Third Edition

Essentials of MEDICAL BIOCHEMISTRY

With Clinical Cases

Chung Eun Ha
N.V. Bhagavan

Essentials of Medical Biochemistry

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- Scenario-based and thought-provoking multiple-choice questions and answers can be found at the end of each chapter in the book. Simpler, more concise multiple-choice questions from the second edition of the book are also available on this site.

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With Clinical Cases

Third Edition

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An imprint of Elsevier

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Academic Press is an imprint of Elsevier
125 London Wall, London EC2Y 5AS, United Kingdom
525 B Street, Suite 1650, San Diego, CA 92101, United States
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom

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ISBN: 978-0-323-88541-6

Publisher: Stacy Masucci
Acquisitions Editor: Peter B. Linsley
Editorial Project Manager: Pat Gonzalez
Production Project Manager: Kiruthika Govindaraju
Cover Designer: Miles Hitchen

Typeset by MPS Limited, Chennai, India

Dedication

This edition is dedicated to the late Nadhipuram V. Bhagavan, Ph.D., who was a great mentor, teacher, scholar, researcher, author, and, above all, a good friend.

Chung Eun Ha, PhD

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Preface

The third edition of *Essentials of Medical Biochemistry* with clinical cases was prepared with a significant focus on the student's perspective on how basic science principles are applied to the understanding of human wellness and diseases. Every chapter starts with a list of learning objectives, which prepares the readers for what to expect when they finish studying the chapter. The body of the text describes basic principles of biochemistry and recent findings to provide students with up-to-date information in the fast-evolving research fields in life sciences.

In particular, new comprehensive figures are added to help students better comprehend the complicated concepts discussed in the text. The majority of figures from the second edition were substantially revised with clearer illustrations. Given the aims of this textbook, which are to provide the essentials of medical biochemistry, many of the discussions on nonessential topics have been omitted. However, with the abundance of online resources, students are encouraged to use web search engines to find omitted details online by using keywords highlighted in the main text.

Biochemistry is the chemistry of life; medical biochemistry mainly deals with biochemical aspects of human diseases. This medical biochemistry textbook focuses on normal biochemical processes and abnormalities in these processes that lead to a diverse array of human diseases. Many details about these human diseases such as symptoms, diagnostic principles, treatment options, and prognosis are presented only for the most widely known and prevalent human diseases. Details about many other human diseases are not discussed but are listed in the text, which students can then use for independent study when necessary.

Each chapter concludes with a list of key points that summarize the major topics discussed. For a better understanding of the subject material, required reading and supplemental reading references are presented. Clinical case studies in each chapter provide real-life cases, not hypothetical cases, to students. Frequently, a real case presentation provides students with the understanding that not every faulty step in a biochemical pathway leads to disease, rather, there are crucial or exclusive steps in these pathways that are typically responsible.

Test-taking skills are important assets given that standardized tests are widely used to measure students' understanding. Knowing the concepts of medical biochemistry and selecting the correct answer on multiple-choice questions are often two distinct challenges and do not always correlate well. To help students learn better and build their test-taking skills, many scenario-based multiple-choice questions are provided at the end of each chapter.

Medical biochemistry is the study of the human body at the molecular level. However, understanding human diseases requires a deep understanding of how multiple organs interact to achieve metabolic homeostasis, which is a major concern for medical biology. Certain aspects of anatomy, biology, pathology, physiology, and pharmacology are addressed throughout the text to improve comprehension of biochemical concepts. In particular, a number of topics in these disciplines are critical for learning medical biochemistry, therefore dedicated chapters exclusively dealing with immunology, the contractile system, and hemostasis are included in the book. A new chapter on drug metabolism is also included to promote understanding of the metabolism and general mechanisms of action of various pharmaceutical agents listed in the other chapters.

Another notable change introduced in the third edition is the combining of several chapters. Chapters 2 and 37, 6 and 7, and 25 and 26 from the previous edition have been combined into single chapters to integrate basic science principles with appropriate topics in medicine.

The authors firmly believe that the new edition will meet curricular requirements and help students pursuing a career in medicine, nursing, pharmacy, and other healthcare disciplines in obtaining a firm foundational knowledge of medical biochemistry.

Chung Eun Ha, PhD

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Acknowledgments

I am grateful to our contributing authors for their significant work on revising their chapters with updated formatting and content. I also appreciate their cooperation and assistance throughout the manuscript preparation and production processes.

Preparing this edition required the collective efforts of many individuals. First of all, a special thank you goes to the sons of the late Dr. Nadhipuram V. Bhagavan, Ram and Kumar Bhagavan, for their continued support.

I would like to thank many other individuals for their collaboration and contributions as consultants and colleagues who provided their valuable suggestions, advice, and academic support. These individuals are Winona K. Lee, Kimberly Yamauchi, Ming lei Tim Sing, Sandy Tsuhako, Kelli-Ann Voloch, Malia Purdy, Celeste M. Wong, Sharleen Chock, Kenton Kramer, and Keawe'aimoku Kaholokula. Many thanks also to the Imi Ho'ola students for inspiring me to look at different aspects of many topics by asking thought-provoking and critical questions on various subjects in class.

I would like to thank the publisher, Elsevier, and senior editor, Peter Linsley, for supporting the project. I would especially like to express my gratitude to Ms. Pat Gonzalez, senior editorial manager, for helping us to maintain the production schedule and providing support in many ways to accommodate the authors' needs.

Lastly, I would like to express my sincere gratitude to my family. First of all, my lifetime partner, Ji Sook Ha, Ph.D., who also teaches medical biochemistry to students in the department of medical technology, for giving me valuable advice on the content of the book and providing a list of errors found in the manuscripts. Jennifer J. Ha for her constant encouragement and cheer throughout the project. Last but not least, James T. Ha, M.D., who is a resident physician, for reviewing every chapter of the new edition and revising Chapter 31. James painstakingly double-checked the added content. His contribution to the new edition is immeasurable and I thank him dearly for his efforts in helping to prepare this edition.

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Chapter 1

The human organism—organ systems, cells, organelles, and microbiota

Learning objectives

After reviewing this chapter, you should be able to:

1. understand 11 different organ systems of human body which provide highly specialized and coordinated functions to maintain normal metabolism and perform essential activities necessary for survival,
2. describe the compartmentalized organelles of human cells which are required to maintain cellular metabolic homeostasis via various catabolism and anabolism,
3. understand certain human diseases caused by defective cellular organelles and their dysfunctions, and
4. understand the diverse roles of gut microbiota in human body and the importance of maintaining healthy microbiota populations to promote health and prevent diseases.

1.1 Organ systems: integrated function at the highest level

The highest level of integrated functionality in humans is the organ system; structurally recognized for thousands of years, but recognized for their functional importance for only a few hundred (Fig. 1.1). The 11 organ systems of humans are as follows:

- Skeletal—bones, cartilage, and ligaments. This system provides the framework and physical form for the body.
- Integumentary—skin, hair, and nails. This system provides a barrier between the outside world and the body.
- Muscular—skeletal, cardiac, and smooth muscle. This system enables movement of the body, skeleton, and internal organs.
- Digestive—mouth, stomach, small and large intestines, colon, and anus. This system provides the pathway for food ingestion and processing to extract nutrients and thus energy, and an environment in which symbiosis occurs with microorganisms that convert foodstuffs into nutrients absent from the foodstuffs but required for survival.
- Cardiovascular or circulator—heart, arteries, veins, and lymphatic vessels. This is the transportation system by which nutrients and oxygen are exchanged with both the lungs and cells within organs.
- Respiratory—lungs with their alveolar sacs, trachea, nasal orifices, and diaphragm. This is the system for oxygen exchange with the atmosphere and specialized muscle that enables breathing.
- Excretory—kidneys and ureters, bladder, and urethra. This is the system for waste removal from nutrient utilization, organ, and cell renewal as well as for maintaining electrolyte balance.
- Nervous—brain, spinal cord, nerves, and some receptors. This system provides cognition and electrical signal/information processing and transmission and acts as the control center for the other organ systems of the human animal.
- Endocrine—glands and secretory tissues. This system provides for chemical signaling via transport using the circulatory system, a second control system.
- Reproductive—testes, ovaries, uterus, and genitalia. This is the system by which the species ensures continuation of itself.
- Immune—white blood cell types, thymus, and spleen. This is the system by which protection from pathogens enables survival.

Organs comprise cells that compartmentalize the lower level functions that are the basis for organ function in living organisms. These, in turn, comprise organelles that further compartmentalize the biochemical reactions and processes that are highly evolved and specialized to produce the chemical substances needed to create these structures and to extract energy to drive the chemical reactions and mechanical actions which the various organ systems provide. Normal health and disease diagnosis are related to the organ systems of humans, and thus the primary sections and chapters of this biochemical text are similarly organized.

Essentials of Medical Biochemistry. DOI: https://doi.org/10.1016/B978-0-323-88541-6.00017-X

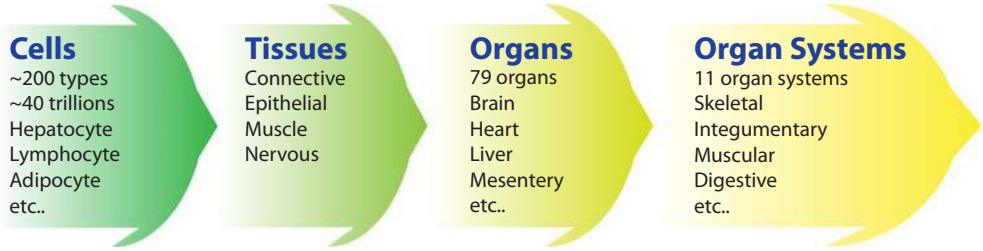

FIGURE 1.1 Hierarchical organization of human body from the simplest cells to the most complexed, multifunctional organ systems.

1.2 Cells: structures and functions

The unifying principle of biology is that all living organisms from the smallest and least complex (bacteria) to the largest (whales) and most complex (humans) are composed of cells. The precise location of cells in the multicellular organisms and the location of intracellular organelles within cells are vital in the normal development and function. During injury, wound repair, or morphogenesis, the precise location and migratory patterns of cells in multicellular organisms involve several strategies that include establishment of gradient of small molecules, regulatory networks, and genetic diversity [1]. The membrane trafficking along with metabolites to correct intracellular locations is precisely regulated. Defects in the membrane trafficking lead to pathological consequences [2].

In the simplest forms of life, such as bacteria, cellular organization and biochemical functions are relatively uncomplicated and are primarily devoted to growth and reproduction. As a consequence, bacteria have evolved to survive and thrive in the widest range of environments imaginable—soil, rivers and oceans, hot springs, and frozen land, as well as in most areas of the human body. The only regions of the body that are normally sterile are the respiratory tract below the vocal cords, the sinus and middle ear, the liver and gall bladder, the urinary tract above the urethra, bones, joints, muscles and blood, the linings around the lungs, and cerebrospinal fluid. Intestinal colon contains numerous microorganisms and they are collectively known as **microbiota**. In particular, microorganisms inhabiting in the gastrointestinal system are called gut microbiota and include bacteria, fungi, viruses, and protozoa. Humans and many microorganism species establish a symbiotic relationship in which the host provides daily nutrients for their survival and in return symbiotic microorganisms aid in supplying (1) nutrients such as short chain fatty acids (SCFAs), (2) various essential metabolites such as essential amino acids and vitamins, (3) processed host metabolites for recycling such as bile acids, and (4) stimulus for secretion of cytokines to boost host immune system and hormones to regulate various host metabolism. Therefore, maintaining a population of normal healthy microbiota is essential for the optimal host health. When the host fails to maintain healthy microbiota and there is dysfunctional microbiota population established in the gut which is referred to as dysbiosis, the host may be susceptible to certain diseases such as irritable bowel syndrome, obesity, and diabetes (Fig. 1.2). Studies have shown that the diet of the host may play a significant role in regulating gut microbiota population. For

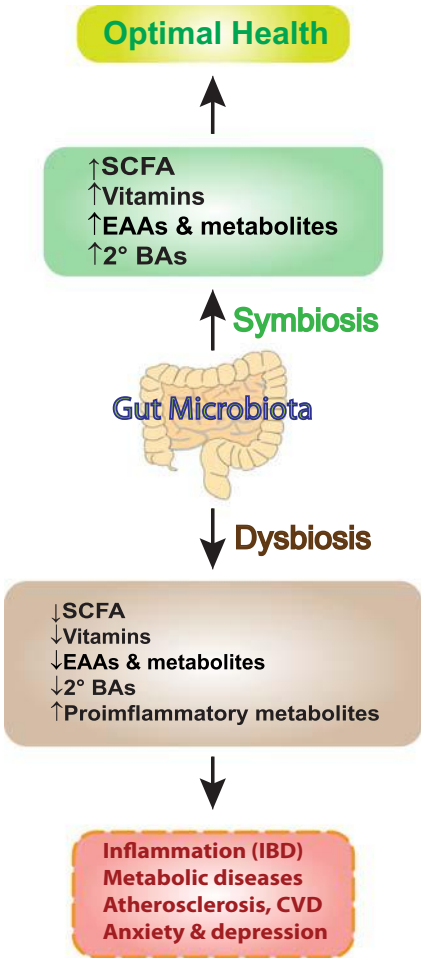

FIGURE 1.2 Gut microbiota in human health and diseases. When the human host and gut microorganisms maintain a symbiotic relationship, the gut microbiota will provide various metabolites essential to the host in maintaining optimal health. On the other hand, if quality (number of microorganism species) and quantity (number of microorganisms) of the gut microbiota are not maintained by the host immune systems, the health of the host will be compromised and various diseases may develop. *2° BAs*, secondary bile acids; *CVD*, cardiovascular disease; *EAAs*, essential amino acids; *IBD*, irritable bowel syndrome; *SCFA*, short chain fatty acid.

example, diet rich in plant fibers by slowing down of gut motility may provide more opportunity for certain species of bacteria to generate various SCFA and metabolites beneficial for the host physiology [3,4]. In germ-free mice, changes in the microbial flora have ameliorated obesity. Fecal flora from healthy persons has been used to reestablish normal microbiota in a recurrent *Clostridium difficile* enteric infection. Diet, the quality of the gut microbiota, and genetic makeup of the host all may play a role in producing a proatherosclerotic metabolite known as trimethylamine-N-oxide (TMAO) leading to cardiovascular disease. Recent studies have revealed that dietary sources of carnitine and phosphatidylcholine (lecithin) are converted to trimethylamine by gut microbes, which in turn enters the enterohepatic blood circulation followed by its conversion to TMAO by hepatic flavin-containing monooxygenases. Colon epithelial barrier damage can provoke infection and inflammation, and may lead to a spectrum of diseases (These aspects, along with required reading references, are discussed in Chapter 11.).

All bacteria belong to the superkingdom called **prokaryotes**. Yeasts, molds, and protozoa are also single-celled organisms, but their cellular structures and functions are more complex than those of bacteria. These organisms belong to the other superkingdom called **eukaryotes**, along with all higher plants and all multicellular animals. A prokaryote cell has no true nucleus or specialized organelles in the cytoplasm. Bacteria reproduce asexually by cell division (fission). As mitochondria (discussed later) have many properties in common with bacteria, it suggests that bacteria-like organisms were assimilated into eukaryotic cells early in their evolution.

All eukaryotic cells have a well-defined nucleus surrounded by a nuclear membrane and cytoplasm containing organelles that perform specialized functions. All eukaryotic somatic cells reproduce by the complex mechanisms of **mitosis** and cytokinesis. Germinal cells (sperm and ova) are formed by a slightly different mechanism called **meiosis**.

Although the size and complexity of eukaryotic organisms differ enormously (ameba, fly, worm, crab, bird, dog, dolphin, chimpanzee, human being), the basic organization and chemistry of their individual cells are quite similar. Sequencing of the nuclear DNA of many different organisms has shown remarkable conservation of key genes and proteins among widely dissimilar organisms. In some cases, a protein produced by a human gene will function just as well when the human gene is swapped for the comparable gene in yeast.

1.2.1 Properties of "living" cells

The human body actually contains trillions of both prokaryotic and eukaryotic cells which perform metabolic functions, many of them are synergistic. Thus, bacteria in the human body are just as essential to the health and survival of a person as are her or his own cells (discussed earlier). Although no single definition serves to distinguish "living" from "nonliving," both prokaryotic and eukaryotic cells share certain properties that distinguish them from nonliving matter.

1.2.1.1 Metabolism

The sum of all chemical reactions in cells that maintain life is metabolism. Living cells extract energy from the environment to fuel their chemical reactions; the metabolism of dead or dying cells is significantly different from that of healthy cells. Bacteria generally extract the chemicals they need from their immediate environment; human cells obtain essential chemicals and nutrients via the circulatory system. The ultimate source of energy for all plants and animals is sunlight, which is used directly by plants and indirectly by animals that consume plants.

1.2.1.2 Growth

As a result of the utilization of energy and the synthesis of new molecules, cells increase in size and weight.

1.2.1.3 Reproduction

All cells reproduce by giving rise to identical copies of themselves. As a result of growth, cells reach a size that triggers reproduction and the production of progeny cells. For example, prokaryotes divide asexually while eukaryotes are capable of sexual reproduction. Key differences in prokaryotic and eukaryotic reproduction are that prokaryotes reproduce when their cell growth in volume cannot be supported efficiently by the cellular interactions with environment due to limited cellular surface area growth (volume of a sphere can be calculated by the equation $(4/3)\pi\ r^3$ vs surface area by $4\pi\ r^2$) and eukaryotes reproduce when they receive signals from other cells.

