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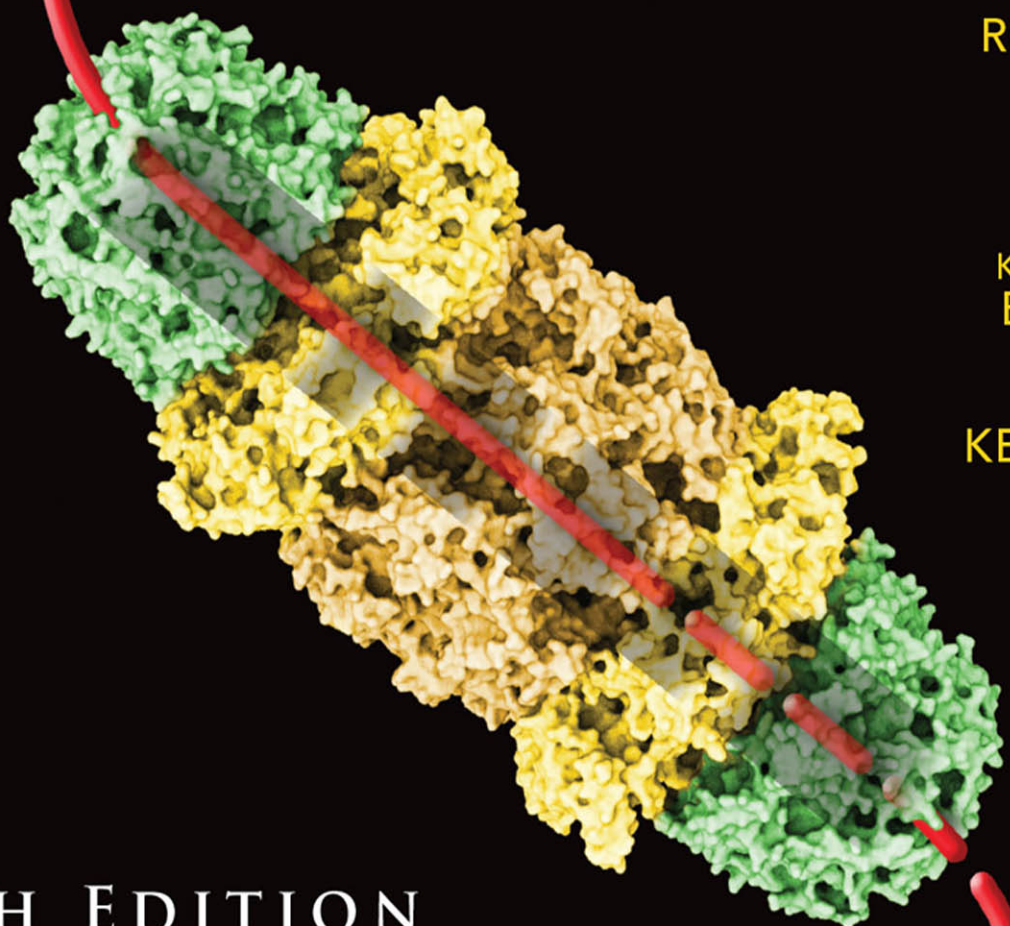

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HARPER'S ILLUSTRATED BIOCHEMISTRY

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Harper's Illustrated Biochemistry

THIRTIETH EDITION

Victor W. Rodwell, PhD
Professor (Emeritus) of Biochemistry
Purdue University
West Lafayette, Indiana

David A. Bender, PhD
Professor (Emeritus) of Nutritional Biochemsitry
University College London
London, United Kingdom

Kathleen M. Botham, PhD, DSc
Emeritus Professor of Biochemistry
Department of Comparative Biomedical Sciences
Royal Veterinary College
University of London
London, United Kingdom

Peter J. Kennelly, PhD
Professor and Head
Department of Biochemistry
Virginia Tech
Blacksburg, Virginia

P. Anthony Weil, PhD
Professor
Department of Molecular Physiology & Biophysics
Vanderbilt University
Nashville, Tennessee

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Co-Authors

Peter L. Gross, MD, MSc, FRCP(C)
Associate Professor
Department of Medicine
McMaster University
Hamilton, Ontario, Canada

Molly Jacob, MBBS, MD, PhD
Professor and Chair
Department of Biochemistry
Christian Medical College
Vellore, Tamil Nadu, India

Peter A. Mayes, PhD, DSc
Emeritus Professor of Veterinary Biochemistry
Royal Veterinary College
University of London
London, United Kingdom

Robert K. Murray, MD, PhD
Emeritus Professor of Biochemistry
University of Toronto
Toronto, Ontario

Margaret L. Rand, PhD
Senior Associate Scientist
The Hospital for Sick Children
Toronto, and Professor
Department of Laboratory Medicine & Pathobiology
University of Toronto, Toronto, Canada

Joe Varghese, MBBS, MD, DNB
Associate Professor
Department of Biochemistry
Christian Medical College
Vellore, Tamil Nadu

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- Review questions follow each of the eleven sections
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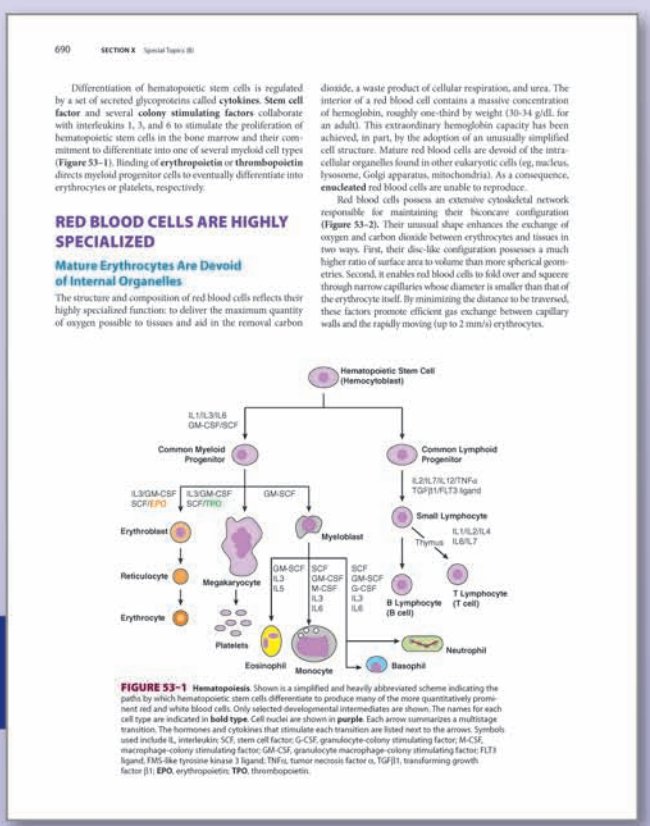
- Applauded by medical students for its engaging style, currency, and comprehensiveness


690 SECTION X Special Topics (II)

Differentiation of hematopoietic stem cells is regulated by a set of secreted glycoproteins called **cytokines**. **Stem cell factor** and several **colony stimulating factors** collaborate with interleukins 1, 3, and 6 to stimulate the proliferation of hematopoietic stem cells in the bone marrow and their commitment to differentiate into one of several myeloid cell types (**Figure 53–1**). Binding of **erythropoietin** or **thrombopoietin** directs myeloid progenitor cells to eventually differentiate into erythrocytes or platelets, respectively.

RED BLOOD CELLS ARE HIGHLY SPECIALIZED

Mature Erythrocytes Are Devoid of Internal Organelles

The structure and composition of red blood cells reflects their highly specialized function: to deliver the maximum quantity of oxygen possible to tissues and aid in the removal carbon dioxide, a waste product of cellular respiration, and urea. The interior of a red blood cell contains a massive concentration of hemoglobin, roughly one-third by weight (30-34 g/dL for an adult). This extraordinary hemoglobin capacity has been achieved, in part, by the adoption of an unusually simplified cell structure. Mature red blood cells are devoid of the intracellular organelles found in other eukaryotic cells (eg, nucleus, lysosome, Golgi apparatus, mitochondria). As a consequence, **enucleated** red blood cells are unable to reproduce.

Red blood cells possess an extensive cytoskeletal network responsible for maintaining their biconcave configuration (**Figure 53–2**). Their unusual shape enhances the exchange of oxygen and carbon dioxide between erythrocytes and tissues in two ways. First, their disc-like configuration possesses a much higher ratio of surface area to volume than more spherical geometries. Second, it enables red blood cells to fold over and squeeze through narrow capillaries whose diameter is smaller than that of the erythrocyte itself. By minimizing the distance to be traversed, these factors promote efficient gas exchange between capillary walls and the rapidly moving (up to 2 mm/s) erythrocytes.

