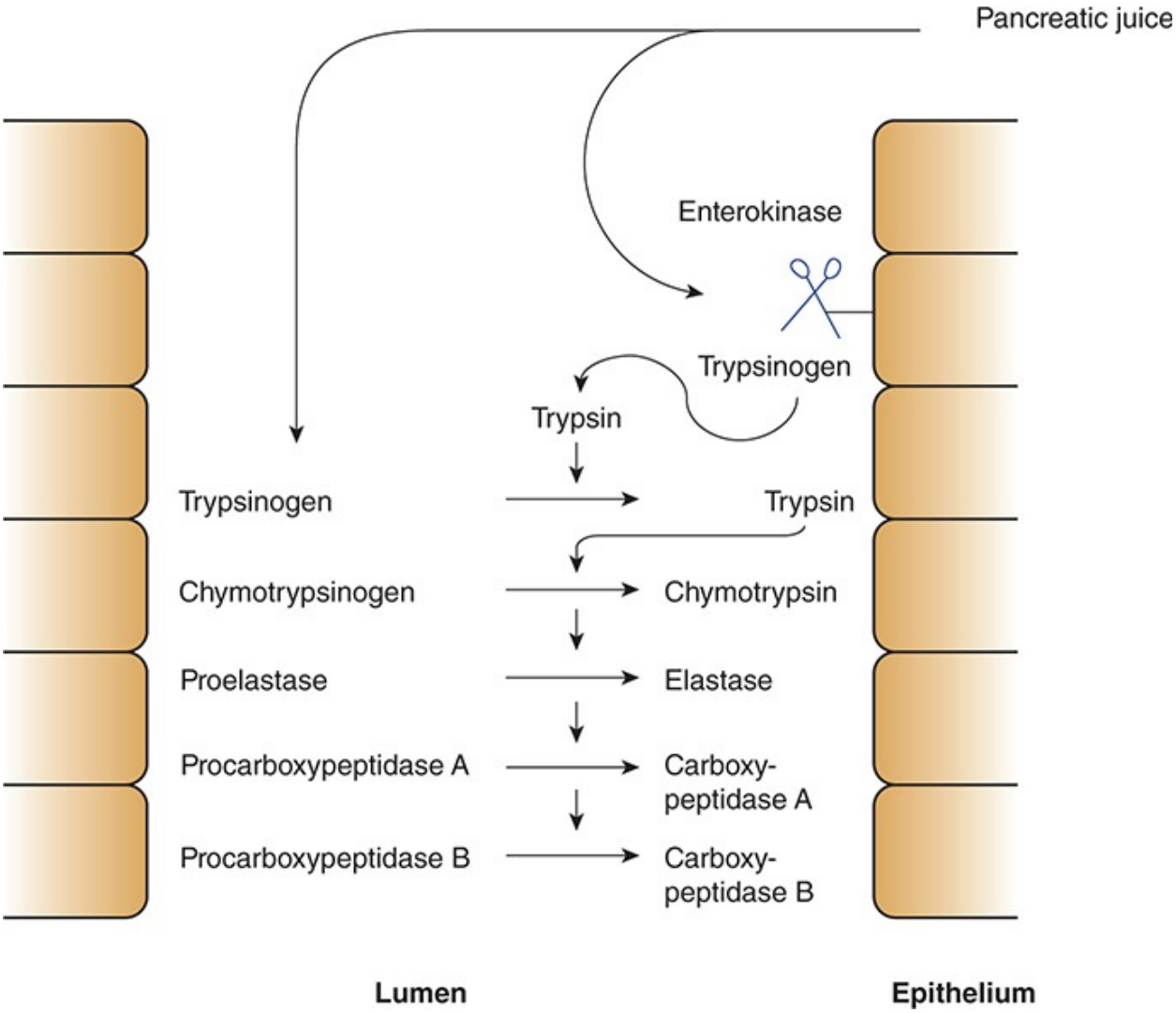


FIGURE 26–3 Mechanism to avoid activation of pancreatic proteases until they are in the duodenal lumen. Pancreatic juice contains proteolytic enzymes in their inactive, precursor forms. When the juice enters the duodenal lumen, trypsinogen contacts enterokinase expressed on the apical surface of enterocytes. Trypsinogen is thereby cleaved to trypsin, which in turn can activate additional trypsin molecules as well as the remaining proteolytic enzymes.

The carboxypeptidases of the pancreas are **exopeptidases** that hydrolyze the amino acids at the carboxyl ends of the polypeptides **(Figure 26–4)**. Some free amino acids are liberated in the intestinal lumen by this mechanism, but others are liberated at the cell surface by aminopeptidases, carboxypeptidases, endopeptidases, and dipeptidases present in the brush border of the mucosal cells. Some dipeptides and tripeptides are actively transported into the intestinal cells and hydrolyzed by intracellular peptidases, with the amino acids entering the bloodstream. Thus, the final digestion to amino acids occurs in three locations: the intestinal lumen, the brush border, and the cytoplasm of the mucosal cells.

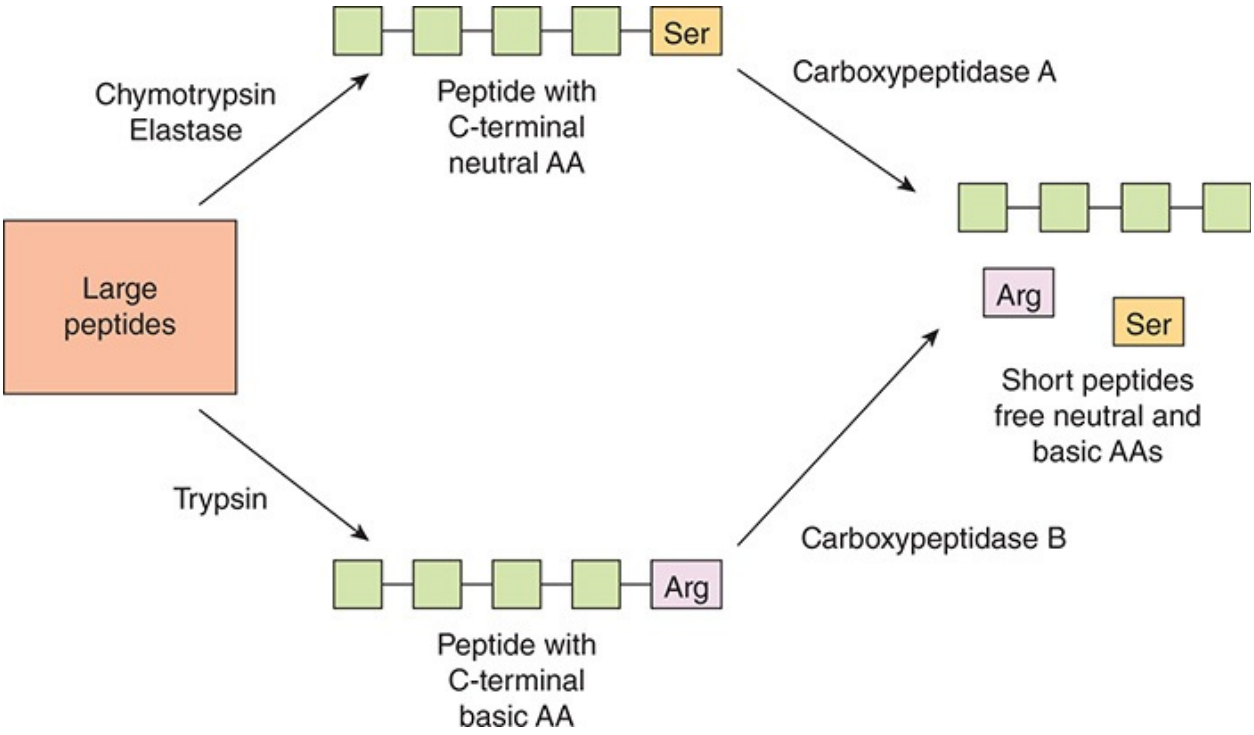


FIGURE 26–4 Luminal digestion of peptides by pancreatic endopeptidases and exopeptidases. Individual amino acids (AAs) are shown as squares.

ABSORPTION

At least seven different transport systems transport amino acids into enterocytes. Five of these require Na^+ and cotransport amino acids and Na^+ in a fashion similar to the cotransport of Na^+ and glucose (Figure 26–2). Two of these five also require Cl^-. In two systems, transport is independent of Na^+.

Dipeptides and tripeptides are transported into enterocytes by a system known

as PepT1 (or peptide transporter 1) that requires H^+ instead of Na^+ **(Figure 26–5)**. There is very little absorption of larger food-derived peptides after the neonatal period. In the enterocytes, amino acids released from the peptides by intracellular hydrolysis plus the amino acids absorbed from the intestinal lumen and brush border are transported out of the enterocytes along their basolateral borders by at least five transport systems. From there, they enter the hepatic portal blood.

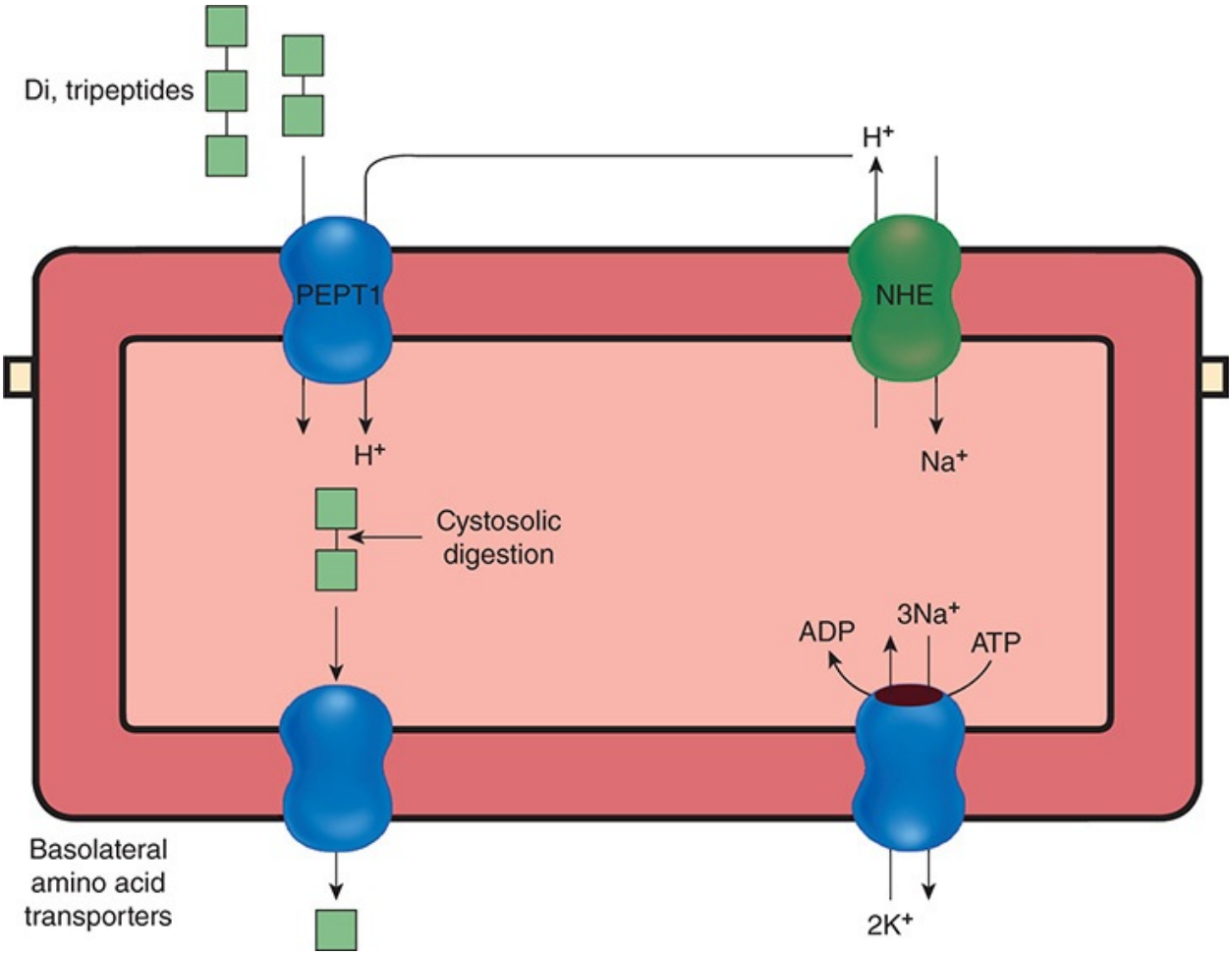


FIGURE 26–5 Disposition of short peptides in intestinal epithelial cells. Peptides are absorbed together with a proton supplied by an apical sodium/hydrogen exchanger (NHE) by the peptide transporter 1 (PepT1). Absorbed peptides are digested by cytosolic proteases, and any amino acids that are surplus to the needs of the epithelial cell are transported into the bloodstream by a series of basolateral transport proteins.

Absorption of amino acids is rapid in the duodenum and jejunum. There is a

little absorption in the ileum in health, because the majority of the free amino acids have already been assimilated at that point. Approximately 50% of the digested protein comes from ingested food, 25% from proteins in digestive juices, and 25% from desquamated mucosal cells. Only 2–5% of the protein in the small intestine escapes digestion and absorption. Some of this is eventually digested by bacterial action in the colon. Almost all of the protein in the stools is not of dietary origin but comes from bacteria and cellular debris. Evidence suggests that the peptidase activities of the brush border and the mucosal cell cytoplasm are increased by resection of part of the ileum and that they are independently altered in starvation. Thus, these enzymes appear to be subject to homeostatic regulation. In humans, a congenital defect in the mechanism that transports neutral amino acids in the intestine and renal tubules causes **Hartnup disease.** A congenital defect in the transport of basic amino acids causes **cystinuria.** However, most patients do not experience nutritional deficiencies of these amino acids because peptide transport compensates.

In infants, moderate amounts of undigested proteins are also absorbed. The protein antibodies in maternal colostrum are largely secretory immunoglobulins (IgAs), the production of which is increased in the breast in late pregnancy. They cross the mammary epithelium by transcytosis and enter the circulation of the infant from the intestine, providing passive immunity against infections. Absorption is by endocytosis and subsequent exocytosis.

Absorption of intact proteins declines sharply after weaning, but adults still absorb small quantities. Foreign proteins that enter the circulation provoke the formation of antibodies, and the antigen–antibody reaction occurring on subsequent entry of more of the same protein may cause allergic symptoms. Thus, absorption of proteins from the intestine may explain the occurrence of allergic symptoms after eating certain foods. The incidence of food allergy in children is said to be as high as 8%. However, in most individuals food allergies do not occur, and there is an evidence for a genetic component in susceptibility.

NUCLEIC ACIDS

Nucleic acids are split into nucleotides in the intestine by the pancreatic nucleases, and the nucleotides are split into the nucleosides and phosphoric acid by enzymes that appear to be located on the luminal surfaces of the mucosal cells. The nucleosides are then split into their constituent sugars and purine and pyrimidine bases. The bases are absorbed by active transport. Families of equilibrative (ie, passive) and concentrative (ie, secondary active) nucleoside

transporters have recently been identified and are expressed on the apical membrane of enterocytes.

LIPIDS

FAT DIGESTION

A lingual lipase is secreted by Ebner glands on the dorsal surface of the tongue in some species, and the stomach also secretes a lipase (Table 26–1). They are of little quantitative significance for lipid digestion other than in the setting of pancreatic insufficiency, but they may generate free fatty acids (FFA) that signal to more distal parts of the gastrointestinal tract (eg, causing the release of CCK; see Chapter 25).

Most fat digestion therefore begins in the duodenum, with pancreatic lipase being one of the most important enzymes involved. This enzyme hydrolyzes the 1- and 3-bonds of the triglycerides (triacylglycerols) with relative ease but acts on the 2-bonds at a very low rate, so the principal products of its action are FFA and 2-monoglycerides (2-monoacylglycerols). It acts on fats that have been emulsified (see below). **Colipase,** an accessory factor, is also secreted in the pancreatic juice, and helps to stabilize pancreatic lipase in an active conformation. Colipase is secreted in an inactive proform (Table 26–1) and is activated in the intestinal lumen by trypsin. Colipase is also critical for the action of lipase because it allows lipase to remain associated with droplets of dietary lipid even in the presence of bile acids.

Another pancreatic lipase that is activated by bile acids has been characterized. This **cholesterol esterase** represents about 4% of the total protein in pancreatic juice. In adults, pancreatic lipase is 10–60 times more active, but unlike pancreatic lipase, cholesterol esterase catalyzes the hydrolysis of cholesterol esters, esters of fat-soluble vitamins, and phospholipids, as well as triglycerides. A very similar enzyme is found in human milk.

Fats are relatively insoluble, which limits their ability to cross the unstirred layer and reach the surface of the mucosal cells. However, they are finely emulsified in the small intestine by the detergent action of bile acids, phosphatidylcholine, and monoglycerides. When the concentration of bile acids in the intestine is high after ingestion of a meal and contraction of the gallbladder, lipids and bile acids interact spontaneously to form **micelles** (**Figure 26–6**). These cylindrical aggregates take up lipids, and although their lipid concentration varies, they generally contain FA, monoglycerides, and cholesterol

in their hydrophobic centers. Micellar formation further solubilizes the lipids and provides a mechanism for their transport to the enterocytes. Thus, the micelles move down their concentration gradient through the unstirred layer to the brush border of the mucosal cells. The lipids diffuse out of the micelles, and a saturated aqueous solution of the lipids is maintained in contact with the brush border of the mucosal cells (Figure 26–6).

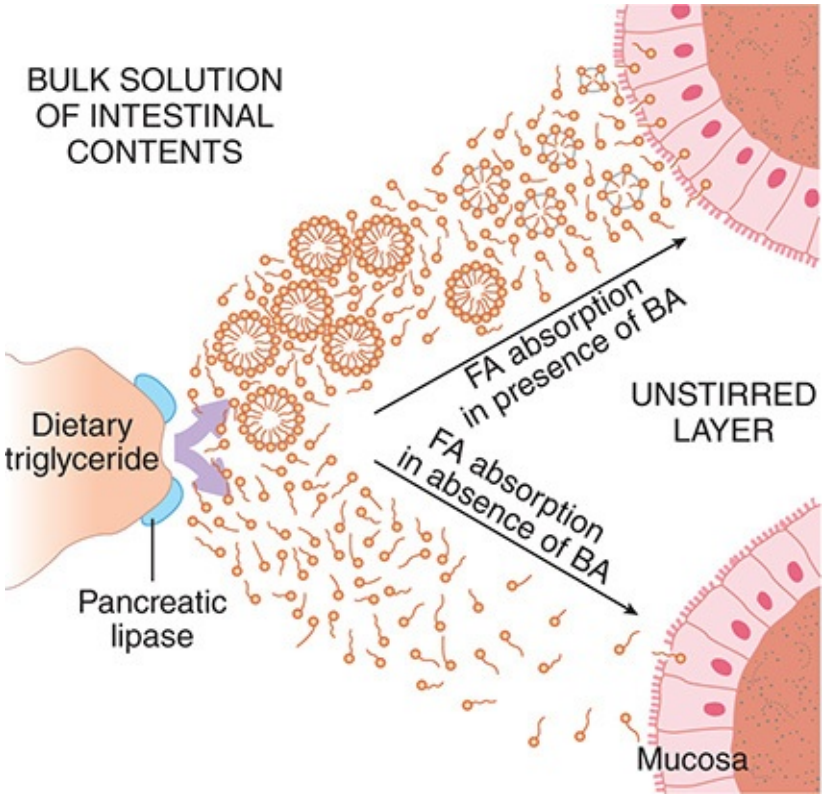


FIGURE 26–6 Lipid digestion and passage to intestinal mucosa. Fatty acids (FA) are liberated by the action of pancreatic lipase on dietary triglycerides and, in the presence of bile acids (BA), form micelles (the circular structures), which diffuse through the unstirred layer to the mucosal surface. Not shown, colipase binds to bile acids on the surface of the triglyceride droplet to anchor lipase to the surface and allow for its lipolytic activity. (Modified with permission from Westergaard H, Dietschy JM: Normal mechanisms of fat absorption and derangements induced by various gastrointestinal diseases. Med Clin North Am Nov; 58(6):1413–1427.)

STEATORRHEA

Pancreatectomized animals and patients with diseases that destroy the exocrine portion of the pancreas have fatty, bulky, clay-colored stools **(steatorrhea)** because of the impaired digestion and absorption of fat. This form of steatorrhea is mostly due to lipase deficiency. However, acid inhibits the lipase, and the lack of alkaline secretion from the pancreas also contributes by lowering the pH of the intestine contents. In some cases, hypersecretion of gastric acid can cause steatorrhea. Another cause of steatorrhea is defective reabsorption of bile acids in the distal ileum (see Chapter 28).

When bile is completely excluded from the intestine, up to 50% of ingested fat may appear in the feces. A severe malabsorption of fat-soluble vitamins also results. When bile acid reabsorption is prevented by resection of the terminal ileum or by disease in this portion of the small intestine, the amount of fat in the stools may also be increased because when the enterohepatic circulation is interrupted, the liver may not be able to increase the rate of bile acid production to a sufficient degree to compensate for the loss. Nevertheless, in some individuals, steatorrhea may not result even in the absence of micelles because the gut has a considerable reserve of absorptive surface area across which lipids may be absorbed in their molecular form, albeit more slowly than in the case of lipids in micellar form. This is referred to as the **anatomic reserve**, and applies also to the assimilation of the digested products of protein and carbohydrate. In individuals who have lost a significant proportion of their bowel and/or absorptive surface area to disease or surgery, however, malabsorption of one or more classes of nutrients may result **(Clinical Box 26–2)**.

FAT ABSORPTION

Traditionally, lipids were thought to enter the enterocytes by passive diffusion, but some evidence now suggests that carriers are involved. Inside the cells, the lipids are rapidly esterified, maintaining a favorable concentration gradient from the lumen into the cells **(Figure 26–7)**. There are also carriers that export certain lipids back into the lumen, thereby limiting their oral availability. This is the case for plant sterols as well as cholesterol.

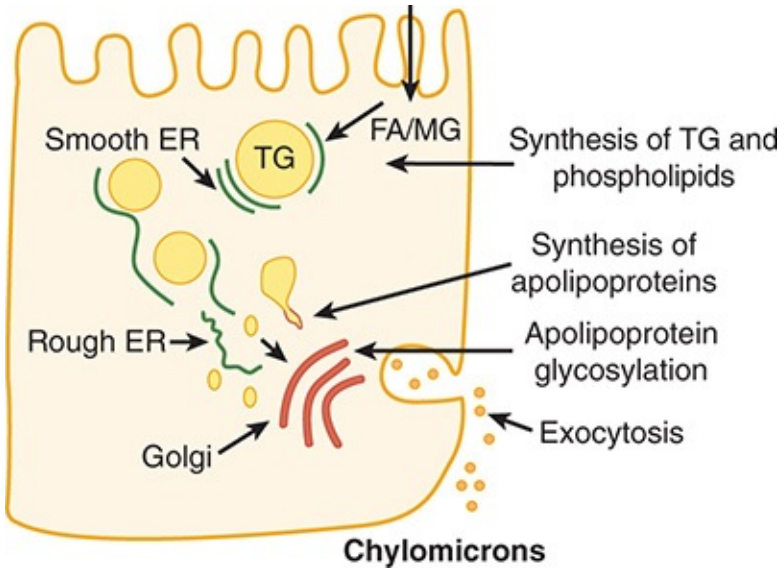


FIGURE 26–7 Intracellular handling of the products of lipid digestion. Absorbed fatty acids (FA) and monoglycerides (MG) are reesterified to form triglyceride (TG) in the smooth endoplasmic reticulum (ER). Apoproteins synthesized in the rough ER are coated around lipid cores, and the resulting chylomicrons are secreted from the basolateral pole of epithelial cells by exocytosis.

The fate of the FA in enterocytes depends on their size. FA containing less than 10–12 carbon atoms are sufficiently water-soluble that they pass through the enterocyte unmodified and are actively transported into the portal blood. They then circulate as free (unesterified) FA. FA containing more than 10–12 carbon atoms are too insoluble for this. They are therefore reesterified to triglycerides in the enterocytes. In addition, some of the absorbed cholesterol is esterified. The triglycerides and cholesterol esters are then coated with a layer of protein, cholesterol, and phospholipid to form chylomicrons. These leave the cell by exocytosis and enter the lymphatics, because they are too large to pass through the junctions between capillary endothelial cells (Figure 26–7).

CLINICAL BOX 26–2

The Malabsorption Syndrome

The digestive and absorptive functions of the small intestine are essential for life. However, the digestive and absorptive capacity of the intestine is larger than needed for normal function (the anatomic reserve). Removal of short

segments of the jejunum or ileum generally does not cause severe symptoms, and compensatory hypertrophy and hyperplasia of the remaining mucosa occur. However, when more than 50% of the small intestine is resected or bypassed **(short gut syndrome),** the absorption of nutrients and vitamins is so compromised that it is very difficult to prevent malnutrition and wasting **(malabsorption).** Resection of the terminal ileum also prevents the absorption of bile acids, and this leads in turn to deficient fat absorption. It also causes diarrhea because the unabsorbed bile acids enter the colon, where they activate chloride secretion (see Chapter 25). Other complications of intestinal resection or bypass include hypocalcemia, arthritis, and possibly fatty infiltration of the liver, followed by cirrhosis. Various disease processes can also impair absorption without a loss of intestinal length. The pattern of deficiencies that results is sometimes called the **malabsorption syndrome.** This pattern varies somewhat with the cause, but it can include deficient absorption of amino acids, with marked body wasting and, eventually, hypoproteinemia and edema. Carbohydrate and fat absorption are also depressed. Because of defective fat absorption, the fat-soluble vitamins (vitamins A, D, E, and K) are not absorbed in adequate amounts. One of the most interesting conditions causing the malabsorption syndrome is the autoimmune disease **celiac disease.** This disease occurs in genetically predisposed individuals in whom gluten and closely related proteins cause intestinal T cells to mount an inappropriate immune response that damages the intestinal epithelial cells and results in a loss of villi and a flattening of the mucosa. The proteins are found in wheat, rye, barley, and to a lesser extent in oats—but not in rice or corn. When grains containing gluten are omitted from the diet, bowel function is generally restored to normal.

THERAPEUTIC HIGHLIGHTS

Treatment of malabsorption depends on the underlying cause. In celiac disease, the mucosa returns to normal if foods containing gluten are strictly excluded from the diet, although this may be difficult to achieve. The diarrhea that accompanies bile acid malabsorption can be treated with a resin (cholestyramine) that binds the bile acids in the lumen and prevents their secretory action on colonocytes. Patients who become deficient in fat-soluble vitamins may be given these compounds as water-soluble derivatives. For serious cases of short bowel syndrome, it may be necessary to supply nutrients parenterally. There is hope that small bowel transplantation may eventually become routine, but of course transplantation carries its own long-term

disadvantages and also requires a reliable supply of donor tissues.

Absorption of long-chain FA is greatest in the upper parts of the small intestine, but appreciable amounts are also absorbed in the ileum. On a moderate fat intake, 95% or more of the ingested fat is absorbed. The processes involved in fat absorption are not fully mature at birth, and infants fail to absorb 10–15% of ingested fat. Thus, they are more susceptible to the ill effects of disease processes that reduce fat absorption.

SHORT-CHAIN FATTY ACIDS IN THE COLON

Short-chain fatty acids (SCFAs) are produced in the colon and absorbed from it. SCFAs are 2–5-carbon weak acids that have an average normal concentration of about 80 mmol/L in the lumen. About 60% of this total is acetate, 25% propionate, and 15% butyrate. They are formed by the action of colonic bacteria (**fermentation**) on complex carbohydrates, resistant starches, and other components of the dietary fiber, that is, the material that escapes digestion in the upper gastrointestinal tract and enters the colon.

Absorbed SCFAs are metabolized and make a significant contribution to the total caloric intake. In addition, they exert a trophic effect on the colonic epithelial cells; combat inflammation; and are absorbed in part by exchange for H^+, helping maintain acid–base equilibrium. SCFAs are absorbed by specific transporters present in colonic epithelial cells. SCFAs also promote the absorption of Na^+.

ABSORPTION OF MINERALS & VITAMINS

MINERALS

A number of minerals must be ingested daily for the maintenance of health. Besides those for which recommended daily dietary allowances have been set, a variety of different trace elements should be included. Trace elements are defined as elements found in tissues in minute amounts. In many cases, the mechanisms that provide for their uptake from the diet are poorly understood. Those believed to be essential for life, at least in experimental animals, are listed in **Table 26–3**. In humans, iron deficiency causes anemia. Cobalt is part of the

vitamin B_{12} molecule, and vitamin B_{12} deficiency leads to megaloblastic anemia (see Chapter 31). Iodine deficiency causes thyroid disorders (see Chapter 19). Zinc deficiency causes skin ulcers, depressed immune responses, and hypogonadal dwarfism. Copper deficiency causes anemia and changes in ossification. Chromium deficiency causes insulin resistance. Fluorine deficiency increases the incidence of dental caries. Sodium and potassium are also essential minerals, but listing them is academic, because it is very difficult to prepare a sodium-free or potassium-free diet. A **low-salt** diet is, however, well tolerated for prolonged periods because of the compensatory mechanisms that conserve Na^+. The mechanisms for sodium and potassium transport from the gut were discussed in Chapter 25.

TABLE 26–3 Trace elements believed essential for life.

Arsenic	Manganese
Chromium	Molybdenum
Cobalt	Nickel
Copper	Selenium
Fluorine	Silicon
Iodine	Vanadium
Iron	Zinc

Conversely, some minerals can be toxic when present in the body in excess. For example, severe iron overload with toxic effects is seen in hemochromatosis (see below). Similarly, copper excess causes brain damage (Wilson disease).

The remainder of this section addresses the intestinal transport mechanisms for two important minerals, calcium and iron.

CALCIUM

A total of 30–80% of ingested calcium is absorbed. The absorptive process and its relation to 1,25-dihydroxycholecalciferol are discussed in Chapter 21. Through this vitamin D derivative, Ca^{2+} absorption is adjusted to body needs; absorption is increased in the presence of Ca^{2+} deficiency and decreased in the presence of Ca^{2+} excess. Ca^{2+} absorption is also facilitated by protein. It is

inhibited by phosphates and oxalates because these anions form insoluble salts with Ca^{2+} in the intestine.

IRON

In adults, the amount of iron lost from the body is relatively small. The losses are generally unregulated, and total body stores of iron are regulated by changes in the rate at which it is absorbed from the intestine. Men lose about 0.6 mg/d, largely in the stools. Premenopausal women have a variable, larger loss averaging about twice this value because of the additional iron lost during menstruation. The average daily iron intake in the United States and Europe is about 20 mg, but the amount absorbed is equal only to the losses. Thus, the amount of iron absorbed is normally about 3–6% of the amount ingested. Various dietary factors affect the availability of iron for absorption; for example, the phytic acid found in cereals reacts with iron to form insoluble compounds in the intestine, as do phosphates and oxalates.

Most of the iron in the diet is in the ferric (Fe^{3+}) form, whereas it is the ferrous (Fe^{2+}) form that is absorbed. Fe^{3+} reductase activity is associated with the iron transporter in the brush borders of the enterocytes **(Figure 26–8)**. Gastric secretions dissolve the iron and permit it to form soluble complexes with ascorbic acid and other substances that aid its reduction to the Fe^{2+} form. The importance of this function in humans is indicated by the fact that iron deficiency anemia is a troublesome and relatively frequent complication of partial gastrectomy.

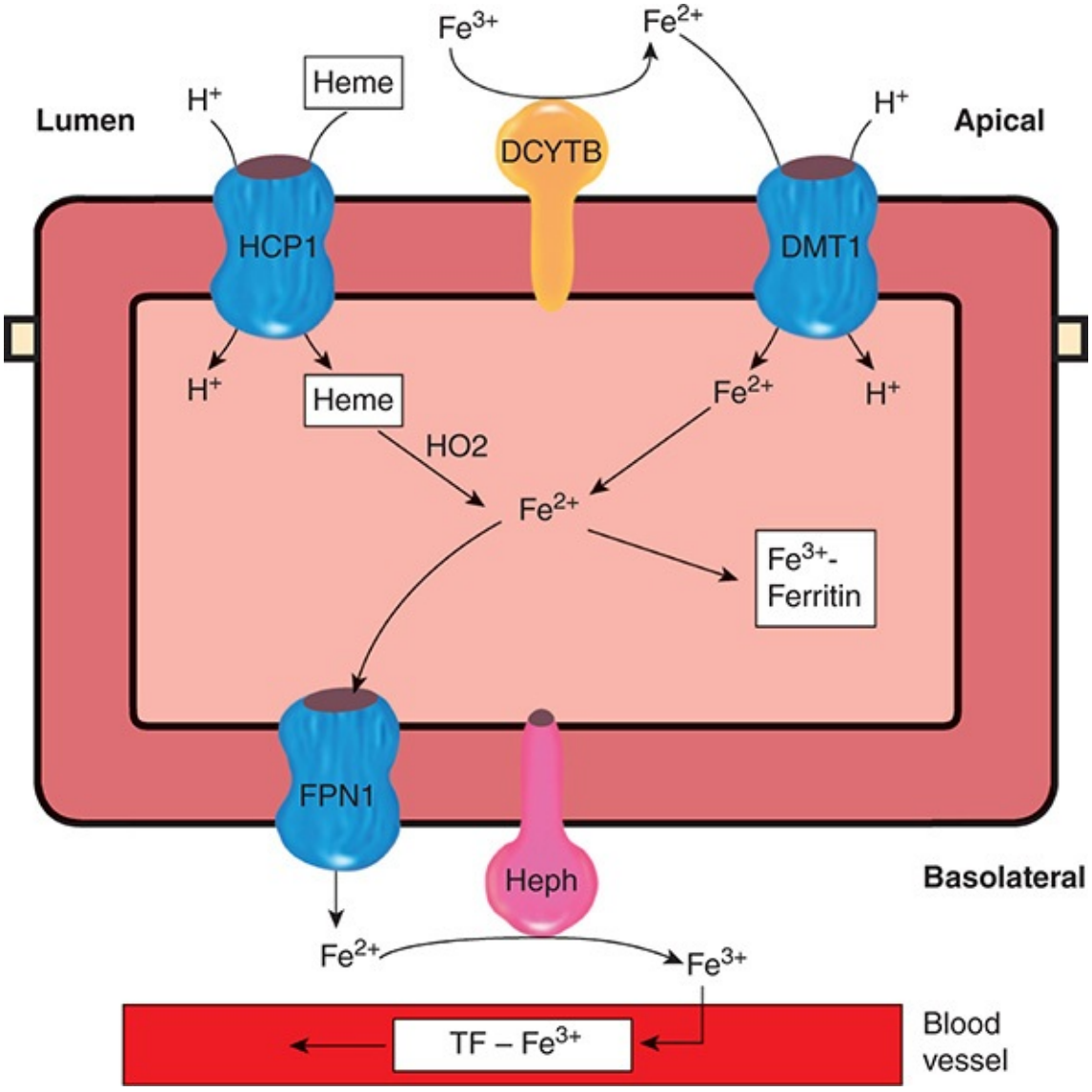


FIGURE 26–8 Intestinal absorption of iron. Fe^{3+} is converted to Fe^{2+} by the ferric reductase DCYTB, and Fe^{2+} is transported into the enterocyte by the apical membrane iron transporter DMT1. Heme is transported into the enterocyte by a separate heme transporter (most likely heme carrier protein 1, HCP1), and heme oxygenase-2 (HO2) releases Fe^{2+} from the heme. Some of the intracellular Fe^{2+} is converted to Fe^{3+} and bound to ferritin. The rest binds to the basolateral Fe^{2+} transporter ferroportin-1 (FPN1) and is transported to the interstitial fluid. The transport is aided by hephaestin (Heph) which converts Fe^{2+} to Fe^{3+}. In plasma, Fe^{3+} is transported bound to the iron transport protein transferrin (TF).

Almost all iron absorption occurs in the duodenum. Transport of Fe^{2+} into the

enterocytes occurs via divalent metal transporter 1 **(DMT1)** (Figure 26–8). Some is stored in ferritin, and the remainder is transported out of the enterocytes by a basolateral transporter named **ferroportin 1.** A protein called **hephaestin (Hp)** is associated with ferroportin 1. It is not a transporter itself, but it facilitates basolateral transport. In the plasma, Fe^{2+} is converted to Fe^{3+} and bound to the iron transport protein **transferrin.** This protein has two iron-binding sites. Normally, transferrin is about 35% saturated with iron, and the normal plasma iron level is about 130 µg/dL (23 µmol/L) in men and 110 µg/dL (19 µmol/L) in women.

Heme (see Chapter 31) binds to an apical transport protein in enterocytes and is carried into the cytoplasm. In the cytoplasm, HO-2, a subtype of heme oxygenase, removes Fe^{2+} from the porphyrin and adds it to the intracellular Fe^{2+} pool.

Seventy percent of the iron in the body is in hemoglobin, 3% in myoglobin, and the rest in ferritin, which is present not only in enterocytes, but also in many other cells. Apoferritin is a globular protein made up of 24 subunits. Ferritin molecules in lysosomal membranes may aggregate in deposits that contain as much as 50% iron. These deposits are called **hemosiderin.**

CLINICAL BOX 26–3

Disorders of Iron Uptake

Iron deficiency causes anemia. Conversely, iron overload causes hemosiderin to accumulate in the tissues, producing **hemosiderosis.** Large amounts of **hemosiderin** can damage tissues, such as is seen in the common genetic disorder of hemochromatosis. This syndrome is characterized by pigmentation of the skin, pancreatic damage with diabetes (“bronze diabetes”), cirrhosis of the liver, a high incidence of hepatic carcinoma, and gonadal atrophy. Hemochromatosis may be hereditary or acquired. The most common cause of the hereditary forms is a mutated *HFE* gene that is common in the white population. It is located on the short arm of chromosome 6 and is closely linked to the HLA-A locus. It is still unknown precisely how mutations in *HFE* cause hemochromatosis, but individuals who are homogenous for *HFE* mutations absorb excess amounts of iron because *HFE* normally inhibits expression of the duodenal transporters that participate in iron uptake. Acquired hemochromatosis occurs when the iron-regulating system is overwhelmed by excess iron loads due to chronic destruction of red

blood cells, liver disease, or repeated transfusions in diseases such as intractable anemia.

THERAPEUTIC HIGHLIGHTS

If hereditary hemochromatosis is diagnosed before excessive amounts of iron accumulate in the tissues, life expectancy can be prolonged substantially by repeated withdrawal of blood.

Intestinal absorption of iron is regulated by three factors: recent dietary intake of iron, the state of the iron stores in the body, and the state of erythropoiesis in the bone marrow. The normal operation of the factors that maintain iron balance is essential for health **(Clinical Box 26–3)**.

VITAMINS

Vitamins were discovered when it was observed that certain diets otherwise adequate in calories, essential amino acids, fats, and minerals failed to maintain health (for example, in sailors engaged in long voyages without access to fresh fruits and vegetables). The term **vitamin** has now come to refer to any organic dietary constituent necessary for life, health, and growth that does not function by supplying energy, and which cannot be synthesized endogenously (at least in adequate amounts).

Most vitamins are absorbed in the upper small intestine, but vitamin B_{12} is absorbed in the ileum. This vitamin binds to intrinsic factor, a protein secreted by the parietal cells of the stomach, and the complex is the form that is absorbed across the ileal mucosa.

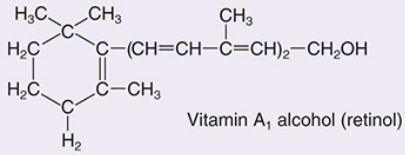
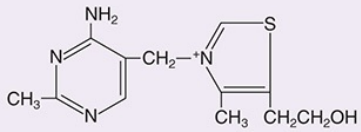
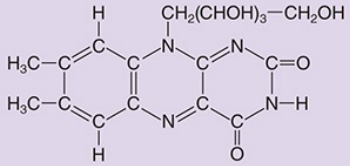
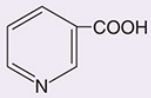
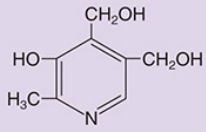
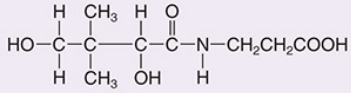
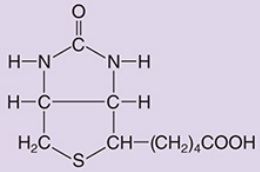
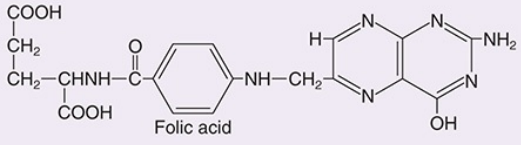
Vitamin B_{12} absorption and folate absorption are Na^+-independent, but all seven of the remaining water-soluble vitamins—thiamin, riboflavin, niacin, pyridoxine, pantothenate, biotin, and ascorbic acid—are absorbed by carriers that are Na^+ cotransporters.

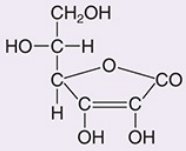
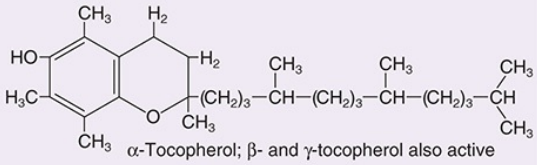
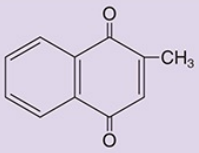
The water-soluble vitamins are easily absorbed, but the fat-soluble vitamins (vitamins A, D, E, and K) are poorly absorbed in the absence of bile and/or pancreatic juice because their absorption depends almost entirely on micellar solubilization. Some dietary fat intake is also necessary for their absorption, and in obstructive jaundice or disease of the exocrine pancreas, deficiencies of the

fat-soluble vitamins can develop even if their intake is adequate. Vitamin A and vitamin D are bound to transfer proteins in the circulation. The α-tocopherol form of vitamin E is normally bound to chylomicrons. In the liver, it is transferred to very low-density lipoprotein (VLDL) and distributed to tissues by an α-tocopherol transfer protein. When this protein is abnormal due to mutation of its gene in humans, there is cellular deficiency of vitamin E and the development of a condition resembling Friedreich ataxia.

Because there are minor differences in metabolism between mammalian species, some substances are vitamins in one species and not in another. The sources and functions of the major vitamins in humans are listed in **Table 26–4**. Most vitamins have important functions in intermediary metabolism or the special metabolism of the various organ systems. The diseases caused by deficiency of each of the vitamins are also listed in Table 26–4.

TABLE 26–4 Vitamins essential or probably essential to human nutrition.[a]

Vitamin	Action	Deficiency Symptoms	Sources	Chemistry
A (A_1, A_2)	Constituents of visual pigments (see Chapter 9: Vision); necessary for fetal development and for cell development throughout life	Night blindness, dry skin	Yellow vegetables and fruit	 Vitamin A_1 alcohol (retinol)
B complex				
Thiamin (vitamin B_1)	Cofactor in decarboxylations	Beriberi, neuritis	Liver, unrefined cereal grains	
Riboflavin (vitamin B_2)	Constituent of flavoproteins	Glossitis, cheilosis	Liver, milk	
Niacin	Constituent of NAD^+ and $NADP^+$	Pellagra	Yeast, lean meat, liver	 Can be synthesized in body from tryptophan
Pyridoxine (vitamin B_6)	Forms prosthetic group of certain decarboxylases and transaminases. Converted in body into pyridoxal phosphate and pyridoxamine phosphate	Convulsions, hyperirritability	Yeast, wheat, corn, liver	
Pantothenic acid	Constituent of CoA	Dermatitis, enteritis, alopecia, adrenal insufficiency	Eggs, liver, yeast	
Biotin	Catalyzes CO_2 "fixation" (in fatty acid synthesis, etc)	Dermatitis, enteritis	Egg yolk, liver, tomatoes	
Folates (folic acid) and related compounds	Coenzymes for "1-carbon" transfer; involved in methylating reactions	Sprue, anemia. Neural tube defects in children born to folate-deficient women	Leafy green vegetables	 Folic acid

Cyanocobalamin (vitamin B_{12})	Coenzyme in amino acid metabolism. Stimulates erythropoiesis	Pernicious anemia (see Chapter 25: Overview of Gastrointestinal Function & Regulation)	Liver, meat, eggs, milk	Complex of four substituted pyrrole rings around a cobalt atom (see Chapter 25: Overview of Gastrointestinal Function & Regulation)
C	Maintains prosthetic metal ions in their reduced form; scavenges free radicals	Scurvy	Citrus fruits, leafy green vegetables	 Ascorbic acid (synthesized in most mammals except guinea pigs and primates, including humans)
D group	Increase intestinal absorption of calcium and phosphate (see Chapter 21: Hormonal Control of Calcium & Phosphate Metabolism & the Physiology of Bone)	Rickets	Fish liver	Family of sterols (see Chapter 21: Hormonal Control of Calcium & Phosphate Metabolism & the Physiology of Bone)
E group	Antioxidants; cofactors in electron transport in cytochrome chain?	Ataxia and other symptoms and signs of spinocerebellar dysfunction	Milk, eggs, meat, leafy vegetables	 α-Tocopherol; β- and γ-tocopherol also active
K group	Catalyze γ carboxylation of glutamic acid residues on various proteins concerned with blood clotting	Hemorrhagic phenomena	Leafy green vegetables	 Vitamin K_3; a large number of similar compounds have biological activity

[a]Choline is synthesized in the body in small amounts, but it has recently been added to the list of essential nutrients.

It is worth remembering, however, that very large doses of the fat-soluble vitamins are definitely toxic. **Hypervitaminosis A** is characterized by anorexia, headache, hepatosplenomegaly, irritability, scaly dermatitis, patchy loss of hair, bone pain, and hyperostosis. Acute vitamin A intoxication was first described by Arctic explorers, who developed headache, diarrhea, and dizziness after eating polar bear liver, which is particularly rich in vitamin A. **Hypervitaminosis D** is associated with weight loss, calcification of many soft tissues, and acute kidney injury. **Hypervitaminosis K** is characterized by gastrointestinal disturbances and anemia. Large doses of water-soluble vitamins have been thought to be less likely to cause problems because they can be rapidly cleared from the body. However, it has been demonstrated that ingestion of megadoses of pyridoxine (vitamin B_6) can produce peripheral neuropathy.

CONTROL OF FOOD INTAKE

The intake of nutrients is under complex control involving signals from both the periphery and the central nervous system. Complicating the picture, higher functions also modulate the response to both central and peripheral cues that either trigger or inhibit food intake. Thus, food preferences, emotions, environment, lifestyle, and circadian rhythms may all have profound effects on whether food is sought, and the type of food that is ingested.

Many of the hormones and other factors that are released coincident with a meal, and may play other important roles in digestion and absorption (see Chapter 25) are also involved in the regulation of feeding behavior **(Figure 26–9)**. For example, CCK either produced by I cells in the intestine, or released by nerve endings in the brain, inhibits further food intake and thus is defined as a **satiety factor** or **anorexin.** CCK and other similar factors have attracted great interest from the pharmaceutical industry in the hopes that derivatives might be useful as aids to dieting, an objective that is lent greater urgency given the current epidemic of obesity in Western countries **(Clinical Box 26–4)**.

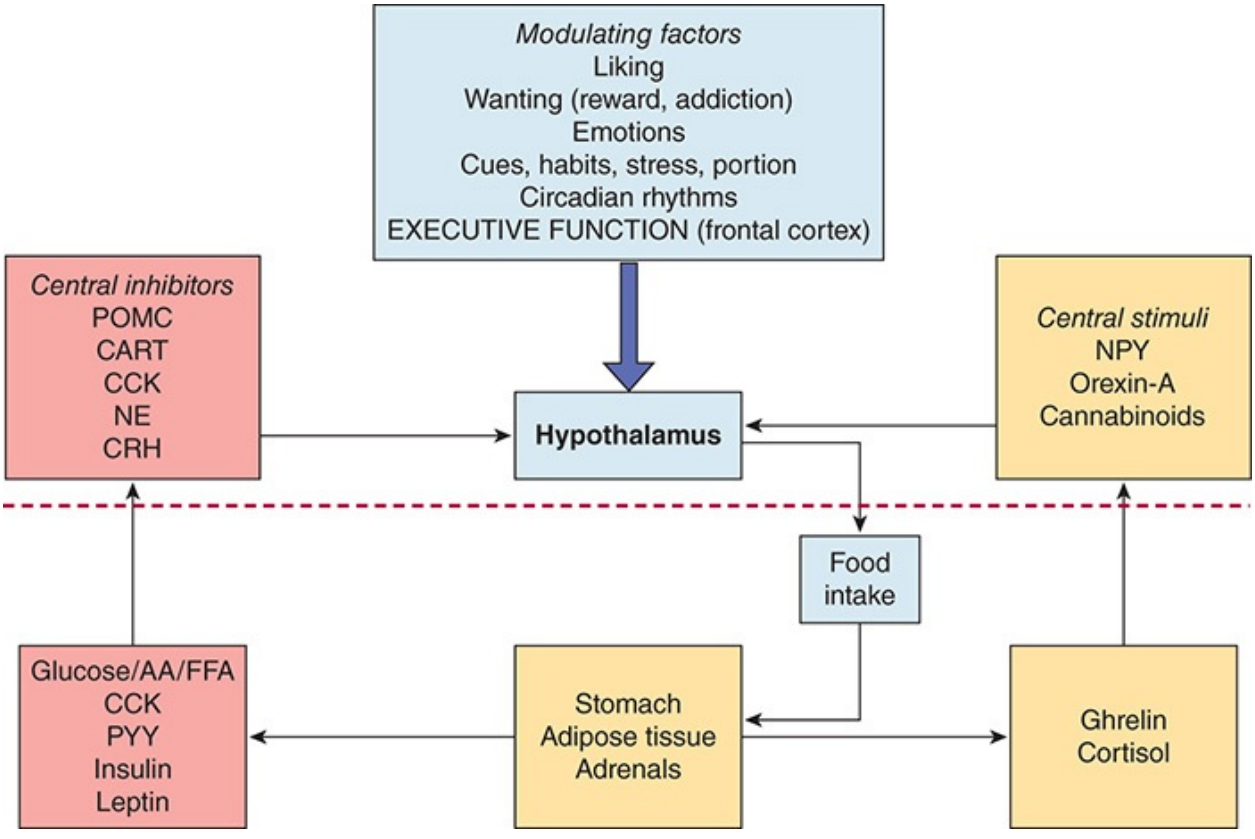


FIGURE 26–9 Summary of mechanisms controlling food intake. Peripheral

stimuli and inhibitors, release in anticipation of or in response to food intake, cross the blood-brain barrier (indicated by the broken red line) and activate the release and/or synthesis of central factors in the hypothalamus that either increase or decrease subsequent food intake. Food intake can also be modulated by signals from higher centers, as shown. Not shown, peripheral orexins can reduce production of central inhibitors, and vice versa. AA, amino acid; CART, cocaine- and amphetamine-regulated transcript; CCK, cholecystokinin; CRH, corticotropin-releasing hormone; FFA, free fatty acids; NE, norepinephrine; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; PYY, Peptide YY. (Used with permission of Dr Samuel Klein, Washington University.)

Leptin and ghrelin are peripheral factors that act reciprocally on food intake, and have emerged as critical regulators in this regard. Both activate their receptors in the hypothalamus that initate signaling cascades leading to changes in food intake. Leptin is produced by adipose tissue, and signals the status of the fat stores therein. As adipocytes increase in size, they release greater quantities of leptin and this tends to decrease food intake, in part by increasing the expression of other anorexigenic factors in the hypothalamus such as pro-opiomelanocortin (POMC), cocaine- and amphetamine-regulated transcript (CART), neurotensin, and corticotropin-releasing hormone (CRH). Leptin also stimulates the metabolic rate. Animal studies have shown that it is possible to become resistant to the effects of leptin, however, and in this setting, food intake persists despite adequate (or even growing) adipose stores—obesity therefore results.

CLINICAL BOX 26–4

Obesity

Obesity is the most common and most expensive nutritional problem in the United States. A convenient and reliable indicator of body fat is the **body mass index (BMI),** which is body weight (in kilograms) divided by the square of height (in meters). Values above 25 are abnormal. Individuals with values of 25–30 are considered overweight, and those with values >30 are obese. In the United States, 34% of the population is overweight and 34% is obese. The incidence of obesity is also increasing in other countries. Indeed, the Worldwatch Institute has estimated that although starvation continues to be a problem in many parts of the world, the number of overweight people in

the world is now as great as the number of underfed. Obesity is a problem because of its complications. It is associated with accelerated atherosclerosis and an increased incidence of gallbladder and other diseases. Its association with type 2 diabetes is especially striking. As weight increases, insulin resistance increases and frank diabetes appears. At least in some cases, glucose tolerance is restored when weight is lost. In addition, the mortality rates from many kinds of cancer are increased in obese individuals. The causes of the high incidence of obesity in the general population are probably multiple. Studies of twins raised apart show a definite genetic component. It has been pointed out that through much of human evolution, famines were common, and mechanisms that permitted increased energy storage as fat had survival value. Now, however, food is plentiful in many countries, and the ability to gain and retain fat has become a liability. As noted above, the fundamental cause of obesity is still an excess of energy intake in food over energy expenditure. If human volunteers are fed a fixed high-calorie diet, some gain weight more rapidly than others, but those with slower weight gain can be shown to exhibit increased energy expenditure in the form of small, fidgety movements **(nonexercise activity thermogenesis; NEAT).** Body weight generally increases at a slow but steady rate throughout adult life. Decreased physical activity is undoubtedly a factor in this increase, but decreased sensitivity to leptin may also play a role.

THERAPEUTIC HIGHLIGHTS

Obesity is such a vexing medical and public health problem because its effective treatment depends so dramatically on lifestyle changes. Long-term weight loss can only be achieved with decreased food intake, increased energy expenditure, or, ideally, some combination of both. Exercise alone is rarely sufficient because it typically induces the patient to ingest more calories. For those who are seriously obese and who have developed serious health complications as a result, a variety of surgical approaches have been developed that reduce the size of the stomach reservoir and/or bypass it altogether. These surgical maneuvers are intended to reduce the size of meals that can be tolerated, but also have dramatic metabolic effects even before significant weight loss occurs, perhaps as a result of reduced production of peripheral orexins, such as ghrelin, by the gut. Pharmaceutical companies are also actively exploring the science of orexins and anorexins to develop drugs that might act centrally to modify food intake (Figure 26–9).

Ghrelin, on the other hand, is a predominantly fast-acting **orexin** that stimulates food intake. It is produced mainly by the stomach, as well as other tissues such as the pancreas and adrenal glands in responses to changes in nutritional status—circulating ghrelin levels increase preprandially, then decrease after a meal. It is believed to be involved primarily in meal initiation, unlike the longer-term effects of leptin. Like leptin, however, the effects of ghrelin are produced mostly via actions in the hypothalamus. It increases synthesis and/or release of central orexins, including neuropeptide Y and cannabinoids, and suppresses the ability of leptin to stimulate the anorexigenic factors discussed above. Loss of the activity of ghrelin may account in part for the effectiveness of gastric bypass procedures for obesity. Its secretion may also be inhibited by leptin, underscoring the reciprocity of these hormones. There is some evidence to suggest, however, that the ability of leptin to reduce ghrelin secretion is lost in the setting of obesity.

CHAPTER SUMMARY

- A balanced diet is important for health, and certain substances obtained from the diet are essential to life. The caloric value of dietary intake must be approximately equal to energy expenditure for homeostasis.
- A typical mixed meal consists of carbohydrates, proteins, and lipids (the latter largely in the form of triglycerides). Each must be digested to allow its uptake into the body. Specific transporters carry the products of digestion into the body.
- In the process of carbohydrate assimilation, the epithelium can only transport monomers, whereas for proteins, short peptides can be absorbed in addition to amino acids.
- The protein assimilation machinery, which rests heavily on the proteases in pancreatic juice, is arranged such that these enzymes are not activated until they reach their substrates in the small intestinal lumen. This is accomplished by the restricted localization of an activating enzyme, enterokinase.
- Lipids face special challenges to assimilation given their hydrophobicity. Bile acids solubilize the products of lipolysis in micelles and accelerate their ability to diffuse to the epithelial surface. The assimilation of triglycerides is enhanced by this mechanism, whereas that of cholesterol and fat-soluble vitamins absolutely requires it.

- Food intake is regulated by a complex network of signals both from the periphery and in the central nervous system. Ghrelin released by the stomach is a critical peripheral orexin that stimulates centers in the hypothalamus to trigger food intake. Leptin is released by adipocytes and signals the status of fat stores. Unless leptin resistance has developed, it stimulates the release of anorexins in the hypothalamus to terminate feeding behavior.
- When energy intake exceeds expenditures, the increasingly prevalent modern disease of obesity results.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. A newborn baby is brought to the pediatrician suffering from severe diarrhea that worsens with meals. The symptoms diminish when nutrients are delivered intravenously. The child most likely has a mutation in which of the following intestinal transporters?

   A. Na^+, K^+ ATPase

   B. NHE3

   C. SGLT-1

   D. H^+, K^+ ATPase

   E. NKCC1

2. An infant that was previously healthy but is displaying symptoms of acute diarrhea and dehydration after a presumed enteric infection is given Pedialyte, a solution of glucose and electrolytes. What membrane protein accounts for the ability of this solution to provide more rapid hydration than plain water?

   A. Sucrase-isomaltase

   B. SGLT-1

   C. CFTR

   D. Chloride-bicarbonate exchanger

   E. Lactose-phlorizin hydrolase

3. In the situation described in question 2, the child's mother does not have any Pedialyte available, so gives the infant some boiled water in which she has dissolved some salt and table sugar. Activity of which enzyme will be needed to make this solution effective?

A. Salivary amylase

B. Pancreatic amylase

C. Glucoamylase

D. Lactose-phlorizin hydrolase

E. Sucrase-isomaltase

4. A decrease in which of the following would be expected in a child exhibiting a congenital absence of enterokinase?

A. Incidence of pancreatitis

B. Glucose absorption

C. Bile acid reabsorption

D. Gastric pH

E. Protein assimilation

5. In Hartnup disease (a defect in the transport of neutral amino acids), patients do not become deficient in these amino acids due to the activity of

A. PepT1.

B. brush border peptidases.

C. Na^+, K^+ ATPase.

D. cystic fibrosis transmembrane conductance regulator (CFTR).

E. trypsin.

6. A patient with hypercholesterolemia is treated with cholestyramine, a resin that binds bile acids in the intestinal lumen. Overall levels of absorption of which of the following is likely to be abnormal in this patient?

A. Long-chain triglyceride

B. Medium-chain triglyceride

C. Starch

D. Vitamin D

E. Vitamin B_6

7. Maximum absorption of short-chain fatty acids produced by bacteria occurs in the

A. stomach.

B. duodenum.

C. jejunum.

D. ileum.

E. colon.

8. A premenopausal woman who is physically active seeks advice from her primary care clinician regarding measures she can take to ensure adequate availability of dietary calcium to ensure bone health later in life. Which of the following dietary components should enhance calcium uptake?

A. Protein

B. Oxalates

C. Iron

D. Vitamin D

E. Sodium

9. A patient with obstructive jaundice who is scheduled for gallbladder surgery is found to have an elevated prothrombin time. This laboratory finding is most likely due to malabsorption of which of the following vitamins?

A. A

B. C

C. B_{12}

D. K

E. E

10. A 40-year-old man undergoes bariatric surgery for the treatment of morbid obesity. Levels of which of the following substances would be expected to be reduced in his brain following recovery?

A. Glucose

B. Ghrelin

C. Leptin

D. Pro-opiomelanocortin

E. Neurotensin

CHAPTER 27

Gastrointestinal Motility

OBJECTIVES

After studying this chapter, you should be able to:

- List the major forms of motility in the gastrointestinal tract and their roles in digestion and excretion; distinguish between peristalsis and segmentation.
- Explain the electrical basis of gastrointestinal contractions and the role of basic electrical activity in governing motility patterns.
- Describe how gastrointestinal motility changes during fasting.
- Understand how food is swallowed and transferred to the stomach.
- Define the factors that govern gastric emptying and the abnormal response of vomiting.
- Define how the motility patterns of the colon subserve its function to desiccate and evacuate the stool.

INTRODUCTION

The digestive and absorptive functions of the gastrointestinal system outlined in the previous chapter depend on a variety of mechanisms that soften the food, propel it through the length of the gastrointestinal tract **(Table 27–1)**, and mix it

with bile from the gallbladder and digestive enzymes secreted by the salivary glands and pancreas. Some of these mechanisms depend on intrinsic properties of the intestinal smooth muscle. Others involve the operation of reflexes involving the neurons intrinsic to the gut, reflexes involving the central nervous system (CNS), paracrine effects of chemical messengers, and gastrointestinal hormones.

TABLE 27–1 Mean lengths of various segments of the gastrointestinal tract as measured by intubation in living humans.

Segment	Length (cm)
Pharynx, esophagus, and stomach	65
Duodenum	25
Jejunum and ileum	260
Colon	110

Data from Hirsch JE, Ahrens EH Jr, Blankenhorn DH: Measurement of human intestinal length in vivo and some causes of variation. Gastroenterology 1956;31:274.

GENERAL PATTERNS OF MOTILITY

PERISTALSIS

Peristalsis is a reflex response that is initiated when the gut wall is stretched by the contents of the lumen, and it occurs in all parts of the gastrointestinal tract from the esophagus to the rectum. The stretch initiates a circular contraction behind the stimulus and an area of relaxation in front of it **(Figure 27–1)**. The wave of contraction then moves in an oral-to-caudal direction, propelling the contents of the lumen forward at rates that vary from 2 to 25 cm/s. Peristaltic activity can be increased or decreased by the autonomic input to the gut, but its occurrence is independent of extrinsic innervation. Indeed, progression of the contents is not blocked by removal and resuture of a segment of intestine in its original position and is blocked only if the segment is reversed before it is sewn back into place. Peristalsis is an excellent example of the integrated activity of the enteric nervous system. It appears that local stretch releases serotonin, which activates sensory neurons that activate the myenteric plexus. Cholinergic neurons passing in a retrograde direction in this plexus activate neurons that release substance P and acetylcholine, causing smooth muscle contraction behind

the bolus. At the same time, cholinergic neurons passing in an anterograde direction activate neurons that secrete NO and vasoactive intestinal polypeptide (VIP), producing the relaxation ahead of the stimulus.

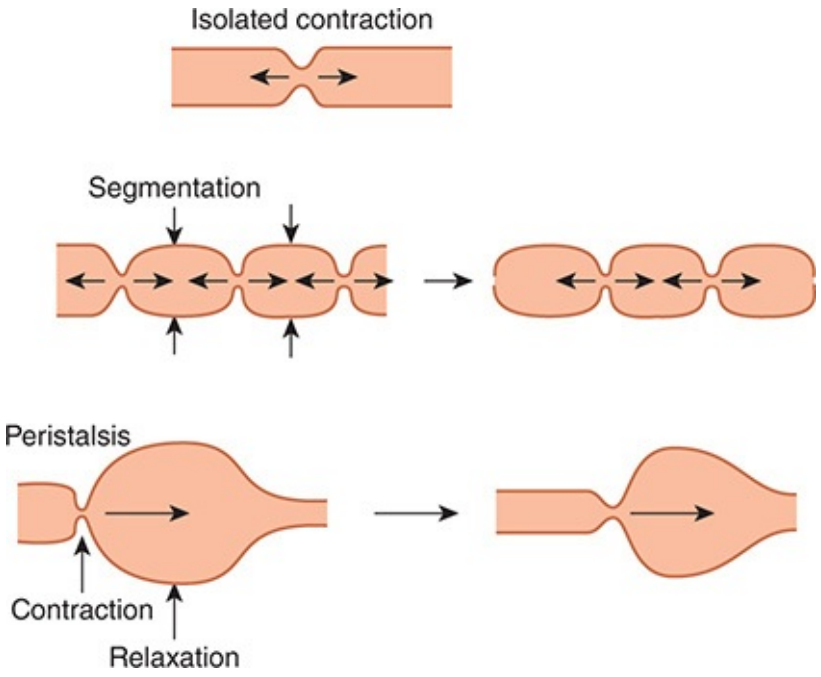


FIGURE 27–1 Patterns of gastrointestinal motility and propulsion. An isolated contraction moves contents orally and aborally. Segmentation mixes contents over a short stretch of intestine, as indicated by the time sequence from left to right. In the diagram on the left, the vertical arrows indicate the sites of subsequent contraction. Peristalsis involves both contraction and relaxation, and moves contents aborally.

SEGMENTATION & MIXING

When the meal is present, the enteric nervous system promotes a motility pattern that is related to peristalsis but is designed to retard the movement of the intestinal contents along the length of the intestinal tract to provide time for digestion and absorption (Figure 27–1). This motility pattern is known as segmentation, and it provides for ample mixing of the intestinal contents (known as **chyme**) with the digestive juices. A segment of bowel contracts at both ends, and then a second contraction occurs in the center of the segment to force the chyme both backward and forward. Unlike peristalsis, therefore, retrograde movement of the chyme occurs routinely in the setting of segmentation. This

mixing pattern persists for as long as nutrients remain in the lumen to be absorbed. It presumably reflects programmed activity of the bowel dictated by the enteric nervous system, and can occur independent of central input, although the latter can modulate it.

BASIC ELECTRICAL ACTIVITY & REGULATION OF MOTILITY

Except in the esophagus and the proximal portion of the stomach, the smooth muscle of the gastrointestinal tract has spontaneous rhythmic fluctuations in membrane potential between about −65 and −45 mV. This **basic electrical rhythm (BER)** is initiated by the **interstitial cells of Cajal,** which are stellate mesenchymal pacemaker cells with smooth muscle-like features that send long multiply branched processes into the intestinal smooth muscle. In the stomach and the small intestine, these cells are located in the outer circular muscle layer near the myenteric plexus; in the colon, they are at the submucosal border of the circular muscle layer. In the stomach and small intestine, there is a descending gradient in pacemaker frequency, and as in the heart, the pacemaker with the highest frequency usually dominates.

The BER itself rarely causes muscle contraction, but **spike potentials** superimposed on the most depolarizing portions of the BER waves do increase muscle tension **(Figure 27–2)**. The depolarizing portion of each spike is due to Ca^{2+} influx, and the repolarizing portion is due to K^{+} efflux. Many polypeptides and neurotransmitters affect the BER. For example, acetylcholine increases the number of spikes and the tension of the smooth muscle, whereas epinephrine decreases the number of spikes and the tension. The rate of the BER is about 4/min in the stomach. It is about 12/min in the duodenum and falls to about 8/min in the distal ileum. In the colon, the BER rate rises from about 2/min at the cecum to about 6/min at the sigmoid. The function of the BER is to coordinate peristaltic and other motor activity, such as setting the rhythm of segmentation; contractions can occur only during the depolarizing part of the waves. After vagotomy or transection of the stomach wall, for example, peristalsis in the stomach becomes irregular and chaotic.

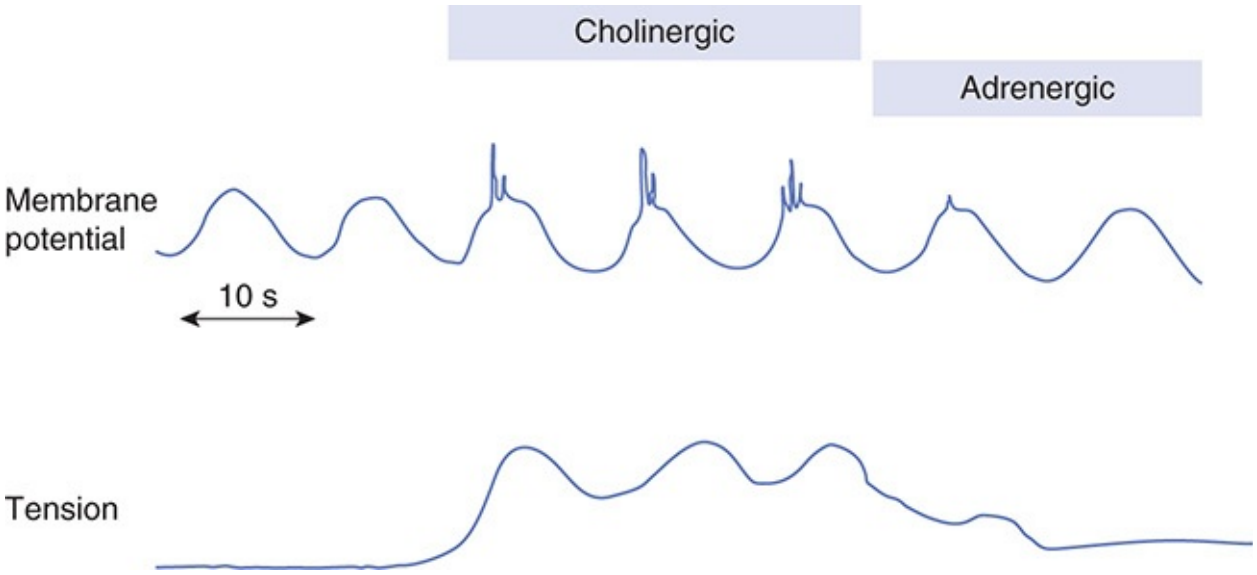


FIGURE 27–2 Basic electrical rhythm (BER) of gastrointestinal smooth muscle. Top: Membrane potential showing spike potentials under conditions of maximal cholinergic tone and inhibition under adrenergic tone. **Bottom:** Associated changes in muscle tension.

MIGRATING MOTOR COMPLEX

During fasting between periods of digestion, the pattern of electrical and motor activity in gastrointestinal smooth muscle becomes modified so that cycles of motor activity migrate from the stomach to the distal ileum. Each cycle, or **migrating motor complex (MMC),** starts with a quiescent period (phase I), continues with a period of irregular electrical and mechanical activity (phase II), and ends with a burst of regular activity (phase III) **(Figure 27–3)**. The MMCs are initiated by motilin. The circulating level of this hormone increases at intervals of approximately 100 min in the interdigestive state, coordinated with the contractile phases of the MMC. The contractions migrate aborally at a rate of about 5 cm/min, and also occur at intervals of approximately 100 min. Gastric secretion, bile flow, and pancreatic secretion increase during each MMC. They likely serve to clear the stomach and small intestine of luminal contents in preparation for the next meal.

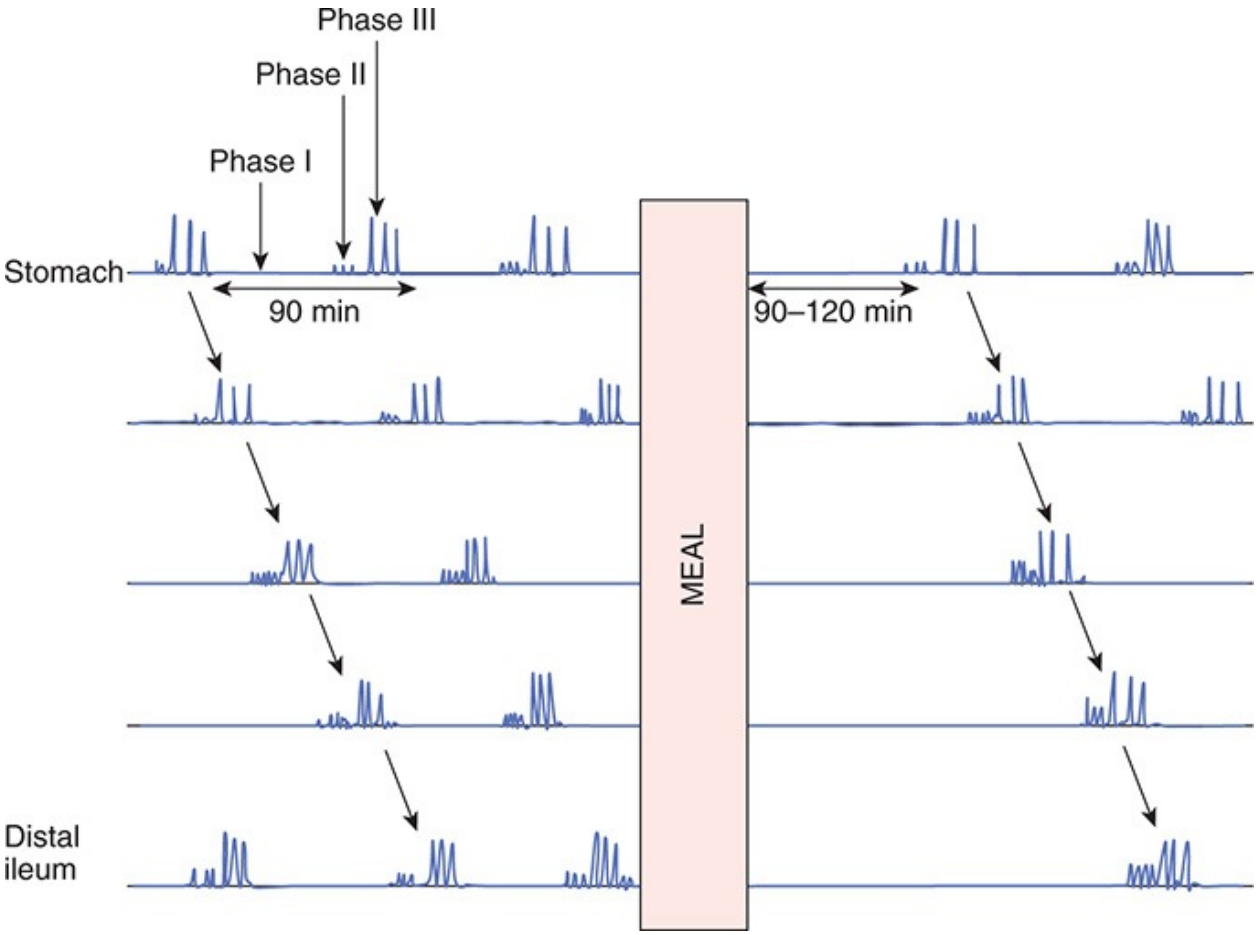


FIGURE 27–3 Migrating motor complexes (MMCs). The three phases include a quiescent phase (phase I); a phase consisting of small, irregular contractions that do not propagate (phase II); and a phase of regular activity lasting about 5 minutes (phase III), which sweeps along the length of the intestine. The entire cycle repeats every 90–100 minutes under fasting conditions. Note that the complexes are completely inhibited by a meal and resume 90–120 min later.

Conversely, when a meal is ingested, secretion of motilin is suppressed (ingestion of food suppresses motilin release via mechanisms that have not yet been elucidated), and the MMC is abolished, until digestion and absorption are complete.

SEGMENT-SPECIFIC PATTERNS OF MOTILITY

MOUTH & ESOPHAGUS

In the mouth, food is mixed with saliva and propelled into the esophagus. Peristaltic waves in the esophagus move the food into the stomach.

MASTICATION

Chewing **(mastication)** breaks up large food particles and mixes the food with the secretions of the salivary glands. This wetting and homogenizing action aids swallowing and subsequent digestion. Large food particles can be digested, but they cause strong and often painful contractions of the esophageal musculature. Particles that are small tend to disperse in the absence of saliva and also make swallowing difficult because they do not form a bolus. The number of chews that is optimal depends on the food, but usually ranges from 20 to 25.

Edentulous patients are generally restricted to a soft diet and have considerable difficulty eating dry food.

SWALLOWING

Swallowing (deglutition) is a reflex response that is triggered by afferent impulses in the trigeminal, glossopharyngeal, and vagus nerves **(Figure 27–4)**. These impulses are integrated in the nucleus of the tractus solitarius and the nucleus ambiguus. The efferent fibers pass to the pharyngeal musculature and the tongue via the trigeminal, facial, and hypoglossal nerves. Swallowing is initiated by the voluntary action of collecting the oral contents on the tongue and propelling them backward into the pharynx. This starts a wave of involuntary contraction in the pharyngeal muscles that pushes the material into the esophagus. Inhibition of respiration and glottic closure are part of the reflex response. A peristaltic ring contraction of the esophageal muscle forms behind the material, which is then swept down the esophagus at a speed of approximately 4 cm/s. When humans are in an upright position, liquids and semisolid foods generally fall by gravity to the lower esophagus ahead of the peristaltic wave. However, if any food remains in the esophagus, it is cleared by a second wave of peristalsis. It is therefore possible to swallow food while standing on one’s head.

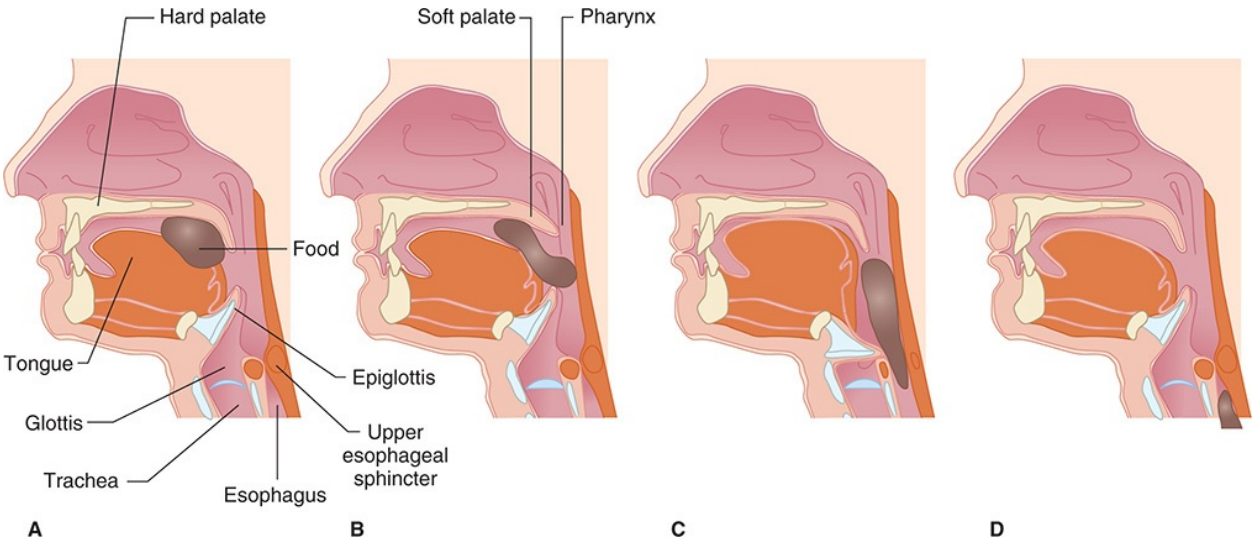


FIGURE 27–4 Movement of food through the pharynx and upper esophagus during swallowing. A) The tongue pushes the food bolus to the back of the mouth. **B)** The soft palate elevates to prevent food from entering the nasal passages. **C)** The epiglottis covers the glottis to prevent food from entering the trachea and the upper esophageal sphincter relaxes. **D)** Food descends into the esophagus.

LOWER ESOPHAGEAL SPHINCTER

Unlike the rest of the esophagus, the musculature of the gastroesophageal junction **(lower esophageal sphincter; LES)** is tonically active but relaxes on swallowing. The tonic activity of the LES between meals prevents reflux of gastric contents into the esophagus. The LES is made up of three components **(Figure 27–5)**. The esophageal smooth muscle is more prominent at the junction with the stomach (intrinsic sphincter). Fibers of the crural portion of the diaphragm, a skeletal muscle, surround the esophagus at this point (extrinsic sphincter) and exert a pinchcock-like action on the esophagus. In addition, the oblique or sling fibers of the stomach wall create a flap valve that helps close off the esophagogastric junction and prevent regurgitation when intragastric pressure rises.

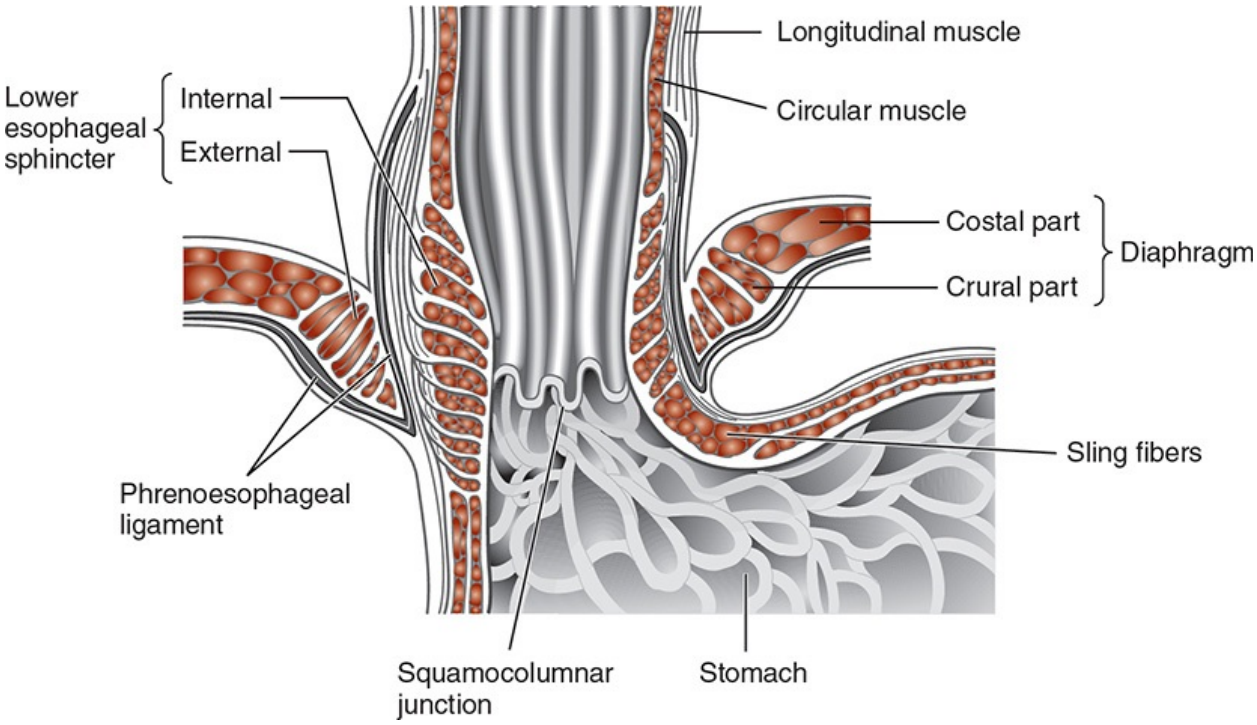


FIGURE 27–5 Esophagogastric junction. Note that the lower esophageal sphincter (intrinsic sphincter) is supplemented by the crural portion of the diaphragm (extrinsic sphincter), and that the two are anchored to each other by the phrenoesophageal ligament. (Reproduced with permission from Mittal RK, Balaban DH: The esophagogastric junction. N Engl J Med 1997; Mar 27; 336(13):924–932.)

The tone of the LES is under neural control. Release of acetylcholine from vagal endings causes the intrinsic sphincter to contract, and release of NO and VIP from interneurons innervated by other vagal fibers causes it to relax. Contraction of the crural portion of the diaphragm, which is innervated by the phrenic nerves, is coordinated with respiration and contractions of chest and abdominal muscles. Thus, the intrinsic and extrinsic sphincters operate together to permit orderly flow of food into the stomach and to prevent reflux of gastric contents into the esophagus **(Clinical Box 27–1)**.

AEROPHAGIA & INTESTINAL GAS

Some air is unavoidably swallowed in the process of eating and drinking **(aerophagia).** Some of the swallowed air is regurgitated (belching), and some of the gases it contains are absorbed, but much of it passes on to the colon. Here,

some of the oxygen is absorbed, and hydrogen, hydrogen sulfide, carbon dioxide, and methane formed by the colonic bacteria from carbohydrates and other substances are added to it. It is then expelled as **flatus.** The smell is largely due to sulfides. The volume of gas normally found in the human gastrointestinal tract is about 200 mL, and the daily production is 500–1500 mL. In some individuals, gas in the intestines causes cramps, **borborygmi** (rumbling noises), and abdominal discomfort.

STOMACH

Food is stored in the stomach; mixed with acid, mucus, and pepsin; and released at a controlled, steady rate into the duodenum.

GASTRIC MOTILITY & EMPTYING

When food enters the stomach, the fundus and upper portion of the body relax and accommodate the food with little if any increase in pressure **(receptive relaxation).** Peristalsis then begins in the lower portion of the body, mixing and grinding the food and permitting small, semiliquid portions of it to pass through the pylorus and enter the duodenum.

Receptive relaxation is, in part, vagally mediated and triggered by movement of the pharynx and esophagus. Intrinsic reflexes also lead to relaxation as the stomach wall is stretched. Peristaltic waves controlled by the gastric BER begin soon thereafter and sweep toward the pylorus. The contraction of the distal stomach caused by each wave is sometimes called **antral systole** and can last up to 10 s. Waves occur 3–4 times per minute.

In the regulation of gastric emptying, the antrum, pylorus, and upper duodenum apparently function as a unit. Contraction of the antrum is followed by sequential contraction of the pyloric region and the duodenum. In the antrum, partial contraction ahead of the advancing gastric contents prevents solid masses from entering the duodenum, and they are mixed and crushed instead. The more liquid gastric contents are squirted a bit at a time into the small intestine. Normally, regurgitation from the duodenum does not occur, because the contraction of the pyloric segment tends to persist slightly longer than that of the duodenum. The prevention of regurgitation may also be due to the stimulating action of cholecystokinin (CCK) and secretin on the pyloric sphincter.

CLINICAL BOX 27–1

Motor Disorders of the Esophagus

Achalasia (literally, failure to relax) is a condition in which food accumulates in the esophagus and the organ can become massively dilated. It is due to increased resting LES tone and incomplete relaxation on swallowing. The myenteric plexus of the esophagus is deficient at the LES and the release of NO and VIP is defective. The opposite condition is LES incompetence, which permits reflux of acid gastric contents into the esophagus **(gastroesophageal reflux disease).** This common condition is the most frequent digestive disorder causing patients to seek care from a clinician. It causes heartburn and esophagitis and can lead to ulceration and stricture of the esophagus due to scarring. In severe cases, the intrinsic sphincter or the extrinsic sphincter, and sometimes both, are weak, but less severe cases are caused by intermittent periods of poorly understood decreases in the neural drive to both sphincters.

THERAPEUTIC HIGHLIGHTS

Achalasia can be treated by pneumatic dilation of the sphincter or incision of the esophageal muscle (myotomy). Inhibition of acetylcholine release by injection of botulinum toxin into the LES is also effective and produces relief that lasts for several months. Gastroesophageal reflux disease can be treated by inhibition of acid secretion with H_2-receptor blockers or proton pump inhibitors (see Chapter 25). Surgical treatment in which a portion of the fundus of the stomach is wrapped around the lower esophagus so that the LES is inside a short tunnel of stomach **(fundoplication)** can also be tried, although in many patients who undergo this procedure the symptoms eventually return.

REGULATION OF GASTRIC MOTILITY & EMPTYING

The rate at which the stomach empties into the duodenum depends on the type of food ingested. Food rich in carbohydrate leaves the stomach in a few hours. Protein-rich food leaves more slowly, and emptying is slowest after a meal containing fat **(Figure 27–6)**. The rate of emptying also depends on the osmotic

pressure of the material entering the duodenum. Hyperosmolality of the duodenal contents is sensed by “duodenal osmoreceptors” that initiate a decrease in gastric emptying, which is probably neural in origin.

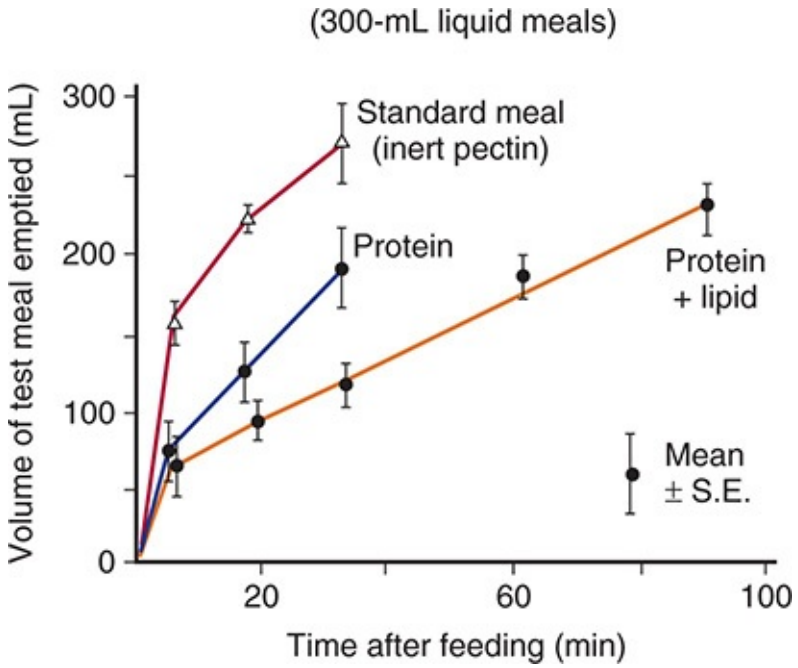


FIGURE 27–6 Effect of protein and fat on the rate of emptying of the human stomach. Persons were fed 300-mL liquid meals. (Reproduced with permission from Brooks FP: Integrative lecture. Response of the GI tract to a meal. *Undergraduate Teaching Project*. American Gastroenterological Association; 1974.)

Fats, carbohydrates, and acid in the duodenum inhibit gastric acid and pepsin secretion and gastric motility via neural and hormonal mechanisms. The messenger involved is probably peptide YY. CCK has also been implicated as an inhibitor of gastric emptying **(Clinical Box 27–2)**.

VOMITING

The protective response of vomiting is an example of central regulation of gut motility functions. Vomiting starts with salivation and the sensation of nausea. Reverse peristalsis empties material from the upper part of the small intestine into the stomach. The glottis closes, preventing aspiration of vomitus into the trachea. The breath is held in mid inspiration. The muscles of the abdominal wall contract, and because the chest is held in a fixed position, the contraction

increases intra-abdominal pressure. The LES and the esophagus relax, and the gastric contents are ejected. The “vomiting center” in the reticular formation of the medulla **(Figure 27–7)** consists of various scattered groups of neurons in this region that control the different components of the vomiting act.

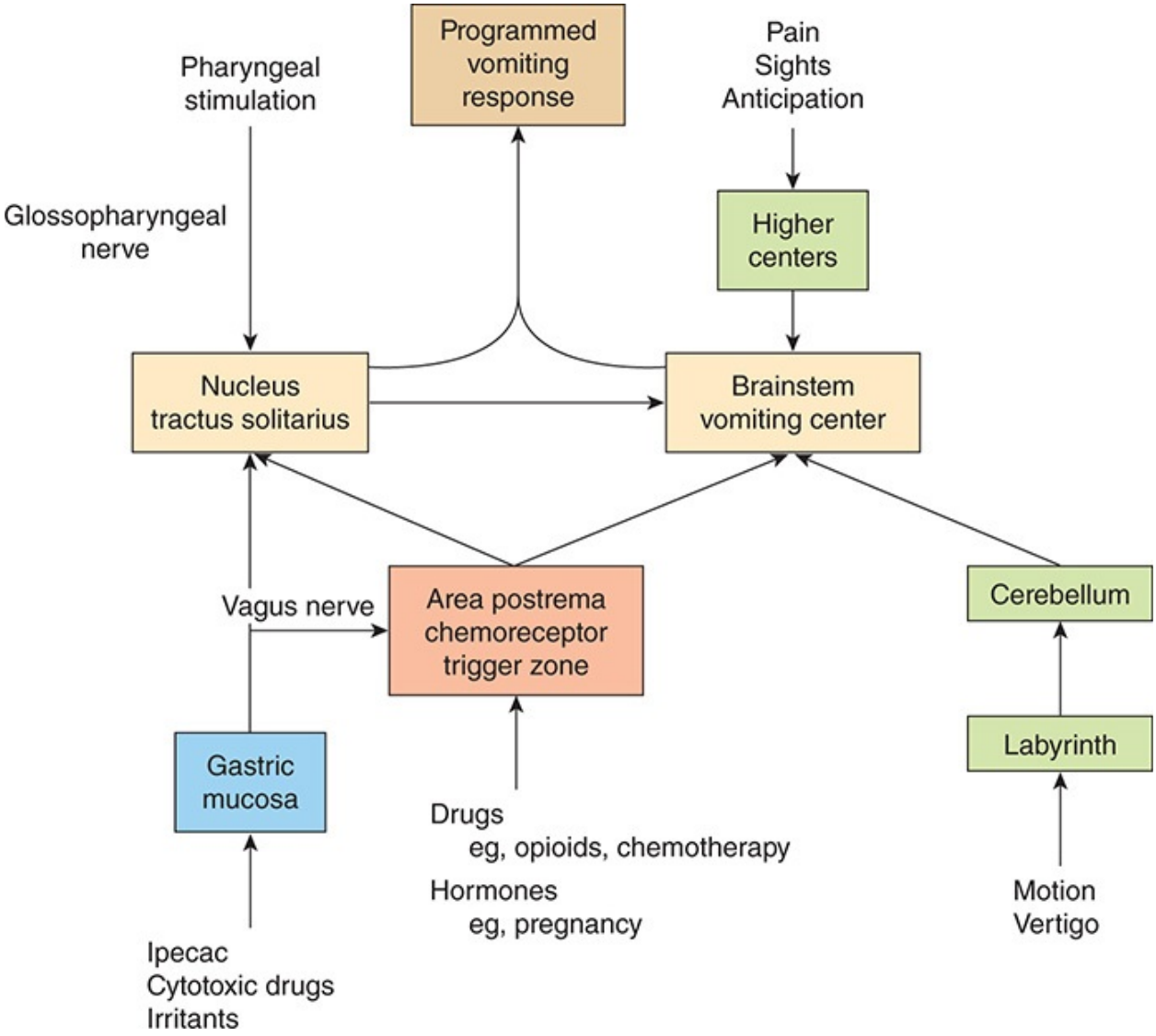


FIGURE 27–7 Neural pathways leading to the initiation of vomiting in response to various stimuli.

CLINICAL BOX 27–2

Consequences of Gastric Bypass Surgery

Patients who are morbidly obese can undergo a surgical procedure in which the stomach is stapled or otherwise reduced so that most of it is bypassed, and thus the reservoir function of the stomach is lost, along with gastric signals, such as ghrelin, that trigger food ingestion. As a result of the loss of the gastric reservoir, patients can eat only small meals. If larger meals are eaten, because of rapid absorption of glucose from the intestine and the resultant hyperglycemia and abrupt rise in insulin secretion, hypoglycemic symptoms sometimes develop about 2 h after meals. This weakness, dizziness, and sweating after meals, due in part to hypoglycemia, are part of the picture of the **"dumping syndrome,"** a distressing syndrome that can also develop in patients in whom portions of the stomach have been removed or the jejunum has been anastomosed to the stomach. Another cause of the symptoms is rapid entry of hypertonic meals into the intestine; this provokes the movement of so much water into the gut that significant hypovolemia and hypotension are produced.

THERAPEUTIC HIGHLIGHTS

There are no treatments, per se, for the dumping syndrome, other than avoiding large meals, and particularly those with high concentrations of simple sugars. Indeed, its occurrence may contribute to the overall success of bypass surgery in reducing food intake, and thus obesity, in many patients who undergo this surgery.

Irritation of the mucosa of the upper gastrointestinal tract is one trigger for vomiting. Impulses are relayed from the mucosa to the medulla over visceral afferent pathways in the sympathetic nerves and vagi. Other causes of vomiting can arise centrally. For example, afferents from the vestibular nuclei mediate the nausea and vomiting of motion sickness. Other afferents presumably reach the vomiting control areas from the diencephalon and limbic system, because emetic responses to emotionally charged stimuli also occur.

Chemoreceptor cells in the medulla can also initiate vomiting when they are stimulated by certain circulating chemical agents. The **chemoreceptor trigger zone** in which these cells are located (Figure 27–7) is in the **area postrema,** a V-shaped band of tissue on the lateral walls of the fourth ventricle near the obex. This structure is one of the circumventricular organs (see Chapter 33) and is not protected by the blood-brain barrier. Lesions of the area postrema have little effect on the vomiting response to gastrointestinal irritation or motion sickness,

but abolish the vomiting that follows injection of apomorphine and a number of other emetic drugs. Such lesions also decrease vomiting in uremia and radiation sickness, both of which may be associated with endogenous production of circulating emetic substances.

Serotonin (5-HT) released from enterochromaffin cells in the small intestine appears to initiate impulses via 5-HT_3 receptors that trigger vomiting. In addition, there are dopamine D_2 receptors and 5-HT_3 receptors in the area postrema and adjacent nucleus of the solitary tract. 5-HT_3 antagonists such as ondansetron and D_2 antagonists such as chlorpromazine and haloperidol are effective antiemetic agents. Corticosteroids, cannabinoids, and benzodiazepines, alone or in combination with 5-HT_3 and D_2 antagonists, are also useful in treatment of the vomiting produced by chemotherapy. The mechanisms of action of corticosteroids and cannabinoids are unknown, whereas the benzodiazepines probably reduce the anxiety associated with chemotherapy.

SMALL INTESTINE

In the small intestine, the intestinal contents are mixed with the secretions of the mucosal cells and with pancreatic juice and bile. The chyme is also retained in the small intestine long enough for nutrient absorption to take place.

INTESTINAL MOTILITY

The MMCs that pass along the intestine at regular intervals in the fasting state, and their replacement by peristaltic and other contractions controlled by the BER, are described above. In the small intestine, there are an average of 12 BER cycles/min in the proximal jejunum, declining to 8/min in the distal ileum. There are three types of smooth muscle contractions: peristaltic waves, segmentation contractions, and tonic contractions. **Peristalsis** is described above. It propels the intestinal contents toward the large intestines. **Segmentation contractions** (Figure 27–1), also described above, move the chyme to and fro and increase its exposure to the mucosal surface. These contractions are initiated by focal increases in Ca^{2+} influx with waves of increased Ca^{2+} concentration spreading from each focus. **Tonic contractions** are relatively prolonged contractions that in effect isolate one segment of the intestine from another. Note that these last two types of contractions slow transit in the small intestine to the point that the transit time is longer in the fed than in the fasted state. This permits longer

contact of the chyme with the enterocytes and fosters absorption **(Clinical Box 27–3)**.

CLINICAL BOX 27–3

Ileus

When the intestines are traumatized, there is a direct inhibition of smooth muscle, which causes a decrease in intestinal motility. It is due in part to activation of opioid receptors. When the peritoneum is irritated, reflex inhibition occurs due to increased discharge of noradrenergic fibers in the splanchnic nerves. Both types of inhibition operate to cause **paralytic (adynamic) ileus** after abdominal surgeries. Because of the diffuse decrease in peristaltic activity in the small intestine, its contents are not propelled into the colon, and it becomes irregularly distended by pockets of gas and fluid. Intestinal peristalsis returns in 6–8 h, followed by gastric peristalsis, but colonic activity takes 2–3 days to return.

THERAPEUTIC HIGHLIGHTS

Adynamic ileus can be relieved by passing a tube through the nose down to the small intestine and aspirating the fluid and gas for a few days until peristalsis returns. The occurrence of ileus has been reduced by more widespread use of minimally invasive (eg, laparoscopic) surgery. Postsurgical regimens also encourage early ambulation, where possible, which tends to enhance intestinal motility. There are also ongoing trials of specific opioid antagonists in this condition.

COLON

The colon serves as a reservoir for the residues of meals that cannot be digested or absorbed **(Figure 27–8)**. Motility in this segment is likewise slowed to allow the colon to absorb water, Na^+, and other minerals. By removing about 90% of the fluid, it converts the 1000–2000 mL of isotonic chyme that enters it each day from the ileum to about 200 mL of semisolid feces.

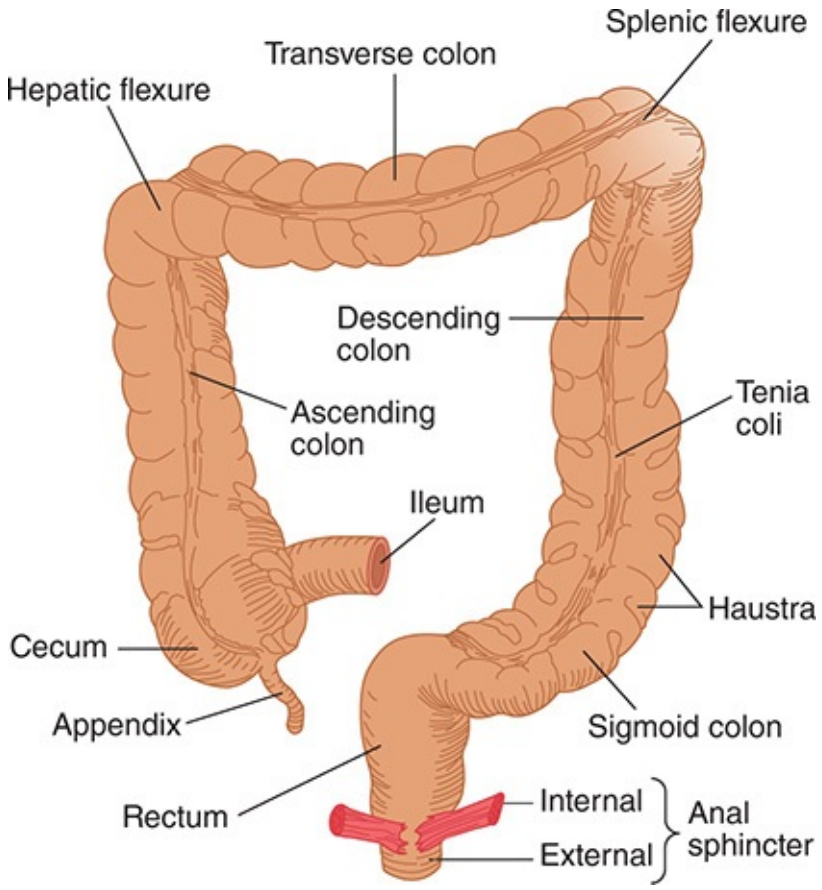


FIGURE 27–8 The human colon.

MOTILITY OF THE COLON

The ileum is linked to the colon by a structure known as the ileocecal valve, which restricts reflux of colonic contents, and particularly the larger numbers of commensal bacteria, into the relatively sterile ileum. The portion of the ileum containing the ileocecal valve projects slightly into the cecum, so that increases in colonic pressure squeeze it shut, whereas increases in ileal pressure open it. It is normally closed. Each time a peristaltic wave reaches it, it opens briefly, permitting some of the ileal chyme to squirt into the cecum. When food leaves the stomach, the cecum relaxes and the passage of chyme through the ileocecal valve increases **(gastroileal reflex).** This is presumably a vagovagal reflex.

The movements of the colon include segmentation contractions and peristaltic waves like those occurring in the small intestine. Segmentation contractions mix the contents of the colon and, by exposing more of the contents to the mucosa, facilitate absorption. Peristaltic waves propel the contents toward the rectum,

although weak antiperistalsis is sometimes seen. A third type of contraction that occurs only in the colon is the **mass action contraction,** occurring about 10 times per day, in which there is simultaneous contraction of the smooth muscle over large confluent areas. These contractions move material from one portion of the colon to another **(Clinical Box 27–4)**. They also move material into the rectum, and rectal distension initiates the defecation reflex (see below).

The movements of the colon are coordinated by the BER of the colon. The frequency of this wave, unlike the wave in the small intestine, increases along the colon, from about 2/min at the ileocecal valve to 6/min at the sigmoid.

TRANSIT TIME IN THE SMALL INTESTINE & COLON

The first part of a test meal reaches the cecum in about 4 h in most individuals, and all the undigested portions have entered the colon in 8 or 9 h. On average, the first remnants of the meal traverse the first third of the colon in 6 h, the second third in 9 h, and reach the terminal part of the colon (the sigmoid colon) in 12 h. From the sigmoid colon to the anus, transport is much slower **(Clinical Box 27–5)**. When small colored beads are fed with a meal, an average of 70% of them are recovered in the stool in 72 h, but total recovery requires more than a week. Transit time, pressure fluctuations, and changes in pH in the gastrointestinal tract can also be observed by monitoring the progress of a small pill that contains sensors and a miniature radio transmitter.

CLINICAL BOX 27–4

Hirschsprung Disease

Some children have a genetically determined condition of abnormal colonic motility known as Hirschsprung disease or **aganglionic megacolon,** which is characterized by abdominal distension, anorexia, and lassitude. The disease is typically diagnosed in infancy, and affects as many as 1 in 5000 live births. It is due to a congenital absence of the ganglion cells in both the myenteric and submucous plexuses of a segment of the distal colon, as a result of failure of the normal cranial-to-caudal migration of neural crest cells during development. The action of endothelins on the endothelin B receptor (see Chapter 7) are necessary for normal migration of certain neural crest cells;

megacolon developed in knockout mice lacking endothelin B receptors. In addition, one cause of congenital aganglionic megacolon in humans appears to be a mutation in the endothelin B receptor gene. The absence of peristalsis in patients with this disorder causes feces to pass the aganglionic region with difficulty, and children with the disease may defecate as infrequently as once every 3 weeks.

THERAPEUTIC HIGHLIGHTS

The symptoms of Hirschsprung disease can be relieved completely if the aganglionic portion of the colon is resected and the portion of the colon above it anastomosed to the rectum. However, this is not possible if an extensive segment is involved. In this case, patients may require a colectomy.

DEFECATION

Distension of the rectum with feces initiates reflex contractions of its musculature and the desire to defecate. In humans, the sympathetic nerve supply to the internal (involuntary) anal sphincter is excitatory, whereas the parasympathetic supply is inhibitory. This sphincter relaxes when the rectum is distended. The nerve supply to the external anal sphincter, a skeletal muscle, comes from the pudendal nerve. The sphincter is maintained in a state of tonic contraction, and moderate distension of the rectum increases the force of its contraction **(Figure 27–9)**. The urge to defecate first occurs when rectal pressure increases to about 18 mm Hg. When this pressure reaches 55 mm Hg, the external as well as the internal sphincter relaxes and there is reflex expulsion of the contents of the rectum. This is why reflex evacuation of the rectum can occur even in the setting of spinal injury.

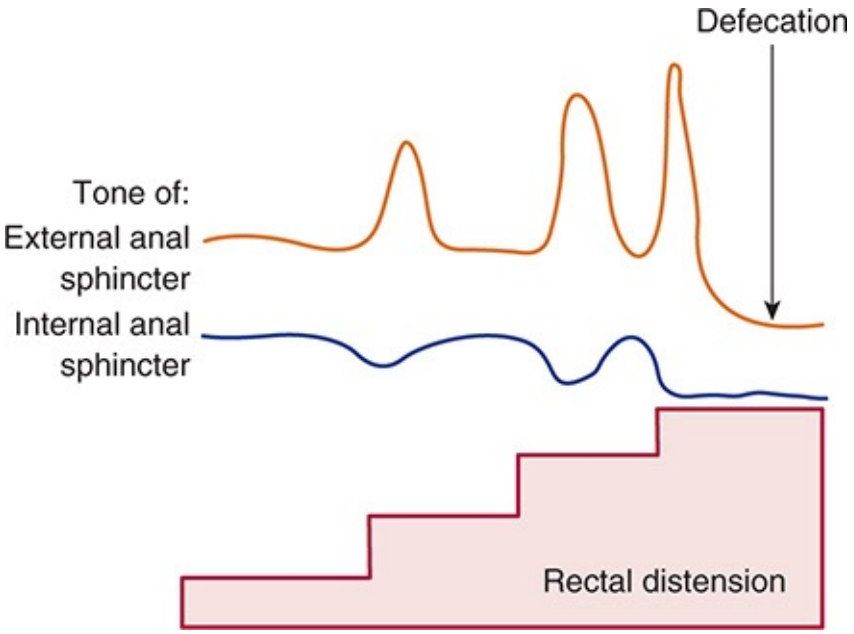


FIGURE 27–9 Motor responses of the anal sphincters to distension of the rectum. Distension produces passive tension due to stretching of the wall of the rectum, and additional active tension when the smooth muscle in the wall contracts. The internal and external sphincters respectively relax and contract, then accommodate, with each stepwise increase in distension until the pressure threshold for defecation is reached.

CLINICAL BOX 27–5

Constipation

Constipation refers to a pathologic decrease in bowel movements. It was previously considered to reflect changes in motility, but the recent success of a drug designed to enhance chloride secretion for the treatment of chronic constipation suggests alterations in the balance between secretion and absorption in the colon could also contribute to symptom generation. Patients with persistent constipation, and particularly those with a recent change in bowel habits, should be examined carefully to rule out underlying organic disease. However, many normal humans defecate only once every 2–3 days, even though others defecate once a day and some as often as three times a day. Furthermore, the only symptoms caused by constipation are slight anorexia and mild abdominal discomfort and distension. These symptoms are not due to absorption of “toxic substances,” because they are promptly

relieved by evacuating the rectum and can be reproduced by distending the rectum with inert material. In western societies, the amount of misinformation and undue apprehension about constipation probably exceeds that about any other health topic. Symptoms other than those described above that are attributed by the lay public to constipation are likely due to anxiety or other causes.

THERAPEUTIC HIGHLIGHTS

Most cases of constipation are relieved by a change in the diet to include more fiber, or the use of laxatives that retain fluid in the colon, thereby increasing the bulk of the stool and promoting reflexes that lead to evacuation. As noted above, lubiprostone has recently joined the armamentarium for the treatment of constipation, and is assumed to act by enhancing chloride, and thus water, secretion into the colon thereby increasing the fluidity of the colonic contents.

Before the pressure that relaxes the external anal sphincter is reached, voluntary defecation can be initiated by straining. Normally, the angle between the anus and the rectum is approximately 90–100° **(Figure 27–10)**, and this, added to contraction of the puborectalis muscle, inhibits defecation. With straining, the abdominal muscles contract, the pelvic floor is lowered 1–3 cm, and the puborectalis muscle relaxes. The anorectal angle is straightened, and when this is combined with relaxation of the external anal sphincter, defecation occurs. Defecation is therefore a spinal reflex that can be voluntarily inhibited by keeping the external sphincter contracted or facilitated by relaxing the sphincter and contracting the abdominal muscles.

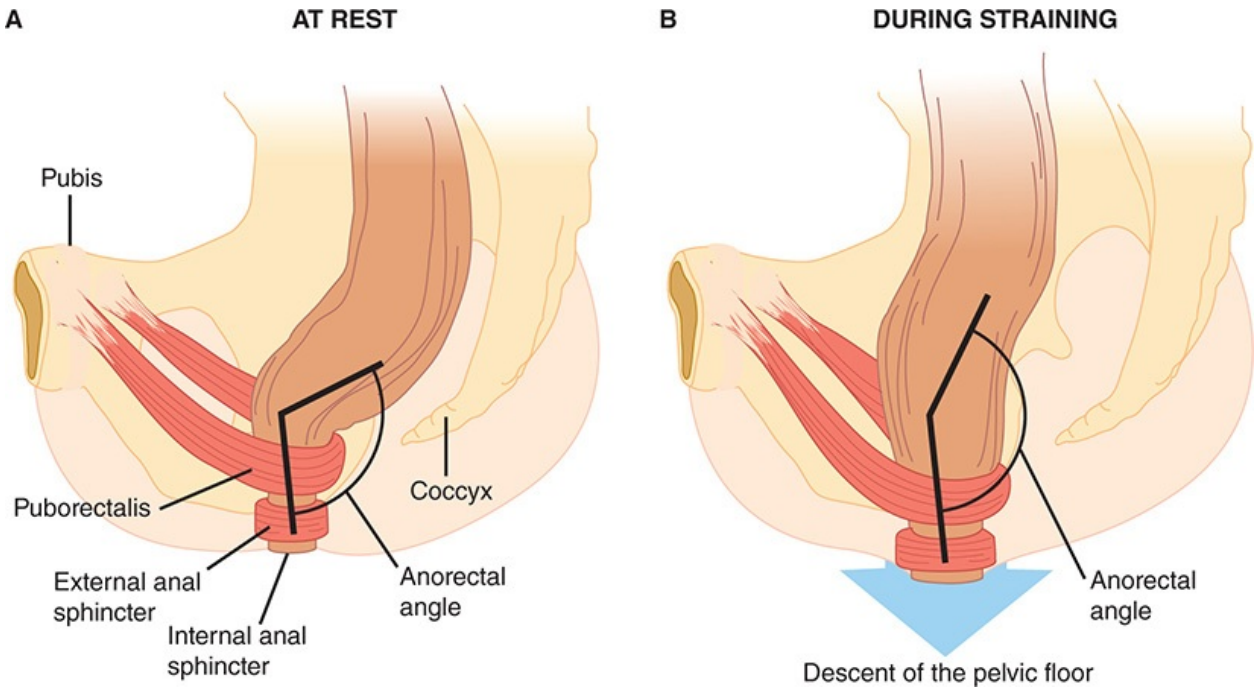


FIGURE 27–10 Sagittal view of the anorectal area at rest (A) and during straining (B). Note the straightening of the anorectal angle and lowering of the pelvic floor during straining. (Modified with permission from Lembo A, Camilleri M: Chronic constipation. N Engl J Med 2003; Oct 2; 349(14):1360–1368.)

Distension of the stomach by food initiates contractions of the rectum and, frequently, a desire to defecate. The response is called the **gastrocolic reflex,** and may be amplified by an action of gastrin on the colon. Because of the response, defecation after meals is frequent in children. In adults, habit and cultural factors play a large role in determining when defecation occurs.

CHAPTER SUMMARY

- The regulatory factors that govern gastrointestinal secretion also regulate its motility to soften the food, mix it with secretions, and propel it along the length of the tract.
- Two major patterns of motility are peristalsis and segmentation, which serve to propel or retard/mix the luminal contents, respectively. Peristalsis involves coordinated contractions and relaxations above and below the food bolus.
- The membrane potential of the majority of gastrointestinal smooth muscle

undergoes rhythmic fluctuations that sweep along the length of the gut. The rhythm varies in different gut segments and is established by pacemaker cells known as interstitial cells of Cajal. This basic electrical rhythm (BER) provides for sites of muscle contraction when stimuli superimpose spike potentials on the depolarizing portion of the BER waves.

- In the period between meals, the intestine is relatively quiescent, but every 90 min or so it is swept through by a large peristaltic wave triggered by the hormone motilin. This migrating motor complex presumably serves a "housekeeping" function.
- Swallowing is triggered centrally and is coordinated with a peristaltic wave along the length of the esophagus that drives the food bolus to the stomach, even against gravity. Relaxation of the lower esophageal sphincter is timed to just precede the arrival of the bolus, thereby limiting reflux of the gastric contents. Nevertheless, gastroesophageal reflux disease is one of the most common gastrointestinal complaints.
- The stomach accommodates the meal by a process of receptive relaxation. This permits an increase in volume without a significant increase in pressure. The stomach then serves to mix and grind the meal and to control its delivery to downstream segments.
- Motility responses in the small intestine mix the meal with pancreatic juice and bile, and propel the meal along the length of the intestine so that its digested constituents are exposed to the epithelial surface, allowing absorption.
- Luminal contents move slowly through the colon, which enhances water recovery. Distension of the rectum causes reflex contraction of the internal anal sphincter and the desire to defecate. After toilet training, defecation can be delayed until a convenient time via voluntary contraction of the external anal sphincter.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. In governing peristalsis, which of the following directions of nerve impulses and neurotransmitter is correctly matched?

   A. Retrograde; VIP

   B. Retrograde; nitric oxide

C. Anterograde; glutamate

D. Anterograde; nitric oxide

E. Anterograde; acetylcholine

2. Conversion of the basic electrical rhythm to an increase in intestinal muscle tension is produced by which of the following?

A. Activation of interstitial cells of Cajal

B. Adrenergic discharge

C. Release of acetylcholine

D. Release of VIP

E. Inhibition of nitric oxide synthase

3. The migrating motor complex is triggered by which of the following?

A. Motilin

B. NO

C. CCK

D. Somatostatin

E. Secretin

4. A patient is referred to a gastroenterologist because of persistent difficulties with swallowing. Endoscopic examination reveals that the lower esophageal sphincter fails to fully open as the bolus reaches it, and a diagnosis of achalasia is made. During the examination, or in biopsies taken from the sphincter region, a decrease would be expected in which of the following?

A. Esophageal peristalsis

B. Expression of neuronal NO synthase

C. Acetylcholine receptors

D. Substance P release

E. Contraction of the crural diaphragm

5. Gastric pressures seldom rise above the levels that breach the lower esophageal sphincter, even when the stomach is filled with a meal, due to which of the following processes?

A. Peristalsis

B. Gastroileal reflex

C. Segmentation

D. Stimulation of the vomiting center

E. Receptive relaxation

6. The physiological gastric pressure response to feeding described in Question 5 could be partly inhibited experimentally by all of the following treatments except

A. cholinergic antagonist.

B. nitric oxide synthase inhibitor.

C. cholecystokinin antagonist.

D. histamine antagonist.

E. VIP antagonist.

7. A patient who has undergone anastomosis of his jejunum to his stomach reports to his primary care physician that he has experienced several episodes of nausea, cramping, dizziness, sweating, and a rapid heart rate an hour or two after ingesting large volumes of sugary beverages. His symptoms are caused in part by

A. increased blood pressure.

B. increased secretion of glucagon.

C. increased secretion of CCK.

D. hypoglycemia.

E. hyperglycemia.

8. For the patient in Question 7, what is the most likely diagnosis?

A. Constipation-predominant irritable bowel syndrome (IBS-C)

B. Hirschsprung disease

C. Achalasia

D. Peptic ulcer disease

E. Dumping syndrome

9. In infants, defecation often follows a meal. The cause of colonic contractions in this situation is

A. histamine.

B. increased circulating levels of CCK.

C. the gastrocolic reflex.

D. increased circulating levels of somatostatin.

E. the enterogastric reflex.

10. Following a forceps delivery of her third child, a woman returns to her physician complaining of minor fecal incontinence when lifting her older children, without any urinary incontinence. Her symptoms are most likely

attributable to injury to which of the following?

A. Anal sensory nerves

B. Internal anal sphincter

C. External anal sphincter

D. Pudendal nerves

E. Puborectalis muscle

CHAPTER 28

Transport & Metabolic Functions of the Liver

OBJECTIVES

After studying this chapter, you should be able to:

- Understand the functional anatomy of the liver and the relative arrangements of hepatocytes, cholangiocytes, endothelial cells, and Kupffer cells.
- Define the characteristics of the hepatic circulation and its role in subserving the liver's functions.
- Describe the major functions of the liver with respect to metabolism, detoxification, and excretion of hydrophobic substances.
- Identify the plasma proteins that are synthesized by the liver.
- Outline the mechanisms by which the liver contributes to whole body ammonia homeostasis and the consequences of the failure of these mechanisms, particularly for brain function.
- Describe the constituents of bile, its formation, and its role in the excretion of cholesterol and bilirubin.
- Identify the mechanisms that permit normal functioning of the gallbladder and the basis of gallstone disease.

INTRODUCTION

The liver is the largest gland in the body. It is essential for life because it conducts a vast array of biochemical and metabolic functions, including ridding the body of substances that would otherwise be injurious if allowed to accumulate, and excreting drug metabolites. It is also the first port of call for most nutrients absorbed across the gut wall, supplies most of the plasma proteins, and synthesizes the bile that optimizes the absorption of fats as well as serving as an excretory fluid. The liver and associated biliary system have therefore evolved an array of structural and physiologic features that underpin this broad range of critical functions.

THE LIVER

FUNCTIONAL ANATOMY

An important function of the liver is to serve as a filter between the blood coming from the gastrointestinal tract and the blood in the rest of the body. Blood from the intestines and other viscera reach the liver via the portal vein. This blood percolates in sinusoids between plates of hepatic cells and eventually drains into the hepatic veins, which in turn empty into the inferior vena cava. Hepatic artery blood also enters the sinusoids. During its passage through the hepatic plates, the blood is extensively modified chemically. Bile is formed on the other side of each plate **(Figure 28–1)**.

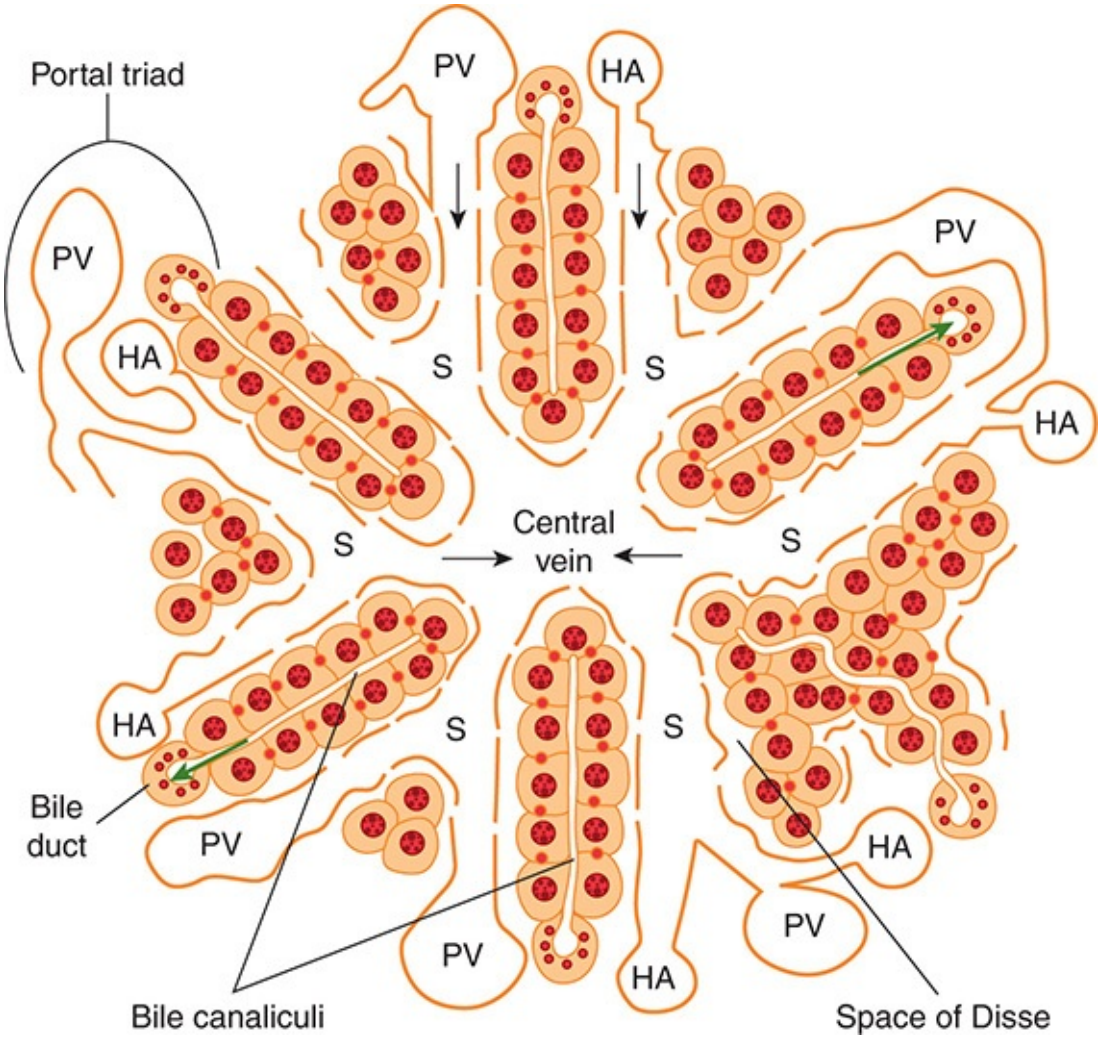


FIGURE 28–1 Schematic anatomy of the liver. Hepatocytes are arranged radially in plates surrounding a central vein. Blood is supplied to the liver by branches of the portal vein (PV) and hepatic artery (HA), which empty into sinusoids (S) surrounding the hepatocytes. The direction of blood flow is indicated with black arrows. The endothelial cells that line the sinusoids are fenestrated and thus provide little hindrance to the transfer of substances from the sinusoids to the space of Disse, which abuts the basolateral membrane of the hepatocytes. The apical membranes of adjacent hepatocytes form bile canaliculi, which transfer bile to the bile ducts lined by cholangiocytes. Bile flows in the opposite direction to blood (green arrows). The bile duct, portal vein, and hepatic artery comprise the "portal triad." (Adapted with permission from Paulsen DF: *Histology and Cell Biology: Examination and Board Review*. 5th edition. New York, NY: McGraw-Hill; 2010.)

In each hepatic lobule, the plates of hepatic cells are usually only one cell

thick. Plasma is in intimate contact with these cells **(Figure 28–2)**. The average transit time for blood across the liver lobule from the portal venule to the central hepatic vein is about 8.4 s. Additional details of the features of the hepatic microcirculation and macrocirculation, which are critical to organ function, are provided below. Numerous macrophages **(Kupffer cells)** are anchored to the endothelium of the sinusoids and project into the lumen. The functions of these phagocytic cells are discussed in Chapter 3.

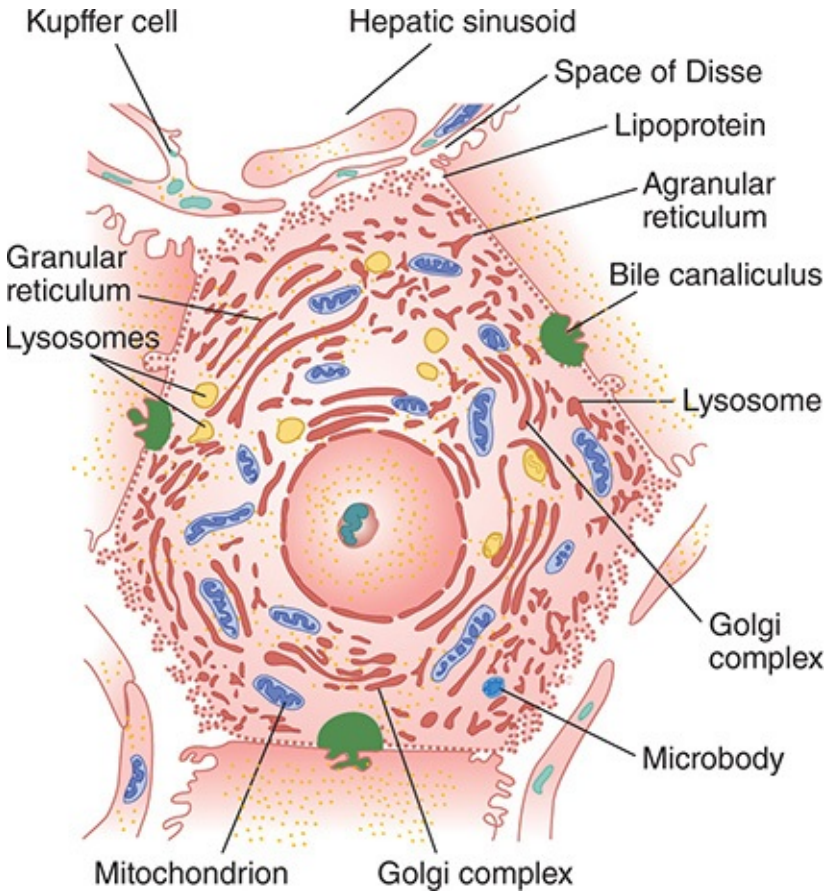


FIGURE 28–2 Hepatocyte. Note the relation of the cell to bile canaliculi and sinusoids. Note also the wide openings (fenestrations) between the endothelial cells next to the hepatocyte. (Used with permission of Sylvia Colard Keene.)

Each liver cell is opposed to several **bile canaliculi** (Figure 28–2). The canaliculi drain into intralobular bile ducts, and these coalesce via interlobular bile ducts to form the right and left hepatic ducts. These ducts join outside the liver to form the common hepatic duct. The cystic duct drains the gallbladder. The hepatic duct unites with the cystic duct to form the common bile duct (Figure 28–1). The common bile duct enters the duodenum at the duodenal

papilla. Its orifice is surrounded by the **sphincter of Oddi,** and it usually unites with the main pancreatic duct just before entering the duodenum. The sphincter is usually closed, but when the gastric contents enter the duodenum, cholecystokinin (CCK) is released and the gastrointestinal hormone relaxes the sphincter and makes the gallbladder contract.

The walls of the extrahepatic biliary ducts and the gallbladder contain fibrous tissue and smooth muscle. They are lined by a layer of columnar cells known as **cholangiocytes** with scattered mucous glands. In the gallbladder, the surface is extensively folded; this increases its surface area and gives the interior of the gallbladder a honeycombed appearance. The cystic duct is also folded to form the so-called spiral valves. This arrangement is believed to increase the turbulence of bile as it flows out of the gallbladder, thereby reducing the risk that it will precipitate and form gallstones.

HEPATIC CIRCULATION

Large gaps occur between endothelial cells in the walls of hepatic sinusoids, and the sinusoids are highly permeable. The way the intrahepatic branches of the hepatic artery and portal vein converge on the sinusoids and drain into the central lobular veins of the liver is shown in Figure 28–1. The functional unit of the liver is the acinus. Each acinus is at the end of a vascular stalk containing terminal branches of portal veins, hepatic arteries, and bile ducts. Blood flows from the center of this functional unit to the terminal branches of the hepatic veins at the periphery **(Figure 28–3)**. This is why the central portion of the acinus, sometimes called zone 1, is well oxygenated, the intermediate zone (zone 2) is moderately well oxygenated, and the peripheral zone (zone 3) is least well oxygenated and most susceptible to anoxic injury. The acini have been likened to grapes or berries, each on a vascular stem. The human liver contains about 100,000 acini.

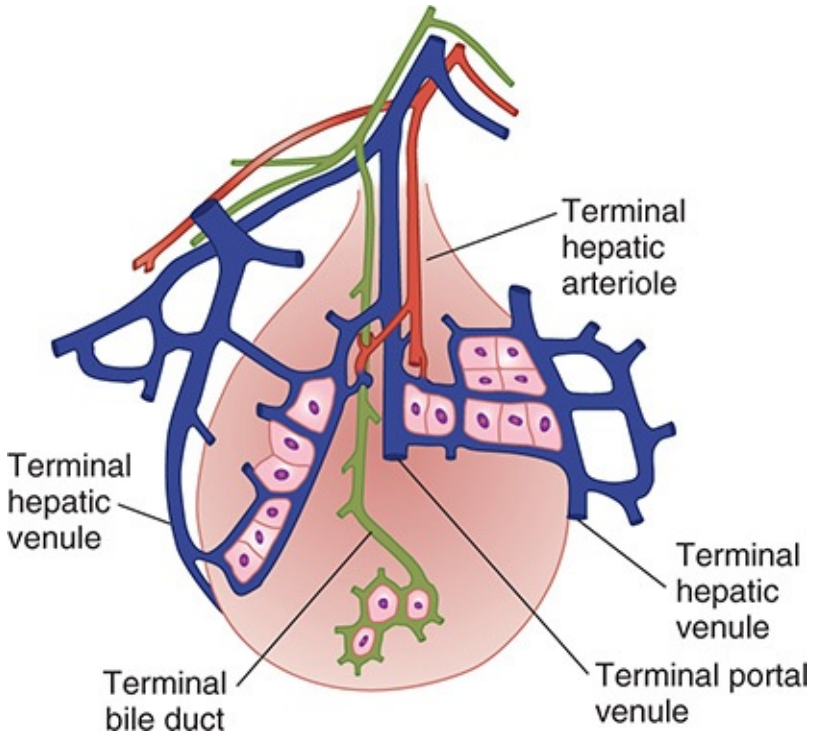


FIGURE 28–3 Concept of the acinus as the functional unit of the liver. In each acinus, blood in the portal venule and hepatic arteriole enters the center of the acinus and flows outward to the hepatic venule. (Adapted with permission from Rappaport AM: The microcirculatory hepatic unit. Microvasc Res 1973 Sep; 6(2):212–228.)

Portal venous pressure is normally about 10 mm Hg in humans, and hepatic venous pressure is approximately 5 mm Hg. The mean pressure in the hepatic artery branches that converge on the sinusoids is about 90 mm Hg, but the pressure in the sinusoids is lower than the portal venous pressure, so a marked pressure drop occurs along the hepatic arterioles. This pressure drop is adjusted so that there is an inverse relationship between hepatic arterial and portal venous blood flow. This inverse relationship may be maintained in part by the rate at which adenosine is removed from the region around the arterioles. According to this hypothesis, adenosine is produced by metabolism at a constant rate. When portal flow is reduced, it is washed away more slowly, and the local accumulation of adenosine dilates the terminal arterioles. In the period between meals, moreover, many of the sinusoids are collapsed. Following a meal, on the other hand, when portal flow to the liver from the intestine increases considerably, these “reserve” sinusoids are recruited. This arrangement means that portal pressures do not increase linearly with portal flow until all sinusoids

have been recruited. This may be important to prevent fluid loss from the highly permeable liver under normal conditions. Indeed, if hepatic pressures are increased in disease states (such as the hardening of the liver that is seen in cirrhosis), many liters of fluid can accumulate in the peritoneal cavity as **ascites.**

The intrahepatic portal vein radicles have smooth muscle in their walls that is innervated by noradrenergic vasoconstrictor nerve fibers reaching the liver via the 3rd to 11th thoracic ventral roots and the splanchnic nerves. The vasoconstrictor innervation of the hepatic artery comes from the hepatic sympathetic plexus. When systemic venous pressure rises, the portal vein radicles are dilated passively and the amount of blood in the liver increases. In heart failure, this hepatic venous congestion may be extreme. Conversely, when diffuse noradrenergic discharge occurs in response to a drop in systemic blood pressure, the intrahepatic portal radicles constrict, portal pressure rises, and blood flow through the liver is brisk, bypassing most of the organ. Most of the blood in the liver enters the systemic circulation. Constriction of the hepatic arterioles diverts blood from the liver, and constriction of the mesenteric arterioles reduces portal inflow. In severe shock, hepatic blood flow may be reduced to such a degree that patchy necrosis of the liver takes place.

FUNCTIONS OF THE LIVER

The liver has many complex functions that are summarized in **Table 28–1**. Several will be touched upon briefly here.

TABLE 28–1 Principal functions of the liver.

Formation and secretion of bile
Nutrient and vitamin metabolism
Glucose and other sugars
Amino acids
Lipids
Fatty acids
Cholesterol
Lipoproteins
Fat-soluble vitamins
Water-soluble vitamins
Inactivation of various substances
Toxins
Steroids
Other hormones
Synthesis of plasma proteins
Acute-phase proteins
Albumin
Clotting factors
Steroid-binding and other hormone-binding proteins
Immunity
Kupffer cells

METABOLISM & DETOXIFICATION

It is beyond the scope of this volume to touch upon all of the metabolic functions of the liver. Instead, this chapter will focus on those aspects most closely aligned to gastrointestinal physiology. First, the liver plays key roles in carbohydrate metabolism, including glycogen storage, conversion of galactose and fructose to glucose, and gluconeogenesis, as well as many of the reactions covered in Chapter 1. The substrates for these reactions derive from the products of carbohydrate digestion and absorption that are transported from the intestine to the liver in the portal blood. The liver also plays a major role in maintaining the stability of blood glucose levels in the postprandial period, removing excess glucose from the blood and returning it as needed—the so-called **glucose buffer**

function of the liver. In liver failure, hypoglycemia is commonly seen. Similarly, the liver contributes to fat metabolism. It supports a high rate of fatty acid oxidation for energy supply to the liver itself and other organs. Amino acids and two carbon fragments derived from carbohydrates are also converted in the liver to fats for storage. The liver also synthesizes most of the lipoproteins required by the body and preserves cholesterol homeostasis by synthesizing this molecule and also converting excess cholesterol to bile acids.

The liver also detoxifies the blood of substances originating from the gut or elsewhere in the body. Part of this function is physical in nature—bacteria and other particulates are trapped in and broken down by the strategically located Kupffer cells. The remaining reactions are biochemical, and mediated in their first stages by the large number of cytochrome P450 enzymes expressed in hepatocytes. These convert xenobiotics and other toxins to inactive, less lipophilic metabolites. Detoxification reactions are divided into phase I (oxidation, hydroxylation, and other reactions mediated by cytochrome P450s) and phase II (esterification). Ultimately, metabolites are secreted into the bile for elimination via the gastrointestinal tract. In this regard, in addition to disposing of drugs, the liver is responsible for metabolism of essentially all steroid hormones. Liver disease can therefore result in the apparent overactivity of the relevant hormone systems.

SYNTHESIS OF PLASMA PROTEINS

The principal proteins synthesized by the liver are listed in Table 28–1. Albumin is quantitatively the most significant, and accounts for the majority of plasma oncotic pressure. Many of the products are **acute-phase proteins,** proteins synthesized and secreted into the plasma on exposure to stressful stimuli (see Chapter 3). Others are proteins that transport steroids and other hormones in the plasma, and still others are clotting factors. Following blood loss, the liver replaces the plasma proteins in days to weeks. The only major class of plasma proteins not synthesized by the liver is the immunoglobulins.

AMMONIA METABOLISM & EXCRETION

The liver is critical for ammonia handling in the body **(Clinical Box 28–1)**. Ammonia levels must be carefully controlled because it is toxic to the central nervous system (CNS), and freely permeable across the blood-brain barrier. The

liver is the only organ in which the complete urea cycle (also known as the Krebs-Henseleit cycle) is expressed (Figure 1–20). This converts circulating ammonia to urea, which can then be excreted in the urine **(Figure 28–4)**.

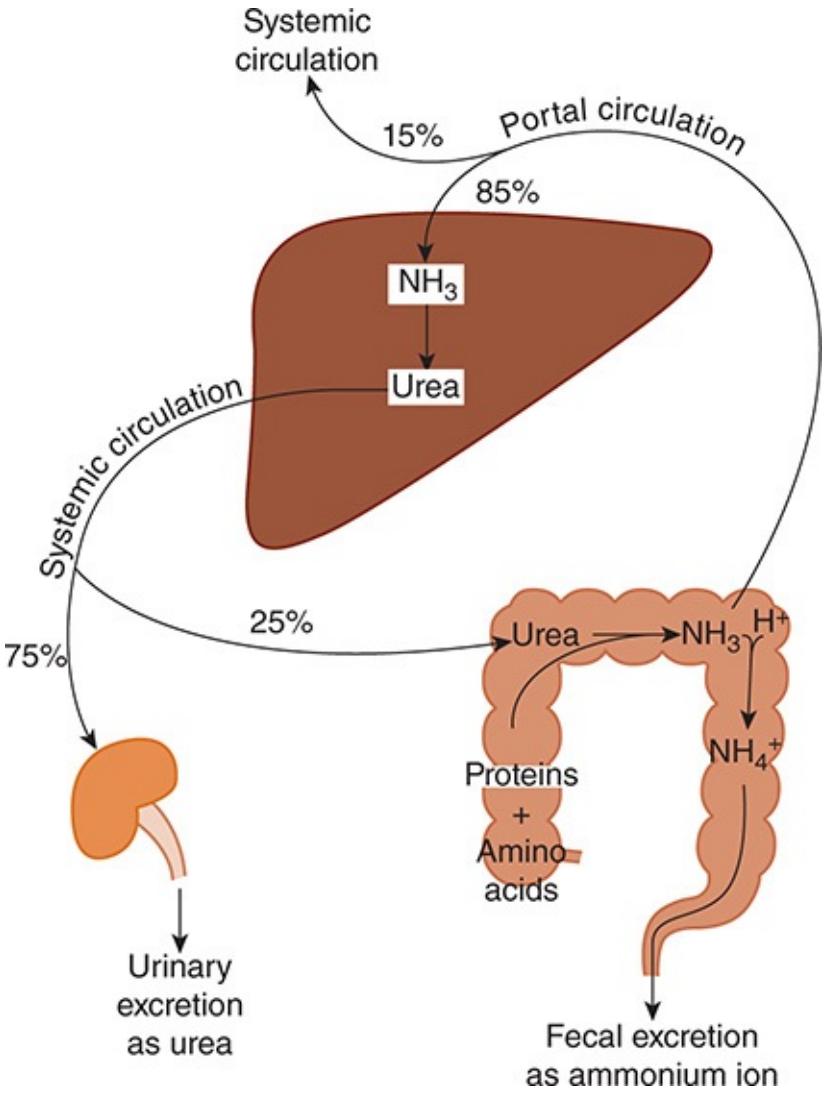


FIGURE 28–4 Whole body ammonia homeostasis in health. The majority of ammonia produced by the body is excreted by the kidneys in the form of urea.

Ammonia in the circulation comes primarily from the colon and kidneys with lesser amounts deriving from the breakdown of red blood cells and from metabolism in the muscles. As it passes through the liver, almost all of the ammonia in the circulation is cleared into the hepatocytes. There, it is converted in the mitochondria to carbamoyl phosphate, which in turn reacts with ornithine to generate citrulline. A series of subsequent cytoplasmic reactions eventually produce arginine, and this can be dehydrated to urea and ornithine. The latter

returns to the mitochondria to begin another cycle, and urea, as a small molecule, diffuses readily back out into the sinusoidal blood. It is then filtered in the kidneys and lost from the body in the urine.

CLINICAL BOX 28–1

Hepatic Encephalopathy

The clinical importance of hepatic ammonia metabolism is seen in liver failure, when increased levels of circulating ammonia cause the condition of hepatic encephalopathy. Initially, patients may seem merely confused, but if untreated, the condition can progress to coma and irreversible changes in cognition. The disease results not only from the loss of functional hepatocytes, but also shunting of portal blood around the hardened liver, meaning that less ammonia is removed from the blood by the remaining hepatic mass. Additional substances that are normally detoxified by the liver likely also contribute to the mental status changes.

THERAPEUTIC HIGHLIGHTS

The cognitive symptoms of advanced liver disease can be minimized by reducing the load of ammonia coming to the liver from the colon (eg, by feeding the nonabsorbable carbohydrate, lactulose, which is converted into short-chain fatty acids in the colonic lumen and thereby traps luminal ammonia in its ionized form). However, in severe disease, the only truly effective treatment is to perform a liver transplant, although the paucity of available organs means that there is great interest in artificial liver assist devices that could clean the blood.

BILE

Bile is made up of the bile acids, bile pigments, and other substances dissolved in an alkaline electrolyte solution that resembles pancreatic juice **(Table 28–2)**. About 500 mL is secreted per day. Some of the components of the bile are reabsorbed in the intestine and then excreted again by the liver **(enterohepatic circulation).** In addition to its role in digestion and absorption of fats (Chapter 26), bile (and subsequently the feces) is the major excretory route for lipid-

soluble waste products.

TABLE 28–2 Composition of human hepatic duct bile.

Water	97.0%
Bile acids	0.7%
Bile pigments	0.2%
Cholesterol	0.06%
Inorganic salts	0.7%
Fatty acids	0.15%
Phosphatidylcholine	0.2%
Fat	0.1%
Alkaline phosphatase	…

The glucuronides of the **bile pigments,** bilirubin and biliverdin, are responsible for the golden yellow color of bile. The formation of these breakdown products of hemoglobin is discussed in detail in Chapter 31, and their excretion is discussed in the following text.

BILIRUBIN METABOLISM & EXCRETION

Most of the bilirubin in the body is formed in the tissues by the breakdown of hemoglobin (see Chapter 31 and **Figure 28–5**). The bilirubin is bound to albumin in the circulation. Most of it is tightly bound, but some of it can dissociate in the liver, and free bilirubin enters liver cells via a member of the organic anion transporting polypeptide (OATP) family, and then becomes bound to cytoplasmic proteins **(Figure 28–6)**. It is next conjugated to glucuronic acid in a reaction catalyzed by the enzyme **glucuronyl transferase** (UDP-glucuronosyltransferase). This enzyme is located primarily in the smooth endoplasmic reticulum. Each bilirubin molecule reacts with two uridine diphosphoglucuronic acid (UDPGA) molecules to form bilirubin diglucuronide. This glucuronide, which is more water-soluble than the free bilirubin, is then transported against a concentration gradient most likely by an active transporter known as multidrug resistance protein-2 (MRP-2) into the bile canaliculi. A small amount of the bilirubin glucuronide escapes into the blood, where it is

bound less tightly to albumin than is free bilirubin, and is excreted in the urine. Thus, the total plasma bilirubin normally includes free bilirubin plus a small amount of conjugated bilirubin. Most of the bilirubin glucuronide passes via the bile ducts to the intestine.

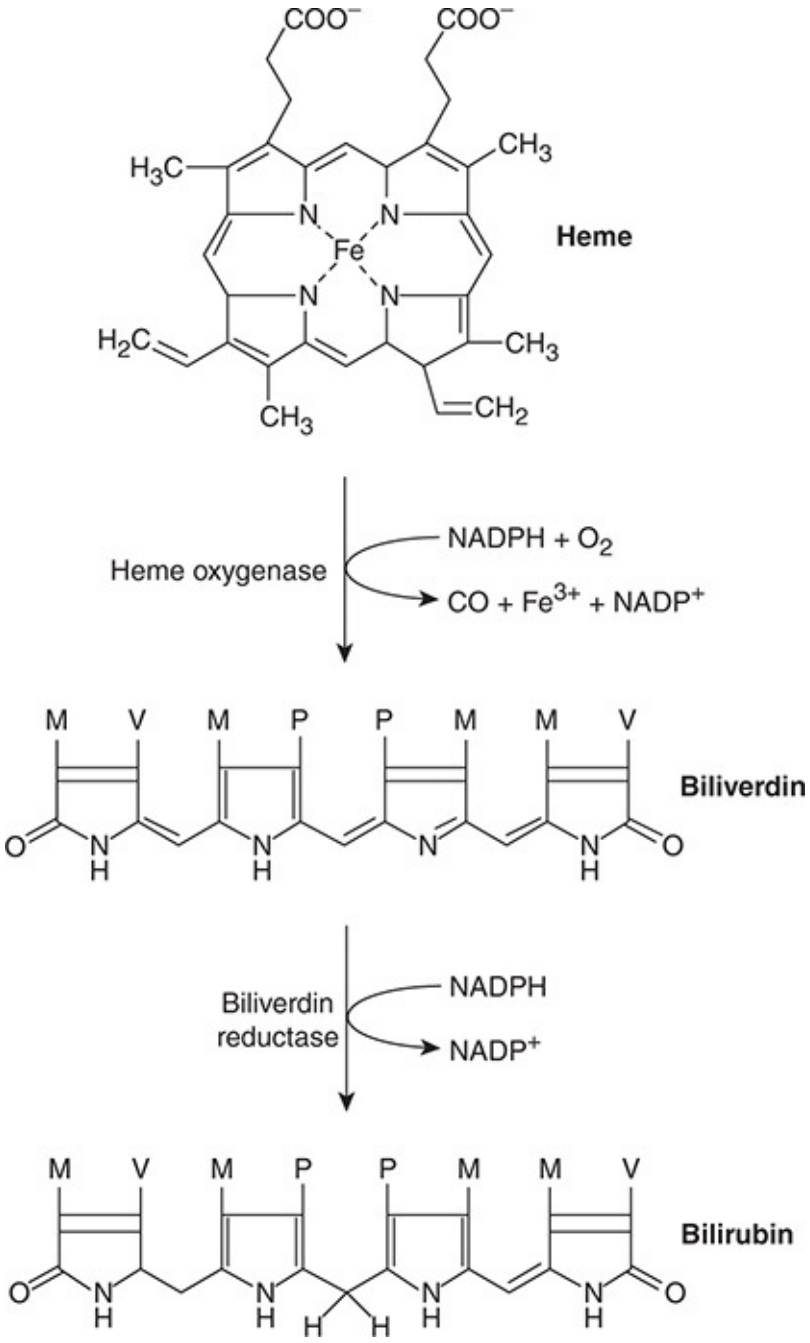


FIGURE 28–5 Conversion of heme to bilirubin is a two-step reaction catalyzed by heme oxygenase and biliverdin reductase. M, methyl; P, propionate; V, vinyl.

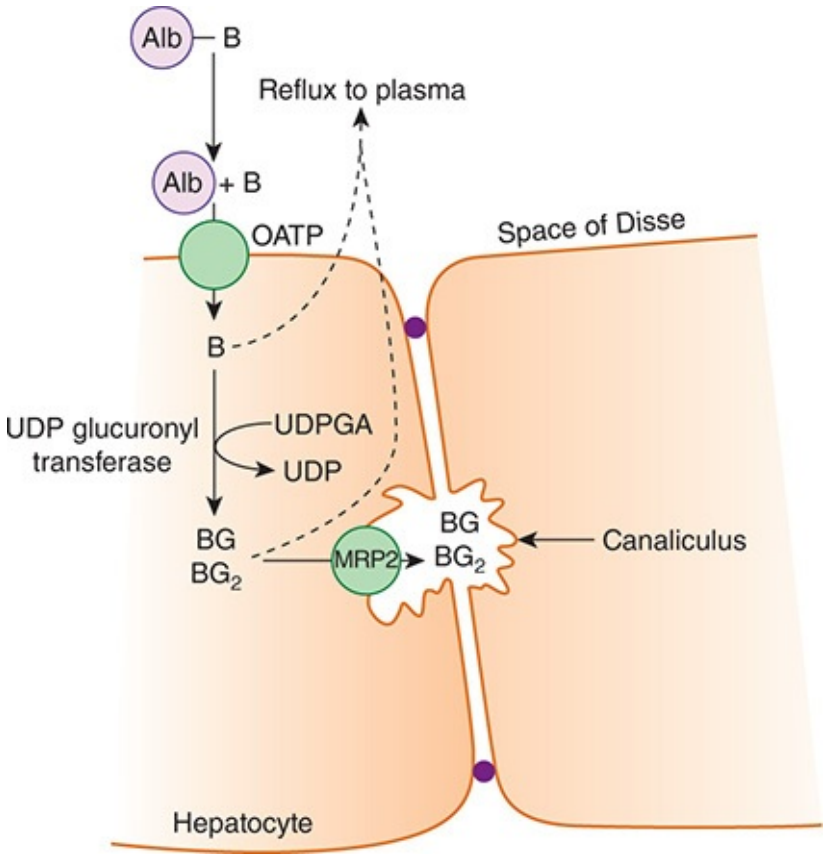


FIGURE 28-6 Handling of bilirubin by hepatocytes. Albumin (Alb)-bound bilirubin (B) enters the space of Disse adjacent to the basolateral membrane of hepatocytes, and bilirubin is selectively transported into the hepatocyte. Here, it is conjugated with glucuronic acid (G). The conjugates are secreted into bile via the multidrug resistance protein 2 (MRP-2). Some unconjugated and conjugated bilirubin also refluxes into the plasma. The purple circles linking the two adjacent cells represent the tight junctions. BG, bilirubin monoglucuronide; BG_2, bilirubin diglucuronide; OATP, organic anion transporting polypeptide.

The intestinal mucosa is relatively impermeable to conjugated bilirubin but is permeable to unconjugated bilirubin and to urobilinogens, a series of colorless derivatives of bilirubin formed by the action of bacteria in the intestine. Consequently, some of the bile pigments and urobilinogens are reabsorbed in the portal circulation. Some of the reabsorbed substances are again excreted by the liver (enterohepatic circulation), but small amounts of urobilinogens enter the general circulation and are excreted in the urine.

JAUNDICE

When free or conjugated bilirubin accumulates in the blood, the skin, scleras, and mucous membranes turn yellow. This yellowness is known as **jaundice** (icterus) and is usually detectable when the total plasma bilirubin is greater than 2 mg/dL (34 µmol/L). Hyperbilirubinemia may be due to (1) excess production of bilirubin (hemolytic anemia, etc; see Chapter 31), (2) decreased uptake of bilirubin into hepatic cells, (3) disturbed intracellular protein binding or conjugation, (4) disturbed secretion of conjugated bilirubin into the bile canaliculi, or (5) intrahepatic or extrahepatic bile duct obstruction. When it is due to one of the first three processes, the free bilirubin rises. When it is due to disturbed secretion of conjugated bilirubin or bile duct obstruction, bilirubin glucuronide regurgitates into the blood, and it is predominantly the conjugated bilirubin in the plasma that is elevated.

OTHER SUBSTANCES CONJUGATED BY GLUCURONYL TRANSFERASE

The glucuronyl transferase system in the smooth endoplasmic reticulum catalyzes the formation of the glucuronides of a variety of substances in addition to bilirubin. As discussed above, the list includes steroids (see Chapter 20) and various drugs. These other compounds can compete with bilirubin for the enzyme system when they are present in appreciable amounts. In addition, several barbiturates, antihistamines, anticonvulsants, and other compounds cause marked proliferation of the smooth endoplasmic reticulum in the hepatic cells, with a concurrent increase in hepatic glucuronyl transferase activity. Phenobarbital has been used successfully for the treatment of a congenital disease in which there is a relative deficiency of glucuronyl transferase (type 2 UDP-glucuronosyltransferase deficiency).

