25th EDITION

哈珀生物化学

英文影印版

Harper's Biochemistry

Robert K. Murray
Daryl K. Granner
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Victor W. Rodwell

科学出版社
McGraw-Hill

Robert K. Murray, Daryl K. Granner, Peter A. Mayes, Victor W. Rodwell: Harper's Biochemistry, 25th Edition

IE ISBN 0-07-118483X

图书在版编目(CIP)数据

哈珀生物化学=Harper's Beochemistry：英文影印版/(美)默里(Murray, R. K.)主编. -25版. -北京：科学出版社，2000.9

ISBN 7-03-008738-0

Ⅰ.哈… Ⅱ.默… Ⅲ.生物化学-医学院校-教材-英文 Ⅳ.Q5

中国版本图书馆CIP数据核字(2000)第67539号

北京市版权局版权登记号：图字01-2000-1764

科学出版社 出版
北京东黄城根北街16号
邮政编码：100717
北京双青印刷厂 印刷
科学出版社发行 各地新华书店经销
*
2000年9月第 一 版 开本：787×1092 1/16
2000年9月第一次印刷 印张：58 3/4
印数：1—3 000 字数：1 374 000
定价：118.00元
(如有印装质量问题，我社负责调换〈新欣〉)

Preface

The authors and publisher are proud to present the twenty-fifth edition of *Harper's Biochemistry*. *Review of Physiological Chemistry* was first published in 1939 and revised in 1944, and it quickly gained a wide readership. In 1951, the third edition appeared with Harold A. Harper, University of California School of Medicine at San Francisco, assuming the duties of authorship. Dr. Harper remained the sole author up to the ninth edition and co-authored eight subsequent editions. Of the present authors, Peter Mayes and Victor Rodwell have been involved with the book since the tenth edition, Daryl Granner since the twentieth edition, and Rob Murray since the twenty-first edition. In this edition we are very pleased to welcome Peter Kennelly as a co-author of the chapters dealing with enzymes.

We believe that *Harper's Biochemistry* has the longest continuous publication record of any biochemistry textbook in the world and has at various times been published in some 15 languages. It is of interest to note that when the first edition was published, the structures of DNA, of the various RNA molecules, and of proteins were unknown.

The overall objective of this silver edition of *Harper's Biochemistry* is to provide concise yet authoritative coverage of the principles of biochemistry and molecular biology. The text offers numerous examples of how a knowledge of biochemistry is essential for understanding the maintenance of health and the causes and rational treatment of many diseases.

CHANGES IN THE TWENTY-FIFTH EDITION

The goals of the authors in preparing this latest edition were first, to provide both medical students and other students of the health sciences with a book that not only describes the basics of biochemistry but also is user-friendly and interesting and second, to reflect the latest advances in biochemistry that are important to medicine. The following summary, which is not intended to be all inclusive, highlights the major changes in the book and points out some of the areas of the text that have been updated:

- Much of the artwork, particularly in sections I, II, and III, has been rendered anew or further improved to facilitate the understanding of concepts and phenomena in the text.
- Every chapter has been revised or updated, some extensively.
- The treatment of the roles of water, hydrogen bonds, and other bonds, and of forces in the stabilization of macromolecules has been expanded, as has the treatment of the pK values of residues in proteins.
- The description of peptide sequencing has been updated.
- The description of the application of X-ray crystallography and nuclear magnetic resonance (NMR) to the determination of three-dimensional structures of proteins has been expanded and several new figures included.
- A new section on the structural basis of the transmissible spongiform encephalopathies or prion diseases (eg, Creutzfeld-Jacob disease, scrapie, and mad cow disease) has been included.
- All four chapters on enzymes, including the discussion of biologic control mechanisms (notably phosphorylation-mediated control of enzyme activity), have been extensively revised.