FIGURE 53–1 **Hematopoiesis**. Shown is a simplified and heavily abbreviated scheme indicating the paths by which hematopoietic stem cells differentiate to produce many of the more quantitatively prominent red and white blood cells. Only selected developmental intermediates are shown. The names for each cell type are indicated in **bold type**. Cell nuclei are shown in **purple**. Each arrow summarizes a multistage transition. The hormones and cytokines that stimulate each transition are listed next to the arrows. Symbols used include IL, interleukin; SCF, stem cell factor; G-CSF, granulocyte-colony stimulating factor; M-CSF, macrophage-colony stimulating factor; GM-CSF, granulocyte macrophage-colony stimulating factor; FLT3 ligand, FMS-like tyrosine kinase 3 ligand; TNFα, tumor necrosis factor α; TGFβ1, transforming growth factor β1; **EPO**, erythropoietin; **TPO**, thrombopoietin.

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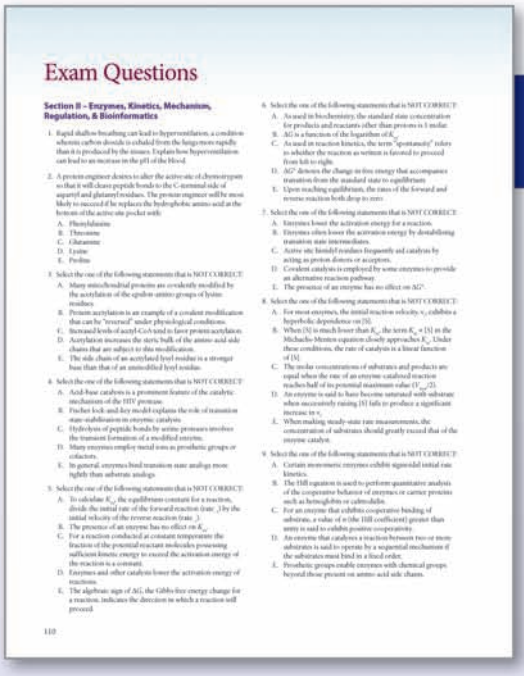


Exam Questions

Section II – Enzymes, Kinetics, Mechanism, Regulation, & Bioinformatics

1. Rapid shallow breathing can lead to hyperventilation, a condition wherein carbon dioxide is exhaled from the lungs more rapidly than it is produced by the tissues. Explain how hyperventilation can lead to an increase in the pH of the blood.

2. A protein engineer desires to alter the active site of chymotrypsin so that it will cleave peptide bonds to the C-terminal side of aspartyl and glutamyl residues. The protein engineer will be most likely to succeed if he replaces the hydrophobic amino acid at the bottom of the active site pocket with:
   A. Phenylalanine
   B. Threonine
   C. Glutamine
   D. Lysine
   E. Proline

3. Select the one of the following statements that is NOT CORRECT:
   A. Many mitochondrial proteins are covalently modified by the acetylation of the epsilon-amino groups of lysine residues.
   B. Protein acetylation is an example of a covalent modification that can be "reversed" under physiological conditions.
   C. Increased levels of acetyl-CoA tend to favor protein acetylation.
   D. Acetylation increases the steric bulk of the amino acid side chains that are subject to this modification.
   E. The side chain of an acetylated lysyl residue is a stronger base than that of an unmodified lysyl residue.

4. Select the one of the following statements that is NOT CORRECT:
   A. Acid-base catalysis is a prominent feature of the catalytic mechanism of the HIV protease.
   B. Fischer lock-and-key model explains the role of transition state-stabilization in enzymic catalysis.
   C. Hydrolysis of peptide bonds by serine proteases involves the transient formation of a modified enzyme.
   D. Many enzymes employ metal ions as prosthetic groups or cofactors.
   E. In general, enzymes bind transition state analogs more tightly than substrate analogs.

5. Select the one of the following statements that is NOT CORRECT:
   A. To calculate K_{eq} the equilibrium constant for a reaction, divide the initial rate of the forward reaction (rate $_1$) by the initial velocity of the reverse reaction (rate $_2$).
   B. The presence of an enzyme has no effect on K_{eq}.
   C. For a reaction conducted at constant temperature the fraction of the potential reactant molecules possessing sufficient kinetic energy to exceed the activation energy of the reaction is a constant.
   D. Enzymes and other catalysts lower the activation energy of reactions.
   E. The algebraic sign of ΔG, the Gibbs free energy change for a reaction, indicates the direction in which a reaction will proceed.

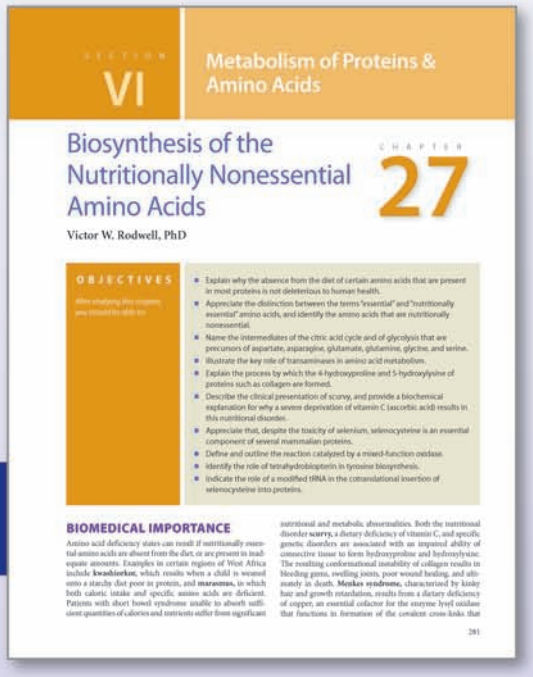
6. Select the one of the following statements that is NOT CORRECT:
   A. As used in biochemistry, the standard state concentration for products and reactants other than protons is 1 molar.
   B. ΔG is a function of the logarithm of K_{eq}.
   C. As used in reaction kinetics, the term "spontaneity" refers to whether the reaction as written is favored to proceed from left to right.
   D. $\Delta G°$ denotes the change in free energy that accompanies transition from the standard state to equilibrium.
   E. Upon reaching equilibrium, the rates of the forward and reverse reaction both drop to zero.

7. Select the one of the following statements that is NOT CORRECT:
   A. Enzymes lower the activation energy for a reaction.
   B. Enzymes often lower the activation energy by destabilizing transition state intermediates.
   C. Active site histidyl residues frequently aid catalysis by acting as proton donors or acceptors.
   D. Covalent catalysis is employed by some enzymes to provide an alternative reaction pathway.
   E. The presence of an enzyme has no effect on $\Delta G°$.

8. Select the one of the following statements that is NOT CORRECT:
   A. For most enzymes, the initial reaction velocity, v_o, exhibits a hyperbolic dependence on [S].
   B. When [S] is much lower than K_m, the term $K_m + [S]$ in the Michaelis-Menten equation closely approaches K_m. Under these conditions, the rate of catalysis is a linear function of [S].
   C. The molar concentrations of substrates and products are equal when the rate of an enzyme-catalyzed reaction reaches half of its potential maximum value ($V_{max}/2$).
   D. An enzyme is said to have become saturated with substrate when successively raising [S] fails to produce a significant increase in v_o.
   E. When making steady-state rate measurements, the concentration of substrates should greatly exceed that of the enzyme catalyst.

9. Select the one of the following statements that is NOT CORRECT:
   A. Certain monomeric enzymes exhibit sigmoidal initial rate kinetics.
   B. The Hill equation is used to perform quantitative analysis of the cooperative behavior of enzymes or carrier proteins such as hemoglobin or calmodulin.
   C. For an enzyme that exhibits cooperative binding of substrate, a value of *n* (the Hill coefficient) greater than unity is said to exhibit positive cooperativity.
   D. An enzyme that catalyzes a reaction between two or more substrates is said to operate by a sequential mechanism if the substrates must bind in a fixed order.
   E. Prosthetic groups enable enzymes with chemical groups beyond those present on amino acid side chains.