OTHER SUBSTANCES EXCRETED IN THE BILE

Cholesterol and alkaline phosphatase are excreted in the bile. In patients with jaundice due to intrahepatic or extrahepatic obstruction of the bile duct, the blood levels of these two substances usually rise. A much smaller rise is generally seen when the jaundice is due to nonobstructive hepatocellular disease. Adrenocortical and other steroid hormones and a number of drugs are excreted in the bile and subsequently reabsorbed (enterohepatic circulation).

THE BILIARY SYSTEM

BILE FORMATION

Each of its major constituents derived from the hepatocytes enters the bile by means of a specific canalicular transporter. It is the active secretion of bile acids, however, that is believed to be the primary driving force for the initial formation of canalicular bile. Because they are osmotically active, the canalicular bile is transiently hypertonic. However, the tight junctions that join adjacent hepatocytes are relatively permeable and thus a number of additional substances passively enter the bile from the plasma by diffusion. These substances include water, glucose, calcium, glutathione, amino acids, and urea.

Phosphatidylcholine that enters the bile forms mixed micelles with the bile acids and cholesterol. The ratio of bile acids:phosphatidylcholine:cholesterol in canalicular bile is approximately 10:3:1. Deviations from this ratio may cause cholesterol to precipitate, leading to one type of gallstones **(Figure 28–7)**.

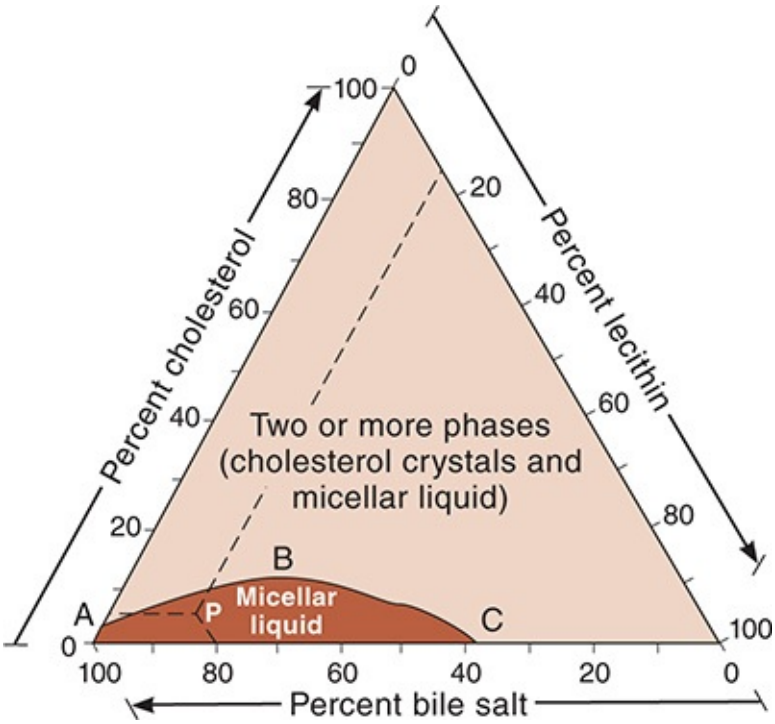


FIGURE 28–7 Cholesterol solubility in bile as a function of the proportions of lecithin (phosphatidylcholine), bile salts (bile acids), and cholesterol. In bile that has a composition described by any point below line ABC (eg, point P), cholesterol is solely in micellar solution; points above line ABC describe bile in which there are cholesterol crystals as well. (Reproduced with permission from

Small DM: Gallstones. N Engl J Med 1968; Sep 12; 279(11):588–593.)

The bile is transferred to progressively larger bile ductules and ducts, where it undergoes modification of its composition. The bile ductules are lined by cholangiocytes, specialized columnar epithelial cells. Their tight junctions are less permeable than those of the hepatocytes, although they remain freely permeable to water and thus bile remains isotonic. The ductules scavenge plasma constituents, such as glucose and amino acids, and return them to the circulation by active transport. Glutathione is also hydrolyzed to its constituent amino acids by an enzyme, gamma glutamyltranspeptidase (GGT), expressed on the apical membrane of the cholangiocytes. Removal of glucose and amino acids is likely important to prevent bacterial overgrowth in the bile, particularly during gallbladder storage (see below). The ductules also secrete bicarbonate in response to secretin in the postprandial period, as well as IgA and mucus for protection.

FUNCTIONS OF THE GALLBLADDER

In normal individuals, bile flows into the gallbladder when the sphincter of Oddi is closed (ie, the period in between meals). In the gallbladder, the bile is concentrated by absorption of water. The degree of this concentration is shown by the increase in the concentration of solids **(Table 28–3)**; hepatic bile is 97% water, whereas the average water content of gallbladder bile is 89%. However, because the bile acids are a micellar solution, the micelles simply become larger, and since osmolarity is a colligative property, bile remains isotonic. However, bile becomes less alkaline as sodium ions are exchanged for protons (although the overall concentration of sodium ions rises with a concomitant loss of chloride and bicarbonate as the bile is concentrated).

TABLE 28–3 Comparison of human hepatic duct bile and gallbladder bile.

	Hepatic Duct Bile	Gallbladder Bile
Percentage of solids	2–4	10–12
Bile acids (mmol/L)	10–20	50–200
Ph	7.8–8.6	7.0–7.4

REGULATION OF BILIARY SECRETION

When food enters the mouth, the resistance of the sphincter of Oddi decreases under both neural and hormonal influences **(Figure 28–8)**. Fatty acids and amino acids in the duodenum release CCK, which causes gallbladder contraction.

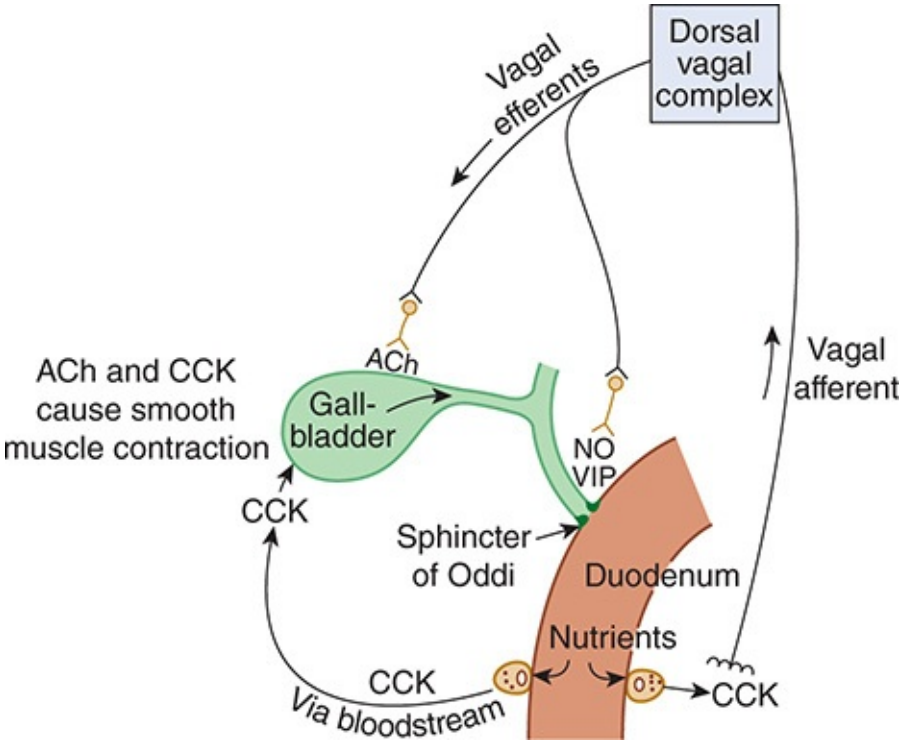


FIGURE 28–8 Neurohumoral control of gallbladder contraction and biliary secretion. Endocrine release of cholecystokinin (CCK) in response to nutrients causes gallbladder contraction. CCK also activates vagal afferents to trigger a vagovagal reflex that reinforces gallbladder contraction (via acetylcholine [ACh]) and relaxation of the sphincter of Oddi to permit bile outflow (via NO and vasoactive intestinal polypeptide [VIP]).

The production of bile is increased by stimulation of the vagus nerves and by the hormone secretin, which increases the water and HCO_3^- content of bile. Substances that increase the secretion of bile are known as **choleretics.** Bile acids themselves are among the most important physiologic choleretics.

EFFECTS OF CHOLECYSTECTOMY

The periodic discharge of bile from the gallbladder aids digestion but is not

essential for it. Cholecystectomized patients maintain good health and nutrition with a constant slow discharge of bile into the duodenum, although eventually the bile duct becomes somewhat dilated, and more bile tends to enter the duodenum after meals than at other times.

VISUALIZING THE GALLBLADDER

Exploration of the right upper quadrant with an ultrasonic beam **(ultrasonography)** and computed tomography (CT) have become the most widely used methods for visualizing the gallbladder and detecting gallstones. A third method of diagnosing gallbladder disease is **nuclear cholescintigraphy.** When administered intravenously, technetium-99m-labeled derivatives of iminodiacetic acid are excreted in the bile and provide excellent gamma camera images of the gallbladder and bile ducts. The response of the gallbladder to CCK can then be observed following intravenous administration of the hormone. The biliary tree can also be visualized by injecting contrast media from an endoscope channel maneuvered into the sphincter of Oddi, in a procedure known as endoscopic retrograde cholangiopancreatography (ERCP). It is even possible to insert small instruments with which to remove gallstone fragments that may be obstructing the flow of bile, the flow of pancreatic juice, or both **(Clinical Box 28–2)**.

CLINICAL BOX 28–2

Gallstones

Cholelithiasis, that is, the presence of gallstones, is a common condition. Its incidence increases with age, so that in the United States, for example, 20% of the women and 5% of the men between the ages of 50 and 65 have gallstones. The stones are of two types: calcium bilirubinate stones and cholesterol stones. In the United States and Europe, 85% of the stones are cholesterol stones. Three factors appear to be involved in the formation of cholesterol stones. One is bile stasis; stones form in the bile that is sequestrated in the gallbladder rather than the bile that is flowing in the bile ducts. A second is supersaturation of the bile with cholesterol. Cholesterol is very insoluble in bile, and it is maintained in solution in micelles only at certain concentrations of bile salts and lecithin. At concentrations above line ABC in Figure 28–7, the bile is supersaturated and contains small crystals of

cholesterol in addition to micelles. However, many healthy individuals in whom gallstones do not develop also have supersaturated bile. The third factor is a mix of nucleation factors that favors formation of stones from the supersaturated bile. Outside the body, bile from patients with cholelithiasis forms stones in 2–3 days, whereas it takes more than 2 weeks for stones to form in bile from normal individuals. The exact nature of the nucleation factors is unsettled, although glycoproteins in gallbladder mucus have been implicated. In addition, it is unsettled whether stones form as a result of excess production of components that favor nucleation or decreased production of antinucleation components that prevent stones from forming in normal individuals.

Gallstones that obstruct bile outflow from the liver can result in **obstructive jaundice.** If the flow of bile out of the liver is completely blocked, substances normally excreted in the bile, such as cholesterol, accumulate in the bloodstream. The interruption of the enterohepatic circulation of bile acids also induces the liver to synthesize bile acids at a greater rate. Some of these bile acids can be excreted by the kidney, and thus represent a mechanism for indirect excretion of at least a portion of cholesterol. However, retained biliary constituents may also cause liver toxicity.

THERAPEUTIC HIGHLIGHTS

The treatment of gallstones depends on their nature and the severity of any symptoms. Many, particularly if small and retained in the gallbladder, may be asymptomatic. Larger stones that cause obstruction may need to be removed surgically, or via ERCP. Oral dissolution agents may dissolve small stones composed of cholesterol, but the effect is slow and stones often return once therapy is stopped. A definitive cure for patients who suffer from recurrent attacks of symptomatic cholelithiasis is to have the gallbladder removed, which is usually now performed laparoscopically.

CHAPTER SUMMARY

- The liver conducts a huge number of metabolic reactions and serves to detoxify and dispose of many exogenous substances, as well as metabolites endogenous to the body that would be harmful if allowed to accumulate.

- The structure of the liver is such that it can filter large volumes of blood and remove even hydrophobic substances that are protein-bound. This function is provided for by a fenestrated endothelium. The liver also receives essentially all venous blood from the intestine prior to its delivery to the remainder of the body.
- The liver serves to buffer blood glucose, synthesize the majority of plasma proteins, contribute to lipid metabolism, and preserve cholesterol homeostasis.
- Bilirubin is an end product of heme metabolism that is glucuronidated by the hepatocyte to permit its excretion in bile. Bilirubin and its metabolites impart color to the bile and stools.
- The liver removes ammonia from the blood and converts it to urea for excretion by the kidneys. An accumulation of ammonia as well as other toxins cause hepatic encephalopathy in the setting of liver failure.
- Bile contains substances actively secreted across the canalicular membrane by hepatocytes, and notably bile acids, phosphatidylcholine, and cholesterol. The composition of bile is modified as it passes through the bile ducts and is stored in the gallbladder. Gallbladder contraction is regulated to coordinate bile availability with the timing of meals.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. Which of the following cell types protects against sepsis secondary to translocation of intestinal bacteria?

   A. Hepatic stellate cell

   B. Cholangiocyte

   C. Kupffer cell

   D. Hepatocyte

   E. Gallbladder epithelial cell

2. A 60-year-old man comes to his physician complaining of a progressive increase in his girth despite attempts to diet. He is also jaundiced and complains of nausea and malaise. When a large needle is inserted into his abdomen, several liters of tan fluid drain out. An increase in which of the following is not involved in this fluid accumulation?

A. Portal pressure
B. Hepatic collagen
C. Plasma albumin
D. Stellate cell activity
E. Plasma transudation

3. P450s (CYPs) are highly expressed in hepatocytes. In which of the following do they *not* play an important role?

A. Bile acid formation
B. Carcinogenesis
C. Steroid hormone formation
D. Detoxification of drugs
E. Glycogen synthesis

4. A surgeon is studying new methods of liver transplantation. She performs a complete hepatectomy in an experimental animal. Before the donor liver is grafted, a rise in the blood level of which of the following would be expected?

A. Glucose
B. Fibrinogen
C. 25-Hydroxycholecalciferol
D. Conjugated bilirubin
E. Estrogens

5. A scientist studying the function of the different cell types in the liver creates a mouse in which the activity of one specific cell type is transiently ablated. The mice exhibit behavioral defects consistent with encephalopathy. The function of which of the following cell types was most likely targeted?

A. Hepatic stellate cell
B. Cholangiocyte
C. Kupffer cell
D. Hepatocyte
E. Gallbladder epithelial cell

6. A patient suffering from severe ulcerative colitis undergoes a total colectomy with formation of a stoma. After a full recovery from surgery, and compared to his condition prior to surgery, which of the following would be expected to be decreased?

A. Ability to absorb lipids

B. Ability to clot the blood

C. Circulating levels of conjugated bile acids

D. Urinary urea

E. Urinary urobilinogen

7. Compared to hepatic bile, gallbladder bile contains a reduced concentration of which of the following?

A. Bile acids

B. Chloride ions

C. Protons

D. Glucose

E. Calcium ions

8. A newborn delivered vaginally is noted to be mildly jaundiced, but no bilirubin is found in the urine. The child's symptoms are most likely attributable to a developmental delay in the expression or establishment of which of the following:

A. Colonic bacterial colonization

B. MDR2

C. UDP glucuronyl transferase

D. Heme oxygenase

E. Biliverdin reductase

9. A 40-year-old woman comes to her primary care clinician complaining of severe, episodic abdominal pain that is particularly intense after she ingests a fatty meal. An imaging procedure reveals that her gallbladder is acutely dilated, and a diagnosis of cholelithiasis is made. A gallstone lodged in which location will also increase her risk of pancreatitis?

A. Left hepatic duct

B. Right hepatic duct

C. Cystic duct

D. Common bile duct

E. Sphincter of Oddi

10. A 45-year-old woman is brought to the emergency room with a 3-day history of colicky epigastric pain that suddenly increased in severity after a large meal. A gallstone is found to be occluding her spincter of Oddi. Which of the following substances would be found at reduced levels in her circulation?

A. Unconjugated bile acids
B. Conjugated bile acids
C. Cholesterol
D. Phosphatidylcholine
E. Amylase

SECTION V

Cardiovascular Physiology

Cells exist within a body fluid compartment known as the interstitial fluid, and the cardiovascular system has evolved to ensure that the composition of the interstitial fluid is maintained within a narrow range. Homeostasis is accomplished by pumping a separate fluid compartment—plasma—around the body, where it can be “conditioned” as it passes through specific organs that add nutrients, oxygen, hormones and needed metabolites, and/or remove waste products. The plasma then delivers needed substances to other organs and tissues. Efficient transfer of substances between the cells and the plasma is accomplished by dense networks of capillaries, which offer little resistance to the transfer of substances across their walls, and provide for short diffusion distances between the capillaries and the sites at which products will be utilized. The pumping function in this system is provided by the heart, a four-chambered organ that drives blood around two circuits in series, one that perfuses the lungs and one that serves the remainder of the body.

In principle, this sounds like a simple system. However, in practice, exquisite minute-to-minute regulation is needed to ensure that organs receive the substances that they need when they need them, particularly in the face of ever-changing demands. For example, when an individual begins to exercise, there is a prompt demand for additional oxygen and glucose in the contracting muscles to sustain muscle activity. In the brain, there is no capacity to store glucose and blood flow must be maintained to ensure consciousness, even in the face of hydrostatic challenges (eg, moving from a recumbent to a standing position). The cardiovascular system must therefore be able to adjust the rate at which the plasma is circulated around the body as a whole and to redirect plasma flow to the places where it is most needed. Further, the body is an “open” system, meaning that some bodily constituents (eg, water) are constantly being lost to the environment. The circulation, and the organs that condition it, must respond

promptly to these threats to homeostasis to ensure the proper functioning of vital body systems, which typically operate within a narrow range of osmolarity, pH, oxygen saturation, etc.

In this section, the components of the cardiovascular system that permit it to serve the body's needs for substance transfer will be considered. First, the electrical activity that allows the chambers of the heart to contract in an ordered fashion, to move the circulation unidirectionally, will be discussed. Then, the properties of blood and its components that suit them to transport dissolved solutes to and from the interstitial fluid will be considered. The properties of the circulatory "plumbing," or blood vessels, will be addressed, along with the mechanisms that regulate them. Finally, the specialized properties of the circulation in areas of the body with unique needs will be discussed.

Obviously, a functional cardiovascular system is vital for life, and irreversible damage quickly ensues in a number of organs if the heart stops beating. Less drastic disorders of the cardiovascular system also represent a considerable burden of disease. In fact, cardiovascular diseases, collectively, are the leading cause of death and responsible for significant disability worldwide. In the United States, heart disease and stroke are the first and third most common causes of death, and an estimated one in three American adults has some form of cardiovascular disorder. Cardiovascular diseases are also leading causes of hospitalization, and account for the highest economic burden of any disease category. Finally, while there have been impressive gains in treatment and prevention of some cardiovascular diseases, the growing epidemic of obesity, and the rise in the proportion of the population with at least one cardiovascular risk factor, has been cause for considerable alarm among public health officials. All of these facts underscore the relevance of a thorough understanding of cardiovascular physiology for the aspiring health professional.

CHAPTER 29

Origin of the Heartbeat & the Electrical Activity of the Heart

OBJECTIVES

After studying this chapter, you should be able to:

- Describe the structure and function of the conduction system of the heart and compare the action potentials in each part.
- Describe the way the electrocardiogram (ECG) is recorded, the waves of the ECG, and the relationship of the ECG to the electrical axis of the heart.
- Name the common cardiac arrhythmias and describe the processes that produce them.
- List the principal early and late ECG manifestations of myocardial infarction and explain the early changes in terms of the underlying ionic events that produce them.
- Describe the ECG changes and the changes in cardiac function produced by alterations in the ionic composition of the body fluids.

INTRODUCTION

The parts of the heart normally beat in orderly sequence: Contraction of the atria

(atrial systole) is followed by contraction of the ventricles **(ventricular systole),** and during **diastole** all four chambers are relaxed (also see Chapter 30). The cardiac electric activity that triggers heartbeat originates in a specialized **cardiac conduction system** and spreads via this system to all parts of the myocardium. The structures that make up the conduction system are the **sinoatrial node (SA node),** the **internodal atrial pathways,** the **atrioventricular node (AV node),** the **bundle of His** and its branches, and the **Purkinje system.** The various parts of the conduction system and, under abnormal conditions, parts of the myocardium, are capable of spontaneous discharge. However, the SA node normally discharges most rapidly, with depolarization spreading from it to the other regions before they discharge spontaneously. The SA node is therefore the normal **cardiac pacemaker,** with its rate of discharge determining the rate at which the heart beats. Impulses generated in the SA node pass through the atrial pathways to the AV node, through this node to the bundle of His, and through the branches of the bundle of His via the Purkinje system to the ventricular muscle. Each of the cell types in the heart contains a unique electrical discharge pattern; the sum of these electrical discharges can be recorded as the electrocardiogram (ECG or EKG).

ORIGIN & SPREAD OF CARDIAC EXCITATION

ANATOMIC CONSIDERATIONS

In the human heart, the SA node is located at the junction of the superior vena cava with the right atrium. The AV node is located in the right posterior portion of the interatrial septum **(Figure 29–1)**. There are three bundles of atrial fibers that contain Purkinje-type fibers and connect the SA node to the AV node: the anterior, middle (tract of Wenckebach), and posterior (tract of Thorel) tracts. Bachmann bundle is sometimes used to identify a branch of the anterior intermodal tract that connects the right and left atria. Conduction also occurs through atrial myocytes, but it is more rapid in these bundles. The AV node is continuous with the bundle of His, which gives off a left bundle branch at the top of the interventricular septum and continues as the right bundle branch. The left bundle branch divides into an anterior fascicle and a posterior fascicle. The branches and fascicles run subendocardially down either side of the septum and come into contact with the Purkinje system, whose fibers spread to all parts of the ventricular myocardium.

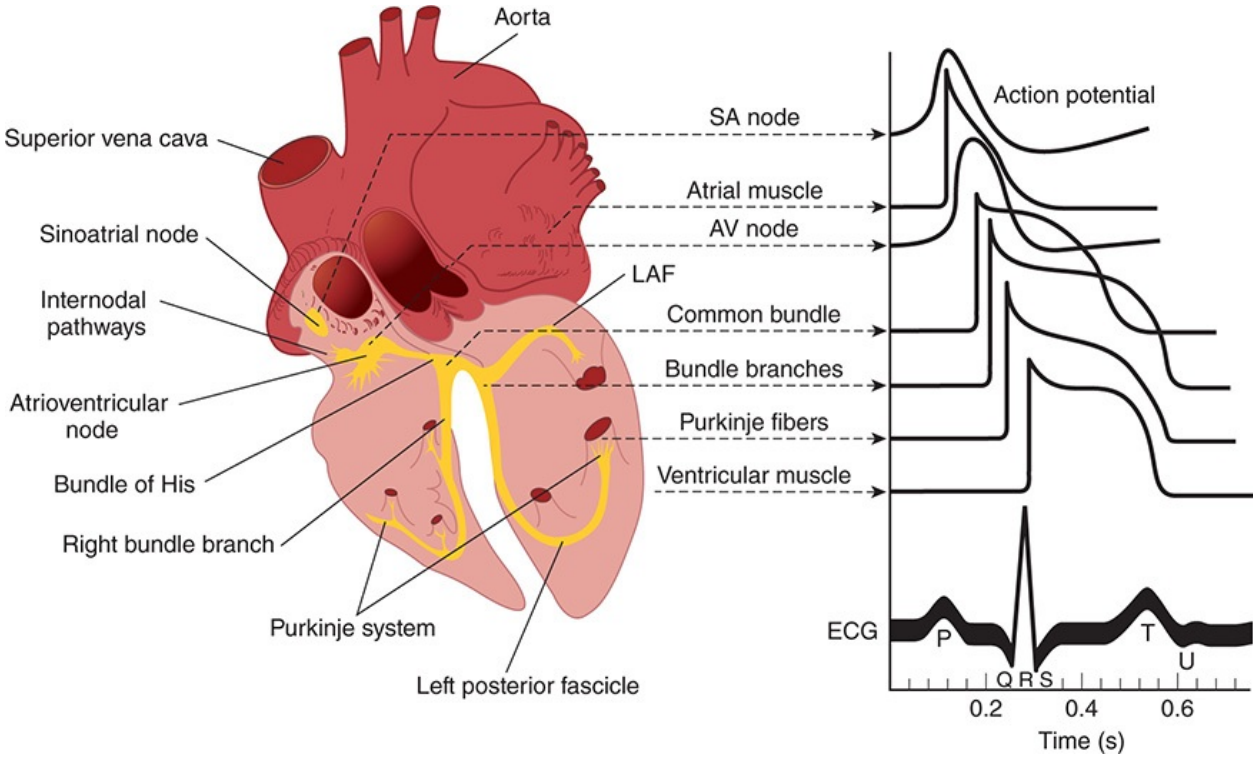


FIGURE 29–1 Conducting system of the heart. Left: Anatomic depiction of the human heart with additional focus on areas of the conduction system. **Right:** Typical transmembrane action potentials for the SA and AV nodes, other parts of the conduction system, and the atrial and ventricular muscles are shown along with the correlation to the extracellularly recorded electrical activity, that is, the electrocardiogram (ECG). The action potentials and ECG are plotted on the same time axis but with different zero points on the vertical scale for comparison. AV, atrioventricular; LAF, left anterior fascicle; SA, sinoatrial. (Data from Donahue JG, Choo PW, Manson JE, et al. The incidence of herpes zoster. Arch Intern Med 1995;155:1605–1609; Choo PW, Galil K, Donahue JG, et al. Risk factors for postherpetic neuralgia. Arch Intern Med 1997;157:1217–1224.)

The histology of a typical cardiac muscle cell (eg, a ventricular myocyte) is described in Chapter 5. For the most part, the conduction system is composed of modified cardiac muscle that has fewer striations and indistinct boundaries. Individual cells within regions of the heart have unique histologic features. Purkinje fibers, specialized conducting cells, are large with fewer mitochondria and striations and distinctly different from a myocyte specialized for contraction. Compared with Purkinje fibers, cells within the SA node and, to a lesser extent, the AV nodes are smaller and sparsely striated and are less conductive due to

their higher internal resistance. The atrial muscle fibers are separated from those of the ventricles by a fibrous tissue ring, and normally the only conducting tissue between the atria and ventricles is the bundle of His.

The SA node develops from structures on the right side of the embryo and the AV node from structures on the left. This is why in the adult the right vagus is distributed mainly to the SA node and the left vagus mainly to the AV node. Similarly, the sympathetic innervation on the right side is distributed primarily to the SA node and the sympathetic innervation on the left side primarily to the AV node. On each side, most sympathetic fibers come from the stellate ganglion. Noradrenergic fibers are epicardial, whereas the vagal fibers are endocardial. However, connections exist for reciprocal inhibitory effects of the sympathetic and parasympathetic innervation of the heart on each other. Thus, acetylcholine acts presynaptically to reduce norepinephrine release from the sympathetic nerves, and conversely, neuropeptide Y released from noradrenergic endings may inhibit the release of acetylcholine.

PROPERTIES OF CARDIAC MUSCLE

The electrical responses of cardiac muscle and nodal tissue and the ionic fluxes that underlie them are discussed in detail in Chapter 5 and are briefly reviewed here for comparison with the pacemaker cells below. Myocardial fibers have a resting membrane potential of approximately −90 mV **(Figure 29–2A)**. The individual fibers are separated by membranes, but membrane depolarization spreads radially through them as if they were a syncytium because of the presence of gap junctions. The transmembrane action potential of single cardiac muscle cells is characterized by rapid depolarization (phase 0), an initial rapid repolarization (phase 1), a plateau (phase 2), and a slow repolarization process (phase 3) that allows return to the resting membrane potential (phase 4). The initial depolarization is due to Na^+ influx through rapidly opening voltage-gated Na^+ channels (the Na^+ current, I_{Na}). The inactivation of voltage-gated Na^+ channels contributes to the rapid repolarization phase. Ca^{2+} influx through more slowly opening voltage-gated Ca^{2+} channels (the Ca^{2+} current, I_{Ca}) produces the plateau phase, and repolarization is due to net K^+ efflux through multiple types of K^+ channels. Recorded extracellularly, the summed electrical activity of all the cardiac muscle fibers is the ECG (discussed below). The timing of the discharge of the individual units relative to the ECG is shown in Figure 29–1 (right panel). Note that the ECG is a combined electrical record and thus the

overall shape reflects electrical activity from cells from different regions of the heart.

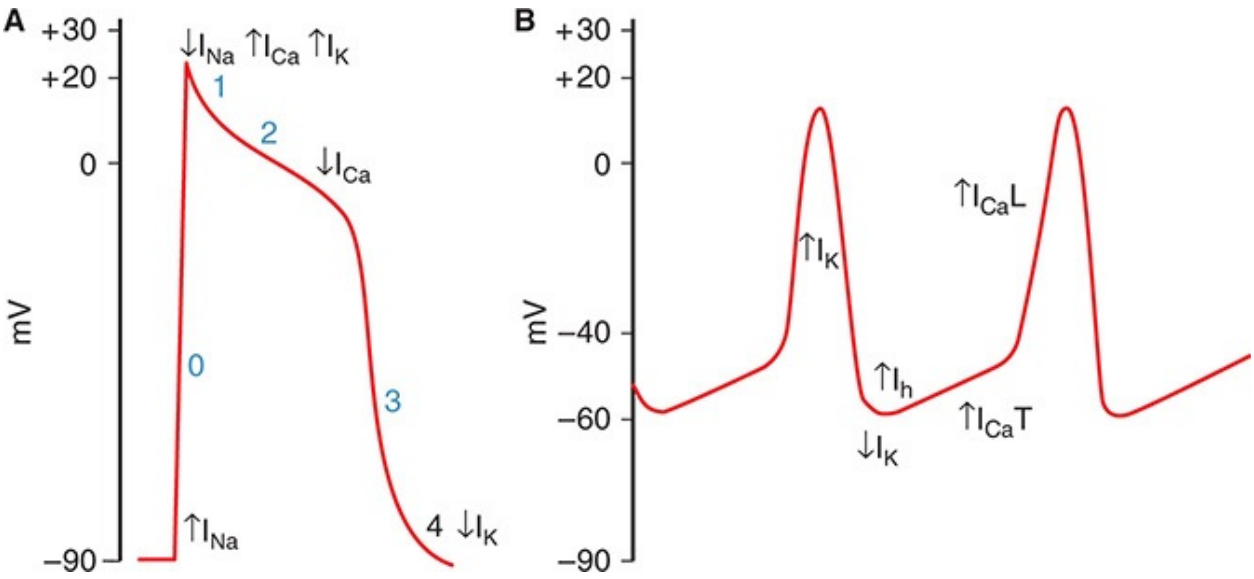


FIGURE 29–2 Comparison of action potentials in ventricular muscle and diagram of the membrane potential of pacemaker tissue. A) Phases of action potential in ventricular myocyte (0–4, see text for details) are superimposed with principal changes in current that contribute to changes in membrane potential. **B)** The principal current responsible for each part of the potential of pacemaker tissue is shown under or beside the component. L, long-lasting; T, transient. Other ion channels contribute to the electrical response. Note that the resting membrane potential of pacemaker tissue is somewhat lower than that of atrial and ventricular muscle.

PACEMAKER POTENTIALS

Rhythmically discharging cells have a membrane potential that, after each impulse, declines to the firing level. Thus, this **prepotential** or **pacemaker potential** (Figure 29–2B) triggers the next impulse. At the peak of each impulse, I_K begins and brings about repolarization. I_K then declines, and a channel permeable to both Na^+ and K^+ is activated. Because this channel is activated following hyperpolarization, it is referred to as an "h" channel and the current through the h channels is called "h" current (I_h); however, because of its unusual (funny) activation it has also been dubbed an "f" channel and the current produced as "funny current." As I_h increases, the membrane begins to

depolarize, forming the first part of the prepotential. When prepotential reaches the activation threshold of voltage-gated Ca^{2+} channels, Ca^{2+} channels then open. There are two types of these voltage-gated Ca^{2+} channels in the heart, the **T** (for transient) **channels** and the **L** (for long-lasting) **channels.** The Ca^{2+} current (I_{Ca}) due to opening of T channels completes the prepotential, and I_{Ca} due to opening of L channels produces the impulse. Other ion channels are also involved, and there is evidence that local Ca^{2+} release from the sarcoplasmic reticulum **(Ca^{2+} sparks)** occurs during the prepotential.

The action potentials in the SA and AV nodes are largely due to Ca^{2+} invlus, with no contribution by Na^+ influx. Consequently, there is no sharp, rapid depolarizing spike before the plateau, as there is in other parts of the conduction system and in the atrial and ventricular fibers. In addition, prepotentials are normally prominent only in the SA and AV nodes. However, “latent pacemakers” are present in other portions of the conduction system that can take over when the SA and AV nodes are depressed or conduction from them is blocked. Atrial and ventricular muscle fibers do not have prepotentials, and they discharge spontaneously only when injured or abnormal.

When the cholinergic vagal fibers to nodal tissue are stimulated, the membrane becomes hyperpolarized and the slope of the prepotentials is decreased **(Figure 29–3)** because the acetylcholine released at the nerve endings increases the K^+ conductance of nodal tissue. This action is mediated by M_2 muscarinic receptors, which, via the βγ subunit of a G protein, open a special set of K^+ channels. The resulting K^+ currents (I_{KAch}) through these G protein-gated K^+ channels slows the depolarizing effect of I_h. In addition, activation of the M_2 receptors decreases cyclic adenosine 3′,5′-monophosphate (cAMP) in the cells, and this slows the opening of Ca^{2+} channels. The result is a decrease in firing rate. Strong vagal stimulation may abolish spontaneous discharge for some time.

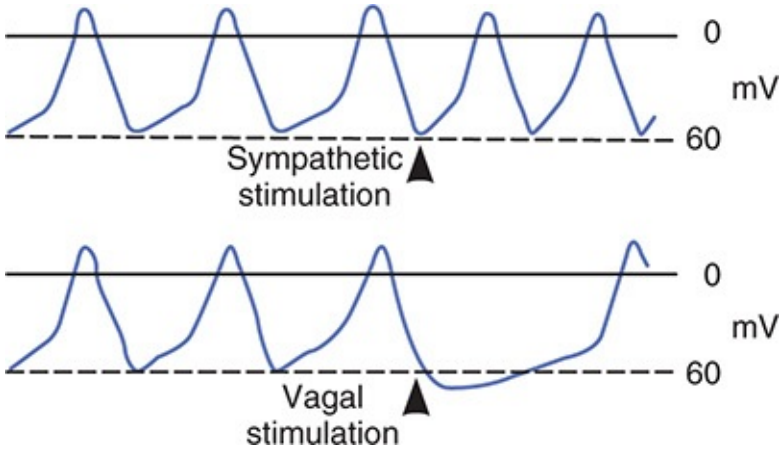


FIGURE 29–3 Effect of sympathetic (noradrenergic) and vagal (cholinergic) stimulation on the membrane potential of the SA node. Note the reduced slope of the prepotential after vagal stimulation and the increased spontaneous discharge after sympathetic stimulation. SA, sinoatrial.

CLINICAL BOX 29–1

Use of Digitalis

Digitalis, or its clinically useful preparations (digoxin and digitoxin), has been described in medical literature for over 200 years. It was originally derived from the foxglove plant. (*Digitalis purpurea* is the name of the common foxglove.) Correct administration can strengthen cardiac contractions through digitalis inhibitory effects on the Na, K ATPase, resulting in greater amounts of Ca^{2+} release and subsequent changes in contraction forces. Digitalis can also have an electrical effect in decreasing AV nodal conduction velocity and thus altering AV transmission to the ventricles.

THERAPEUTIC HIGHLIGHTS

Digitalis has been used for treatment of systolic heart failure. It augments contractility, thereby improving cardiac output, improving left ventricle emptying, and decreasing ventricular filling pressures. Digitalis has also been used to treat atrial fibrillation and atrial flutter. In this scenario, digitalis reduces the number of impulses transmitted through the AV node and thus, provides effective rate control.

In both these instances alternative treatments developed over the past 20 years and the need to tightly regulate dose due to significant potential for side effects have reduced the use of digitalis. However, with better understanding of mechanism and toxicity, digitalis and its clinically prepared derivatives remain important drugs in modern medicine.

Conversely, stimulation of the sympathetic cardiac nerves speeds the depolarizing effect of I_h, and the rate of spontaneous discharge increases (Figure 29–3). Norepinephrine secreted by the sympathetic endings binds to β_1 receptors, and the resulting increase in intracellular cAMP facilitates the opening of L channels, increasing I_{Ca} and the rapidity of the depolarization phase of the impulse.

The rate of discharge of the SA node and other nodal tissue is influenced by temperature and by drugs. The discharge frequency is increased when the temperature rises, and this may contribute to the tachycardia associated with fever. Digitalis depresses nodal tissue and exerts an effect like that of vagal stimulation, particularly on the AV node **(Clinical Box 29–1;** also see Clinical Box 5–6).

SPREAD OF CARDIAC EXCITATION

Depolarization initiated in the SA node spreads radially through the atria, then converges on the AV node. Atrial depolarization is complete in about 0.1 s. Because conduction in the AV node is slow **(Table 29–1)**, a delay of about 0.1 s **(AV nodal delay)** occurs before excitation spreads to the ventricles. It is interesting to note here that when there is a lack of contribution of I_{Na} in the depolarization (phase 0) of the action potential, a marked loss of conduction is observed. This delay is shortened by stimulation of the sympathetic nerves to the heart and lengthened by stimulation of the vagi. From the top of the septum, the wave of depolarization spreads in the rapidly conducting Purkinje fibers to all parts of the ventricles in 0.08–0.1 s. In humans, depolarization of the ventricular muscle starts at the left side of the interventricular septum and moves first to the right across the mid portion of the septum. The wave of depolarization then spreads down the septum to the apex of the heart. It returns along the ventricular walls to the AV groove, proceeding from the endocardial to the epicardial surface **(Figure 29–4)**. The last parts of the heart to be depolarized are the

posterobasal portion of the left ventricle, the pulmonary conus, and the uppermost portion of the septum.

TABLE 29–1 Conduction speeds in cardiac tissue.

Tissue	Conduction Rate (m/s)
SA node	0.05
Atrial pathways	1
AV node	0.05
Bundle of His	1
Purkinje system	4
Ventricular muscle	1

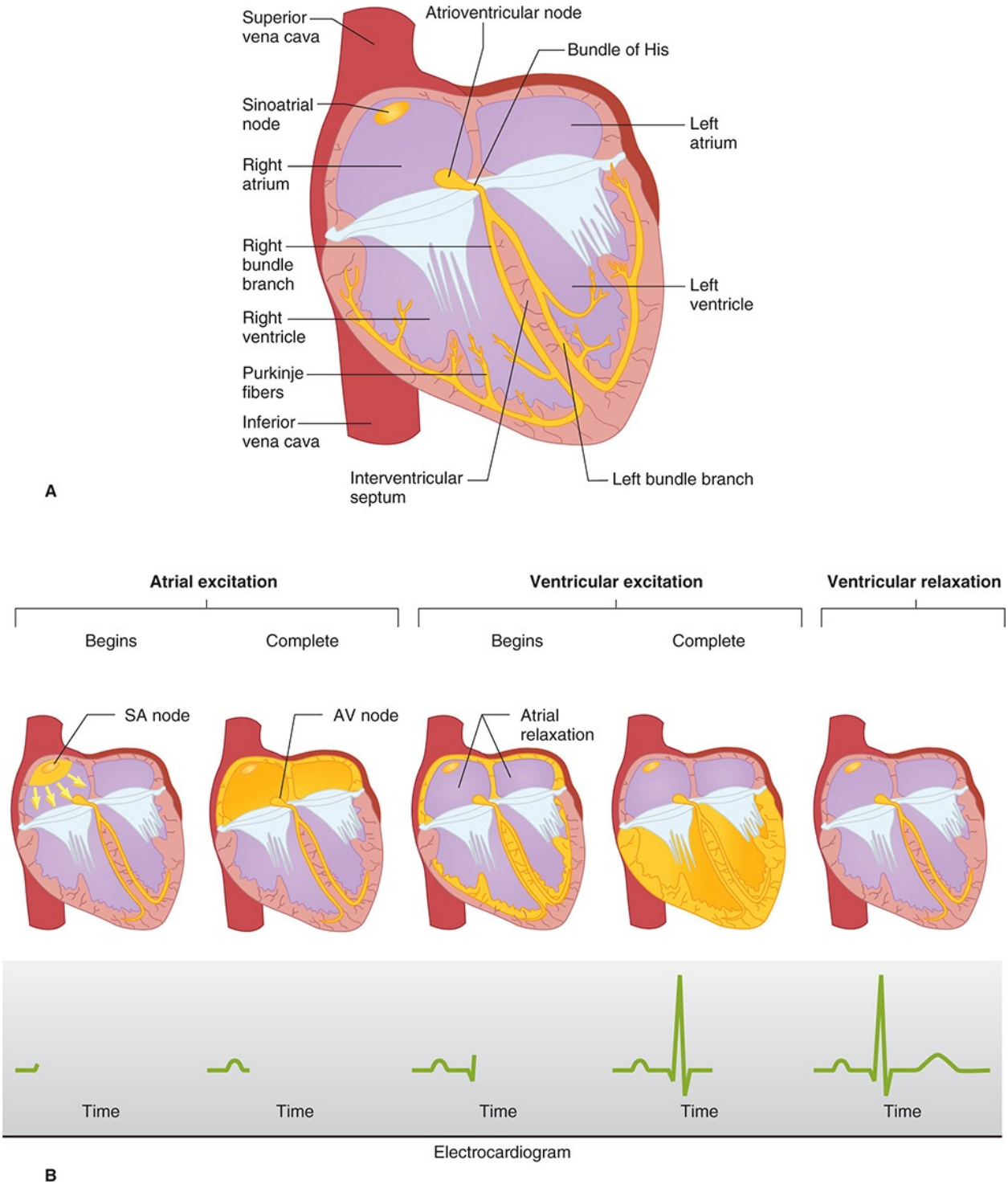


FIGURE 29–4 Normal spread of electrical activity in the heart. A) Conducting system of the heart. **B)** Sequence of cardiac excitation. **Top:** Anatomic position of electrical activity. **Bottom:** Corresponding electrocardiogram. The yellow color denotes areas that are depolarized. AV, atrioventricular; SA, sinoatrial. (Reproduced with permission from Goldman MJ:

Principles of Clinical Electrocardiography, 12th ed. Originally published by Appleton & Lange. Copyright © 1986 by McGraw-Hill.)

THE ELECTROCARDIOGRAM

Because the body fluids are good conductors (ie, because the body is a **volume conductor**), fluctuations in potential, representing the algebraic sum of the action potentials of myocardial fibers, can be recorded extracellularly. The record of these fluctuations in potential during the cardiac cycle is the **ECG or EKG.**

The ECG may be recorded by using an **active** or **exploring electrode** connected to an indifferent electrode at zero potential **(unipolar recording)** or by using two active electrodes **(bipolar recording).** In a volume conductor, the sum of the potentials at the points of an equilateral triangle with a current source in the center is zero at all times. A triangle with the heart at its center **(Einthoven triangle,** see below) can be approximated by placing electrodes on both arms and on the left leg. These are the three **standard limb leads** used in electrocardiography. If these electrodes are connected to a common terminal, an indifferent electrode that stays near zero potential is obtained. Depolarization moving toward an active electrode in a volume conductor produces a positive deflection, whereas depolarization moving in the opposite direction produces a negative deflection.

The names of the various waves and segments of the ECG in humans are shown in **Figure 29–5**. By convention, an upward deflection is written when the active electrode becomes positive relative to the indifferent electrode, and a downward deflection is written when the active electrode becomes negative. As can be seen in Figure 29–1, the P wave is primarily produced by atrial depolarization, the QRS complex is dominated by ventricular depolarization, and the T wave by ventricular repolarization. The U wave is an inconstant finding that may be due to ventricular myocytes with long action potentials. However, the contributions to this segment are still undetermined. The intervals between the various waves of the ECG and the events in the heart that occur during these intervals are shown in **Table 29–2**.

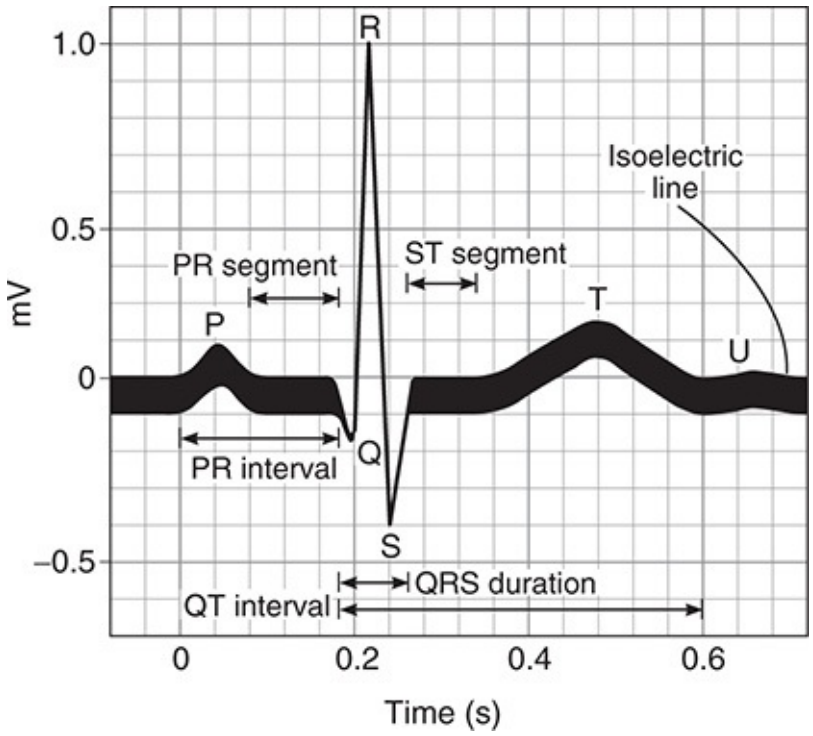


FIGURE 29–5 Waves of the ECG. Standard names for individual waves and segments that make up the ECG are shown. Electrical activity that contributes the observed deflections are discussed in the text and in Table 29–2. ECG, electrocardiogram.

TABLE 29–2 ECG intervals.

Intervals	Normal Durations (second, s)		Events in the Heart during Interval
	Average	Range	
PR interval[a]	0.18[b]	0.12–0.20	Atrioventricular conduction
QRS duration	0.08	to 0.10	Ventricular depolarization
QT interval	0.40[c]	to 0.43	Ventricular action potential
ST interval (QT minus QRS)	0.32	…	Plateau portion of the ventricular action potential

[a]Measured from the beginning of the P wave to the beginning of the QRS complex.
[b]Shortens as heart rate increases from average of 0.18 s at a rate of 70 beats/min to 0.14 s at a rate of 130 beats/min.
[c]Can be lower (0.35 s) depending on the heart rate.

BIPOLAR LEADS

Bipolar leads were used before unipolar leads were developed. The **standard limb leads** each record the differences in potential between two limbs. Because current flows only in the body fluids, the records obtained are those that would be obtained if the electrodes were at the points of attachment of the limbs, no

matter where on the limbs the electrodes are placed. In lead I, the electrodes are connected so that an upward deflection is inscribed when the left arm becomes positive relative to the right (left arm positive). In lead II, the electrodes are on the right arm and left leg, with the leg positive; and in lead III, the electrodes are on the left arm and left leg, with the leg positive.

UNIPOLAR (V) LEADS

An additional nine unipolar leads, that is, leads that record the potential difference between an **exploring electrode** and an **indifferent electrode,** are commonly used in clinical electrocardiography. There are six unipolar chest leads (precordial leads) designated V_1–V_6 **(Figure 29–6)** and three unipolar limb leads: VR (right arm), VL (left arm), and VF (left foot). The indifferent electrode is constructed by connecting electrodes placed on the two arms and the left leg to a central terminal. This "V" lead effectively records a "zero" potential because they are situated such that the electrical activity should be cancelled out. **Augmented limb leads,** designated by the letter a (aVR, aVL, aVF), are generally used. The augmented limb leads do not use the "V" electrode as the zero, rather, they are recordings between the one, augmented limb and the other two limbs. This increases the size of the potentials by 50% without any change in configuration from the nonaugmented record.

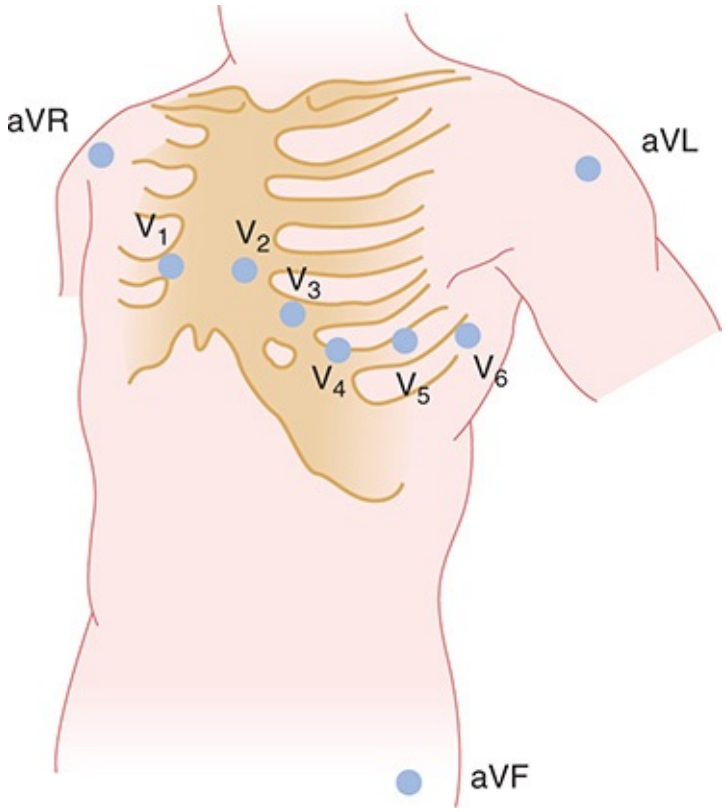


FIGURE 29–6 Unipolar electrocardiographic leads. Positional for standard unipolar leads are shown. The augmented extremity leads (aVR, aVL, and aVF) are shown on the right arm, left arm, and left leg, respectively. The six chest leads (V_1–V_6) are shown in their proper placement.

Unipolar leads can also be placed at the tips of catheters and inserted into the esophagus or heart. Although sensitivity can be increased, this is obviously more invasive and thus, not a first step in obtaining electrical readings.

NORMAL ECG

The ECG tracings of a normal individual are shown in Figure 29–4b and **Figure 29–7**. The sequence in which the parts of the heart are depolarized (Figure 29–4) and the position of the heart relative to the electrodes (Figure 29–7) are the important considerations in interpreting the configurations of the waves in each lead. The atria are located posteriorly in the chest. The ventricles form the base and anterior surface of the heart, and the right ventricle is anterolateral to the left. Thus, aVR "looks at" the cavities of the ventricles. Atrial depolarization, ventricular depolarization, and ventricular repolarization move away from the

exploring electrode, and the P wave, QRS complex, and T wave are therefore all negative (downward) deflections; aVL and aVF look at the ventricles, and the deflections are therefore predominantly positive or biphasic. There is no Q wave in V_1 and V_2, and the initial portion of the QRS complex is a small upward deflection because ventricular depolarization first moves across the midportion of the septum from left to right toward the exploring electrode. The wave of excitation then moves down the septum and into the left ventricle away from the electrode, producing a large S wave. Finally, it moves back along the ventricular wall toward the electrode, producing the return to the isoelectric line. Conversely, in the left ventricular leads (V_4–V_6) there may be an initial small Q wave (left to right septal depolarization), and there is a large R wave (septal and left ventricular depolarization) followed in V_4 and V_5 by a moderate S wave (late depolarization of the ventricular walls moving back toward the AV junction). It should be noted that there is considerable variation in the position of the normal heart, and the position affects the configuration of the electrocardiographic complexes in the various leads.

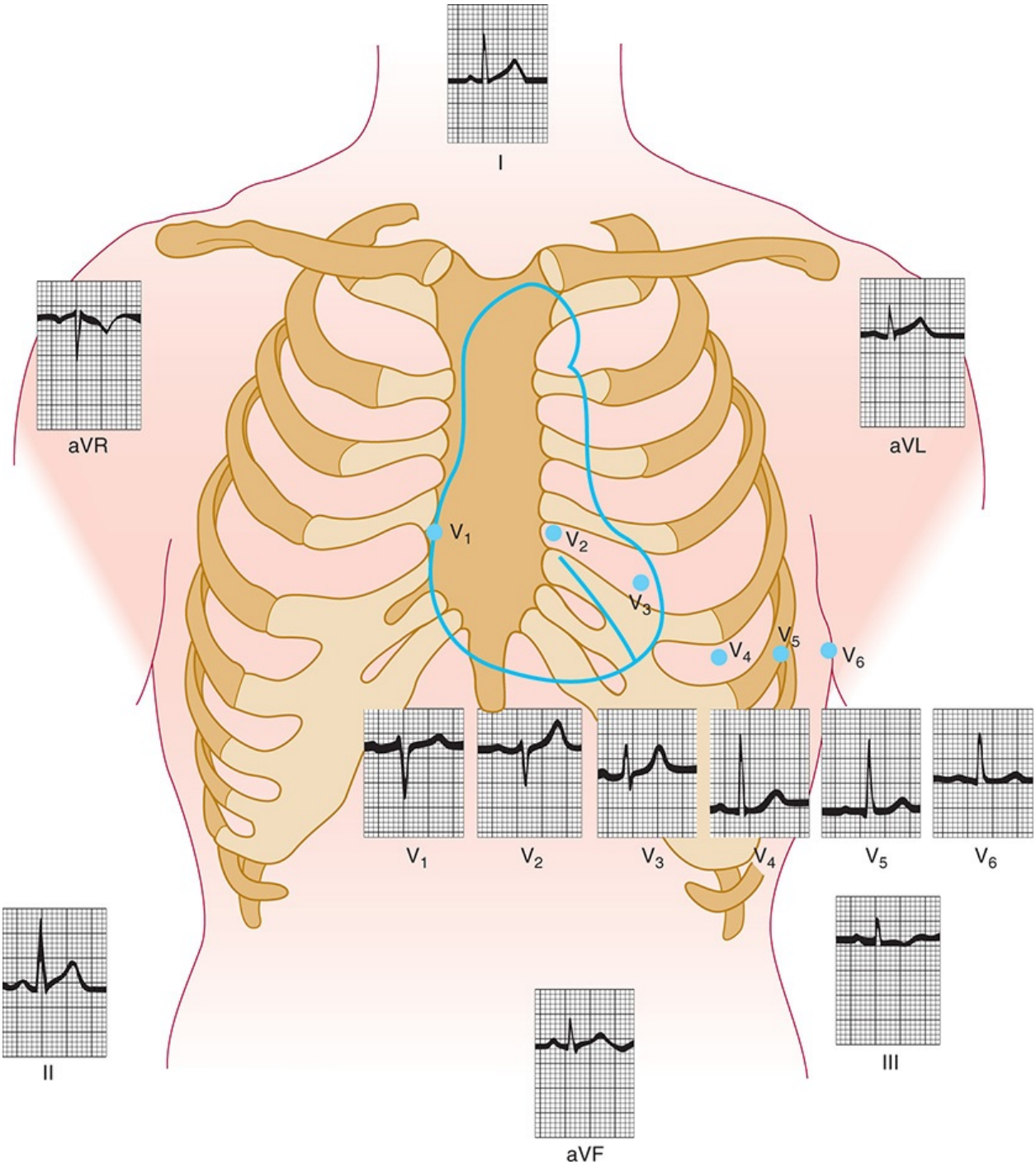


FIGURE 29–7 Normal ECG. Tracings from individual electrodes (positions marked in figure) are shown for a normal ECG. See text for additional details. ECG, electrocardiogram. (Reproduced with permission from Goldman MJ: *Principles of Clinical Electrocardiography*, 12th ed. Originally published by Appleton & Lange. Copyright © 1986 by McGraw-Hill.)

BIPOLAR LIMB LEADS & THE CARDIAC VECTOR

Because the standard limb leads are records of the potential differences between two points, the deflection in each lead at any instant indicates the magnitude and direction of the electromotive force generated in the heart in the axis of the lead **(cardiac vector** or **axis).** The vector at any given moment in the two dimensions of the frontal plane can be calculated from any two standard limb leads **(Figure 29–8)** if it is assumed that the three electrode locations form the points of an equilateral triangle (Einthoven triangle) and that the heart lies in the center of the triangle. These assumptions are not completely warranted, but calculated vectors are useful approximations. An approximate **mean QRS vector** (“electrical axis of the heart”) is often plotted by using the average QRS deflection in each lead, as shown in Figure 29–8. This is a **mean** vector as opposed to an **instantaneous** vector, and the average QRS deflections should be measured by integrating the QRS complexes. However, they can be approximated by measuring the net differences between the positive and negative peaks of the QRS. The normal direction of the mean QRS vector is generally said to be from −30 to +110° on the coordinate system shown in Figure 29–8. **Left** or **right axis deviation** is said to be present if the calculated axis falls to the left of −30° or to the right of +110°, respectively. Right axis deviation suggests right ventricular hypertrophy. Left axis deviation may be due to left ventricular hypertrophy, but there are better and more reliable electrocardiographic criteria for this condition.

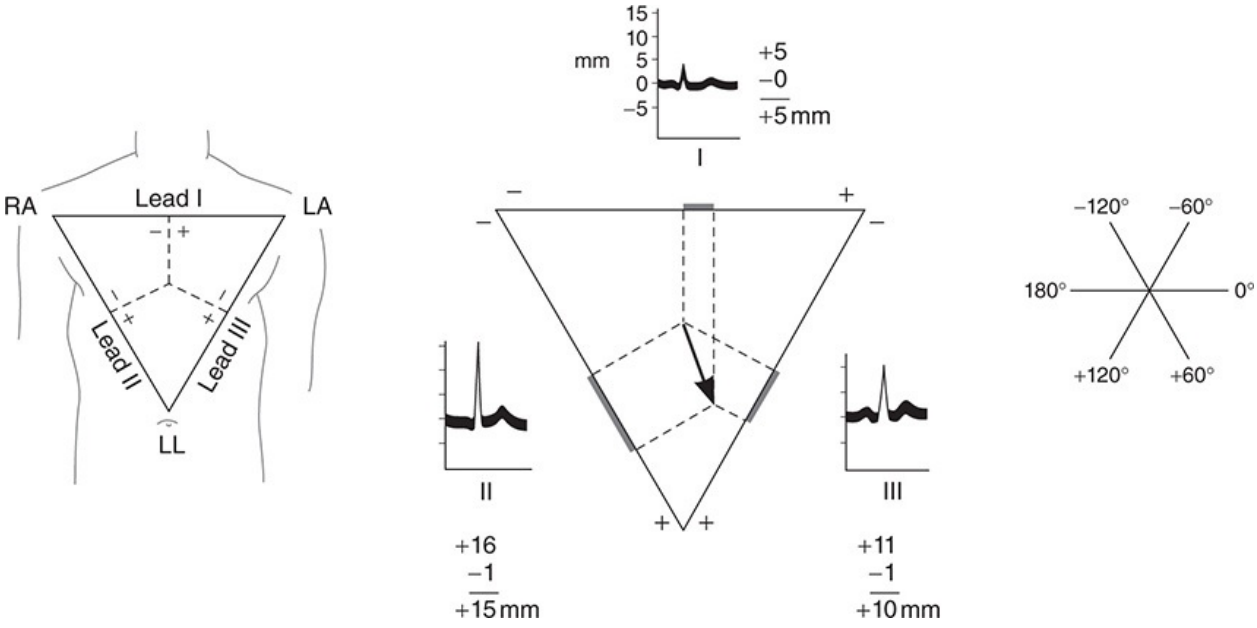


FIGURE 29–8 Cardiac vector. Left: Einthoven triangle. Perpendiculars dropped from the midpoints of the sides of the equilateral triangle intersect at the center of electrical activity. RA, right arm; LA, left arm; LL, left leg. **Center:** Calculation of mean QRS vector. In each lead, distances equal to the height of the R wave minus the height of the largest negative deflection in the QRS complex are measured off from the midpoint of the side of the triangle representing that lead. An arrow drawn from the center of electrical activity to the point of intersection of perpendiculars extended from the distances measured off on the sides represents the magnitude and direction of the mean QRS vector. **Right:** Reference axes for determining the direction of the vector.

HIS BUNDLE ELECTROGRAM

In patients with heart block, the electrical events in the AV node, bundle of His, and Purkinje system are frequently studied with a catheter containing an electrode at its tip that is passed through a vein to the right side of the heart and manipulated into a position close to the tricuspid valve. Three or more standard electrocardiographic leads are recorded simultaneously. The record of the electrical activity obtained with the catheter **(Figure 29–9)** is the **His bundle electrogram (HBE).** It normally shows an A deflection when the AV node is activated, an H spike during transmission through the His bundle, and a V deflection during ventricular depolarization. With the HBE and the standard electrocardiographic leads, it is possible to time three intervals accurately: (1) the PA interval, the time from the first appearance of atrial depolarization to the A wave in the HBE, which represents conduction time from the SA node to the AV node; (2) the AH interval, from the A wave to the start of the H spike, which represents the AV nodal conduction time; and (3) the HV interval, the time from the start of the H spike to the start of the QRS deflection in the ECG, which represents conduction in the bundle of His and the bundle branches. The approximate normal values for these intervals in adults are PA, 27 ms; AH, 92 ms; and HV, 43 ms. These values illustrate the relative slowness of conduction in the AV node.

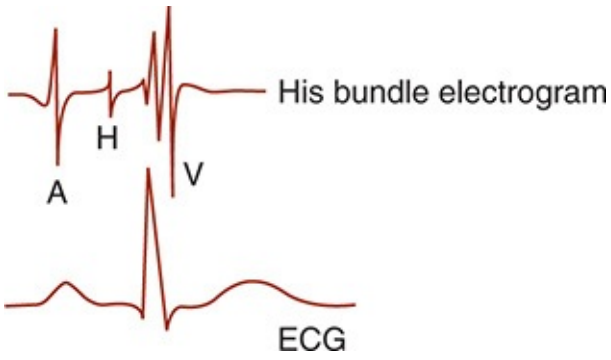


FIGURE 29–9 Normal His bundle electrogram (HBE) with simultaneously recorded ECG. An HBE recorded with an invasive electrode is superimposed on a standard ECG reading. Timing of depolarizations of the HBE are described in the text. ECG, electrocardiogram.

MONITORING

The ECG has long been used in patient care. In the past, it was often recorded continuously in hospital coronary care units, with alarms arranged to sound at the onset of life-threatening arrhythmias. Using a small portable tape recorder **(Holter monitor),** it is also possible to record the ECG in ambulatory individuals as they go about their normal activities. The recording is later played back at high speed and analyzed. Recordings obtained with monitors have proved valuable in the diagnosis of arrhythmias and in planning the treatment of patients recovering from myocardial infarctions. Currently, modern systems can be hooked up to individuals and obtain and store heart rhythm data over days to better evaluate long-term electrical activity.

CLINICAL APPLICATIONS: CARDIAC ARRHYTHMIAS

NORMAL CARDIAC RATE

In the normal human heart, each beat originates in the SA node **(normal sinus rhythm, NSR).** The heart beats about 70 times a minute at rest. The rate is slowed **(bradycardia)** during sleep and accelerated **(tachycardia)** by emotion, exercise, fever, and many other stimuli. In healthy young individuals breathing at a normal rate, the heart rate varies with the phases of respiration: It accelerates during inspiration and decelerates during expiration, especially if the depth of

breathing is increased. This **sinus arrhythmia** **(Figure 29–10)** is a normal phenomenon and is primarily due to fluctuations in parasympathetic output to the heart. During inspiration, impulses in the vagi from the stretch receptors in the lungs inhibit the cardio-inhibitory area in the medulla oblongata. The tonic vagal discharge that keeps the heart rate slow decreases, and the heart rate rises. Disease processes affecting the sinus node lead to marked bradycardia accompanied by dizziness and syncope **(Clinical Box 29–2)**.

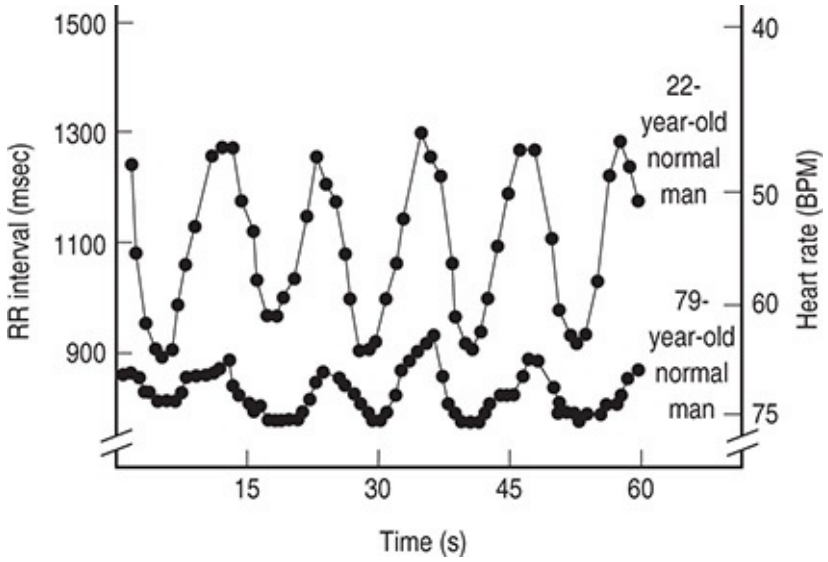


FIGURE 29–10 Sinus arrhythmia in a young man and an old man. Each subject breathed five times per minute. With each inspiration the RR interval (the interval between R waves) declined, indicating an increase in heart rate. Note the marked reduction in the magnitude of the arrhythmia in the older man. These records were obtained after β-adrenergic blockade but would have been generally similar in its absence. (Reproduced with permission from Pfeifer MA, Weinberg CR, Cook D, Best JD, Reenan A, Halter JB: Differential changes of autonomic nervous system function with age in man. Am J Med 1983;75:249.)

ABNORMAL PACEMAKERS

The AV node and other portions of the conduction system can, in abnormal situations, become the cardiac pacemaker. In addition, diseased atrial and ventricular muscle fibers can have their membrane potentials reduced and discharge repetitively.

As noted above, the discharge rate of the SA node is more rapid than that of the other parts of the conduction system, and this is why the SA node normally

controls the heart rate. When conduction from the atria to the ventricles is completely interrupted, **complete (third-degree) heart block** results, and the ventricles beat at a low rate **(idioventricular rhythm)** independently of the atria **(Figure 29–11)**. The block may be due to disease in the AV node **(AV nodal block)** or in the conducting system below the node **(infranodal block).** In patients with AV nodal block, the remaining nodal tissue becomes the pacemaker and the rate of the idioventricular rhythm is approximately 45 beats/min. In patients with infranodal block due to disease in the bundle of His, the ventricular pacemaker is located more peripherally in the conduction system and the ventricular rate is lower; it averages 35 beats/min, but in individual cases it can be as low as 15 beats/min. In such individuals, there may also be periods of asystole lasting a minute or more. The resultant cerebral ischemia causes dizziness and fainting **(Stokes–Adams syndrome).** Causes of third-degree heart block include septal myocardial infarction and damage to the bundle of His during surgical correction of congenital interventricular septal defects.

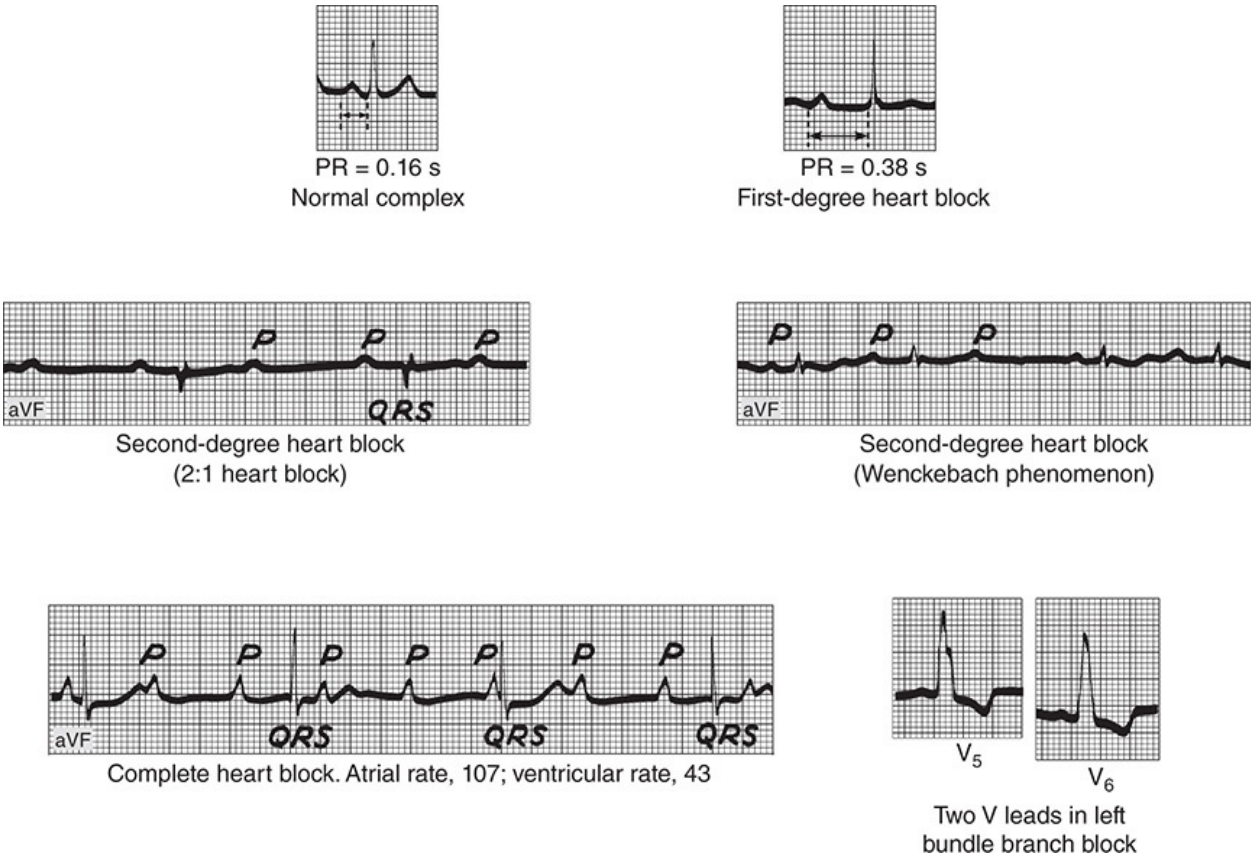


FIGURE 29–11 ECG with heart block. Individual traces that depict various forms of heart block are shown. When appropriate, unipolar leads are noted. See text for further details. ECG, electrocardiogram.

When conduction between the atria and ventricles is slowed but not completely interrupted, **incomplete heart block** is present. In the form called **first-degree heart block,** all the atrial impulses reach the ventricles but the PR interval is abnormally long. In the form called **second-degree heart block,** not all atrial impulses are conducted to the ventricles. For example, a ventricular beat may follow every second or every third atrial beat (2:1 block, 3:1 block, etc). In another form of incomplete heart block, there are repeated sequences of beats in which the PR interval lengthens progressively until a ventricular beat is dropped **(Wenckebach phenomenon)**. The PR interval of the cardiac cycle that follows each dropped beat is usually normal or only slightly prolonged (Figure 29–11).

CLINICAL BOX 29–2

Sick Sinus Syndrome

Sick sinus syndrome (bradycardia-tachycardia syndrome; sinus node dysfunction) is a collection of heart rhythm disorders that include **sinus bradycardia** (slow heart rates from the natural pacemaker of the heart), **tachycardias** (fast heart rates), and **bradycardia-tachycardia** (alternating slow and fast heart rhythms). Sick sinus syndrome is relatively uncommon and is usually found in people older than 50, in whom the cause is often a nonspecific, scar-like degeneration of the heart's conduction system. When found in younger people, especially in children, a common cause of sick sinus syndrome is heart surgery, especially on the upper chambers. Holter monitoring is an effective tool for diagnosing sick sinus syndrome because of the episodic nature of the disorder. Extremely slow heart rate and prolonged pauses may be seen during Holter monitoring, along with episodes of atrial tachycardias.

THERAPEUTIC HIGHLIGHTS

Treatment depends on the severity and type of disease. Tachycardias are frequently treated with medication. When there is marked bradycardia in patients with sick sinus syndrome or third-degree heart block, an electronic pacemaker is frequently implanted. These devices, which have become sophisticated and reliable, are useful in patients with sinus node dysfunction, AV block, and bifascicular or trifascicular block. They are useful also in patients with severe neurogenic syncope, in whom carotid sinus stimulation

produces pauses of more than 3 s between heartbeats.

Sometimes one branch of the bundle of His is interrupted, causing **right** or **left bundle branch block.** In bundle branch block, excitation passes normally down the bundle on the intact side and then sweeps back through the muscle to activate the ventricle on the blocked side. The ventricular rate is therefore normal, but the QRS complexes are prolonged and deformed (Figure 29–11). Block can also occur in the anterior or posterior fascicle of the left bundle branch, producing the condition called **hemiblock** or **fascicular block.** Left anterior hemiblock produces abnormal left axis deviation in the ECG, whereas left posterior hemiblock produces abnormal right axis deviation. It is not uncommon to find combinations of fascicular and branch blocks **(bifascicular** or **trifascicular block).** The HBE permits detailed analysis of the site of block when there is a defect in the conduction system.

ECTOPIC FOCI OF EXCITATION

Normally, myocardial cells do not discharge spontaneously, and the possibility of spontaneous discharge of the His bundle and Purkinje system is low because the normal pacemaker discharge of the SA node is more rapid than their rate of spontaneous discharge. However, in abnormal conditions, the His–Purkinje fibers or the myocardial fibers may discharge spontaneously. In these conditions, **increased automaticity** of the heart is said to be present. If an irritable **ectopic focus** discharges once, the result is a beat that occurs before the expected next normal beat and transiently interrupts the cardiac rhythm (atrial, nodal, or ventricular **extrasystole** or **premature beat**). If the focus discharges repetitively at a rate higher than that of the SA node, it produces rapid, regular tachycardia (atrial, ventricular, or nodal **paroxysmal tachycardia** or **atrial flutter**).

REENTRY

A more common cause of paroxysmal arrhythmias is a defect in conduction that permits a wave of excitation to propagate continuously within a closed circuit **(circus movement).** For example, if a transient block is present on one side of a portion of the conducting system, the impulse can go down the other side. If the block then wears off, the impulse may conduct in a retrograde direction in the

previously blocked side back to the origin and then descend again, establishing a circus movement. An example of this in a ring of tissue is shown in **Figure 29–12**. If the reentry is in the AV node, the reentrant activity depolarizes the atrium, and the resulting atrial beat is called an echo beat. In addition, the reentrant activity in the node propagates back down to the ventricle, producing paroxysmal nodal tachycardia. Circus movements can also become established in the atrial or ventricular muscle fibers. In individuals with an abnormal extra bundle of conducting tissue connecting the atria to the ventricles (bundle of Kent), the circus activity can pass in one direction through the AV node and in the other direction through the bundle, thus involving both the atria and the ventricles.

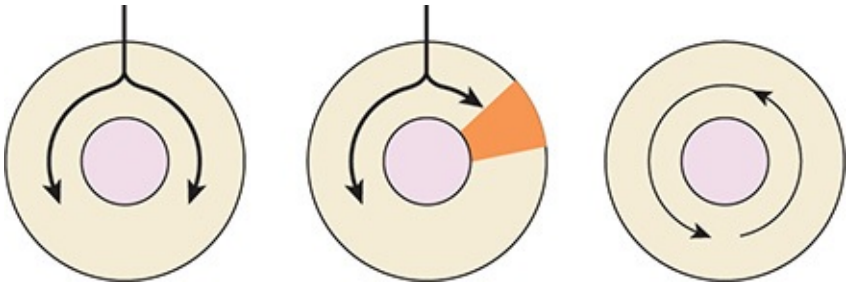


FIGURE 29–12 Depolarization of a ring of cardiac tissue. Normally, the impulse spreads in both directions in the ring **(left)** and the tissue immediately behind each branch of the impulse is refractory. When a transient block occurs on one side **(center),** the impulse on the other side goes around the ring, and if the transient block has now worn off **(right),** the impulse passes this area and continues to circle indefinitely (circus movement).

ATRIAL ARRHYTHMIAS

Excitation spreading from an independently discharging focus in the atria stimulates the AV node prematurely and is conducted to the ventricles. The P waves of atrial extrasystoles are abnormal, but the QRST configurations are usually normal **(Figure 29–13)**. The excitation may depolarize the SA node, which must repolarize and then depolarize to the firing level before it can initiate the next normal beat. Consequently, a pause occurs between the extrasystole and the next normal beat that is usually equal in length to the interval between the normal beats preceding the extrasystole, and the rhythm is “reset” (see below).

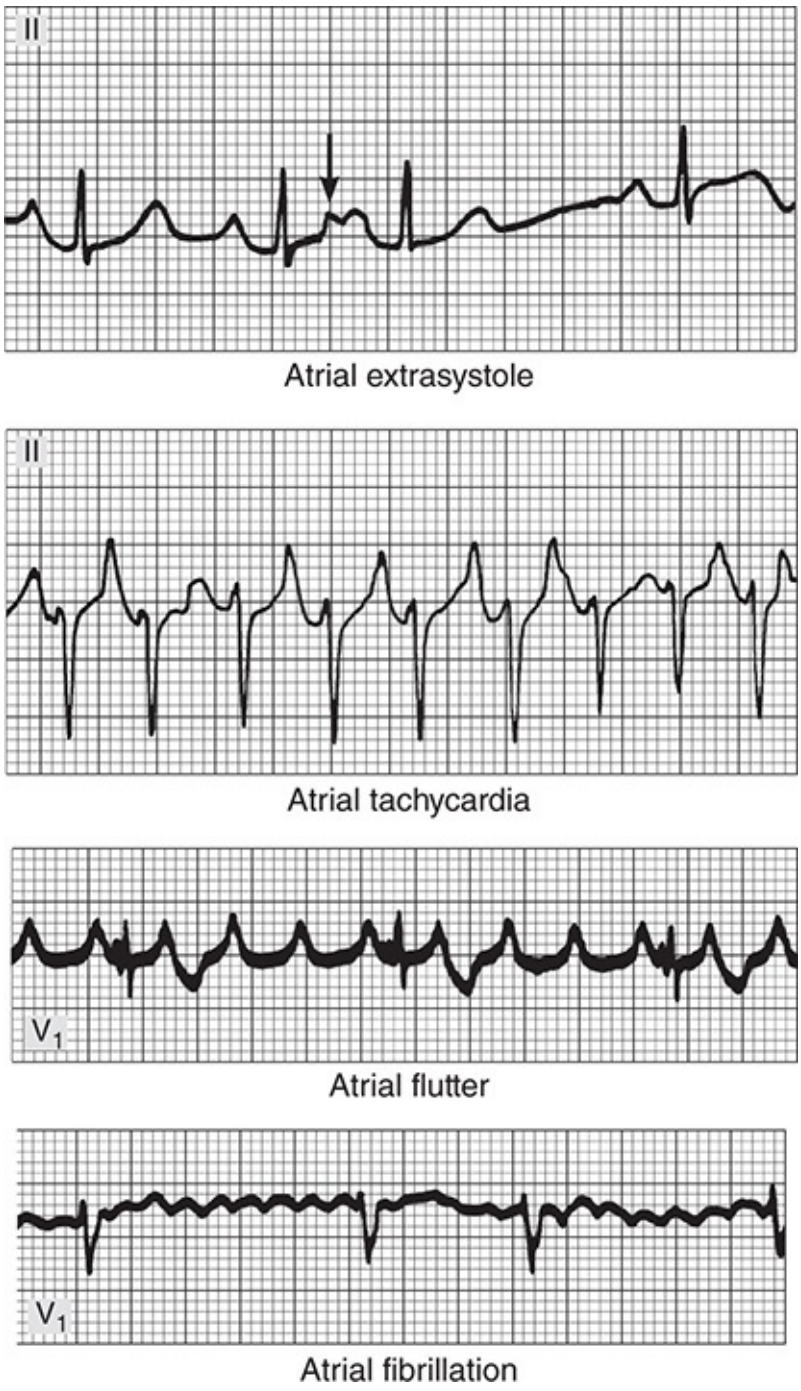


FIGURE 29–13 Atrial arrhythmias. The illustration shows an atrial premature beat with its P wave superimposed on the T wave of the preceding beat (*arrow*); atrial tachycardia; atrial flutter with 4:1 AV block; and atrial fibrillation with a totally irregular ventricular rate. Leads used to capture electrical activity are marked in each trace. AV, atrioventricular. (Tracings reproduced with permission from Goldschlager N, Goldman MJ: *Principles of Clinical Electrocardiography*, 13th ed. Originally published by Appleton & Lange. Copyright © 1989 by

McGraw-Hill.)

Atrial tachycardia occurs when an atrial focus discharges regularly or there is reentrant activity producing atrial rates up to 220/min. Sometimes, especially in digitalized patients, some degree of atrioventricular block is associated with the tachycardia **(paroxysmal atrial tachycardia with block).**

In atrial flutter, the atrial rate is 200–350/min (Figure 29–13). In the most common form of this arrhythmia, there is large counterclockwise circus movement in the right atrium. This produces a characteristic sawtooth pattern of flutter waves due to atrial contractions. It is almost always associated with 2:1 or greater AV block, because in adults the AV node cannot conduct more than about 230 impulses per minute.

In **atrial fibrillation,** the atria beat very rapidly (300–500/min) in a completely irregular and disorganized fashion. Because the AV node discharges at irregular intervals, the ventricles also beat at a completely irregular rate, usually 80–160/min (Figure 29–13). The condition can be paroxysmal or chronic, and in some cases there appears to be a genetic predisposition. The cause of atrial fibrillation is still a matter of debate, but in most cases it appears to be due to multiple concurrently circulating reentrant excitation waves in both atria. However, some cases of paroxysmal atrial fibrillation seem to be produced by discharge of one or more ectopic foci. Many of these foci appear to be located in the pulmonary veins as much as 4 cm from the heart. Atrial muscle fibers extend along the pulmonary veins and are the origin of these discharges.

CONSEQUENCES OF ATRIAL ARRHYTHMIAS

Occasional atrial extrasystoles occur from time to time in most normal humans and have no pathological significance. In paroxysmal atrial tachycardia and flutter, the ventricular rate may be so high that diastole is too short for adequate filling of the ventricles with blood between contractions. Consequently, cardiac output is reduced and symptoms of heart failure appear. Heart failure may also complicate atrial fibrillation when the ventricular rate is high. Acetylcholine liberated at vagal endings depresses conduction in the atrial musculature and AV node. This is why stimulating reflex vagal discharge by pressing on the eyeball **(oculocardiac reflex)** or massaging the carotid sinus often converts tachycardia and sometimes converts atrial flutter to normal sinus rhythm. Alternatively, vagal stimulation increases the degree of AV block, abruptly lowering the ventricular rate. Digitalis also depresses AV conduction and is used to lower a

rapid ventricular rate in atrial fibrillation.

VENTRICULAR ARRHYTHMIAS

Premature beats that originate in an ectopic ventricular focus usually have bizarrely shaped prolonged QRS complexes **(Figure 29–14)** because of the slow spread of the impulse from the focus through the ventricular muscle to the rest of the ventricle. They are usually incapable of exciting the bundle of His, and retrograde conduction to the atria therefore does not occur. In the meantime, the next succeeding normal SA nodal impulse depolarizes the atria. The P wave is usually buried in the QRS of the extrasystole. If the normal impulse reaches the ventricles, they are still in the refractory period following depolarization from the ectopic focus.

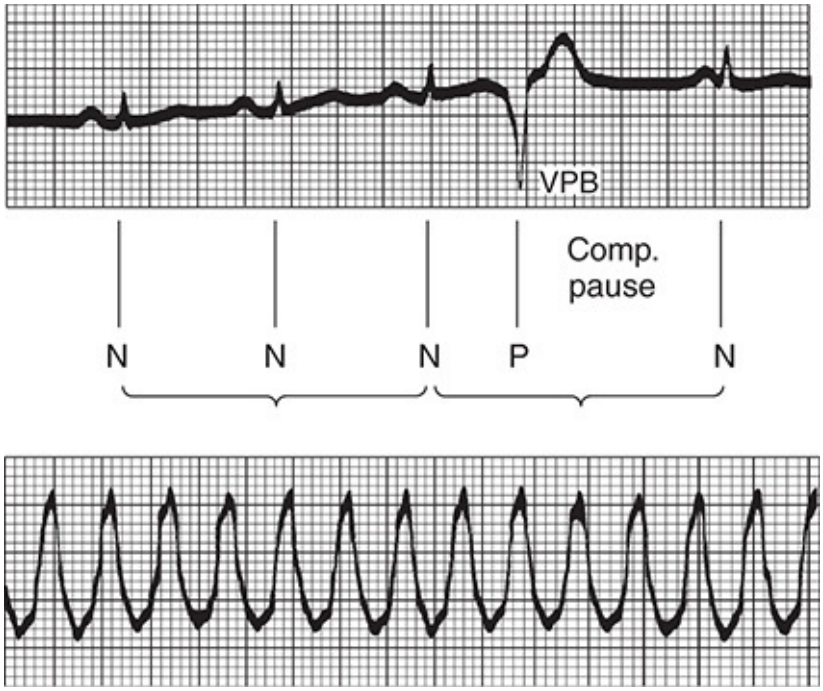


FIGURE 29–14 Top: Ventricular premature beats (VPB). The lines under the tracing illustrate the compensatory pause and show that the duration of the premature beat plus the preceding normal beat is equal to the duration of two normal beats. **Bottom:** Ventricular tachycardia.

However, the second succeeding impulse from the SA node produces a normal beat. Thus, ventricular premature beats are followed by a **compensatory pause** that is often longer than the pause after an atrial extrasystole.

Furthermore, ventricular premature beats do not interrupt the regular discharge of the SA node, whereas atrial premature beats often interrupt and “reset” the normal rhythm.

Atrial and ventricular premature beats are not strong enough to produce a pulse at the wrist if they occur early in diastole, when the ventricles have not had time to fill with blood and the ventricular musculature is still in its relatively refractory period. They may not even open the aortic and pulmonary valves, in which case there is, in addition, no second heart sound.

Paroxysmal ventricular tachycardia (Figure 29–14) is in effect a series of rapid, regular ventricular depolarizations usually due to a circus movement involving the ventricles. **Torsades de pointes** is a form of ventricular tachycardia in which the QRS morphology varies **(Figure 29–15)**. Tachycardias originating above the ventricles (supraventricular tachycardias such as paroxysmal nodal tachycardia) can be distinguished from paroxysmal ventricular tachycardia by use of the HBE; in supraventricular tachycardias, a His bundle H deflection is present, whereas in ventricular tachycardias, there is none. Ventricular premature beats are not uncommon and, in the absence of ischemic heart disease, usually benign. Ventricular tachycardia is more serious because cardiac output is decreased, and ventricular fibrillation is an occasional complication of ventricular tachycardia.

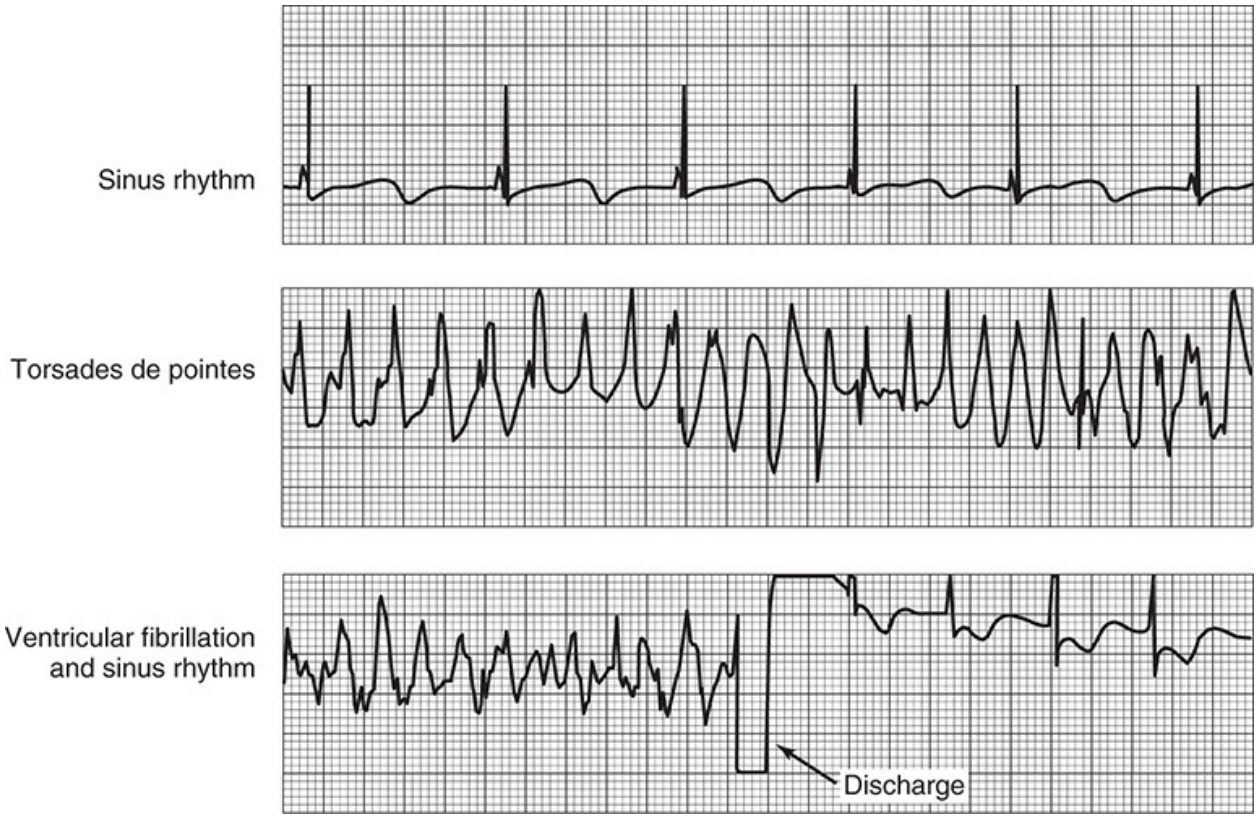


FIGURE 29–15 Record obtained from an implanted cardioverter–defibrillator in a 12-year-old boy with congenital long QT syndrome who collapsed while answering a question in school. Top: Normal sinus rhythm with long QT interval. **Middle:** Torsades de pointes. **Bottom:** Ventricular fibrillation with discharge of defibrillator, as programmed 7.5 s after the start of ventricular tachycardia, converting the heart to normal sinus rhythm. The boy recovered consciousness in 2 min and had no neurologic sequelae. (Reproduced with permission from Moss AJ, Daubert JP: Images in clinical medicine. Internal ventricular fibrillation. N Engl J Med 2000;342:398.)

In **ventricular fibrillation** (Figure 29–15), the ventricular muscle fibers contract in a totally irregular and ineffective way because of the very rapid discharge of multiple ventricular ectopic foci or a circus movement. The fibrillating ventricles, like the fibrillating atria, look like a quivering “bag of worms.” Ventricular fibrillation can be produced by an electric shock or an extrasystole during a critical interval, the **vulnerable period.** The vulnerable period coincides in time with the midportion of the T wave; that is, it occurs at a time when some of the ventricular myocardium is depolarized, some is incompletely repolarized, and some is completely repolarized. These are excellent conditions in which to establish reentry and a circus movement. The

fibrillating ventricles cannot pump blood effectively, and circulation of the blood stops. Therefore, in the absence of emergency treatment, ventricular fibrillation that lasts more than a few minutes is fatal. The most frequent cause of sudden death in patients with myocardial infarcts is ventricular fibrillation.

LONG QT SYNDROME

An indication of vulnerability of the heart during repolarization is the fact that in patients in whom the QT interval is prolonged, cardiac repolarization is irregular and the incidence of ventricular arrhythmias and sudden death increases. The syndrome can be caused by a number of different drugs, by electrolyte abnormalities, and by myocardial ischemia. It can also be congenital. Mutations of eight different genes have been reported to cause the syndrome. Six cause reduced function of various K^+ channels by alterations in their structure; one inhibits a K^+ channel by reducing the amount of the ankyrin isoform that links it to the cytoskeleton; and one increases the function of the cardiac Na^+ channel. Long QT syndrome is discussed in Clinical Box 5–5.

ACCELERATED AV CONDUCTION

An interesting condition seen in some otherwise normal individuals who are prone to attacks of paroxysmal atrial arrhythmias is **accelerated AV conduction (Wolff-Parkinson-White syndrome).** Normally, the only conducting pathway between the atria and the ventricles is the AV node. Individuals with Wolff-Parkinson-White syndrome have an additional aberrant muscular or nodal tissue connection **(bundle of Kent)** between the atria and ventricles. This conducts more rapidly than the slowly conducting AV node, and one ventricle is excited early. The manifestations of its activation merge with the normal QRS pattern, producing a short PR interval and a prolonged QRS deflection slurred on the upstroke **(Figure 29–16)**, with a normal interval between the start of the P wave and the end of the QRS complex (“PJ interval”). The paroxysmal atrial tachycardias seen in this syndrome often follow an atrial premature beat. This beat conducts normally down the AV node but spreads to the ventricular end of the aberrant bundle, and the impulse is transmitted retrograde to the atrium. A circus movement is thus established. Less commonly, an atrial premature beat finds the AV node refractory but reaches the ventricles via the bundle of Kent, setting up a circus movement in which the impulse passes from the ventricles to

the atria via the AV node.

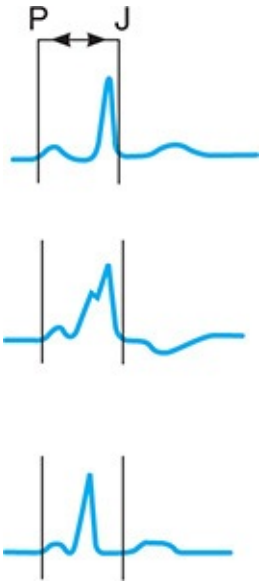


FIGURE 29–16 Accelerated AV conduction. Top: Normal sinus beat. **Middle:** Short PR interval; wide, slurred QRS complex; normal PJ interval (Wolff-Parkinson-White syndrome). **Bottom:** Short PR interval, normal QRS complex (Lown-Ganong-Levine syndrome). (Reproduced with permission from Goldschlager N, Goldman MJ: *Principles of Clinical Electrocardiography*, 13th ed. Originally published by Appleton & Lange. Copyright © 1989 by McGraw-Hill.)

In some instances, the Wolff-Parkinson-White syndrome is familial. In two such families, there is a mutation in a gene that codes for an AMP-activated protein kinase. Presumably, this kinase is normally involved in suppressing abnormal atrioventricular pathways during fetal development.

Attacks of paroxysmal supraventricular tachycardia, usually nodal tachycardia, are seen in individuals with short PR intervals and normal QRS complexes **(Lown-Ganong-Levine syndrome).** In this condition, depolarization presumably passes from the atria to the ventricles via an aberrant bundle that bypasses the AV node but enters the intraventricular conducting system distal to the node.

TREATMENTS FOR ARRHYTHMIAS

Many different drugs used in the treatment of arrhythmias slow conduction in

the conduction system and the myocardium. This depresses ectopic activity and reduces the discrepancy between normal and reentrant paths so that reentry does not occur. Drugs that target Na^+ channels (eg, quinidine) can slow I_{Na} and prolong refractoriness (eg, quinidine, disopy-ramide), decrease I_{Na} with minimal prolongation of refractoriness (eg, flecainide, propafenone) or shorten refractoriness in depolarized cells (eg, lidocaine, mexiletine). Drugs that target K^+ channels can prolong refractoriness (eg, amiodarone, sotalol, dofetilide). Drugs that block L-type voltage-gated Ca^{2+} channels can slow SA pacemaker and AV conduction (eg, nifedipine, verapamil, diltiazem). Finally, drugs that block β-adrenergic receptors thus reduce the activation of I_{CaL} (eg, propanolol, metoprolol). Interestingly, it has become clear that in some patients any of these drugs can be **proarrhythmic** rather than antiarrhythmic—that is, they can also cause various arrhythmias. Therefore, careful monitoring and alternative procedures are extremely important when using antiarrhythmic drugs.

An alternative treatment is radiofrequency catheter ablation of reentrant pathways. Catheters with electrodes at the tip can be inserted into the chambers of the heart and its environs and used to map the exact location of an ectopic focus or accessory bundle responsible for the production of reentry and supraventricular tachycardia. The pathway can then be ablated by passing radiofrequency current with the catheter tip placed close to the bundle or focus. In skilled hands, this form of treatment can be very effective and is associated with few complications. It is particularly useful in conditions that cause supraventricular tachycardias, including Wolff-Parkinson-White syndrome and atrial flutter. It has also been used with success to ablate foci in the pulmonary veins causing paroxysmal atrial fibrillation.

ELECTROCARDIOGRAPHIC FINDINGS IN OTHER CARDIAC & SYSTEMIC DISEASES

MYOCARDIAL INFARCTION

When the blood supply to part of the myocardium is interrupted, profound changes take place in the myocardium that lead to irreversible changes and death of muscle cells. The ECG is very useful for diagnosing ischemia and locating areas of infarction. The underlying electrical events and the resulting electrocardiographic changes are complex, and only a brief review can be presented here.

The three major abnormalities that cause electrocardiographic changes in acute myocardial infarction are summarized in **Table 29–3**. The first change—abnormally rapid repolarization after discharge of the infarcted muscle fibers as a result of accelerated opening of K^+ channels—develops seconds after occlusion of a coronary artery in experimental animals. It lasts only a few minutes, but before it is over the resting membrane potential of the infarcted fibers declines because of the loss of intracellular K^+. Starting about 30 min later, the infarcted fibers also begin to depolarize more slowly than the surrounding normal fibers.

TABLE 29–3 Summary of the three major abnormalities of membrane polarization associated with acute myocardial infarction.

Defect in Infarcted Cells	Current Flow	Resultant ECG Change in Leads over Infarct
Rapid repolarization	Out of infarct	ST segment elevation
Decreased resting membrane potential	Into infarct	TQ segment depression (manifested as ST segment elevation)
Delayed depolarization	Out of infarct	ST segment elevation

All three of these changes cause current flow that produces elevation of the ST segment in electrocardiographic leads recorded with electrodes over the infarcted area **(Figure 29–17)**. Because of the rapid repolarization in the infarct, the membrane potential of the area is greater than it is in the normal area during the latter part of repolarization, making the normal region negative relative to the infarct. Extracellularly, current therefore flows out of the infarct into the normal area (since, by convention, current flow is from positive to negative). This current flows toward electrodes over the injured area, causing increased positivity between the S and T waves of the ECG. Similarly, the delayed depolarization of the infarcted cells causes the infarcted area to be positive relative to the healthy tissue (Table 29–3) during the early part of repolarization, and the result is also ST segment elevation. The remaining change—the decline in resting membrane potential during diastole—causes a current flow into the infarct during ventricular diastole. The result of this current flow is a depression

of the TQ segment of the ECG. However, the electronic arrangement in electrocardiographic recorders is such that a TQ segment depression is recorded as an ST segment elevation. Thus, the hallmark of acute myocardial infarction is elevation of the ST segments in the leads overlying the area of infarction (Figure 29–17). Leads on the opposite side of the heart show ST segment depression.

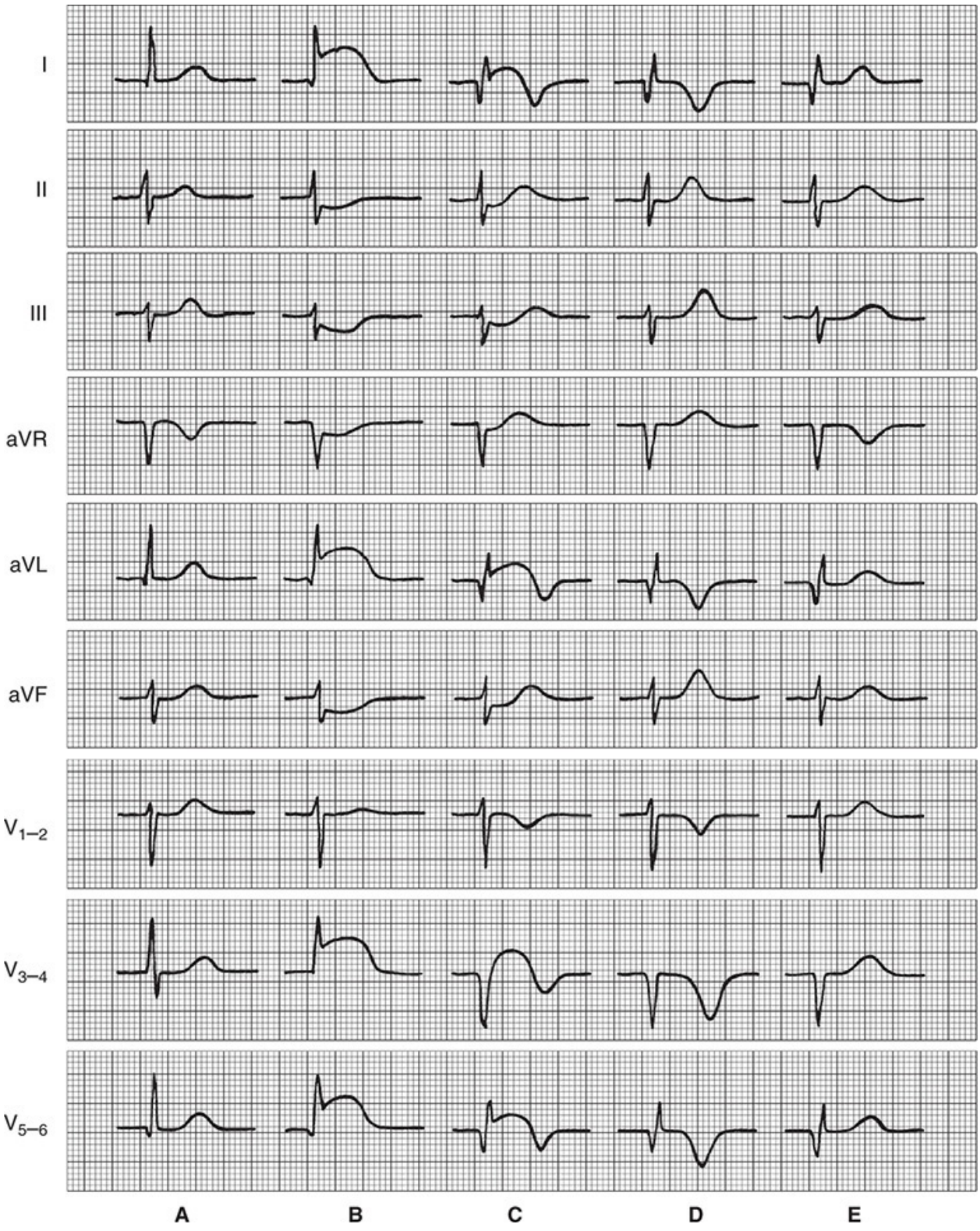


FIGURE 29–17 Diagrammatic illustration of serial electrocardiographic patterns in anterior infarction. A) Normal tracing. **B)** Very early pattern (hours

after infarction): ST segment elevation in I, aVL, and $V_{3–6}$; reciprocal ST depression in II, III, and aVF. **C)** Later pattern (many hours to a few days): Q waves have appeared in I, aVL, and $V_{5–6}$. QS complexes are present in $V_{3–4}$. This indicates that the major transmural infarction is underlying the area recorded by $V_{3–4}$; ST segment changes persist but are of lesser degree, and the T waves are beginning to invert in the leads in which the ST segments are elevated. **D)** Late established pattern (many days to weeks): The Q waves and QS complexes persist, the ST segments are isoelectric, and the T waves are symmetric and deeply inverted in leads that had ST elevation and tall in leads that had ST depression. This pattern may persist for the remainder of the patient's life. **E)** Very late pattern: This may occur many months to years after the infarction. The abnormal Q waves and QS complexes persist. The T waves have gradually returned to normal. (Reproduced with permission from Goldschlager N, Goldman MJ: *Principles of Clinical Electrocardiography,* 13th ed. Originally published by Appleton & Lange. Copyright © 1989 by McGraw-Hill.)

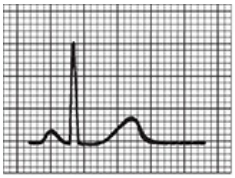
After some days or weeks, the ST segment abnormalities subside. The dead muscle and scar tissue become electrically silent. The infarcted area is therefore negative relative to the normal myocardium during systole, and it fails to contribute its share of positivity to the electrocardiographic complexes. The manifestations of this negativity are multiple and subtle. Common changes include the appearance of a Q wave in some of the leads in which it was not previously present and an increase in the size of the normal Q wave in some of the other leads, although so-called non–Q-wave infarcts are also seen. These latter infarcts tend to be less severe, but there is a high incidence of subsequent reinfarction. Another finding in infarction of the anterior left ventricle is "failure of progression of the R wave;" that is, the R wave fails to become successively larger in the precordial leads as the electrode is moved from right to left over the left ventricle. If the septum is infarcted, the conduction system may be damaged, causing bundle branch block or other forms of heart block.

Myocardial infarctions are often complicated by serious ventricular arrhythmias, with the threat of ventricular fibrillation and death. In experimental animals, and presumably in humans, ventricular arrhythmias occur during three periods. During the first 30 min of an infarction, arrhythmias due to reentry are common. There follows a period relatively free from arrhythmias but, starting 12 h after infarction, arrhythmias occur as a result of increased automaticity. Arrhythmias occurring 3 days to several weeks after infarction are once again

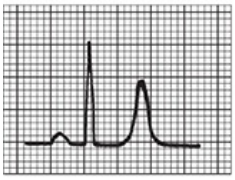
usually due to reentry. It is worth noting in this regard that infarcts that damage the epicardial portions of the myocardium interrupt sympathetic nerve fibers, producing denervation super-sensitivity to catecholamines in the area beyond the infarct. Alternatively, endocardial lesions can selectively interrupt vagal fibers, leaving the actions of sympathetic fibers unopposed.

EFFECTS OF CHANGES IN THE IONIC COMPOSITION OF THE BLOOD

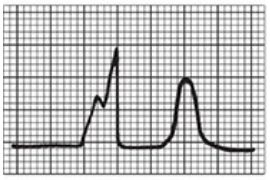
Changes in the Na^+ and K^+ concentrations of the extracellular fluids would be expected to affect the potentials of the myocardial fibers because the electrical activity of the heart depends on the distribution of these ions across the muscle cell membranes. Clinically, a fall in the plasma level of Na^+ may be associated with low-voltage electrocardiographic complexes, but changes in the plasma K^+ level produce severe cardiac abnormalities. Hyperkalemia is a very dangerous and potentially lethal condition because of its effects on the heart. As the plasma K^+ level rises, the first change in the ECG is the appearance of tall peaked T waves, a manifestation of altered repolarization **(Figure 29–18)**. At higher K^+ levels, paralysis of the atria and prolongation of the QRS complexes occur. Ventricular arrhythmias may develop. The resting membrane potential of the muscle fibers decreases as the extracellular K^+ concentration increases. The fibers eventually become unexcitable, and the heart stops in diastole. Conversely, a decrease in the plasma K^+ level causes prolongation of the PR interval, prominent U waves, and, occasionally, late T wave inversion in the precardial leads. If the T and U waves merge, the apparent QT interval is often prolonged; if the T and U waves are separated, the true QT interval is seen to be of normal duration. Hypokalemia is a serious condition, but it is not as rapidly fatal as hyperkalemia.



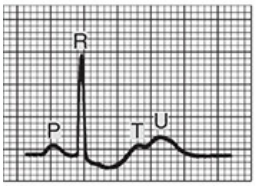
Normal tracing (plasma K^+ 4–5.5 mEq/L). PR interval = 0.16 s; QRS interval = 0.06 s; QT interval = 0.4 s (normal for an assumed heart rate of 60).



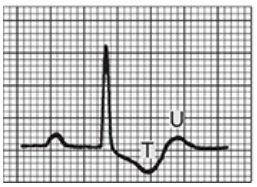
Hyperkalemia (plasma K^+ ±7.0 mEq/L). The PR and QRS intervals are within normal limits. Very tall, slender peaked T waves are now present.



Hyperkalemia (plasma K^+ ±8.5 mEq/L). There is no evidence of atrial activity; the QRS complex is broad and slurred and the QRS interval has widened to 0.2 s. The T waves remain tall and slender. Further elevation of the plasma K^+ level may result in ventricular tachycardia and ventricular fibrillation.



Hypokalemia (plasma K^+ ±3.5 mEq/L). PR interval = 0.2 s; QRS interval = 0.06 s; ST segment depression. A prominent U wave is now present immediately following the T. The actual QT interval remains 0.4 s. If the U wave is erroneously considered a part of the T, a falsely prolonged QT interval of 0.6 s will be measured.



Hypokalemia (plasma K^+ ±2.5 mEq/L). The PR interval is lengthened to 0.32 s; the ST segment is depressed; the T wave is inverted; a prominent U wave is seen. The true QT interval remains normal.

FIGURE 29–18 Correlation of plasma K^+ level and the ECG, assuming that the plasma Ca^{2+} level is normal. The diagrammed complexes are left ventricular epicardial leads. ECG, electrocardiogram. (Reproduced with permission from Goldman MJ: *Principles of Clinical Electrocardiography*, 12th ed. Originally published by Appleton & Lange. Copyright © 1986 by McGraw-Hill.)

Increases in extracellular Ca^{2+} concentration enhance myocardial contractility. When large amounts of Ca^{2+} are infused into experimental animals, the heart relaxes less during diastole and eventually stops in systole **(calcium rigor).** However, in clinical conditions associated with hypercalcemia, the plasma calcium level is rarely if ever high enough to affect the heart. Hypocalcemia causes prolongation of the ST segment and consequently of the QT interval, a change that is also produced by phenothiazines and tricyclic antidepressant drugs and by various diseases of the central nervous system.

CHAPTER SUMMARY

- Contractions in the heart are controlled via a well-regulated electrical signaling cascade that originates in pacemaker cells in the sinoatrial (SA) node and is passed via internodal atrial pathways to the atrioventricular (AV) node, the bundle of His, the Purkinje system, and to all parts of the ventricle.
- Most cardiac cells have an action potential that includes a rapid depolarization, an initial rapid repolarization, a plateau, and a slow repolarization process to return to resting potential. These changes are defined by sequential activation, inactivation and deactivation of Na^+, Ca^{2+}, and K^+ channels.
- Compared to typical myocytes, pacemaker cells have a slightly different sequence of events. After repolarization to the resting potential, there is a slow depolarization that occurs due to a channel that can pass both Na^+ and K^+. As this "funny" current continues to depolarize the cell the activation threshold of the voltage-gated Ca^{2+} channels, Ca^{2+} channels are activated to rapidly depolarize the cell. The hyperpolarization phase is again dominated by K^+ current.
- Spread of the electrical signal from cell to cell is via gap junctions. The rate of spread is dependent on anatomic features, but also can be altered (to a certain extent) via neural input.
- The electrocardiogram (ECG or EKG) is an algebraic sum of the electrical activity in the heart. The normal ECG includes well-defined waves and segments, including the P wave (atrial depolarization), the QRS complex (ventricular depolarization), and the T wave (ventricular repolarization). Various arrhythmias can be detected in irregular ECG recordings.
- Because of the contribution of ionic movement to cardiac muscle contraction, heart tissue is sensitive to ionic composition of the blood. Most serious are increases in $[K^+]$ that can produce severe cardiac abnormalities, including paralysis of the atria and ventricular arrhythmias.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. Which part of the ECG (eg, Figure 29–5) corresponds to ventricular

repolarization?

A. The P wave

B. The QRS duration

C. The T wave

D. The U wave

E. The PR interval

2. Which of the following normally has a slowly depolarizing “prepotential”?

A. Sinoatrial node

B. Atrial muscle cells

C. Bundle of His

D. Purkinje fibers

E. Ventricular muscle cells

3. In second-degree heart block

A. the ventricular rate is lower than the atrial rate.

B. the ventricular ECG complexes are distorted.

C. there is a high incidence of ventricular tachycardia.

D. stroke volume is decreased.

E. cardiac output is increased.

4. Currents caused by opening of which of the following channels contribute to the repolarization phase of the action potential of ventricular muscle fibers?

A. Na^+ channels

B. Cl^- channels

C. Ca^{2+} channels

D. K^+ channels

E. HCO^{3-} channels

5. In complete heart block

A. fainting may occur because the atria are unable to pump blood into the ventricles.

B. ventricular fibrillation is common.

C. the atrial rate is lower than the ventricular rate.

D. fainting may occur because of prolonged periods during which the ventricles fail to contract.

CHAPTER 30

The Heart as a Pump

OBJECTIVES

After studying this chapter, you should be able to:

- Describe how the sequential pattern of contraction and relaxation in the heart results in a normal pattern of blood flow.
- Understand the pressure, volume, and flow changes that occur during the cardiac cycle.
- Explain the basis of the arterial pulse, heart sounds, and murmurs.
- Understand methods that can be used to measure cardiac output.
- Describe how the pumping action of the heart can be compromised in the setting of specific disease states such as heart failure and shock.
- Delineate the ways by which cardiac output can be upregulated in the setting of specific physiologic demands for increased oxygen supply to the tissues, such as exercise, and how the heart's own demand for oxygen is adjusted.

INTRODUCTION

Of course, the electrical activity of the heart discussed in the previous chapter is

designed to subserve the heart's primary physiologic role—to pump blood through the lungs, where gas exchange can occur, and thence to the remainder of the body. This is accomplished when the orderly depolarization process described in the previous chapter triggers a wave of contraction that spreads through the myocardium. In single muscle fibers, contraction starts just after depolarization and lasts until about 50 ms after repolarization is completed (see Figure 5–15). Atrial systole starts after the P wave of the electrocardiogram (ECG); ventricular systole starts near the end of the R wave and ends just after the T wave. This chapter will cover the ways in which these changes in contraction produce sequential changes in pressures and flows in the heart chambers and blood vessels, thereby propelling blood appropriately as needed by whole body demands for oxygen and nutrients. As an aside, it should be noted that the term **systolic pressure** in the vascular system refers to the peak pressure reached during systole, not the mean pressure; similarly, the **diastolic pressure** refers to the lowest pressure during diastole.

MECHANICAL EVENTS OF THE CARDIAC CYCLE

EVENTS IN LATE DIASTOLE

Late in diastole, the mitral (bicuspid) and tricuspid valves between the atria and ventricles (atrioventricular [AV] valves) are open and the aortic and pulmonary valves are closed. Blood flows into the heart throughout diastole, filling the atria and ventricles. The rate of filling declines as the ventricles become distended and, especially when the heart rate is low, the cusps of the AV valves drift toward the closed position **(Figure 30–1)**. The pressure in the ventricles remains low. About 70% of the ventricular filling occurs passively during diastole.

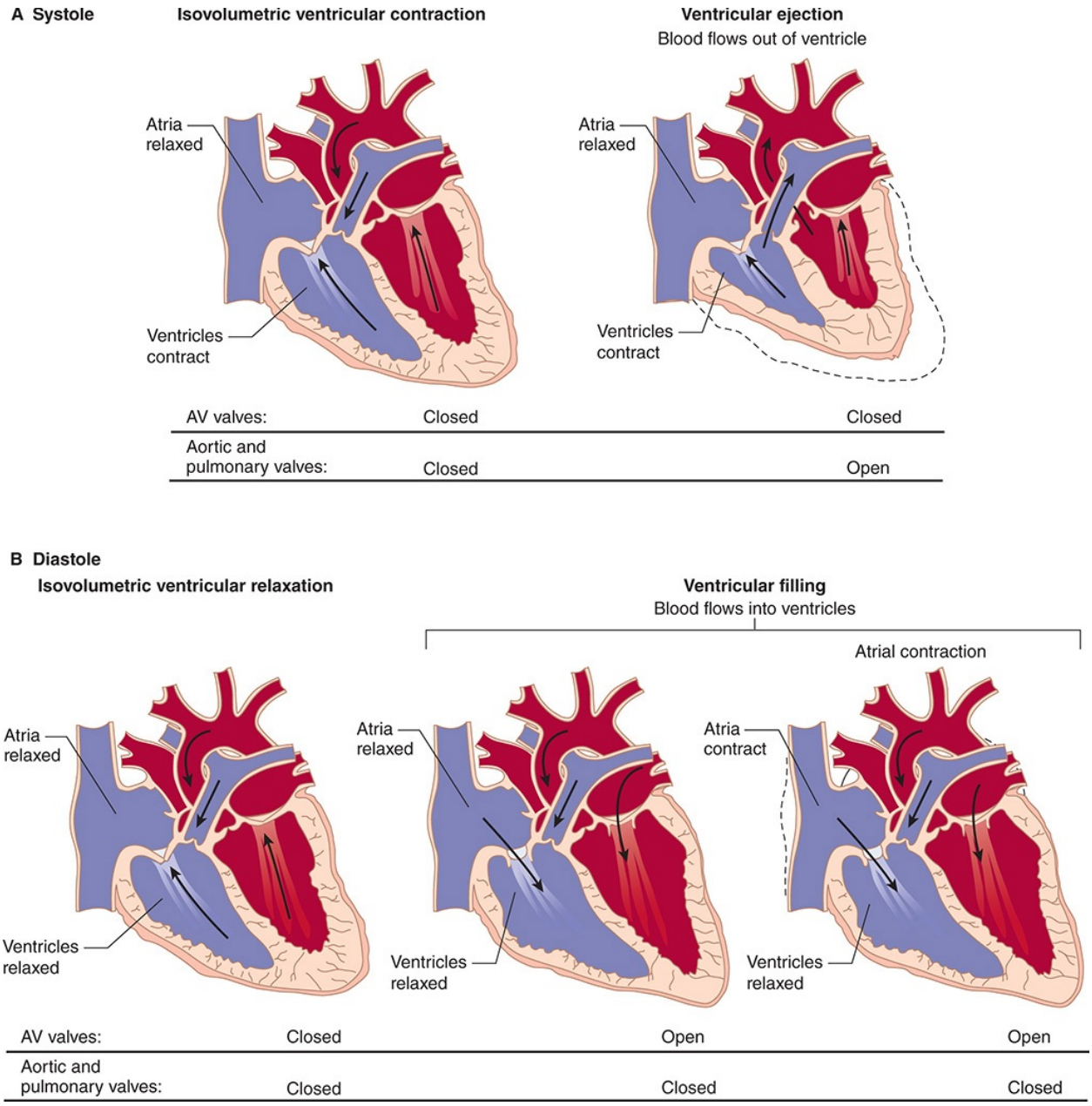


FIGURE 30–1 Divisions of the cardiac cycle: A) systole and B) diastole. The phases of the cycle are identical in both halves of the heart. The direction in which the pressure difference favors flow is denoted by an arrow; note, however, that flow will not actually occur if a valve prevents it. AV, atrioventricular. Note that the rightmost panel of those shown in B corresponds to atrial systole, even though the cardiac cycle overall remains in diastole.

ATRIAL SYSTOLE

Contraction of the atria propels some additional blood into the ventricles. Contraction of the atrial muscle narrows the orifices of the superior and inferior vena cava and pulmonary veins, and the inertia of the blood moving toward the heart tends to keep blood in it. However, despite these inhibitory influences, there is some regurgitation of blood into the veins.

VENTRICULAR SYSTOLE

At the start of ventricular systole, the AV valves close. Ventricular muscle initially shortens relatively little, but intraventricular pressure rises sharply as the myocardium presses on the blood in the ventricle **(Figure 30–2)**. This period of **isovolumetric (isovolumic, isometric) ventricular contraction** lasts about 0.05 s, until the pressures in the left and right ventricles exceed the pressures in the aorta (80 mm Hg; 10.6 kPa) and pulmonary artery (10 mm Hg) and the aortic and pulmonary valves open. During isovolumetric contraction, the AV valves bulge into the atria, causing a small but sharp rise in atrial pressure **(Figure 30–3)**.

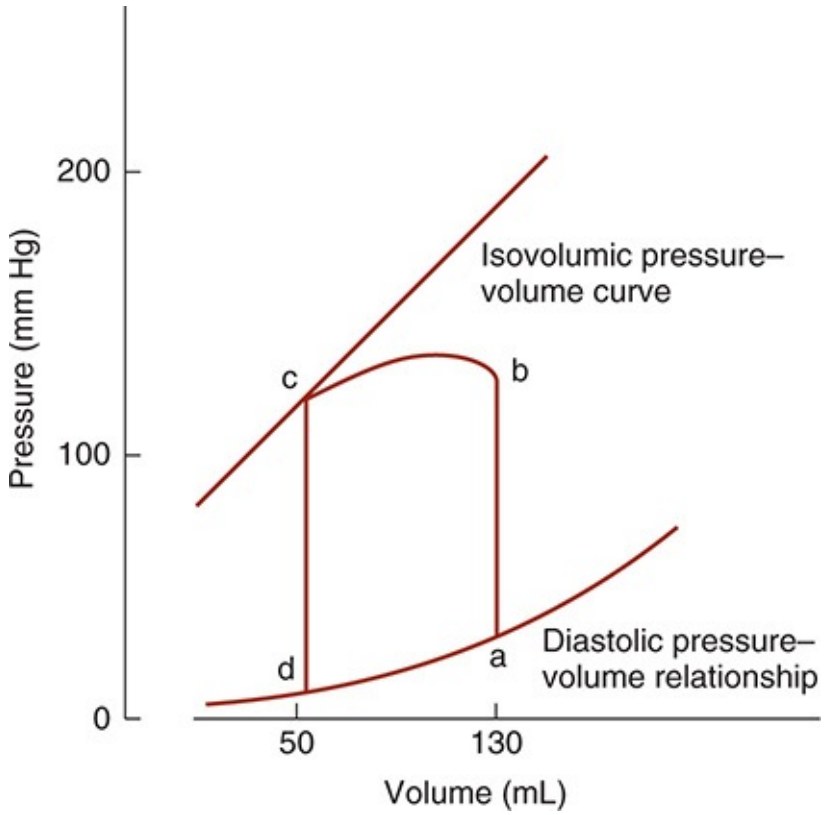


FIGURE 30–2 Normal pressure–volume loop of the left ventricle. During

diastole, the ventricle fills and pressure increases from d to a. Pressure then rises sharply from a to b during isovolumetric contraction and from b to c during ventricular ejection. At c, the aortic valves close and pressure falls during isovolumetric relaxation from c back to d. (Reproduced with permission from McPhee SJ, Lingappa VR, Ganong WF [editors]: *Pathophysiology of Disease*, 6th ed. New York, NY: McGraw-Hill; 2010.)

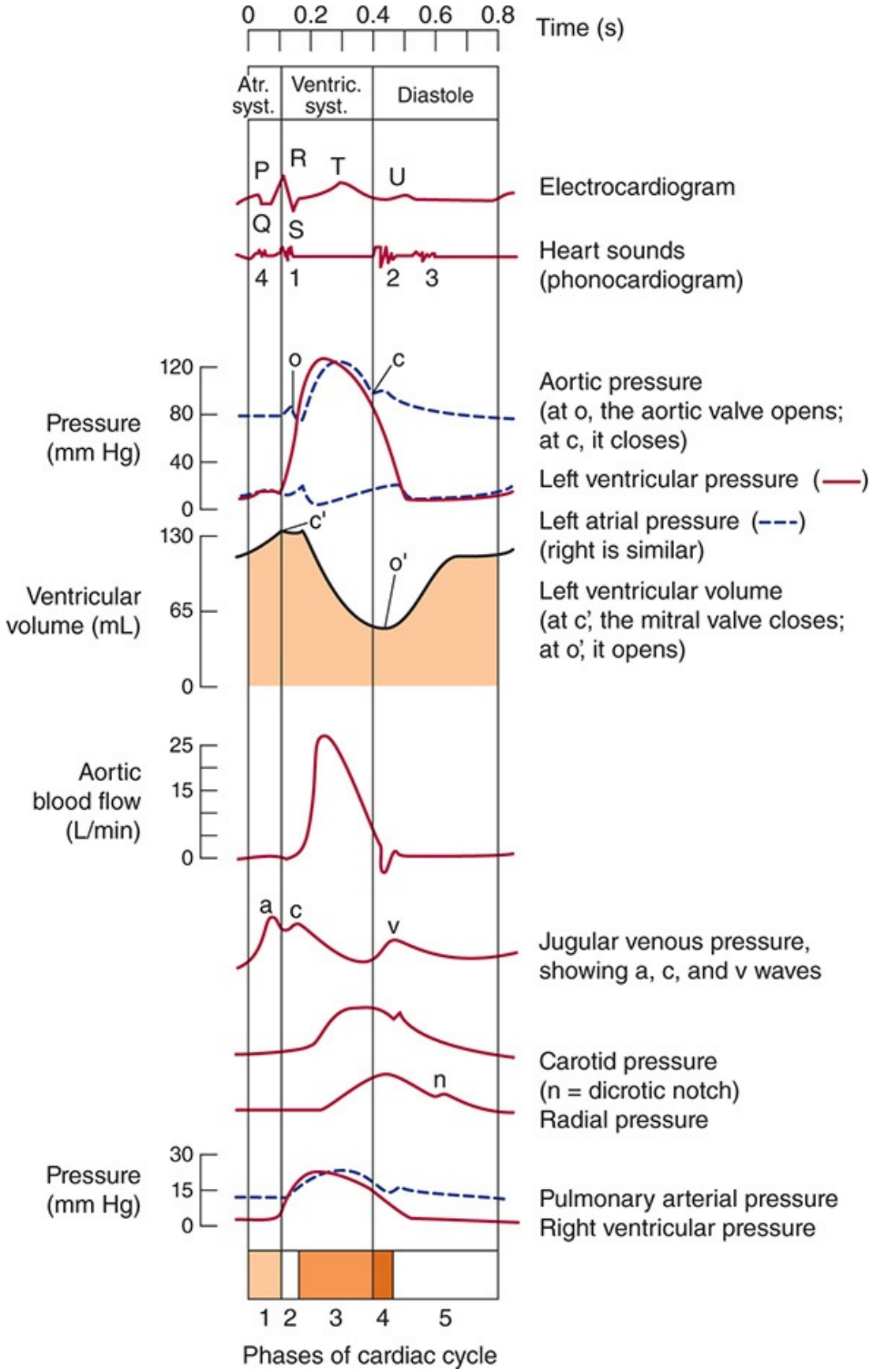

0
0.2
0.4
0.6
0.8
Time (s)
Atr. syst.
Ventric. syst.
Diastole
P
R
T
U
Q
S
Electrocardiogram
4
1
2
3
Heart sounds (phonocardiogram)
Pressure (mm Hg)
120
80
40
0
o
c
Aortic pressure (at o, the aortic valve opens; at c, it closes)
Left ventricular pressure (——)
Left atrial pressure (- - -) (right is similar)
Ventricular volume (mL)
130
65
0
c'
o'
Left ventricular volume (at c', the mitral valve closes; at o', it opens)
Aortic blood flow (L/min)
25
15
0
a
c
v
Jugular venous pressure, showing a, c, and v waves
n
Carotid pressure (n = dicrotic notch)
Radial pressure
Pressure (mm Hg)
30
15
0
Pulmonary arterial pressure
Right ventricular pressure
1 2 3 4 5
Phases of cardiac cycle

FIGURE 30–3 Events of the cardiac cycle at a heart rate of 75 beats/min. The phases of the cardiac cycle identified by the numbers at the bottom are as follows: 1, atrial systole; 2, isovolumetric ventricular contraction; 3, ventricular ejection; 4, isovolumetric ventricular relaxation; 5, ventricular filling. Note that late in systole, aortic pressure actually exceeds left ventricular pressure. However, the momentum of the blood keeps it flowing out of the ventricle for a short period. The pressure relationships in the right ventricle and pulmonary artery are similar.

When the aortic and pulmonary valves open, the phase of **ventricular ejection** begins. Ejection is rapid at first, slowing down as systole progresses. The intraventricular pressure rises to a maximum and then declines somewhat before ventricular systole ends. Peak pressures in the left and right ventricles are about 120 and 25 mm Hg, respectively. Late in systole, pressure in the aorta actually exceeds that in the left ventricle, but for a short period momentum keeps the blood moving forward. The AV valves are pulled down by the contractions of the ventricular muscle, and atrial pressure drops. The amount of blood ejected by each ventricle per stroke at rest is 70–90 mL. The **end-diastolic ventricular volume** is about 130 mL. Thus, about 50 mL of blood remains in each ventricle at the end of systole **(end-systolic ventricular volume),** and the **ejection fraction,** the percentage of the end-diastolic ventricular volume that is ejected with each stroke, is about 65%. The ejection fraction is a valuable index of ventricular function. It can be measured by injecting radionuclide-labeled red blood cells and imaging the cardiac blood pool at the end of diastole and the end of systole (equilibrium radionuclide angiocardiography), or by computed tomography.

EARLY DIASTOLE

Once the ventricular muscle is fully contracted, the already falling ventricular pressures drop more rapidly. This is the period of **protodiastole,** which lasts about 0.04 s. It ends when the momentum of the ejected blood is overcome and the aortic and pulmonary valves close, setting up transient vibrations in the blood and blood vessel walls. After the valves are closed, pressure continues to drop rapidly during the period of **isovolumetric ventricular relaxation.** Isovolumetric relaxation ends when the ventricular pressure falls below the atrial pressure and the AV valves open, permitting the ventricles to fill. Filling is rapid

at first, then slows as the next cardiac contraction approaches. Atrial pressure continues to rise after the end of ventricular systole until the AV valves open, then drops and slowly rises again until the next atrial systole.

TIMING

Although events on the two sides of the heart are similar, they are somewhat asynchronous. Right atrial systole precedes left atrial systole, whereas contraction of the right ventricle starts after that of the left (see Chapter 29). However, since pulmonary arterial pressure is lower than aortic pressure, right ventricular ejection begins before that of the left. During expiration, the pulmonary and aortic valves close at the same time; but during inspiration, the aortic valve closes slightly before the pulmonary valve. The slower closure of the pulmonary valve is due to lower impedance of the pulmonary vascular tree. When measured over a period of minutes, the outputs of the two ventricles are, of course, equal, but transient differences in output during the respiratory cycle occur in normal individuals.

LENGTH OF SYSTOLE & DIASTOLE

Cardiac muscle has the unique property of contracting and repolarizing faster when the heart rate is high (see Chapter 5), and the duration of systole decreases from 0.27 s at a heart rate of 65 beats/min to 0.16 s at a rate of 200 beats/min **(Table 30–1)**. The reduced time interval is mainly due to a decrease in the duration of systolic ejection. However, the duration of systole is much more fixed than that of diastole, so when the heart rate is increased, diastole is shortened to a much greater degree. For example, at a heart rate of 65 beats/min, the duration of diastole is 0.62 s, whereas at a heart rate of 200 beats/min, it is only 0.14 s. This fact has important physiologic and clinical implications. It is during diastole that the heart muscle rests, and coronary blood flow to the subendocardial portions of the left ventricle also can only occur during diastole (see Chapter 33). Furthermore, most of the ventricular filling occurs in diastole. At heart rates up to about 180 beats/min, filling is adequate as long as there is ample venous return, and cardiac output per minute is increased by an increase in rate. However, at very high heart rates, filling may be compromised to such a degree that cardiac output per minute falls.

TABLE 30–1 Variation in length of action potential and associated phenomena with cardiac rate.[a]

	Heart Rate 75/min	Heart Rate 200/min	Skeletal Muscle
Duration, each cardiac cycle	0.80	0.30	…
Duration of systole	0.27	0.16	…
Duration of action potential	0.25	0.15	0.007
Duration of absolute refractory period	0.20	0.13	0.004
Duration of relative refractory period	0.05	0.02	0.003
Duration of diastole	0.53	0.14	…

[a]All values are in seconds.

Used with permission of AC Barger and GS Richardson.

Because it has a prolonged action potential, cardiac muscle cannot contract in response to a second stimulus until near the end of the initial contraction (see Figure 5–16). Therefore, cardiac muscle cannot be tetanized like skeletal muscle. The highest rate at which the ventricles can contract is theoretically about 400/min, but in adults the AV node will not conduct more than about 230 impulses/min because of its long refractory period. A ventricular contraction rate of more than 230/min is seen only in paroxysmal ventricular tachycardia (see Chapter 29).

Exact measurement of the duration of isovolumetric ventricular contraction is difficult in clinical situations, but it is relatively easy to measure the duration of **total electromechanical systole (QS2),** the **preejection period (PEP),** and the **left ventricular ejection time (LVET)** by recording the ECG, phonocardiogram, and carotid pulse simultaneously. QS_2 is the period from the onset of the QRS complex to the closure of the aortic valves, as determined by the onset of the second heart sound. LVET is the period from the beginning of the carotid pressure rise to the dicrotic notch (discussed later). PEP is the difference between QS_2 and LVET and represents the time for the electrical as well as the mechanical events that precede systolic ejection. The ratio PEP/LVET is normally about 0.35, and it increases without a change in QS_2

when left ventricular performance is compromised in a variety of cardiac diseases.

ARTERIAL PULSE

The blood forced into the aorta during systole not only moves the blood in the vessels forward but also sets up a pressure wave that travels along the arteries. The pressure wave expands the arterial walls as it travels, and the expansion is palpable as the **pulse.** The rate at which the wave travels, which is independent of and much higher than the velocity of blood flow, is about 4 m/s in the aorta, 8 m/s in the large arteries, and 16 m/s in the small arteries of young adults. Consequently, the pulse is felt in the radial artery at the wrist about 0.1 s after the peak of systolic ejection into the aorta (Figure 30–3). With advancing age, the arteries become more rigid, and the pulse wave moves faster.

The strength of the pulse is determined by the pulse pressure and bears little relation to the mean pressure. The pulse is weak ("thready") in shock. It is strong when stroke volume is large; for example, during exercise or after the administration of histamine. When the pulse pressure is high, the pulse waves may be large enough to be felt or even heard by the individual (palpitation, "pounding heart"). When the aortic valve is incompetent (aortic regurgitation), the pulse is particularly strong, and the force of systolic ejection may be sufficient to make the head nod with each heartbeat. The pulse in aortic regurgitation is called a **Corrigan** or **water-hammer pulse.**

The **dicrotic notch,** a small oscillation in the falling phase of the pulse wave caused by vibrations set up when the aortic valve snaps shut (Figure 30–3), is visible if the pressure wave is recorded but is not palpable at the wrist. The pulmonary artery pressure curve also has a dicrotic notch produced by the closure of the pulmonary valves.

ATRIAL PRESSURE CHANGES & THE JUGULAR PULSE

Atrial pressure rises during atrial systole and continues to rise during isovolumetric ventricular contraction when the AV valves bulge into the atria. When the AV valves are pulled down by the contracting ventricular muscle, pressure falls rapidly and then rises as blood flows into the atria until the AV valves open early in diastole. The return of the AV valves to their relaxed

position also contributes to this pressure rise by reducing atrial capacity. The atrial pressure changes are transmitted to the great veins, producing three characteristic waves in the record of jugular pressure (Figure 30–3). The **a wave** is due to atrial systole. As noted above, some blood regurgitates into the great veins when the atria contract. In addition, venous inflow stops, and the resultant rise in venous pressure contributes to the **a wave**. The **c wave** is the transmitted manifestation of the rise in atrial pressure produced by the bulging of the tricuspid valve into the atria during isovolumetric ventricular contraction. The **v wave** mirrors the rise in atrial pressure before the tricuspid valve opens during diastole. The jugular pulse waves are superimposed on the respiratory fluctuations in venous pressure. Venous pressure falls during inspiration as a result of the increased negative intrathoracic pressure and rises again during expiration.

HEART SOUNDS

Two sounds are normally heard through a stethoscope during each cardiac cycle. The first is a low, slightly prolonged “lub” **(first sound),** caused by vibrations set up by the sudden closure of the AV valves at the start of ventricular systole (Figure 30–3). The second is a shorter, high-pitched “dup” **(second sound),** caused by vibrations associated with closure of the aortic and pulmonary valves just after the end of ventricular systole. A soft, low-pitched **third sound** is heard about one-third of the way through diastole in many normal young individuals. It coincides with the period of rapid ventricular filling and is probably due to vibrations set up by the inrush of blood. A **fourth sound** can sometimes be heard immediately before the first sound when atrial pressure is high or the ventricle is stiff in conditions such as ventricular hypertrophy. It is due to ventricular filling and is rarely heard in normal adults.

The first sound has a duration of about 0.15 s and a frequency of 25–45 Hz. It is soft when the heart rate is low, because the ventricles are well filled with blood and the leaflets of the AV valves float together before systole. The second sound lasts about 0.12 s, with a frequency of 50 Hz. It is loud and sharp when the diastolic pressure in the aorta or pulmonary artery is elevated, causing the respective valves to shut briskly at the end of systole. The interval between aortic and pulmonary valve closure during inspiration is frequently long enough for the second sound to be reduplicated (physiologic splitting of the second sound). Splitting also occurs in various diseases. The third sound, when present, has a duration of 0.1 s.

MURMURS

Murmurs, or **bruits,** are abnormal sounds heard in various parts of the vascular system. The two terms are used interchangeably, though "murmur" is more commonly used to denote noise heard over the heart than over blood vessels. As discussed in detail in Chapter 31, blood flow is laminar, nonturbulent, and silent up to a critical velocity; above this velocity (such as beyond an obstruction), blood flow is turbulent and creates sounds. Blood flow speeds up when an artery or a heart valve is narrowed.

Examples of vascular sounds outside the heart are the bruit heard over a large, highly vascular goiter, the bruit heard over a carotid artery when its lumen is narrowed and distorted by atherosclerosis, and the murmurs heard over an aneurysmal dilation of one of the large arteries, an arteriovenous (A-V) fistula, or a patent ductus arteriosus.

The major—but certainly not the only—cause of cardiac murmurs is disease of the heart valves. When the orifice of a valve is narrowed **(stenosis),** blood flow through it is accelerated and turbulent. When a valve is incompetent, blood flows through it backward **(regurgitation),** again through a narrow orifice that accelerates flow. The timing (systolic or diastolic) of a murmur due to any particular valve **(Table 30–2)** can be predicted from a knowledge of the mechanical events of the cardiac cycle. Murmurs due to disease of a particular valve can generally be heard best when the stethoscope is directly over the valve. There are also other aspects of the duration, character, accentuation, and transmission of the sound that help locate its origin in one valve or another. Most murmurs can be heard only with the aid of the stethoscope, but one of the loudest murmurs is that produced when blood flows backward in diastole through a hole in a cusp of the aortic valve. This high-pitched musical diastolic murmur is sometimes audible to the unaided ear several feet from the patient.

TABLE 30–2 Heart murmurs.

Valve	Abnormality	Timing of Murmur
Aortic or pulmonary	Stenosis	Systolic
	Regurgitation	Diastolic
Mitral or tricuspid	Stenosis	Diastolic
	Regurgitation	Systolic

In patients with congenital interventricular septal defects, flow from the left to the right ventricle causes a systolic murmur. Soft murmurs may also be heard in patients with interatrial septal defects, although they are not a constant finding.

Soft systolic murmurs are also common in individuals, especially children, who have no cardiac disease. Systolic murmurs are also heard in anemic patients as a result of the low viscosity of the blood and associated rapid flow (see Chapter 31).

ECHOCARDIOGRAPHY

Wall movement and other aspects of cardiac function can be evaluated by the noninvasive technique of **echocardiography.** Pulses of ultrasonic waves are emitted from a transducer that also functions as a receiver to detect waves reflected back from various parts of the heart. Reflections occur wherever acoustic impedance changes, and a recording of the echoes displayed against time on an oscilloscope provides a record of the movements of the ventricular wall, septum, and valves during the cardiac cycle. When combined with Doppler techniques, echocardiography can be used to measure velocity and volume of flow through valves. It has considerable clinical usefulness, particularly in evaluating and planning therapy in patients with valvular lesions.

CARDIAC OUTPUT

METHODS OF MEASUREMENT

In experimental animals, cardiac output can be measured with an electromagnetic flow meter placed on the ascending aorta. Two methods of measuring output that are applicable to humans, in addition to Doppler combined with echocardiography, are the **direct Fick's method** and the **indicator dilution method.**

The **Fick's principle** states that the amount of a substance taken up by an organ (or by the whole body) per unit of time is equal to the arterial level of the substance minus the venous level **(A-V difference)** times the blood flow. This principle can be applied, of course, only in situations in which the arterial blood is the sole source of the substance taken up. The principle can be used to determine cardiac output by measuring the amount of O_2 consumed by the body

in a given period and dividing this value by the A-V difference across the lungs. Because systemic arterial blood has effectively the same O_2 content in all parts of the body, the arterial O_2 content can be measured in a sample obtained from any convenient artery. A sample of venous blood in the pulmonary artery is obtained by means of a cardiac catheter. It has now become commonplace to insert a long catheter through a forearm vein and to guide its tip into the heart with the aid of a fluoroscope. Catheters can thus be inserted through the right atrium and ventricle into the small branches of the pulmonary artery. An example of the calculation of cardiac output using a typical set of values is as follows:

$$\text{Output of left ventricle}$$

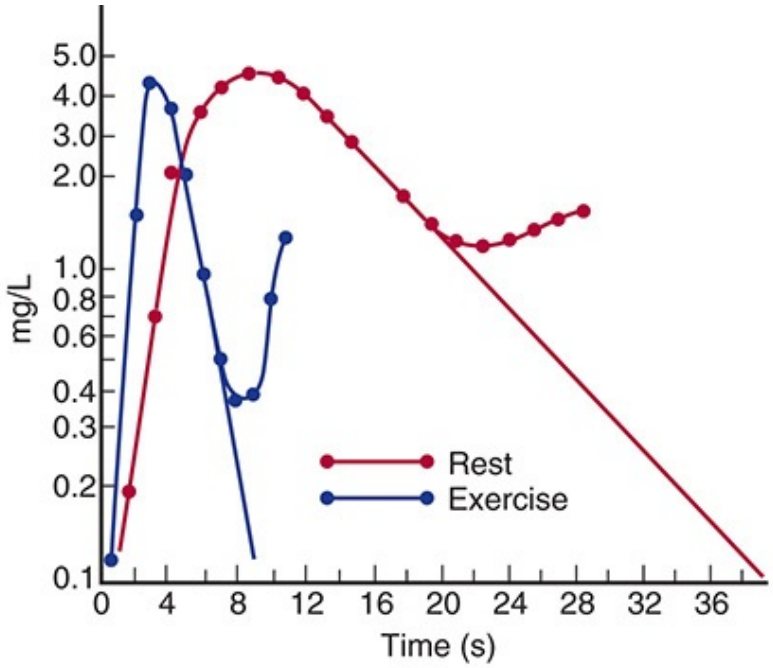
$$= \frac{O_2 \text{ consumption (mL/min)}}{[A_{O_2}] - [V_{O_2}]}$$

$$= \frac{250 \text{ mL/min}}{190 \text{ mL/L arterial blood} - 140 \text{ mL/L venous blood in pulmonary artery}}$$

$$= \frac{250 \text{ mL/min}}{50 \text{ mL/L}}$$

$$= 5 \text{ L/min}$$

In the indicator dilution technique, a known amount of a substance such as a dye or, more commonly, a radioactive isotope is injected into an arm vein and the concentration of the indicator in serial samples of arterial blood is determined. The output of the heart is equal to the amount of indicator injected divided by its average concentration in arterial blood after a single circulation through the heart **(Figure 30–4)**. The indicator must, of course, be a substance that stays in the bloodstream during the test and has no harmful or hemodynamic effects. In practice, the log of the indicator concentration in the serial arterial samples is plotted against time as the concentration rises, falls, and then rises again as the indicator recirculates. The initial decline in concentration, linear on a semilog plot, is extrapolated to the abscissa, giving the time for first passage of the indicator through the circulation. The cardiac output for that period is calculated (Figure 30–4) and then converted to output per minute.



$$F = \frac{E}{\int_0^{\alpha} C\,dt}$$

F = flow
E = amount of indicator injected
C = instantaneous concentration of indicator in arterial blood

In the **rest** example above,

$$\text{Flow in 39 s (time of first passage)} = \frac{\text{5 mg injection}}{\text{1.6 mg/L (avg concentration)}}$$

Flow = 3.1 L in 39 s

$$\text{Flow (cardiac output)/min} = 3.1 \times \frac{60}{39} = 4.7 \text{ L}$$

For the **exercise** example,

$$\text{Flow in 9 s} = \frac{5 \text{ mg}}{1.51 \text{ mg/L}} = 3.3 \text{ L}$$

$$\text{Flow/min} = 3.3 \times \frac{60}{9} = 22.0 \text{ L}$$

FIGURE 30–4 Determination of cardiac output by indicator (dye) dilution. Two examples are shown—at rest and during exercise.

A popular indicator dilution technique is **thermodilution,** in which the indicator used is cold saline. The saline is injected into the right atrium through one channel of a double-lumen catheter, and the temperature change in the blood is recorded in the pulmonary artery, using a thermistor in the other, longer side of the catheter. The temperature change is inversely proportional to the amount of blood flowing through the pulmonary artery; that is, to the extent that the cold saline is diluted by blood. This technique has two important advantages: (1) the saline is completely innocuous and (2) the cold is dissipated in the tissues so recirculation is not a problem, and it is easy to make repeated determinations.

CARDIAC OUTPUT IN VARIOUS CONDITIONS

The amount of blood pumped out of the heart per beat, the **stroke volume,** is about 70 mL from each ventricle in a resting man of average size in the supine position. The output of the heart per unit of time is the **cardiac output.** In a resting, supine man, it averages about 5.0 L/min (70 mL × 72 beats/min). There is a correlation between resting cardiac output and body surface area. The output per minute per square meter of body surface (the **cardiac index**) averages 3.2 L. The effects of various conditions on cardiac output are summarized in **Table 30–3**.

TABLE 30–3 Effect of various conditions on cardiac output.

	Condition or Factor[a]
No change	Sleep
	Moderate changes in environmental temperature
Increase	Anxiety and excitement (50–100%)
	Eating (30%)
	Exercise (up to 700%)
	High environmental temperature
	Pregnancy
	Epinephrine
Decrease	Sitting or standing from lying position (20–30%)
	Rapid arrhythmias
	Heart disease

[a]Approximate percentage changes are shown in parentheses.

FACTORS CONTROLLING CARDIAC OUTPUT

Predictably, changes in cardiac output that are called for by physiologic conditions can be produced by changes in cardiac rate, or stroke volume, or both **(Figure 30–5)**. The cardiac rate is controlled primarily by the autonomic nerves, with sympathetic stimulation increasing the rate and parasympathetic stimulation decreasing it (see Chapter 29). Stroke volume is also determined in part by neural input, with sympathetic stimuli making the myocardial muscle fibers contract with greater strength at any given length and parasympathetic stimuli having the opposite effect. When the strength of contraction increases without an increase in fiber length, more of the blood that normally remains in the ventricles is expelled; that is, the ejection fraction increases. The cardiac accelerator action of the catecholamines liberated by sympathetic stimulation is referred to as their **chronotropic action,** whereas their effect on the strength of cardiac contraction is called their **inotropic action.**

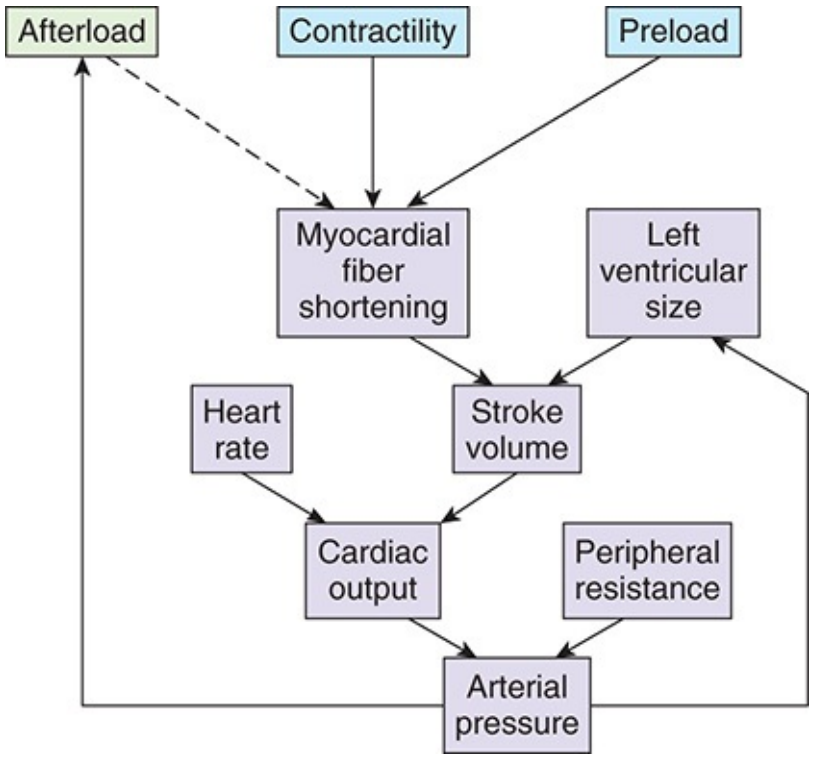


FIGURE 30–5 Interactions between the components that regulate cardiac output and arterial pressure. Solid arrows indicate increases, and the dashed arrow indicates a decrease.

The force of contraction of cardiac muscle depends on its preloading and its afterloading. These factors are illustrated in **Figure 30–6**, in which a muscle strip is stretched by a load (the **preload**) that rests on a platform. The initial phase of the contraction is isometric; the elastic component in series with the contractile element is stretched, and tension increases until it is sufficient to lift the load. The tension at which the load is lifted is the **afterload.** The muscle then contracts isotonically without developing further tension. In vivo, the preload is the degree to which the myocardium is stretched before it contracts and the afterload is the resistance against which blood is expelled.

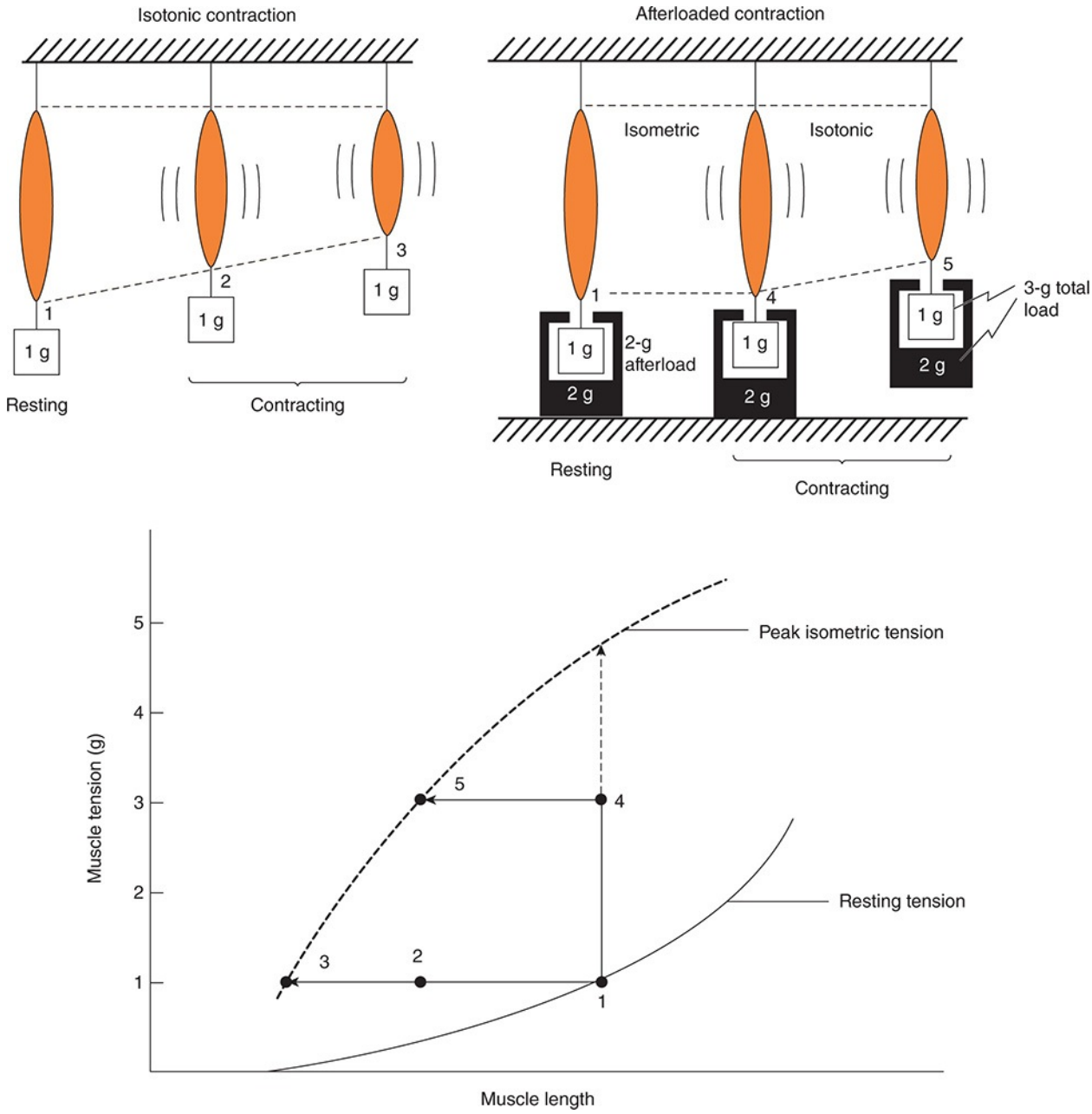


FIGURE 30–6 Model for contraction of afterloaded muscles. The figure depicts isotonic and afterloaded contractions within the constraints of the cardiac muscle length–tension diagram. The numbered points on the diagram correspond to the various conditions shown in the upper part. (Adapted with permission from Mohrman DE, Heller LJ: *Cardiovascular Physiology*, 8th ed. New York, NY: McGraw-Hill; 2014.)