1.2.1.4 Mutation

In the process of growth and reproduction, cells occasionally undergo a mutation which is a permanent heritable change in the genetic information in the cell's DNA. Mutations that arise in sperm or ova are called **germinal mutations** and these may lead to hereditary disorders. Mutations that arise in body cells other than sperm or ova are called **somatic mutations**; these mutations may alter normal cell growth and reproduction and may underlie the development of cancer (unregulated cell growth), aging, or other derangements of cellular functions.

1.2.1.5 Response

All living cells and multicellular organisms respond to environmental stimuli that change chemical reactions and behaviors. Stimuli that evoke cellular responses include

light, nutrients, noxious chemicals, stress, and other environmental factors.

1.2.2 Evolution

As a result of mutation and other genetic mechanisms, the genetic information, chemical reactions, and other properties of organisms change (evolve) over time. Some of the inherited changes that occur in organisms make some individuals better able to survive and reproduce in particular environments. Evolution causes populations of organisms to evolve over time; some become extinct and others develop into new species.

First proposed in the 1800s, the cell theory of life is now well integrated into biological sciences and medicine. In general, the **cell theory** states that (1) all organisms consist of one or more cells; (2) cells are the smallest units, characteristic of life; and (3) all cells arise from preexisting cells. How the first cells(s) arose on Earth is still matter of intense scientific research, speculation, and controversy.

1.2.3 Structures and organelles in eukaryotic cells

1.2.3.1 Cytosol

The intracellular aqueous compartment that surrounds all of the subcellular organelles is known as **cytosol**. The content of cytosol includes water, dissolved ions, and small and large water-soluble organic molecules. The cytosolic components participate in the osmoregulation, extracellular transduction events, transport and delivery of metabolites to selected cellular locations, and several metabolic pathways. The metabolic pathways of cytosol involve interdependence of organelles. For example, synthesis of glucose (gluconeogenesis), heme, urea, and pyrimidines require enzymes located in both cytosol and mitochondria. Some metabolic pathways occur entirely in the cytosol, such as glycolysis and hexose monophosphate (HMP) shunt pathways.

The metabolic pathways are organized and located at specific sites of the cytosol. The organization and integration of feedback loops of proteins that participate in specific pathways and signaling networks are facilitated by scaffolding proteins [5,6]. Cytosolic metabolic pathways are regulated depending upon the availability of nutrients and oxygen supply. This is illustrated in the circulating red blood cells (RBCs). During relative deoxygenated state of hemoglobin, glycolysis operates to produce adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG). ATP provides energy and 2,3-DPG facilitates oxygen delivery to tissues from oxyhemoglobin. During oxygenated state of hemoglobin, glucose oxidation is shunted to HMP pathway for the production of nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione, both of which are required to protect the RBCs from oxidative stress (discussed in Chapters 13 and 25).

All eukaryotic cells possess characteristic structures and organelles (Fig. 1.3). The shape and size of eukaryotic cells differ markedly depending on their functions, but all are much larger than the most prokaryote. Most eukaryotic cells contain all of the structures shown in Fig. 1.3 but there are exceptions. Nonmotile cells usually lack a flagellum or cilia; the nuclei of RBCs are extruded after being synthesized in bone marrow before they enter the circulation; the nuclei also are digested in the outermost layer of skin cells. Cilia are sensory organelles and possess hair-like microtubular structures. They participate in signaling pathways, which include detections of external signals and their integrations into metabolic functions. Ciliary dysfunction due to genetic defects can result in a wide variety of developmental and degenerative disorders known as **ciliopathies** [7]. Most cells in the body are in a dynamic state of degradation and renewal. Skin cells and gastrointestinal epithelial cells, for example, are destroyed and replaced on a regular basis. If portions of the liver are damaged by disease or surgical removal, the organ will regrow to its original size. Table 1.1 lists the primary functions of the specialized structures and organelles in a generalized animal cell. The size of cellular organelles is subject to nutrient availabilities, metabolic demands, and stressful conditions [8].

Cells of every adult multicellular organism trace their ancestry to the **zygote**, the first cell of a new individual that is formed by union of sperm and egg. During development, cells undergo repeated mitosis and division and ultimately differentiate into specialized cells that have structures and functions specific to the needs of each tissue or organ in the body.

1.2.4 The plasma membrane and cytoskeleton

The plasma membrane and cytoskeleton determine a cell's morphology and transport of molecules. The **plasma membrane** consists of a complex lipid bilayer, phosphate and carbohydrate components, cholesterol, and a large number of proteins embedded in the membrane that connect the inner milieu of the cell with the external environment. The plasma membrane maintains the physical integrity of the cell and prevents the contents of the cell from leaking into the fluid environment. At the same time, it facilitates the entry of nutrients, ions, and other molecules from the outside. The pattern of proteins and carbohydrate−lipid complexes exposed on the cell surface also are specific to a particular cell type and individual. For example, skin cells of one individual are recognized as "foreign" by another individual's immune system,

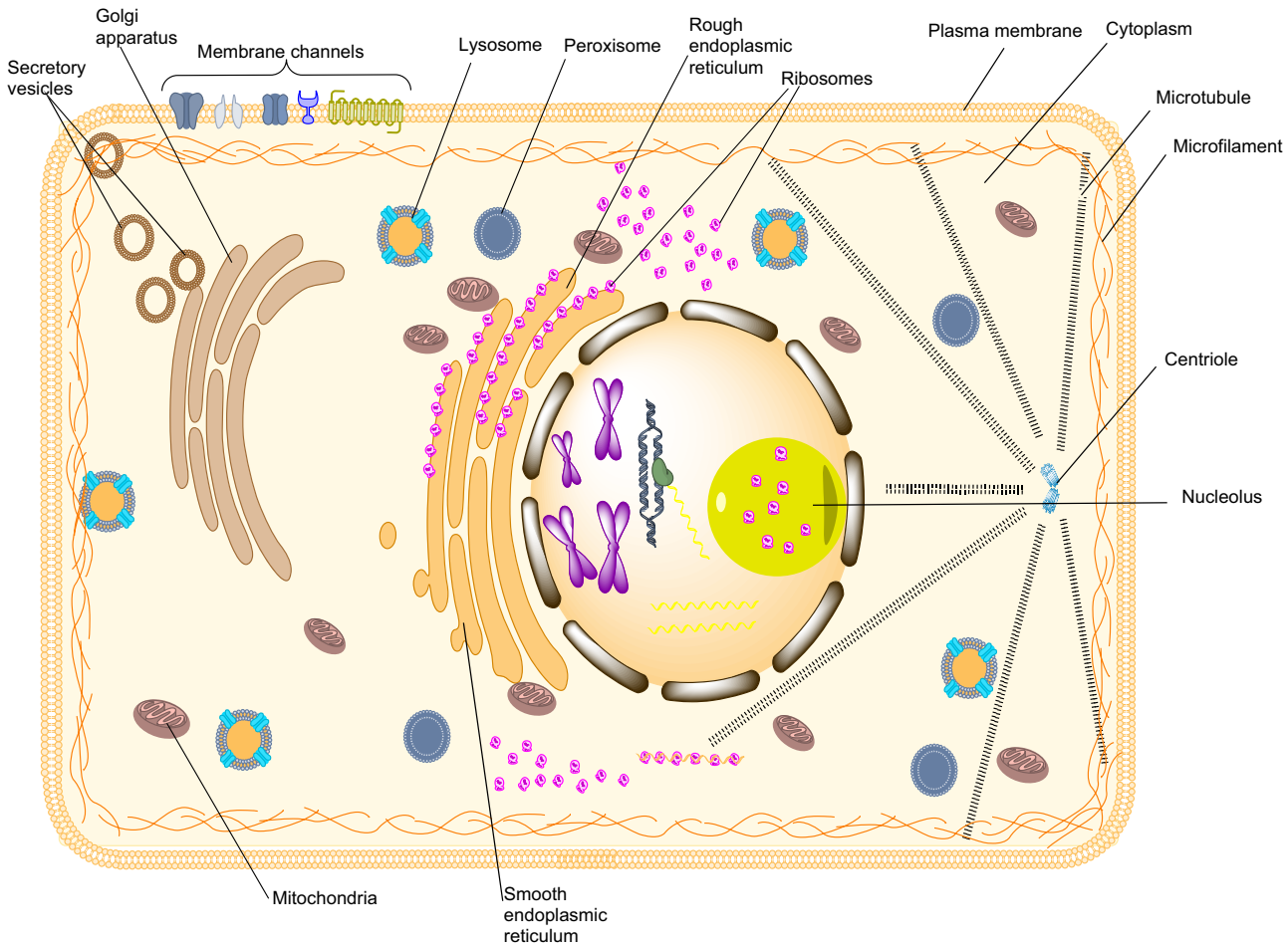

FIGURE 1.3 Schematic representation of a eukaryotic animal cell showing characteristic structures and organelles.

although cells from both individuals function as skin cells.

The functions of the plasma membrane are coordinated by specialized adhesion receptors called **integrins**. The complex modular structure of the extracellular portion of an integrin is shown in Fig. 1.4. Integrins represent important cell receptors that regulate fundamental cellular processes such as attachment, movement, growth, and differentiation. As integrins play such vital roles in the functions of the plasma membranes, they are also involved in many disease processes: they contribute to the initiation and progression of neoplasia, tumor metastasis, immune dysfunction, ischemia-reperfusion injury, viral infections, and osteoporosis.

The **cytoskeleton** of a cell is a constantly changing array of components (**microfilaments** and **microtubules**) that give a cell its structure and motility. Microtubules are involved in intracellular transport of organelles, vesicles, and enzymes. In particular, movement of cellular components along the axonal axis of microtubules, referred to as axonal transport, is crucial for the survival of nerve cells. Depending on the direction of the movement, the transport can be defined as anterograde, which transport the loads toward growing end or + end of microtubules or as retrograde, which transport cargos toward shrinking end or − end of microtubules. Anterograde axonal transport is carried out by cytoplasmic motor proteins called kinesin and retrograde transport is by another motor protein called dynein (Fig. 1.5) [3,4]. Defects in microtubule transport lead to neurological and ciliary diseases [9]. The cytoskeleton also plays an important role in cell division and the transport of molecules across the plasma membrane. Microfilaments consist of long, very thin strands of the protein **actin**, which is also a main component of muscle. Strands of microfilaments form spontaneously in high concentrations of Ca^{2+} and Mg^{2+} within the cell. Microtubules are long, thin tubes composed of the protein **tubulin**. They also assemble and disassemble in response to the ionic environment. Microtubules comprise the **spindle fibers** that separate chromosomes prior to cell division. **Centrioles** are composed of microtubules and function as the organizing center for the formation of spindle fibers.

TABLE 1.1 Functions of components and organelles in eukaryotic cells.

Component	Function
Plasma membrane	Lipid bilayer in which proteins are embedded. Regulates influx and efflux of nutrients and chemicals; also cell−cell recognition
Cytoskeleton	An array of microfilaments and microtubules in dynamic rearrangement. Determine shapes of various cells; participate in the movement of cellular organelles and vesicles & importation/exportation of various molecules & metabolites via processes such as endocytosis, exocytosis, & pinocytosis
Flagellum and cilia	Motility of cell or movement of fluids over tissues
ER	Internal membranes (rough and smooth) that serve as sites of synthesis for proteins, lipids (phospholipids and steroids), and carbohydrates; also modify and transport proteins to other cellular organelles; store calcium ions; *de novo* synthesis of peroxisomes
Nucleus	Structure with double membrane that contains chromosomes (DNA) that express genes and control chemical activities of the cell
Nucleolus	Site of ribosome synthesis and assembly
Golgi apparatus	Site of packaging of proteins for transport from the cell; posttranslational modification of proteins such as glycosylation, sulfation, phosphorylation, etc.; transport of lipids and proteins to lysosome, peroxisome, and plasma membrane via lipid vesicles; synthesis of glycosaminoglycan and assembly of proteoglycans; play role in apoptosis
Lysosomes	Structures containing enzymes that degrade unwanted cellular products and foreign substances; maintain internal acidic pH by active proton pumps on the membrane
Mitochondria	Organelles that produce cellular energy (ATP); contain circular DNA molecules (mitochondrial DNA, 37 genes), tRNAs, and ribosomes for own protein expression; double-membrane system for oxidative phosphorylation, lipid modifications, electron transport system; participate cellular quality control processes such as mitophagy, autophagy, and apoptosis; harbors unique proteins, which can trigger immune responses when they are released outside cells because the proteins resemble bacterial proteins; site of cardiolipin synthesis; participate in cellular Ca^{2+} signaling along with ER through contact sites
Peroxisomes	Very long-chain fatty acid oxidation and α-oxidation of phytanic acid; etherphospholipid (plasmalogens) synthesis; produce hydrogen peroxide to oxidize amino acids and lipids
Ribosomes	Sites of protein synthesis in cells; active ribosomes bound to rough ER are responsible for the synthesis of transmembrane and secretory proteins; cytosolic proteins are made by free ribosomes in cytosol
Microfilaments	Long, thin filaments of actin; form and degrade in response to changes in intracellular ion concentrations
Microtubules	Composed of tubulin; found in flagella, cilia, and spindle fibers used to separate chromosomes in mitosis and meiosis

ATP, Adenosine triphosphate; *ER*, endoplasmic reticulum.

1.2.5 Structures and organelles involved in synthesis, transport, and degradation of molecules

The **endoplasmic reticulum (ER)** appears as an intricate, complex, folded net in the cytoplasm. A portion of the ER has ribosomes bound to it, which give it a "rough" appearance when viewed by electron microscopy. The rough ER is the site for synthesis of proteins that are destined to be exported from the cell. The ER also has mechanisms for maintaining the quality of the proteins synthesized, especially those destined for transport. The ER has sensor molecules that monitor the amounts of unfolded or improperly folded proteins that accumulate. **Parkinson's disease** and **Alzheimer's disease** are both characterized by synthesis of excessive amounts of mutant proteins that are folded incorrectly. In general, accumulation of nonfunctional proteins in the ER contributes to a situation referred to as ER stress, which is associated with diabetes and cancer, as well as neurodegenerative diseases. ER is one of the largest cellular organelles in animal cells and has extensive intracellular network, which includes membrane contact sites with other cellular organelles such as mitochondria, lysosomes, plasma membrane, etc. Through these contacts, ER can directly exchange transmembrane proteins, membrane lipids, and other metabolite and ions (e.g., Ca^{2+}). ER is the site of most *de novo* synthesis of cellular organelles such as

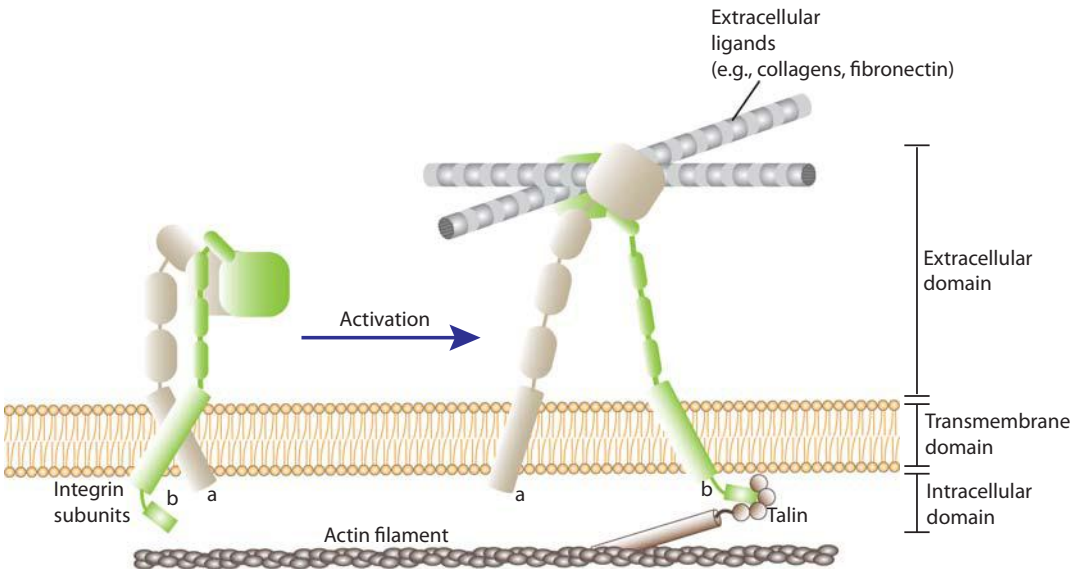

FIGURE 1.4 Diagram of integrin heterodimer modules. Extracellular domain upon activation recognizes extracellular ligands such as fibronectin to induce cell migration and extracellular matrix remodeling (signal transduction from inside to outside cell, or inside-out signal transduction). Also, intracellular domain upon activation interacts with intracellular components such as a cytoskeleton protein, talin to regulate cellular processes (signal transduction from outside to inside cell, outside-in signal transduction).