NEW! Review questions follow each section



SECTION VI

Metabolism of Proteins & Amino Acids

Biosynthesis of the Nutritionally Nonessential Amino Acids

CHAPTER 27

Victor W. Rodwell, PhD

OBJECTIVES

After studying this chapter, you should be able to:

- Explain why the absence from the diet of certain amino acids that are present in most proteins is not deleterious to human health.
- Appreciate the distinction between the terms "essential" and "nutritionally essential" amino acids, and identify the amino acids that are nutritionally nonessential.
- Name the intermediates of the citric acid cycle and of glycolysis that are precursors of aspartate, asparagine, glutamate, glutamine, glycine, and serine.
- Illustrate the key role of transaminases in amino acid metabolism.
- Explain the process by which the 4-hydroxyproline and 5-hydroxylysine of proteins such as collagen are formed.
- Describe the clinical presentation of scurvy, and provide a biochemical explanation for why a severe deprivation of vitamin C (ascorbic acid) results in this nutritional disorder.
- Appreciate that, despite the toxicity of selenium, selenocysteine is an essential component of several mammalian proteins.
- Define and outline the reaction catalyzed by a mixed-function oxidase.
- Identify the role of tetrahydrobiopterin in tyrosine biosynthesis.
- Indicate the role of a modified tRNA in the cotranslational insertion of selenocysteine into proteins.

BIOMEDICAL IMPORTANCE

Amino acid deficiency states can result if nutritionally essential amino acids are absent from the diet, or are present in inadequate amounts. Examples in certain regions of West Africa include **kwashiorkor**, which results when a child is weaned onto a starchy diet poor in protein, and **marasmus**, in which both caloric intake and specific amino acids are deficient. Patients with short bowel syndrome unable to absorb sufficient quantities of calories and nutrients suffer from significant nutritional and metabolic abnormalities. Both the nutritional disorder **scurvy**, a dietary deficiency of vitamin C, and specific genetic disorders are associated with an impaired ability of connective tissue to form hydroxyproline and hydroxylysine. The resulting conformational instability of collagen results in bleeding gums, swelling joints, poor wound healing, and ultimately in death. **Menkes syndrome**, characterized by kinky hair and growth retardation, results from a dietary deficiency of copper, an essential cofactor for the enzyme lysyl oxidase that functions in formation of the covalent cross-links that

Boxed OBJECTIVES define the goals of each chapter

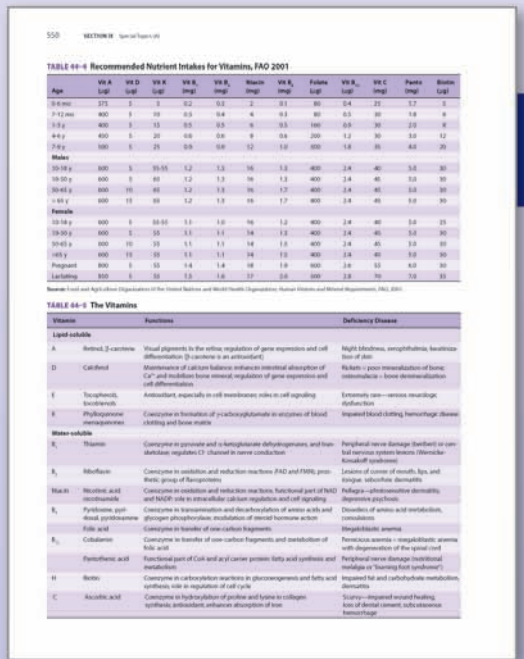


TABLE 44–4 Recommended Nutrient Intakes for Vitamins, FAO 2001

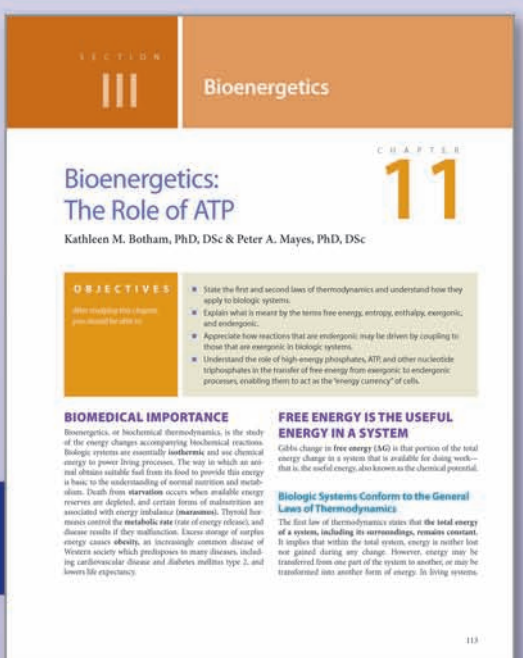
Age	Vit A (µg)	Vit D (µg)	Vit K (µg)	Vit B_1 (mg)	Vit B_2 (mg)	Niacin (mg)	Vit B_6 (mg)	Folate (µg)	Vit B_{12} (µg)	Vit C (mg)	Panto (mg)	Biotin (µg)
0–6 mo	375	5	5	0.2	0.3	2	0.1	80	0.4	25	1.7	5
7–12 mo	400	5	10	0.3	0.4	4	0.3	80	0.5	30	1.8	6
1–3 y	400	5	15	0.5	0.5	6	0.5	160	0.9	30	2.0	8
4–6 y	450	5	20	0.6	0.6	9	0.6	200	1.2	30	3.0	12
7–9 y	500	5	25	0.9	0.9	12	1.0	300	1.8	35	4.0	20
Males												
10–18 y	600	5	35–55	1.2	1.3	16	1.3	400	2.4	40	5.0	30
19–50 y	600	5	65	1.2	1.3	16	1.3	400	2.4	45	5.0	30
50–65 y	600	10	65	1.2	1.3	16	1.7	400	2.4	45	5.0	30
> 65 y	600	10	65	1.2	1.3	16	1.7	400	2.4	45	5.0	30
Female												
10–18 y	600	5	55–55	1.1	1.0	16	1.2	400	2.4	40	5.0	25
19–50 y	600	5	55	1.1	1.1	14	1.3	400	2.4	45	5.0	30
50–65 y	600	10	55	1.1	1.1	14	1.5	400	2.4	45	5.0	30
>65 y	600	10	55	1.1	1.1	14	1.5	400	2.4	45	5.0	30
Pregnant	800	5	55	1.4	1.4	18	1.9	600	2.6	55	6.0	30
Lactating	850	5	55	1.5	1.6	17	2.0	500	2.8	70	7.0	35

Source: Food and Agriculture Organization of the United Nations and World Health Organization, Human Vitamin and Mineral Requirements, FAO, 2001.

TABLE 44–5 The Vitamins

Vitamin		Functions	Deficiency Disease
Lipid-soluble			
A	Retinol, β-carotene	Visual pigments in the retina; regulation of gene expression and cell differentiation (β-carotene is an antioxidant)	Night blindness, xerophthalmia, keratinization of skin
D	Calciferol	Maintenance of calcium balance; enhances intestinal absorption of Ca^{2+} and mobilizes bone mineral; regulation of gene expression and cell differentiation	Rickets = poor mineralization of bone; osteomalacia = bone demineralization
E	Tocopherols, tocotrienols	Antioxidant, especially in cell membranes; roles in cell signaling	Extremely rare—nervous neurologic dysfunction
K	Phylloquinone menaquinones	Coenzyme in formation of γ-carboxyglutamate in enzymes of blood clotting and bone matrix	Impaired blood clotting, hemorrhagic disease
Water-soluble			
B_1	Thiamin	Coenzyme in pyruvate and α-ketoglutarate dehydrogenases, and transketolase; regulates Cl^- channel in nerve conduction	Peripheral nerve damage (beriberi) or central nervous system lesions (Wernicke-Korsakoff syndrome)
B_2	Riboflavin	Coenzyme in oxidation and reduction reactions (FAD and FMN); prosthetic group of flavoproteins	Lesions of corner of mouth, lips, and tongue; seborrheic dermatitis
Niacin	Nicotinic acid, nicotinamide	Coenzyme in oxidation and reduction reactions; functional part of NAD and NADP; role in intracellular calcium regulation and cell signaling	Pellagra—photosensitive dermatitis, depressive psychosis
B_6	Pyridoxine, pyridoxal, pyridoxamine	Coenzyme in transamination and decarboxylation of amino acids and glycogen phosphorylase; modulation of steroid hormone action	Disorders of amino acid metabolism, convulsions
	Folic acid	Coenzyme in transfer of one-carbon fragments	Megaloblastic anemia
B_{12}	Cobalamin	Coenzyme in transfer of one-carbon fragments and metabolism of folic acid	Pernicious anemia = megaloblastic anemia with degeneration of the spinal cord
	Pantothenic acid	Functional part of CoA and acyl carrier protein; fatty acid synthesis and metabolism	Peripheral nerve damage (nutritional metalgia or "burning-foot syndrome")
H	Biotin	Coenzyme in carboxylation reactions in gluconeogenesis and fatty acid synthesis; role in regulation of cell cycle	Impaired fat and carbohydrate metabolism, dermatitis
C	Ascorbic acid	Coenzyme in hydroxylation of proline and lysine in collagen synthesis; antioxidant; enhances absorption of iron	Scurvy—impaired wound healing, loss of dental cement, subcutaneous hemorrhage