RELATION OF TENSION TO LENGTH IN

CARDIAC MUSCLE

The length–tension relationship in cardiac muscle (see Figure 5–17) is similar to that in skeletal muscle (see Figure 5–10); when the muscle is stretched, the developed tension increases to a maximum and then declines as stretch becomes more extreme. Starling pointed this out when he stated that the "energy of contraction is proportional to the initial length of the cardiac muscle fiber" **(Starling's law of the heart** or the **Frank-Starling law).** For the heart, the length of the muscle fibers (ie, the extent of the preload) is proportional to the end-diastolic volume. The relation between ventricular stroke volume and end-diastolic volume is called the Frank-Starling curve.

When cardiac output is regulated by changes in cardiac muscle fiber length, this is referred to as **heterometric regulation.** Conversely, regulation due to changes in contractility independent of length is sometimes called **homometric regulation.**

FACTORS AFFECTING END-DIASTOLIC VOLUME

Alterations in systolic and diastolic function have different effects on the heart **(Clinical Box 30–1)**. When systolic contractions are reduced, there is a primary reduction in stroke volume. Diastolic function also affects stroke volume, but in a different way.

The myocardium is covered by a fibrous layer known as the epicardium. This, in turn, is surrounded by the pericardium, which separates the heart from the rest of the thoracic viscera. The space between the epicardium and pericardium (the pericardial sac) normally contains 5–30 mL of clear fluid, which lubricates the heart and permits it to contract with minimal friction.

An increase in intrapericardial pressure (eg, as a result of infection or pressure from a tumor) limits the extent to which the ventricle can fill, as does a decrease in ventricular compliance (ie, an increase in ventricular stiffness produced by myocardial infarction, infiltrative disease, and other abnormalities). Atrial contractions aid ventricular filling. Factors affecting the amount of blood returning to the heart likewise influence the degree of cardiac filling during diastole. An increase in total blood volume increases venous return **(Clinical Box 30–2)**. Constriction of the veins reduces the size of the venous reservoirs, decreasing venous pooling, and thus increasing venous return. An increase in the

normal negative intrathoracic pressure increases the pressure gradient along which blood flows to the heart, whereas a decrease impedes venous return. Standing decreases venous return, and muscular activity increases it as a result of the pumping action of skeletal muscle.

The effects of systolic and diastolic dysfunction on the pressure–volume loop of the left ventricle are summarized in **Figure 30–7**.

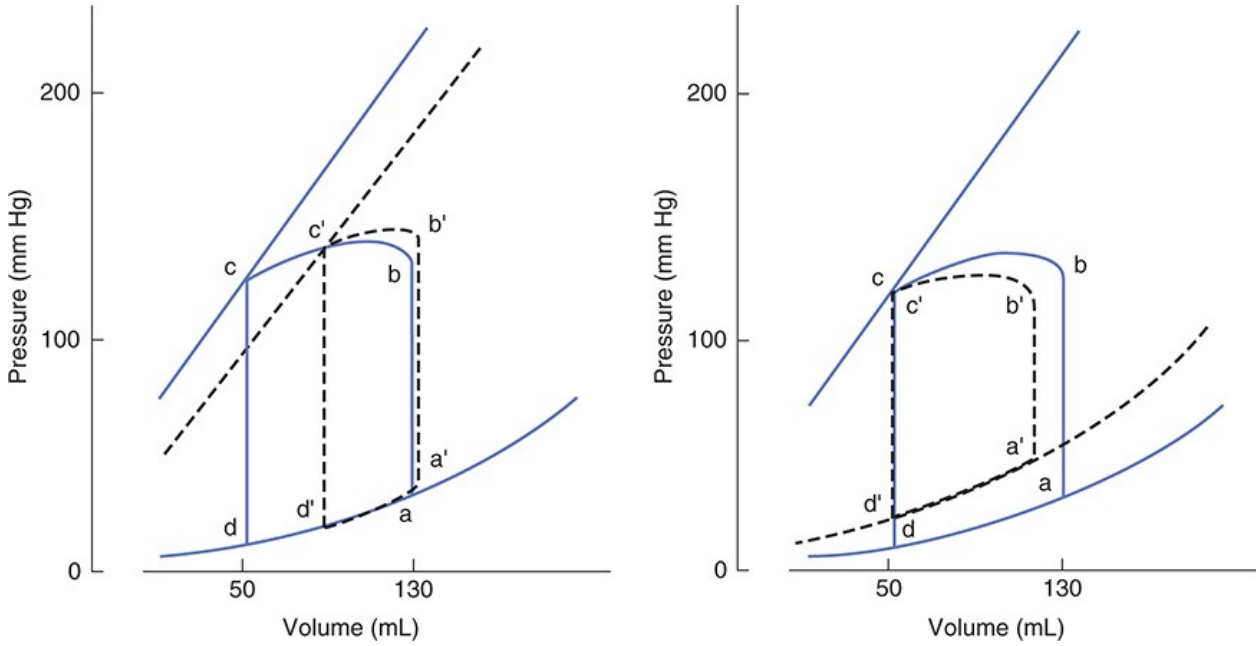


FIGURE 30–7 Effect of systolic and diastolic dysfunction on the pressure–volume loop of the left ventricle. In both panels, the solid lines represent the normal pressure–volume loop (equivalent to that shown in Figure 30–2) and the dashed lines show how the loop is shifted by the disease process represented. **Left:** Systolic dysfunction shifts the isovolumic pressure–volume curve to the right, decreasing the stroke volume from b–c to b'–c'. **Right:** Diastolic dysfunction increases end-diastolic volume and shifts the diastolic pressure–volume relationship upward and to the left. This reduces the stroke volume from b–c to b'–c'. (Reproduced with permission from McPhee SJ, Lingappa VR, Ganong WF [editors]: *Pathophysiology of Disease*, 6th ed. New York, NY: McGraw-Hill; 2010.)

MYOCARDIAL CONTRACTILITY

The contractility of the myocardium exerts a major influence on stroke volume. When the sympathetic nerves to the heart are stimulated, the whole length–

tension curve shifts upward and to the left **(Figure 30–8)**. The positive inotropic effect of norepinephrine liberated at the nerve endings is augmented by circulating norepinephrine, and epinephrine has a similar effect. Conversely, there is a negative inotropic effect of vagal stimulation on both atrial and (to a lesser extent) ventricular muscle.

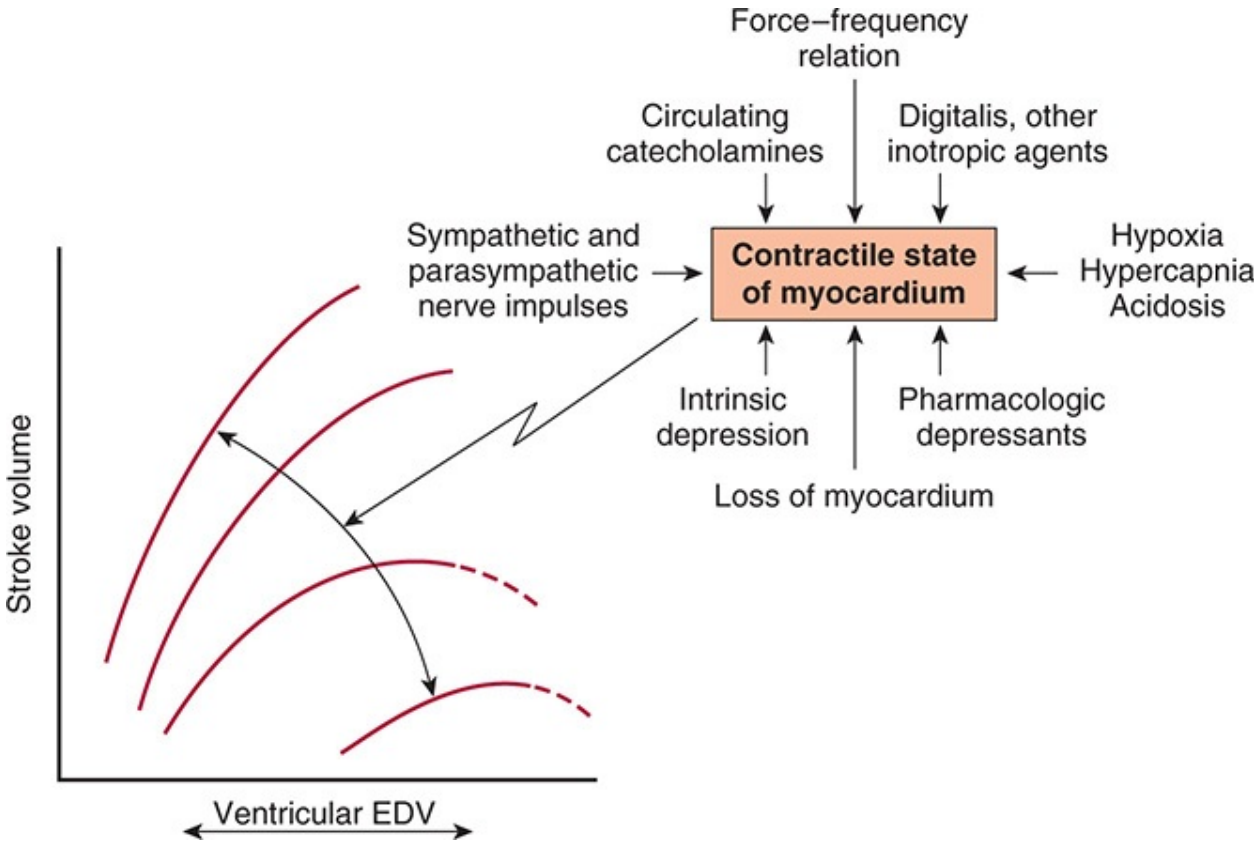


FIGURE 30–8 Effect of changes in myocardial contractility on the Frank-Starling curve. The curve shifts downward and to the right as contractility is decreased. The major factors influencing contractility are summarized on the right. The dashed lines indicate portions of the ventricular function curves where maximum contractility has been exceeded; that is, they identify points on the "descending limb" of the Frank-Starling curve. EDV, end-diastolic volume. (Reproduced with permission from Braunwald E, Ross J, Sonnenblick EH: Mechanisms of contraction of the normal and failing heart. N Engl J Med 1967; October 12; 277(15):794–800.)

Changes in cardiac rate and rhythm also affect myocardial contractility (known as the force–frequency relation, Figure 30–8). Ventricular extrasystoles condition the myocardium in such a way that the next succeeding contraction is

stronger than the preceding normal contraction. This **postextrasystolic potentiation** is independent of ventricular filling, since it occurs in isolated cardiac muscle and is due to increased availability of intracellular Ca^{2+}. A sustained increment in contractility can be produced therapeutically by delivering paired electrical stimuli to the heart in such a way that the second stimulus is delivered shortly after the refractory period of the first. It has also been shown that myocardial contractility increases as the heart rate increases, although this effect is relatively small.

Catecholamines exert their inotropic effect via an action on cardiac β_1-adrenergic receptors and Gs, with resultant activation of adenylyl cyclase and increased intracellular cyclic adenosine 3′,5′-monophosphate (cAMP). Xanthines such as caffeine and theophylline that inhibit the breakdown of cAMP are predictably positively inotropic. The positively inotropic effect of digitalis and related drugs (Figure 30–8), on the other hand, is due to their inhibitory effect on the Na^+, K^+ ATPase in the myocardium, and a subsequent decrease in calcium removal from the cytosol by Na^+/Ca^{2+} exchange (see Chapter 5). Hypercapnia; hypoxia; acidosis; and drugs, such as quinidine, procainamide, and barbiturates, depress myocardial contractility. The contractility of the myocardium is also reduced in heart failure (intrinsic depression). The causes of this depression are not fully understood but may reflect down-regulation of β-adrenergic receptors and associated signaling pathways and impaired calcium liberation from the sarcoplasmic reticulum. In acute heart failure, such as that associated with sepsis, this response could be considered an appropriate adaptation (so-called “myocardial hibernation”) to a situation where energy supply to the heart is limited, thereby reducing energy expenditure and avoiding cell death.

CLINICAL BOX 30–1

Heart Failure

Heart failure occurs when the heart is unable to put out an amount of blood that is adequate for the needs of the tissues. It can be acute and associated with sudden death, or chronic. The failure may involve primarily the right ventricle (cor pulmonale), but much more commonly it involves the larger, thicker left ventricle or both ventricles. Heart failure may also be systolic or diastolic. In **systolic failure,** stroke volume is reduced because ventricular contraction is weak. This causes an increase in the end-systolic ventricular volume, so that the **ejection fraction** falls from 65% to as low as 20%. The

initial response to failure is activation of the genes that cause cardiac myocytes to hypertrophy, and thickening of the ventricular wall **(cardiac remodeling).** The incomplete filling of the arterial system leads to increased discharge of the sympathetic nervous system and increased secretion of renin and aldosterone, so Na^+ and water are retained. These responses are initially compensatory, but eventually the failure worsens and the ventricles dilate.

In **diastolic failure,** the ejection fraction is initially maintained, but the elasticity of the myocardium is reduced so filling during diastole is reduced. This leads to inadequate stroke volume and the same cardiac remodeling and Na^+ and water retention that occur in systolic failure. It should be noted that the inadequate cardiac output in heart failure may be relative rather than absolute. When a large arteriovenous fistula is present, in thyrotoxicosis or in thiamine deficiency, cardiac output may be elevated in absolute terms but still be inadequate to meet the needs of the tissues **(high-output failure)**.

THERAPEUTIC HIGHLIGHTS

Treatment of heart failure is aimed at improving cardiac contractility, treating the symptoms, and decreasing the load on the heart. Currently, the most effective treatment in general use is inhibition of the production of angiotensin II with angiotensin-converting enzyme (ACE) inhibitors. Blockade of the effects of angiotensin II on AT_1 receptors with nonpeptide antagonists is also of value. Blocking the production of angiotensin II or its effects also reduces the circulating aldosterone level and decreases blood pressure, reducing the afterload against which the heart pumps. The effects of aldosterone can be further reduced by administering aldosterone receptor blockers. Reducing venous tone with nitrates or hydralazine increases venous capacity so that the amount of blood returned to the heart is reduced, lowering the preload. Diuretics reduce the fluid overload. Drugs that block β-adrenergic receptors have been shown to decrease mortality and morbidity. Digitalis derivatives such as digoxin have classically been used to treat heart failure because of their ability to increase stores of intracellular Ca^{2+} and hence exert a positive inotropic effect, but they are now used in a secondary role to treat systolic dysfunction and slow the ventricular rate in patients with atrial fibrillation.

INTEGRATED CONTROL OF CARDIAC OUTPUT

The mechanisms listed above operate in an integrated way to maintain cardiac output. For example, during muscular exercise, there is increased sympathetic discharge, so that myocardial contractility is increased and the heart rate rises. The increase in heart rate is particularly prominent in normal individuals, and there is only a modest increase in stroke volume **(see Table 30–4 and Clinical Box 30–3)**. However, patients with transplanted hearts are able to increase their cardiac output during exercise in the absence of cardiac innervation through the operation of the Frank-Starling mechanism **(Figure 30–9)**. Circulating catecholamines also contribute. If venous return increases and there is no change in sympathetic tone, venous pressure rises, diastolic inflow is greater, ventricular end-diastolic pressure increases, and the heart muscle contracts more forcefully. During muscular exercise, venous return is increased by the pumping action of the muscles and the increase in respiration (see Chapter 32). In addition, because of vasodilation in the contracting muscles, peripheral resistance, and, consequently, afterload are decreased. The end result in both normal and transplanted hearts is thus a prompt and marked increase in cardiac output.

TABLE 30–4 Changes in cardiac function with exercise. Note that stroke volume levels off, then falls somewhat (as a result of the shortening of diastole) when the heart rate rises to high values.

Work (kg-m/min)	O_2 Usage (mL/min)	Pulse Rate (per min)	Cardiac Output (L/min)	Stroke Volume (mL)	A-V O_2 Difference (mL/dL)
Rest	267	64	6.4	100	4.3
288	910	104	13.1	126	7.0
540	1430	122	15.2	125	9.4
900	2143	161	17.8	110	12.3
1260	3007	173	20.9	120	14.5

Reproduced with permission from Asmussen E, Nielsen M: The cardiac output in rest and work determined by the acetylene and the dye injection methods. Acta Physiol Scand 1952;27(2–3):217–230.

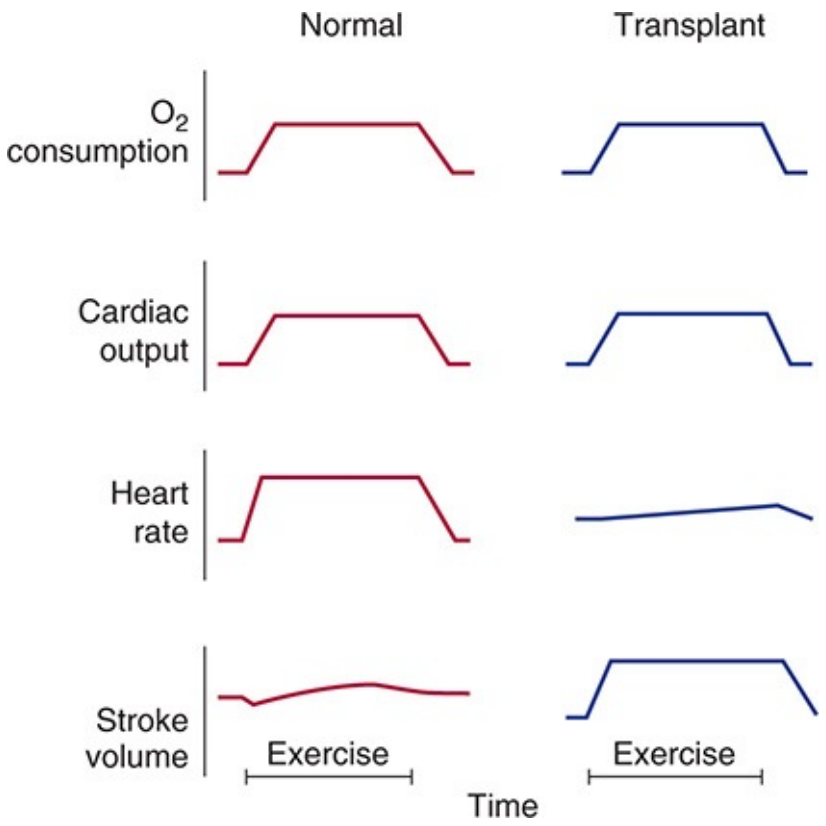


FIGURE 30–9 Cardiac responses to moderate supine exercise in normal humans and patients with transplanted and hence denervated hearts. Note that the transplanted heart, without the benefit of neural input, relies primarily on an increase in stroke volume rather than heart rate to raise cardiac output in the setting of exercise. (Reproduced with permission from Kent KM, Cooper T: The denervated heart. N Engl J Med 1974; Nov7; 291(19):1017–1021.)

One of the differences between untrained individuals and trained athletes is that the athletes have lower heart rates, greater end-systolic ventricular volumes, and greater stroke volumes at rest. Therefore, they can potentially achieve a given increase in cardiac output by further increases in stroke volume without increasing their heart rate to as great a degree as an untrained individual.

CLINICAL BOX 30–2

Shock

Circulatory shock comprises a collection of different entities that share certain common features; however, the feature that is common to all the entities is

inadequate tissue perfusion with a relatively or absolutely inadequate cardiac output. The cardiac output may be inadequate because the amount of fluid in the vascular system is inadequate to fill it **(hypovolemic shock).** Alternatively, it may be inadequate in the relative sense because the size of the vascular system is increased by vasodilation even though the blood volume is normal **(distributive, vasogenic,** or **low-resistance shock).** Shock may also be caused by inadequate pumping action of the heart as a result of myocardial abnormalities **(cardiogenic shock),** and by inadequate cardiac output as a result of obstruction of blood flow in the lungs or heart **(obstructive shock).**

Hypovolemic shock is also called “cold shock.” It is characterized by hypotension; a rapid, thready pulse; cold, pale, clammy skin; intense thirst; rapid respiration; and restlessness or, alternatively, torpor. None of these findings, however, are invariably present. Hypovolemic shock is commonly subdivided into categories on the basis of cause. Of these, it is useful to consider the effects of hemorrhage in some detail because of the multiple compensatory reactions that come into play to defend extracellular fluid (ECF) volume. Thus, the decline in blood volume produced by bleeding decreases venous return, and cardiac output falls. The heart rate is increased, and with severe hemorrhage, a fall in blood pressure always occurs. With moderate hemorrhage (5–15 mL/kg body weight), pulse pressure is reduced but mean arterial pressure may be normal. The blood pressure changes vary from individual to individual, even when exactly the same amount of blood is lost. The skin is cool and pale and may have a grayish tinge because of stasis in the capillaries and a small amount of cyanosis. Inadequate perfusion of the tissues leads to increased anaerobic glycolysis, with the production of large amounts of lactic acid. In severe cases, the blood lactate level rises from the normal value of about 1 mmol/L to 9 mmol/L or more. The resulting **lactic acidosis** depresses the myocardium, decreases peripheral vascular responsiveness to catecholamines, and may be severe enough to cause coma. When blood volume is reduced and venous return is decreased, moreover, stimulation of arterial baroreceptors is reduced, increasing sympathetic output. Even if there is no drop in mean arterial pressure, the decrease in pulse pressure decreases the rate of discharge in the arterial baroreceptors, and reflex tachycardia and vasoconstriction result.

With more severe blood loss, tachycardia is replaced by bradycardia; this occurs while shock is still reversible. The bradycardia is presumably due to unmasking a vagally mediated depressor reflex, and the response may have

evolved as a mechanism for stopping further blood loss. With even greater hemorrhage, the heart rate rises again. Vasoconstriction is generalized, sparing only the vessels of the brain and heart. A widespread reflex venoconstriction also helps maintain the filling pressure of the heart. In the kidneys, both afferent and efferent arterioles are constricted, but the efferent vessels are constricted to a greater degree. The glomerular filtration rate is depressed, but renal plasma flow is decreased to a greater extent, so that the filtration fraction increases. Na^+ retention is marked, and the nitrogenous products of metabolism are retained in the blood **(azotemia** or **uremia).** If the hypotension is prolonged, renal tubular damage may be severe **(acute kidney injury).** After a moderate hemorrhage, the circulating plasma volume is restored in 12–72 h. Preformed albumin also enters rapidly from extravascular stores, but most of the tissue fluids that are mobilized are protein-free. After the initial influx of preformed albumin, the rest of the plasma protein losses are replaced, presumably by hepatic synthesis, over a period of 3–4 days. Erythropoietin appears in the circulation, and the reticulocyte count increases, reaching a peak in 10 days. The red cell mass is restored to normal in 4–8 weeks.

THERAPEUTIC HIGHLIGHTS

The treatment of shock is aimed at correcting the cause and helping the physiologic compensatory mechanisms to restore an adequate level of tissue perfusion. If the primary cause of the shock is blood loss, the treatment should include early and rapid transfusion of adequate amounts of compatible whole blood. In shock due to burns and other conditions in which there is hemoconcentration, plasma is the treatment of choice to restore the fundamental defect, the loss of plasma. Concentrated human serum albumin and other hypertonic solutions expand the blood volume by drawing fluid out of the interstitial spaces. They are valuable in emergency treatment but have the disadvantage of further dehydrating the tissues of an already dehydrated patient.

OXYGEN CONSUMPTION BY THE HEART

Basal O_2 consumption by the myocardium is about 2 mL/100 g/min. This value is considerably higher than that of resting skeletal muscle. O_2 consumption by the beating heart is about 9 mL/100 g/min at rest. Increases occur during

exercise and in a number of different states. Cardiac venous O_2 tension is low, and little additional O_2 can be extracted from the blood in the coronaries, so increases in O_2 consumption require increases in coronary blood flow. The regulation of coronary flow is discussed in Chapter 33.

CLINICAL BOX 30–3

Circulatory Changes during Exercise

The blood flow of resting skeletal muscle is low (2–4 mL/100 g/min). When a muscle contracts, it compresses the vessels in it if it develops more than 10% of its maximal tension; when it develops more than 70% of its maximal tension, blood flow is completely stopped. Between contractions, however, flow is so greatly increased that blood flow per unit of time in a rhythmically contracting muscle is increased as much as 30-fold. Local mechanisms maintaining a high blood flow in exercising muscle include a fall in tissue P_{O_2}, a rise in tissue P_{CO_2}, and accumulation of K^+ and other vasodilator metabolites. The temperature rises in active muscle, and this further dilates the vessels. Dilation of the arterioles and precapillary sphincters causes a 10- to 100-fold increase in the number of open capillaries. The average distance between the blood and the active muscle cells—and the distance O_2 and metabolic products must diffuse—is thus greatly decreased. The dilation increases the cross-sectional area of the vascular bed, and the velocity of flow therefore decreases.

The systemic cardiovascular response to exercise that provides for the additional blood flow to contracting muscle depends on whether the muscle contractions are primarily isometric or primarily isotonic with the performance of external work. With the start of an isometric muscle contraction, the heart rate rises, probably as a result of psychic stimuli acting on the medulla oblongata. The increase is largely due to decreased vagal tone, although increased discharge of the cardiac sympathetic nerves plays some role. Within a few seconds of the onset of an isometric muscle contraction, systolic and diastolic blood pressures rise sharply. Stroke volume changes relatively little, and blood flow to the steadily contracting muscles is reduced as a result of compression of their blood vessels. The response to exercise involving isotonic muscle contraction is similar in that there is a prompt increase in heart rate, but different in that a marked increase in stroke volume

occurs. In addition, there is a net fall in total peripheral resistance due to vasodilation in exercising muscles. Consequently, systolic blood pressure rises only moderately, whereas diastolic pressure usually remains unchanged or falls.

The difference in response to isometric and isotonic exercise is explained in part by the fact that the active muscles are tonically contracted during isometric exercise and consequently contribute to increased total peripheral resistance. Cardiac output is increased during isotonic exercise to values that may exceed 35 L/min, the amount being proportional to the increase in O_2 consumption. The maximal heart rate achieved during exercise decreases with age. In children, it rises to 200 or more beats/min; in adults it rarely exceeds 195 beats/min, and in elderly individuals the rise is even smaller. Both at rest and at any given level of exercise, trained athletes have a larger stroke volume and lower heart rate than untrained individuals and they tend to have larger hearts. Training increases the maximal oxygen consumption ($V \cdot O_{2max}$) that can be produced by exercise in an individual. $V \cdot O_{2max}$ averages about 38 mL/kg/min in active healthy men and about 29 mL/kg/min in active healthy women. It is lower in sedentary individuals. $V \cdot O_{2max}$ is the product of maximal cardiac output and maximal O_2 extraction by the tissues, and both increase with training.

A great increase in venous return also takes place with exercise, although the increase in venous return is not the primary cause of the increase in cardiac output. Venous return is increased by the activity of the muscle and thoracic pumps; by mobilization of blood from the viscera; by increased pressure transmitted through the dilated arterioles to the veins; and by noradrenergically mediated venoconstriction, which decreases the volume of blood in the veins. Blood mobilized from the splanchnic area and other reservoirs may increase the amount of blood in the arterial portion of the circulation by as much as 30% during strenuous exercise. After exercise, the blood pressure may transiently drop to subnormal levels, presumably because accumulated metabolites keep the muscle vessels dilated for a short period. However, the blood pressure soon returns to the preexercise level. The heart rate returns to normal more slowly.

O_2 consumption by the heart is determined primarily by the intramyocardial tension, the contractile state of the myocardium, and the heart rate. Ventricular

work per beat correlates with O_2 consumption. The work is the product of stroke volume and mean arterial pressure in the pulmonary artery or the aorta (for the right and left ventricle, respectively). Because aortic pressure is seven times greater than pulmonary artery pressure, the stroke work of the left ventricle is approximately seven times the stroke work of the right. In theory, a 25% increase in stroke volume without a change in arterial pressure should produce the same increase in O_2 consumption as a 25% increase in arterial pressure without a change in stroke volume. However, for reasons that are incompletely understood, pressure work produces a greater increase in O_2 consumption than volume work. In other words, an increase in afterload causes a greater increase in cardiac O_2 consumption than does an increase in preload. This is why angina pectoris due to deficient delivery of O_2 to the myocardium is more common in aortic stenosis than in aortic regurgitation. In aortic stenosis, intraventricular pressure must be increased to force blood through the stenotic valve, whereas in aortic regurgitation, an increase in stroke volume occurs with little change in aortic impedance.

It is worth noting that the increase in O_2 consumption produced by increased stroke volume when the myocardial fibers are stretched is an example of the operation of the law of Laplace. This law, which is discussed in detail in Chapter 31, states that the tension developed in the wall of a hollow viscus is proportional to the radius of the viscus. When the heart is dilated, its radius is increased. O_2 consumption per unit time increases when the heart rate is increased by sympathetic stimulation because of the increased number of beats and the increased velocity and strength of each contraction. However, this is somewhat offset by the decrease in end-systolic volume and hence in the radius of the heart.

CHAPTER SUMMARY

- Blood flows into the atria and then the ventricles of the heart during diastole and atrial systole, and is ejected during systole when the ventricles contract and pressure exceeds the pressures in the pulmonary artery and aorta.
- Careful timing of the opening and closing of the atrioventricular (AV), pulmonary, and aortic valves allows blood to move in an appropriate direction through the heart with minimal regurgitation.
- The proportion of blood leaving the ventricles in each cardiac cycle is called

the ejection fraction and is a sensitive indicator of cardiac health.

- The arterial pulse represents a pressure wave set up when blood is forced into the aorta; it travels much faster than the blood itself.
- Heart sounds reflect the normal vibrations set up by abrupt valve closures; heart murmurs can arise from abnormal flow often (although not exclusively) caused by diseased valves.
- Changes in cardiac output reflect variations in heart rate, stroke volume, or both; these are controlled, in turn, by neural and hormonal input to cardiac myocytes.
- Cardiac output can be measured by assessing the amount of oxygen consumed by the body during a given period divided by the difference in oxygen concentration between arterial blood and the pulmonary artery (Fick's principle). Alternatively, dilution of an inert dye or thermodilution of cold saline can be used.
- In heart failure, the ejection fraction of the heart is reduced due to impaired contractility in systole or reduced filling during diastole; this results in inadequate blood supplies to meet the body's needs. Initially, this is manifested only during exercise, but eventually the heart will not be able to supply sufficient blood flow even at rest.
- Cardiac output is strikingly increased during exercise.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. A 75-year-old woman with a history of chronic congestive heart failure undergoes cardiac catheterization to determine her extent of cardiac dysfunction. During estimation of her systolic function, in what phase of the cardiac cycle should her peak left ventricular pressure occur?

   A. Rapid filling

   B. Isovolumetric contraction

   C. Ventricular ejection

   D. Atrial systole

   E. Isovolumetric relaxation

2. The work performed by the left ventricle is substantially greater than that performed by the right ventricle, because in the left ventricle

A. the contraction is slower.
B. the wall is thicker.
C. the stroke volume is greater.
D. the preload is greater.
E. the afterload is greater.

3. The dicrotic notch on the aortic pressure curve is caused by
A. closure of the mitral valve.
B. closure of the tricuspid valve.
C. closure of the aortic valve.
D. closure of the pulmonary valve.
E. rapid filling of the left ventricle.

4. The second heart sound is caused by
A. closure of the aortic and pulmonary valves.
B. vibrations in the ventricular wall during systole.
C. ventricular filling.
D. closure of the mitral and tricuspid valves.
E. retrograde flow in the vena cava.

5. The fourth heart sound is caused by
A. closure of the aortic and pulmonary valves.
B. vibrations in the ventricular wall during systole.
C. ventricular filling.
D. closure of the mitral and tricuspid valves.
E. retrograde flow in the vena cava.

6. During exercise, a man consumes 1.8 L of oxygen per minute. His arterial O_2 content is 190 mL/L, and the O_2 content of his mixed venous blood is 134 mL/L. His cardiac output is approximately
A. 3.2 L/min.
B. 16 L/min.
C. 32 L/min.
D. 54 L/min.
E. 160 mL/min.

7. Starling’s law of the heart
A. does not operate in the failing heart.

B. does not operate during exercise.

C. explains the increase in heart rate produced by exercise.

D. explains the increase in cardiac output that occurs when venous return is increased.

E. explains the increase in cardiac output when the sympathetic nerves supplying the heart are stimulated.

8. A 65-year-old woman comes to her physician complaining of chest pain on exertion and is diagnosed with angina pectoris. An examination reveals an aortic abnormality. This is more likely to be aortic stenosis as opposed to aortic regurgitation because:

A. An increase in preload causes a greater increase in cardiac O_2 consumption than an increase in afterload.

B. Cardiac O_2 consumption is unrelated to ventricular work per beat.

C. Increased pressure work causes a greater increase in cardiac O_2 consumption than an increase in volume work.

D. Cardiac venous O_2 tension is low.

E. Aortic stenosis decreases pressure in the aortic valve.

CHAPTER 31

Blood as a Circulatory Fluid & the Dynamics of Blood & Lymph Flow

OBJECTIVES

After studying this chapter, you should be able to:

- Describe the cellular components of blood, and their origins, how the fragility of red blood cells is controlled, the role of hemoglobin in transporting oxygen in red blood cells, and the basis of hemoglobinopathies.
- Define the composition of plasma and lymph and identify the sources of their constituents.
- Understand the molecular basis of blood groups and the reasons for transfusion reactions.
- Delineate the process of hemostasis that restricts blood loss when vessels are damaged, the adverse consequences of intravascular thrombosis, and the basis of clotting disorders.
- Identify the types of blood and lymphatic vessels that make up the circulatory system and the regulation and function of their primary constituent cell types.
- Describe how physical principles dictate the flow of blood and lymph around the body.

- Understand the basis of methods used to measure blood flow and blood pressure in various vascular segments.
- Understand the basis and consequences of a sustained elevation of systemic arterial pressure (hypertension).

INTRODUCTION

The **circulatory system** supplies inspired O_2 as well as substances absorbed from the gastrointestinal tract to the tissues, returns CO_2 to the lungs and other products of metabolism to the kidneys, functions in the regulation of body temperature, and distributes hormones and other agents that regulate cell function. The blood, the carrier of these substances, is pumped through a closed system of blood vessels by the heart. From the left ventricle, blood is pumped through the arteries and arterioles to the capillaries, where it equilibrates with the interstitial fluid. The capillaries drain through venules into the veins and back to the right atrium. Some tissue fluids enter another system of closed vessels, the lymphatics, which drain lymph via the thoracic duct and the right lymphatic duct into the venous system. The circulation is controlled by multiple regulatory systems that function in general to maintain adequate capillary blood flow when possible in all organs, but particularly in the heart and brain.

Blood flows through the circulation primarily because of the forward motion imparted to it by the pumping of the heart, although in the case of the systemic circulation, diastolic recoil of the walls of the arteries, compression of the veins by skeletal muscles during exercise, and the negative pressure in the thorax during inspiration also move the blood forward. The resistance to flow depends to a minor degree on the viscosity of the blood but mostly on the diameter of the vessels, and principally that of the arterioles. The blood flow to each tissue is regulated by local chemical and general neural and humoral mechanisms that dilate or constrict its vessels. All of the blood flows through the lungs, but the systemic circulation is made up of numerous different circuits in parallel **(Figure 31–1)**. The arrangement permits wide variations in regional blood flow without changing total systemic flow.

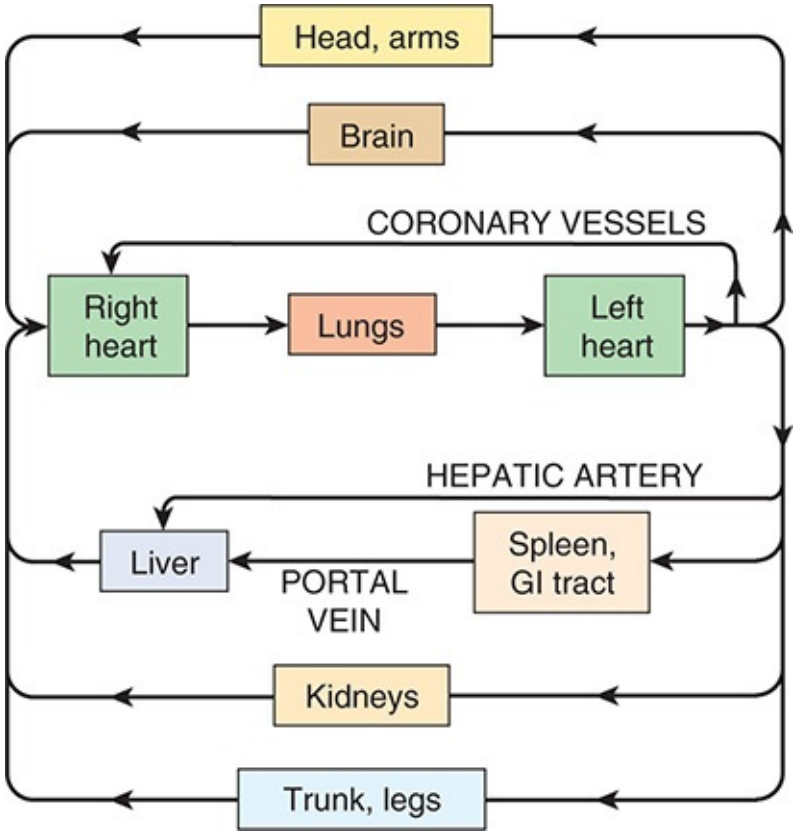


FIGURE 31–1 Diagram of the circulation in the adult.

This chapter is concerned with blood and lymph and with the multiple functions of the cells they contain. It will also address general principles that apply to all parts of the circulation and pressure and flow in the systemic circulation. The homeostatic mechanisms operating to adjust flow are the subject of Chapter 32. The special characteristics of the pulmonary and renal circulations are discussed in Chapters 34 and 37. Likewise, the role of blood as the carrier of many immune effector cells will not be discussed here, but rather was covered in Chapter 3.

BLOOD AS A CIRCULATORY FLUID

Blood consists of a protein-rich fluid known as plasma, in which are suspended cellular elements: white blood cells, red blood cells, and platelets. The normal total circulating blood volume is about 8% of the body weight (5600 mL in a 70-kg man). About 55% of this volume is plasma.

BONE MARROW

In the adult, red blood cells, many white blood cells, and platelets are formed in the bone marrow. In the fetus, blood cells are also formed in the liver and spleen, and in adults such **extramedullary hematopoiesis** may occur in diseases in which the bone marrow becomes destroyed or fibrosed. In children, blood cells are actively produced in the marrow cavities of all the bones. By age 20, the marrow in the cavities of the long bones, except for the upper humerus and femur, has become inactive **(Figure 31–2)**.

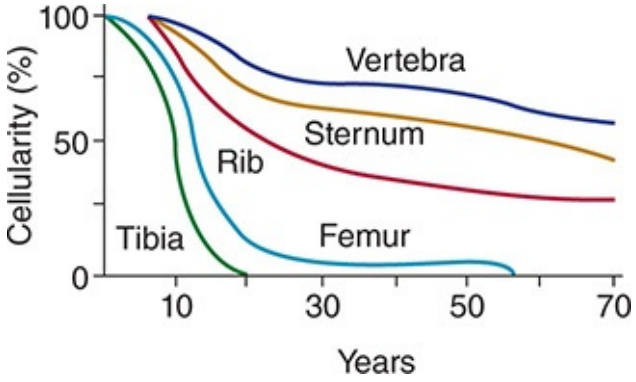


FIGURE 31–2 Changes in red bone marrow cellularity in various bones with age. Hundred percent equals the degree of cellularity at birth. (Reproduced with permission from Whitby LEH, Britton CJC: *Disorders of the Blood*, 10th ed. Churchill Livingstone; 1969.)

The bone marrow is actually one of the largest organs in the body, approaching the size and weight of the liver. It is also one of the most active. Normally, 75% of the cells in the marrow belong to the white blood cell–producing myeloid series and only 25% are maturing red cells, even though there are over 500 times as many red cells in the circulation as there are white cells. This difference in the marrow reflects the fact that the average life span of white cells is short, whereas that of red cells is long.

Hematopoietic stem cells (HSCs) are bone marrow cells that are capable of producing all types of blood cells. They differentiate into one or another type of committed stem cells **(progenitor cells).** These in turn form the various differentiated types of blood cells. There are separate pools of progenitor cells for megakaryocytes, lymphocytes, erythrocytes, eosinophils, and basophils; neutrophils and monocytes arise from a common precursor. The bone marrow stem cells are also the source of osteoclasts (see Chapter 21), Kupffer cells (see Chapter 28), mast cells, dendritic cells, and Langerhans cells. The HSCs are few

in number but are capable of completely replacing the bone marrow when injected into a patient whose own bone marrow has been entirely destroyed.

The HSCs are derived from uncommitted, totipotent stem cells that can be stimulated to form any cell in the body. Adults have a few of these, but they are more readily obtained from the blastocysts of embryos. There is not surprisingly immense interest in stem cell research due to its potential to regenerate diseased tissues, but ethical issues are involved, and debate on these issues will undoubtedly continue.

WHITE BLOOD CELLS

Normally, human blood contains 4000–11,000 white blood cells per microliter **(Table 31–1)**. Of these, the **granulocytes (polymorphonuclear leukocytes, PMNs)** are the most numerous. Young granulocytes have horseshoe-shaped nuclei that become multilobed as the cells grow older **(Figure 31–3)**. Most of them contain neutrophilic granules **(neutrophils),** but a few contain granules that stain with acidic dyes **(eosinophils),** and some have basophilic granules **(basophils).** The other two cell types found normally in peripheral blood are **lymphocytes,** which have large round nuclei and scanty cytoplasm, and **monocytes,** which have abundant agranular cytoplasm and kidney-shaped nuclei (Figure 31–3). Acting together, these cells provide the body with the powerful defenses against tumors and viral, bacterial, and parasitic infections that were discussed in Chapter 3.

TABLE 31–1 Normal values for the cellular elements in human blood.

Cell	Cells/μL (average)	Approximate Normal Range	Percentage of Total White Cells
Total white blood cells	9000	4000–11,000	…
Granulocytes			
Neutrophils	5400	3000–6000	50–70
Eosinophils	275	150–300	1–4
Basophils	35	0–100	0.4
Lymphocytes	2750	1500–4000	20–40
Monocytes	540	300–600	2–8
Erythrocytes			
Females	4.8×10^6	…	…
Males	5.4×10^6	…	…
Platelets	300,000	200,000–500,000	…

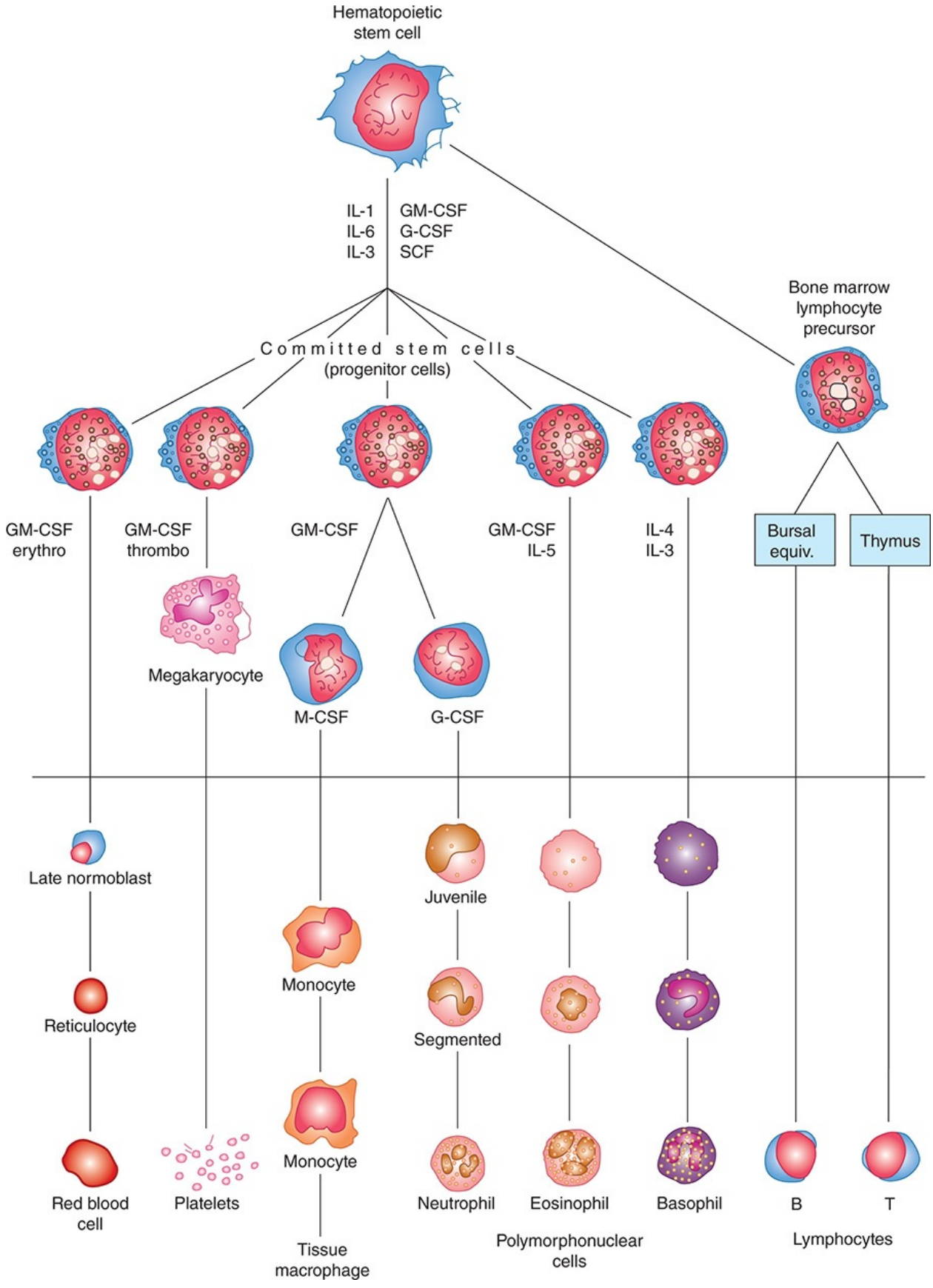

Hematopoietic stem cell
IL-1
IL-6
IL-3
GM-CSF
G-CSF
SCF
Committed stem cells (progenitor cells)
Bone marrow lymphocyte precursor
GM-CSF erythro
GM-CSF thrombo
GM-CSF
GM-CSF IL-5
IL-4 IL-3
Bursal equiv.
Thymus
Megakaryocyte
M-CSF
G-CSF
Late normoblast
Reticulocyte
Red blood cell
Platelets
Monocyte
Monocyte
Tissue macrophage
Juvenile
Segmented
Neutrophil
Eosinophil
Basophil
Polymorphonuclear cells
B
T
Lymphocytes

FIGURE 31–3 Development of various formed elements of the blood from bone marrow cells. Cells below the horizontal line are found in normal peripheral blood. The principal sites of action of erythropoietin (erythro) and the various colony-stimulating factors (CSFs) that stimulate the differentiation of the components are indicated. G, granulocyte; M, macrophage; IL, interleukin; thrombo, thrombopoietin; erythro, erythropoietin; SCF, stem cell factor.

PLATELETS

Platelets are small, granulated bodies that aggregate at sites of vascular injury. They lack nuclei and are 2–4 µm in diameter (Figure 31–3). There are about 300,000/µL of circulating blood, and they normally have a half-life of about 4 days. The **megakaryocytes,** giant cells in the bone marrow, form platelets by pinching off bits of cytoplasm and extruding them into the circulation. Between 60% and 75% of the platelets that have been extruded from the bone marrow are in the circulating blood, and the remainder are mostly in the spleen. Splenectomy causes an increase in the platelet count **(thrombocytosis).**

RED BLOOD CELLS

The red blood cells **(erythrocytes)** carry hemoglobin in the circulation. They are biconcave disks **(Figure 31–4)** that are manufactured in the bone marrow. In mammals, they lose their nuclei before entering the circulation. In humans, they survive in the circulation for an average of 120 days. The average normal red blood cell count is 5.4 million/µL in men and 4.8 million/µL in women. The number of red cells is also conveniently expressed as the **hematocrit,** or the percentage of the blood, by volume, that is occupied by erythrocytes. Each human red blood cell is about 7.5 µm in diameter and 2 µm thick, and each contains approximately 29 pg of hemoglobin **(Table 31–2)**. There are thus about 3×10^{13} red blood cells and about 900 g of hemoglobin in the circulating blood of an adult man **(Figure 31–5)**.

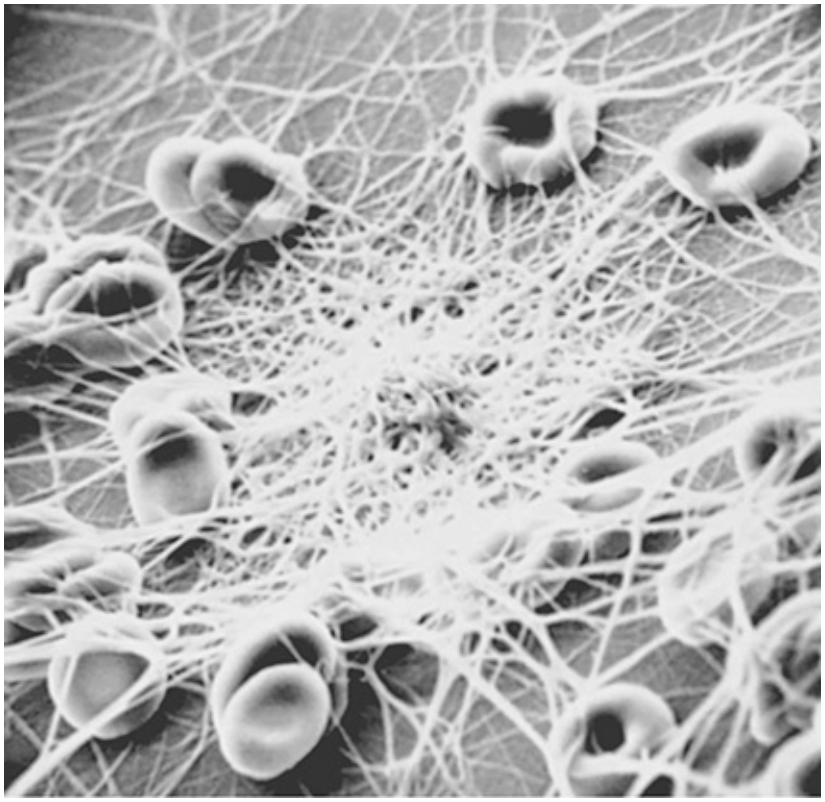


FIGURE 31–4 Human red blood cells and fibrin fibrils. Blood was placed on a polyvinyl chloride surface, fixed, and photographed with a scanning electron microscope. Reduced from ×2590. (Used with permission of NF Rodman.)

TABLE 31–2 Characteristics of human red cells.[a]

		Male	Female
Hematocrit (Hct) (%)		47	42
Red blood cells (RBC) (10^6/μL)		5.4	4.8
Hemoglobin (Hb) (g/dL)		16	14
Mean corpuscular volume (MCV) (fL)	$= \frac{\text{Hct} \times 10}{\text{RBC } (10^6/\mu\text{L})}$	87	87
Mean corpuscular hemoglobin (MCH) (pg)	$= \frac{\text{Hb} \times 10}{\text{RBC } (10^6/\mu\text{L})}$	29	29
Mean corpuscular hemoglobin concentration (MCHC) (g/dL)	$= \frac{\text{Hb} \times 100}{\text{Hct}}$	34	34
Mean cell diameter (MCD) (μm)	= Mean diameter of 500 cells in smear	7.5	7.5

[a]Cells with MCVs > 95 fL are called macrocytes; cells with MCVs < 80 fL are called microcytes; cells with MCHCs < 25 g/dL are called hypochromic.

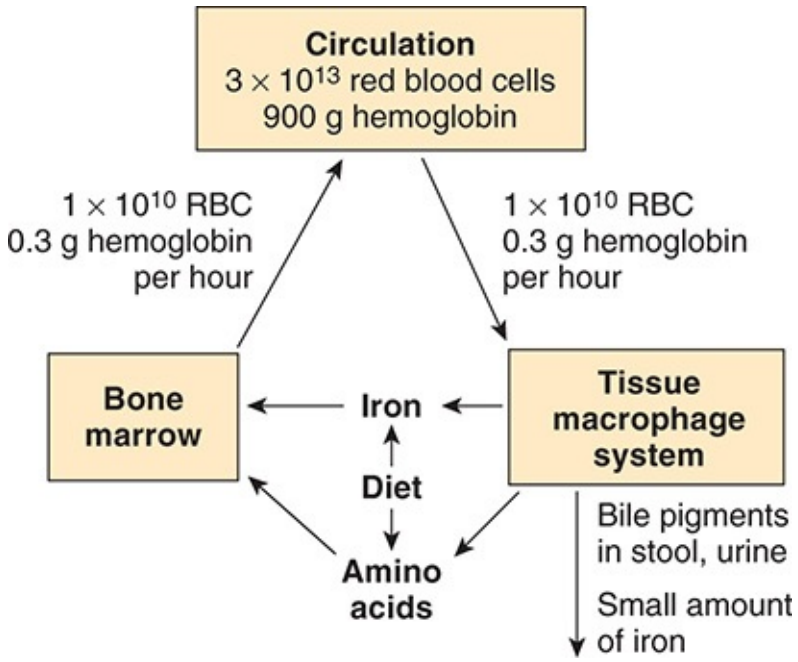


FIGURE 31–5 Red cell formation and destruction. RBC, red blood cells.

The feedback control of erythropoiesis by erythropoietin is discussed in Chapter 38, and the role of IL-1, IL-3, IL-6 (interleukin), and GM-CSF (granulocyte-macrophage colony-stimulating factor) in development of the relevant erythroid stem cells is shown in Figure 31–3.

ROLE OF THE SPLEEN

The spleen is an important blood filter that removes aged or abnormal red cells. It also contains many platelets and plays a significant role in the immune system. Abnormal red cells are removed if they are not as flexible as normal red cells and consequently are unable to squeeze through the slits between the endothelial cells that line the splenic sinuses **(Clinical Box 31–1)**.

HEMOGLOBIN

The red, oxygen-carrying pigment in the red blood cells of vertebrates is the protein **hemoglobin**. Hemoglobin is a globular molecule made up of four

subunits **(Figure 31–6)**. Each subunit contains a **heme** moiety conjugated to a polypeptide. Heme is an iron-containing porphyrin derivative **(Figure 31–7)**. The polypeptides are referred to collectively as the **globin** portion of the hemoglobin molecule. There are two pairs of polypeptides in each hemoglobin molecule. In normal adult human hemoglobin **(hemoglobin A),** the two polypeptides are called α chains and β chains. Thus, hemoglobin A is designated $\alpha_2\beta_2$. Not all the hemoglobin in the blood of normal adults is hemoglobin A. About 2.5% of the hemoglobin is hemoglobin A_2, in which β chains are replaced by δ chains ($\alpha_2\delta_2$).

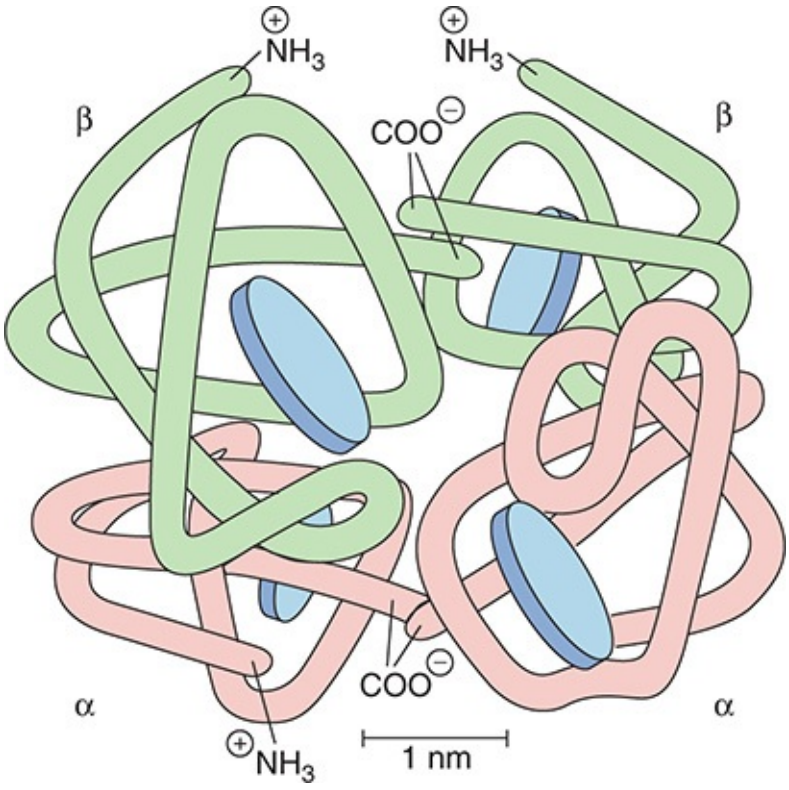


FIGURE 31–6 Diagrammatic representation of a molecule of hemoglobin A, showing the four subunits. There are two α and two β polypeptide chains, each containing a heme moiety. These moieties are represented by the blue disks. (Reproduced with permission from Harper HA, et al: *Physiologische Chemie*. Springer; 1975.)

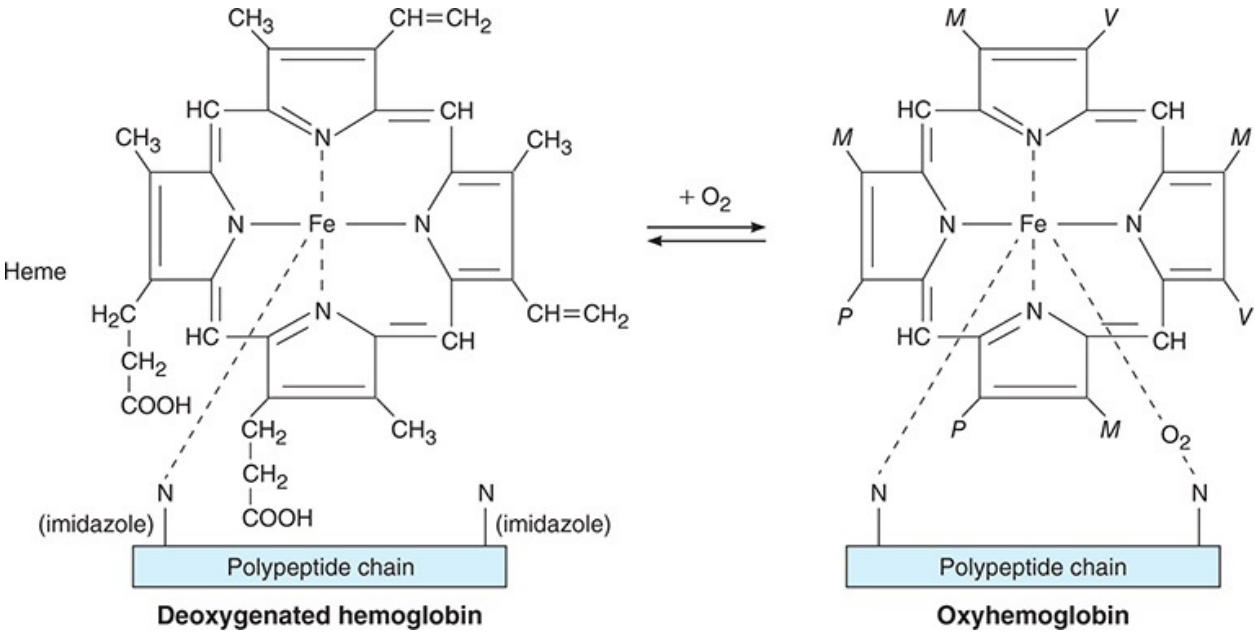


FIGURE 31–7 Reaction of heme with O_2. The abbreviations *M*, *V*, and *P* stand for the groups shown on the molecule on the left.

There are small amounts of hemoglobin A derivatives closely associated with hemoglobin A that represent glycated hemoglobins. One of these, hemoglobin A_{1c} (HbA_{1c}), has a glucose attached to the terminal valine in each β chain and is of special interest because it increases in the blood of patients with poorly controlled diabetes mellitus (see Chapter 24), and is measured clinically as a marker of the progression of that disease and/or the effectiveness of treatment.

REACTIONS OF HEMOGLOBIN

O_2 binds to the Fe^{2+} in the heme moiety of hemoglobin to form **oxyhemoglobin.** The affinity of hemoglobin for O_2 is affected by pH, temperature, and the concentration in the red cells of 2,3-bisphosphoglycerate (2,3-BPG). 2,3-BPG and H^+ compete with O_2 for binding to deoxygenated hemoglobin, decreasing the affinity of hemoglobin for O_2 by shifting the positions of the four peptide chains (quaternary structure). The details of the oxygenation and deoxygenation of hemoglobin and the physiologic role of these reactions in O_2 transport are discussed in Chapter 35.

CLINICAL BOX 31–1

Red Cell Fragility

Red blood cells, like other cells, shrink in solutions with an osmotic pressure greater than that of normal plasma. In solutions with a lower osmotic pressure they swell, become spherical rather than disk-shaped, and eventually lose their hemoglobin **(hemolysis).** The hemoglobin of hemolyzed red cells dissolves in the plasma, coloring it red. A 0.9% sodium chloride solution is isotonic with plasma. When **osmotic fragility** is normal, red cells begin to hemolyze when suspended in 0.5% saline; 50% lysis occurs in 0.40–0.42% saline, and lysis is complete in 0.35% saline. In **hereditary spherocytosis** (congenital hemolytic icterus), the cells are spherocytic in normal plasma and hemolyze more readily than normal cells in hypotonic sodium chloride solutions. Abnormal spherocytes are also trapped and destroyed in the spleen, meaning that hereditary spherocytosis is one of the most common causes of **hereditary hemolytic anemia.** The spherocytosis is caused by mutations in proteins that make up the membrane skeleton of the erythrocyte, which normally maintain the shape and flexibility of the red cell membrane, including **spectrin,** the transmembrane protein **band 3,** and the linker protein, **ankyrin.** Red cells can also be lysed by drugs (especially penicillin and sulfa drugs) and infections. The susceptibility of red cells to hemolysis by these agents is increased by deficiency of the enzyme glucose-6-phosphate dehydrogenase (G6PD), which catalyzes the initial step in the oxidation of glucose via the hexose monophosphate pathway (see Chapter 1). This pathway generates dihydronicotinamide adenine dinucleotide phosphate (NADPH), which is needed for the maintenance of normal red cell fragility. Severe G6PD deficiency also inhibits the killing of bacteria by granulocytes and predisposes to severe infections.

THERAPEUTIC HIGHLIGHTS

Severe cases of hereditary spherocytosis can be treated by splenectomy, but this is not without other risks, such as sepsis. Milder cases can be treated with dietary folate supplementation and/or blood transfusions. Treatment of other forms of hemolytic anemia depends on the underlying cause. Some forms are autoimmune in nature, and have been shown to benefit from treatment with corticosteroids.

When blood is exposed to various drugs and other oxidizing agents in vitro or in vivo, the ferrous iron (Fe^{2+}) that is normally present in hemoglobin is converted to ferric iron (Fe^{3+}), forming **methemoglobin.** Methemoglobin is dark-colored, and when it is present in large quantities in the circulation, it causes a dusky discoloration of the skin resembling cyanosis (see Chapter 35). Some oxidation of hemoglobin to methemoglobin occurs normally, but an enzyme system in the red cells, the dihydronicotinamide adenine dinucleotide (NADH)-methemoglobin reductase system, converts methemoglobin back to hemoglobin. Congenital absence of this system is one cause of hereditary methemoglobinemia.

Carbon monoxide reacts with hemoglobin to form **carbon monoxyhemoglobin (carboxyhemoglobin).** The affinity of hemoglobin for O_2 is much lower than its affinity for carbon monoxide, which consequently displaces O_2 on hemoglobin, reducing the oxygen-carrying capacity of blood (see Chapter 35).

HEMOGLOBIN IN THE FETUS

The blood of the human fetus normally contains **fetal hemoglobin (hemoglobin F).** Its structure is similar to that of hemoglobin A except that the β chains are replaced by γ chains; that is, hemoglobin F is $\alpha_2\gamma_2$. Fetal hemoglobin is normally replaced by adult hemoglobin soon after birth **(Figure 31–8)**. In certain individuals, it fails to disappear and persists throughout life. In the body, its O_2 content at a given PO_2 is greater than that of adult hemoglobin because it binds 2,3-BPG less avidly. Hemoglobin F is therefore critical to facilitate movement of O_2 from the maternal to the fetal circulation, particularly at later stages of gestation where oxygen demand increases (see Chapter 33). In young embryos there are, in addition, ζ and ε chains, forming Gower 1 hemoglobin ($\zeta_2\varepsilon_2$) and Gower 2 hemoglobin ($\alpha_2\varepsilon_2$). Switching from one form of hemoglobin to another during development seems to be regulated largely by oxygen availability, with relative hypoxia favoring the production of hemoglobin F both via direct effects on globin gene expression, as well as upregulated production of erythropoietin.

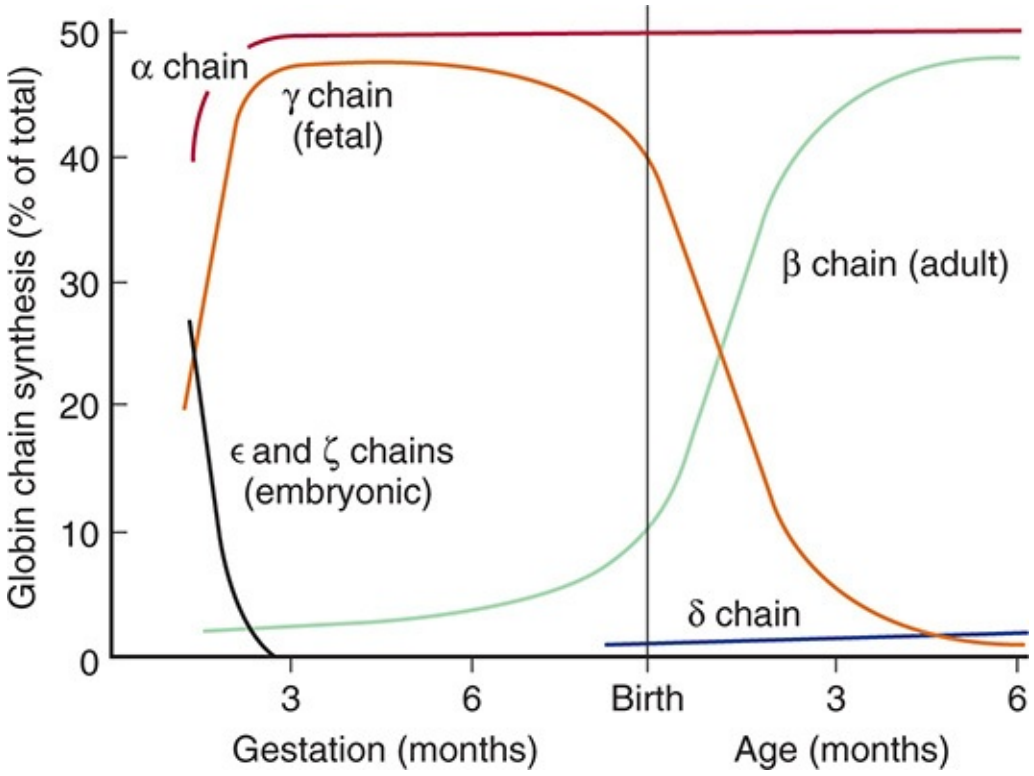


FIGURE 31–8 Development of human hemoglobin chains. The graph shows the normal rate of synthesis of the various hemoglobin chains in utero, and how these change after birth.

SYNTHESIS OF HEMOGLOBIN

The average normal hemoglobin content of blood is 16 g/dL in men and 14 g/dL in women, all of it in red cells. In the body of a 70-kg man, there are about 900 g of hemoglobin, and 0.3 g of hemoglobin is destroyed and 0.3 g synthesized every hour (Figure 31–5). The heme portion of the hemoglobin molecule is synthesized from glycine and succinyl-CoA **(Clinical Box 31–2)**.

CATABOLISM OF HEMOGLOBIN

When old red blood cells are destroyed by tissue macrophages, the globin portion of the hemoglobin molecule is split off, and the heme is converted to **biliverdin.** The enzyme involved is a subtype of heme oxygenase (see Figure 28–4), and CO is formed in the process. CO is an intercellular messenger, like NO (see Chapters 2 and 3). In humans, most of the biliverdin is converted to

bilirubin and excreted in the bile (see Chapter 28). The iron from the heme is reused for hemoglobin synthesis.

Exposure of the skin to white light converts bilirubin to lumirubin, which has a shorter half-life than bilirubin. **Phototherapy** (exposure to light) is of value in treating infants with jaundice due to hemolysis. Iron is essential for hemoglobin synthesis; if blood is lost from the body and the iron deficiency is not corrected, **iron deficiency anemia** results.

CLINICAL BOX 31–2

Abnormalities of Hemoglobin Production

There are two major types of inherited disorders of hemoglobin in humans: the **hemoglobinopathies,** in which abnormal globin polypeptide chains are produced, and the **thalassemias** and related disorders, in which the chains are normal in structure but produced in decreased amounts or absent because of defects in the regulatory portion of the globin genes. Mutant genes that cause the production of abnormal hemoglobins are widespread, and over 1000 abnormal hemoglobins have been described in humans. In one of the most common examples, hemoglobin S, the α chains are normal but the β chains have a single substitution of a valine residue for one glutamic acid, leading to **sickle cell anemia** (Table 31–3). When an abnormal gene inherited from one parent dictates formation of an abnormal hemoglobin (ie, when the individual is heterozygous), half the circulating hemoglobin is abnormal and half is normal. When identical abnormal genes are inherited from both parents, the individual is homozygous and all the hemoglobin is abnormal. It is theoretically possible to inherit two different abnormal hemoglobins, one from the father and one from the mother. Studies of the inheritance and geographic distribution of abnormal hemoglobins have made it possible in some cases to decide where the mutant gene originated and approximately how long ago the mutation occurred. In general, harmful mutations tend to die out, but mutant genes that confer traits with survival value persist and spread in the population. Many of the abnormal hemoglobins are harmless; however, some have abnormal O_2 equilibriums, while others cause anemia. For example, hemoglobin S polymerizes at low O_2 tensions, and this causes the red cells to become sickle-shaped, hemolyze, and form aggregates that block blood vessels. The sickle cell gene is an example of a gene that has persisted and spread in the population due to its beneficial effect when present

in heterozygous form. It originated in Africa, and confers resistance to one type of malaria. In some parts of Africa, 40% of the population is heterozygous for hemoglobin S. There is a corresponding prevalence of 10% among African Americans in the United States.

THERAPEUTIC HIGHLIGHTS

Hemoglobin F decreases the polymerization of deoxygenated hemoglobin S, and hydroxyurea stimulates production of hemoglobin F in children and adults. Hydroxyurea has therefore proven to be a very valuable agent for the treatment of sickle cell disease. In patients with severe sickle cell disease, hematopoietic stem cell transplantation has also been shown to have some benefit, and prophylactic treatment with antibiotics has also been shown to be helpful. The clinically important thalassemias result in severe anemia, often requiring repeated blood transfusions. However, these run the risk of iron overload, and often must be accompanied by treatment with drugs that chelate iron. Hematopoietic stem cell transplantation is also being explored for treatment of the thalassemias.

TABLE 31–3 Partial amino acid composition of normal human β chain, and some hemoglobins with abnormal β chains.[a]

	Positions on Polypeptide Chain of Hemoglobin						
Hemoglobin	**123**	**67**	**26**	**63**	**67**	**121**	**146**
A (normal)	Val-His-Leu	Glu-Glu	Glu	His	Val	Glu	His
S (sickle cell)		Val					
C		Lys					
$G_{San\ Jose}$		Gly					
E			Lys				
$M_{Saskatoon}$				Tyr			
$M_{Milwaukee}$					Glu		
O_{Arabia}						Lys	

[a]Other hemoglobins have abnormal α chains. Abnormal hemoglobins that are very similar electrophoretically but differ slightly in composition are indicated by the same letter and a subscript indicating the geographic location where they were first discovered; hence, $M_{Saskatoon}$ and $M_{Milwaukee}$.

PLASMA

The fluid portion of the blood, the **plasma,** is a remarkable solution containing an immense number of ions, inorganic molecules, and organic molecules that are in transit to various parts of the body or aid in the transport of other substances. Normal plasma volume is about 5% of body weight, or roughly 3500 mL in a 70-kg man. Plasma clots on standing, remaining fluid only if an anticoagulant is added. If whole blood is allowed to clot and the clot is removed, the remaining fluid is called **serum.** Serum has essentially the same composition as plasma, except that its fibrinogen and clotting factors II, V, and VIII **(Table 31–4)** have been removed and it has a higher serotonin content because of the breakdown of platelets during clotting.

TABLE 31–4 System for naming blood-clotting factors.

Factor[a]	Names
I	Fibrinogen
II	Prothrombin
III	Thromboplastin
IV	Calcium
V	Proaccelerin, labile factor, accelerator globulin
VII	Proconvertin, SPCA, stable factor
VIII	Antihemophilic factor (AHF), antihemophilic factor A, antihemophilic globulin (AHG)
IX	Plasma thromboplastic component (PTC), Christmas factor, antihemophilic factor B
X	Stuart-Prower factor
XI	Plasma thromboplastin antecedent (PTA), antihemophilic factor C
XII	Hageman factor, glass factor
XIII	Fibrin-stabilizing factor, Laki-Lorand factor
HMW-K	High-molecular-weight kininogen, Fitzgerald factor
Pre-Ka	Prekallikrein, Fletcher factor
Ka	Kallikrein
PL	Platelet phospholipid

[a]Factor VI is not a separate entity and has been dropped.

PLASMA PROTEINS

The plasma proteins consist of **albumin, globulin,** and **fibrinogen** fractions. Most capillary walls are relatively impermeable to the proteins in plasma, and the proteins therefore exert an osmotic force of about 25 mm Hg across the capillary wall (**oncotic pressure;** see Chapter 1) that pulls water into the blood. The plasma proteins are also responsible for 15% of the buffering capacity of proteins in the blood (including hemoglobin; see Chapter 39) because of the

weak ionization of their substituent COOH and NH_2 groups. At the normal plasma pH of 7.40, the proteins are mostly in the anionic form (see Chapter 1). Plasma proteins may have specific functions (eg, antibodies and the proteins concerned with blood clotting), whereas others function as nonspecific carriers for various hormones, other solutes, and drugs.

ORIGIN OF PLASMA PROTEINS

Circulating antibodies are manufactured by lymphocytes. Most of the other plasma proteins are synthesized in the liver. These proteins and their principal functions are listed in **Table 31–5**.

TABLE 31–5 Some of the proteins synthesized by the liver: Physiologic functions and properties.

Name	Principal Function	Binding Characteristics	Serum or Plasma Concentration
Albumin	Binding and carrier protein; osmotic regulator	Hormones, amino acids, steroids, vitamins, fatty acids	4500–5000 mg/dL
Orosomucoid	Uncertain; may have a role in inflammation		Trace; rises in inflammation
α_1-Antiprotease	Trypsin and general protease inhibitor	Proteases in serum and tissue secretions	1.3–1.4 mg/dL
α-Fetoprotein	Osmotic regulation; binding and carrier protein[a]	Hormones, amino acids	Found normally in fetal blood
α_2-Macroglobulin	Inhibitor of serum endoproteases	Proteases	150–420 mg/dL
Antithrombin-III	Protease inhibitor of intrinsic coagulation system	1:1 binding to proteases	17–30 mg/dL
Ceruloplasmin	Transport of copper	Six atoms copper/molecule	15–60 mg/dL
C-reactive protein	Uncertain; has role in tissue inflammation	Complement C1q	<1 mg/dL; rises in inflammation
Fibrinogen	Precursor to fibrin in hemostasis		200–450 mg/dL
Haptoglobin	Binding, transport of cell-free hemoglobin	Hemoglobin 1:1 binding	40–180 mg/dL
Hemopexin	Binds to porphyrins, particularly heme for heme recycling	1:1 with heme	50–100 mg/dL
Transferrin	Transport of iron	Two atoms iron/molecule	3.0–6.5 mg/dL
Apolipoprotein B	Assembly of lipoprotein particles	Lipid carrier	
Angiotensinogen	Precursor to pressor peptide angiotensin II		
Proteins, coagulation factors II, VII, IX, X	Blood clotting		20 mg/dL
Antithrombin C, protein C	Inhibition of blood clotting		
Insulin-like growth factor I	Mediator of anabolic effects of growth hormone	IGF-I receptor	
Steroid hormone-binding globulin	Carrier protein for steroids in bloodstream	Steroid hormones	3.3 mg/dL
Thyroxine-binding globulin	Carrier protein for thyroid hormone in bloodstream	Thyroid hormones	1.5 mg/dL
Transthyretin (thyroid-binding prealbumin)	Carrier protein for thyroid hormone in bloodstream	Thyroid hormones	25 mg/dL

[a]The function of α-fetoprotein is uncertain, but because of its structural homology to albumin it is often assigned these functions.

Data on the turnover of albumin show that synthesis plays an important role in the maintenance of normal levels. In normal adult humans, the plasma albumin level is 3.5–5.0 g/dL, and the total exchangeable albumin pool is 4.0–5.0 g/kg body weight; 38–45% of this albumin is intravascular, and much of the rest of it is in the skin. Between 6% and 10% of the exchangeable pool is degraded per day, and the degraded albumin is replaced by hepatic synthesis of 200–400 mg/kg/day. The albumin is probably transported to the extravascular areas by vesicular transport across the walls of the capillaries (see Chapter 2). Albumin synthesis is carefully regulated. It is decreased during fasting and increased in conditions such as nephrosis in which there is excessive albumin

loss.

HYPOPROTEINEMIA

Plasma protein levels are maintained during starvation until body protein stores are markedly depleted. However, in prolonged starvation and in malabsorption syndromes due to intestinal diseases, plasma protein levels are low **(hypoproteinemia).** They are also low in liver disease, because hepatic protein synthesis is depressed, and in nephrosis, because large amounts of albumin are lost in the urine. Because of the decrease in the plasma oncotic pressure, edema tends to develop. Rarely, there is congenital absence of one or another plasma protein. An example of congenital protein deficiency is the congenital form of **afibrinogenemia,** characterized by defective blood clotting.

LYMPH

Lymph is tissue fluid that enters the lymphatic vessels. It drains into the venous blood via the thoracic and right lymphatic ducts. It contains clotting factors and clots on standing in vitro. In most locations, it also contains proteins that have traversed capillary walls and can then return to the blood via the lymph. Nevertheless, its protein content is generally lower than that of plasma, which contains about 7 g/dL, but lymph protein content varies with the region from which the lymph drains **(Table 31–6)**. Water-insoluble fats are absorbed from the intestine into the lymphatics, and the lymph in the thoracic duct after a meal is milky because of its high fat content (see Chapter 26). Lymphocytes also enter the circulation principally through the lymphatics, and there are appreciable numbers of lymphocytes in thoracic duct lymph.

TABLE 31–6 Approximate protein content of lymph in humans.

Source of Lymph	Protein Content (g/dL)
Choroid plexus	0
Ciliary body	0
Skeletal muscle	2
Skin	2
Lung	4
Gastrointestinal tract	4.1
Heart	4.4
Liver	6.2

Data from JN Diana.

BLOOD TYPES

The membranes of human red cells contain a variety of **blood group antigens,** which are also called **agglutinogens.** The most important and best known of these are the A and B antigens, but there are many more.

THE ABO SYSTEM

The A and B antigens are inherited as mendelian dominants, and individuals are divided into four major **blood types** on this basis. Type A individuals have the A antigen, type B have the B, type AB have both, and type O have neither. The A and B antigens are complex oligosaccharides that differ in their terminal sugar. An *H* gene codes for a fucose transferase that adds a terminal fucose, forming the H antigen that is usually present in individuals of all blood types **(Figure 31–9)**. Individuals who are type A also express a second transferase that catalyzes placement of a terminal N-acetylgalactosamine on the H antigen, whereas individuals who are type B express a transferase that places a terminal galactose. Individuals who are type AB have both transferases. Individuals who are type O have neither, so the H antigen persists.

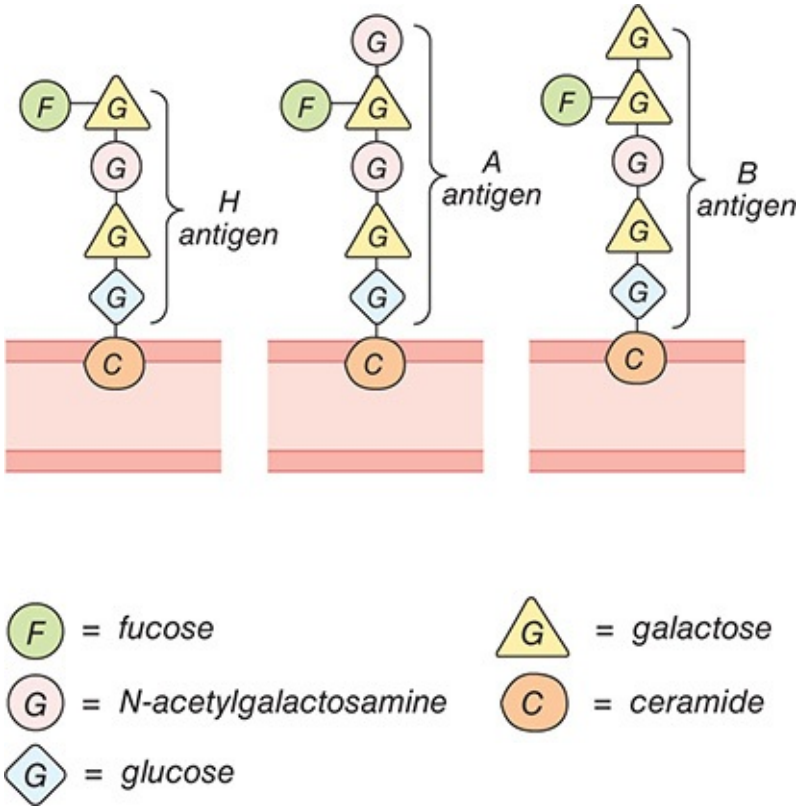


FIGURE 31–9 Antigens of the ABO system on the surface of red blood cells.

Antibodies against red cell agglutinogens are called **agglutinins.** Antigens very similar to A and B are common in intestinal bacteria and possibly in foods to which newborn individuals are exposed. Therefore, infants rapidly develop antibodies against the antigens not present in their own cells. Thus, type A individuals develop anti-B antibodies, type B individuals develop anti-A antibodies, type O individuals develop both, and type AB individuals develop neither **(Table 31–7)**. When the plasma of a type A individual is mixed with type B red cells, the anti-B antibodies cause the type B red cells to clump (agglutinate), as shown in **Figure 31–10**. The other agglutination reactions produced by mismatched plasma and red cells are summarized in Table 31–7. ABO **blood typing** is performed by mixing an individual's red blood cells with antisera containing the various agglutinins on a slide and seeing whether agglutination occurs.

TABLE 31–7 Summary of ABO system.

Blood Type	Agglutinins in Plasma	Frequency in United States (%)	Plasma Agglutinates Red Cells of Type:
O	Anti-A, anti-B	45	A, B, AB
A	Anti-B	41	B, AB
B	Anti-A	10	A, AB
AB	None	4	None

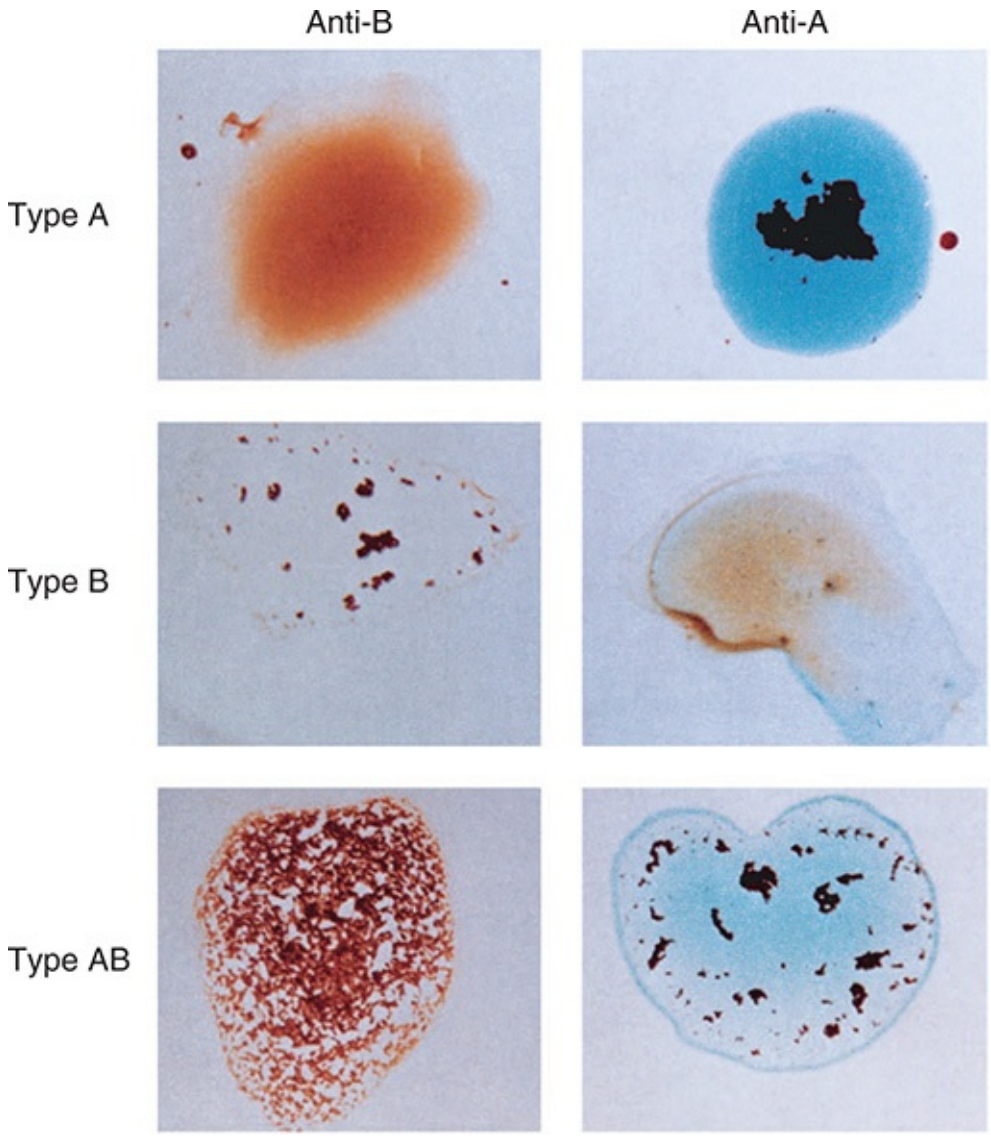


FIGURE 31–10 Red cell agglutination in incompatible plasma.

TRANSFUSION REACTIONS

Dangerous **hemolytic transfusion reactions** occur when blood is transfused into an individual with an incompatible blood type; that is, an individual who has agglutinins against the red cells in the transfusion. The plasma in the transfusion is usually so diluted in the recipient that it rarely causes agglutination even when the titer of agglutinins against the recipient's cells is high. However, when the recipient's plasma has agglutinins against the donor's red cells, the cells agglutinate and hemolyze. Free hemoglobin is liberated into the plasma. The severity of the resulting transfusion reaction may vary from an asymptomatic minor rise in the plasma bilirubin level to severe jaundice and renal tubular damage leading to anuria and death.

Incompatibilities in the ABO blood group system are summarized in Table 31–7. Persons with type AB blood are "universal recipients" because they have no circulating agglutinins and can be given blood of any type without developing a transfusion reaction due to ABO incompatibility. Type O individuals are "universal donors" because they lack A and B antigens, and type O blood can be given to anyone without producing a transfusion reaction due to ABO incompatibility. This does not mean, however, that blood should ever be transfused without being cross-matched except in the most extreme emergencies, since the possibility of reactions or sensitization due to incompatibilities in systems other than ABO systems always exists. In cross-matching, donor red cells are mixed with recipient plasma on a slide and checked for agglutination.

A procedure that has recently become popular is to withdraw the patient's own blood in advance of elective surgery and then infuse this blood back **(autologous transfusion)** if a transfusion is needed during the surgery. With iron treatment, 1000–1500 mL can be withdrawn over a 3-week period. The popularity of banking one's own blood is primarily due to fear of transmission of infectious diseases by heterologous transfusions, but of course another advantage is elimination of the risk of transfusion reactions.

OTHER AGGLUTINOGENS

In addition to the ABO system of antigens in human red cells, there are systems such as the Rh, MNSs, Lutheran, Kell, Kidd, and many others. There are over 500 billion possible known blood group phenotypes, and because undiscovered antigens undoubtedly exist, it has been calculated that the number of phenotypes is actually in the trillions.

The number of blood groups in animals is as large as it is in humans. An interesting question is why this degree of polymorphism developed and has persisted through evolution. Certain diseases are more common in individuals with one blood type or another, but the differences are not great. The significance of a recognition code of this complexity is therefore unknown.

THE RH GROUP

Aside from the antigens of the ABO system, those of the Rh system are of the greatest clinical importance. The Rh factor, named for the rhesus monkey because it was first studied using the blood of this animal, is a system composed primarily of the C, D, and E antigens, although it actually contains many more. Unlike the ABO antigens, the system has not been detected in tissues other than red cells. D is by far the most antigenic component, and the term Rh-positive as it is generally used means that the individual has agglutinogen D. The Rh-negative individual has no D antigen and forms the anti-D agglutinin when injected with D-positive cells. The Rh typing serum used in routine blood typing is anti-D serum. Eighty-five percent of whites are D-positive and 15% are D-negative; over 99% of Asians are D-positive. Unlike the antibodies of the ABO system, anti-D antibodies do not develop without exposure of a D-negative individual to D-positive red cells by transfusion or entrance of fetal blood into the maternal circulation. However, D-negative individuals who have received a transfusion of D-positive blood (even years previously) can have appreciable anti-D titers and thus may develop transfusion reactions when transfused again with D-positive blood.

HEMOLYTIC DISEASE OF THE NEWBORN

Another complication due to Rh incompatibility arises when an Rh-negative mother carries an Rh-positive fetus. Small amounts of fetal blood leak into the maternal circulation at the time of delivery, and some mothers develop significant titers of anti-Rh agglutinins during the postpartum period. During the next pregnancy, the mother’s agglutinins cross the placenta to the fetus. When anti-Rh agglutinins cross the placenta to an Rh-positive fetus, they can cause hemolysis and various forms of **hemolytic disease of the newborn (erythroblastosis fetalis).** If hemolysis in the fetus is severe, the infant may die in utero or may develop anemia, severe jaundice, and edema **(hydrops fetalis). Kernicterus,** a neurologic syndrome in which unconjugated bilirubin is

deposited in the basal ganglia, may also develop, especially if birth is complicated by a period of hypoxia. Bilirubin rarely penetrates the brain in adults, but it does in infants with erythroblastosis, possibly in part because the blood-brain barrier is more permeable in infancy. However, the main reasons that the concentration of unconjugated bilirubin is very high in this condition are that production is increased and the bilirubin-conjugating system is not yet mature.

About 50% of Rh-negative individuals are sensitized (develop an anti-Rh titer) by transfusion of Rh-positive blood. Because sensitization of Rh-negative mothers by carrying an Rh-positive fetus generally occurs at birth, the first child is usually normal. However, hemolytic disease occurs in about 17% of the Rh-positive fetuses born to Rh-negative mothers who have previously been pregnant one or more times with Rh-positive fetuses. Fortunately, it is usually possible to prevent sensitization from occurring the first time by administering a single dose of anti-Rh antibodies in the form of Rh immune globulin during the postpartum period. Such passive immunization does not harm the mother and has been demonstrated to prevent active antibody formation by the mother. In obstetric clinics, the institution of such treatment on a routine basis to unsensitized Rh-negative women who have delivered an Rh-positive baby has reduced the overall incidence of hemolytic disease by more than 90%. In addition, fetal Rh typing with material obtained by amniocentesis or chorionic villus sampling is now possible, and treatment with a small dose of Rh immune serum will prevent sensitization during pregnancy.

HEMOSTASIS

Hemostasis is the process of forming clots in the walls of damaged blood vessels and preventing blood loss while maintaining blood in a fluid state within the vascular system. A collection of complex interrelated systemic mechanisms operates to maintain a balance between coagulation and anticoagulation.

RESPONSE TO INJURY

When a small blood vessel is transected or damaged, the injury initiates a series of events **(Figure 31–11)** that lead to the formation of a clot. This seals off the damaged region and prevents further blood loss. A temporary **hemostatic plug** of platelets is triggered when platelets bind to collagen and aggregate. There is also constriction of the vessel, with constriction of an injured arteriole or small

artery occasionally so marked that its lumen is obliterated, at least temporarily. The vasoconstriction is due to serotonin (5-HT) and other vasoconstrictors liberated from the platelets adhered to the walls of the damaged vessel. This is then followed by conversion of the plug into the definitive clot.

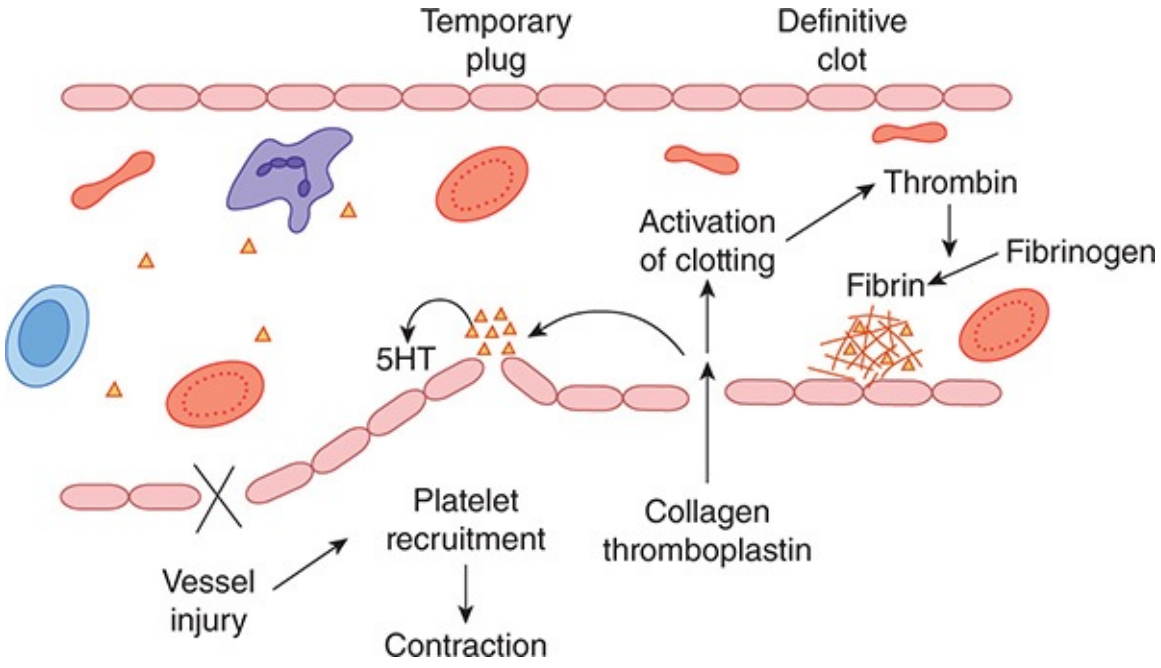


FIGURE 31–11 Summary of reactions involved in hemostasis. Injury to a blood vessel exposes collagen and thromboplastin, recruiting platelets to the site of injury to form a temporary plug. Platelets release 5-hydroxytryptamine, among other factors, resulting in smooth muscle contraction and vasoconstriction. Activation of the clotting cascade in response to collagen and thromboplastin activates thrombin, which converts circulating fibrinogen to fibrin monomers. Fibrin monomers polymerize and are cross-linked and accumulate with platelets at the site of injury to form the definitive clot.

THE CLOTTING MECHANISM

The loose aggregation of platelets in the temporary plug is bound together and converted into the definitive clot by **fibrin.** Fibrin formation involves a cascade of enzymatic reactions and a series of numbered clotting factors (Table 31–4). The fundamental reaction is conversion of the soluble plasma protein fibrinogen to insoluble fibrin **(Figure 31–12)**. The process involves the release of two pairs of polypeptides from each fibrinogen molecule. The remaining portion, **fibrin monomer,** then polymerizes with other monomer molecules to form **fibrin.** The

fibrin is initially a loose mesh of interlacing strands. It is converted by the formation of covalent cross-linkages to a dense, tight aggregate (stabilization). This latter reaction is catalyzed by activated factor XIII and requires Ca^{2+}.

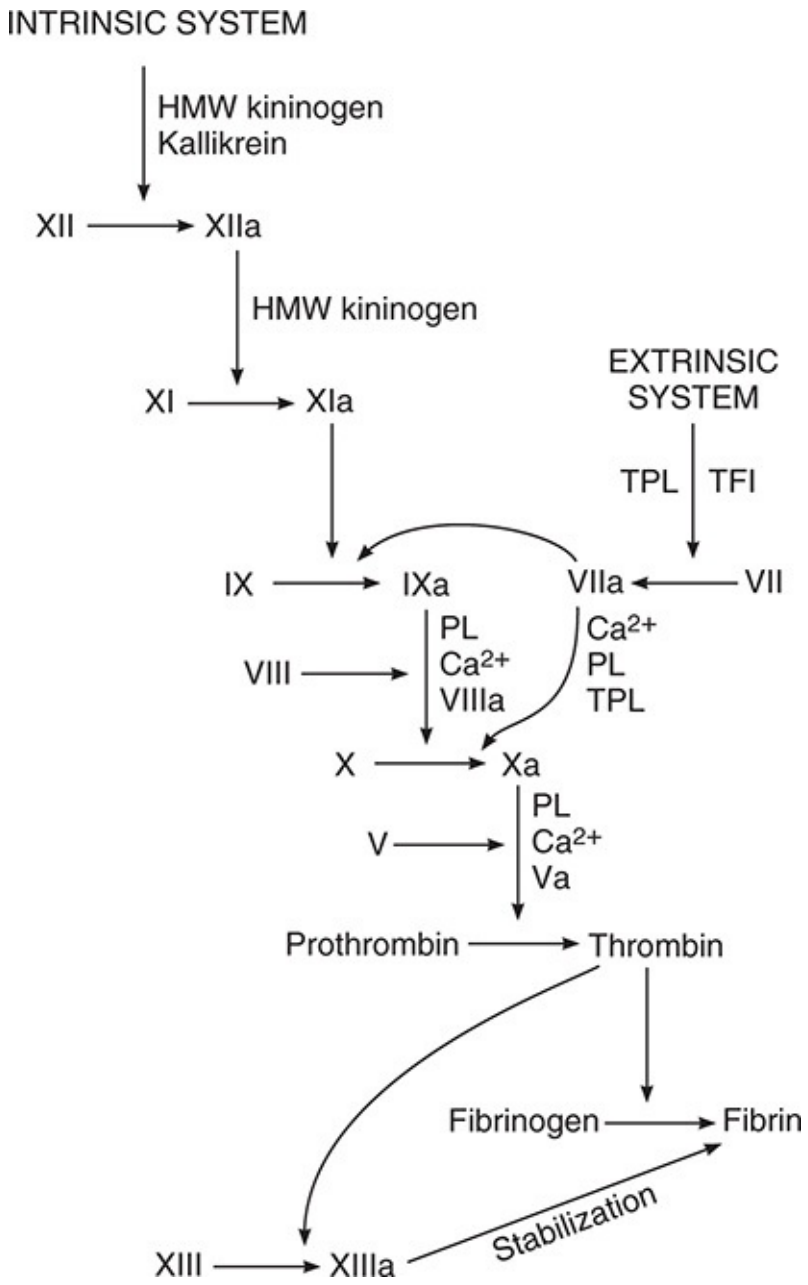


FIGURE 31–12 The clotting mechanism. a, active form of clotting factor. TFI, tissue factor pathway inhibitor; TPL, tissue thromboplastin. For other abbreviations, see Table 31–4.

The conversion of fibrinogen to fibrin is catalyzed by thrombin. Thrombin is

a serine protease that is formed from its circulating precursor, prothrombin, by the action of activated factor X. It has additional actions, including activation of platelets, endothelial cells, and leukocytes via so-called proteinase activated receptors, which are G-protein-coupled.

Factor X can be activated by either of two systems, known as intrinsic and extrinsic (Figure 31–12). The initial reaction in the **intrinsic system** is conversion of inactive factor XII to active factor XII (XIIa). This activation, which is catalyzed by high-molecular-weight kininogen and kallikrein (see Chapter 32), can be brought about in vitro by exposing the blood to glass, or in vivo by collagen fibers underlying the endothelium. Active factor XII then activates factor XI, and active factor XI activates factor IX. Activated factor IX forms a complex with active factor VIII, which is activated when it is separated from von Willebrand factor. The complex of IXa and VIIIa activate factor X. Phospholipids from aggregated platelets (PL) and Ca^{2+} are necessary for full activation of factor X. The **extrinsic system** is triggered by the release of tissue thromboplastin (TPL), a protein–phospholipid mixture that activates factor VII. Tissue thromboplastin and factor VII activate factors IX and X. In the presence of PL, Ca^{2+}, and factor V, activated factor X catalyzes the conversion of prothrombin to thrombin. The extrinsic pathway is inhibited by a **tissue factor pathway inhibitor** (TFI) that forms a quaternary structure with TPL, factor VIIa, and factor Xa.

ANTICLOTTING MECHANISMS

The tendency of blood to clot is balanced in vivo by reactions that prevent clotting inside the blood vessels, break down any clots that do form, or both. These reactions include the interaction between the platelet-aggregating effect of thromboxane A_2 and the antiaggregating effect of prostacyclin, which causes clots to form at the site when a blood vessel is injured but keeps the vessel lumen free of clot (see Chapter 32 and **Clinical Box 31–3**).

Antithrombin III is a circulating protease inhibitor that binds to serine proteases in the coagulation system, blocking their activity as clotting factors. This binding is facilitated by **heparin,** a naturally occurring anticoagulant that is a mixture of sulfated polysaccharides. The clotting factors that are inhibited are the active forms of factors IX, X, XI, and XII.

CLINICAL BOX 31–3

Abnormalities of Hemostasis

In addition to clotting abnormalities due to platelet disorders, hemorrhagic diseases can be produced by selective deficiencies of most of the clotting factors **(Table 31–8)**. Hemophilia A, which is caused by factor VIII deficiency, is relatively common. von Willebrand factor deficiency likewise causes a bleeding disorder (von Willebrand disease) by reducing platelet adhesion and by lowering plasma factor VIII. The condition can be congenital or acquired. The large von Willebrand molecule is subject to cleavage and resulting inactivation by the plasma metalloprotease ADAM-13 in vascular areas where fluid shear stress is elevated. Finally, when absorption of vitamin K is depressed along with absorption of other fat-soluble vitamins (see Chapter 26), the resulting clotting factor deficiencies may cause the development of a significant bleeding tendency.

Formation of clots inside blood vessels is called **thrombosis** to distinguish it from the normal extravascular clotting of blood. Thromboses are a major medical problem. They are particularly prone to occur where blood flow is sluggish because the slow flow permits activated clotting factors to accumulate instead of being washed away. They also occur in vessels when the intima is damaged by atherosclerotic plaques, and over areas of damage to the endocardium. They frequently occlude the arterial supply to the organs in which they form, and bits of thrombus **(emboli)** sometimes break off and travel in the bloodstream to distant sites, damaging other organs. An example is obstruction of the pulmonary artery or its branches by thrombi from the leg veins **(pulmonary embolism).** Congenital absence of protein C leads to uncontrolled intravascular coagulation and, in general, death in infancy. If this condition is diagnosed and treatment is instituted, the coagulation defect disappears. Resistance to activated protein C is another cause of thrombosis, and this condition is common. It is due to a point mutation in the gene for factor V, which prevents activated protein C from inactivating the factor.

Disseminated intravascular coagulation is another serious complication of septicemia, extensive tissue injury, and other diseases in which fibrin is deposited in the vascular system and many small- and medium-sized vessels are thrombosed. The increased consumption of platelets and coagulation factors causes bleeding to occur at the same time. The cause of the condition appears to be increased generation of thrombin due to increased TPL activity without adequate TFI activity.

THERAPEUTIC HIGHLIGHTS

Hemophilia has been treated with factor VIII–rich preparations made from plasma, or, more recently, factor VIII produced by recombinant DNA techniques. Some patients with von Willebrand disease are treated with desmopressin, which stimulates production of factor VIII, particularly prior to dental procedures or surgery. Thrombotic disorders, on the other hand, are treated with anticoagulants such as heparin.

TABLE 31–8 Examples of diseases due to deficiency of clotting factors.

Deficiency of Factor	Clinical Syndrome	Cause
I	Afibrinogenemia	Depletion during pregnancy with premature separation of placenta; also congenital (rare)
II	Hypoprothrombinemia (hemorrhagic tendency in liver disease)	Decreased hepatic synthesis, usually secondary to vitamin K deficiency
V	Parahemophilia	Congenital
VII	Hypoconvertinemia	Congenital
VIII	Hemophilia A (classic hemophilia)	Congenital defect due to various abnormalities of the gene on X chromosome that codes for factor VIII; disease is therefore inherited as a sex-linked characteristic
IX	Hemophilia B (Christmas disease)	Congenital
X	Stuart-Prower factor deficiency	Congenital
XI	PTA deficiency	Congenital
XII	Hageman trait	Congenital

The endothelium of the blood vessels also plays an active role in preventing the extension of clots. All endothelial cells except those in the cerebral microcirculation produce **thrombomodulin,** a thrombin-binding protein, on their surfaces. In circulating blood, thrombin is a procoagulant that activates factors V and VIII, but when it binds to thrombomodulin, it becomes an anticoagulant in that the thrombomodulin-thrombin complex activates protein C **(Figure 33–13)**. Activated protein C (APC), along with its cofactor protein S, inactivates factors V and VIII and inactivates an inhibitor of tissue plasminogen activator,

increasing the formation of plasmin.

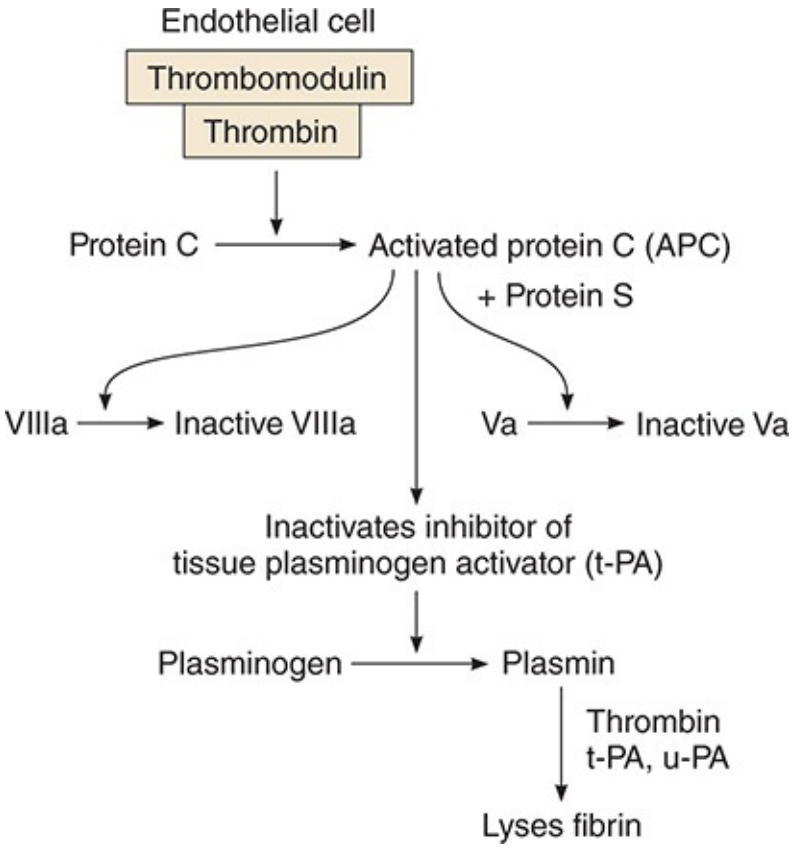


FIGURE 31–13 The fibrinolytic system and its regulation by protein C. u-PA, urokinase-type plasminogen activator.

Plasmin (fibrinolysin) is the active component of the **plasminogen (fibrinolytic) system** (Figure 31–13). This enzyme lyses fibrin and fibrinogen, with the production of fibrinogen degradation products (FDPs) that inhibit thrombin. Plasmin is formed from its inactive precursor, plasminogen, by the action of thrombin and **tissue-type plasminogen activator (t-PA).** Plasminogen is converted to active plasmin when t-PA hydrolyzes the bond between Arg 560 and Val 561. It is also activated by **urokinase-type plasminogen activator (u-PA).** If the t-PA gene or the u-PA gene is knocked out in mice, some fibrin deposition occurs and clot lysis is slowed. However, when both are knocked out, spontaneous fibrin deposition is extensive.

Plasminogen receptors are located on the surfaces of many different types of cells and are plentiful on endothelial cells. When plasminogen binds to its receptors, it becomes activated, so intact blood vessel walls are provided with a mechanism that discourages clot formation.

Human t-PA is produced by recombinant DNA techniques for clinical use in myocardial infarction and stroke. Streptokinase, a bacterial enzyme, is also fibrinolytic and is also used in the treatment of early myocardial infarction (see Chapter 33).

ANTICOAGULANTS

As noted above, heparin is a naturally occurring anticoagulant that facilitates the action of antithrombin III. Low-molecular-weight fragments have been produced from unfractionated heparin, and these are seeing increased clinical use because they have a longer half-life and produce a more predictable anticoagulant response than unfractionated heparin. The highly basic protein protamine forms an irreversible complex with heparin and is used clinically to neutralize heparin.

In vivo, a plasma Ca^{2+} level low enough to interfere with blood clotting is incompatible with life, but clotting can be prevented in vitro if Ca^{2+} is removed from the blood by the addition of substances such as oxalates, which form insoluble salts with Ca^{2+}, or **chelating agents,** which bind Ca^{2+}. Coumarin derivatives such as **dicumarol** and **warfarin** are also effective anticoagulants. They inhibit the action of vitamin K, which is a necessary cofactor for the enzyme that catalyzes the conversion of glutamic acid residues to γ-carboxyglutamic acid residues. Factors II (prothrombin), VII, IX, and X, protein C, and protein S (see above) require conversion of a number of glutamic acid residues to γ-carboxyglutamic acid residues before being released into the circulation, and hence all six are vitamin K–dependent.

STRUCTURAL FEATURES OF THE CIRCULATION

Here, we will discuss the two major cell types that make up the blood vessels and how they are arranged into the various vessel types that subserve the needs of the circulation.

ENDOTHELIUM

Located between the circulating blood and the media and adventitia of the blood vessels, the endothelial cells constitute a large and important organ. They

respond to flow changes, stretch, a variety of circulating substances, and inflammatory mediators. They secrete growth regulators and vasoactive substances (see below and Chapter 32).

VASCULAR SMOOTH MUSCLE

The smooth muscle in blood vessel walls has been one of the most-studied forms of visceral smooth muscle because of its importance in the regulation of blood pressure and hypertension. The membranes of the muscle cells contain various types of K^+, Ca^{2+}, and Cl^- channels. Contraction is produced primarily by the myosin light chain mechanism described in Chapter 5. However, vascular smooth muscle also undergoes prolonged contractions that determine vascular tone. These may be due in part to the latch-bridge mechanism (see Chapter 5), but other factors also play a role. Some of the molecular mechanisms that appear to be involved in contraction and relaxation are shown in **Figure 31–14**.

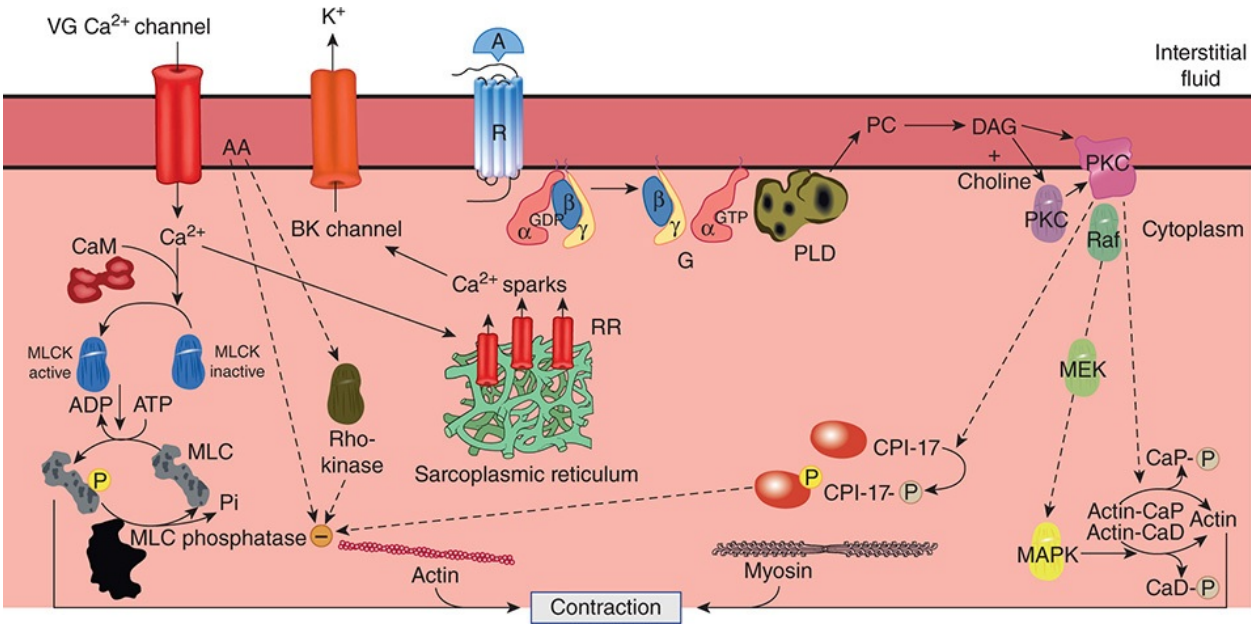


FIGURE 31–14 Some of the established and postulated mechanisms involved in the contraction and relaxation of vascular smooth muscle. A, agonist; AA, arachidonic acid; BK, Ca^+-activated K^+ channel; G, heterotrimeric G-protein; MLC, myosin light chain; MLCK, myosin light chain kinase; PLD, phospholipase D; R, receptor; RR, ryanodine receptors; SR, sarcoplasmic reticulum; VGCC, voltage-gated Ca^{2+} channel. For other abbreviations, see Chapter 2.

Vascular smooth muscle cells provide an interesting example of the way high and low cytosolic Ca^{2+} can have different and even opposite effects (see Chapter 2). In these cells, influx of Ca^{2+} via voltage-gated Ca^{2+} channels produces a diffuse increase in cytosolic Ca^{2+} that initiates contraction. However, the Ca^{2+} influx also initiates Ca^{2+} release from the sarcoplasmic reticulum via ryanodine receptors (see Chapter 5), and the high local Ca^{2+} concentration produced by these Ca^{2+} sparks increases the activity of **Ca^{2+}-activated K^+ channels** in the cell membrane. These are also known as big K or **BK channels** because K^+ flows through them at a high rate. The resulting K^+ efflux increases the membrane potential, shutting off voltage-gated Ca^{2+} channels and producing relaxation. The site of action of the Ca^{2+} sparks is the β_1-subunit of the BK channel, and mice in which this subunit is knocked out develop increased vascular tone and blood pressure.

ARTERIES & ARTERIOLES

The characteristics of the various types of blood vessels are listed in **Table 31–9**. The walls of all arteries are made up of an outer layer of connective tissue, the adventitia; a middle layer of smooth muscle, the media; and an inner layer, the intima, made up of the endothelium and underlying connective tissue **(Figure 31–15)**. The walls of the aorta and other large diameter arteries contain a relatively large amount of elastic tissue, primarily located in the inner and external elastic laminas. They are stretched during systole and recoil on the blood during diastole. The walls of the arterioles contain less elastic tissue but much more smooth muscle. The muscle is innervated by noradrenergic nerve fibers, which function as constrictors, and in some instances by cholinergic fibers, which dilate the vessels. The arterioles are the major site of the resistance to blood flow, and small changes in their caliber cause large changes in the total peripheral resistance.

TABLE 31–9 Characteristics of various types of blood vessels in humans.

Vessel	Lumen Diameter	Wall Thickness	All Vessels of Each Type	
			Approximate Total Cross-Sectional Area (cm^2)	Percentage of Blood Volume Contained[a]
Aorta	2.5 cm	2 mm	4.5	2
Artery	0.4 cm	1 mm	20	8
Arteriole	30 µm	20 µm	400	1
Capillary	5 µm	1 µm	4500	5
Venule	20 µm	2 µm	4000	
Vein	0.5 cm	0.5 mm	40	54
Vena cava	3 cm	1.5 mm	18	

[a]In systemic vessels; there is an additional 12% in the heart and 18% in the pulmonary circulation.

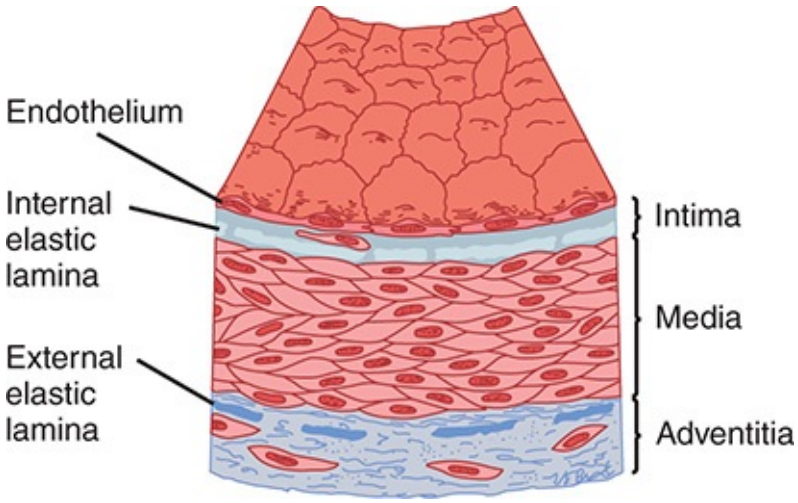


FIGURE 31–15 Structure of a normal muscle artery. (Reproduced with permission from Ross R, Glomset JA: The pathogenesis of atherosclerosis. N Engl J Med 1976; August 12; 295(7):369–377.)

CAPILLARIES

The arterioles divide into smaller muscle-walled vessels called **metarterioles,** and these in turn feed into capillaries **(Figure 31–16)**. The openings of the capillaries are surrounded on the upstream side by minute smooth muscle **precapillary sphincters.** It is unsettled whether the metarterioles are innervated, and it appears that the precapillary sphincters are not. However, they can of course respond to local or circulating vasoconstrictor substances. The capillaries are about 5 µm in diameter at the arterial end and 9 µm in diameter at the venous end. When the sphincters are dilated, the diameter of the capillaries is just sufficient to permit red blood cells to squeeze through in “single file.” As they

pass through the capillaries, the red cells become thimble- or parachute-shaped, with the flow pushing the center ahead of the edges. This configuration appears to be simply due to the pressure in the center of the vessel, whether or not the edges of the red blood cell are in contact with the capillary walls.

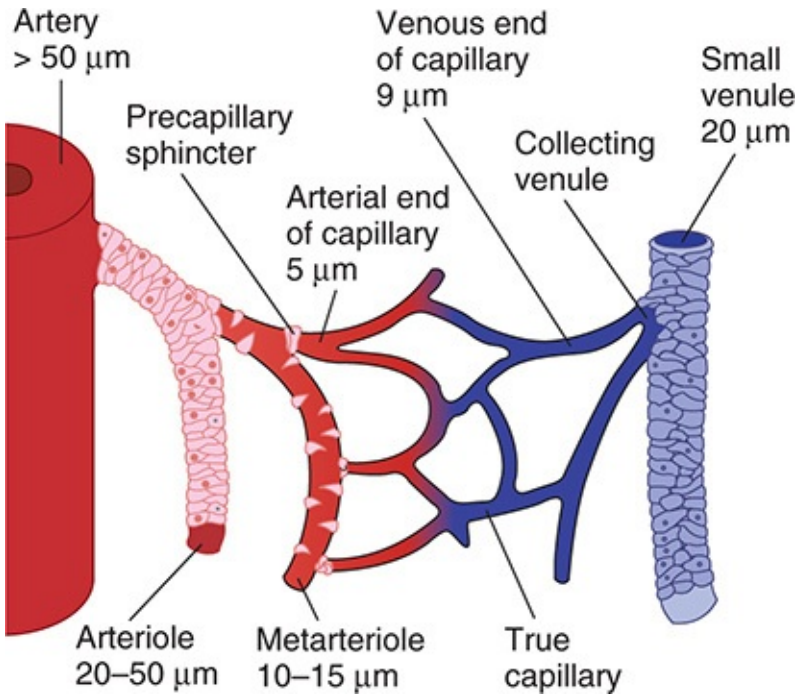


FIGURE 31–16 The microcirculation. Arterioles give rise to metarterioles, which give rise to capillaries. The capillaries drain via short collecting venules to the venules. The walls of the arteries, arterioles, and small venules contain relatively large amounts of smooth muscle. There are scattered smooth muscle cells in the walls of the metarterioles, and the openings of the capillaries are guarded by muscular precapillary sphincters. The diameters of the various vessels are also shown. (Used with permission of JN Diana.)

The total area of all the capillary walls in the body exceeds 6300 m^2 in the adult. The walls, which are about 1 µm thick, are made up of a single layer of endothelial cells. The structure of the walls varies from organ to organ. In many beds, including those in skeletal, cardiac, and smooth muscle, the junctions between the endothelial cells **(Figure 31–17)** permit the passage of molecules up to 10 nm in diameter. It also appears that plasma and its dissolved proteins can be taken up by endocytosis, transported across the endothelial cells, and discharged by exocytosis (**vesicular transport;** see Chapter 2). However, this process can account for only a small portion of the transport across the

endothelium. In the brain, the capillaries resemble the capillaries in muscle, but the junctions between endothelial cells are tighter, and transport across them is largely limited to small molecules (although pathological conditions may open these junctions). In most endocrine glands, the intestinal villi, and parts of the kidneys, on the other hand, the cytoplasm of the endothelial cells is attenuated to form gaps called **fenestrations.** These fenestrations are 20–100 nm in diameter and may be opened to permit the passage of larger molecules, although their permeability is likely significantly reduced under normal circumstances by a thick layer of endothelial glycocalyx. An exception to this, however, is found in the liver, where the sinusoidal capillaries are extremely porous, the endothelium is discontinuous, and gaps occur between endothelial cells that are not closed by membranes (see Figure 28–2). Some of the gaps are 600 nm in diameter, and others may be as large as 3000 nm. They therefore permit the passage of large molecules, including plasma proteins, which is important for hepatic function (see Chapter 28). The permeability of capillaries in various parts of the body, expressed in terms of their hydraulic conductivity, are summarized in **Table 31–10**.

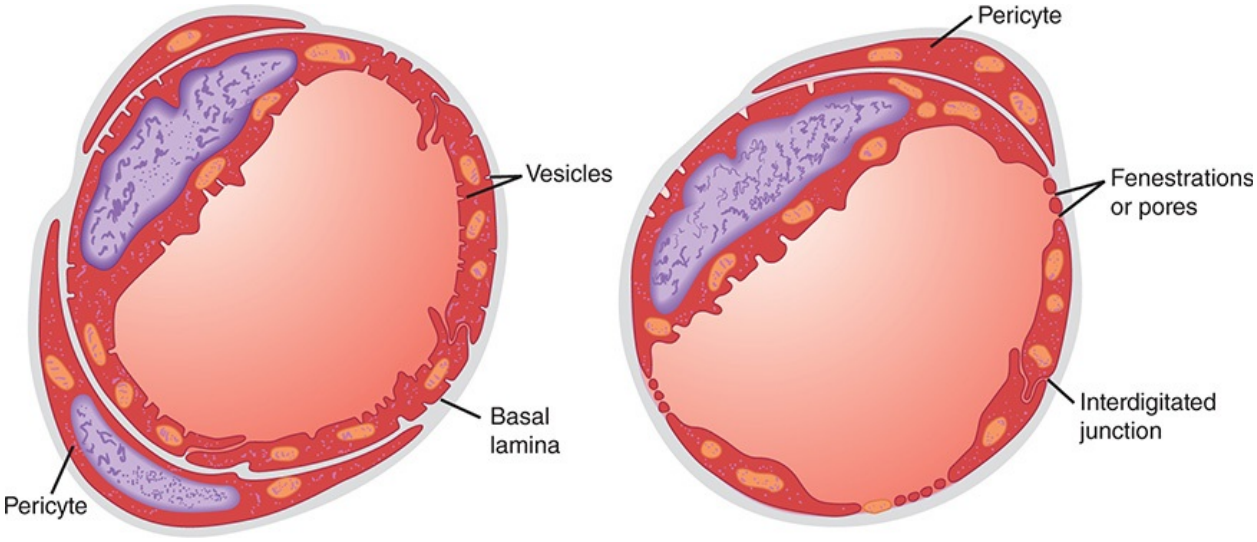


FIGURE 31–17 Cross-sections of capillaries. Left: Type of capillary found in muscle. **Right:** Fenestrated type of capillary. (Reproduced with permission from Orbison JL and D Smith, editors: *Peripheral Blood Vessels*. Baltimore: Williams & Wilkins, 1962.)

TABLE 31–10 Hydraulic conductivity of capillaries in various parts of the body.

Organ	Conductivity[a]	Type of Endothelium
Brain (excluding circumventricular organs)	3	Continuous
Skin	100	
Skeletal muscle	250	
Lung	340	
Heart	860	
Gastrointestinal tract (intestinal mucosa)	13,000	Fenestrated
Glomerulus in kidney	15,000	

[a]Units of conductivity are 10^{-13} cm^3 s^{-1} $dyne^{-1}$.

Data from JN Diana.

Capillaries and postcapillary venules have **pericytes** around their endothelial cells (Figure 31–17). These cells have long processes that wrap around the vessels. They are contractile and release a wide variety of vasoactive agents. They also synthesize and release constituents of the basement membrane and extracellular matrix. One of their physiologic functions appears to be regulation of flow through the junctions between endothelial cells, particularly in the presence of inflammation. They are closely related to the mesangial cells in the renal glomeruli (see Chapter 37).

LYMPHATICS

The lymphatics serve to collect plasma and its constituents that have exuded from the capillaries into the interstitial space (ie, the lymph). They drain from the body tissues via a system of vessels that coalesce and eventually enter the right and left subclavian veins at their junctions with the respective internal jugular veins. The lymph vessels contain valves and regularly traverse lymph nodes along their course. The ultrastructure of the small lymph vessels differs from that of the capillaries in several details: No fenestrations are visible in the lymphatic endothelium; very little if any basal lamina is present under the endothelium; and the junctions between endothelial cells are open, with no tight intercellular connections.

ARTERIOVENOUS ANASTOMOSES

In the fingers, palms, and ear lobes, short channels connect arterioles to venules, bypassing the capillaries. These **arteriovenous (A-V) anastomoses,** or **shunts,** have thick, muscular walls and are abundantly innervated, presumably by vasoconstrictor nerve fibers.

VENULES & VEINS

The walls of the venules are only slightly thicker than those of the capillaries. The walls of the veins are also thin and easily distended. They contain relatively little smooth muscle, but considerable venoconstriction is produced by activity in the noradrenergic nerves to the veins and by circulating vasoconstrictors such as endothelins. Variations in venous tone are important in circulatory adjustments.

The intima of the limb veins is folded at intervals to form **venous valves** that prevent retrograde flow. However, no valves are present in very small veins, the great veins (superior and inferior vena cava plus the pulmonary veins), or the veins from the brain and viscera.

ANGIOGENESIS

When tissues grow, blood vessels must proliferate if the tissue is to maintain a normal blood supply. Therefore, angiogenesis, the formation of new blood vessels, is important during fetal life and growth to adulthood. It is also important in adulthood for processes such as wound healing, formation of the corpus luteum after ovulation, and formation of new endometrium after menstruation. Abnormally, it is important in tumor growth since tumors that do not develop a blood supply cannot grow.

During embryonic development, a network of leaky capillaries is formed in tissues from angioblasts: this process is sometimes called **vasculogenesis.** Branches from nearby vessels then hook up with the capillaries and provide them with smooth muscle, which brings about their maturation. Angiogenesis in adults is presumably similar, but consists of new vessel formation by branching from preexisting vessels rather than from angioblasts.

Many factors are involved in angiogenesis. A key compound is **vascular endothelial growth factor (VEGF).** VEGF is primarily responsible for

vasculogenesis, whereas the budding of vessels that connect to the immature capillary network is regulated by other as yet unidentified factors. Some VEGF isoforms and receptors may play a more prominent role in the formation of lymphatic vessels **(lymphangiogenesis)** than that of blood vessels.

The actions of VEGF and related factors have received considerable attention in recent years because of the requirement for angiogenesis in the development of tumors. VEGF antagonists and other angiogenesis inhibitors have entered clinical practice as adjunctive therapies for malignancies and are being tested as first-line therapies as well.

BIOPHYSICAL CONSIDERATIONS FOR CIRCULATORY PHYSIOLOGY

FLOW, PRESSURE, & RESISTANCE

Blood always flows, of course, from areas of high pressure to areas of low pressure, except in certain situations when momentum transiently sustains flow (see Figure 30–3). The relationship between mean flow, mean pressure, and resistance in the blood vessels is analogous in a general way to the relationship between the current, electromotive force (voltage), and resistance in an electrical circuit as expressed in Ohm law:

$$\text{Current (I)} = \frac{\text{Electromotive force (E)}}{\text{Resistance (R)}}$$

$$\text{Flow (F)} = \frac{\text{Pressure (P)}}{\text{Resistance (R)}}$$

Flow in any portion of the vascular system is therefore equal to the **effective perfusion pressure** in that portion divided by the **resistance.** The effective perfusion pressure is the mean intraluminal pressure at the arterial end minus the mean pressure at the venous end. The units of resistance (pressure divided by flow) are dyne·s/cm^5. To avoid dealing with such complex units, resistance in the cardiovascular system is sometimes expressed in **R units,** which are obtained by dividing pressure in mm Hg by flow in mL/s (see also Table 33–1). Thus, for example, when the mean aortic pressure is 90 mm Hg and the left ventricular output is 90 mL/s, the total peripheral resistance is

$$\frac{90 \text{ mm Hg}}{90 \text{ mL/s}} = 1 \text{ R unit}$$

METHODS FOR MEASURING BLOOD FLOW

Various noninvasive devices have been developed to measure blood flow. Most commonly, blood velocity can be measured with **Doppler flow meters.** Ultrasonic waves are sent into a vessel diagonally, and the waves reflected from the red and white blood cells are picked up by a downstream sensor. The frequency of the reflected waves is higher by an amount that is proportional to the rate of flow toward the sensor because of the Doppler effect.

Indirect methods for measuring the blood flow in various organs in humans include adaptations of the Fick and indicator dilution techniques described in Chapter 30. One example is the use of the Kety N_2O method for measuring cerebral blood flow (see Chapter 33). Another is determination of the renal blood flow by measuring the clearance of para-aminohippuric acid (see Chapter 37). A considerable amount of data on blood flow in the extremities have been obtained by **plethysmography.** The forearm, for example, is sealed in a watertight chamber **(plethysmograph).** Changes in the volume of the forearm, reflecting changes in the amount of blood and interstitial fluid it contains, displace the water, and this displacement is measured with a volume recorder. When the venous drainage of the forearm is occluded, the rate of increase in the volume of the forearm is a function of the arterial blood flow **(venous occlusion plethysmography).**

APPLICABILITY OF PHYSICAL PRINCIPLES TO FLOW IN BLOOD VESSELS

Physical principles and equations that describe the behavior of perfect fluids in rigid tubes have often been used indiscriminately to explain the behavior of blood in blood vessels, whereas blood vessels are not rigid tubes, and the blood is not a perfect fluid but a two-phase system of liquid and cells. Therefore, the behavior of the circulation deviates, sometimes markedly, from that predicted by physical principles. However, these principles are of value when used as an aid to understanding what goes on in the body.

LAMINAR FLOW

The flow of blood in straight blood vessels, like the flow of liquids in narrow rigid tubes, is normally **laminar.** Within the blood vessels, an infinitely thin layer of blood in contact with the wall of the vessel does not move. The next layer within the vessel has a low velocity, the next a higher velocity, and so forth, velocity being greatest in the center of the stream **(Figure 31–18)**. Laminar flow occurs at velocities up to a certain **critical velocity.** At or above this velocity, flow is turbulent. The probability of turbulence is also related to the diameter of the vessel and the viscosity of the blood. This probability can be expressed by the ratio of inertial to viscous forces as follows:

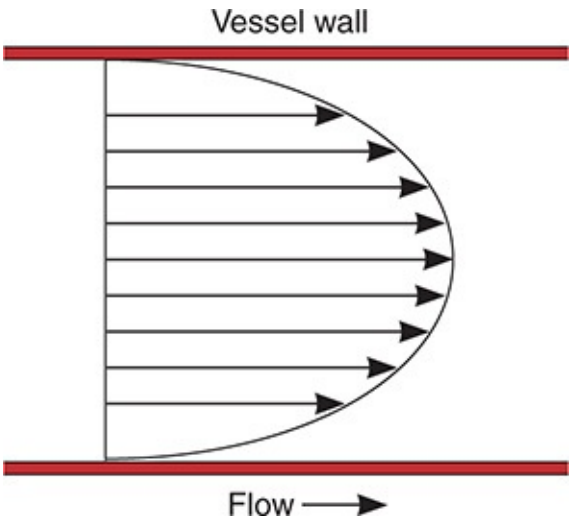


FIGURE 31–18 Diagram of the velocities of concentric laminas of a viscous fluid flowing in a tube, illustrating the parabolic distribution of velocities.

$$Re = \frac{\rho D \dot{V}}{\eta}$$

where Re is the Reynolds number; ρ is the density of the fluid; D is the diameter of the tube; V. is the velocity of the flow; and η is the viscosity of the fluid. The higher the value of Re, the greater the probability of turbulence. When D is in cm, V. is in cm/s^{-1}, and η is in poise; flow is usually not turbulent if Re is less than 2000. When Re is more than 3000, turbulence is almost always present. Laminar flow can be disturbed at the branching points of arteries, and the resulting turbulence may increase the likelihood that atherosclerotic plaques will be deposited. Constriction of an artery likewise increases the velocity of blood flow through the constriction, producing turbulence and sound beyond the

constriction **(Figure 31–19)**. Examples are bruits heard over arteries constricted by atherosclerotic plaques and the sounds of Korotkoff heard when measuring blood pressure (see below). In healthy humans, the critical velocity is sometimes exceeded in the ascending aorta at the peak of systolic ejection, but it is usually exceeded only when an artery is constricted.

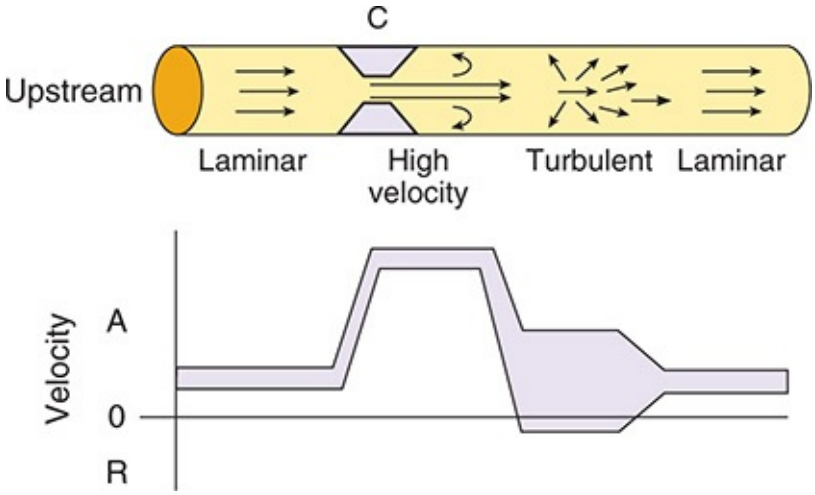


FIGURE 31–19 **Top:** Effect of constriction (C) on the profile of velocities in a blood vessel. The arrows indicate direction of velocity components, and their length is proportional to their magnitude. **Bottom:** Range of velocities at each point along the vessel. In the area of turbulence, there are many different anterograde (A) and some retrograde (R) velocities. (Modified with permission from Richards KE: Doppler echocardiographic diagnosis and quantification of vascular heart disease. Curr Probl Cardiol 1985 February; 10(2):1–49.)

SHEAR STRESS & GENE ACTIVATION

Flowing blood creates a force on the endothelium that is parallel to the long axis of the vessel. This **shear stress** (γ) is proportional to viscosity (η) times the shear rate (dy/dr), which is the rate at which the axial velocity increases from the vessel wall toward the lumen.

$$\gamma = \eta\ (dy/dr)$$

Change in shear stress and other physical variables, such as cyclic strain and stretch, produce marked changes in the expression of genes by endothelial cells. The genes that are activated include those that produce growth factors, integrins, and related molecules **(Figure 31–20)**. Recent evidence has implicated

endothelial surface structures known as **primary cilia** as the sensors of shear stress, which in turn activate signaling pathways to alter cell function. However, other components may also be involved, such as ion channels, adhesion molecules, and the cytoskeleton.

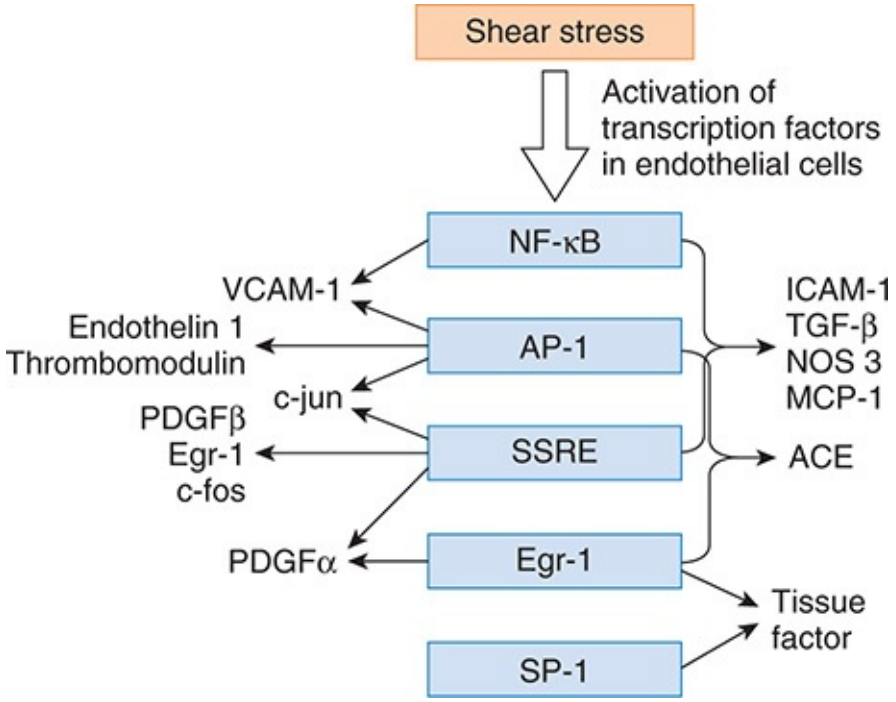


FIGURE 31–20 Endothelial transcription factors and target genes activated by shear stress. ACE, angiotensin-converting enzyme; AP-1, activator protein-1; Egr-1, early growth response protein-1; ICAM-1; intercellular adhesion molecule-1; MCP-1, monocyte chemoattractant protein-1; NF-κB, nuclear factor-κB; NOS 3, nitric oxide synthase 3; PDGF, platelet-derived growth factor; SP1, specificity protein 1; SSRE, shear stress responsive element; TGF-β, transforming growth factor-β; VCAM-1, vascular cell adhesion molecule-1. (Data from Braddock M, et al: Fluid shear stress modulation of gene expression in endothelial cells. News Physiol Sci 1998;13:241.)

AVERAGE VELOCITY

When considering flow in a system of tubes, it is important to distinguish between velocity, which is displacement per unit time (eg, cm/s), and flow, which is volume per unit time (eg, cm^3/s). Velocity ($\dot{V}$) is proportional to flow (Q) divided by the area of the conduit (A):

$$\dot{V} = \frac{Q}{A}$$

Therefore, $Q = A \times \dot{V}$, and if flow stays constant, velocity increases in direct proportion to any decrease in A (Figure 31–19).

The average velocity of fluid movement at any point in a system of tubes in parallel is inversely proportional to the *total* cross-sectional area at that point. Therefore, the average velocity of the blood is high in the aorta, declines steadily in the smaller vessels, and is lowest in the capillaries, which have 1000 times the *total* cross-sectional area of the aorta (Table 31–9). The average velocity of blood flow increases again as the blood enters the veins and is relatively high in the vena cava, although not so high as in the aorta. Clinically, the velocity of the circulation can be measured by injecting a bile salt preparation into an arm vein and timing the first appearance of the bitter taste it produces **(Figure 31–21)**. The average normal arm-to-tongue **circulation time** is 15 s.

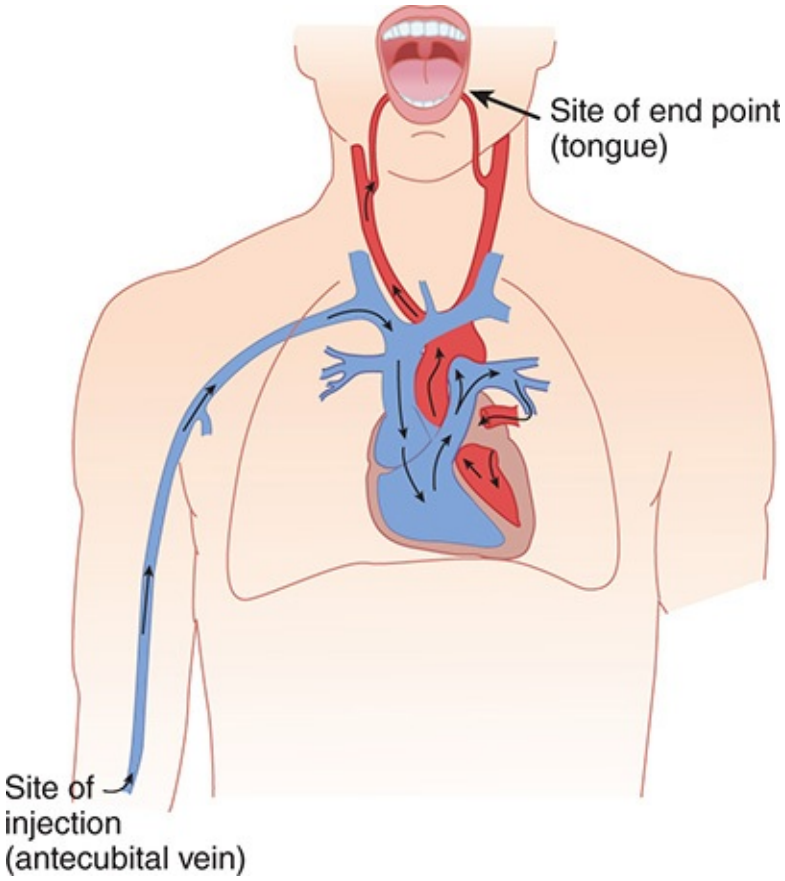


FIGURE 31–21 Pathway traversed by the injected material when the arm-to-tongue circulation time is measured.

POISEUILLE-HAGEN FORMULA

The relationship between the flow in a long narrow tube, the viscosity of the fluid, and the radius of the tube is expressed mathematically in the **Poiseuille-Hagen formula:**

$$F = (P_A - P_B) \times \left(\frac{\pi}{8}\right) \times \left(\frac{1}{\eta}\right) \times \left(\frac{r^4}{L}\right)$$

where

F = flow

$P_A - P_B$ = pressure difference between two ends of the tube

η = viscosity

r = radius of tube

L = length of tube

Because flow is equal to pressure difference divided by resistance (R),

$$R = \frac{8\eta L}{\pi r^4}$$

Because flow varies directly and resistance inversely with the fourth power of the radius, blood flow and resistance in vivo are markedly affected by small changes in the caliber of the vessels. Thus, for example, flow through a vessel is doubled by an increase of only 19% in its radius; and when the radius is doubled, resistance is reduced to 6% of its previous value. This is why organ blood flow is so effectively regulated by small changes in the caliber of the arterioles and why variations in arteriolar diameter have such a pronounced effect on systemic arterial pressure.

VISCOSITY & RESISTANCE

The resistance to blood flow is determined not only by the radius of the blood vessels but also by the viscosity of the blood. Plasma is about 1.8 times as viscous as water, whereas whole blood is 3–4 times as viscous as water. Thus, viscosity depends for the most part on the **hematocrit.** The effect of viscosity in vivo deviates from that predicted by the Poiseuille-Hagen formula. In large vessels, increases in hematocrit cause appreciable increases in viscosity. However, in vessels smaller than 100 μm in diameter—that is, in arterioles,

capillaries, and venules—the viscosity change per unit change in hematocrit is much less than it is in large-bore vessels. This is due to a difference in the nature of flow through the small vessels, known as the Fahraeus-Lindqvist effect. In small vessels, erythrocytes move to the center of the vessel, leaving cell-free plasma at the vessel wall. Therefore, the net change in viscosity per unit change in hematocrit is considerably smaller in the body than if it is measured in vitro **(Figure 31–22)**. This is why hematocrit changes have relatively little effect on the peripheral resistance except when the changes are large. In severe polycythemia (excessive production of red blood cells), the increase in resistance does increase the work of the heart. Conversely, in marked anemia, peripheral resistance is decreased, in part because of the decline in viscosity. Of course, the decrease in hemoglobin decreases the O_2-carrying ability of the blood, but the improved blood flow due to the decrease in viscosity partially compensates for this.

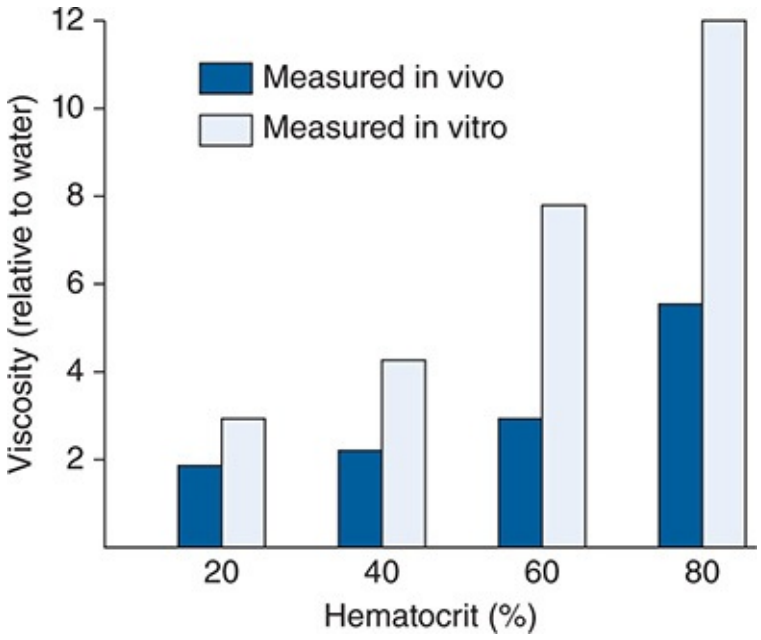


FIGURE 31–22 Effect of changes in hematocrit on the relative viscosity of blood measured in vivo and in vitro. (Data simplified and replotted from Whittaker SRF, Winton FR: The apparent viscosity of blood flowing in the isolated hind limb of the dog, and its variation with corpuscular concentration. J Physiol [Lond] 1933; 78:338.)

Viscosity is also affected by the composition of the plasma and the resistance of the cells to deformation. Clinically significant increases in viscosity are seen in diseases in which plasma proteins such as immunoglobulins are markedly

elevated as well as when red blood cells are abnormally rigid (hereditary spherocytosis).

CRITICAL CLOSING PRESSURE

In rigid tubes, the relationship between pressure and flow of homogeneous fluids is linear, but in thin-walled blood vessels in vivo it is not. When the pressure in a small blood vessel is reduced, a point is reached at which no blood flows, even though the pressure is not zero **(Figure 31–23)**. This is because the vessels are surrounded by tissues that exert a small but definite pressure on them, and when the intraluminal pressure falls below the tissue pressure, they collapse. In inactive tissues, for example, the pressure in many capillaries is low because the precapillary sphincters and metarterioles are constricted, and many of these capillaries are collapsed. The pressure at which flow ceases is called the **critical closing pressure.**

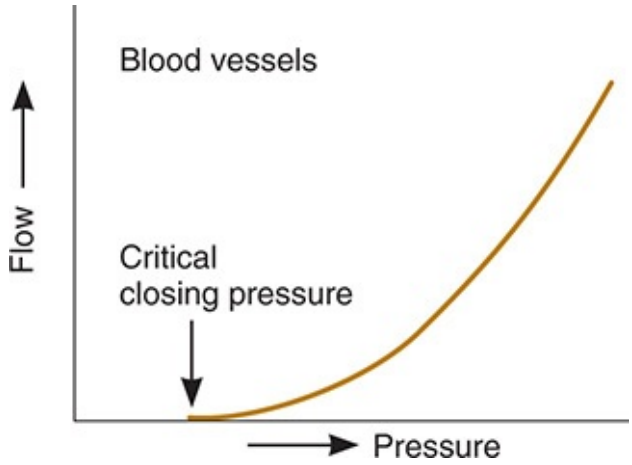


FIGURE 31–23 Relation of pressure to flow in thin-walled blood vessels.

LAW OF LAPLACE

It is perhaps surprising that structures as thin-walled and delicate as the capillaries are not more prone to rupture. The principal reason for their relative invulnerability is their small diameter. The protective effect of small size in this case is an example of the operation of the **law of Laplace,** an important physical principle with several other applications in physiology. This law states that tension in the wall of a cylinder (T) is equal to the product of the transmural pressure (P) and the radius (r) divided by the wall thickness (w):

$$T = Pr/w$$

The **transmural pressure** is the pressure inside the cylinder minus the pressure outside the cylinder, but because tissue pressure in the body is low, it can generally be ignored and P equated to the pressure inside the blood vessel. In a thin-walled vessel, w is very small and it too can be ignored, but it becomes a significant factor in vessels such as arteries. Therefore, in a thin-walled vessel, P = T divided by the two principal radii of curvature of the structure:

$$P = T\left(\frac{1}{r_1} + \frac{1}{r_2}\right)$$

In a cylinder such as a blood vessel, one radius is infinite, so

$$P = \frac{T}{r}$$

Consequently, the smaller the radius of a blood vessel, the lower the tension in the wall necessary to balance the distending pressure. In the human aorta, for example, the tension at normal pressures is about 170,000 dynes/cm, and in the vena cava it is about 21,000 dynes/cm; but in the capillaries, it is approximately 16 dynes/cm.