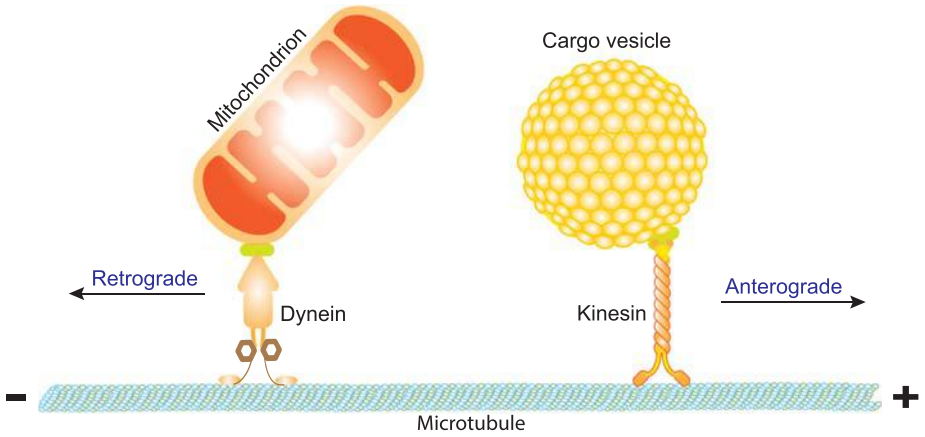

FIGURE 1.5 Schematic illustration of anterograde and retrograde axonal cargo transport mediated by motor proteins, kinesin and dynein.

peroxisomes and also participates in the maintenance of other cellular organelles through supplying newly synthesized proteins and lipids. For example, ER can initiate the removal of dysfunctional mitochondria by activating autophagy process exclusively for the organelle termed mitophagy. If the maintenance of cellular organelles failed or ER itself is subjected under ER stress, ER can initiate programmed cell death known as apoptosis collaborating with mitochondria and other organelles [10].

Other ribosomes are not attached to the ER and are "free" in the cell, although they are usually attached to the cytoskeleton. These ribosomes synthesize proteins used intracellularly. The smooth ER is the site of lipid synthesis, which does not require ribosomes.

The **Golgi apparatus** (named for its discoverer, Camillo Golgi) is a specialized organelle in which proteins are processed, modified, and prepared for export from the cell. The Golgi apparatus resembles a stack of 10−20 hollow, flat structures with the smallest being attached to the plasma membrane. Proteins are received from the ER and passed through layers of the Golgi apparatus where polysaccharides are synthesized and attached to proteins to make **glycoproteins**, or to lipids to make **glycolipids**. During the transit from the ER side of Golgi (called cis face) to the plasma membrane side of Golgi

(called trans face), the newly synthesized proteins and lipids are modified and sorted out for their respective destinations. For example, during the cis to trans transit, new lysosomal proteins are subjected to the modifications of N-linked sugar moieties by series of enzymatic actions, which convert mannose residues to mannose 6-phosphates and directed into a particular space in the Golgi for the later transport to lysosomes. If these processes become dysfunctional due to enzyme deficiencies, an autosomal recessive disease called I-cell disease develops. The Golgi apparatus also participate in apoptosis coordinated by ER and mitochondria.

The **lysosomes** are membrane-bounded sacs containing enzymes capable of destroying the cell if they were not confined to the lysosome. Lysosomes contain as many as forty different hydrolytic enzymes and a eukaryotic cell (especially liver and kidney) may contain several hundred lysosomes. The hydrolytic enzymes found in lysosomes include proteases, nucleases, glycosidases, lipases, phosphatases, and sulfatases; all of these enzymes function at the acidic pH (<pH 5), which is mainly established by active proton pump in the lysosomal membrane.

Lysosomes assist in cell renewal by digesting old or damaged cellular components. During development, lysosomes play an important role in the formation of specialized tissues such as fingers and toes. For example, lysosomes digest the webbed tissues that join fingers and toes in the embryo. White blood cells protect the body from infectious disease by engulfing pathogenic microorganisms and isolating them in a membranous sac called a **phagosome**. A lysosome then fuses with the phagosome and digests the pathogen. A similar process involving the formation of autophagosome and fusion with a lysosome, referred to as autophagy, is used to eliminate dysfunctional or damaged cellular organelles, thereby enabling the cells to maintain healthy cellular organelles (discussed in Chapter 4).

Peroxisomes are similar to lysosomes, in that they are single membranous sacs containing enzymes. However, peroxisomes contain enzymes that are used for detoxification rather than for hydrolysis. One of the most important functions of peroxisomes is the detoxification of alcohol in liver cells. Other peroxisome enzymes remove the amine group from amino acids and convert it to ammonia prior to excretion. Liver peroxisomes contain three important detoxification enzymes: **catalase**, **urate oxidase**, and **D-amino acid oxidase**. These enzymes use molecular oxygen to remove hydrogen atoms from specific substrates in oxidation reactions. The enzyme content of cellular peroxisomes varies according to the needs of the tissue. Peroxisomes also participate in degradation of very long-chain fatty acids. Peroxisome defects lead to disorders such as **adrenoleukodystrophy**, **Zellweger's syndrome**, and **Refsum's disease** [11].

The **ubiquitin−proteasome system** and **autophagosomes**, which are located in the cytosol, are required for the intracellular proteolysis. The ubiquitin−proteasome system, which is also present in the nucleus, consists of organelles called proteasomes; an individual proteasome is a multiprotein subunits barrel-like structure with a central hollow pore. Ubiquitin, so named because it occurs ubiquitously in all eukaryotes, is a small 76 amino acid residue protein (8.5 kDa), which tags misfolded proteins as well as normal proteins destined for destruction. The polyubiquitinated protein is eventually surrendered to proteasome for proteolysis, while ubiquitin molecules escape proteolysis and are reused. The overall process is regulated, requires several enzymes, and energy is provided by ATP hydrolysis. The autophagosome (self-digestion) system consists of sequestration of misfolded proteins, followed by their integration with lysosomes and proteolysis by lysosomal enzymes. The ubiquitin−proteasome and autophagosome systems are required for several normal cellular functions (e.g., cell cycle and division, immune response, biogenesis of organelles). Disruption of ubiquitin−proteasome and autophagosome systems is associated with several disorders. A chemotherapeutic agent, **bortezomib** inhibits ubiquitin−proteasomal proteolysis and promotes apoptosis of rapidly growing monoclonal plasma cells and thus is used in the treatment of multiple myeloma (Chapter 4).

Almost all vertebrate cells have a primary or many specialized projections on the cell surface that are called **cilia**; some cells also have a larger, more complex projection called a **flagellum** (plural flagella) that is used for cell movement. Some cilia are also involved in motility, but most have other functions and are immotile. Cilia are also specialized cellular organelles.

Cilia play essential roles in the olfactory system, in visual photoreceptors, and in mechanosensation. Cilia play vital roles in intercellular communication and signaling. Ciliary defects are associated with many human disorders, including retinal degeneration, polycystic kidney disease, and neural tube defects.

1.2.6 Mitochondria provide cells with energy

Mitochondria are organelles in eukaryotic cells that supply energy for all cellular metabolic activities. The number of mitochondria in cells varies as do their energy needs, muscle cells, especially those in the heart, contain the largest number of mitochondria. The overall production of energy in body cells is expressed by the equation:

$$\text{Glucose} + \text{oxygen} \Rightarrow \text{carbon dioxide} + \text{water} + \text{energy}$$

Mitochondria are characterized by an outer membrane and an inner membrane that is intricately folded and organized for the transport of electrons along the **respiratory chain**. The

inner membrane is the site of **oxidative phosphorylation** which generates most of a cell's energy in the form of ATP. Mitochondria are in a dynamic state in the cells; they are mobile and constantly change from oval- to rod-shaped.

In addition, mitochondria are in a continual state of fission and fusion, so that the identity of any given mitochondrion is transient. At any point in the cell's cycle, a mitochondrion may undergo fission to give rise to two separate mitochondria (Fig. 1.6). Concurrently, other mitochondria are undergoing fusion in which both the inner and outer membranes of the mitochondria break and rejoin to form a single intact mitochondrion. At least three different GTPases [mitofusin-1 (Mfn1), -2 (Mfn2), and optic atrophy factor 1 (Opa1)] are involved in mitochondrial fusion. For fission, another GTPase called **dynamin-related protein 1** (**DRP1**) is required [12]. Mitochondria maintain their quantity and quality in a cell through the series of fusions and fissions. If there is dysfunctional or defective mitochondrion due to the accumulated defective proteins and lipids, fusion with healthy mitochondrion enables the faulty one to recover with the resupply of normal proteins and lipids or divide by fission into two separate mitochondria, one healthy mitochondrion survives and the other one with defects will be destroyed by mitophagy. At least one serious disease involving microcephaly and metabolic abnormalities has been ascribed to mutations in DRP1 (see Chapter 12 and case study 12.1).

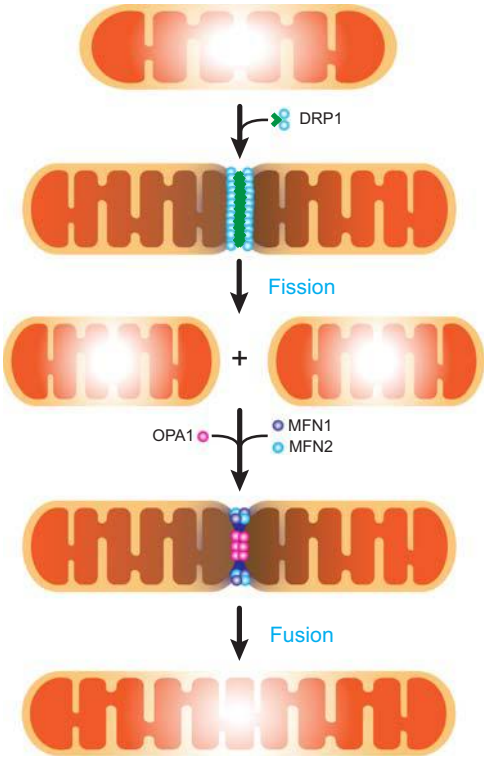

FIGURE 1.6 Mitochondrial fusion and fission.

Mitochondria contain small, circular DNA molecules (mtDNA) containing 37 genes that are essential for mitochondrial functions. The ribosomes in the mitochondria also resemble prokaryotic ribosomes rather than eukaryotic ribosomes found in the cytoplasm, which further supports their derivation from bacteria that were assimilated early in the evolution of eukaryotic cells.

Mutations in mtDNA are responsible for a number of diseases (**mitochondriopathies**) that can be inherited, although not in the Mendelian pattern that is characteristic of nuclear genes (see clinical study 12−2 for an exception). Inheritance of mtDNA defects is through a maternal lineage, as mitochondria are present in large numbers in the ovum that forms the zygote. Male mitochondria are not present in the head of the sperm that fertilizes the ovum because they are eliminated by programmed mitophagy processes. In some patients, exercise intolerance and muscle fatigue are due to mutations in mtDNA. Mitochondria also participate in other metabolic pathways in conjunction with the cytoplasmic enzymes; these include heme biosynthesis, urea formation, fatty acid oxidation, and initiation of apoptosis by releasing of cytochrome C.

1.2.6.1 Lipid droplets

Lipid droplets store triacylglycerol (triglyceride) and cholesterol esters as discrete organelles. Adipocytes and other cells that store triacylglycerols and cholesteryl esters contain lipid droplets which are organized by proteins known as **perilipins**. The perilipin family of proteins and other associated proteins provide structural organization of scaffolding and metabolic functions. In the white adipose tissue cells, 90% of the cell volume consists of unilocular lipid droplets (Chapter 17).

1.2.7 The nucleus controls a cell's development and chemical activities

The nucleus is the control center of the cell; it contains the chromosomes that carry all of the individual's genetic information. The nucleus is encased in a double membrane called the **nuclear envelope**. The outer membrane is fused with the ER at multiple sites and forms **nuclear pores** that facilitate transport of molecules between the cytoplasm and the nucleus. The inner membrane is supported by fibrillar protein called **lamina**, which interacts with nuclear membrane proteins and the chromatin inside nucleus. The importance of the mesh network of the lamin proteins in nucleus is evidenced in genetic conditions causing premature aging diseases, also known as Hutchinson Gilford progeria syndrome, which is caused by mutations in one of the lamin proteins, lamin A (Fig. 1.7). Also, there are usually two or more organelles called **nucleoli** that surround a region of the DNA

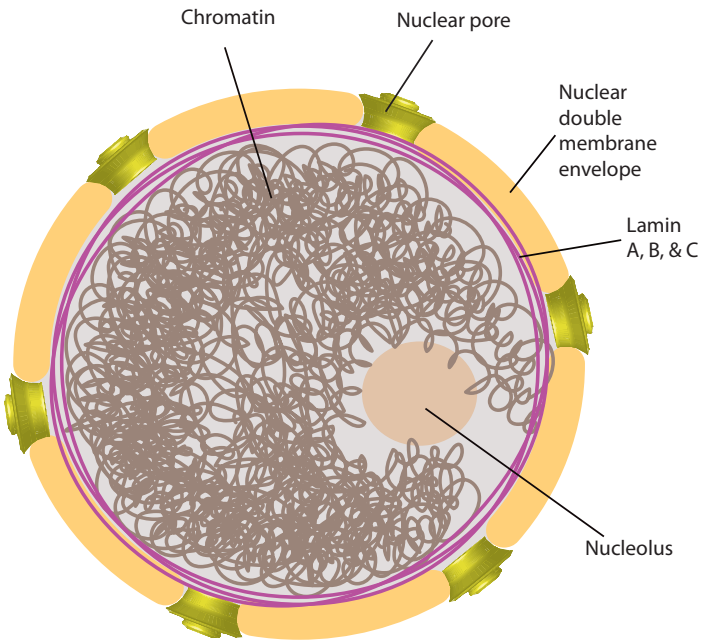

FIGURE 1.7 Structure of an animal cell nucleus. The inner membrane of nuclear double envelope encases the lamina layer, which consists of three lamin proteins, A, B, and C. The lamina layer provides important structural support for nuclear structure and also interacts with chromatin to participate in various nuclear functions.

containing genes for making ribosomal RNA (rRNA). The many copies of rRNA synthesized in the nucleoli are bound to ribosomal proteins that are transported to the nucleus from the cytoplasm. After assembly of ribosomes in nucleoli, they are exported through the nuclear pores back into the cytoplasm.

During most of the cell cycle, the chromosomes are in a diffuse, extended state in the nucleus referred to as euchromatin and are not visible. During mitosis when a cell is preparing to divide, the chromosomes condense into visible structures, which is termed heterochromatin, the movements of which can be observed in the light microscope. All of the genes whose products are essential for the functions of the particular cell are regulated and expressed (transcribed) as messenger RNA (mRNA) molecules that are subsequently transported to the cytoplasm, where they are translated into proteins. However, prior to being transported to the cytoplasm, the mRNAs are extensively processed. RNA-splicing enzymes remove segments of the mRNA (introns) and rejoin segments that are to be translated (exons). Nucleotides are also added to both ends of the mRNA that facilitate its transport and prevent premature degradation.

Each of the 46 chromosomes in a human cell contains a linear, continuous strand of double helical DNA. Each DNA molecule contains multiple sites of replication, a centromere that functions in separating chromosomes during mitosis and repeated nucleotide sequences (TTAGGG) at both ends of the DNA called **telomeres**. These special structures at the ends of DNA molecules prevent the loss of genetic information at each cycle of DNA replication. Because of the mechanics of DNA replication, some bases are lost at the ends of each molecule of DNA after each cycle of DNA replication. Thus, the telomeres protect against the loss of genetic information at each cycle of replication by binding to telomere binding proteins called **shelterin** proteins and forming shelterin complex, which protect telomere end structure, while the length of the telomeric repeats becomes shorter.

The DNA in telomeres can be restored and elongated by an enzyme called **telomerase**. Most somatic cells in an adult no longer produce active telomerase. As a result, telomeres are gradually lost during repeated cell divisions until the cell reaches a stage when it is no longer viable. Recent studies demonstrate that telomeres and telomerase play essential roles in the biology of cancer, stem cells, aging, and an inherited disorder, **dyskeratosis congenital (DKC)**. Known causes of DKC include mutations in telomere binding proteins, which result in the dysfunction of telomere protection mechanisms. Cells that contain serious defects in telomere length or in telomerase activity are destroyed by **apoptosis** (programmed cell death). Apoptosis is believed to be necessary during normal differentiation of tissues in development and for replacement of aging cells in the adult. Abnormalities in apoptosis may play a role in cancer; as many as 90% of cancer cells have reactivated telomerase which may contribute to the unregulated growth of tumors.

Apoptosis is a normal part of embryonic and postnatal development of the nervous system. Many cells become "gratuitous" after serving their function during development. After performing their necessary functions, they are removed by apoptosis. Thus, apoptosis is essential for normal development and for the removal of aging cells in the adult. However, failure to inhibit apoptotic activity in adult neural cells that are not replaced may be one of the causes of neurological diseases. Many chronic, neurological diseases are associated with neural cell death, including **amyotrophic lateral sclerosis**, **Alzheimer's disease**, **Parkinson's disease**, and **Huntington's disease**.

1.2.8 The body of an adult consists of more than 200 specialized types of cells

During the development of a human being (and other vertebrates), cells undergo **differentiation** and become specialized in both form and function as they assemble into specialized tissues and organs. Examples of major tissues in the body are epithelial tissue, connective tissue, muscle tissue, and nervous tissue. **Epithelial cells** aggregate into sheets of cells that line the inner and outer surfaces of the body. Depending on their location in the body, epithelial cells differentiate into secretory cells, ciliated cells, absorptive cells, and other cell types. The spaces between tissues and organs are filled with **connective cells** that provide a matrix for other organs and tissues. **Nervous tissue** consists of highly specialized cells in the brain and central nervous system that control all of the organism's activities and capabilities. **Muscle tissue** provides the mechanical force that allows movement.

In addition to these tissue systems, organs such as the liver, heart, kidney, ovary, testis, and pancreas possess specialized cells whose biochemical activities are specific to each organ. All cellular activities must be regulated and integrated with the functions of other tissues and organs for optimal health of the individual. **Understanding the chemical activities of specialized cells and tissues, their molecular interactions with other cells and tissues, and how aberrations produce disease is the subject of medical biochemistry**.