Tables encapsulate important information



SECTION III

Bioenergetics

Bioenergetics: The Role of ATP

CHAPTER 11

Kathleen M. Botham, PhD, DSc & Peter A. Mayes, PhD, DSc

OBJECTIVES

After studying this chapter, you should be able to:

- State the first and second laws of thermodynamics and understand how they apply to biologic systems.
- Explain what is meant by the terms free energy, entropy, enthalpy, exergonic, and endergonic.
- Appreciate how reactions that are endergonic may be driven by coupling to those that are exergonic in biologic systems.
- Understand the role of high-energy phosphates, ATP, and other nucleotide triphosphates in the transfer of free energy from exergonic to endergonic processes, enabling them to act as the "energy currency" of cells.

BIOMEDICAL IMPORTANCE

Bioenergetics, or biochemical thermodynamics, is the study of the energy changes accompanying biochemical reactions. Biologic systems are essentially **isothermic** and use chemical energy to power living processes. The way in which an animal obtains suitable fuel from its food to provide this energy is basic to the understanding of normal nutrition and metabolism. Death from **starvation** occurs when available energy reserves are depleted, and certain forms of malnutrition are associated with energy imbalance (**marasmus**). Thyroid hormones control the **metabolic rate** (rate of energy release), and disease results if they malfunction. Excess storage of surplus energy causes **obesity**, an increasingly common disease of Western society which predisposes to many diseases, including cardiovascular disease and diabetes mellitus type 2, and lowers life expectancy.

FREE ENERGY IS THE USEFUL ENERGY IN A SYSTEM

Gibbs change in **free energy (ΔG)** is that portion of the total energy change in a system that is available for doing work—that is, the useful energy, also known as the chemical potential.

Biologic Systems Conform to the General Laws of Thermodynamics

The first law of thermodynamics states that **the total energy of a system, including its surroundings, remains constant.** It implies that within the total system, energy in neither lost nor gained during any change. However, energy may be transferred from one part of the system to another, or may be transformed into another form of energy. In living systems,

Every chapter includes a section on the biomedical importance of a given topic

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Preface

The authors and publishers are pleased to present the thirtieth edition of *Harper's Illustrated Biochemistry*. The first edition, entitled Harper's Biochemistry, was published in 1939 under the sole authorship of Dr Harold Harper at the University of California School of Medicine, San Francisco, California. Presently entitled *Harpers Illustrated Biochemistry*, the book continues, as originally intended, to provide a concise survey of aspects of biochemistry most relevant to the study of medicine. Various authors have contributed to subsequent editions of this medically oriented biochemistry text, which is now observing its 75th year.

Cover Illustration for the Thirtieth Edition

The illustration on the cover depicts the proteasome and the initial proteolytic degradation of an ubiquitinated intracellular protein. The proteosome consists of a macromolecular complex of 14 α and 14 β subunits (shown green and yellow, respectively) arranged as four stacked $\alpha_7\beta_7\beta_7\alpha_7$ rings. These form a hollow, tube-like chamber that contains immobilized proteases. A polypeptide tagged for degradation (shown red) enters the proteasome (top left) and is hydrolyzed into peptide fragments by internal proteases of the proteosome. Following their exit from the proteosome (bottom, right), extracellular proteases degrade these peptide fragments to amino acids.

The timely and controlled degradation of intracellular proteins is critical to such fundamental biological processes as cell differentiation and division. The ability to recognize and dispose of denatured or damaged proteins is essential to health, since the accumulation of protein aggregates contributes significantly to the etiology of a variety of human diseases, including numerous neurological disorders. For the discovery of ubiquitin-mediated protein degradation, Aaron Ciechanover and Avram Hershko of Israel and Irwin Rose of the United States were awarded the 2004 Nobel Prize in Chemistry.

Changes in the Thirtieth Edition

The 30th Anniversary edition of *Harper's Illustrated Biochemistry* continues its timely, integrated updating of biochemical knowledge, with repeated emphasis on its relationship to genetic diseases, clinical information, and the practice of medicine. This edition includes new full-color illustrations and tables, and numerous medically-relevant examples that present a clear and succinct review of those fundamentals of biochemistry that a student needs to understand for success in medical school. In addition to timely updating of content, the order of presentation of concepts has undergone major revision. The present 58 chapters are organized under an expanded list of eleven Sections. Chapters and topics in these sections emphasize integrated coverage of biochemical disease and clinical information. A major change has been that following the retirement of Dr. Murray, authorship and revision of his thirteen chapters have been assumed by Drs. Bender, Botham, Kennelly and Rodwell. For example, Section X contains a new chapter on white blood cells, and Section XI features nine entirely new, open-ended clinical case problems that emphasize clinical relevance and test both knowledge and understanding. To facilitate a student's grasp of each group of concepts, Question Sets now appear after each of the eleven new Sections. Many new questions have been added, and an Answer Bank follows the last chapter. New to this edition is the Answer Bank's inclusion of comprehensive explanations of many answers.

Organization of the Book

All 58 chapters of the thirtieth edition place major emphasis on the medical relevance of biochemistry. Topics are organized under eleven major headings. To facilitate retention of the contained information, Questions follow each Section and an Answer Bank follows the Appendix.

Section I includes a brief history of biochemistry and emphasizes the interrelationships between biochemistry and medicine. Water and pH are reviewed, and the various orders of proteins structure are addressed.

Section II begins with a chapter on hemoglobin, three chapters address the kinetics, mechanism of action, and metabolic regulation of enzymes. A chapter on Bioinformatics and Computational Biology reflects the ever-increasing importance of these topics in modern biochemistry, biology, and medicine.

Section III addresses bioenergetics and the role of high energy phosphates in energy capture and transfer, the oxidation–reduction reactions involved in biologic oxidation, and metabolic details of energy capture via the respiratory chain and oxidative phosphorylation.

Section IV considers the metabolism of carbohydrates via glycolysis, the citric acid cycle, the pentose phosphate pathway, glycogen metabolism, gluconeogenesis, and the control of blood glucose.

Section V outlines the nature of simple and complex lipids, lipid transport and storage, the biosynthesis and degradation of fatty acids and more complex lipids, and the reactions and metabolic regulation of cholesterol biosynthesis and transport in human subjects.

Section VI discusses protein catabolism, urea biosynthesis, and the catabolism of amino acids and stresses the medically significant metabolic disorders associated with their incomplete catabolism. The final chapter considers the biochemistry of the porphyrins and bile pigments.

Section VII first outlines the structure and function of nucleotides and nucleic acids, then details DNA replication and repair, RNA synthesis and modification, protein synthesis, the principles of recombinant DNA technology, and the regulation of gene expression.

Section VIII considers aspects of extracellular and intracellular communication. Specific topics include membrane structure and function, the molecular bases of the actions of hormones, and signal transduction.

Sections IX, X, & XI address fourteen topics of significant medical importance.

Section IX discusses nutrition, digestion, and absorption, micronutrients including, vitamins, free radicals and antioxidants, glycoproteins, the metabolism of xenobiotics, and clinical biochemistry.

Section X addresses intracellular traffic and the sorting of proteins, the extracellular matrix, muscle and the cytoskeleton, plasma proteins and immunoglobulins, and the biochemistry of red cells and of white cells.

Section XI includes hemostasis and thrombosis, an overview of cancer, and the biochemistry of aging.

Acknowledgments

The authors thank Michael Weitz for his role in the planning of this edition and Regina Y. Brown for her key role in preparing it for publication. We also thank Shruti Awasthi of Cenveo Publisher Services for her efforts in editing, typesetting, and artwork. Suggestions from students and colleagues around the world have been most helpful in the formulation of this edition. We look forward to receiving similar input in the future. Finally, we acknowledge Robert Murray for his leadership and contributions to prior editions of this book.