The law of Laplace also makes clear a disadvantage faced by dilated hearts. When the radius of a cardiac chamber is increased, a greater tension must be developed in the myocardium to produce any given pressure; consequently, a dilated heart must do more work than a nondilated heart.

RESISTANCE & CAPACITANCE VESSELS

In vivo, the veins are an important blood reservoir. Normally, they are partially collapsed and oval in cross-section. A large amount of blood can be added to the venous system before the veins become distended to the point where further increments in volume produce a large rise in venous pressure. The veins are therefore called **capacitance vessels.** The small arteries and arterioles are referred to as **resistance vessels** because they are the principal site of the peripheral resistance (see below).

At rest, at least 50% of the circulating blood volume is in the systemic veins, 12% is in the heart cavities, and 18% is in the low-pressure pulmonary circulation. Only 2% is in the aorta, 8% in the arteries, 1% in the arterioles, and

5% in the capillaries (Table 31–9). When extra blood is administered by transfusion, less than 1% of it is distributed in the arterial system (the **"high-pressure system"**), and all the rest is found in the systemic veins, pulmonary circulation, and heart chambers other than the left ventricle (the **"low-pressure system").**

ARTERIAL & ARTERIOLAR CIRCULATION

The pressure and velocities of the blood in the various parts of the systemic circulation are summarized in **Figure 31–24**. The general relationships in the pulmonary circulation are similar, but the pressure in the pulmonary artery is 25/10 mm Hg or less.

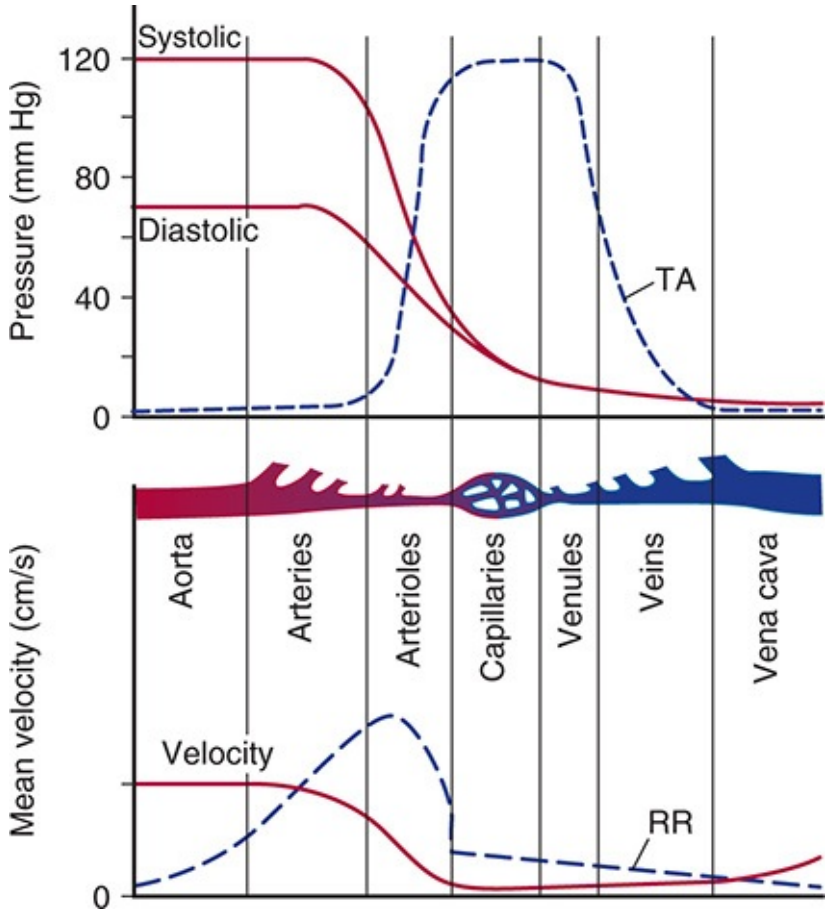


FIGURE 31–24 Diagram of the changes in pressure and velocity as blood flows through the systemic circulation. TA, total cross-sectional area of the vessels, which increases from 4.5 cm^2 in the aorta to 4500 cm^2 in the capillaries (Table 31–9). RR, relative resistance, which is highest in the arterioles.

PULSATILE FLOW OF BLOOD

Although the mean velocity of the blood in the proximal portion of the aorta is 40 cm/s, the flow is phasic, and velocity ranges from 120 cm/s during systole to a negative value at the time of the transient backflow before the aortic valve closes in diastole. In the distal portions of the aorta and in the large arteries, velocity is also much greater in systole than it is in diastole. However, the vessels are elastic, and forward flow is continuous because of the recoil during diastole of the vessel walls that have been stretched during systole **(Figure 31–25)**. Pulsatile flow appears to maintain optimal function of the tissues, apparently via distinct effects on gene transcription.

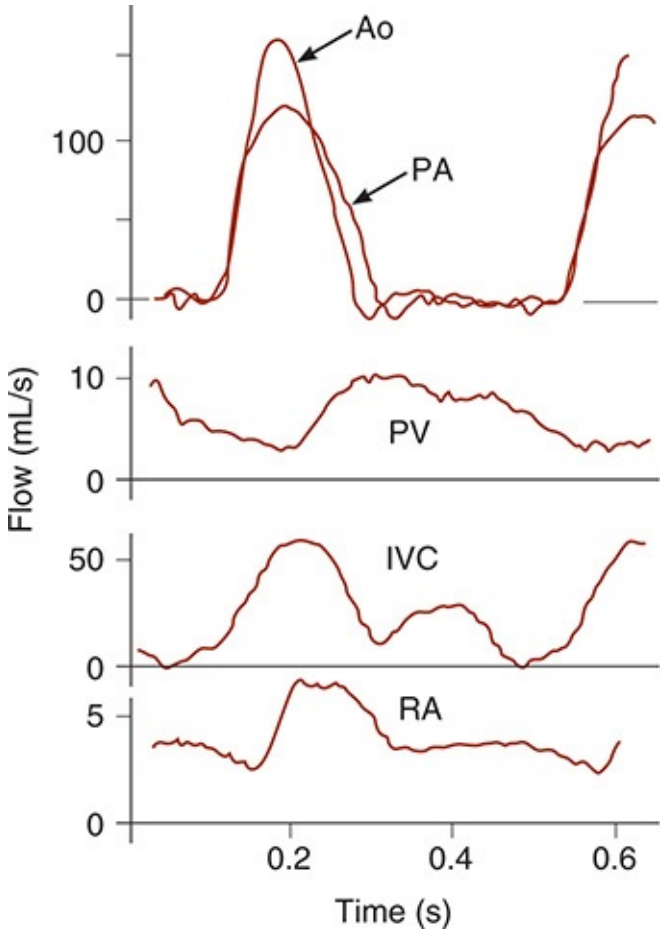


FIGURE 31–25 Changes in blood flow during the cardiac cycle in the dog. Diastole is followed by systole starting at 0.1 and again at 0.5 s. Flow patterns in humans are similar. Ao, aorta; IVC, inferior vena cava; PA, pulmonary artery; PV, pulmonary vein; RA, renal artery. (Reproduced with permission from Milnor WR: Pulsatile blood flow. N Engl J Med 1972; July 6; 287(1):27–34.)

ARTERIAL PRESSURE

The pressure in the aorta and in the brachial and other large arteries in a young adult human rises to a peak value **(systolic pressure)** of about 120 mm Hg during each heart cycle and falls to a minimum **(diastolic pressure)** of about 70 mm Hg. The arterial pressure is conventionally written as systolic pressure over diastolic pressure, for example, 120/70 mm Hg. The **pulse pressure,** the difference between the systolic and diastolic pressures, is normally about 50 mm Hg. The **mean pressure** is the average pressure throughout the cardiac cycle. Because systole is shorter than diastole, the mean pressure is slightly less than the value halfway between systolic and diastolic pressure. It can be determined only by integrating the area of the pressure curve **(Figure 31–26)**; however, as an approximation, mean pressure equals the diastolic pressure plus one-third of the pulse pressure.

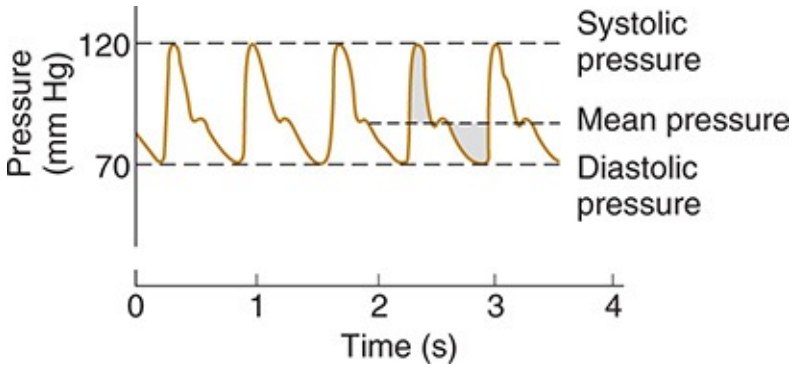


FIGURE 31–26 Brachial artery pressure curve of a normal young human, showing the relation of systolic and diastolic pressure to mean pressure. The shaded area above the mean pressure line is equal to the shaded area below it.

The pressure falls very slightly in the large- and medium-sized arteries because their resistance to flow is small, but it falls rapidly in the small arteries and arterioles, which are the main sites of the peripheral resistance against which the heart pumps. The mean pressure at the end of the arterioles is 30–38 mm Hg. Pulse pressure also declines rapidly to about 5 mm Hg at the ends of the arterioles. The magnitude of the pressure drop along the arterioles varies considerably depending on whether they are constricted or dilated.

EFFECT OF GRAVITY

The pressures in Figure 31–24 are those in blood vessels at heart level. The pressure in any vessel below heart level is increased and that in any vessel above heart level is decreased by the effect of gravity. The magnitude of the gravitational effect is 0.77 mm Hg/cm of vertical distance above or below the heart. Thus, in an adult human in the upright position, when the mean arterial pressure at heart level is 100 mm Hg, the mean pressure in a large artery in the head (50 cm above the heart) is 62 mm Hg (100 − [0.77 × 50]) and the pressure in a large artery in the foot (105 cm below the heart) is 180 mm Hg (100 + [0.77 × 105]). The effect of gravity on venous pressure is similar **(Figure 31–27)**.

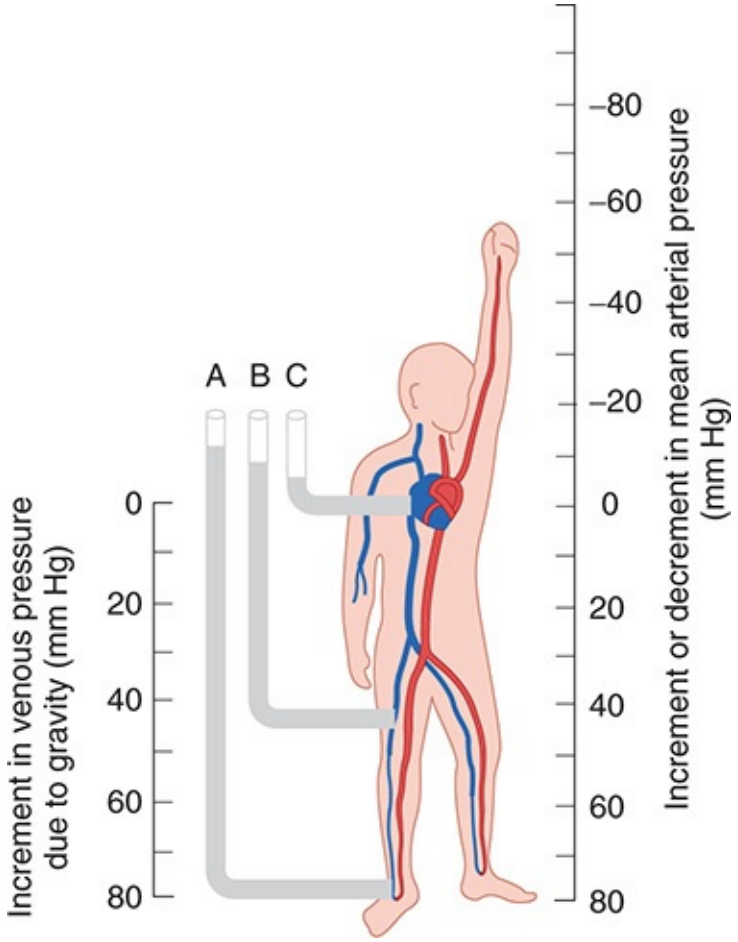


FIGURE 31–27 Effects of gravity on arterial and venous pressure. The scale on the right indicates the increment (or decrement) in mean pressure in a large artery at each level. The mean pressure in all large arteries is approximately 100 mm Hg when they are at the level of the left ventricle. The scale on the left indicates the increment in venous pressure at each level due to gravity. The manometers on the left of the figure indicate the height to which a column of blood in a tube would rise if connected to an ankle vein (A), the femoral vein

(B), or the right atrium (C), with the subject in the standing position. The approximate pressures in these locations in the recumbent position; that is, when the ankle, thigh, and right atrium are at the same level, are A, 10 mm Hg; B, 7.5 mm Hg; and C, 4.6 mm Hg.

METHODS OF MEASURING BLOOD PRESSURE

If a cannula is inserted into an artery, the arterial pressure can be measured directly with a mercury manometer or a suitably calibrated strain gauge. When an artery is tied off beyond the point at which the cannula is inserted, an **end pressure** is recorded, flow in the artery is interrupted, and all the kinetic energy of flow is converted into pressure energy. If, alternatively, a T tube is inserted into a vessel and the pressure is measured in the side arm of the tube, the recorded **side pressure,** under conditions where pressure drop due to resistance is negligible, is lower than the end pressure by the kinetic energy of flow. This is because in a tube or a blood vessel the total energy—the sum of the kinetic energy of flow and the potential energy—is constant **(Bernoulli principle).**

It is worth noting that the pressure drop in any segment of the arterial system is due both to resistance and to conversion of potential into kinetic energy. The pressure drop due to energy lost in overcoming resistance is irreversible, since the energy is dissipated as heat; but the pressure drop due to conversion of potential to kinetic energy as a vessel narrows is reversed when the vessel widens out again **(Figure 31–28)**.

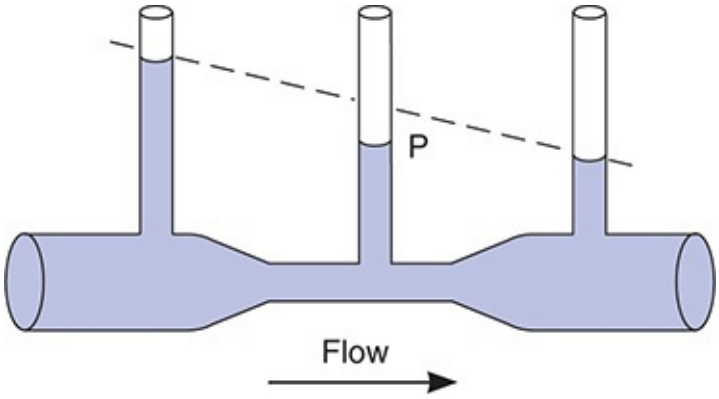


FIGURE 31–28 Bernoulli principle. When fluid flows through the narrow portion of the tube, the kinetic energy of flow is increased as the velocity increases, and the potential energy is reduced. Consequently, the measured pressure (P) is lower than it would have been at that point if the tube had not

been narrowed. The dashed line indicates what the pressure drop due to frictional forces would have been if the tube had been of uniform diameter.

The Bernoulli principle also has a significant application in pathophysiology. According to the principle, the greater the velocity of flow in a vessel, the lower the lateral pressure distending its walls. When a vessel is narrowed, the velocity of flow in the narrowed portion increases and the distending pressure decreases. Therefore, when a vessel is narrowed by a pathologic process such as an atherosclerotic plaque, the lateral pressure at the constriction is decreased and the narrowing tends to maintain itself.

AUSCULTATORY METHOD

The arterial blood pressure in humans is routinely measured by the **auscultatory method.** An inflatable cuff attached to a mercury manometer **(sphygmomanometer)** is wrapped around the arm and a stethoscope is placed over the brachial artery at the elbow. The cuff is rapidly inflated until the pressure is well above the expected systolic pressure in the brachial artery. The artery is occluded by the cuff, and no sound is heard with the stethoscope. The pressure in the cuff is then lowered slowly. At the point at which systolic pressure in the artery just exceeds the cuff pressure, a spurt of blood passes through with each heartbeat and, synchronously with each beat, a tapping sound is heard below the cuff. The cuff pressure at which the sounds are first heard is the systolic pressure. As the cuff pressure is lowered further, the sounds become louder, then dull and muffled. These are the **sounds of Korotkoff.** Finally, in most individuals, they disappear. When direct and indirect blood pressure measurements are made simultaneously, the diastolic pressure in resting adults correlates best with the pressure at which the sound disappears. However, in adults after exercise and in children, the diastolic pressure correlates best with the pressure at which the sounds become muffled. This is also true in diseases such as hyperthyroidism and aortic regurgitation.

The sounds of Korotkoff are produced by turbulent flow in the brachial artery. When the artery is narrowed by the cuff, the velocity of flow through the constriction exceeds the **critical velocity** and turbulent flow results (Figure 31–19). At cuff pressures just below the systolic pressure, flow through the artery occurs only at the peak of systole, and the intermittent turbulence produces a tapping sound. As long as the pressure in the cuff is above the diastolic pressure in the artery, flow is interrupted at least during part of diastole, and the

intermittent sounds have a staccato quality. When the cuff pressure is near the arterial diastolic pressure, the vessel is still constricted, but the turbulent flow is continuous. Continuous sounds have a muffled rather than a staccato quality.

CHANGES IN ARTERIAL BLOOD PRESSURE

Because the arterial pressure is the product of the cardiac output and the peripheral resistance, it is affected by conditions that affect either or both of these factors. Emotion increases the cardiac output and peripheral resistance, and about 20% of hypertensive patients have blood pressures that are higher in the doctor's office than at home ("white coat hypertension"). Blood pressure normally falls up to 20 mm Hg during sleep. This fall is reduced or absent in hypertension.

There is general agreement that blood pressure rises with advancing age, but the magnitude of this rise is uncertain because hypertension is a common disease and its incidence also increases with advancing age **(Clinical Box 31–4)**. Individuals who have systolic blood pressures < 120 mm Hg at age 50–60 and never develop clinical hypertension still have systolic pressures that rise throughout life **(Figure 31–29)**. This rise may be the closest approximation to the rise in normal individuals. Diastolic pressure also rises, but then starts to fall in middle age as the stiffness of arteries increases. Consequently, pulse pressure rises with advancing age. It is interesting that systolic and diastolic blood pressures are lower in women than in men until age 55–65, after which they become comparable. Because there is a positive correlation between blood pressure and the incidence of heart attacks and strokes (see below), the lower blood pressure before menopause in women may be one reason that, on average, they live longer than men.

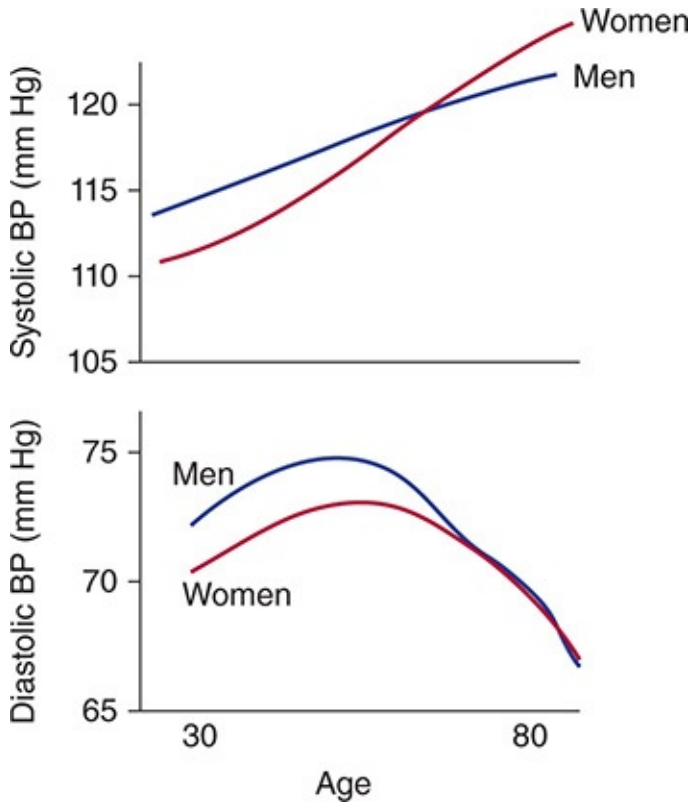


FIGURE 31–29 Effects of age and sex on arterial pressure components in humans. Data are from a large group of individuals who were studied every 2 years throughout their adult lives and who were found not be hypertensive (ie, they had systolic blood pressures < 120 mm Hg) at age 50–60. (Data replotted from Franklin SS, et al: Hemodynamic patterns of age-related changes in blood pressure: The Framingham Heart Study. Circulation 1997; 96:308 as well as the NHANES III study.)

CAPILLARY CIRCULATION

At any one time, only 5% of the circulating blood is in the capillaries, but this 5% is in a sense the most important part of the blood volume because it is the only pool from which O_2 and nutrients can enter the interstitial fluid and into which CO_2 and waste products can enter the bloodstream. Exchange across the capillary walls is essential to the survival of the tissues.

METHODS OF STUDY

It is difficult to obtain accurate measurements of capillary pressures and flows.

Experimentally, capillary pressure has been estimated by determining the amount of external pressure necessary to occlude the capillaries or the amount of pressure necessary to make saline start to flow through a micropipette inserted so that its tip faces the arteriolar end of the capillary.

CAPILLARY PRESSURE & FLOW

Capillary pressures vary considerably, but typical values in human nail bed capillaries are 32 mm Hg at the arteriolar end and 15 mm Hg at the venous end. The pulse pressure is approximately 5 mm Hg at the arteriolar end and zero at the venous end. The capillaries are short, but blood moves slowly (about 0.07 cm/s) because the total cross-sectional area of the capillary bed is large. Transit time from the arteriolar to the venular end of an average-sized capillary is 1–2 s.

CLINICAL BOX 31–4

Hypertension

Hypertension is a sustained elevation of the systemic arterial pressure. It is most commonly due to increased peripheral resistance and is a very common abnormality in humans. It can be produced by many diseases **(Table 31–11)** and causes a number of serious disorders. When the resistance against which the left ventricle must pump (afterload) is elevated for a long period, the cardiac muscle hypertrophies. The initial response is activation of immediate-early genes in the ventricular muscle, followed by activation of a series of genes involved in growth during fetal life. Left ventricular hypertrophy is associated with a poor prognosis. The total O_2 consumption of the heart, already increased by the work of expelling blood against a raised pressure (see Chapter 30), is increased further because there is more muscle. Therefore, any decrease in coronary blood flow has more serious consequences in hypertensive patients than it does in normal individuals, and degrees of coronary vessel narrowing that do not produce symptoms when the size of the heart is normal may produce myocardial infarction when the heart is enlarged.

The incidence of atherosclerosis increases in hypertension, and myocardial infarcts are common even when the heart is not enlarged. Eventually, the ability to compensate for the high peripheral resistance is exceeded, and the

heart fails. Hypertensive individuals are also predisposed to thromboses of cerebral vessels and cerebral hemorrhage. An additional complication is chronic kidney disease. However, the incidence of heart failure, strokes, and chronic kidney disease can be markedly reduced by active treatment of hypertension, even when the hypertension is relatively mild. In most patients with elevated blood pressure, the cause of the hypertension is unknown, and they are said to have **essential hypertension** (Table 31–11). At present, essential hypertension is treatable but not curable. In other, less common forms of hypertension, the cause is known. A review of these is helpful because it emphasizes ways disordered physiology can lead to disease. Pathology that compromises the renal blood supply leads to renal hypertension, as does narrowing (coarctation) of the thoracic aorta, which both increases renin secretion and increases peripheral resistance. Pheochromocytomas, adrenal medullary tumors that secrete norepinephrine and epinephrine, can cause sporadic or sustained hypertension (see Chapter 20). Estrogens increase angiotensinogen secretion, and contraceptive pills containing large amounts of estrogen cause hypertension (pill hypertension) on this basis (see Chapter 22). Increased secretion of aldosterone or other mineralocorticoids causes renal Na^+ retention, which leads to hypertension. A primary increase in plasma mineralocorticoids inhibits renin secretion. For unknown reasons, plasma renin is also low in 10–15% of patients with essential hypertension and normal circulating mineralocortical levels (low renin hypertension). Mutations in a number of single genes are also known to cause hypertension. These cases of monogenic hypertension are rare but informative. One of these is glucocorticoid-remediable aldosteronism (GRA), in which a hybrid gene encodes an adrenocorticotropic hormone (ACTH)-sensitive aldosterone synthase, with resulting hyperaldosteronism (see Chapter 20). 11-β Hydroxylase deficiency also causes hypertension by increasing the secretion of deoxycorticosterone (see Chapter 20). Normal blood pressure is restored when ACTH secretion is inhibited by administering a glucocorticoid. Mutations that decrease 11-β hydroxysteroid dehydrogenase cause loss of specificity of the mineralocorticoid receptors (see Chapter 20) with stimulation of them by cortisol and, in pregnancy, by the elevated circulating levels of progesterone. Finally, mutations of the genes for ENaCs that reduce degradation of the β or γ subunits increase ENaC activity and lead to excess renal Na^+ retention and hypertension (Liddle syndrome; see Chapter 37).

THERAPEUTIC HIGHLIGHTS

Effective lowering of the blood pressure can be produced by drugs that block α-adrenergic receptors, either in the periphery or in the central nervous system; drugs that block β-adrenergic receptors; drugs that inhibit the activity of angiotensin-converting enzyme; and calcium channel blockers that relax vascular smooth muscle.

TABLE 31–11 Estimated frequency of various forms of hypertension in the general hypertensive population.

	Percentage of Population
Essential hypertension	88
Renal hypertension	
Renovascular	2
Parenchymal	3
Endocrine hypertension	
Primary aldosteronism	5
Cushing syndrome	0.1
Pheochromocytoma	0.1
Other adrenal forms	0.2
Estrogen treatment ("pill hypertension")	1
Miscellaneous (Liddle syndrome, coarctation of the aorta, etc)	0.6

Data from Braunwald E, et al (eds): *Harrison's Principles of Internal Medicine*, 15th ed. New York, NY: McGraw-Hill; 2001.

EQUILIBRATION WITH INTERSTITIAL FLUID

As noted above, the capillary wall is a thin membrane made up of endothelial cells. Substances pass through the junctions between endothelial cells and through fenestrations when they are present. Some also pass through the cells by vesicular transport.

The factors other than vesicular transport that are responsible for transport

across the capillary wall are diffusion and filtration (see Chapter 1). Diffusion is quantitatively much more important. O_2 and glucose are in higher concentration in the bloodstream than in the interstitial fluid and diffuse into the interstitial fluid, whereas CO_2 diffuses in the opposite direction.

The rate of filtration at any point along a capillary depends on a balance of forces sometimes called the **Starling forces**. One of these is the **hydrostatic pressure gradient** (the hydrostatic pressure in the capillary minus the hydrostatic pressure of the interstitial fluid). The interstitial fluid pressure varies from one organ to another. It is positive in the liver and kidneys and as high as 6 mm Hg in the brain. The other force is the **osmotic pressure gradient** across the capillary wall (colloid osmotic pressure of plasma minus colloid osmotic pressure of interstitial fluid). This component is directed inward.

Thus:

$$\text{Fluid movement} = k\,[(P_c - P_i) - (\pi_c - \pi_i)]$$

where

k = capillary filtration coefficient

P_c = capillary hydrostatic pressure

P_i = interstitial hydrostatic pressure

π_c = capillary colloid osmotic pressure

π_i = interstitial colloid osmotic pressure

π_i is usually negligible, so the osmotic pressure gradient ($\pi_c - \pi_i$) usually equals the oncotic pressure. The capillary filtration coefficient takes into account, and is proportional to, the permeability of the capillary wall and the area available for filtration. The magnitude of the Starling forces along a typical muscle capillary is shown in **Figure 31–30**. Fluid moves into the interstitial space at the arteriolar end of the capillary and into the capillary at the venular end. In other capillaries, the balance of Starling forces may be different. For example, fluid moves out of almost the entire length of the capillaries in the renal glomeruli. On the other hand, fluid moves into the capillaries through almost their entire length in the intestines. About 24 L of fluid is filtered through the capillaries per day. This is about 0.3% of the cardiac output. About 85% of the filtered fluid is reabsorbed into the capillaries, and the remainder returns to the circulation via the lymphatics.

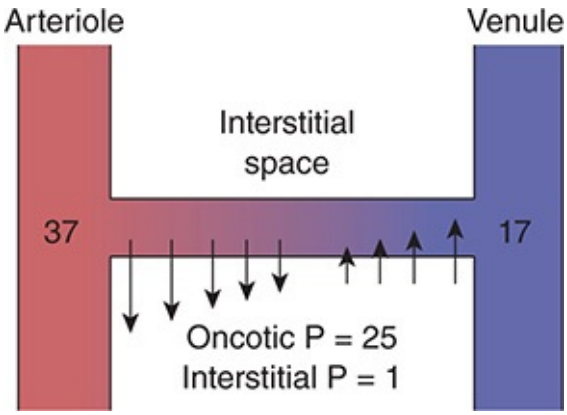


FIGURE 31–30 Schematic representation of pressure gradients across the wall of a muscle capillary. The numbers at the arteriolar and venular ends of the capillary are the hydrostatic pressures in mm Hg at these locations. The arrows indicate the approximate magnitude and direction of fluid movement. In this example, the pressure differential at the arteriolar end of the capillary is 11 mm Hg ([37 − 1] −25) outward; at the opposite end, it is 9 mm Hg (25 − [17 − 1]) inward.

It is worth noting that small molecules often equilibrate with the tissues near the arteriolar end of each capillary. In this situation, total diffusion can be increased by increasing blood flow; that is, exchange is **flow-limited** (Figure 31–31). Conversely, transfer of substances that do not reach equilibrium with the tissues during their passage through the capillaries is said to be **diffusion-limited.**

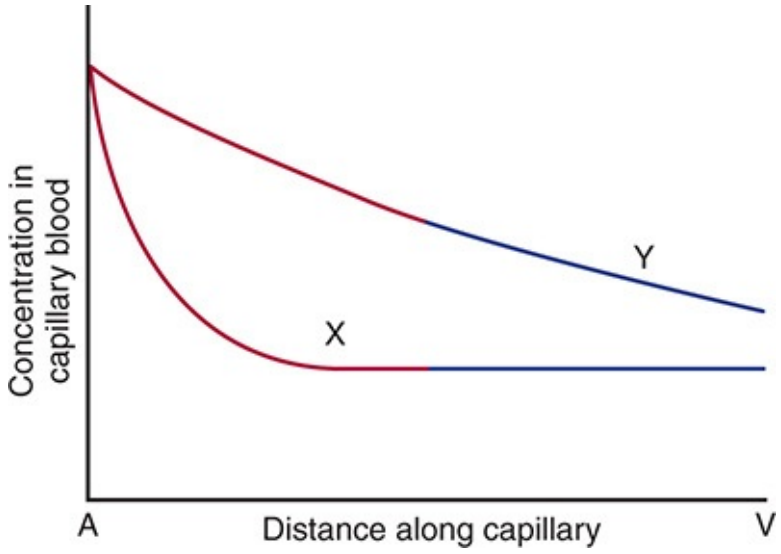


FIGURE 31–31 Flow-limited and diffusion-limited exchange across

capillary walls. A and V indicate the arteriolar and venular ends of the capillary. Substance X equilibrates with the tissues (movement into the tissues equals movement out) well before the blood leaves the capillary, whereas substance Y does not equilibrate. If other factors stay constant, the amount of X entering the tissues can be increased only by increasing blood flow; that is, it is flow-limited. The movement of Y is diffusion-limited.

ACTIVE & INACTIVE CAPILLARIES

In resting tissues, most of the capillaries are collapsed. In active tissues, the metarterioles and the precapillary sphincters dilate. The intracapillary pressure rises, overcoming the critical closing pressure of the vessels, and blood flows through all of the capillaries. Relaxation of the smooth muscle of the metarterioles and precapillary sphincters is due to the action of vasodilator metabolites formed locally in active tissue (see Chapter 32).

After noxious stimulation, substance P released by the axon reflex (see Chapter 33) increases capillary permeability. Bradykinin and histamine also increase capillary permeability. When capillaries are stimulated mechanically, they empty (white reaction; see Chapter 33), probably due to contraction of the precapillary sphincters.

VENOUS CIRCULATION

Blood flows through the blood vessels, including the veins, primarily because of the pumping action of the heart. However, venous flow is aided by the heartbeat, the increase in the negative intrathoracic pressure during each inspiration, and contractions of skeletal muscles that compress the veins **(muscle pump).**

VENOUS PRESSURE & FLOW

The pressure in the venules is 12–18 mm Hg. It falls steadily in the larger veins to about 5.5 mm Hg in the great veins outside the thorax. The pressure in the great veins at their entrance into the right atrium **(central venous pressure)** averages 4.6 mm Hg, but fluctuates with respiration and heart action.

Peripheral venous pressure, like arterial pressure, is affected by gravity. It is increased by 0.77 mm Hg for each centimeter below the right atrium and decreased by a like amount for each centimeter above the right atrium the

pressure is measured (Figure 31–27). Thus, on a proportional basis, gravity has a greater effect on venous than on arterial pressures.

When blood flows from the venules to the large veins, its average velocity increases as the total cross-sectional area of the vessels decreases. In the great veins, the velocity of blood is about one-fourth that in the aorta, averaging about 10 cm/s.

THORACIC PUMP

During inspiration, the intrapleural pressure falls from −2.5 to −6 mm Hg. This negative pressure is transmitted to the great veins and, to a lesser extent, the atria, so that central venous pressure fluctuates from about 6 mm Hg during expiration to approximately 2 mm Hg during quiet inspiration. The drop in venous pressure during inspiration aids venous return. When the diaphragm descends during inspiration, intra-abdominal pressure rises, and this also squeezes blood toward the heart because backflow into the leg veins is prevented by the venous valves.

EFFECTS OF HEARTBEAT

The variations in atrial pressure are transmitted to the great veins, producing the **a, c,** and **v waves** of the venous pressure-pulse curve (see Chapter 30). Atrial pressure drops sharply during the ejection phase of ventricular systole because the atrioventricular valves are pulled downward, increasing the capacity of the atria. This action sucks blood into the atria from the great veins. The sucking of the blood into the atria during systole contributes appreciably to venous return, especially at rapid heart rates.

Close to the heart, venous flow becomes pulsatile. When the heart rate is slow, two periods of peak flow are detectable, one during ventricular systole, due to pulling down of the atrioventricular valves, and one in early diastole, during the period of rapid ventricular filling (Figure 31–25).

MUSCLE PUMP

In the limbs, the veins are surrounded by skeletal muscles, and contraction of these muscles during activity compresses the veins. Pulsations of nearby arteries may also compress veins. Because the venous valves prevent reverse flow, the

blood moves toward the heart. During quiet standing, when the full effect of gravity is manifest, venous pressure at the ankle is 85–90 mm Hg (Figure 31–27). Pooling of blood in the leg veins reduces venous return, with the result that cardiac output is reduced, sometimes to the point where fainting occurs. Rhythmic contractions of the leg muscles while the person is standing serve to lower the venous pressure in the legs to less than 30 mm Hg by propelling blood toward the heart. This heartward movement of the blood is decreased in patients with **varicose veins** because their valves are incompetent. These patients may develop stasis and ankle edema. However, even when the valves are incompetent, muscle contractions continue to produce a basic heartward movement of the blood because the resistance of the larger veins in the direction of the heart is less than the resistance of the small vessels away from the heart.

VENOUS PRESSURE IN THE HEAD

In the upright position, the venous pressure in the parts of the body above the heart is decreased by the force of gravity. The neck veins collapse above the point where the venous pressure is close to zero. However, the dural sinuses have rigid walls and cannot collapse. The pressure in them in the standing or sitting position is therefore subatmospheric. The magnitude of the negative pressure is proportional to the vertical distance above the top of the collapsed neck veins, and in the superior sagittal sinus may be as much as −10 mm Hg. This fact must be kept in mind by neurosurgeons. Neurosurgical procedures are sometimes performed with the patient seated. If one of the sinuses is opened during such a procedure it sucks air, causing **air embolism.**

AIR EMBOLISM

Because air, unlike fluid, is compressible, its presence in the circulation has serious consequences. The forward movement of the blood depends on the fact that blood is incompressible. Large amounts of air fill the heart and effectively stop the circulation, causing sudden death because most of the air is compressed by the contracting ventricles rather than propelled into the arteries. Small amounts of air are swept through the heart with the blood, but the bubbles lodge in the small blood vessels. The surface capillarity of the bubbles markedly increases the resistance to blood flow, and flow is reduced or abolished. Blockage of small vessels in the brain leads to serious and even fatal neurologic abnormalities. Treatment with hyperbaric oxygen (see Chapter 35) is of value

because the pressure reduces the size of the gas emboli. In experimental animals, the amount of air that produces fatal air embolism varies considerably, depending in part on the rate at which it enters the veins. Sometimes as much as 100 mL can be injected without ill effects, whereas at other times as little as 5 mL is lethal.

MEASURING VENOUS PRESSURE

Central venous pressure can be measured directly by inserting a catheter into the thoracic great veins. **Peripheral venous pressure** correlates well with central venous pressure in most conditions. To measure peripheral venous pressure, a needle attached to a manometer containing sterile saline is inserted into an arm vein. The peripheral vein should be at the level of the right atrium (a point half the chest diameter from the back in the supine position). The values obtained in millimeters of saline can be converted into millimeters of mercury (mm Hg) by dividing by 13.6 (the density of mercury). The amount by which peripheral venous pressure exceeds central venous pressure increases with the distance from the heart along the veins. The mean pressure in the antecubital vein is normally 7.1 mm Hg, compared with a mean pressure of 4.6 mm Hg in the central veins.

Central venous pressure is decreased during negative pressure breathing and shock. It is increased by positive pressure breathing, straining, expansion of the blood volume, and heart failure. In advanced heart failure or obstruction of the superior vena cava, the pressure in the antecubital vein may reach values of 20 mm Hg or more.

LYMPHATIC CIRCULATION & INTERSTITIAL FLUID VOLUME

LYMPHATIC CIRCULATION

Fluid efflux normally exceeds influx across the capillary walls, but the extra fluid enters the lymphatics and drains through them back into the blood. This keeps the interstitial fluid pressure from rising and promotes the turnover of tissue fluid. The normal 24-h lymph flow is 2–4 L.

Lymphatic vessels can be divided into two types: initial lymphatics and collecting lymphatics **(Figure 31–32)**. The former lack valves and smooth

muscle in their walls, and they are found in regions such as the intestine or skeletal muscle. Tissue fluid enters them through loose junctions between the endothelial cells that form their walls. The fluid in them apparently is massaged by muscle contractions of the organs and contraction of arterioles and venules, with which they are often associated. They drain into the collecting lymphatics, which have valves and smooth muscle in their walls and contract in a peristaltic manner, propelling the lymph along the vessels. Flow in the collecting lymphatics is further aided by movements of skeletal muscle, the negative intrathoracic pressure during inspiration, and the suction effect of high velocity flow of blood in the veins in which the lymphatics terminate. However, the contractions are the principal factor propelling the lymph.

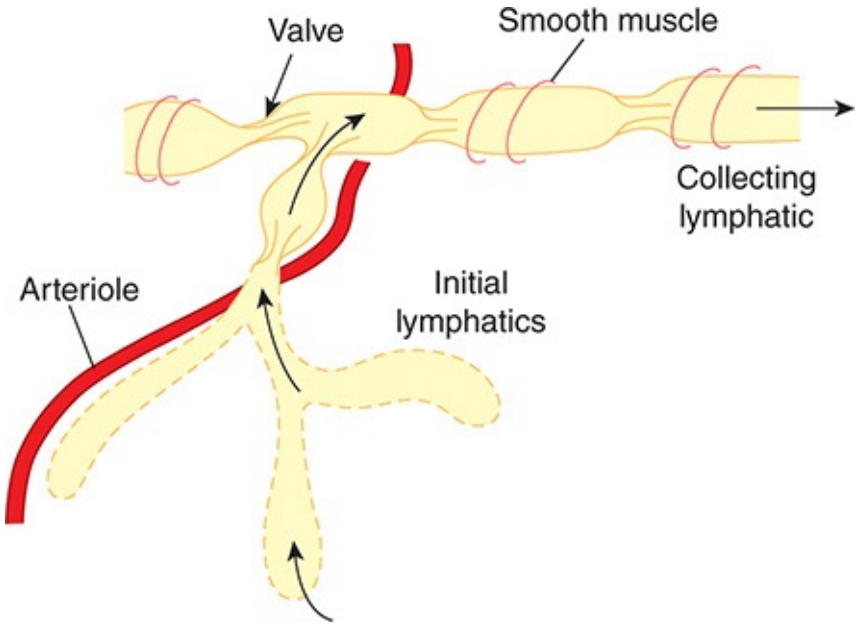


FIGURE 31–32 Schematic of the lymphatic system. Initial lymphatics are highly permeable structures without smooth muscle or valves. Unidirectional flow is accomplished in the collecting lymphatics, which appear like a string of beads due to the regular valves. Lymphatics are often close to blood vessels, whose contractions also encourage flow in the lymphatic system.

OTHER FUNCTIONS OF THE LYMPHATIC SYSTEM

Appreciable quantities of protein enter the interstitial fluid in the liver and intestine, and smaller quantities enter from the blood in other tissues. The

macromolecules enter the lymphatics, presumably at the junctions between the endothelial cells, and the proteins are returned to the bloodstream via the lymphatics. The amount of protein returned in this manner in 1 day is equal to 25–50% of the total circulating plasma protein. The transport of lipids absorbed from the intestine via the lymphatics has been discussed in Chapter 26.

INTERSTITIAL FLUID VOLUME

The amount of fluid in the interstitial spaces depends on the capillary pressure, the interstitial fluid pressure, the oncotic pressure, the capillary filtration coefficient, the number of active capillaries, the lymph flow, and the total extracellular fluid (ECF) volume. The ratio of precapillary to postcapillary venular resistance is also important. Precapillary constriction lowers filtration pressure, whereas postcapillary constriction raises it. Changes in any of these variables lead to changes in the volume of interstitial fluid. Factors promoting an increase in this volume are summarized in **Table 31–12**. **Edema** is the accumulation of interstitial fluid in abnormally large amounts.

TABLE 31–12 Causes of increased interstitial fluid volume and edema.

Increased filtration pressure
Venular constriction
Increased venous pressure (heart failure, incompetent valves, venous obstruction, increased total ECF volume, effect of gravity, etc)
Decreased osmotic pressure gradient across capillary
Decreased plasma protein level
Accumulation of osmotically active substances in interstitial space
Increased capillary permeability
Substance P
Histamine and related substances
Kinins, etc
Inadequate lymph flow

In active tissues, capillary pressure rises, often to the point where it exceeds the oncotic pressure throughout the length of the capillary. In addition, osmotically active metabolites may temporarily accumulate in the interstitial

fluid because they cannot be washed away as rapidly as they are formed. To the extent that they accumulate, they exert an osmotic effect that decreases the magnitude of the osmotic gradient due to the oncotic pressure. The amount of fluid leaving the capillaries is therefore markedly increased and the amount entering them reduced. Lymph flow is increased, decreasing the degree to which the fluid would otherwise accumulate, but exercising muscle, for example, still increases in volume by as much as 25%.

Interstitial fluid tends to accumulate in dependent parts because of the effect of gravity. In the upright position, the capillaries in the legs are protected from the high arterial pressure by the arterioles, but the high venous pressure is transmitted to them through the venules. Skeletal muscle contractions keep the venous pressure low by pumping blood toward the heart (see above) when the individual moves about; however, if one stands still for long periods, fluid accumulates and edema eventually develops. The ankles also swell during long trips when travelers sit for prolonged periods with their feet in a dependent position. Whenever there is abnormal retention of salt in the body, water is also retained. The salt and water are distributed throughout the ECF, and since the interstitial fluid volume is therefore increased, there is a predisposition to edema. Salt and water retention is a factor in the edema seen in heart failure, nephrosis, and cirrhosis, but there are also variations in the mechanisms that govern fluid movement across the capillary walls in these diseases. In heart failure, for example, venous pressure is usually elevated, with a consequent elevation in capillary pressure. In cirrhosis of the liver, oncotic pressure is low because hepatic synthesis of plasma proteins is depressed; and in nephrosis, oncotic pressure is low because large amounts of protein are lost in the urine.

Another cause of edema is inadequate lymphatic drainage. Edema caused by lymphatic obstruction is called **lymphedema,** and the edema fluid has a high protein content. If it persists, it causes a chronic inflammatory condition that can lead to fibrosis of the interstitial tissue. One cause of lymphedema is radical mastectomy, during which removal of the axillary lymph nodes leads to reduced lymph drainage.

CHAPTER SUMMARY

- Blood consists of a suspension of red blood cells (erythrocytes), white blood cells, and platelets. The cells arise in the bone marrow and are subject to regular renewal. Mutations in proteins that make up the cytoskeleton of the red cells make them more fragile and susceptible to lysis.

- Hemoglobin, stored in red blood cells, transports oxygen to peripheral tissues. Fetal hemoglobin is specialized to facilitate diffusion of oxygen from mother to fetus during development. Mutated forms of hemoglobin lead to red cell abnormalities and anemia.
- The blood cells are suspended in a protein-rich fluid known as plasma. The majority of plasma proteins are synthesized by the liver. Lymph is an exudate of plasma with lower protein concentration that serves to recover fluids from the tissues.
- Complex oligosaccharide structures on the membrane of red blood cells, specific to groups of individuals, form the basis of the ABO blood group system. AB blood group oligosaccharides, as well as other blood group molecules, can trigger the production of antibodies in naïve individuals following inappropriate transfusions, with potentially serious consequences due to erythrocyte agglutination.
- When a blood vessel is injured, a complex cascade of molecular interactions among clotting factors ensures that the vessel is sealed and blood loss is minimized (hemostasis). Loss of specific clotting factors may compromise this process and lead to excessive bleeding and bruising. Abnormal clotting that occurs inside an intact vessel is called thrombosis and may occlude blood supply to vital regions.
- Blood flows from the heart to arteries and arterioles, thence to capillaries, and eventually to venules and veins and back to the heart. Each segment of the vasculature has specific contractile properties and regulatory mechanisms that subserve its physiologic functions. Transfer of oxygen and nutrients from the blood to tissues, as well as collection of metabolic wastes, occurs exclusively in the capillary beds.
- Fluid also leaves the circulation across the walls of capillaries. Some is reabsorbed; the remainder enters the lymphatic system, which eventually drains into the subclavian veins to return fluid to the bloodstream.
- Physical principles of pressure, wall tension, and vessel caliber govern the flow of blood through each segment of the circulation. These same physical principles can be applied to measure blood flow and blood pressure.
- Hypertension is an increase in mean blood pressure that is usually chronic and is common in humans. Hypertension can result in serious health consequences if left untreated. The majority of hypertension is of unknown cause, but several gene mutations underlie rare forms of the disease and are informative about mechanisms that control the dynamics of the circulatory

system.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. A 2-year-old-African-American boy is brought to his pediatrician because of acute fever, apparent bone pain, and painful swelling of his hands and feet. A physical examination reveals splenomegaly and a blood test reveals the presence of hemoglobin S. After the acute symptoms subside with rest, hydroxyurea is prescribed. This drug would be expected to reduce the risk of further acute symptoms by which mechanism?

   A. Increased synthesis of hemoglobin S

   B. Increasing the oxygen-carrying capacity of hemoglobin

   C. Increasing expression of hypoxia-inducible factor 1 alpha

   D. Decreasing the oxygen-carrying capacity of hemoglobin

   E. Increasing the synthesis of hemoglobin F

2. A patient is brought to the emergency room suffering from severe blood loss following a motor vehicle accident. Because of a natural disaster, the lab is closed and it is not possible to determine the patient's blood type before initiating a blood transfusion to save his life. However, banked blood is available. Blood of which type would be least likely to provoke a dangerous hemolytic transfusion reaction?

   A. A

   B. B

   C. AB

   D. O

   E. A mixture of A and B

3. Which of the following is not required for hemostasis following injury to a blood vessel?

   A. Platelets

   B. 5-Hydroxytryptamine

   C. Nitric oxide

   D. Collagen

   E. Fibrin monomers

4. A pharmacologist discovers a drug that stimulates the production of VEGF

receptors. He is excited because the drug might be of value in the treatment of

A. coronary artery disease.

B. cancer.

C. emphysema.

D. diabetes insipidus.

E. dysmenorrhea.

5. Which of the following has the highest *total* cross-sectional area in the body?

A. Arteries

B. Arterioles

C. Capillaries

D. Venules

E. Veins

6. The velocity of blood flow

A. is higher in the capillaries than the arterioles.

B. is higher in the veins than in the venules.

C. is higher in the veins than the arteries.

D. falls to zero in the descending aorta during diastole.

E. is reduced in a constricted area of a blood vessel.

7. When the radius of the resistance vessels is increased, which of the following is increased?

A. Systolic blood pressure

B. Diastolic blood pressure

C. Viscosity of the blood

D. Hematocrit

E. Capillary blood flow

8. The pressure in a capillary in skeletal muscle is 35 mm Hg at the arteriolar end and 14 mm Hg at the venular end. The interstitial pressure is 0 mm Hg. The colloid osmotic pressure is 25 mm Hg in the capillary and 1 mm Hg in the interstitium. The net force producing fluid movement across the capillary wall at its arteriolar end is

A. 3 mm Hg out of the capillary.

B. 3 mm Hg into the capillary.

C. 10 mm Hg out of the capillary.

D. 11 mm Hg out of the capillary.

E. 11 mm Hg into the capillary.

9. A 40-year-old man with long-standing diabetes comes to his primary care physician complaining of swelling in his feet and ankles that is so bad that he cannot don his shoes. He states that his urine has developed a foamy appearance and tests reveal that his blood urea nitrogen is decreased. The swelling in this patient is most likely primarily caused by a reduction in which of the following:

A. Lymph flow

B. Barrier function of the capillaries

C. Venous hydrostatic pressure

D. Capillary oncotic pressure

E. Interstitial oncotic pressure

10. A 30-year-old patient comes to her primary care clinician complaining of headaches and vertigo. A blood test reveals a hematocrit of 65%, and a diagnosis of polycythemia is made. Which of the following would also be increased?

A. Mean blood pressure

B. Radius of the resistance vessels

C. Radius of the capacitance vessels

D. Central venous pressure

E. Capillary blood flow

11. Lymph flow from the foot is

A. increased when an individual rises from the supine to the standing position.

B. increased by massaging the foot.

C. increased when capillary permeability is decreased.

D. decreased when the valves of the leg veins are incompetent.

E. decreased by exercise.

12. A 60-year-old woman is evaluated for a new finding of chest pain on mild exertion and is incidentally found to be suffering from primary hypertension. Evaluation of her coronary arteries reveals only minimal obstruction. This patient most likely suffers from angina on mild exertion despite only modest coronary artery disease because, compared with a normotensive individual, which of the following would be increased?

A. Oxygen supply to the coronary muscle

B. The size of heart
C. The risk of stroke
D. The propensity to thrombosis
E. Nitric oxide production by the vascular endothelium

CHAPTER 32

Cardiovascular Regulatory Mechanisms

OBJECTIVES

After studying this chapter, you should be able to:

- Contrast the sympathetic and parasympathetic control of the heart and vasculature.
- Identify the components and neurotransmitters in the central neural pathways that control arterial blood pressure and heart rate and list factors that determine the activity within the pathways.
- Identify the locations of the receptors and afferent and efferent pathways, the effector responses, and the functions of the baroreceptor reflex pathway.
- Describe the role of atrial and cardiopulmonary receptors in the control of the cardiovascular system in health and disease.
- Contrast the locations, properties, and functions of the peripheral and central chemoreceptor reflexes.
- Define how the process of autoregulation contributes to control of vascular caliber.
- Identify the paracrine factors, particularly those secreted by the endothelium, that regulate vascular tone and their mechanisms of action.
- List circulating vasodilators and vasoconstrictors.

INTRODUCTION

Multiple cardiovascular regulatory mechanisms exist that help increase the blood supply to active tissues and increase or decrease heat loss from the body by redistributing the blood. In the face of challenges such as hemorrhage, they maintain blood flow to the heart and brain. When the challenge faced is severe, flow to these vital organs is maintained at the expense of the circulation to the rest of the body.

Circulatory adjustments are effected by altering the output of the pump (the heart), changing the diameter of the resistance vessels (primarily the arterioles), or altering the amount of blood pooled in the capacitance vessels (the veins). Regulation of cardiac output is discussed in Chapter 30. The caliber of the arterioles is adjusted in part by autoregulation **(Table 32–1)**. It is also increased in active tissues by locally produced vasodilator metabolites and substances secreted by the endothelium. The diameter of the arterioles is also regulated by circulating vasoactive substances and the sympathetic nerves that innervate them. Circulating vasoactive substances and sympathetic nerves also determine the caliber of the capacitance vessels. The systemic regulatory mechanisms synergize with the local mechanisms and adjust vascular responses throughout the body.

TABLE 32–1 Summary of factors affecting the caliber of the arterioles.

Vasoconstriction	Vasodilation
Local factors	
Decreased local temperature	Increased CO_2 and decreased O_2
Autoregulation	Increased K^+, adenosine, lactate
	Decreased local pH
	Increased local temperature
Endothelial products	
Endothelin-1	Nitric oxide
Locally released platelet serotonin	Kinins
Thromboxane A_2	Prostacyclin
Circulating neurohumoral agents	
Epinephrine (except in skeletal muscle and liver)	Epinephrine in skeletal muscle and liver
Norepinephrine	Calcitonin G-related protein
Arginine vasopressin	Substance P
Angiotensin II	Histamine
Endogenous digitalis-like substance	Atrial natriuretic peptide
Neuropeptide Y	Vasoactive intestinal polypeptide
Neural factors	
Increased discharge of sympathetic nerves	Decreased discharge of sympathetic nerves
	Activation of β_2-adrenoceptors on the vasculature of skeletal muscles of the limbs

The terms **vasoconstriction** and **vasodilation** are generally used to refer to constriction and dilation of the resistance vessels. Changes in the caliber of the veins are referred to as **venoconstriction** or **venodilation.**

NEURAL CONTROL OF THE CARDIOVASCULAR SYSTEM

INNERVATION OF THE BLOOD VESSELS

Most of the vasculature is an example of an autonomic effector organ that receives innervation from the sympathetic but not the parasympathetic division of the autonomic nervous system (see Chapter 13). Sympathetic postganglionic fibers terminate on vascular smooth muscle in all parts of the body and release norepinephrine to act on α_1-adrenoceptors to mediate vasoconstriction. In the vasculature of exercising muscles, activation of the sympathetic nervous system and release of epinephrine from the adrenal medulla can also mediate vasodilation by the activation of β_2-adrenoceptors on skeletal muscle blood vessels.

Although the arterioles and the other resistance vessels are most densely innervated, all blood vessels except capillaries and venules contain smooth muscle and receive motor nerve fibers from the sympathetic division of the autonomic nervous system. The fibers to the resistance vessels regulate tissue blood flow and arterial pressure. The fibers to the venous capacitance vessels vary the volume of blood "stored" in the veins. The innervation of most veins is sparse, but the splanchnic veins are well innervated. Venoconstriction is produced by stimuli that also activate the vasoconstrictor nerves to the arterioles. The resultant decrease in venous capacity increases venous return, shifting blood to the arterial side of the circulation.

When the sympathetic nerves are damaged **(sympathectomy** or **neuropathy**), the blood vessels dilate. A change in the level of activity (increase or decrease) in sympathetic nerves is just one of the many factors that mediate vasoconstriction or vasodilation (Table 32–1).

INNERVATION OF THE HEART

The heart is one example of an effector organ that receives opposing influences from the sympathetic and parasympathetic divisions of the autonomic nervous system (see Chapter 13). Release of norepinephrine from postganglionic sympathetic nerves activates β_1-adrenoceptors in the heart, notably on the sinoatrial (SA) node, atrioventricular (AV) node, His-Purkinje conductive tissue, and atrial and ventricular contractile tissue. Stimulation of sympathetic nerves leads to an increase in heart rate **(chronotropy),** rate of transmission in the cardiac conductive tissue **(dromotropy),** and the force of ventricular contraction **(inotropy).** On the other hand, release of acetylcholine from postganglionic

parasympathetic (vagus) nerves activates muscarinic receptors in the heart, notably on the SA and AV nodes and atrial muscle. Stimulation of the vagus nerve reduces the heart rate, the rate of transmission through the AV node, and atrial contractility.

The above description presents an oversimplified explanation of autonomic control of cardiac function. There are adrenergic and cholinergic receptors on autonomic nerve terminals that modulate transmitter release from nerve endings. For example, release of acetylcholine from vagal nerve terminals inhibits the release of norepinephrine from sympathetic nerve terminals, so this can enhance the effects of vagal nerve activation on the heart.

There is a moderate amount of tonic discharge in the cardiac sympathetic nerves at rest, but there is considerable tonic vagal discharge **(vagal tone)** in humans and other large animals. After the administration of muscarinic receptor antagonists such as atropine, the heart rate in humans increases from 70 beats/min, its normal resting value, to 150–180 beats/min because the sympathetic tone is unopposed. In humans in whom both noradrenergic and cholinergic systems are blocked, the heart rate is approximately 100 beats/min.

CENTRAL NEURAL CONTROL OF THE CARDIOVASCULAR SYSTEM

The cardiovascular system is under neural influences coming from several parts of the brainstem, forebrain, and insular cortex. The brainstem receives feedback from sensory receptors in the vasculature (eg, baroreceptors and chemoreceptors). A simplified model of the feedback control circuit is shown in **Figure 32–1**. An increase in neural output from the brainstem to sympathetic nerves leads to a decrease in blood vessel diameter (arteriolar vasoconstriction) and increases in stroke volume and heart rate, which contribute to a rise in blood pressure. This in turn causes an increase in baroreceptor activity, which signals the brainstem to reduce the neural output to sympathetic nerves. The baroreceptor reflex is described in detail below.

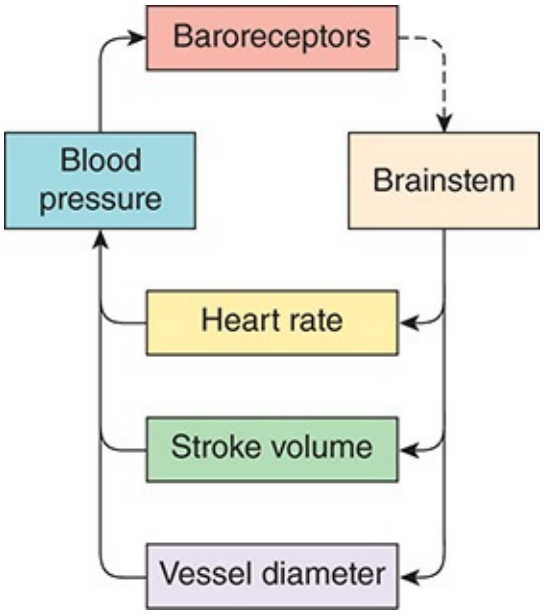


FIGURE 32–1 Feedback control of blood pressure. Brainstem excitatory input to sympathetic nerves to the heart and vasculature increases heart rate and stroke volume and reduces vessel diameter. Together these increase blood pressure, which activates the baroreceptor reflex to reduce the activity in the brainstem.

Venoconstriction and a decrease in the stores of blood in the venous reservoirs usually accompany increases in arteriolar constriction, although changes in the capacitance vessels do not always parallel changes in the resistance vessels. In the presence of an increase in sympathetic nerve activity to the heart and vasculature, there is usually an associated decrease in the activity of vagal fibers to the heart. Conversely, a decrease in sympathetic activity causes vasodilation, a fall in blood pressure, and an increase in the storage of blood in the venous reservoirs. There is usually a concomitant decrease in heart rate, but this is mostly due to stimulation of the vagal innervation of the heart.

One of the major sources of excitatory input to sympathetic nerves controlling the vasculature is a group of neurons located near the pial surface of the medulla in the **rostral ventrolateral medulla (RVLM; Figure 32–2)**. The axons of RVLM neurons course dorsally and medially and then descend in the lateral column of the spinal cord to the thoracolumbar intermediolateral cell column (IML). They contain phenylethanolamine-N-methyltransferase (PNMT; see Chapter 7), but glutamate is the excitatory transmitter they secrete to activate preganglionic sympathetic neurons. Neurovascular compression of the RVLM has been linked to some cases of **essential hypertension** in humans **(Clinical**

Box 32–1).

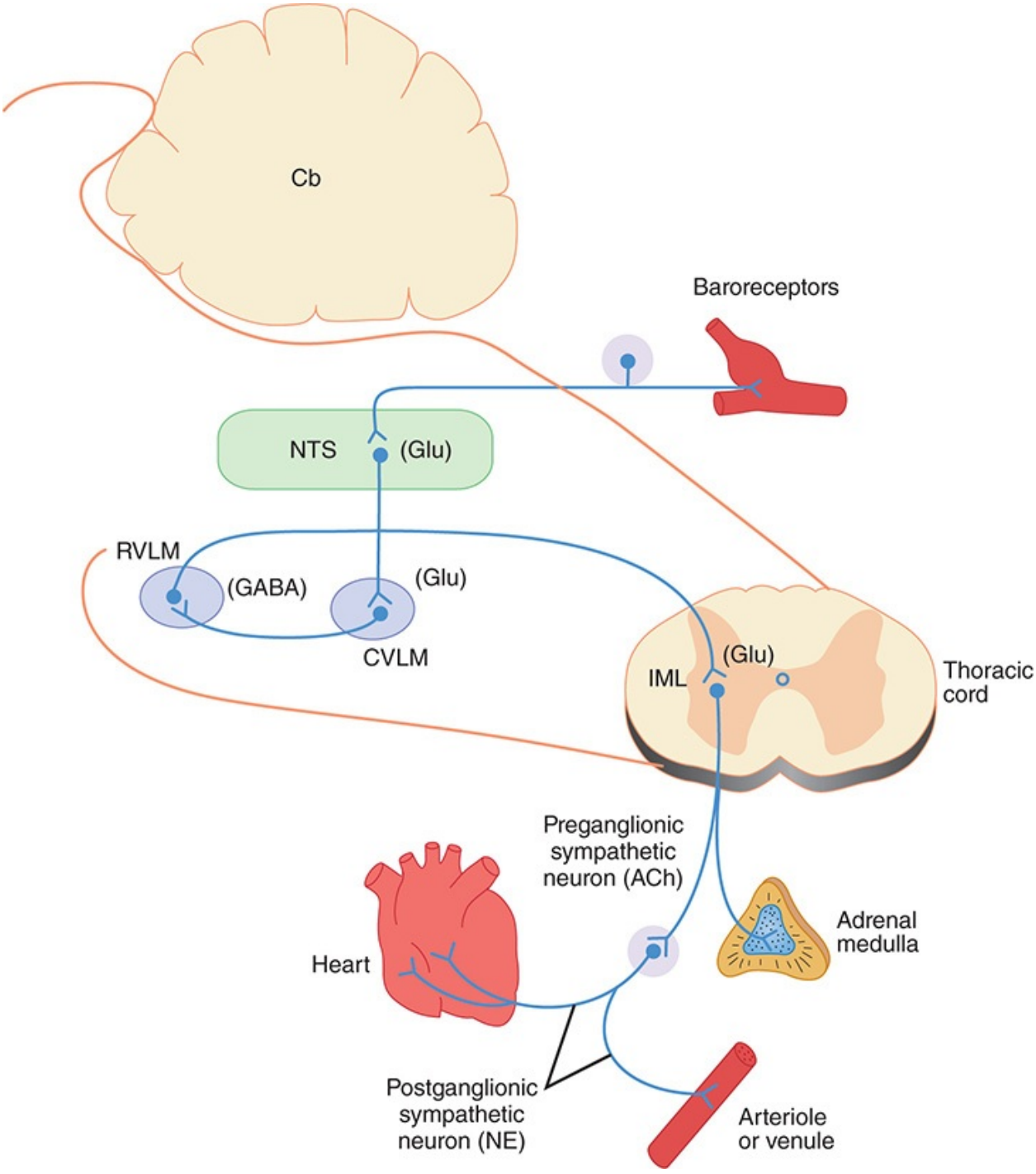


FIGURE 32–2 Basic neural pathways involved in the control of blood pressure. The drawing shows a parasagittal section of the brainstem and its connections to the thoracic spinal cord. The rostral ventrolateral medulla (RVLM) is one of the major sources of excitatory input to sympathetic nerves

controlling the vasculature. These neurons receive inhibitory input from the baroreceptors via an inhibitory (GABAergic) neuron in the caudal ventrolateral medulla (CVLM). The nucleus of the tractus solitarius (NTS) is the site of termination of baroreceptor afferent fibers that release glutamate. ACh, acetylcholine; GABA, γ-aminobutyric acid; Glu, glutamate; IML, intermediolateral cell column; NE, norepinephrine.

The activity of RVLM neurons is determined by many factors **(see Table 32–2)**. They include input from a pathway that originates in the cerebral cortex (particularly the limbic cortex) and has a relay in the hypothalamus. This pathway is responsible for the blood pressure rise and tachycardia produced by emotions such as stress, sexual excitement, and anger. The RVLM also receives input from other brain regions and from arterial baroreceptors and the carotid and aortic chemoreceptors. In addition, some stimuli act directly on RVLM neurons to increase their firing rate.

TABLE 32–2 Factors affecting the activity of the RVLM.

Direct stimulation
CO_2
Hypoxia
Excitatory inputs
Cortex via hypothalamus
Mesencephalic periaqueductal gray
Brainstem reticular formation
Pain pathways
Somatic afferents (somatosympathetic reflex)
Carotid and aortic chemoreceptors
Inhibitory inputs
Cortex via hypothalamus
Caudal ventrolateral medulla
Caudal medullary raphe nuclei
Lung inflation afferents

Carotid, aortic, and cardiopulmonary baroreceptors

Inflation of the lungs causes vasodilation and a decrease in blood pressure. This response is mediated via vagal afferents from the lungs that inhibit RVLM and sympathetic nerve activities. Pain usually causes a rise in blood pressure via afferent impulses converging in the RVLM. However, prolonged severe pain may cause vasodilation and fainting. The activity in afferents from exercising muscles probably exerts a similar pressor effect via a pathway to the RVLM. The pressor response to stimulation of somatic afferent nerves is called the **somatosympathetic reflex.**

The medulla is also a major site of origin of excitatory input to cardiac vagal motor neurons in the nucleus ambiguus **(Figure 32–3)**. **Table 32–3** is a summary of factors that affect heart rate. In general, stimuli that increase heart rate also increase blood pressure, whereas those that decrease heart rate lower blood pressure. However, there are exceptions, such as combined hypotension and tachycardia with stimulation of atrial stretch receptors and hypertension and bradycardia during increased intracranial pressure.

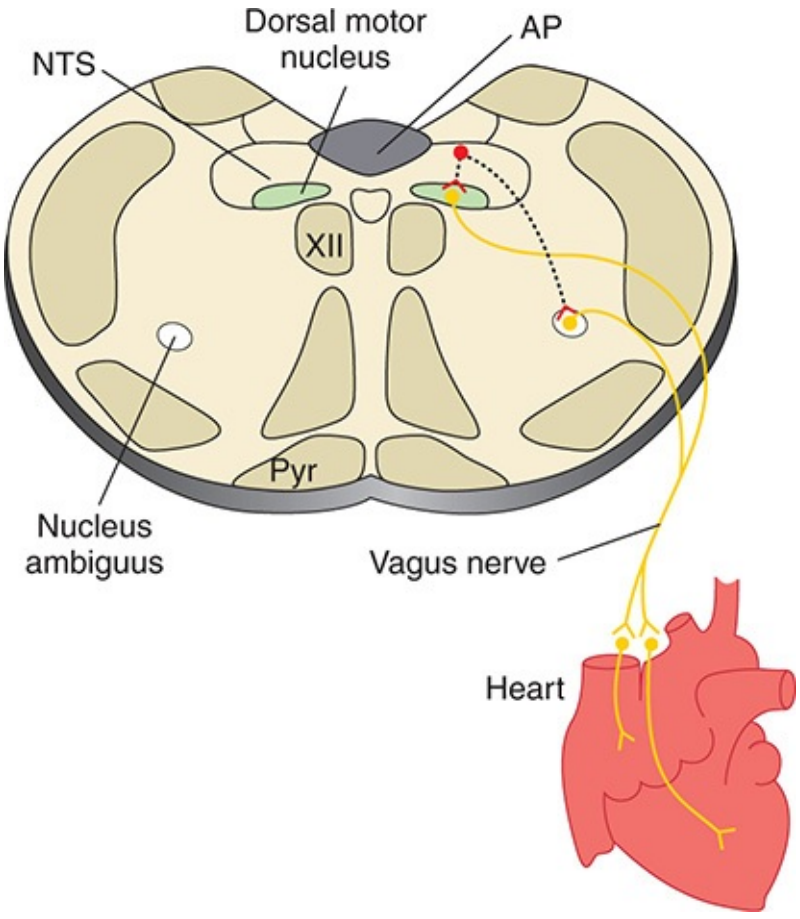


FIGURE 32–3 Basic pathways involved in the control of heart rate by the vagus nerves. Neurons in the nucleus of the tractus solitarius (NTS) project to and excite cardiac preganglionic parasympathetic neurons primarily in the nucleus ambiguus. Some are also located in the dorsal motor nucleus of the vagus; however, this nucleus primarily contains vagal motor neurons that project to the gastrointestinal tract. AP, area postrema; Pyr, pyramid; XII, hypoglossal nucleus.

TABLE 32–3 Factors affecting heart rate.

Heart rate accelerated by:
Decreased activity of arterial baroreceptors
Increased activity of atrial stretch receptors
Inspiration
Excitement
Anger
Most painful stimuli
Hypoxia
Exercise
Thyroid hormones
Fever
Heart rate slowed by:
Increased activity of arterial baroreceptors
Expiration
Fear
Grief
Stimulation of pain fibers in trigeminal nerve
Increased intracranial pressure

BARORECEPTORS

The **baroreceptors** are stretch receptors in the walls of the heart and blood vessels. The **carotid sinus** and **aortic arch** receptors monitor the arterial

circulation. There are also stretch receptors in the low-pressure part of the circulation **(cardiopulmonary receptors).** These include receptors in the walls of the right and left atria at the entrance of the superior and inferior vena cava, in the pulmonary veins, and in the pulmonary circulation.

The carotid sinus is a small dilation of the internal carotid artery just above the bifurcation of the common carotid into external and internal carotid branches **(Figure 32–4)**. Baroreceptors are in the adventitia of the vessel within this dilation as well as in the wall of the arch of the aorta. The afferent nerve fibers from the carotid sinus form a distinct branch of the glossopharyngeal nerve, the **carotid sinus nerve.** The fibers from the aortic arch form a branch of the vagus nerve, the **aortic depressor nerve.**

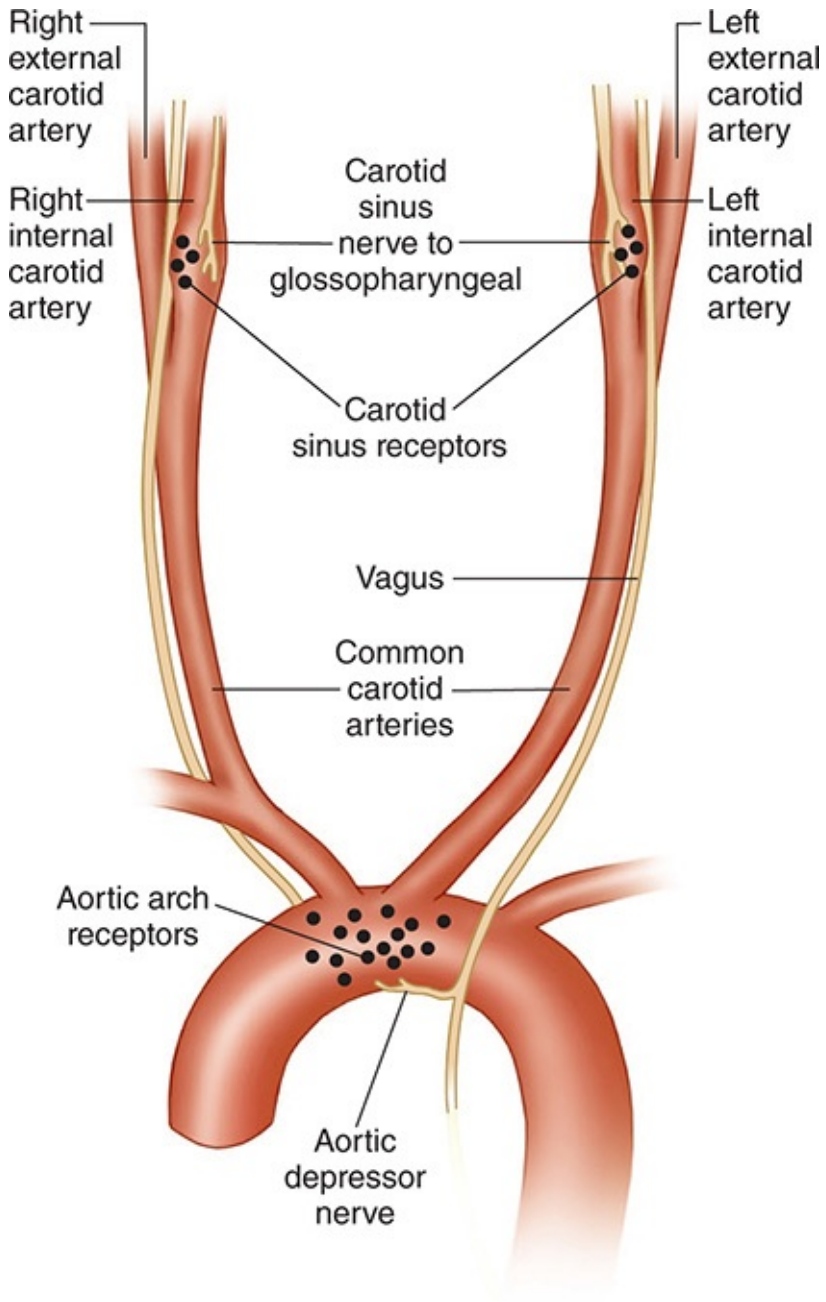


FIGURE 32–4 Baroreceptor areas in the carotid sinus and aortic arch. One set of baroreceptors (stretch receptors) is located in the carotid sinus, a small dilation of the internal carotid artery just above the bifurcation of the common carotid into external and internal carotid branches. These receptors are innervated by a branch of the glossopharyngeal nerve, the carotid sinus nerve. A second set of baroreceptors is located in the wall of the arch of the aorta. These receptors are innervated by a branch of the vagus nerve, the aortic depressor nerve.

The baroreceptors are stimulated by distension of the structures in which they are located; they discharge at an increased rate when the pressure in the carotid sinus and aortic arch rises. Their afferent fibers pass via the glossopharyngeal and vagus nerves to the medulla. These fibers release glutamate to excite neurons in the **nucleus of the tractus solitarius** (NTS) as the first synapse in the baroreceptor reflex (Figure 32–2). These NTS neurons then excite neurons in the **caudal ventrolateral medulla** (CVLM). These CVLM neurons release the inhibitory neurotransmitter γ-aminobutyric acid (GABA) into the RVLM to reduce the firing rate of these. Excitatory projections also extend from the NTS to the vagal motor neurons in the nucleus ambiguus and dorsal motor nucleus (Figure 32–3). Thus, increased baroreceptor discharge *inhibits* the tonic discharge of sympathetic nerves and *excites* the vagal innervation of the heart. These neural changes produce vasodilation, venodilation, hypotension, bradycardia, and a decrease in cardiac output.

BARORECEPTOR NERVE ACTIVITY

Baroreceptors are more sensitive to pulsatile pressure than to constant pressure. A decline in pulse pressure without any change in mean pressure decreases the rate of baroreceptor discharge and provokes a rise in systemic blood pressure and tachycardia. At normal blood pressure levels (about 100 mm Hg mean pressure), a burst of action potentials appears in a single baroreceptor fiber during systole, but there are few action potentials in early diastole **(Figure 32–5)**. At lower mean pressures, this phasic change in firing is even more dramatic with activity only occurring during systole. At these lower pressures, the overall firing rate is considerably reduced. The threshold for eliciting activity in the carotid sinus nerve is approximately 50 mm Hg; maximal activity occurs at approximately 200 mm Hg, with activity throughout the cardiac cycle.

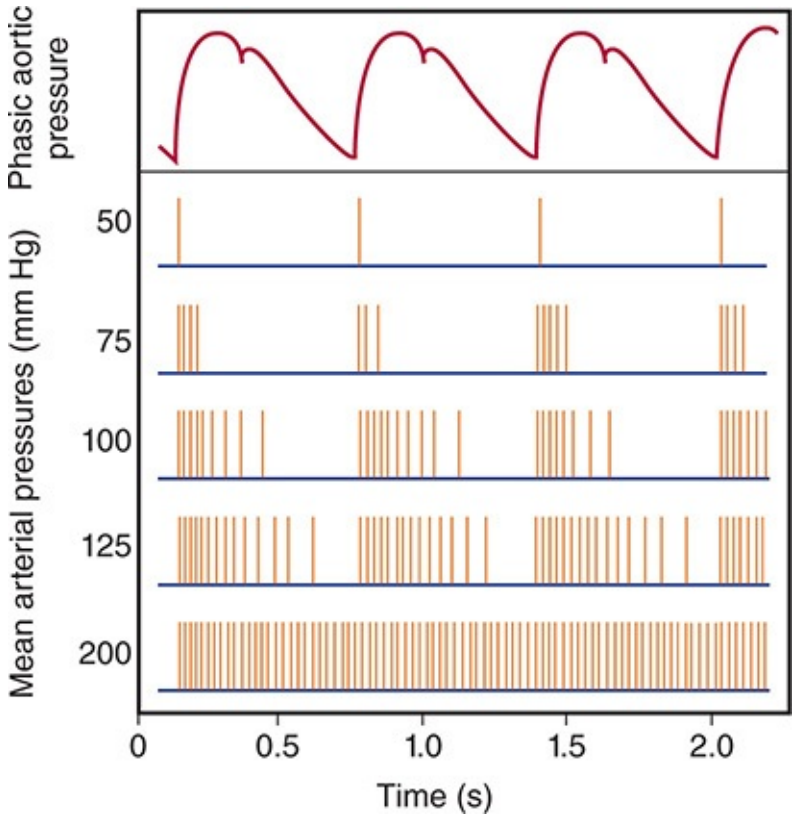


FIGURE 32–5 Discharges (vertical lines) in a single afferent nerve fiber from the carotid sinus at various levels of mean arterial pressures, plotted against changes in aortic pressure with time. Baroreceptors are very sensitive to changes in pulse pressure as shown by the record of phasic aortic pressure. (Reproduced with permission from Levy MN, Pappano AJ: *Cardiovascular Physiology*, 9th ed. Mosby; 2007.)

From the foregoing discussion, it is apparent that the baroreceptors on the arterial side of the circulation, their afferent connections to the medullary cardiovascular areas, and the efferent pathways from these areas constitute a reflex feedback mechanism that operates to stabilize blood pressure and heart rate. Any drop in systemic arterial pressure decreases the discharge in the buffer nerves, and there is a compensatory rise in blood pressure and cardiac output. Any rise in pressure produces dilation of the arterioles and decreases cardiac output until the blood pressure returns to its previous baseline level.

Clinical Box 32–1

Essential Hypertension & Neurovascular Compression of the RVLM

In about 88% of patients with elevated blood pressure, the cause of the hypertension is unknown, and they are said to have **essential hypertension** (see Chapter 31). **Neurovascular compression** of the RVLM is associated with essential hypertension in some persons. For example, patients with a schwannoma (acoustic neuroma) or meningioma lying close to the RVLM also have hypertension. Magnetic resonance angiography (MRA) has been used to compare the incidence of neurovascular compression in hypertensive and normotensive individuals and to correlate indices of increased sympathetic nerve activity with the presence or absence of compression. Some of these studies showed a higher incidence of coexistence of neurovascular compression with essential hypertension than in other forms of hypertension or normotension, but others showed the presence of a compression in normotensive subjects. On the other hand, there was a strong positive relationship between the presence of neurovascular compression and increased sympathetic nerve activity.

THERAPEUTIC HIGHLIGHTS

In the 1970s, Dr. Peter Jannetta, a neurosurgeon in Pittsburgh, PA, developed a technique for “microvascular decompression” of the medulla to treat trigeminal neuralgia and hemifacial spasm, which he attributed to pulsatile compression of the vertebral and posterior inferior cerebellar arteries impinging on the fifth and seventh cranial nerves. Moving the arteries away from the nerves led to reversal of the neurologic symptoms in many cases. Some of these patients were also hypertensive, and they showed reductions in blood pressure postoperatively. Later, a few human studies showed that surgical decompression of the RVLM could sometimes relieve hypertension. There are also reports that hypertension is relieved after surgical decompression in patients with a schwannoma or meningioma in the vicinity of the RVLM.

ROLE OF BARORECEPTORS IN SHORT-TERM CONTROL OF BLOOD PRESSURE

The changes in heart rate and blood pressure that occur on standing up or lying down are due for the most part to baroreceptor reflexes. Baroreceptors are very

important in short-term control of arterial pressure. Activation of the reflex allows for rapid adjustments in blood pressure in response to abrupt changes in posture, blood volume, cardiac output, or peripheral resistance during exercise. Removal of the baroreceptor reflex prevents an individual from adjusting their blood pressure in response to stimuli that cause abrupt changes in blood volume, cardiac output, or peripheral resistance, including exercise and postural changes.

In chronic hypertension, the baroreceptor reflex mechanism is “reset” to maintain an elevated rather than a normal blood pressure. In perfusion studies on hypertensive experimental animals, raising the pressure in the isolated carotid sinus lowers the elevated systemic pressure; and decreasing the perfusion pressure raises the elevated pressure. A long-term change in blood pressure resulting from loss of baroreceptor reflex control is called **neurogenic hypertension.**

ATRIAL STRETCH & CARDIOPULMONARY RECEPTORS

The stretch receptors in the atria are of two types: those that discharge primarily during atrial systole (type A), and those that discharge primarily in late diastole, at the time of peak atrial filling (type B). The discharge of type B baroreceptors is increased when venous return is increased and decreased by positive-pressure breathing, indicating that these baroreceptors respond primarily to distension of the atrial walls. The reflex circulatory adjustments initiated by activation of these receptors include vasodilation and a fall in blood pressure. However, the heart rate is increased rather than decreased.

Receptors in the endocardial surfaces of the ventricles are activated during ventricular distension. The response is a vagal bradycardia and hypotension, comparable to a baroreceptor reflex. Left ventricular stretch receptors may play a role in the maintenance of vagal tone that keeps the heart rate low at rest. Various chemicals are known to elicit reflexes due to activation of cardiopulmonary chemoreceptors and may play a role in various cardiovascular disorders **(Clinical Box 32–2)**.

VALSALVA MANEUVER

The function of the receptors can also be tested by monitoring the changes in pulse pressure and blood pressure that occur in response to brief periods of

straining (forced expiration against a closed glottis: the **Valsalva maneuver**). Valsalva maneuvers occur regularly during coughing, defecation, and heavy lifting. The blood pressure rises at the onset of straining **(Figure 32–6)** because the increase in intrathoracic pressure is added to the pressure of the blood in the aorta. It then falls because the high intrathoracic pressure compresses the veins, decreasing venous return and cardiac output. The decreases in arterial pressure and pulse pressure inhibit the baroreceptors, causing tachycardia and a rise in peripheral resistance. When the glottis is opened and the intrathoracic pressure returns to normal, cardiac output is restored but the peripheral vessels are constricted. The blood pressure therefore rises above normal, and this stimulates the baroreceptors, causing bradycardia and a drop in pressure to normal levels.

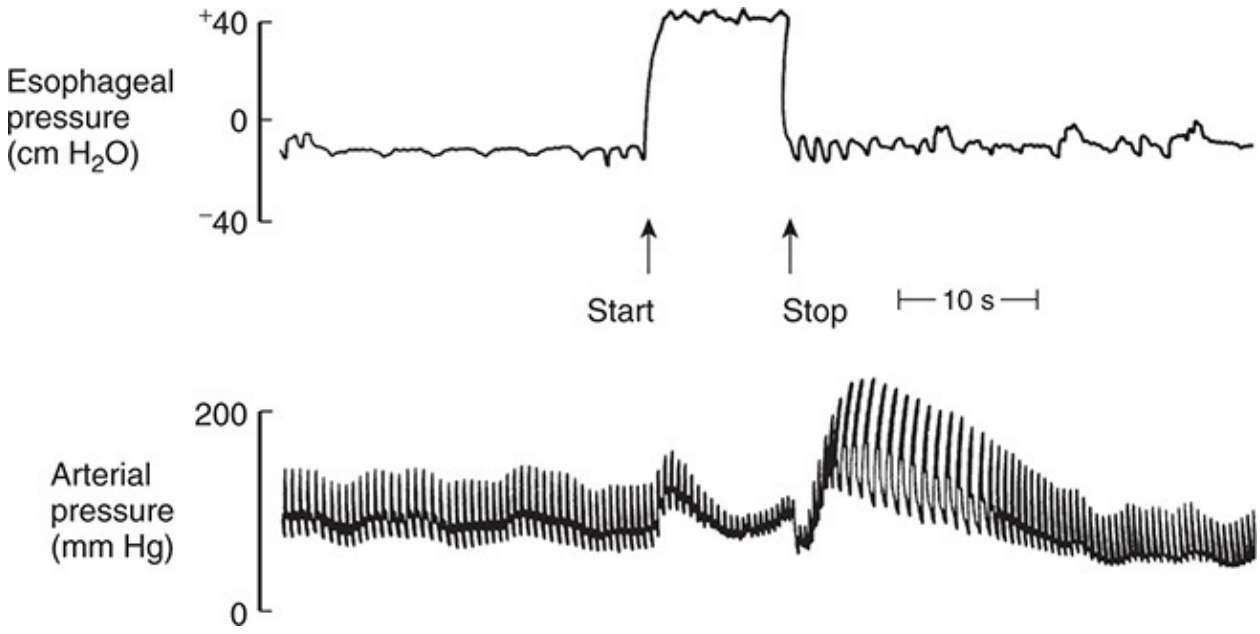


FIGURE 32–6 Diagram of the response to straining (the Valsalva maneuver) in a healthy man, recorded with a needle in the brachial artery. Blood pressure rises at the onset of straining because increased intrathoracic pressure is added to the pressure of the blood in the aorta. It then falls because the high intrathoracic pressure compresses veins, decreasing venous return and cardiac output. (Used with permission of M Mcllroy.)

In patients whose sympathetic nervous system is not functional, heart rate changes still occur because the baroreceptors and the vagi are intact. However, in patients with autonomic insufficiency, a syndrome in which autonomic function is widely disrupted, the heart rate changes are absent. For unknown reasons, patients with primary hyperaldosteronism also fail to show the heart rate changes

and the blood pressure rise when the intrathoracic pressure returns to normal. Their response to the Valsalva maneuver returns to normal after removal of the aldosterone-secreting tumor.

PERIPHERAL CHEMORECEPTOR REFLEX

Peripheral arterial chemoreceptors in the **carotid** and **aortic bodies** are exposed to very high rates of blood flow. These receptors are primarily activated by a reduction in arterial partial pressure of oxygen (PaO_2), but they also respond to an increase in the arterial partial pressure of carbon dioxide ($PaCO_2$) and pH. Chemoreceptors exert their main effects on respiration; however, their activation also leads to vasoconstriction. Heart rate changes are variable and depend on various factors, including changes in respiration. A direct effect of chemoreceptor activation is to increase vagal nerve activity. However, hypoxia also produces hyperpnea and increased catecholamine release from the adrenal medulla, both of which produce tachycardia and an increase in cardiac output. Hemorrhage that produces hypotension leads to chemoreceptor stimulation due to decreased blood flow to the chemoreceptors and consequent stagnant anoxia of these organs. Chemoreceptor activity may also contribute to the production of **Mayer waves,** which are slow, regular oscillations in arterial pressure that occur at the rate of about one every 20–40 s during hypotension. Under these conditions, hypoxia stimulates the chemoreceptors to increase blood pressure; this improves the blood flow in the receptor organs and eliminates the stimulus to the chemoreceptors; blood pressure then falls and a new cycle is initiated. Mayer waves should not be confused with **Traube–Hering waves,** which are fluctuations in blood pressure synchronized with respiration.

Clinical Box 32–2

Cardiopulmonary Chemosensitive Receptors

Activation of chemosensitive vagal C fibers in the cardiopulmonary region (eg, juxtacapillary region of alveoli, ventricles, atria, great veins, and pulmonary artery) causes profound bradycardia, hypotension, and a brief period of apnea followed by rapid shallow breathing. This response pattern is called the **Bezold–Jarisch reflex** and can be elicited by a variety of substances including capsaicin, serotonin, phenylbiguanide, and veratridine.

The Bezold–Jarisch reflex is activated during certain pathophysiologic conditions. For example, this reflex may be activated during myocardial ischemia and reperfusion as a result of increased production of oxygen radicals and by agents used as radiocontrast for coronary angiography. This can contribute to the hypotension that is frequently a stubborn complication of heart disease. Activation of cardiopulmonary chemosensitive receptors may also be part of a defense mechanism protecting individuals from toxic chemical hazards. Activation of cardiopulmonary reflexes may help reduce the amount of inspired pollutants that gets absorbed into the blood, protecting vital organs from potential toxicity of these pollutants, and facilitating their elimination. Finally, the syndrome of cardiac slowing with hypotension **(vasovagal syncope)** has also been attributed to activation of the Bezold–Jarisch reflex. Vasovagal syncope can occur after prolonged upright posture that results in pooling of blood in the lower extremities and diminished intracardiac blood volume (also called **postural syncope**). This phenomenon is exaggerated if combined with dehydration. The resultant arterial hypotension is sensed in the carotid sinus baroreceptors, and afferent fibers from these receptors trigger autonomic signals that increase cardiac rate and contractility. However, pressure receptors in the wall of the left ventricle respond by sending signals that trigger paradoxical bradycardia and decreased contractility, resulting in sudden marked hypotension. The individual also feels lightheaded and may experience a brief episode of loss of consciousness.

THERAPEUTIC HIGHLIGHTS

The most critical intervention for individuals who experience episodes of neurogenic syncope is to avoid dehydration and to avoid situations that trigger the adverse event. Episodes of syncope may be reduced or prevented by an increased dietary salt intake or administration of mineralocorticoids. Vasovagal syncope has been treated with the use of **β-adrenoceptor antagonists** and **disopyramide,** an antiarrhythmic agent that blocks Na^+ channels. Cardiac pacemakers have also been used to stabilize the heart rate during episodes that normally trigger bradycardia.

CENTRAL CHEMORECEPTORS

When intracranial pressure is increased, the blood supply to the brain is compromised, and the local hypercapnia activates central chemoreceptors located along the ventrolateral medullary surface. Their activation induces a rise in systemic arterial pressure **(Cushing reflex)** that tends to restore the blood flow to the brain. Over a considerable range, the blood pressure rise is proportional to the increase in intracranial pressure. The rise in blood pressure causes a reflex decrease in heart rate via the arterial baroreceptors. This is why bradycardia rather than tachycardia is characteristically seen in patients with increased intracranial pressure.

Hypercapnia directly stimulates the RVLM (Table 32–3), but the direct peripheral effect of hypercapnia is vasodilation. Therefore, the peripheral and central actions tend to cancel each other out. Moderate hyperventilation that significantly lowers the CO_2 tension of the blood causes cutaneous and cerebral vasoconstriction, but there is little change in blood pressure. Exposure to high concentrations of CO_2 is associated with marked cutaneous and cerebral vasodilation, but vasoconstriction occurs elsewhere and usually there is a slow rise in blood pressure.

LOCAL REGULATION

AUTOREGULATION

The capacity of tissues to regulate their own blood flow is referred to as **autoregulation.** Most vascular beds have an intrinsic capacity to compensate for moderate changes in perfusion pressure by changes in vascular resistance, so that blood flow remains relatively constant. This capacity is well developed in the kidneys (see Chapter 37), but it has also been observed in the mesentery, skeletal muscle, brain, liver, and myocardium. It is due in part to the intrinsic contractile response of smooth muscle to stretch **(myogenic theory of autoregulation).** As the pressure rises, the blood vessels are distended and the vascular smooth muscle fibers that surround the vessels contract. If it is postulated that the muscle responds to the tension in the vessel wall, this theory explains the greater degree of contraction at higher pressures; the wall tension is proportional to the distending pressure times the radius of the vessel (law of Laplace; see Chapter 31), and the maintenance of a stable wall tension as the pressure rises would require a decrease in radius. Vasodilator substances tend to accumulate in active tissues, and these “metabolites” also contribute to autoregulation **(metabolic theory of autoregulation).** When blood flow decreases, they accumulate and the

vessels dilate; when blood flow increases, they tend to be washed away.

VASODILATOR METABOLITES

The metabolic changes that produce vasodilation include, in most tissues, decreases in O_2 tension and pH. These changes cause relaxation of the arterioles and precapillary sphincters. A local fall in O_2 tension, in particular, can initiate a program of vasodilatory gene expression secondary to production of hypoxia-inducible factor-1α (HIF-1α), a transcription factor with multiple targets. Increases in CO_2 tension and osmolality also dilate the vessels. The direct dilator action of CO_2 is most pronounced in the skin and brain. The neutrally mediated vasoconstrictor effects of systemic, as opposed to local, hypoxia and hypercapnia have been discussed above. A rise in temperature exerts a direct vasodilator effect, and the temperature rise in active tissues (due to the heat of metabolism) may contribute to vasodilation. K^+ is another substance that accumulates locally, and has demonstrated dilator activity secondary to the hyperpolarization of vascular smooth muscle cells. Lactate may also contribute to the dilation. In injured tissues, histamine released from damaged cells increases capillary permeability. Thus, it is probably responsible for some of the swelling in areas of inflammation. Adenosine plays a vasodilator role in cardiac muscle but not in skeletal muscle. It also inhibits the release of norepinephrine.

LOCALIZED VASOCONSTRICTION

Injured arteries and arterioles constrict strongly. The constriction appears to be due in part to the local liberation of serotonin from platelets that stick to the vessel wall in the injured area. Injured veins also constrict.

A drop in tissue temperature causes vasoconstriction, and this local response to cold plays a part in temperature regulation (see Chapter 17).

SUBSTANCES SECRETED BY THE ENDOTHELIUM

ENDOTHELIAL CELLS

As noted in Chapter 31, the endothelial cells constitute a large and important

tissue. They secrete many growth factors and vasoactive substances. The vasoactive substances include prostaglandins and thromboxanes, nitric oxide (NO), and endothelins.

PROSTACYCLIN & THROMBOXANE A_2

Prostacyclin is produced by endothelial cells and thromboxane A_2 by platelets from their common precursor, arachidonic acid, via the cyclooxygenase pathway. Thromboxane A_2 promotes platelet aggregation and vasoconstriction, whereas prostacyclin inhibits platelet aggregation and promotes vasodilation. The balance between thromboxane A_2 and prostacyclin fosters localized platelet aggregation and consequent clot formation (see Chapter 31) while preventing excessive extension of the clot and maintaining blood flow around it.

The thromboxane A_2–prostacyclin balance can be shifted toward prostacyclin by administration of low doses of aspirin. Aspirin produces irreversible inhibition of cyclooxygenase by acetylating a serine residue in its active site. Obviously, this reduces production of both thromboxane A_2 and prostacyclin. However, endothelial cells produce new cyclooxygenase in a matter of hours, whereas platelets cannot manufacture the enzyme, and the level rises only as new platelets enter the circulation. This is a slow process because platelets have a half-life of about 4 days. Therefore, administration of small amounts of aspirin for prolonged periods reduces clot formation and has been shown to be of value in preventing myocardial infarctions, unstable angina, transient ischemic attacks, and stroke.

NITRIC OXIDE

Many different stimuli act on endothelial cells to produce **endothelium-derived relaxing factor (EDRF),** a substance that is now known to be **nitric oxide.** NO is synthesized from arginine **(Figure 32–7)** in a reaction catalyzed by nitric oxide synthase (NO synthase, NOS). Three isoforms of NOS have been identified: NOS 1, found in the nervous system; NOS 2, found in macrophages and other immune cells; and NOS 3, found in endothelial cells. NOS 1 and NOS 3 are activated by agents that increase intracellular Ca^{2+} concentrations, including the vasodilators acetylcholine and bradykinin. The NOS in immune cells is not activated by Ca^{2+} but is induced by cytokines. NO formed in the

endothelium diffuses to smooth muscle cells, where it activates soluble guanylyl cyclase, producing cyclic 3,5-guanosine monophosphate (cGMP; see Figure 32–7), which in turn mediates the relaxation of vascular smooth muscle. NO is short-lived and is inactivated by hemoglobin.

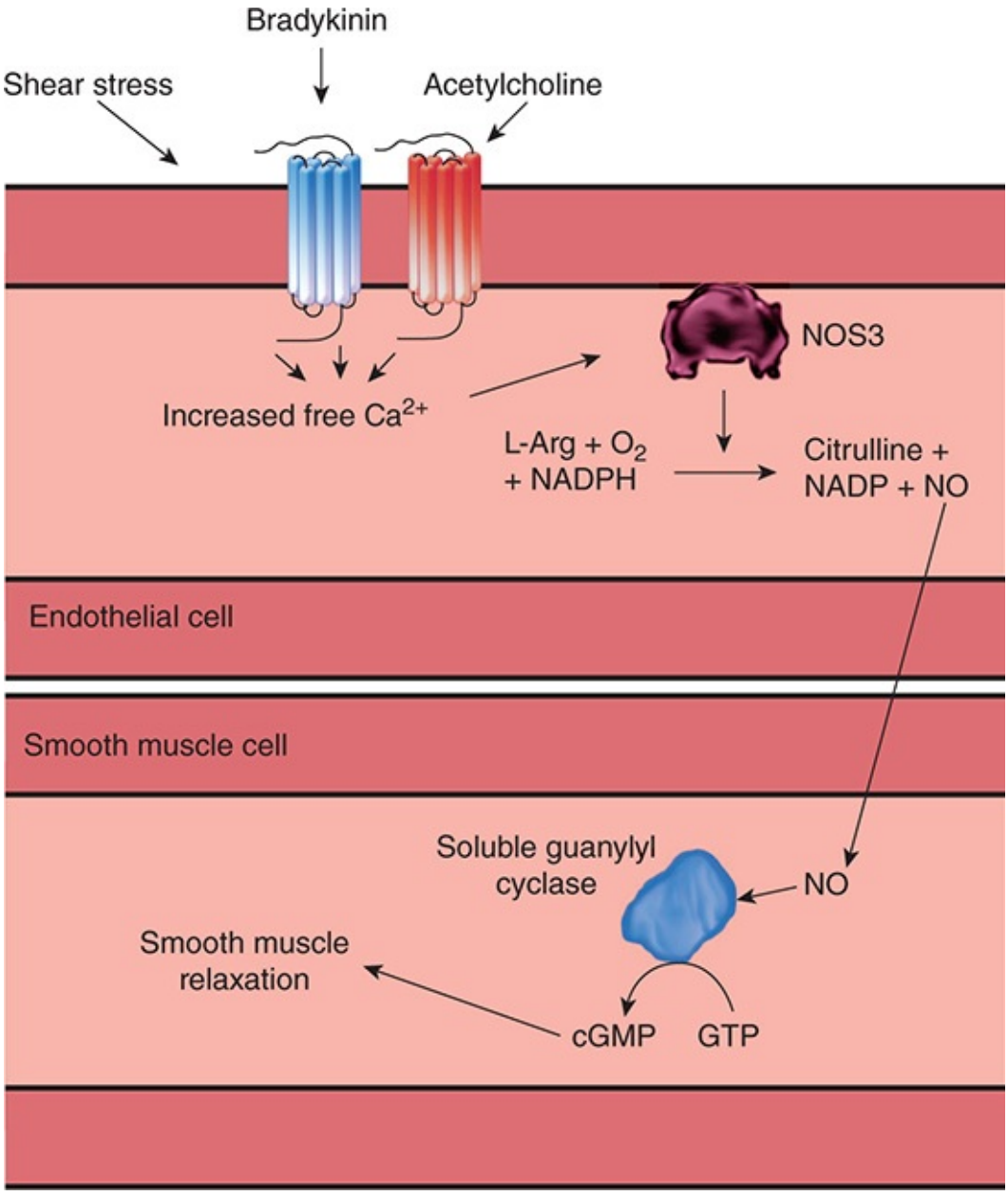


FIGURE 32–7 Endothelial-derived NO leads to smooth muscle relaxation. The endothelial form of nitric oxide synthase (NOS3) is activated by increased free Ca^{2+} and can be induced by a variety of extracellular signals. NOS3 acts on L-arginine, NADPH, and O_2 to yield citrulline, NADP, and NO. A variety of

cofactors are required for this reaction (not shown). NO can diffuse to adjacent smooth muscle cells where it activates soluble guanylyl cyclase. This leads to the production of cGMP and subsequent smooth muscle relaxation.

Adenosine, atrial natriuretic peptide (ANP), and histamine via H_2 receptors produce relaxation of vascular smooth muscle that is independent of the endothelium. However, acetylcholine, histamine via H_1 receptors, bradykinin, vasoactive intestinal peptide (VIP), substance P, and some other polypeptides act via the endothelium, and various vasoconstrictors that act directly on vascular smooth muscle would produce much greater constriction if their effects were not limited by their ability simultaneously to cause release of NO. When flow to a tissue is suddenly increased by arteriolar dilation, the large arteries to the tissue also dilate. This flow-induced dilation is due to local release of NO. Products of platelet aggregation also cause release of NO, and the resulting vasodilation helps keep blood vessels with an intact endothelium patent. This is in contrast to injured blood vessels, where the endothelium is damaged at the site of injury and platelets therefore aggregate and produce vasoconstriction (see Chapter 31).

Further evidence for a physiologic role of NO is the observation that mice lacking NOS 3 are hypertensive. This suggests that tonic release of NO is necessary to maintain normal blood pressure.

NO is also involved in vascular remodeling and angiogenesis, and NO may be involved in the pathogenesis of atherosclerosis. It is interesting in this regard that in some patients with heart transplants, an accelerated form of atherosclerosis develops in the vessels of the transplant, and there is reason to believe that this is triggered by endothelial damage. Nitroglycerin and other nitrovasodilators that are of great value in the treatment of angina act by stimulating guanylyl cyclase in the same manner as NO.

Penile erection is also produced by release of NO, with consequent vasodilation and engorgement of the corpora cavernosa (see Chapter 23). This accounts for the efficacy of drugs such as Viagra that slow the breakdown of cGMP.

OTHER FUNCTIONS OF NO

NO is present in the brain and, acting via cGMP, it is important in brain function (see Chapter 7). NO is also necessary for the antimicrobial and cytotoxic activity of various inflammatory cells, although the net effect of NO in inflammation and tissue injury depends on the amount and kinetics of release, which in turn may

depend on the specific NOS isoform involved. In the gastrointestinal tract, NO is important in the relaxation of smooth muscle. Other functions of NO are mentioned in other parts of this book.

CARBON MONOXIDE

The production of carbon monoxide (CO) from heme is shown in Figure 28–4. Heme oxygenase-2 (HO-2), the enzyme that catalyzes the reaction, is also present in cardiovascular tissues, and there is growing evidence that CO as well as NO produces local dilation in blood vessels. Interestingly, hydrogen sulfide is likewise emerging as a third gas transmitter that regulates vascular tone, although the relative roles of NO, CO, and H_2S have yet to be established.

ENDOTHELINS

Endothelial cells also produce **endothelin-1,** one of the most potent vasoconstrictor agents yet isolated. Endothelin-1 (ET-1), endothelin-2 (ET-2), and endothelin-3 (ET-3) are the members of a family of three similar 21-amino-acid polypeptides. Each is encoded by a different gene.

ENDOTHELIN-1

In endothelial cells, the product of the endothelin-1 gene is processed to a 39-amino-acid prohormone, **big endothelin-1,** which has about 1% of the activity of endothelin-1. The prohormone is cleaved by **endothelin-converting enzyme** at a tryptophan-valine (Trp-Val) bond to form endothelin-1. Small amounts of big endothelin-1 and endothelin-1 are secreted into the blood, but for the most part, they are secreted locally and act in a paracrine fashion.

Two different endothelin receptors have been cloned, both of which are coupled via G-proteins to phospholipase C (see Chapter 2). The ET_A receptor, which is specific for endothelin-1, is found in many tissues and mediates the vasoconstriction produced by endothelin-1. The ET_B receptor responds to all three endothelins and is coupled to G_i. It may mediate vasodilation, and it appears to mediate the developmental effects of the endothelins (see below).

REGULATION OF SECRETION

Endothelin-1 is not stored in secretory granules, and most regulatory factors alter the transcription of its gene, with changes in secretion occurring promptly thereafter. Factors activating and inhibiting the gene are summarized in **Table 32–4**.

TABLE 32–4 Regulation of endothelin-1 secretion via transcription of its gene.

Stimulators
Angiotensin II
Catecholamines
Growth factors
Hypoxia
Insulin
Oxidized LDL
HDL
Shear stress
Thrombin
Inhibitors
NO
ANP
PGE_2
Prostacyclin

ANP, atrial natriuretic peptide; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NO, nitric oxide; PGE_2, prostaglandin E_2; VIP, vasoactive intestinal polypeptide.

CARDIOVASCULAR FUNCTIONS

As noted above, endothelin-1 appears primarily to be a paracrine regulator of vascular tone. However, endothelin-1 is not increased in hypertension, and in mice in which one allele of the endothelin-1 gene is knocked out, blood pressure

is actually elevated rather than reduced. The concentration of circulating endothelin-1 is, however, elevated in heart failure and after myocardial infarction, so it may play a role in the pathophysiology of these diseases.

OTHER FUNCTIONS OF ENDOTHELINS

Endothelin-1 is found in the brain and kidneys as well as the endothelial cells. Endothelin-2 is produced primarily in the kidneys and intestine. Endothelin-3 is present in the blood and is found in high concentrations in the brain. It is also found in the kidneys and gastrointestinal tract. In the brain, endothelins are abundant and, in early life, are produced by both astrocytes and neurons. They are found in the dorsal root ganglia, ventral horn cells, the cortex, the hypothalamus, and cerebellar Purkinje cells. They also play a role in regulating transport across the blood-brain barrier. There are endothelin receptors on mesangial cells (see Chapter 37), and the polypeptide participates in tubuloglomerular feedback.

SYSTEMIC REGULATION BY NEUROHUMORAL AGENTS

Many circulating substances affect the vascular system. The vasodilator regulators include kinins, VIP, and ANP. Circulating vasoconstrictor hormones include vasopressin, norepinephrine, epinephrine, and angiotensin II.

KININS

Two related vasodilator peptides called **kinins** are found in the body. One is the nonapeptide **bradykinin,** and the other is the decapeptide **kallidin,** also known as **lysylbradykinin** **(Figure 32–8)**. Kallidin can be converted to bradykinin by aminopeptidase. Both peptides are metabolized to forms that are active at the type 1 bradykinin receptor by **kininase I,** a carboxypeptidase that removes the carboxyl terminal arginine (Arg). In addition, the dipeptidylcarboxypeptidase **kininase II** inactivates bradykinin and kallidin by removing phenylalanine-arginine (Phe-Arg) from the carboxyl terminal as well as additional cleavages. Kininase II is the same enzyme as **angiotensin-converting enzyme (ACE),** which removes histidine-leucine (His-Leu) from the carboxyl terminal end of angiotensin I.

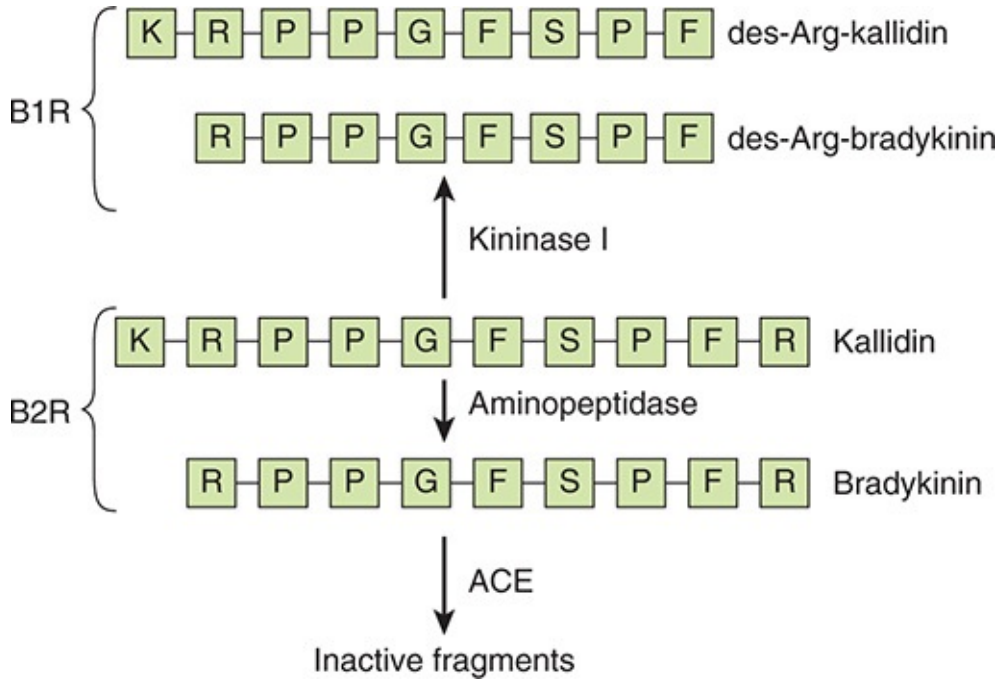


FIGURE 32–8 Kinins. Kallidin (lysylbradykinin) can be converted to bradykinin by aminopeptidase. Both kallidin and bradykinin act at type 2 bradykinin receptors (B2R). The peptides are converted by kininase I to their des-Arg derivatives that are ligands for type 1 bradykinin receptors (B1R). They can also be cleaved by angiotensin-converting enzyme (ACE, also known as kininase II) to shorter fragments that lack biologic activity.

Bradykinin and kallidin are formed from two precursor proteins: **high-molecular-weight kininogen** and **low-molecular-weight kininogen** **(Figure 32–9)**. They are formed by alternative splicing of a single gene located on chromosome 3. Proteases called **kallikreins** release the peptides from their precursors. There are two types of kallikreins: **plasma kallikrein,** which circulates in an inactive form, and **tissue kallikrein,** which appears to be located primarily on the apical membranes of cells concerned with transcellular electrolyte transport. Tissue kallikrein is found in many tissues, including sweat and salivary glands, the pancreas, the prostate, the intestine, and the kidneys. Tissue kallikrein acts on high-molecular-weight kininogen to form bradykinin and low-molecular-weight kininogen to form kallidin. When activated, plasma kallikrein acts on high-molecular-weight kininogen to form bradykinin.

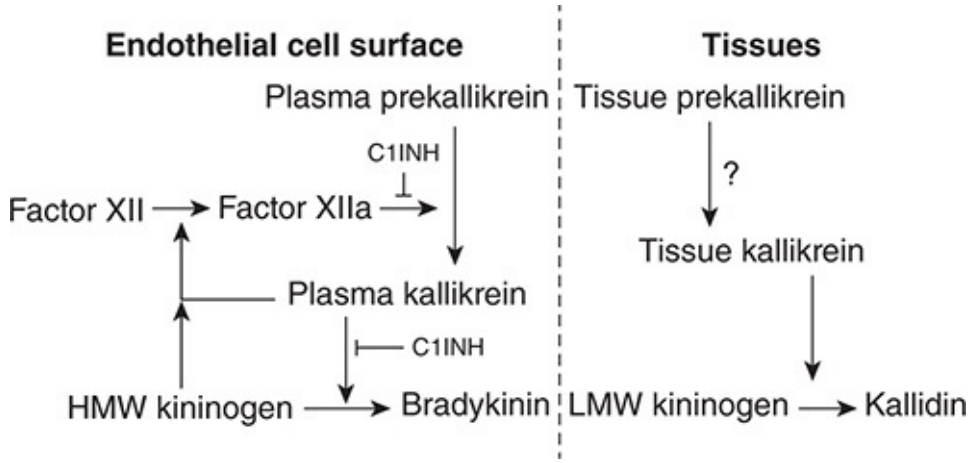


FIGURE 32–9 Formation of kinins from high-molecular-weight (HMW) and low-molecular-weight (LMW) kininogens. Note that the formation of bradykinin is subject to a positive feedback loop that can be interrupted in two places by C1-esterase inhibitor (C1INH).

Inactive plasma kallikrein **(prekallikrein)** is converted to the active form, kallikrein, by active factor XII, the factor that initiates the intrinsic blood clotting cascade. Kallikrein also activates factor XII in a positive feedback loop, and high-molecular-weight kininogen has a factor XII–activating action (see Figure 31–12). This system is normally kept in check by the C1-esterase inhibitor (C1INH), which is a member of the serpin protein family that inhibits several enzymes in the clotting cascade by binding irreversibly to their active sites.

The actions of both kinins resemble those of histamine. They are primarily paracrines, although small amounts are also found in the circulating blood. They cause contraction of visceral smooth muscle, but they relax vascular smooth muscle via NO, lowering blood pressure. They also significantly increase vascular permeability resulting in edema, attract leukocytes, and cause pain upon injection under the skin. They are formed during active secretion in sweat glands, salivary glands, and the exocrine portion of the pancreas, and they are probably responsible for the increase in blood flow when these tissues are actively secreting their products. In keeping with the scheme for bradykinin production outlined above, mutations in C1INH lead to the rare bradykinin-mediated swelling disorder known as hereditary angioedema.

Two bradykinin receptors, B1R and B2R, have been identified. The B1R may mediate the pain-producing effects of the kinins, and antagonists have entered clinical trials for the treatment of pain and inflammation. The B2 receptor has strong homology to the histamine H_2-receptor and is found in many different

tissues.

NATRIURETIC HORMONES

A family of natriuretic peptides is also involved in vascular regulation, including ANP secreted by the heart, B-type natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). They are released in response to hypervolemia. ANP and BNP circulate, whereas CNP acts predominantly in a paracrine fashion. In general, these peptides antagonize the action of various vasoconstrictor agents and lower blood pressure. ANP and BNP also serve to coordinate the control of vascular tone with fluid and electrolyte homeostasis via actions on the kidney.

CIRCULATING VASOCONSTRICTORS

Vasopressin is a potent vasoconstrictor, but when it is injected in healthy individuals, there is a compensating decrease in cardiac output, so that there is little change in blood pressure. Its role in blood pressure regulation is discussed in Chapter 17.

Norepinephrine has a generalized vasoconstrictor action, whereas epinephrine dilates the vessels in skeletal muscle and the liver. The relative unimportance of circulating norepinephrine, as opposed to norepinephrine released from vasomotor nerves, is pointed out in Chapter 19, where the cardiovascular actions of catecholamines are discussed in detail.

Angiotensin II has a generalized vasoconstrictor action. It is formed by the action of ACE on angiotensin I, which itself is liberated by the action of renin from the kidney on circulating angiotensinogen (see Chapter 38). Renin secretion, in turn, is increased when the blood pressure falls or extracellular fluid (ECF) volume is reduced, and angiotensin II therefore helps maintain blood pressure. Angiotensin II also increases water intake and stimulates aldosterone secretion, and increased formation of angiotensin II is part of a homeostatic mechanism that operates to maintain ECF volume (see Chapter 19). In addition, there are renin–angiotensin systems in many different organs, including the walls of blood vessels. Angiotensin II produced in blood vessel walls could be important in some forms of clinical hypertension. The role of angiotensin II in cardiovascular regulation is also amply demonstrated in the widespread use of ACE inhibitors as antihypertensive medications.

Urotensin-II, a polypeptide first isolated from the spinal cord of fish, is

present in human cardiac and vascular tissue. It is one of the most potent mammalian vasoconstrictors known, and is being explored for its role in a large range of different human disease states. For example, levels of both urotensin-II and its receptor have been shown to be elevated in hypertension and heart failure, and may be markers of disease in these and other conditions.

CHAPTER SUMMARY

- The vasculature is innervated by sympathetic nerves that mediate vasoconstriction; the heart is innervated both by sympathetic nerves that mediate an increase in heart rate, AV conduction, and ventricular contractility and by parasympathetic (vagal) nerves that produce a reduction in heart rate and conduction through the AV node.
- RVLM neurons project to the thoracolumbar IML and release glutamate on preganglionic sympathetic neurons that innervate the heart and vasculature. The NTS is the major excitatory input to cardiac vagal motor neurons in the nucleus ambiguus. RVLM and NTS neurons receive input from other brain regions, baroreceptors, chemoreceptors, and other sources.
- Carotid sinus and aortic depressor baroreceptors are innervated by branches of the ninth and tenth cranial nerves, respectively (glossopharyngeal and aortic depressor nerves). These receptors are most sensitive to changes in pulse pressure but also respond to changes in mean arterial pressure. Baroreceptor nerves terminate in the NTS and release glutamate. NTS neurons project to the CVLM and nucleus ambiguus and release glutamate. CVLM neurons project to RVLM and release GABA. This leads to a reduction in sympathetic activity and an increase in vagal activity (ie, the baroreceptor reflex).
- The reflex circulatory adjustments initiated by activation of atrial stretch receptors include a reduction in blood pressure and an increase in heart rate. Activation of ventricular stretch receptors induces hypotension and bradycardia. Activation of cardiopulmonary chemoreceptors induces the Bezold–Jarisch reflex that plays a role in various cardiovascular disorders (eg, myocardial ischemia and reperfusion and vasovagal syncope).
- Activation of peripheral chemoreceptors in the carotid and aortic bodies, primarily by a reduction in PaO_2, leads to an increase in vasoconstriction. Heart rate changes are variable and depend on various factors including changes in respiration. Central chemoreceptors are activated primarily by

hypercapnia; activation leads to an increase in blood pressure and a baroreceptor reflex-induced bradycardia.

- Most vascular beds have a capacity to respond to changes in blood pressure within a certain range by altering vascular resistance to maintain stable blood flow. This property is known as autoregulation and is related to intrinsic properties of the vascular smooth muscle as well as the actions of local vasoactive metabolites.
- Local factors such as oxygen tension, pH, temperature, and metabolic products contribute to vascular regulation; many produce vasodilation to restore blood flow.
- The endothelium is an important source of vasoactive mediators that act to either contract or relax vascular smooth muscle. In particular, three gaseous mediators—NO, CO, and H_2S—are important regulators of vasodilation.
- Endothelins and angiotensin II induce vasoconstriction and may be involved in the pathogenesis of some forms of hypertension.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. When a pheochromocytoma (tumor of the adrenal medulla) suddenly discharges a large amount of epinephrine into the circulation, the patient's heart rate would be expected to

   A. increase because the increase in blood pressure stimulates the carotid and aortic baroreceptors.

   B. increase because epinephrine has a direct chronotropic effect on the heart.

   C. increase because of increased tonic parasympathetic discharge to the heart.

   D. decrease because the increase in blood pressure stimulates the carotid and aortic chemoreceptors.

   E. decrease because of increased tonic parasympathetic discharge to the heart.

2. A 45-year-old woman had a blood pressure of 155/95 mm Hg when she was at her physician's office for a physical. She was worried as this was her first physical in over 10 years. The clinician suggested that she begin monitoring her blood pressure at home. Stress-induced activation of which neurons might contribute to the higher BP while in her physician's office?

   A. Limbic cortex and NTS

B. Hypothalamus and CVLM

C. NTS and CVLM

D. Hypothalamus and RVLM

E. Limbic cortex and nucleus ambiguus

3. Which group of neurons within central autonomic pathways release glutamate on their postsynaptic targets?

A. CVLM neurons projecting to the IML and NTS neurons projecting to the nucleus ambiguus.

B. RVLM neurons projecting to the IML and NTS neurons projecting to the CVLM.

C. NTS neurons projecting to the nucleus ambiguus and CVLM neurons projecting to the RVLM.

D. NTS neurons projecting to the RVLM and CVLM neurons projecting to the RVLM.

E. CVLM neurons projecting to the NTS and RVLM neurons projecting to the IML.

4. List the location of the arterial baroreceptors, their afferent innervation, and the central site of termination of the afferent fibers.

A. Atria and pulmonary veins, vagus nerve, and nucleus ambiguus

B. Carotid body and aortic body, glossopharyngeal nerve and vagus nerve, and NTS

C. Carotid sinus and aortic arch, carotid sinus nerve and aortic depressor nerve, and NTS

D. Carotid sinus and atria, glossopharyngeal and vagus nerves, and nucleus ambiguus

E. Carotid body and ventricle, dorsal root fibers, and RVLM

5. A 30-year-old woman experienced profound bradycardia, hypotension, and a brief period of apnea followed by rapid shallow breathing. This description is consistent with the activation of which of the following reflexes?

A. Cushing reflex

B. Central chemoreceptor reflex

C. Peripheral chemoreceptor reflex

D. Atrial stretch receptor reflex

E. Bezold–Jarisch reflex

6. A 53-year-old woman with chronic lung disease was experiencing difficulty

breathing. Her PaO_2 and $PaCO_2$ were 50 mm Hg and 60 mm Hg, respectively. Peripheral chemoreceptors are very sensitive to ____ and central chemoreceptors are very sensitive to ____. Fill in the missing information

A. Small increases in $PaCO_2$ and small decreases in local partial pressure of O_2.

B. Small decreases in $PaCO_2$ and small increases in local partial pressure of O_2.

C. Small decreases in PaO_2 and small increases in local partial pressure of CO_2.

D. Small increases in PaO_2 and small decreases in local partial pressure of CO_2.

E. Small decreases in arterial pH and small increases in local pH.

7. Due to a partial vascular occlusion, a portion of the small bowel becomes hypoxic. Factors that would likely contribute to an adaptive increase in blood flow in the affected segment would include all of the following except

A. HIF-1α

B. Increased local tension of CO_2

C. Relaxation of precapillary sphincters

D. Increased local concentration of K^+

E. Changes in gene expression

8. A 55-year-old man comes to his primary care clinician complaining of erectile dysfunction. He is given a prescription for Viagra, and on follow-up, reports that his ability to sustain an erection has been improved markedly by this treatment. The action of which of the following vasoactive mediators would primarily be increased in this patient?

A. Histamine

B. Endothelin-1

C. Prostacyclin

D. Nitric oxide

E. Atrial natriuretic peptide

9. Why is the dilator response to injected acetylcholine changed to a constrictor response when the endothelium is damaged?

A. More Na^+ is generated.

B. More bradykinin is generated.
C. The damage lowers the pH of the remaining layers of the artery.
D. The damage augments the production of endothelin by the endothelium.
E. The damage interferes with the production of NO by the endothelium.

CHAPTER 33

Circulation Through Special Regions

OBJECTIVES

After studying this chapter, you should be able to:

- Define the special features of the circulation in the brain. Describe how cerebrospinal fluid (CSF) is formed and reabsorbed, and its role in protecting the brain from injury. Understand how the blood-brain barrier impedes the entry of specific substances into the brain. Explain how cerebral blood flow can be measured, how it is regulated by intracranial pressure, and how it protects the supply of oxygen to the brain.
- Understand the anatomy of the coronary circulation of the heart and how the cardiac cycle regulates flow in the coronary blood vessels. Delineate how the oxygen needs of the contracting myocardium are met by the coronary arteries, and the consequences of their occlusion.
- Describe how the cutaneous circulation participates in heat loss from the body and how this function is regulated. List the vascular reactions of the skin and the reflexes that mediate them.
- Describe the uterine circulation and how it changes during the course of pregnancy and parturition. Understand how the fetus is supplied with oxygen and nutrients in utero, and the circulatory events required for a transition to independent life after birth.

INTRODUCTION

The distribution of the cardiac output to various parts of the body at rest in a normal man is shown in **Table 33–1**. The general principles described in preceding chapters apply to the circulation of all these regions, but the vascular supplies of many organs have additional special features that are important to their physiology. This chapter is concerned with the special circulations of the brain, the heart, and the skin, as well as the placenta and fetus. In addition, the portal circulation of the anterior pituitary is discussed in Chapter 18; the pulmonary circulation in Chapter 34; the renal circulation in Chapter 37; and the circulation of the splanchnic area, particularly the intestines and liver, in Chapters 25 and 28.

TABLE 33–1 Resting blood flow and O_2 consumption of various organs in a 63-kg adult man with a mean arterial blood pressure of 90 mm Hg and an O_2 consumption of 250 mL/min.

Region	Mass (kg)	Blood Flow		Arteriovenous Oxygen Difference (mL/L)	Oxygen Consumption		Resistance (R units)[a]		Percentage of Total	
		mL/min	mL/100 g/min		mL/min	mL/100 g/min	Absolute	per kg	Cardiac Output	Oxygen Consumption
Liver	2.6	1500	57.7	34	51	2.0	3.6	9.4	27.8	20.4
Kidneys	0.3	1260	420.0	14	18	6.0	4.3	1.3	23.3	7.2
Brain	1.4	750	54.0	62	46	3.3	7.2	10.1	13.9	18.4
Skin	3.6	462	12.8	25	12	0.3	11.7	42.1	8.6	4.8
Skeletal muscle	31.0	840	2.7	60	50	0.2	6.4	198.4	15.6	20.0
Heart muscle	0.3	250	84.0	114	29	9.7	21.4	6.4	4.7	11.6
Rest of body	23.8	336	1.4	129	44	0.2	16.1	383.2	6.2	17.6
Whole body	63.0	5400	8.6	46	250	0.4	1.0	63.0	100.0	100.0

[a]R units are pressure (mm Hg) divided by blood flow (mL/s).

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CEREBRAL CIRCULATION: ANATOMIC CONSIDERATIONS

VESSELS

The principal arterial inflow to the brain in humans is via four arteries: two internal carotid arteries and two vertebral arteries. In humans, the carotid arteries are quantitatively the most significant. The vertebral arteries unite to form the basilar artery, and the basilar artery and the carotids form the **circle of Willis** below the hypothalamus. The circle of Willis is the origin of the six large vessels

supplying the cerebral cortex. Substances injected into one carotid artery are distributed almost exclusively to the cerebral hemisphere on that side. Normally no crossing over occurs, probably because the pressure is equal on both sides. Even when it is not, the anastomotic channels in the circle do not permit a very large flow. Occlusion of one carotid artery, particularly in older patients, often causes serious symptoms of cerebral ischemia. There are precapillary anastomoses between the cerebral vessels, but flow through these channels is generally insufficient to maintain the circulation and prevent infarction when a cerebral artery is occluded.

Venous drainage from the brain by way of the deep veins and dural sinuses empties principally into the internal jugular veins in humans, although a small amount of venous blood drains through the ophthalmic and pterygoid venous plexuses, through emissary veins to the scalp, and down the system of paravertebral veins in the spinal canal.

The cerebral vessels have a number of unique anatomic features. In the choroid plexuses, there are gaps between the endothelial cells of the capillary wall, but the choroid epithelial cells that separate them from the cerebrospinal fluid (CSF) are connected to one another by tight junctions. The capillaries in the brain substance resemble the nonfenestrated capillaries found in muscle (see Chapter 31), and there are tight junctions between the endothelial cells that limit the passage of substances via the paracellular route. In addition, there are relatively few vesicles in the endothelial cytoplasm, and presumably little vesicular transport. However, multiple transport systems are present in the capillary cells. The brain capillaries are surrounded by the endfeet of astrocytes **(Figure 33–1)**. These endfeet are closely applied to the basal lamina of the capillaries, but they do not cover the entire capillary wall, and gaps of about 20 nm occur between endfeet **(Figure 33–2)**. However, the endfeet induce formation of tight junctions in the capillaries (see Chapter 31).

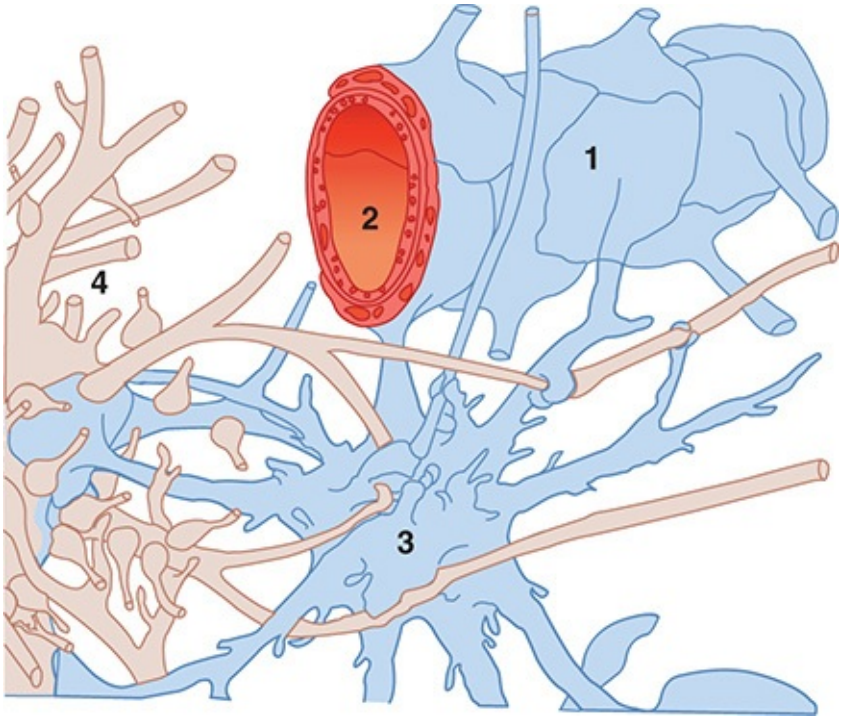


FIGURE 33–1 Relation of fibrous astrocyte (3) to a capillary (2) and neuron (4) in the brain. The endfeet of the astrocyte processes form a discontinuous membrane around the capillary (1). Astrocyte processes also envelop the neuron. (Adapted with permission from Krstic RV: *Die Gewebe des Menschen und der Säugetiere*. Springer; 1978.)

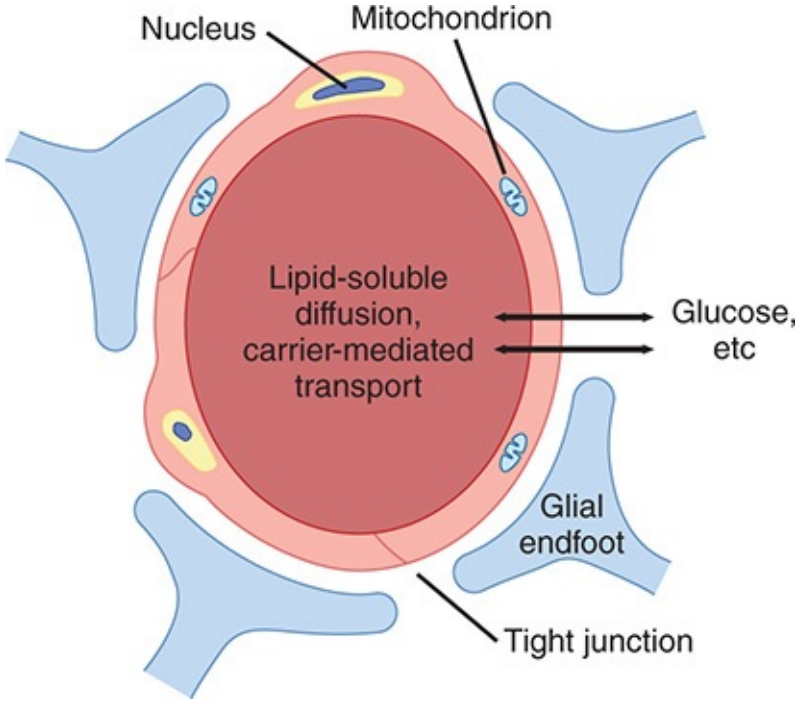


FIGURE 33–2 Transport across cerebral capillaries. Only free lipid-soluble substances can move passively across the endothelial cells. Water-soluble solutes, such as glucose, require active transport mechanisms. Proteins and protein-bound lipids are excluded.

INNERVATION

Three systems of nerves innervate the cerebral blood vessels. Postganglionic sympathetic neurons have their cell bodies in the superior cervical ganglia, and their endings contain norepinephrine. Many also contain neuropeptide Y. Cholinergic neurons that probably originate in the sphenopalatine ganglia also innervate the cerebral vessels, and the postganglionic cholinergic neurons acting on the blood vessels contain acetylcholine. Many also contain vasoactive intestinal peptide (VIP) (see Chapter 7) and peptide histidyl methionine (PHM-27). These nerves end primarily on large arteries. Sensory nerves are found on more distal arteries. They have their cell bodies in the trigeminal ganglia and contain substance P, neurokinin A, and calcitonin gene-related peptide (CGRP). Substance P, CGRP, VIP, and PHM-27 cause vasodilation, whereas neuropeptide Y is a vasoconstrictor. Touching or pulling on the cerebral vessels causes pain.

CEREBROSPINAL FLUID

FORMATION & ABSORPTION

CSF fills the ventricles and subarachnoid space. In humans, the volume of CSF is about 150 mL, and the rate of CSF production is about 550 mL/d. Thus, the CSF turns over about 3.7 times a day. It has been estimated that 50–70% of the CSF is formed in the choroid plexuses and the remainder is formed around blood vessels and along the ventricular walls. The CSF in the ventricles flows through the foramens of Magendie and Luschka to the subarachnoid space and is absorbed through the **arachnoid villi** into veins, primarily the cerebral venous sinuses. The villi consist of projections of the fused arachnoid membrane and endothelium of the sinuses into low pressure venous sinuses. Similar, smaller villi project into veins around spinal nerve routes. These projections may contribute to the outflow of CSF into venous blood by a process known as **bulk flow,** which is unidirectional. An additional and perhaps more important route for CSF reabsorption into the bloodstream in health is via the cribriform plate above the nose and thence into the cervical lymphatics. However, reabsorption

via one-way valves (of uncertain structural basis) in the arachnoid villi may assume a greater role if CSF pressure is elevated. Likewise, when CSF builds up abnormally, aquaporin water channels may be expressed in the choroid plexus and brain microvessels as a compensatory adaptation.

CSF is formed continuously by the choroid plexus in two stages. First, plasma is passively filtered across the choroidal capillary endothelium. Next, secretion of water and ions across the choroidal epithelium provides for active control of CSF composition and quantity. Bicarbonate, chloride, and potassium ions enter the CSF via channels in the epithelial cell apical membranes. Aquaporins provide for water movement to balance osmotic gradients. The composition of CSF **(Table 33–2)** is essentially the same as that of brain extracellular fluid (ECF), which in living humans makes up 15% of the brain volume. In adults, free communication appears to take place between the brain interstitial fluid and CSF, although the diffusion distances from some parts of the brain to the CSF are appreciable. Consequently, equilibration may take some time to occur, and local areas of the brain may have extracellular microenvironments that are transiently different from CSF.

TABLE 33–2 Concentration of various substances in human cerebrospinal fluid (CSF) and plasma.

	Units	CSF	Plasma	Ratio CSF/Plasma
Na^+	(mEq/kg H_2O)	147.0	150.0	0.98
K^+	(mEq/kg H_2O)	2.9	4.6	0.62
Mg^{2+}	(mEq/kg H_2O)	2.2	1.6	1.39
Ca^{2+}	(mEq/kg H_2O)	2.3	4.7	0.49
Cl^-	(mEq/kg H_2O)	113.0	99.0	1.14
HCO_3^-	(mEq/L)	25.1	24.8	1.01
PCO_2	(mm Hg)	50.2	39.5	1.28
pH		7.33	7.40	…
Osmolality	(mOsm/kg H_2O)	289.0	289.0	1.00
Protein	(mg/dL)	20.0	6000.0	0.003
Glucose	(mg/dL)	64.0	100.0	0.64
Inorganic phosphate	(mg/dL)	3.4	4.7	0.73
Urea	(mg/dL)	12.0	15.0	0.80
Creatinine	(mg/dL)	1.5	1.2	1.25
Uric acid	(mg/dL)	1.5	5.0	0.30
Cholesterol	(mg/dL)	0.2	175.0	0.001

Lumbar CSF pressure is normally 70–180 mm H_2O. Up to pressures well above this range, the rate of CSF formation is independent of intraventricular pressure. However, absorption is proportional to the pressure **(Figure 33–3)**. At

a pressure of 112 mm H_2O, which is the average normal CSF pressure, formation and absorption are equal. Below a pressure of approximately 68 mm H_2O, absorption stops. Large amounts of fluid accumulate when the capacity for CSF reabsorption is decreased **(external hydrocephalus, communicating hydrocephalus).** Fluid also accumulates proximal to the block and distends the ventricles when the foramens of Luschka and Magendie are blocked or there is obstruction within the ventricular system **(internal hydrocephalus, noncommunicating hydrocephalus).**

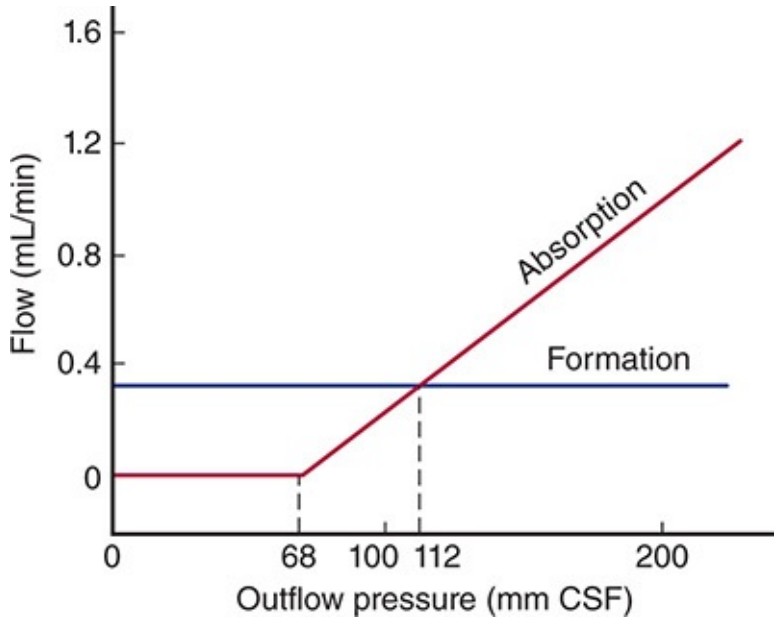


FIGURE 33–3 CSF formation and absorption in humans at various CSF pressures. Note that at 112 mm CSF, formation and absorption are equal, and at 68 mm CSF, absorption is zero. CSF, cerebrospinal fluid. (Modified with permission from Cutler RWP, et al: Formation and absorption of cerebrospinal fluid in man. Brain 1968;91(4):707-720.)

PROTECTIVE FUNCTION

The most critical role for CSF (and the meninges) is to protect the brain. The dura is attached firmly to bone. Normally, there is no “subdural space,” with the arachnoid being held to the dura by the surface tension of the thin layer of fluid between the two membranes. As shown in **Figure 33–4**, the brain itself is supported within the arachnoid by the blood vessels and nerve roots and by the multiple fine fibrous **arachnoid trabeculae.** The brain weighs about 1400 g in

air, but in its “water bath” of CSF it has a net weight of only 50 g. The buoyancy of the brain in the CSF permits its relatively flimsy attachments to suspend it very effectively. When the head receives a blow, the arachnoid slides on the dura and the brain moves, but its motion is gently checked by the CSF cushion and by the arachnoid trabeculae.

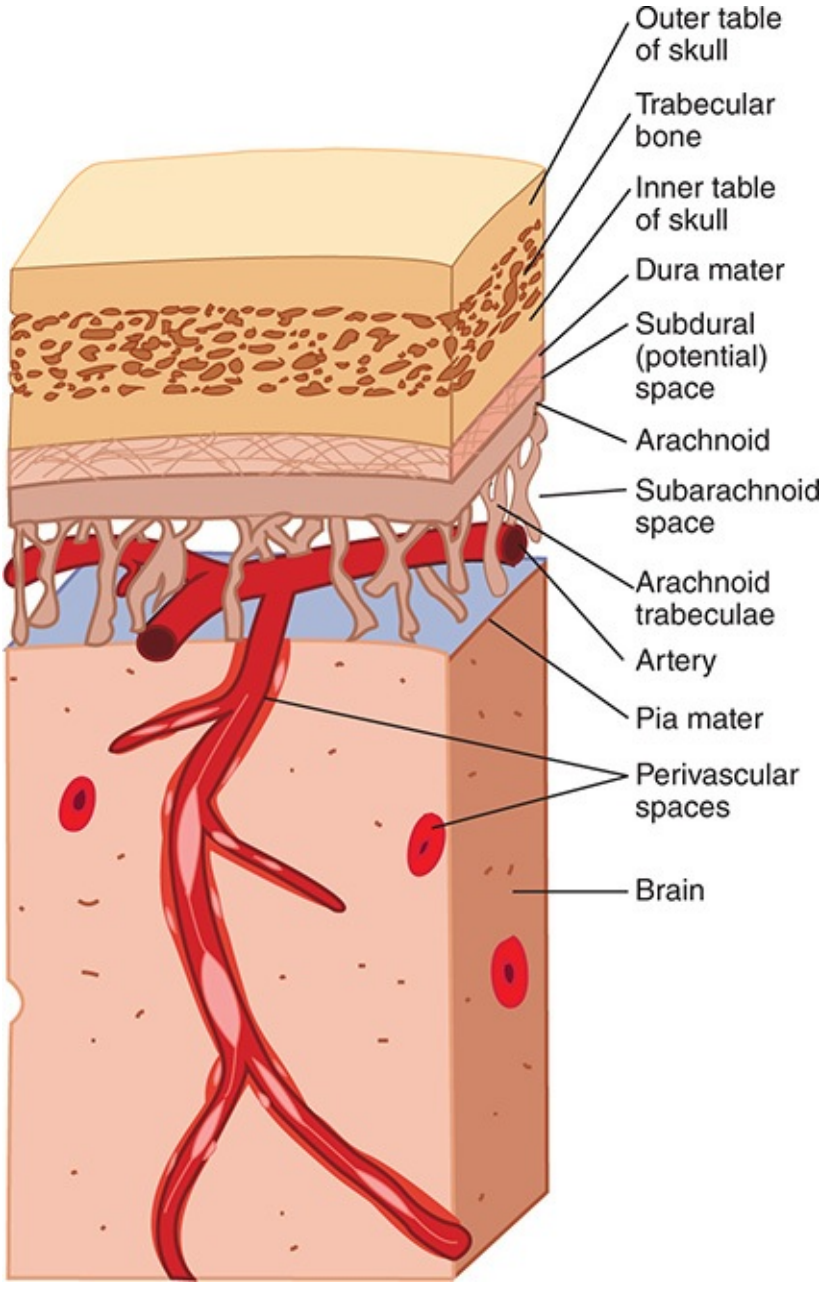


FIGURE 33–4 Investing membranes of the brain, showing their relation to the skull and to brain tissue. (Reproduced with permission from Young B, Heath JW: *Wheater’s Functional Histology*, 4th ed. Churchill Livingstone;

2000.)

The pain produced by spinal fluid deficiency illustrates the importance of CSF in supporting the brain. Removal of CSF during lumbar puncture can cause a severe headache after the fluid is removed, because the brain hangs on the vessels and nerve roots, and traction on them stimulates pain fibers. The pain can be relieved by intrathecal injection of sterile isotonic saline.

HEAD INJURIES

Without the protection of the spinal fluid and the meninges, the brain would probably be unable to withstand even the minor traumas of everyday living; but with the protection afforded, it takes a fairly severe blow to produce cerebral damage. The brain is damaged most commonly when the skull is fractured and bone is driven into neural tissue (depressed skull fracture), when the brain moves far enough to tear the delicate bridging veins from the cortex to the bone, or when the brain is accelerated by a blow on the head and is driven against the skull or the tentorium at a point opposite where the blow was struck **(contrecoup injury).**

THE BLOOD-BRAIN BARRIER

The tight junctions between capillary endothelial cells in the brain and between the epithelial cells in the choroid plexus effectively prevent proteins from entering the brain in adults and slow the penetration of some smaller molecules as well. An example is the slow penetration of urea **(Figure 33–5)**. This uniquely limited exchange of substances into the brain is referred to as the **blood-brain barrier,** a term most commonly used to encompass this barrier overall and more specifically the barrier in the choroid epithelium between blood and CSF.

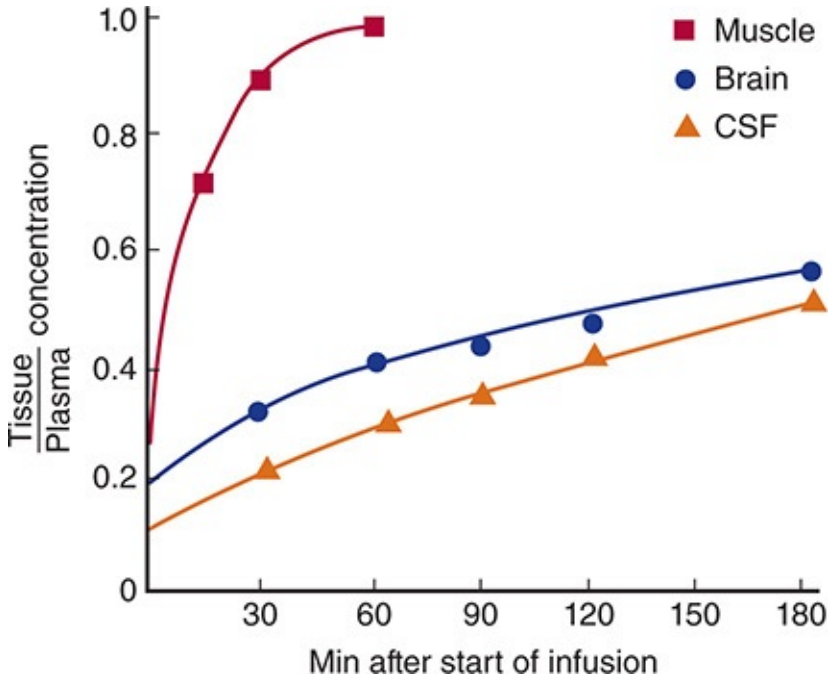


FIGURE 33–5 Penetration of urea into muscle, brain, and cerebrospinal fluid (CSF). Urea was administered by constant infusion.

Passive diffusion across the tight cerebral capillaries is very limited, and little vesicular transport takes place. However, there are numerous carrier-mediated and active transport systems in the cerebral capillaries.

PENETRATION OF SUBSTANCES INTO THE BRAIN

Water, CO_2, and O_2 penetrate the brain with ease, as do the lipid-soluble free forms of steroid hormones, whereas their protein-bound forms and, in general, all proteins and polypeptides do not. The rapid passive penetration of CO_2 contrasts with the regulated transcellular penetration of H^+ and HCO_3^- and has physiologic significance in the regulation of respiration (see Chapter 35).

Glucose is the major ultimate source of energy for nerve cells. Its diffusion across the blood-brain barrier would be very slow, but the rate of transport into the CSF is markedly enhanced by the presence of specific transporters, including the glucose transporter 1 (GLUT1). The brain contains two forms of GLUT1: GLUT1 55K and GLUT1 45K. Both are encoded by the same gene, but they differ in the extent to which they are glycosylated. GLUT1 55K is present in

high concentration in brain capillaries **(Figure 33–6)**. Infants with congenital GLUT1 deficiency have low CSF glucose concentrations in the presence of normal plasma glucose, and they have seizures and delayed development. In addition, transporters for thyroid hormones; several organic acids; choline; nucleic acid precursors; and neutral, basic, and acidic amino acids are present at the blood-brain barrier.

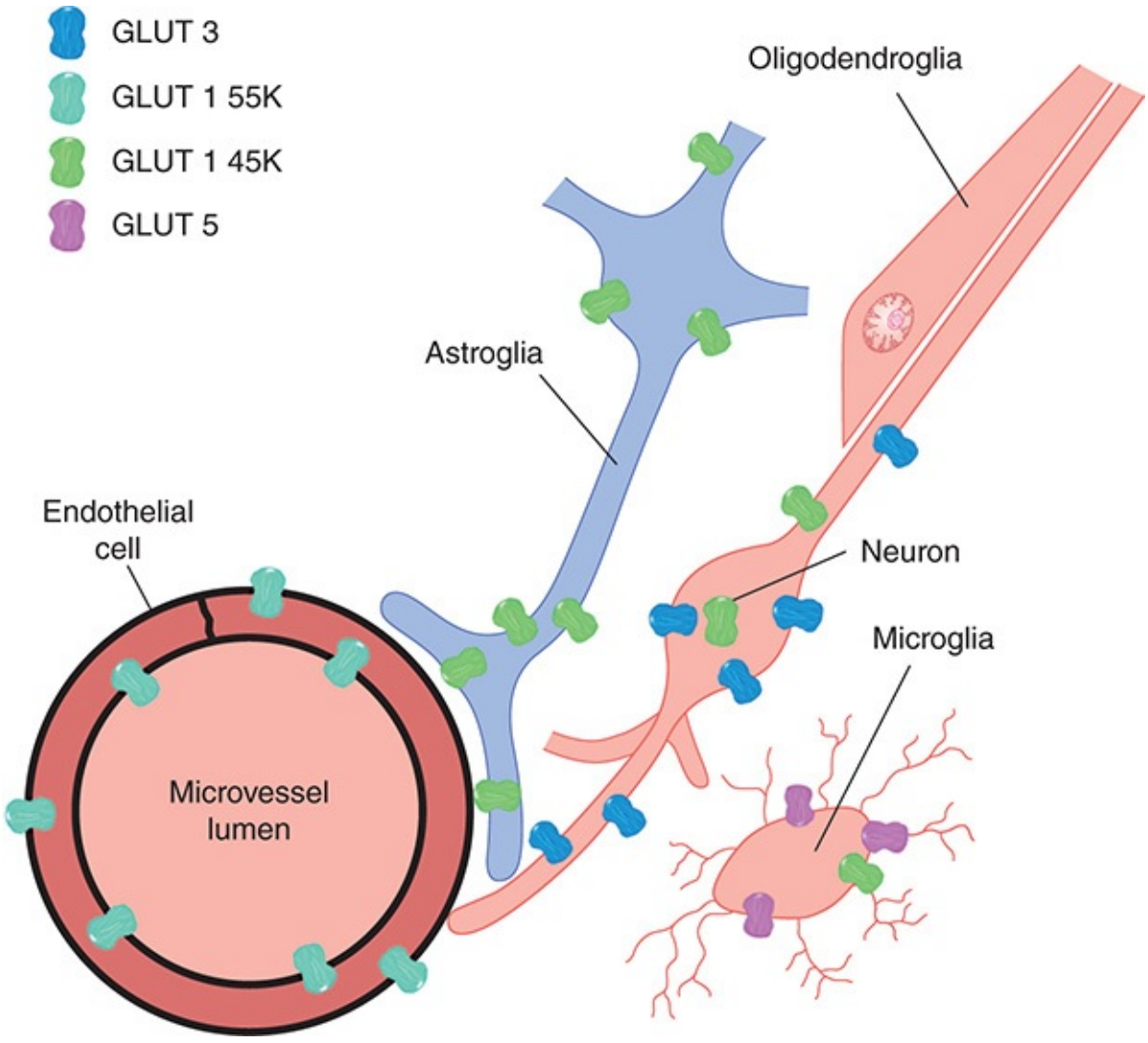


FIGURE 33–6 Localization of the various GLUT transporters in the brain. (Adapted with permission from Maher F, Vannucci SJ, Simpson IA: Glucose transporter proteins in brain. FASEB J 1994 Oct; 8(13):1003-1011.)