1.2.9 Stem cells are a renewable source of specialized cells

Embryonic stem cells are derived from the inner cell mass of 5- to 10-day-old blastocysts. These cells are pluripotent and are capable of differentiating into any specialized cell in the body. Human embryonic stem cells have been obtained by transferring a somatic cell (skin cells) nucleus into denucleated human oocytes. Stem cells can also be derived from fetuses, umbilical cord blood, the placenta, or from adult tissues. Stem cells from these sources may be less than pluripotent. When cultured under favorable conditions, embryonic stem cells have the potential to develop into stable lines of differentiated cell types. The major goal of all stem cell research is to develop techniques for propagating specific cells from an individual that can be used to treat diseases, for example, replacing pancreatic islet cells from a person with type 1 diabetes.

Stem cells have three characteristics that distinguish them from other cells: (1) the capacity for self-renewal; at cell division one or both daughter cells have the same biological properties as the parent cell; (2) the capacity to develop into multiple types of cell lineages; (3) the potential to proliferate indefinitely. Hematopoietic stem cells migrate through the blood, across endothelial vasculature, and end up in specific organs and bone marrow. Migration of stem cells from blood to destined tissue is a complex process called **homing**, which involves stress signals and other factors.

The controversy over stem cell research derives from the origin of cells used for research or treatment, namely, embryos created *in vitro* or aborted fetuses. However, a noncontroversial source of stem cells is umbilical cord blood, which is usually discarded after birth. Stem cells harvested from cord blood have been used in the treatment of leukemia and lymphoma. Multifaceted and multidisciplinary approaches are underway to produce pluripotent stem cells from noncontroversial sources.

One of the methods involves the following steps: the induction of pluripotent stem cells from fibroblasts (iPSCs) obtained from a person with a genetic defect, correction of the genetic defect, reprogramming of the corrected stem cells to the desired somatic cells, and finally the reintroduction of repaired somatic cells to the original person from which the fibroblasts were obtained (Fig. 1.8). This concept has been accomplished in part in α_1-antitrypsin (α_1-AT) deficiency. Fibroblasts obtained from α_1-AT deficient human are converted to iPSCs and, after correction of the genetic defect, the cells are reprogrammed to hepatocytes and injected into mice. In the injected mice with corrected human hepatocytes, they expressed normal α_1-AT [13].

Another avenue of research involves obtaining stem cells from human tooth pulp. Injection of these cells into transected rat spinal cord showed functional improvements [14].

In studies using rodents [15,16], it has been shown that the fibroblasts of injured myocardium can be reprogrammed into becoming normal cardiomyocytes by administration of exogenous transcription factors and micro-RNA (Chapter 22).

All of these studies are aimed at obtaining readily available immunologically compatible stem cells by various methods for the therapy of many diseases.

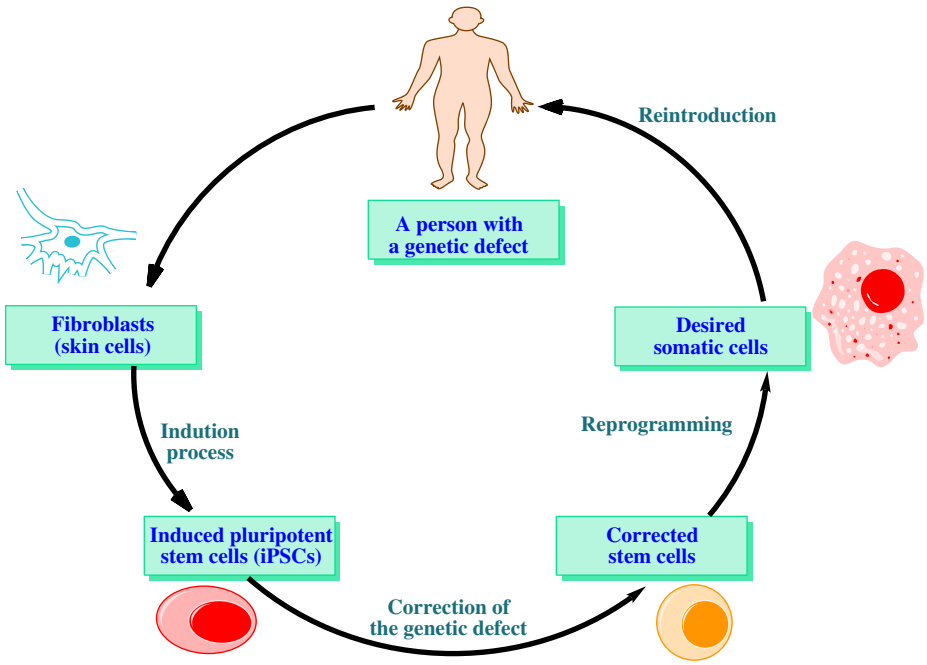

FIGURE 1.8 A model for correction of monogenic defect using patients' own fibroblasts. This method uses iPSCs derived from human α1-antitrypsin (α_1-AT) fibroblasts after the correction of the genetic defect and converts them to hepatocytes, the physiologic source of α_1-AT. The injected reprogrammed hepatocytes into mice were found in the liver and synthesized α_1-AT (9).

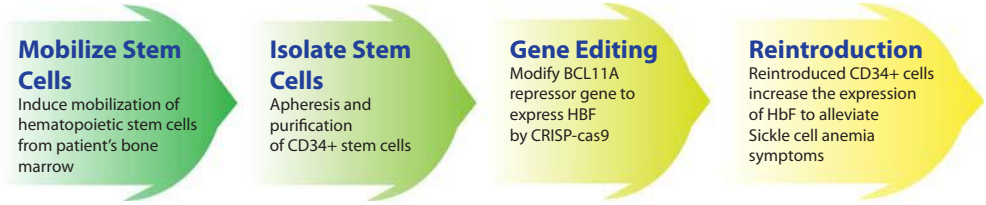

FIGURE 1.9 Steps involved in the activation of gamma-globin gene by using CRISPR-cas9 method. *CRISPR*, Clustered regularly interspaced short palindromic repeat.

With the advancement of new gene-editing technologies using prokaryotic defense system against bacteriophage infections commonly referred to as clustered regularly interspaced short palindromic repeats (CRISPR) sequence guided DNA scissors, new attempts have been made to alleviate genetic diseases such as sickle cell disease (SCD) and beta-thalassemia (BT). Recent studies with SCD and BT patients reported that CRISPR-cas9 method was successfully used to disable a repressor gene (BCL11A) for gamma-globin subunits and induced the activation of gamma-globin gene expression, thereby increasing the production of fetal hemoglobin (HbF) to relieve the symptoms of SCD and BT. (Fig. 1.9) [17,18].

Key points

1. The human organism is hierarchically organized; at the highest level, it is organized into organ systems classically related to functions and anatomical structures.
2. Distinctions among organs are the consequence of their specialized tissues and cells that are produced during embryonic differentiation, a process that begins after fertilization of an ovum by a spermatozoon and continues for some organs for a time postpartum.
3. Cells are the basic building blocks for organs and tissues in all living systems.
4. Biochemical processes within cells, and thus within organ systems, include metabolism, growth, reproduction, mutation, response, self-destruction, and evolution.
5. The body of an adult consists of more than 200 differentiated and thus specialized cell types.
6. Specialized structures with functional distinctions, organelles, exist with cells and constrain particular biological processes that create unique functions that confer metabolic efficiency and cellular integrity. Intracellular

organelles work interdependently to degrade, synthesize, transport, and excrete intracellular products.

7. Cellular shape and ability to exchange substances with the circulatory and other transport systems are derived from the cellular membranes and intracellular membranous structures.
   a. Mitochondria are the primary sources of energy for cells, organs, and the living animal.
   b. The nucleus provides the genetic and genetic expression systems that enable growth, cell division, and cell differentiation.
8. Stem cells, a unique precursor to all cell types, tissues, and organ types, are capable of repairing damaged or defective cells, tissues, and organs.
9. The human body is colonized by microorganisms at several locations. The microbiota is primarily in the distal intestine within the gastrointestinal tract. Gastrointestinal microbes contribute to the host as both symbiotic and pathogenic agents in many physiological processes.

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Multiple-choice questions

1. Cerebrohepatorenal syndrome, also known as Zellweger's syndrome, is one of a family of disorders called leukodystrophies and caused by defective cellular organelle functions. Which of the following cellular organelles is responsible for the formation of the defective organelles commonly found in Zellweger's syndrome?

   A. Mitochondria
   B. Lysosome
   C. Peroxisome
   D. Endoplasmic reticulum (ER)
   E. Ribosome

2. Mitochondrial fusion and fission processes are important in maintaining the quality and quantity of cellular mitochondria pool. Many proteins participate in the processes of fusion and fission. Which of the following GTPase proteins is a key player in the mitochondrial fission process and its defects causes various metabolic abnormalities found in diseases such as Alzheimer's and Parkinson's diseases?

A. Fibronectin
B. Voltage-dependent anion transporter
C. Mitofusin 1 (Mfn1)
D. Optic atrophy a (Opa1)
E. Dynamin-related protein 1 (Drp1)

3. The one major difference between prokaryotes and eukaryotes is that eukaryotic cells contain highly compartmentalized cellular organelles. The compartmentalization provided the advantages of having very efficient separation of metabolite synthesis and breakdown. However, many cellular metabolite synthesis processes rely on the reactions taking places at different compartments. Which of the following biochemical pathways takes places partly in the mitochondria and partly in the cytosol?

A. Beta-oxidation of fatty acids
B. Glycolysis
C. Gluconeogenesis
D. Glycogen synthesis
E. Glycogen breakdown

4. Dyskeratosis congenita (DKC), also known as Zinsser−Cole−Engman syndrome, is a rare genetically inherited disorder, which is caused by mutations in DKC1 gene. The normal DKC1 proteins are involved in the maintenance of telomere by recruiting telomerase, which is responsible for adding extra telomere repeat sequence by using its internal RNA template RNA sequence. Which of the following repeat sequences is extended by the enzyme telomerase?

A. TTAGGG
B. GAATTC
C. GGGAAT
D. GGGTTA
E. CAGCAG

5. Transmembrane or secretory proteins are synthesized in the rough ER and transported to the Golgi apparatus for further glycosylation and modifications. Which of the following modifications happen in the Golgi for the proteins destined to be transported into lysosomes?

A. Phosphorylation
B. Addition of glucose 6-phosphate
C. Addition of fructose 6-phosphoate
D. Addition of mannose 6-phosphate
E. Addition of glucose 1-phosphate

Answers: 1. D, 2. E, 3. C, 4. A, 5. D

Chapter 2

Water, acid-base, buffers, and homeostatic control systems of body fluids

Learning objectives

After studying this chapter, you will be able to:

1. understand the colligative properties of water and why they are essential for providing the vital environment for life;
2. understand the structure of water and the nature of hydrogen bonding, which is crucial in forming molecular structures and hydrophilic and hydrophobic interactions;
3. understand the definition of pH and important buffer systems of the body, which resist the changes of pH to provide a stable environment for various metabolic processes;
4. understand the Henderson–Hasselbalch equation, the relationship of pH changes to the differences in the ratio of conjugate base and acid, and be able to apply the equation to determine pH values based on a given concentration of buffering agents;
5. understand how the bicarbonate buffer system of the blood works, and its role in oxygen delivery to tissues and carbon dioxide delivery to the lungs;
6. understand the homeostatic control systems of the body to maintain constant water, electrolyte, and acid–base balance; and
7. understand the effects of the dysfunctional homeostasis in maintaining blood volume, osmolality, and acid–base balance.

2.1 Properties of water

Acid and base concentrations in living systems are carefully regulated to maintain conditions compatible with life. Biochemical reactions involving acids and bases occur in the body water, whereas buffer systems protect the body from significant variations in the concentrations of acids and bases. This chapter introduces basic concepts of the properties of water, acids, bases, and buffers.

Life cannot be sustained without water. Water constitutes 45%–73% of total human body weight. It is distributed in intracellular (55%) and extracellular (45%) compartments and provides a continuous solvent phase between body compartments. As the biological solvent, water plays a major role in all aspects of metabolism: absorption, transport, digestion, and excretion of inorganic and organic substances, as well as maintenance of body temperature. The unique properties of water are inherent in its structure.

2.1.1 Hydrogen bonding

Water (H_2O) is a hydride of oxygen in which the highly electronegative oxygen atom attracts the bonding electrons from two hydrogen atoms. This leads to polar H–O bonds in which the hydrogen atoms have a slightly positive charge (δ^+) and the oxygen atom has a slightly negative charge (δ^-) (Fig. 2.1). Water molecules have a relatively high dipole moment because of the angle (104.5 degrees) of the H–O–H bond and the polarity of the bonds. Neighboring liquid water molecules interact with one another to form an extensive lattice-like structure, similar to the structure of ice. The intermolecular bonding between water molecules arises from the attraction between the partial negative charge on the oxygen atom and the partial positive charge on the hydrogen atom of adjacent water molecules. This type of attraction involving a hydrogen atom is known as a **hydrogen bond** (Fig. 2.2).

Hydrogen bonds contain a hydrogen atom between two electronegative atoms (e.g., O and N). One is the formal hydrogen donor; the other is the hydrogen acceptor. The amount of energy required to break a hydrogen bond (bond energy) is estimated to be 2–5 kcal/mol (8.4–20.9 kJ/mol) in the gas phase. Covalent bonds have bond energies of 50–100 kcal/mol (209–418 kJ/mol). The cumulative effect of many hydrogen bonds is equivalent to the stabilizing effect of covalent bonds. In proteins, nucleic acids, and water, hydrogen bonds are essential to stabilize overall structure. In ice, each water molecule forms a hydrogen bond with four other water molecules, giving rise to a rigid tetrahedral arrangement (Fig. 2.3). In the liquid state, water maintains a tetrahedrally coordinated structure over short ranges and for short time periods.

Essentials of Medical Biochemistry. DOI: https://doi.org/10.1016/B978-0-323-88541-6.00034-X

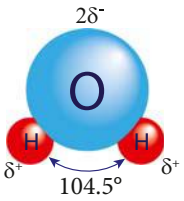

FIGURE 2.1 Structure of the water molecule.

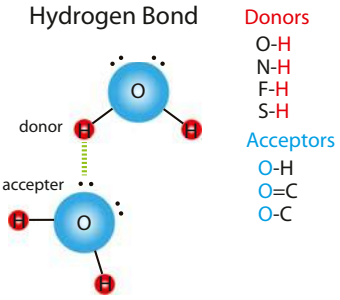

FIGURE 2.2 A hydrogen bond between water molecules. Possible hydrogen bond donors and acceptors are also listed.

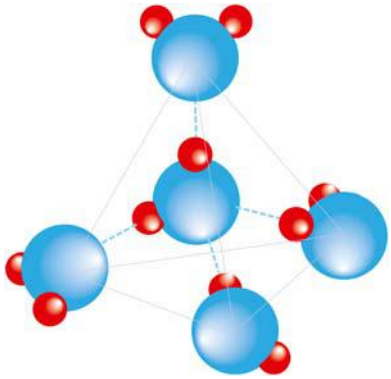

FIGURE 2.3 Tetrahedral hydrogen-bonded structure of water molecules in ice. The tetrahedral arrangement is due to the fact that each water molecule has four fractional charges as a consequence of the polarity of the O−H bonds: two partial negative charges on the oxygen atom and one partial positive charge on each of the two hydrogen atoms. In the liquid phase this tetrahedral array occurs transiently.

2.1.2 Physical properties

Properties of water uniquely suited to biological systems include the melting point, boiling point, heat of vaporization (quantity of heat energy required to transform 1 g of liquid to vapor at the boiling point), heat of fusion (quantity of heat energy required to convert 1 g of solid to liquid at the melting point), specific heat (the amount of heat required to raise the temperature of 1 g of substance by 1°C), and surface tension. All these values for water are much higher than those for other low-molecular-weight substances, because of the strong intermolecular hydrogen bonding of water. These properties contribute to the maintenance of temperature and dissipation of heat in living systems. Thus, water plays a major role in thermoregulation in living systems. The optimal body temperature is a balance between heat production and heat dissipation. Impaired thermoregulation causes either **hypothermia or hyperthermia**, and has serious metabolic consequences; if uncorrected, impaired thermoregulation may lead to death. Maintenance of therapeutic hypothermia at 32°C−34°C for 12−24 hours has been employed to improve neurologic outcomes after cardiac arrest [1].

Water is transported across cell membranes in one of two ways: by simple diffusion through the phospholipid bilayer and by the action of membrane-spanning transport proteins known as **aquaporins** (Fig. 2.4).

Thus, the concentration of water is in thermodynamic equilibrium across the cell membrane. In the renal collecting duct, water is reabsorbed through a specific aquaporin channel protein (aquaporin 2). This reabsorption of water is regulated by the antidiuretic hormone (ADH, also known as **vasopressin**). A defect or lack of functional aquaporin 2, vasopressin, or its receptor leads to enormous loss of water in the urine, causing the disease known as **diabetes insipidus**. Water plays a significant role in enzyme functions, molecular assembly of macromolecules, and allosteric regulation of proteins. For example, the effect of protein solvation in allosteric regulation is implicated in the transition of deoxyhemoglobin to oxyhemoglobin. During this process about 60 extra water molecules bind to oxyhemoglobin.