Victor W. Rodwell
David A. Bender
Kathleen M. Botham
Peter J. Kennelly
P. Anthony Weil

SECTION I

Structures & Functions of Proteins & Enzymes

CHAPTER 1

Biochemistry & Medicine

Victor W. Rodwell, PhD & Robert K. Murray, MD, PhD

OBJECTIVES

After studying this chapter, you should be able to:

- Understand the importance of the ability of cell-free extracts of yeast to ferment sugars, an observation that enabled discovery of the intermediates of fermentation, glycolysis, and other metabolic pathways.
- Appreciate the scope of biochemistry and its central role in the life sciences, and that biochemistry and medicine are intimately related disciplines.
- Appreciate that biochemistry integrates knowledge of the chemical processes in living cells with strategies to maintain health, understand disease, identify potential therapies, and enhance our understanding of the origins of life on earth.
- Describe how genetic approaches have been critical for elucidating many areas of biochemistry, and how the Human Genome Project has furthered advances in numerous aspects of biology and medicine.

BIOMEDICAL IMPORTANCE

Biochemistry and medicine enjoy a mutually cooperative relationship. Biochemical studies have illuminated many aspects of health and disease, and the study of various aspects of health and disease has opened up new areas of biochemistry. The medical relevance of biochemistry both in normal and abnormal situations is emphasized throughout this book. Biochemistry makes significant contributions to the fields of cell biology, physiology, immunology, microbiology, pharmacology, and toxicology, as well as the fields of inflammation, cell injury, and cancer. These close relationships emphasize that life, as we know it, depends on biochemical reactions and processes.

BIOCHEMISTRY BEGAN WITH THE DISCOVERY THAT A CELL-FREE EXTRACT OF YEAST CAN FERMENT SUGAR

The knowledge that yeast can convert the sugars to ethyl alcohol predates recorded history. It was not, however, until the earliest years of the 20th century that this process led directly to the science of biochemistry. Despite his insightful investigations of brewing and wine making, the great French microbiologist Louis Pasteur maintained that the process of fermentation could only occur in intact cells. His error was shown in 1899 by the brothers Büchner, who discovered that

fermentation can indeed occur in cell-free extracts. This revelation resulted from storage of a yeast extract in a crock of concentrated sugar solution added as a preservative. Overnight, the contents of the crock fermented, spilled over the laboratory bench and floor, and dramatically demonstrated that fermentation can proceed in the absence of an intact cell. This discovery made possible a rapid and highly productive series of investigations in the early years of the 20th century that initiated the science of biochemistry. These investigations revealed the vital role of inorganic phosphate, ADP, ATP, and NAD(H), and ultimately identified the phosphorylated sugars and the chemical reactions and enzymes (Gk "in yeast") that convert glucose to pyruvate (glycolysis) or to ethanol and CO_2 (fermentation). Subsequent research in the 1930s and 1940s identified the intermediates of the citric acid cycle and of urea biosynthesis, and provided insight into the essential roles of certain vitamin-derived cofactors or "coenzymes" such as thiamin pyrophosphate, riboflavin, and ultimately coenzyme A, coenzyme Q, and cobamide coenzymes. The 1950s revealed how complex carbohydrates are synthesized from, and broken down to simple sugars, and delineated the pathways for biosynthesis of pentoses and the breakdown of amino acids and lipids.

Animal models, perfused intact organs, tissue slices, cell homogenates and their subfractions, and purified enzymes all were used to isolate and identify metabolites and enzymes. These advances were made possible by the development in the late 1930s and early 1940s of techniques such as analytical ultracentrifugation, paper and other forms of chromatography, and the post-World War II availability of radioisotopes, principally ^{14}C, ^{3}H and ^{32}P, as "tracers" to identify the intermediates in complex pathways such as that leading to the biosynthesis of cholesterol and other isoprenoids and the pathways of amino acid biosynthesis and catabolism. X-ray crystallography was then used to solve the three-dimensional structure, first of myoglobin, and subsequently of numerous proteins, polynucleotides, enzymes, and viruses including that of the common cold. Genetic advances that followed the realization that DNA was a double helix include the polymerase chain reaction, and transgenic animals or those with gene knockouts. The methods used to prepare, analyze, purify, and identify metabolites and the activities of natural and recombinant enzymes and their three-dimensional structures are discussed in the following chapters.

BIOCHEMISTRY & MEDICINE HAVE STIMULATED MUTUAL ADVANCES

The two major concerns for workers in the health sciences—and particularly physicians—are the understanding and maintenance of health and the understanding and effective treatment of disease. Biochemistry impacts both of these fundamental concerns, and the interrelationship of biochemistry and medicine is a wide, two-way street. Biochemical studies have illuminated many aspects of health and disease, and conversely, the study of various aspects of health and disease has opened up new areas of biochemistry (**Figure 1–1**). Knowledge of protein structure and function was necessary to identify and understand the single difference in amino acid sequence between normal hemoglobin and sickle cell hemoglobin, and analysis of numerous variant sickle cell and other hemoglobins has contributed significantly to our understanding of the structure and function both of normal hemoglobin and of other proteins. During the early 1900s the English physician Archibald Garrod studied patients with the relatively rare disorders of alkaptonuria, albinism, cystinuria, and pentosuria and established that these conditions were genetically determined. Garrod designated these conditions as **inborn errors of metabolism.** His insights provided a foundation for the development of the field of human biochemical genetics. A more recent example was investigation of the genetic and molecular basis of familial hypercholesterolemia, a disease that results in early onset atherosclerosis. In addition to clarifying different genetic mutations responsible for this disease, this provided a deeper understanding of cell receptors and mechanisms of uptake, not only of cholesterol, but of how other molecules' cross cell membranes. Studies of **oncogenes** and **tumor suppressor genes** in cancer cells have directed

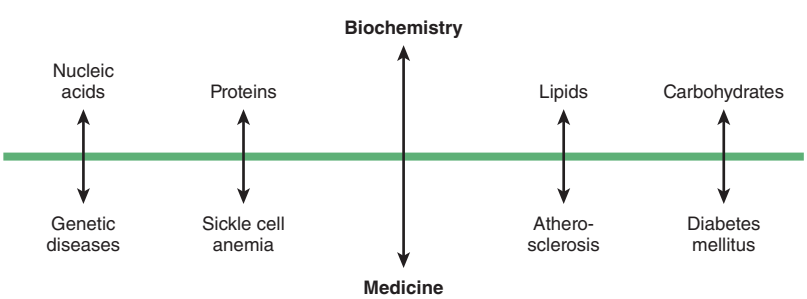

FIGURE 1–1 A two-way street connects biochemistry and medicine. Knowledge of the biochemical topics listed above the green line of the diagram has clarified our understanding of the diseases shown below the green line. Conversely, analyses of the diseases have casted light on many areas of biochemistry. Note that sickle cell anemia is a genetic disease, and that both atherosclerosis and diabetes mellitus have genetic components.

attention to the molecular mechanisms involved in the control of normal cell growth. These examples illustrate how the study of disease can open up areas of basic biochemical research. Science provides physicians and other workers in health care and biology with a foundation that impacts practice, stimulates curiosity, and promotes the adoption of scientific approaches for continued learning. So long as medical treatment is firmly grounded in the knowledge of biochemistry and other basic sciences, the practice of medicine will have a rational basis capable of accommodating and adapting to new knowledge.

NORMAL BIOCHEMICAL PROCESSES ARE THE BASIS OF HEALTH

Biochemical Research Impacts Nutrition & Preventive Medicine

The World Health Organization (WHO) defines health as a state of "complete physical, mental, and social well-being and not merely the absence of disease and infirmity." From a biochemical viewpoint, health may be considered that situation in which all of the many thousands of intra- and extracellular reactions that occur in the body are proceeding at rates commensurate with the organism's survival under pressure from both internal and external challenges. The maintenance of health requires optimal dietary intake of a number of chemicals, chief among which are **vitamins,** certain **amino acids** and **fatty acids,** various **minerals,** and **water.** Understanding nutrition depends to a great extent on knowledge of biochemistry, and the sciences of biochemistry and nutrition share a focus on these chemicals. Recent increasing emphasis on systematic attempts to maintain health and forestall disease, or **preventive medicine**, includes nutritional approaches to the prevention of diseases such as atherosclerosis and cancer.

