BIOCHEMICAL ENGINEERING FUNDAMENTALS

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PREFACE

Biochemical engineering is the domain of microbial and enzyme processes, natural or artificial. The field encompasses many topics including industrial fermentation, enzyme utilization, and wastewater treatment. Our intention has been to treat the majority of these topics by covering the biochemical and engineering principles upon which these processes are based. We have provided an inclusive base of fundamentals and applications in the hope that the broad subject of biochemical engineering may soon be incorporated into many more engineering curricula than the few presently offering this course.

Biochemical engineering courses for juniors, seniors, and graduate students in chemical, environmental, civil, or food engineering may be taught from appropriate portions of this text. The authors, both chemical engineers, have presented undergraduate and graduate versions of a biochemical engineering course on quarter or semester bases over the last five years at Princeton University and the University of Houston. Portions of our notes from which the text has been developed have been used in biochemical engineering courses at the University of California at Berkeley, Iowa State University, the University of Massachusetts, and the University of Virginia.

Topics covered by the text include biochemistry, microbiology, reactor design and analysis, and transport phenomena. Our approach varies from traditional presentations in several ways. We have tried to interweave descriptive material on the life sciences together with engineering processes and analytical techniques. The implications of life science fundamentals for engineering processes are frequently discussed in sections dealing with biological principles. For example, the introduction of molecular genetics, viruses, mutation and genetic manipulation is followed by a discussion of recent applications of microbial genetics in developing especially productive microorganisms for several fermentations. The appropriate analytical techniques are presented after initial descriptive and background material has been given. Thus, enzyme kinetics and technology are introduced after the description of biochemicals including proteins; pure culture reactor dynamics are analyzed prior to the introduction of multiple species interactions.

Both text examples and end-of-chapter problems provide the student with opportunities to apply the concepts presented and to broaden understanding of the subject. More than 130 problems, spanning a range of difficulty, require discussions, derivations, and/or calculations from the student. A teacher's manual for this text, which provides many solutions and additional suggestions, is available.

Acknowledgments are due to Peter Reilly and Murray Moo-Young for their criticisms, to Elmer Gaden and Harold Bungay for copies of their own course notes, and to George Tsao, whose 1970 Chemical Engineering Education articles identified the need for a broad ranging text such as the present effort. Peter Reilly also kindly donated several homework problems. In addition, we are indebted to J. F. Andrews, E. L. Cussler, A. C. Payatakes, W. Phillips, D. A. Saville, and C. J. Shearer for valuable discussions. The authors take full responsibility for any errors and welcome comments and suggestions from readers.

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We are pleased to thank several established contributors in biochemical engineering including Elmer Gaden, Arthur E. Humphrey, and Daniel I. C. Wang for warmly encouraging our entry in this field several years ago. Unrestricted financial support provided by the Camille and Henry Dreyfus Foundation to both authors freed the time needed to complete this text.

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CONTENTS

CHAPTER

ONE

A LITTLE MICROBIOLOGY

Small living creatures called *microorganisms* interact in numerous ways with human activities. On the large scale of the biosphere, which consists of all regions of the earth containing life, microorganisms play a primary role in the capture of energy from the sun. Their biological activities also complete critical segments of the cycles of carbon, oxygen, nitrogen, and other elements essential for life. Microbes are also responsible for many human diseases. It has been argued that plagues caused by microorganisms have altered the course of history.

In this text, however, we concentrate on the purposeful utilization of microorganisms. A wide spectrum of profitable examples can be cited, including food processing, the manufacture of alcoholic beverages, and production of such complex organic molecules as vitamins and hormones. Moreover, microbial action provides an indispensable contribution in the treatment of sewage and many industrial wastes. Our principal objective is to understand and analyze such processes so that we can design and operate them in a rational way.

To reach this goal, however, a basic working knowledge of microbial growth and function is required. These factors and others peculiar to biological systems usually dominate biochemical process engineering. Consider for a moment that a living microorganism may be viewed in an approximate conceptual sense as an expanding chemical reactor which takes in chemical species called *nutrients* from its environment, grows, reproduces, and releases products into its surroundings. In instances such as sewage treatment, consumption of nutrients (here the organic sewage material) is the engineering objective. When microbes are grown for food sources or supplements, it is the mass of microbial matter produced which is desired. For a sewage-treatment process, on the other hand, this microbial matter produced by nutrient consumption constitutes an undesirable solid waste, and its amount should be minimized. Finally, the products formed and released during biological activity are of major concern in many industrial and natural contexts, including penicillin and ethanol manufacture. The relative rates of nutrient utilization, growth, and release of products depend strongly on the type of microorganism involved and on the temperature and composition of its environment. Understanding these interactions requires a foundation built upon biochemistry, biophysics, and microbiology. Since study of these subjects is not traditionally included in engineering education, a substantial portion of our efforts must be dedicated to them.