2.1.3 Solutes, micelles, and hydrophobic interactions

Water is an excellent solvent for both ionic compounds (e.g., NaCl) and low-molecular-weight nonionic polar compounds (e.g., sugars and alcohols). Ionic compounds are soluble because water can overcome the electrostatic attraction between ions through solvation of the ions. Nonionic polar compounds are soluble because water molecules can form hydrogen bonds to polar groups (e.g., −OH).

Amphipathic compounds, which contain both large nonpolar hydrocarbon chains (hydrophobic groups) and polar or ionic groups (hydrophilic groups), may associate with each other in submicroscopic aggregations called **micelles** (Fig. 2.5). Micelles have hydrophilic (water-liking) groups on their exterior (bonding with solvent water), and hydrophobic (water-disliking) groups clustered in their interior. They occur in spherical, cylindrical, or ellipsoidal shapes. Micelle structures are stabilized by hydrogen bonding with water, by van der Waals attractive forces between hydrocarbon groups in the interior, and by the energy of hydrophobic reactions (Fig. 2.6). As with

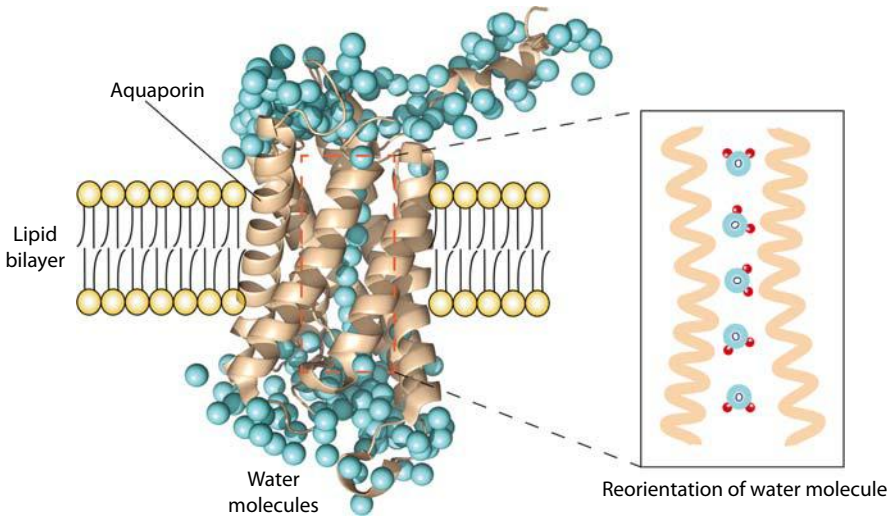

FIGURE 2.4 Water transport across the plasma membrane through aquaporin. Aquaporin consists of six transmembrane helices and forms a very distinctive channel geometry, which only allows a single column of water molecules to pass through. When water molecules move through the aquaporin transmembrane channel, hydrogen bonding interactions with amino acids positioned throughout the channel force the water molecules to reorient their direction in the middle of the channel, which prevents proton transfer between them. The aquaporin structure in the figure was based on the pdb file pdb ID:3zoj.

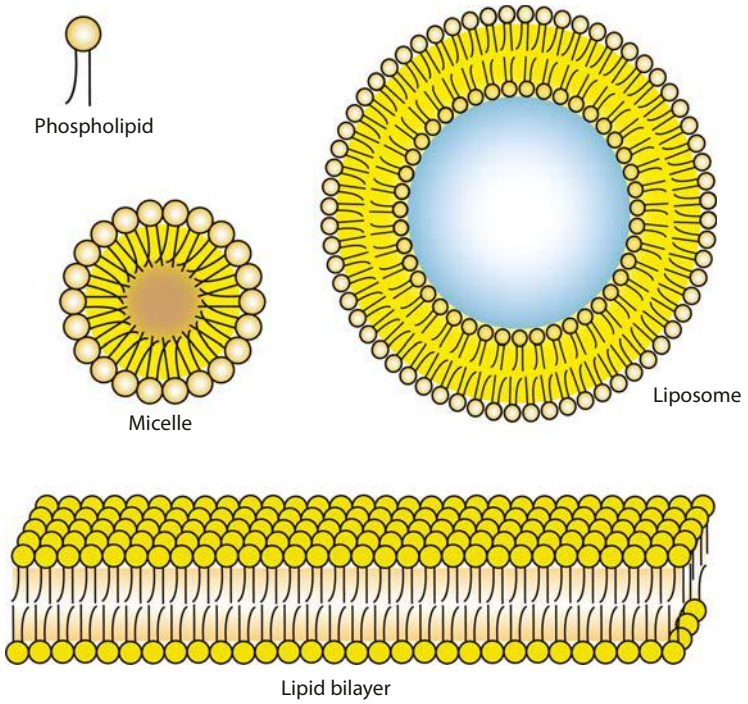

FIGURE 2.5 Schematic illustration of micelle, liposome, and lipid bilayer. Amphipathic phospholipid molecules can form a micelle when a single layer of phospholipids is exposed to an aqueous environment. On the other hand, a double layer or bilayer of phospholipids forms a liposome structure in water.

hydrogen bonds, each hydrophobic interaction is very weak, but many such interactions result in the formation of large, stable structures.

Hydrophobic interaction plays a major role in maintaining the structure and function of cell membranes, the activity of proteins, the anesthetic action of nonpolar compounds such as chloroform and nitrous oxide, the absorption of digested fats, and the circulation of hydrophobic molecules in the interior of micelles in blood plasma.

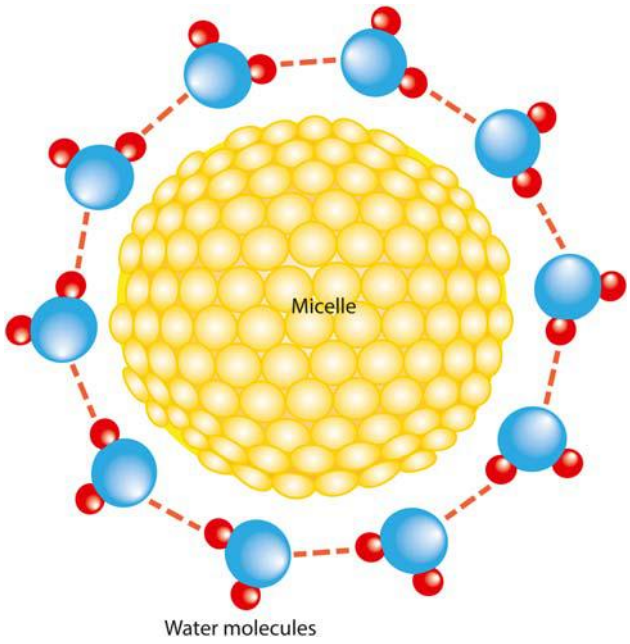

FIGURE 2.6 Hydrophobic and hydrophilic interactions between a micelle and water molecules.

2.1.4 Colligative properties

The colligative properties of a solution depend on the concentration of solute particles. These properties include freezing point depression, vapor pressure depression, osmotic pressure, and boiling point elevation. The freezing point of water is depressed by 1.86°C when 1 mol of nonvolatile solute, which neither dissociates nor associates in solution, is dissolved in 1 kg of water. The same concentration of solute elevates the boiling point by 0.543°C. Osmotic pressure is a measure of the tendency of water molecules to migrate from a dilute to a concentrated solution through a semipermeable membrane. This migration of water molecules is termed **osmosis**. A solution containing 1 mol of solute particles in 1 kg of water is a 1 osmolal solution. When 1 mol of a solute (such as NaCl) that dissociates into two ions (Na^+ and Cl^-) is dissolved in 1 kg of water, the solution is 2 osmolal.

Measurement of colligative properties is useful in estimating solute concentrations in biological fluids. For example, in blood plasma, the normal total concentration of solutes is remarkably constant (275–295 milliosmolal). Pathological conditions (e.g., dehydration, renal failure) involving abnormal plasma osmolality are discussed later in this chapter.

2.1.5 Dissociation of water and the pH scale

Water dissociates to yield a hydrogen ion (H^+) and a hydroxyl ion (OH^-).

$$H_2O \rightleftharpoons H^+ + OH^- \tag{2.1}$$

The H^+ bonds to the oxygen atom of an undissociated H_2O molecule to form a hydronium ion (H_3O^+).

$$H_2O + H_2O \rightleftharpoons H_3O^+ + OH^-$$

Thus, water functions as both an acid (donor of H^+ or proton) and as a base (acceptor of H^+ or proton). This description of an acid and a base follows from the Bronsted–Lowry theory. According to the Lewis theory, acids are electron pair acceptors and bases are electron pair donors. The equilibrium constant, K, for the dissociation reaction in Eq. (2.1) is:

$$K = \frac{[H^+][OH^-]}{[H_2O]} \tag{2.2}$$

where the square brackets refer to the molar concentrations of the ions involved. K can be determined by measurement of the electrical conductivity of pure water, which has the value of 1.8×10^{-16} M at 25°C, indicative of a very small ion concentration, where M (molar) is the units of moles per liter. Therefore, the concentration of undissociated water is essentially unchanged by the dissociation reaction.

Since 1 L of water weighs 1000 g and 1 mol of water weighs 18 g, the molar concentration of pure water is 55.5 M. Substitution for K and $[H_2O]$ in Eq. (2.2) yields:

$$[H^+][OH^-] = (55.5\ \text{M}) \times \left(1.8 \times 10^{-16}\text{M}\right)$$

$$[H^+][OH^-] = 1.0 \times 10^{-14}\text{M}^2 = K_w$$

K_w is known as the **ion product of water**. In pure water, $[H^+]$ and $[OH^-]$ are equal, so that:

$$[OH^-] = [H^+] = 1.0 \times 10^{-7}\text{M}$$

pH is employed to express these ion concentrations in a convenient form, where the "p" of pH symbolizes "negative logarithm (base 10)" of the concentration in question (see Table 2.1). Thus,

$$\text{pH} = -\log_{10}[H^+] = \log\frac{1}{[H^+]}$$

Similarly,

$$\text{pOH} = -\log_{10}[OH^-] = \log\frac{1}{[OH^-]}$$

Therefore, for water:

$$\log[H^+] + \log[OH^-] = \log\ 10^{-14}$$

or

$$\text{pH} + \text{pOH} = 14.$$

The pH value of 7 for pure water at 25°C is considered to be neutral, values below 7 are considered acidic, and values above 7 are considered basic. It is

TABLE 2.1 The pH scale.

$[H^+]$ (M)	pH	$[OH^-]$ (M)
Acidic		
10.0	−1	10^{-15}
1.0	0	10^{-14}
0.1	1	10^{-13}
0.01 (10^{-2})	2	10^{-12}
10^{-3}	3	10^{-11}
10^{-4}	4	10^{-10}
10^{-5}	5	10^{-9}
10^{-6}	6	10^{-8}
Neutral		
10^{-7}	7	10^{-7}
Basic		
10^{-8}	8	10^{-6}
10^{-9}	9	10^{-5}
10^{-10}	10	10^{-4}
10^{-11}	11	10^{-3}
10^{-12}	12	0.01
10^{-13}	13	0.1
10^{-14}	14	1
10^{-15}	15	10

important to recognize that as the pH decreases, $[H^+]$ increases. A decrease in one pH unit reflects a 10-fold increase in H^+ concentration. In discussions of acid−base problems in human biochemistry, it is often preferable to express H^+ concentration as nanomoles per liter (nmol/L).

2.2 Buffers

Buffers resist changes in pH in solutions when acids or bases are added. They are either a mixture of a weak acid (HA) and its conjugate base (A^-) or a mixture of a weak base (B) and its conjugate acid (HB^+).

2.2.1 Henderson−Hasselbalch equation

The Henderson−Hasselbalch equation was developed independently by the American biological chemist L. J. Henderson and the Swedish physiologist K. A. Hasselbalch, for relating the pH to the bicarbonate buffer system of the blood (see next). In its general form, the Henderson−Hasselbalch equation is a useful expression for buffer calculations.

It can be derived from the equilibrium constant expression for a dissociation reaction of the general weak acid (HA) in Eq. (2.3):

$$K = \frac{[H^+][A^-]}{[HA]} \tag{2.3}$$

where K is the equilibrium constant at a given temperature. For a defined set of experimental conditions, this equilibrium constant is designated as K′ (K prime) and referred to as an apparent dissociation constant. The higher the value of K′, the greater the number of H^+ ions liberated per mole of acid in solution and hence the stronger the acid. K′ is thus a measure of the strength of an acid. Rearrangement of Eq. (2.3) yields

$$[H^+] = \frac{K'[HA]}{[A^-]} \tag{2.4}$$

Taking logarithms of both sides of Eq. (2.4) and multiplying throughout by −1 gives

$$-\log[H^+] = -\log K' - \log[HA] + \log[A^-] \tag{2.5}$$

Substituting pH for $-\log[H^+]$ and pK′ for $-\log K'$ yields

$$pH = pK' + \log\frac{[A^-]}{[HA]} \tag{2.6}$$

or

$$pH = pK' + \log\frac{[\text{conjugate base}]}{[\text{acid}]} \tag{2.7}$$

This relationship represents the Henderson−Hasselbalch equation.

Since a buffer is intended to give only a small change in pH with added H^+ or OH^-, the best buffer for a given pH is the one that gives the smallest change. As may be seen from the Henderson−Hasselbalch equation, when the pH of the solution equals the pK′ of the buffer, [conjugate base] = [acid], the buffer can respond equally to both added acid and added base. The Henderson−Hasselbalch relation for a weak base can be derived and applied using similar reasoning.

For a weak acid, if the pH is one unit below its pK′ value, the solution contains approximately 91% of the unionized species (protonated, HA). Conversely, if the pH is one unit above its pK′ value, the solution contains 91% of the ionized species (unprotonated, A^-). For a weak base, if the pH is one unit below its pK′ value, the solution contains approximately 91% of the ionized species (protonated, BH^+). Conversely, if the pH is one unit above its pK′ value, the solution contains approximately 91% of unionized species (unprotonated, B).

2.2.2 Buffer systems of blood and exchange of O_2 and CO_2

If the H^+ concentration deviates significantly from its normal level in blood, the health and survival of the human body are in jeopardy. H^+ is the smallest ion, and it combines with many negatively charged and neutral functional groups. Changes of $[H^+]$ therefore, affect the charged regions of many molecular structures, such as enzymes, cell membranes, and nucleic acids, and dramatically alter their physiological activity. If the plasma pH reaches either 6.8 or 7.8, death may be unavoidable. Despite the fact that large amounts of acidic and basic metabolites are produced and eliminated from the body, buffer systems maintain a fairly constant pH in body fluids.

The major metabolic product from oxidation of ingested carbon compounds is CO_2. Hydration of CO_2 dissolved in water yields the weak acid H_2CO_3 (carbonic acid). Depending on the type of food ingested and oxidized, 0.7−1.0 mol of CO_2 is produced per mole of O_2 consumed. This results in the metabolic production of about 13 mol of hydrated CO_2 each day in a normal person.

For efficient transport of relatively insoluble CO_2 from the tissues where it is formed to the lungs where it must be exhaled, the carbonic anhydrase of red blood cells converts CO_2 to the very soluble anionic form HCO_3^- (bicarbonate ion). The principal buffers in blood are bicarbonate−carbonic acid in plasma, hemoglobin in red blood cells, and protein functional groups in both. The normal balance between rates of elimination and production of CO_2 yields a steady-state concentration of CO_2 in the body fluids and a relatively constant pH.

Other acids that are products of metabolism are lactic acid, acetoacetic acid, β-hydroxybutyric acid, phosphoric acid, sulfuric acid, and hydrochloric acid. The organic acids (e.g., lactate, acetoacetate, and β-hydroxybutyrate) are normally oxidized further to form CO_2 and H_2O. The hydrogen ions and anions contributed by mineral acids and any unmetabolized organic acids are eliminated via the excretory system of the kidneys. Thus, although body metabolism produces a large amount of acid, a constant pH is maintained by transport of H^+ ions and other acid anions in buffer systems, elimination of CO_2 through alveolar ventilation in the lungs, and excretion of aqueous acids in the urine.

Metabolic activities continuously release CO_2 to the blood (Fig. 2.7), and the lungs continuously eliminate CO_2 (Fig. 2.8). As oxygen is consumed in peripheral tissues, CO_2 is formed and its pressure (P_{CO_2}) builds to about 50 mmHg, whereas the blood entering the tissue capillaries has a P_{CO_2} of about 40 mmHg. Because of this difference in P_{CO_2} values, CO_2 diffuses through the cell membranes of the capillary endothelium and the blood P_{CO_2} rises to 45−46 mmHg. Despite this increase in P_{CO_2}, the blood pH value drops by only about 0.03 during the flow from the arterial capillary (pH 7.41) to the venous capillary (pH 7.38) as a consequence of buffering.