Most Diseases Have a Biochemical Basis

Apart from infectious organisms and environmental pollutants, many diseases are manifestations of abnormalities in genes, proteins, chemical reactions, or biochemical processes, each of which can adversely affect one or more critical biochemical functions. Examples of disturbances in human biochemistry responsible for diseases or other debilitating conditions include electrolyte imbalance, defective nutrient ingestion or absorption, hormonal imbalances, toxic chemicals or biologic agents, and DNA-based genetic disorders. To address these challenges, biochemical research continues to be interwoven with studies in disciplines such as genetics, cell biology, immunology, nutrition, pathology, and pharmacology. In addition, many biochemists are vitally interested in contributing to solutions to key issues such as the ultimate survival of mankind, and educating the public to support use of the scientific method in solving environmental and other major problems that confront us.

Impact of the Human Genome Project on Biochemistry, Biology, & Medicine

Initially unanticipated rapid progress in the late 1990s in sequencing the human genome led in mid-2000 to the announcement that over 90% of the genome had been sequenced. This effort was headed by the International Human Genome Sequencing Consortium and by Celera Genomics, a private company. Except for a few gaps, the sequence of the entire human genome was completed in 2003, just 50 years after the description of the double-helical nature of DNA by Watson and Crick. The implications for biochemistry, medicine, and indeed for all of biology, are virtually unlimited. For example, the ability to isolate and sequence a gene and to investigate its structure and function by sequencing and "gene knockout" experiments have revealed previously unknown genes and their products, and new insights have been gained concerning human evolution and procedures for identifying disease-related genes.

Major advances in biochemistry and understanding human health and disease continue to be made by mutation of the genomes of model organisms such as yeast and of eukaryotes such as the fruit fly *Drosophila melanogaster* and the round worm *Caenorhabditis elegans*. Each organism has a short generation time and can be genetically manipulated to provide insight into the functions of individual genes. These advances can potentially be translated into approaches that help humans by providing clues to curing human diseases such as cancer and Alzheimer disease. **Figure 1–2** highlights areas that have developed or accelerated as a direct result of progress made in the Human Genome Project (HGP). New "**-omics"** fields have blossomed, each of which focuses on comprehensive study of the structures and functions of the molecules with which each is concerned. Definitions of these -omics fields mentioned below appear in the Glossary of this chapter. The products of genes (RNA molecules and proteins) are being studied using the techniques of **transcriptomics** and **proteomics.** A spectacular example of the speed of progress in transcriptomics is the explosion of knowledge about small RNA molecules as regulators of gene activity. Other -omics fields include **glycomics, lipidomics, metabolomics, nutrigenomics,** and **pharmacogenomics**. To keep pace with the information generated, **bioinformatics** has received much attention. Other related fields to which the impetus from the HGP has carried over are **biotechnology, bioengineering, biophysics,** and **bioethics. Nanotechnology** is an active area, which, for example, may provide novel methods of diagnosis and treatment for cancer and other disorders. **Stem cell biology** is at the center of much current research. **Gene therapy** has yet to deliver the promise that it appears to offer, but it seems probable that ultimately will occur. Many new **molecular diagnostic tests** have developed in areas such as genetic, microbiologic, and immunologic testing and diagnosis. **Systems biology** is also burgeoning. The outcomes of research in the various areas mentioned above will impact tremendously the future of biology, medicine, and the health sciences. **Synthetic biology** offers the potential for

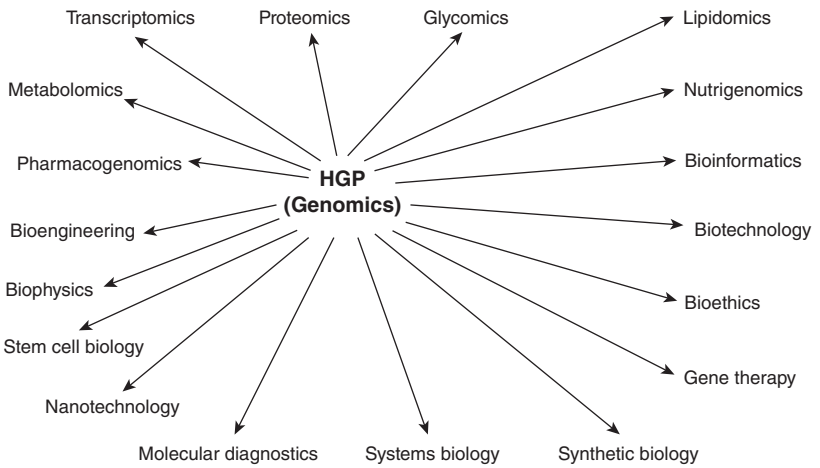

FIGURE 1–2 The Human Genome Project (HGP) has influenced many disciplines and areas of research. Biochemistry is not listed since it predates commencement of the HGP, but disciplines such as bioinformatics, genomics, glycomics, lipidomics, metabolomics, molecular diagnostics, proteomics, and transcriptomics are nevertheless active areas of biochemical research.

creating living organisms, initially small bacteria, from genetic material in vitro that might carry out specific tasks such as cleansing petroleum spills. All of the above make the 21st century an exhilarating time to be directly involved in biology and medicine.

SUMMARY

- Biochemistry is the science concerned with studying the various molecules that occur in living cells and organisms, the individual chemical reactions and their enzyme catalysts, and the expression and regulation of each metabolic process. Because life depends on biochemical reactions, biochemistry has become the basic language of all biologic sciences.
- Despite the focus on human biochemistry in this text, biochemistry concerns the entire spectrum of life forms, from relatively simple viruses and bacteria and plants to complex eukaryotes such as human beings.
- Biochemistry, medicine and other health care disciplines are intimately related. Health in all species depends on a harmonious balance of the biochemical reactions occurring in the body, while disease reflects abnormalities in biomolecules, biochemical reactions, or biochemical processes.
- Advances in biochemical knowledge have illuminated many areas of medicine, and the study of diseases has often revealed previously unsuspected aspects of biochemistry.
- Biochemical approaches are often fundamental in illuminating the causes of diseases and in designing appropriate therapies, and various biochemical laboratory tests represent an integral component of diagnosis and monitoring of treatment.
- A sound knowledge of biochemistry and of other related basic disciplines is essential for the rational practice of medicine and related health sciences.
- Results of the HGP and of research in related areas will have a profound influence on the future of biology, medicine, and other health sciences.
- Genomic research on model organisms such as yeast, the fruit fly *D. melanogaster*, and the round worm *C. elegans* provides insight into understanding human diseases

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GLOSSARY

Bioengineering: The application of engineering to biology and medicine.

Bioethics: The area of ethics that is concerned with the application of moral and ethical principles to biology and medicine.

Bioinformatics: The discipline concerned with the collection, storage, and analysis of biologic data, mainly DNA and protein sequences (see Chapter 10).

Biophysics: The application of physics and its techniques to biology and medicine.

Biotechnology: The field in which biochemical, engineering, and other approaches are combined to develop biological products of use in medicine and industry.

Gene Therapy: Applies to the use of genetically engineered genes to treat various diseases.

Genomics: The genome is the complete set of genes of an organism, and genomics is the in-depth study of the structures and functions of genomes.

Glycomics: The glycome is the total complement of simple and complex carbohydrates in an organism. Glycomics is the systematic study of the structures and functions of glycomes such as the human glycome.

Lipidomics: The lipidome is the complete complement of lipids found in an organism. Lipidomics is the in-depth study of the structures and functions of all members of the lipidome and of their interactions, in both health and disease.

Metabolomics: The metabolome is the complete complement of metabolites (small molecules involved in metabolism) present in an organism. Metabolomics is the in-depth study of their structures, functions, and changes in various metabolic states.

Molecular Diagnostics: Refers to the use of molecular approaches such as DNA probes to assist in the diagnosis of various biochemical, genetic, immunologic, microbiologic, and other medical conditions.

Nanotechnology: The development and application to medicine and to other areas of devices such as nanoshells which are only a few nanometers in size (10^{-9} m = 1 nm).

Nutrigenomics: The systematic study of the effects of nutrients on genetic expression and of the effects of genetic variations on the metabolism of nutrients.

Pharmacogenomics: The use of genomic information and technologies to optimize the discovery and development of new drugs and drug targets.

Proteomics: The proteome is the complete complement of proteins of an organism. Proteomics is the systematic study of the structures and functions of proteomes and their variations in health and disease.

Stem Cell Biology: Stem cells are undifferentiated cells that have the potential to self-renew and to differentiate into any of the adult cells of an organism. Stem cell biology concerns the biology of stem cells and their potential for treating various diseases.

Synthetic Biology: The field that combines biomolecular techniques with engineering approaches to build new biological functions and systems.