Whenever possible we shall extend our study of biological processes beyond qualitative understanding to determine quantitative mathematical representations. These mathematical models will often be extremely oversimplified and idealized, since even a single microorganism is a very complicated system. Nevertheless, basic concepts in microbiology will serve as a guide in formulating models and checking their validity, just as basic knowledge in fluid mechanics is useful when correlating the friction factor with the Reynolds number.

1.1. BIOPHYSICS AND THE CELL DOCTRINE

Microbiology is the study of living organisms too small to be seen clearly by the naked eye. As a rough rule of thumb, most microorganisms have a diameter of 0.1 mm or less. For many years, microbiology and other avenues of biological science were considered disciplines distinct from the physical sciences. It was thought that living things contained a "vital force" not governed by the laws of physics and chemistry.

In retrospect this is not surprising, for present knowledge indicates that even the simplest microorganism houses chemical reactors, information and control systems, and mass-transfer operations of amazing sophistication, efficiency, and organization. These conclusions have been reached in numerous experimental studies involving methods adapted from the physical sciences. Since this approach has proven so fruitful, the applicability of the principles of chemistry and physics to biological systems is now a widespread working hypothesis within the life sciences. The term *biophysics* is sometimes used to indicate explicitly the union of the biological and physical sciences.

A development critical to the understanding of living systems started in 1838, when Schleiden and Schwann first proposed the *cell theory*. This theory stated that all living systems are composed of cells and their products. Thus, the concept of a basic module, or building block, for life emerged. This notion of a common denominator permits an important decomposition in the analysis of living systems: first the component parts, the cells, can be studied, and then this knowledge is used to try to understand the complete organism.

The value of this decomposition rests on the fact that cells from a wide variety of organisms share many common features in their structure and function. In many instances this permits successful extrapolation of knowledge gained from experiments on cells from one organism to cells of other types. This existence of common cellular characteristics also simplifies our task of learning how microorganisms behave. By concentrating on the apparently universal features of cellular function, a basic framework for understanding all living systems can be established.

We should not leave this section with the impression that all cells are alike, however. Muscle cells are clearly different from those found in the eye or brain. Equally, there are many different types of single-celled organisms. These in turn can be classified in terms of the two major types of cellular organization described next.

1.2. THE STRUCTURE OF CELLS

Observations with the electron microscope have revealed two markedly different kinds of microbial cells. Although still linked by certain common features, these two classes are sufficiently distinct in their organization and function to warrant individual consideration here. So far as is known today, all cells belong to one of these groups.

1.2.1. Procaryotic Cells

Procaryotic cells, or *procaryotes*, are relatively small and simple cells. They usually exist alone, not associated with other cells. The typical dimension of these cells, which may be spherical, rodlike, or spiral, is from 0.5 to 3 μm.† In order to gain a qualitative feel for such dimensions, it is instructive to compare the relative sizes of cells with other components of the universe. As Fig. 1.1 reveals, the size of a procaryote relative to a man is approximately equal to the size of a man relative to the earth and less than the size of the hydrogen atom compared with that of a cell. These size relationships are very significant considerations when the details of cell function are investigated as we shall see later. The volume of procaryotes is on the order of 10^{-12} ml per cell, of which 50 to 80 percent is water. As a rough estimate, the mass of a single procaryote is 10^{-12} g.

Cells of this type gr[illegible] are widespread in the biosphere. Some, for example, can d[illegible] in 20 min. Typically, procaryotes are biochemically [illegible] can accept a wide variety of nutrients and further are capable of selecting the best nutrient from among several available in their environment. This feature and others to b[illegible] later make procaryotic cells adaptable to a wide range of environ[illegible] procaryotes usually exist as isolated single-celled organisms, they have little [illegible]

† 1 m (meter) = 10^3 mm (millimeter) = 10^6 μm (micrometer, formerly known as micron) = 10^{10} Å (angstrom units).