- Color has been added, particularly in metabolic diagrams, both to enhance comprehension of the mechanisms underlying metabolic diseases, such as diabetes mellitus and atherosclerosis, and to foster the student's appreciation of important metabolic concepts underpinning current nutritional advice and practice.
- Updated material in the chapters on metabolism incorporates new aspects of the role of insulin in regulating glycogen metabolism; the mechanisms of inhibition of prostaglandin biosynthesis by aspirin, non-steroidal anti-inflammatory drugs, and anti-inflammatory corticosteroids; the mechanism of reverse cholesterol transport including the role of pre-β high-density lipoproteins; the role of triacylglycerol transfer protein in the formation of very low density lipoprotein; the functioning of signaling metabolites in the regulation of gene expression; and the roles of various glucose transporter proteins in the absorption of sugars from the intestine.
- New information on telomeres, telomerase, and their relationship to aging and cancer has been introduced.
- Discussion of the role of DNA triplet repeat sequences in various diseases is an added feature.
- Explanations of DNA replication and DNA repair have been updated.
- General features of DNA and RNA synthesis are compared.
- The discussions of the assembly of the transcription preinitiation complex and of the initial steps involved in transcription initiation have been revised, and the two prevailing models of the assembly of the preinitiation complex are explained in detail.
- The descriptions of RNA processing and RNA modification are updated and expanded.
- The role of eIF-4E in the initiation of protein synthesis is explained and the mechanisms by which some viruses co-opt host protein synthesis are discussed.
- Information on the regulation of gene expression has been extensively updated.
- The roles of coregulators and histone acetylation/deacetylation in hormone action are discussed and the concept of hormone response units is described.
- Appropriate revisions have been made in all of the chapters dealing with the actions of the various classes of hormones.
- Concerning membranes, new material on the nuclear import of proteins and on ion channels has been included.
- The recently announced updated aims of the U.S. Human Genome Project are described.
- The roles of protein aggregation and other factors in the genesis of certain chronic neurodegenerative diseases are discussed.
- A new case history on hemochromatosis is introduced, and the discussion of muscular dystrophy has been updated.
- In a number of areas of the text, the use of Online Mendelian Inheritance in Man numbers to refer to genetic diseases has been introduced to facilitate reference to these diseases by readers.
- A listing of useful Web sites in biochemistry and molecular biology has been included.

ORGANIZATION OF THE BOOK

The text is divided into three introductory chapters followed by six main sections.

Section I deals with proteins and enzymes, the workhorses of the body. Because most reactions in the human body are catalyzed by enzymes, it is vital to understand the properties of enzymes before moving on to other topics.

Section II explains how various cellular reactions either utilize or produce energy and traces the pathways by which carbohydrates and lipids are synthesized and degraded. The many functions of these two classes of molecules are also described.

Section III deals with the amino acids and their fates and shows how the metabolism of amino acids also uses and yields energy.

Section IV describes the structures and functions of the nucleic acids, covering the representation DNA → RNA → proteins. This section also describes the principles of recombinant DNA technology, a topic with tremendous implications for biomedical science and all of biology.

Section V discusses hormones and their key roles in intercellular communication and metabolic regulation. To affect cells, hormones must first interact with the plasma membranes of cells; thus membrane structure and function are addressed initially in this section.

Section VI consists of fourteen special topics: water- and lipid-soluble vitamins, nutrition, digestion and absorption, glycoproteins, the extracellular matrix, muscle, plasma proteins (and immunoglobulins and blood coagulation), red and white blood cells, metabolism of xenobiotics, cancer and growth factors, the biochemical and genetic bases of diseases, various neuropsychiatric disorders, and ten biochemical case histories.

The **Appendix** contains a list of the major biochemical laboratory tests and their reference ranges used in clinical medicine.

ACKNOWLEDGMENTS

The authors thank David Barnes for his keen interest, advice, and support. We are particularly grateful to Jim Ransom for his masterly editorial work; his skills and commitment will have played a major role in any success that this edition enjoys. The excellent art work and cooperation of Maggie Darrow and her colleagues are gratefully acknowledged, as are the superb editorial skills of Harriet Lebowitz and Jeanmarie Roche. Suggestions for improvements and corrections from students and colleagues around the world have been most helpful in formulating this edition; we look forward to receiving similar input regarding the next edition. VWR wishes to thank Peter Kennelly for the pleasure of working with him on chapters 8-11. RKM thanks Drs. Inka Brockhausen, W. Robert Bruce, and Paul Fraser for their generous assistance in revising Chapters 56, 62, and 64, respectively, and is also grateful to Drs. Fred Keeley and Margaret Rand for their ongoing roles as co-authors of Chapters 57 and 59, respectively. The authors are grateful for the broad base of acceptance and support this book has received in many countries. Several editions of the English language version have been reprinted in Japan, Lebanon, Taiwan, the Philippines, and Korea. In addition, there are translations in Italian, Spanish, Portuguese, Japanese, Polish, German, Indonesian, Serbo-Croatian, and Greek.

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April 1999

Contents

Biochemistry and Medicine

1

Robert K. Murray, MD, PhD

INTRODUCTION

Biochemistry is the science concerned with the various molecules that occur in living cells and organisms and with their chemical reactions. Anything more than a superficial comprehension of life—in all its diverse manifestations—demands a knowledge of biochemistry. Medical students who acquire a sound knowledge of biochemistry will be in a strong position to deal with two central concerns of the health sciences: (1) the understanding and maintenance of health and (2) the understanding and effective treatment of disease.

Biochemistry Is the Chemistry of Life

Biochemistry can be defined more formally as *the science concerned with the chemical basis of life* (Gk *bios* "life").