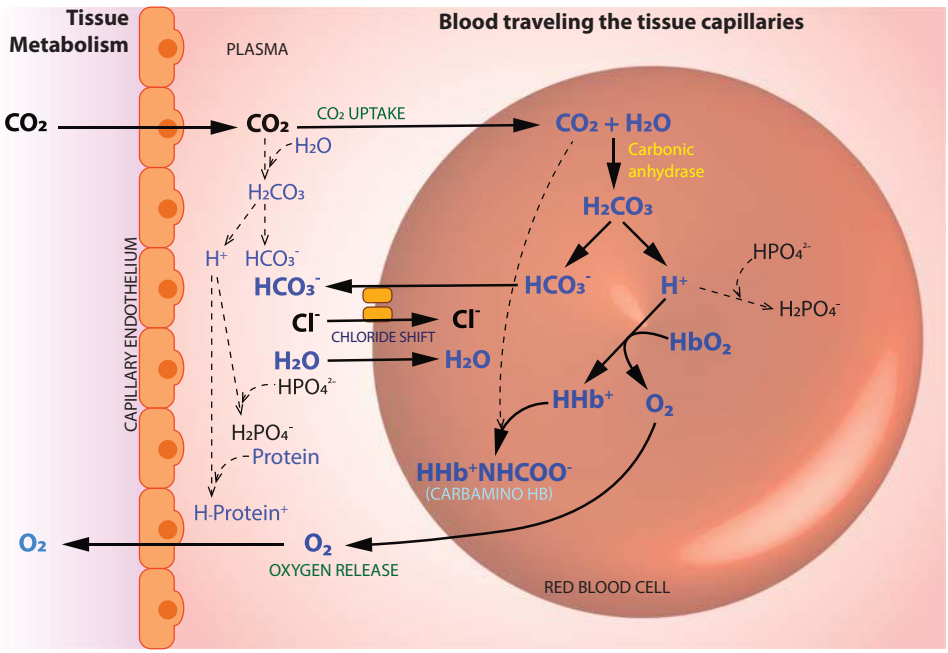

FIGURE 2.7 Schematic representation of the transport of CO_2 from the tissues to the blood. Note that most of the CO_2 is transported as HCO_3^- in the plasma, and that the principal buffer in the red blood cell is hemoglobin. Solid lines refer to major pathways, and broken lines refer to minor pathways. *Hb*, Hemoglobin.

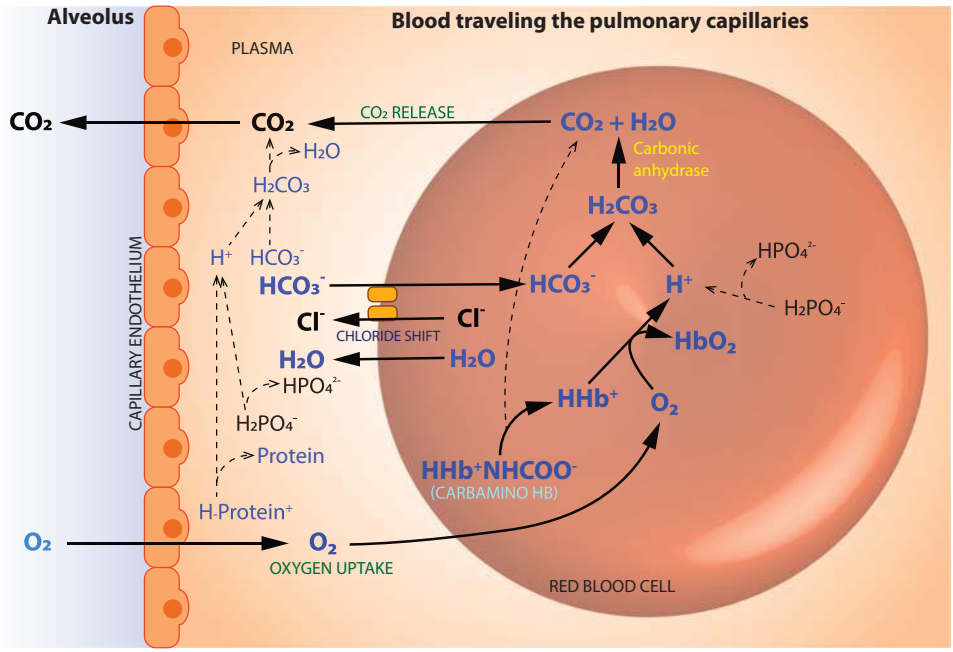

FIGURE 2.8 Schematic representation of the transfer of CO_2 from the alveolus (and its loss in the expired air in the lungs) and oxygenation of hemoglobin. Note that the sequence of events occurring in the pulmonary capillaries is the opposite of the process taking place in the tissue capillaries (Fig. 2.7). Solid lines indicate major pathways and broken lines indicate minor pathways. *Hb*, Hemoglobin.

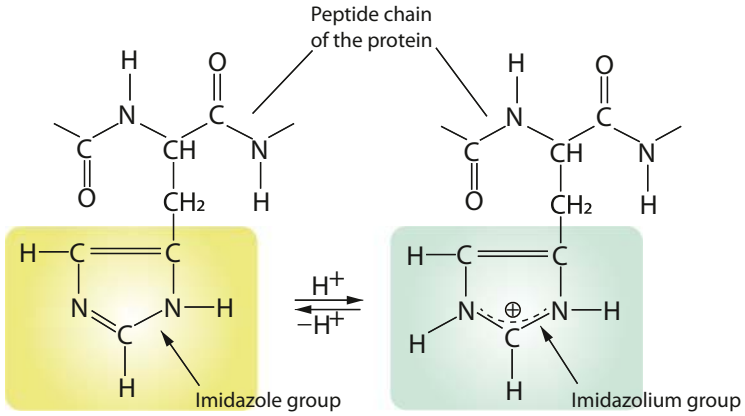

FIGURE 2.9 Buffer function of the imidazole/imidazolium groups of histidine residues in protein.

About 95% of the CO_2 entering the blood diffuses into the red blood cells where the enzyme carbonic anhydrase catalyzes the conversion of most of the CO_2 to H_2CO_3:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3$$

H_2CO_3 dissociates into H^+ and HCO_3^-. Although H_2CO_3 is a weak acid, its dissociation is essentially 100% because of the removal of H^+ ions by the buffering action of hemoglobin. The presence of CO_2 and the production of H^+ cause a reduction in the affinity of hemoglobin for oxygen. Oxyhemoglobin (HbO_2) consequently dissociates into oxygen and deoxyhemoglobin (Hb). This effect of pH on the binding of O_2 to hemoglobin is known as the **Bohr effect**.

Oxygen diffuses into the tissues because the P_{O_2} in the blood is greater than the P_{O_2} in tissue cells, and because protonated deoxyhemoglobin (HHb^+) is a weaker acid than HbO_2 and thereby binds H^+ more strongly than HbO_2. When purified HbO_2 dissociates at pH 7.4 to yield oxygen and Hb, the Hb binds 0.7 mol of H^+ per mole of oxygen released. However, under physiological conditions in whole blood, the Hb binds 0.31 mol of H^+ per mole of oxygen released. This process is reversible. The remainder of the H^+ is buffered by phosphate and proteins other than hemoglobin. The main buffering group involved in the transport of H^+ is an imidazolium group of a histidine residue in hemoglobin (Fig. 2.9). The imidazolium group has a pK′ value of about 6.5 (Chapter 3). The difference

in acid–base properties between the two forms of hemoglobin molecules is explained by the conformational change that accompanies conversion from HbO_2 to HHb^+ (Chapters 5 and 25).

As the concentration of HCO_3^- (i.e., of metabolic CO_2) in red blood cells increases, an imbalance occurs between the bicarbonate ion concentrations in the red blood cell and plasma. This osmotic imbalance causes a marked efflux of HCO_3^- to plasma and a consequent influx of Cl^- from plasma, in order to maintain the balance of electrostatic charges. The latter osmotic influx, known as the **chloride shift**, is accompanied by the migration of water to red blood cells. Thus, transport of metabolic CO_2 in the blood occurs primarily in the form of plasma bicarbonate formed after CO_2 diffuses into red blood cells.

A small percentage of CO_2 entering the red blood cells combines reversibly with an unionized amino group ($-NH_2$) of hemoglobin:

$$\begin{array}{l}\text{hemoglobin}\\ \quad|\\ \text{NH}_2\end{array} + CO_2 \rightleftharpoons \begin{array}{l}\text{hemoglobin}\\ \quad|\\ \text{NH}-\text{COO}^-\end{array} + H^+$$

Hemoglobin–NH–COO^-, commonly known as **carbaminohemoglobin**, is more correctly named hemoglobin carbamate. The formation of this compound also causes a lowering of the affinity of hemoglobin for oxygen. Thus, an elevated concentration of CO_2 favors dissociation of oxyhemoglobin to oxygen and deoxyhemoglobin. Conversely, CO_2 binds more tightly to deoxyhemoglobin than to oxyhemoglobin. All of these processes occurring in the red blood cells of peripheral capillaries are functionally reversed in the lungs (Fig. 2.4). Since alveolar P_{O_2} is higher than that of the incoming deoxygenated blood, oxygenation of hemoglobin and release of H^+ occur. The H^+ release takes place because HbO_2 is a stronger acid (i.e., has a lower pK′) than deoxyhemoglobin. The released bicarbonate, which is transported to the red blood cells with the corresponding efflux of Cl^-, combines with the released H^+ to form H_2CO_3. Cellular carbonic anhydrase catalyzes dehydration of H_2CO_3 and release of CO_2 from the red blood cells.

Thus, **red blood cell carbonic anhydrase, which catalyzes the reversible hydration of CO_2, plays a vital role in carbon dioxide transport and elimination**. Carbonic anhydrase is a monomeric (Molecular Weight, MW 29,000) zinc metalloenzyme and is present in several different isoenzyme forms (Fig. 2.10). The most prevalent red blood cell isoenzyme of carbonic anhydrase is type I (CA I). The zinc ion, held by coordinate covalent linkages with three imidazole groups of three histidine residues, is involved in the catalytic mechanism of carbonic anhydrase. Water bound to the zinc ion reacts with CO_2 bound to the nearby catalytic site of carbonic anhydrase to produce H_2CO_3. The action of carbonic anhydrase is essential for a number of metabolic functions. Some include the formation

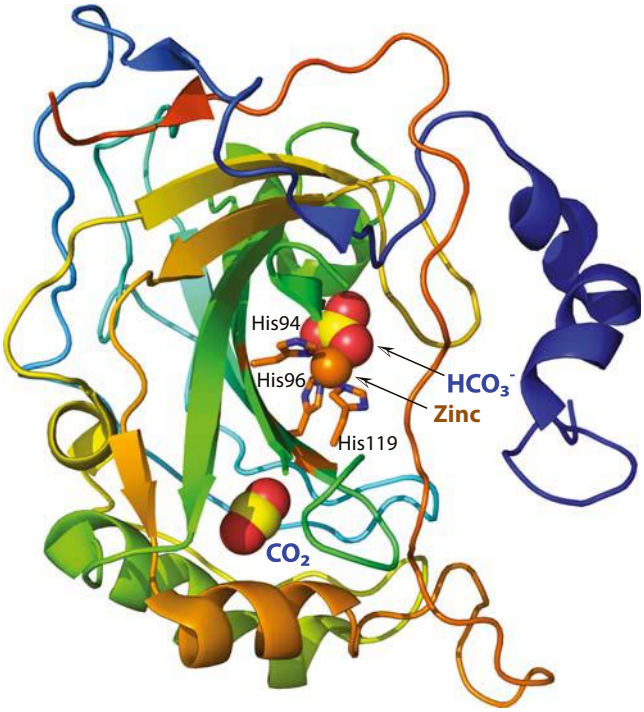

FIGURE 2.10 Structure of carbonic anhydrase. The zinc ion in the catalytic center is held by three histidine residues and plays a key role in coordinating water and CO_2 to form H_2CO_3. The enzyme structure was generated by using pdb file ID:2vvb.

of H^+ ion in stomach parietal cells (Chapter 10), bone resorption by osteoclasts (Chapter 33), and reclamation of HCO_3^- in renal tubule cells discussed later in this chapter. In osteoclasts and in the renal tubule cells, the isoenzyme carbonic anhydrase II (CA II) catalyzes the hydration reaction of CO_2. CAII deficiency is an autosomal recessive disorder that results in osteopetrosis (marble bone disease), renal tubular acidosis, and cerebral calcification.

The diffusion of CO_2 from venous blood into the alveoli is facilitated by a pressure gradient of CO_2 between the venous blood (45 mmHg) and the alveoli (40 mmHg), and by the high permeability of the pulmonary membrane to CO_2. Blood leaving the lungs has a P_{CO_2} of about 40 mmHg; thus, essentially complete equilibration occurs between alveolar CO_2 and blood CO_2.

2.2.3 Blood buffer calculations

Carbonic acid has the following pK values:

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \quad pK_1' = 3.8 \qquad (2.8)$$

$$HCO_3^- \rightleftharpoons CO_3^{2-} + H^+ \quad pK_2' = 10.2 \qquad (2.9)$$

It is apparent from the pK′ values that neither equilibrium can serve as a buffer system at the physiological pH of 7.4. However, carbonic acid (the proton donor) is in

equilibrium with dissolved CO_2, which in turn is in equilibrium with gaseous CO_2:

$$H_2O + CO_2(\text{aqueous}) \rightleftharpoons H_2CO_3 \tag{2.10}$$

The hydration reaction (2.10), coupled with the first dissociation of carbonic acid (2.8), produces an apparent pK′ of 6.1 for bicarbonate formation. Thus, the summation of Eqs. (2.8) and (2.10) yields:

$$H_2O + CO_2 \rightleftharpoons H^+ + HCO_3^- \tag{2.11}$$

$$\text{pK}'(\text{apparent}) = \frac{[HCO_3^-][H^+]}{[H_2CO_3]} = 6.1 \tag{2.12}$$

The ratio of HCO_3^- to H_2CO_3 at a physiological pH of 7.4 can be calculated by means of employing the Henderson–Hasselbalch equation:

$$7.4 = 6.1 + \log\frac{[HCO_3^-]}{[H_2CO_3]} \tag{2.13}$$

$$\log\frac{[HCO_3^-]}{[H_2CO_3]} = 1.3$$

Taking antilogarithms:

$$\frac{[HCO_3^-]}{[H_2CO_3]} = \frac{20}{1} = \frac{\text{Proton acceptor}}{\text{Proton donor}}$$

This ratio is large because the pH is greater than the pK′. At pH 7.4, the bicarbonate system is a good buffer toward acid (i.e., it can neutralize large amounts of acid), but a poor buffer for alkali. However, blood H_2CO_3 is in rapid equilibrium with a relatively large (about 1000 times as much) reservoir of cellular CO_2, and can function as an effective buffer against increases in alkalinity. The HCO_3^-/H_2CO_3 ratio in blood is coupled to the partial pressure of CO_2, that is, to the metabolic production of CO_2 and to the loss of CO_2 during respiration. In the equilibrium expression for the bicarbonate–carbonic acid buffer system at pH 7.4, the carbonic acid term can be replaced by a pressure term, because the carbonic acid concentration is proportional to P_{CO_2} in the blood.

$$\text{pH} = 6.1 + \log\frac{[HCO_3^-]}{aP_{CO_2}} \tag{2.14}$$

where a, a proportionality constant (for normal plasma at 37°C, 0.0301), is defined by the equation:

$$[H_2CO_3] = aP_{CO_2} \tag{2.15}$$

The HCO_3^-/H_2CO_3 buffer system effectively maintains a constant blood pH of 7.4 if bicarbonate and H_2CO_3 concentrations are maintained at a ratio of 20:1. The concentration of HCO_3^- is regulated by selective excretion and absorption by the membranes of the renal tubular epithelial cell. Levels of P_{CO_2} and $[H_2CO_3]$ in the blood can be altered by changes in the rate and depth of respiration. Respiration is controlled by a skeletal muscle known as the diaphragm. It contains both type 1 and type IIa fibers. The former are fatigue-resistant, slow-twitch and the latter are fast-twitch fibers (Chapter 18). The diaphragm structure is dome-shaped and separates thoracic and abdominal cavities. Phrenic nerves whose roots originate in the neck region (C3–C5) control diaphragm muscle and thus respiration. Dysfunctional breathing can occur due to many causes and affects blood P_{CO_2} [2]. For example, **hypoventilation** (slow, shallow breathing) leads to increased blood P_{CO_2}, whereas **hyperventilation** (rapid, deep breathing) has the opposite effect. P_{CO_2} changes mediated by the lungs are more rapid than $[HCO_3^-]$ changes effected through the kidneys (discussed later).

2.2.4 Nonbicarbonate buffers in blood

Other important nonbicarbonate blood buffers are protein and phosphate. The predominant buffer system in the red blood cells is hemoglobin. Protein amino acid side chains (R-groups) that act as buffers are carboxylate groups of glutamate and aspartate, and the weakly basic groups of lysine, arginine, cysteine, and histidine. To be effective, the pK′ value of a buffer should be close to the pH of the system to be buffered. Except for the imidazolium group of histidine and the sulfhydryl group of cysteine, the pK′ values of the other amino acids mentioned above are not close enough to the physiological pH of blood to be effective buffers (Fig. 2.9). The imidazolium group has a pK′ value of 6.5 in an alanine pentapeptide, but it can vary from 2.4 to 9.2 depending on differences in its electrostatic environment, either within the same protein molecule or in different proteins. Although the sulfhydryl group of cysteine has a pK′ value of 8.6 in an alanine pentapeptide and can have pK′ values from 2.5 to 11.1 in various folded proteins, it normally participates in disulfide bonds and is not available as free sulfhydryl group [3]. Another potential buffering group in proteins is the α-amino group of the amino acid residues at the amino terminus of the protein. This group has a pK′ value ranging from 6.8 to 9.1, with a typical value of about 8.

In plasma, the protein buffer system has a limited role; the principal plasma buffer is the bicarbonate–carbonic acid system, and in the red blood cells, hemoglobin buffers a major portion of the hydrogen ions produced by the dissociation of H_2CO_3 generated by the hydration of CO_2 (Fig. 2.11).