A variety of drugs and peptides actually permeate into the endothelial cells of the cerebral capillaries but are promptly transported back into the blood by a nonspecific multidrug transporter in the apical membrane. This **P-glycoprotein**

is a member of the family of adenosine triphosphate (ATP) binding cassette proteins that transport various proteins and lipids across cell membranes (see Chapter 2). In the absence of this transporter in mice, much larger proportions of systemically administered doses of various chemotherapeutic drugs, analgesics, and opioid peptides are found in the brain than in controls. If pharmacologic agents that inhibit this transporter can be developed, they could be of value in the treatment of brain tumors and other central nervous system (CNS) diseases in which it is difficult to introduce adequate amounts of therapeutic agents into the brain.

CIRCUMVENTRICULAR ORGANS

When dyes that bind to proteins in the plasma are injected, they stain many tissues but spare most of the brain. However, four small areas in or near the brainstem do take up the stain. These areas are: (1) the **posterior pituitary** (neurohypophysis) and the adjacent ventral part of the **median eminence** of the hypothalamus, (2) the **area postrema,** (3) the **organum vasculosum of the lamina terminalis (OVLT,** supraoptic crest), and (4) the **subfornical organ (SFO).**

These areas are referred to collectively as the **circumventricular organs (Figure 33–7)**. All have fenestrated capillaries, and because of their permeability they are said to be "outside the blood-brain barrier." Some of them function as **neurohemal organs;** that is, areas in which polypeptides secreted by neurons enter the circulation. Others contain receptors for many different peptides and other substances, and function as chemoreceptor zones in which substances in the circulating blood can act to trigger changes in brain function without penetrating the blood-brain barrier. For example, the area postrema is a chemoreceptor trigger zone that initiates vomiting in response to chemical changes in the plasma (see Chapter 27). It is also concerned with cardiovascular control, where circulating angiotensin II acts to produce a neurally mediated increase in blood pressure. Angiotensin II also acts on the SFO and possibly on the OVLT to increase water intake. In addition, it appears that the OVLT is the site of the osmoreceptor controlling vasopressin secretion (see Chapter 38), and evidence suggests that circulating interleukin-1 (IL-1) produces fever by acting here too.

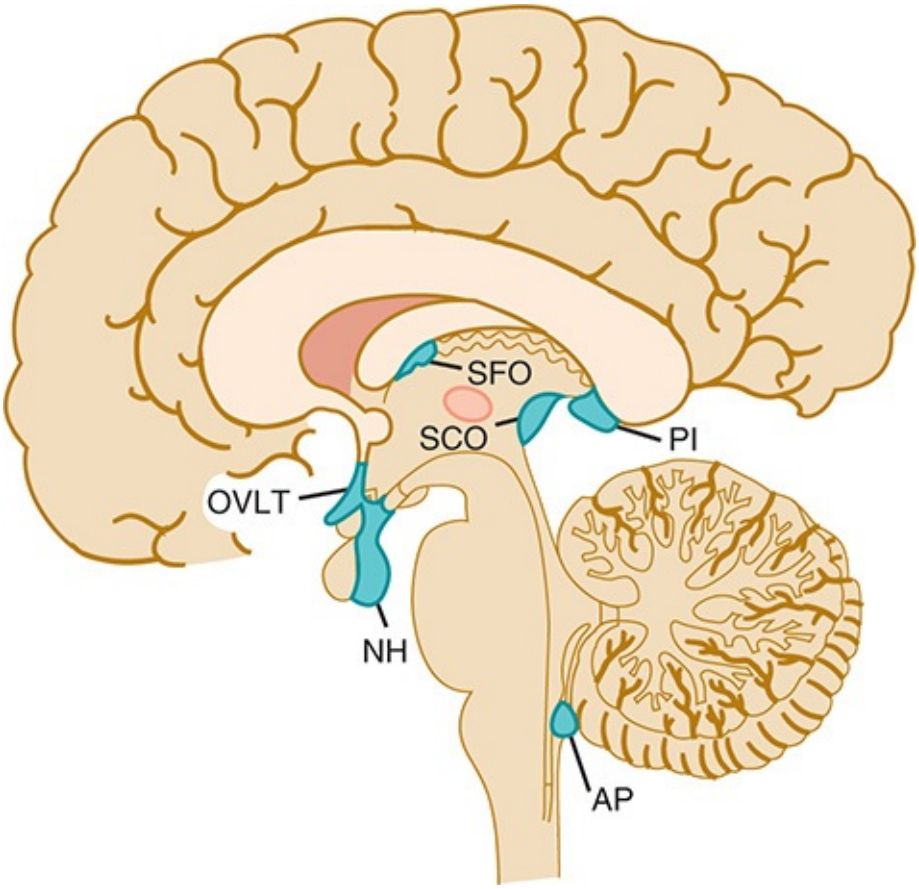


FIGURE 33–7 Circumventricular organs. The neurohypophysis (NH), organum vasculosum of the lamina terminalis (OVLT, organum vasculosum of the lamina terminalis), subfornical organ (SFO), and area postrema (AP) are shown projected on a sagittal section of the human brain. PI, pineal; SCO, subcommissural organ.

The subcommissural organ (Figure 33–7) is closely associated with the pineal gland and histologically resembles the circumventricular organs. However, it does not have fenestrated capillaries, and is not highly permeable. It may, instead, be involved in guiding axons during brain development. Conversely, the pineal and the anterior pituitary do have fenestrated capillaries and are outside the blood-brain barrier, but both are endocrine glands and are not part of the brain.

FUNCTION OF THE BLOOD-BRAIN BARRIER

The blood-brain barrier strives to maintain the constancy of the environment of

the neurons in the CNS **(Clinical Box 33–1)**. Even minor variations in the concentrations of K^+, Ca^{2+}, Mg^{2+}, H^+, and other ions can have far-reaching consequences. The constancy of the composition of the ECF in all parts of the body is maintained by multiple homeostatic mechanisms (see Chapters 1 and 38), but because of the sensitivity of the cortical neurons to ionic change, it is not surprising that an additional defense has evolved to protect them. Other functions of the blood-brain barrier include protection of the brain from endogenous and exogenous toxins in the blood and prevention of the escape of neurotransmitters into the general circulation.

DEVELOPMENT OF THE BLOOD-BRAIN BARRIER

In experimental animals, many small molecules penetrate the brain more readily during the fetal and neonatal period than they do in the adult. On this basis, it is often stated that the blood-brain barrier is immature at birth. Humans are more mature at birth, and not surprisingly, detailed data on passive permeability of the normal human neonatal blood-brain barrier are not available. However, in severely jaundiced infants with high plasma levels of free bilirubin and an immature hepatic bilirubin-conjugating system, free bilirubin enters the brain and, in the presence of asphyxia, damages the basal ganglia **(kernicterus).** The counterpart of this situation in later life is the Crigler-Najjar syndrome in which there is a congenital deficiency of glucuronyl transferase. These individuals can have very high free bilirubin levels in the blood and develop encephalopathy. In other conditions, however, free bilirubin levels are generally not high enough to produce brain damage.

CLINICAL BOX 33–1

Clinical Implications of the Blood-Brain Barrier

Clinicians must know the degree to which drugs penetrate the brain in order to treat diseases of the nervous system intelligently. For example, it is clinically relevant that the amines dopamine and serotonin penetrate brain tissue to a very limited degree but their corresponding acid precursors, L-dopa and 5-hydroxytryptophan, respectively, enter with relative ease (see Chapters 7 and 12). Another important clinical consideration is the fact that

the blood-brain barrier tends to break down in areas of infection or injury. Tumors develop new blood vessels, and the capillaries that are formed lack contact with normal astrocytes. Therefore, there are no tight junctions, and the vessels may even be fenestrated. The lack of a barrier helps in identifying the location of tumors; substances such as radioactive iodine-labeled albumin penetrate normal brain tissue very slowly, but they enter tumor tissue, making the tumor stand out as an island of radioactivity in the surrounding normal brain. The blood-brain barrier can also temporarily be disrupted by sudden marked increases in blood pressure or by intravenous injection of hypertonic fluids.

CEREBRAL BLOOD FLOW & ITS REGULATION

KETY METHOD

According to the **Fick's principle** (see Chapter 30), the blood flow of any organ can be measured by determining the amount of a given substance (Q_x) removed from the bloodstream by the organ per unit of time and dividing that value by the difference between the concentration of the substance in arterial blood and the concentration in the venous blood from the organ ($[A_x] - [V_x]$). Thus:

$$\text{Cerebral blood flow (CBF)} = \frac{Q_x}{[A_x] - [V_x]}$$

This can be applied clinically using inhaled nitrous oxide (N_2O) **(Kety method).** The average cerebral blood flow in young adults is 54 mL/100 g/min. The average adult brain weighs about 1400 g, so the flow for the whole brain is about 756 mL/min. Note that the Kety method provides an average value for perfused areas of brain and gives no information about regional differences in blood flow. It also can only measure flow to perfused parts of the brain. If the blood flow to a portion of the brain is occluded, the measured flow does not change because the nonperfused area does not take up any N_2O.

In spite of the marked local fluctuations in brain blood flow with neural activity, the cerebral circulation is regulated in such a way that total blood flow remains relatively constant. The factors involved in regulating the flow are summarized in **Figure 33–8**.

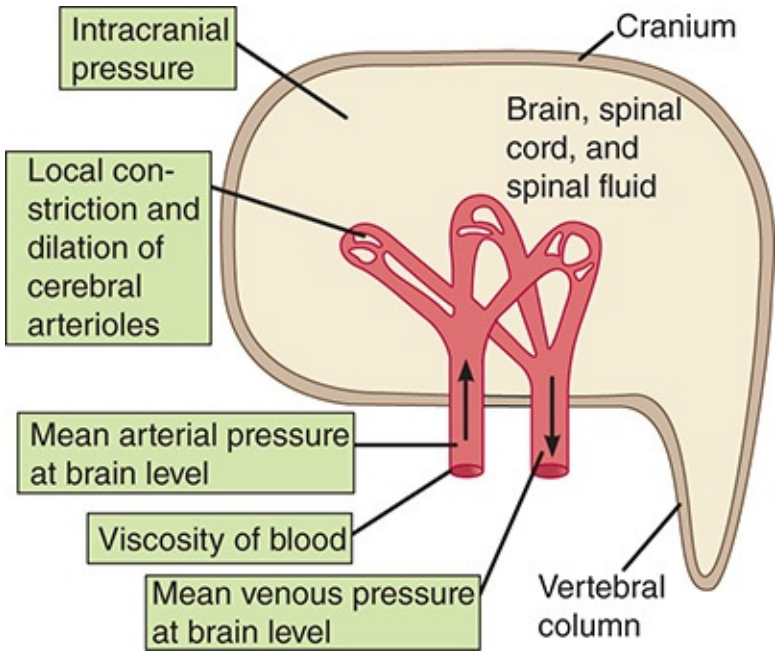


FIGURE 33–8 Diagrammatic summary of the factors affecting overall cerebral blood flow.

ROLE OF INTRACRANIAL PRESSURE

In adults, the brain, spinal cord, and spinal fluid are encased, along with the cerebral vessels, in a rigid bony enclosure. The cranial cavity normally contains a brain weighing approximately 1400 g, 75 mL of blood, and 75 mL of spinal fluid. Because brain tissue and spinal fluid are essentially incompressible, the volume of blood, spinal fluid, and brain in the cranium at any time must be relatively constant **(Monro-Kellie doctrine).** More importantly, the cerebral vessels are compressed whenever the intracranial pressure rises. Any change in venous pressure promptly causes a similar change in intracranial pressure. Thus, a rise in venous pressure decreases cerebral blood flow both by decreasing the effective perfusion pressure and by compressing the cerebral vessels. This relationship helps compensate for changes in arterial blood pressure at the level of the head. For example, if the body is accelerated upward (positive *g*), blood moves toward the feet and arterial pressure at the level of the head decreases. However, venous pressure also falls and intracranial pressure falls, so that the pressure on the vessels decreases and blood flow is much less severely compromised than it would otherwise be. Conversely, during acceleration downward, force acting toward the head (negative *g*) increases arterial pressure at head level, but intracranial pressure also rises, so that the vessels are

supported and do not rupture. The cerebral vessels are protected during the straining associated with defecation or delivery in the same way.

AUTOREGULATION

As seen in other vascular beds, autoregulation is prominent in the brain **(Figure 33–9)**. This process, by which the flow to many tissues is maintained at relatively constant levels despite variations in perfusion pressure, is discussed in Chapter 32. In the brain, autoregulation maintains a constant cerebral blood flow at arterial pressures of 65–140 mm Hg.

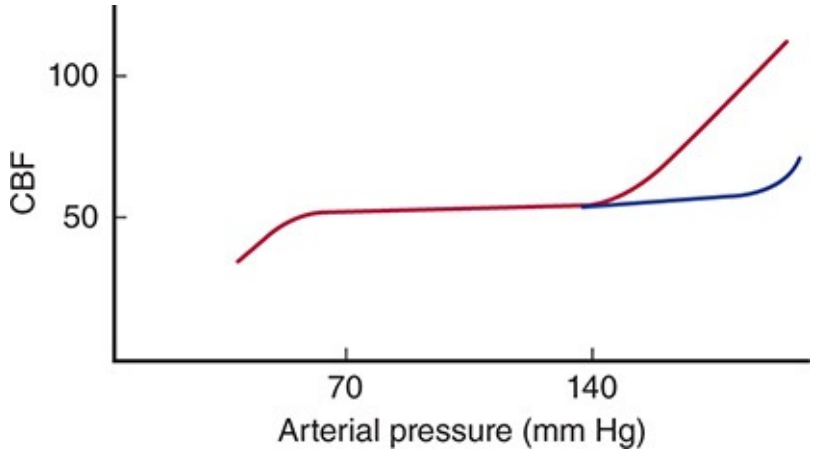


FIGURE 33–9 Autoregulation of cerebral blood flow (CBF) during steady-state conditions. The blue line shows the alteration produced by sympathetic stimulation during autoregulation.

ROLE OF VASOMOTOR & SENSORY NERVES

The innervation of large cerebral blood vessels by postganglionic sympathetic and parasympathetic nerves and the additional distal innervation by sensory nerves have been described above. The nerves may also modulate tone indirectly, via the release of paracrine substances from astrocytes. The precise role of these nerves, however, remains a matter of debate. It has been argued that noradrenergic discharge occurs when the blood pressure is markedly elevated. This reduces the resultant passive increase in blood flow and helps protect the blood-brain barrier from the disruption that could otherwise occur (see above). Thus, vasomotor discharges affect autoregulation. With sympathetic stimulation, the constant-flow, or plateau, part of the pressure-flow curve is extended to the

right (Figure 33–9); that is, greater increases in pressure can occur without an increase in flow. On the other hand, the vasodilator hydralazine and the angiotensin-converting enzyme (ACE) inhibitor captopril reduce the length of the plateau. Finally, neurovascular coupling may adjust local perfusion in response to changes in brain activity (see below).

BLOOD FLOW IN VARIOUS PARTS OF THE BRAIN

A major advance in recent decades has been the development of techniques for monitoring regional blood flow in living, conscious humans. Among the most valuable methods are **positron emission tomography (PET)** and related techniques in which a short-lived radioisotope is used to label a compound and the compound is injected. The arrival and clearance of the tracer are monitored by scintillation detectors placed over the head. Because blood flow is tightly coupled to brain metabolism, local uptake of 2-deoxyglucose is also a good index of blood flow (see below and Chapter 1). If the 2-deoxyglucose is labeled with a short half-life positron emitter such as ^{18}F, ^{11}O, or ^{15}O, its concentration in any part of the brain can be monitored.

Another valuable technique involves magnetic resonance imaging (MRI). MRI is based on detecting resonant signals from different tissues in a magnetic field. **Functional magnetic resonance imaging (fMRI)** measures the amount of blood in a tissue area. When neurons become active, their increased discharge alters the local field potential. A still unsettled mechanism triggers an increase in local blood flow and oxygen. The increase in oxygenated blood is detected by fMRI. PET scanning can be used to measure not only blood flow but also the concentration of molecules, such as dopamine, in various regions of the living brain. On the other hand, fMRI does not involve the use of radioactivity. Consequently, it can be used at frequent intervals to measure changes in regional blood flow in a single individual.

In resting humans, the average blood flow in gray matter is 69 mL/100 g/min compared with 28 mL/100 g/min in white matter. A striking feature of cerebral function is the marked variation in local blood flow with changes in brain activity. In persons who are awake but at rest, blood flow is greatest in the premotor and frontal regions. This is the part of the brain that is believed to be concerned with decoding and analyzing afferent input and with intellectual activity. During voluntary clenching of the right hand, flow is increased in the

hand area of the left motor cortex and the corresponding sensory areas in the postcentral gyrus. Especially when the movements being performed are sequential, the flow is also increased in the supplementary motor area. When persons talk, there is a bilateral increase in blood flow in the face, tongue, and mouth-sensory and motor areas and the upper premotor cortex in the categorical (usually the left) hemisphere. When the speech is stereotyped, Broca and Wernicke areas do not show increased flow, but when the speech is creative—that is, when it involves ideas—flow increases in both these areas. Reading produces widespread increases in blood flow. Problem solving, reasoning, and motor ideation without movement produce increases in selected areas of the premotor and frontal cortex. In anticipation of a cognitive task, many of the brain areas that will be activated during the task are activated beforehand, as if the brain produces an internal model of the expected task. In right-handed individuals, blood flow to the left hemisphere is greater when a verbal task is being performed and blood flow to the right hemisphere is greater when a spatial task is being performed **(Clinical Box 33–2)**. An example of how blood flow changes are regionalized during a mental task is shown in **Figure 33–10**.

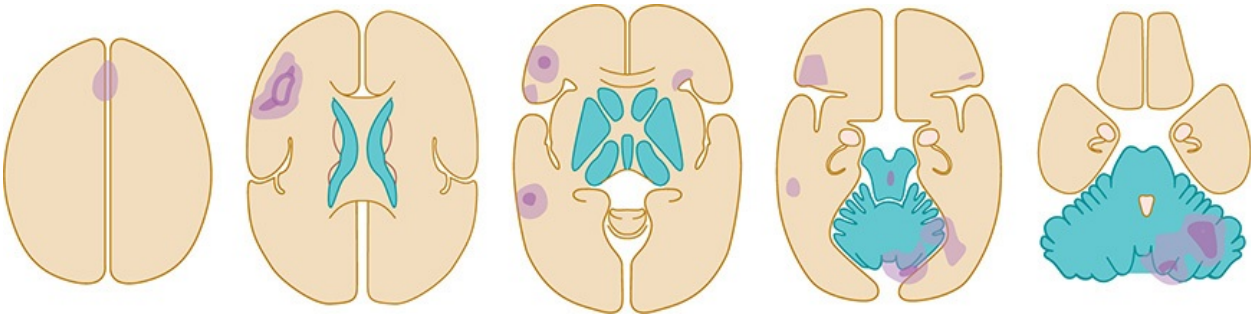


FIGURE 33–10 Activity in the human brain at five different horizontal levels while a subject generates a verb that is appropriate for each noun presented by an examiner. This mental task activates the frontal cortex (slices 1–4), anterior cingulate gyrus (slice 1), and posterior temporal lobe (slice 3) on the left side and the cerebellum (slices 4 and 5) on the right side. Light purple, moderate activation; dark purple, marked activation. (Based on PET scans in Posner MI, Raichle ME: *Images of Mind*. Scientific American Library, 1994.)

CLINICAL BOX 33–2

Changes in Cerebral Blood Flow in Disease

Several disease states are known to be associated with localized or general

changes in cerebral blood flow, as revealed by PET scanning and fMRI. For example, epileptic foci are hyperemic during seizures, whereas flow is reduced in other parts of the brain. Between seizures, flow is sometimes reduced in the foci that generate the seizures. Parietooccipital flow is decreased in patients with symptoms of agnosia (see Chapter 15). In Alzheimer disease, the earliest change is decreased metabolism and blood flow in the superior parietal cortex, with later spread to the temporal and finally the frontal cortex. The precentral and postcentral gyri, basal ganglia, thalamus, brainstem, and cerebellum are relatively spared. In Huntington disease, blood flow is reduced bilaterally in the caudate nucleus, and this alteration in flow occurs early in the disease. In manic depressives (but interestingly, not in patients with unipolar depression), there is a general decrease in cortical blood flow when the patients are depressed. In schizophrenia, some evidence suggests decreased blood flow in the frontal lobes, temporal lobes, and basal ganglia. Finally, during the aura in patients with migraine, a bilateral decrease in blood flow starts in the occipital cortex and spreads anteriorly to the temporal and parietal lobes.

BRAIN METABOLISM & OXYGEN REQUIREMENTS

UPTAKE & RELEASE OF SUBSTANCES BY THE BRAIN

If the cerebral blood flow is known, it is possible to calculate the consumption or production by the brain of O_2, CO_2, glucose, or any other substance present in the bloodstream by multiplying the cerebral blood flow by the difference between the concentration of the substance in arterial blood and cerebral venous blood **(Table 33–3)**. When calculated in this manner, a negative value indicates that the brain is producing the substance.

TABLE 33–3 Utilization and production of substances by the adult human brain in vivo.

Substance	Uptake (+) or Output (–) per 100 g of Brain/min	Total/min
Substances utilized		
Oxygen	+3.5 mL	+49 mL
Glucose	+5.5 mg	+77 mg
Glutamate	+0.4 mg	+5.6 mg
Substances produced		
Carbon dioxide	–3.5 mL	–49 mL
Glutamine	–0.6 mL	–8.4 mg

Substances not used or produced in the fed state: lactate, pyruvate, total ketones, and α-ketoglutarate.

OXYGEN CONSUMPTION

O_2 consumption by the human brain (**cerebral** metabolic rate for O_2, $CMRO_2$) averages approximately 20% of the total body resting O_2 consumption (Table 33–1). The brain is extremely sensitive to hypoxia, and occlusion of its blood supply produces unconsciousness in a period as short as 10 s. The vegetative structures in the brainstem are more resistant to hypoxia than the cerebral cortex, and patients may recover from accidents such as cardiac arrest and other conditions causing fairly prolonged hypoxia with normal vegetative functions but severe, permanent intellectual deficiencies. The basal ganglia use O_2 at a very high rate, and symptoms of Parkinson disease as well as intellectual deficits can be produced by chronic hypoxia. The thalamus and the inferior colliculus are also very susceptible to hypoxic damage **(Clinical Box 33–3)**.

ENERGY SOURCES

Glucose is the major ultimate source of energy for the brain; under normal conditions, 90% of the energy needed to maintain ion gradients across cell membranes and transmit electrical impulses comes from this source. Glucose enters the brain via GLUT1 in cerebral capillaries (see above). Other transporters

then distribute it to neurons and glial cells.

Glucose is taken up from the blood in large amounts, and the RQ (respiratory quotient; see Chapter 26) of cerebral tissue is 0.95–0.99 in normal individuals. Importantly, insulin is not required for most cerebral cells to utilize glucose. In general, glucose utilization at rest parallels blood flow and O_2 consumption. This does not mean that the total source of energy is always glucose. During prolonged starvation, appreciable utilization of other substances occurs. Indeed, evidence indicates that as much as 30% of the glucose taken up under normal conditions is converted to amino acids, lipids, and proteins, and those substances other than glucose are metabolized for energy during convulsions. Some utilization of amino acids from the circulation may also take place even though the amino acid arteriovenous difference across the brain is normally minute.

The consequences of hypoglycemia in terms of neural function are discussed in Chapter 24.

GLUTAMATE & AMMONIA REMOVAL

The brain's uptake of glutamate is approximately balanced by its output of glutamine. Glutamate entering the brain associates with ammonia and leaves as glutamine. The glutamate-glutamine conversion in the brain—the opposite of the reaction in the kidney that produces some of the ammonia entering the tubules—serves as a detoxifying mechanism to keep the brain free of ammonia. Ammonia is very toxic to nerve cells, and ammonia intoxication is believed to be at least among the causes of neurologic symptoms that may emerge in advanced liver disease (**hepatic encephalopathy**, see Chapter 28).

CORONARY CIRCULATION

ANATOMIC CONSIDERATIONS

The two coronary arteries that supply the myocardium arise from sinuses behind two of the cusps of the aortic valve at the root of the aorta **(Figure 33–11)**. Most of the venous blood returns to the heart through the coronary sinus and anterior cardiac veins **(Figure 33–12)**, which drain into the right atrium. In addition, there are other vessels that empty directly into the heart chambers. These include **arteriosinusoidal vessels,** sinusoidal capillary-like vessels that connect arterioles to the chambers; **Thebesian veins** that connect capillaries to the

chambers; and a few **arterioluminal vessels** that are small arteries draining directly into the chambers. A few anastomoses occur between the coronary arterioles and extracardiac arterioles, especially around the mouths of the great veins.

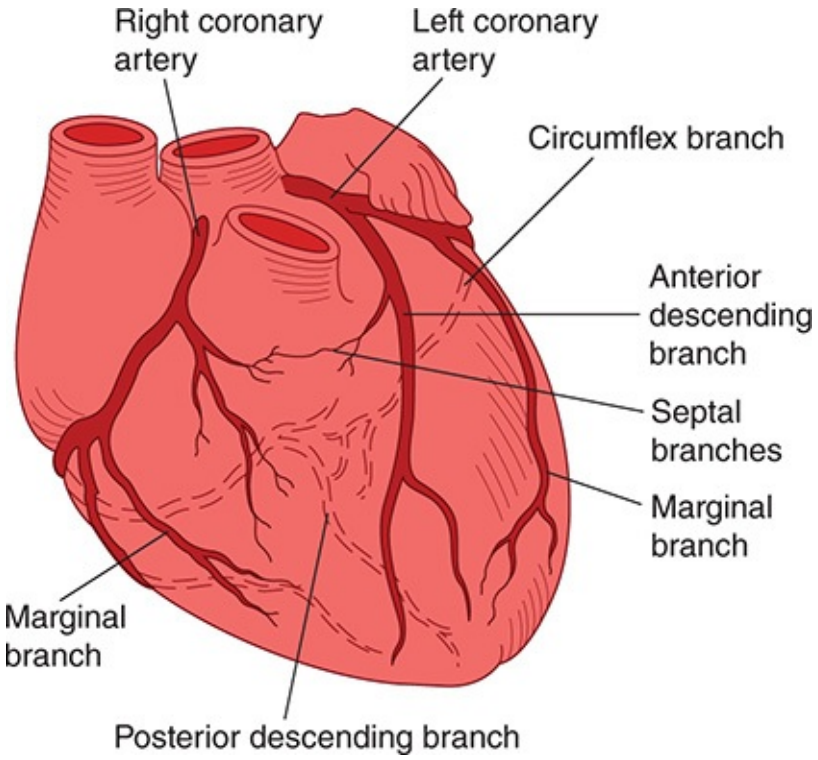


FIGURE 33–11 Coronary arteries and their principal branches in humans. (Reproduced with permission from Ross G: *Essentials of Human Physiology*. Year Book Medical Publishers; 1978.)

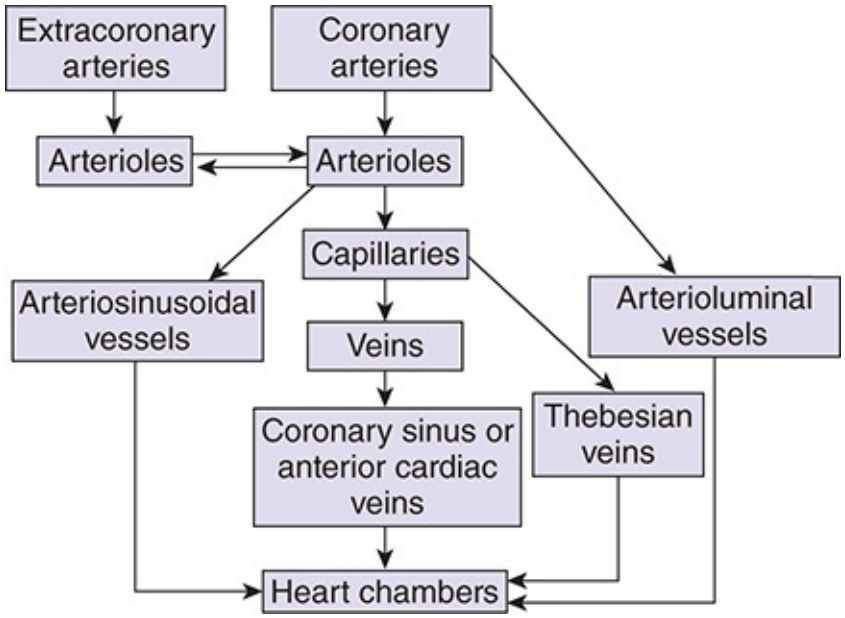


FIGURE 33–12 Diagram of the coronary circulation.

CLINICAL BOX 33–3

Stroke

When the blood supply to a part of the brain is interrupted, ischemia damages or kills the cells in the area, producing the signs and symptoms of a stroke. There are two general types of strokes: hemorrhagic and ischemic. Hemorrhagic stroke occurs when a cerebral artery or arteriole ruptures, sometimes but not always at the site of a small aneurysm. Ischemic stroke occurs when flow in a vessel is compromised by atherosclerotic plaques on which thrombi form. Thrombi may also be produced elsewhere (eg, in the atria in patients with atrial fibrillation) and pass to the brain as emboli where they then lodge and interrupt flow. In the past, little could be done to modify the course of a stroke and its consequences. However, it has become clear that in the penumbra (the area surrounding the most severe brain damage) ischemia reduces glutamate uptake by astrocytes, and the increase in local glutamate causes excitotoxic damage and death to neurons (see Chapter 7).

THERAPEUTIC HIGHLIGHTS

The clot-lysing drug, tissue-type plasminogen activator (t-PA) (see Chapter 31) is of great benefit in ischemic strokes. Drugs that prevent excitotoxic damage

have had equivocal effects in human clinical trials, but growing insights into the mechanism of injury are yielding more targeted agents that may be of benefit via this pathway in the future. However, t-PA (and presumably any effective antiexcitotoxic treatment) must be given early in the course of a stroke to be of maximum benefit. This is why stroke has become a condition in which rapid diagnosis and treatment are extremely important. In addition, of course, it is important to determine if a stroke is thrombotic or hemorrhagic, since clot lysis is contraindicated in the latter. Trials are ongoing of therapies that enhance hemostasis in hemorrhagic stroke.

PRESSURE GRADIENTS & FLOW IN THE CORONARY VESSELS

The heart is a muscle that, like skeletal muscle, compresses its blood vessels when it contracts. The pressure inside the left ventricle is slightly higher than in the aorta during systole **(Table 33–4)**. Consequently, in the arteries supplying the subendocardial portion of the left ventricle, flow occurs only during diastole, although the force is sufficiently dissipated in the more superficial portions of the left ventricular myocardium to permit some flow in this region throughout the cardiac cycle. Because the relative length of diastole is shorter when the heart rate is high, left ventricular coronary flow is reduced during tachycardia. On the other hand, the pressure differential between the aorta and the right ventricle, and between the aorta and the atria, is somewhat greater during systole than during diastole. Consequently, coronary flow in those parts of the heart is not appreciably reduced during systole. Flow in the right and left coronary arteries is shown in **Figure 33–13**. Because no blood flow occurs during systole in the subendocardial portion of the left ventricle, this region is prone to ischemic damage and is the most common site of myocardial infarction. Blood flow to the left ventricle is decreased in patients with stenotic aortic valves because the pressure in the left ventricle must be much higher than that in the aorta to eject the blood. Consequently, the coronary vessels are more severely compressed during systole. Patients with aortic stenosis are particularly prone to develop symptoms of myocardial ischemia, in part because of this compression and in part because the myocardium requires more O_2 to expel blood through the stenotic aortic valve. Coronary flow is also decreased when the aortic diastolic pressure is low. The rise in venous pressure in conditions such as heart failure

reduces coronary flow because it decreases effective coronary perfusion pressure **(Clinical Box 33–4)**.

TABLE 33–4 Pressure in aorta and left and right ventricles (vent) in systole and diastole.

	Pressure (mm Hg) in			Pressure Differential (mm Hg) between Aorta and	
	Aorta	Left Vent	Right Vent	Left Vent	Right Vent
Systole	120	121	25	–1	95
Diastole	80	0	0	80	80

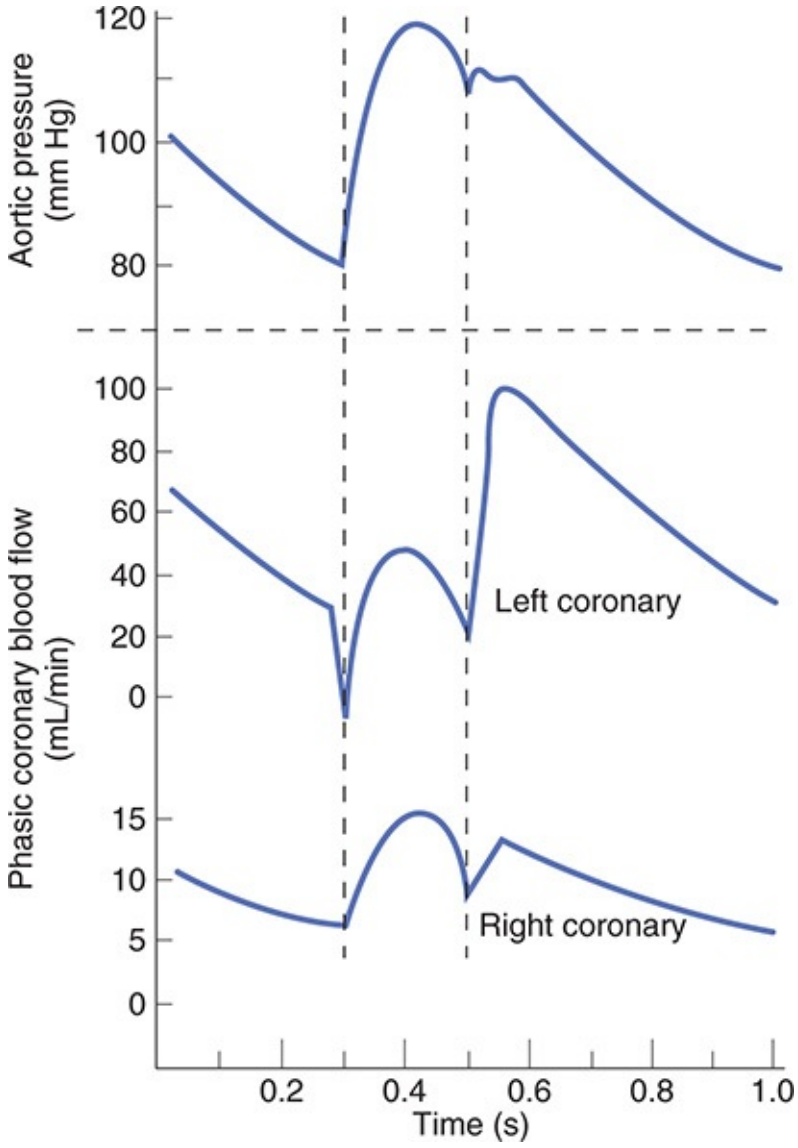


FIGURE 33–13 Blood flow in the left and right coronary arteries during various phases of the cardiac cycle. Systole occurs between the two vertical dashed lines. (Reproduced with permission from Berne RM, Levy MN: *Physiology*, 2nd ed. Mosby; 1988.)

Coronary blood flow has been measured by inserting a catheter into the coronary sinus and applying the Kety method to the heart on the assumption that the N_2O content of coronary venous blood is typical of the entire myocardial effluent. Coronary flow at rest in humans is about 250 mL/min (5% of the cardiac output). A number of techniques utilizing **radionuclides** detected with radiation detectors over the chest have been used to study regional blood flow in the heart and to detect areas of ischemia and infarct as well as to evaluate

ventricular function. Radionuclides such as thallium-201 (^{201}Tl) are pumped into cardiac muscle cells by Na^+, K^+ ATPase and equilibrate with the intracellular K^+ pool. For the first 10–15 min after intravenous injection, ^{201}Tl distribution is directly proportional to myocardial blood flow, and areas of ischemia can be detected by their low uptake. The uptake of this isotope is often determined soon after exercise and again several hours later to bring out areas in which exertion leads to compromised flow. Conversely, radiopharmaceuticals such as technetium-99m stannous pyrophosphate (^{99m}Tc-PYP) are selectively taken up by infarcted tissue and make infarcts stand out as “hot spots” on scintigrams of the chest. Coronary angiography can be combined with measurement of ^{133}Xe washout (see above) to provide detailed analysis of coronary blood flow. Radiopaque contrast medium is first injected into the coronary arteries, and radiographs are used to outline their distribution. The angiographic camera is then replaced with a scintillation camera, and ^{133}Xe washout is measured.

VARIATIONS IN CORONARY FLOW

At rest, the heart extracts 70–80% of the O_2 from each unit of blood delivered to it (Table 33–1). O_2 delivery can only be increased significantly by increasing blood flow. Therefore, it is not surprising that blood flow increases when the metabolism of the myocardium is increased. The caliber of the coronary vessels, and consequently the rate of coronary blood flow, is influenced not only by pressure changes in the aorta but also by chemical and neural factors. The coronary circulation also shows considerable autoregulation.

CHEMICAL FACTORS

The close relationship between coronary blood flow and myocardial O_2 consumption implies that one or more metabolic products causes coronary vasodilation. Factors suspected of playing this role include a lack of O_2 and increased local concentrations of CO_2, H^+, K^+, lactate, prostaglandins, adenine nucleotides, and adenosine. Likely several or all of these vasodilator metabolites act in an integrated manner, redundant manner, or both. Asphyxia, hypoxia, and intracoronary injections of cyanide all increase coronary blood flow 200–300% in denervated as well as intact hearts, and the feature common to these three stimuli is hypoxia of the myocardial fibers. A similar increase in flow is

produced in the area supplied by a coronary artery if the artery is occluded and then released. This **reactive hyperemia** is similar to that seen in the skin (see below) and appears to be due to the release of adenosine.

CLINICAL BOX 33–4

Coronary Artery Disease

When flow through a coronary artery is reduced to the point that the myocardium it supplies becomes hypoxic, **angina pectoris** develops (see Chapter 30). If the myocardial ischemia is severe and prolonged, irreversible changes occur in the muscle, and the result is **myocardial infarction.** Many individuals have angina only on exertion, and coronary blood flow is normal at rest. Others have more severe restriction of blood flow and have anginal pain at rest as well. Partially occluded coronary arteries can be constricted further by vasospasm, producing myocardial infarction. However, it is now clear that the most common cause of myocardial infarction is rupture of an **atherosclerotic plaque,** or hemorrhage into it, which triggers the formation of a coronary-occluding clot at the site of the plaque. The electrocardiographic changes in myocardial infarction are discussed in Chapter 29. When myocardial cells die, they leak enzymes into the circulation, and measuring the rise in serum enzymes and isoenzymes produced by infarcted myocardial cells also plays an important role in the diagnosis of myocardial infarction. The enzymes most commonly measured are the MB isomer of creatine kinase (CK-MB), troponin T, and troponin I. Myocardial infarction is a very common cause of death in developed countries because of the widespread occurrence of atherosclerosis. In addition, there is a relation between atherosclerosis and circulating levels of **lipoprotein(a) (Lp[a]).** Lp(a) has an outer coat consisting of apo(a). It interferes with fibrinolysis by down-regulating plasmin generation (see Chapter 31). It now appears that atherosclerosis has an important inflammatory component as well. The lesions of the disease contain inflammatory cells, and there is a positive correlation between increased levels of C-reactive protein and other **inflammatory markers** in the circulation with subsequent myocardial infarction.

THERAPEUTIC HIGHLIGHTS

Treatment of myocardial infarction aims to restore flow to the affected area as

rapidly as possible while minimizing reperfusion injury. Needless to say, it should be started as promptly as possible to avoid irreversible changes in heart function. In acute disease, antithrombotic agents are often given, but these can be problematic, leading to increased mortality due to bleeding if cardiac surgery is subsequently needed. Mechanical/surgical approaches to coronary artery disease include balloon angioplasty and/or implantation of stents to hold the vessels open, or coronary artery bypass grafting (CABG).

NEURAL FACTORS

The coronary arterioles contain α-adrenergic receptors, which mediate vasoconstriction, and β-adrenergic receptors, which mediate vasodilation. Activity in the noradrenergic nerves to the heart and injections of norepinephrine cause coronary vasodilation. Mechanistically, norepinephrine increases the heart rate and the force of cardiac contraction, and the vasodilation is due to production of vasodilator metabolites by the myocardium secondary to the increase in its activity. When the inotropic and chronotropic effects of noradrenergic discharge are blocked by a β-adrenergic blocking drug, stimulation of the noradrenergic nerves or injection of norepinephrine in unanesthetized animals elicits coronary vasoconstriction. Thus, the direct effect of noradrenergic stimulation is constriction rather than dilation of the coronary vessels. On the other hand, stimulation of vagal fibers to the heart dilates the coronaries.

When the systemic blood pressure falls, the overall effect of the reflex increase in noradrenergic discharge is increased coronary blood flow secondary to the metabolic changes in the myocardium at a time when the cutaneous, renal, and splanchnic vessels are constricted. In this way the circulation of the heart, like that of the brain, is preserved when flow to other organs is compromised.

CUTANEOUS CIRCULATION

The amount of heat lost from the body is regulated to a large extent by varying the amount of blood flowing through the skin. The fingers, toes, palms, and earlobes contain well-innervated anastomotic connections between arterioles and venules (arteriovenous anastomoses; see Chapter 31). Blood flow in response to thermoregulatory stimuli can vary from 1 to as much as 150 mL/100 g of

skin/min, and it has been postulated that these wide variations are possible because blood can be shunted through the anastomoses. The subdermal capillary and venous plexus is a blood reservoir of some importance, and the skin is one of the few places where the reactions of blood vessels can be observed visually.

WHITE REACTION

When a pointed object is drawn lightly over the skin, the stroke lines become pale **(white reaction).** The mechanical stimulus apparently initiates contraction of the precapillary sphincters, and blood drains out of the capillaries and small veins. The response appears in about 15 s.

TRIPLE RESPONSE

When the skin is stroked more firmly with a pointed instrument, instead of the white reaction there is reddening at the site that appears in about 10 s **(red reaction).** This is followed in a few minutes by local swelling and diffuse, mottled reddening around the site. The initial redness is due to capillary dilation, a direct response of the capillaries to pressure. The swelling **(wheal)** is local edema due to increased permeability of the capillaries and postcapillary venules, with consequent extravasation of fluid. The redness spreading out from the site **(flare)** is due to arteriolar dilation. This three-part response—the red reaction, wheal, and flare—is called the **triple response** and is part of the normal reaction to injury (see Chapter 3). It persists after total sympathectomy. On the other hand, the flare is absent in locally anesthetized skin and in denervated skin after the sensory nerves have degenerated, but it is present immediately after nerve block or section above the site of the injury. This, plus other evidence, indicates that it is due to an **axon reflex,** a response in which impulses initiated in sensory nerves by the injury are relayed antidromically down other branches of the sensory nerve fibers **(Figure 33–14)**. This is the one situation in the body in which there is substantial evidence for a physiologic effect due to antidromic conduction. The transmitter released at the central termination of the sensory C fiber neurons is substance P (see Chapter 7), and substance P and CGRP are present in all parts of the neurons. Both dilate arterioles and, in addition, substance P cause extravasation of fluid. Effective nonpeptide antagonists to substance P have been developed, and they reduce the extravasation. Thus, it appears that substance P produces the wheal.

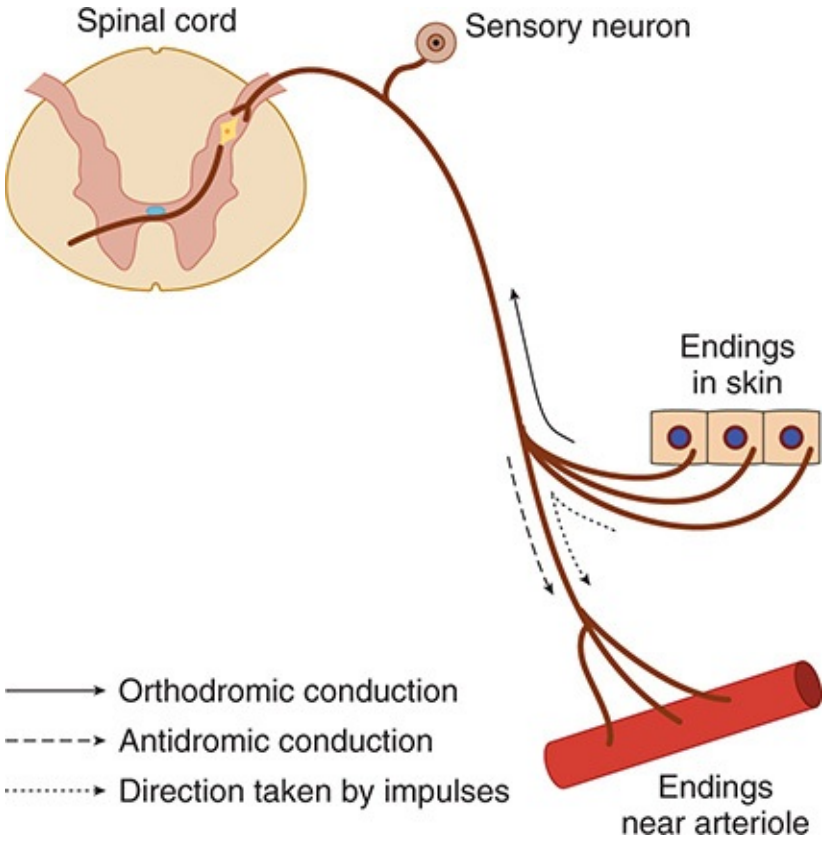


FIGURE 33–14 Axon reflex.

REACTIVE HYPEREMIA

A response of the blood vessels that occurs in many organs but is visible in the skin is **reactive hyperemia,** an increase in the amount of blood in a region when its circulation is reestablished after a period of occlusion. When the blood supply to a limb is occluded, the cutaneous arterioles below the occlusion dilate. When the circulation is reestablished, blood flowing into the dilated vessels makes the skin fiery red. O_2 in the atmosphere can diffuse a short distance through the skin, and reactive hyperemia is prevented if the circulation of the limb is occluded in an atmosphere of 100% O_2. Therefore, the arteriolar dilation is apparently due to a local effect of hypoxia.

GENERALIZED RESPONSES

Noradrenergic nerve stimulation and circulating epinephrine and norepinephrine constrict cutaneous blood vessels. No known vasodilator nerve fibers extend to

the cutaneous vessels, and thus vasodilation is brought about by a decrease in constrictor tone as well as the local production of vasodilator metabolites. Skin color and temperature also depend on the state of the capillaries and venules. A cold blue or gray skin is one in which the arterioles are constricted and the capillaries dilated; a warm red skin is the one in which both are dilated.

Because painful stimuli cause diffuse noradrenergic discharge, a painful injury causes generalized cutaneous vasoconstriction in addition to the local triple response. When the body temperature rises during exercise, the cutaneous blood vessels dilate in spite of continuing noradrenergic discharge in other parts of the body. Dilation of cutaneous vessels in response to a rise in hypothalamic temperature overcomes other reflex activity. Shock is more profound in patients with elevated temperatures because of cutaneous vasodilation, and patients in shock should not be warmed to the point that their body temperature rises. Conversely, cold causes cutaneous vasoconstriction; however, with severe cold, superficial vasodilation may supervene. This latter vasodilation is the cause of the ruddy complexion seen on a cold day.

PLACENTAL & FETAL CIRCULATION

UTERINE CIRCULATION

The blood flow of the uterus parallels the metabolic activity of the myometrium and endometrium and undergoes cyclic fluctuations that correlate with the menstrual cycle in nonpregnant women. The function of the spiral and basilar arteries of the endometrium in menstruation is discussed in Chapter 22. During pregnancy, blood flow increases rapidly as the uterus increases in size **(Figure 33–15)**. Vasodilator metabolites are undoubtedly produced in the uterus, as they are in other active tissues. In early pregnancy, the arteriovenous O_2 difference across the uterus is small, and it has been suggested that estrogens act on the blood vessels to increase uterine blood flow in excess of tissue O_2 needs. Corticotrophin-releasing hormone also appears to play an important role in up-regulating uterine blood flow, as well as in the eventual timing of birth. However, even though uterine blood flow increases 20-fold during pregnancy, the size of the conceptus increases much more, changing from a single cell to a fetus plus a placenta that can weigh 4–5 kg at term in humans. Consequently, more O_2 is extracted from the uterine blood during the latter part of pregnancy, and the O_2 saturation of uterine blood falls.

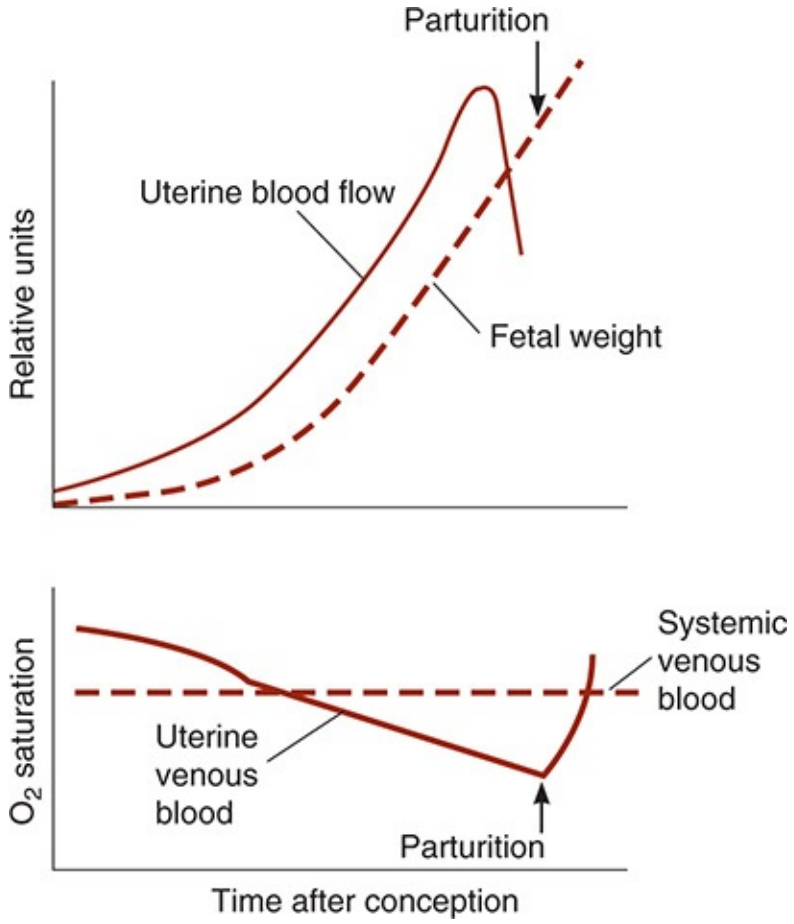


FIGURE 33–15 Changes in uterine blood flow and the amount of O_2 in uterine venous blood during pregnancy. (Modified with permission from Keele CA, Neil *E: Samson Wright's Applied Physiology*, 12th ed. Oxford University Press; 1971.)

PLACENTA

The placenta is the "fetal lung" (**Figures 33–16** and **33–17**). Its maternal portion is in effect a large blood sinus. Into this "lake" project the villi of the fetal portion containing the small branches of the fetal umbilical arteries and vein (Figure 33–16). O_2 is taken up by the fetal blood and CO_2 is discharged into the maternal circulation across the walls of the villi in a manner analogous to O_2 and CO_2 exchange in the lungs (see Chapter 35). However, the cellular layers covering the villi are thicker and less permeable than the alveolar membranes in the lungs, and exchange is much less efficient. The placenta is also the route by which all nutritive materials enter the fetus and by which fetal wastes are

discharged to the maternal blood.

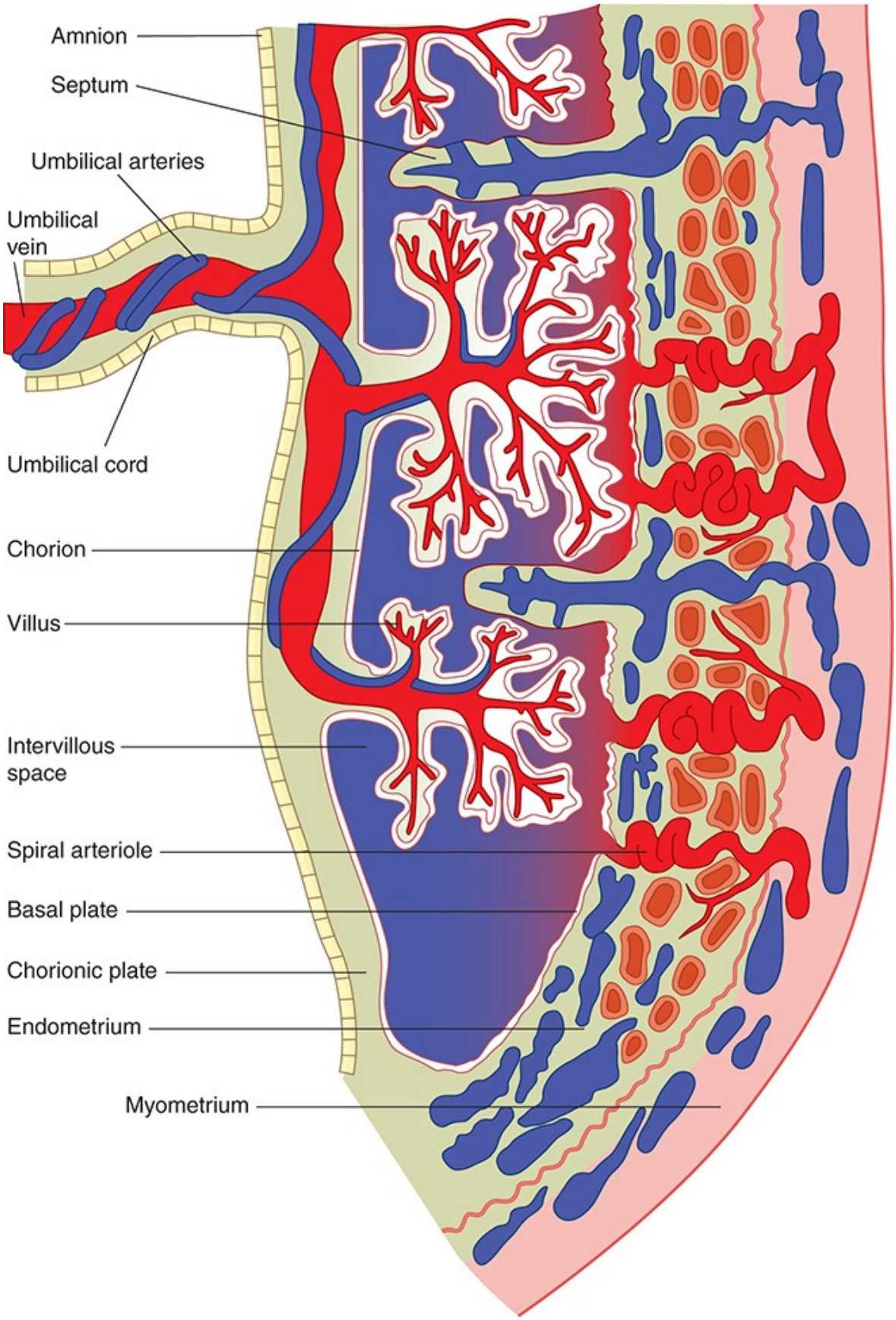

Amnion
Septum
Umbilical arteries
Umbilical vein
Umbilical cord
Chorion
Villus
Intervillous space
Spiral arteriole
Basal plate
Chorionic plate
Endometrium
Myometrium

FIGURE 33–16 Diagram of a section through the human placenta, showing the way the fetal villi project into the maternal sinuses. (©Elsevier, Inc., Netterimages.com.)

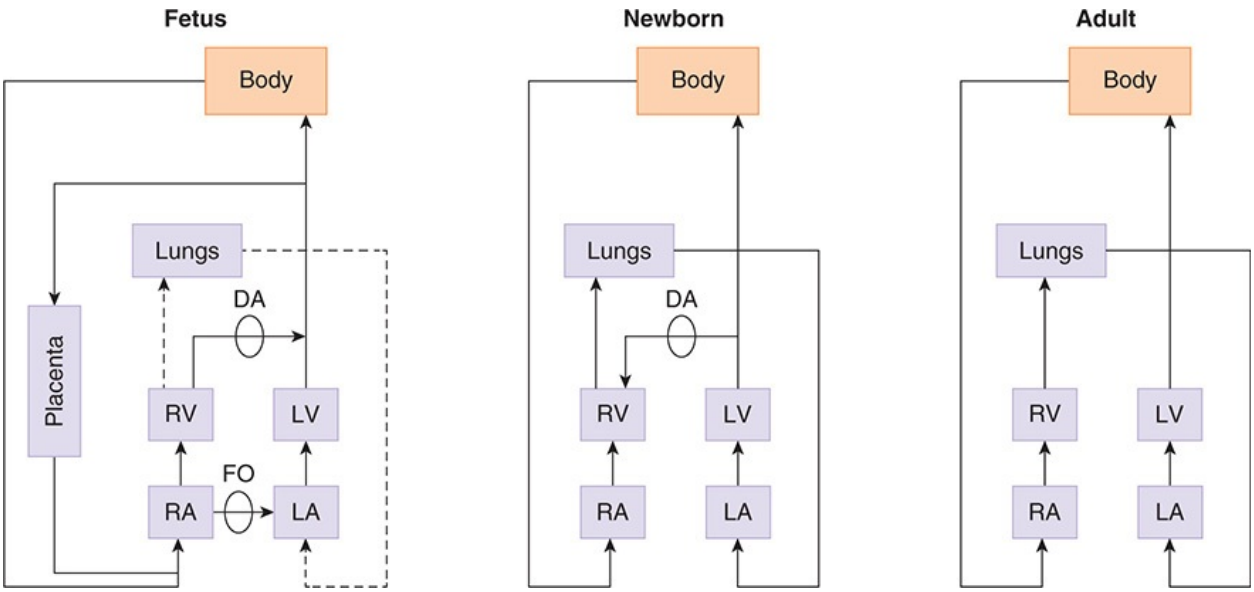


FIGURE 33–17 Diagram of the circulation in the fetus, the newborn infant, and the adult. DA, ductus arteriosus; FO, foramen ovale; LA, left atrium; LV, left ventricle; RA, right atrium; RV, right ventricle. Note that circulation to the fetal liver is not shown for simplicity (see Figure 33–18).

FETAL CIRCULATION

The arrangement of the circulation in the fetus is shown diagrammatically in Figure 33–17. The blood in the umbilical vein in humans is believed to be about 80% saturated with O_2, compared with 98% saturation in the arterial circulation of the adult. The ductus venosus **(Figure 33–18)** diverts some of this blood directly to the inferior vena cava, and the remainder mixes with the portal blood of the fetus. The portal and systemic venous blood of the fetus is only 26% saturated, and the saturation of the mixed blood in the inferior vena cava is approximately 67%. Most of the blood entering the heart through the inferior vena cava is diverted directly to the left atrium via the patent foramen ovale. Most of the blood from the superior vena cava enters the right ventricle and is expelled into the pulmonary artery. The resistance of the collapsed lungs is high, and the pressure in the pulmonary artery is several mm Hg higher than it is in the

aorta, so that most of the blood in the pulmonary artery passes through the **ductus arteriosus** to the aorta. In this manner, the relatively unsaturated blood from the right ventricle is diverted to the trunk and lower body of the fetus, while the head of the fetus receives the better-oxygenated blood from the left ventricle. From the aorta, some of the blood is pumped into the umbilical arteries and back to the placenta. The O_2 saturation of the blood in the lower aorta and umbilical arteries of the fetus is approximately 60%.

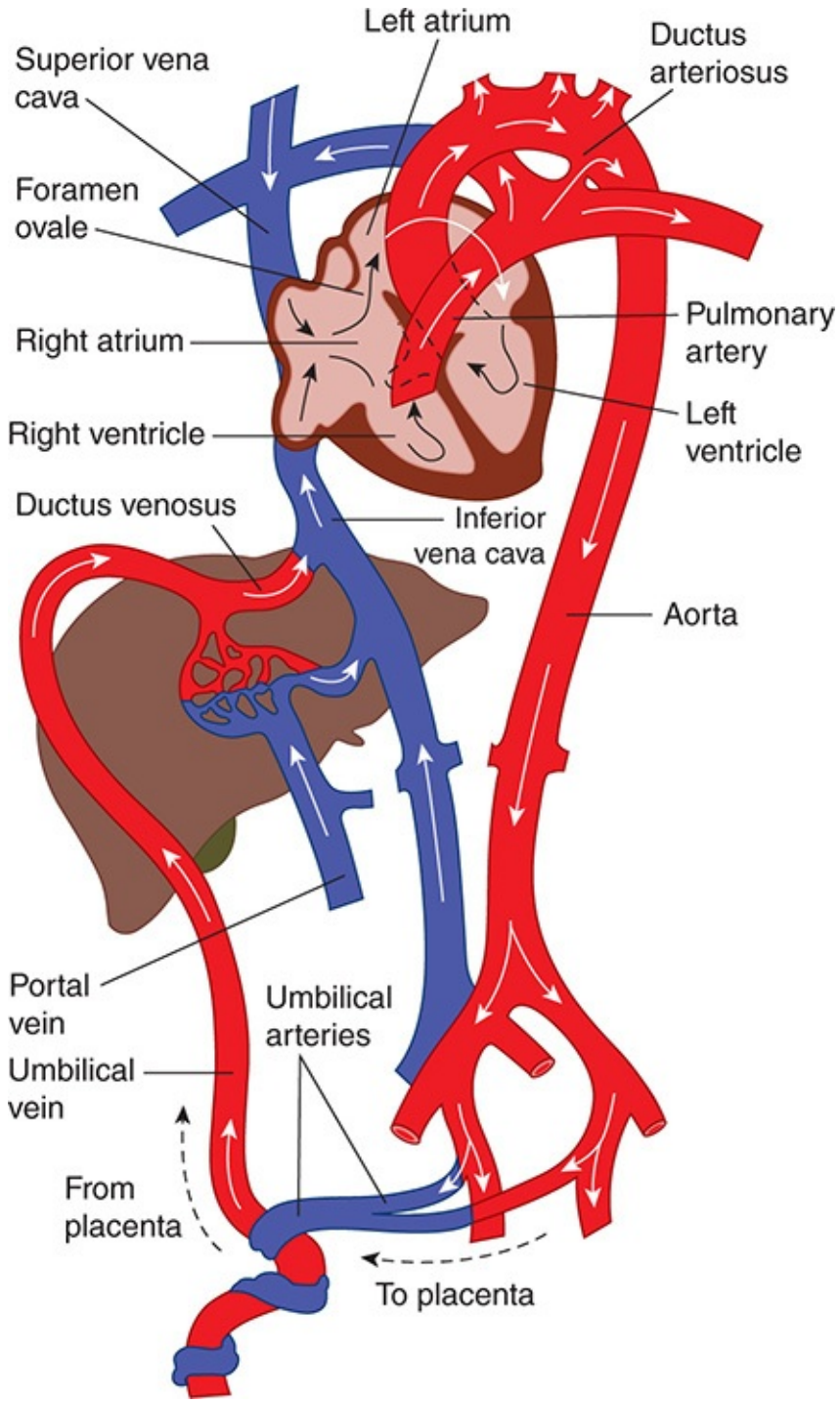


FIGURE 33–18 Circulation in the fetus. Most of the oxygenated blood reaching the heart via the umbilical vein and inferior vena cava is diverted through the foramen ovale and pumped out the aorta to the head, while the deoxygenated blood returned via the superior vena cava is mostly pumped through the pulmonary artery and ductus arteriosus to the feet and the umbilical arteries.

FETAL RESPIRATION

The tissues of fetal and newborn mammals have a remarkable but poorly understood resistance to hypoxia. However, the O_2 saturation of the maternal blood in the placenta is so low that the fetus might suffer hypoxic damage if fetal red cells did not have a greater O_2 affinity than adult red cells **(Figure 33–19)**. The fetal red cells contain fetal hemoglobin (hemoglobin F), whereas the adult cells contain adult hemoglobin (hemoglobin A). The cause of the difference in O_2 affinity between the two is that hemoglobin F binds 2, 3-DPG less effectively than hemoglobin A does. The decrease in O_2 affinity due to the binding of 2, 3-DPG is discussed in Chapter 31.

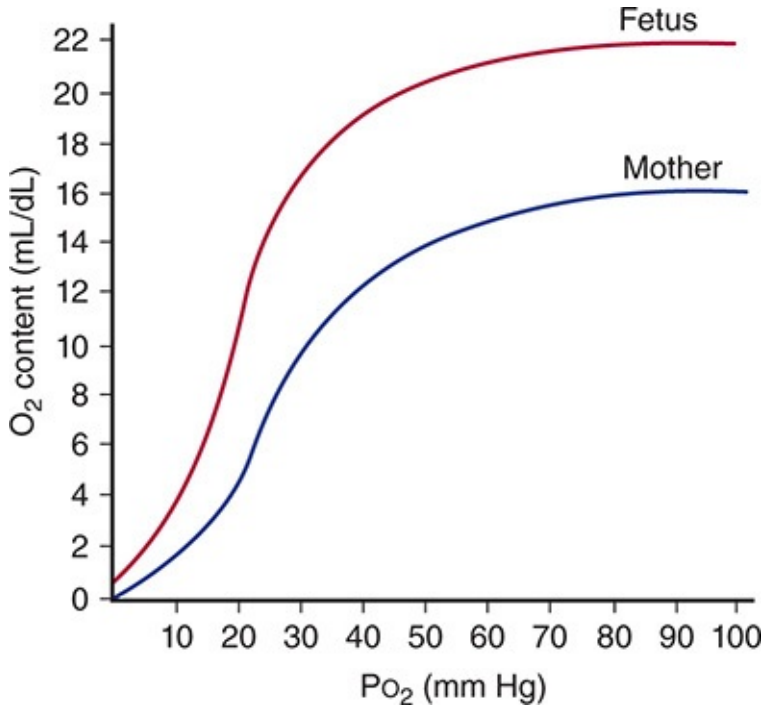


FIGURE 33–19 Dissociation curves of hemoglobin in human maternal and fetal blood.

CHANGES IN FETAL CIRCULATION & RESPIRATION AT BIRTH

Because of the patent ductus arteriosus and foramen ovale (Figure 33–18), the left heart and right heart pump in parallel in the fetus rather than in series as they

do in the adult. At birth the circulation has to change from a parallel to a serial system and from placental to pulmonary gas exchange. This has to be largely completed within minutes of birth, although the complete changes may not be achieved for many hours. The essential element initiating the changes is the fall in the pulmonary vascular resistance when the lungs are first filled with a gas. The pulmonary vascular resistance falls to less than 20% of the value in utero after the lungs are expanded by the first few breaths.

The stimulus for the baby to initiate breathing after birth is not fully understood. In a normal labor, there is no evidence that hypoxia plays a significant role and it is likely that the sudden exposure to light, sound, cold air, and other tactile stimuli are the most important triggers. Oxygen is a strong vasodilator of the pulmonary vascular tree, operating through the production of nitric oxide (NO). The high arterial oxygen tension and bradykinin, which is released from the lungs during their initial inflation, are vasoconstrictors for the umbilical arteries and ductus arteriosus.

Blood returning from the lungs raises the pressure in the left atrium, closing the foramen ovale by pushing the valve that guards it against the interatrial septum. The ductus arteriosus constricts and this, together with the fall in pulmonary vascular resistance, directs most of the output of the right ventricle through the lungs. The ductus arteriosus is functionally closed within a few hours of birth and permanent anatomic closure follows in the next 24–48 h due to extensive intimal thickening. In addition, relatively high concentrations of vasodilators are present in the ductus in utero—especially prostaglandin $F_{2\alpha}$—and synthesis of this prostaglandin is blocked by inhibition of cyclooxygenase at birth. In many premature infants the ductus fails to close spontaneously but closure can be produced by drugs that inhibit cyclooxygenase.

After pulmonary respiration is established, highly oxygenated blood containing pulmonary bradykinin reaches the umbilical arteries, initiating constriction. Further constriction is also stimulated by cold and handling of the cord and the arteries are completely constricted within 3−5 minutes after birth. Venous flow continues as a result of the increased placental pressure within the contracted uterus and the negative intrathoracic pressure of the baby’s respiratory efforts (initially −30 to −50 mm Hg). This redistribution of blood within the fetoplacental circulation after birth is called the “placental transfusion.” Within 5 minutes, however, flow ceases within the umbilical cord and there is little residual blood remaining within the placenta.

Finally, in the fetal circulation the right ventricular output is slightly greater than that of the left. This is possible because of the parallel pump system and the

shunts. After birth the serial system requires both ventricular outputs to be equal before the shunts can close.

CHAPTER SUMMARY

- Blood flow to the brain is via four arteries that ultimately unite to form the circle of Willis; from which six arteries supply the cerebral cortex. There is little cross-over of substances from one side of the arterial system to the other side of the brain. Venous drainage is via the deep veins and dural sinuses into the internal jugular veins.
- Cerebrospinal fluid is produced predominantly in the choroid plexus of the brain, driven mainly by active transport mechanisms in the choroid epithelial cells. Fluid is reabsorbed into the bloodstream to maintain appropriate pressure in the setting of continuous production.
- The permeation of circulating substances into the brain is tightly controlled. Water, CO_2, and O_2 permeate freely. Other substances (such as glucose) require specific transport mechanisms, whereas entry of macromolecules is negligible. The effectiveness of the blood-brain barrier in preventing entry of xenobiotics is bolstered by active efflux mediated by P-glycoprotein.
- The brain consumes approximately 20% of the total body resting O_2 consumption, and occlusion of its blood supply can produce unconsciousness in seconds. Glucose is also critical to supply energy for brain function. When blood flow to a part of the brain is interrupted, the local injury results in a stroke.
- The coronary circulation supplies oxygen to the contracting myocardium. Metabolic products and neural input induce vasodilation as needed for oxygen demand.
- Blockage of coronary arteries may lead to irreversible injury to heart tissue. Blood flows to the subendocardial portion of the left ventricle only during diastole, making this region most susceptible to myocardial infarction in the setting of coronary artery disease.
- Control of cutaneous blood flow is a key facet of temperature regulation, and is underpinned by varying levels of shunting through arteriovenous anastomoses. Hypoxia, axon reflexes, and sympathetic input are all important determinants of flow through the cutaneous vasculature.
- The fetal circulation cooperates with that of the placenta and uterus to

deliver oxygen and nutrients to the growing fetus, as well as carrying away waste products. Unique anatomic features of the fetal circulation as well as biochemical properties of fetal hemoglobin serve to ensure adequate O_2 supply, particularly to the head.

- At birth, the circulation must shift rapidly to a serial system where the right and left heart no longer pump in parallel. The foramen ovale and the ductus arteriosus close such that the neonatal lungs take over as the site for oxygen exchange. Following the establishment of pulmonary respiration, flow in the umbilical system rapidly ceases due to vasoconstrictive signals.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. Which of the following organs has the greatest blood flow per 100 g of tissue?

   A. Brain

   B. Heart muscle

   C. Skin

   D. Liver

   E. Kidneys

2. A baby boy is brought to the hospital because of convulsions. In the course of a workup, his body temperature and plasma glucose are found to be normal, but his cerebrospinal fluid glucose is 12 mg/dL (normal, 65 mg/dL). A possible explanation of his condition is

   A. constitutive activation of GLUT3 in neurons.

   B. SGLT-1 deficiency in astrocytes.

   C. GLUT5 deficiency in cerebral capillaries.

   D. GLUT1 55K deficiency in cerebral capillaries.

   E. GLUT1 45K deficiency in microglia.

3. A scientist injects a rat intravenously with Evans blue, a dye that binds to serum albumin, then euthanizes the animal one hour later. When she examines sections of the brain, where would blue staining be expected?

   A. Medulla

   B. Area postrema

   C. Motor area

D. Parietal lobe

E. Thalamus

4. The pressure differential between the heart and the aorta is least in the

A. left ventricle during systole.

B. left ventricle during diastole.

C. right ventricle during systole.

D. right ventricle during diastole.

E. left atrium during systole.

5. Injection of tissue plasminogen activator (t-PA) would probably be most beneficial

A. after at least 1 year of uncomplicated recovery following occlusion of a coronary artery.

B. after at least 2 months of rest and recuperation following occlusion of a coronary artery.

C. during the second week after occlusion of a coronary artery.

D. during the second day after occlusion of a coronary artery.

E. during the second hour after occlusion of a coronary artery.

6. Which of the following does *not* dilate arterioles in the skin?

A. Increased body temperature

B. Acetylcholine

C. Bradykinin

D. Substance P

E. Epinephrine

7. Blood in which of the following vessels normally has the lowest P_{O_2}?

A. Maternal artery

B. Maternal uterine vein

C. Maternal femoral vein

D. Umbilical artery

E. Umbilical vein

8. A female infant is delivered at a gestational age of 32 weeks and weighing 1 kg. Forty-eight hours after birth, she is noted to have a heart murmur and a bounding pulse, and is feeding poorly. Her symptoms resolve with administration of indomethacin. What is the most likely diagnosis?

A. Necrotizing enterocolitis
B. Bronchopulmonary dysplasia
C. Apnea
D. Patent ductus arteriosus
E. Respiratory distress syndrome

SECTION VI

Respiratory Physiology

Respiration, or the uptake of O_2 and removal of CO_2 from the body as a whole, is the primary goal of the lungs. At rest, a normal human breathes 12–15 times a minute. With each breath containing ~500 mL of air, this translates to 6–8 L of air that is inspired and expired every minute. Once the air reaches the depths of the lungs in the alveoli, simple diffusion allows O_2 to enter the blood in the pulmonary capillaries and CO_2 to enter the alveoli, from where it can be expired. Using some basic math, on average, 250 mL of O_2 enters the body per minute and 200 mL of CO_2 is excreted. In addition to the O_2 that enters the respiratory system, inspired air also contains a variety of particulates that must be properly filtered and/or removed to maintain lung health. Finally, although humans have a certain amount of control over breathing, most of the minute to minute function, including the fine adjustments necessary for proper lung function, are accomplished independent of voluntary control. The goal of this section is to review basic concepts that underlie important aspects of the control and outcome of breathing as well as to highlight other important functions in respiratory physiology.

The respiratory system is connected to the outside world by the upper airway that leads down a set of conduits before reaching the gas-exchanging areas (the alveoli). The function of the lungs is supported by a variety of anatomic features that serve to inflate/deflate the lungs, thereby allowing the movement of gases to and from the rest of the body. Supporting features include the chest wall, the respiratory muscles (which increase and decrease the size of the thoracic cavity), the areas in the brain that control the muscles, and the tracts and nerves that connect the brain to the muscles. Finally, the lungs support the pulmonary circulation, which allows for movement of gases to other organs and tissues of the body. In the first chapter of this section, the unique anatomic, histological,

and cellular makeup of the respiratory system and how the intricate structure of the lungs contributes to respiratory physiology will be explored. This examination will lead into basic lung measurements that both define and allow for lung inflation/deflation as well as some of the nonrespiratory functions essential for lung health.

The discussion will continue with an overview of the primary function of the respiratory system—the capture of O_2 from the outside environment and its delivery to tissues, as well as the simultaneous removal of CO_2 from the tissues to the outside environment. During this discussion, the critical role of pH in gas exchange as well as the ability of the lungs to contribute to pH regulation of the blood is examined. A discussion of respiratory responses to altered O_2 or CO_2 concentrations, caused by environmental and/or pathophysiological changes, is used to better understand the overall control of coordinated uptake of O_2 and excretion of CO_2.

Control of breathing is quite complex and includes not only the repetitive neuronal firing that controls muscle movements that inflate/deflate the lungs but also a series of feedback loops that increase/decrease deflation depending on the gas content of the blood. The final chapter in this section begins with an overview of some of the key factors that aid in this regulation of respiration. Specific examples of common respiratory abnormalities and how they relate to altered regulation of breathing are also discussed to better understand the intricate feedback loops that help regulate breathing.

Due to the complexity of the lungs and thus the variety of working parts that can be compromised, there is a wide-ranging list of diseases that impact its function. Such diseases include common (and uncommon) respiratory infections, asthma, chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome, pulmonary hypertension, lung cancer, and many more. The health burden from such a diverse collection of disorders cannot be overstated. Using COPD as an example, current conservative estimates hold that over 12 million adults in the United States suffer from the condition. In fact, COPD is the fourth leading cause of death (and rising) in the world and a contributory factor in an equal number of non-COPD deaths. Although treatment strategies for COPD, largely based on continuing research efforts and understanding, have contributed to an improved lifestyle, the underlying causes are, as of yet, untreatable. The continued and improved understanding of respiratory physiology and lung

function (and its dysfunction) will provide opportunities to develop new strategies for treatment of COPD, as well as the myriad of other lung diseases.

CHAPTER 34

Introduction to Pulmonary Structure & Mechanics

OBJECTIVES

After studying this chapter, you should be able to:

- List the passages through which air passes from the exterior to the alveoli, and describe the cells that line each of them.
- List the major muscles involved in respiration, and state the role of each.
- Define the basic measures of lung volume and give approximate values for each in a normal adult.
- Define lung compliance and airway resistance.
- Compare the pulmonary and systemic circulations and list some major differences between them.
- Describe basic lung defense and metabolic functions.
- Define partial pressure and calculate the partial pressure of each of the important gases in the atmosphere at sea level.

INTRODUCTION

The structure of the respiratory system is uniquely suited to its primary function, the transport of gases in (O_2) and out (CO_2) of the body. In addition, the respiratory system provides a large volume of tissue that is constantly exposed to the outside environment, and thus, to potential infection and injury. Finally, the pulmonary system includes a unique circulation that must handle the blood flow; the lungs are the only organ that receives the whole cardiac output in the body. This chapter begins with the basic anatomy and cellular physiology that contribute to the respiratory system and their unique features. The chapter also includes discussion of how the anatomic features contribute to the basic mechanics of breathing, as well as highlights of nonrespiratory functions in the pulmonary system.

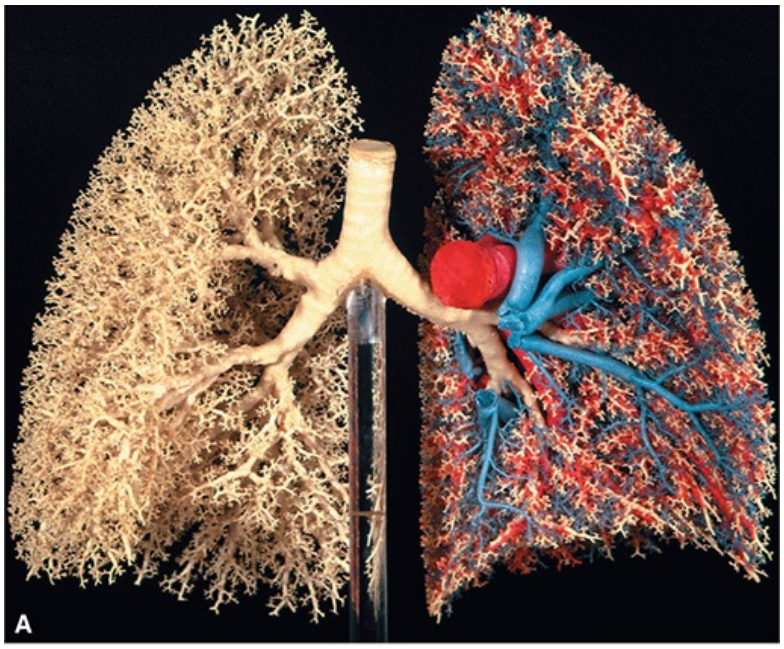
ANATOMY OF THE LUNGS

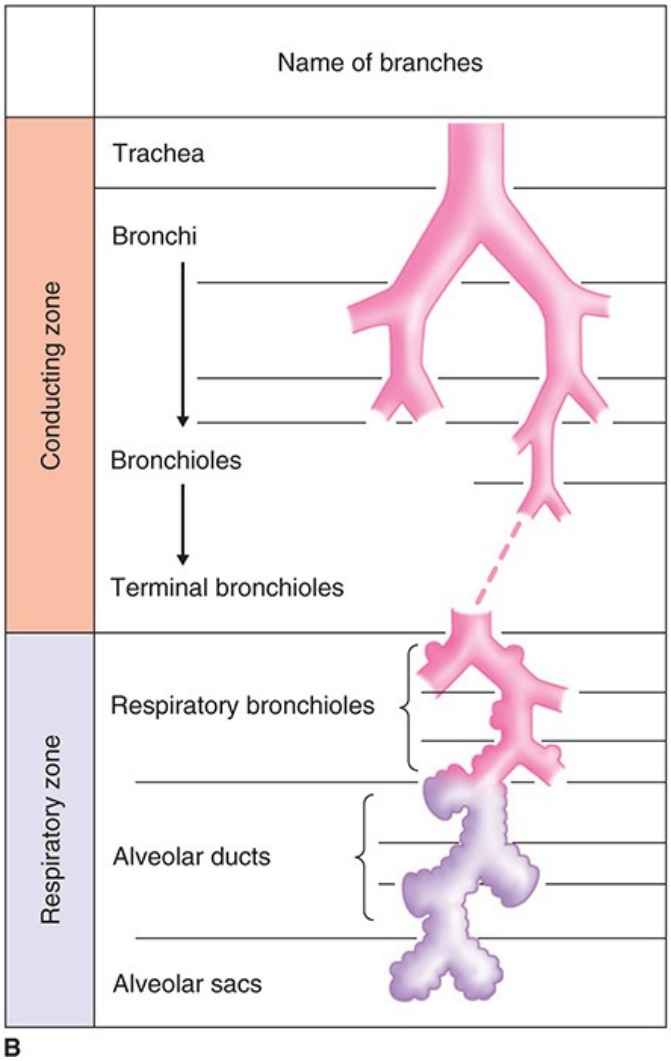
REGIONS OF THE RESPIRATORY TRACT

Airflow through the respiratory system can be broken down into three interconnected regions: the **upper airway,** the **conducting airway,** and the **alveolar airway** (also known as the **lung parenchyma** or **acinar tissue**). The upper airway consists of the entry systems, the nose/nasal cavity and mouth that lead into the pharynx. The larynx extends from the lower part of the pharynx to complete the upper airway. The nose is the primary point of entry for inhaled air; therefore, the mucosal epithelium lining the nasopharyngeal airways is exposed to the highest concentration of inhaled allergens, toxicants, and particulate matter. With this in mind, it is easy to understand that in addition to olfaction, the nose and upper airway provides two additional crucial functions in airflow: (1) filtering out large particulates to prevent them from reaching the conducting and alveolar airways and (2) serving to warm and humidify air as it enters the body. Particulates larger than 30–50 μm in size (diameter) tend to not to be inhaled through the nose whereas particulates on the order of 5–10 μm impact on the nasopharynx and do enter the conducting airway. Most of these latter particles settle on mucous membranes in the nose and pharynx. Because of their momentum, they do not follow the airstream as it curves downward into the lungs, and they impact on or near the **tonsils** and **adenoids,** large collections of immunologically active lymphoid tissue in the back of the pharynx.

CONDUCTING AIRWAY

The conducting airway begins at the trachea and branches dichotomously to greatly expand the surface area of the tissue in the lungs. The first 16 generations of passages form the conducting zone of the airways that transports gas from and to the upper airway described above **(Figure 34–1)**. These branches are made up of bronchi, bronchioles, and terminal bronchioles. The conducting airway is made up of a variety of specialized cells that provide more than simply a conduit for air to reach the lungs **(Figure 34–2)**. The mucosal epithelium is attached to a thin basement membrane, and beneath this, the lamina propria. Collectively these are referred to as the "airway mucosa." Smooth muscle cells are found beneath the epithelium and an enveloping connective tissue is likewise interspersed with cartilage that is more predominant in the portions of the conducting airway of greater caliber. The epithelium is organized as a pseudostratified epithelium and contains several cell types, including ciliated and secretory cells (eg, goblet cells and glandular acini) that provide key components for airway innate immunity, and basal cells that can serve as progenitor cells for repair during injury. As the conducting airway transitions to terminal and transitional bronchioles, the histological appearance of the conducting tubes change. Secretory glands are absent from the epithelium of the bronchioles and terminal bronchioles, smooth muscle plays a more prominent role and cartilage is largely absent from the underlying tissue. Club cells (formerly termed "Clara cells"), nonciliated cuboidal epithelial cells that secrete important defense markers and serve as progenitor cells after injury, make up a large portion of the epithelial lining in the latter portions of the conducting airway.


A


Name of branches
Conducting zone
Trachea
Bronchi
Bronchioles
Terminal bronchioles
Respiratory zone
Respiratory bronchioles
Alveolar ducts
Alveolar sacs

B

FIGURE 34–1 Conducting and respiratory zones in the airway. A) Resin cast of the human airway tree shows dichotomous branching beginning at the trachea. Note the added pulmonary arteries (red) and veins (blue) displayed in the left lung. **B)** The branching pattern of the airway is sketched with individual regions of the conducting and respiratory airways identified. (Reproduced with permission from Fishman AP et al (eds): *Fishman's Pulmonary Diseases and Disorders*. 4th ed. New York: McGraw-Hill; 2008.)

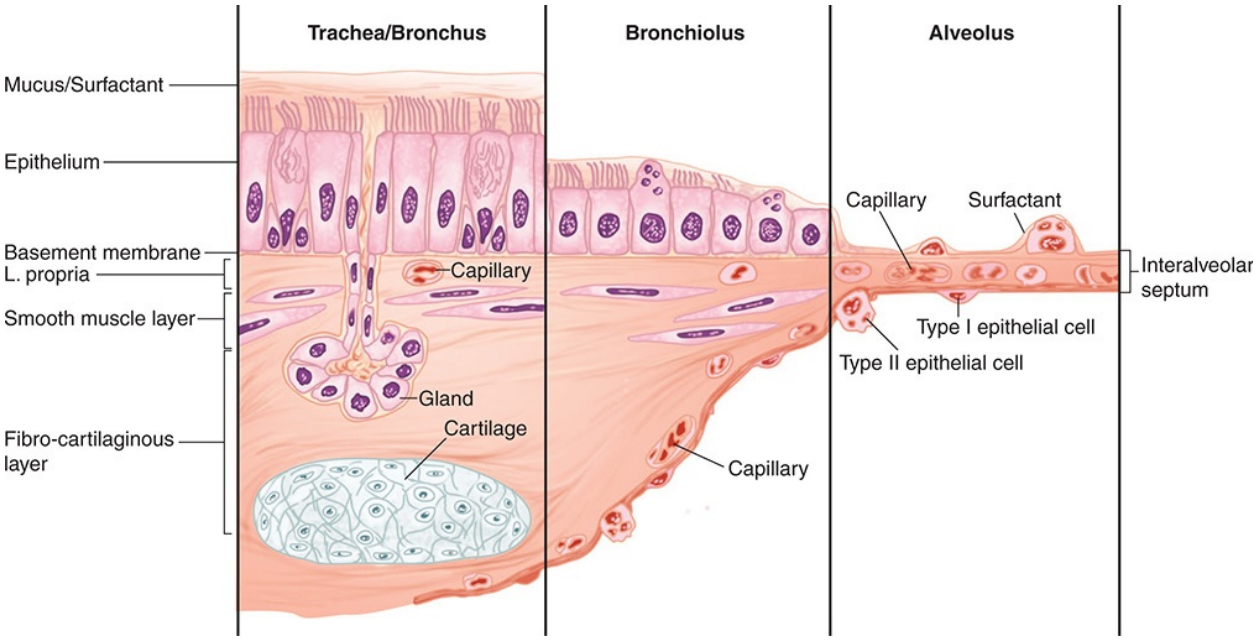


FIGURE 34–2 Cellular transition from conducting airway to the alveolus. The epithelial layer transitions from pseudostratified layer with submucosal glands to a cuboidal epithelium and then to a squamous epithelium. The underlying mesynchyme tissue and capillary structure also changes with the airway transition. (Adapted with permission from Fishman AP et al (eds): *Fishman's Pulmonary Diseases and Disorders*. 4th ed. New York: McGraw-Hill; 2008.)

Epithelial cells in the conducting airway can secrete a variety of molecules that aid in lung defense. Secretory immunoglobulins (IgA), collectins (including surfactant protein (SP) A and SP-D), defensins and other peptides and proteases, reactive oxygen species (ROS), and reactive nitrogen species are all generated by airway epithelial cells. These secretions can act directly as antimicrobials to help keep the airway free of infection. Airway epithelial cells also secrete a variety of chemokines and cytokines that recruit traditional immune cells and

other immune effector cells to site of infections. The smaller particles that make it through the upper airway, ~2–5 µm in diameter, generally fall on the walls of the bronchi as the airflow slows in the smaller passages. There they can initiate reflex bronchial constriction and coughing. Alternatively, they can be moved away from the lungs by the "mucociliary escalator." The epithelium of the respiratory passages from the anterior third of the nose to the beginning of the respiratory bronchioles is ciliated (Figure 34–2). The cilia are bathed in a periciliary fluid where they typically beat at rates of 10–15 Hz. On top of the periciliary layer and the beating cilia rests a mucus layer, a complex mixture of proteins and polysaccharides secreted from specialized cells, glands, or both in the conducting airway. This combination allows for the trapping of foreign particles (in the mucus) and their transport out of the airway (powered by ciliary beat). The ciliary mechanism is capable of moving particles away from the lungs at a rate of at least 16 mm/min. When ciliary motility is defective, as can occur in smokers, or as a result of other environmental conditions or genetic deficiencies, mucus transport is virtually absent. This can lead to chronic sinusitis, recurrent lung infections, and bronchiectasis. Some of these symptoms are evident in cystic fibrosis **(Clinical Box 34–1)**.

The walls of the bronchi and bronchioles are innervated by the autonomic nervous system. Nerve cells in the airways sense mechanical stimuli or the presence of unwanted substances in the airways such as inhaled dusts, cold air, noxious gases, and cigarette smoke. These neurons can signal the respiratory centers to contract the respiratory muscles and initiate sneeze or cough reflexes. The receptors show rapid adaptation when they are continuously stimulated to limit sneeze and cough under normal conditions. The β_2-adrenergic receptors help mediate bronchodilation. They also increase bronchial secretions (eg, mucus), while α_1-adrenergic receptors inhibit secretions.

ALVEOLAR AIRWAY

Between the trachea and the alveolar sacs, the airways divide 23 times. The last seven generations form the transitional and respiratory zones where gas exchange occurs are made up of transitional and respiratory bronchioles, alveolar ducts, and alveoli (Figure 34–1). These multiple divisions greatly increase the total cross-sectional area of the airways, from 2.5 cm^2 in the trachea to 11,800 cm^2 in the alveoli. Consequently, the velocity of airflow in the small airways declines to very low values. The transition from the conducting to the

respiratory region that ends in the alveoli also includes a change in cellular arrangements (Figure 34–2 and **Figure 34–3**). There are 300 million alveoli in humans, and the total area of the alveolar walls in contact with capillaries in both lungs is about 70 m^2.

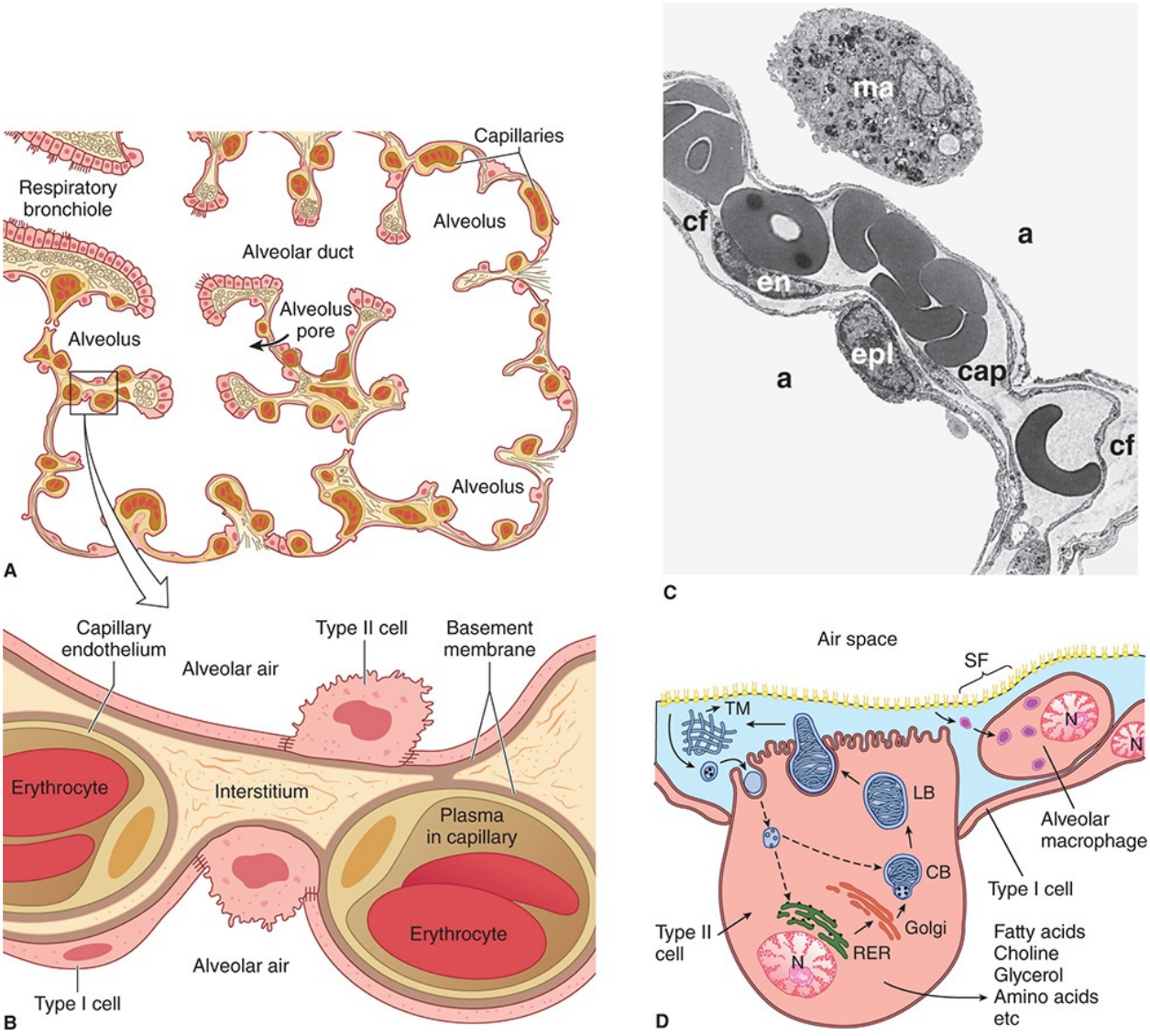


FIGURE 34–3 Prominent cells in the adult human alveolus. A) A cross-section of the respiratory zone shows the relationship between capillaries and the airway epithelium. Only 4 of the 18 alveoli are labeled. **B)** Enlargement of the boxed area from (A) displaying intimate relationship between capillaries, the interstitium, and the alveolar epithelium. **C)** Electron micrograph displaying a typical area depicted in (B). The pulmonary capillary (cap) in the septum contains plasma with red blood cells. Note the closely apposed endothelial and

pulmonary epithelial cell membranes separated at places by additional connective tissue fibers (cf); en, nucleus of endothelial cell; epl, nucleus of type I alveolar epithelial cell; a, alveolar space; ma, alveolar macrophage. **D)** Type II cell formation and metabolism of surfactant. Lamellar bodies (LBs) are formed in type II alveolar epithelial cells and secreted by exocytosis into the fluid lining the alveoli. The released lamellar body material is converted to tubular myelin (TM), and the TM is the source of the phospholipid surface film (SF). Surfactant is taken up by endocytosis into alveolar macrophages and type II epithelial cells. CB, composite body; Golgi, Golgi apparatus; N, nucleus; RER, rough endoplasmic reticulum. (For **(A)** Reproduced with permission from Greep RO, Weiss L: *Histology*, 3rd ed. New York: New York, NY: McGraw-Hill; 1973; **(B)** Adapted with permission from Gong H, Drage CW: *The Respiratory System: A Core Curriculum*. Norwalk, CT: Appleton-Century-Crofts: 1982; **(C)** Reproduced with permission from Burri PA: Development and growth of the human lung. In: *Handbook of Physiology*, Section 3, *The Respiratory System*. Fishman AP, Fisher AB [editors]. American Physiological Society, 1985; and **(D)** Reproduced with permission from Wright JR: Metabolism and turnover of lung surfactant. Am Rev Respir Dis1987 Aug; 136(2):444, 1987.)

The alveoli are lined by two types of epithelial cells. **Type I cells** are flat cells with large cytoplasmic extensions and are the primary lining cells of the alveoli, covering approximately 95% of the alveolar epithelial surface area. **Type II cells (granularpneumocytes)** are thicker and contain numerous lamellar inclusion bodies. Although these cells make up only 5% of the surface area, they represent approximately 60% of the epithelial cells in the alveoli. Type II cells are important in alveolar repair as well as other lung cellular functions. One prime function of the type II cell is the production of **surfactant** (Figure 34–3D). Typical **lamellar bodies,** membrane-bound organelles containing whorls of phospholipid, are formed in these cells and secreted into the alveolar lumen by exocytosis. Tubes of lipid called **tubular myelin** form from the extruded bodies, and the tubular myelin in turn forms a phospholipid film. Following secretion, the phospholipids of surfactant line up in the alveoli with their hydrophobic fatty acid tails facing the alveolar lumen. This surfactant layer plays an important role in maintaining alveolar structure by reducing surface tension (see below). Surface tension is inversely proportional to the surfactant concentration per unit area. The surfactant molecules move further apart as the alveoli enlarge during inspiration, and surface tension increases, whereas it decreases when they move closer together during expiration. Some of the protein–lipid complexes in surfactant are taken up by endocytosis in type II alveolar cells and recycled.

CLINICAL BOX 34–1

Cystic Fibrosis

Among whites, cystic fibrosis is one of the most common genetic disorders; greater than 3% of the United States population are carriers for this autosomal recessive disease.

The gene that is abnormal in cystic fibrosis is located on the long arm of chromosome 7 and encodes the **cystic fibrosis transmembrane conductance regulator (CFTR),** a regulated Cl^- channel located on the apical membrane of various secretory and absorptive epithelia. The number of reported mutations in the *CFTR* gene that cause cystic fibrosis is large (> 1000) and the mutations are now grouped into five classes (I–V) based on their effects on cellular function. Class I mutations do not allow for synthesis of the protein. Class II mutations have protein processing defects. Class III mutations have a block in their channel regulation. Class IV mutations display altered conductance of the ion channel. Class V mutations display reduced synthesis of the protein. The severity of the defect varies with the class and the individual mutation. The most common mutation causing cystic fibrosis is loss of the phenylalanine residue at amino acid position 508 of the protein (ΔF508), a Class II mutation that limits the amount of CFTR protein that gets to the plasma membrane.

One outcome of cystic fibrosis is repeated pulmonary infections, particularly with *Pseudomonas aeruginosa*, and progressive, eventually fatal destruction of the lungs. There is also suppressed chloride secretion across the wall of the airways. One would expect Na^+ reabsorption to be depressed as well, and indeed in sweat glands it is. However, in the lungs, it is enhanced, so that the Na^+ and water move out of airways, leaving their other secretions inspissated and sticky. This results in a reduced periciliary layer that inhibits function of the mucociliary and escalator and alters the local environment to reduce the effectiveness of antimicrobial secretions.

THERAPEUTIC HIGHLIGHTS

Traditional treatments of cystic fibrosis address the various symptoms. Chest physiotherapy and mucolytics are used to loosen thick mucus and aid lung clearance. Antibiotics are used to prevent new infections and keep chronic infections in check. Bronchodilators and anti-inflammatory medications are

used to help expand and clear air passages. Pancreatic enzymes and nutritive supplements are used to increase nutrient absorption and promote weight gain. Because of the “single gene” mutation of this disease, gene therapy has been closely examined; however, results have not been successful. More recently, drugs that target the molecular defects have been advancing in clinical trials and are showing great promise for better treatments. The newly developed genome editing tool, CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats (CRISPR) associated nuclease 9), will become a highly promising in vivo gene therapy for cystic fibrosis by correcting mutation(s) of *CFTR* gene.

The alveoli are surrounded by pulmonary capillaries. In most areas, air and blood are separated only by the alveolar epithelium and the capillary endothelium, so they are about 0.5 µm apart (Figure 34–3). The alveoli also contain other specialized cells, including pulmonary alveolar macrophages (PAMs, or AMs), lymphocytes, plasma cells, neuroendocrine cells, and mast cells. PAMs are an important component of the pulmonary defense system. Like other macrophages, these cells come originally from the bone marrow. PAMs are actively phagocytic and ingest small particles that evade the mucociliary escalator and reach the alveoli. They also help process inhaled antigens for immunologic attack, and they secrete substances that attract granulocytes to the lungs as well as substances that stimulate granulocyte and monocyte formation in the bone marrow. PAM function can also be detrimental—when they ingest large amounts of the substances in cigarette smoke or other irritants, they may release lysosomal products into the extracellular space to cause inflammation.

RESPIRATORY MUSCLES

The lungs are positioned within the thoracic cavity, which is defined by the rib cage and the spinal column. The lungs are surrounded by a variety of muscles that contribute to breathing **(Figure 34–4)**. Movement of the diaphragm accounts for 75% of the change in intrathoracic volume during quiet inspiration. Attached around the bottom of the thoracic cage, this muscle arches over the liver and moves downward like a piston when it contracts. The distance it moves ranges from 1.5 cm to as much as 7 cm with deep inspiration.

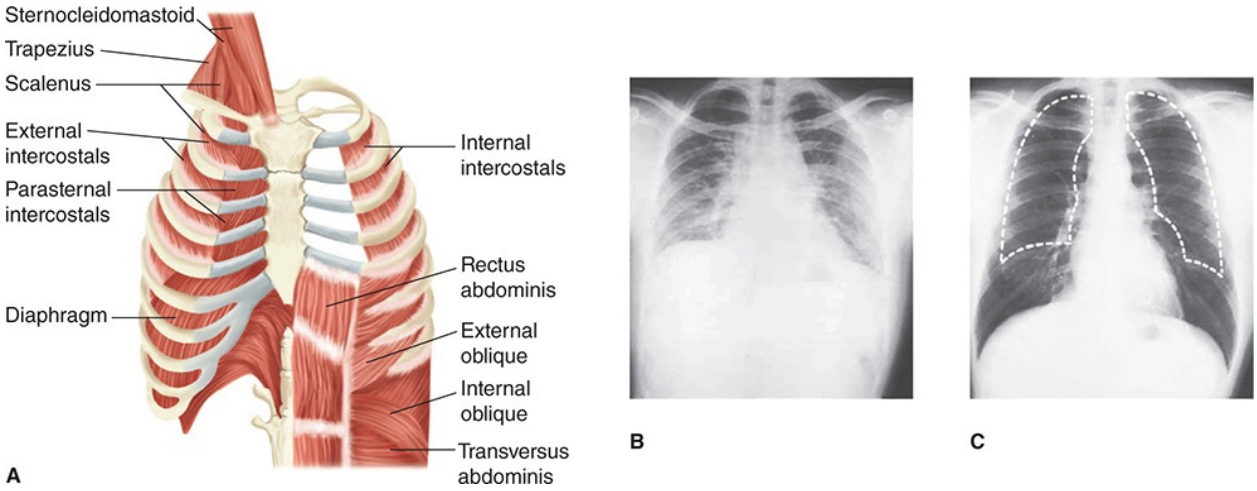


FIGURE 34–4 Muscles and movement in respiration. A) An idealized diagram of respiratory muscles surrounding the rib cage. The diaphragm and intercostals play prominent roles in respiration. **B)** and **C)** Radiograph of chest in full expiration (B) and full inspiration (C). The dashed white line in C is an outline of the lungs in full expiration. Note the difference in intrathoracic volume. (For **(A)** Reproduced with permission from Fishman AP et al (eds): *Fishman's Pulmonary Diseases and Disorders*. 4th ed. New York: McGraw-Hill; 2008; **(B, C)** Reproduced with permission from Comroe JH Jr: *Physiology of Respiration*, 2nd ed., Year Book; 1975.)

The diaphragm has three parts: the portion, made up of muscle fibers that are attached to the ribs around the bottom of the thoracic cage; the crural portion, made up of fibers that are attached to the ligaments along the vertebrae; and the central tendon, into which the costal and the crural fibers insert. The central tendon is also the inferior part of the pericardium. The crural fibers pass on either side of the esophagus and can compress it when they contract. The costal and crural portions are innervated by different parts of the phrenic nerve and can contract separately. For example, during vomiting and eructation, intra-abdominal pressure is increased by contraction of the costal fibers but the crural fibers remain relaxed, allowing material to pass from the stomach into the esophagus.

The other important **inspiratory muscles** are the **external intercostal muscles,** which run obliquely downward and forward from rib to rib. The ribs pivot as if hinged at the back, so that when the external intercostals contract they elevate the lower ribs. This pushes the sternum outward and increases the anteroposterior diameter of the chest. The transverse diameter also increases but to a lesser degree. Either the diaphragm or the external intercostal muscles alone

can maintain adequate ventilation at rest. Transection of the spinal cord above the third cervical segment is fatal without artificial respiration, but transection below the fifth cervical segment is not because it leaves the phrenic nerves that innervate the diaphragm intact; the phrenic nerves arise from cervical segments 3–5. Conversely, in patients with bilateral phrenic nerve palsy but intact innervation of their intercostal muscles, respiration is somewhat labored but adequate to maintain life. The scalene and sternocleidomastoid muscles in the neck are accessory inspiratory muscles that help elevate the thoracic cage during deep labored respiration.

A decrease in intrathoracic volume and forced expiration result when the **expiratory muscles** contract. The internal intercostals have this action because they pass obliquely downward and posteriorly from rib to rib and therefore pull the rib cage downward when they contract. Contractions of the muscles of the anterior abdominal wall also aid expiration by pulling the rib cage downward and inward and by increasing the intra-abdominal pressure, which pushes the diaphragm upward.

In order for air to get into the conducting airway it must pass through the **glottis,** defined as the area including and between the vocal folds within the larynx. The abductor muscles in the larynx contract early in inspiration, pulling the vocal cords apart and opening the glottis. During swallowing or gagging, a reflex contraction of the adductor muscles closes the glottis and prevents aspiration of food, fluid, or vomitus into the lungs. In unconscious or anesthetized patients, glottic closure may be incomplete and vomitus may enter the trachea, causing an inflammatory reaction in the lungs **(aspiration pneumonia).**

LUNG PLEURA

The **pleural cavity** and its infoldings serve as a lubricating fluid/area that allows for lung movement within the thoracic cavity **(Figure 34–5A)**. There are two layers that contribute to the pleural cavity: the **parietal pleura** and the **visceral pleura.** The parietal pleura is a membrane that lines the chest cavity containing the lungs. The **visceral pleura** is a membrane that lines the lung surface. The pleural fluid (∼15–20 mL) forms a thin layer between the pleural membranes and prevents friction between surfaces during inspiration and expiration.

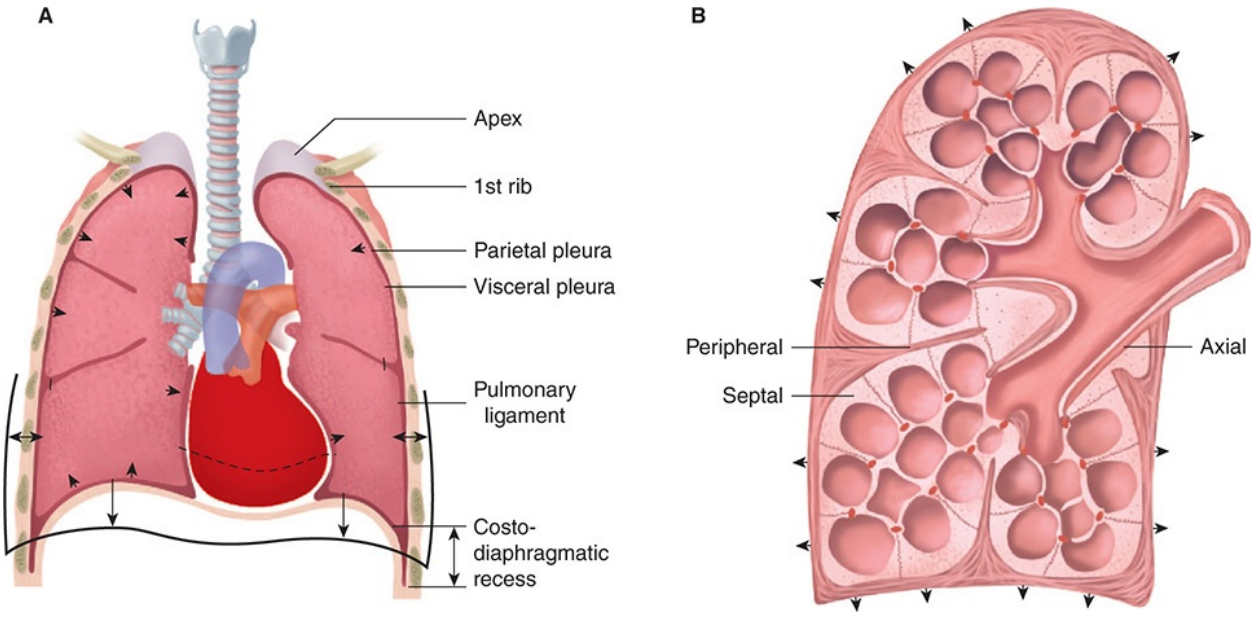


FIGURE 34–5 Pleural space and connective fibers. A) Front sectional drawing of lungs within the rib cage. Note the parietal and visceral pleura and the infoldings around the lung lobes that include pleural space. **B)** Connective fiber tracts of the lung are highlighted. Note the axial fibers along the airways, the peripheral fibers in the pleura, and the septal fibers. (Adapted with permission from Fishman AP et al (eds): *Fishman's Pulmonary Diseases and Disorders*. 4th ed. New York: McGraw-Hill; 2008.)

The lungs themselves contain a vast amount of free space—it is ~80% air. Although this maximizes surface area for gas exchange, it also requires an extensive support network to maintain lung shape and function. The **connective tissue** within the visceral pleura contains three layers that help support the lungs. Elastic fibers that follow the mesothelium effectively wrap the three lobes of the right lung and the two lobes of the left lung **(Figure 34–5B)**. A deep sheet of fine fibers that follow the outline of the alveoli provide support to individual air sacks. Between these two separate sheets lies connective tissue that is interspersed with individual cells for support and lung maintenance/function.

Blood & Lymph in the Lungs

Both the **pulmonary circulation** and the **bronchial circulation** contribute to blood flow in the lungs. In the pulmonary circulation **(Figure 34–6)**, almost all the blood in the body passes via the pulmonary artery to the pulmonary capillary bed, where it is oxygenated and returned to the left atrium via the pulmonary

veins. The pulmonary arteries strictly follow the branching of the bronchi down to the respiratory bronchioles. The pulmonary veins, however, are spaced between the bronchi on their return to the heart. The separate and much smaller bronchial circulation includes the bronchial arteries that come from systemic arteries. They form capillaries, which drain into bronchial veins or anastomose with pulmonary capillaries or veins. The bronchial veins drain into the azygos vein. The bronchial circulation nourishes the trachea down to the terminal bronchioles and also supplies the pleura and hilar lymph nodes. It should be noted that lymphatic channels are more abundant in the lungs than in any other organ. Lymph nodes are arranged along the bronchial tree and extend down until the bronchi are ~5 mm in diameter. Lymph node sizes can range from 1 mm at the bronchial periphery to 10 mm along the trachea. The nodes are connected by lymph vessels and allow for unidirectional flow of lymph to the subclavian veins.

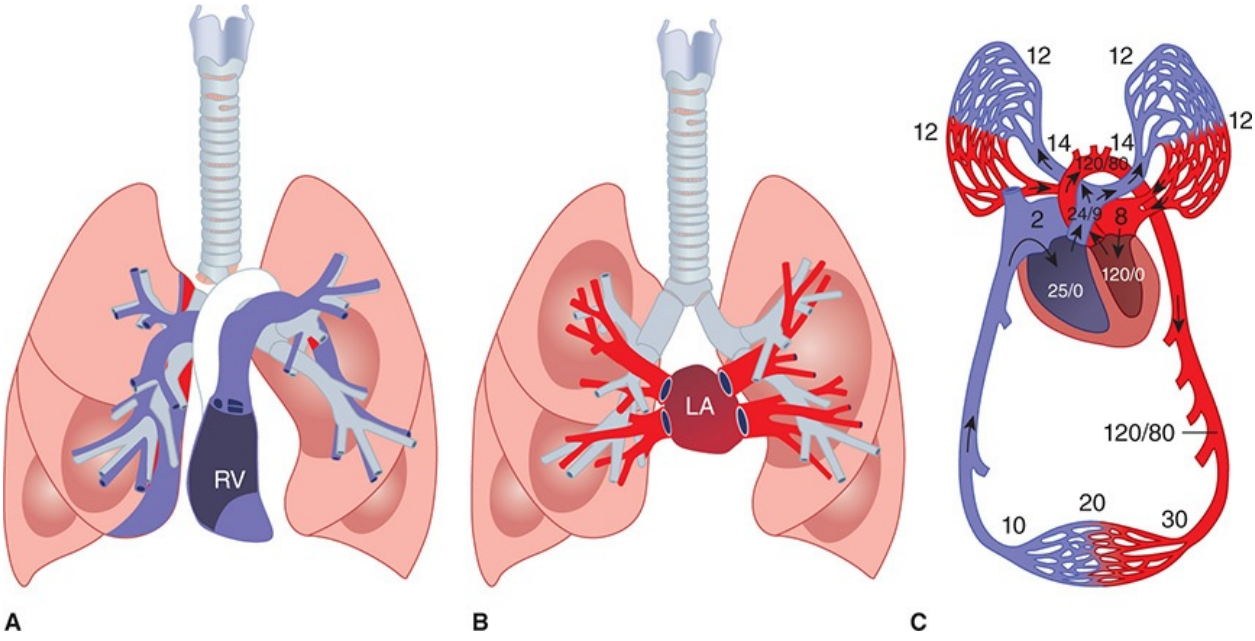


FIGURE 34–6 Pulmonary circulation. A) Schematic diagram of the relation of the main branches of the pulmonary arteries to the bronchial tree. **B)** Schematic of the pulmonary veins in relation to the bronchial tree. **C)** Representative areas of blood flow are labeled with corresponding blood pressure (mm Hg). In all diagrams, blue represents deoxygenated blood and red represents oxygenated blood. The deoxygenated/oxygenated shift in **C** occurs as blood traverses capillaries that wrap individual alveoli and is not as abrupt as shown. LA, left atrium; RV, right ventricle. (For **(A, B)** Reproduced with permission from Fishman AP et al (eds): *Fishman's Pulmonary Diseases and Disorders*. 4th ed.

New York: McGraw-Hill; 2008; **(C)** Modified with permission from Comroe JH Jr: *Physiology of Respiration*, 2nd ed. Year Book; 1974.)

MECHANICS OF RESPIRATION

INSPIRATION & EXPIRATION

The lungs and the chest wall are elastic structures. Normally, no more than a thin layer of fluid is present between the lungs and the chest wall (intrapleural space). The lungs slide easily on the chest wall, but resist being pulled away from it in the same way that two moist pieces of glass slide on each other but resist separation. The pressure in the “space” between the lungs and chest wall (intrapleural pressure) is subatmospheric **(Figure 34–7)**. The lungs are stretched when they expand at birth, and at the end of quiet expiration their tendency to recoil from the chest wall is just balanced by the tendency of the chest wall to recoil in the opposite direction. If the chest wall is opened, the lungs collapse; and if the lungs lose their elasticity, the chest expands and becomes barrel-shaped.

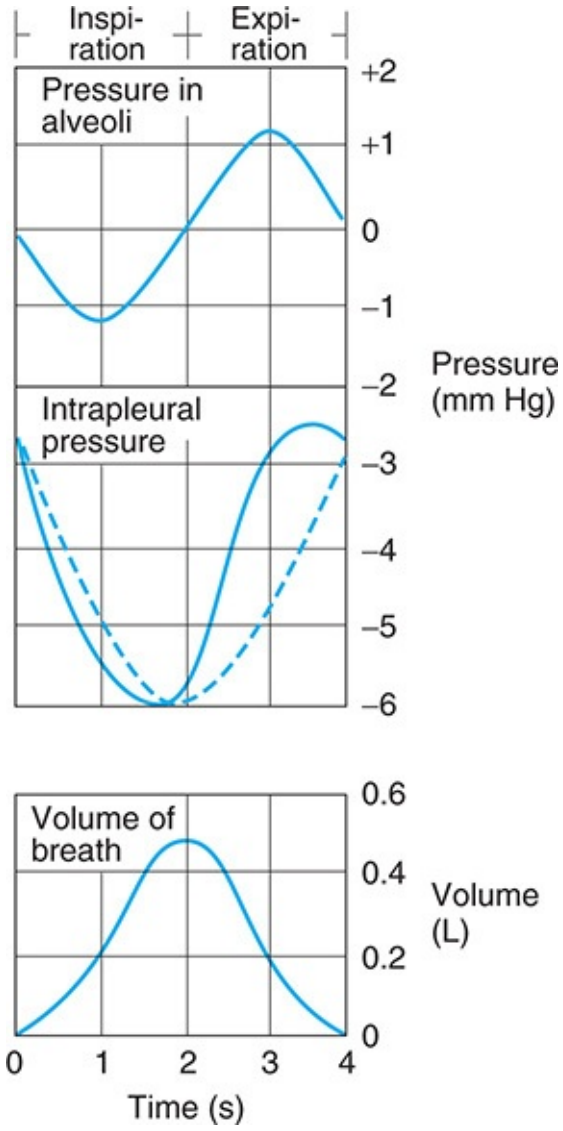


FIGURE 34–7 Pressure in the alveoli and the pleural space relative to atmospheric pressure during inspiration and expiration. The dashed line indicates what the intrapleural pressure would be in the absence of airway and tissue resistance; the actual curve (solid line) is skewed to the left by the resistance. Volume of breath during inspiration/expiration is graphed for comparison.

Inspiration is an active process. The contraction of the inspiratory muscles increases intrathoracic volume. The intrapleural pressure at the base of the lungs, which is normally about –2.5 mm Hg (relative to atmospheric) at the start of inspiration, decreases to about –6 mm Hg. The lungs are pulled into a more expanded position. The pressure in the airway becomes slightly negative, and air flows into the lungs. At the end of inspiration, the lung recoil begins to pull the

chest back to the expiratory position, where the recoil pressures of the lungs and chest wall balance (see below). The pressure in the airway becomes slightly positive, and air flows out of the lungs. Expiration during quiet breathing is passive in the sense that no muscles that decrease intrathoracic volume contract. However, some contraction of the inspiratory muscles occurs in the early part of expiration. This contraction exerts a braking action on the recoil forces and slows expiration. Strong inspiratory efforts reduce intrapleural pressure to values as low as –30 mm Hg, producing correspondingly greater degrees of lung inflation. When ventilation is increased, the extent of lung deflation is also increased by active contraction of expiratory muscles that decrease intrathoracic volume.

QUANTITATING RESPIRATORY PHENOMENA

Modern spirometers permit direct measurement of gas intake and output. Since gas volumes vary with temperature and pressure and since the amount of water vapor in them varies, these devices have the ability to correct respiratory measurements involving volume to a stated set of standard conditions. It should be noted that correct measurements are highly dependent on the ability for the practitioner to properly encourage the patient to fully utilize the device. Modern techniques for gas analysis make possible rapid, reliable measurements of the composition of gas mixtures and the gas content of body fluids. For example, O_2 and CO_2 electrodes, small probes sensitive to O_2 or CO_2, can be inserted into the airway or into blood vessels or tissues and the P_{O_2} and P_{CO_2} recorded continuously. Chronic assessment of oxygenation is carried out noninvasively with a **pulse oximeter,** which can be easily placed on a fingertip.

Lung Volumes & Capacities

Important quantitation of lung function can be gleaned from the displacement of air volume during inspiration and/or expiration. Lung capacities refer to subdivisions that contain two or more volumes. Volumes and capacities recorded on a spirometer from a healthy individual are shown in **Figure 34–8**. Diagnostic spirometry is used to assess a patient's lung function for purposes of comparison with a normal population, or with previous measures from the same patient. The amount of air that moves into the lungs with each inspiration (or the amount that moves out with each expiration) during quiet breathing is called the **tidal**

volume (TV). Typical values for TV are on the order of 500–750 mL. The air inspired with a maximal inspiratory effort in excess of the TV is the **inspiratory reserve volume** (IRV; ∼2 L). The volume expelled by an active expiratory effort after passive expiration is the **expiratory reserve volume** (ERV; ∼1 L), and the air left in the lungs after a maximal expiratory effort is the **residual volume** (RV; ∼1.3 L). When all four of the above components are taken together, they make up the **total lung capacity** (TLC, ∼5 L). The TLC can be broken down into alternative capacities that help define functioning lungs. The **vital capacity** (VC, ∼3.5 L) refers to the maximum amount of air expired from the fully inflated lungs, or maximum inspiratory level (this represents TV + IRV + ERV). The **inspiratory capacity** (IC, ∼2.5 L) is the maximum amount of air inspired from the end-expiratory level (IRV + TV). The **functional residual capacity** (FRC; ∼2.5 L) represents the volume of the air remaining in the lungs after expiration of a normal breath (RV + ERV).

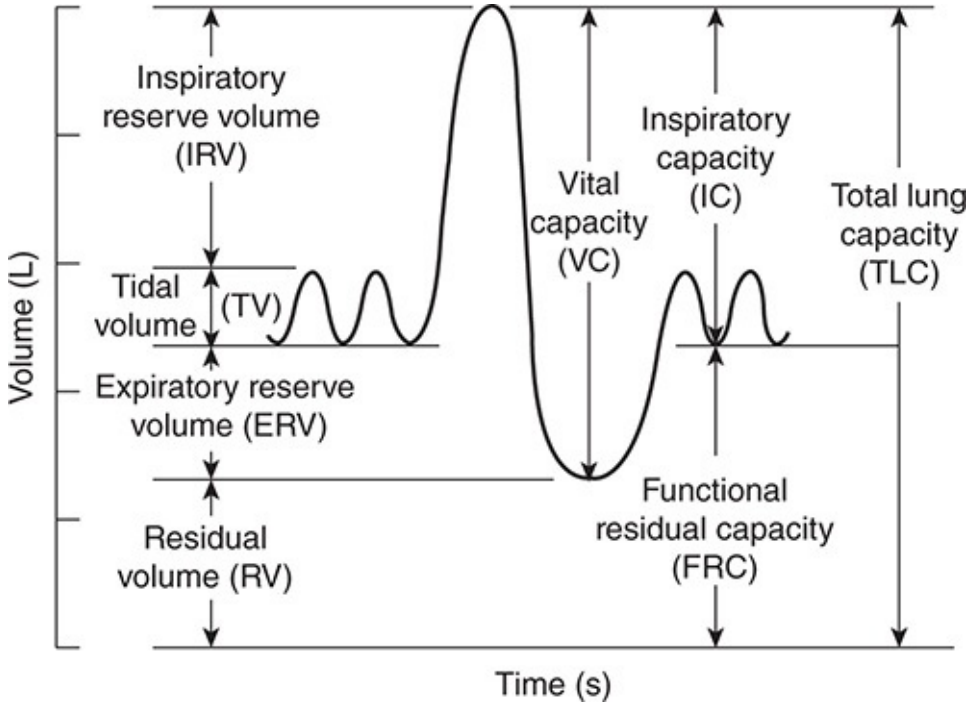


FIGURE 34–8 Lung volumes and capacity measurements. Lung volumes recorded by a spirometer. Lung capacities are determined from volume recordings. See text for definitions. (Reproduced with permission from Fishman AP et al (eds): *Fishman's Pulmonary Diseases and Disorders*. 4th ed. New York: McGraw-Hill; 2008.)

Dynamic measurements of lung volumes and capacities have been used to

determine lung dysfunction. The **forced vital capacity (FVC),** the largest amount of air that can be expired after a maximal inspiratory effort, is frequently measured clinically as an index of pulmonary function. It gives useful information about the strength of the respiratory muscles and other aspects of pulmonary function. The fraction of the VC expired during the first second of a forced expiration is referred to as **FEV_1** (forced expiratory volume in the first second; **Figure 34–9**). The FEV_1 to FVC ratio (FEV_1/FVC) is a useful tool in the recognizing classes of airway disease **(Clinical Box 34–2)**. Other dynamic measurements include the **respiratory minute volume (RMV)** and the **maximal voluntary ventilation (MVV).** RMV is normally ~6 L (500 mL/breath × 12 breaths/min). The **MVV** is the largest volume of gas that can be moved into and out of the lungs in 1 min by voluntary effort. Typically this is measured over a 15-s period and prorated to a minute; normal values range from 140 L/min to 180 L/min for healthy adult men. Changes in RMV and MVV in a patient can be indicative of lung dysfunction.

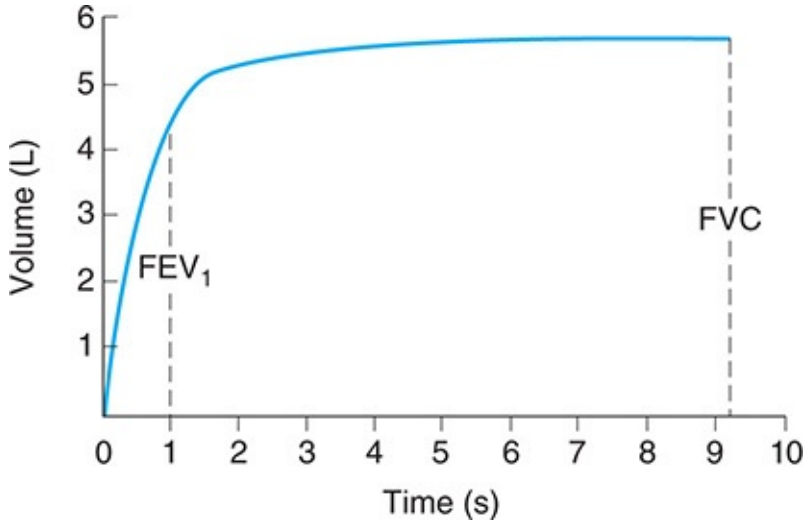


FIGURE 34–9 Volume of gas expired by a normal adult man during a forced expiration, demonstrating the FEV_1 and the FVC. From the graph, the forced expiratory volume in the first second (FEV_1) to the forced vital capacity (FVC) ration (FEV_1/FVC) can be calculated (4L/5L = 80%). (Reproduced with permission from Crapo RO: Pulmonary-function testing, N Engl J Med 1994; July 7; 331(1):25–30.)

COMPLIANCE OF THE LUNGS & CHEST WALL

Compliance is developed due to the tendency for tissue to resume its original position after an applied force has been removed. After an expiration during quiet breathing (eg, at the FRC), the lungs have a tendency to collapse and the chest wall has a tendency to expand. The interaction between the recoil of the lungs and recoil of the chest can be measured through a spirometer that has a valve just beyond the mouthpiece. The mouthpiece contains a pressure-measuring device. After the person inhales a given amount, the valve is shut, closing off the airway. The respiratory muscles are then relaxed while the pressure in the airway is recorded. The procedure is repeated after inhaling or actively exhaling various volumes. The curve of airway pressure obtained in this way, plotted against volume, is the **pressure–volume curve** of the total respiratory system (P_{TR} in **Figure 34–10**). The pressure is zero at a lung volume that corresponds to the volume of gas in the lungs at **FRC (**ie, **relaxation volume).** As can be noted from Figure 34–10, this relaxation pressure is the sum of slightly negative pressure component from the chest wall (P_w) and a slightly positive pressure from the lungs (P_L). P_{TR} is positive at greater volumes and negative at smaller volumes. **Compliance** of the lungs and chest wall is measured as the slope of the P_{TR} curve, or, as a change in lung volume per unit change in airway pressure ($\Delta V/\Delta P$). It is normally measured in the pressure range where the relaxation pressure curve is steepest, and normal values are ~0.2 L/cm H_2O in a healthy adult man. However, compliance depends on lung volume and thus can vary. In an extreme example, an individual with only one lung has approximately half the ΔV for a given ΔP. Compliance is also slightly greater when measured during deflation than when measured during inflation. Consequently, it is more informative to examine the whole pressure–volume curve. The curve is shifted downward and to the right (compliance is decreased) by pulmonary edema and interstitial pulmonary fibrosis **(Figure 34–11)**. Pulmonary fibrosis is a progressive restrictive airway disease in which there is stiffening and scarring of the lungs. The curve is shifted upward and to the left (compliance is increased) in emphysema.

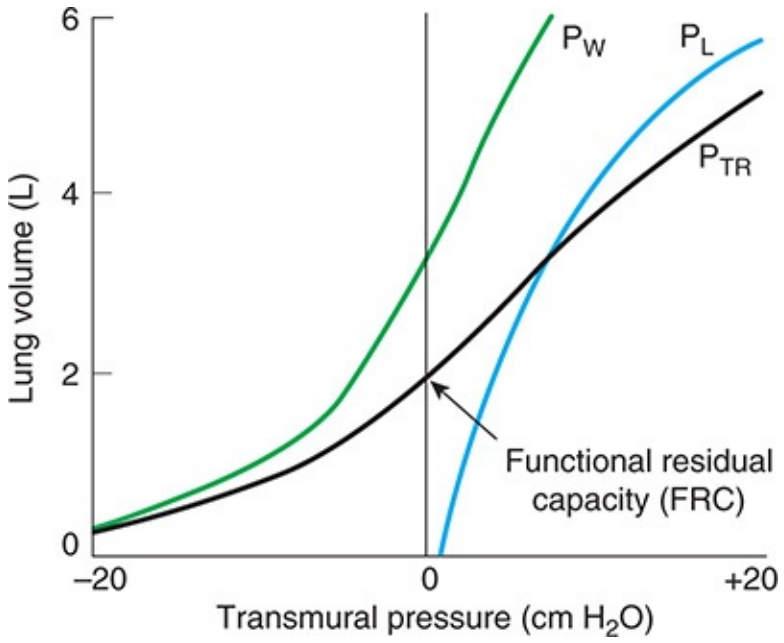


FIGURE 34–10 Pressure–volume curves in the lungs. The pressure–volume curves of the total respiratory system (P_{TR}, black line), the lungs (P_L, blue line), and the chest (P_W, green line) are plotted together with standard volumes for functional residual capacity and tidal volume. The transmural pressure is intrapulmonary pressure minus intrapleural pressure in the case of the lungs, intrapleural pressure minus outside (barometric) pressure in the case of the chest wall, and intrapulmonary pressure minus barometric pressure in the case of the total respiratory system. From these curves, the total and actual elastic work associated with breathing can be derived. (Modified with permission from Mines AH: *Respiratory Physiology*, 3rd ed. New York, NY: Raven Press; 1993.)

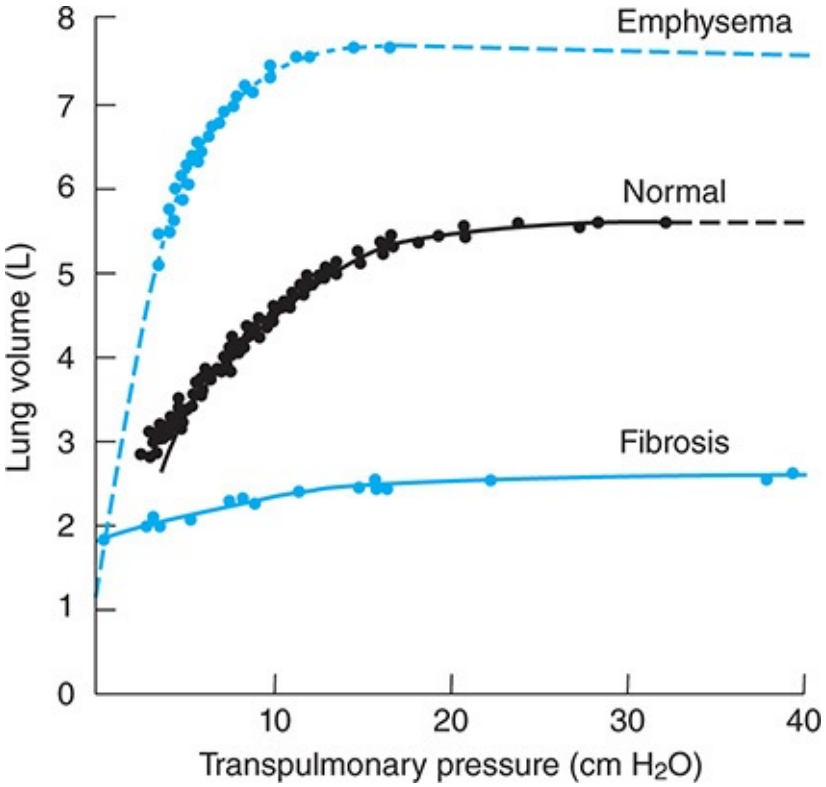
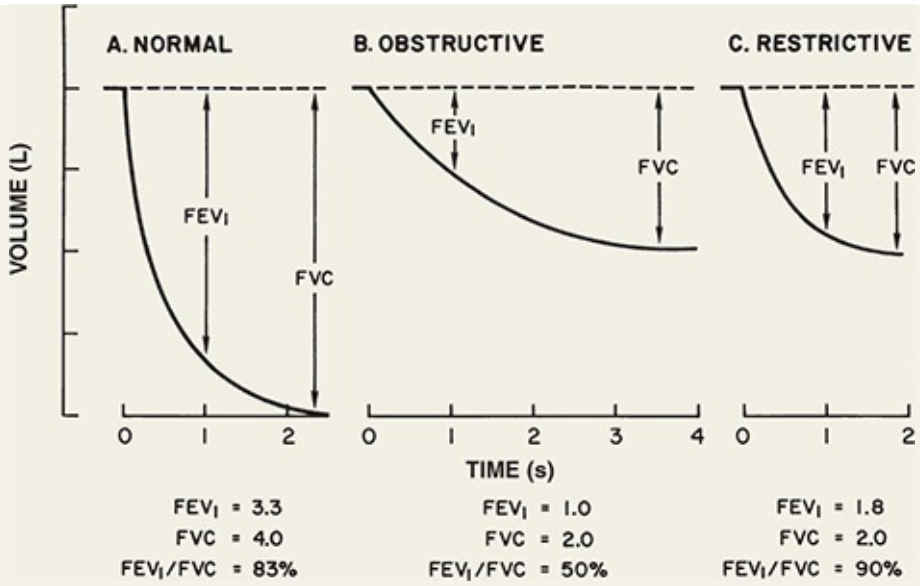


FIGURE 34–11 Static expiratory pressure–volume curves of lungs in healthy persons and persons with severe emphysema and pulmonary fibrosis. (Modified with permission from Pride NB, Macklem PT: Lung mechanics in disease. In: *Handbook of Physiology*. Section 3, *The Respiratory System*. Vol III, part 2. Fishman AP [editor]. American Physiological Society, 1986.)

CLINICAL BOX 34–2

Altered Airflow in Disease:



Representative lung volume expired over time in normal and diseased lungs during respiratory testing or pulmonary function test (PFT). A) Healthy normal subject. **B)** Patient with obstructive lung disease. **C)** Patient with restrictive lung disease. Note the differences in FEV_1, FVC and FEV_1/FVC at the bottom of the figure. FEV_1, forced expiratory volume in the first second; FVC, forced vital capacity. (Reproduced with permission from Fishman AP et al (eds): *Fishman's Pulmonary Diseases and Disorders*. 4th ed. New York: McGraw-Hill; 2008.)

Airflow Measurements of Obstructive & Restrictive Disease

In the example above, a healthy or normal FVC is ~4.0 L and a healthy FEV_1 is ~3.3 L. The calculated FEV_1/FVC is ~80%. Patients with obstructive or restrictive lung diseases can display reduced FVC, on the order of 2.0 L in the example above. Measurement of FEV_1, however, tends to vary significantly between the two diseases. In obstructive lung disorders, patients tend to show a slow, steady slope to the FVC, resulting in a small FEV_1, on the order of 1.0 L in the example. However, in the restrictive lung disorder, patient's airflow tends to be fast at first, and then quickly level out to approach FVC. The resultant FEV_1 is much greater, on the order of 1.8 L in the example, even though FVC is equivalent (compare B, C above). A quick calculation of FEV_1/FVC for patients with obstructive (50%) versus restrictive (90%) patterns defines the hallmark measurements in evaluating these two diseases. Obstructive disorders result in a marked decrease in both FVC and

FEV_1/FVC, whereas restrictive disorders result in a loss of FVC without loss in FEV_1/FVC. It should be noted that these examples are idealized and several disorders can show mixed readings.

Obstructive Disease—Asthma

Asthma is characterized by episodic or chronic wheezing, cough, and a feeling of tightness in the chest as a result of bronchoconstriction. Although the disease is not fully understood, three airway abnormalities are present: **airway obstruction** that is at least partially reversible, airway inflammation, and airway hyperresponsiveness to a variety of stimuli. A link to allergy has long been recognized, and plasma IgE levels are often elevated. Proteins released from eosinophils in the inflammatory reaction may damage the airway epithelium and contribute to the hyperresponsiveness. Leukotrienes are released from eosinophils and mast cells, and can enhance bronchoconstriction. Numerous other amines, neuropeptides, chemokines, and interleukins have effects on bronchial smooth muscle or produce inflammation, and they may be involved in asthma.

Restrictive Disease—Idiopathic Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (IPF), a type of pulmonary fibrosis, is a rare and irreversible interstitial lung disease that usually affects middle-aged and older adults (> 50 years old). The most common cause of death related to IPF is respiratory failure. Other causes of death include pulmonary hypertension (PH) and PH-associated right heart failure, pulmonary embolism and lung cancer. Up to 132,000 people in the United States are currently affected by IPF, and there are 30,000–40,000 new cases each year. Alveolar epithelial injury due to epithelial cell apoptosis and endoplasmic reticulum (ER) stress and epithelial-to-mesenchymal transition (EMT) have been implicated as cellular mechanisms involved in the development of IPF.

THERAPEUTIC HIGHLIGHTS

Asthma

Because β_2-adrenergic receptors mediate bronchodilation, β_2-adrenergic agonists have long been the mainstay of “rescue” treatment for mild to moderate asthma attacks. Inhaled steroids are used even in mild to moderate cases to reduce inflammation; they are very effective, but their side effects can be a problem. Agents that block synthesis of leukotrienes or their $CysLT_1$

receptor inhibit acetylcholine-mediated activation of muscarinic (M) receptors and blockade Cl^- channels and Ca^{2+} channels in mast cells have also proved useful in certain cases.

IPF

Clinical management and treatment have been focused on suppressing the inflammatory and fibrotic process of IPF. Agents with anti-fibrotic, anti-inflammation, and anti-oxidant effects (eg, nintedanib, pirfenidone, thalidomide) are often used for treatment of IPF. Lung transplantation (replacement of one or both lungs) is a surgical therapeutic intervention of proven benefits in IPF, which is often reserved for patients at the advanced stage of IPF; the 5-year survival rate is approximately 50% for lung transplant patients.

Airway Resistance

Airway resistance is defined as the change of pressure (ΔP) from the alveoli to the mouth divided by the change in flow rate ($\dot{V}$). Because of the structure of the bronchial tree, and thus the pathway for air that contributes to its resistance, it is difficult to apply mathematical estimates of the movement through the bronchial tree. However, measurements where alveolar and intrapleural pressure can be compared to actual pressure (eg, Figure 34–7 middle panel) show the contribution of airway resistance. Airway resistance is significantly increased as lung volume is reduced. Also, bronchi and bronchioles significantly contribute to airway resistance. Thus, contraction of the smooth muscle that lines the bronchial airways will increase airway resistance and make breathing more difficult.

Role of Surfactant in Alveolar Surface Tension

An important factor affecting the compliance of the lungs is the surface tension of the film of fluid that lines the alveoli. The magnitude of this component at various lung volumes can be measured by removing the lungs from the body of an experimental animal and distending them alternately with saline and with air while measuring the intrapulmonary pressure. Because saline reduces the surface tension to nearly zero, the pressure–volume curve obtained with saline measures

only the tissue elasticity **(Figure 34–12)**, whereas the curve obtained with air measures both tissue elasticity and surface tension. The difference between the saline and air curves is much smaller when lung volumes are small. Differences are also obvious in the curves generated during inflation and deflation. This difference is termed **hysteresis,** and notably is not present in the saline generated curves (Figure 34–12). The alveolar environment, and specifically the secreted factors that help reduce surface tension and keep alveoli from collapsing, contribute to hysteresis.

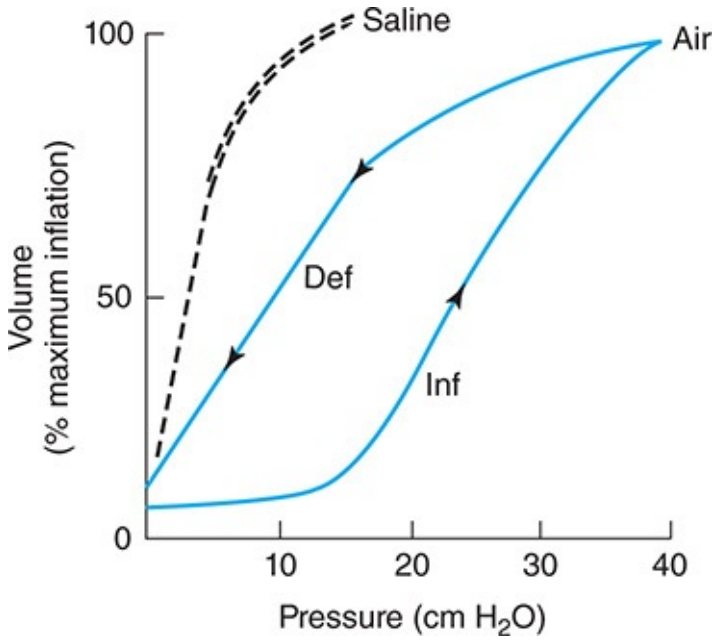


FIGURE 34–12 Pressure–volume curves in the lungs of a cat after removal from the body. Saline: lungs inflated and deflated with saline to reduce surface tension, resulting in a measurement of tissue elasticity. **Air:** lungs inflated (Inf) and deflated (Def) with air results in a measure of both tissue elasticity and surface tension. (Reproduced with permission from Morgan TE: Pulmonary surfactant. N Engl J Med 1971; May 27; 284(21):1185–1193.)

The low surface tension when the alveoli are small is due to the presence of **surfactant** in the fluid lining the alveoli. Surfactant is a mixture of dipalmitoylphosphatidylcholine (DPPC), other lipids, and proteins. If the surface tension is not kept low when the alveoli become smaller during expiration, they collapse in accordance with the law of Laplace. In spherical structures like an alveolus, the distending pressure (P) equals two times the tension (T) divided by the radius (r): P = 2T/r, if T is not reduced as r is reduced, the tension overcomes the distending pressure. Surfactant also helps prevent pulmonary edema. It has

been calculated that if it were not present, the unopposed surface tension in the alveoli would produce a 20 mm Hg force; such a force would greatly favor transudation of fluid from the blood into the alveoli.

Formation of the phospholipid film is greatly facilitated by the proteins in surfactant. This material contains four unique proteins: surfactant protein (SP)-A, SP-B, SP-C, and SP-D. SP-A is a large glycoprotein and has a collagen-like domain within its structure. It has multiple functions, including regulation of the feedback uptake of surfactant by the type II alveolar epithelial cells that secrete it. SP-B and SP-C are smaller proteins, which are the key protein members of the monomolecular film of surfactant. Like SP-A, SP-D is a glycoprotein. Its full function is uncertain; however, it plays an important role in the organization of SP-B and SP-C into the surfactant layer. Both SP-A and SP-D are members of the collectin family of proteins that are involved in innate immunity in the conducting airway as well as in the alveoli. Some clinical aspects of surfactant are discussed in **Clinical Box 34–3**.

WORK OF BREATHING

Work is performed by the respiratory muscles in stretching the elastic tissues of the chest wall and lungs (elastic work; approximately 65% of the total work), moving inelastic tissues (viscous resistance; 7% of total), and moving air through the respiratory passages (airway resistance; 28% of total). Because the dimensions of pressure × volume ($g/cm^2 \times cm^3 = g \times cm$) has the same dimensions as work (force × distance; g × cm), the work of breathing can be calculated from the previously presented pressure-volume curve (Figure 34–10). Note that the relaxation pressure curve of the total respiratory system (P_{TR}) differs from that of the lungs alone (P_L). The amount of elastic work required to inflate the whole respiratory system is less than the amount required to inflate the lungs alone because part of the work comes from elastic energy stored in the thorax.

CLINICAL BOX 34–3

Surfactant

Surfactant is important at birth. The fetus makes respiratory movements in utero, but the lungs remain collapsed until birth. After birth, the infant makes

several strong inspiratory movements and the lungs expand. Surfactant keeps them from collapsing again. Surfactant deficiency is an important cause of **infant respiratory distress syndrome** (IRDS, also known as **hyaline membrane disease**), the serious pulmonary disease that develops in infants born before their surfactant system is functional. Surface tension in the lungs of these infants is high, and the alveoli are collapsed in many areas **(atelectasis).** An additional factor in IRDS is retention of fluid in the lungs. During fetal life, Cl^- is secreted with fluid by the pulmonary epithelial cells. At birth, there is a shift to Na^+ absorption by these cells via the epithelial Na^+ channels (ENaCs), and fluid is absorbed with the Na^+. Prolonged immaturity of the ENaCs contributes to the pulmonary abnormalities in IRDS.

Overproduction/dysregulation of surfactant proteins can also lead to respiratory distress and is the cause of pulmonary alveolar proteinosis.

THERAPEUTIC HIGHLIGHTS

Treatment of IRDS is commonly done with surfactant replacement therapy. Interestingly, such surfactant replacement therapy has not been as successful in clinical trials for adults experiencing respiratory distress due to surfactant dysfunction.

Estimates of the total work of quiet breathing range from 0.3 to 0.8 kg-m/min. The value rises markedly during exercise, but the energy cost of breathing in normal individuals represents less than 3% of the total energy expenditure during exercise. The work of breathing is greatly increased in diseases such as emphysema, asthma, and heart failure with dyspnea and orthopnea. The respiratory muscles have length-tension relations like those of other skeletal and cardiac muscles, and when they are severely stretched, they contract with less strength. They can also become fatigued and fail (pump failure), leading to inadequate ventilation.

DIFFERENCES IN VENTILATION & BLOOD FLOW IN DIFFERENT PARTS OF THE LUNG

In the upright position, ventilation ($\dot{V}$) per unit lung volume is greater at the base of the lung than at the apex. The reason for this is that at the start of inspiration,

intrapleural pressure is less negative at the base than at the apex, and since the intrapulmonary intrapleural pressure difference is less than at the apex, the lung is less expanded. Conversely, at the apex, the lung is more expanded; that is, the percentage of maximum lung volume is greater. Because of the stiffness of the lung, the increase in lung volume per unit increase in pressure is smaller when the lung is initially more expanded, and ventilation is consequently greater at the base. Blood flow is also greater at the base than the apex. The relative change in blood flow from the apex to the base is greater than the relative change in ventilation, so the ventilation/perfusion ($\dot{V}/\dot{Q}$) ratio is low at the base and high at the apex.

The ventilation and perfusion differences from the apex to the base of the lungs have usually been attributed to gravity: they tend to disappear in the supine position, and the weight of the lungs would be expected to create pressure at the base in the upright position. However, the inequalities of ventilation and blood flow in human lungs were found to persist to a remarkable degree in the weightlessness of space. Therefore, other factors also play a role in producing the inequalities.

DEAD SPACE & UNEVEN VENTILATION

Because gaseous exchange in the respiratory system occurs only in the terminal portions of the airways, the gas that occupies the rest of the respiratory system is not available for gas exchange with pulmonary capillary blood. Normally, the volume (in mL) of this **anatomic dead space** is approximately equal to the body weight in pounds. As an example, in a man who weighs 150 lb (68 kg), only the first 350 mL of the 500 mL inspired with each breath at rest mixes with the air in the alveoli. Conversely, with each expiration, the first 150 mL expired is gas that occupied the dead space, and only the last 350 mL is gas from the alveoli. Consequently, the **alveolar ventilation,** that is, the amount of air reaching the alveoli per minute, is less than the RMV. Note that because of the dead space, rapid shallow breathing produces much less alveolar ventilation than slow deep breathing at the same RMV **(Table 34–1)**.

TABLE 34–1 Effect of variations in respiratory rate and depth on alveolar ventilation.

Respiratory rate	30/min	10/min
Tidal volume	200 mL	600 mL
Minute volume	6 L	6 L
Alveolar ventilation	(200 – 150) × 30 = 1500 mL	(600 – 150) × 10 = 4500 mL

It is important to distinguish between the **anatomic dead space** (respiratory system volume exclusive of alveoli) and the **total (physiological) dead space** (volume of gas not equilibrating with blood; ie, wasted ventilation). In healthy individuals, the two dead spaces are identical and can be estimated by body weight. However, in disease states, no exchange may take place between the gas in some of the alveoli and the blood, and some of the alveoli may be overventilated. The volume of gas in nonperfused alveoli and any volume of air in the alveoli in excess of that necessary to oxygenate the blood in the alveolar capillaries is part of the dead space (nonequilibrating) gas volume. The anatomic dead space can be measured by analysis of the single-breath N_2 curve **(Figure 34–13)**. From mid-inspiration, the patient takes as deep a breath as possible of pure O_2, then exhales steadily while the N_2 content of the expired gas is continuously measured. The initial gas exhaled (phase I) is the gas that filled the dead space and that consequently contains no N_2. This is followed by a mixture of dead space and alveolar gas (phase II) and then by alveolar gas (phase III). The volume of the dead space is the volume of the gas expired from peak inspiration to the midportion of phase II.

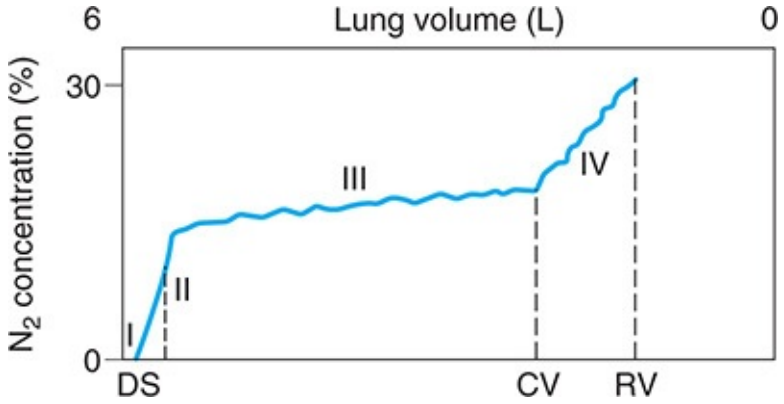


FIGURE 34–13 Single-breath N_2 curve. From mid-inspiration, the patient takes a deep breath of pure O_2 then exhales steadily. The changes in the N_2

concentration of expired gas during expiration are shown, with the various phases of the curve indicated by roman numerals. Notably, region I is representative of the dead space (DS); from I–II is a mixture of DS and alveolar gas; the transition from III–IV is the closing volume (CV), and the end of IV is the residual volume (RV).

Phase III of the single-breath N_2 curve terminates at the **closing volume (CV)** and is followed by phase IV, during which the N_2 content of the expired gas is increased. The CV is the lung volume above RV at which airways in the lower, dependent parts of the lungs begin to close off because of the lesser transmural pressure in these areas. The gas in the upper portions of the lungs is richer in N_2 than the gas in the lower, dependent portions because the alveoli in the upper portions are more distended at the start of the inspiration of O_2 and, consequently, the N_2 in them is less diluted with O_2. It is also worth noting that in most normal individuals, phase III has a slight positive slope even before phase IV is reached. This indicates that even during phase III there is a gradual increase in the proportion of the expired gas coming from the relatively N_2-rich upper portions of the lungs.

The total dead space can be calculated from the P_{CO_2} of expired air, the P_{CO_2} of arterial blood, and the TV. The tidal volume (V_T) times the P_{CO_2} of the expired gas (P_{ECO_2}) equals the arterial P_{CO_2} (Pa_{CO_2}) times the difference between the TV and the dead space (V_D) plus the P_{CO_2} of inspired air (P_{ICO_2}) times V_D **(Bohr equation):**

$$P_{ECO_2} \times V_T = Pa_{CO_2} \times (V_T - V_D) + P_{ICO_2} \times V_D$$

The term $P_{ICO_2} \times V_D$ is so small that it can be ignored and the equation solved for V_D, where $V_D = V_T - (P_{ECO_2} \times V_T)/(Pa_{CO_2})$

If, for example: P_{ECO_2} = 28 mm Hg; Pa_{CO_2} = 40 mm Hg; and V_T = 500 mL, then V_D = 150 mL.