Systems Biology: The field concerns complex biologic systems studied as integrated entities.

Transcriptomics: The comprehensive study of the transcriptome, the complete set of RNA transcripts produced by the genome during a fixed period of time.

CHAPTER 2

Water & pH

Peter J. Kennelly, PhD & Victor W. Rodwell, PhD

OBJECTIVES

After studying this chapter, you should be able to:

- Describe the properties of water that account for its surface tension, viscosity, liquid state at ambient temperature, and solvent power.
- Use structural formulas to represent several organic compounds that can serve as hydrogen bond donors or acceptors.
- Explain the role played by entropy in the orientation, in an aqueous environment, of the polar and nonpolar regions of macromolecules.
- Indicate the quantitative contributions of salt bridges, hydrophobic interactions, and van der Waals forces to the stability of macromolecules.
- Explain the relationship of pH to acidity, alkalinity, and the quantitative determinants that characterize weak and strong acids.
- Calculate the shift in pH that accompanies the addition of a given quantity of acid or base to the pH of a buffered solution.
- Describe what buffers do, how they do it, and the conditions under which a buffer is most effective under physiologic or other conditions.
- Illustrate how the Henderson-Hasselbalch equation can be used to calculate the net charge on a polyelectrolyte at a given pH.

BIOMEDICAL IMPORTANCE

Water is the predominant chemical component of living organisms. Its unique physical properties, which include the ability to solvate a wide range of organic and inorganic molecules, derive from water's dipolar structure and exceptional capacity for forming hydrogen bonds. The manner in which water interacts with a solvated biomolecule influences the structure both of the biomolecule and of water itself. An excellent nucleophile, water is a reactant or product in many metabolic reactions. Regulation of water balance depends upon hypothalamic mechanisms that control thirst, on antidiuretic hormone (ADH), on retention or excretion of water by the kidneys, and on evaporative loss. Nephrogenic diabetes insipidus, which involves the inability to concentrate urine or adjust to subtle changes in extracellular fluid osmolarity, results from the unresponsiveness of renal tubular osmoreceptors to ADH.

Water has a slight propensity to dissociate into hydroxide ions and protons. The concentration of protons, or **acidity,** of aqueous solutions is generally reported using the logarithmic pH scale. Bicarbonate and other buffers normally maintain the pH of extracellular fluid between 7.35 and 7.45. Suspected disturbances of acid-base balance are verified by measuring the pH of arterial blood and the CO_2 content of venous blood. Causes of acidosis (blood pH <7.35) include diabetic ketosis and lactic acidosis. Alkalosis (pH >7.45) may follow vomiting of acidic gastric contents.

WATER IS AN IDEAL BIOLOGIC SOLVENT

Water Molecules Form Dipoles

A water molecule is an irregular, slightly skewed tetrahedron with oxygen at its center (**Figure 2–1**). The two hydrogens and the unshared electrons of the remaining two sp^3-hybridized orbitals occupy the corners of the tetrahedron. The 105° angle between the two hydrogen atoms differs slightly from the ideal tetrahedral angle, 109.5°. Ammonia is also tetrahedral, with a 107° angle between its three hydrogens. The strongly electronegative oxygen atom in a water molecule attracts electrons

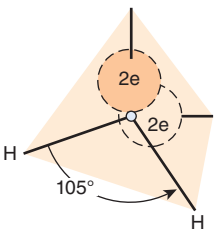

FIGURE 2–1 **The water molecule has tetrahedral geometry.**

away from the hydrogen nuclei, leaving them with a partial positive charge, while its two unshared electron pairs constitute a region of local negative charge.

A molecule with electrical charge distributed asymmetrically about its structure is referred to as a **dipole.** Water's strong dipole is responsible for its high **dielectric constant.** As described quantitatively by Coulomb's law, the strength of interaction *F* between oppositely charged particles is inversely proportionate to the dielectric constant ε of the surrounding medium. The dielectric constant for a vacuum is essentially unity; for hexane it is 1.9; for ethanol, 24.3; and for water at 25°C, 78.5. Water therefore greatly decreases the force of attraction between charged and polar species relative to water-free environments with lower dielectric constants. Its strong dipole and high dielectric constant enable water to dissolve large quantities of charged compounds such as salts.

Water Molecules Form Hydrogen Bonds

A partially unshielded hydrogen nucleus covalently bound to an electron-withdrawing oxygen or nitrogen atom can interact with an unshared electron pair on another oxygen or nitrogen atom to form a **hydrogen bond.** Since water molecules contain both of these features, hydrogen bonding favors the self-association of water molecules into ordered arrays (**Figure 2–2**). Hydrogen bonding profoundly influences the physical properties of water and accounts for its relatively high viscosity, surface tension, and boiling point. On average, each molecule in liquid water associates through hydrogen bonds with 3.5 others. These bonds are both relatively weak and transient, with a half-life of a few picoseconds. Rupture of a hydrogen bond in liquid water requires only about 4.5 kcal/mol, less than 5% of the energy required to rupture a covalent O—H bond.

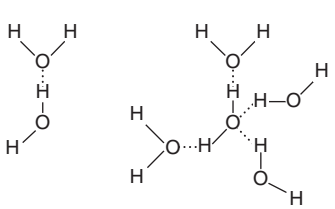

FIGURE 2–2 **Water molecules self-associate via hydrogen bonds.** Shown are the association of two water molecules (**left**) and a hydrogen-bonded cluster of four water molecules (**right**). Notice that water can serve simultaneously both as a hydrogen donor and as a hydrogen acceptor.

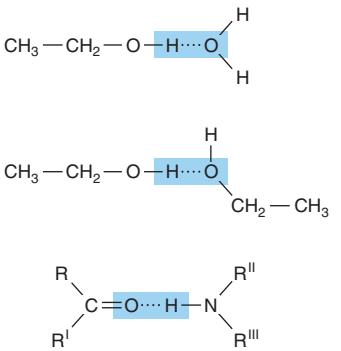

FIGURE 2–3 **Additional polar groups participate in hydrogen bonding.** Shown are hydrogen bonds formed between alcohol and water, between two molecules of ethanol, and between the peptide carbonyl oxygen and the peptide nitrogen hydrogen of an adjacent amino acid.

Hydrogen bonding enables water to dissolve many organic biomolecules that contain functional groups which can participate in hydrogen bonding. The oxygen atoms of aldehydes, ketones, and amides, for example, provide lone pairs of electrons that can serve as hydrogen acceptors. Alcohols, carboxylic acids, and amines can serve both as hydrogen acceptors and as donors of unshielded hydrogen atoms for formation of hydrogen bonds (**Figure 2–3**).

INTERACTION WITH WATER INFLUENCES THE STRUCTURE OF BIOMOLECULES

Covalent and Noncovalent Bonds Stabilize Biologic Molecules

The covalent bond is the strongest force that holds molecules together (**Table 2–1**). Noncovalent forces, while of lesser magnitude, make significant contributions to the structure, stability, and functional competence of macromolecules in living

TABLE 2–1 **Bond Energies for Atoms of Biologic Significance**

Bond Type	Energy (kcal/mol)	Bond Type	Energy (kcal/mol)
O — O	34	O═O	96
S — S	51	C — H	99
C — N	70	C═S	108
S — H	81	O — H	110
C — C	82	C═C	147
C — O	84	C═N	147
N — H	94	C═O	164

cells. These forces, which can be either attractive or repulsive, involve interactions both within the biomolecule and between it and the water that forms the principal component of the surrounding environment.

Biomolecules Fold to Position Polar & Charged Groups on Their Surfaces

Most biomolecules are **amphipathic;** that is, they possess regions rich in charged or polar functional groups as well as regions with hydrophobic character. Proteins tend to fold with the R-groups of amino acids with hydrophobic side chains in the interior. Amino acids with charged or polar amino acid side chains (eg, arginine, glutamate, serine, see Table 3–1) generally are present on the surface in contact with water. A similar pattern prevails in a phospholipid bilayer where the charged "head groups" of phosphatidylserine or phosphatidylethanolamine contact water while their hydrophobic fatty acyl side chains cluster together, excluding water (see Figure 40–5). This pattern maximizes the opportunities for the formation of energetically favorable charge-dipole, dipole-dipole, and hydrogen bonding interactions between polar groups on the biomolecule and water. It also minimizes energetically unfavorable contacts between water and hydrophobic groups.