2.3 H^+ concentration and pH

The use of pH to represent $[H^+]$ is due to the fact that a broad range of $[H^+]$ can be compressed within the numerical scale of 0–14. However, in clinical acid–base problems, use of the pH scale has some disadvantages. Since the pH is the logarithm of the reciprocal of $[H^+]$,

significant variations of $[H^+]$ in a patient may not be fully appreciated. For example, if the blood pH decreases from 7.4 to 7.1, $[H^+]$ is doubled, or if the pH increases from 7.4 to 7.7, $[H^+]$ is halved (Fig. 2.12). In addition, the use of the pH scale masks the relationship between $[H^+]$ and the concentrations of other cations, for example, Na^+ and K^+. Thus, in clinical situations, it is preferable to express $[H^+]$ directly as nanomoles per liter to better evaluate acid–base changes and interpret laboratory tests.

A blood pH of 7.4 corresponds to 40 nM $[H^+]$, which is the mean of the normal range (Fig. 2.9). The normal range is 7.36–7.44 on the pH scale, or 44–36 nM $[H^+]$. If the pH of blood falls below 7.36 ($[H^+] > 44$ nM), the condition is called **acidemia**. Conversely, if the pH rises above pH 7.44 ($[H^+] < 36$ nM), the condition is called **alkalemia**. The suffix **"-emia"** refers to blood, and usually to an abnormal concentration in blood. Over the pH range of 7.20–7.50, for every change of 0.01 pH unit, there is a change of approximately 1 nM $[H^+]$ in the opposite direction.

Since the Henderson–Hasselbalch equation uses pH terms, its utility in clinical situations is less than optimal. Kassirer and Bleich have derived a modified Henderson–Hasselbalch expression that relates $[H^+]$, instead of pH, to P_{CO_2} and HCO_3^-, as follows:

$$[H^+] = \frac{K'a \times P_{CO_2}}{[HCO_3^-]} \tag{2.16}$$

In Eq. (2.16), K′ and a are constants, and the numerical value of K′a is 24 when P_{CO_2} is expressed in mmHg, $[HCO_3^-]$ in mM, and $[H^+]$ in nM. Therefore

$$[H^+] = \frac{24 \times P_{CO_2}}{[HCO_3^-]} \tag{2.17}$$

This equation expresses the interdependence of three factors; if two of them are known, the third can be calculated. For example, at a blood $[HCO_3^-]$ of 24 mM and a P_{CO_2} of 40 mmHg, $[H^+]$ is 40 nM. Clinical applications of Eq. (2.17) are discussed later in this chapter.

2.4 Water metabolism in the human body

Water is the most abundant body constituent; it comprises 45%–60% of total body weight (Fig. 2.13). In a lean person, it accounts for a larger fraction of the body mass than in an overweight person. Since most biochemical reactions take place in an aqueous environment, the control of water balance is an important requirement for homeostasis.

Water permeates cell membranes through water channels consisting of integral membrane proteins known as **aquaporins**. Solute concentrations are regulated because of barriers imposed by membrane systems. These barriers give rise to fluid pools or compartments of different but rather constant composition (Fig. 2.14).

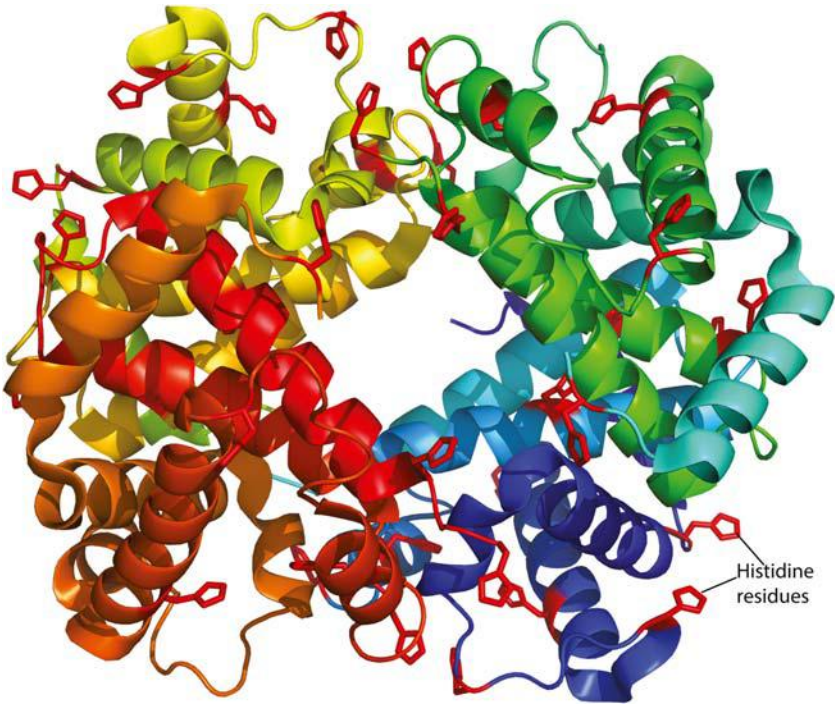

FIGURE 2.11 Structure of hemoglobin with histidine residues. The imidazole side chain of histidine can serve as a buffering agent in red blood cells. Many histidine residues in hemoglobin play a major role as a buffer in red blood cells. PDB file ID: 2hhb.

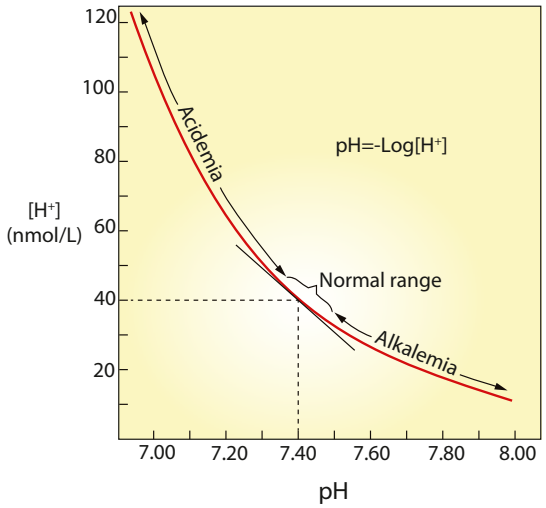

FIGURE 2.12 The relationship of pH to hydrogen ion concentration (in nanomoles per liter). The normal blood pH of 7.4 corresponds to 40 nmol/L of H^+. The solid straight line is drawn to show the linear relationship between the concentration of H^+ and pH, over the pH range of 7.2–7.5. A 0.01-unit change in pH is equivalent to about 1.0 nmol/L $[H^+]$ change in the opposite direction.

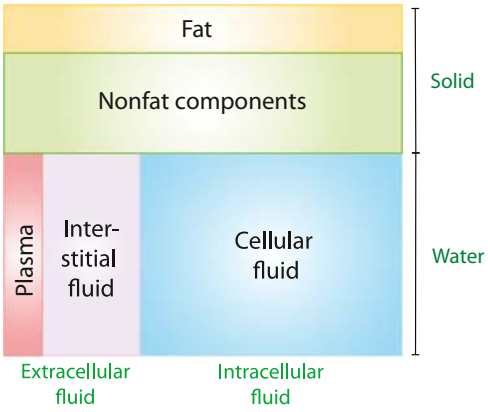

FIGURE 2.13 Proportional distribution of solids and water in a healthy adult.

Intracellular fluid makes up 30%–40% of the body weight, or about two-thirds of total body water. Potassium and magnesium are the predominant cations. The anions are mainly proteins and organic phosphates, with chloride and bicarbonate at low concentrations.

Extracellular fluid contains sodium as the predominant cation and accounts for 20%–25% of body weight, or one-third of total body water. It makes up the vascular, interstitial, transcellular, and dense connective tissue fluid pools. Vascular fluid is the circulating portion, is rich in protein, and does not readily cross endothelial membranes. Interstitial fluid surrounds cells and accounts for 18%–20% of total body water. It exchanges with vascular fluid via the lymph system. Transcellular fluid is present in digestive juices, intraocular fluid, cerebrospinal fluid

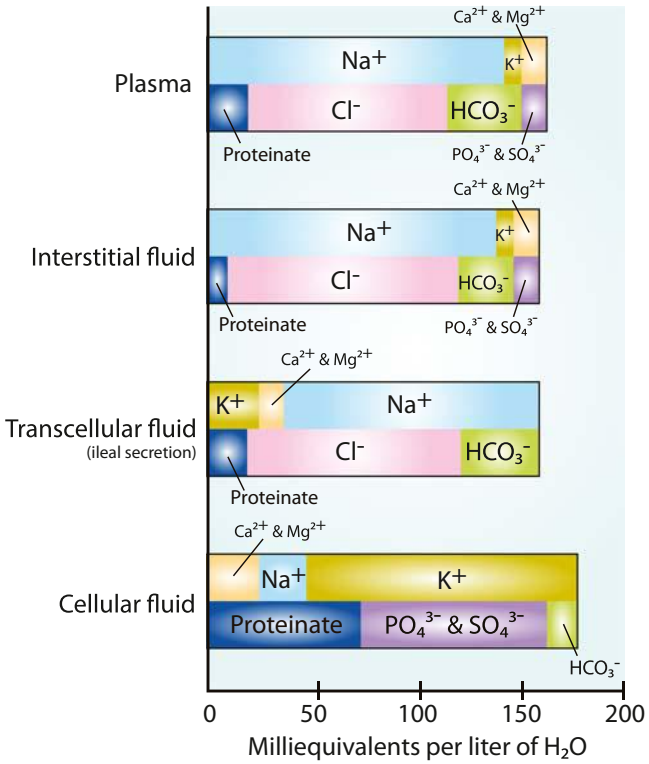

FIGURE 2.14 Composition of body fluids.

(CSF), and synovial (joint) fluid. These fluids are secretions of specialized cells. Their composition differs considerably from that of the rest of the extracellular fluid, with which they rapidly exchange contents under normal conditions. Dense connective tissue (bone, cartilage) fluid exchanges slowly with the rest of the extracellular fluid and accounts for 15% of total body water.

Movements of water occur mainly via aquaporins affected by osmosis and filtration. In osmosis, water moves to the area of highest solute concentration. Thus, active movement of salts into an area creates a concentration gradient down which water flows passively. In filtration, hydrostatic pressure in arterial blood moves water and nonprotein solutes through specialized membranes to produce an almost protein-free filtrate: this process occurs in the formation of the renal glomerular filtrate. Filtration also accounts for movement of water from the vascular space into the interstitial compartment, which is opposed by the osmotic (oncotic) pressure of plasma proteins. Cells move ions (especially Na^+ and K^+) against a concentration gradient by a "sodium pump" that actively transports sodium across the plasma membranes (Chapter 10).

The kidneys are the major organs that regulate extracellular fluid composition and volume via their functional units known **nephrons**. The average number of nephrons present in an adult per kidney is about 1 million and this number declines with the normal aging process. Low

birth−weight infants, due to intrauterine growth retardation, have a decreased number of nephrons. Three main processes occur in the nephrons:

1. Formation of a virtually protein-free ultrafiltrate at the glomerulus;
2. Active reabsorption (principally in the proximal tubule) of solutes from the glomerular filtrate; and
3. Active excretion of substances such as hydrogen ions into the tubular lumen, usually in the distal portion of the tubule (Fig. 2.15).

The normal **glomerular filtration rate** (GFR) is 100−120 mL/min; about 150 L of fluid passes through the renal tubules each day. Since the average daily urine volume is 1−1.5 L, 99% of the glomerular filtrate is reabsorbed. Approximately 80% of the water is reabsorbed in the proximal tubule, a consequence of active absorption of solutes. Reabsorption in the rest of the tubule varies according to the individual's water balance, in contrast to the **obligatory** reabsorption that occurs in the proximal tubule.

The facultative absorption of water depends on the establishment of an osmotic gradient by the reabsorption of Na^+ from the ascending loop, which decreases the osmolality of the filtrate and Na^+ uptake by the descending loop in the loop of Henle, which increases the osmolality of the filtrate. As a result, the proximal end of the loop is hyperosmotic (1200 mosm/kg) and the distal end hypo-osmotic with respect to blood. The collecting ducts run through the hyperosmotic region (Fig. 2.12). In the absence of ADH (see Chapter 28), the cells of the collecting ducts are relatively impermeable to water. They become permeable to water in the presence of ADH, however, and the urine becomes hyperosmotic with respect to blood.

2.5 Homeostatic controls

The composition and volume of extracellular fluid are regulated by complex hormonal and nervous mechanisms that interact to control its osmolality, volume, and pH (Fig. 2.16). The osmolality of extracellular fluid is due mainly to Na^+ and accompanying anions. It is kept within narrow limits (285−295 mosm/kg) by regulation of water intake (via a thirst center located in the hypothalamus) and water excretion by the kidney through the action of ADH. The volume is kept relatively constant, provided the individual's weight remains constant to within ± 1 kg. Volume receptors, located in the walls of certain blood vessels, sense the effective circulating blood volume, which, when decreased, stimulates the renin−angiotensin−aldosterone

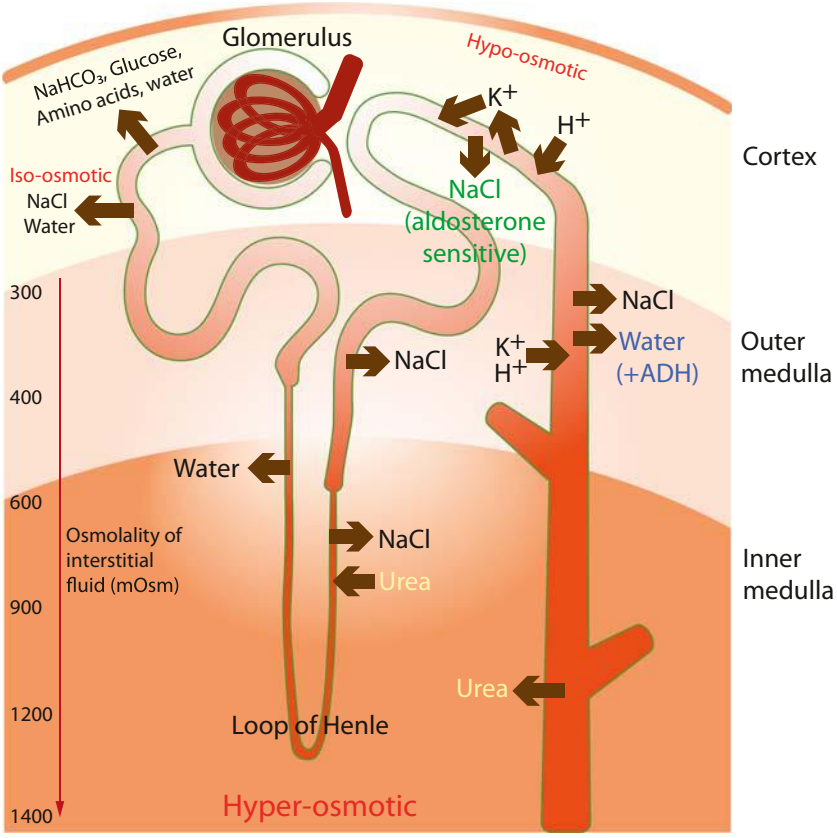

FIGURE 2.15 Principal transport processes in the renal nephron. *ADH*, Antidiuretic hormone.

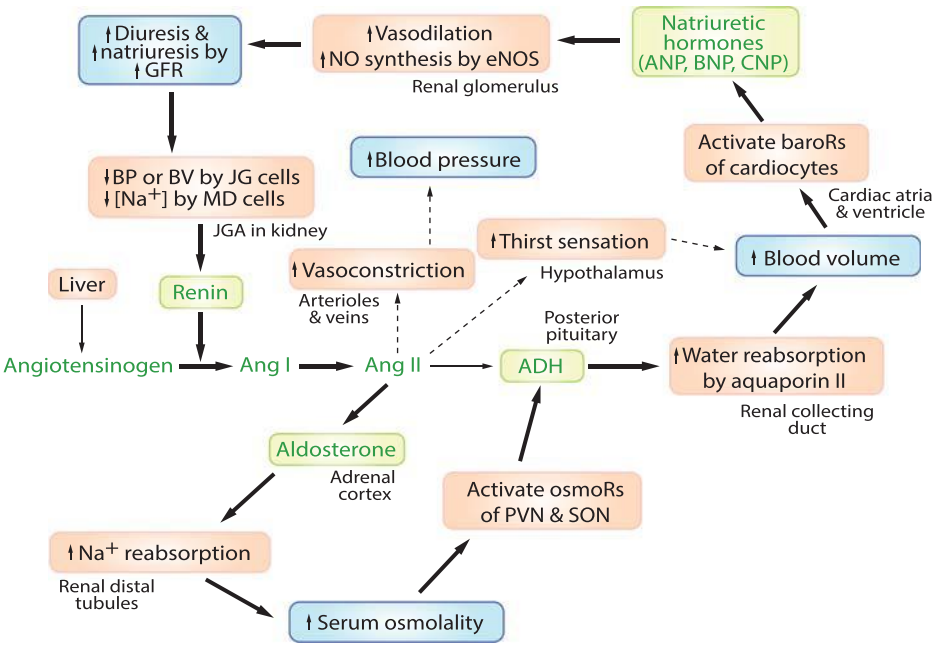

FIGURE 2.16 Homeostatic controls of the composition and volume of ECF. The renin–angiotensin–aldosterone system increases serum osmolality and activates osmoreceptors in the hypothalamus to secrete ADH, which increases water reabsorption in the collecting ducts resulting in an increase in blood volume. The increased blood volume triggers a counter response, which involves natriuretic peptide secretion from cardiac myocytes. The natriuretic hormones increase the GFR by triggering vasodilation of the blood vessels in the kidneys, which leads to increased urine output and decreased sodium reabsorption and blood volume. Thick arrows indicate major mechanisms in ECF volume and composition regulation. The dotted arrows indicate other actions of Ang II. *ADH*, Antidiuretic hormone; *Ang I*, angiotensin I; *Ang II*, angiotensin II; *ANP*, atrial natriuretic peptide; *baroRs*, baroreceptors; *BNP*, brain natriuretic peptide; *BP*, blood pressure, *BV*, blood volume; *CNP*, C-type natriuretic peptide; *ECF*, extracellular fluid; *GFR*, glomerular filtration rate; *JG*, juxtaglomerular; *MD*, macula densa; *osmoRs*, osomoreceptors; *NO*, nitric oxide; *eNOS*, endothelial nitric oxide synthetase; *PVN*, paraventricular nucleus of the hypothalamus; *SON*, supraoptic nucleus of the hypothalamus.

system and results in retention of Na^+ (Chapter 28). The increased Na^+ level leads to a rise in osmolality and secretion of ADH, with a resultant increase in water retention. Antagonistic systems exist which results in increased Na^+ excretion. Natriuretic peptides (NP, also called **natriuretic hormones**), atrial natriuretic peptide (ANP), and brain natriuretic peptide (BNP) are released by the cardiocytes of the cardiac atria and ventricles, respectively, in response to mechanical stretch caused by the plasma volume expansion. NP induces diuresis and natriuresis. These effects result from renal hemodynamic changes associated with increases in GFR and inhibition of Na^+ reabsorption from the inner medullary collecting ducts. ANP and BNP are 28- and 32-amino acid peptides, respectively. They both have a single disulfide linkage and are derived from precursors by intracellular proteolysis. Some of the stimuli other than blood volume which function as secretagogues of NP include high blood pressure, elevated serum osmolality, increased heart rate, and elevated levels of plasma catecholamines. Activation of the NP genes in cardiocytes by glucocorticoids leads to increased synthesis. NP also regulates Na^+ and water homeostasis by different mechanisms that include inhibition of steps in the renin–angiotensin–aldosterone pathway and inhibition of ADH secretion from the posterior pituitary cells.