The **cell** is the structural unit of living systems. Consideration of this concept leads to a functional definition of biochemistry as *the science concerned with the chemical constituents of living cells and with the reactions and processes that they undergo.* By this definition, biochemistry encompasses large areas of **cell biology,** of **molecular biology,** and of **molecular genetics.**

The Aim of Biochemistry Is to Describe and Explain, in Molecular Terms, All Chemical Processes of Living Cells

The major objective of biochemistry is the complete understanding at the molecular level of all of the chemical processes associated with living cells. To achieve this objective, biochemists have sought to isolate the numerous molecules found in cells, determine their structures, and analyze how they function. To give one example, the efforts of many biochemists to understand the molecular basis of **contractility**—a process associated primarily, but not exclusively, with muscle cells—have entailed purification of many molecules, both simple and complex, followed by detailed structure-function studies. Through these efforts, some of the features of the molecular basis of **muscle contraction** have been revealed.

A further objective of biochemistry is to attempt to understand how life began. Knowledge of this fascinating subject is still embryonic.

The scope of biochemistry is as wide as life itself. Wherever there is life, chemical processes are occurring. Biochemists study the chemical processes that occur in microorganisms, plants, insects, fish, birds, mammals, and human beings. Students in the biomedical sciences will be particularly interested in the biochemistry of the two latter groups. However, an appreciation of the biochemistry of less complex forms of life is often of direct relevance to human biochemistry. For instance, contemporary theories on the regulation of the activities of genes and of enzymes in humans emanate from pioneering studies on bread molds and on bacteria. The field of **recombinant DNA** emerged from studies on bacteria and their viruses; their rapid multiplication times and the ease of extracting their genetic material make them suitable for genetic analyses and manipulations. Knowledge gained from the study of viral genes responsible for certain types of cancer in animals **(viral oncogenes)** has provided profound insights into how human cells become cancerous.

A Knowledge of Biochemistry Is Essential to All Life Sciences

The biochemistry of the nucleic acids lies at the heart of genetics; in turn, the use of genetic approaches has been critical for elucidating many areas of biochemistry. Physiology, the study of body function, overlaps with biochemistry almost completely. Immunology employs numerous biochemical techniques, and many immunologic approaches have found wide use by biochemists. Pharmacology and pharmacy rest on a sound knowledge of biochemistry and physiology; in particular, most drugs are metabolized by enzyme-catalyzed reactions, and the complex interactions among drugs are best understood biochemically. Poisons act on biochemical reactions or processes; this is the subject matter of toxicology. Biochemical approaches are being used increasingly to study basic aspects of pathology (the study of disease), such as inflammation, cell injury, and cancer.

Many workers in microbiology, zoology, and botany employ biochemical approaches almost exclusively. These relationships are not surprising, because life as we know it depends on biochemical reactions and processes. In fact, the old barriers among the life sciences are breaking down, and biochemistry is increasingly becoming their common language.

A Reciprocal Relationship Between Biochemistry and Medicine Has Stimulated Mutual Advances

As stated at the beginning of this chapter, 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 diseases. Biochemistry impacts enormously on both of these fundamental concerns of medicine. In fact, 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. Some examples of this two-way street are shown in Figure 1–1. For instance, a knowledge of protein structure and function was necessary to elucidate the single biochemical difference between normal and sickle cell hemoglobin. On the other hand, analysis of sickle cell hemoglobin has contributed significantly to our understanding of the structure and function of both normal hemoglobin and other proteins. Analogous examples of reciprocal benefit between biochemistry and medicine could be cited for the other paired items shown in Figure 1–1. Another example is the pioneering work of Garrod, a physician in England during the early years of this century. He studied patients with a number of relatively rare disorders (alkaptonuria, albinism, cystinuria, and pentosuria; these are described in later chapters) and established that these conditions were genetically determined. Garrod designated these conditions as **inborn errors of metabolism.** His insights provided a major foundation for the development of the field of human biochemical genetics.

This relationship between medicine and biochemistry has important philosophical implications for the former. As long as medical treatment is firmly grounded in a knowledge of biochemistry and other relevant basic sciences (eg, physiology, microbiology, nutrition), the practice of medicine will have a rational basis that can be adapted to accommodate new knowledge. This contrasts with unorthodox health cults, which are often founded on little more than myth and wishful thinking and generally lack any intellectual basis.

NORMAL BIOCHEMICAL PROCESSES ARE THE BASIS OF HEALTH

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 strictly 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 its maximal survival in the physiologic state. However, this is an extremely reductionist view, and it should be apparent that caring for the health of patients requires not only a wide knowledge of biologic principles but also of psychologic and social principles.