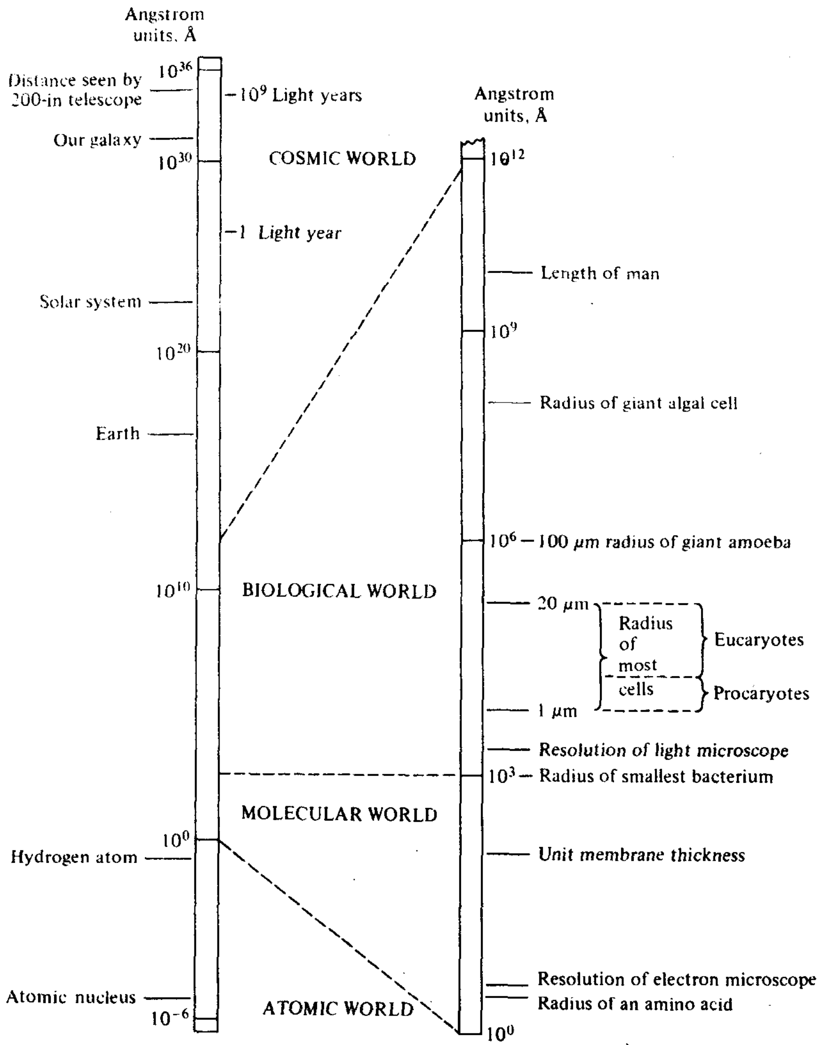

Figure 1.1 Characteristic dimensions of the universe. The biological world encompasses a broad spectrum of sizes. (*From "Cell Structure and Function," 2d ed., p. 35, by Ariel G. Loewy and Philip Siekevitz. Copyright © 1963, 1969 by Holt, Rinehart and Winston Inc. Reprinted by permission of Holt, Rinehart and Winston.*)

of controlling their surroundings. Therefore the nutrient flexibility they exhibit is an essential characteristic for their survival. The rapid growth and biochemical versatility of procaryotes make them obvious choices for biological research and biochemical processing.

In Fig. 1.2 the basic features of a procaryotic cell are illustrated. The cell is surrounded by a rigid *wall*, approximately 200 Å thick. This wall lends structural

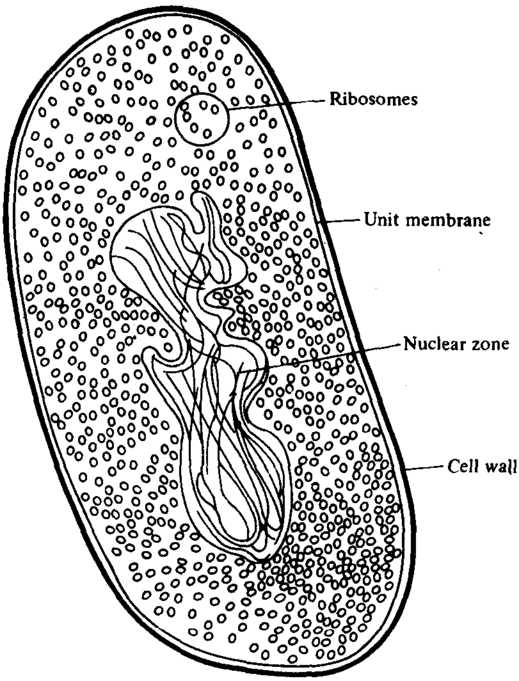

Figure 1.2 A sketch of a procaryote, *Escherichia coli*. This bacterium is native to man's intestinal tract and is sometimes simply called the intestinal bacterium. It is the most thoroughly investigated cell at present. Much of our knowledge of genetics at the molecular level is derived from studies of *E. coli*.