The mechanism of action of NP on target cells involves the formation of cyclic guanosine monophosphate (cGMP) via the activation of plasma membrane receptors. The NP receptor itself is a guanylyl cyclase with its ligand-binding domain located in the extracellular space and its catalytic domain in the cytosolic domain. The receptor has only one membrane-spanning domain. This NP receptor–activated guanylyl cyclase complex is unique and does not involve any G-proteins. There is also a soluble cytosolic guanylyl cyclase that is activated by nitric oxide binding to the heme group of the enzyme, which causes vascular relaxation (Chapter 14). The intracellular formation of cGMP causes activation of cGMP-dependent protein kinases which mediate the actions of ANP. Blood levels of BNP or its other circulatory peptide fragments are used in the diagnosis of heart failure because the increased level of plasma BNP correlates well with the severity of heart failure. A recombinant human BNP (nesiritide), approved by the US Food and Drug Administration in 2001, is also used therapeutically to treat acute and chronic heart failure patients. A combination of inhibitors of two systems (renin–angiotensin–aldosterone and natriuretic peptide systems) that promotes optimal cardiovascular function and ameliorates neurohormonal overactivation in subjects with congestive heart

failure has been valuable as a synergistic therapeutic agent. This combined therapy uses the two inhibitors Sacubitril and Valsartan. Valsartan is an angiotensin II receptor blocker, which inhibits vasoconstriction of blood vessels and lower blood pressure. Sacubitril inhibit the enzyme **neprilysin**, which is responsible for degrading peptides such as natriuretic peptides and bradykinin [4,5].

The pH of extracellular fluid is kept within very narrow limits (7.35−7.45) by buffering mechanisms, the lungs, and the kidneys. These three systems do not act independently. For example, in acute blood loss, release of ADH and aldosterone restores the blood volume, and renal regulation of the pH leads to shifts in K^+ and Na^+ levels.

2.6 Water and osmolality controls

Despite considerable variation in fluid intake, an individual maintains water balance and a constant composition of body fluids. The homeostatic regulation of water is summarized in Fig. 2.17. Body water is derived from 2−4 L of water consumed daily in food and drink and 300 mL of metabolic water formed daily by oxidation of lipids and carbohydrates. Water loss occurs by perspiration, expiration of air (∼1 L/day), defecation (∼200 mL) (Chapter 10), and urination (1−2 L/day).

Water balance is regulated to maintain a constant osmolality of body fluids. This osmolality is directly related to the number of particles present per unit weight of solvent. A solution that contains 1 mol of particles in 22.4 kg of water (22.4 L at 4°C) exerts an osmotic pressure of 1 atm and has an osmolality of 0.0446. Conversely, the osmotic pressure of 1 osmolal solution (1 mol of particles/kg of water) is 22.4 atm. In this sense, "number of particles" is roughly defined as the number of noninteracting molecular or ionic groups present. Since glucose does not readily dissociate, 1 mol dissolved in 1 kg of water (a molal solution) produces 1 mol of "particles," and has an osmolality of 1. Sodium chloride dissociates completely in water to form two particles from each molecule of NaCl so that a molal solution of NaCl is a 2 osmolal solution. Similarly, a molal solution of Na_2SO_4 or $(NH_4)_2SO_4$ is a 3 osmolal solution. In practice, the milliosmole (mosm) is the unit used.

With aqueous solutions, *osmolarity* is sometimes used interchangeably with *osmolality*. Although this practice is not strictly correct (moles of particles per liter of solution vs moles of particles per kilogram of solvent), in water at temperatures of biological interest the error is fairly small unless solute concentrations are high (i.e., when an appreciable fraction of the solution is not water). Thus, with urine the approximation is acceptable, whereas with serum it is not, because of the large amount of protein present. Although osmolarity is more readily measured, it is temperature dependent, unlike osmolality. Osmolality is commonly measured by freezing point or vapor pressure depression. In terms of vapor pressure (P^v), the osmotic pressure (π) is defined as:

$$\pi = P^v_{\text{pure solvent}} - P^v_{\text{solution}}$$

As defined previously, osmolality = $\pi/22.4$, where π is measured in atmospheres. In one instrument, solution and solvent vapor pressures are measured by the use of sensitive thermistors to detect the difference in temperature

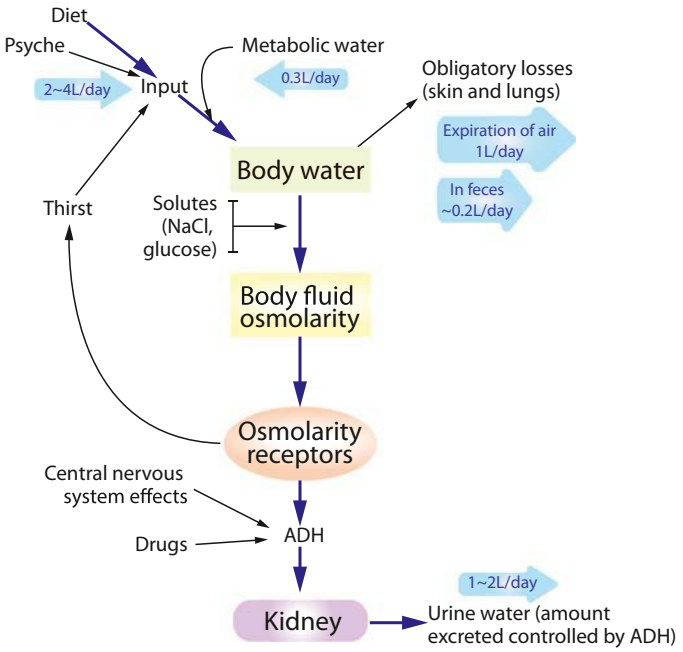

FIGURE 2.17 Regulation of osmolality in the body.

decrease caused by evaporation of solvent from a drop of pure solvent and a drop of solution. Because the rate of evaporation (vapor pressure) of the solution is lower, the temperature change will be less, and the vapor pressure difference can be calculated.

The freezing point of a solution is always lower than that of the solvent. The exact difference depends on the solvent and the osmolality of the solution. For water:

$$\text{Osmolality} = \frac{\Delta T}{1.86}$$

where ΔT is the freezing point depression in °C. Instruments that measure the freezing point of a sample are used in clinical laboratories to determine serum and urine osmolality.

Since water passes freely through most biological membranes, all body fluids are in osmotic equilibrium; consequently, the osmolality of plasma is representative of the osmolality of other body fluids. The osmotic pressure of extracellular fluid is due primarily to its principal cation Na^+ and the anions Cl^- and HCO_3^-. Taking twice the Na^+ concentration gives a good estimate of serum osmolality. Thus, normal plasma contains 135−145 mEq of Na^+/L (3.1−3.3 g/L), and normal plasma osmolality is about 270−290 mosm/kg (this corresponds to an osmotic pressure of 6.8−7.3 atm and a freezing point depression of 0.50°C−0.54°C). Glucose contributes only 5−6 mosm/kg (or 0.1 atm) to the osmotic pressure. Plasma protein contributes approximately 10.8 mosm/kg. Because of their size and general inability to pass through biological membranes, proteins are important determinants of fluid balance between intravascular and extravascular spaces. That portion of the osmotic pressure which is due to proteins is often referred to as the **oncotic pressure**.

Since many molecules in plasma interact, the measured osmolality of a sample is an effective osmolality and is lower than the value calculated from the concentrations of all the ions and molecules it contains. A solution that has the same effective osmolality as plasma is said to be **isotonic**, for example, 0.9% saline, 5% glucose, and Ringer's and Locke's solutions. If a solute can permeate a membrane freely, then a solution of that solute will behave like pure water with respect to the membrane. Thus, a solution of urea will cause red cells to swell and burst as pure water does, because urea moves freely across erythrocyte membranes.

The osmolality of urine can differ markedly from that of plasma because of active concentration processes in the renal tubules. The membranes of renal collecting ducts show varying degrees of water permeability and permit removal of certain solutes without simultaneous uptake of water. Plasma osmolality can be calculated from the concentrations of plasma Na^+, glucose, and serum urea nitrogen:

$$\text{Osmolality} = 1.86(Na^+ \text{ mEq/L}) + \frac{\text{glucose (mg/dL)}}{18} + \frac{\text{serum urea nitrogen (mg/dL)}}{2.8}$$

The numerical denominators for glucose and urea nitrogen convert the concentrations to moles per kilogram. Such an estimated osmolality is usually 6−9 mosm less than the value determined by freezing point or vapor pressure measurements. If the latter value is much greater than the estimated value, molecules **other** than Na^+, glucose, and urea must account for the difference. Such "osmolal gaps" occur in individuals suffering from drug toxicity (alcohol, barbiturates, salicylates), acute poisoning due to unknown substances, and acidosis (keto-, lactic, or renal). Determination of osmolality is helpful in the management of patients with fluid and electrolyte disorders, for example, chronic renal disease, nonketotic diabetic coma, hypo- and hypernatremia, hyperglycemia, hyperlipidemia, burns, sequelae to major surgery or severe trauma (particularly serious head injuries), hemodialysis, or diabetes insipidus [6]. Changes of about 2% or more are detected by hypothalamic osmoreceptors (Chapter 28), which elicit a sensation of thirst and production of hypertonic urine. Under conditions of fluid restriction, urine osmolality can reach 800−1200 mosm/kg (normal is 390−1090 mosm/kg), or three to four times the plasma levels. A decrease in plasma osmolarity (as in excessive water intake) results in urine with decreased osmolality. Water losses from skin and lungs are not subject to controls of this type.

ADH acts at the renal tubules and collecting ducts to raise cyclic adenosine monophosphate (cAMP) levels, followed by aquaporin-mediated water uptake. Urinary levels of cAMP are increased by ADH. Factors other than plasma hypertonicity may stimulate ADH secretion. For example, in acute hemorrhage, extracellular fluid volume drops abruptly, and ADH is secreted to increase the volume at the expense of a drop in osmolarity. Biological actions of ADH (also known as **vasopressin**) are mediated by its cell membrane receptor subtypes. Receptor subtype V1a causes vasoconstriction, V1b causes secretion of adrenocorticotropic hormone, and V2 causes water reabsorption and secretion of von Willebrand factor and factor VIII (Chapter 32).

Inappropriate ADH secretion can occur in the presence of water overload and a decline in plasma Na^+ concentration and osmolality. Fear, pain, and certain hormone-secreting tumors can cause inappropriate ADH secretion, which leads to hyponatremia and water retention. Morphine and barbiturates increase, while ethanol decreases secretion of ADH.

In **diabetes insipidus**, due to defective ADH receptors or to diminished ADH secretion, renal tubules fail to recover the water from the glomerular filtrate. In cases of deficiency

of ADH, hormone replacement with 8-lysine vasopressin or 1-deamino-8-D-arginine vasopressin (administered as a nasal spray or subcutaneously) is effective. In osmotic diuresis, for example, in **diabetes mellitus** with severe glycosuria, the solute load increases the osmolality of the glomerular filtrate and impairs the ability of the kidney to concentrate the urine. Extracellular fluid volume in a normal adult is kept constant; body weight does not vary by more than a pound per day despite fluctuations in food and fluid intake. A decrease in extracellular fluid volume lowers the effective blood volume and compromises the circulatory system. An increase may lead to hypertension, edema, or both. Volume control is centered on renal regulation of Na^+ balance. When the extracellular fluid volume decreases, less Na^+ is excreted; when it increases, more Na^+ is lost. Na^+ retention leads to expansion of extracellular fluid volume, since Na^+ is confined to this region and causes increased water retention. Renal Na^+ flux is controlled by the renin–angiotensin–aldosterone system (Chapter 28) and natriuretic peptides (discussed earlier).

2.7 Electrolyte balance

The major electrolytes are Na^+, K^+, Cl^-, and HCO_3^- (HCO_3^- is discussed next, under Section 2.8).

2.7.1 Sodium

The average Na^+ content of the human body is 60 mEq/kg, of which 50% is in extracellular fluid, 40% in bone, and 10% is intracellular Na^+ [7]. The chief dietary source of sodium is salt added in cooking. Excess sodium is largely excreted in the urine, although some is lost in perspiration. Gastrointestinal losses are small except in diarrhea.

Sodium balance is integrated with the regulation of extracellular fluid volume. Depletional **hyponatremia** (sodium loss greater than water loss) may result from inadequate Na^+ intake, excessive fluid loss from vomiting or diarrhea, diuretic abuse, and adrenal insufficiency (see Clinical Case Study 2.1). Hyponatremia can also be triggered by extracellular fluid volume overload, as it occurs

CLINICAL CASE STUDY 2.1 Hyponatremia and hyperkalemia in a neonate

This case was abstracted from: S.P. Paul, B.A. Smith, T.M. Taylor, J. Walker, Take with a grain of salt, Clin. Chem. 59 (2013) 348–352.

Synopsis

A 5-day-old girl was admitted to the hospital for 15% loss of her original birth weight. The patient was born at term after an uncomplicated pregnancy and delivery. She was fed normal-term formula milk and was found to be mildly dehydrated on initial exam. Electrolytes were within normal limits. She was started on a feeding regimen with close monitoring of weight, but after five days the patient's weight remained unchanged, and her laboratory results showed hyponatremia and hyperkalemia. Further biochemical and endocrine testing revealed a markedly elevated plasma renin level of 854 mIU/L (reference range 4–190 mIU/L) and a markedly elevated serum aldosterone level of >5786 ng/L (reference range 300–2000 ng/L). These laboratory results were consistent with the diagnosis of pseudohypoaldosteronism type 1 (PHA1) as a cause of her hyponatremia and hyperkalemia.

Teaching points

1. There are several classifications and etiologies of hyponatremia (see supplemental Ref. [4]). The presence of both hyponatremia and hyperkalemia in a neonate with excessive weight loss suggests a problem with sodium chloride metabolism, most commonly congenital adrenal hyperplasia due to salt-wasting 21-hydroxylase deficiency (SW21-OHD). However, SW21-OHD has other manifestations such as female virilization, genitourinary anomalies, and abnormal serum levels of 17-hydroxyprogesterone, cortisol, renin, and aldosterone. Other causes include adrenal hypoplasia, cystic fibrosis, cerebral salt-wasting syndrome, and aldosterone insensitivity secondary to urinary tract infections, pyelonephritis, or obstructive uropathy.
2. PHA is a group of electrolyte imbalance disorders caused by **aldosterone resistance of the aldosterone receptor**. PHA1 is caused by mutations of the gene coding for the luminal epithelial sodium channel (coupled with the Na^+/K^+-ATPase pump) of many organs including kidney, lung, colon, and sweat and salivary glands. Receptor dysfunction and unresponsiveness to aldosterone leads to excretion of sodium (hyponatremia) and retention of potassium (hyperkalemia).
3. The electrolyte abnormalities of PHA1 may present after the patient has already developed symptoms of excessive weight loss, failure to thrive, feeding difficulties, vomiting, and dehydration within the first two weeks of life.
4. Treatment for PHA1 is sodium chloride replacement therapy. For the management of other causes of hyponatremia, see supplemental Refs. [1] and [2]. Severe hyponatremia requires urgent infusion of hypertonic saline to correct cerebral edema; however, rapid correction of chronic hyponatremia may result in serious neurologic injury in some cases. Patients with asymptomatic hyponatremia do not need immediate therapy to acutely correct serum sodium.

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