Biochemical Research Has Impact on Nutrition and Preventive Medicine

One major prerequisite for the maintenance of health is that there be optimal dietary intake of a number of chemicals; the chief of these are **vitamins,** cer-

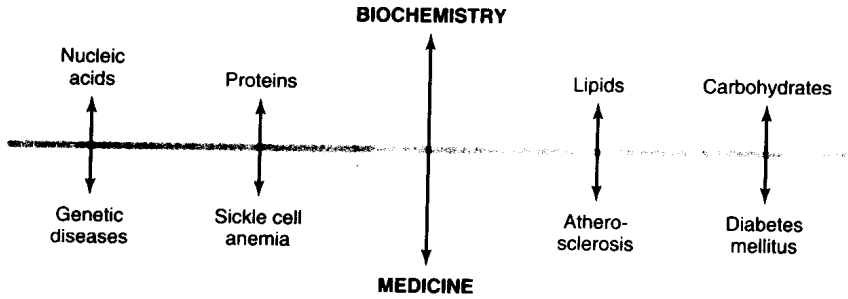

Figure 1–1. Examples of the two-way street connecting biochemistry and medicine. Knowledge of the biochemical compounds shown in the top part of the diagram has clarified our understanding of the diseases shown in the bottom half; conversely, analyses of the diseases shown below have cast light on many areas of biochemistry. Note that sickle cell anemia is a genetic disease and that both atherosclerosis and diabetes mellitus have genetic components.

tain **amino acids,** certain **fatty acids,** various **minerals,** and **water.** Because much of the subject matter of both biochemistry and nutrition is concerned with the study of various aspects of these chemicals, there is a close relationship between these two sciences. Moreover, as attempts are made to curb the rising costs of medical care, more emphasis is being placed on systematic attempts to maintain health and forestall disease, ie, on **preventive medicine.** Thus, nutritional approaches to—for example—the prevention of atherosclerosis and cancer are receiving increased emphasis. Understanding nutrition depends to a great extent on a knowledge of biochemistry.

All Disease Has a Biochemical Basis

All diseases are manifestations of abnormalities of molecules, chemical reactions, or processes. The major factors responsible for causing diseases in animals and humans are listed in Table 1–1. All of them affect one or more critical chemical reactions or molecules in the body. The majority of diseases discussed in this text are due to causes 5, 7, and 8.

Biochemical Studies Contribute to Diagnosis, Prognosis, and Treatment

There is a wealth of documentation of the uses of biochemistry in prevention, diagnosis, and treatment of disease; many examples will be cited throughout this text. Here, seven brief examples are given to illustrate the breadth of the subject and stimulate the reader's interest. Further information on these diseases is presented in the chapters listed in Table 1–2.

(1) Humans must ingest a number of complex organic molecules called vitamins in order to maintain health. If a particular vitamin is deficient in the diet, the reactions in which it is involved are compromised. This situation may be manifested as a deficiency disease such as **scurvy** or **rickets** (due to lack of intake of vitamin C and D, respectively). The elucidation of the roles played by the vitamins or their biologically active derivatives in animal and human cells has been a concern of biochemists and nutritionists since the turn of the century. Once a disease was established as resulting from a vitamin deficiency, it became rational to treat it by administration of the appropriate vitamin.

(2) The fact that many plants in Africa are deficient in one or more essential amino acids (ie, amino acids that must be supplied in the diet in order to maintain health) helps explain the debilitating malnutrition **(kwashiorkor)** suffered by infants who depend on such plants as major dietary sources of protein. Treatment of deficiencies of essential amino acids is rational but, unfortunately, not always feasible. It consists of providing a well-balanced diet containing adequate amounts of all of the essential amino acids.

(3) Greenland Inuit consume large quantities of fish oils rich in certain polyunsaturated fatty acids and are known to have low plasma levels of cholesterol and a low incidence of **atherosclerosis.** These observations have stimulated interest in the use of polyunsaturated fatty acids to reduce plasma levels of cholesterol.

The vitamin deficiency diseases and the essential amino acid deficiencies are examples of nutritional imbalances (Table 1–1). Atherosclerosis may be considered as a nutritional imbalance, but other important factors (eg, genetic) are also involved.

Table 1–1. The major causes of diseases. All of the causes listed act by influencing the various biochemical mechanisms in the cell or in the body.[1]

1. Physical agents: Mechanical trauma, extremes of temperature, sudden changes in atmospheric pressure, radiation, electric shock.
2. Chemical agents, including drugs: Certain toxic compounds, therapeutic drugs, etc.
3. Biologic agents: Viruses, bacteria, fungi, higher forms of parasites.
4. Oxygen lack: Loss of blood supply, depletion of the oxygen-carrying capacity of the blood, poisoning of the oxidative enzymes.
5. Genetic disorders: Congenital, molecular.
6. Immunologic reactions: Anaphylaxis, autoimmune disease.
7. Nutritional imbalances: Deficiencies, excesses.
8. Endocrine imbalances: Hormonal deficiencies, excesses.

[1]Adapted, with permission, from Robbins SL, Cotram RS, Kumar V: *The Pathologic Basis of Disease,* 3rd ed. Saunders, 1984.

Table 1–2. Some examples of diseases whose biochemical aspects are discussed in this text.