Hydrophobic Interactions

Hydrophobic interaction refers to the tendency of nonpolar compounds to self-associate in an aqueous environment. This self-association is driven neither by mutual attraction nor by what are sometimes incorrectly referred to as "hydrophobic bonds." Self-association minimizes the disruption of energetically favorable interactions between the surrounding water molecules.

While the hydrogens of nonpolar groups such as the methylene groups of hydrocarbons do not form hydrogen bonds, they do affect the structure of the water that surrounds them. Water molecules adjacent to a hydrophobic group are restricted in the number of orientations (degrees of freedom) that permit them to participate in the maximum number of energetically favorable hydrogen bonds. Maximal formation of multiple hydrogen bonds, which maximizes enthalpy, can be maintained only by increasing the order of the adjacent water molecules, with an accompanying decrease in entropy.

It follows from the second law of thermodynamics that the optimal free energy of a hydrocarbon-water mixture is a function of both maximal enthalpy (from hydrogen bonding) and highest entropy (maximum degrees of freedom). Thus, nonpolar molecules tend to form droplets that minimize exposed surface area and reduce the number of water molecules whose motional freedom becomes restricted. Similarly, in the aqueous environment of the living cell the hydrophobic portions of biopolymers tend to be buried inside the structure of the molecule, or within a lipid bilayer, minimizing contact with water.

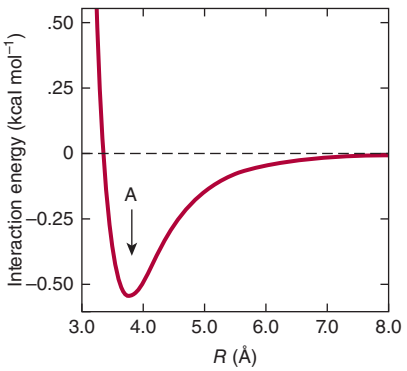

FIGURE 2–4 The strength of van der Waals interactions varies with the distance, *R*, between interacting species. The force of interaction between interacting species increases with decreasing distance between them until they are separated by the van der Waals contact distance (see arrow marked A). Repulsion due to interaction between the electron clouds of each atom or molecule then supervenes. While individual van der Waals interactions are extremely weak, their cumulative effect is nevertheless substantial for macromolecules such as DNA and proteins which have many atoms in close contact.

Electrostatic Interactions

Interactions between charged groups help shape biomolecular structure. Electrostatic interactions between oppositely charged groups within or between biomolecules are termed **salt bridges.** Salt bridges are comparable in strength to hydrogen bonds but act over larger distances. They therefore often facilitate the binding of charged molecules and ions to proteins and nucleic acids.

van der Waals Forces

van der Waals forces arise from attractions between transient dipoles generated by the rapid movement of electrons of all neutral atoms. Significantly weaker than hydrogen bonds but potentially extremely numerous, van der Waals forces decrease as the sixth power of the distance separating atoms (**Figure 2–4**). Thus, they act over very short distances, typically 2 to 4 Å.

Multiple Forces Stabilize Biomolecules

The DNA double helix illustrates the contribution of multiple forces to the structure of biomolecules. While each individual DNA strand is held together by covalent bonds, the two strands of the helix are held together exclusively by noncovalent interactions such as hydrogen bonds between nucleotide bases (Watson-Crick base pairing) and van der Waals interactions between the stacked purine and pyrimidine bases. The double helix presents the charged phosphate groups and polar hydroxyl groups from the ribose sugars of the DNA backbone to water while burying the relatively hydrophobic nucleotide bases inside. The extended backbone maximizes the distance between negatively charged phosphates, minimizing unfavorable electrostatic interactions (see Figure 34–2).

WATER IS AN EXCELLENT NUCLEOPHILE

Metabolic reactions often involve the attack by lone pairs of electrons residing on electron-rich molecules termed **nucleophiles** upon electron-poor atoms called **electrophiles.** Nucleophiles and electrophiles do not necessarily possess a formal negative or positive charge. Water, whose two lone pairs of sp^3 electrons bear a partial negative charge (see Figure 2–1), is an excellent nucleophile. Other nucleophiles of biologic importance include the oxygen atoms of phosphates, alcohols, and carboxylic acids; the sulfur of thiols; and the nitrogen atom of amines and of the imidazole ring of histidine. Common electrophiles include the carbonyl carbons in amides, esters, aldehydes, and ketones and the phosphorus atoms of phosphoesters.

Nucleophilic attack by water typically results in the cleavage of the amide, glycoside, or ester bonds that hold biopolymers together. This process is termed **hydrolysis.** Conversely, when monomer units are joined together to form biopolymers, such as proteins or glycogen, water is a product, for example, during the formation of a peptide bond between two amino acids.

While hydrolysis is a thermodynamically favored reaction, the amide and phosphoester bonds of polypeptides and oligonucleotides are stable in the aqueous environment of the cell. This seemingly paradoxical behavior reflects the fact that the thermodynamics that govern the equilibrium point of a reaction do not determine the *rate* at which it will proceed toward its equilibrium point. In the cell, protein catalysts called **enzymes** accelerate the rate of hydrolytic reactions when needed. **Proteases** catalyze the hydrolysis of proteins into their component amino acids, while **nucleases** catalyze the hydrolysis of the phosphoester bonds in DNA and RNA. Careful control of the activities of these enzymes is required to ensure that they act only at appropriate times.

Many Metabolic Reactions Involve Group Transfer

Many of the enzymic reactions responsible for synthesis and breakdown of biomolecules involve the transfer of a chemical group G from a donor D to an acceptor A to form an acceptor group complex, A—G:

$$\mathrm{D{-}G + A \rightleftarrows A{-}G + D}$$

The hydrolysis and phosphorolysis of glycogen, for example, involve the transfer of glucosyl groups to water or to orthophosphate. The equilibrium constant for the hydrolysis of covalent bonds strongly favors the formation of split products. Conversely, many group transfer reactions responsible for the biosynthesis of macromolecules involve the thermodynamically unfavored formation of covalent bonds. Enzyme catalysts play a critical role in surmounting these barriers by virtue of their capacity to directly link two normally separate reactions together. By linking an energetically unfavorable group transfer reaction with a thermodynamically favorable reaction, such as the hydrolysis of ATP, a new coupled reaction can be generated whose net *overall* change in free energy favors biopolymer synthesis.

Given the nucleophilic character of water and its high concentration in cells, why are biopolymers such as proteins and DNA relatively stable? And how can synthesis of biopolymers occur in an aqueous environment that favors hydrolysis? Central to both questions are the properties of enzymes. In the absence of enzymic catalysis, even reactions that are highly favored thermodynamically do not necessarily take place rapidly. Precise and differential control of enzyme activity and the sequestration of enzymes in specific organelles determine the physiologic circumstances under which a given biopolymer will be synthesized or degraded. The ability of enzyme active sites to sequester substrates in an environment from which water can be excluded facilitates biopolymer synthesis.

Water Molecules Exhibit a Slight but Important Tendency to Dissociate

The ability of water to ionize, while slight, is of central importance for life. Since water can act both as an acid and as a base, its ionization may be represented as an intermolecular proton transfer that forms a hydronium ion (H_3O^+) and a hydroxide ion (OH^-):

$$\mathrm{H_2O + H_2O \rightleftarrows H_3O + OH^-}$$

The transferred proton is actually associated with a cluster of water molecules. Protons exist in solution not only as H_3O^+, but also as multimers such as $H_5O_2^+$ and $H_7O_3^+$. The proton is nevertheless routinely represented as H^+, even though it is in fact highly hydrated.

Since hydronium and hydroxide ions continuously recombine to form water molecules, an *individual* hydrogen or oxygen cannot be stated to be present as an ion or as part of a water molecule. At one instant it is an ion; an instant later it is part of a water molecule. Individual ions or molecules are therefore not considered. We refer instead to the *probability* that at any instant in time a given hydrogen will be present as an ion or as part of a water molecule. Since 1 g of water contains 3.46×10^{22} molecules, the ionization of water can be described statistically. To state that the probability that a hydrogen exists as an ion is 0.01 means that at any given moment in time, a hydrogen atom has 1 chance in 100 of being an ion and 99 chances out of 100 of being part of a water molecule. The actual probability of a hydrogen atom in pure water existing as a hydrogen ion is approximately 1.8×10^{-9}. The probability of its being part of a water molecule thus is almost unity. Stated another way, for every hydrogen ion or hydroxide ion in pure water, there are 0.56 billion or 0.56×10^9 water molecules. Hydrogen ions and hydroxide ions nevertheless contribute significantly to the properties of water.

For dissociation of water,

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