strength to the cell so that it can withstand a wide variety of external surroundings. Immediately inside this wall is the *cell membrane*, which typically has a thickness of about 70 Å. This membrane has a general structure common to membranes found in all cells. It is sometimes called a *plasma membrane*. These membranes play a critical role: they largely determine which chemical species can be transferred between the cell and its environment as well as the net rate of such transfer. Within the cell is a large, ill-defined region called the *nuclear zone*, which is the dominant control center for cell operation. The grainy dark spots apparent in the cell interior are the *ribosomes*, the sites of important biochemical reactions. The *cytoplasm* is the fluid occupying the remainder of the cell. Finally the *cytosol* is a colloidal suspension of large organic molecules. Not apparent here but visible in other photographs are clear, bubblelike regions called *storage granules*. We shall explore the composition and function of these structures within the procaryotic cell in greater detail after establishing the necessary background and defining some terms.

In order to bring out the similarities and differences between procaryotes, we consider another member of this family in Fig. 1.3. Again ribosomes, a nuclear zone, and cell wall are evident. This microorganism, however, is equipped with the biochemical machinery to utilize sunlight as an energy source, a capability illustrated by the presence of photosynthetic membranes.

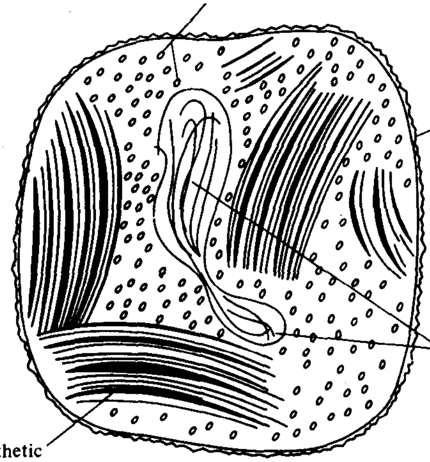

Figure 1.3 Another procaryote, a blue-green alga. This cell contains membranes capable of conducting *photosynthesis*, a complex process which captures light energy from the sun, provides the cell with organic molecules suitable for its reactions, and releases oxygen into the atmosphere.

1.2.2. Eucaryotic Cells

Eucaryotic cells, or *eucaryotes*, make up the other major class of cell types. As a rule these cells are 1000 to 10,000 times larger than procaryotes. All cells of higher organisms belong to this family. In order to meet the many specialized needs of animals, for example, eucaryotic cells exist in a wide variety of forms, as illustrated in Fig. 1.4. By coexisting and interacting in a cooperative manner in a higher organism, these cells can avoid the necessity for biochemical flexibility and adaptability so essential to procaryotes. Eucaryotic cells are not confined to plants and animals, however. In the next section we shall see several examples of eucaryotes which exist as single-celled organisms.

The internal structure of eucaryotes is considerably more complex than that in procaryotic cells, as can be seen in Figs. 1.5 and 1.6. Here there is a substantial degree of spatial organization and differentiation. The internal region is divided into a number of distinct compartments, which we shall explore in greater detail later; they have special structures and functions for conducting the business of the cell. At this point we shall only consider the general features of eucaryotic cells.

The cell is surrounded by a plasma, or unit, membrane similar to that found in procaryotes. On the exterior surface of this membrane may be a cell coat, or wall. The nature of the outer covering depends on the particular cell. For example, cells of higher animals usually have a thin cell coat. The specific adhesive properties of this coat are important in binding like cells to form specialized tissues and organs such as the liver. Plant cells, on the other hand, are often enclosed in a very strong,