Disease[1]	Causes	Chapters
Scurvy (MIM 240400), rickets	Deficiencies of vitamins C and D, respectively	52, 53
Kwashiorkor	Deficiency of dietary protein	65
Atherosclerosis	Genetic, dietary, and environmental factors	28
Phenylketonuria (MIM 261600)	Mainly mutations in the *gene encoding phenylalanine hydroxylase*	32
Cystic fibrosis (MIM 219700)	Mutations in the gene encoding the CFTR protein	65
Cholera	Exotoxin of *Vibrio cholerae*	65
Diabetes mellitus, type 1 (MIM 222100)	Genetic and environmental factors resulting in a deficiency of insulin	51, 65

[1]The numbers in parentheses are the Mendelian Inheritance in Man numbers (see References); where no number is listed, the condition is not listed in that work, probably because it is not predominantly a genetic condition (eg, rickets, kwashiorkor, cholera) or because it is a pathologic process (eg, atherosclerosis) as opposed to a distinct disease entity.

(4) The condition known as **phenylketonuria,** if untreated, may lead to severe mental retardation in infancy. The biochemical basis of phenylketonuria has been known since 1953; the disorder is genetically determined and results from low or absent activity of the enzyme that converts the amino acid phenylalanine to the amino acid tyrosine. This in turn causes an elevation of the level of phenylalanine in the blood, resulting in damage to the developing central nervous system. When the nature of the biochemical lesion in phenylketonuria was revealed, it became rational to treat the disease by placing affected infants on a diet low in phenylalanine. Once biochemical screening tests for diagnosing phenylketonuria at birth became available, effective treatment could be started immediately.

(5) **Cystic fibrosis** is a common genetic disease affecting the exocrine glands and the eccrine sweat glands. It is characterized by abnormally viscous secretions that plug up the secretory ducts of the pancreas and the bronchioles. In addition, patients with cystic fibrosis exhibit elevated amounts of chloride in their sweat. Victims often die at an early age from lung infections. The isolation and complete sequence of the gene responsible for this disease was reported in 1989. The normal gene codes for a transmembrane protein (the cystic fibrosis transmembrane conductance regulator), 1480 amino acids in length, which functions as a chloride channel. The abnormality in approximately 70% of patients with cystic fibrosis is a deletion of three bases in the gene, resulting in the transmembrane protein lacking amino acid number 508, a phenylalanine residue. How this deletion impairs the function of the transmembrane protein and results in the excessively thick mucus is being determined. This important work should facilitate the detection of carriers of the cystic fibrosis gene and, it is hoped, lead to more rational treatment of the disease than exists at present. For instance, it may be possible to design drugs that can correct the abnormality in the transmembrane protein; likewise, it may be possible to introduce the normal gene into lung cells by gene therapy. Phenylketonuria and cystic fibrosis are examples of genetic diseases (Table 1–1).

(6) Analysis of the mechanism of action of the bacterial toxin that causes **cholera** has provided important insights into how the clinical manifestations of this disease (copious diarrhea and loss of salt and water) are brought about.

(7) **Diabetes mellitus** is prevalent in many parts of the world. One fundamental aspect of diabetes is an abnormality of the metabolism of glucose, resulting in elevated blood levels (hyperglycemia). Two major types are recognized: type 1 (insulin-dependent) and type 2 (non-insulin-dependent). To understand diabetes mellitus and treat it effectively, one must be familiar with the metabolism of glucose and the many effects of insulin in the human body.

Many Biochemical Studies Illuminate Disease Mechanisms, and Diseases Inspire Biochemical Research

The initial observations made by Garrod on a small group of inborn errors of metabolism in the early 1900s stimulated the investigation of the biochemical pathways affected in these conditions. Efforts to understand the basis of the genetic disease known as **familial hypercholesterolemia,** which results in severe atherosclerosis at an early age, have led to dramatic progress in knowledge of cell receptors and of mechanisms of uptake of cholesterol into cells. The ongoing studies of **oncogenes** in cancer cells have directed attention to the molecular mechanisms involved in the control of normal cell growth. These and many other possible examples illustrate how the study of disease can open up whole areas of cell function for basic biochemical research.

THIS TEXT WILL HELP RELATE BIOCHEMICAL KNOWLEDGE TO CLINICAL PROBLEMS

Brief descriptions of the biochemical mechanisms underlying many diseases are interspersed throughout the text. However, Chapters 63, 64, and 65 specifically describe the biochemical bases of a number of important diseases. The Appendix briefly dis-

Table 1–3. Some uses of biochemical investigations and laboratory tests in relation to diseases.