The equation can also be used to measure the anatomic dead space if one replaces Pa_{CO_2} with alveolar P_{CO_2} (P_{ACO_2}), which is the P_{CO_2} of the last 10 mL of expired gas. P_{CO_2} is an average of gas from different alveoli in proportion to their ventilation regardless of whether they are perfused. This is in contrast to Pa_{CO_2}, which is gas equilibrated only with perfused alveoli, and consequently, in individuals with underperfused alveoli, is greater than P_{ACO_2}.

GAS EXCHANGE IN THE LUNGS

PARTIAL PRESSURES

Unlike liquids, gases expand to fill the volume available to them, and the volume occupied by a given number of gas molecules at a given temperature and pressure is (ideally) the same regardless of the composition of the gas. **Partial pressures** are frequently used to describe gases in respiration. The pressure of a gas is proportional to its temperature and number of moles occupying a certain volume **(Table 34–2)**. The pressure exerted by any one gas in a mixture of gases (its partial pressure) is equal to the total pressure times the fraction of the total amount of gas it represents.

TABLE 34–2 Properties of gases.

$P = \frac{nRT}{V}$ (from equation of state of ideal gas)

The pressure (P) of a gas is proportional to its temperature (T) and the number of moles (n) per volume (V); R, Gas constant.

The composition of dry air, or the fraction (F) of gas molecules in the dry air, is 20.98% O_2 (FO_2), 0.04% CO_2 (FCO_2), 78.06% N_2 (FN_2), and 0.92% other inert constituents such as argon and helium. The barometric pressure (P_B) at sea level is 760 mm Hg (1 atmosphere). The partial pressure (indicated by the symbol P) of O_2 (ie, P_{O_2}) in dry air is therefore 0.21 × 760, or 160 mm Hg at sea level. The P_{N_2} and the other inert gases is 0.79 × 760, or 600 mm Hg; and the P_{CO_2} is 0.0004 × 760, or 0.3 mm Hg. The water vapor in the air in most climates reduces these percentages, and therefore the partial pressures, to a slight degree. Air equilibrated with water is saturated with water vapor, and inspired air is saturated by the time it reaches the lungs. The P_{H_2O} at body temperature (37°C) is 47 mm Hg. Therefore, the partial pressures at sea level of the other gases in the air reaching the lungs are P_{O_2}, 150 mm Hg; P_{CO_2}, 0.3 mm Hg; and P_{N_2} (including the other inert gases), 563 mm Hg.

Gas diffuses from areas of high partial pressure to areas of low partial pressure, with the rate of diffusion depending on the concentration gradient and the nature of the barrier between the two areas. When a mixture of gases is in

contact with, and permitted to, equilibrate with a liquid, each gas in the mixture dissolves in the liquid to an extent determined by its partial pressure and its solubility in the fluid. The partial pressure of a gas in a liquid is the pressure that, in the gaseous phase in equilibrium with the liquid, would produce the concentration of gas molecules found in the liquid.

SAMPLING ALVEOLAR AIR

Theoretically, all but the first 150 mL expired from a healthy 150 lb man (ie, the dead space) with each expiration is the gas that was in the alveoli **(alveolar air),** but some mixing always occurs at the interface between the dead-space gas and the alveolar air (Figure 34–13). A later portion of expired air is therefore the portion taken for analysis. Using modern apparatus with a suitable automatic valve, it is possible to collect the last 10 mL expired gas during quiet breathing. The composition of alveolar gas is compared with that of inspired and expired air in **Figure 34–14**.

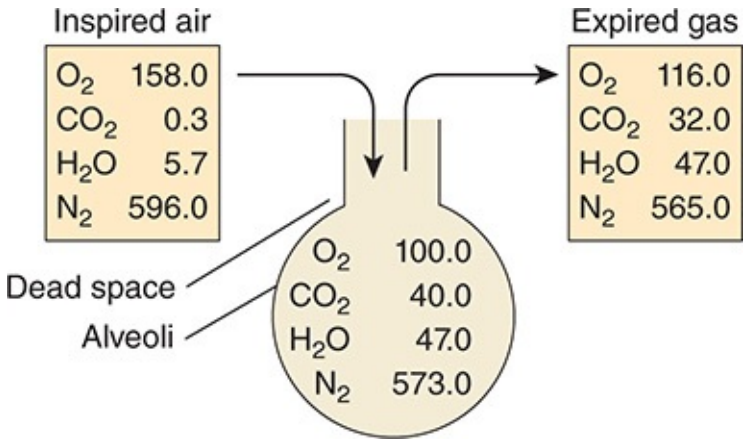


FIGURE 34–14 Partial pressures of gases (mm Hg) in various parts of the respiratory system. Typical partial pressures for inspired air, alveolar air, and expired air are given. See text for additional details.

P_{AO_2} can also be calculated from the **alveolar gas equation:**

$$P_{AO_2} = P_{IO_2} - P_{ACO_2}\left(F_{IO_2} + \frac{1 - F_{IO_2}}{R}\right)$$

where F_{IO_2} is the fraction of O_2 molecules in the inspired (I) dry gas, P_{IO_2} is the

inspired P_{O_2}, and R is the respiratory exchange ratio; that is, the flow of CO_2 molecules across the alveolar membrane per minute divided by the flow of O_2 molecules across the membrane per minute.

COMPOSITION OF ALVEOLAR AIR

Oxygen continuously diffuses out of the gas in the alveoli into the bloodstream, and CO_2 continuously diffuses into the alveoli from the blood. In the steady state, inspired air mixes with the alveolar gas, replacing the O_2 that has entered the blood and diluting the CO_2 that has entered the alveoli. Part of this mixture is expired. The O_2 content of the alveolar gas then falls and its CO_2 content rises until the next inspiration. Because the volume of gas in the alveoli is about 2 L at the end of expiration (FRC), each 350 mL increment of inspired and expired air has relatively little effect on P_{O_2} and P_{CO_2}. Indeed, the composition of alveolar gas remains remarkably constant, not only at rest but also under a variety of other conditions.

DIFFUSION ACROSS THE ALVEOLOCAPILLARY MEMBRANE

Gases diffuse from the alveoli to the blood in the pulmonary capillaries or vice versa across the thin alveolocapillary membrane made up of the pulmonary epithelium, the capillary endothelium, and their fused basement membranes (Figure 34–3). Whether or not substances passing from the alveoli to the capillary blood reach equilibrium in the 0.75 s that blood takes to traverse the pulmonary capillaries at rest depends on their reaction with substances in the blood. Thus, for example, the anesthetic gas nitrous oxide (N_2O) does not react and reaches equilibrium in about 0.1 s **(Figure 34–15)**. In this situation, the amount of N_2O taken up is not limited by diffusion but by the amount of blood flowing through the pulmonary capillaries; that is, it is **flow-limited.** On the other hand, carbon monoxide (CO) is taken up by hemoglobin in the red blood cells at such a high rate that the partial pressure of CO in the capillaries stays very low and equilibrium is not reached in the 0.75 s the blood is in the pulmonary capillaries. Therefore, the transfer of CO is not limited by perfusion at rest and instead is **diffusion-limited.** O_2 is intermediate between N_2O and CO;

it is taken up by hemoglobin, but much less avidly than CO, and it reaches equilibrium with capillary blood in about 0.3 s. Thus, its uptake is **perfusion-limited.**

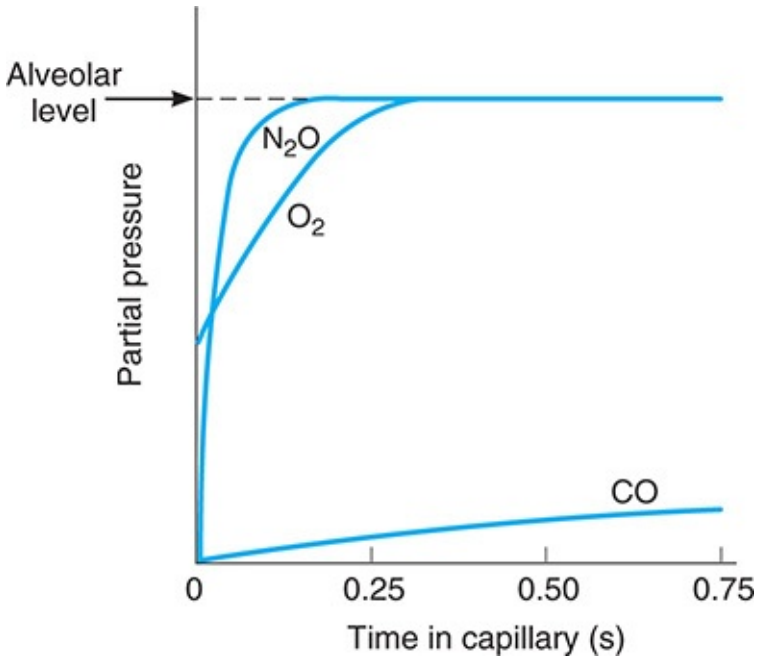


FIGURE 34–15 Uptake of various substances during the 0.75 s they are in transit through a pulmonary capillary. N_2O is not bound in blood, so its partial pressure in blood rises rapidly to its partial pressure in the alveoli. Conversely, CO is avidly taken up by red blood cells, so its partial pressure reaches only a fraction of its partial pressure in the alveoli. O_2 is intermediate between the two.

The **diffusing capacity** of the lungs for a given gas is directly proportional to the surface area of the alveolo-capillary membrane and inversely proportional to its thickness. The diffusing capacity for CO (D_{LCO}) is measured as an index of diffusing capacity because its uptake is diffusion-limited. D_{LCO} is proportional to the amount of CO entering the blood ($\dot{V}_{CO}$) divided by the partial pressure of CO in the alveoli minus the partial pressure of CO in the blood entering the pulmonary capillaries. Except in habitual cigarette smokers, this latter term is close to zero, so it can be ignored and the equation becomes:

$$D_{LCO} = \frac{\dot{V}_{CO}}{P_{ACO}}$$

The normal value of D_{LCO} at rest is about 25 mL/min/mm Hg. It increases up to threefold during exercise because of capillary dilation and an increase in the number of active capillaries. The P_{O_2} of alveolar air is normally 100 mm Hg, and the P_{O_2} of the blood entering the pulmonary capillaries is 40 mm Hg. The diffusing capacity for O_2, like that for CO at rest, is about 25 mL/min/mm Hg, and the P_{O_2} of blood is raised to 97 mm Hg, a value just under the alveolar P_{O_2}.

The P_{CO_2} of venous blood is 46 mm Hg, whereas that of alveolar air is 40 mm Hg, and CO_2 diffuses from the blood into the alveoli along this gradient. The P_{CO_2} of blood leaving the lungs is 40 mm Hg. CO_2 passes through all biological membranes with ease, and the diffusing capacity of the lungs for CO_2 is much greater than the capacity for O_2. It is for this reason that CO_2 retention is rarely a problem in patients with alveolar fibrosis even when the reduction in diffusing capacity for O_2 is severe.

PULMONARY CIRCULATION

PULMONARY BLOOD VESSELS

The pulmonary vascular bed resembles the systemic one, except that the walls of the pulmonary artery and its large branches are about 30% as thick as the wall of the aorta, and the small arterial vessels, unlike the systemic arterioles, contain relatively little smooth muscle in their walls. The walls of the pulmonary venous vessels also contain some smooth muscle. The pulmonary capillaries are large, and there are multiple anastomoses, so that each alveolus sits in a capillary basket.

PRESSURE, VOLUME, & FLOW

With two quantitatively minor exceptions, the blood put out by the left ventricle returns to the right atrium and is ejected by the right ventricle, making the pulmonary vasculature unique in that it accommodates a blood flow that is almost equal to that of all the other organs in the body.

One of the exceptions is part of the bronchial blood flow. There are anastomoses between the bronchial capillaries and the pulmonary capillaries and veins, and although some of the bronchial blood enters the bronchial veins, some enters the pulmonary capillaries and veins, bypassing the right ventricle. The

other exception is blood that flows from the coronary arteries into the chambers of the left side of the heart. Because of the small **physiological shunt** created by those two exceptions, the blood in systemic arteries has a P_{O_2} about 2 mm Hg lower than that of blood that has equilibrated with alveolar air, and the saturation of hemoglobin is 0.5% less.

The pressure in the various parts of the pulmonary portion of the pulmonary circulation is shown in Figure 34–6C. The pressure gradient in the pulmonary circulation system is about 7 mm Hg, compared with a gradient of about 90 mm Hg in the systemic circulation. Pulmonary capillary pressure is about 10 mm Hg, whereas the oncotic pressure is 25 mm Hg, so that an inward-directed pressure gradient of about 15 mm Hg keeps the alveoli free of all but a thin film of fluid. When the pulmonary capillary pressure is more than 25 mm Hg, pulmonary congestion and edema result.

The volume of blood in the pulmonary vessels at any one time is about 1 L, of which less than 100 mL is in the capillaries. The mean velocity of the blood in the root of the pulmonary artery is the same as that in the aorta (about 40 cm/s). It falls off rapidly, then rises slightly again in the larger pulmonary veins. It takes a red cell about 0.75 s to traverse the pulmonary capillaries at rest and 0.3 s or less during exercise.

EFFECT OF GRAVITY

Gravity has a relatively marked effect on the pulmonary circulation. In the upright position, the upper portions of the lungs are well above the level of the heart, and the bases are at or below it. Consequently, in the upper part of the lungs, the blood flow is less, the alveoli are larger, and ventilation is less than at the base **(Figure 34–16)**. The pressure in the capillaries at the top of the lungs is close to the atmospheric pressure in the alveoli. Pulmonary arterial pressure is normally just sufficient to maintain perfusion, but if it is reduced or if alveolar pressure is increased, some of the capillaries collapse. Under these circumstances, no gas exchange takes place in the affected alveoli and they become part of the physiological dead space.

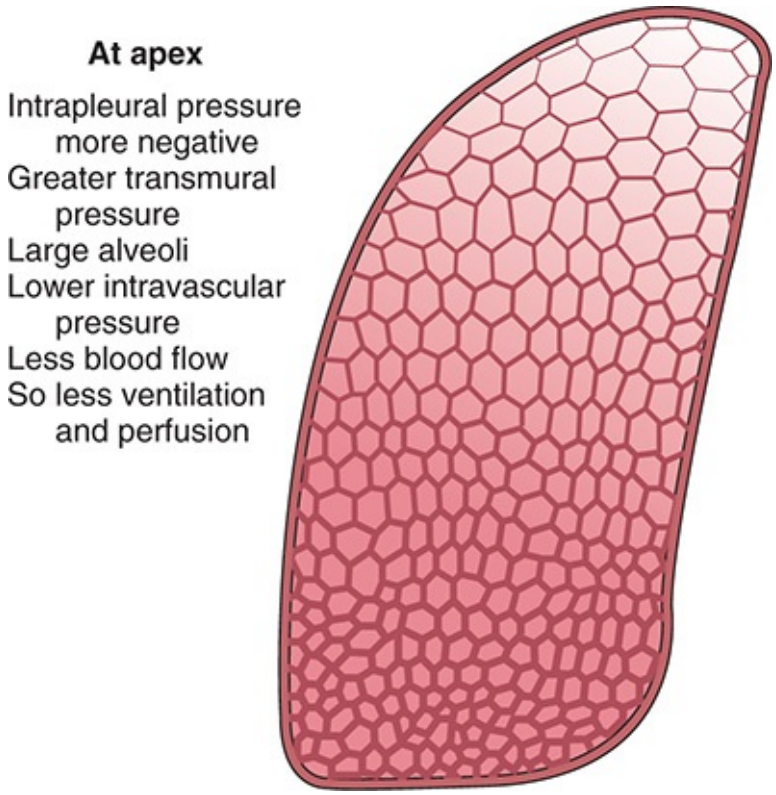


FIGURE 34–16 Diagram of normal differences in ventilation and perfusion of the lung in the upright position. Outlined areas are representative of changes in alveolar size (not actual size). Note the gradual change in alveolar size from top (apex) to bottom. Characteristic differences of alveoli at the apex of the lung are stated. (Modified with permission from Levitzky MG: *Pulmonary Physiology*, 6th ed. New York, NY: McGraw-Hill; 2003.)

In the middle portions of the lungs, the pulmonary arterial pressure and capillary pressure exceed alveolar pressure, but the pressure in the pulmonary venules may be lower than alveolar pressure during normal expiration, so they are collapsed. Under these circumstances, blood flow is determined by the difference between pulmonary arterial pressure and alveolar pressure rather than the difference between pulmonary arterial pressure and pulmonary venous pressure. Beyond the constriction, blood "falls" into the pulmonary veins, which are compliant and take whatever amount of blood the constriction lets flow into them. This has been called the **waterfall effect.** Obviously, the compression of vessels produced by alveolar pressure decreases and pulmonary blood flow increases as the arterial pressure increases toward the base of the lungs. In the lower portions of the lungs, alveolar pressure is lower than the pressure in all parts of the pulmonary circulation and blood flow is determined by the arterial-

venous pressure difference. Examples of diseases affecting the pulmonary circulation are given in **Clinical Box 34–4**.

VENTILATION/PERFUSION RATIOS

The ratio of pulmonary ventilation ($\dot{V}$) to pulmonary blood flow or perfusion ($\dot{Q}$) for the whole lungs at rest is about 0.8 (4.2 L/min ventilation divided by 5.5 L/min blood flow). However, relatively marked differences occur in this **ventilation/perfusion Q($\dot{V}/\dot{Q}$) ratio** in various parts of the normal lungs as a result of the effect of gravity, and local changes in the ventilation/perfusion ratio are common in disease. If the ventilation to an alveolus is reduced relative to its perfusion, the P_{O_2} in the alveolus falls because less O_2 is delivered to it and the P_{CO_2} rises because less CO_2 is expired. Conversely, if perfusion is reduced relative to ventilation, the P_{CO_2} falls because less CO_2 is delivered and the P_{O_2} rises because less O_2 enters the blood.

As noted above, ventilation, as well as perfusion in the upright position, declines in a linear fashion from the bases to the apices of the lungs. However, the ventilation/perfusion ratios are high in the upper portions of the lungs. When widespread, nonuniformity of ventilation and perfusion in the lungs can cause CO_2 retention and lowers systemic arterial P_{O_2}.

CLINICAL BOX 34–4

Diseases Affecting the Pulmonary Circulation

Pulmonary Hypertension

Pulmonary hypertension (PH) is classified into five groups: pulmonary arterial hypertension (PAH; including persistent PH of the newborn), PH associated with left heart disease, PH due to lung diseases and/or hypoxia, chronic thromboembolic pulmonary hypertension (CTEPH), and PH with unclear multifactorial mechanisms. PH, as a syndrome with multiple causes, can occur at any age, but idiopathic PAH (a fatal and progressive form of PAH) predominantly affects young women. The causes for PH or PAH are different from those causing systemic arterial hypertension. For example, acute hypoxia causes pulmonary vasoconstriction and chronic hypoxia causes pulmonary hypertension, whereas hypoxia causes systemic vasodilation.

Other unique causes for PH include inhalation of cocaine, treatment with dexfenfluramine and related appetite-suppressing drugs, HIV/HPV infection, systemic lupus erythematosus and scleroderma, and mutations of the genes encoding the bone morphogenetic protein receptor II (BMPRII), the pH-sensitive two-pore-domain K^+ channel (KCNK3), the serotonin transporter (SERT), the activin receptor kinase-like 1 (ALK1), the transient receptor potential canonical channel 6 (TRPC6), the glycoprotein endoglin (ENG), and the scaffolding protein caveolin 1 (CAV1). Sustained pulmonary vasoconstriction, concentric pulmonary vascular remodeling, in situ pulmonary thrombosis, and increased pulmonary vascular wall stiffness all contribute to the increased pulmonary vascular resistance and pulmonary arterial pressure in patients with PH or PAH. If appropriate therapy is not initiated, the increased right ventricular afterload can lead eventually to right heart failure and death.

THERAPEUTIC HIGHLIGHTS

Treatment with vasodilators and antiproliferative agents, such as continuous intravenous infusion of prostacyclin (Flolan) and oral intake or inhalation of prostacyclin analogs (eg, beroprost, ilorost, treprostinil), is effective for patients with severe PH (eg, idiopathic, heritable and associated PAH). Conventional treatment includes diuretics, oxygen therapy, anticoagulation, digoxin, and exercise. Inhaled nitric oxide (NO) is effective for many pediatric patients. Advanced treatment includes voltage-gated Ca^{2+} channel blockers (eg, nifedipine, diltiazem, verapamil, amlodipine), prostanoids (eg, epoprostenol/PGI_2, treprostinil, iloprost), endothelin receptor antagonists (eg, bosentan, ambrisentan), phosphodiesterase (PDE) inhibitors (eg, sildenafil/Viagra, tadalafil/Cialis), and combination therapy. Surgical treatment includes atrial septostomy, whole lung transplantation, living-donor lobar lung transplantation, pulmonary endarterectomy, and pulmonary arterioplasty.

A unique mechanism that ensures the optimal ventilation/perfusion ratio in the lungs is hypoxic pulmonary vasoconstriction (HPV). HPV is a homeostatic mechanism that, in the adult men, diverts desaturated, mixed venous blood in the pulmonary artery from poorly ventilated (hypoxic) areas of the lungs to other better ventilated (normoxic) areas of the lungs. The alveolar hypoxia-induced vasoconstriction helps maintain an optimal ventilation/perfusion ratio and

maximize the oxygenation of the mixed venous blood in the lungs.

REGULATION OF PULMONARY BLOOD FLOW

It is unsettled whether pulmonary veins and pulmonary arteries are regulated separately, although constriction of the veins increases pulmonary capillary pressure and constriction of pulmonary arteries increases the load on the right side of the heart.

Pulmonary blood flow is affected by both active and passive factors. There is an extensive autonomic innervation of the pulmonary vessels, and stimulation of the cervical sympathetic ganglia reduces pulmonary blood flow by as much as 30%. The vessels also respond to circulating humoral agents. A diversity of the receptors involved in the regulation of pulmonary vasomotor tone and their effect on pulmonary smooth muscle are summarized in **Table 34–3**. Many of the dilator responses are endothelium-dependent and presumably operate via release of nitric oxide (NO).

TABLE 34–3 Receptors affecting smooth muscle in pulmonary arteries and veins.

Receptor	Subtype	Response	Endothelium Dependency
Autonomic			
Adrenergic	α_1	Contraction	No
	α_2	Relaxation	Yes
	β_2	Relaxation	Yes
Muscarinic	M_3	Relaxation	Yes
Purinergic	P2X	Contraction	No
	P2Y	Relaxation	Yes
Tachykinin	NK_1	Relaxation	Yes
	NK_2	Contraction	No
VIP	?	Relaxation	?
CGRP	?	Relaxation	No
Humoral			
Adenosine	A_1	Contraction	No
	A_2	Relaxation	No
Angiotensin II	AT_1	Contraction	No
ANP	ANP_A	Relaxation	No
	ANP_B	Relaxation	No
Bradykinin	B_1?	Relaxation	Yes
	B_2	Relaxation	Yes
Endothelin	ET_A	Contraction	No
	ET_B	Relaxation	Yes
Histamine	H_1	Relaxation	Yes
	H_2	Relaxation	No
5-HT	5-HT_1	Contraction	No
	5-HT_1C	Relaxation	Yes
Prostacyclin (PGI_2)	IP	Relaxation	No
Thromboxane	TP	Contraction	No
Vasopressin	V_1	Relaxation	Yes

Modified and reproduced with permission from Barnes PJ, Lin SF: Regulation of pulmonary vascular tone. Pharmacol Rev 1995;47:88.

Passive factors such as cardiac output and gravitational forces also have significant effects on pulmonary blood flow. Local adjustments of perfusion to

ventilation occur with local changes in O_2. With exercise, cardiac output increases and pulmonary arterial pressure rises. More red cells move through the lungs without any reduction in the O_2 saturation of the hemoglobin in them, and consequently, the total amount of O_2 delivered to the systemic circulation is increased. Capillaries dilate (also referred as capillary extension), and previously underperfused capillaries are "recruited" (also referred to as capillary recruitment) to carry blood. The net effect is a marked increase in pulmonary blood flow with few, if any, alterations in autonomic outflow to the pulmonary vessels.

When a bronchus or a bronchiole is obstructed, hypoxia develops in the underventilated alveoli beyond the obstruction. The O_2 reduction apparently acts directly on vascular smooth muscle in the area to produce constriction (ie, HPV), shunting blood away from the hypoxic area. Accumulation of CO_2 leads to a drop in pH in the area, and a decline in pH also produces vasoconstriction in the lungs, as opposed to the vasodilation it produces in other tissues. Conversely, reduction of the blood flow to a portion of the lung lowers the alveolar P_{CO_2} in that area, and this leads to constriction of the bronchi supplying it, shifting ventilation away from the poorly perfused area. Systemic hypoxia also causes the pulmonary arterioles to constrict, with a resultant increase in pulmonary arterial pressure.

METABOLIC & ENDOCRINE FUNCTIONS OF THE LUNGS

In addition to their functions in gas exchange, the lungs have a number of metabolic functions. They manufacture surfactant for local use, as noted above. They also contain a fibrinolytic system that lyses clots in the pulmonary vessels. They release a variety of substances that enter the systemic arterial blood **(Table 34–4)**, and they remove other substances from the systemic venous blood that reach them via the pulmonary artery. Prostaglandins are removed from the circulation, but they are also synthesized in the lungs and released into the blood when lung tissue is stretched.

TABLE 34–4 Biologically active substances metabolized by the lungs.

Synthesized and used in the lungs
Surfactant

Synthesized or stored and released into the blood
Prostaglandins Histamine Kallikrein
Partially removed from the blood
Prostaglandins Bradykinin Adenine nucleotides Serotonin Norepinephrine Acetylcholine
Activated in the lungs
Angiotensin II (from angiotensin I by the angiotensin-converting enzyme, ACE)

The lungs play an important role in activating angiotensin. The physiologically inactive decapeptide angiotensin I is converted to the pressor, aldosterone-stimulating octapeptide angiotensin II in the pulmonary circulation. The reaction occurs in other tissues as well, but it is particularly prominent in the lungs. Large amounts of the angiotensin-converting enzyme (ACE) responsible for this activation are located on the surface of the endothelial cells of the pulmonary capillaries. The converting enzyme also inactivates bradykinin, an endothelium-dependent vasodilator. Circulation time through the pulmonary capillaries is less than 1 s, yet 70% of the angiotensin I reaching the lungs is converted to angiotensin II in a single trip through the capillaries. Four other peptidases have been identified on the surface of the pulmonary endothelial cells, but their full physiological role is unsettled.

Removal of serotonin and norepinephrine reduces the amounts of these vasoactive substances reaching the systemic circulation. However, many other vasoactive hormones pass through the lungs without being metabolized. These include epinephrine, dopamine, oxytocin, vasopressin, and angiotensin II. In addition, various amines and polypeptides are secreted by neuroendocrine cells

in the lungs.

CHAPTER SUMMARY

- Air enters the respiratory system in the upper airway, proceeds to the conducting airway and then on to the respiratory airway that ends in the alveoli. The cross-sectional area of the airway gradually increases through the conducting zone, and then rapidly increases during the transition from conducting to respiratory zones.
- The mucociliary escalator in the conducting airway helps keep particulates out of the respiratory zone.
- There are several important measures of lung volume, including tidal volume (TV), inspiratory reserve volume (IRV), expiratory reserve volume (ERV), forced vital capacity (FVC), the forced expiratory volume in first second (FEV_1), respiratory minute volume (RMV), and maximal voluntary ventilation.
- Net "driving pressure" for air movement into the lungs includes the force of muscle contraction, lung compliance ($\Delta P/\Delta V$), and airway resistance ($\Delta P/\Delta$ .V).
- Surfactant decreases surface tension in the alveoli and helps keep them from deflating.
- Not all air that enters the airway is available for gas exchange. The regions where gas is not exchanged in the airway are termed "dead space." The conducting airway represents anatomic dead space. Increased dead space can occur in response to disease that affects air exchange in the respiratory zone (physiological dead space).
- The pressure gradient in the pulmonary circulation system is much less than that in the systemic circulation.
- Hypoxic pulmonary vasoconstriction is a unique mechanism that directs the mixed venous blood from poorly ventilated (hypoxic) areas of the lungs to well (normoxic) ventilated areas to assure maximal oxygenation of the venous blood.
- There are a variety of biologically activated substances that are metabolized in the lungs. These include substances that are made and function in the lungs (eg, surfactant), substances that are released or removed from the blood (eg, prostaglandins), and substances that are activated as they pass

through the lungs (eg, angiotensin II).

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. On the summit of Mt. Everest, where the barometric pressure is about 250 mm Hg, the partial pressure of O_2 in mm Hg is about

   A. 0.1.
   B. 0.5.
   C. 5.
   D. 50.
   E. 100.

2. The forced vital capacity is

   A. the amount of air that normally moves into (or out of) the lungs with each respiration.
   B. the amount of air that enters the lungs but does not participate in gas exchange.
   C. the amount of air expired after maximal expiratory effort.
   D. the largest amount of gas that can be moved into and out of the lungs in 1 min.

3. The tidal volume is

   A. the amount of air that normally moves into (or out of) the lungs with each respiration.
   B. the amount of air that enters the lungs but does not participate in gas exchange.
   C. the amount of air expired after maximal expiratory effort.
   D. the amount of gas that can be moved into and out of the lungs in 1 min.

4. Which of the following is responsible for the movement of O_2 from the alveoli into the blood in the pulmonary capillaries?

   A. Active transport
   B. Filtration
   C. Secondary active transport
   D. Facilitated diffusion
   E. Passive diffusion

5. Airway resistance
   A. is increased if the lungs are removed and inflated with saline.
   B. does not affect the work of breathing.
   C. is increased in paraplegic patients.
   D. is increased following bronchial smooth muscle contraction.
   E. makes up 80% of the work of breathing.
6. Surfactant lining the alveoli
   A. helps prevent alveolar collapse.
   B. is produced in alveolar type I cells and secreted into the alveolus.
   C. is increased in the lungs of heavy smokers.
   D. is a glycolipid complex.

CHAPTER 35

Gas Transport & pH

OBJECTIVES

After studying this chapter, you should be able to:

- Describe the manner in which O_2 flows "downhill" from the lungs to the tissues and CO_2 flows "downhill" from the tissues to the lungs.
- List the important factors affecting the affinity of hemoglobin for O_2 and the physiological significance of each.
- List the reactions that increase the amount of CO_2 in the blood and draw the CO_2 dissociation curve for arterial and venous blood.
- Define alkalosis and acidosis and list typical causes and compensatory responses to each.
- Define hypoxia and describe differences in subtypes of hypoxia.
- Describe the effects of hypercapnia and hypocapnia and give examples of conditions that can cause them.

INTRODUCTION

The concentrations for O_2 and CO_2 (measured as partial pressures, or P_{O_2} and

P_{CO_2}) change within each region of the lung, allowing for these gases to flow "downhill" or from higher partial pressures to lower partial pressures. For example, P_{O_2} is highest in the alveoli upon inspiration and lowest in the deoxygenated blood, whereas, P_{CO_2} is exactly opposite. This allows for O_2 to cross the alveoli and reoxygenate the blood in the pulmonary vasculature, while CO_2 leaves the bloodstream and enters the alveoli where it is expired. However, the amount of both of these gases transported to and from the tissues would be grossly inadequate if it were not for the fact that about 99% of the O_2 that dissolves in the blood combines with the O_2-carrying protein hemoglobin and that about 94.5% of the CO_2 that dissolves enters into a series of reversible chemical reactions that convert it into other compounds. Thus, the presence of hemoglobin increases the O_2-carrying capacity of the blood 70-fold, and the reactions of CO_2 increase the blood CO_2 content 17-fold. In this chapter, physiological details that underlie O_2 and CO_2 movement under various conditions are discussed.

OXYGEN TRANSPORT

OXYGEN DELIVERY TO THE TISSUES

Oxygen delivery, or by definition, the volume of oxygen delivered to the systemic vascular bed per minute, is the product of the cardiac output and the arterial oxygen concentration. The ability to deliver O_2 in the body depends on both the respiratory and the cardiovascular systems. O_2 delivery to a particular tissue depends on the amount of O_2 entering the lungs, the adequacy of pulmonary gas exchange, the blood flow to the tissue, and the capacity of the blood to carry O_2. Blood flow to an individual tissue depends on cardiac output and the degree of constriction of the vascular bed in the tissue. The amount of O_2 in the blood is determined by the amount of dissolved O_2, the amount of hemoglobin in the blood, and the affinity of the hemoglobin for O_2.

REACTION OF HEMOGLOBIN & OXYGEN

The dynamics of the reaction of hemoglobin with O_2 make it a particularly

suitable O_2 carrier. Hemoglobin is a protein made up of four subunits, each of which contains a **heme** moiety attached to a polypeptide chain. In normal adults, most of the hemoglobin molecules contain two α and two β chains. Heme (see Figure 31–7) is a porphyrin ring complex that includes one atom of ferrous iron. Each of the four iron atoms in hemoglobin can reversibly bind one O_2 molecule. The iron stays in the ferrous state, so that the reaction is **oxygenation** (not oxidation). It has been customary to write the reaction of hemoglobin with O_2 as $Hb + O_2 \rightleftarrows HbO_2$. Because it contains four deoxyhemoglobin (Hb) units, the hemoglobin molecule can also be represented as Hb_4, and it actually reacts with four molecules of O_2 to form Hb_4O_8.

$$Hb_4 + O_2 \rightleftarrows Hb_4O_2$$
$$Hb_4O_2 + O_2 \rightleftarrows Hb_4O_4$$
$$Hb_4O_4 + O_2 \rightleftarrows Hb_4O_6$$
$$Hb_4O_6 + O_2 \rightleftarrows Hb_4O_8$$

The reaction is rapid, requiring less than 0.01 s. The deoxygenation of Hb_4O_8 is also very rapid.

The quaternary structure of hemoglobin determines its affinity for O_2. In deoxyhemoglobin, the globin units are tightly bound in a **tense (T) configuration,** which reduces the affinity of the molecule for O_2. When O_2 is first bound, the bonds holding the globin units are released, producing a **relaxed (R) configuration,** which exposes more O_2-binding sites. The net result is a 500-fold increase in O_2 affinity. In tissues, these reactions are reversed, resulting in O_2 release. The transition from one state to another has been calculated to occur about 10^8 times in the life of a red blood cell.

The **oxygen–hemoglobin dissociation curve** relates percentage saturation of the O_2-carrying power of hemoglobin (abbreviated as SaO_2) to the PO_2 **(Figure 35–1)**. This curve has a characteristic sigmoid shape due to the T–R configuration interconversion. Combination of the first heme in the Hb molecule with O_2 increases the affinity of the second heme for O_2, and oxygenation of the second increases the affinity of the third, and so on, so that the affinity of Hb for the fourth O_2 molecule is many times that for the first. Especially note that small changes at low PO_2 lead to large changes in SaO_2.

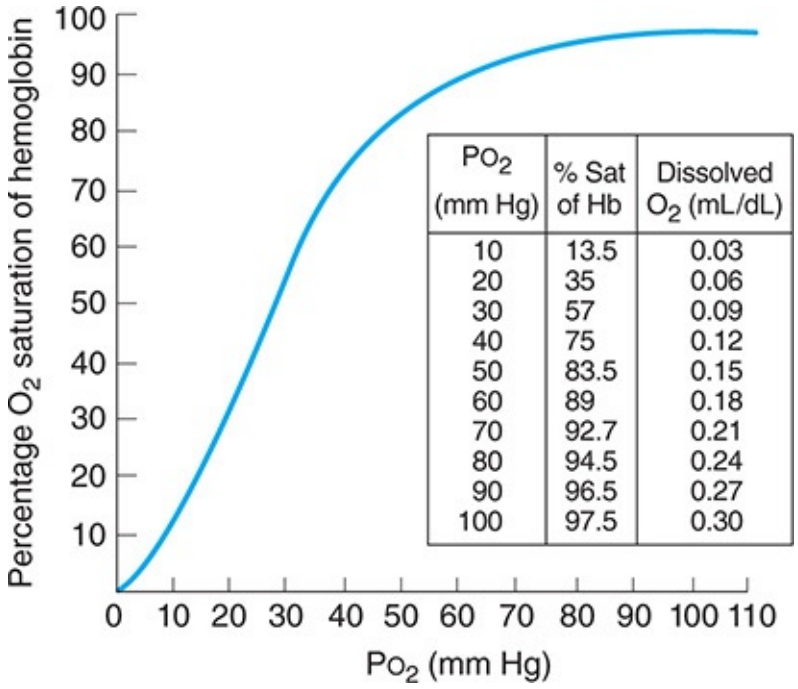


FIGURE 35–1 Oxygen–hemoglobin dissociation curve. pH 7.40, temperature 38°C. Inset table relates the percentage of saturated hemoglobin (SaO_2) to Po_2 and dissolved O_2. (Modified with permission from Comroe JH Jr, et al: *The Lung: Clinical Physiology and Pulmonary Function Tests*, 2nd ed. Year Book; 1962.)

When blood is equilibrated with 100% O_2, the normal hemoglobin becomes 100% saturated. When fully saturated, each gram (g) of normal hemoglobin contains 1.39 mL of O_2. However, blood normally contains small quantities of inactive hemoglobin derivatives, and the measured value in vivo is thus slightly lower. Using the traditional estimate of saturated hemoglobin in vivo, 1.34 mL of O_2, the hemoglobin concentration in normal blood is about 15 g/dL (14 g/dL in women and 16 g/dL in men). Therefore, 1 dL of blood contains 20.1 mL (1.34 mL × 15) of O_2 bound to hemoglobin when the hemoglobin is 100% saturated. The amount of dissolved O_2 is a linear function of the Po_2 (0.003 mL/dL blood/mm Hg Po_2).

In vivo, the hemoglobin in the blood at the ends of the pulmonary capillaries is about 97.5% saturated with O_2 (Po_2 = 100 mm Hg). Because of a slight admixture with venous blood that bypasses the pulmonary capillaries (ie, physiological shunt), the hemoglobin in systemic arterial blood is only 97% saturated. The arterial blood therefore contains a total of about 19.8 mL of O_2

per dL: 0.29 mL in solution and 19.5 mL bound to hemoglobin. In venous blood at rest, the hemoglobin is 75% saturated and the total O_2 content is about 15.2 mL/dL: 0.12 mL in solution and 15.1 mL bound to hemoglobin. Thus, at rest the tissues remove about 4.6 mL of O_2 from each deciliter (dL) of blood passing through them **(Table 35–1)**; 0.17 mL of this total represents O_2 that was in solution in the blood, and the remainder represents O_2 that was liberated from hemoglobin. In this way, 250 mL of O_2 per minute is transported from the blood to the tissues at rest.

TABLE 35–1 Gas content of blood.

	mL/dL of Blood Containing 15 g of Hemoglobin			
	Arterial Blood (Po_2 95 mm Hg; Pco_2 40 mm Hg; Hb 97% Saturated)		Venous Blood (Po_2 40 mm Hg; Pco_2 46 mm Hg; Hb 75% Saturated)	
Gas	Dissolved	Combined	Dissolved	Combined
O_2	0.29	19.5	0.12	15.1
CO_2	2.62	46.4	2.98	49.7
N_2	0.98	0	0.98	0

FACTORS AFFECTING THE AFFINITY OF HEMOGLOBIN FOR OXYGEN

Three important conditions affect the oxygen–hemoglobin dissociation curve: the **pH,** the **temperature,** and the concentration of **2,3-diphosphoglycerate (DPG; 2,3-DPG).** A rise in temperature or a fall in pH shifts the curve to the right **(Figure 35–2)**. When the curve is shifted in this direction, a higher P_{O_2} is required for hemoglobin to bind a given amount of O_2. Conversely, a fall in temperature or a rise in pH shifts the curve to the left, and a lower P_{O_2} is required to bind a given amount of O_2. A convenient index for comparison of such shifts is the P_{50}, the P_{O_2} at which hemoglobin is half saturated with O_2. The higher the P_{50}, the lower the affinity of hemoglobin for O_2.

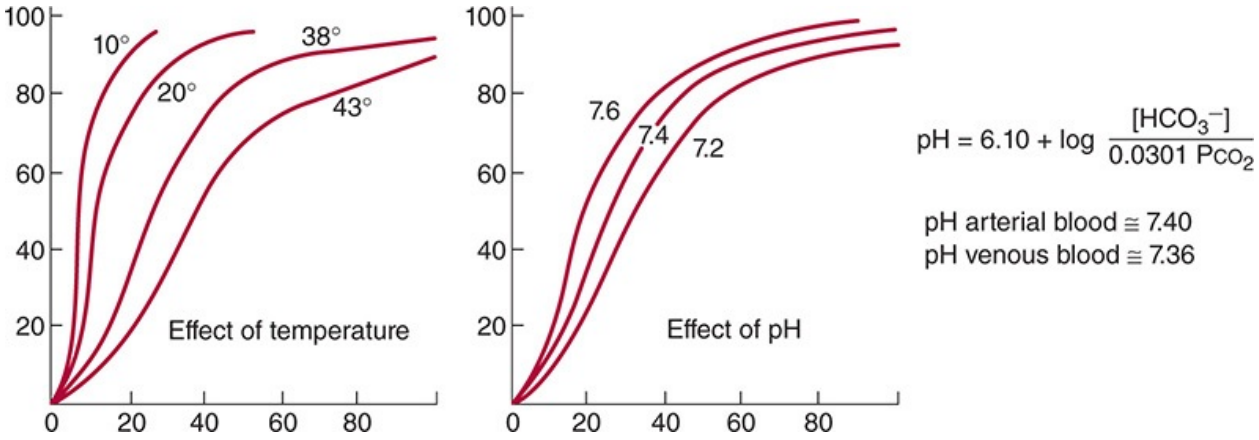


FIGURE 35–2 Effects of temperature and pH on the oxygen–hemoglobin dissociation curve. Both changes in temperature **(left)** and pH **(right)** can alter the affinity of hemoglobin for O_2. Plasma pH can be estimated using the modified Henderson–Hasselbalch equation, as shown. (Modified with permission from Comroe JH Jr, et al: *The Lung: Clinical Physiology and Pulmonary Function Tests*, 2nd ed. Year Book; 1962.)

The decrease in O_2 affinity of hemoglobin when the pH of blood falls is called the **Bohr effect** and is closely related to the fact that deoxygenated hemoglobin (deoxyhemoglobin) binds H^+ more actively than does oxygenated hemoglobin (oxyhemoglobin). The pH of blood falls as its CO_2 content increases, so that when the P_{CO_2} rises, the curve shifts to the right and the P_{50} rises. Most of the unsaturation of hemoglobin that occurs in the tissues is secondary to the decline in the P_{O_2}, but an extra 1–2% unsaturation is due to the rise in P_{CO_2} and consequent shift of the dissociation curve to the right.

2,3-DPG is very plentiful in red cells. It is formed from 3-phosphoglyceraldehyde, which is a product of glycolysis via the Embden–Meyerhof pathway. It is a highly charged anion that binds to the β chains of deoxyhemoglobin. One mole of deoxyhemoglobin binds 1 mol of 2,3-DPG. In effect,

$$HbO_2 + \text{2,3-DPG} \rightleftarrows Hb - \text{2,3-DPG} + O_2$$

In this equilibrium, an increase in the concentration of 2,3-DPG shifts the reaction to the right, causing more O_2 to be liberated.

Because acidosis inhibits red cell glycolysis, the 2,3-DPG concentration falls when the pH is low. Conversely, thyroid hormones, growth hormones, and

androgens can all increase the concentration of 2,3-DPG and the P_{50}.

Exercise has been reported to produce an increase in 2,3-DPG within 60 min (although the rise may not occur in trained athletes). The P_{50} is also increased during exercise, because the temperature rises in active tissues and CO_2 and metabolites accumulate, lowering the pH. In addition, much more O_2 is removed from each unit of blood flowing through active tissues because the tissue P_{O_2} declines. Finally, at low P_{O_2} values, the oxygen–hemoglobin dissociation curve is steep, and large amounts of O_2 are liberated per unit drop in P_{O_2}. Some clinical features of hemoglobin are discussed in **Clinical Box 35–1**.

An interesting contrast to hemoglobin is **myoglobin,** an iron-containing pigment found in skeletal muscle. Myoglobin resembles hemoglobin but binds 1 rather than 4 mol of O_2 per mole protein. The lack of cooperative binding is reflected in the myoglobin dissociation curve, a rectangular hyperbola rather than the sigmoid curve observed for hemoglobin **(Figure 35–3)**. Additionally, the leftward shift of the myoglobin O_2 binding curve when compared with hemoglobin demonstrates a higher affinity for O_2, and thus promotes a favorable transfer of O_2 from hemoglobin in the blood. The steepness of the myoglobin curve also shows that O_2 is released only at low P_{O_2} values (eg, during exercise). The myoglobin content is greatest in muscles specialized for sustained contraction. The muscle blood supply is compressed during such contractions, and myoglobin can continue to provide O_2 under reduced blood flow and/or reduced P_{O_2} in the blood.

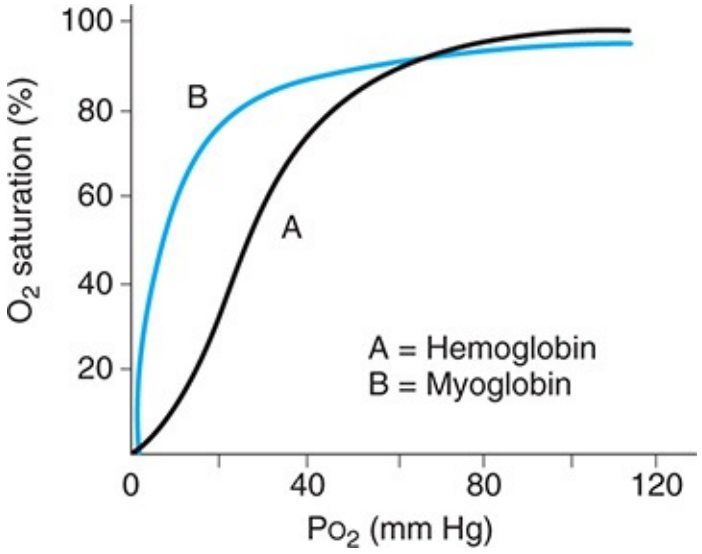


FIGURE 35–3 Comparison of dissociation curves for hemoglobin and myoglobin. The myoglobin binding curve (B) lacks the sigmoidal shape of the hemoglobin binding curve (A) because of the single O_2-binding site in each molecule. Myoglobin also has greater affinity for O_2 than hemoglobin (curve shifted left) and thus can release O_2 in muscle when P_{O_2} in blood is low (eg, during exercise).

CARBON DIOXIDE TRANSPORT

MOLECULAR FATE OF CARBON DIOXIDE IN BLOOD

The solubility of CO_2 in blood is about 20 times that of O_2; therefore, considerably more CO_2 than O_2 is present in simple solution at equal partial pressures. The CO_2 that diffuses into red blood cells is rapidly hydrated to H_2CO_3 because of the presence of carbonic anhydrase **(Figure 35–4)**. The H_2CO_3 dissociates to H^+ and HCO_3^-, and the H^+ is buffered, primarily by hemoglobin, while the HCO_3^- enters the plasma. Some of the CO_2 in the red cells reacts with the amino groups of hemoglobin and other proteins (R), forming **carbamino compounds.**

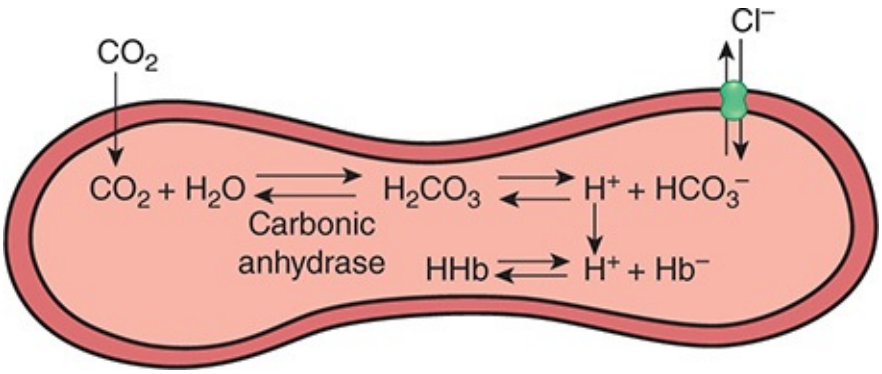
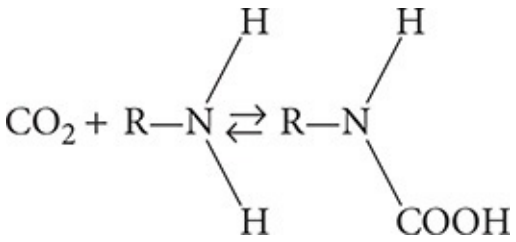


FIGURE 35–4 Fate of CO_2 in the red blood cell. Upon entering the red blood cell, CO_2 is rapidly hydrated to H_2CO_3 by carbonic anhydrase. H_2CO_3 is in equilibrium with H^+ and its conjugate base, HCO_3^-. H^+ can interact with deoxyhemoglobin, whereas HCO_3^- can be transported outside of the cell via anion exchanger 1 (AE1 or Band 3). In effect, for each CO_2 molecule that enters

the red cell, there is an additional HCO_3^- or Cl^- in the cell.



CLINICAL BOX 35–1

Hemoglobin & O_2 Binding In Vivo

Cyanosis

Reduced hemoglobin has a dark color, and a dusky bluish discoloration of the tissues, called **cyanosis,** appears when the reduced hemoglobin concentration of the blood in the capillaries is more than 5 g/dL. Its occurrence depends on the total amount of hemoglobin in the blood, the degree of hemoglobin unsaturation, and the state of the capillary circulation. Cyanosis is most easily seen in the nail beds and mucous membranes and in the earlobes, lips, and fingers, where the skin is thin and there are plenty capillaries. Although visible observation is indicative of cyanosis, it is not fully reliable. Further tests of arterial oxygen tension and saturation, blood and hemoglobin counts can provide more reliable diagnoses.

Effects of 2,3-DPG on Fetal & Stored Blood

The affinity of fetal hemoglobin (hemoglobin F) for O_2, which is greater than that for adult hemoglobin (hemoglobin A), facilitates the movement of O_2 from the mother to the fetus. The cause of this greater affinity is the poor binding of 2,3-DPG by the γ polypeptide chains that replace β chains in fetal hemoglobin. Some abnormal hemoglobins in adults have low P_{50} values, and the resulting high O_2 affinity of the hemoglobin causes enough tissue hypoxia to stimulate increased red cell formation, with resulting polycythemia. It is interesting to speculate that these hemoglobins may not bind 2,3-DPG.

Red cell 2,3-DPG concentration is increased in anemia and in a variety of diseases in which there is chronic hypoxia. This facilitates the delivery of O_2 to the tissues by raising the P_{O_2} at which O_2 is released in peripheral capillaries. In banked blood that is stored, the 2,3-DPG level falls and the

ability of this blood to release O_2 to the tissues is reduced. This decrease, which obviously limits the benefit of the blood if it is transfused into a hypoxic patient, is less if the blood is stored in citrate-phosphate-dextrose solution rather than the usual acid-citrate-dextrose solution.

THERAPEUTIC HIGHLIGHTS

Cyanosis is an indication of poorly oxygenated hemoglobin rather than a disease, and thus can have many causes, from cold exposure to drug overdose to chronic lung disease. As such, proper treatment depends on the underlying cause. For cyanosis caused by exposure to cold, maintaining a warm environment can be effective, whereas supplemental oxygen administration may be required under conditions of chronic and hypoxic disease.

Because deoxyhemoglobin binds more H^+ than oxyhemoglobin and forms carbamino compounds more readily, binding of O_2 to hemoglobin reduces its affinity for CO_2. The **Haldane effect** refers to the increased capacity of deoxygenated hemoglobin to bind and carry CO_2. Consequently, venous blood carries more CO_2 than arterial blood, CO_2 uptake is facilitated in the tissues, and CO_2 release is facilitated in the lungs. About 11% of the CO_2 added to the blood in the systemic capillaries is carried to the lungs as carbamino-CO_2.

CHLORIDE SHIFT

Because the rise in the HCO_3^- content of red cells is much greater than that in plasma as the blood passes through the capillaries, about 70% of the HCO_3^- formed in the red cells enters the plasma. The excess HCO_3^- leaves the red cells in exchange for Cl^- (Figure 35–4). This process is mediated by **anion exchanger 1 (AE1;** also called Band 3), a major membrane protein in the red blood cell. Because of this **chloride shift,** the Cl^- content of the red cells in venous blood is significantly greater than that in arterial blood. The chloride shift occurs rapidly and is essentially complete within 1 s.

Note that for each CO_2 molecule added to a red cell, there is an increase of one osmotically active particle in the cell—either an HCO_3^- or a Cl^- (Figure 35–

4). Consequently, the red cells take up water and increase in size. For this reason, plus the fact that a small amount of fluid in the arterial blood returns via the lymphatics rather than the veins, the hematocrit of venous blood is normally 3% greater than that of the arterial blood. In the lungs, the Cl^- moves back out of the cells and they shrink.

SPATIAL DISTRIBUTION OF CARBON DIOXIDE IN BLOOD

For convenience, the various fates of CO_2 in the plasma and red cells are summarized in **Table 35–2**. The extent to which they increase the capacity of the blood to carry CO_2 is indicated by the difference between the lines indicating the dissolved CO_2 and the total CO_2 in the dissociation curves for CO_2 shown in **Figure 35–5**.

TABLE 35–2 Fate of CO_2 in blood.

In plasma
1. Dissolved
2. Formation of carbamino compounds with plasma protein
3. Hydration, H^+ buffered, HCO_3^- in plasma
In red blood cells
1. Dissolved
2. Formation of carbamino-Hb
3. Hydration, H^+ buffered, 70% of HCO_3^- enters the plasma
4. Cl^- shifts into cells; mOsm in cells increases

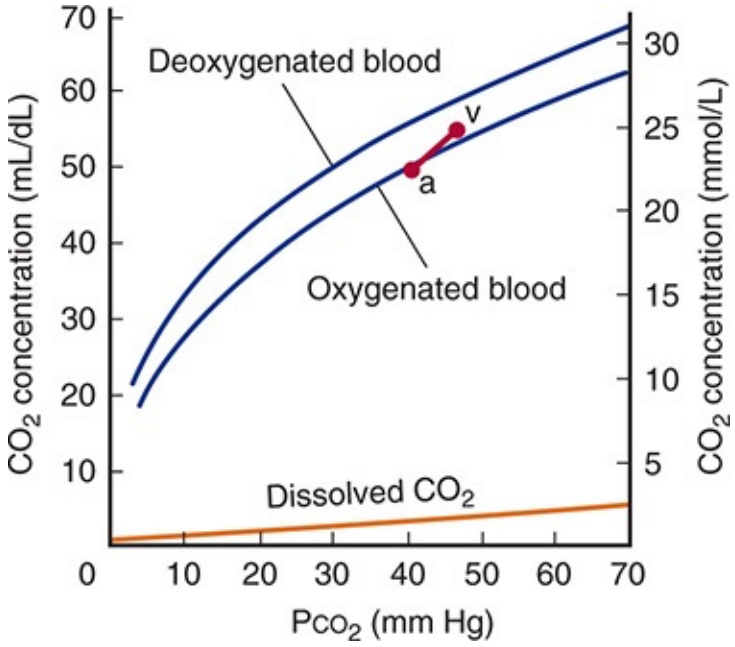


FIGURE 35–5 CO_2 dissociation curves. The arterial point (a) and the venous point (v) indicate the total CO_2 content found in arterial blood and venous blood of normal resting humans. Note the low amount of CO_2 that is dissolved (orange trace) compared to that which can be carried by other means (Table 35–2). (Modified with permission from Schmidt RF, Thews G [editors]: *Human Physiology*. Springer; 1983.)

Of the approximately 49 mL of CO_2 in each deciliter of arterial blood (Table 35–1), 2.6 mL is dissolved, 2.6 mL is in carbamino compounds, and 43.8 mL is in HCO_3^-. In the tissues, 3.7 mL of CO_2 per deciliter of blood is added, 0.4 mL stays in solution, 0.8 mL forms carbamino compounds, and 2.5 mL forms HCO_3^-. The pH of the blood drops from 7.40 to 7.36. In the lungs, the processes are reversed, and the 3.7 mL of CO_2 is discharged into the alveoli. In this manner, 200 mL of CO_2 per minute at rest and much larger amounts during exercise are transported from the tissues to the lungs and excreted. It is worth noting that this amount of CO_2 is equivalent in 24 h to over 12,500 mEq of H^+.

ACID–BASE BALANCE & GAS TRANSPORT

The major source of acids in the blood under normal conditions is through cellular metabolism. The CO_2 formed by metabolism in the tissues is in large

part hydrated to H_2CO_3, resulting in the large total H^+ load noted above (> 12,500 mEq/day). However, most of the CO_2 is excreted in the lungs, and the small quantities of the remaining H^+ are excreted by the kidneys.

BUFFERING IN THE BLOOD

Acid and base shifts in the blood are largely controlled by three main buffers in blood: (1) proteins, (2) hemoglobin, and (3) the carbonic acid-bicarbonate system. Plasma **proteins** are effective buffers because both their free carboxyl and their free amino groups dissociate:

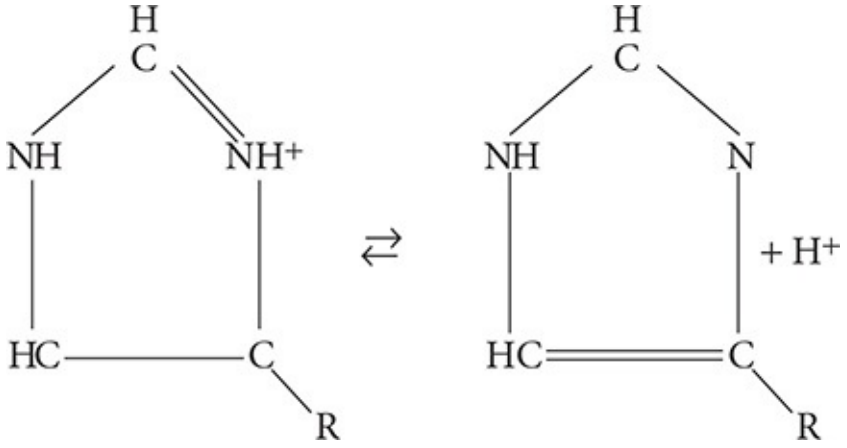
$$RCOOH \rightleftarrows RCOO^- + H^+$$

$$pH = pK'_{RCOOH} + \log \frac{[RCOO^-]}{[RCOOH]}$$

$$RNH_3^+ \rightleftarrows RNH_2 + H^+$$

$$pH = pK'_{RNH_3} + \log \frac{[RNH_2]}{[RNH_3^+]}$$

The second buffer system is provided by the dissociation of the imidazole groups of the histidine residues in **hemoglobin.**



In the pH 7.0–7.7 range, the free carboxyl and amino groups of hemoglobin contribute relatively little to its buffering capacity. However, the hemoglobin molecule contains 38 histidine residues, and on this basis—plus the fact that hemoglobin is present in large amounts—the hemoglobin in blood has six times

the buffering capacity of the plasma proteins. In addition, the action of hemoglobin is unique because the imidazole groups of deoxyhemoglobin (Hb) dissociate less than those of oxyhemoglobin (HbO_2), making Hb a weaker acid and therefore a better buffer than HbO_2. The titration curves for Hb and HbO_2 **(Figure 35–6)** illustrate the differences in H^+ buffering capacity.

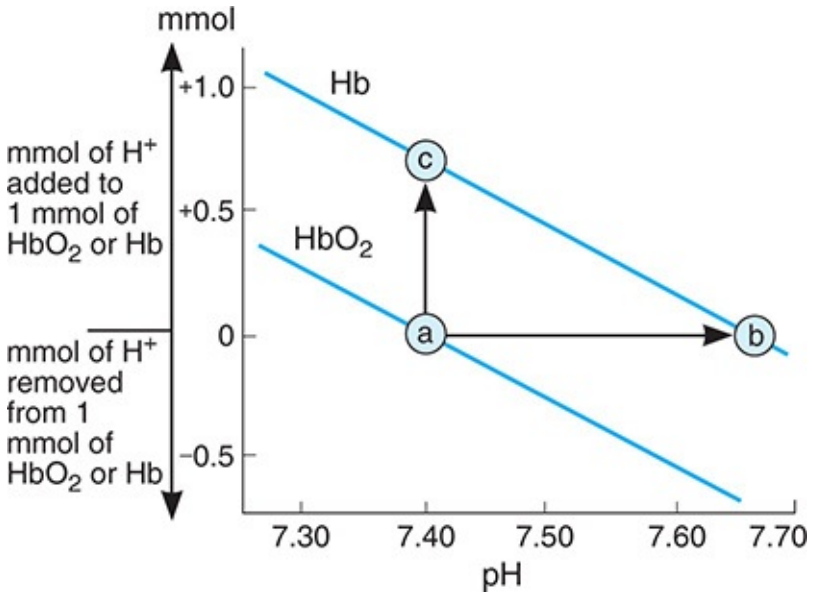


FIGURE 35–6 Comparative titration curves for oxygenated hemoglobin (HbO_2) and deoxyhemoglobin (Hb). The arrow from a to c indicates the number of additional millimoles of H^+ that Hb can buffer compared with a similar concentration of HbO_2 (ie, no shift in pH). The arrow from a to b indicates the pH shift that would occur on deoxygenation of HbO_2 without additional H^+.

The third and major buffer system in blood is the **carbonic acid-bicarbonate system:**

$$H_2CO_3 \rightleftarrows H^+ + HCO_3^-$$

The Henderson–Hasselbalch equation for this system is

$$pH = pK + \log \frac{[HCO_3^-]}{[H_2CO_3]}$$

The pK for this system in an ideal solution is low (about 3), and the amount

of H_2CO_3 is small and hard to measure accurately. However, in the body, H_2CO_3 is in equilibrium with CO_2:

$$H_2CO_3 \rightleftarrows CO_2 + H_2O$$

If the pK is changed to pK′ (apparent ionization constant; distinguished from the true pK due to less than ideal conditions for the solution) and $[CO_2]$ is substituted for $[H_2CO_3]$, the pK′ is 6.1:

$$pH = 6.10 + \log \frac{[HCO_3^-]}{[CO_2]}$$

The clinically relevant form of this equation is:

$$pH = 6.10 + \log \frac{[HCO_3^-]}{0.0301\ P_{CO_2}}$$

since the amount of dissolved CO_2 is proportional to the partial pressure of CO_2 and the solubility coefficient of CO_2 in mmol/L/mm Hg is 0.0301. $[HCO_3^-]$ cannot be measured directly, but pH and P_{CO_2} can be measured with suitable accuracy with pH and P_{CO_2} glass electrodes, and $[HCO_3^-]$ can then be calculated.

The pK' of this system is still low relative to the pH of the blood, but the system is one of the most effective buffer systems in the body because the amount of dissolved CO_2 is controlled by respiration (ie, it is an "open" system). Additional control of the plasma concentration of HCO_3^- is provided by the kidneys. When H^+ is added to the blood, HCO_3^- declines as more H_2CO_3 is formed. If the extra H_2CO_3 were not converted to CO_2 and H_2O and the CO_2 excreted in the lungs, the H_2CO_3 concentration would rise. Without CO_2 removal to reduce H_2CO_3, sufficient H^+ addition that would halve the plasma HCO_3^- would alter the pH 7.4 to 6.0. However, such a H^+ concentration increase is tolerated because: (1) extra H_2CO_3 that is formed is removed and (2) the H^+ rise stimulates respiration and therefore produces a drop in P_{CO_2}, so that some additional H_2CO_3 is removed. The net pH after such an increase in H^+ concentration is actually 7.2 or 7.3.

There are two additional factors that make the carbonic acid-bicarbonate system such a good biological buffer. First, the reaction $CO_2 + H_2O \rightleftarrows H_2CO_3$ proceeds slowly in either direction unless the enzyme **carbonic anhydrase** is present. There is no carbonic anhydrase in plasma, but there is an abundant supply in red blood cells, spatially confining and controlling the reaction. Second, the presence of hemoglobin in the blood increases the buffering of the system by binding free H^+ produced by the hydration of CO_2 and allowing for movement of the HCO_3^- into the plasma.

ACIDOSIS & ALKALOSIS

The pH of the arterial plasma is normally 7.40 and that of venous plasma slightly lower. A decrease in pH below the norm **(acidosis)** is technically present whenever the arterial pH is below 7.40 and an increase in pH **(alkalosis)** is technically present whenever pH is above 7.40. In practice, variations of up to 0.05 pH unit occur without untoward effects. Acid–base disorders are split into four categories: respiratory acidosis, respiratory alkalosis, metabolic acidosis, and metabolic alkalosis. In addition, these disorders can occur in combination. Some causes of acid–base disturbances are shown in **Table 35–3**.

TABLE 35–3 Plasma pH, HCO_3^-, and PCO_2 values in various typical disturbances of acid–base balance.[a]

Condition	Arterial Plasma pH	HCO_3^- (mEq/L)	P_{CO_2} (mm Hg)	Cause
Normal	7.40	24.1	40	
Metabolic acidosis	7.28	18.1	40	NH_4Cl ingestion
	6.96	5.0	23	Diabetic acidosis
Metabolic alkalosis	7.50	30.1	40	$NaHCO_3$ ingestion
	7.56	49.8	58	Prolonged vomiting
Respiratory acidosis	7.34	25.0	48	Breathing 7% CO_2
	7.34	33.5	64	Emphysema
Respiratory alkalosis	7.53	22.0	27	Voluntary hyper-ventilation
	7.48	18.7	26	Three-week residence at 4000-m altitude

[a]In the diabetic acidosis and prolonged vomiting examples, respiratory compensation for primary metabolic acidosis and alkalosis has occurred, and the P_{CO_2} has shifted from 40 mm Hg. In the emphysema and high-altitude examples, renal compensation for primary respiratory acidosis and alkalosis has occurred and has made the deviations from normal of the plasma HCO_3^- larger than they would otherwise be.

RESPIRATORY ACIDOSIS

Any short-term rise in arterial P_{CO_2} (ie, above 40 mm Hg, due to hypoventilation) results in **respiratory acidosis.** Recall that CO_2 that is retained is in equilibrium with H_2CO_3, which in turn is in equilibrium with HCO_3^-. The effective rise in plasma HCO_3^- means that a new equilibrium is reached at a lower pH. This can be indicated graphically on a plot of plasma HCO_3^- concentration versus pH **(Figure 35–7)**. The pH change observed at any increase in P_{CO_2} during respiratory acidosis depends on the buffering capacity of the

blood. The initial changes shown in Figure 35–7 are those that occur independently of any compensatory mechanism; that is, they are those of **uncompensated respiratory acidosis.**

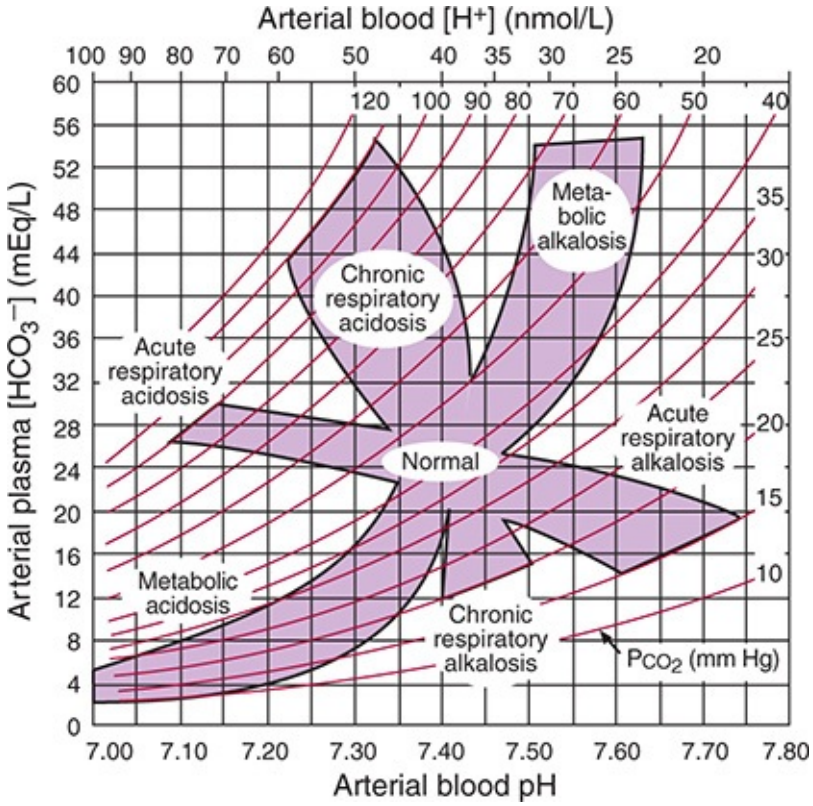


FIGURE 35–7 Acid–base nomogram. Changes in the P_{CO_2} (curved lines), plasma HCO_3^-, and pH (or $[H^+]$) of arterial blood in respiratory and metabolic acidosis are shown. Note the shifts in HCO_3^- and pH as acute respiratory acidosis and alkalosis are compensated, producing their chronic counterparts. (Reproduced with permission from Brenner BM, Rector FC Jr. [editors]: *Brenner & Rector's The Kidney*, 7th ed. St. Louis, MO: Saunders; 2004.)

RESPIRATORY ALKALOSIS

Any short-term lowering of P_{CO_2} below what is needed for proper CO_2 exchange (ie, below 35 mm Hg, as can occur during hyperventilation) results in **respiratory alkalosis.** The decreased CO_2 shifts the equilibrium of the carbonic acid-bicarbonate system to effectively lower $[H^+]$ and increase pH. As in respiratory acidosis, initial pH changes corresponding to respiratory alkalosis

(Figure 35–7) are those that occur independently of any compensatory mechanism and are thus **uncompensated respiratory alkalosis.**

METABOLIC ACIDOSIS & ALKALOSIS

Blood pH changes can also arise by nonrespiratory mechanism. **Metabolic acidosis** (or nonrespiratory acidosis) occurs when strong acids are added to blood. If, for example, a large amount of acid is ingested (eg, aspirin overdose), acids in the blood are quickly increased. The H_2CO_3 that is formed is converted to H_2O and CO_2, and the CO_2 is rapidly excreted via the lungs. This is the situation in **uncompensated metabolic acidosis** (Figure 35–7). Note that in contrast to respiratory acidosis, metabolic acidosis does not include a change in P_{CO_2}; the shift toward metabolic acidosis occurs along an isobar line **(Figure 35–8)**. When the free $[H^+]$ level falls as a result of addition of alkali, or more commonly, the removal of large amounts of acid (eg, following vomiting), **metabolic alkalosis** results. In uncompensated metabolic alkalosis the pH rises along the isobar line (Figures 35–7 and 35–8).

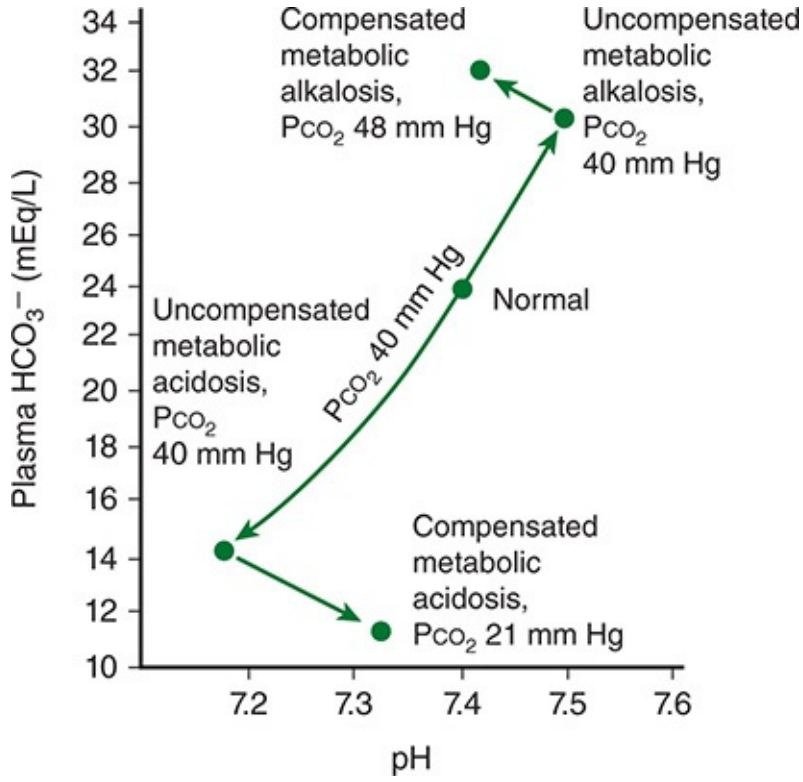


FIGURE 35–8 Acid–base paths during metabolic acidosis. Changes in true

plasma pH, HCO_3^-, and P_{CO_2} at rest, during metabolic acidosis and alkalosis, and following respiratory compensation are plotted. Metabolic acidosis or alkalosis causes changes in pH along the P_{CO_2} isobar line (middle line). Respiratory compensation moves pH toward normal by altering P_{CO_2} (top and bottom arrows). (Modified with permission from Davenport HW: *The ABC of Acid-Base Chemistry*, 6th ed. University of Chicago Press; 1974.)

RESPIRATORY & RENAL COMPENSATION

Uncompensated acidosis and alkalosis as described above are seldom seen because of compensation systems. The two main compensatory systems are **respiratory compensation** and **renal compensation.**

The respiratory system compensates for metabolic acidosis or alkalosis by altering ventilation, and consequently, the P_{CO_2}, which can directly change blood pH. Respiratory mechanisms are fast. In response to metabolic acidosis, ventilation is increased, resulting in a decrease of P_{CO_2} (eg, from 40 mm Hg to 20 mm Hg) and a subsequent increase in pH toward normal (Figure 35–8). In response to metabolic alkalosis, ventilation is decreased, P_{CO_2} is increased, and a subsequent decrease in pH occurs. Because respiratory compensation is a quick response, the graphical representation in Figure 35–8 overstates the two-step adjustment in blood pH. In actuality, as soon as metabolic acidosis begins, respiratory compensation is invoked and the large shifts in pH depicted do not occur.

For complete compensation from respiratory or metabolic acidosis/alkalosis, renal compensatory mechanisms are invoked. The kidney responds to acidosis by actively secreting fixed acids while retaining filtered HCO_3^-. In contrast, the kidney responds to alkalosis by decreasing H^+ secretion and by decreasing the retention of filtered HCO_3^-.

Renal tubule cells in the kidney have active carbonic anhydrase and thus can produce H^+ and HCO_3^- from CO_2. In response to acidosis, these cells secrete H^+ into the tubular fluid in exchange for Na^+ while the HCO_3^- is actively reabsorbed into the peritubular capillary; for each H^+ secreted, one Na^+ and one HCO_3^- are added to the blood. The result of this renal compensation for respiratory acidosis is shown graphically in the shift from acute to chronic respiratory acidosis in Figure 35–7. Conversely, in response to alkalosis, the

kidney decreases H^+ secretion and depresses HCO_3^- reabsorption. The result of this renal compensation for respiratory alkalosis is shown graphically in the shift from acute to chronic respiratory alkalosis in Figure 35–7. Clinical evaluations of acid–base status are discussed in **Clinical Box 35–2**, and the role of the kidneys in acid–base homeostasis is discussed in more detail in Chapter 38.

HYPOXIA

Hypoxia is O_2 deficiency at the tissue level. It is a more correct term than **anoxia** (lack of O_2), since there is rarely no O_2 at all left in the tissues.

Numerous classifications for hypoxia have been used, but the more traditional four-type system still has considerable utility if the definitions of the terms are kept clearly in mind. The four categories are (1) **hypoxemia** (sometimes termed **hypoxic hypoxia**), in which the P_{O_2} of the arterial blood is reduced; (2) **anemic hypoxia,** in which the arterial P_{O_2} is normal but the amount of hemoglobin available to carry O_2 is reduced; (3) **ischemic** or **stagnant hypoxia,** in which the blood flow to a tissue is so low that adequate O_2 is not delivered to it despite a normal P_{O_2} and hemoglobin concentration; and (4) **histotoxic hypoxia,** in which the amount of O_2 delivered to a tissue is adequate but, because of the action of a toxic agent, the tissue cells cannot make use of the O_2 supplied to them. Some specific effects of hypoxia on cells and tissues are discussed in **Clinical Box 35–3**.

CLINICAL BOX 35–2

Clinical Evaluation of Acid–Base Status

In evaluating disturbances of acid–base balance, it is important to know the pH and HCO_3^- content of arterial plasma. Reliable pH determinations can be made with a pH meter and a glass pH electrode. Using pH and a direct measurement of the P_{CO_2} with a CO_2 electrode, HCO_3^- concentration can be calculated. The P_{CO_2} is ~8 mm Hg higher and the pH 0.03–0.04 unit lower in venous than arterial plasma because venous blood contains the CO_2 being carried from the tissues to the lungs. Therefore, the calculated HCO_3^- concentration is about 2 mmol/L higher. However, if this is kept in mind, free-

flowing venous blood can be substituted for arterial blood in most clinical situations.

A measurement that is of some value in the differential diagnosis of metabolic acidosis is the **anion gap.** This gap, which is something of a misnomer, refers to the difference between the concentration of cations other than Na^+ and the concentration of anions other than Cl^- and HCO_3^- in the plasma. It consists for the most part of proteins in anionic form, HPO_4^{2-}, SO_4^{2-}, and organic acids; a normal value is about 12 mEq/L. It is increased when the plasma concentration of K^+, Ca^{2+}, or Mg^+ is decreased; when the concentration of (or the charge on) plasma proteins is increased; or when organic anions such as lactate or foreign anions accumulate in blood. It is decreased when cations are increased or when plasma albumin is decreased. The anion gap is increased in metabolic acidosis due to ketoacidosis, lactic acidosis, and other forms of acidosis in which organic anions are increased.

HYPOXEMIA

By definition, hypoxemia is a condition of reduced arterial P_{O_2}. Hypoxemia is a problem in normal individuals at high altitudes and is a complication of pneumonia and a variety of other diseases of the respiratory system.

CLINICAL BOX 35–3

Effects of Hypoxia on Cells and Selected Tissues

Effects on Cells

Hypoxia causes the production of transcription factors (hypoxia-inducible factors; HIFs). These are made up of α and β subunits. In normally oxygenated tissues, the α subunits are rapidly ubiquitinated and destroyed. However, in hypoxic cells, the α subunits dimerize with β subunits, and the dimers activate genes that produce several proteins including angiogenic factors and erythropoietin, among others.

Effects on the Brain

In hypoxymia and the other generalized forms of hypoxia, the brain is

affected first. A sudden drop in the inspired P_{O_2} to less than 20 mm Hg, which occurs, for example, when cabin pressure is suddenly lost in a plane flying above 16,000 m, causes loss of consciousness in 10–20 s and death in 4–5 min. Less severe hypoxia causes a variety of mental aberrations not unlike those produced by alcohol: impaired judgment, drowsiness, dulled pain sensibility, excitement, disorientation, loss of time sense, and headache. Other symptoms include anorexia, nausea, vomiting, tachycardia, and, when the hypoxia is severe, hypertension. The rate of ventilation is increased in proportion to the severity of the hypoxia of the carotid chemoreceptor cells.

Respiratory Stimulation

Dyspnea is by definition difficult or labored breathing in which the person is conscious of shortness of breath; **hyperpnea** is the general term for an increase in the rate or depth of breathing regardless of the patient's subjective sensations. **Tachypnea** is rapid, shallow breathing. In general, a normal individual is not conscious of respiration until ventilation is doubled, and breathing is not uncomfortable until ventilation is tripled or quadrupled. Whether or not a given level of ventilation is uncomfortable also appears to depend on a variety of other factors. Hypercapnia and, to a lesser extent, hypoxia cause dyspnea. An additional factor is the effort involved in moving the air in and out of the lungs (the work of breathing).

EFFECTS OF DECREASED BAROMETRIC PRESSURE

The composition of air stays the same, but the total barometric pressure falls with increasing altitude **(Figure 35–9)**, and thus, the P_{O_2} also falls. At 3000 m (~10,000 ft) above sea level, the alveolar P_{O_2} is about 60 mm Hg and there is enough hypoxic stimulation of the chemoreceptors under normal breathing to cause increased ventilation. As one ascends higher, the alveolar P_{O_2} falls less rapidly and the alveolar P_{CO_2} declines because of the hyperventilation. The resulting fall in arterial P_{CO_2} produces respiratory alkalosis. A number of compensatory mechanisms operate over a period of time to increase altitude tolerance **(acclimatization),** but in unacclimatized persons, mental symptoms such as irritability appear at about 3700 m. At 5500 m, the hypoxic symptoms are severe; and at altitudes above 6100 m (20,000 ft), consciousness is usually

lost.

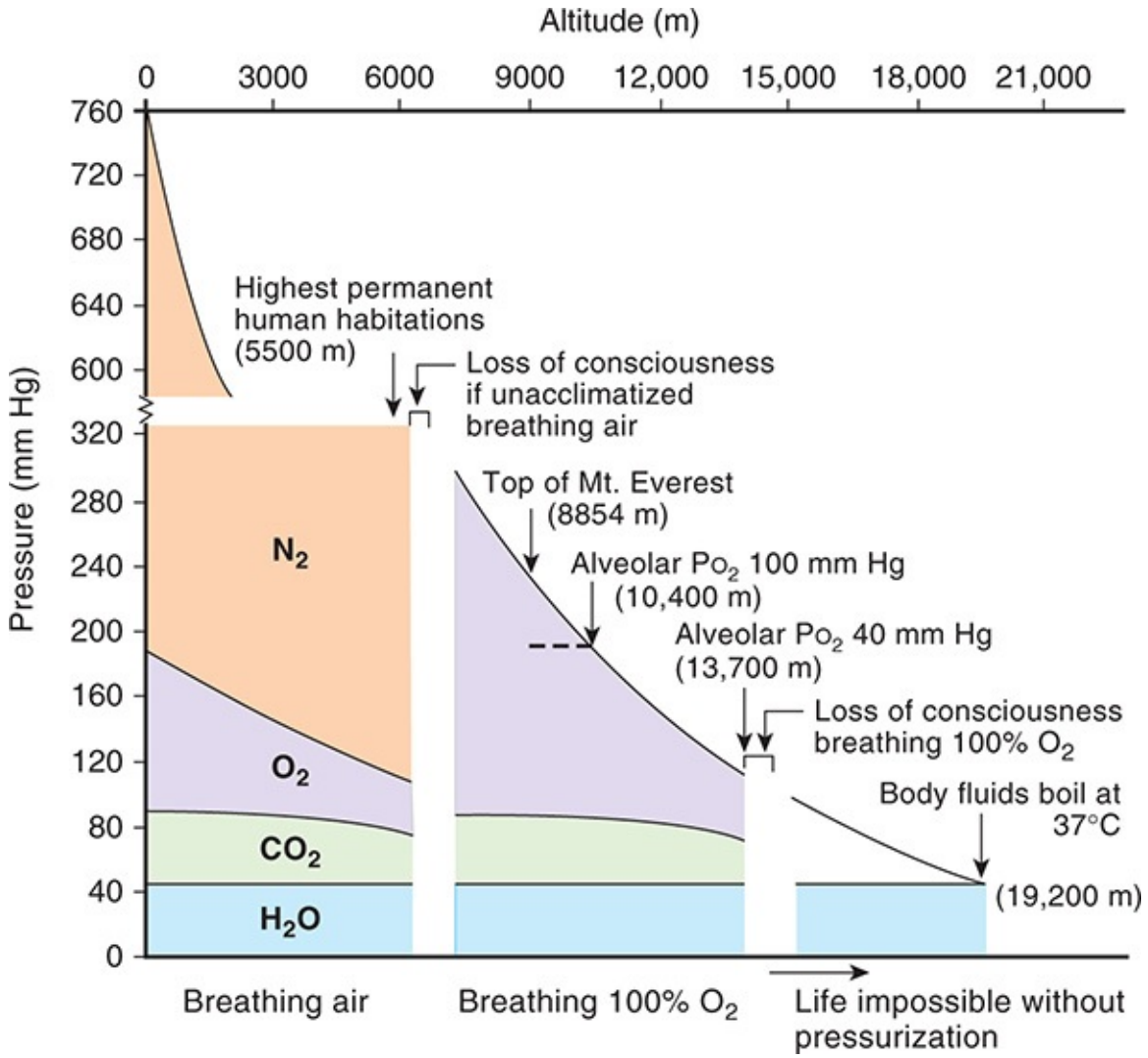


FIGURE 35–9 Composition of alveolar air in individuals breathing air (0–6100 m) and 100% O_2 (6100–13,700 m). The minimal alveolar P_{O_2} that an unacclimatized person can tolerate without loss of consciousness is about 35–40 mm Hg. Note that with increasing altitude, the alveolar P_{CO_2} drops because of the hyperventilation due to hypoxic stimulation of the carotid and aortic chemoreceptors. The fall in barometric pressure with increasing altitude is not linear because air is compressible.

HYPOXIC SYMPTOMS & BREATHING OXYGEN

Some of the effects of high altitude can be offset by breathing 100% O_2. Under

these conditions, the total atmospheric pressure becomes the limiting factor in altitude tolerance.

The partial pressure of water vapor in the alveolar air is constant at 47 mm Hg, and that of CO_2 is normally 40 mm Hg, so that the lowest barometric pressure at which a normal alveolar P_{O_2} of 100 mm Hg is possible is 187 mm Hg, the pressure at about 10,400 m (34,000 ft). At greater altitudes, the increased ventilation due to the decline in alveolar P_{O_2} lowers the alveolar P_{CO_2} somewhat, but the maximum alveolar P_{O_2} that can be attained when breathing 100% O_2 at the ambient barometric pressure of 100 mm Hg at 13,700 m is ~40 mm Hg. At ~14,000 m, consciousness is lost in spite of the administration of 100% O_2. At 19,200 m, the barometric pressure is 47 mm Hg, and at or below this pressure the body fluids boil at body temperature. The point is largely academic, however, because any individual exposed to such a low pressure would be dead of hypoxia before the bubbles of body fluids could cause death.

Of course, an artificial atmosphere can be created around an individual; in a pressurized suit or cabin supplied with O_2 and a system to remove CO_2, it is possible to ascend to any altitude and to live in the vacuum of interplanetary space. Some delayed effects of high altitude are discussed in **Clinical Box 35–4**.

ACCLIMATIZATION

Acclimatization to altitude is due to the operation of a variety of compensatory mechanisms. The respiratory alkalosis produced by the hyperventilation shifts the oxygen–hemoglobin dissociation curve to the left, but a concomitant increase in red blood cell 2,3-DPG tends to decrease the O_2 affinity of hemoglobin. The net effect is a small increase in P_{50}. The decrease in O_2 affinity makes more O_2 available to the tissues. However, the value of the increase in P_{50} is limited because when the arterial P_{O_2} is markedly reduced, the decreased O_2 affinity also interferes with O_2 uptake by hemoglobin in the lungs.

The initial ventilatory response to increased altitude is relatively small, because the alkalosis tends to counteract the stimulating effect of hypoxia. However, ventilation steadily increases over the next 4 days **(Figure 35–10)** because the active transport of H^+ into cerebrospinal fluid (CSF), or possibly a developing lactic acidosis in the brain, causes a fall in CSF pH that increases the response to hypoxia. After 4 days, the ventilatory response begins to decline slowly, but it takes years of residence at higher altitudes for it to decline to the

initial level, if it is reached at all.

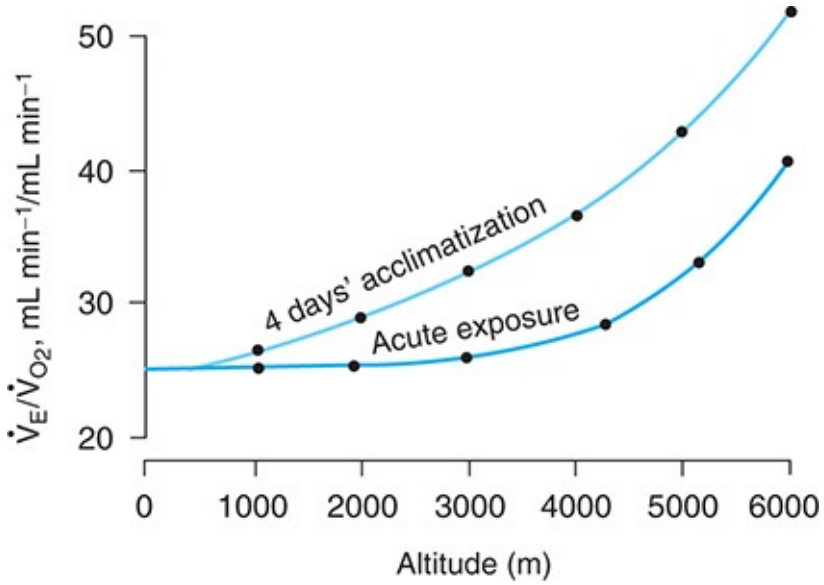


FIGURE 35–10 Effect of acclimatization on the ventilatory response at various altitudes. $\dot{V}_E/\dot{V}_{O_2}$ is the ventilatory equivalent, the ratio of expired minute volume $(\dot{V}_E)$ to the O_2 consumption $(\dot{V}_{O_2})$. (Reproduced with permission from Lenfant C, Sullivan K: Adaptation to high altitude. N Engl J Med 1971; June 10; 284(23):1298–1309.)

CLINICAL BOX 35–4

Delayed Effects of High Altitude

When they first arrive at a high altitude, many individuals develop acute "mountain sickness." This syndrome develops 8–24 h after arrival at altitude and lasts 4–8 days. It is characterized by headache, irritability, insomnia, breathlessness, and nausea and vomiting. Its cause is unsettled, but it appears to be associated with cerebral edema. The low P_{O_2} at high altitude causes systemic arteriolar dilation, and if cerebral autoregulation does not compensate, there is an increase in capillary pressure that favors increased transudation of fluid into brain tissue.

Two more serious syndromes that are associated with high-altitude illness: **high-altitude cerebral edema** and **high-altitude pulmonary edema.** In high-altitude cerebral edema, the capillary leakage in mountain sickness progresses to frank brain swelling, with ataxia, disorientation, and in some

cases coma and death due to herniation of the brain through the tentorium. High-altitude pulmonary edema is a patchy edema of the lungs that is related to the marked pulmonary hypertension that develops at high altitude. It has been argued that it occurs because not all pulmonary arteries have enough smooth muscle to constrict in response to hypoxia, and in the capillaries supplied by those arteries, the general rise in pulmonary arterial pressure causes a capillary pressure increase that disrupts their walls (stress failure).

THERAPEUTIC HIGHLIGHTS

All forms of high-altitude illness are benefited by descent to lower altitude and by treatment with the diuretic acetazolamide. This drug inhibits carbonic anhydrase and results in stimulated respiration, increased $PaCO_2$, and reduced formation of CSF. When cerebral edema is marked, large doses of glucocorticoids are often administered as well. Their mechanism of action is unsettled. In high-altitude pulmonary edema, prompt treatment with O_2 is essential—and, if available, use of a hyperbaric chamber. Portable hyperbaric chambers are now available in a number of mountain areas. Nifedipine, a Ca^{2+} channel blocker that lowers pulmonary artery pressure, can also be useful.

Erythropoietin secretion increases promptly on ascent to high altitude and then falls somewhat over the following 4 days as the ventilatory response increases and the arterial P_{O_2} rises. The increase in circulating red blood cells triggered by the erythropoietin begins in 2–3 days and is sustained as long as the individual remains at high altitude. Compensatory changes also occur in the tissues. The mitochondria, which are the site of oxidative reactions, increase in number, and myoglobin increases, which facilitates the movement of O_2 into the tissues. The tissue content of cytochrome oxidase also increases.

The effectiveness of the acclimatization process is indicated by the fact that permanent human habitations exist in the Andes and Himalayas at elevations above 5500 m (18,000 ft). The natives who live in these villages are barrel-chested and markedly polycythemic. They have low alveolar P_{O_2} values, but in most other ways they are remarkably normal.

DISEASES CAUSING HYPOXEMIA

Hypoxemia is the most common form of hypoxia seen clinically. The diseases that cause it can be roughly divided into those in which the gas exchange apparatus fails, those such as congenital heart disease in which large amounts of blood are shunted from the venous to the arterial side of the circulation, and those in which the respiratory pump fails. Lung failure occurs when conditions such as pulmonary fibrosis produce alveolar-capillary block, or there is ventilation–perfusion imbalance. Pump failure can be due to fatigue of the respiratory muscles in conditions in which the work of breathing is increased or to a variety of mechanical defects such as pneumothorax or bronchial obstruction that limit ventilation. It can also be caused by abnormalities of the neural mechanisms that control ventilation, such as depression of the respiratory neurons in the medulla by morphine and other drugs. Some specific causes of hypoxemia are discussed in the following text.

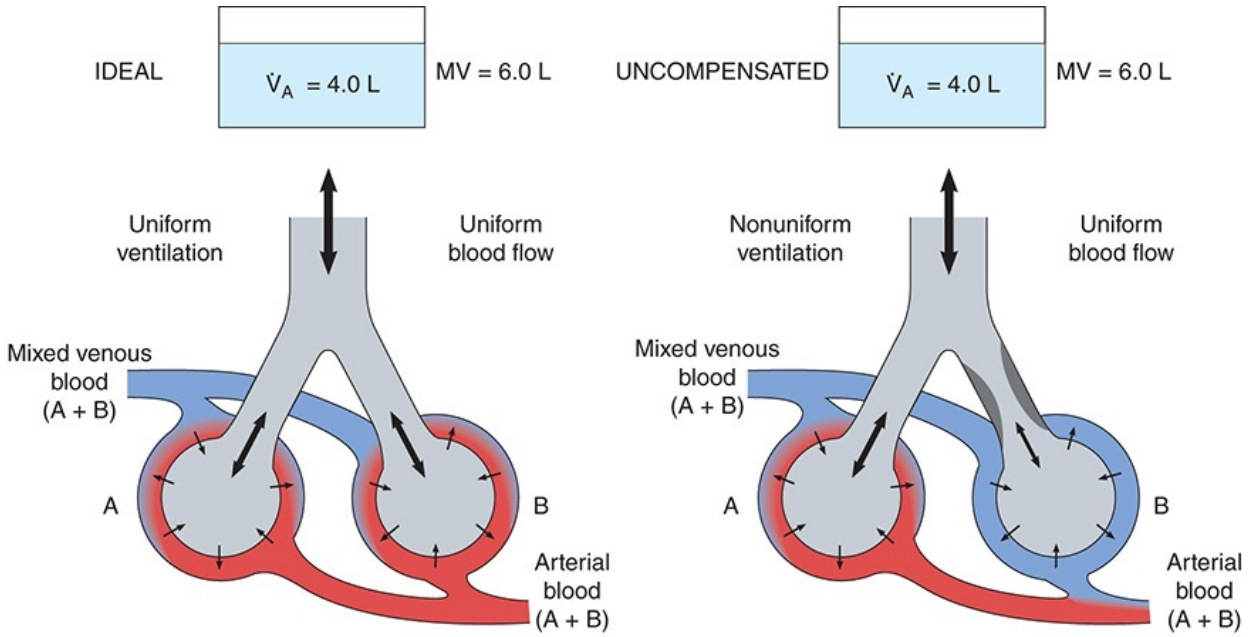
VENOUS-TO-ARTERIAL SHUNTS

When a cardiovascular abnormality such as an interatrial septal defect permits large amounts of unoxygenated venous blood to bypass the pulmonary capillaries and dilute the oxygenated blood in the systemic arteries (“right-to-left shunt”), chronic hypoxemia and cyanosis **(cyanotic congenital heart disease)** result. Administration of 100% O_2 raises the O_2 content of alveolar air but has little effect on hypoxia due to venous-to-arterial shunts. This is because the deoxygenated venous blood does not have the opportunity to get to the lung to be oxygenated.

VENTILATION–PERFUSION IMBALANCE

Patchy ventilation–perfusion imbalance is by far the most common cause of hypoxemia in clinical situations. In disease processes that prevent ventilation of some of the alveoli, the ventilation-blood flow ratios in different parts of the lung determine the extent to which systemic arterial P_{O_2} declines. If nonventilated alveoli are perfused, the nonventilated but perfused portion of the lung is in effect a right-to-left shunt, dumping unoxygenated blood into the left side of the heart. Lesser degrees of ventilation–perfusion imbalance are more common. In the example illustrated in **Figure 35–11**, the balanced ventilation–perfusion example on the left illustrates a uniform distribution throughout gas exchange. However, when ventilation is not in balance with perfusion, O_2

exchange is compromised. Note that the underventilated alveoli (B) have a low alveolar P_{O_2}, whereas the overventilated alveoli (A) have a high alveolar P_{O_2} while both have the same blood flow. The unsaturation of the hemoglobin of the blood coming from B is not completely compensated by the slightly greater saturation of the blood coming from A, because hemoglobin is normally nearly saturated in the lungs and the higher alveolar P_{O_2} adds only a little more O_2 to the hemoglobin than it normally carries. Consequently, the arterial blood is unsaturated. The CO_2 content of the arterial blood is generally normal in such situations, since extra loss of CO_2 in overventilated regions can balance diminished loss in underventilated areas.



	A	B	A + B
Alveolar ventilation (L/min)	2.0	2.0	4.0
Pulmonary blood flow (L/min)	2.5	2.5	5.0
Ventilation/blood flow ratio	0.8	0.8	0.8
Mixed venous O_2 saturation (%)	75.0	75.0	75.0
Arterial O_2 saturation (%)	97.4	97.4	97.4
Mixed venous O_2 tension (mm Hg)	40.0	40.0	40.0
Alveolar O_2 tension (mm Hg)	104.0	104.0	104.0
Arterial O_2 tension (mm Hg)	104.0	104.0	104.0

	A	B	A + B
Alveolar ventilation (L/min)	3.2	0.8	4.0
Pulmonary blood flow (L/min)	2.5	2.5	5.0
Ventilation/blood flow ratio	1.3	0.3	0.8
Mixed venous O_2 saturation (%)	75.0	75.0	75.0
Arterial O_2 saturation (%)	98.2	91.7	95.0
Mixed venous O_2 tension (mm Hg)	40.0	40.0	40.0
Alveolar O_2 tension (mm Hg)	116.0	66.0	106.0
Arterial O_2 tension (mm Hg)	116.0	66.0	84.0

FIGURE 35–11 Comparison of ventilation/blood flow relationships in health and disease. Left: "Ideal" ventilation/blood flow relationship. **Right:** Nonuniform ventilation and uniform blood flow, uncompensated. $\dot{V}_A$, alveolar ventilation; MV, respiratory minute volume. See text for details. (Reproduced with permission from Comroe JH Jr, et al: *The Lung: Clinical Physiology and*

Pulmonary Function Tests, 2nd ed. Year Book; 1962.)

OTHER FORMS OF HYPOXIA

ANEMIC HYPOXIA

Hypoxia due to anemia is not severe at rest unless the hemoglobin deficiency is marked, because 2,3-DPG increases in the red blood cells. However, anemic patients may have considerable difficulty during exercise because of a limited ability to increase O_2 delivery to the active tissues **(Figure 35–12)**.

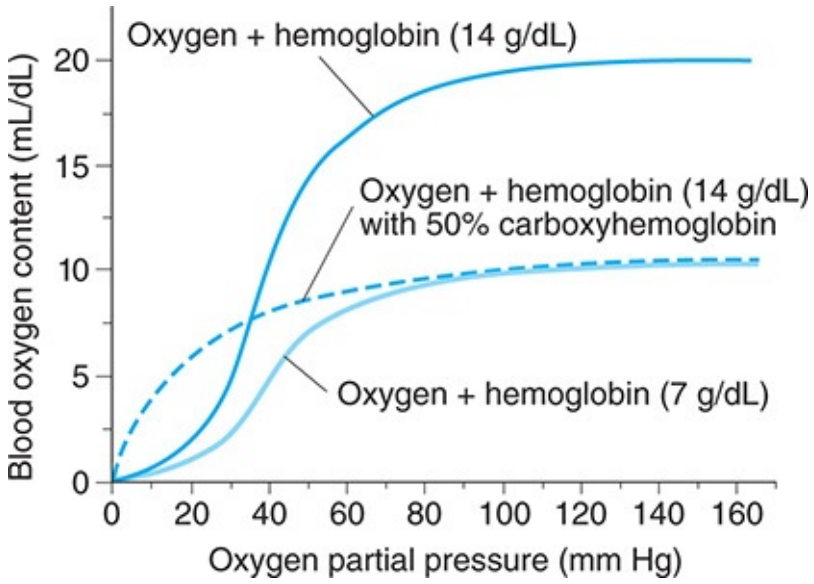


FIGURE 35–12 Effects of anemia and CO on hemoglobin binding of O_2. Normal oxyhemoglobin (14 g/dL hemoglobin) dissociation curve compared with anemia (7 g/dL hemoglobin) and with oxyhemoglobin dissociation curves in CO poisoning (50% arboxy-hemoglobin). Note that the CO-poisoning curve is shifted to the left of the anemia curve. (Reproduced with permission from Leff AR, Schumacker PT: *Respiratory Physiology: Basics and Applications*. Saunders; 1993.)

CARBON MONOXIDE POISONING

Small amounts of carbon monoxide (CO) are formed in the body, and this gas may function as a chemical messenger in the brain and elsewhere. In larger amounts, it is poisonous. Outside the body, it is formed by incomplete

combustion of carbon. It was used by the Greeks and Romans to execute criminals, and today it causes more deaths than any other gas. CO poisoning has become less common in the United States, since natural gas replaced other gases such as coal gas, which contain large amounts of CO. CO is, however, still readily available, as the exhaust of gasoline engines is 6% or more CO.

CO is toxic because it reacts with hemoglobin to form **carbon monoxyhemoglobin (carboxyhemoglobin, COHb),** and COHb does not take up O_2 (Figure 35–12). CO poisoning is often listed as a form of anemic hypoxia because the amount of hemoglobin that can carry O_2 is reduced, but the total hemoglobin content of the blood is unaffected by CO. The affinity of hemoglobin for CO is 210 times its affinity for O_2, and COHb liberates CO very slowly. An additional difficulty is that when COHb is present, the dissociation curve of the remaining HbO_2 shifts to the left, decreasing the amount of O_2 released. This is why an anemic individual who has 50% of the normal amount of HbO_2 may be able to perform moderate work, whereas an individual with HbO_2 reduced to the same level because of the formation of COHb is seriously incapacitated.

Because of the affinity of CO for hemoglobin, progressive COHb formation occurs when the alveolar P_{CO} is greater than 0.4 mm Hg. However, the amount of COHb formed depends on the duration of exposure to CO as well as the concentration of CO in the inspired air and the alveolar ventilation.

CO is also toxic to the cytochromes in the tissues, but the amount of CO required to poison the cytochromes is 1000 times the lethal dose; tissue toxicity thus plays no role in clinical CO poisoning.

The symptoms of CO poisoning are those of any type of hypoxia, especially headache and nausea, but there is little stimulation of respiration, since in the arterial blood, P_{O_2} remains normal and the carotid and aortic chemoreceptors are not stimulated. The cherry-red color of COHb is visible in the skin, nail beds, and mucous membranes. Death results when about 70–80% of the circulating hemoglobin is converted to COHb. The symptoms produced by chronic exposure to sublethal concentrations of CO are those of progressive brain damage, including mental changes and, sometimes, a parkinsonism-like state.

Treatment of CO poisoning consists of immediate termination of the exposure and adequate ventilation, by artificial respiration if necessary. Ventilation with O_2 is preferable to ventilation with fresh air, since O_2 hastens the dissociation of COHb. Hyperbaric oxygenation (see below) is useful in this condition.

ISCHEMIC HYPOXIA

Ischemic hypoxia, or stagnant hypoxia, is due to slow circulation and is a problem in organs such as the kidneys and heart during shock. The liver and possibly the brain are damaged by ischemic hypoxia in heart failure. The blood flow to the lung is normally very large, and it takes prolonged hypotension to produce significant damage. However, acute respiratory distress syndrome (ARDS) can develop when there is prolonged circulatory collapse.

HISTOTOXIC HYPOXIA

Hypoxia due to inhibition of tissue oxidative processes is most commonly the result of cyanide poisoning. Cyanide inhibits cytochrome oxidase and possibly other enzymes. Methylene blue or nitrites are used to treat cyanide poisoning. They act by forming **methemoglobin,** which then reacts with cyanide to form **cyanmethemoglobin,** a nontoxic compound. The extent of treatment with these compounds is, of course, limited by the amount of methemoglobin that can be safely formed. Hyperbaric oxygenation may also be useful.

OXYGEN TREATMENT OF HYPOXIA

Administration of oxygen-rich gas mixtures is of very limited value in hypoperfusion, anemic, and histotoxic hypoxia because all that can be accomplished in this way is an increase in the amount of dissolved O_2 in the arterial blood. This is also true in hypoxemia when it is due to shunting of unoxygenated venous blood past the lungs. In other forms of hypoxemia, O_2 is of great benefit. Treatment regimens that deliver less than 100% O_2 are of value both acutely and chronically, and administration of O_2 24 h/day for 2 years in this manner has been shown to significantly decrease the mortality of chronic obstructive pulmonary disease. O_2 toxicity and therapy are discussed in **Clinical Box 35–5**.

HYPERCAPNIA & HYPOCAPNIA

HYPERCAPNIA

Retention of CO_2 in the body **(hypercapnia)** initially stimulates respiration. Retention of larger amounts produces such symptoms as confusion, diminished sensory acuity and, eventually, coma with respiratory depression and death due to depression of the central nervous system. In patients with these symptoms, the P_{CO_2} is markedly elevated and severe respiratory acidosis is present. Large amounts of HCO_3^- are excreted, but more HCO_3^- is reabsorbed, raising the plasma HCO_3^- and partially compensating for the acidosis.

CO_2 is so much more soluble than O_2 that hypercapnia is rarely a problem in patients with pulmonary fibrosis. However, it does occur in ventilation–perfusion inequality and when for any reason alveolar ventilation is inadequate in the various forms of pump failure. It is exacerbated when CO_2 production is increased. For example, in febrile patients there is a 13% increase in CO_2 production for each 1°C rise in temperature, and a high carbohydrate intake increases CO_2 production because of the increase in the respiratory quotient. Normally, alveolar ventilation increases and the extra CO_2 is expired, but it accumulates when ventilation is compromised.

HYPOCAPNIA

Hypocapnia is the result of hyperventilation. During voluntary hyperventilation, the arterial P_{CO_2} falls from 40 mm Hg to as low as 15 mm Hg while the alveolar P_{O_2} rises to 120–140 mm Hg.

The more chronic effects of hypocapnia are seen in neurotic patients who chronically hyperventilate. Cerebral blood flow may be reduced 30% or more because of the direct constrictor effect of hypocapnia on the cerebral vessels. The cerebral ischemia causes light-headedness, dizziness, and paresthesias. Hypocapnia also increases cardiac output. It has a direct constrictor effect on many peripheral vessels, but it depresses the vasomotor center, so that the blood pressure is usually unchanged or only slightly elevated.

Other consequences of hypocapnia are due to the associated respiratory alkalosis, the blood pH being increased to 7.5 or 7.6. The plasma HCO_3^- level is low, but HCO_3^- reabsorption is decreased because of the inhibition of renal acid secretion by the low P_{CO_2}. The plasma total calcium level does not change, but the plasma Ca^{2+} level falls and hypocapnic individuals develop carpopedal spasm, a positive Chvostek sign, and other signs of tetany.

CLINICAL BOX 35–5

Administration of Oxygen & Its Potential Toxicity

It is interesting that while O_2 is necessary for life in aerobic organisms, it is also toxic. Indeed, 100% O_2 has been demonstrated to exert toxic effects not only in animals but also in bacteria, fungi, cultured animal cells, and plants. The toxicity seems to be due to the production of reactive oxygen species including superoxide anion (O_2^-) and H_2O_2. When 80–100% O_2 is administered to humans for periods of 8 h or more, the respiratory passages become irritated, causing substernal distress, nasal congestion, sore throat, and coughing.

A chronic condition characterized by lung cysts and densities **(bronchopulmonary dysplasia)** develops in some infants treated with O_2 for respiratory distress syndrome. This syndrome may be a manifestation of O_2 toxicity. Another complication in these infants is **retinopathy of prematurity (retrolental fibroplasia),** the formation of opaque vascular tissue in the eyes, which can lead to serious visual defects. The retinal receptors mature from the center to the periphery of the retina, and they use considerable O_2. This causes the retina to become vascularized in an orderly manner. Oxygen treatment before maturation is complete provides the needed O_2 to the photoreceptors, and consequently the normal vascular pattern fails to develop. Evidence indicates that this condition can be prevented or ameliorated by treatment with vitamin E, which exerts an anti-oxidant effect and, in animals, by growth hormone inhibitors.

Administration of 100% O_2 at increased pressure accelerates the onset of O_2 toxicity, with the production not only of tracheobronchial irritation but also of muscle twitching, ringing in the ears, dizziness, convulsions, and coma. The speed with which these symptoms develop is proportional to the pressure at which the O_2 is administered; for example, at 4 atm, symptoms develop in 30 min in half the patients, whereas at 6 atm, convulsions develop in a few minutes.

On the other hand, exposure to 100% O_2 at 2–3 atm can increase dissolved O_2 in arterial blood to the point that arterial O_2 tension is greater than 2000 mm Hg and tissue O_2 tension is 400 mm Hg. If exposure is limited to 5 h or

less at these pressures, O_2 toxicity is not a problem. Therefore, **hyperbaric O_2** therapy in closed tanks is used to treat diseases in which improved oxygenation of tissues cannot be achieved in other ways. It is of demonstrated value in carbon monoxide poisoning, radiation-induced tissue injury, gas gangrene, very severe blood loss anemia, diabetic leg ulcers, and other wounds that are slow to heal, and rescue of skin flaps and grafts in which the circulation is marginal. It is also the primary treatment for decompression sickness and air embolism.

In hypercapnic patients in severe pulmonary failure, the CO_2 level may be so high that it depresses rather than stimulates respiration. Some of these patients keep breathing only because the carotid and aortic chemoreceptors drive the respiratory center. If the hypoxic drive is withdrawn by administering O_2, breathing may stop. During the resultant apnea, the arterial P_{O_2} drops but breathing may not start again, as P_{CO_2} further depresses the respiratory center. Therefore, O_2 therapy in this situation must be started with care.

CHAPTER SUMMARY

- Partial pressure differences between air and blood for O_2 and CO_2 dictate a net flow of O_2 into the blood and CO_2 out of the blood in the pulmonary system.
- The amount of O_2 in the blood is determined by the amount dissolved (minor) and the amount bound (major) to hemoglobin. Each hemoglobin molecule contains four subunits that each can bind O_2. Hemoglobin O_2 binding is cooperative and also affected by pH, temperature, and the concentration of 2,3-diphosphoglycerate (2,3-DPG).
- CO_2 in blood is rapidly converted into H_2CO_3 due to the activity of carbonic anhydrase. CO_2 also readily forms carbamino compounds with blood proteins (including hemoglobin). The rapid net loss of CO_2 allows more CO_2 to dissolve in blood.
- The pH of plasma is 7.4. A decrease in plasma pH is termed **acidosis** and an increase of plasma pH is termed **alkalosis.** A short-term change in arterial P_{CO_2} due to decreased ventilation results in respiratory acidosis. A short-

term change in arterial P_{CO_2} due to increased ventilation results in respiratory alkalosis. Metabolic acidosis occurs when strong acids are added to the blood, and metabolic alkalosis occurs when strong bases are added to (or strong acids are removed from) the blood.

- Respiratory compensation to acidosis or alkalosis involves quick changes in ventilation. Such changes effectively change the P_{CO_2} in the blood plasma. Renal compensation mechanisms are much slower and involve H^+ secretion or HCO_3^- reabsorption.
- Hypoxia is a deficiency of O_2 at the tissue level. Hypoxia has powerful consequences at the cellular, tissue, and organ level: It can alter cellular transcription factors and thus protein expression; it can quickly alter brain function and produce symptoms similar to alcohol (eg, dizziness, impaired mental function, drowsiness, headache); and it can affect ventilation. Long-term hypoxia results in cell and tissue death.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. Most of the CO_2 transported in the blood is

   A. dissolved in plasma.

   B. in carbamino compounds formed from plasma proteins.

   C. in carbamino compounds formed from hemoglobin.

   D. bound to Cl^-.

   E. in HCO_3^-.

2. Which of the following has the greatest effect on the ability of blood to transport oxygen?

   A. Capacity of the blood to dissolve oxygen

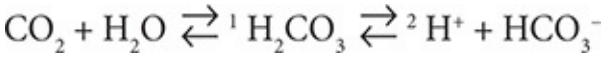
   B. Amount of hemoglobin in the blood

   C. pH of plasma

   D. CO_2 content of red blood cells

   E. Temperature of the blood

3. Which of the following is true of the system?



A. Reaction 2 is catalyzed by carbonic anhydrase.

B. Because of reaction 2, the pH of blood declines during hyperventilation.

C. Reaction 1 occurs in the red blood cell.

D. Reaction 1 occurs primarily in plasma.

E. The reactions move to the right when there is excess H^+ in the tissues.

4. In comparing uncompensated respiratory acidosis and uncompensated metabolic acidosis which one of the following is true?

A. Plasma pH change is always greater in uncompensated respiratory acidosis compared to uncompensated metabolic acidosis.

B. There are no compensation mechanisms for respiratory acidosis, whereas there is respiratory compensation for metabolic acidosis.

C. Uncompensated respiratory acidosis involves changes in plasma [HCO_3^-], whereas plasma [HCO_3^-] is unchanged in uncompensated metabolic acidosis.

D. Uncompensated respiratory acidosis is associated with a change in P_{CO_2}, whereas in uncompensated metabolic acidosis P_{CO_2} is constant.

CHAPTER 36

Regulation of Respiration

OBJECTIVES

After studying this chapter, you should be able to:

- Locate the pre-Bötzinger complex and describe its role in producing spontaneous respiration.
- Identify the location and probable functions of the dorsal and ventral groups of respiratory neurons, the pneumotaxic center, and the apneustic center in the brainstem.
- List the specific respiratory functions of the vagus nerves and the respiratory receptors in the carotid body, the aortic body, and the ventral surface of the medulla oblongata.
- Describe and explain the ventilatory responses to increased CO_2 concentrations in the inspired air.
- Describe and explain the ventilatory responses to decreased O_2 concentrations in the inspired air.
- Describe the effects of each of the main nonchemical factors that influence respiration.
- Describe the effects of exercise on ventilation and O_2 exchange in the tissues.

- Define periodic breathing and explain its occurrence in various disease states.

INTRODUCTION

Spontaneous respiration is produced by rhythmic discharge of motor neurons that innervate the respiratory muscles. This discharge is totally dependent on nerve impulses from the brain; breathing stops if the spinal cord is transected above the origin of the phrenic nerves. The rhythmic discharges from the brain that produce spontaneous respiration are regulated by alterations in arterial P_{O_2}, P_{CO_2}, and H^+ concentration, and this chemical control of breathing is supplemented by a number of nonchemical influences. The overall respiration control system thus consists of a network of neurons in cortex and medulla/pons that exert voluntary control and automatic control, respectively. The system adjusts the rate of ventilation as environmental conditions change (eg, from normoxic to hypoxic condition) and arterial P_{O_2}, P_{CO_2}, and H^+ concentration ($[H^+]$) alter. The mechano- and chemoreceptors in the system sense the changes in force/displacement and arterial levels of gases and metabolites, and adjust the rate of ventilation to ensure optimal gas exchange in the lungs **(Figure 36–1)**. The physiological bases for these phenomena are discussed in this chapter.

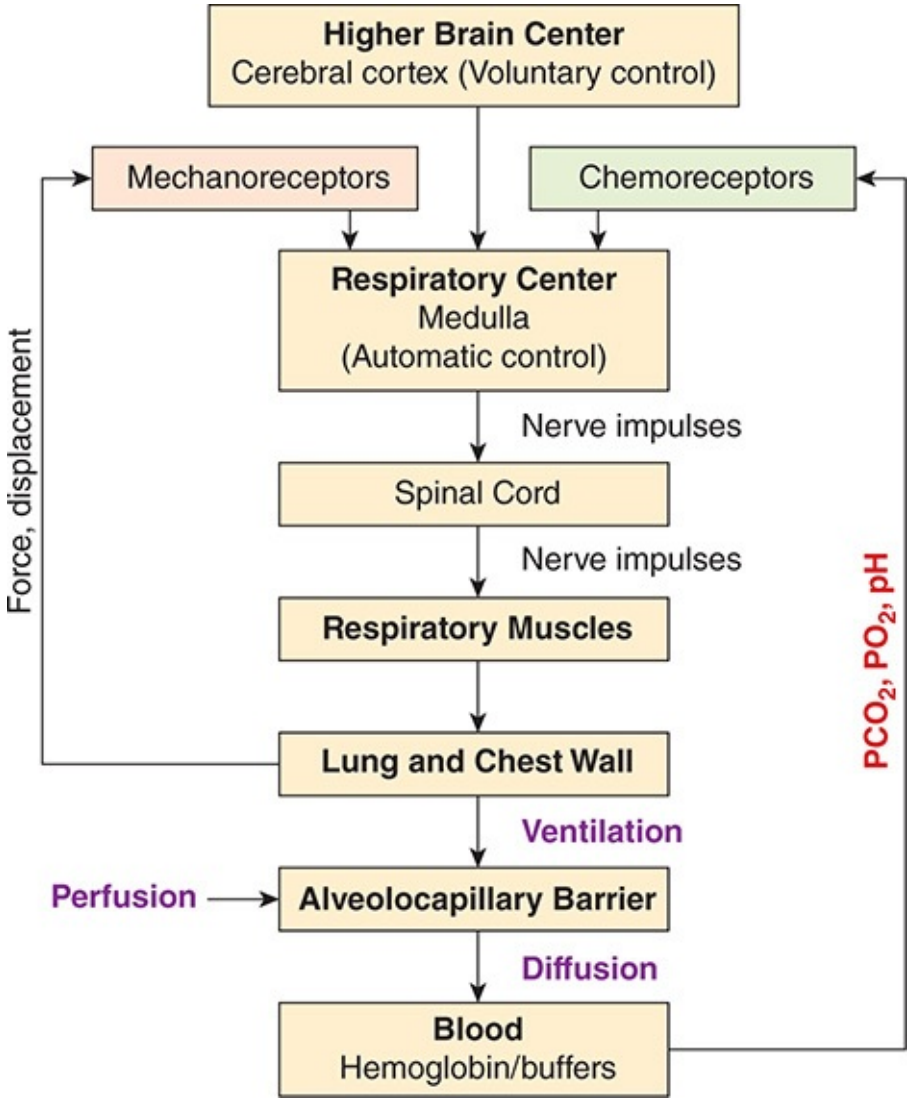


FIGURE 36–1 Diagram of the overall respiratory control system. The volunteer control center is in cerebral cortex and the automatic control centers are in medulla and pons. The nerve impulses or electrical signals from the respiratory neurons in these centers regulate the activity of respiratory muscles (including diaphragm) and thus the rate and depth of breathing (ventilation). The mechanoreceptors and chemoreceptors (central and peripheral) then sense the physical changes in the lungs and chest wall and the chemical changes in the blood (eg, arterial P_{CO_2}, arterial P_{O_2} and $[H^+]$) to further adjust the control of breathing. (Modified from Berne RM, Levy MN (eds): *Physiology*, 2nd ed. Mosby, 1988.)

NEURAL CONTROL OF BREATHING

CONTROL SYSTEMS

Two separate neural mechanisms regulate respiration. One is responsible for **voluntary control** and the other for **automatic control.** The voluntary control system is located in the cerebral cortex and sends impulses to the respiratory motor neurons via the corticospinal tracts. The automatic system is driven by a group of pacemaker cells in the medulla. Impulses from these cells activate motor neurons in the cervical and thoracic spinal cord that innervate inspiratory muscles. Those in the cervical cord activate the diaphragm via the phrenic nerves, and those in the thoracic spinal cord activate the external intercostal muscles. However, the impulses also reach the innervation of the internal intercostal muscles and other expiratory muscles.

The motor neurons to the expiratory muscles are inhibited when those supplying the inspiratory muscles are active, and vice versa. Although spinal reflexes contribute to this **reciprocal innervation,** it is due primarily to activity in descending pathways. Impulses in these descending pathways excite agonists and inhibit antagonists. The one exception to the reciprocal inhibition is a small amount of activity in phrenic axons for a short period after inspiration. The function of this postinspiratory output appears to be to brake the lung's elastic recoil and make respiration smooth.

MEDULLARY SYSTEMS

The main components of the **respiratory control pattern generator** responsible for automatic respiration are located in the medulla. Rhythmic respiration is initiated by a small group of synaptically coupled pacemaker cells in the **pre-Bötzinger complex** (pre-BÖTC) on either side of the medulla between the nucleus ambiguus and the lateral reticular nucleus **(Figure 36–2)**. These neurons discharge rhythmically, and they produce rhythmic discharges in phrenic motor neurons that are abolished by sections between the pre-BÖTC complex and these motor neurons. They also contact the hypoglossal nuclei, and the tongue is involved in the regulation of airway resistance.

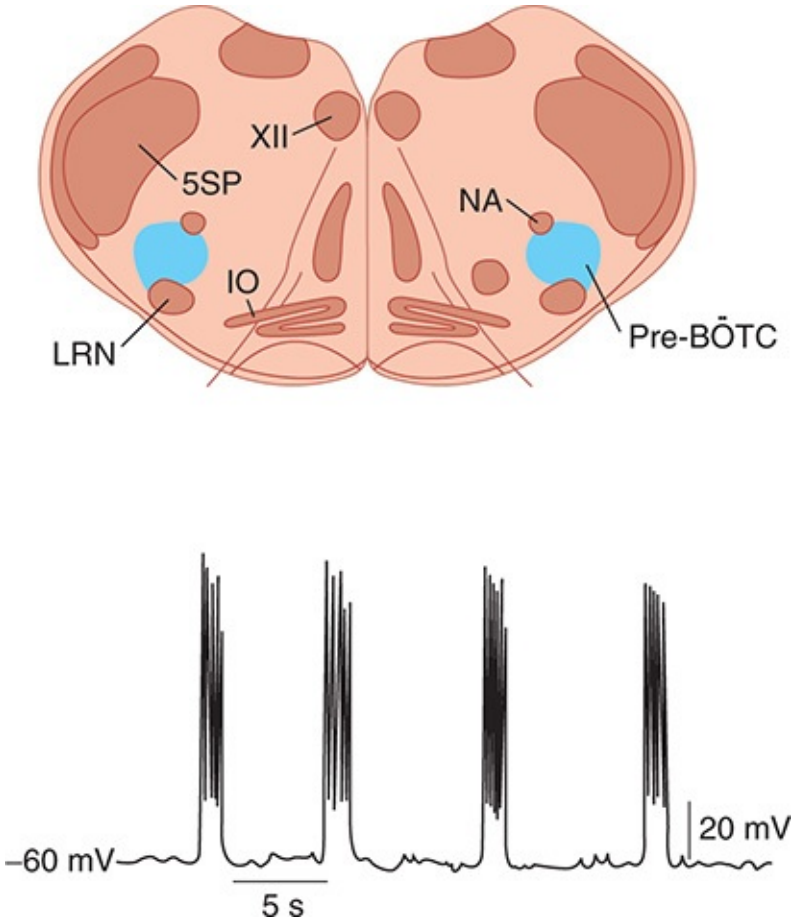


FIGURE 36–2 Pacemaker cells in the pre-Bötzinger complex (pre-BÖTC). **Top:** Anatomic diagram of the pre-BÖTC from a neonatal rat. **Bottom:** Sample rhythmic discharge tracing of neurons in the pre-BÖTC complex from a brain slice of a neonatal rat. IO, inferior olive; LRN, lateral reticular nucleus; NA, nucleus ambiguus; XII, nucleus of 12th cranial nerve; 5SP, spinal nucleus of trigeminal nerve. (Modified with permission from Feldman JC, Gray PA: Sighs and gasps in a dish. Nat Neurosci 2000;3(6):531–532.)

Neurons in the pre-BÖTC complex discharge rhythmically in brain slice preparations in vitro, and if the slices become hypoxic, discharge changes to one associated with gasping. Addition of cadmium to the slices causes occasional sigh-like discharge patterns. There are NK1 receptors and μ-opioid receptors on these neurons and, in vivo, substance P stimulates and opioids inhibit respiration. Depression of respiration is a side effect that limits the use of opioids in the treatment of pain. However, it is now known that the 5-hydroxytryptamine receptor 4 (5-HT_4 receptor) is present in the pre-BÖTC complex and treatment with 5-HT_4 agonists blocks the inhibitory effect of opioids on respiration in

experimental animals, without inhibiting their analgesic effect.

In addition, dorsal and ventral groups of respiratory neurons are present in the medulla **(Figure 36–3)**. However, lesions of these neurons do not abolish respiratory activity, and they apparently project to the pre-BÖTC pacemaker neurons.

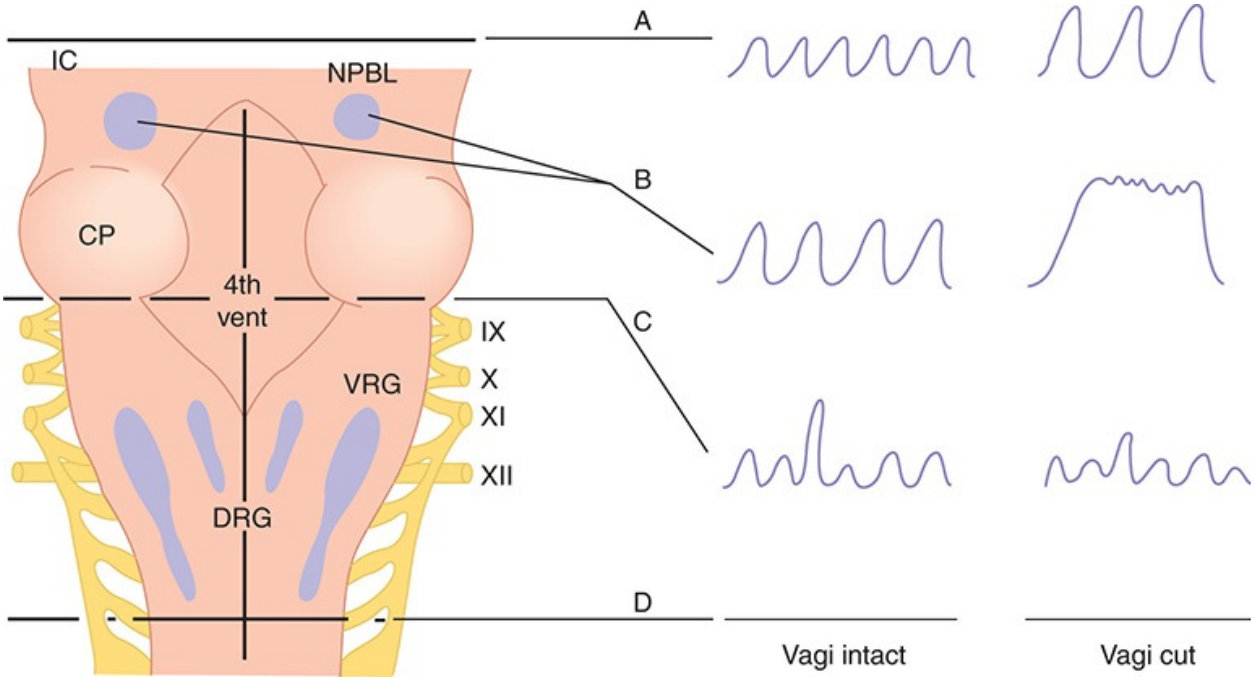


FIGURE 36–3 Respiratory neurons in the brainstem. Dorsal view of brainstem; cerebellum removed. The effects of various lesions and brainstem transections are shown; the spirometer tracings at the right indicate the depth and rate of breathing. If a lesion is introduced at D, breathing ceases. The effects of higher transections, with and without vagus nerves transection, are shown (see text for details). CP, middle cerebellar peduncle; DRG, dorsal group of respiratory neurons; IC, inferior colliculus; NPBL, nucleus parabrachialis (pneumotaxic center); VRG, ventral group of respiratory neurons; 4th vent, fourth ventricle. The roman numerals identify cranial nerves. (Modified with permission from Mitchell RA, Berger A: State of the art: Review of neural regulation of respiration. Am Rev Respir Dis 1975 Feb;111(2):206-224.)

PONTINE & VAGAL INFLUENCES

Although the rhythmic discharge of medullary neurons concerned with respiration is spontaneous, it is modified by neurons in the pons and afferents in

the vagus from receptors in the airways and lungs. An area known as the **pneumotaxic center** in the medial parabrachial and Kölliker-Fuse nuclei of the dorsolateral pons contains neurons active during inspiration and neurons active during expiration. When this area is damaged, respiration becomes slower and tidal volume greater, and when the vagi are also cut in anesthetized animals, there are prolonged inspiratory spasms that resemble breath holding (**apneusis;** section B in Figure 36–3). The normal function of the pneumotaxic center is unknown, but it may play a role in switching between inspiration and expiration.

Stretching of the lungs during inspiration initiates impulses in afferent pulmonary vagal fibers. These impulses inhibit inspiratory discharge. This is why the depth of inspiration is increased after vagotomy (Figure 36–3) and apneusis develops if the vagi are cut after damage to the pneumotaxic center. Vagal feedback activity does not alter the rate of rise of the neural activity in respiratory motor neurons **(Figure 36–4)**.

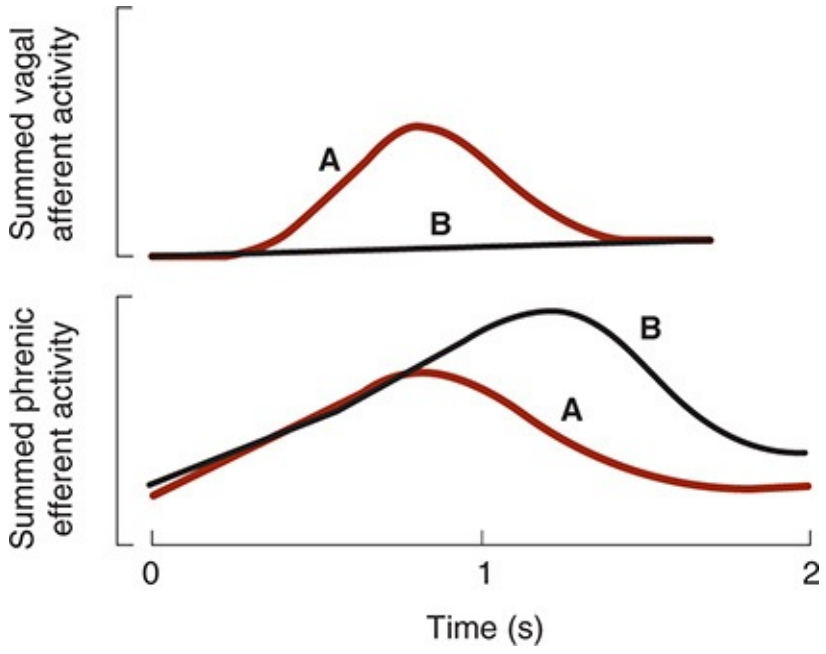


FIGURE 36–4 Afferent vagal fibers inhibit inspiratory discharge. Superimposed records of two breaths: **A)** with and **B)** without feedback vagal afferent activity from stretch receptors in the lungs. Note that the rate of rise in phrenic nerve activity to the diaphragm is unaffected but the discharge is prolonged in the absence of vagal input.

When the activity of the inspiratory neurons is increased in intact animals, the rate and the depth of breathing are increased. The depth of respiration is

increased because the lungs are stretched to a greater degree before the amount of vagal and pneumotaxic center inhibitory activity is sufficient to overcome the more intense inspiratory neuron discharge. The respiratory rate is increased because the after-discharge in the vagal and possibly the pneumotaxic afferents to the medulla is rapidly overcome.

REGULATION OF RESPIRATORY ACTIVITY

A rise in the P_{CO_2} or H^+ concentration of arterial blood or a drop in its P_{O_2} increases the level of respiratory neuron activity in the medulla, and changes in the opposite direction have a slight inhibitory effect. The effects of variations in blood chemistry on ventilation are mediated via respiratory **chemoreceptors**—the carotid and aortic bodies and collections of cells in the medulla and elsewhere that are sensitive to changes in the chemistry of the blood. They initiate impulses that stimulate the respiratory center. Superimposed on this basic **chemical control of respiration,** other afferents provide nonchemical controls that affect breathing in particular situations **(Table 36–1)**.

TABLE 36–1 Stimuli affecting the respiratory center.

Chemical control
CO_2 (via CSF and brain interstitial fluid H^+ concentration)
O_2, H^+ (via carotid and aortic bodies)
Nonchemical control
Vagal afferents from receptors in the airways and lungs
Afferents from the pons, hypothalamus, and limbic system
Afferents from proprioceptors
Afferents from baroreceptors: arterial, atrial, ventricular, pulmonary

CHEMICAL CONTROL OF BREATHING

The chemical regulatory mechanisms adjust ventilation in such a way that the alveolar P_{CO_2} is normally held constant, the effects of excess H^+ in the blood are combated, and the P_{O_2} is raised when it falls to a potentially dangerous level. The respiratory minute volume is proportional to the metabolic rate, but the link

between metabolism and ventilation is CO_2, not O_2. The receptors in the carotid and aortic bodies are stimulated by a rise in the P_{CO_2} or H^+ concentration of arterial blood or a decline in its P_{O_2}. After denervation of the carotid chemoreceptors, the response to a drop in P_{O_2} is abolished; the predominant effect of hypoxia after denervation of the carotid bodies is a direct depression of the respiratory center. The response to changes in arterial blood H^+ concentration in the pH 7.3–7.5 range is also abolished, although larger changes exert some effect. The response to changes in arterial P_{CO_2}, on the other hand, is affected only slightly; it is reduced no more than 30–35%.

CAROTID & AORTIC BODIES

There is a carotid body near the carotid bifurcation on each side, and there are usually two or more aortic bodies near the arch of the aorta **(Figure 36–5)**. Each carotid and aortic body **(glomus)** contains islands of two types of cells, type I and type II cells, surrounded by fenestrated sinusoidal capillaries. The type I or **glomus cells** are closely associated with cuplike endings of the afferent nerves **(Figure 36–6)**. The glomus cells resemble adrenal chromaffin cells and have dense-core granules containing catecholamines that are released upon exposure to hypoxia and cyanide. The cells are excited by hypoxia (potentially by hypoxia-induced inhibition of O_2-sensitive K^+ channels), and the principal transmitter appears to be dopamine, which excites the nerve endings by way of D_2 receptors. The type II cells are glia-like, and each surrounds four to six type I cells. The function of type II cells is not fully defined.

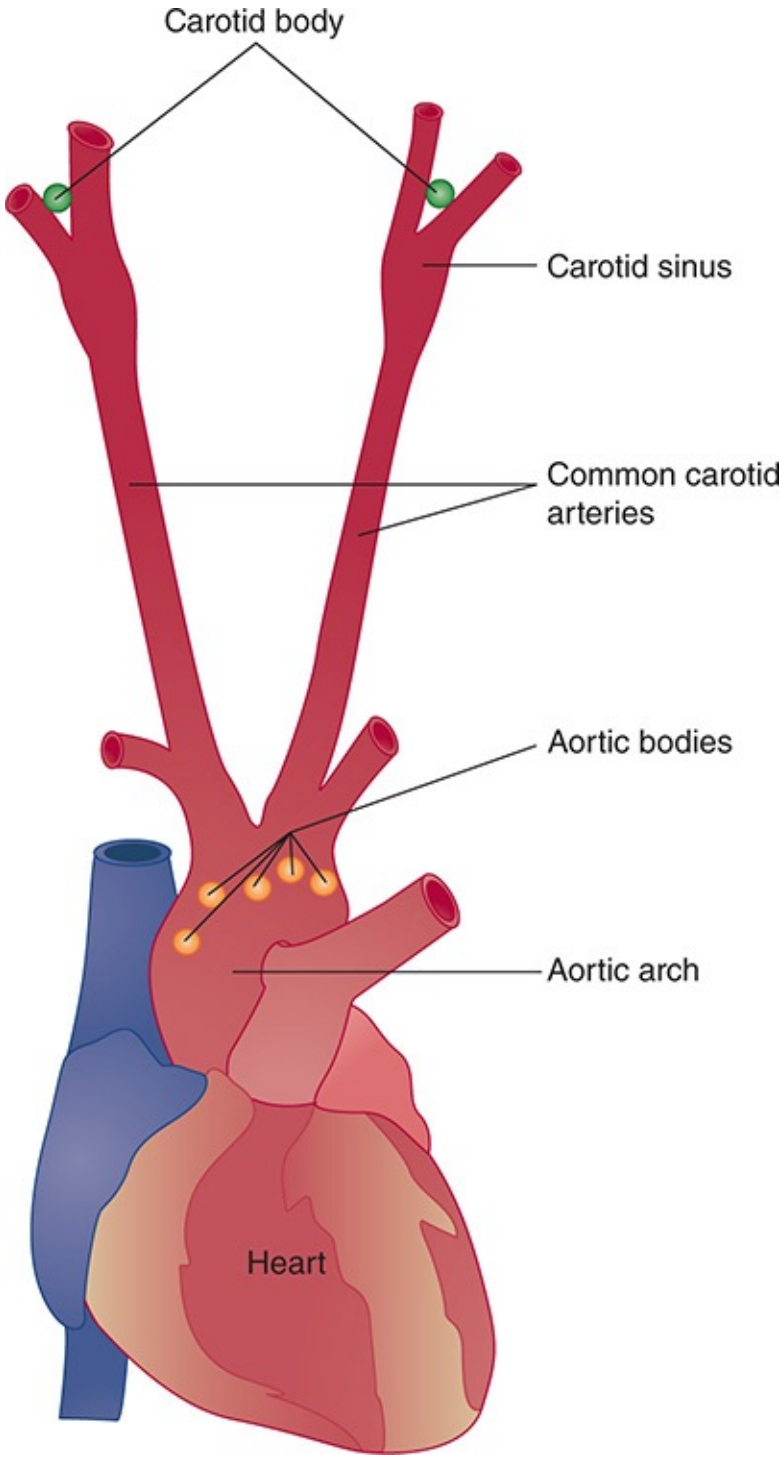


FIGURE 36–5 Location of carotid and aortic bodies. Carotid bodies are positioned near a major arterial baroreceptor, the carotid sinus. Aortic bodies are shown near the aortic arch.

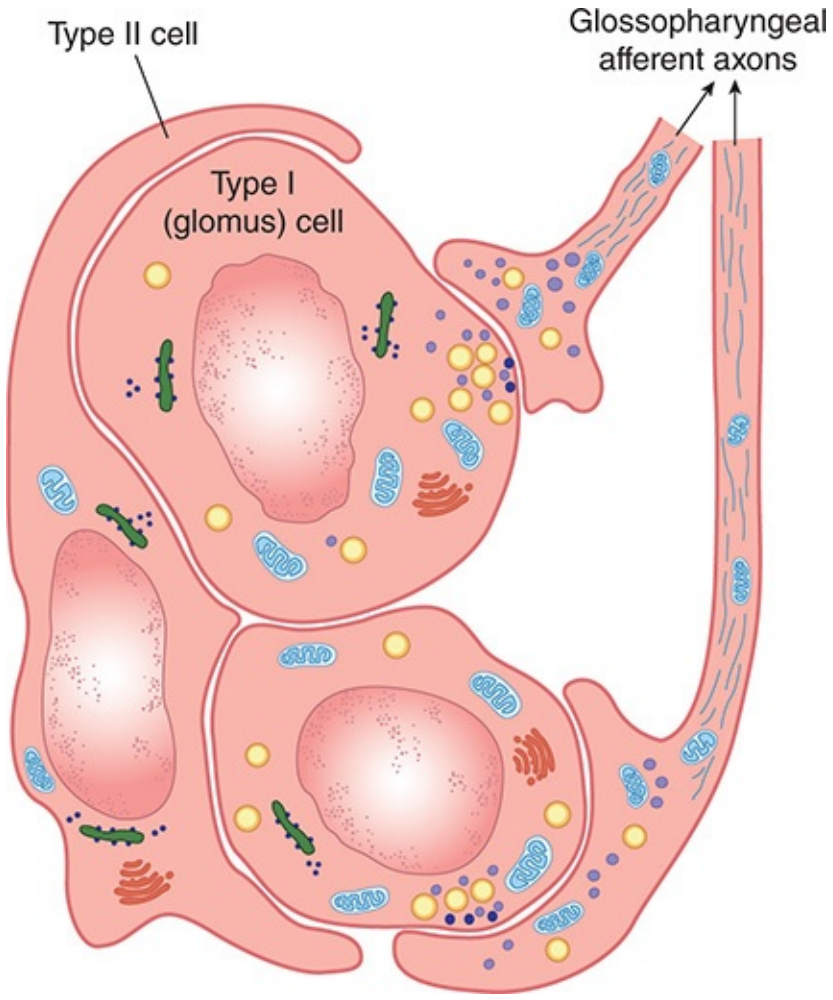


FIGURE 36–6 Organization of the carotid body. Type I (glomus) cells contain catecholamines. When exposed to hypoxia, they release their catecholamines, which stimulate the cuplike endings of the carotid sinus nerve fibers in the glossopharyngeal nerve. The glia-like type II cells surround the type I cells and probably have a sustentacular function.

Outside the capsule of each body, the nerve fibers acquire a myelin sheath; however, they are only 2–5 µm in diameter and conduct at the relatively low rate of 7–12 m/s. Afferents from the carotid bodies ascend to the medulla via the carotid sinus and glossopharyngeal nerves, and fibers from the aortic bodies ascend in the vagi. Studies in which one carotid body has been isolated and perfused while recordings are being taken from its afferent nerve fibers show that there is a graded increase in impulse traffic in these afferent fibers as the P_{O_2} of the perfusing blood is lowered **(Figure 36–7)** or the P_{CO_2} is raised.

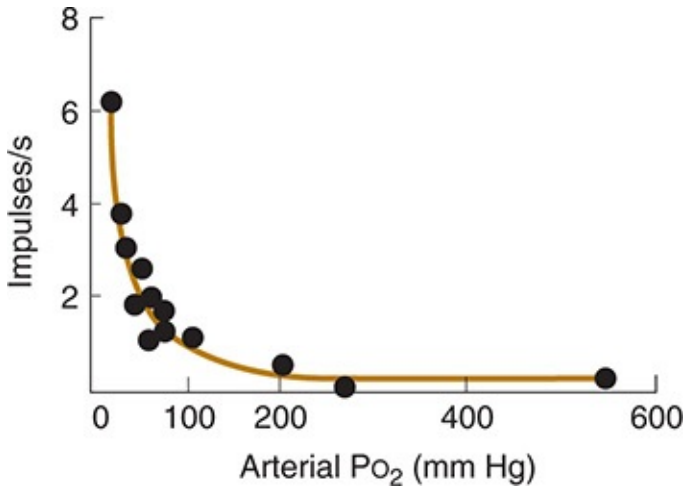


FIGURE 36–7 Effect of P_{CO_2} on afferent nerve firing. The rate of discharge of a single afferent fiber from the carotid body (circles) is plotted at several P_{O_2} values and fitted to a line. A sharp increase in firing rate is observed as P_{O_2} falls below normal resting levels (ie, near 100 mm Hg). (Used with permission of S. Sampson.)

Type I glomus cells have O_2-sensitive K^+ channels, whose conductance is reduced in proportion to the degree of hypoxia to which they are exposed. This reduces the K^+ efflux, depolarizing the cell and causing Ca^{2+} influx, primarily via L-type voltage-gated Ca^{2+} channels. The Ca^{2+} influx triggers action potentials and transmitter release, with consequent excitation of the afferent nerve endings. The smooth muscle cells of pulmonary arteries contain similar O_2-sensitive K^+ channels, which mediate the vasoconstriction caused by hypoxia. This is in contrast to systemic arteries, which contain ATP-sensitive K^+ channels that permit more K^+ efflux with hypoxia and consequently cause vasodilation instead of vasoconstriction.

The blood flow in each 2 mg carotid body is about 0.04 mL/min, or 2000 mL/100 g of tissue/min compared with a blood flow of 54 mL or 420 mL per 100 g/min in the brain and kidneys, respectively. Because the blood flow per unit of tissue is so enormous, the O_2 needs of the cells can be met largely by dissolved O_2 alone. Therefore, the receptors are not stimulated in conditions such as anemia or carbon monoxide poisoning, in which the amount of dissolved O_2 in the blood reaching the receptors is generally normal, even though the combined O_2 in the blood is markedly decreased. The receptors are stimulated when the arterial P_{O_2} is low or when, because of vascular stasis, the amount of

O_2 delivered to the receptors per unit time is decreased. Powerful stimulation is also produced by cyanide, which prevents O_2 utilization at the tissue level. In sufficient doses, nicotine and lobeline activate the chemoreceptors. It has also been reported that infusion of K^+ increases the discharge rate in chemoreceptor afferents, and because the plasma K^+ level is increased during exercise, the increase may contribute to exercise-induced hyperpnea.

Because of their anatomic location, the aortic bodies have not been studied in as detail as the carotid bodies. Their responses are probably similar but of lesser magnitude. In humans in whom both carotid bodies have been removed but the aortic bodies left intact, the responses are essentially the same as those following denervation of both carotid and aortic bodies in animals: little change in ventilation at rest, but the ventilatory response to hypoxia is lost and the ventilatory response to CO_2 is reduced by 30%.

Neuroepithelial bodies composed of innervated clusters of amine-containing cells are found in the airways. These cells have an outward K^+ current that is reduced by hypoxia, and this would be expected to produce depolarization. However, the function of these hypoxia-sensitive cells is uncertain because, as noted above, removal of the carotid bodies alone abolishes the respiratory response to hypoxia.

CHEMORECEPTORS IN THE BRAINSTEM

The chemoreceptors that mediate the hyperventilation produced by increases in arterial P_{CO_2} after the carotid and aortic bodies are denervated are located in the medulla oblongata and consequently are called **medullary chemoreceptors.** They are separate from the dorsal and ventral respiratory neurons and are located on the ventral surface of the medulla **(Figure 36–8)**. Recent evidence indicates that additional chemoreceptors are located in the vicinity of the solitary tract nuclei, the locus coeruleus, and the hypothalamus.

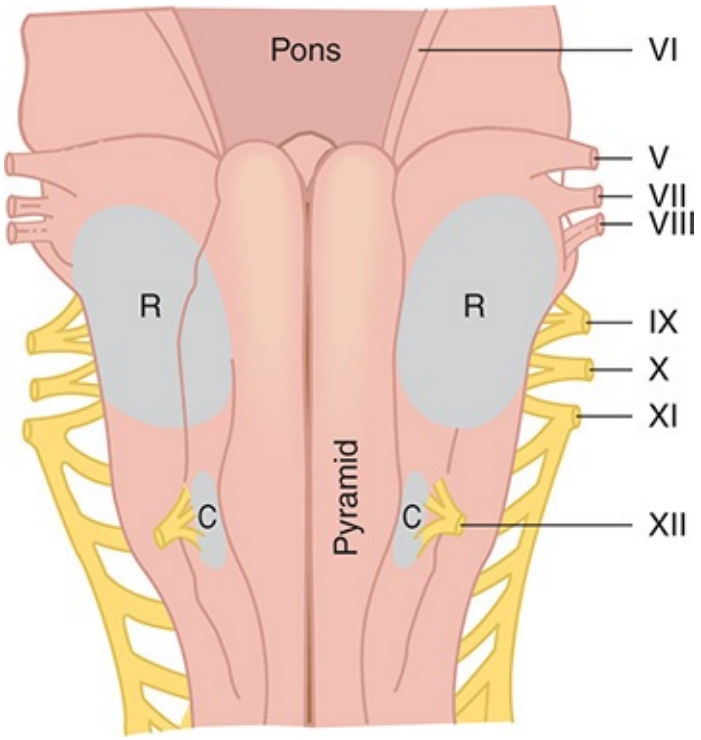


FIGURE 36–8 Rostral (R) and caudal (C) chemosensitive areas on the ventral surface of the medulla. Cranial nerves, pyramid, and pons are labeled for reference.

The chemoreceptors monitor the H^+ concentration of cerebrospinal fluid (CSF), including the brain interstitial fluid. CO_2 readily penetrates membranes, including the blood-brain barrier, whereas H^+ and HCO_3^- penetrate slowly. The CO_2 that enters the brain and CSF is promptly hydrated. The H_2CO_3 dissociates, so that the local H^+ concentration rises. The H^+ concentration in brain interstitial fluid parallels the arterial P_{CO_2}. Experimentally produced changes in the P_{CO_2} of CSF have minor, variable effects on respiration as long as the H^+ concentration is held constant, but any increase in spinal fluid H^+ concentration stimulates respiration. The magnitude of the stimulation is proportional to the rise in H^+ concentration. Thus, the effects of CO_2 on respiration are mainly due to its movement into the CSF and brain interstitial fluid, where it increases the H^+ concentration and stimulates receptors sensitive to H^+.

VENTILATORY RESPONSES TO CHANGES IN

ACID–BASE BALANCE

In metabolic acidosis due, for example, to the accumulation of acid ketone bodies in the circulation in diabetes mellitus, there is pronounced respiratory stimulation (Kussmaul breathing). The hyperventilation decreases alveolar P_{CO_2} ("blows off CO_2") and thus produces a compensatory fall in blood H^+ concentration. Conversely, in metabolic alkalosis due, for example, to protracted vomiting with loss of HCl from the body, ventilation is depressed and the arterial P_{CO_2} rises, raising the H^+ concentration toward normal. If there is an increase in ventilation that is not secondary to a rise in arterial H^+ concentration, the drop in P_{CO_2} lowers the H^+ concentration below normal **(respiratory alkalosis);** conversely, hypoventilation that is not secondary to a fall in plasma H^+ concentration causes **respiratory acidosis.**

VENTILATORY RESPONSES TO CO_2

The arterial P_{CO_2} is normally maintained at 40 mm Hg. When arterial P_{CO_2} rises as a result of increased tissue metabolism, ventilation is stimulated and the rate of pulmonary excretion of CO_2 increases until the arterial P_{CO_2} falls to normal, shutting off the stimulus. The operation of this feedback mechanism keeps CO_2 excretion and production in balance.

When a gas mixture containing CO_2 is inhaled, the alveolar P_{CO_2} rises, elevating the arterial P_{CO_2} and stimulating ventilation as soon as the blood that contains more CO_2 reaches the medulla. CO_2 elimination is increased, and the alveolar P_{CO_2} drops toward normal. This is why relatively large increments in the P_{CO_2} of inspired air (eg, 15 mm Hg) produce relatively slight increments in alveolar P_{CO_2} (eg, 3 mm Hg). However, the P_{CO_2} does not drop to normal, and a new equilibrium is reached at which the alveolar P_{CO_2} is slightly elevated and the hyperventilation persists as long as CO_2 is inhaled. The essentially linear relationship between respiratory minute volume and the alveolar P_{CO_2} is shown in **Figure 36–9**.