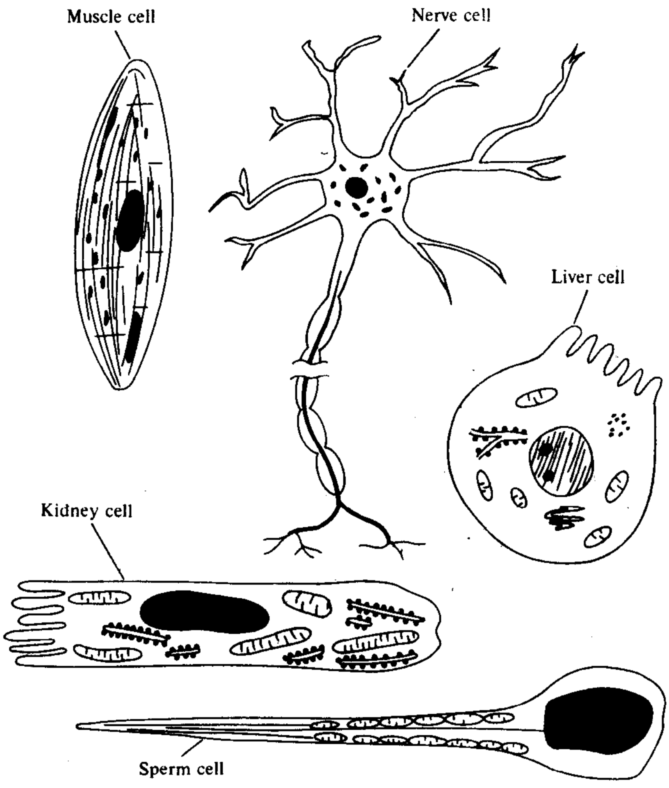

Figure 1.4 Several varieties of eucaryotes found in man. *(From "Cell Structure and Function," 2d ed., p. 6, by Ariel G. Loewy and Philip Siekevitz. Copyright © 1963, 1969 by Holt, Rinehart and Winston Inc. Reprinted by permission of Holt, Rinehart and Winston.)*

thick wall, which can be seen in Fig. 1.7. Wood consists for the most part of the walls of dead tree cells.

Important to the internal specialization of eucaryotic cells is the presence of unit membranes within the cell. A complex, convoluted membrane system, called the *endoplasmic reticulum*, leads from the cell membrane into the cell. The *nucleus* here is surrounded by a porous membrane. *Ribosomes*, reaction sites seen before in procaryotes, are embedded in the surface of much of the endoplasmic reticulum. (Ribosomes in procaryotes are smaller, however.) Ribosomes are in a sense analogous to the metal crystallites impregnated within porous supports to catalyze reactions in the classical process industry. A highly convoluted and twisting endoplasmic reticulum serves the same end as a very porous support for catalytic metals: it increases the available surface area per unit volume. The resemblance with such fabricated systems cannot be extended much further, however, because the living cell is more complex and sophisticated by many orders of magnitude.

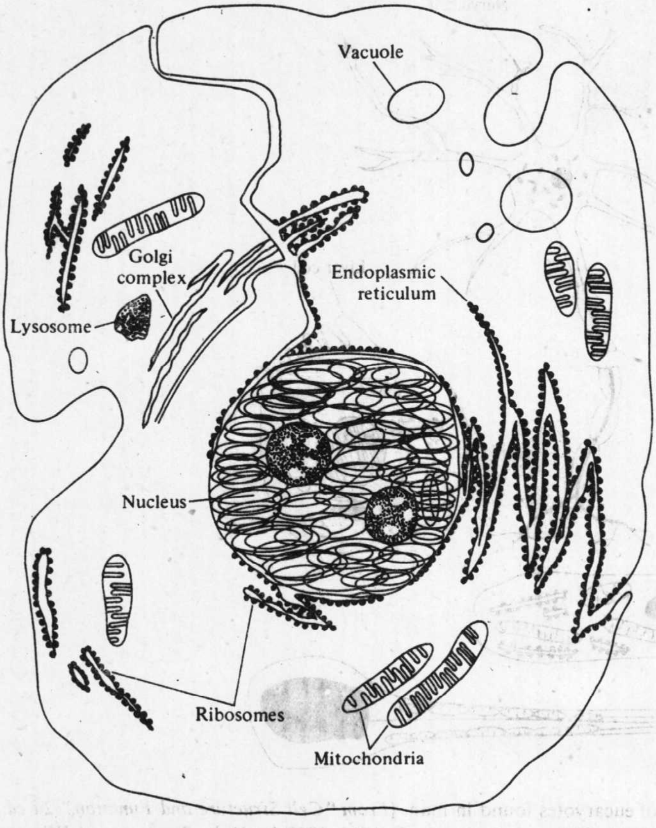

Figure 1.5 A typical eucaryotic cell. Such a typical cell is an imaginary construct, for there are wide variations between different eucaryotes. Many of these cells share common features and components, making the typical eucaryote a convenient and useful concept.