Use	Example
1. To reveal the fundamental causes and mechanisms of diseases	Demonstration of the nature of the genetic defects in cystic fibrosis.
2. To suggest rational treatments of diseases based on (1) above	Use of a diet low in phenylalanine for the treatment of phenylketonuria.
3. To assist in the diagnosis of specific diseases	Use of the plasma enzyme creatine kinase MB (CK-MB) in the diagnosis of myocardial infarction.
4. To act as screening tests for the early diagnosis of certain diseases	Use of measurement of blood thyroxine or thyroid-stimulating hormone (TSH) in the neonatal diagnosis of congenital hypothyroidism.
5. To assist in monitoring the progress (eg, recovery, worsening, remission, or relapse) of certain diseases	Use of the plasma enzyme alanine aminotransferase (ALT) in monitoring the progress of infectious hepatitis.
6. To assist in assessing the response of diseases to therapy	Use of measurement of blood carcinoembryonic antigen (CEA) in certain patients who have been treated for cancer of the colon.

cusses some basic considerations used in the interpretation of the results of the biochemical laboratory tests and lists the most widely used tests along with their ranges of normal values. The overall purpose of these final chapters and the Appendix is to assist and encourage the reader to translate knowledge of biochemistry into effective clinical use.

Some major uses of biochemical investigations and of laboratory tests in relation to diseases are summarized in Table 1–3. Additional examples of many of these uses are presented in various sections of this text.

SUMMARY

Biochemistry is the science concerned with studying the various molecules that occur in living cells and organisms and with their chemical reactions. Because life depends on biochemical reactions, biochemistry has become the basic language of all biologic sciences.

Biochemistry is concerned with the entire spectrum of life forms, from relatively simple viruses and bacteria to complex human beings.

Biochemistry and medicine are intimately related. Health depends on a harmonious balance of biochemical reactions occurring in the body, and disease reflects abnormalities in biomolecules, biochemical reactions, or biochemical processes.

Advances in biochemical knowledge have illuminated many areas of medicine. Conversely, 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.

The judicious use of various biochemical laboratory tests is 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 medical and related health sciences.

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(The numbers assigned to the entries in MIM and OMIM will be cited in selected chapters of this work. Consulting this extensive collection of diseases and other relevant entries—specific proteins, enzymes, etc—will greatly expand the reader's knowledge and understanding of various topics referred to and discussed in this text. The online version is updated almost daily.)

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2 Biomolecules and Biochemical Methods

Robert K. Murray, MD, PhD

INTRODUCTION

This chapter has five objectives. The first is to indicate **the composition of the body and the major classes of molecules** found in it. These molecules make up the principal subjects of this text.

The cell is the major structural and functional unit of biology. Most chemical reactions occurring in the body take place within cells. Thus, the second objective is to give a concise account of the **components of cells** and how they may be isolated; the details of how these components function form much of the fabric of this text.

The third objective concerns the fact that biochemistry is an experimental science. It is important to have an understanding and appreciation of the **experimental approach and methods** used in biochemistry, lest its study become an exercise in rote learning. Moreover, biochemistry is not an immutable corpus of knowledge but a constantly evolving field. Advances, like those in other biomedical areas, depend on the experimental approach and technologic innovation.

The fourth objective is to summarize briefly the **principal achievements** that have been made in biochemistry. The concise view of the science that is presented will assist in imparting to the reader a sense of the overall direction of the remainder of the text.

The fifth objective is to indicate **how little is known about certain areas,** eg, about development, differentiation, brain function, cancer, and many other human diseases. Perhaps this will serve as a stimulus to some readers to contribute to research in these areas.

THE HUMAN BODY IS COMPOSED OF A FEW ELEMENTS THAT COMBINE TO FORM A GREAT VARIETY OF MOLECULES

Carbon, Hydrogen, Oxygen, and Nitrogen Are the Major Elements

The elementary composition of the human body has been determined, and the major findings are listed in Table 2–1. Carbon, oxygen, hydrogen, and nitrogen are the major constituents of most biomolecules. **Phosphate** is a component of the nucleic acids and other molecules and is also widely distributed in its ionized form in the human body. **Calcium** plays a key role in innumerable biologic processes and is the focus of much current research. The elements listed in the third column of the table fulfill diverse roles. Most are encountered on an almost daily basis in medical practice in dealing with patients with electrolyte imbalances (K^+, Na^+, Cl^-, and Mg^{2+}), iron-deficiency anemia (Fe^{2+}), and thyroid diseases (I^-).

The Five Major Complex Biomolecules Are DNA, RNA, Proteins, Polysaccharides, and Complex Lipids

As shown in Table 2–2, the major complex biomolecules found in the cells and tissues of higher animals (including humans) are **DNA, RNA, proteins, polysaccharides,** and **lipids.** These complex molecules are constructed from simple biomolecules, which are also listed. The building blocks of DNA and RNA (collectively known as the nucleic acids) are **deoxynucleotides** and **ribonucleotides,** respectively. The building blocks of proteins are **amino acids.** Polysaccharides are built up from simple carbohydrates; in the case of **glycogen** (the principal polysaccharide found in human tissues), the carbohydrate is **glucose. Fatty acids** may be considered to be the building blocks of many lipids, although lipids are not polymers of fatty acids. DNA, RNA, proteins, and polysaccharides are referred to as **biopolymers** because they are composed of repeating units of their building blocks (the monomers). The above molecules essentially make up the "stuff of life"; most of this text will be largely concerned with descriptions of their various biochemical features and of their building blocks. The same complex molecules are also generally found in lower organisms, although the building blocks in certain cases may differ from those shown in Table 2–2. For instance, bacteria do not contain glycogen or triacylglycerols, but they do contain other polysaccharides and lipids.