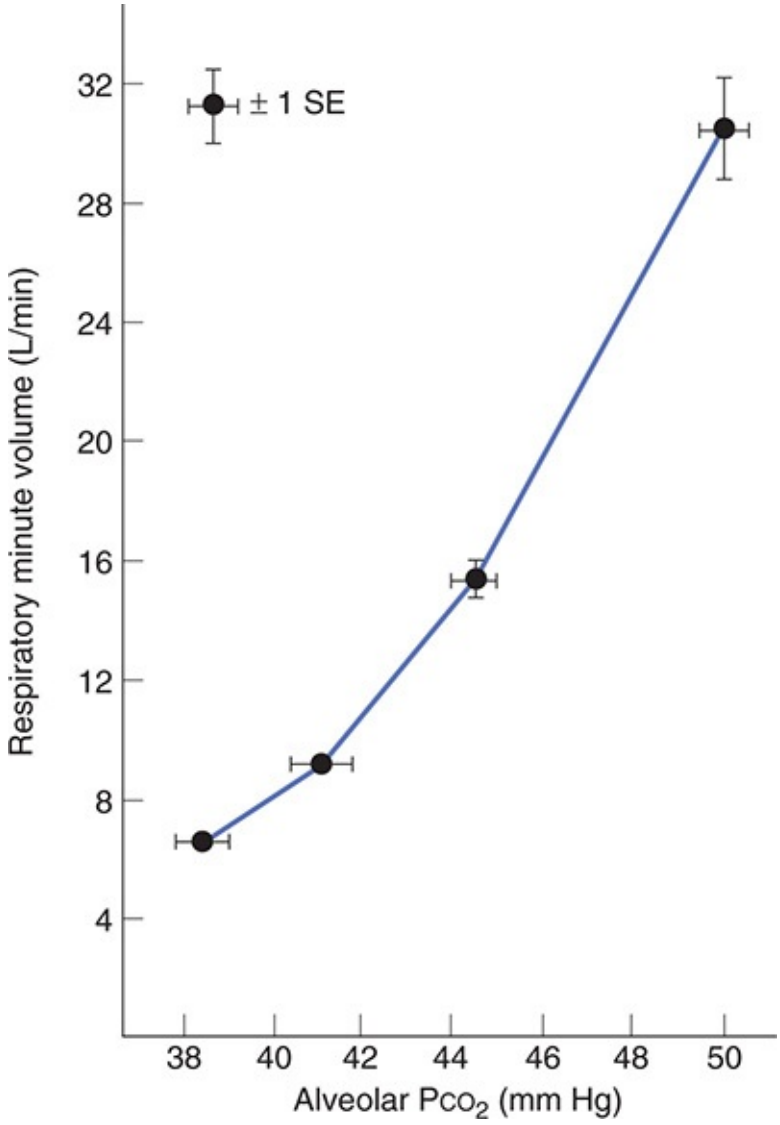


FIGURE 36–9 Responses of normal subjects to inhaling O_2 and approximately 2, 4, and 6% CO_2. The relatively linear increase in respiratory minute volume in response to increased CO_2 is due to an increase in both the depth and rate of respiration. (Reproduced with permission from Mountcastle VB (ed): *Medical Physiology*, 13th ed. Mosby; 1974.)

Of course, this linearity has an upper limit. When the P_{CO_2} of the inspired gas is close to the alveolar P_{CO_2}, elimination of CO_2 becomes difficult. When the CO_2 content of the inspired gas is more than 7%, the alveolar and arterial P_{CO_2} begin to rise abruptly in spite of hyperventilation. The resultant accumulation of CO_2 in the body **(hypercapnia)** depresses the central nervous system, including

the respiratory center, and produces headache, confusion, and eventually coma **(CO_2 narcosis).**

VENTILATORY RESPONSE TO OXYGEN DEFICIENCY

When the O_2 content of the inspired air is decreased, respiratory minute volume is increased. The stimulation is slight when the P_{O_2} of the inspired air is more than 60 mm Hg, and marked stimulation of respiration occurs only at lower P_{O_2} values **(Figure 36–10)**. However, any decline in arterial P_{O_2} below 100 mm Hg produces increased discharge in the nerves from the carotid and aortic chemoreceptors. There are two reasons why this increase in impulse traffic does not increase ventilation to any extent in normal individuals until the P_{O_2} is less than 60 mm Hg. First, because Hb is a weaker acid than HbO_2, there is a slight decrease in the H^+ concentration of arterial blood when the arterial P_{O_2} falls and hemoglobin becomes less saturated with O_2. The fall in H^+ concentration tends to inhibit respiration. In addition, any increase in ventilation that does occur lowers the alveolar P_{CO_2}, and this also tends to inhibit respiration. Therefore, the stimulatory effects of hypoxia on ventilation are not clearly manifest until they become strong enough to override the counterbalancing inhibitory effects of a decline in arterial H^+ concentration and P_{CO_2}.

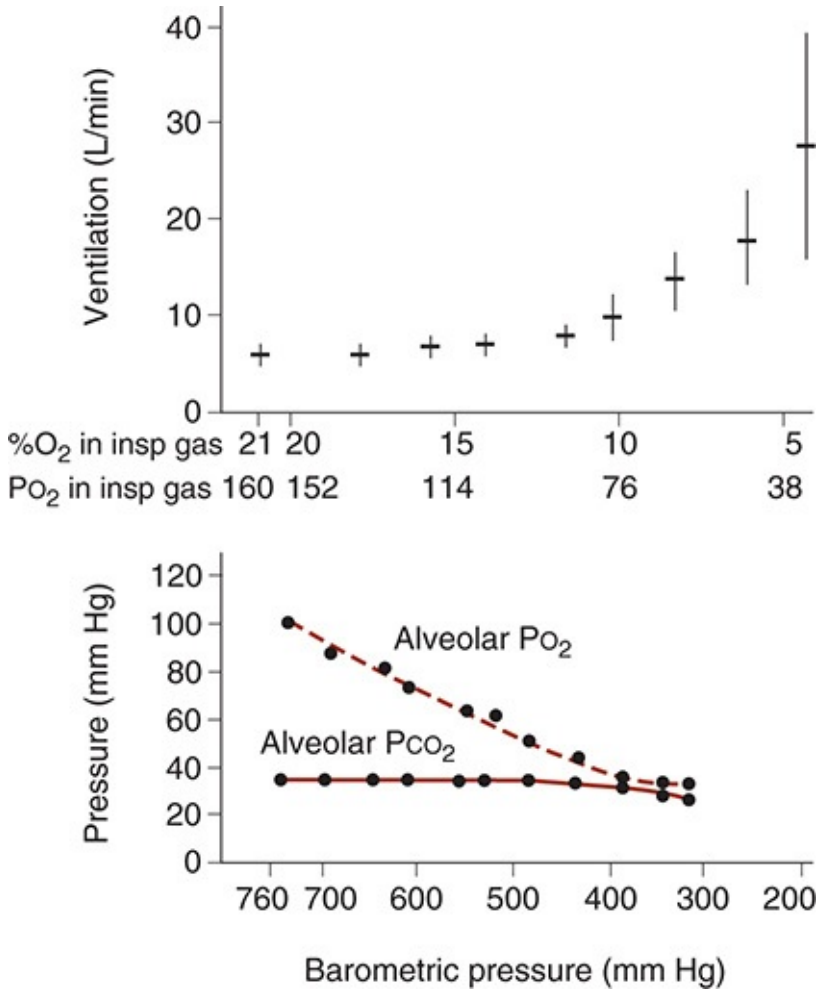


FIGURE 36–10 Effects of changes in inspired Po_2 on ventilation and alveolar gases. Top: Average respiratory minute volume during the first half hour of exposure to gases containing various amounts of O_2. Marked changes in ventilation occur at P_{O_2} values lower than 60 mm Hg. The horizontal line in each case indicates the mean; the vertical bar indicates one standard deviation. **Bottom:** Alveolar P_{O_2} and P_{CO_2} values when breathing air at various barometric pressures. The two graphs are aligned so that the P_{O_2} of the inspired gas mixtures in the upper graph correspond to the P_{O_2} at the various barometric pressures in the lower graph. (Used with permission of RH Kellogg.)

The effects on ventilation of decreasing the alveolar P_{O_2} while holding the alveolar P_{CO_2} constant are shown in **Figure 36–11**. When the alveolar P_{CO_2} is stabilized at a level 2–3 mm Hg above normal, there is an inverse relationship between ventilation and the alveolar P_{O_2} even in the 90– 110 mm Hg range; but

when the alveolar P_{CO_2} is fixed at lower than normal values, there is no stimulation of ventilation by hypoxia until the alveolar P_{O_2} falls below 60 mm Hg.

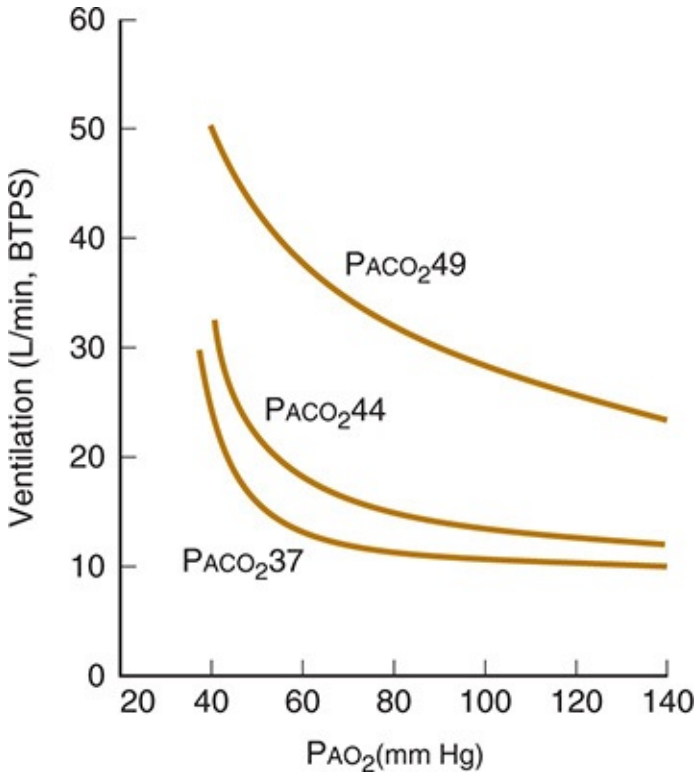


FIGURE 36–11 Ventilation at various alveolar Po_2 values when Pco_2 is held constant. Note the dramatic effect on the ventilatory response to P_{AO_2} when P_{ACO_2} is increased. (Data from Loeschke HH and Gertz KH.)

EFFECTS OF HYPOXIA ON THE CO_2 RESPONSE CURVE

When the converse experiment is performed—that is, when the alveolar P_{O_2} is held constant while the response to varying amounts of inspired CO_2 is tested—a linear response is obtained **(Figure 36–12)**. When the CO_2 response is tested at different fixed P_{O_2} values, the slope of the response curve changes, with the slope increased when alveolar P_{O_2} is decreased. In other words, hypoxia makes the individual more sensitive to an increase in arterial P_{CO_2}. However, the

alveolar P_{CO_2} level at which the curves in Figure 36–12 intersect is unaffected. In the normal individual, this threshold value is just below the normal alveolar P_{CO_2}, indicating that normally there is a very slight but definite "CO_2 drive" of the respiratory area.

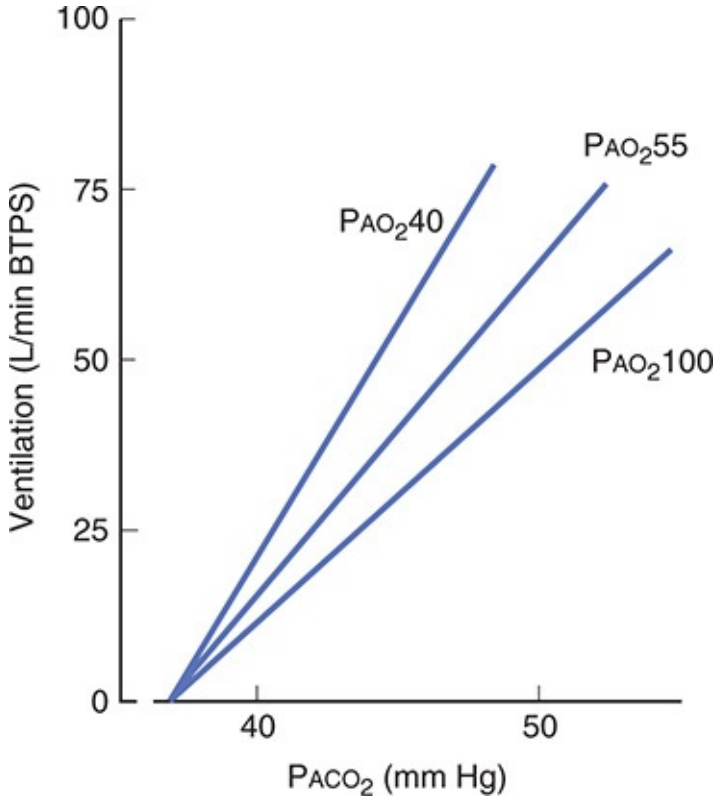


FIGURE 36–12 Fan of lines showing CO_2 response curves at various fixed values of alveolar Po_2. Decreased P_{AO_2} results in a more sensitive response to P_{ACO_2}.

EFFECT OF H^+ ON THE CO_2 RESPONSE

The stimulatory effects of H^+ and CO_2 on respiration appear to be additive and not, like those of CO_2 and O_2, complexly interrelated. In metabolic acidosis, the CO_2 response curves are similar to those in Figure 36–12, except that they are shifted to the left. In other words, the same amount of respiratory stimulation is produced by lower arterial P_{CO_2} levels. It has been calculated that the CO_2 response curve shifts 0.8 mm Hg to the left for each nanomole rise in arterial H^+.

About 40% of the ventilatory response to CO_2 is removed if the increase in arterial H^+ produced by CO_2 is prevented. As noted above, the remaining 60% is probably due to the effect of CO_2 on spinal fluid or brain interstitial fluid H^+ concentration.

BREATH HOLDING

Respiration can be voluntarily inhibited for some time, but eventually the voluntary control is overridden. The point at which breathing can no longer be voluntarily inhibited is called the **breaking point.** Breaking is due to the rise in arterial P_{CO_2} and the fall in P_{O_2}. Individuals can hold their breath longer after removal of the carotid bodies. Breathing 100% oxygen before breath holding raises alveolar P_{O_2} initially, so that the breaking point is delayed. The same is true of hyperventilating room air, because CO_2 is blown off and arterial P_{CO_2} is lower at the start. Reflex or mechanical factors appear to influence the breaking point, since persons who hold their breath as long as possible and then breathe a gas mixture low in O_2 and high in CO_2 can hold their breath for an additional 20 s or more. Psychological factors also play a role, and persons can hold their breath longer when they are told their performance is very good than when they are not.

NONCHEMICAL INFLUENCES ON RESPIRATION

RESPONSES MEDIATED BY RECEPTORS IN THE AIRWAYS & LUNGS

Receptors in the airways and lungs are innervated by myelinated and unmyelinated vagal fibers. The unmyelinated fibers are C fibers. The receptors innervated by myelinated fibers are commonly divided into **slowly adapting receptors** and **rapidly adapting receptors** on the basis of whether sustained stimulation leads to prolonged or transient discharge in their afferent nerve fibers **(Table 36–2)**. The other group of receptors presumably consists of the endings of C fibers, and they are divided into pulmonary and bronchial subgroups on the basis of their location.

TABLE 36–2 Airway and lung receptors.

Vagal Innervation	Type	Location in Interstitium	Stimulus	Response
Myelinated	Slowly adapting	Among airway smooth muscle cells (?)	Lung inflation	Inspiratory time shortening Hering-Breuer inflation and deflation reflexes Bronchodilation Tachycardia Hyperpnea
	Rapidly adapting	Among airway epithelial cells	Lung hyperinflation Exogenous and endogenous substances (eg, histamine, prostaglandins)	Cough Bronchoconstriction Mucus secretion
Unmyelinated C fibers	Pulmonary C fibers Bronchial C fibers	Close to blood vessels	Lung hyperinflation Exogenous and endogenous substances (eg, capsaicin, bradykinin, serotonin)	Apnea followed by rapid breathing Bronchoconstriction Bradycardia Hypotension Mucus secretion

Data from Berger AJ, Hornbein TF: Control of respiration. In: *Textbook of Physiology,* 21st ed, Vol 2. Patton HD, et al (editors). Saunders, 1989.

The shortening of inspiration produced by vagal afferent activity (Figure 36–4) is mediated by slowly adapting receptors, as are the **Hering-Breuer reflexes.** The Hering-Breuer inflation reflex is an increase in the duration of expiration produced by steady lung inflation, and the Hering-Breuer deflation reflex is a decrease in the duration of expiration produced by marked deflation of the lung. Because the rapidly adapting receptors are stimulated by chemicals such as histamine, they have been called **irritant receptors.** Activation of rapidly adapting receptors in the trachea causes coughing, bronchoconstriction, and mucus secretion, and activation of rapidly adapting receptors in the lung may produce hyperpnea.

Because the C fiber endings are close to pulmonary vessels, they have been called J (juxtacapillary) receptors. They are stimulated by hyperinflation of the lung, but they respond as well to intravenous or intracardiac administration of chemicals such as capsaicin. The reflex response that is produced is apnea followed by rapid breathing, bradycardia, and hypotension **(pulmonary chemoreflex)**. A similar response is produced by receptors in the heart (**Bezold-Jarisch reflex** or the **coronary chemoreflex)**. The physiological role of this reflex is uncertain, but it probably occurs in pathologic states such as pulmonary congestion or embolization, in which it is produced by endogenously released substances.

COUGHING & SNEEZING

Coughing begins with a deep inspiration followed by forced expiration against a closed glottis. This increases the intrapleural pressure to 100 mm Hg or more. The glottis is then suddenly opened, producing an explosive outflow of air at velocities up to 965 km (600 mi) per hour. Sneezing is a similar expiratory effort with a continuously open glottis. These reflexes help expel irritants and keep airways clear. Other aspects of innervation are considered in a special case **(Clinical Box 36–1)**.

AFFERENTS FROM PROPRIOCEPTORS

Carefully controlled experiments have shown that active and passive movements of joints stimulate respiration, presumably because impulses in afferent pathways from proprioceptors in muscles, tendons, and joints stimulate the inspiratory neurons. This effect probably helps increase ventilation during exercise. Other afferents are considered in **Clinical Box 36–2**.

RESPIRATORY COMPONENTS OF VISCERAL REFLEXES

Inhibition of respiration and closure of the glottis during vomiting, swallowing, and sneezing not only prevent the aspiration of food or vomitus into the trachea but, in the case of vomiting, fix the chest so that contraction of the abdominal muscles increases the intra-abdominal pressure. Similar glottic closure and inhibition of respiration occur during voluntary and involuntary straining.

Hiccup is a spasmodic contraction of the diaphragm and other inspiratory muscles that produces an inspiration during which the glottis suddenly closes. The glottic closure is responsible for the characteristic sensation and sound. Hiccups occur in the fetus in utero as well as throughout extrauterine life. Their function is unknown. Most attacks of hiccups are usually of short duration, and they often respond to breathholding or other measures that increase arterial P_{CO_2}. Intractable hiccups, which can be debilitating, sometimes respond to dopamine antagonists and perhaps to some centrally acting analgesic compounds.

Yawning is a peculiar “infectious” respiratory act whose physiological basis and significance are uncertain. Like hiccupping, it occurs in utero, and it occurs in fish and tortoises as well as mammals. The view that it is needed to increase O_2 intake has been discredited. Underventilated alveoli have a tendency to collapse, and it has been suggested that the deep inspiration and stretching them

open prevents the development of atelectasis. However, in actual experiments, no atelectasis-preventing effect of yawning could be demonstrated. Yawning increases venous return to the heart, which may benefit the circulation. It has been suggested that yawning is a nonverbal signal used for communication between monkeys in a group, and one could argue that on a different level, the same thing is true in humans.

CLINICAL BOX 36–1

Lung Innervation & Patients with Heart-Lung Transplants

Transplantation of the lung and/or heart is an established treatment for severe pulmonary disease and other conditions. In individuals with transplants, the recipient's right atrium is sutured to the donor heart, and the donor heart does not reinnervate, so the resting heart rate is elevated. The donor trachea is sutured to the recipient's just above the carina, and afferent fibers from the lungs do not regrow. Consequently, healthy patients with heart-lung transplants provide an opportunity to evaluate the role of lung innervation in normal physiology. Their cough responses to stimulation of the trachea are normal because the trachea remains innervated, but their cough responses to stimulation of the smaller airways are absent. Their bronchi tend to be dilated to a greater degree than normal. In addition, they have the normal number of yawns and sighs, indicating that these do not depend on innervation of the lungs. Finally, they lack Hering-Breuer reflexes, but their pattern of breathing at rest is normal, indicating that these reflexes do not play an important role in the regulation of resting respiration in humans.

CLINICAL BOX 36–2

Afferents from "Higher Centers"

Pain and emotional stimuli affect respiration, suggesting that afferents from the limbic system and hypothalamus signal to the respiratory neurons in the brainstem. In addition, even though breathing is not usually a conscious event, both inspiration and expiration are under voluntary control. The pathways for voluntary control pass from the neocortex to the motor neurons innervating the respiratory muscles, bypassing the medullary neurons.

Because voluntary and automatic control of respiration are separate, automatic control is sometimes disrupted without loss of voluntary control. The clinical condition that results has been called **Ondine's curse.** In German legend, Ondine was a water nymph who had an unfaithful mortal lover. The king of the water nymphs punished the lover by casting a curse on him that took away all his automatic functions. In this state, he could stay alive only by staying awake and remembering to breathe. He eventually fell asleep from sheer exhaustion, and his respiration stopped. Patients with this intriguing condition generally have bulbar poliomyelitis or disease processes that compress the medulla.

RESPIRATORY EFFECTS OF BARORECEPTOR STIMULATION

Afferent fibers from the baroreceptors in the carotid sinuses, aortic arch, atria, and ventricles relay to the respiratory neurons, as well as the vasomotor and cardioinhibitory neurons in the medulla. Impulses in them inhibit respiration, but the inhibitory effect is slight and of little physiological importance. The hyperventilation in shock is due to chemoreceptor stimulation caused by acidosis and hypoxia secondary to local stagnation of blood flow, and is not baroreceptor-mediated. The activity of inspiratory neurons affects blood pressure and heart rate, and activity in the vasomotor and cardiac areas in the medulla may have minor effects on respiration.

EFFECTS OF SLEEP

Respiration is less rigorously controlled during sleep than in the waking state, and brief periods of apnea occur in normal sleeping adults. Changes in the ventilatory response to hypoxia vary. If the P_{CO_2} falls during the waking state, various stimuli from proprioceptors and the environment maintain respiration, but during sleep, these stimuli are decreased and a decrease in P_{CO_2} can cause apnea. During rapid eye movement (REM) sleep, breathing is irregular and the CO_2 response is highly variable.

RESPIRATORY ABNORMALITIES

ASPHYXIA

In asphyxia produced by occlusion of the airway, acute hypercapnia and hypoxia develop together. Stimulation of respiration is pronounced, with violent respiratory efforts. Blood pressure and heart rate rise sharply, catecholamine secretion is increased, and blood pH drops. Eventually the respiratory efforts cease, the blood pressure falls, and the heart slows. Asphyxiated animals can still be revived at this point by artificial respiration, although they are prone to ventricular fibrillation, probably because of the combination of hypoxic myocardial damage and high circulating catecholamine levels. If artificial respiration is not started, cardiac arrest occurs in 4–5 min.

DROWNING

Drowning is asphyxia caused by immersion, usually in water. In about 10% of drownings, the first gasp of water after the losing struggle not to breathe triggers laryngospasm, and death results from asphyxia without any water in the lungs. In the remaining cases, the glottic muscles eventually relax and fluid enters the lungs. Fresh water is rapidly absorbed, diluting the plasma and causing intravascular hemolysis. Ocean water is markedly hypertonic and draws fluid from the vascular system into the lungs, decreasing plasma volume. The immediate goal in the treatment of drowning is, of course, resuscitation, but long-term treatment must also consider the circulatory effects of the water in the lungs.

PERIODIC BREATHING

The acute effects of voluntary hyperventilation demonstrate the interaction of the chemical mechanisms regulating respiration. When a normal individual hyperventilates for 2–3 min, then stops and permits respiration to continue without exerting any voluntary control over it, a period of apnea occurs. This is followed by a few shallow breaths and then by another period of apnea, followed again by a few breaths **(periodic breathing)**. The cycles may last for some time before normal breathing is resumed **(Figure 36–13)**. The apnea apparently is due to a lack of CO_2 because it does not occur following hyperventilation with gas mixtures containing 5% CO_2. During the apnea, the alveolar P_{O_2} falls and the P_{CO_2} rises. Breathing resumes because of hypoxic stimulation of the carotid and

aortic chemoreceptors before the CO_2 level has returned to normal. A few breaths eliminate the hypoxic stimulus and breathing stops until the alveolar P_{O_2} falls again. Gradually, however, the P_{CO_2} returns to normal, and normal breathing resumes. Changes in breathing patterns can be symptomatic of disease **(Clinical Box 36–3)**.

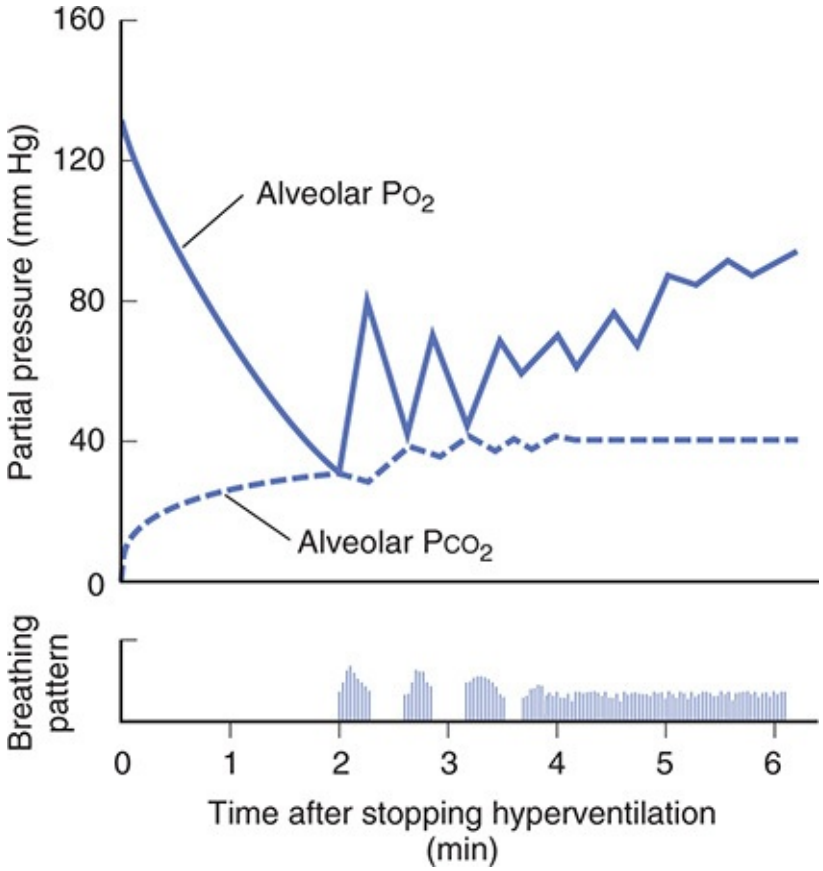


FIGURE 36–13 Changes in breathing and composition of alveolar air after forced hyperventilation for 2 min. Bars in bottom indicate breathing, whereas blank spaces are indicative of apnea.

EFFECTS OF EXERCISE

Exercise provides a physiological example to explore many of the control systems discussed above. Of course, many cardiovascular and respiratory mechanisms must operate in an integrated fashion if the O_2 needs of the active tissue are to be met and the extra CO_2 and heat removed from the body during exercise. Circulatory changes increase muscle blood flow while maintaining

adequate circulation in the rest of the body. In addition, there is an increase in the extraction of O_2 from the blood in exercising muscles and an increase in ventilation. This provides extra O_2, eliminates some of the heat, and excretes extra CO_2. A focus on regulation of ventilation and tissue O_2 is presented below, as many other aspects of regulation have been presented in previous chapters.

CLINICAL BOX 36–3

Periodic Breathing in Disease

Cheyne-Stokes Respiration

Periodic breathing occurs in various disease states and is often called Cheyne-Stokes respiration. It is seen most commonly in patients with heart failure and uremia, but it occurs also in patients with brain disease and during sleep in some normal individuals. Some of the patients with Cheyne-Stokes respiration have increased sensitivity to CO_2. The increased response is apparently due to disruption of neural pathways that normally inhibit respiration. In these individuals, CO_2 causes relative hyperventilation, lowering the arterial P_{CO_2}. During the resultant apnea, the arterial P_{CO_2} again rises to normal, but the respiratory mechanism again overresponds to CO_2. Breathing ceases, and the cycle repeats.

Another cause of periodic breathing in patients with cardiac disease is prolongation of the lung-to-brain circulation time, so that it takes longer for changes in arterial gas tensions to affect the respiratory area in the medulla. When individuals with a slower circulation hyperventilate, they lower the P_{CO_2} of the blood in their lungs, but it takes longer than normal for the blood with a low P_{CO_2} to reach the brain. During this time, the P_{CO_2} in the pulmonary capillary blood continues to be lowered, and when this blood reaches the brain, the low P_{CO_2} inhibits the respiratory area, producing apnea. In other words, the respiratory control system oscillates because the negative feedback loop from lungs to brain is abnormally long.

Sleep Apnea

Episodes of apnea during sleep can be central in origin (ie, due to failure of discharge in the nerves producing respiration) or they can be due to airway obstruction **(obstructive sleep apnea)**. Apnea can occur at any age and is

produced when the pharyngeal muscles relax during sleep. In some cases, failure of the genioglossus muscles to contract during inspiration contributes to the blockage. The genioglossus muscles pull the tongue forward, and without (or with weak) contraction the tongue can obstruct the airway. After several increasingly strong respiratory efforts, the patient wakes up, takes a few normal breaths, and falls back to sleep. Apneic episodes are most common during REM sleep, when the muscles are most hypotonic. The symptoms are loud snoring, morning headaches, fatigue, and daytime sleepiness. When severe and prolonged, the condition can lead to hypertension and its complications. Frequent apneas can lead to numerous brief awakenings during sleep and to sleepiness during waking hours. With this in mind, it is not surprising to find that the incidence of motor vehicle accidents in sleep apnea patients is seven times greater than it is in the general driving population. In addition, sleep apnea is associated with many cardiovascular diseases, such as hypertension, arrhythmia, stroke and heart failure.

THERAPEUTIC HIGHLIGHTS

Treatment of sleep apnea depends on the patient and on the cause (if known). Treatments range from mild to moderate interventions to surgery. Interventions including positional therapy, dental appliances that rearrange the architecture of the airway, avoidance of muscle relaxants (eg, alcohol) or drugs that reduce respiratory drive, or continuous positive airway pressure (C-PAP, a standard of care in the management of symptoms of moderate to severe obstructive sleep apnea). Because sleep apnea is increased in overweight or obese individuals, weight loss can also be effective.

CHANGES IN VENTILATION

During exercise, the amount of O_2 entering the blood in the lungs is increased because the amount of O_2 added to each unit of blood and the pulmonary blood flow per minute are increased. The P_{O_2} of blood flowing into the pulmonary capillaries falls from 40 to 25 mm Hg or less, so that the alveolar-capillary P_{O_2} gradient is increased and more O_2 enters the blood. Blood flow per minute is increased from 5.5 L/min to as much as 20–35 L/min. The total amount of O_2

entering the blood therefore increases from 250 mL/min at rest to values as high as 4000 mL/min. The amount of CO_2 removed from each unit of blood is increased, and CO_2 excretion increases from 200 mL/min to as much as 8000 mL/min. The increase in O_2 uptake is proportional to work load, up to a maximum. Above this maximum, O_2 consumption levels off and the blood lactate level continues to rise **(Figure 36–14)**. The lactate comes from muscles in which aerobic resynthesis of energy stores cannot keep pace with their utilization, and an **oxygen debt** is being incurred.

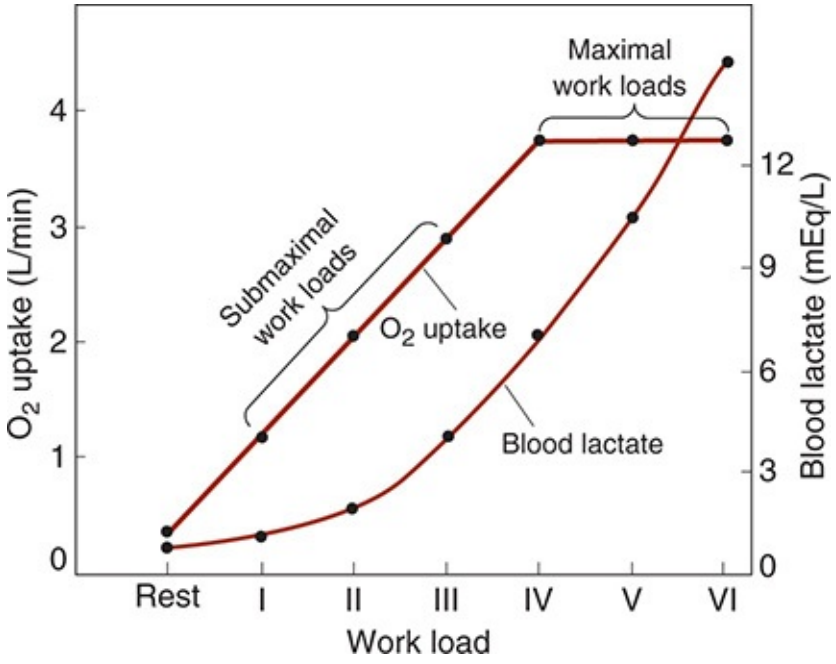


FIGURE 36–14 Relation between work load, blood lactate level, and O_2 uptake. I–VI, increasing work loads produced by increasing the speed and grade of a treadmill on which the subjects worked. (Reproduced with permission from Mitchell JH, Blomqvist G: Maximal oxygen uptake. N Engl J Med 1971 May 6;284(18):1018–1022.)

Ventilation increases abruptly with the onset of exercise, which is followed after a brief pause by a further, more gradual increase **(Figure 36–15)**. With moderate exercise, the increase is due mostly to an increase in the depth of respiration; this is accompanied by an increase in the respiratory rate when the exercise is more strenuous. Ventilation abruptly decreases when exercise ceases, which is followed after a brief pause by a more gradual decline to preexercise values. The abrupt increase at the start of exercise is presumably due to psychic

stimuli and afferent impulses from proprioceptors in muscles, tendons, and joints. The more gradual increase is presumably humoral, even though arterial pH, P_{CO_2}, and P_{O_2} remain constant during moderate exercise. The increase in ventilation is proportional to the increase in O_2 consumption, but the mechanisms responsible for the stimulation of respiration are still the subject of much debate. The increase in body temperature may play a role. Exercise increases the plasma K^+ level, and this increase may stimulate the peripheral chemoreceptors. In addition, it may be that the sensitivity of the neurons controlling the response to CO_2 is increased or that the respiratory fluctuations in arterial P_{CO_2} increase so that, even though the mean arterial P_{CO_2} does not rise, it is CO_2 that is responsible for the increase in ventilation. O_2 also seems to play some role, despite the lack of a decrease in arterial P_{O_2}, since during the performance of a given amount of work, the increase in ventilation while breathing 100% O_2 is 10–20% less than the increase while breathing air. Thus, it currently appears that a number of different factors combine to produce the increase in ventilation seen during moderate exercise.

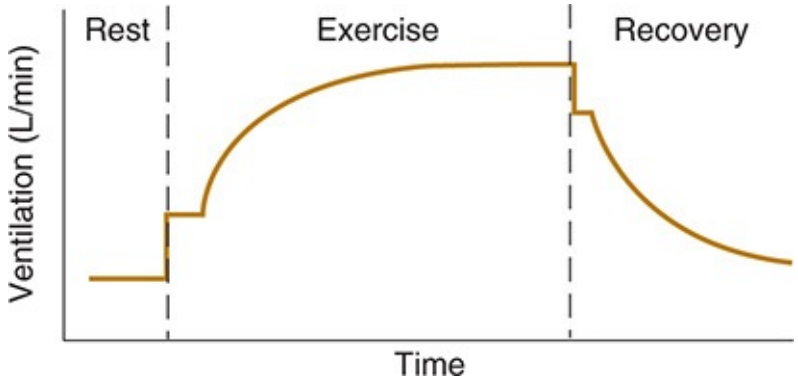


FIGURE 36–15 Diagrammatic representation of changes in ventilation during exercise. See text for details.

When exercise becomes more vigorous, buffering of the increased amounts of lactic acid that are produced liberates more CO_2, and this further increases ventilation. The response to graded exercise is shown in **Figure 36–16**. With increased production of acid, the increases in ventilation and CO_2 production remain proportional, so alveolar and arterial CO_2 change relatively little **(isocapnic buffering)**. Because of the hyperventilation, alveolar P_{O_2} increases. With further accumulation of lactic acid, the increase in ventilation outstrips CO_2

production and alveolar P_{CO_2} falls, as does arterial P_{CO_2}. The decline in arterial P_{CO_2} provides respiratory compensation for the metabolic acidosis produced by the additional lactic acid. The additional increase in ventilation produced by the acidosis depends on the carotid bodies and does not occur if they are removed.

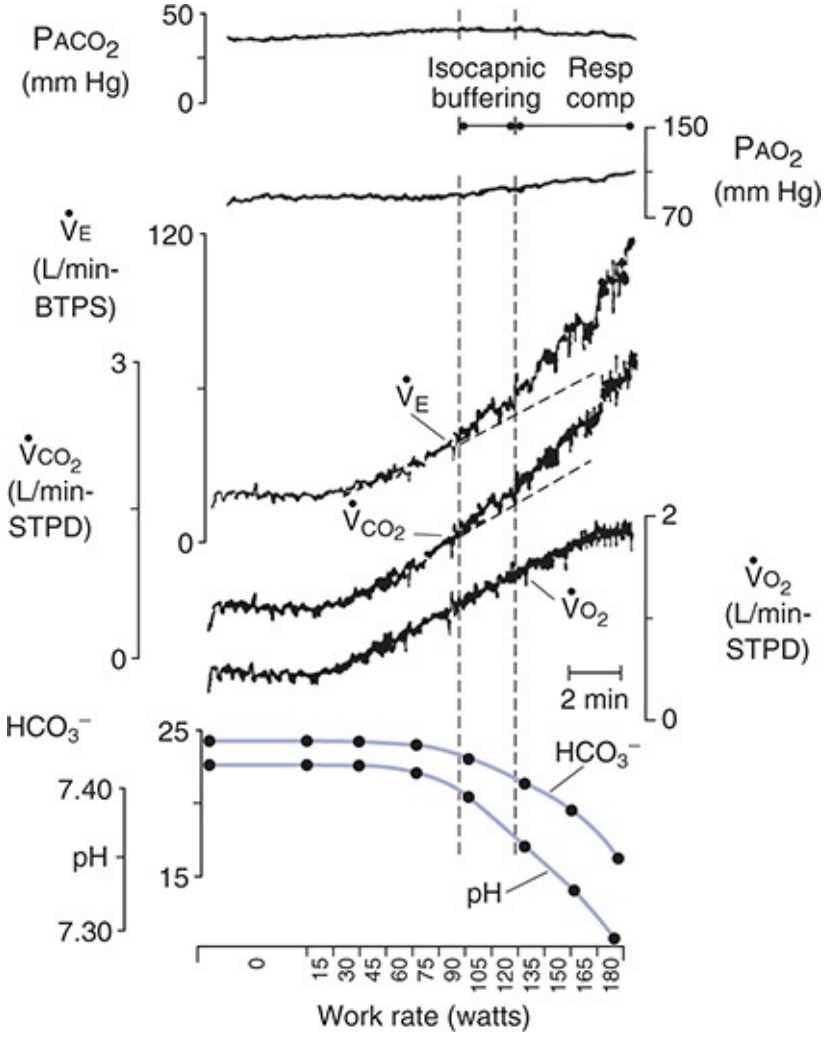


FIGURE 36–16 Physiological responses to work rate during exercise. Changes in alveolar P_{CO_2} (P_{ACO_2}), alveolar P_{O_2} (P_{AO_2}), ventilation ($\dot{V}_E$), CO_2 production ($\dot{V}_{CO_2}$), O_2 consumption ($\dot{V}_{O_2}$), arterial HCO_3^-, and arterial pH with graded increases in work by an adult male on a bicycle ergometer. Resp comp, respiratory compensation. BTPS, body temperature (BT) and pressure (760 mm Hg), saturated; STPD, standard temperature (0°C) and pressure (760 mm Hg), dry. Dashed lines emphasize deviation from linear response. See text for additional details. (Reproduced with permission from Wasserman K: Breathing during exercise. N Engl J Med 1978 Apr 6;298(14):780–785.)

The respiratory rate after exercise does not reach basal levels until the O_2 debt is repaid. This may take as long as 90 min. The stimulus to ventilation after exercise is not the arterial P_{CO_2}, which is normal or low, or the arterial P_{O_2}, which is normal or high, but the elevated arterial H^+ concentration due to the lactic acidemia. The magnitude of the O_2 debt is the amount by which O_2 consumption exceeds basal consumption from the end of exertion until the O_2 consumption has returned to preexercise basal levels. During repayment of the O_2 debt, the O_2 concentration in muscle myoglobin rises slightly. ATP and phosphorylcreatine are resynthesized, and lactic acid is removed. Eighty percent of the lactic acid is converted to glycogen and 20% is metabolized to CO_2 and H_2O.

CHANGES IN THE TISSUES

Maximum O_2 uptake during exercise is limited by the maximum rate at which O_2 is transported to the mitochondria in the exercising muscle. However, this limitation is not normally due to deficient O_2 uptake in the lungs, and hemoglobin in arterial blood is saturated even during the most severe exercise.

During exercise, the contracting muscles use more O_2, and the tissue P_{O_2} and the P_{O_2} in venous blood from exercising muscle fall nearly to zero. More O_2 diffuses from the blood, the blood P_{O_2} in the muscles drops, and more O_2 is removed from hemoglobin. Because the capillary bed of contracting muscle is dilated and many previously closed capillaries are open (capillary recruitment and extension), the mean distance from the blood to the tissue cells is greatly decreased; this facilitates the movement of O_2 from blood to cells. The oxygen–hemoglobin dissociation curve is steep in the P_{O_2} range below 60 mm Hg, and a relatively large amount of O_2 is supplied for each drop of 1 mm Hg in P_{O_2} (see Figure 35–2). Additional O_2 is supplied because, as a result of the accumulation of CO_2 and the rise in temperature in active tissues—and perhaps because of a rise in red blood cell 2,3-diphosphoglycerate (2,3-DPG)—the dissociation curve shifts to the right. The net effect is a 3-fold increase in O_2 extraction from each unit of blood (see Figure 35–3). Because this increase is accompanied by a 30-fold or greater increase in blood flow, it permits the metabolic rate of muscle to rise as much as 100-fold during exercise.

EXERCISE TOLERANCE & FATIGUE

What determines the maximum amount of exercise that can be performed by an individual? Obviously, exercise tolerance has a time as well as an intensity dimension. For example, a fit young man can produce a power output on a bicycle of about 700 W for 1 min, 300 W for 5 min, and 200 W for 40 min. It used to be argued that the limiting factors in exercise performance were the rate at which O_2 could be delivered to the tissues or the rate at which O_2 could enter the body in the lungs. These factors play a role, but it is clear that other factors also contribute, and that exercise stops when the sensation of **fatigue** progresses to the sensation of exhaustion. Fatigue is produced in part by bombardment of the brain by neural impulses from muscles, and the decline in blood pH produced by lactic acidosis also makes one feel tired, as does the rise in body temperature, dyspnea, and, perhaps, the uncomfortable sensations produced by activation of the J receptors in the lungs.

CHAPTER SUMMARY

- Breathing is under both voluntary control (located in the cerebral cortex) and automatic control (driven by pacemaker cells in the medulla). There is a reciprocal innervation to expiratory and inspiratory muscles in that motor neurons supplying expiratory muscles are inactive when motor neurons supplying inspiratory muscles are active, and vice versa.
- The pre-Bötzinger complex on either side of the medulla contains synaptically coupled pacemaker cells that allow for rhythmic generation of breathing. The spontaneous activity of these neurons can be altered by neurons in the pneumotaxic center, although the full regulatory function of these neurons on normal breathing is not understood.
- Breathing patterns are sensitive to chemicals in the blood through activation of respiratory chemoreceptors. There are chemoreceptors in the carotid and aortic bodies and in collections of cells in the medulla. These chemoreceptors respond to changes in P_{O_2} and P_{CO_2} as well as H^+ to regulate breathing.
- Receptors in the airway are additionally innervated by slowly adapting and rapidly adapting myelinated vagal fibers. Slowly adapting receptors can be activated by lung inflation. Rapidly adapting receptors, or irritant receptors, can be activated by chemicals such as histamine and result in cough or even

hyperpnea.

- Receptors in the airway are also innervated by unmyelinated vagal fibers (C fibers) that are typically found next to pulmonary vessels. They are stimulated by hyperinflation (or exogenous substances including capsaicin) and lead to the pulmonary chemoreflex. The physiological role for this response is not fully understood.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. The main respiratory control neurons
   A. send out regular bursts of impulses to expiratory muscles during quiet respiration.
   B. are unaffected by stimulation of pain receptors.
   C. are located in the pons.
   D. send out regular bursts of impulses to inspiratory muscles during quiet respiration.
   E. are unaffected by impulses from the cerebral cortex.
2. Intravenous lactic acid increases ventilation. The receptors responsible for this effect are located in the
   A. medulla oblongata.
   B. carotid bodies.
   C. lung parenchyma.
   D. aortic baroreceptors.
   E. trachea and large bronchi.
3. Spontaneous respiration ceases after
   A. transection of the brainstem above the pons.
   B. transection of the brainstem at the caudal end of the medulla.
   C. bilateral vagotomy.
   D. bilateral vagotomy combined with transection of the brainstem at the superior border of the pons.
   E. transection of the spinal cord at the level of the first thoracic segment.
4. The following physiological events that occur in vivo are listed in random order: (1) decreased CSF pH; (2) increased arterial P_{CO_2}; (3) increased CSF

P_{CO_2}; (4) stimulation of medullary chemoreceptors; and (5) increased alveolar P_{CO_2}.

What is the usual sequence in which they occur when they affect respiration?

A. 1, 2, 3, 4, 5

B. 4, 1, 3, 2, 5

C. 3, 4, 5, 1, 2

D. 5, 2, 3, 1, 4

E. 5, 3, 2, 4, 1

5. The following events that occur in the carotid bodies when they are exposed to hypoxia are listed in random order: (1) depolarization of type I glomus cells; (2) excitation of afferent nerve endings; (3) reduced conductance of hypoxia-sensitive K^+ channels in type I glomus cells; (4) Ca^{2+} entry into type I glomus cells; (5) decreased K^+ efflux.

What is the usual sequence in which they occur on exposure to hypoxia?

A. 1, 3, 4, 5, 2

B. 1, 4, 2, 5, 3

C. 3, 4, 5, 1, 2

D. 3, 1, 4, 5, 2

E. 3, 5, 1, 4, 2

6. Injection of a drug that stimulates the carotid bodies would be expected to cause

A. a decrease in the pH of arterial blood.

B. a decrease in the P_{CO_2} of arterial blood.

C. an increase in the HCO_3^- concentration of arterial blood.

D. an increase in urinary Na^+ excretion.

E. an increase in plasma Cl^-.

7. Variations in which of the following components of blood or CSF do *not* affect respiration?

A. Arterial HCO_3^- concentration

B. Arterial H^+ concentration

C. Arterial Na^+ concentration

D. CSF CO_2 concentration

E. CSF H^+ concentration

SECTION VII

Renal Physiology

The kidneys, the bladder, and the ureters make up the urinary system. Within the kidney, the functional unit is the nephron and each human kidney has approximately 1 million nephrons. The kidneys play an essential role in the regulation of water homeostasis, electrolyte composition (eg, Na, Cl, K, HCO_3), regulation of extracellular volume (thus blood pressure), and acid–base homeostasis (Chapter 39). The kidneys filter the plasma of the blood and produce urine, which allows the kidneys to excrete metabolic waste products (eg, urea; ammonium; and foreign chemicals, such as drug metabolites) from the body. The kidneys are responsible for the reabsorption of glucose and amino acids from the plasma filtrate in addition to regulated calcium and phosphate uptake (high in children). The kidneys play a role in gluconeogenesis and during fasting can synthesize and release glucose into the blood, producing almost 20% of the liver's glucose capacity. The kidneys are also endocrine organs, making kinins (see Chapter 32), 1,25-dihydroxycholecalciferol (see Chapter 21), erythropoietin (see Chapter 37), and making and secreting renin (see Chapter 38).

Specifically, in the kidneys, a fluid that resembles plasma is filtered through the glomerular capillaries into the renal tubules **(glomerular filtration).** As this glomerular filtrate passes down the tubules, its volume is reduced and its composition altered by the processes of **tubular reabsorption** (removal of water and solutes from the tubular fluid) and **tubular secretion** (secretion of solutes into the tubular fluid) to form the urine that enters the renal pelvis. From the renal pelvis, the urine passes to the bladder and is expelled to the exterior by the process of urination, or **micturition.**

Diseases of the kidney are numerous. Commonly occurring clinical conditions include acute kidney injury, chronic kidney disease (CKD), diabetic kidney

disease, nephritic and nephrotic syndromes, polycystic kidney disease, urinary tract obstruction, urinary tract infection, and renal cancer. When renal function decreases such that the kidneys are no longer functioning to maintain health, patients sometimes undergo dialysis and eventually kidney transplantation.

The prevalence of kidney disease is increasing across the world as is the cost of treating the diseases that cause kidney damage; thus kidney disease represents a large threat to healthcare resources worldwide. The two diseases that cause the largest prevalence of kidney dysfunction are diabetes and hypertension. Nearly one billion people worldwide have high blood pressure and that number is expected to increase to 1.56 billion by 2025. There are currently over 240 million people with diabetes worldwide and this figure is anticipated to rise to 380 million by 2025. Of those with diabetes, about 40% will develop CKD, which means that their risk of cardiovascular complications also increases. The cumulative global cost for dialysis and transplantation over the next decade is predicted to exceed one-trillion dollars.

CHAPTER 37

Renal Function & Micturition

OBJECTIVES

After studying this chapter, you should be able to:

- Describe the morphology of a typical nephron and its blood supply.
- Explain autoregulation and how it regulates renal function.
- Define glomerular filtration rate (GFR), describe how it can be measured, and list the major factors that alter GFR.
- Outline how each tubular segment of the nephron handles Na^+ and water.
- Explain tubular reabsorption and secretion of glucose and K^+.
- Describe how the kidney operates to produce hypertonic or hypotonic urine.
- Define the major classes of diuretics and explain where each operates to increase urine flow.
- Describe the voiding reflex and draw a cystometrogram.

FUNCTIONAL ANATOMY

THE NEPHRON

Each individual renal tubule and its glomerulus is a unit **(nephron).** The size of

the kidneys between species varies, as does the number of nephrons they contain. Each human kidney has approximately 1 million nephrons. The specific structures of the nephron are shown in diagrammatic manner in **Figure 37–1**.

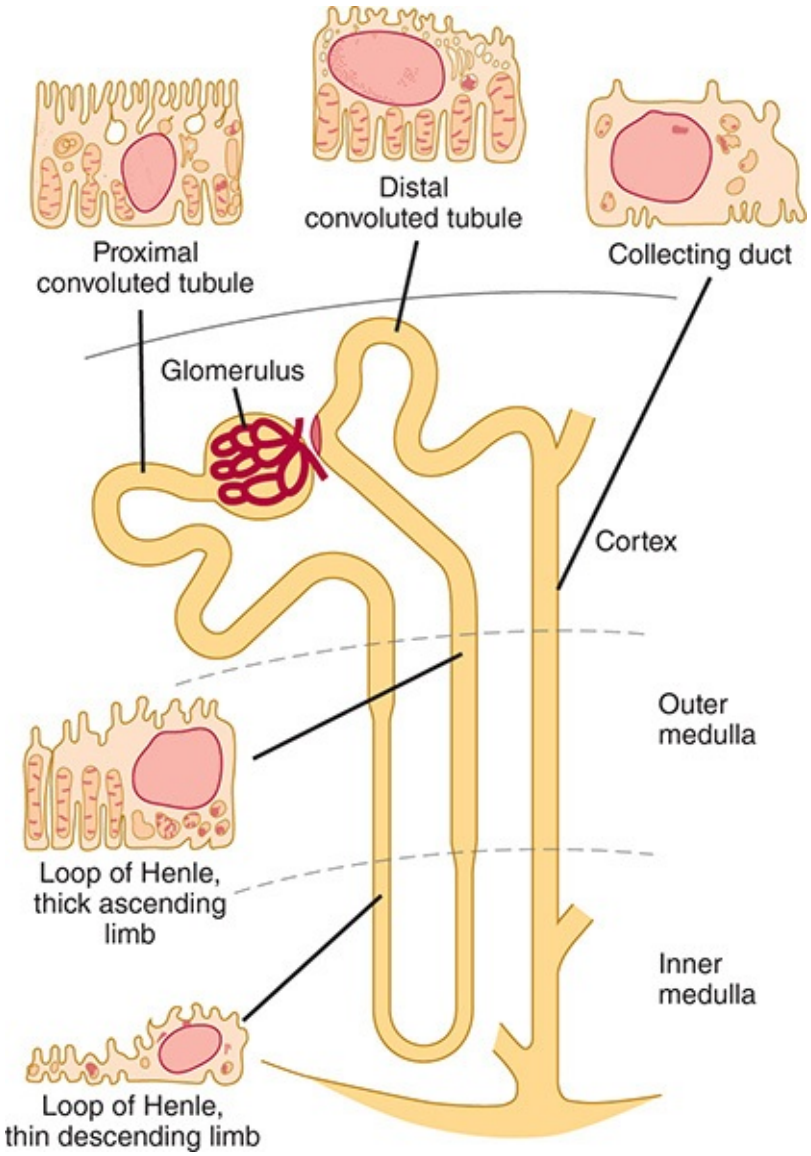


FIGURE 37–1 Diagram of a nephron. The main histologic features of the cells that make up each portion of the tubule are also shown.

The glomerulus, which is about 200 µm in diameter, is formed by the invagination of a tuft of capillaries into the dilated, blind end of the nephron **(Bowman's capsule).** The capillaries are supplied by an **afferent arteriole** and drained by the **efferent arteriole** **(Figure 37–2)**, and it is from the glomerulus that the filtrate is formed. The diameter of the afferent arteriole is larger than the

efferent arteriole. Two cellular layers separate the blood from the glomerular filtrate in Bowman's capsule: the capillary endothelium and the specialized epithelium of the capsule. The endothelium of the glomerular capillaries is fenestrated, with pores that are 70–90 nm in diameter. The endothelium of the glomerular capillaries is completely surrounded by the glomerular basement membrane along with specialized cells called podocytes. **Podocytes** have numerous pseudopodia that interdigitate (Figure 37–2) to form **filtration slits** along the capillary wall. The slits are approximately 25 nm wide, and each is closed by a thin membrane. The glomerular basement membrane, the basal lamina, does not contain visible gaps or pores. Stellate cells called **mesangial cells** are located between the basal lamina and the endothelium. They are similar to cells called **pericytes,** which are found in the walls of capillaries elsewhere in the body. Mesangial cells are especially common between two neighboring capillaries, and in these locations the basal membrane forms a sheath shared by both capillaries (Figure 37–2). The mesangial cells are contractile and play a role in the regulation of glomerular filtration. Mesangial cells secrete the extracellular matrix, take up immune complexes, and are involved in the progression of glomerular disease.

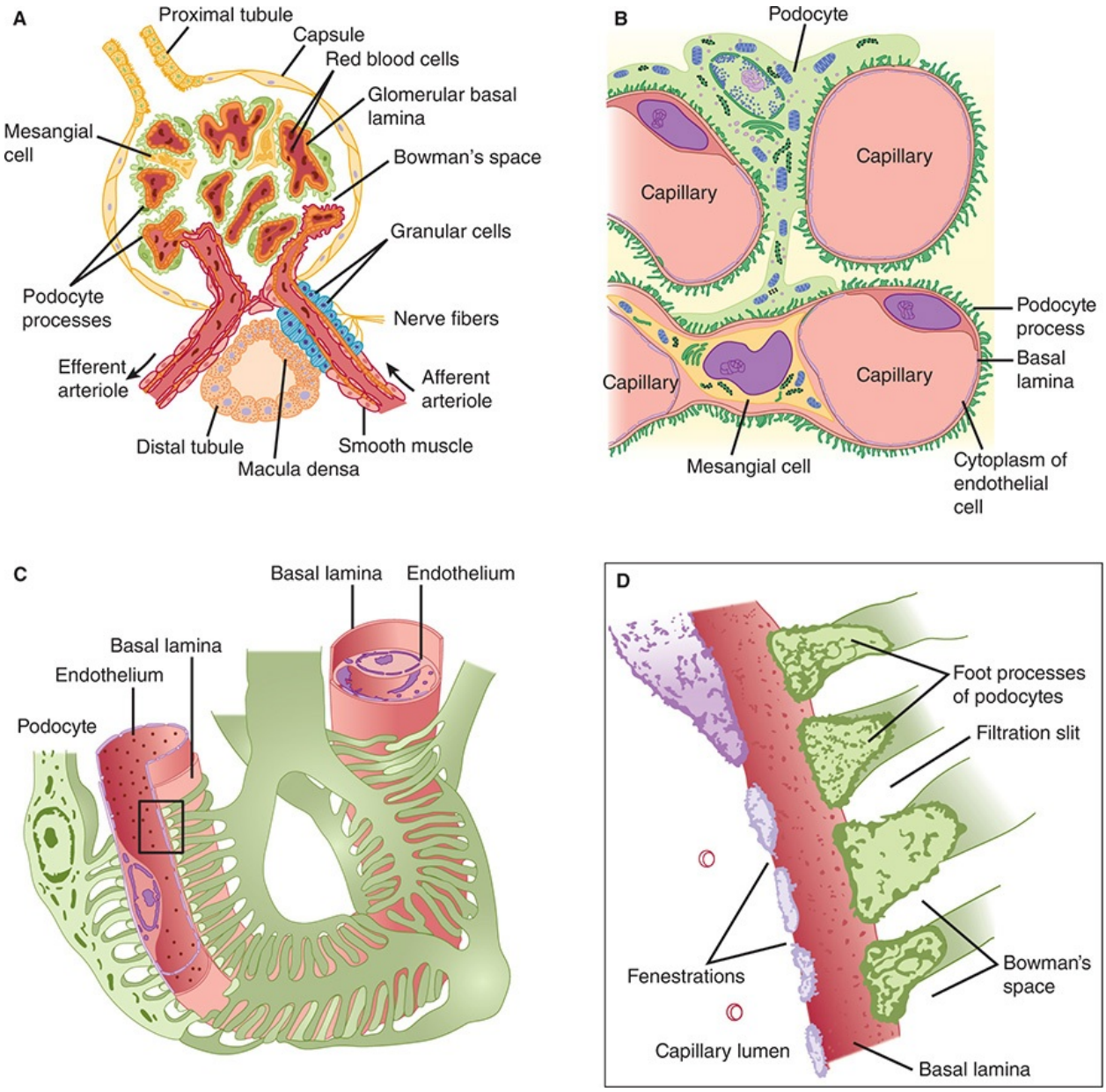


FIGURE 37–2 Structural details of glomerulus. A) Section through vascular pole, showing capillary loops. **B)** Relation of mesangial cells and podocytes to glomerular capillaries. **C)** Detail of the way podocytes form filtration slits on the basal lamina, and the relation of the lamina to the capillary endothelium. **D)** Enlargement of the rectangle in C to show the podocyte processes. The fuzzy material on their surfaces is glomerular polyanion.

Functionally, the glomerular membrane permits the free passage of neutral substances up to 4 nm in diameter and almost totally excludes those with diameters greater than 8 nm. However, the charge on molecules as well as their

diameters affects their passage into Bowman's capsule. The total area of glomerular capillary endothelium across which filtration occurs in humans is about 0.8 m^2.

The general features of the cells that make up the walls of the tubules are shown in Figure 37–1; however, there are cell subtypes in all segments, and the anatomic differences between them correlate with differences in function.

The human **proximal convoluted tubule** is about 15 mm long and 55 µm in diameter. Its wall is made up of a single layer of cells that interdigitate with one another and are united by apical tight junctions. Between the cells are extensions of the extracellular space called the **lateral intercellular spaces.** The luminal edges of the cells have a striated **brush border** due to the presence of many microvilli.

The convoluted proximal tubule straightens and the next portion of each nephron is the **loop of Henle.** The descending portion of the loop and the proximal portion of the ascending limb are made up of thin, permeable cells. On the other hand, the thick portion of the ascending limb (Figure 37–1) is made up of thick cells containing many mitochondria. The nephrons with glomeruli in the outer portions of the renal cortex have short loops of Henle **(cortical nephrons),** whereas those with glomeruli in the juxtamedullary region of the cortex **(juxtamedullary nephrons)** have long loops extending down into the medullary pyramids. In humans, only 15% of the nephrons have long loops.

The thick end of the ascending limb of the loop of Henle reaches the glomerulus of the nephron from which the tubule arose and nestles between its afferent and efferent arterioles. Specialized cells at the end form the **macula densa,** which is close to the efferent and particularly the afferent arteriole (Figure 37–2). The macula, the neighboring **lacis cells,** and the renin-secreting **granular cells** in the afferent arteriole form the **juxtaglomerular apparatus** (see Figure 38–8).

The **distal convoluted tubule,** which starts at the macula densa, is about 5 mm long. Its epithelium is lower than that of the proximal tubule, and although a few microvilli are present, there is no distinct brush border. The distal tubules coalesce to form **collecting ducts** that are about 20 mm long and pass through the renal cortex and medulla to empty into the pelvis of the kidney at the apexes of the medullary pyramids. The epithelium of the collecting ducts is made up of **principal cells (P cells)** and **intercalated cells (I cells).** The P cells, which predominate, are relatively tall and have few organelles. They are involved in Na^+ reabsorption and vasopressin-stimulated water reabsorption. The I cells, which are present in smaller numbers and are also found in the distal tubules,

have more microvilli, cytoplasmic vesicles, and mitochondria. They are concerned with acid secretion and HCO_3^- transport. The total length of the nephrons, including the collecting ducts, ranges from 45 to 65 mm.

Cells in the kidneys that appear to have a secretory function include not only the granular cells in the juxtaglomerular apparatus but also some of the cells in the interstitial tissue of the medulla. These cells are called **renal medullary interstitial cells (RMICs)** and are specialized fibroblast-like cells. They contain lipid droplets and are a major site of cyclooxygenase 2 (COX-2) and prostaglandin synthase (PGES) expression. PGE_2 is the major prostanoid synthesized in the kidney and is an important paracrine regulator of salt and water homeostasis. PGE_2 is secreted by the RMICs, by the macula densa, and by cells in the collecting ducts; prostacyclin (PGI_2) and other prostaglandins are secreted by the arterioles and glomeruli.

BLOOD VESSELS

The renal circulation is diagrammed in **Figure 37–3**. The **afferent arterioles** are short, straight branches of the interlobular arteries. Each divides into multiple capillary branches to form the tuft of vessels in the glomerulus. The capillaries coalesce to form the **efferent arteriole,** which in turn breaks up into capillaries that supply the tubules **(peritubular capillaries)** before draining into the interlobular veins. The arterial segments between glomeruli and tubules are thus technically a portal system, and the glomerular capillaries are the only capillaries in the body that drain into arterioles. However, there is relatively little smooth muscle in the efferent arterioles.

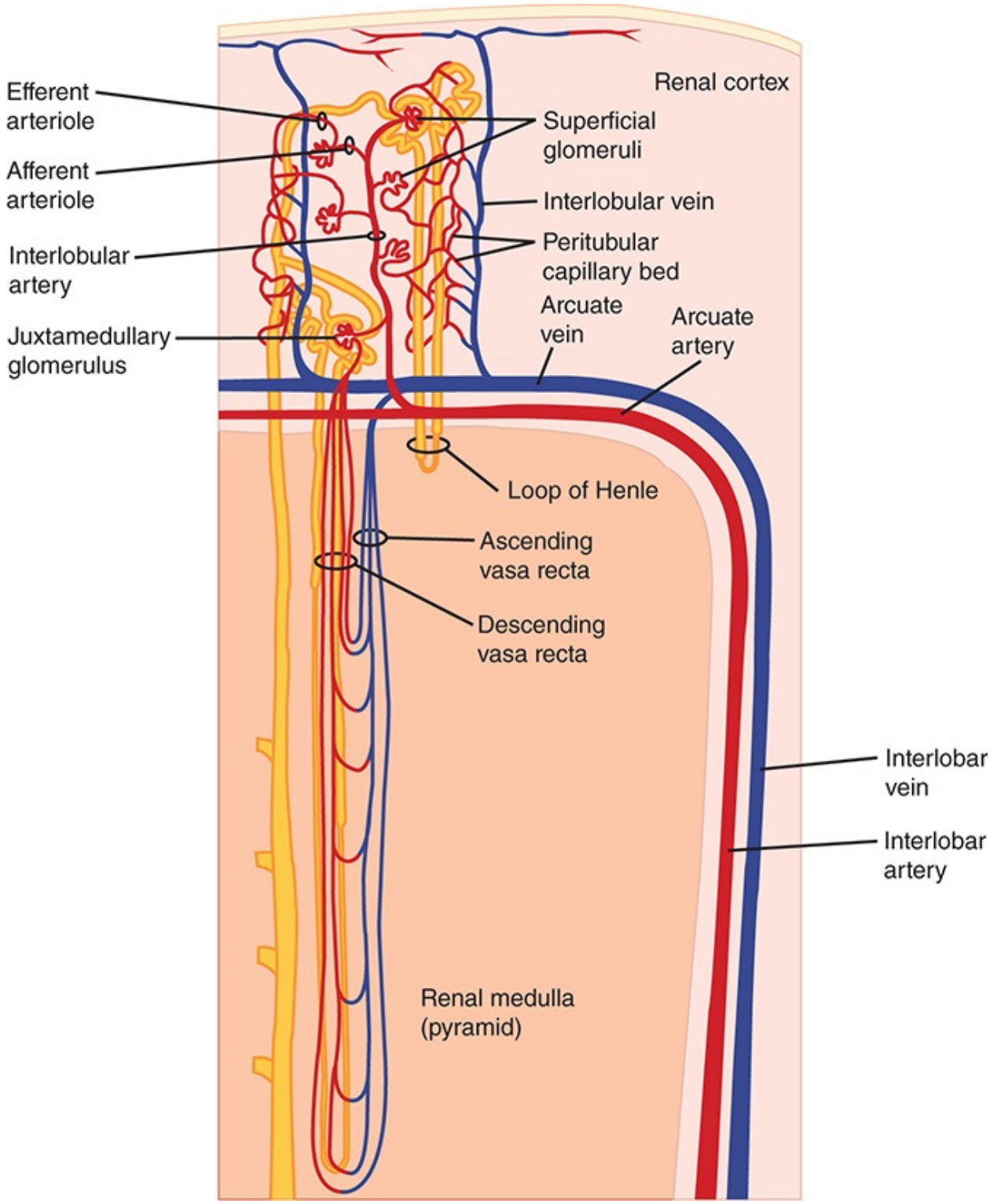


FIGURE 37–3 Renal circulation. Interlobar arteries divide into arcuate arteries, which give off interlobular arteries in the cortex. The interlobular arteries provide an afferent arteriole to each glomerulus. The efferent arteriole from each

glomerulus breaks up into capillaries that supply blood to the renal tubules. Venous blood enters interlobular veins, which in turn flow via arcuate veins to the interlobar veins. (Modified with permission from Boron WF, Boulpaep EL: *Medical Physiology*. Saunders, 2009.)

The capillaries draining the tubules of the cortical nephrons form a peritubular network, whereas the efferent arterioles from the juxtamedullary glomeruli drain not only into a peritubular network, but also into vessels that form hairpin loops (the **vasa recta**). These loops dip into the medullary pyramids alongside the loops of Henle (Figure 37–3). The descending vasa recta have a nonfenestrated endothelium that contains a facilitated transporter for urea, and the ascending vasa recta have a fenestrated endothelium, consistent with their function in conserving solutes.

The efferent arteriole from each glomerulus breaks up into capillaries that supply a number of different nephrons. Thus, the tubule of each nephron does not necessarily receive blood solely from the efferent arteriole of the same nephron. In humans, the total surface of the renal capillaries is approximately equal to the total surface area of the tubules, both being about 12 m^2. The volume of blood in the renal capillaries at any given time is 30–40 mL.

LYMPHATICS

The kidneys have an abundant lymphatic supply that drains via the thoracic duct into the venous circulation in the thorax.

CAPSULE

The renal capsule is thin but tough. If the kidney becomes edematous, the capsule limits the swelling, and the tissue pressure **(renal interstitial pressure)** rises. This decreases the glomerular filtration rate (GFR) and is claimed to enhance and prolong anuria in acute kidney injury (AKI).

INNERVATION OF THE RENAL VESSELS

The renal nerves travel along the renal blood vessels as they enter the kidney. They contain many postganglionic sympathetic efferent fibers and a few afferent fibers. There also appears to be a cholinergic innervation via the vagus nerve, but

its function is uncertain. The sympathetic preganglionic innervation comes primarily from the lower thoracic and upper lumbar segments of the spinal cord, and the cell bodies of the postganglionic neurons are in the sympathetic ganglion chain, in the superior mesenteric ganglion, and along the renal artery. The sympathetic fibers are distributed primarily to the afferent and efferent arterioles, the proximal and distal tubules, and the juxtaglomerular apparatus (see Chapter 38). In addition, there is a dense noradrenergic innervation of the thick ascending limb of the loop of Henle.

Nociceptive afferents that mediate pain in kidney disease parallel the sympathetic efferents and enter the spinal cord in the thoracic and upper lumbar dorsal roots. Other renal afferents presumably mediate a **renorenal reflex** by which an increase in ureteral pressure in one kidney leads to a decrease in efferent nerve activity to the contralateral kidney. This decrease permits an increase in its excretion of Na^+ and water.

RENAL CIRCULATION

BLOOD FLOW

In a resting adult, the kidneys receive 1.2–1.3 L of blood per minute, or just under 25% of the cardiac output. Renal blood flow can be measured with electromagnetic or other types of flow meters, or it can be determined by applying the Fick principle (see Chapter 30) to the kidney; that is, by measuring the amount of a given substance taken up per unit of time and dividing this value by the arteriovenous difference for the substance across the kidney. Because the kidney filters plasma, the **renal plasma flow** (RPF) equals the amount of a substance excreted per unit of time divided by the renal arteriovenous difference as long as the amount in the red cells is unaltered during passage through the kidney. Any excreted substance can be used if its concentration in arterial and renal venous plasma can be measured and if it is not metabolized, stored, or produced by the kidney and does not itself affect blood flow.

RPF can be measured by infusing p-aminohippuric acid (PAH) and determining its urine and plasma concentrations. PAH is filtered by the glomeruli and secreted by the tubular cells, so that its **extraction ratio** (arterial concentration minus renal venous concentration divided by arterial concentration) is high. For example, when PAH is infused at low doses, 90% of the PAH in arterial blood is removed in a single circulation through the kidney. It has therefore become commonplace to calculate the “RPF” by dividing the

amount of PAH in the urine by the plasma PAH level, ignoring the level in renal venous blood. Peripheral venous plasma can be used because its PAH concentration is essentially identical to that in the arterial plasma reaching the kidney. The value obtained should be called the **effective renal plasma flow (ERPF)** to indicate that the level in renal venous plasma was not measured. In humans, ERPF averages about 625 mL/min.

$$\text{ERPF} = \frac{U_{PAH}\dot{V}}{P_{PAH}} = \text{Clearance of PAH } (C_{PAH})$$

Example:

Concentration of PAH in urine (U_{PAH}): 14 mg/mL

Urine flow ($\dot{V}$): 0.9 mL/min

Concentration of PAH in plasma (P_{PAH}): 0.02 mg/mL

$$\text{ERPF} = \frac{14 \times 0.9}{0.02} = 630 \text{ mL/min}$$

It should be noted that the ERPF determined in this way is the **clearance** of PAH. The concept of clearance is discussed in detail below.

ERPF can be converted to actual renal plasma flow (RPF):

Average PAH extraction ratio: 0.9

$$\frac{\text{ERP}}{\text{Extraction ration}} = \frac{630}{0.9} = \text{Actual RPF} = 700 \text{ mL/min}$$

From the renal plasma flow, the renal blood flow can be calculated by dividing by 1 minus the hematocrit:

Hematocrit (Hct): 45%

$$\begin{aligned}\text{Renal blood flow} &= \text{RPF} \times \frac{1}{1 - \text{Hct}} \\ &= 700 \times \frac{1}{0.55} \\ &= 1273 \text{ mL/min}\end{aligned}$$

PRESSURE IN RENAL VESSELS

The pressure in the glomerular capillaries has been measured directly in rats and has been found to be considerably lower than predicted on the basis of indirect measurements. When the mean systemic arterial pressure is 100 mm Hg, the glomerular capillary pressure is about 45 mm Hg. The pressure drop across the glomerulus is only 1–3 mm Hg, but a further drop occurs in the efferent arteriole so that the pressure in the peritubular capillaries is about 8 mm Hg. The pressure in the renal vein is about 4 mm Hg. Pressure gradients are similar in squirrel monkeys and presumably in humans, with a glomerular capillary pressure that is about 40% of systemic arterial pressure.

REGULATION OF THE RENAL BLOOD FLOW

Norepinephrine (noradrenaline) constricts the renal vessels, with the greatest effect of injected norepinephrine being exerted on the interlobular arteries and the afferent arterioles. Dopamine is made in the kidney and causes renal vasodilation and natriuresis. Angiotensin II exerts a constrictor effect on both the afferent and efferent arterioles. Prostaglandins increase blood flow in the renal cortex and decrease blood flow in the renal medulla. Acetylcholine also produces renal vasodilation. A high-protein diet raises glomerular capillary pressure and increases renal blood flow.

FUNCTIONS OF THE RENAL NERVES

Stimulation of the renal nerves increases renin secretion by a direct action of released norepinephrine on β_1-adrenergic receptors on the juxtaglomerular cells (see Chapter 38), and it increases Na^+ reabsorption, probably by a direct action of norepinephrine on renal tubular cells. The proximal and distal tubules and the thick ascending limb of the loop of Henle are richly innervated. When the renal nerves are stimulated to increasing extents in experimental animals, the first response is an increase in the sensitivity of the granular cells in the juxtaglomerular apparatus **(Table 37–1)**, followed by increased renin secretion, then increased Na^+ reabsorption, and finally, at the highest threshold, renal vasoconstriction with decreased glomerular filtration and renal blood flow. It is still unsettled whether the effect on Na^+ reabsorption is mediated via α- or β-adrenergic receptors, and it may be mediated by both. The physiologic role of the renal nerves in Na^+ homeostasis is also unsettled, in part because most renal functions appear to be normal in patients with transplanted kidneys, and it takes

some time for transplanted kidneys to acquire a functional innervation.

TABLE 37–1 Renal responses to graded renal nerve stimulation.

Renal Nerve Stimulation Frequency (Hz)	RSR	$U_{Na}V$	GFR	RBF
0.25	No effect on basal values; augments RSR mediated by nonneural stimuli	0	0	0
0.50	Increased without changing $U_{Na}V$, GFR, or RBF	0	0	0
1.0	Increased with decreased $U_{Na}V$, without changing GFR or RBF	↓	0	0
2.50	Increased with decreased $U_{Na}V$, GFR, and RBF	↓	↓	↓

GFR, glomerular filtration rate; RBF, renal blood flow; RSR, renin secretion rate; $U_{Na}V$, urinary sodium excretion.

Reproduced from DiBona GF: Neural control of renal function: Cardiovascular implications. Hypertension 1989;13:539. By permission of the American Heart Association.

Strong stimulation of the sympathetic noradrenergic nerves to the kidneys causes a marked decrease in renal blood flow. This effect is mediated by α_1-adrenergic receptors and to a lesser extent by postsynaptic α_2-adrenergic receptors. Some tonic discharge takes place in the renal nerves at rest in animals and humans. When systemic blood pressure falls, the vasoconstrictor response produced by decreased discharge in the baroreceptor nerves includes renal vasoconstriction. Renal blood flow is decreased during exercise and, to a lesser extent, on rising from the supine position.

AUTOREGULATION OF RENAL BLOOD FLOW

When the kidney is perfused at moderate pressures (90–220 mm Hg in the dog), the renal vascular resistance varies with the pressure so that renal blood flow is

relatively constant **(Figure 37–4)**. Autoregulation of this type occurs in other organs, and several factors contribute to it (see Chapter 32). Renal autoregulation is present in denervated and in isolated, perfused kidneys but is prevented by the administration of drugs that paralyze vascular smooth muscle. It is probably produced in part by a direct contractile response to stretch of the smooth muscle of the afferent arteriole. NO may also be involved. At low perfusion pressures, angiotensin II also appears to play a role by constricting the efferent arterioles, thus maintaining the GFR. This is believed to be the explanation of the renal failure that sometimes develops in patients with poor renal perfusion who are treated with drugs that inhibit angiotensin-converting enzyme.

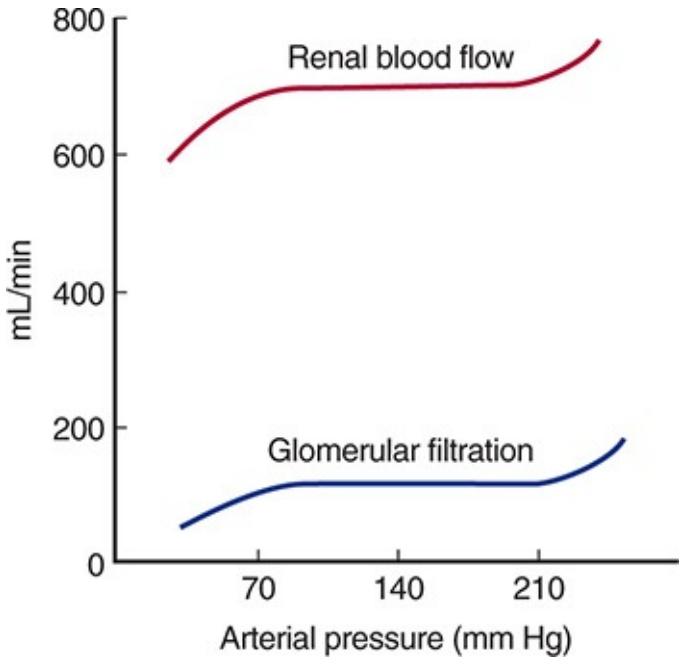


FIGURE 37–4 Autoregulation in the kidneys.

REGIONAL BLOOD FLOW & OXYGEN CONSUMPTION

The main function of the renal cortex is filtration of large volumes of blood through the glomeruli, so it is not surprising that the renal cortical blood flow is relatively great and little oxygen is extracted from the blood. Cortical blood flow is about 5 mL/g of kidney tissue/min (compared with 0.5 mL/g/min in the brain), and the arteriovenous oxygen difference for the whole kidney is only 14 mL/L of blood, compared with 62 mL/L for the brain and 114 mL/L for the heart (see

Table 33–1). The P_{O_2} of the cortex is about 50 mm Hg. On the other hand, maintenance of the osmotic gradient in the medulla requires a relatively low blood flow. It is not surprising, therefore, that the blood flow is about 2.5 mL/g/min in the outer medulla and 0.6 mL/g/min in the inner medulla. However, metabolic work is being done, particularly to reabsorb Na^+ in the thick ascending limb of Henle, so relatively large amounts of O_2 are extracted from the blood in the medulla. The P_{O_2} of the medulla is about 15 mm Hg. This makes the medulla vulnerable to hypoxia if flow is reduced further. NO, prostaglandins, and many cardiovascular peptides in this region function in a paracrine manner to maintain the balance between low blood flow and metabolic needs.

GLOMERULAR FILTRATION

MEASURING GFR

Glomerular filtration rate (GFR) is the amount of plasma ultrafiltrate formed each minute and can be measured in intact experimental animals and humans by measuring the plasma level of a substance and the amount of that substance that is excreted. A substance to be used to measure GFR must be freely filtered through the glomeruli and must be neither secreted nor reabsorbed by the tubules.

In addition to the requirement that it be freely filtered and neither reabsorbed nor secreted in the tubules, a substance suitable for measuring the GFR should be nontoxic and not metabolized by the body. Inulin, a polymer of fructose with a molecular weight of 5200, meets these criteria in humans and most animals and can be used to measure GFR.

Renal plasma clearance is the *volume of plasma* from which a substance is completely removed by the kidney in a given amount of time (usually minutes). The amount of that substance that appears in the urine per unit of time is the result of the renal filtering of a certain number of milliliters of plasma that contained this amount. GFR and clearance are measured in mL/min.

Therefore, if the substance is designated by the letter X, the GFR is equal to the concentration of X in urine (U_X) times the **urine flow** per unit of time ($\dot{V}$) divided by the **arterial plasma level** of X (P_X), or $\dot{V}/P_X$. **This value is called the clearance of X (CX).**

In practice, a loading dose of inulin is administered intravenously, followed by a sustaining infusion to keep the arterial plasma level constant. After the

inulin has equilibrated with body fluids, an accurately timed urine specimen is collected and a plasma sample obtained halfway through the collection. Plasma and urinary inulin concentrations are determined and the clearance is calculated:

$$U_{IN} = 35 \text{ mg/mL}$$
$$\dot{V} = 0.9 \text{ mL/min}$$
$$P_{IN} = 0.25 \text{ mg/mL}$$
$$C_{IN} = \frac{U_{IN}\,\dot{V}}{P_{IN}} = \frac{35 \times 0.9}{0.25}$$
$$C_{IN} = 126 \text{ mL/min}$$

Clearance of creatinine (C_{Cr}) can also be used to determine GFR; however, some creatinine is secreted by the tubules, thus the clearance of creatinine will be slightly higher than inulin. In spite of this, the clearance of endogenous creatinine is a reasonable estimate of GFR as the values agree quite well with the GFR values measured with inulin **(see Table 37–2)**. More common though is the use of **P_{Cr} values as an index of renal function (normal = 1 mg/dL).**

TABLE 37–2 Normal clearance values of different solutes.

Substance	Clearance (mL/min)
Glucose	0
Sodium	0.9
Chloride	1.3
Potassium	12
Phosphate	25
Urea	75
Inulin	125
Creatinine	140
PAH	560

NORMAL GFR

The GFR in a healthy adult of average size is approximately 125 mL/min. Its magnitude correlates fairly well with surface area, but values in women are 10% lower than those in men even after correction for surface area. A rate of 125 mL/min is 7.5 L/h, or 180 L/d, whereas the normal urine volume is about 1 L/d. Thus, 99% or more of the filtrate is normally reabsorbed. At the rate of 125 mL/min, in 1 day the kidneys filter an amount of fluid equal to four times the total body water, 15 times the ECF volume, and 60 times the plasma volume.

CONTROL OF GFR

The factors governing filtration across the glomerular capillaries are the same as those governing filtration across all other capillaries (see Chapter 31), that is, the size of the capillary bed, the permeability of the capillaries, and the hydrostatic and osmotic pressure gradients across the capillary wall. For each nephron:

$$GFR = K_f [(P_{GC} - P_T) - (\pi_{GC} - \pi_T)]$$

where K_f, the glomerular ultrafiltration coefficient, is the product of the glomerular capillary wall hydraulic conductivity (ie, its permeability) and the effective filtration surface area. P_{GC} is the mean hydrostatic pressure in the glomerular capillaries, P_T the mean hydrostatic pressure in the tubule (Bowman's space), π_{GC} the oncotic pressure of the plasma in the glomerular capillaries, and π_T the oncotic pressure of the filtrate in the tubule (Bowman's space).

PERMEABILITY

The permeability of the glomerular capillaries is about 50 times that of the capillaries in skeletal muscle. Neutral substances with effective molecular diameters of less than 4 nm are freely filtered, and the filtration of neutral substances with diameters of more than 8 nm approaches zero. Between these values, filtration is inversely proportional to diameter. However, sialoproteins in the glomerular capillary wall are negatively charged, and studies with negatively and positively charged dextrans indicate that the negative charges repel negatively charged substances in blood, with the result that filtration of anionic substances 4 nm in diameter is less than half that of neutral substances of the same size. This probably explains why albumin, with an effective molecular

diameter of approximately 7 nm, normally has a glomerular concentration only 0.2% of its plasma concentration rather than the higher concentration that would be expected on the basis of diameter alone; circulating albumin is negatively charged. Conversely, filtration of cationic substances is greater than that of neutral substances.

The amount of protein in the urine is normally less than 100 mg/day, and most of this is not filtered but comes from shed tubular cells. The presence of significant amounts of albumin in the urine is called **albuminuria.** In nephritis, the negative charges in the glomerular wall are dissipated, and albuminuria can occur for this reason without an increase in the size of the "pores" in the membrane.

SIZE OF THE CAPILLARY BED

K_f can be altered by the mesangial cells, with contraction of these cells producing a decrease in K_f that is largely due to a reduction in the area available for filtration. Contraction of points where the capillary loops bifurcate probably shifts flow away from some of the loops, and elsewhere, contracted mesangial cells distort and encroach on the capillary lumen. Agents that have been shown to affect the mesangial cells are listed in Table 37–3. Angiotensin II is an important regulator of mesangial contraction, and there are angiotensin II receptors in the glomeruli. In addition, some evidence suggests that mesangial cells make renin.

TABLE 37–3 Agents causing contraction or relaxation of mesangial cells.

Contraction	Relaxation
Endothelins	ANP
Angiotensin II	Dopamine
Vasopressin	PGE_2
Norepinephrine	cAMP
Platelet-activating factor	
Platelet-derived growth factor	
Thromboxane A_2	
PGF_2	
Leukotrienes C_4 and D_4	
Histamine	

HYDROSTATIC & OSMOTIC PRESSURE

The pressure in the glomerular capillaries is higher than that in other capillary beds because the afferent arterioles are short, straight branches of the interlobular arteries. Furthermore, the vessels “downstream” from the glomeruli, the efferent arterioles, have a relatively high resistance. The capillary hydrostatic pressure is opposed by the hydrostatic pressure in Bowman’s capsule. It is also opposed by the oncotic pressure gradient across the glomerular capillaries ($\pi_{GC} - \pi_T$). π_T is normally negligible, and the gradient is essentially equal to the oncotic pressure of the plasma proteins.

The actual pressures in one strain of rats are shown in **Figure 37–5**. The net filtration pressure (P_{UF}) is 15 mm Hg at the afferent end of the glomerular capillaries, but it falls to zero—that is, filtration equilibrium is reached—proximal to the efferent end of the glomerular capillaries. This is because fluid leaves the plasma and the oncotic pressure rises as blood passes through the glomerular capillaries. The calculated change in $\Delta\pi$ along an idealized glomerular capillary is also shown in Figure 37–5. It is apparent that portions of the glomerular capillaries do not normally contribute to the formation of the glomerular ultrafiltrate; that is, exchange across the glomerular capillaries is flow-limited rather than diffusion-limited. It is also apparent that a decrease in the rate of rise of the Δ curve produced by an increase in RPF would increase filtration because it would increase the distance along the capillary in which filtration was taking place.

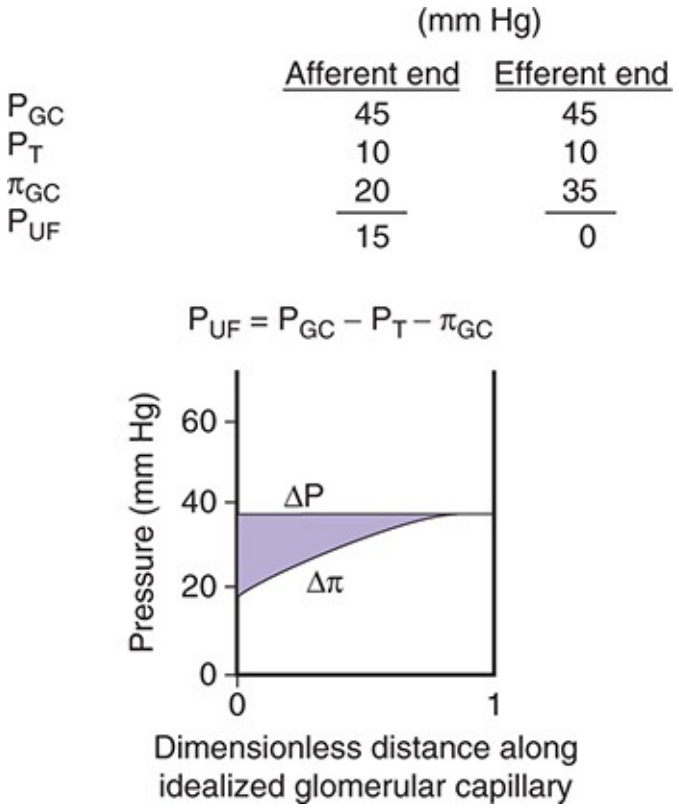


FIGURE 37–5 Hydrostatic pressure (P_{GC}) and osmotic pressure (π_{GC}) in a glomerular capillary in the rat. P_T, pressure in Bowman's capsule; P_{UF}, net filtration pressure. π_T is normally negligible, so $\Delta\pi = \pi_{GC}$. $\Delta P = P_{GC} - P_T$. (Reproduced with permission from Mercer PF, Maddox DA, Brenner BM: Current concepts of sodium chloride and water transport by the mammalian nephron. West J Med 1974; Jan; 120(1):33–45.)

There is considerable species variation in whether filtration equilibrium is reached, and some uncertainties are inherent in the measurement of K_f. It is uncertain whether filtration equilibrium is reached in humans.

CHANGES IN GFR

Variations in the factors discussed in the preceding paragraphs and listed in **Table 37–4** have predictable effects on the GFR. Changes in renal vascular resistance as a result of autoregulation tend to stabilize filtration pressure, but when the mean systemic arterial pressure drops below the autoregulatory range (Figure 37–4), GFR drops sharply. The GFR tends to be maintained when efferent arteriolar constriction is greater than afferent constriction, but either type

of constriction decreases blood flow to the tubules.

TABLE 37–4 Factors affecting the glomerular filtration rate.

Changes in renal blood flow
Changes in glomerular capillary hydrostatic pressure
Changes in systemic blood pressure
Afferent or efferent arteriolar constriction
Changes in hydrostatic pressure in Bowman capsule
Ureteral obstruction
Edema of kidney inside tight renal capsule
Changes in concentration of plasma proteins: dehydration, hypoproteinemia, etc (minor factors)
Changes in K_f
Changes in glomerular capillary permeability
Changes in effective filtration surface area

FILTRATION FRACTION

The ratio of the GFR to the RPF, the **filtration fraction,** is normally 0.16–0.20. The GFR varies less than the RPF. When there is a fall in systemic blood pressure, the GFR falls less than the RPF because of efferent arteriolar constriction, and consequently the filtration fraction rises.

TUBULAR FUNCTION

GENERAL CONSIDERATIONS

The amount of any substance (X) that is filtered is the product of the GFR and

the plasma level of the substance ($C_{ln}P_X$). The tubular cells may add more of the substance to the filtrate (tubular secretion), may remove some or all of the substance from the filtrate (tubular reabsorption), or may do both. The amount of the substance excreted per unit of ($U_X\dot{V}$) time equals the amount filtered plus the **net amount transferred** by the tubules. This latter quantity is conveniently indicated by the symbol T_X **(Figure 37–6)**. The clearance of the substance equals the GFR if there is no net tubular secretion or reabsorption, exceeds the GFR if there is net tubular secretion, and is less than the GFR if there is net tubular reabsorption.

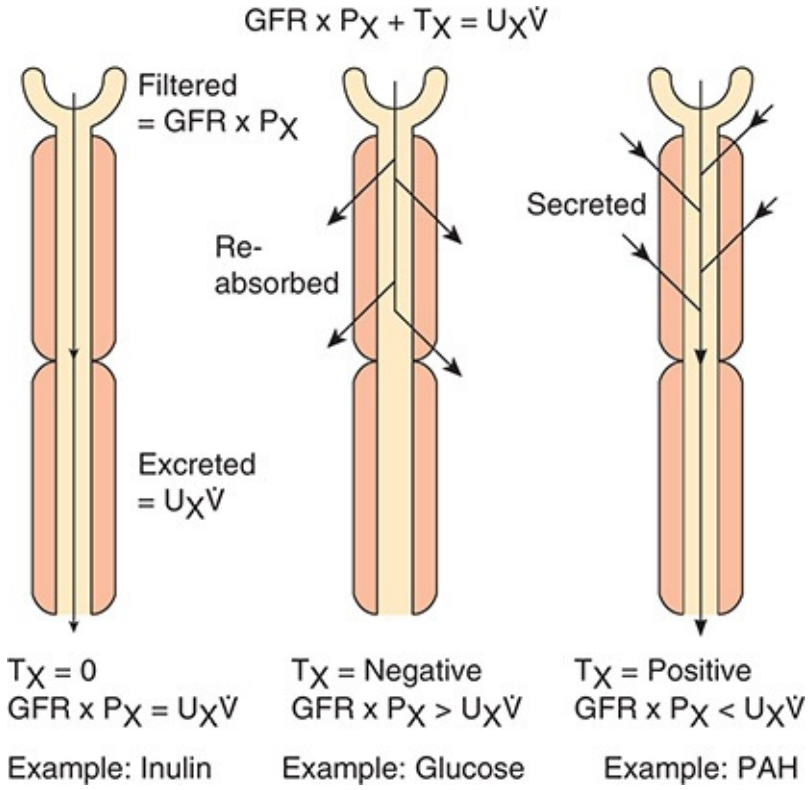


FIGURE 37–6 Tubular function. For explanation of symbols, see text.

Much of the knowledge about glomerular filtration and tubular function has been obtained by using micropuncture techniques. Micropipettes can be inserted into the tubules of the living kidney and the composition of aspirated tubular fluid determined by the use of microchemical techniques. In addition, two pipettes can be inserted in a tubule and the tubule perfused in vivo. Alternatively, isolated perfused segments of tubules can be studied in vitro, and tubular cells can be grown and studied in culture.

MECHANISMS OF TUBULAR REABSORPTION & SECRETION

Small proteins and some peptide hormones are reabsorbed in the proximal tubules by endocytosis. Other substances are secreted or reabsorbed in the tubules by passive diffusion between cells and through cells by facilitated diffusion down chemical or electrical gradients or active transport against such gradients. Movement is by way of ion channels, exchangers, cotransporters, and pumps. Many of these have now been cloned, and their regulation is being studied.

It is important to note that the pumps and other transporters in the luminal membrane are different from those in the basolateral membrane. As was discussed for the gastrointestinal epithelium, it is this polarized distribution that makes possible net movement of solutes across the epithelia.

Like transport systems elsewhere, renal active transport systems have a maximal rate, or **transport maximum (Tm),** at which they can transport a particular solute. Thus, the amount of a particular solute transported is proportional to the amount present up to the Tm for the solute, but at higher concentrations, the transport mechanism is **saturated** and there is no appreciable increment in the amount transported. However, the Tms for some systems are high, and it is difficult to saturate them.

It should also be noted that the tubular epithelium, like that of the small intestine, is a **leaky epithelium** in that the tight junctions between cells permit the passage of some water and electrolytes. The degree to which leakage by this **paracellular pathway** contributes to the net flux of fluid and solute into and out of the tubules is controversial since it is difficult to measure, but current evidence seems to suggest that it is a significant factor in the proximal tubule. One indication of this is that paracellin-1, a protein localized to tight junctions, is related to Mg^{2+} reabsorption, and a loss-of-function mutation of its gene causes severe Mg^{2+} and Ca^{2+} loss in the urine.

NA^{+} REABSORPTION

The reabsorption of Na^{+} and Cl^{-} plays a major role in body electrolyte and water homeostasis. In addition, Na^{+} transport is coupled to the movement of H^{+}, glucose, amino acids, organic acids, phosphate, and other electrolytes and substances across the tubule walls. The principal cotransporters and exchangers

in the various parts of the nephron are listed in **Table 37–5**. In the proximal tubules, the thick portion of the ascending limb of the loop of Henle, the distal tubules, and the collecting ducts, Na^+ moves by cotransport or exchange from the tubular lumen into the tubular epithelial cells down its concentration and electrical gradients, and is then actively pumped from these cells into the interstitial space. Na^+ is pumped into the interstitium by Na, K ATPase in the basolateral membrane. Thus, Na^+ is actively transported out of all parts of the renal tubule except the thin portions of the loop of Henle. The operation of the ubiquitous Na^+ pump is considered in detail in Chapter 2. It extrudes three Na^+ in exchange for two K^+ that are pumped into the cell.

TABLE 37–5 Transport proteins involved in the movement of Na^+ and Cl^- across the apical membranes of renal tubular cells.[a]

Site	Apical Transporter	Function
Proximal tubule	Na/glucose CT	Na^+ uptake, glucose uptake
	Na^+/P_i CT	Na^+ uptake, P_i uptake
	Na^+ amino acid CT	Na^+ uptake, amino acid uptake
	Na/lactate CT	Na^+ uptake, lactate uptake
	Na/H exchanger	Na^+ uptake, H^+ extrusion
	Cl/base exchanger	Cl^- uptake
Thick ascending limb	Na–K–2Cl CT	Na^+ uptake, Cl^- uptake, K^+ uptake
	Na/H exchanger	Na^+ uptake, H^+ extrusion
	K^+ channels	K^+ extrusion (recycling)
Distal convoluted tubule	NaCl CT	Na^+ uptake, Cl^- uptake
Collecting duct	Na^+ channel (ENaC)	Na^+ uptake

[a]Uptake indicates movement from tubular lumen to cell interior, extrusion is movement from cell interior to tubular lumen. CT, cotransporter; P_i, inorganic phosphate.

Data from Schnermann JB, Sayegh EI: *Kidney Physiology*. Lippincott-Raven, 1998.

The tubular cells along the nephron are connected by tight junctions at their luminal edges, but there is space between the cells along the rest of their lateral borders. Much of the Na^+ is actively transported into these **lateral intercellular spaces** (Figure 37–7).

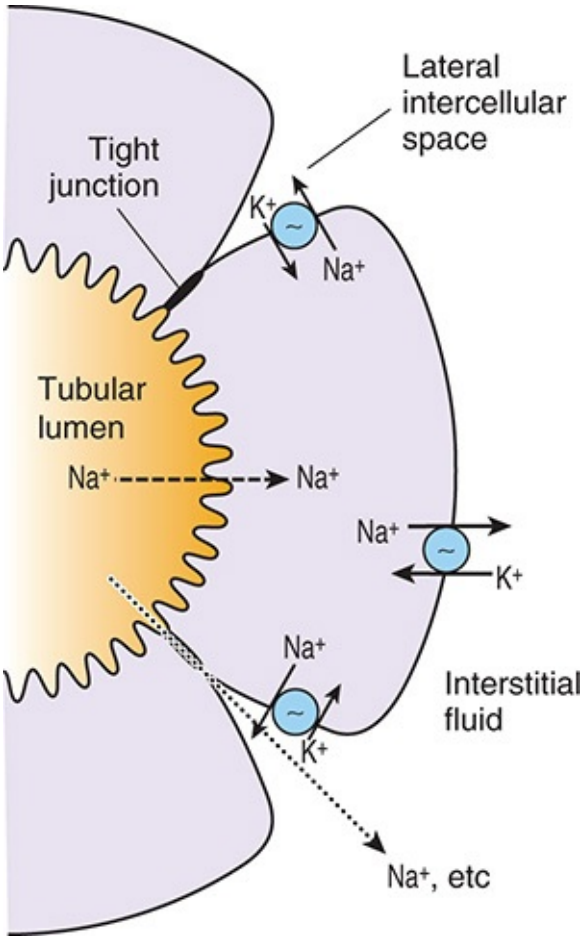


FIGURE 37–7 Mechanism of Na^+ reabsorption in the proximal tubule. Na^+ moves out of the tubular lumen by cotransport and exchange mechanism through the apical membrane of the tubule (dashed line). The Na^+ is then actively transported into the interstitial fluid by Na, K ATPase in the basolateral membrane (solid lines). K^+ enters the interstitial fluid via K^+ channels. A small amount of Na^+, other solutes, and H_2O reenter the tubular lumen by passive transport through the tight junctions (dotted lines).

Normally about 60% of the filtered Na^+ is reabsorbed in the proximal tubule, primarily by Na–H exchange. Another 30% is absorbed via the Na–2Cl–K cotransporter in the thick ascending limb of the loop of Henle. In both of these

segments of the nephron, passive paracellular movement of Na^+ also contributes to overall Na^+ reabsorption. In the distal convoluted tubule 7% of the filtered Na^+ is absorbed by the Na–Cl cotransporter. The remainder of the filtered Na^+, about 3%, is absorbed via ENaC channels in the collecting ducts, and this is the portion that is regulated by aldosterone to permit homeostatic adjustments in Na^+ balance.

GLUCOSE REABSORPTION

Glucose, amino acids, and bicarbonate are reabsorbed along with Na^+ in the early portion of the proximal tubule **(Figure 37–8)**. Glucose is typical of substances removed from the urine by secondary active transport. It is filtered at a rate of approximately 100 mg/min (80 mg/dL of plasma × 125 mL/min). Essentially all of the glucose is reabsorbed, and no more than a few milligrams appear in the urine per 24 h. The amount reabsorbed is proportional to the amount filtered and hence to the plasma glucose level (P_G) times the GFR up to the transport maximum (Tm_G). When the Tm_G is exceeded, the amount of glucose in the urine rises **(Figure 37–9)**. The Tm_G is about 375 mg/min in men and 300 mg/min in women.

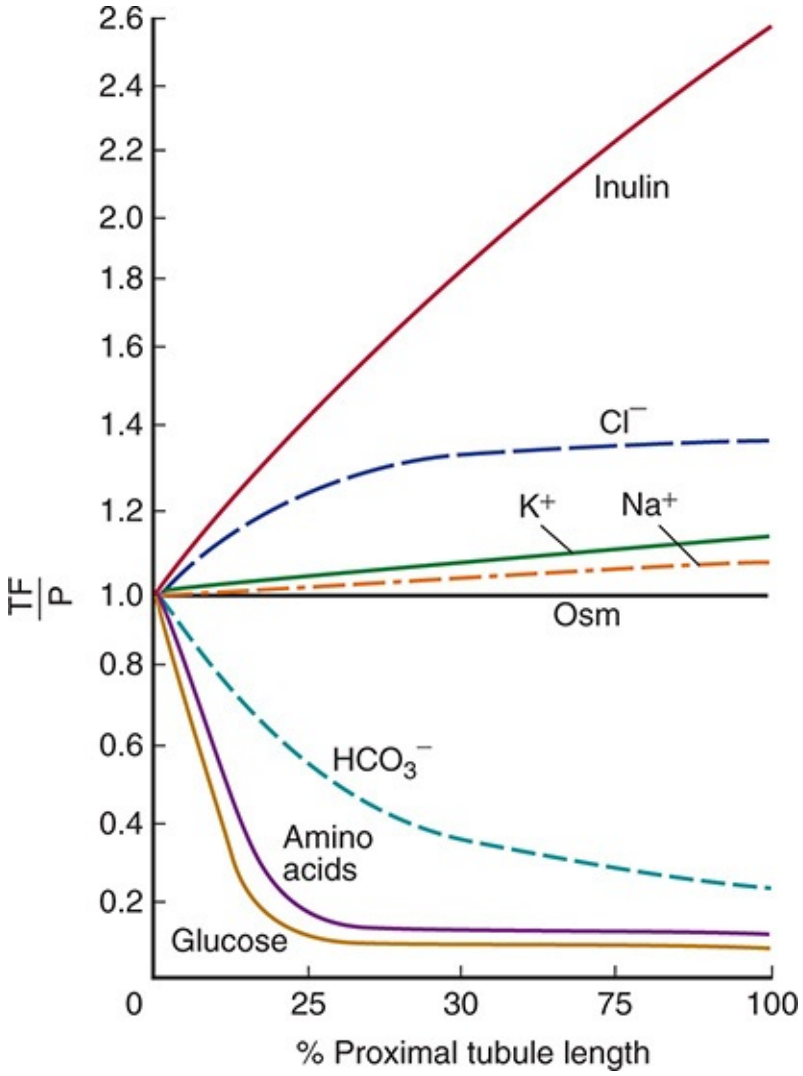


FIGURE 37–8 Reabsorption of various solutes in the proximal tubule. TF/P, tubular fluid:plasma concentration ratio. (Used with permission of FC Rector Jr.)

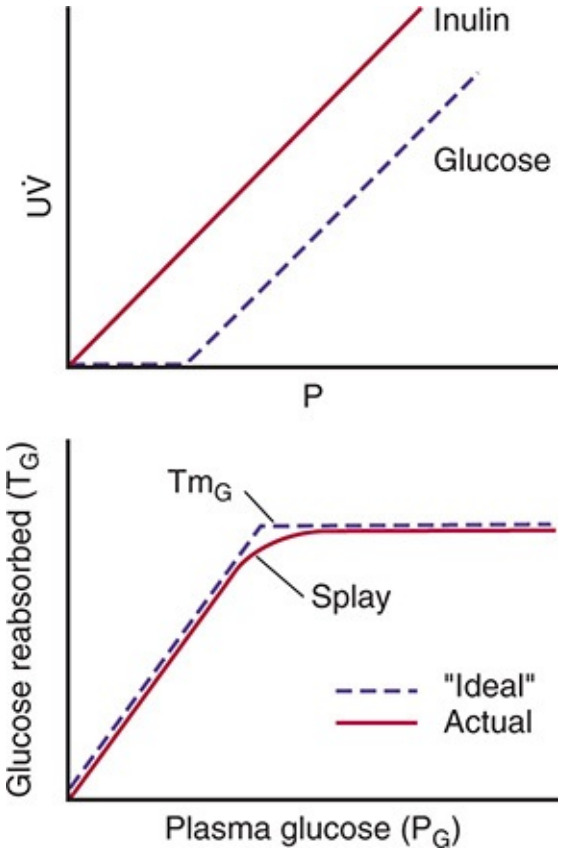


FIGURE 37–9 Renal glucose transport. Top: Relation between the plasma level (P) and excretion (UV) of glucose and inulin. **Bottom:** Relation between the plasma glucose level (P_G) and amount of glucose reabsorbed (T_G).

The **renal threshold** for glucose is the plasma level at which the glucose first appears in the urine in more than the normal minute amounts. One would predict that the renal threshold would be about 300 mg/dL, that is, 375 mg/min (Tm_G) divided by 125 mL/min (GFR). However, the actual renal threshold is about 200 mg/dL of arterial plasma, which corresponds to a venous level of about 180 mg/dL. Figure 37–9 shows why the actual renal threshold is less than the predicted threshold. The "ideal" curve shown in this diagram would be obtained if the Tm_G in all the tubules was identical and if all the glucose were removed from each tubule when the amount filtered was below the Tm_G. This is not the case, and in humans, for example, the actual curve is rounded and deviates considerably from the "ideal" curve. This deviation is called **splay.** The magnitude of the splay is inversely proportional to the avidity with which the transport mechanism binds the substance it transports.

GLUCOSE TRANSPORT MECHANISM

Glucose reabsorption in the kidneys is similar to glucose reabsorption in the intestine (see Chapter 26). Glucose and Na^+ bind to the sodium-dependent glucose transporter (SGLT)-2 in the apical membrane, and glucose is carried into the cell as Na^+ moves down its electrical and chemical gradient. The Na^+ is then pumped out of the cell into the interstitium, and the glucose exits by facilitated diffusion via glucose transporter (GLUT)-2 into the interstitial fluid. At least in the rat, there is some transport by SGLT-1 and GLUT-1 as well.

SGLT-2 specifically binds the D isomer of glucose, and the rate of transport of D-glucose is many times greater than that of L-glucose. Glucose transport in the kidneys is inhibited, as it is in the intestine, by the plant glucoside **phlorhizin,** which competes with D-glucose for binding to the carrier.

ADDITIONAL EXAMPLES OF SECONDARY ACTIVE TRANSPORT

Like glucose reabsorption, amino acid reabsorption is most marked in the early portion of the proximal convoluted tubule. Absorption in this location resembles absorption in the intestine (see Chapter 26). The main carriers in the apical membrane cotransport Na^+, whereas the carriers in the basolateral membranes are not Na^+-dependent. Na^+ is pumped out of the cells by Na, K ATPase and the amino acids leave by passive or facilitated diffusion to the interstitial fluid.

Some Cl^- is reabsorbed with Na^+ and K^+ in the thick ascending limb of the loop of Henle. In addition, two members of the family of **Cl channels** have been identified in the kidney. Mutations in the gene for one of the renal channels is associated with Ca^{2+}-containing kidney stones and hypercalciuria **(Dent disease),** but how tubular transport of Ca^{2+} and Cl^- are linked is still unsettled.

PAH TRANSPORT

The dynamics of PAH transport illustrate the operation of the active transport mechanisms that secrete substances into the tubular fluid **(Clinical Box 37–1)**. The filtered load of PAH is a linear function of the plasma level, but PAH secretion increases as P_{PAH} rises only until a maximal secretion rate (Tm_{PAH}) is reached **(Figure 37–10)**. When P_{PAH} is low, C_{PAH} is high; but as P_{PAH} rises

above Tm_{PAH}, C_{PAH} falls progressively. It eventually approaches the clearance of inulin (C_{In}) **(Figure 37–11)** because the amount of PAH secreted becomes a smaller and smaller fraction of the total amount excreted. Conversely, the clearance of glucose is essentially zero at P_G levels below the renal threshold; but above the threshold, C_G rises to approach C_{In} as P_G is raised.

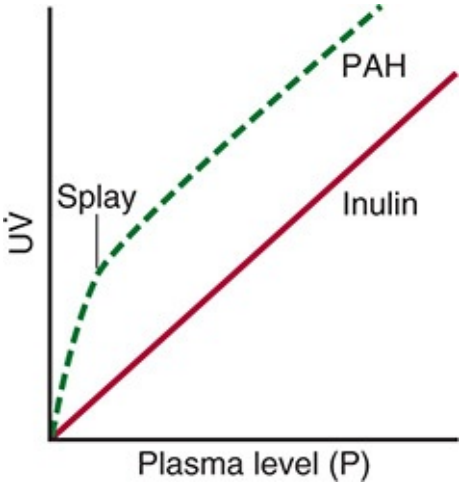


FIGURE 37–10 Relation between plasma levels and excretion ($U\dot{V}$) of PAH and inulin.

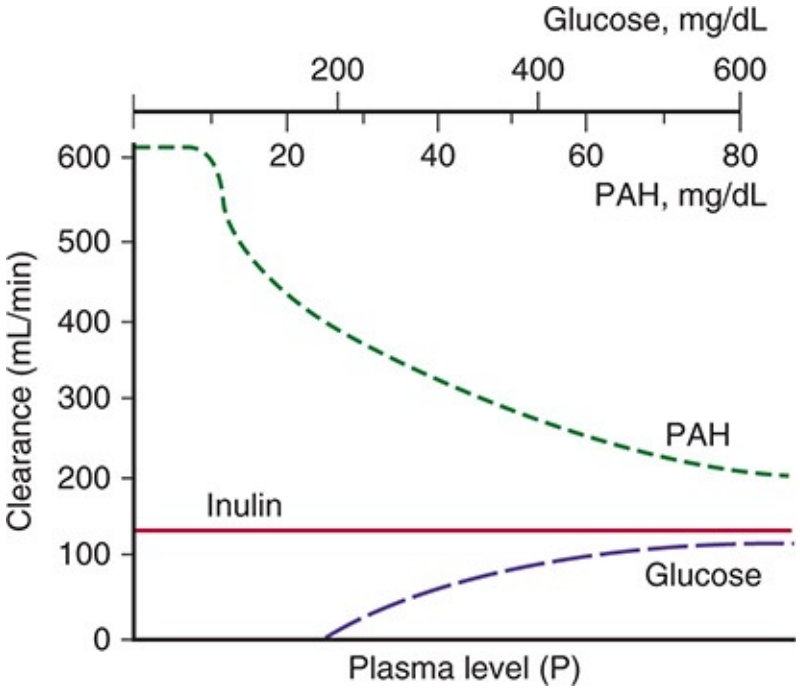


FIGURE 37–11 Clearance of inulin, glucose, and PAH at various plasma

levels of each substance in humans.

CLINICAL BOX 37–1

Substances Secreted by the Tubules

Derivatives of hippuric acid in addition to PAH, phenol red and other sulfonphthalein dyes, penicillin, and a variety of iodinated dyes are actively secreted into the tubular fluid. Substances that are normally produced in the body and secreted by the tubules include various ethereal sulfates, steroid and other glucuronides, and 5-hydroxyindoleacetic acid, the principal metabolite of serotonin.

THERAPEUTIC HIGHLIGHTS

The loop diuretic, furosemide, and the thiazide diuretics are organic anions that gain access to their tubular sites of action (thick ascending limb and distal convoluted tubule, respectively) when they are secreted into the urine by the proximal tubule.

The use of C_{PAH} to measure ERPF is discussed above.

TUBULOGLOMERULAR FEEDBACK & GLOMERULOTUBULAR BALANCE

Signals from the renal tubule in each nephron feed back to affect filtration in its glomerulus. As the rate of flow through the ascending limb of the loop of Henle and first part of the distal tubule increases, glomerular filtration in the same nephron decreases, and, conversely, a decrease in flow increases the GFR **(Figure 37–12)**. This process, which is called **tubuloglomerular feedback,** tends to maintain the constancy of the load delivered to the distal tubule.

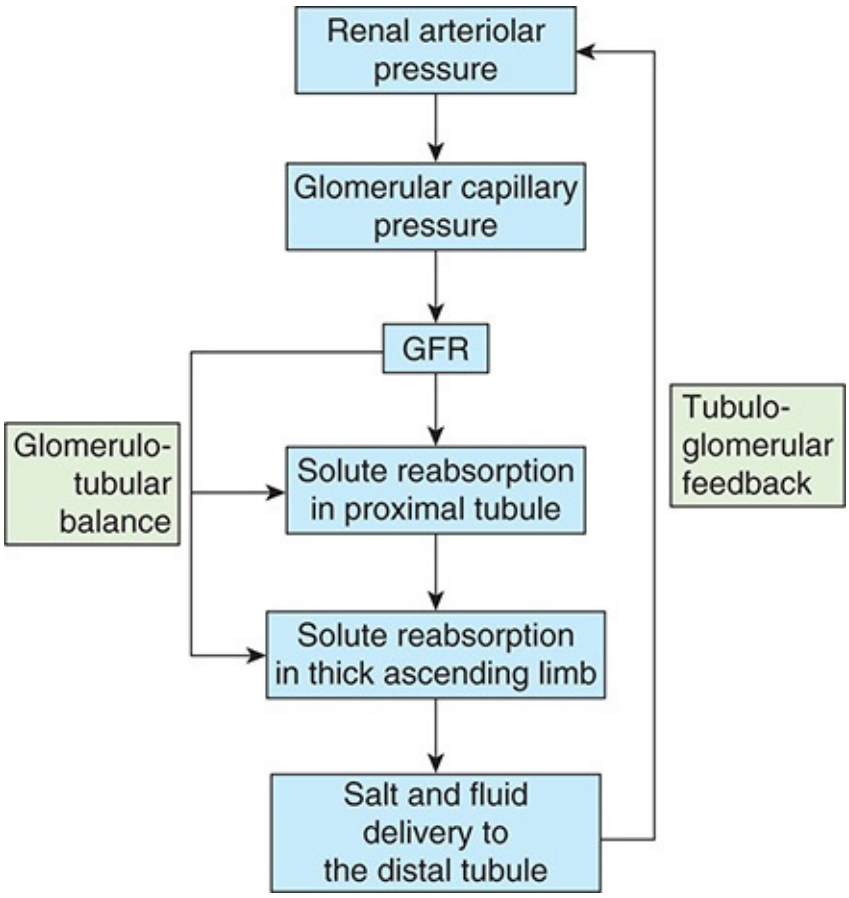


FIGURE 37–12 Mechanisms of glomerulotubular balance and tubuloglomerular feedback.

The sensor for this response is the **macula densa.** The amount of fluid entering the distal tubule at the end of the thick ascending limb of the loop of Henle depends on the amount of Na^+ and Cl^- in it. The Na^+ and Cl^- enter the macula densa cells via the Na–K–2Cl cotransporter in their apical membranes. The increased Na^+ causes increased Na, K ATPase activity and the resultant increased ATP hydrolysis causes more adenosine to be formed. Presumably, adenosine is secreted from the basal membrane of the cells. It acts via adenosine A_1 receptors on the macula densa cells to increase their release of Ca^{2+} to the vascular smooth muscle in the afferent arterioles. This causes afferent vasoconstriction and a resultant decrease in GFR. Presumably, a similar mechanism generates a signal that decreases renin secretion by the adjacent juxtaglomerular cells in the afferent arteriole (see Chapter 38), but this remains unsettled.

Conversely, an increase in GFR causes an increase in the reabsorption of

solutes, and consequently of water, primarily in the proximal tubule, so that in general the percentage of the solute reabsorbed is held constant. This process is called **glomerulotubular balance,** and it is particularly prominent for Na^+. The change in Na^+ reabsorption occurs within seconds after a change in filtration, so it seems unlikely that an extrarenal humoral factor is involved. Alternatively, one mediating factor is the oncotic pressure in the peritubular capillaries. When the GFR is high, there is a relatively large increase in the oncotic pressure of the plasma leaving the glomeruli via the efferent arterioles and hence in their capillary branches. This increases the reabsorption of Na^+ from the tubule. However, other as yet unidentified intrarenal mechanisms are likely also involved.

WATER TRANSPORT

Normally, 180 L of fluid is filtered through the glomeruli each day, while the average daily urine volume is about 1 L. The same load of solute can be excreted per 24 h in a urine volume of 500 mL with a concentration of 1400 mOsm/kg or in a volume of 23.3 L with a concentration of 30 mOsm/kg **(Table 37–6)**. These figures demonstrate two important facts: First, at least 87% of the filtered water is reabsorbed, even when the urine volume is 23 L; and second, the reabsorption of the remainder of the filtered water can be varied without affecting total solute excretion. Therefore, when the urine is concentrated, water is retained in excess of solute; and when it is dilute, water is lost from the body in excess of solute. Both facts have great importance in the regulation of the osmolality of the body fluids. A key regulator of water output is vasopressin acting on the collecting ducts.

TABLE 37–6 Alterations in water metabolism produced by vasopressin in humans. In each case, the osmotic load excreted is 700 mOsm/day.

	GFR (mL/min)	Percentage of Filtered Water Reabsorbed	Urine Volume (L/day)	Urine Concentration (mOsm/kg H_2O)	Gain or Loss of Water in Excess of Solute (L/day)
Urine isotonic to plasma	125	98.7	2.4	290	…
Vasopressin (maximal antidiuresis)	125	99.7	0.5	1400	1.9 gain
No vasopressin ("complete" diabetes insipidus)	125	87.1	23.3	30	20.9 loss

AQUAPORINS

Rapid diffusion of water across cell membranes depends on the presence of water channels, integral membrane proteins called **aquaporins.** To date, 13 aquaporins have been cloned; however, only four aquaporins (aquaporin-1, -2, -3, and -4) play a key role in the kidney. The roles played by aquaporin-1 and aquaporin-2 in renal water transport are discussed below.

PROXIMAL TUBULE

Active transport of many substances occurs from the fluid in the proximal tubule, but micropuncture studies have shown that the fluid remains essentially iso-osmotic until the end of the proximal tubule (Figure 37–8). **Aquaporin-1** is localized to both the basolateral and apical membrane of the proximal tubules and its presence allows water to move rapidly out of the tubule along the osmotic gradients set up by active transport of solutes, and isotonicity is maintained. Because the ratio of the concentration in tubular fluid to the concentration in plasma (TF/P) of the nonreabsorbable substance inulin is 2.5 to 3.3 at the end of the proximal tubule, it follows that 60–70% of the filtered solute and 60–70% of the filtered water have been removed by the time the filtrate reaches this point **(Figure 37–13)**.

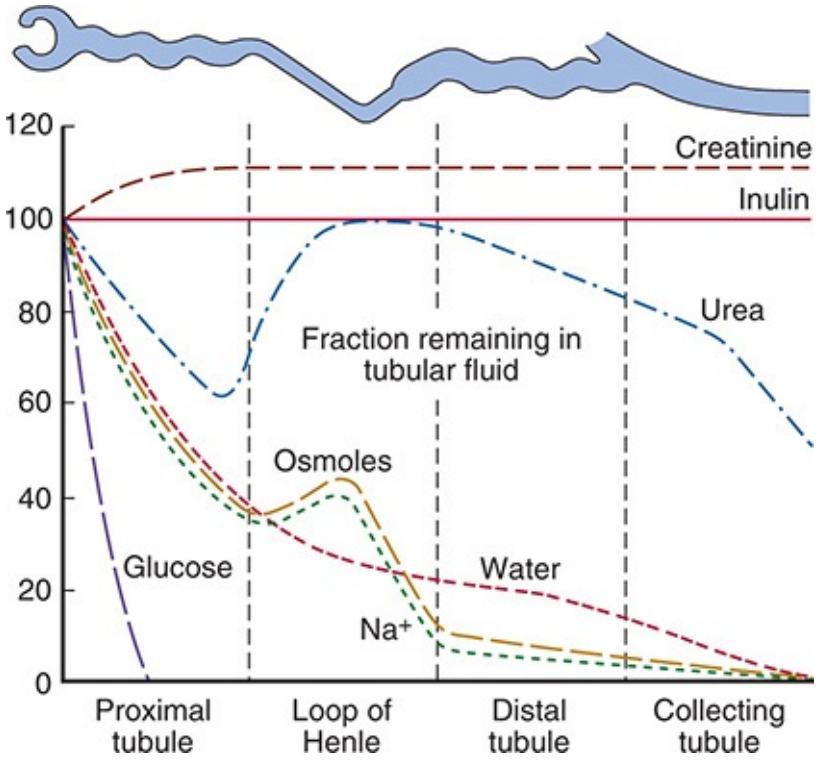


FIGURE 37–13 Changes in the percentage of the filtered amount of substances remaining in the tubular fluid along the length of the nephron in the presence of vasopressin. (Modified with permission from Sullivan LP, Grantham JJ: *Physiology of the Kidney*, 2nd ed. Lea & Febiger, 1982.)

When aquaporin-1 was knocked out in mice, proximal tubular water permeability was reduced by 80%. When the mice were subjected to dehydration, their urine osmolality did not increase (<700 mOsm/kg), even though other renal aquaporins were present. In humans with mutations that eliminate aquaporin-1 activity, the defect in water homeostasis is not as severe, though their response to dehydration is defective.

LOOP OF HENLE

As noted above, the loops of Henle of the juxtamedullary nephrons dip deeply into the medullary pyramids before draining into the distal convoluted tubules in the cortex, and all the collecting ducts descend back through the medullary pyramids to drain at the tips of the pyramids into the renal pelvis. There is a graded increase in the osmolality of the interstitium of the pyramids in humans: The osmolality at the tips of the papillae can reach about 1200 mOsm/kg of H_2O, approximately four times that of plasma. The descending limb of the loop of Henle is permeable to water, due to the presence of **aquaporin-1** in both the apical and basolateral membranes, but the ascending limb is impermeable to water. Na^+, K^+, and Cl^- are cotransported out of the thick segment of the ascending limb. Therefore, the fluid in the descending limb of the loop of Henle becomes **hypertonic** as water moves out of the tubule into the hypertonic interstitium. In the ascending limb it becomes more dilute because of the movement of Na^+ and Cl^- out of the tubular lumen, and when fluid reaches the top of the ascending limb (called the **diluting segment**) it is now **hypotonic** to plasma. In passing through the descending loop of Henle, another 15% of the filtered water is removed, so approximately 20% of the filtered water enters the distal tubule, and the TF/P of inulin at this point is about 5.

In the thick ascending limb, a carrier cotransports one Na^+, one K^+, and $2Cl^-$ from the tubular lumen into the tubular cells. This is another example of secondary active transport; the Na^+ is actively transported from the cells into the interstitium by Na, K ATPase in the basolateral membranes of the cells, keeping the intracellular Na^+ low. The Na–K–2Cl cotransporter has 12 transmembrane domains with intracellular amino and carboxyl terminals. It is a member of a

family of transporters found in many other locations, including salivary glands, the gastrointestinal tract, and the airways.

The K^+ diffuses back into the tubular lumen and back into the interstitium via ROMK and other K^+ channels. The Cl^- moves into the interstitium via ClC–Kb channels **(Figure 37–14)**.

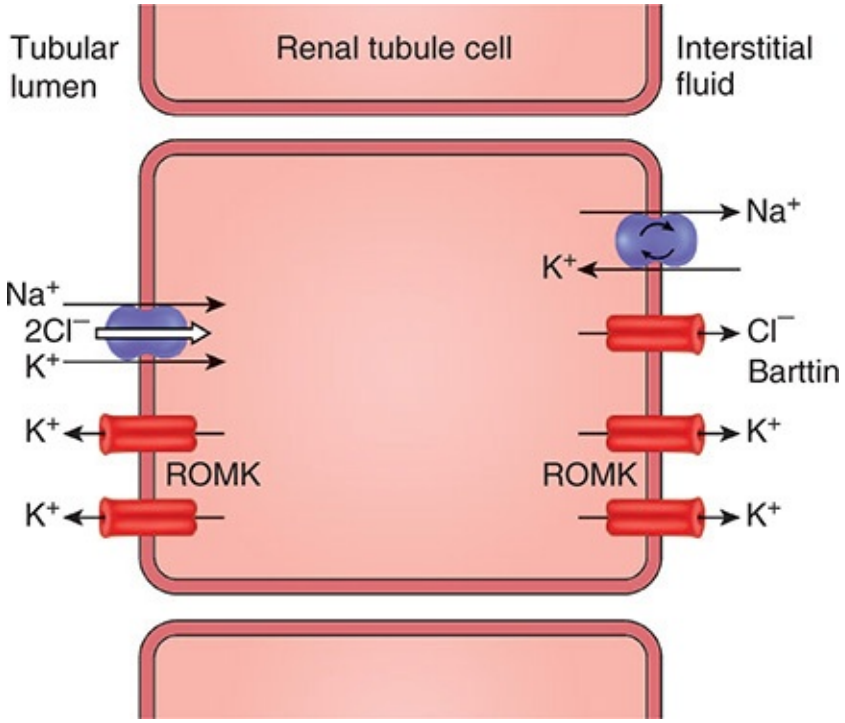


FIGURE 37–14 NaCl transport in the thick ascending limb of the loop of Henle. The Na–K–2Cl cotransporter moves these ions into the tubular cell by secondary active transport. Na^+ is transported out of the cell into the interstitium by Na, K ATPase in the basolateral membrane of the cell. Cl^- exits in basolateral ClC–Kb Cl^- channels. Barttin, a protein in the cell membrane, is essential for normal ClC–Kb function. K^+ moves from the cell to the interstitium and the tubular lumen by ROMK and other K^+ channels **(Clinical Box 37–2)**.

CLINICAL BOX 37–2

Genetic Mutations in Renal Transporters

Mutations of individual genes for many renal sodium transporters and channels cause specific syndromes such as Bartter syndrome, Liddle syndrome, and Dent disease. A large number of mutations have been

described.

Bartter syndrome is a rare but interesting condition that is due to defective transport in the thick ascending limb. It is characterized by chronic Na^+ loss in the urine, with resultant hypovolemia causing stimulation of renin and aldosterone secretion without hypertension, plus hyperkalemia and alkalosis. The condition can be caused by loss-of-function mutations in the gene for any of four key proteins: the Na–K–2Cl cotransporter, the ROMK K^+ channel, the ClC–Kb Cl^- channel, or **barttin,** a recently described integral membrane protein that is necessary for the normal function of ClC–Kb Cl^- channels.

The stria vascularis in the inner ear is responsible for maintaining the high K^+ concentration in the scala media that is essential for normal hearing. It contains both ClC–Kb and ClC–Ka Cl^- channels. Bartter syndrome associated with mutated ClC–Kb channels is not associated with deafness because the Clc–Ka channels can carry the load. However, both types of Cl^- channels are barttin-dependent, so patients with Bartter syndrome due to mutated barttin are also deaf.

Another interesting example involves the proteins polycystin-1 (PKD-1) and polycystin-2 (PKD-2). PKD-1 appears to be a Ca^{2+} receptor that activates a nonspecific ion channel associated with PKD-2. The normal function of this apparent ion channel is unknown, but both proteins are abnormal in **autosomal dominant polycystic kidney disease,** in which the renal parenchyma is progressively replaced by fluid-filled cysts until there is complete renal failure.

DISTAL TUBULE

The distal tubule, particularly its first part, is in effect an extension of the thick segment of the ascending limb. It is relatively impermeable to water, and continued removal of the solute in excess of solvent further dilutes the tubular fluid.

COLLECTING DUCTS

The collecting ducts have two portions: a cortical portion and a medullary portion. The changes in osmolality and volume in the collecting ducts depend on

the amount of vasopressin acting on the ducts. This antidiuretic hormone from the posterior pituitary gland increases the permeability of the collecting ducts to water. The key to the action of vasopressin on the collecting ducts is aquaporin-2. Unlike the other aquaporins, this aquaporin is stored in vesicles in the cytoplasm of principal cells. Vasopressin causes rapid insertion of these vesicles into the apical membrane of cells. The effect is mediated via the vasopressin V_2 receptor, cyclic adenosine 5-monophosphate (cAMP), and protein kinase A. Cytoskeletal elements are involved, including microtubule-based motor proteins (dynein and dynactin) as well as actin filament-binding proteins such as myosin-1.

In the presence of enough vasopressin to produce maximal antidiuresis, water moves out of the hypotonic fluid entering the cortical collecting ducts into the interstitium of the cortex, and the tubular fluid becomes isotonic. In this manner, as much as 10% of the filtered water is removed. The isotonic fluid then enters the medullary collecting ducts with a TF/P inulin of about 20. An additional 4.7% or more of the filtrate is reabsorbed into the hypertonic interstitium of the medulla, producing a concentrated urine with a TF/P inulin of over 300. In humans, the osmolality of urine may reach 1400 mOsm/kg of H_2O, almost five times the osmolality of plasma, with a total of 99.7% of the filtered water being reabsorbed (Table 37–6). In other species, the ability to concentrate urine is even greater. Maximal urine osmolality is about 2500 mOsm/kg in dogs, about 3200 mOsm/kg in laboratory rats, and as high as 5000 mOsm/kg in certain desert rodents.

When vasopressin is absent, the collecting duct epithelium is relatively impermeable to water. The fluid therefore remains hypotonic, and large amounts flow into the renal pelvis. In humans, the urine osmolality may be as low as 30 mOsm/kg of H_2O. The impermeability of the distal portions of the nephron is not absolute; along with the salt that is pumped out of the collecting duct fluid, about 2% of the filtered water is reabsorbed in the absence of vasopressin. However, as much as 13% of the filtered water may be excreted, and urine flow may reach 15 mL/min or more.

THE COUNTERCURRENT MECHANISM

The concentrating mechanism depends on the maintenance of a gradient of **increasing osmolality** along the medullary pyramids. This gradient is produced by the operation of the loops of Henle as **countercurrent multipliers** and

maintained by the operation of the vasa recta as **countercurrent exchangers.** A countercurrent system is a system in which the inflow runs parallel to, counter to, and in close proximity to the outflow for some distance. This occurs for both the loops of Henle and the vasa recta in the renal medulla (Figure 37–3).

The operation of each loop of Henle as a countercurrent multiplier depends on the high permeability of the thin descending limb to water (via aquaporin-1), the active transport of Na^+ and Cl^- out of the thick ascending limb, and the inflow of tubular fluid from the proximal tubule, with outflow into the distal tubule. The process can be explained using hypothetical steps leading to the normal equilibrium condition, although the steps do not occur in vivo. It is also important to remember that the equilibrium is maintained unless the osmotic gradient is washed out. These steps are summarized in **Figure 37–15** for a cortical nephron with no thin ascending limb. Assume first a condition in which osmolality is 300 mOsm/kg of H_2O throughout the descending and ascending limbs and the medullary interstitium (Figure 37–15A). Assume in addition that the pumps in the thick ascending limb can pump 100 mOsm/kg of Na^+ and Cl^- from the tubular fluid to the interstitium, increasing interstitial osmolality to 400 mOsm/kg of H_2O. Water then moves out of the thin descending limb, and its contents equilibrate with the interstitium (Figure 37–15B). However, fluid containing 300 mOsm/kg of H_2O is continuously entering this limb from the proximal tubule (Figure 37–15C), so the gradient against which the Na^+ and Cl^- are pumped is reduced and more enters the interstitium (Figure 37–15D). Meanwhile, hypotonic fluid flows into the distal tubule, and isotonic and subsequently hypertonic fluid flows into the ascending thick limb. The process keeps repeating, and the final result is a gradient of osmolality from the top to the bottom of the loop.

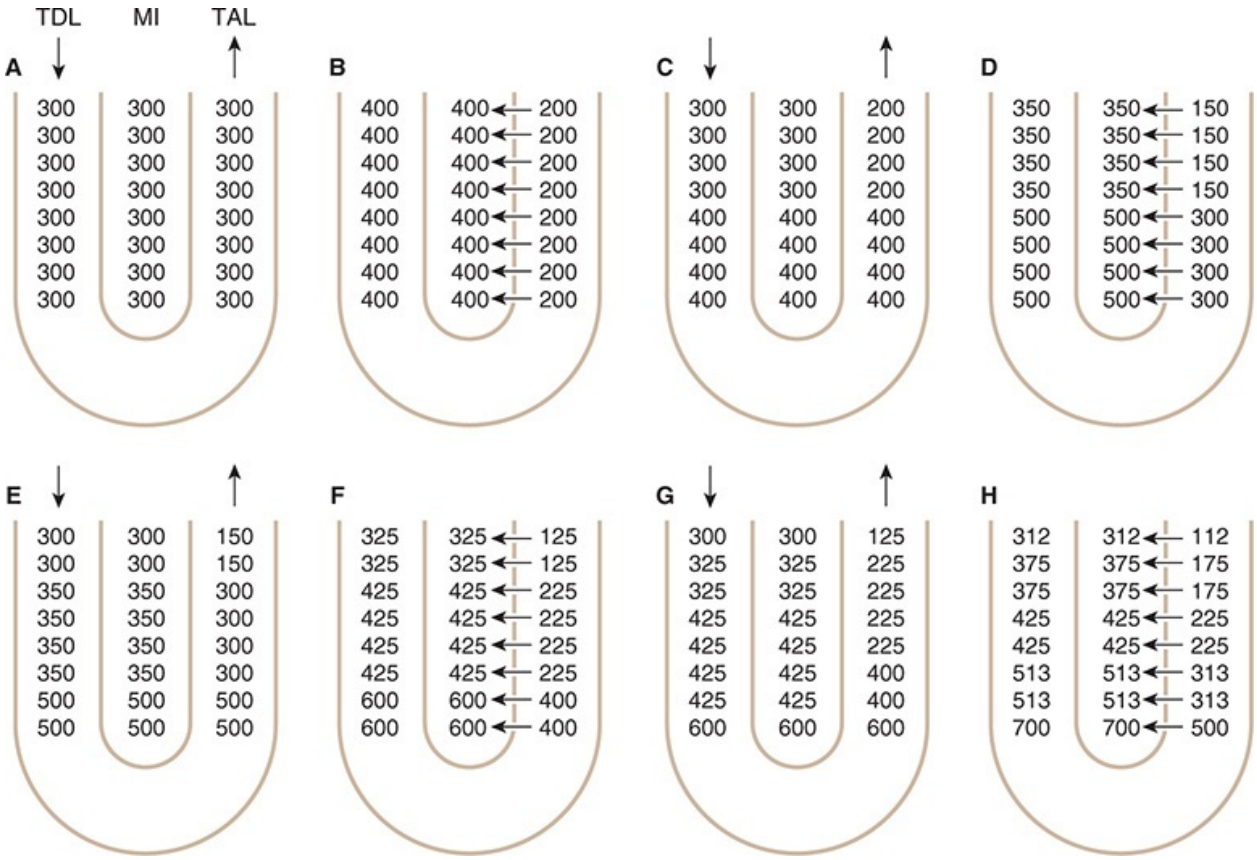


FIGURE 37–15 Operation of the loop of Henle as a countercurrent multiplier producing a gradient of hyperosmolarity in the medullary interstitium (MI). The process of generation of the gradient is illustrated as occurring in hypothetical steps, starting at A, where osmolality in both limbs and the interstitium is 300 mOsm/kg of water. The pumps in the thick ascending limb move Na^+ and Cl^- into the interstitium, increasing its osmolality to 400 mOsm/kg, and this equilibrates with the fluid in the thin descending limb. However, isotonic fluid continues to flow into the thin descending limb and hypotonic fluid out of the thick ascending limb. Continued operation of the pumps makes the fluid leaving the thick ascending limb even more hypotonic, while hypertonicity accumulates at the apex of the loop. TAL, thick ascending limb; TDL, thin descending limb.

In juxtamedullary nephrons with longer loops and thin ascending limbs, the osmotic gradient is spread over a greater distance and the osmolality at the tip of the loop is greater. This is because the thin ascending limb is relatively impermeable to water but permeable to Na^+ and Cl^-. Therefore, Na^+ and Cl^- move down their concentration gradients into the interstitium, and there is additional passive countercurrent multiplication. The greater the length of the

loop of Henle, the greater the osmolality that can be reached at the tip of the medulla.

The osmotic gradient in the medullary pyramids would not last long if the Na^+ and urea in the interstitial spaces were removed by the circulation. These solutes remain in the pyramids primarily because the vasa recta operate as countercurrent exchangers **(Figure 37–16)**. The solutes diffuse out of the vessels conducting blood toward the cortex and into the vessels descending into the pyramid. Conversely, water diffuses out of the descending vessels and into the fenestrated ascending vessels. Therefore, the solutes tend to recirculate in the medulla and water tends to bypass it, so that hypertonicity is maintained. The water removed from the collecting ducts in the pyramids is also removed by the vasa recta and enters the general circulation. Countercurrent exchange is a passive process; it depends on movement of water and could not maintain the osmotic gradient along the pyramids if the process of counter-current multiplication in the loops of Henle were to cease.

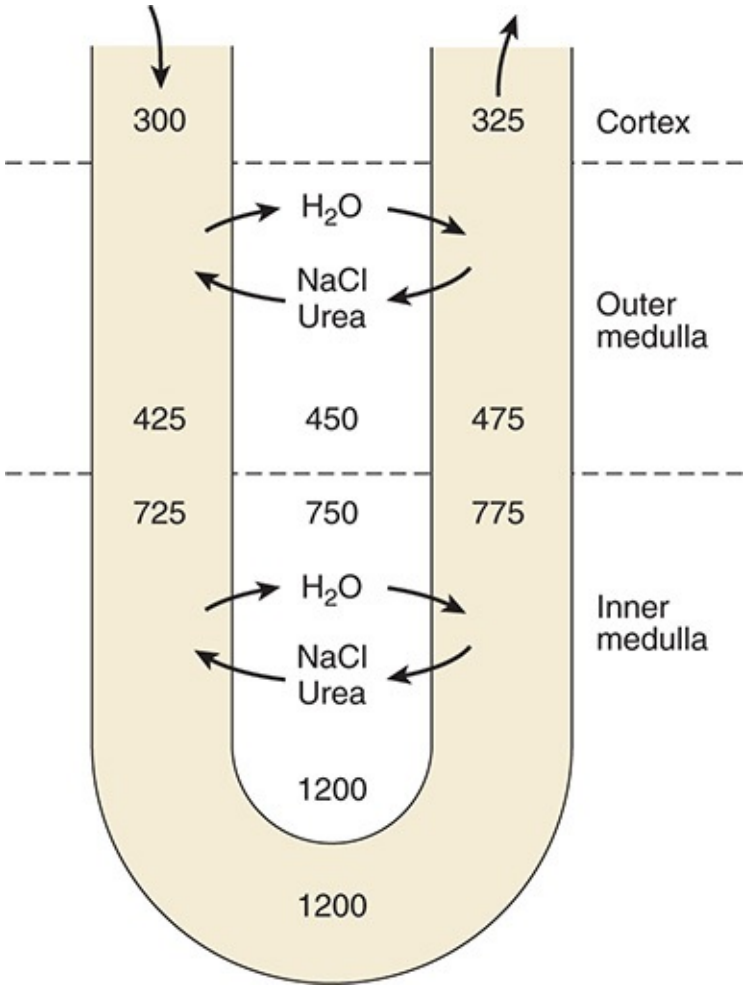


FIGURE 37–16 Operation of the vasa recta as countercurrent exchangers in the kidney. NaCl and urea diffuse out of the ascending limb of the vessel and into the descending limb, whereas water diffuses out of the descending and into the ascending limb of the vascular loop. (Modified with permission from Pitts RF: *Physiology of the Kidney and Body Fluid*, 3rd ed. Chicago: Yearbook Medical Publications, 1974.)

It is worth noting that there is a very large osmotic gradient in the loop of Henle and, in the presence of vasopressin, in the collecting ducts. It is the countercurrent system that makes this gradient possible by spreading it along a system of tubules 1 cm or more in length, rather than across a single layer of cells that is only a few micrometers thick. There are other examples of the operation of countercurrent exchangers in animals. One is the heat exchange between the arteries and venae comitantes of the limbs. To a minor degree in humans, but to a major degree in mammals living in cold water, heat is transferred from the arterial blood flowing into the limbs to the adjacent veins draining blood back into the body, making the tips of the limbs cold while conserving body heat (see Chapter 33).

ROLE OF UREA

Urea contributes to the establishment of the osmotic gradient in the medullary pyramids and to the ability to form a concentrated urine in the collecting ducts. Urea transport is mediated by urea transporters, presumably by facilitated diffusion. There are at least four isoforms of the transport protein UT-A in the kidneys (UT-A1 to UT-A4); UT-B is found in erythrocytes and in the descending limbs of the vasa recta. Urea transport in the collecting duct is mediated by UT-A1 and UT-A3, and both are regulated by vasopressin. During antidiuresis, when vasopressin is high, the amount of urea deposited in the medullary interstitium increases, thus increasing the concentrating capacity of the kidney. In addition, the amount of urea in the medullary interstitium and, consequently, in the urine varies with the amount of urea filtered, and this in turn varies with the dietary intake of protein. Therefore, a high-protein diet increases the ability of the kidneys to concentrate the urine and a low-protein diet reduces the kidneys' ability to concentrate the urine.

OSMOTIC DIURESIS

The presence of large quantities of unreabsorbed solutes in the renal tubules causes an increase in urine volume called **osmotic diuresis.** Solutes that are not reabsorbed in the proximal tubules exert an appreciable osmotic effect as the volume of tubular fluid decreases and their concentration rises. Therefore, they "hold water in the tubules." In addition, the concentration gradient against which Na^+ can be pumped out of the proximal tubules is limited. Normally, the movement of water out of the proximal tubule prevents any appreciable gradient from developing, but Na^+ concentration in the fluid falls when water reabsorption is decreased because of the presence in the tubular fluid of increased amounts of unreabsorbable solutes. The limiting concentration gradient is reached, and further proximal reabsorption of Na^+ is prevented; more Na^+ remains in the tubule, and water stays with it. The result is that the loop of Henle is presented with a greatly increased volume of isotonic fluid. This fluid has a decreased Na^+ concentration, but the total amount of Na^+ reaching the loop per unit time is increased. In the loop, reabsorption of water and Na^+ is decreased because the medullary hypertonicity is decreased. The decrease is due primarily to decreased reabsorption of Na^+, K^+, and Cl^- in the ascending limb of the loop because the limiting concentration gradient for Na^+ reabsorption is reached. More fluid passes through the distal tubule, and because of the decrease in the osmotic gradient along the medullary pyramids, less water is reabsorbed in the collecting ducts. The result is a marked increase in urine volume and excretion of Na^+ and other electrolytes.

Osmotic diuresis is produced by the administration of compounds such as mannitol and related polysaccharides that are filtered but not reabsorbed. It is also produced by naturally occurring substances when they are present in amounts exceeding the capacity of the tubules to reabsorb them. For example, in **diabetes mellitus,** if blood glucose is high, glucose in the glomerular filtrate is high, thus the filtered load will exceed the Tm_G and glucose will remain in the tubules causing polyuria. Osmotic diuresis can also be produced by the infusion of large amounts of sodium chloride or urea.

It is important to recognize the difference between osmotic diuresis and water diuresis. In water diuresis, the amount of water reabsorbed in the proximal portions of the nephron is normal, and the maximal urine flow that can be produced is about 16 mL/min. In osmotic diuresis, increased urine flow is due to decreased water reabsorption in the proximal tubules and loops and very large urine flows can be produced. As the load of excreted solute is increased, the concentration of the urine approaches that of plasma **(Figure 37–17)** in spite of maximal vasopressin secretion, because an increasingly large fraction of the

excreted urine is isotonic proximal tubular fluid. If osmotic diuresis is produced in an animal with diabetes insipidus, the urine concentration rises for the same reason.

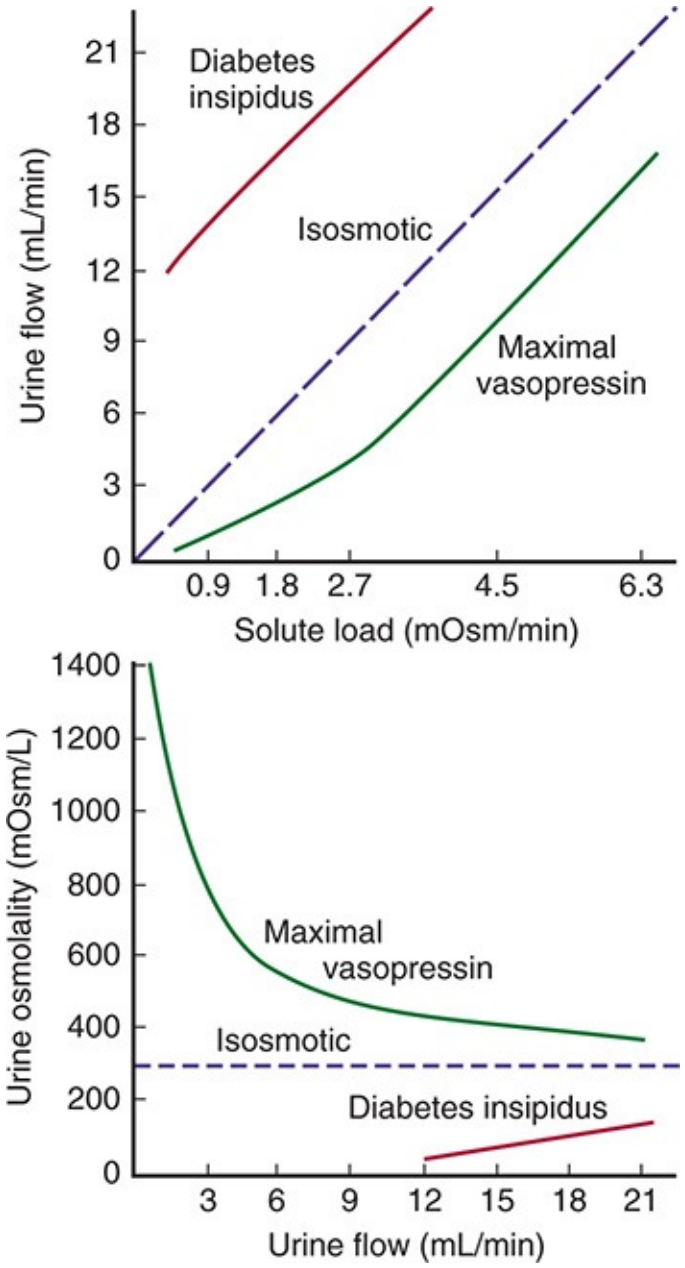


FIGURE 37–17 Approximate relationship between urine concentration and urine flow in osmotic diuresis in humans. The dashed line in the lower diagram indicates the concentration at which the urine is isosmotic with plasma. (Reproduced with permission from Brobeck JR (ed): *Best and Taylor's Physiological Basis of Medical Practice*, 9th ed. Philadelphia, PA: Williams &

Wilkins; 1979.)

RELATION OF URINE CONCENTRATION TO GFR

The magnitude of the osmotic gradient along the medullary pyramids is increased when the rate of flow of fluid through the loops of Henle is decreased. A reduction in GFR such as that caused by dehydration produces a decrease in the volume of fluid presented to the countercurrent mechanism, so that the rate of flow in the loops declines and the urine becomes more concentrated. When the GFR is low, the urine can become quite concentrated in the absence of vasopressin. If one renal artery is constricted in an animal with diabetes insipidus, the urine excreted on the side of the constriction becomes hypertonic because of the reduction in GFR, whereas that excreted on the opposite side remains hypotonic.

"FREE WATER CLEARANCE"

In order to quantitate the gain or loss of water by excretion of a concentrated or dilute urine, the "free water clearance" (C_{H2O}) is sometimes calculated. This is the difference between the urine volume and the clearance of osmoles (C_{Osm}):

$$C_{H_2O} = \dot{V} - \frac{U_{Osm}\,\dot{V}}{P_{Osm}}$$

where $\dot{V}$ is the urine flow rate and U_{Osm} and P_{Osm} the urine and plasma osmolality, respectively. C_{Osm} is the amount of water necessary to excrete the osmotic load in a urine that is isotonic with plasma. Therefore, C_{H2O} is negative when the urine is hypertonic and positive when the urine is hypotonic. For example, using the data in Table 37–6, the values for C_{H2O} are –1.3 mL/min (–1.9 L/d) during maximal antidiuresis and 14.5 mL/min (20.9 L/d) in the absence of vasopressin.

REGULATION OF NA^+ EXCRETION

Na^+ is filtered in large amounts, but it is actively transported out of all portions of the tubule except the descending thin limb of the loop of Henle. Normally about 99% of the filtered Na^+ is reabsorbed. Because Na^+ is the most abundant cation in ECF and because Na^+ salts account for over 90% of the osmotically active solute in the plasma and interstitial fluid, the amount of Na^+ in the body is a prime determinant of the ECF volume. Therefore, it is not surprising that multiple regulatory mechanisms have evolved in terrestrial animals to control the excretion of this ion. Through the operation of these regulatory mechanisms, the amount of Na^+ excreted is adjusted to equal the amount ingested over a wide range of dietary intakes, and the individual stays in Na^+ balance. When Na intake is high, or saline is infused, natriuresis occurs, whereas when ECF is reduced (eg, fluid loss following vomiting or diarrhea) a decrease in Na^+ excretion occurs. Thus, urinary Na^+ output ranges from less than 1 mEq/day on a low-salt diet to 400 mEq/day or more when the dietary Na^+ intake is high.

MECHANISMS

Variations in Na^+ excretion are brought about by changes in GFR **(Table 37–7)** and changes in tubular reabsorption, primarily in the 3% of filtered Na^+ that reaches the collecting ducts. The factors affecting GFR, including tubuloglomerular feedback, have been discussed previously. Factors affecting Na^+ reabsorption include the circulating level of aldosterone and other adrenocortical hormones, the circulating level of ANP and other natriuretic hormones, and the rate of tubular secretion of H^+ and K^+.

TABLE 37–7 Changes in Na^+ excretion that would occur as a result of changes in GFR if there were no concomitant changes in Na^+ reabsorption.

GFR (mL/min)	Plasma Na^+ (µEq/mL)	Amount Filtered (µEq/min)	Amount Reabsorbed (µEq/min)	Amount Excreted (µEq/min)
125	145	18,125	18,000	125
127	145	18,415	18,000	415
124.1	145	18,000	18,000	0

GFR, glomerular filtration rate.

EFFECTS OF ADRENOCORTICAL STEROIDS

Adrenal mineralocorticoids such as aldosterone increase tubular reabsorption of Na^+ in association with secretion of K^+ and H^+ and also Na^+ reabsorption with Cl^-. When these hormones are injected into adrenalectomized animals, a latent period of 10–30 min occurs before their effects on Na^+ reabsorption become manifest, because of the time required for the steroids to alter protein synthesis via their action on DNA. Mineralocorticoids may also have more rapid membrane-mediated effects, but these are not apparent in terms of Na^+ excretion in the whole animal. The mineralocorticoids act primarily in the collecting ducts to increase the number of active epithelial sodium channels (ENaCs) in this part of the nephron. The molecular mechanisms believed to be involved are discussed in Chapter 20 and summarized in **Figure 37–18**.