For example, a major function of the nucleus is to control the catalytic activity at the ribosomes. Not only are the reaction rates regulated, but the particular reactions which occur are determined by chemical messengers manufactured in the nucleus.

The nucleus is one of several interior regions surrounded by unit membranes. These specialized membrane-enclosed domains are known collectively as *organelles*. Catalyzing reactions whose products are the major energy supply of the cell, the *mitochondria* are organelles with an extremely specialized and organized internal structure. They are found in all eucaryotic cells which utilize oxygen in the process of energy generation. In *phototrophic cells*, which are those using light as a primary energy source, the *chloroplast* (see Fig. 1.7) is the organelle serving as the major cell powerhouse. Chloroplasts and mitochondria are the sites of many other important biochemical reactions in addition to their role in energy production.

The Golgi complex, lysosomes, and vacuoles are the remaining organelles illustrated in Figs. 1.5 to 1.7. In general, they serve to isolate chemical reactions or

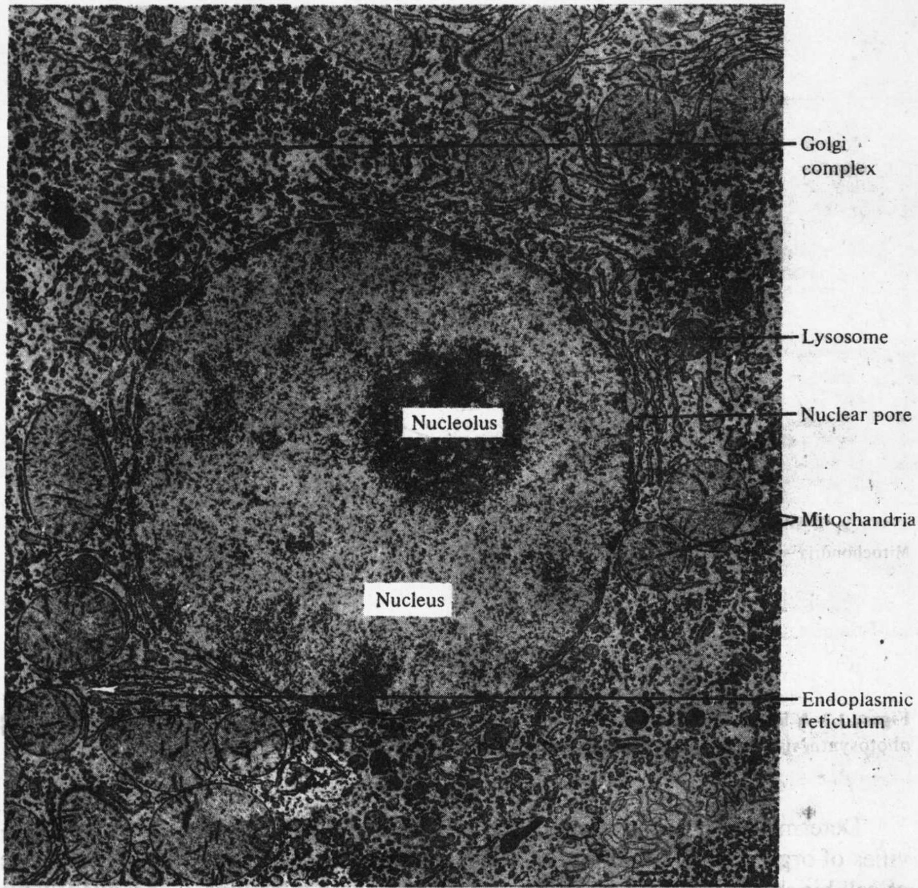

Figure 1.6 Electron micrograph of a rat liver cell (×11,000). (*Courtesy of George E. Palade, Yale University.*)

certain chemical compounds from the cytoplasm. This isolation is desirable either from the standpoint of reaction efficiency or protection of other cell components from the contents of the organelle.

Other interior features of eucaryotic cells include components involved in cell division and cell motion. Since such aspects of cell operation are not central to our purposes, we leave details on these matters to the references listed at the end of the chapter.

The discovery of similar organelles in a wide variety of cells allows a refinement of the major working advantages of the cell doctrine. The activities of the cell itself can now be decomposed conceptually into the activities of its component organelles, which in turn can be studied in isolation. In the absence of contrary evidence, similar organelles are assumed to perform similar operations and functions, regardless of the type of cell in which they are found.