Table 2–1. Approximate elementary composition of the human body (dry weight basis).[1]

Element	Percent	Element	Percent
Carbon	50	Potassium	1
Oxygen	20	Sulfur	0.8
Hydrogen	10	Sodium	0.4
Nitrogen	8.5	Chlorine	0.4
Calcium	4	Magnesium	0.1
Phosphorus	2.5	Iron	0.01
		Manganese	0.001
		Iodine	0.00005

[1]Reproduced, with permission, from West ES, Todd WR: *Textbook of Biochemistry,* 3rd ed. Macmillan, 1961.

Protein, Fat, Carbohydrate, Water, and Minerals Are the Chief Components of the Human Body

The elementary composition of the human body is given above. Its chemical composition is shown in Table 2–3; protein, fat, carbohydrate, water, and minerals are the chief components. Water constitutes the major component, although its amount varies widely among different tissues. Its polar nature and ability to form hydrogen bonds render water ideally suited for its function as the solvent of the body. A detailed account of the properties of water is presented in Chapter 3.

THE CELL IS THE BASIC UNIT OF BIOLOGY

The cell was established as the fundamental unit of biologic activity by Schleiden and Schwann and other pioneers, such as Virchow, in the 19th century. However, in the years immediately after World War II, three developments helped usher in a period of unparalleled activity in biochemistry and cell biology. These were (1) the increasing availability of the **electron microscope;** (2) the introduction of methods permitting **disruption of cells** under relatively mild conditions that preserved function; and (3) the increasing availability of the high-speed, refrigerated **ultracentrifuge,** capable of generating centrifugal forces sufficient to separate the constituents of disrupted cells from one another without overheating them. Use of the electron microscope revealed many previously unknown or poorly observable cellular components, while disruption and ultracentrifugation permitted their isolation and analysis in vitro.

Table 2–2. The major complex organic biomolecules of cells and tissues. The nucleic acids, proteins, and polysaccharides are biopolymers, constructed from the building blocks shown. The lipids are not generally biopolymers, and not all lipids have fatty acids as building blocks.

Biomolecule	Building Block	Major Functions
DNA	Deoxynucleotide	Genetic material
RNA	Ribonucleotide	Template for protein synthesis
Proteins	Amino acids	Numerous; usually they are the molecules of the cell that perform work (eg, enzymes, contractile elements)
Polysaccharide (glycogen)	Glucose	Short-term storage of energy as glucose
Lipids	Fatty acids	Numerous, eg, membrane components and long-term storage of energy as triacylglycerols

Table 2–3. Normal chemical composition for a man weighing 65 kg.[1]

	kg	Percent
Protein	11	17.0
Fat	9	13.8
Carbohydrate	1	1.5
Water[2]	40	61.6
Minerals	4	6.1

[1]Reproduced, with permission, from Davidson SD, Passmore R, Brock JF: *Human Nutrition and Dietetics,* 5th ed. Churchill Livingstone, 1973.
[2]The value of water can vary widely among different tissues, being as low as 22.5% for marrow-free bone. The percentage consisting of water also tends to diminish as body fat increases.

A Rat Hepatocyte Illustrates Features Common to Many Eukaryotic Cells

A diagram of the structure of a liver cell (hepatocyte) of a rat is shown in Figure 2–1; this cell is one of the most extensively studied of all cells from a biochemical viewpoint, partly because of its availability in relatively large amounts, suitability for fractionation studies, and diversity of functions. The hepatocyte contains the major **organelles** found in eukaryotic cells (Table 2–4); these include the nucleus, mitochondria, endoplasmic reticulum, free ribosomes, Golgi apparatus, lysosomes, peroxisomes, plasma membrane, and certain cytoskeletal elements.

Physical Techniques Are Used to Disrupt Cells and to Isolate Intracellular Molecules and Subcellular Organelles

In order to study the function of any organelle in depth, it is first necessary to isolate it in relatively pure form, free of significant contamination by other organelles. The usual process by which this is achieved is called **subcellular fractionation** and generally entails three procedures: extraction, homogenization, and centrifugation. Much of the pioneering work in this area was done using rat liver.