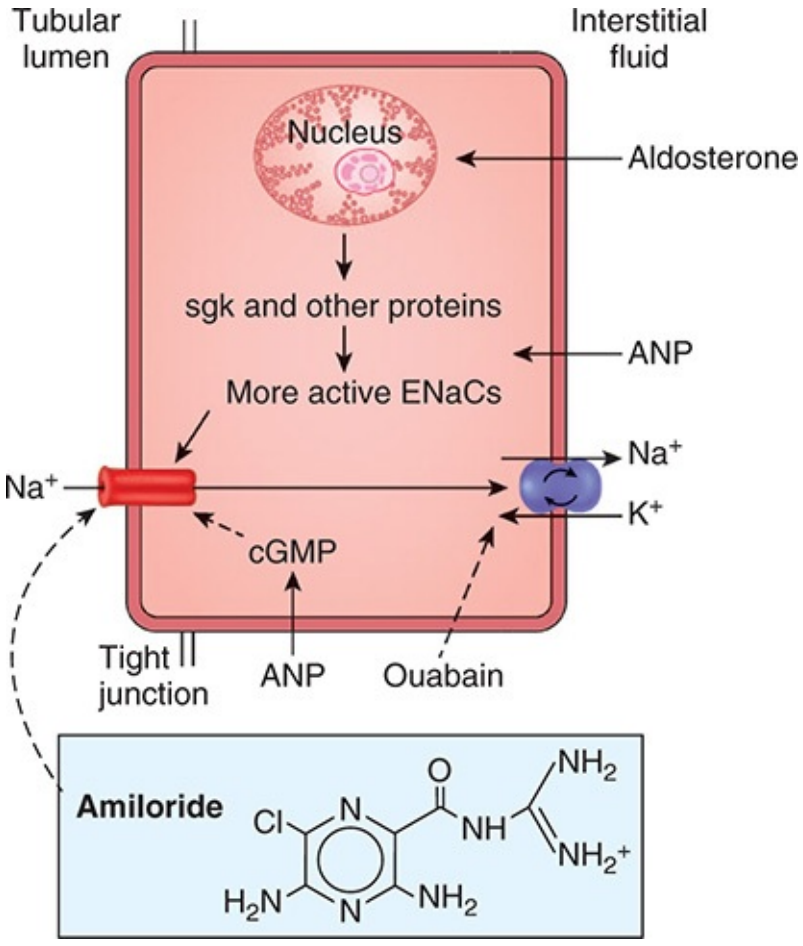


FIGURE 37–18 Renal principal cell. Na^+ enters via the ENaCs in the apical membrane and is pumped into the interstitial fluid by Na, K ATPases in the

basolateral membrane. Aldosterone activates the genome to produce serum- and glucocorticoid-regulated kinase (sgk) and other proteins, and the number of active ENaCs is increased.

In Liddle syndrome, mutations in the genes that code for the β subunit and less commonly the γ subunit of ENaC cause the channels to become constitutively active in the kidney. This leads to Na^+ retention and hypertension.

OTHER HUMORAL EFFECTS

Reduction of dietary intake of salt increases aldosterone secretion (see Figure 20–24), producing marked but slowly developing decreases in Na^+ excretion. A variety of other humoral factors affect Na^+ reabsorption. PGE_2 causes a natriuresis, possibly by inhibiting Na, K ATPase and possibly by increasing intracellular Ca^{2+}, which in turn inhibits Na^+ transport via ENaCs. Endothelin and IL-1 cause natriuresis, probably by increasing the formation of PGE_2. ANP and related molecules increase intracellular cyclic 3′,5′-guanosine monophosphate (cGMP), and this inhibits transport via ENaC. Inhibition of Na, K ATPase by another natriuretic hormone, which appears to be endogenously produced ouabain, also increases Na^+ excretion. Angiotensin II increases reabsorption of Na^+ and HCO_3^- by an action on the proximal tubules. There is an appreciable amount of angiotensin-converting enzyme in the kidneys, and the kidneys convert 20% of the circulating angiotensin I to angiotensin II. In addition, angiotensin I is generated in the kidneys.

Prolonged exposure to high levels of circulating mineralocorticoids does not cause edema in otherwise normal individuals because eventually the kidneys escape from the effects of the steroids. This **escape phenomenon,** which may be due to increased secretion of ANP, is discussed in Chapter 20. It appears to be reduced or absent in nephrosis, cirrhosis, and heart failure, and patients with these diseases continue to retain Na^+ and become edematous when exposed to high levels of mineralocorticoids.

REGULATION OF WATER EXCRETION

WATER DIURESIS

The feedback mechanism controlling vasopressin secretion and the way

vasopressin secretion is stimulated by a rise and inhibited by a drop in the effective osmotic pressure of the plasma are discussed in Chapter 17. The **water diuresis** produced by drinking large amounts of hypotonic fluid begins about 15 min after ingestion of a water load and reaches its maximum in about 40 min. The act of drinking produces a small decrease in vasopressin secretion before the water is absorbed, but most of the inhibition is produced by the decrease in plasma osmolality after the water is absorbed.

WATER INTOXICATION

During excretion of an average osmotic load, the maximal urine flow that can be produced during a water diuresis is about 16 mL/min. If water is ingested at a higher rate than this for any length of time, swelling of the cells becomes severe because of the uptake of water from the hypotonic ECF and, rarely, the symptoms of **water intoxication** may develop. Swelling of the cells in the brain causes convulsions and coma and leads eventually to death. Water intoxication can also occur when water intake is not reduced after administration of exogenous vasopressin or when secretion of endogenous vasopressin occurs in response to nonosmotic stimuli such as surgical trauma. Administration of oxytocin after parturition (to contract the uterus) can also lead to water intoxication if water intake is not monitored carefully.

REGULATION OF K^+ EXCRETION

Much of the filtered K^+ is removed from the tubular fluid by active reabsorption in the proximal tubules, and K^+ is then secreted into the fluid by the distal tubular cells. The rate of K^+ secretion is proportional to the rate of flow of the tubular fluid through the distal portions of the nephron, because with rapid flow there is less opportunity for the tubular K^+ concentration to rise to a value that stops further secretion. In the absence of complicating factors, the amount secreted is approximately equal to the K^+ intake, and K^+ balance is maintained. In the collecting ducts, Na^+ is generally reabsorbed and K^+ is secreted. There is no rigid one-for-one exchange, and much of the movement of K^+ is passive. However, there is electrical coupling in the sense that intracellular migration of Na^+ from the lumen tends to lower the potential difference across the tubular cell, and this favors movement of K^+ into the tubular lumen. K^+ excretion is decreased when the amount of Na^+ reaching the distal tubule is small. In

addition, if H^+ secretion is increased, K^+ excretion will decrease as K^+ is reabsorbed in collecting duct cells in exchange for H^+, via the action of the H, K-ATPase.

DIURETICS

Although a detailed discussion of diuretic agents is beyond the scope of this book, consideration of their mechanisms of action constitutes an informative review of the factors affecting urine volume and electrolyte excretion. These mechanisms are summarized in **Table 37–8**. Water, alcohol, osmotic diuretics, xanthines, and acidifying salts have limited clinical usefulness, and the vasopressin antagonists are currently undergoing clinical trials. However, many of the other agents on the list are used extensively in medical practice.

TABLE 37–8 Mechanism of action of various diuretics.

Agent	Mechanism of Action
Water	Inhibits vasopressin secretion
Ethanol	Inhibits vasopressin secretion
Antagonists of V_2 vasopressin receptors such as astolvaptan	Inhibit action of vasopressin on collecting duct
Large quantities of osmotically active substances such as mannitol and glucose	Produce osmotic diuresis
Xanthines such as caffeine and theophylline	Decrease tubular reabsorption of Na^+ and increase GFR
Acidifying salts such as $CaCl_2$ and NH_4Cl	Supply acid load; H^+ is buffered, but an anion is excreted with Na^+ when the ability of the kidneys to replace Na^+ with H^+ is exceeded
Carbonic anhydrase inhibitors such as acetazolamide (Diamox)	Decrease H^+ secretion, with resultant increase in Na^+ and K^+ excretion
Metolazone (Zaroxolyn), thiazides such as chlorothiazide (Diuril)	Inhibit the Na–Cl cotransporter in the early portion of the distal tubule
Loop diuretics such as furosemide (Lasix), ethacrynic acid (Edecrin), and bumetanide	Inhibit the Na–K–2Cl cotransporter in the medullary thick ascending limb of the loop of Henle
K^+-retaining natriuretics such as spironolactone (Aldactone), triamterene (Dyrenium), and amiloride (Midamor)	Inhibit Na^+–K^+ "exchange" in the collecting ducts by inhibiting the action of aldosterone (spironolactone) or by inhibiting the ENaCs (amiloride)

The carbonic anhydrase-inhibiting drugs are only moderately effective as diuretic agents, but because they inhibit acid secretion by decreasing the supply of carbonic acid, they have far-reaching effects. Not only is Na^+ excretion increased because H^+ secretion is decreased, but HCO_3^- reabsorption is also depressed; and because H^+ and K^+ compete with each other and with Na^+, the decrease in H^+ secretion facilitates the secretion and excretion of K^+.

Furosemide and the other loop diuretics inhibit the Na–K–2Cl cotransporter in the thick ascending limb of the loop of Henle. They cause a marked natriuresis and kaliuresis. Thiazides act by inhibiting Na–Cl cotransport in the distal tubule. The diuresis they cause is less marked, but both loop diuretics and thiazides cause increased delivery of Na^+ (and fluid) to the collecting ducts, facilitating K^+ excretion. Thus, over time, K^+ depletion and hypokalemia are common complications in those who use them if they do not supplement their K^+ intake. On the other hand, the so-called K^+-sparing diuretics act in the collecting duct by inhibiting the action of aldosterone or blocking ENaCs.

EFFECTS OF DISORDERED KIDNEY FUNCTION

A number of abnormalities are common to many different types of renal disease. The secretion of renin by the kidneys and the relation of the kidneys to hypertension are discussed in Chapter 38. A frequent finding in various forms of renal disease is the presence in the urine of protein, leukocytes, red cells, and **casts,** which are proteinaceous material precipitated in the tubules and washed into the bladder. Other important consequences of renal disease are loss of the ability to concentrate or dilute the urine, uremia, acidosis, and abnormal retention of Na^+ **(Clinical Box 37–3)**.

LOSS OF CONCENTRATING & DILUTING ABILITY

In kidney disease, the urine becomes less concentrated and urine volume is often increased, producing the symptoms of **polyuria** and **nocturia** (waking up at night to void). The ability to form a dilute urine is often retained, but in advanced kidney disease, the osmolality of the urine becomes fixed at about that of plasma, indicating that the diluting and concentrating functions of the kidney have both been lost. The loss is due in part to disruption of the countercurrent mechanism, but a more important cause is a loss of functioning nephrons. When one kidney is removed surgically, the number of functioning nephrons is halved. The number of osmoles to be excreted is not reduced to this extent, and so the remaining nephrons must each be filtering and excreting more osmotically active substances, producing what is in effect an osmotic diuresis. In osmotic diuresis, the osmolality of the urine approaches that of plasma. The same thing happens when the number of functioning nephrons is reduced by disease. The increased

filtration in the remaining nephrons eventually damages them, and thus more nephrons are lost. The damage resulting from increased filtration may be due to progressive fibrosis in the proximal tubule cells, but this is unsettled. However, the eventual result of this positive feedback is loss of so many nephrons that complete kidney failure with **oliguria,** or even **anuria,** results.

CLINICAL BOX 37–3

Proteinuria

In many kidney diseases and in one benign condition, the permeability of the glomerular capillaries is increased, and protein is found in the urine in more than the usual trace amounts **(proteinuria).** Most of this protein is **albumin,** and the defect is commonly called albuminuria. The relation of charges on the glomerular membrane to albuminuria has been discussed above. The amount of protein in the urine may be very large, and especially in nephrosis, the urinary protein loss may exceed the rate at which the liver can synthesize plasma proteins. The resulting hypoproteinemia reduces the oncotic pressure, and the plasma volume declines, sometimes to dangerously low levels, while edema fluid accumulates in the tissues.

A benign condition that causes proteinuria is a poorly understood change in renal hemodynamics, which in some otherwise normal individuals, causes protein to appear in urine when they are in the standing position **(orthostatic albuminuria).** Urine formed when these individuals are lying down is protein-free.

UREMIA

When the breakdown products of protein metabolism accumulate in the blood, the syndrome known as **uremia** develops. The symptoms of uremia include lethargy, anorexia, nausea and vomiting, mental deterioration and confusion, muscle twitching, convulsions, and coma. The blood urea nitrogen (BUN) and creatinine levels are high, and the blood levels of these substances are used as an index of the severity of the uremia. It probably is not the accumulation of urea and creatinine per se but rather the accumulation of other toxic substances—possibly organic acids or phenols—that produces the symptoms of uremia.

The toxic substances that cause the symptoms of uremia can be removed by

dialyzing the blood of uremic patients against a bath of suitable composition in an artificial kidney **(hemodialysis).** Patients can be kept alive and in reasonable health for many months on dialysis, even when they are completely anuric or have had both kidneys removed. However, the treatment of choice today is certainly transplantation of a kidney from a suitable donor.

Other features of chronic kidney disease include anemia, which is caused primarily by failure to produce erythro- poietin, and secondary hyperparathyroidism due to 1,25- dihydroxycholecalciferol deficiency (see Chapter 21).

ACIDOSIS

Acidosis is common in chronic kidney disease because of failure to excrete the acid products of digestion and metabolism (see Chapter 39). In the rare syndrome of **renal tubular acidosis,** there is specific impairment of the ability to make the urine acidic, and other renal functions are usually normal. However, in most cases of chronic kidney disease the urine is maximally acidified, and acidosis develops because the total amount of H^+ that can be secreted is reduced because of impaired renal tubular production of NH_4^+.

ABNORMAL NA^+ HANDLING

Many patients with kidney disease retain excessive amounts of Na^+ and become edematous. Na^+ retention in kidney disease has at least three causes. In acute glomerulonephritis, a disease that affects primarily the glomeruli, the amount of Na^+ filtered is decreased markedly. In the nephrotic syndrome, an increase in aldosterone secretion contributes to salt retention. The plasma protein level is low in this condition, and so fluid moves from the plasma into the interstitial spaces and the plasma volume falls. The decline in plasma volume triggers an increase in aldosterone secretion via the renin–angiotensin system. A third cause of Na^+ retention and edema in kidney disease is **heart failure.** Kidney disease predisposes to heart failure, partly because of the hypertension it frequently produces.

THE BLADDER

FILLING

The walls of the ureters contain smooth muscle arranged in spiral, longitudinal, and circular bundles, but distinct layers of muscle are not seen. Regular peristaltic contractions occurring one to five times per minute move the urine from the renal pelvis to the bladder, where it enters in spurts synchronous with each peristaltic wave. The ureters pass obliquely through the bladder wall and, although there are no ureteral sphincters as such, the oblique passage tends to keep the ureters closed except during peristaltic waves, preventing reflux of urine from the bladder.

EMPTYING

The smooth muscle of the bladder, like that of the ureters, is arranged in spiral, longitudinal, and circular bundles. Contraction of the circular muscle, which is called the **detrusor muscle,** is mainly responsible for emptying the bladder during urination (micturition). Muscle bundles pass on either side of the urethra, and these fibers are sometimes called the **internal urethral sphincter,** although they do not encircle the urethra. Farther along the urethra is a sphincter of skeletal muscle, the sphincter of the membranous urethra **(external urethral sphincter).** The bladder epithelium is made up of a superficial layer of flat cells and a deep layer of cuboidal cells. The innervation of the bladder is summarized in **Figure 37–19**.

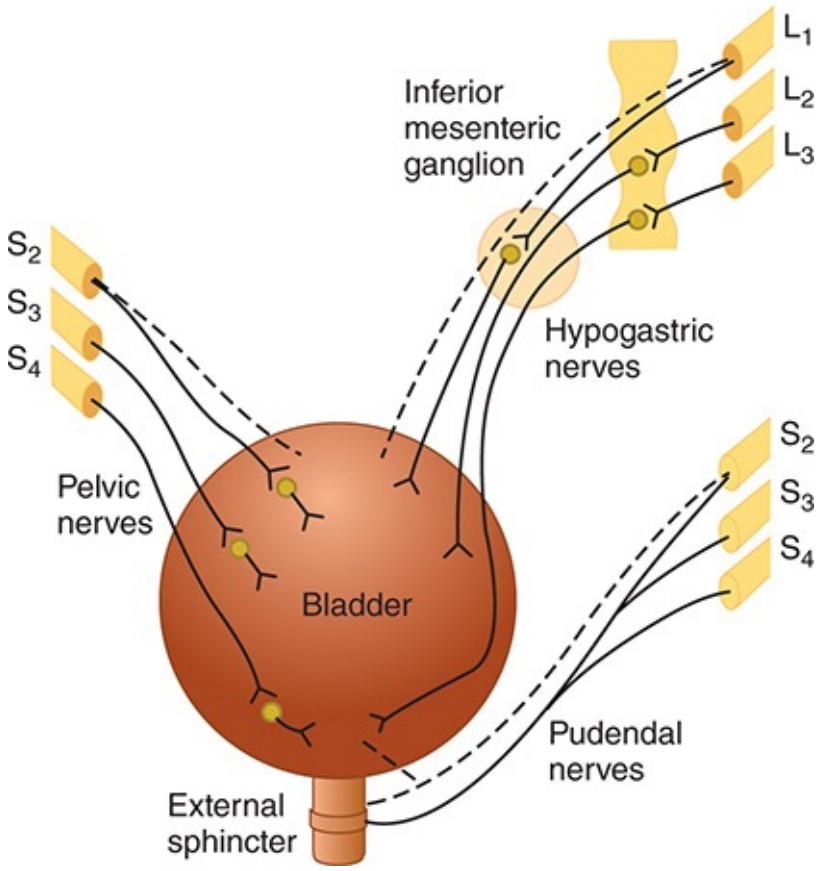


FIGURE 37–19 Innervation of the bladder. Dashed lines indicate sensory nerves. Parasympathetic innervation is shown at the left, sympathetic at the upper right, and somatic at the lower right.

The physiology of bladder emptying and the physiologic basis of its disorders are subjects about which there is much confusion. Micturition is fundamentally a spinal reflex facilitated and inhibited by higher brain centers and, like defecation, subject to voluntary facilitation and inhibition. Urine enters the bladder without producing much increase in intravesical pressure until the viscus is well filled. In addition, like other types of smooth muscle, the bladder muscle has the property of plasticity; when it is stretched, the tension initially produced is not maintained. The relation between intravesical pressure and volume can be studied by inserting a catheter and emptying the bladder, then recording the pressure while the bladder is filled with 50-mL increments of water or air **(cystometry).** A plot of intravesical pressure against the volume of fluid in the bladder is called a **cystometrogram** **(Figure 37–20)**. The curve shows an initial slight rise in pressure when the first increments in volume are produced; a long, nearly flat segment as further increments are produced; and a sudden, sharp rise

in pressure as the micturition reflex is triggered. These three components are sometimes called segments Ia, Ib, and II. The first urge to void is felt at a bladder volume of about 150 mL, and a marked sense of fullness at about 400 mL. The flatness of segment Ib is a manifestation of the law of Laplace. This law states that the pressure in a spherical viscus is equal to twice the wall tension divided by the radius. In the case of the bladder, the tension increases as the organ fills, but so does the radius. Therefore, the pressure increase is slight until the organ is relatively full.

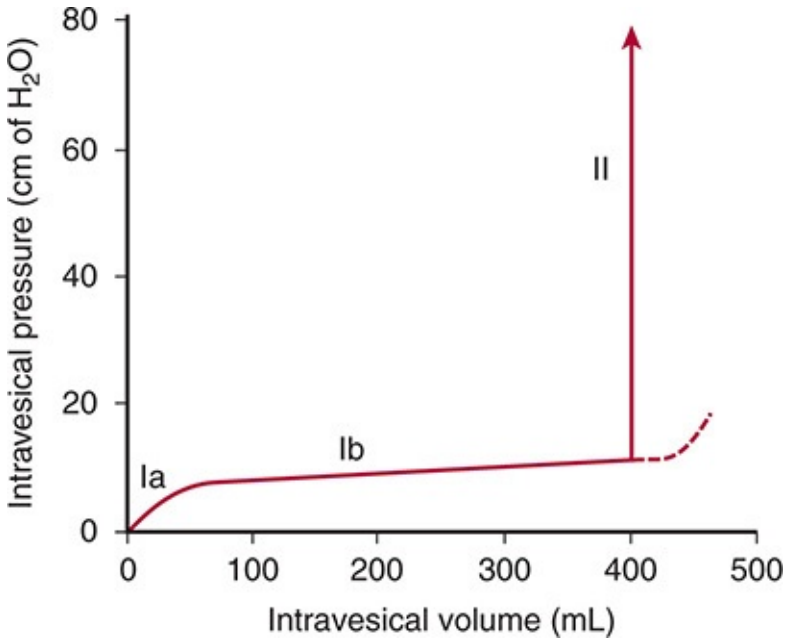


FIGURE 37–20 Cystometrogram in a normal human. The numerals identify the three components of the curve described in the text. The dashed line indicates the pressure–volume relations that would have been found had micturition not occurred and produced component II. (Modified and reproduced with permission from Tanagho EA, McAninch JW: *Smith's General Urology*, 15th ed. New York, NY: McGraw-Hill; 2000.)

During micturition, the perineal muscles and external urethral sphincter are relaxed, the detrusor muscle contracts, and urine passes out through the urethra. The bands of smooth muscle on either side of the urethra apparently play no role in micturition, and their main function in males is believed to be the prevention of reflux of semen into the bladder during ejaculation.

The mechanism by which voluntary urination is initiated remains unsettled. One of the initial events is relaxation of the muscles of the pelvic floor, and this may cause a sufficient downward tug on the detrusor muscle to initiate its

contraction. The perineal muscles and external sphincter can be contracted voluntarily, preventing urine from passing down the urethra or interrupting the flow once urination has begun. It is through the learned ability to maintain the external sphincter in a contracted state that adults are able to delay urination until the opportunity to void presents itself. After urination, the female urethra empties by gravity. Urine remaining in the urethra of the male is expelled by several contractions of the bulbocavernosus muscle.

REFLEX CONTROL

The bladder smooth muscle has some inherent contractile activity; however, when its nerve supply is intact, stretch receptors in the bladder wall initiate a reflex contraction that has a lower threshold than the inherent contractile response of the muscle. Fibers in the pelvic nerves are the afferent limb of the voiding reflex, and the parasympathetic fibers to the bladder that constitute the efferent limb also travel in these nerves. The reflex is integrated in the sacral portion of the spinal cord. In the adult, the volume of urine in the bladder that normally initiates a reflex contraction is about 300–400 mL. The sympathetic nerves to the bladder play no part in micturition, but in males they do mediate the contraction of the bladder muscle that prevents semen from entering the bladder during ejaculation.

The stretch receptors in the bladder wall have no small motor nerve system. However, the threshold for the voiding reflex, like the stretch reflexes, is adjusted by the activity of facilitatory and inhibitory centers in the brainstem. There is a facilitatory area in the pontine region and an inhibitory area in the midbrain. After transection of the brainstem just above the pons, the threshold is lowered and less bladder filling is required to trigger it, whereas after transection at the top of the midbrain, the threshold for the reflex is essentially normal. There is another facilitatory area in the posterior hypothalamus. Humans with lesions in the superior frontal gyrus have a reduced desire to urinate and difficulty in stopping micturition once it has commenced. However, stimulation experiments in animals indicate that other cortical areas also affect the process. The bladder can be made to contract by voluntary facilitation of the spinal voiding reflex when it contains only a few milliliters of urine. Voluntary contraction of the abdominal muscles aids the expulsion of urine by increasing the intra-abdominal pressure, but voiding can be initiated without straining even when the bladder is nearly empty.

EFFECTS OF DEAFFERENTATION

When the sacral dorsal roots are cut in experimental animals or interrupted by diseases of the dorsal roots, such as **tabes dorsalis** in humans, all reflex contractions of the bladder are abolished. The bladder becomes distended, thin-walled, and hypotonic, but some contractions occur because of the intrinsic response of the smooth muscle to stretch.

EFFECTS OF DENERVATION

When the afferent and efferent nerves are both destroyed, as they may be by tumors of the cauda equina or filum terminale, the bladder is flaccid and distended for a while. Gradually, however, the muscle of the "decentralized bladder" becomes active, with many contraction waves that expel dribbles of urine out of the urethra. The bladder becomes shrunken and the bladder wall hypertrophied. The reason for the difference between the small, hypertrophic bladder seen in this condition and the distended, hypotonic bladder seen when only the afferent nerves are interrupted is not known. The hyperactive state in the former condition suggests the development of denervation hypersensitization even though the neurons interrupted are preganglionic rather than postganglionic **(Clinical Box 37–4)**.

EFFECTS OF SPINAL CORD TRANSECTION

During spinal shock, the bladder is flaccid and unresponsive. It becomes overfilled, and urine dribbles through the sphincters **(overflow incontinence).** After spinal shock has passed, the voiding reflex returns, although there is, of course, no voluntary control and no inhibition or facilitation from higher centers when the spinal cord is transected. Some paraplegic patients train themselves to initiate voiding by pinching or stroking their thighs, provoking a mild mass reflex (see Chapter 12). In some instances, the voiding reflex becomes hyperactive, bladder capacity is reduced, and the wall becomes hypertrophied. This type of bladder is sometimes called the **spastic neurogenic bladder.** The reflex hyperactivity is made worse by, and may be caused by, infection in the bladder wall.

CLINICAL BOX 37–4

Abnormalities of Micturition

Three major types of bladder dysfunction are due to neural lesions: (1) due to interruption of the afferent nerves from the bladder, (2) due to interruption of both afferent and efferent nerves, and (3) due to interruption of facilitatory and inhibitory pathways descending from the brain. The bladder contracts in all three types, but the contractions are generally not sufficient to empty the viscus completely, and residual urine is left in the bladder.

CHAPTER SUMMARY

- Plasma enters the kidneys and is filtered in the glomerulus. As the filtrate passes down the nephron and through the tubules its volume is reduced and water and solutes are removed (tubular reabsorption) and waste products are secreted (tubular secretion).
- A nephron consists of an individual renal tubule and its glomerulus. Each tubule has several segments, beginning with the proximal tubule, followed by the loop of Henle (descending and ascending limbs), the distal convoluted tubule, the connecting tubule, and the collecting duct.
- The kidneys receive just under 25% of the cardiac output and renal plasma flow can be measured by infusing p-aminohippuric acid (PAH) and determining its urine and plasma concentrations.
- Renal blood flow enters the glomerulus via the afferent arteriole and leaves via the efferent arteriole (whose diameter is smaller). Renal blood flow is regulated by norepinephrine (constriction, reduction of flow), dopamine (vasodilation, increases flow), angiotensin II (constricts), prostaglandins (dilation in the renal cortex and constriction in the renal medulla), and acetylcholine (vasodilation).
- Glomerular filtration rate can be measured by a substance that is freely filtered and neither reabsorbed nor secreted in the tubules, is nontoxic, and is not metabolized by the body. Inulin meets these criteria and is extensively used to measure GFR.
- Urine is stored in the bladder before voiding (micturition). The micturition response involves reflex pathways but is under voluntary control.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. In the presence of vasopressin, the greatest fraction of filtered water is absorbed in the
   A. proximal tubule.
   B. loop of Henle.
   C. distal tubule.
   D. cortical collecting duct.
   E. medullary collecting duct.
2. In the absence of vasopressin, the greatest fraction of filtered water is absorbed in the
   A. proximal tubule.
   B. loop of Henle.
   C. distal tubule.
   D. cortical collecting duct.
   E. medullary collecting duct.
3. If the clearance of a substance which is freely filtered is less than that of inulin,
   A. there is net reabsorption of the substance in the tubules.
   B. there is net secretion of the substance in the tubules.
   C. the substance is neither secreted nor reabsorbed in the tubules.
   D. the substance becomes bound to protein in the tubules.
   E. the substance is secreted in the proximal tubule to a greater degree than in the distal tubule.
4. Glucose reabsorption occurs in the
   A. proximal tubule.
   B. loop of Henle.
   C. distal tubule.
   D. cortical collecting duct.
   E. medullary collecting duct.
5. On which of the following does aldosterone exert its greatest effect?
   A. Glomerulus

B. Proximal tubule

C. Thin portion of the loop of Henle

D. Thick portion of the loop of Henle

E. Cortical collecting duct

6. What is the clearance of a substance when its concentration in the plasma is 10 mg/dL, its concentration in the urine is 100 mg/dL, and urine flow is 2 mL/min?

A. 2 mL/min

B. 10 mL/min

C. 20 mL/min

D. 200 mL/min

E. Clearance cannot be determined from the information given

7. As urine flow increases during osmotic diuresis

A. the osmolality of urine falls below that of plasma.

B. the osmolality of urine increases because of the increased amounts of nonreabsorbable solute in the urine.

C. the osmolality of urine approaches that of plasma because plasma leaks into the tubules.

D. the osmolality of urine approaches that of plasma because an increasingly large fraction of the excreted urine is isotonic proximal tubular fluid.

E. the action of vasopressin on the renal tubules is inhibited.

CHAPTER 38

Regulation of Extracellular Fluid Composition & Volume

OBJECTIVES

After studying this chapter, you should be able to:

- Describe how the tonicity (osmolality) of the extracellular fluid is maintained by alterations in water intake and vasopressin secretion.
- Discuss the effects of vasopressin, the receptors on which it acts, and how its secretion is regulated.
- Describe how the volume of the extracellular fluid is maintained by alterations in renin and aldosterone secretion.
- Define the stimuli and the reactions that lead to the formation of angiotensin II
- List the functions of angiotensin II, and the receptors on which it acts.
- Describe the function of atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), and C-type natriuretic peptide (CNP) and detail the receptors on which they act.
- Describe the site and mechanism of action of erythropoietin, and the feedback regulation of its secretion.

INTRODUCTION

This chapter is a review of the major homeostatic mechanisms that operate, primarily through the kidneys and the lungs, to maintain the **tonicity,** the **volume,** and the **specific ionic composition** of the extracellular fluid (ECF). The interstitial portion of this fluid is the fluid environment of the cells, and life depends on the constancy of this "internal sea" (see Chapter 1).

DEFENSE OF TONICITY

The defense of the tonicity of the ECF is primarily the function of the vasopressin-secreting and thirst mechanisms. The total body osmolality is directly proportional to the total body sodium plus the total body potassium divided by the total body water, so that changes in the osmolality of the body fluids occur when a mismatch exists between the amount of these electrolytes and the amount of water ingested or lost from the body. When the effective osmotic pressure of the plasma rises, vasopressin secretion is increased and the thirst mechanism is stimulated; water is retained in the body, diluting the hypertonic plasma; and water intake is increased **(Figure 38–1)**. Conversely, when the plasma becomes hypotonic, vasopressin secretion is decreased and "solute-free water" (water in excess of solute) is excreted. In this way, the tonicity of the body fluids is maintained within a narrow normal range. In health, plasma osmolality ranges from 280 mOsm/kg of H_2O to 295 mOsm/kg of H_2O, with vasopressin secretion maximally inhibited at 285 mOsm/kg and stimulated at higher values **(Figure 38–2)**.

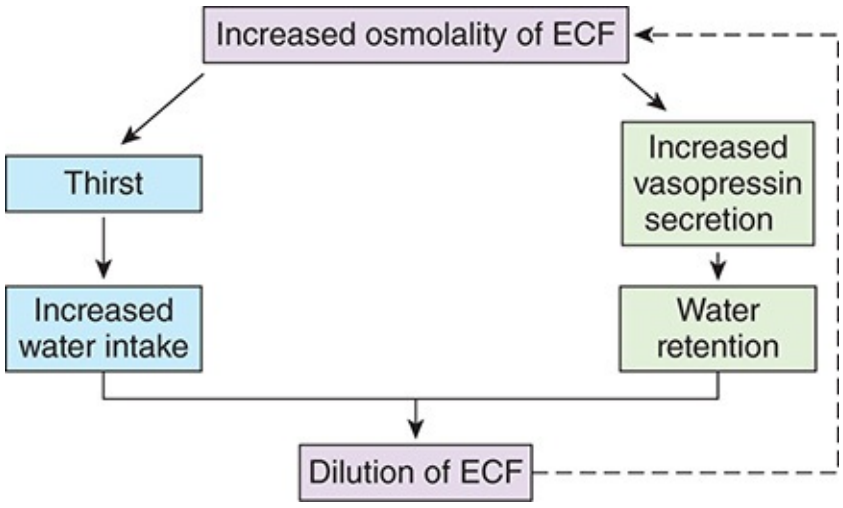


FIGURE 38–1 Mechanisms for defending extracellular fluid (ECF) tonicity. The dashed arrow indicates inhibition. (Used with permission of J Fitzsimmons.)

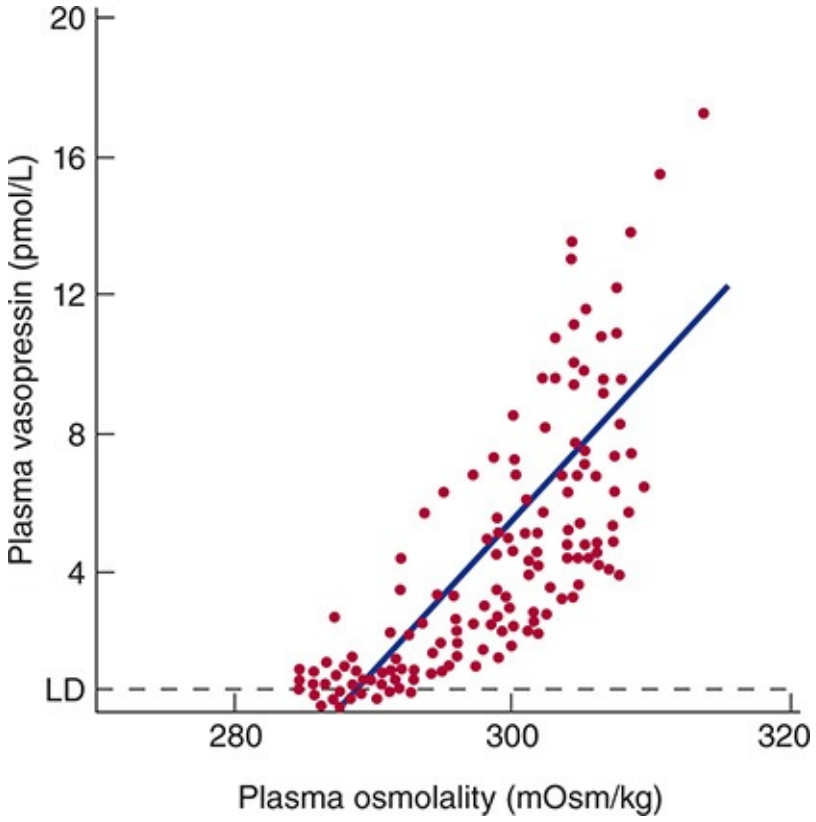


FIGURE 38–2 Relation between plasma osmolality and plasma vasopressin in healthy adult humans during infusion of hypertonic saline. LD, limit of detection. (Reproduced with permission from Thompson CJ, et al: The osmotic thresholds for thirst and vasopressin are similar in healthy humans. Clin Sci [Colch] 1986; Dec; 71:(6):651–656.)

VASOPRESSIN RECEPTORS

There are at least three kinds of vasopressin receptors: V_{1A}, V_{1B}, and V_2. All are G-protein-coupled. The V_{1A} and V_{1B} receptors act through phosphatidylinositol hydrolysis to increase the intracellular Ca^{2+} concentration. The V_2 receptors act through G_s to increase cyclic adenosine 3′, 5′-monophosphate (cAMP) levels.

EFFECTS OF VASOPRESSIN

Because one of its principal physiologic effects is the retention of water by the kidney, vasopressin is often called the **antidiuretic hormone (ADH).** It increases the permeability of the collecting ducts of the kidney, so that water enters the hypertonic interstitium of the renal pyramids. The urine becomes concentrated, and its volume decreases. The overall effect is therefore retention of water in excess of solute; consequently, the effective osmotic pressure of the body fluids is decreased. In the absence of vasopressin, the urine is hypotonic to plasma, urine volume is increased, and there is a net water loss. Consequently, the osmolality of the body fluid rises.

The mechanism by which vasopressin exerts its antidiuretic effect is activated by **V_2 receptors** and involves the insertion of aquaporin 2 into the apical (luminal) membranes of the principal cells of the collecting ducts. Movement of water across membranes by simple diffusion is now known to be augmented by movement through these water channels. These channels are stored in endosomes inside the cells, and vasopressin causes their rapid translocation to the luminal membranes.

V_{1A} receptors mediate the vasoconstrictor effect of vasopressin, and vasopressin is a potent stimulator of vascular smooth muscle in vitro. However, relatively large amounts of vasopressin are needed to raise blood pressure in vivo, because vasopressin also acts on the brain to decrease in cardiac output. The site of this action is the **area postrema,** one of the circumventricular organs (see Chapter 33). Hemorrhage is a potent stimulus for vasopressin secretion, and the blood pressure fall after hemorrhage is more marked in animals that have been treated with synthetic peptides that block the pressor action of vasopressin. Consequently, it appears that vasopressin does play a role in blood pressure homeostasis.

V_{1A} receptors are also found in the liver and the brain. Vasopressin causes glycogenolysis in the liver, and, as noted above, it is a neurotransmitter in the brain and spinal cord.

The V_{1B} receptors (also called V_3 receptors) appear to be unique to the anterior pituitary, where they mediate increased secretion of adrenocorticotropic hormone (ACTH) from the corticotropes.

METABOLISM

Circulating vasopressin is rapidly inactivated, principally in the liver and kidneys. It has a **biologic half-life** of approximately 18 min in humans.

CONTROL OF VASOPRESSIN SECRETION: OSMOTIC STIMULI

Vasopressin is stored in the posterior pituitary and released into the bloodstream in response to impulses in the nerve fibers that contain the hormone. The factors affecting its secretion are summarized in **Table 38–1**. When the effective osmotic pressure of the plasma is increased above 285 mOsm/kg, the rate of discharge of neurons containing vasopressin increases and vasopressin secretion occurs (Figure 38–2). At 285 mOsm/kg, plasma vasopressin is at or near the limits of detection by available assays, but its levels probably decrease when plasma osmolality is below this level. Vasopressin secretion is regulated by osmoreceptors located in the anterior hypothalamus. They are outside the blood-brain barrier and appear to be located in the circumventricular organs, primarily the organum vasculosum of the lamina terminalis (OVLT) (see Chapter 33). The osmotic threshold for thirst (Figure 38–1) is the same as or slightly greater than the threshold for increased vasopressin secretion (Figure 38–2), and it is still uncertain whether the same osmoreceptors mediate both effects.

TABLE 38–1 Summary of stimuli affecting vasopressin secretion.

Vasopressin Secretion Increased	Vasopressin Secretion Decreased
Increased effective osmotic pressure of plasma	Decreased effective osmotic pressure of plasma
Decreased ECF volume	Increased ECF volume
Pain, emotion, "stress," exercise	Alcohol
Nausea and vomiting	
Standing	
Clofibrate, carbamazepine	
Angiotensin II	

ECF, extracellular fluid.

Vasopressin secretion is thus controlled by a delicate feedback mechanism that operates continuously to defend the osmolality of the plasma. Significant changes in secretion occur when osmolality is changed as little as 1%. In this way, the osmolality of the plasma in normal individuals is maintained very close to 285 mOsm/L.

VOLUME EFFECTS ON VASOPRESSIN SECRETION

ECF volume also affects vasopressin secretion. Vasopressin secretion is increased when ECF volume is low and decreased when ECF volume is high (Table 38–1). There is an inverse relationship between the rate of vasopressin secretion and the rate of discharge in afferents from stretch receptors in the low- and high-pressure portions of the vascular system. The low-pressure receptors are those in the great veins, right and left atria, and pulmonary vessels; the high-pressure receptors are those in the carotid sinuses and aortic arch (see Chapter 32). The exponential increases in plasma vasopressin produced by decreases in blood pressure are documented in **Figure 38–3**. However, the low-pressure receptors monitor the fullness of the vascular system, and moderate decreases in blood volume that reduce central venous pressure without lowering arterial pressure can also increase plasma vasopressin.

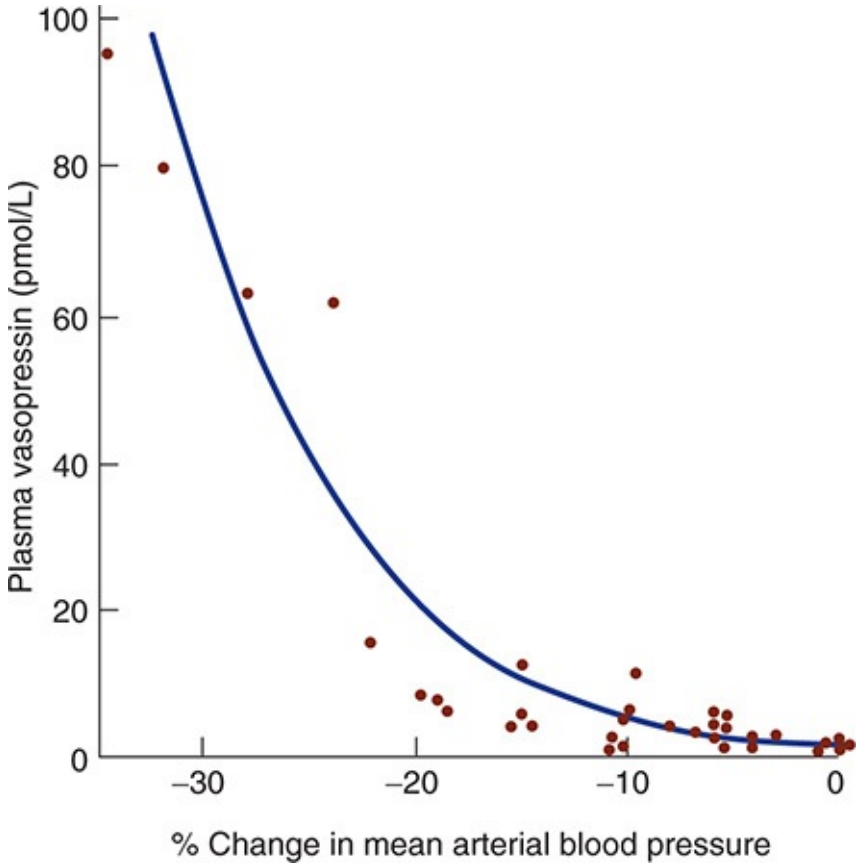


FIGURE 38–3 Relation of mean arterial blood pressure to plasma vasopressin in healthy adult humans in whom a progressive decline in blood

pressure was induced by infusion of graded doses of the ganglionic blocking drug trimethaphan. The relation is exponential rather than linear. (Data from Baylis PH: Osmoregulation and control of vasopressin secretion in healthy humans. Am J Physiol 1987;253:R671.)

Thus, the low-pressure receptors are the primary mediators of volume effects on vasopressin secretion. Impulses pass from them via the vagi to the nucleus of the tractus solitarius (NTS). An inhibitory pathway projects from the NTS to the caudal ventrolateral medulla (CVLM), and there is a direct excitatory pathway from the CVLM to the hypothalamus. Angiotensin II reinforces the response to hypovolemia and hypotension by acting on the circumventricular organs to increase vasopressin secretion (see Chapter 33).

Hypovolemia and hypotension produced by conditions such as hemorrhage release large amounts of vasopressin, and in the presence of hypovolemia, the osmotic response curve is shifted to the left **(Figure 38–4)**. Its slope is also increased. The result is water retention and reduced plasma osmolality. This includes hyponatremia, since Na^+ is the most abundant osmotically active component of the plasma.

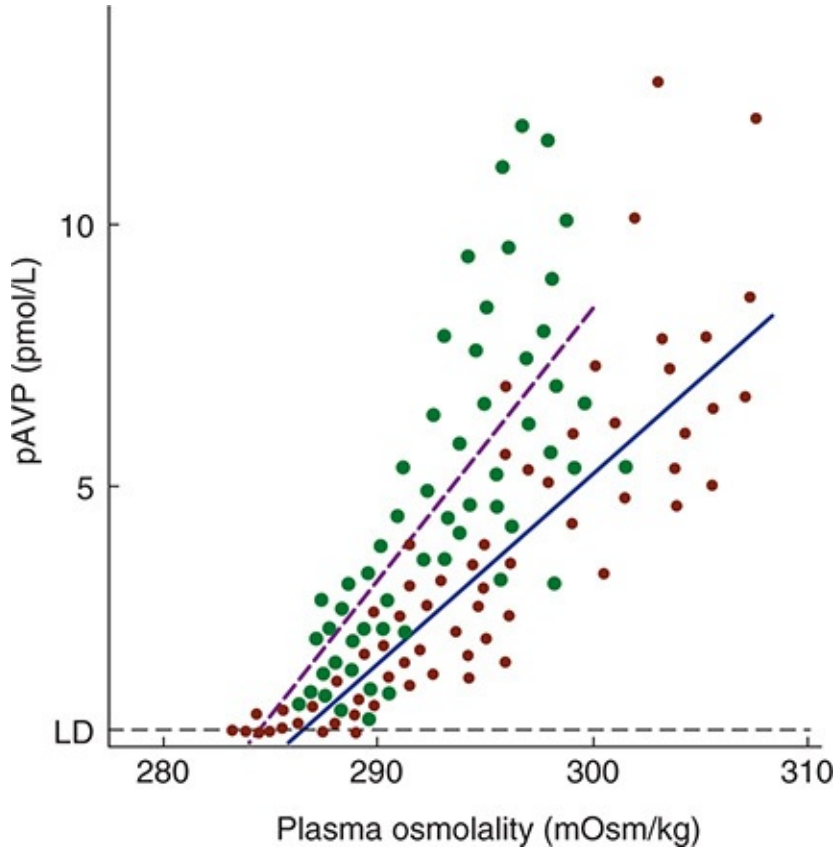


FIGURE 38–4 Effect of hypovolemia and hypervolemia on the relation between plasma vasopressin (pAVP) and plasma osmolality. Seven blood samples were drawn at various times from 10 normal men when hypovolemia was induced by water deprivation (green circles, dashed line) and again when hypervolemia was induced by infusion of hypertonic saline (red circles, solid line). Linear regression analysis defined the relationship pAVP = 0.52 (plasma osmolality—283.5) for water deprivation and pAVP = 0.38 (plasma osmolality—285.6) for hypertonic saline. LD, limit of detection. Note the steeper curve as well as the shift of the intercept to the left during hypovolemia. (Used with permission of CJ Thompson.)

OTHER STIMULI AFFECTING VASOPRESSIN SECRETION

A variety of stimuli in addition to osmotic pressure changes and ECF volume aberrations increase vasopressin secretion. These include pain, nausea, surgical stress, and some emotions (Table 38–1). Nausea is associated with particularly large increases in vasopressin secretion. Alcohol decreases vasopressin secretion.

CLINICAL IMPLICATIONS

In various clinical conditions, volume and other nonosmotic stimuli bias the osmotic control of vasopressin secretion. For example, patients who have had surgery may have elevated levels of plasma vasopressin because of pain and hypovolemia, and this may cause low plasma osmolality and dilutional hyponatremia **(see Clinical Box 38–1**).

Diabetes insipidus is the syndrome that results when there is a vasopressin deficiency **(central diabetes insipidus)** or when the kidneys fail to respond to the hormone **(nephrogenic diabetes insipidus).**

Causes of vasopressin deficiency include disease processes in the supraoptic and paraventricular nuclei, the hypothalamohypophysial tract, or the posterior pituitary gland. It has been estimated that 30% of clinical cases are due to neoplastic lesions of the hypothalamus, either primary or metastatic; 30% are posttraumatic; 30% are idiopathic; and the remainder are due to vascular lesions, infections, systemic diseases such as sarcoidosis that affect the hypothalamus, or mutations in the gene for prepropressophysin. Disease that develops after

surgical removal of the posterior lobe of the pituitary may be temporary if the distal ends of the supraoptic and paraventricular fibers are only damaged, because the fibers recover, make new vascular connections, and begin to secrete vasopressin again.

The symptoms of diabetes insipidus are passage of large amounts of dilute urine **(polyuria)** and the drinking of large amounts of fluid **(polydipsia),** provided the thirst mechanism is intact. It is the polydipsia that keeps these patients healthy. If their sense of thirst is depressed for any reason and their intake of dilute fluid decreases, dehydration that can be fatal develops.

Another cause of diabetes insipidus is inability of the kidneys to respond to vasopressin **(nephrogenic diabetes insipidus).** Two forms of this disease have been described. In one form, the gene for the V_2 receptor is mutated, making the receptor unresponsive. The V_2 receptor gene is on the X chromosome, thus this condition is X-linked and inheritance is sex-linked recessive. In the other form of the condition, mutations occur in the autosomal gene for aquaporin-2 and produce nonfunctional versions of this water channel, many of which do not reach the apical membrane of the collecting duct but are trapped in intracellular locations.

CLINICAL BOX 38–1

Syndrome of Inappropriate Antidiurectic Hormone

The **syndrome of "inappropriate" hypersecretion of antidiuretic hormone (SIADH)** occurs when vasopressin is inappropriately high relative to serum osmolality. Vasopressin is responsible not only for dilutional **hyponatremia** (serum sodium < 135 mmol/L) but also for loss of salt in the urine when water retention is sufficient to expand the ECF volume, reducing aldosterone secretion (see Chapter 20). This occurs in patients with cerebral disease ("cerebral salt wasting") and pulmonary disease ("pulmonary salt wasting"). Hypersecretion of vasopressin in patients with pulmonary diseases such as lung cancer may be due in part to the interruption of inhibitory impulses in vagal afferents from the stretch receptors in the atria and great veins.

A significant number of lung tumors and some other cancers secrete vasopressin. There is a process called **"vasopressin escape"** that counteracts the water-retaining action of vasopressin to limit the degree of hyponatremia in SIADH. Studies in rats have demonstrated that prolonged exposure to elevated levels of vasopressin can lead eventually to down-regulation of the

production of aquaporin-2. This permits urine flow suddenly to increase and plasma osmolality to fall despite exposure of the collecting ducts to elevated levels of the hormone; that is, the individual escapes from the renal effects of vasopressin.

THERAPEUTIC HIGHLIGHTS

Patients with inappropriate hypersecretion of vasopressin have been successfully treated with demeclocycline, an antibiotic that reduces the renal response to vasopressin.

SYNTHETIC AGONISTS & ANTAGONISTS

Synthetic peptides that have selective actions and are more active than naturally occurring vasopressin have been produced by altering the amino acid residues. For example, 1-deamino-8-D-arginine vasopressin (desmopressin; dDAVP) has very high antidiuretic activity with little pressor activity, making it valuable in the treatment of vasopressin deficiency.

DEFENSE OF VOLUME

The volume of the ECF is determined primarily by the total amount of osmotically active solute in the ECF. The composition of the ECF is discussed in Chapter 1. Because Na^+ and Cl^- are by far the most abundant osmotically active solutes in ECF, and because changes in Cl^- are to a great extent secondary to changes in Na^+, the amount of Na^+ in the ECF is the most important determinant of ECF volume. Therefore, the mechanisms that control Na^+ balance are the major mechanisms defending ECF volume. However, there is volume control of water excretion as well; a rise in ECF volume inhibits vasopressin secretion, and a decline in ECF volume produces an increase in the secretion of this hormone. Volume stimuli override the osmotic regulation of vasopressin secretion. Angiotensin II stimulates aldosterone and vasopressin secretion. It also causes thirst and constricts blood vessels, which help maintain blood pressure. Thus, angiotensin II plays a key role in the body's response to hypovolemia **(Figure 38–5)**. In addition, expansion of the ECF volume increases the secretion of atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) by the heart, and

this causes natriuresis and diuresis.

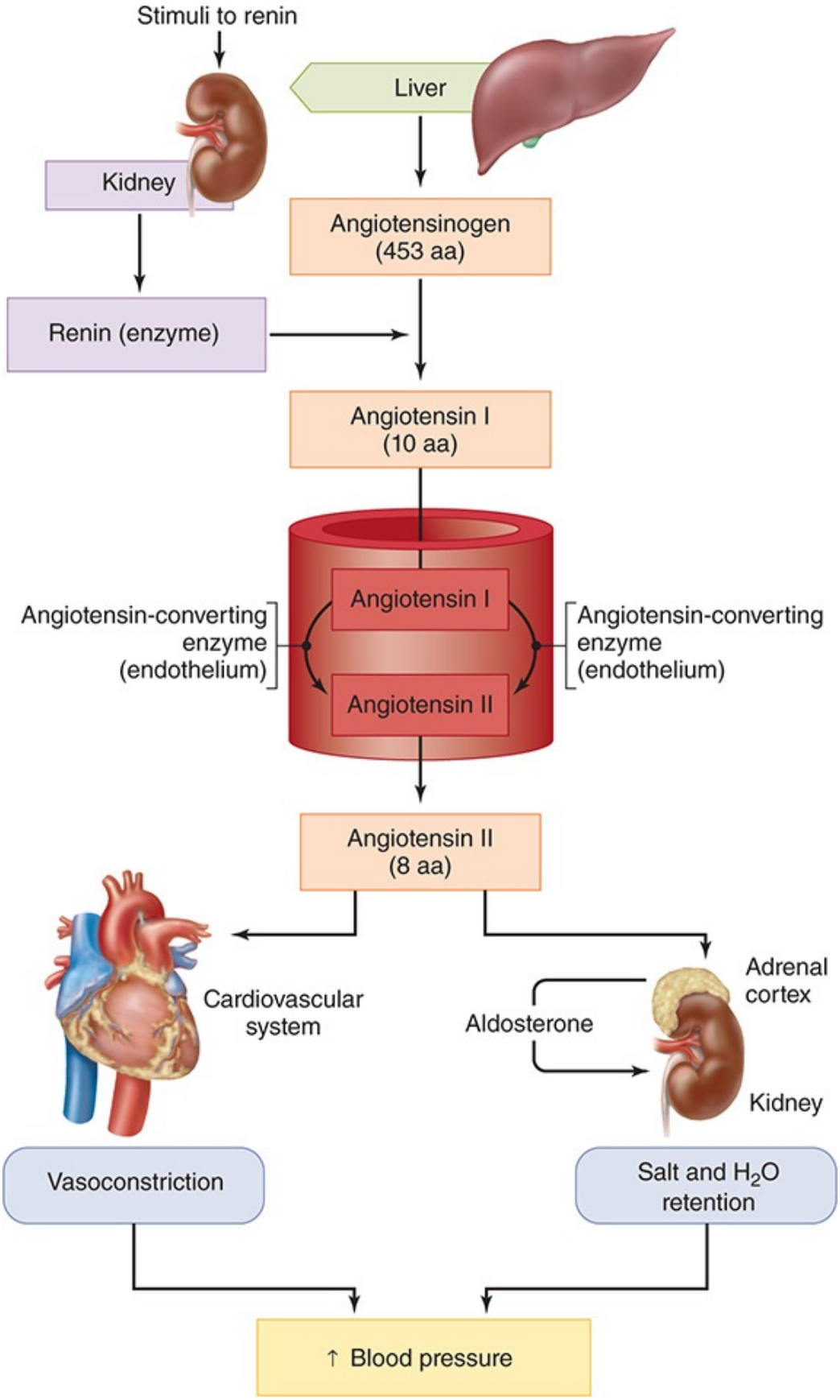

Stimuli to renin
Liver
Kidney
Angiotensinogen
(453 aa)
Renin (enzyme)
Angiotensin I
(10 aa)
Angiotensin I
Angiotensin II
Angiotensin-converting
enzyme
(endothelium)
Angiotensin-converting
enzyme
(endothelium)
Angiotensin II
(8 aa)
Cardiovascular
system
Adrenal
cortex
Aldosterone
Kidney
Vasoconstriction
Salt and H_2O
retention
↑ Blood pressure

FIGURE 38–5 Summary of the renin–angiotensin system and the stimulation of aldosterone secretion by angiotensin II. The plasma concentration of renin is the rate-limiting step in the renin–angiotensin system; therefore, it is the major determinant of plasma angiotensin II concentration.

In disease states, loss of water from the body **(dehydration)** causes a moderate decrease in ECF volume, because water is lost from both the intracellular and ECF compartments; but excessive loss of Na^+ in the stools (diarrhea), urine (severe acidosis, adrenal insufficiency), or sweat (heat prostration) decreases ECF volume markedly and eventually leads to shock. The immediate compensations in shock operate principally to maintain intravascular volume, but they also affect Na^+ balance. In adrenal insufficiency, the decline in ECF volume is not only due to loss of Na^+ in the urine but also to its movement into cells. Because of the key position of Na^+ in volume homeostasis, it is not surprising that more than one mechanism has evolved to control the excretion of this ion.

The filtration and reabsorption of Na^+ in the kidneys and the effects of these processes on Na^+ excretion are discussed in Chapter 37. When ECF volume is decreased, blood pressure falls, glomerular capillary pressure declines, and the glomerular filtration rate (GFR) therefore falls, reducing the amount of Na^+ filtered. Tubular reabsorption of Na^+ is increased, in part because the secretion of aldosterone is increased. Aldosterone secretion is controlled in part by a feedback system in which the change that initiates increased secretion is a decline in mean intravascular pressure. Other changes in Na^+ excretion occur too rapidly to be solely due to changes in aldosterone secretion. For example, rising from the supine to the standing position increases aldosterone secretion. However, Na^+ excretion is decreased within a few minutes, and this rapid change in Na^+ excretion occurs in adrenalectomized subjects. It is probably due to hemodynamic changes and possibly to decreased ANP secretion.

The kidneys produce three hormones: 1,25-dihydroxycholecalciferol (see Chapter 21), renin, and erythropoietin. Natriuretic peptides, substances secreted by the heart and other tissues, increase excretion of sodium by the kidneys, and an additional natriuretic hormone (endogenous ouabain) inhibits Na, K ATPase.

THE RENIN–ANGIOTENSIN SYSTEM

RENIN

The rise in blood pressure produced by injection of kidney extracts is due to **renin,** an acid protease secreted by the kidneys into the bloodstream. This enzyme acts in concert with angiotensin-converting enzyme (ACE) to form angiotensin II **(Figure 38–6)**. It is a glycoprotein with a molecular weight of 37,326 in humans. The molecule is made up of two lobes, or domains, between which the active site of the enzyme is located in a deep cleft. Two aspartic acid residues, one at position 104 and one at position 292 (residue numbers from human preprorenin), are juxtaposed in the cleft and are essential for activity. Thus, renin is an aspartyl protease.

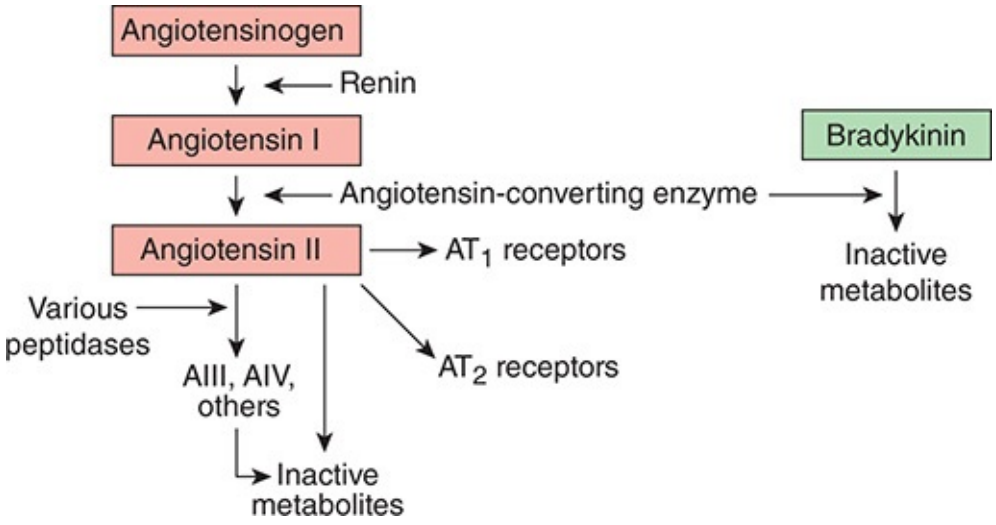


FIGURE 38–6 Formation and metabolism of circulating angiotensins.

Like other hormones, renin is synthesized as a large preprohormone. Human **preprorenin** contains 406 amino acid residues. The **prorenin** that remains after removal of a leader sequence of 23 amino acid residues from the amino terminal contains 383 amino acid residues, and after removal of the pro sequence from the amino terminal of prorenin, active **renin** contains 340 amino acid residues. Prorenin has little if any biologic activity.

Some prorenin is converted to renin in the kidneys, and some is secreted. Prorenin is also secreted by other organs, including the ovaries. After nephrectomy, the prorenin level in the circulation is usually only moderately reduced and may actually rise, but the level of active renin falls to essentially zero. Thus, very little prorenin is converted to renin in the circulation, and active renin is a product primarily, if not exclusively, of the kidneys. Prorenin is

secreted constitutively, whereas active renin is formed in the secretory granules of the granular cells in the juxtaglomerular apparatus, the same cells that produce renin (see below). Active renin has a half-life in the circulation of 80 min or less. Its only known function is to cleave the decapeptide **angiotensin I** from the amino terminal end of **angiotensinogen (renin substrate)** (Figure 38–7).

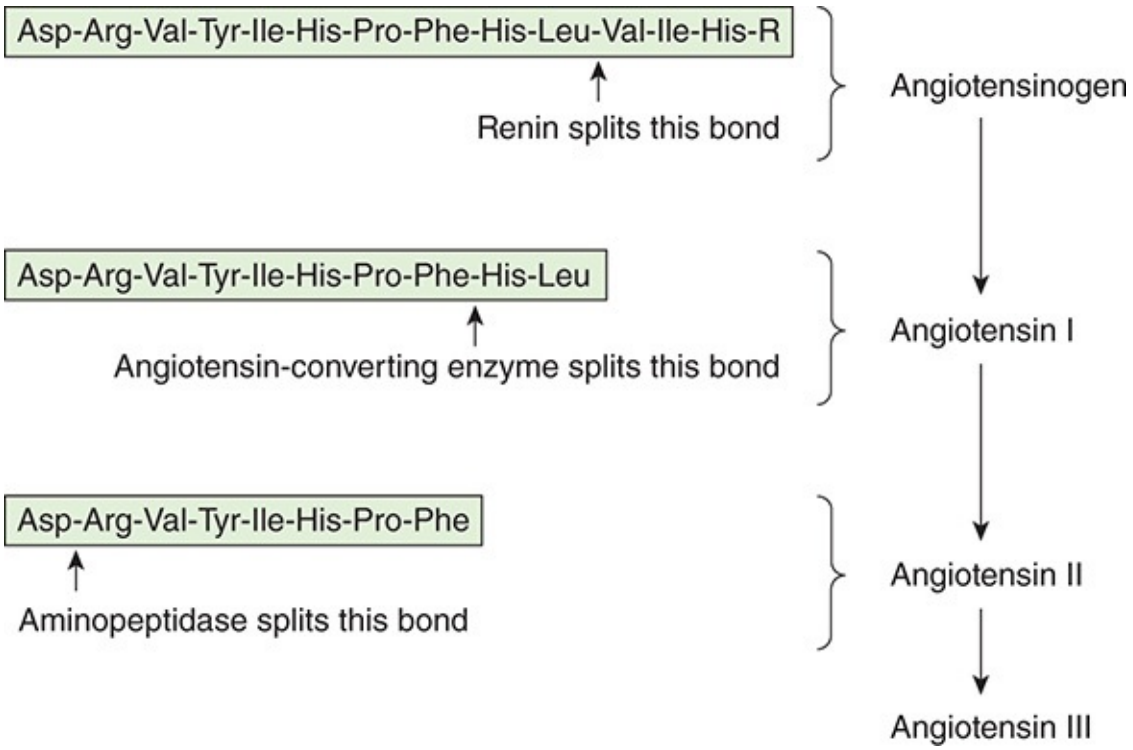


FIGURE 38–7 Structure of the amino terminal end of angiotensinogen and angiotensins I, II, and III in humans. R, remainder of protein. After removal of a 24-amino-acid leader sequence, angiotensinogen contains 453 amino acid residues. The structure of angiotensin II in dogs, rats, and many other mammals is the same as that in humans. Bovine and ovine angiotensin II have valine instead of isoleucine at position 5.

ANGIOTENSINOGEN

Circulating angiotensinogen is found in the α_2-globulin fraction of the plasma (Figure 38–6). It contains about 13% carbohydrate and is made up of 453 amino acid residues. It is synthesized in the liver with a 32-amino-acid signal sequence that is removed in the endoplasmic reticulum. Its circulating level is increased by

glucocorticoids, thyroid hormones, estrogens, several cytokines, and angiotensin II.

ANGIOTENSIN-CONVERTING ENZYME & ANGIOTENSIN II

ACE is a dipeptidyl carboxypeptidase that splits off histidyl-leucine from the physiologically inactive angiotensin I, forming the octapeptide **angiotensin II** (Figure 38–7). The same enzyme inactivates bradykinin (Figure 38–6). Increased tissue bradykinin produced when ACE is inhibited acts on B_2 receptors to produce the cough that is an annoying side effect in up to 20% of patients treated with ACE inhibitors **(see Clinical Box 38–2**). Most of the converting enzyme that forms angiotensin II in the circulation is located in endothelial cells. Much of the conversion occurs as the blood passes through the lungs, but conversion also occurs in many other parts of the body.

ACE is an ectoenzyme that exists in two forms: a **somatic** form found throughout the body and a **germinal** form found solely in postmeiotic spermatogenic cells and spermatozoa (see Chapter 23). Both forms have a single transmembrane domain and a short cytoplasmic tail. However, somatic ACE is a 170-kDa protein with two homologous extracellular domains, each containing an active site. Germinal ACE is a 90-kDa protein that has only one extracellular domain and active site. Both enzymes are formed from a single gene. However, the gene has two different promoters, producing two different mRNAs. In male mice in which the ACE gene has been knocked out, blood pressure is lower than normal, but in females it is normal. In addition, fertility is reduced in males but not in females.

CLINICAL BOX 38–2

Pharmacologic Manipulation of the Renin–Angiotensin System

It is now possible to inhibit the secretion or the effects of renin in a variety of ways. Inhibitors of prostaglandin synthesis such as **indomethacin** and β-adrenergic blocking drugs such as **propranolol** reduce renin secretion. The peptide **pepstatin** and newly developed renin inhibitors such as **enalkiren** prevent renin from generating angiotensin I. ACE inhibitors such as **captopril** and **enalapril** prevent conversion of angiotensin I to angiotensin II. **Saralasin**

and several other analogs of angiotensin II are competitive inhibitors of the action of angiotensin II on both AT_1 and AT_2 receptors. **Losartan** (DuP-753) selectively blocks AT_1 receptors, and PD-123177 and several other drugs selectively block AT_2 receptors.

METABOLISM OF ANGIOTENSIN II

Angiotensin II is metabolized rapidly; its half-life in the circulation in humans is 1–2 min. It is metabolized by various peptidases. An aminopeptidase removes the aspartic acid (Asp) residue from the amino terminal of the peptide (Figure 38–7). The resulting heptapeptide has physiologic activity and is sometimes called **angiotensin III.** Removal of a second amino terminal residue from angiotensin III produces the hexapeptide sometimes called angiotensin IV, which is also said to have some activity. Most, if not all, of the other peptide fragments that are formed are inactive. In addition, aminopeptidase can act on angiotensin I to produce (des-Asp^1) angiotensin I, and this compound can be converted directly to angiotensin III by the action of ACE. Angiotensin-metabolizing activity is found in red blood cells and many tissues. In addition, angiotensin II appears to be removed from the circulation by some sort of trapping mechanism in the vascular beds of tissues other than the lungs.

Renin is usually measured by incubating the sample to be assayed and measuring by immunoassay the amount of angiotensin I generated. This measures the **plasma renin activity (PRA)** of the sample. Deficiency of angiotensinogen as well as renin can cause low PRA values, and to avoid this problem, exogenous angiotensinogen is often added, so that **plasma renin concentration (PRC)** rather than PRA is measured. The normal PRA in supine subjects eating a normal amount of sodium is approximately 1 ng of angiotensin I generated per milliliter per hour. The plasma angiotensin II concentration in such subjects is about 25 pg/mL (approximately 25 pmol/L).

ACTIONS OF ANGIOTENSINS

Angiotensin I appears to function solely as the precursor of angiotensin II and does not have any other established action.

Angiotensin II produces arteriolar constriction and a rise in systolic and diastolic blood pressure. It is one of the most potent vasoconstrictors known,

being four to eight times as active as norepinephrine on a weight basis in normal individuals. However, its pressor activity is decreased in Na^+-depleted individuals and in patients with cirrhosis and some other diseases. In these conditions, circulating angiotensin II is increased, and this down-regulates the angiotensin receptors in vascular smooth muscle. Consequently, there is less response to injected angiotensin II.

Angiotensin II also acts directly on the adrenal cortex to increase the secretion of aldosterone, and the renin–angiotensin system is a major regulator of aldosterone secretion. Additional actions of angiotensin II include facilitation of the release of norepinephrine by a direct action on postganglionic sympathetic neurons, contraction of mesangial cells with a resultant decrease in GFR (see Chapter 37), and a direct effect on the renal tubules to increase Na^+ reabsorption.

Angiotensin II also acts on the brain to decrease the sensitivity of the baroreflex, and this potentiates the pressor effect of angiotensin II. In addition, it acts on the brain to increase water intake and increase the secretion of vasopressin and ACTH. It does not penetrate the blood-brain barrier, but it triggers these responses by acting on the circumventricular organs, four small structures in the brain that are outside the blood-brain barrier (see Chapter 33). One of these structures, the area postrema, is primarily responsible for the pressor potentiation, whereas two of the others, the subfornical organ (SFO) and the OVLT, are responsible for the increase in water intake (dipsogenic effect). It is not certain which of the circumventricular organs are responsible for the increases in vasopressin and ACTH secretion.

Angiotensin III [(des-Asp^1) angiotensin II] has about 40% of the pressor activity of angiotensin II, but 100% of the aldosterone-stimulating activity. It has been suggested that angiotensin III is the natural aldosterone-stimulating peptide, whereas angiotensin II is the blood pressure–regulating peptide. However, this appears not to be the case, and instead angiotensin III is simply a breakdown product with some biologic activity. The same is probably true of angiotensin IV, though some researchers have argued that it has unique effects in the brain.

TISSUE RENIN–ANGIOTENSIN SYSTEMS

In addition to the system that generates circulating angiotensin II, many different tissues contain independent renin–angiotensin systems that generate angiotensin II, apparently for local use. Components of the renin–angiotensin system are found in the walls of blood vessels and in the uterus, the placenta, and the fetal membranes. Amniotic fluid has a high concentration of prorenin. In addition,

tissue renin–angiotensin systems, or at least several components of the renin–angiotensin system, are present in the eyes, exocrine portion of the pancreas, heart, fat, adrenal cortex, testis, ovary, anterior and intermediate lobes of the pituitary, pineal, and brain. Tissue renin contributes very little to the circulating renin pool, because PRA falls to undetectable levels after the kidneys are removed. The functions of these tissue renin–angiotensin systems are unsettled, though evidence is accumulating that angiotensin II is a significant growth factor in the heart and blood vessels. ACE inhibitors or AT_1 receptor blockers are now the treatment of choice for heart failure, and part of their value may be due to inhibition of the growth effects of angiotensin II.

ANGIOTENSIN II RECEPTORS

There are at least two classes of angiotensin II receptors. AT_1 receptors are serpentine receptors coupled by a G-protein (G_q) to phospholipase C, and angiotensin II increases the cytosolic free Ca^{2+} level. It also activates numerous tyrosine kinases. In vascular smooth muscle, AT_1 receptors are associated with caveolae (see Chapter 2), and AII increases production of caveolin-1, one of the three isoforms of the protein that is characteristic of caveolae. In rodents, two different but closely related AT_1 subtypes, AT_{1A} and AT_{1B}, are coded by two separate genes. The AT_{1A} subtype is found in blood vessel walls, the brain, and many other organs. It mediates most of the known effects of angiotensin II. The AT_{1B} subtype is found in the anterior pituitary and the adrenal cortex. In humans, an AT_1 receptor gene is present on chromosome 3. There may be a second AT_1 type, but it is still unsettled whether distinct AT_{1A} and AT_{1B} subtypes occur.

There are also AT_2 receptors, which are coded in humans by a gene on the X chromosome. Like the AT_1 receptors, they have seven transmembrane domains, but their actions are different. They act via a G-protein to activate various phosphatases which in turn antagonize growth effects and open K^+ channels. In addition, AT_2 receptor activation increases the production of NO and therefore increases intracellular cyclic 3,5-guanosine monophosphate (cGMP). The overall physiologic consequences of these second-messenger effects are unsettled. AT_2 receptors are more plentiful in fetal and neonatal life, but they persist in the brain and other organs in adults.

The AT_1 receptors in the arterioles and the AT_1 receptors in the adrenal cortex

are regulated in opposite ways: an excess of angiotensin II down-regulates the vascular receptors, but it up-regulates the adrenocortical receptors, making the gland more sensitive to the aldosterone-stimulating effect of the peptide.

THE JUXTAGLOMERULAR APPARATUS

The renin in kidney extracts and the bloodstream is produced by the **juxtaglomerular cells (JG cells).** These epitheloid cells are located in the media of the afferent arterioles as they enter the glomeruli **(Figure 38–8)**. The membrane-lined secretory granules in them have been shown to contain renin. Renin is also found in agranular **lacis cells** that are located in the junction between the afferent and efferent arterioles, but its significance in this location is unknown.

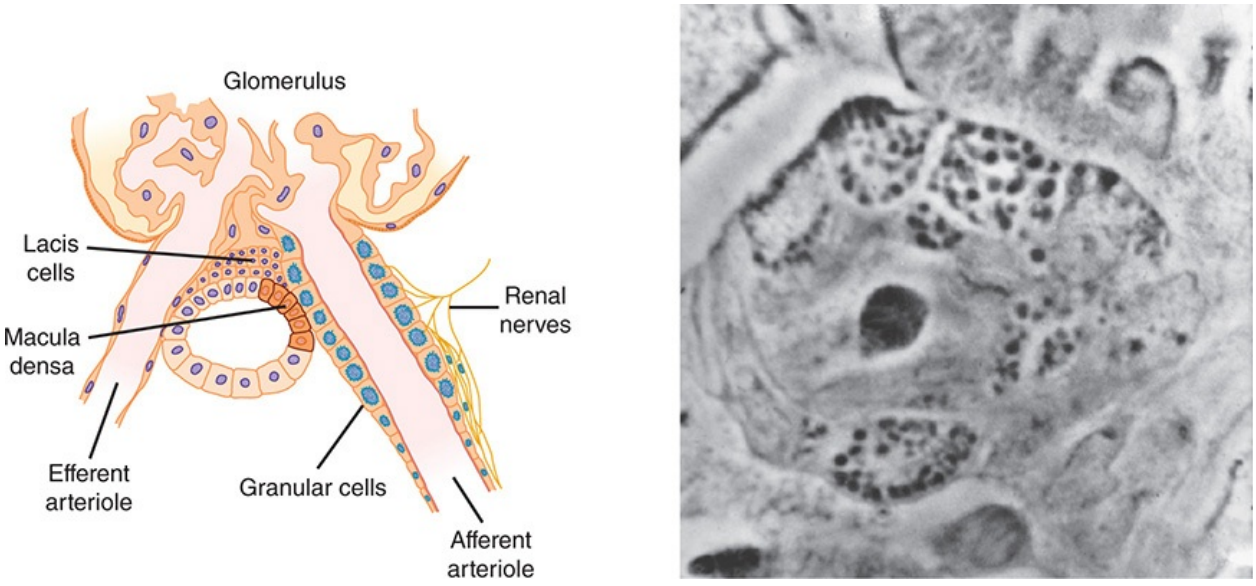


FIGURE 38–8 Left: Diagram of glomerulus, showing the juxtaglomerular apparatus. Right: Phase contrast photomicrograph of afferent arteriole in an unstained, freeze-dried preparation of the kidney of a mouse. Note the red blood cell in the lumen of the arteriole and the granular cells in the wall. (Used with permission of C Peil.)

At the point where the afferent arteriole enters the glomerulus and the efferent arteriole leaves it, the tubule of the nephron touches the arterioles of the glomerulus from which it arose. At this location, which marks the start of the distal convolution, there is a modified region of tubular epithelium called the

macula densa (Figure 38–8). The macula densa is in close proximity to the JG cells. The lacis cells, the JG cells, and the macula densa constitute the **juxtaglomerular apparatus.**

REGULATION OF RENIN SECRETION

Several different factors regulate renin secretion **(Table 38–2)**, and the rate of renin secretion at any given time is determined by the summed activity of these factors. One factor is an intra-renal baroreceptor mechanism that causes renin secretion to decrease when arteriolar pressure at the level of the JG cells increases and to increase when arteriolar pressure at this level falls. Another renin-regulating sensor is in the macula densa. Renin secretion is inversely proportional to the amount of Na^+ and Cl^- entering the distal renal tubules from the loop of Henle. Presumably, these electrolytes enter the macula densa cells via the Na–K–$2Cl^-$ transporters in their apical membranes, and the increase in some fashion triggers a signal that decreases renin secretion in the JG cells in the adjacent afferent arterioles. A possible mediator is NO, but the identity of the signal remains unsettled. Renin secretion also varies inversely with the plasma K^+ level, but the effect of K^+ appears to be mediated by the changes it produces in Na^+ and Cl^- delivery to the macula densa.

TABLE 38–2 Factors that affect renin secretion.

Stimulatory
Increased sympathetic activity via renal nerves
Increased circulating catecholamines
Prostaglandins
Inhibitory
Increased Na^+ and Cl^- reabsorption across macula densa
Increased afferent arteriolar pressure
Angiotensin II
Vasopressin

Angiotensin II feeds back to inhibit renin secretion by a direct action on the JG cells. Vasopressin also inhibits renin secretion in vitro and in vivo, although

there is some debate about whether its in vivo effect is direct or indirect.

Finally, increased activity of the sympathetic nervous system increases renin secretion. The increase is mediated both by increased circulating catecholamines and by norepinephrine secreted by postganglionic renal sympathetic nerves. The catecholamines act mainly on β_1-adrenergic receptors on the JG cells and renin release is mediated by an increase in intracellular cAMP.

The principal conditions that increase renin secretion in humans are listed in **Table 38–3**. Most of the listed conditions decrease central venous pressure, which triggers an increase in sympathetic activity, and some also decrease renal arteriolar pressure **(see Clinical Box 38–3)**. Renal artery constriction and constriction of the aorta proximal to the renal arteries produces a decrease in renal arteriolar pressure. Psychological stimuli increase the activity of the renal nerves.

TABLE 38–3 Conditions that increase renin secretion.

Na^+ depletion
Diuretics
Hypotension
Hemorrhage
Upright posture
Dehydration
Cardiac failure
Cirrhosis
Constriction of renal artery or aorta
Various psychological stimuli

HORMONES OF THE HEART & OTHER NATRIURETIC FACTORS

STRUCTURE

The existence of various **natriuretic hormones** has been postulated for some time. Two of these are secreted by the heart. The muscle cells in the atria and, to a much lesser extent in the ventricles, contain secretory granules **(Figure 38–9)**. The granules increase in number when NaCl intake is increased and ECF

expanded, and extracts of atrial tissue cause natriuresis.

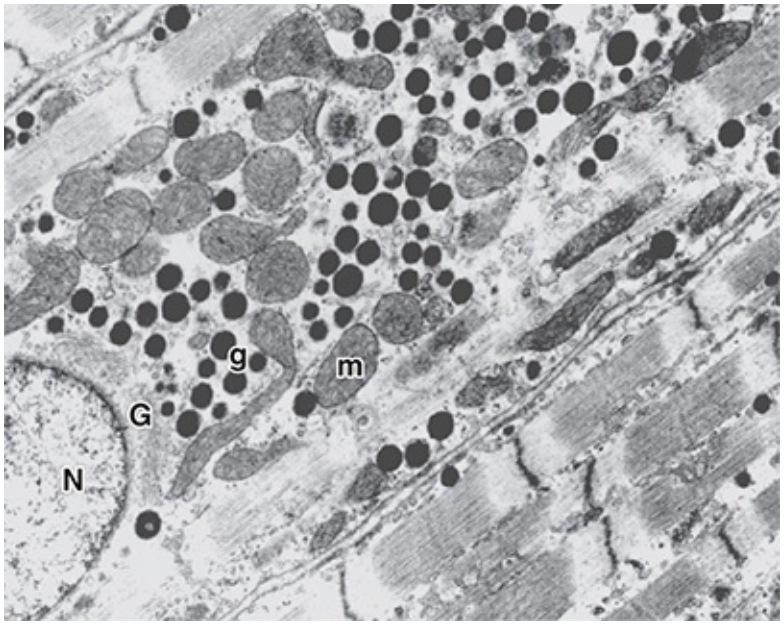


FIGURE 38–9 ANP granules (g) interspersed between mitochondria (m) in rat atrial muscle cell. G, Golgi complex; N, nucleus. The granules in human atrial cells are similar (× 17,640). (Used with permission of M Cantin.)

The first natriuretic hormone isolated from the heart was **ANP,** a polypeptide with a characteristic 17-amino-acid ring formed by a disulfide bond between two cysteines. The circulating form of this polypeptide has 28 amino acid residues. It is formed from a large precursor molecule that contains 151 amino acid residues, including a 24-amino-acid signal peptide. ANP was subsequently isolated from other tissues, including the brain, where it exists in two forms that are smaller than circulating ANP. A second natriuretic polypeptide was isolated from porcine brain and named **B-type natriuretic peptide** (also known as **brain natriuretic peptide**). It is also present in the brain in humans, but more is present in the human heart, including the ventricles. The circulating form of this hormone contains 32 amino acid residues. It has the same 17-member ring as ANP, though some of the amino acid residues in the ring are different. A third member of this family has been named **C-type natriuretic peptide (CNP)** because it was the third in the sequence to be isolated. It contains 22 amino acid residues, and there is also a larger 53-amino-acid form. CNP is present in the brain, the pituitary, the kidneys, and vascular endothelial cells. However, very little is present in the heart and the circulation, and it appears to be primarily a paracrine mediator.

CLINICAL BOX 38–3

Role of Renin in Clinical Hypertension

Constriction of one renal artery causes a prompt increase in renin secretion and the development of sustained hypertension **(renal** or **Goldblatt hypertension).** Removal of the ischemic kidney or the arterial constriction cures the hypertension if it has not persisted too long. In general, the hypertension produced by constricting one renal artery with the other kidney intact (one-clip, two-kidney Goldblatt hypertension) is associated with increased circulating renin. The clinical counterpart of this condition is **renal hypertension** due to atheromatous narrowing of one renal artery or other abnormalities of the renal circulation. However, plasma-renin activity is usually normal in one-clip one-kidney Goldblatt hypertension. The explanation of the hypertension in this situation is unsettled. However, many patients with hypertension respond to treatment with ACE inhibitors or losartan even when their renal circulation appears to be normal and they have normal or even low plasma-renin activity.

ACTIONS

ANP and BNP in the circulation act on the kidneys to increase Na^+ excretion and injected CNP has a similar effect. They appear to produce this effect by dilating afferent arterioles and relaxing mesangial cells. Both of these actions increase glomerular filtration (see Chapter 37). In addition, they act on the renal tubules to inhibit Na^+ reabsorption (see Chapter 37). Other actions include an increase in capillary permeability, leading to extravasation of fluid and a decline in blood pressure. In addition, they relax vascular smooth muscle in arterioles and venules. CNP has a greater dilator effect on veins than ANP and BNP. These peptides also inhibit renin secretion and counteract the pressor effects of catecholamines and angiotensin II.

In the brain, ANP is present in neurons, and an ANP-containing neural pathway projects from the anteromedial part of the hypothalamus to the areas in the lower brainstem that are concerned with neural regulation of the cardiovascular system. In general, the effects of ANP in the brain are opposite to those of angiotensin II, and ANP-containing neural circuits appear to be involved in counteracting the effects of vasopressin and angiotensin II,

promoting natriuresis.

NATRIURETIC PEPTIDE RECEPTORS

Three different natriuretic peptide receptors (NPRs) have been isolated and characterized. The NPR-A and NPR-B receptors both span the cell membrane and have cytoplasmic domains that are guanylyl cyclases. ANP has the greatest affinity for the NPR-A receptor, and CNP has the greatest affinity for the NPR-B receptor. The third receptor, NPR-C, binds all three natriuretic peptides but has a markedly truncated cytoplasmic domain. Some evidence suggests that it acts via G-proteins to activate phospholipase C and inhibit adenylyl cyclase. However, it has also been argued that this receptor does not trigger any intracellular change and is instead a **clearance receptor** that removes natriuretic peptides from the bloodstream and then releases them later, helping to maintain a steady blood level of the hormones.

SECRETION & METABOLISM

The concentration of ANP in plasma is about 5 fmol/mL in normal humans ingesting moderate amounts of NaCl. ANP secretion is increased when the ECF volume is increased by infusion of isotonic saline and when the atria are stretched. BNP secretion is increased when the ventricles are stretched. ANP secretion is also increased by immersion in water up to the neck **(Figure 38–10)**, a procedure that counteracts the effect of gravity on the circulation and increases central venous and consequently atrial pressure. Note that immersion also decreases the secretion of renin and aldosterone. Conversely, a small but measurable decrease in plasma ANP occurs in association with a decrease in central venous pressure on rising from the supine to the standing position. Thus, it seems clear that the atria respond directly to stretch in vivo and that the rate of ANP secretion is proportional to the degree to which the atria are stretched by increases in central venous pressure. Similarly, BNP secretion is proportional to the degree to which the ventricles are stretched. Plasma levels of both hormones are elevated in heart failure, and their measurement is being used increasingly in the diagnosis of this condition.

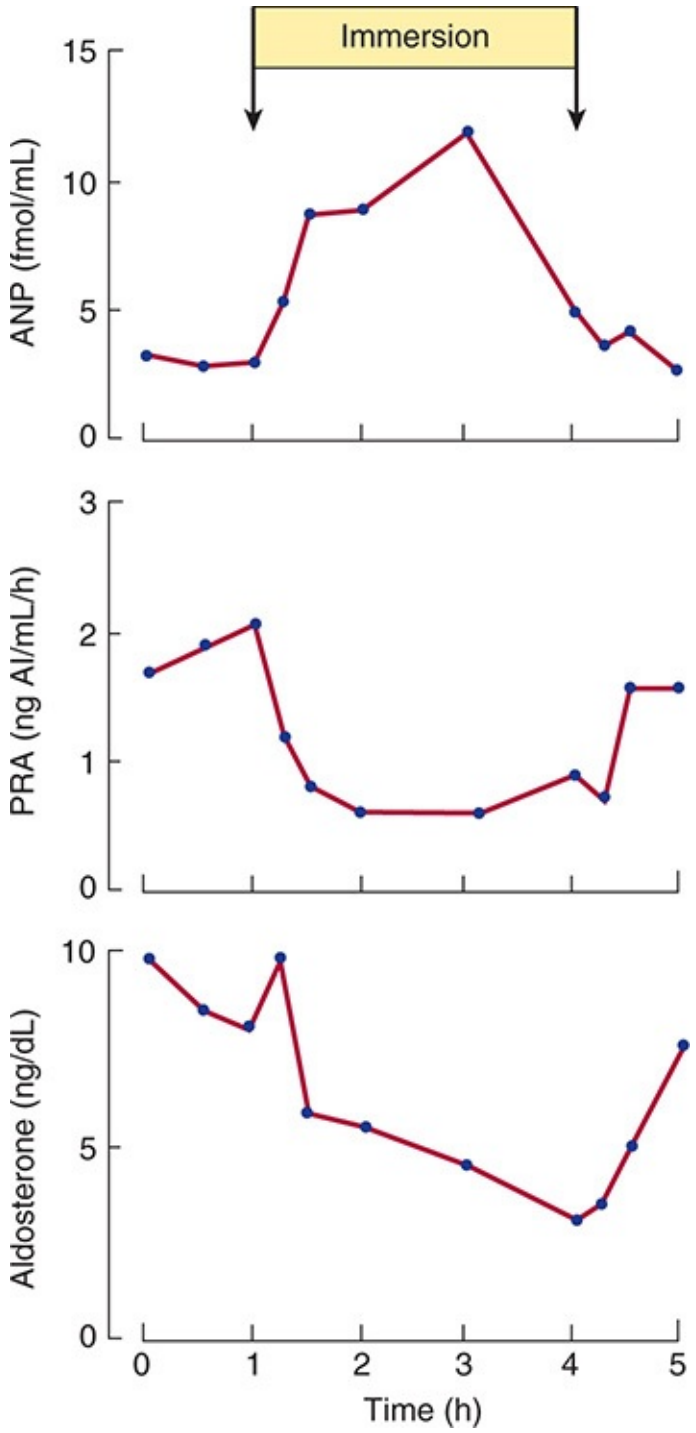


FIGURE 38–10 Effect of immersion in water up to the neck for 3 h on plasma concentrations of ANP, PRA, and aldosterone. ANP, atrial natriuretic peptide; PRA, plasma renin activity. (Reproduced with permission from Epstein M, et al: Increases in circulating atrial natriuretic factor during immersion-induced central hypervolaemia in normal humans. J Hypertension Suppl 1986 June; 4(2):S93–S99.))

Circulating ANP has a short half-life. It is metabolized by neutral endopeptidase (NEP), which is inhibited by thiorphan. Therefore, administration of thiorphan increases circulating ANP.

NA, K ATPase-INHIBITING FACTOR

Another natriuretic factor is present in blood. This factor produces natriuresis by inhibiting Na, K ATPase and raises rather than lowers blood pressure. Current evidence indicates that it may well be the digitalis-like steroid **ouabain** and that it comes from the adrenal glands. However, its physiologic significance is not yet known.

DEFENSE OF SPECIFIC IONIC COMPOSITION

Special regulatory mechanisms maintain the levels of certain specific ions in the ECF as well as the levels of glucose and other nonionized substances important in metabolism (see Chapter 1). The feedback of Ca^{2+} on the parathyroids and the calcitonin-secreting cells to adjust their secretion maintains the ionized calcium level of the ECF (see Chapter 21). The Mg^{2+} concentration is subject to close regulation, but the mechanisms controlling Mg^{+} homeostasis are incompletely understood.

The mechanisms controlling Na^{+} and K^{+} content are part of those determining the volume and tonicity of ECF and have been discussed above. The levels of these ions are also dependent on the H^{+} concentration, and pH is one of the major factors affecting the anion composition of ECF. This will be discussed in Chapter 39.

ERYTHROPOIETIN

STRUCTURE & FUNCTION

When an individual bleeds or becomes hypoxic, hemoglobin synthesis is enhanced, and production and release of red blood cells from the bone marrow **(erythropoiesis)** are increased (see Chapter 31). Conversely, when the red cell volume is increased above normal levels by transfusion, the erythropoietic activity of the bone marrow decreases. These adjustments are brought about by changes in the circulating level of **erythropoietin,** a circulating glycoprotein that

contains 165 amino acid residues and four oligosaccharide chains that are necessary for its activity in vivo. Its blood level is markedly increased in anemia **(Figure 38–11)**.

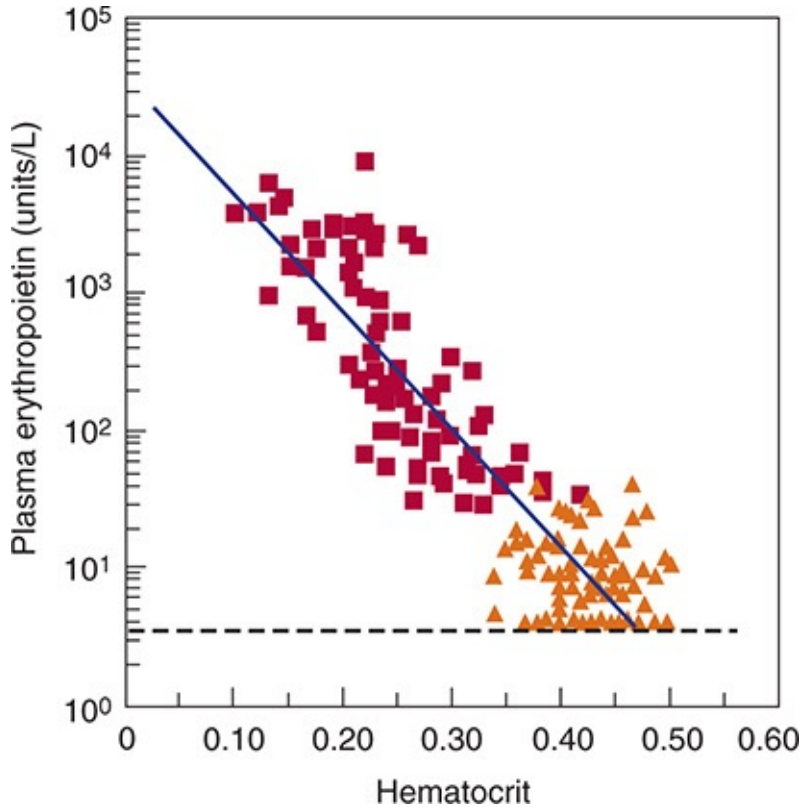


FIGURE 38–11 Plasma erythropoietin levels in normal blood donors (triangles) and patients with various forms of anemia (squares). (Reproduced with permission from Erslev AJ: Erythropoietin. N Engl J Med 1991; May 9; 324(19):1339–1344.)

Erythropoietin increases the number of erythropoietin-sensitive committed stem cells in the bone marrow that are converted to red blood cell precursors and subsequently to mature erythrocytes (see Chapter 31). The receptor for erythropoietin is a linear protein with a single transmembrane domain that is a member of the cytokine receptor superfamily (see Chapter 3). The receptor has tyrosine kinase activity, and it activates a cascade of serine and threonine kinases, resulting in inhibited apoptosis of red cells and their increased growth and development.

The principal site of inactivation of erythropoietin is the liver, and the hormone has a half-life in the circulation of about 5 h. However, the increase in circulating red cells that it triggers takes 2–3 days to appear, since red cell

maturation is a relatively slow process.

SOURCES

In adults, about 85% of the erythropoietin comes from the kidneys and 15% from the liver. Both these organs contain the mRNA for erythropoietin. Erythropoietin can also be extracted from the spleen and salivary glands, but these tissues do not contain its mRNA and consequently do not appear to manufacture the hormone. When renal mass is reduced in adults by kidney disease or nephrectomy, the liver cannot compensate and anemia develops.

Erythropoietin is produced by interstitial cells in the peritubular capillary bed of the kidneys and by perivenous hepatocytes in the liver. It is also produced in the brain, where it exerts a protective effect against excitotoxic damage triggered by hypoxia; and in the uterus and oviducts, where it is induced by estrogen and appears to mediate estrogen-dependent angiogenesis.

The gene for the hormone has been cloned, and recombinant erythropoietin produced in animal cells is available for clinical use as epoetin alfa. The recombinant erythropoietin is of value in the treatment of the anemia associated with kidney failure; 90% of the patients with end-stage renal disease who are undergoing dialysis are anemic as a result of erythropoietin deficiency. Erythropoietin is also used to stimulate red cell production in individuals who are banking a supply of their own blood in preparation for autologous transfusions during elective surgery (see Chapter 31).

REGULATION OF SECRETION

The usual stimulus for erythropoietin secretion is hypoxia, but secretion of the hormone can also be stimulated by cobalt salts and androgens. Recent evidence suggests that the O_2 sensor regulating erythropoietin secretion in the kidneys and the liver is a heme protein that in the deoxy form stimulates and in the oxy form inhibits transcription of the erythropoietin gene to form erythropoietin mRNA. Secretion of the hormone is also facilitated by the alkalosis that develops at high altitudes. Like renin secretion, erythropoietin secretion is facilitated by catecholamines via a β-adrenergic mechanism, although the renin–angiotensin system is totally separate from the erythropoietin system.

CHAPTER SUMMARY

- Total body osmolality is directly proportional to the total body sodium plus the total body potassium divided by the total body water. Changes in the osmolality of the body fluids occur when a disproportion exists between the amount of these electrolytes and the amount of water ingested or lost from the body.
- Vasopressin's main physiologic effect is the retention of water by the kidney by increasing the water permeability of the renal collecting ducts. Water is absorbed from the urine, the urine becomes concentrated, and its volume decreases.
- Vasopressin is stored in the posterior pituitary and released into the bloodstream in response to the stimulation of osmoreceptors or baroreceptors. Increases in secretion occur when osmolality is changed as little as 1%, thus keeping the osmolality of the plasma very close to 285 mOsm/L.
- The amount of Na^+ in the ECF is the most important determinant of ECF volume, and mechanisms that control Na^+ balance are the major mechanisms defending ECF volume. The main mechanism regulating sodium balance is the renin–angiotensin system, a hormone system that regulates blood pressure.
- The kidneys secrete the enzyme renin and renin acts in concert with angiotensin-converting enzyme to form angiotensin II. Angiotensin II acts directly on the adrenal cortex to increase the secretion of aldosterone. Aldosterone increases the retention of sodium from the urine via action on the renal collecting duct.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. Dehydration increases the plasma concentration of all the following hormones *except*

   A. vasopressin.

   B. angiotensin II.

   C. aldosterone.

   D. norepinephrine.

E. atrial natriuretic peptide.

2. In a patient who has become dehydrated, body water should be replaced by intravenous infusion of

A. distilled water.

B. 0.9% sodium chloride solution.

C. 5% glucose solution.

D. hyperoncotic albumin.

E. 10% glucose solution.

3. Renin is secreted by

A. cells in the macula densa.

B. cells in the proximal tubules.

C. cells in the distal tubules.

D. granular cells in the juxtaglomerular apparatus.

E. cells in the peritubular capillary bed.

4. Erythropoietin is secreted by

A. cells in the macula densa.

B. cells in the proximal tubules.

C. cells in the distal tubules.

D. granular cells in the juxtaglomerular apparatus.

E. cells in the peritubular capillary bed.

5. When a woman who has been on a low-sodium diet for 8 days is given an intravenous injection of captopril, a drug that inhibits angiotensin-converting enzyme, which of the following would be expected?

A. Blood pressure to rise because cardiac output would fall

B. Blood pressure to rise because peripheral resistance would fall

C. Blood pressure to fall because cardiac output would fall

D. Blood pressure to fall because peripheral resistance would fall

E. Plasma renin activity to fall because circulating angiotensin I level would rise

6. Which of the following would *not* be expected to increase renin secretion?

A. Administration of a drug that blocks angiotensin-converting enzyme

B. Administration of a drug that blocks AT1 receptors

C. Administration of a drug that blocks β-adrenergic receptors

D. Constriction of the aorta between the celiac artery and the renal arteries

E. Administration of a drug that reduces ECF volume

7. Which of the following is *least* likely to contribute to the beneficial effects of angiotensin-converting enzyme inhibitors in the treatment of heart failure?

A. Vasodilation

B. Decreased cardiac growth

C. Decreased cardiac afterload

D. Increased plasma renin activity

E. Decreased plasma aldosterone

CHAPTER 39

Acidification of the Urine & Bicarbonate Excretion

OBJECTIVES

After studying this chapter, you should be able to:

- Explain the three processes involved in the secretion of H^+ into the tubules and discuss the significance of these processes in the regulation of acid-base balance.
- Define acidosis and alkalosis, and give (in mEq/L and pH) the normal mean and the range of H^+ concentrations in blood that are compatible with health.
- List the principal buffers in blood, interstitial fluid, and intracellular fluid, and, using the Henderson-Hasselbalch equation, describe what is unique about the bicarbonate buffer system.
- Define the changes in blood chemistry that occur during metabolic acidosis and metabolic alkalosis. Explain the integration of respiratory and renal compensations for these conditions.
- Define the changes in blood chemistry that occur during respiratory acidosis and respiratory alkalosis, and how the kidneys compensate in these conditions.

INTRODUCTION

The kidneys play a key role in the maintenance of acid-base balance and to do this they must excrete acid in the amount equivalent to the production of nonvolatile acids in the body. The production of nonvolatile acids will vary with diet, metabolism, and disease. The kidneys must also filter and reabsorb plasma bicarbonate, and thus prevent the loss of bicarbonate in the urine. Both processes are linked physiologically, due to the nephron's ability to secrete H^+ ions into the filtrate.

RENAL H^+ SECRETION

The cells of the proximal and distal tubules, like the cells of the gastric glands (see Chapter 25), secrete hydrogen ions. Hydrogen secretion also occurs in the collecting ducts. The transporter that is responsible for H^+ secretion in the proximal tubules is the Na–H exchanger (primarily NHE3) **(Figure 39–1)**. This is an example of secondary active transport; Na^+ is moved from the inside of the cell to the interstitium by Na, K ATPase on the basolateral membrane, which keeps intracellular Na^+ low, thus establishing the drive for Na^+ to enter the cell, via the Na–H exchanger, from the tubular lumen. The Na–H exchanger secretes H^+ into the lumen in exchange for Na^+.

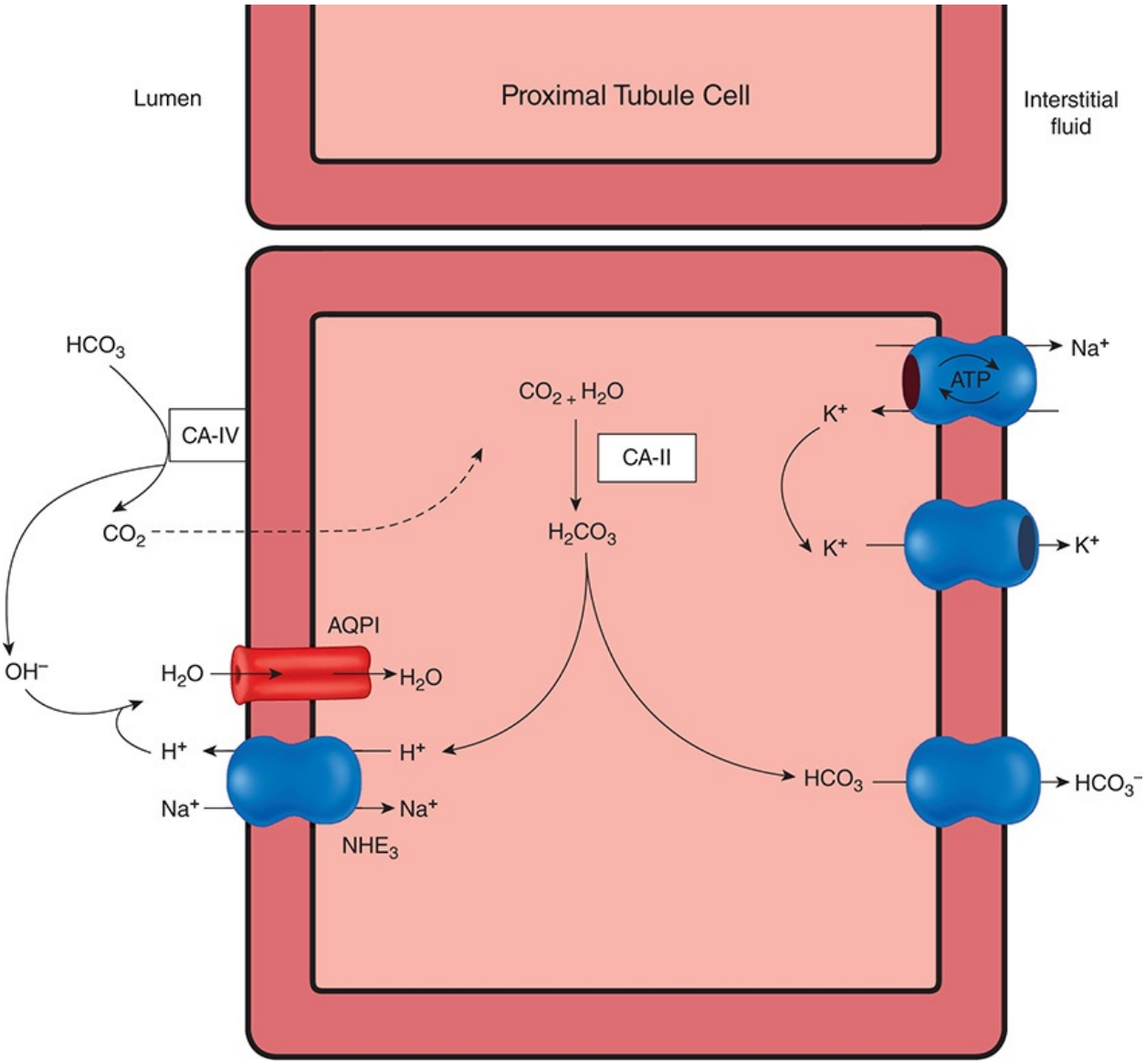


FIGURE 39–1 Secretion of acid and reabsorption of filtered bicarbonate by proximal tubular cells in the kidney. H^+ is transported into the tubular lumen by NHE_3 in exchange for Na^+. Active transport by Na, K ATPase is indicated by arrows. Dashed arrows indicate diffusion.

The secreted H^+ ion combines with filtered HCO_3^- to form H_2CO_3 and the presence of **carbonic anhydrase** on the apical membrane of the proximal tubule catalyzes the formation of H_2O and CO_2 from H_2CO_3. The apical membrane of epithelial cells lining the proximal tubule is permeable to CO_2 and H_2O, and they enter the tubule rapidly. Eighty percent of the filtered load of HCO_3^- is reabsorbed in the proximal tubule.

Inside the cell, carbonic anhydrase is also present and can catalyze the formation of H_2CO_3 from CO_2 and H_2O. H_2CO_3 dissociates into H^+ ions and HCO_3^-; the H^+ is secreted into the tubular lumen, as mentioned above, and the HCO_3^- that is formed diffuses into the interstitial fluid. Thus, for each H^+ ion secreted, one Na^+ ion and one HCO_3^- ion enter the interstitial fluid. Because carbonic anhydrase catalyzes the formation of H_2CO_3, drugs that inhibit carbonic anhydrase depress both secretion of acid by the proximal tubules and the reactions that depend on it.

Some evidence suggests that H^+ is secreted in the proximal tubules by other types of transporters, but the evidence for these additional transporters is controversial, and in any case, their contribution is small relative to that of the Na–H exchanger mechanism.

This is in contrast to what occurs in the distal tubules and collecting ducts, where H^+ secretion is relatively independent of Na^+ in the tubular lumen. In this part of the tubule, most H^+ is secreted by an ATP-driven proton pump. Aldosterone acts on this pump to increase distal H^+ secretion. The I cells in this part of the renal tubule secrete acid and, like the parietal cells in the stomach, contain abundant carbonic anhydrase and numerous tubulovesicular structures. There is evidence that the H^+-translocating ATPase that produces H^+ secretion is located in these vesicles as well as in the apical cell membrane and that, in acidosis, the number of H^+ pumps is increased by insertion of these tubulovesicles into the apical cell membrane. Some of the H^+ is additionally secreted by $H–K^+$ ATPase. The I cells also contain **anion exchanger 1 (AE1,** formerly known as **Band 3**), an anion exchange protein, in their basolateral cell membranes. This protein may function as a Cl/HCO_3 exchanger for the transport of HCO_3^- to the interstitial fluid.

FATE OF H^+ IN THE URINE

The amount of acid secreted depends on the subsequent events that modify the composition of the tubular urine. The maximal H^+ gradient against which the transport mechanisms can secrete in humans corresponds to a urine pH of about 4.5; that is, an H^+ concentration in the urine that is 1000 times the concentration in plasma. pH 4.5 is thus the **limiting pH.** This is normally reached in the collecting ducts. If there were no buffers that “tied up” H^+ in the urine, this pH

would be reached rapidly, and H^+ secretion would stop. However, three important reactions in the tubular fluid remove free H^+, permitting more acid to be secreted **(Figure 39–2)**. These are the reactions of H^+ with HCO_3^- to form CO_2 and H_2O (discussed above), with HPO_4^{2-} to form $H_2PO_4^-$ (titratable acids), and with NH_3 to form NH_4^+.

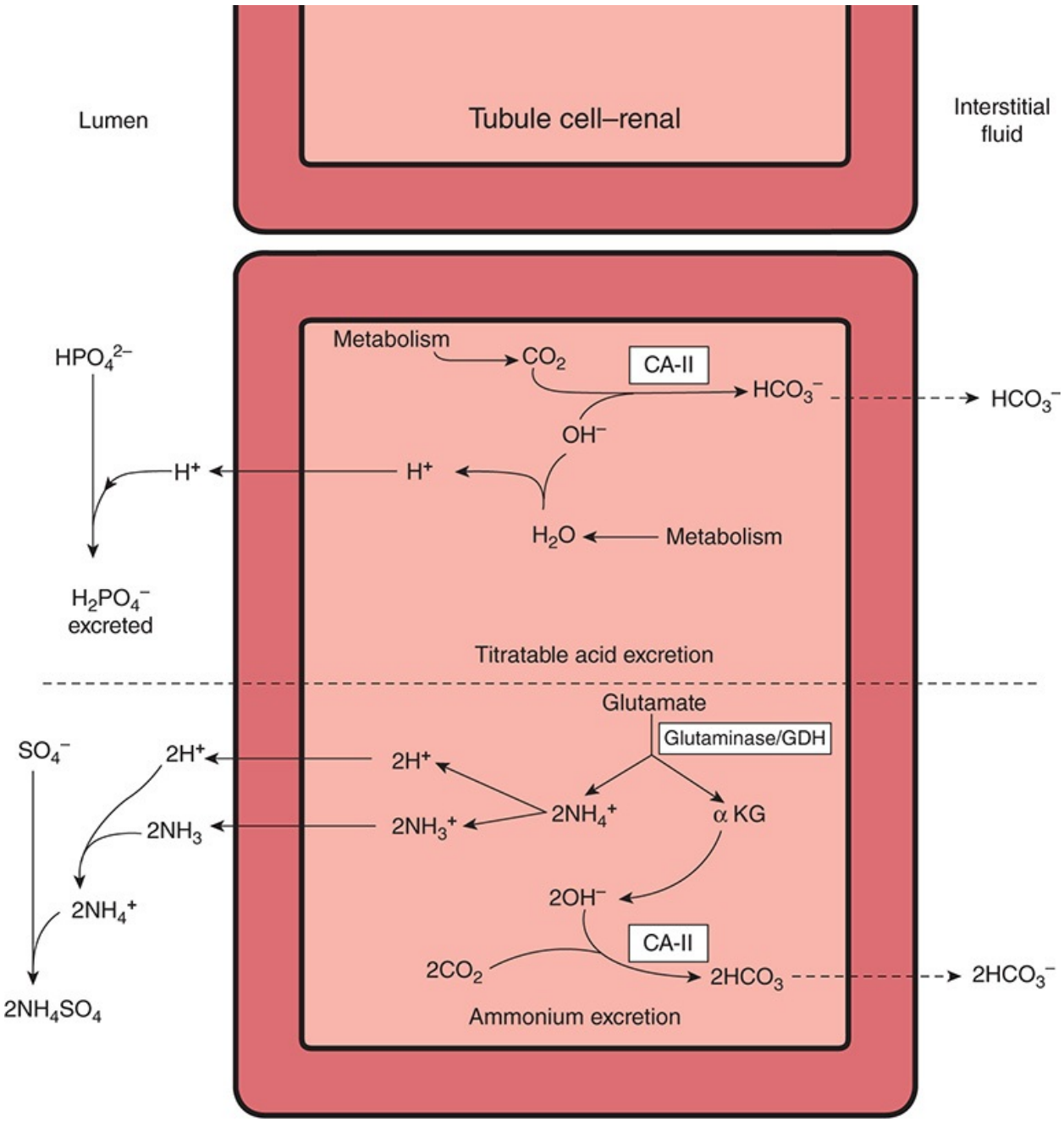


FIGURE 39–2 Titratable acid and ammonium formation. Top: Formation of

monobasic phosphate. **Bottom:** Ammonium formation. Note that in each instance one Na^+ ion and one HCO_3^- ion enter the bloodstream for each H^+ ion secreted.

REACTION WITH BUFFERS

The three buffers of importance in the renal handling of acid and its secretion into the lumen are thus bicarbonate, dibasic phosphate, and ammonia. On an average diet, approximately 40% of nonvolatile acids (about 30 mEq/day), produced by the body in the course of various metabolic reactions is excreted as **titratable acid** (ie, phosphate system) and 60% of nonvolatile acid (about 50 mEq/day) is excreted as NH_4^+. The pK′ of the bicarbonate system is 6.1, that of the dibasic phosphate system is 6.8, and that of the ammonia system is 9.0. The concentration of HCO_3^- in the plasma, and consequently in the glomerular filtrate, is normally about 24 mEq/L, whereas that of phosphate is only 1.5 mEq/L. Therefore, in the proximal tubule, most of the secreted H^+ reacts with HCO_3^- as described above, to form H_2CO_3 (Figure 39–2) and this enters the cell as CO_2 and H_2O following the action of carbonic anhydrase in the brush border of the proximal tubule cells. The CO_2 entering the tubular cells adds to the pool of CO_2 available to form H_2CO_3. Because most of the H^+ is removed from the tubule, the pH of the fluid is changed very little. This is the mechanism by which HCO_3^- is reabsorbed; for each mole of HCO_3^- removed from the tubular fluid, 1 mol of HCO_3^- diffuses from the tubular cells into the blood, although it is important to note that it is not the same mole that disappeared from the tubular fluid. About 4500 mEq of HCO_3^- are filtered and reabsorbed each day.

Secreted H^+ also reacts with dibasic phosphate (HPO_4^{2-}) to form monobasic phosphate ($H_2PO_4^-$). This happens to the greatest extent in the distal tubules and collecting ducts, because it is here that the phosphate that escapes proximal reabsorption is greatly concentrated by the reabsorption of water. H^+ ions are also known to combine to a minor degree with other buffer anions.

The ammonia buffering system allows secreted H^+ to combine with NH_3, and this occurs in the proximal tubule (where NH_3 is made, see below) and in the distal tubules. The pK′ of the ammonia system is 9.0, and the ammonia system is titrated only from the pH of the urine to pH 7.4, so it contributes very little to the

titratable acidity. Each H^+ ion that reacts with the buffers contributes to the urinary **titratable acidity,** which is measured by determining the amount of alkali that must be added to the urine to return its pH to 7.4, the pH of the glomerular filtrate. However, the titratable acidity obviously measures only a fraction of the acid secreted, since it does not account for the H_2CO_3 that has been converted to H_2O and CO_2.

The reabsorption of HCO_3^- is crucial to the maintenance of acid-base balance, as a loss of a single HCO_3^- ion in the urine would be the equivalent of adding a H^+ ion to the blood. However, the kidneys have the ability to replenish the body with new bicarbonate ions. This occurs when H^+ ions are removed from the body as NH_4^+ or titratable acid, as there is formation of new bicarbonate within the cells, and this enters the blood (ie, these bicarbonate ions are not those originally filtered, and yet they still enter the blood).

AMMONIA SECRETION

As mentioned above, reactions in the renal tubular cells produce NH_4^+ and HCO_3^-. NH_4^+ is in equilibrium with NH_3 and H^+ in the cells. Because the pK′ of this reaction is 9.0, the ratio of NH_3 to NH_4^+ at pH 7.0 is 1:100 **(Figure 39–3)**. However, NH_3 is lipid-soluble and diffuses across the cell membranes down its concentration gradient into the interstitial fluid and tubular urine. In the urine it reacts with H^+ to form NH_4^+, and the NH_4^+ remains "trapped" in the urine.

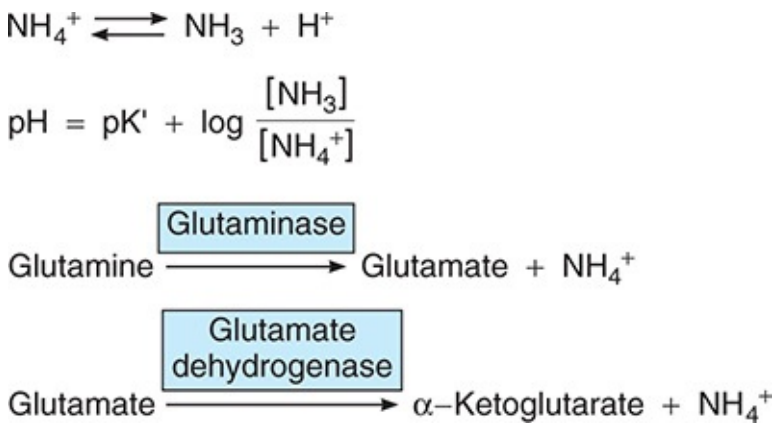


FIGURE 39–3 Major reactions involved in ammonia production in the kidneys.

The principal reaction producing NH_4^+ in cells is conversion of glutamine to glutamate. This reaction is catalyzed by the enzyme **glutaminase,** which is abundant in renal tubular cells (Figure 39–3). **Glutamate dehydrogenase** catalyzes the conversion of glutamate to α-ketoglutarate, with the production of more NH_4^+. Subsequent metabolism of α-ketoglutarate utilizes $2H^+$, freeing $2HCO_3^-$.

In chronic acidosis, the amount of NH_4^+ excreted at any given urine pH also increases, because more NH_3 enters the tubular urine. The effect of this **adaptation** of NH_3 secretion, the cause of which is unsettled, is further removal of H^+ from the tubular fluid and consequently a further enhancement of H^+ secretion by the renal tubules and excretion in the urine. Because the amount of phosphate buffer filtered at the glomerulus cannot be increased, urinary excretion of acid via the phosphate buffer system is limited. The production of NH_4^+ by the renal tubules is the only way the kidneys can remove even the normal amount, much less an increased amount, of nonvolatile acid produced in the body.

In the inner medullary cells of the collecting duct, the main process by which NH_3 is secreted into the urine and then changed to NH_4^+ is called **nonionic diffusion** (see Chapter 2), thereby maintaining the concentration gradient for diffusion of NH_3. In the proximal tubule, nonionic diffusion of NH_4^+ is less important because NH_4^+ can be secreted into the lumen, often by replacing H^+ on the Na–H exchanger.

Salicylates and a number of other drugs that are weak bases or weak acids are also secreted by nonionic diffusion. They diffuse into the tubular fluid at a rate that depends on the pH of the urine, so the amount of each drug excreted varies with the pH of the urine.

pH CHANGES ALONG THE NEPHRON

A moderate drop in pH occurs in the proximal tubular fluid, but, as noted above, most of the secreted H^+ has little effect on luminal pH because of the formation of CO_2 and H_2O from H_2CO_3. In contrast, the distal tubule has less capacity to secrete H^+, but secretion in this segment has a greater effect on urinary pH.

FACTORS AFFECTING ACID SECRETION

Renal acid secretion is altered by changes in the intracellular PCO_2, K^+ concentration, carbonic anhydrase level, and adrenocortical hormone concentration. When the PCO_2 is high **(respiratory acidosis),** more intracellular H_2CO_3 is available to buffer the hydroxyl ions and acid secretion is enhanced, whereas the reverse is true when the PCO_2 falls. K^+ depletion enhances acid secretion, apparently because the loss of K^+ causes intracellular acidosis even though the plasma pH may be elevated. Conversely, K^+ excess in the cells inhibits acid secretion. When carbonic anhydrase is inhibited, acid secretion is inhibited because the formation of H_2CO_3 is decreased. Aldosterone and the other adrenocortical steroids that enhance tubular reabsorption of Na^+ also increase the secretion of H^+ and K^+.

BICARBONATE EXCRETION

Although the process of HCO_3^- reabsorption does not involve actual transport of this ion into the tubular cells, HCO_3^- reabsorption is proportional to the amount filtered over a relatively wide range. There is no demonstrable Tm, but HCO_3^- reabsorption is decreased by an unknown mechanism when the extracellular fluid (ECF) volume is expanded **(Figure 39–4)**. When the plasma HCO_3^- concentration is low, all the filtered HCO_3^- is reabsorbed; but when the plasma HCO_3^- concentration is high; that is, above 26–28 mEq/L (the renal threshold for HCO_3^-), HCO_3^- appears in the urine and the urine becomes alkaline. Conversely, when the plasma HCO_3^- falls below about 26 mEq/L, the value at which all the secreted H^+ is being used to reabsorb HCO_3^-, more H^+ becomes available to combine with other buffer anions. Therefore, the lower the plasma HCO_3^- concentration drops, the more acidic the urine becomes and the greater its NH_4^+ content **(Clinical Box 39–1)**.

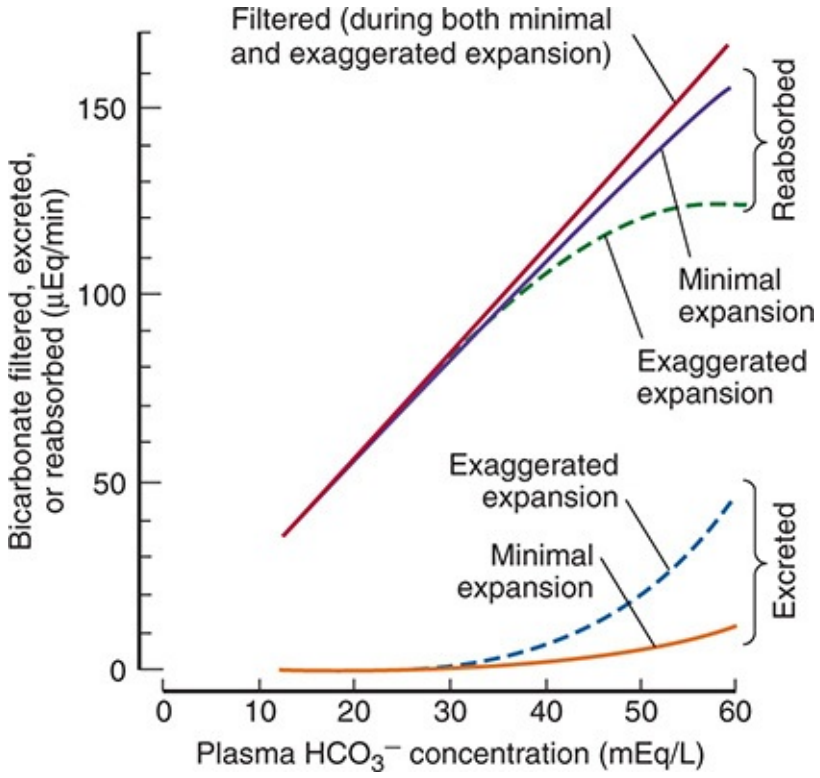


FIGURE 39–4 Effect of ECF volume on HCO_3^- filtration, reabsorption, and excretion in rats. The pattern of HCO_3^- excretion is similar in humans. The plasma HCO_3^- concentration is normally about 24 mEq/L. ECF, extracellular fluid. (Reproduced with permission from Valtin H: *Renal Function*, 2nd ed. Little, Brown; 1983.)

DEFENSE OF H^+ CONCENTRATION

The mystique that envelopes the subject of acid-base balance makes it necessary to point out that the core of the problem is not "buffer base" or "fixed cation" or the like, but simply the maintenance of the H^+ concentration of the ECF. The mechanisms regulating the composition of the ECF are particularly important as far as this specific ion is concerned, because the machinery of the cells is very sensitive to changes in H^+ concentration. Intracellular H^+ concentration, which can be measured by using microelectrodes, pH-sensitive fluorescent dyes, and phosphorus magnetic resonance, is distinct from extracellular pH and appears to be regulated by a variety of intracellular processes. However, it is sensitive to changes in ECF H^+ concentration.

The pH notation is a useful means of expressing H^+ concentrations in the

body, because the H^+ concentrations are very low relative to those of other cations. Thus, the normal Na^+ concentration of arterial plasma that has been equilibrated with red blood cells is about 140 mEq/L, whereas the H^+ concentration is 0.00004 mEq/L **(Table 39–1)**. The pH, the negative logarithm of 0.00004, is therefore 7.4. Of course, a decrease in pH of 1 unit, for example, from 7.0 to 6.0, represents a 10-fold increase in H^+ concentration. It is important to remember that the pH of blood is the pH of **true plasma**—plasma that has been in equilibrium with red cells—because the red cells contain hemoglobin, which is quantitatively one of the most important blood buffers (see Chapter 36).

TABLE 39–1 H^+ concentration and pH of body fluids.

		H^+ Concentration		
		mEq/L	mol/L	pH
Gastric HCl		150	0.15	0.8
Maximal urine acidity		0.03	3×10^{-5}	4.5
Plasma	Extreme acidosis	0.0001	1×10^{-7}	7.0
	Normal	0.00004	4×10^{-8}	7.4
	Extreme alkalosis	0.00002	2×10^{-8}	7.7
Pancreatic juice		0.00001	1×10^{-8}	8.0

CLINICAL BOX 39–1

Implications of Urinary pH Changes

Depending on the rates of the interrelated processes of acid secretion, NH_4^+ production, and HCO_3^- excretion, the pH of the urine in humans varies from 4.5 to 8.0. Excretion of urine that is at a pH different from that of the body fluids has important implications for the body's electrolyte and acid–base economy. Acids are buffered in the plasma and cells, the overall reaction being $HA + NaHCO_3 \rightarrow NaA + H_2CO_3$. The H_2CO_3 forms CO_2 and H_2O, and the CO_2 is expired, while the NaA appears in the glomerular filtrate. To the extent that the Na^+ is replaced by H^+ in the urine, Na^+ is conserved in the body. Furthermore, for each H^+ ion excreted with phosphate or as NH_4^+, there

is a net gain of one HCO_3^- ion in the blood, replenishing the supply of this important buffer anion. Conversely, when base is added to the body fluids, the OH^- ions are buffered, raising the plasma HCO_3^-. When the plasma level exceeds 28 mEq/L, the urine becomes alkaline and the extra HCO_3^- is excreted in the urine. Because the rate of maximal H^+ secretion by the tubules varies directly with the arterial PCO_2. HCO_3^- reabsorption is also affected by the PCO_2. This relationship has been discussed in more detail in the text.

THERAPEUTIC HIGHLIGHTS

Sulfonamides inhibit carbonic anhydrase and sulfonamide derivatives have been used clinically as diuretics because of their inhibitory effects on carbonic anhydrase in the kidney (see Chapter 37).

H^+ BALANCE

The pH of the arterial plasma is normally 7.40 and that of venous plasma slightly lower. Technically, **acidosis** is present whenever the arterial pH is below 7.40, and **alkalosis** is present whenever it is above 7.40, although variations of up to 0.05 pH unit occur without untoward effects. The H^+ concentrations in the ECF that are compatible with life cover an approximately fivefold range, from 0.00002 mEq/L (pH 7.70) to 0.0001 mEq/L (pH 7.00).

Amino acids are utilized in the liver for gluconeogenesis, leaving NH_4^+ and HCO_3^- as products from their amino and carboxyl groups **(Figure 39–5)**. The NH_4^+ is incorporated into urea (see Chapter 28) and the protons that are formed are buffered intracellularly by HCO_3^-, so little NH_4^+ and HCO_3^- escape into the circulation. However, metabolism of sulfur-containing amino acids produces H_2SO_4, and metabolism of phosphorylated amino acids such as phosphoserine produces H_3PO_4. These strong acids enter the circulation and present a major H^+ load to the buffers in the ECF. The H^+ load from amino acid metabolism is normally about 50 mEq/day. The CO_2 formed by metabolism in the tissues is in large part hydrated to H_2CO_3 (see Chapter 36), and the total H^+ load from this source is over 12,500 mEq/day. However, most of the CO_2 is excreted in the

lungs, and only small quantities of the H^+ remain to be excreted by the kidneys. Common sources of extra acid loads are strenuous exercise (lactic acid), diabetic ketosis (acetoacetic acid and β-hydroxybutyric acid), and ingestion of acidifying salts such as NH_4Cl and $CaCl_2$, which in effect add HCl to the body. A failure of diseased kidneys to excrete normal amounts of acid is also a cause of acidosis. Fruits are the main dietary source of alkali. They contain Na^+ and K^+ salts of weak organic acids, and the anions of these salts are metabolized to CO_2, leaving $NaHCO_3$ and $KHCO_3$ in the body. $NaHCO_3$ and other alkalinizing salts are sometimes ingested in large amounts, but a more common cause of alkalosis is loss of acid from the body as a result of vomiting of gastric juice rich in HCl. This is, of course, equivalent to adding alkali to the body.

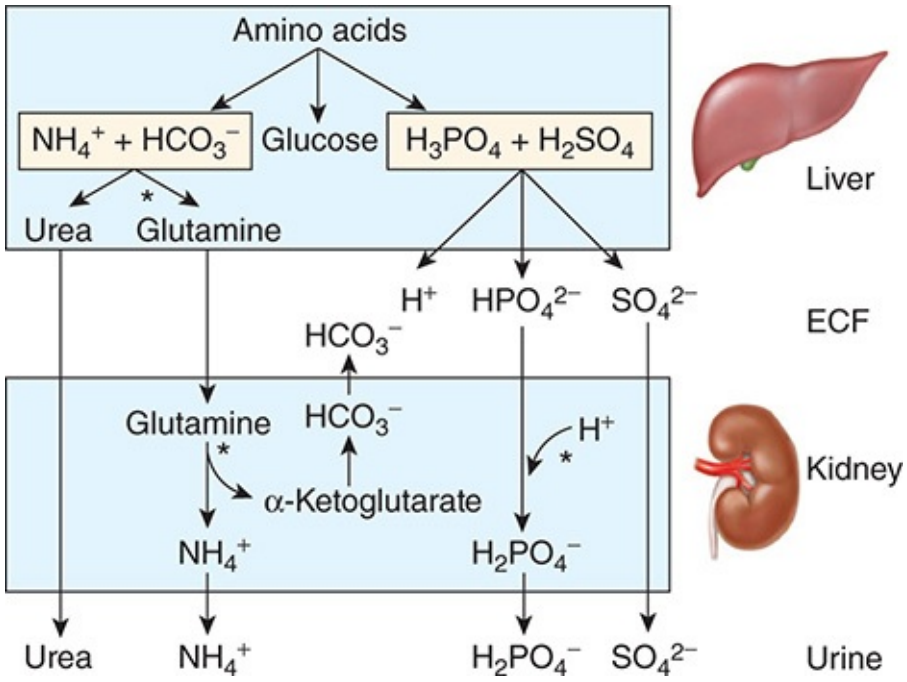


FIGURE 39–5 Role of the liver and kidneys in the handling of metabolically produced acid loads. Sites where regulation occurs are indicated by asterisks. (Modified with permission from Knepper MA, et al: Ammonium, urea, and systemic pH regulation. Am J Physiol 1987; July; 253(1 Pt 2): F199–F202.)

BUFFERING

Buffering is of key importance in maintaining H^+ homeostasis. It is defined in Chapter 1 and discussed in Chapter 36 in the context of gas transport, with an

emphasis on roles for proteins, hemoglobin, and the carbonic anhydrase system in the blood. Carbonic anhydrase is also found in high concentration in gastric acid–secreting cells (see Chapter 25) and in renal tubular cells (see Chapter 37). Carbonic anhydrase is a protein with a molecular weight of 30,000 that contains an atom of zinc in each molecule. It is inhibited by cyanide, azide, and sulfide. In vivo, buffering is, of course, not limited to the blood. The principal buffers in the blood, interstitial fluid, and intracellular fluid are listed in **Table 39–2**. The principal buffers in cerebrospinal fluid and urine are the bicarbonate and phosphate systems. In metabolic acidosis, only 15–20% of the acid load is buffered by the H_2CO_3–HCO_3^- system in the ECF, and most of the remainder is buffered in cells. In metabolic alkalosis, about 30–35% of the OH^- load is buffered in cells, whereas in respiratory acidosis and alkalosis, almost all of the buffering is intracellular.

TABLE 39–2 Principal buffers in body fluids.

Blood	$H_2CO_3 \rightleftarrows H^+ + HCO_3^-$ $HProt \rightleftarrows H^+ + Prot^-$ $HHb \rightleftarrows H^+ + Hb^-$
Interstitial fluid	$H_2CO_3 \rightleftarrows H^+ + HCO_3^-$
Intracellular fluid	$HProt \rightleftarrows H^+ + Prot^-$ $H_2PO_4^- \rightleftarrows H^+ + HPO_4^{2-}$

In animal cells, the principal regulators of intracellular pH are HCO_3^- transporters. Those characterized to date include the Cl^-–HCO_3^- exchanger **AE1,** three Na^+–HCO_3^- cotransporters, and a K^+–HCO_3^- cotransporter.

SUMMARY

When a strong acid is added to the blood, the major buffer reactions are driven to the left. The blood levels of the three "buffer anions" Hb^- (hemoglobin), $Prot^-$ (protein), and HCO_3^- consequently drop. The anions of the added acid are filtered into the renal tubules. They are accompanied ("covered") by cations, particularly Na^+, because electrochemical neutrality is maintained. By processes that have been discussed above, the tubules replace the Na^+ with H^+ and in so

doing reabsorb equimolar amounts of Na^+ and HCO_3^-, thus conserving the cations, eliminating the acid, and restoring the supply of buffer anions to normal. When CO_2 is added to the blood, similar reactions occur, except that since it is H_2CO_3 that is formed, the plasma HCO_3^- rises rather than falls.

RENAL COMPENSATION TO RESPIRATORY ACIDOSIS & ALKALOSIS

As noted in Chapter 35, a rise in arterial PCO_2 due to decreased ventilation causes **respiratory acidosis** and conversely, a decline in PCO_2 causes **respiratory alkalosis.** The initial changes shown in Figure 35–7 are those that occur independently of any compensatory mechanism; that is, they are those of **uncompensated** respiratory acidosis or alkalosis. In either situation, changes are produced in the kidneys, which then tend to **compensate** for the acidosis or alkalosis, adjusting the pH toward normal.

HCO_3^- reabsorption in the renal tubules depends not only on the filtered load of HCO_3^-, which is the product of the glomerular filtration rate and the plasma HCO_3^- level, but also on the rate of H^+ secretion by the renal tubular cells, since HCO_3^- is reabsorbed by exchange for H^+. The rate of H^+ secretion—and hence the rate of HCO_3^- reabsorption—is proportional to the arterial PCO_2, probably because the more CO_2 that is available to form H_2CO_3 in the tubular cells, the more H^+ that can be secreted. Furthermore, when the PCO_2 is high, the interior of most cells becomes more acidic. In respiratory acidosis, renal tubular H^+ secretion is therefore increased, removing H^+ from the body; and even though the plasma HCO_3^- is elevated, HCO_3^- reabsorption is increased, further raising the plasma HCO_3^-. This renal compensation for respiratory acidosis is shown graphically in the shift from acute to chronic respiratory acidosis in Figure 35–7. Cl^- excretion is increased, and plasma Cl^- falls as plasma HCO_3^- is increased. Conversely, in respiratory alkalosis, the low PCO_2 hinders renal H^+ secretion, HCO_3^- reabsorption is depressed, and HCO_3^- is excreted, further reducing the already low plasma HCO_3^- and lowering the pH toward normal.

METABOLIC ACIDOSIS

When acids stronger than Hb and the other buffer acids are added to blood, **metabolic acidosis** is produced; and when the free H^+ level falls as a result of addition of alkali or removal of acid, **metabolic alkalosis** results. Following the example from Chapter 35, if H_2SO_4 is added, the H^+ is buffered and the Hb^-, $Prot^-$, and HCO_3^- levels in plasma drop. The H_2CO_3 formed is converted to H_2O and CO_2, and the CO_2 is rapidly excreted via the lungs. This is the situation in **uncompensated** metabolic acidosis. Actually, the rise in plasma H^+ stimulates respiration, so that the PCO_2, instead of rising or remaining constant, is reduced. This **respiratory compensation** raises the pH even further. The **renal** compensatory mechanisms then bring about the excretion of the extra H^+ and return the buffer systems to normal.

RENAL COMPENSATION

The anions that replace HCO_3^- in the plasma in metabolic acidosis are filtered, each with a cation (principally Na^+), thus maintaining electrical neutrality. The renal tubular cells secrete H^+ into the tubular fluid in exchange for Na^+; and for each H^+ secreted, one Na^+ and one HCO_3^- are added to the blood. The limiting urinary pH of 4.5 would be reached rapidly and the total amount of H^+ secreted would be small if no buffers were present in the urine to "tie up" H^+. However, secreted H^+ reacts with HCO_3^- to form CO_2 and H_2O (bicarbonate reabsorption); with HPO_4^{2-} to form $H_2PO_4^-$; and with NH_3 to form NH_4^+. In this way, large amounts of H^+ can be secreted, permitting correspondingly large amounts of HCO_3^- to be returned to (in the case of bicarbonate reabsorption) or added to the depleted body stores and large numbers of the cations to be reabsorbed. It is only when the acid load is very large that cations are lost with the anions, producing diuresis and depletion of body cation stores. In chronic acidosis, glutamine synthesis in the liver is increased, using some of the NH_4^+ that usually is converted to urea (Figure 39–5), and the glutamine provides the kidneys with an additional source of NH_4^+. NH_3 secretion increases over a period of days (adaptation of NH_3 secretion), further improving the renal compensation for acidosis. In addition, the metabolism of glutamine in the kidneys produces α-

ketoglutarate, and this in turn is decarboxylated, producing HCO_3^-, which enters the bloodstream and helps buffer the acid load (Figure 39–5).

The overall reaction in blood when a strong acid such as H_2SO_4 is added is:

$$2NaHCO_3 + H_2SO_4 \rightarrow Na_2SO_4 + 2H_2CO_3$$

For each mole of H^+ added, 1 mole of $NaHCO_3$ is lost. The kidney in effect reverses the reaction:

$$Na_2SO_4 + 2\,H_2CO_3 \rightarrow 2NaHCO_3 + 2H^+ + SO_4^{2-}$$

and the H^+ and SO_4^{2-} are excreted. Of course, H_2SO_4 is not excreted as such, the H^+ appearing in the urine as titratable acidity and NH_4^+.

In metabolic acidosis, the respiratory compensation tends to inhibit the renal response in the sense that the induced drop in PCO_2 hinders acid secretion, but it also decreases the filtered load of HCO_3^- and so its net inhibitory effect is not great.

METABOLIC ALKALOSIS

In metabolic alkalosis, the plasma HCO_3^- level and pH rise **(Figure 39–6)**. The respiratory compensation is a decrease in ventilation produced by the decline in H^+ concentration, and this elevates the PCO_2. This brings the pH back toward normal while elevating the plasma HCO_3^- level still further. The magnitude of this compensation is limited by the carotid and aortic chemoreceptor mechanisms, which drive the respiratory center if any appreciable fall occurs in the arterial PO_2. In metabolic alkalosis, more renal H^+ secretion is expended in reabsorbing the increased filtered load of HCO_3^-; and if the HCO_3^- level in plasma exceeds 26–28 mEq/L, HCO_3^- appears in the urine. The rise in PCO_2 inhibits the renal compensation by facilitating acid secretion, but its effect is relatively slight.

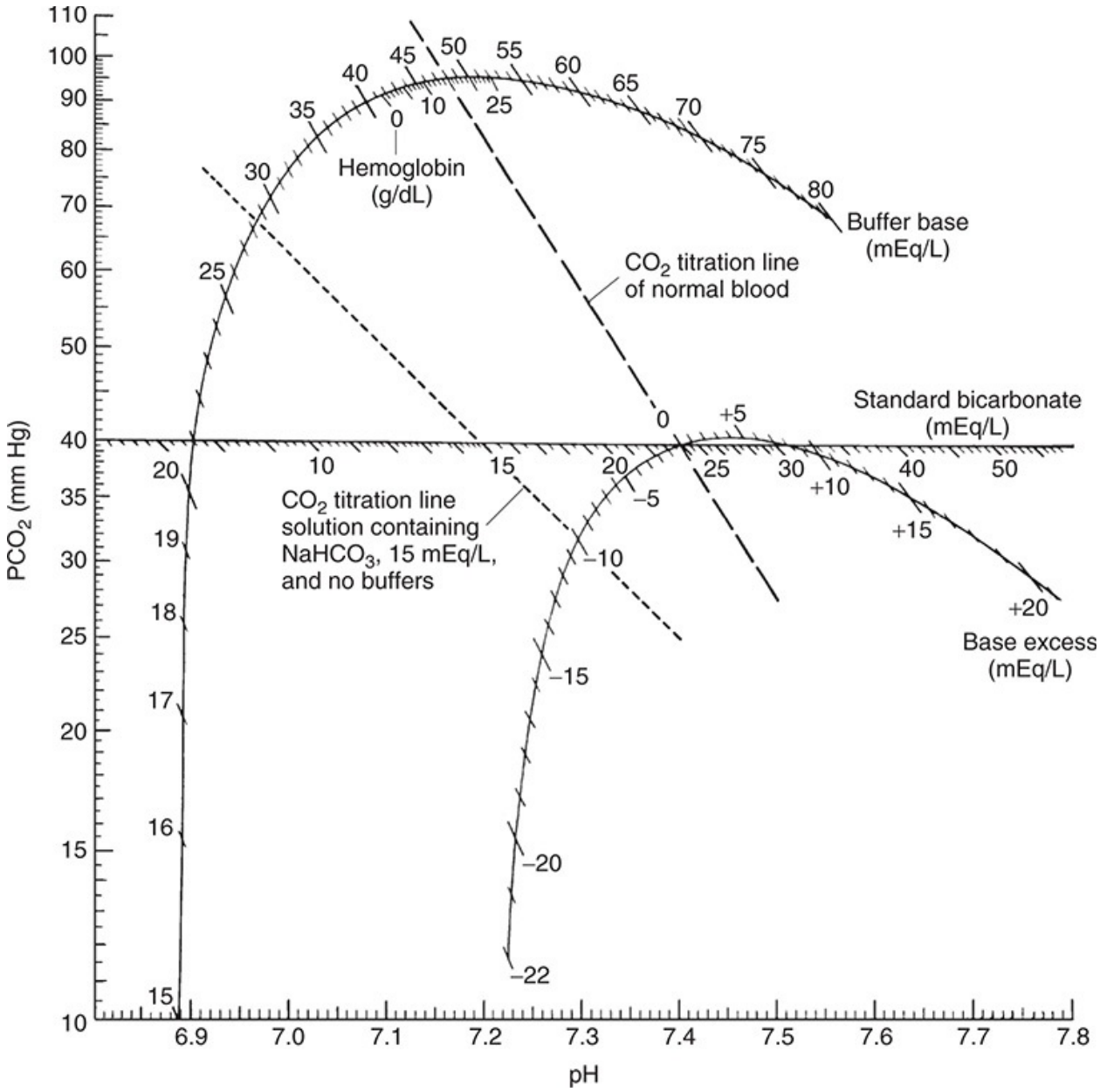


FIGURE 39–6 Siggaard–Andersen curve nomogram. (Used with permission of O Siggaard–Andersen and Radiometer, Copenhagen, Denmark.)

THE SIGGAARD–ANDERSEN CURVE NOMOGRAM

Use of the Siggaard-Andersen curve nomogram (Figure 39–6) to plot the acid–base characteristics of arterial blood is helpful in clinical situations. This nomogram has PCO_2 plotted on a log scale on the vertical axis and pH on the

horizontal axis. Thus, any point to the left of a vertical line through pH 7.40 indicates acidosis, and any point to the right indicates alkalosis. The position of the point above or below the horizontal line through a PCO_2 of 40 mm Hg defines the effective degree of hypoventilation or hyperventilation.

If a solution containing $NaHCO_3$ and no buffers were equilibrated with gas mixtures containing various amounts of CO_2, the pH and PCO_2 values at equilibrium would fall along the dashed line on the left in Figure 39–6 or a line parallel to it. If buffers were present, the slope of the line would be greater; and the greater the buffering capacity of the solution, the steeper the line. For normal blood containing 15 g of hemoglobin/dL, the CO_2 **titration line** passes through the 15-g/dL mark on the hemoglobin scale (on the underside of the upper curved scale) and the point where the PCO_2 = 40 mm Hg and pH = 7.40 lines intersect, as shown in Figure 39–6. When the hemoglobin content of the blood is low, there is significant loss of buffering capacity, and the slope of the CO_2 titration line diminishes. However, blood of course contains buffers in addition to hemoglobin, so that even the line drawn from the zero point on the hemoglobin scale through the normal PCO_2–pH intercept is steeper than the curve for a solution containing no buffers.

For clinical use, arterial blood or arterialized capillary blood is drawn anaerobically and its pH measured. The pHs of the same blood after equilibration with each of two gas mixtures containing different known amounts of CO_2 are also determined. The pH values at the known PCO_2 levels are plotted and connected to provide the CO_2 titration line for the blood sample. The pH of the blood sample before equilibration is plotted on this line, and the PCO_2 of the sample is read off the vertical scale. The **standard bicarbonate** content of the sample is indicated by the point at which the CO_2 titration line intersects the bicarbonate scale on the PCO_2 = 40 mm Hg line. The standard bicarbonate is not the actual bicarbonate concentration of the sample but, rather, what the bicarbonate concentration would be after elimination of any respiratory component. It is a measure of the alkali reserve of the blood, except that it is measured by determining the pH rather than the total CO_2 content of the sample after equilibration. Like the alkali reserve, it is an index of the degree of metabolic acidosis or alkalosis present.

Additional graduations on the upper curved scale of the nomogram (Figure 39–6) are provided for measuring **buffer base** content; the point where the CO_2 calibration line of the arterial blood sample intersects this scale shows the mEq/L

of buffer base in the sample. The buffer base is equal to the total number of buffer anions (principally $Prot^-$, HCO_3^-, and Hb^-) that can accept hydrogen ions in the blood. The normal value in an individual with 15 g of hemoglobin per deciliter of blood is 48 mEq/L.

The point at which the CO_2 calibration line intersects the lower curved scale on the nomogram indicates the **base excess.** This value, which is positive in alkalosis and negative in acidosis, is the amount of acid or base that would restore 1 L of blood to normal acid–base composition at a PCO_2 of 40 mm Hg. It should be noted that a base deficiency cannot be completely corrected simply by calculating the difference between the normal standard bicarbonate (24 mEq/L) and the actual standard bicarbonate and administering this amount of $NaHCO_3$ per liter of blood; some of the added HCO_3^- is converted to CO_2 and H_2O, and the CO_2 is lost in the lungs. The actual amount that must be added is roughly 1.2 times the standard bicarbonate deficit, but the lower curved scale on the nomogram, which has been developed empirically by analyzing many blood samples, is more accurate.

In treating acid–base disturbances, one must, of course, consider not only the blood but also all the body fluid compartments. The other fluid compartments have markedly different concentrations of buffers. It has been determined empirically that administration of an amount of acid (in alkalosis) or base (in acidosis) equal to 50% of the body weight in kilograms times the blood base excess per liter will correct the acid–base disturbance in the whole body. At least when the abnormality is severe, however, it is unwise to attempt such a large correction in a single step; instead, about half the indicated amount should be given and the arterial blood acid–base values determined again. The amount required for final correction can then be calculated and administered. It is also worth noting that, at least in lactic acidosis, $NaHCO_3$ decreases cardiac output and lowers blood pressure, so it should be used with caution.

CHAPTER SUMMARY

- The cells of the proximal and distal tubules secrete hydrogen ions. Acidification also occurs in the collecting ducts. The reaction that is primarily responsible for H^+ secretion in the proximal tubules is Na^+–H^+ exchange. Na is absorbed from the lumen of the tubule and H is excreted.
- The maximal H^+ gradient against which the transport mechanisms can

secrete in humans corresponds to a urine pH of about 4.5. However, three important reactions in the tubular fluid remove free H^+, permitting more acid to be secreted. These are the reactions with HCO_3^- to form CO_2 and H_2O, with HPO_4^{2-} to form $H_2PO_4^-$, and with NH_3 to form NH_4^+.

- Carbonic anhydrase catalyzes the formation of H_2CO_3, and drugs that inhibit carbonic anhydrase depress secretion of acid by the proximal tubules.
- Renal acid secretion is altered by changes in the intracellular PCO_2, K^+ concentration, carbonic anhydrase level, and adrenocortical hormone concentration.

MULTIPLE-CHOICE QUESTIONS

For all questions, select the single best answer unless otherwise directed.

1. Which of the following is the principal buffer in interstitial fluid?

   A. Hemoglobin
   B. Other proteins
   C. Carbonic acid
   D. H_2PO_4
   E. Compounds containing histidine

2. Increasing alveolar ventilation increases the blood pH because

   A. it activates neural mechanisms that remove acid from the blood.
   B. it makes hemoglobin a stronger acid.
   C. it increases the PO_2 of the blood.
   D. it decreases the PCO_2 in the alveoli.
   E. the increased muscle work of increased breathing generates more CO_2.

3. In uncompensated metabolic alkalosis

   A. the plasma pH, the plasma HCO_3^- concentration, and the arterial PCO_2 are all low.
   B. the plasma pH is high and the plasma HCO_3^- concentration and arterial PCO_2 are low.
   C. the plasma pH and the plasma HCO_3^- concentration are low and the arterial PCO_2 is normal.

D. the plasma pH and the plasma HCO_3^- concentration are high and the arterial PCO_2 is normal.

E. the plasma pH is low, the plasma HCO_3^- concentration is high, and the arterial PCO_2 is normal.

4. In a patient with a plasma pH of 7.10, the $[HCO_3^-]/[H_2CO_3]$ ratio in plasma is

A. 20.

B. 10.

C. 2.

D. 1.

E. 0.1.

Answers to Multiple Choice Questions

Chapter 1

1. B **2.** C **3.** B **4.** C **5.** C **6.** D **7.** E **8.** E

Chapter 2

1. A **2.** D **3.** D **4.** B **5.** C **6.** C **7.** B **8.** A

Chapter 3

1. C **2.** B **3.** D **4.** C **5.** B **6.** B **7.** E **8.** D

Chapter 4

1. C **2.** E **3.** E **4.** E **5.** C **6.** B **7.** E **8.** C **9.** D **10.** D

Chapter 5

1. B **2.** D **3.** B **4.** C **5.** C

Chapter 6

1. C **2.** C **3.** A **4.** E **5.** B **6.** D **7.** B **8.** D **9.** E

Chapter 7

1. D **2.** B **3.** D **4.** D **5.** B **6.** E **7.** E **8.** C **9.** E

Chapter 8

1. A **2.** C **3.** D **4.** E **5.** E **6.** C **7.** B **8.** D **9.** A **10.** E **11.** D

Chapter 9

1. D **2.** B **3.** D **4.** D **5.** A **6.** C **7.** E **8.** D

Chapter 10

1. D **2.** B **3.** C **4.** B **5.** E **6.** E **7.** D **8.** B **9.** D **10.** A **11.** A **12.** B **13.** B

Chapter 11

1. E **2.** D **3.** B **4.** E **5.** A **6.** C **7.** D **8.** D **9.** E **10.** D **11.** B **12.** D

Chapter 12

1. D **2.** E **3.** C **4.** C **5.** A **6.** E **7.** E **8.** B **9.** A **10.** E **11.** C **12.** D **13**. E

Chapter 13

1. A **2.** D **3.** C **4.** E **5.** D **6.** C **7.** B **8**. D

Chapter 14

1. D **2.** D **3.** C **4.** D **5.** B **6.** C **7.** A **8.** E **9.** B

Chapter 15

1. D **2.** B **3.** B **4.** A **5.** E **6.** D **7.** D **8.** B

Chapter 16

No multiple choice questions

Chapter 17

1. B **2.** E **3.** B **4.** A **5.** A **6.** B **7.** D **8.** D

Chapter 18

1. E **2.** E **3.** D **4.** C **5.** B **6.** E **7.** C **8.** A

Chapter 19

1. D **2.** B **3.** E **4.** D **5.** C **6.** D **7.** D **8.** A **9.** A

Chapter 20

1. C **2.** B **3.** A **4.** E **5.** C **6.** C **7.** A **8.** D **9.** D **10.** C

Chapter 21

1. C **2.** E **3.** D **4.** D **5.** A **6.** C **7.** E **8.** D

Chapter 22

1. C **2.** D **3.** C **4.** A

Chapter 23

1. E **2.** A **3.** C **4.** B

Chapter 24

1. E **2.** D **3.** D **4.** C **5.** E **6.** D **7.** C

Chapter 25

1. C **2.** D **3.** D **4.** C **5.** D **6.** B **7.** B **8.** C **9.** E

Chapter 26

1. C **2.** B **3.** E **4.** E **5.** A **6.** D **7.** E **8.** D **9.** D **10.** B

Chapter 27

1. D **2.** C **3.** A **4.** B **5.** E **6.** D **7.** D **8.** E **9.** C **10.** C

Chapter 28

1. C **2.** C **3.** E **4.** E **5.** D **6.** E **7.** B **8.** C **9.** E **10.** A

Chapter 29

1. C **2.** A **3.** A **4.** D **5.** D

Chapter 30

1. C **2.** E **3.** C **4.** A **5.** C **6.** C **7.** D **8.** C

Chapter 31

1. E **2.** D **3.** C **4.** A **5.** C **6.** B **7.** E **8.** D **9.** D **10.** A **11.** B **12.** B

Chapter 32

1. B **2.** D **3.** B **4.** C **5.** E **6.** C **7.** D **8.** D **9.** E

Chapter 33

1. E **2.** D **3.** B **4.** A **5.** E **6.** E **7.** D **8.** D

Chapter 34

1. D **2.** C **3.** A **4.** E **5.** D **6.** A

Chapter 35

1. E **2.** B **3.** D **4.** D

Chapter 36

1. D **2.** B **3.** B **4.** D **5.** E **6.** B **7.** C

Chapter 37

1. A **2.** A **3.** A **4.** A **5.** E **6.** C **7.** D

Chapter 38

1. E **2.** C **3.** D **4.** E **5.** D **6.** C **7.** D

Chapter 39

1. C **2.** D **3.** D **4.** B

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