A. Extraction: As a first step toward isolating a specific organelle (or molecule), it is necessary to extract it from the cells in which it is located. Most organelles and many biomolecules are labile and sub-

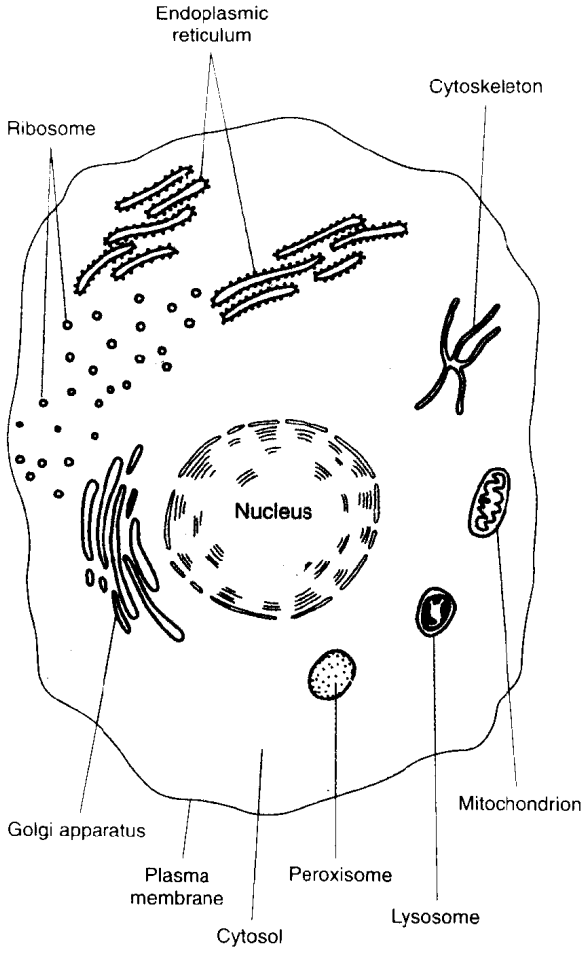

Figure 2–1. Schematic representation of a rat liver cell with its major organelles.

ject to loss of biologic activities: they must be extracted using mild conditions (ie, employment of aqueous solutions and avoidance of extremes of pH and osmotic pressure and of high temperatures). In fact, most procedures for isolating organelles are performed at about 0–4 °C (eg, in a cold room or using material kept on ice). Significant losses of activity can occur at room temperature, partly owing to the action of various digestive enzymes (proteases, nucleases, etc) liberated when cells are disrupted. A common solution for extraction of organelles consists of sucrose, 0.25 mol/L (isosmotic), adjusted to pH 7.4 by TRIS (tris-[hydroxymethyl]aminomethane) hydrochloric acid buffer, 0.05 mol/L, containing K^+ and Mg^{2+} ions at near physiologic concentrations; this solution is conveniently called STKM. Not all solvents used for extraction are as mild as STKM; eg, organic solvents are used for the extraction of lipids and of nucleic acids.

B. Homogenization: To extract an organelle (or biomolecule) from cells, it is first necessary to disrupt the cells under mild conditions. Organs (eg, liver, kidney, brain) and their contained cells may be conveniently disrupted by the process of homogenization, in which a manually operated or motor-driven pestle is rotated within a glass tube of suitable dimensions containing minced fragments of the organ under study and a suitable homogenizing medium, such as STKM. The controlled rotation of the pestle exerts mechanical shearing forces on cells and disrupts them, liberating their constituents into the sucrose. The resulting suspension, containing many intact organelles, is known as a **homogenate.**

C. Centrifugation: Subfractionation of the contents of a homogenate by differential centrifugation has been a technique of central importance in biochemistry. The classic method uses a series of three different centrifugation steps at successively greater speeds (Figure 2–2), each yielding a pellet and a supernatant. The supernatant from each step is subjected to centrifugation in the next step. This procedure provides three pellets, named the nuclear, mitochondrial, and microsomal fractions. None of these fractions are composed of absolutely pure organelles. However, it has been well established by the use of the electron microscope and by measurements of suitable "marker" enzymes and chemical components (eg, DNA and RNA) that the major constituents of each of these three fractions are nuclei, mitochondria, and microsomes, respectively. A "marker" enzyme or chemical is one that is almost exclusively confined to one particular organelle, eg, acid phosphatase to lysosomes and DNA to the nucleus (Table 2–4). The marker can thus serve to indicate the presence or absence in any particular fraction of the organelle in which it is contained. The **microsomal fraction (microsomes)** contains mostly a mixture of smooth endoplasmic reticulum, rough endoplasmic reticulum (ie, endoplasmic reticulum with attached ribosomes), and free ribosomes. The contents of the final supernatant correspond approximately to those of the **cell sap (cytosol).** Modifications of this basic approach, using different homogenization media or different protocols or methods of centrifugation (eg, the use of gradients—either continuous or discontinuous—of sucrose), have permitted the isolation in more or less pure form of all of the organelles illustrated in Figure 2–1 and listed in Table 2–4. The scheme described above is applicable in general terms to most organs and cells; however, cell fractionations of this type must be assessed by the use of measurements of marker enzymes and chemicals and by the electron microscope until the overall procedure can be considered to be standardized.

The importance of subcellular fractionation studies in the development of biochemistry and cell biology cannot be overemphasized. It has been one of the major components of the experimental approach (see below), and—largely because of its application—the