Essentials of Medical Microbiology

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Preface

The advent of a new medically-oriented microbiology text to compete with what seems to be an abundance of such texts may seem foolhardy, indeed. But this book, like many other texts, was written with a specific objective and for a fairly specific audience. The objective was a comprehensive text containing the essentials of medical microbiology that could be read in its entirety during a one-term course. Within the confines of this objective every effort has been made to support scientific facts with experimental data, explanations, and illustrations. This text is directed to a reader who has had at least an elementary introduction to biochemistry, but it does not require a background in microbiology.

Who, then, are the expected readers? This text is modeled, in part, after the microbiology course taught to medical students at the University of Virginia, in which the author has taught for over a quarter of a century. It is also designed for upper-level undergraduates and graduate students who wish to complete a one-term course in medical microbiology.

The book is divided into five units. Unit One provides an introduction to the microbial world, and for those individuals who are not new to the field it should provide an easy review of basic microbiology. Unit Two is concerned with immunology and contains a detailed discussion of nonspecific host resistance as well as humoral and cellular immune reactions. This unit describes the structure and synthesis of antibodies, and the role of antibodies, complement, and cells in host protection, allergy, and autoimmune reactions. Unit Three is devoted to the medically important bacteria and fungi, while Unit Four describes the structure, growth, and characteristics of animal viruses. Both of these units emphasize the epidemiology, mechanism of disease production, and laboratory diagnosis for the etiologic agents of human disease. Unit Five contains a brief survey of human disease caused by protozoa and worms. All chapters include a list of current references which direct the reader to additional information on the material covered.

Throughout this book, illustrations and tables are used to illuminate the text. These could not have been included without the generosity of individuals and publishers who provided photographs and permissions, and

deepest thanks are extended for them. All but a few figures make their textbook debut here, and the quality of the micrographs should result in a level of interest and attractiveness unattainable by words alone. To maintain a crisp, student-oriented presentation, acknowledgments do not accompany each figure or table; rather, the reader is encouraged to refer to the complete list of credits after the last chapter.

During the writing of this text none of my colleagues escaped my questions, but final responsibility for any errors that may appear must be my own. I would like to acknowledge especially the following persons from the University of Virginia, who read entire units or large portions thereof: D. C. Benjamin, S. U. Emerson, R. J. Kadner, G. L. Mandell, and D. E. Normansell. In addition, the following individuals from other institutions read single chapters or parts of chapters of the manuscript: C. G. Alexander, San Francisco State University; D. F. Bainton, University of California, San Francisco; P. Bodel, Yale University; M. D. Little, Tulane University; S. Madoff, Massachusetts General Hospital; L. A. McGonagle, University of Washington; G. E. Michaels, University of Georgia; L. A. Page, National Animal Disease Center; J. T. Sinski, University of Arizona; E. J. Stanbridge, University of California, Irvine; and H. S. Wessenberg, San Francisco State University. Blocks of chapters were reviewed by J. W. Goodman, University of California, San Francisco, and J. L. Pate, University of Wisconsin, Madison. Entire units were read by C. Albin, Stanford University; A. A. Blazkovec, University of Wisconsin; C. R. Goodheart, Biolabs; R. C. Johnson, University of Minnesota; C. E. Schwerdt, Stanford University; M. Stone, Stanford University; and M. Voge, University of California, Los Angeles. The entire manuscript was reviewed by E. D. Weinberg, Indiana University. My warmest appreciation is extended to all of these reviewers who contributed to this text by their comments.

Wesley A. Volk

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WHERE, when, and how did life originate? How can a single cell divide and differentiate into the incredible complexity of the human body?

Is disease a random event, a punishment, or a result of something real, something solid, something controllable? Is there a general unity or theme of life that is essentially the same in a tree, a human, a bacterium, a virus? Can we learn about ourselves from knowledge of the metabolism and genetic inheritance of organisms so small that literally millions of them could "dance on the head of a pin"?

These questions have occupied the minds of philosophers and scientists for centuries. A number have been answered, others are well on the way toward a solution, and a few seem destined to remain forever unknown.

But we now know that many diseases are caused by microorganisms, and we are continually learning how such organisms cause disease at the molecular level and how the diseases can be prevented, treated, and controlled. Furthermore, we can see a biochemical unity in the world to allow us to extrapolate our knowledge of bacterial genetics toward a better understanding of the regulation and control of human physiology.

The growth of biological knowledge during the past century is analogous to the advances in physics that have taken us off the horse and to the moon. This knowledge allows us to question the role of viruses in human cancer, it permits us to discuss the feasibility of genetic engineering to correct heritable defects, and it brings us closer to an understanding of many hitherto mysterious maladies such as multiple sclerosis, juvenile diabetes, and autoimmune diseases.

Is this what microbiology is all about? In part, yes. Microbiology is that part of biology set aside as a separate science because it deals primarily with the biology of organisms too small for the naked eye to see. Medical microbiology is simply a subdivision concerned with the biology of the microorganisms that cause disease. Thus, medical microbiology includes a study of microorganisms that can grow on or in a host organism and produce disease. It encompasses the responses the host makes to the infection, and it seeks answers to questions concerning the control of infectious diseases as well as diseases resulting from genetic disorders.

This text will not concern itself with a detailed classification of microorganisms. Rather, its objective will be to describe the characteristics of those organisms that cause disease, to discuss (insofar as possible) how they produce the disease in question, and to outline protective measures available to us. However, before these objectives can become comprehensible, we must learn about what bacteria are, how they grow, and how they can be controlled. Unit One is designed to provide some of the answers to these questions.

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1

The Microbial World

The word "microbiology" is a broad term meaning the study of living organisms that are individually too small to be seen with the naked eye. It includes the study of bacteria (bacteriology), viruses (virology), yeasts and molds (mycology), protozoa (protozoology), and algae (phycology). Such minute forms of life are given the name microorganisms, and sometimes they are called microbes or, in the vernacular, germs.

Considering the vast knowledge we now possess concerning microorganisms, it is difficult to imagine that hardly more than a century ago Louis Pasteur and a few of his colleagues were trying to convince the medical profession that these little organisms actually cause disease or even that one kind of microorganism turns fruit juice to wine while a different organism turns it to vinegar. Once a few such seminal ideas were proven and accepted, the study of microorganisms and their metabolic processes has grown rapidly into an important science.

The information acquired from microbiology has made possible great advances in our ability to control many infectious diseases. In addition, many biochemical processes first understood in microorganisms have subsequently been shown to occur in higher forms of life. Thus, many metabolic pathways of human metabolism were first observed in microorganisms. The field of molecular genetics and current models of gene action and gene regulation had much of their origin in the study of microorganisms.

It is therefore clear that the field of microbiology includes more than just a study of disease-producing microorganisms; it is the study of all biological activities of microbes. Perhaps the time is not far distant when we can both understand and control diseases such as cancer and those resulting from genetic defects, and it is certain that the continued study of microbiology will contribute to that knowledge.

PRACTICAL APPLICATIONS OF MICROBIOLOGY

A practical knowledge of microbiology is immediately and vitally important in medicine and related fields. For example, at a most basic level a primary responsibility of hospital personnel is to safeguard patients, and a large part of this responsibility is to protect the patient from the injurious effects of microorganisms. Under normal hospital conditions, a patient is always in some peril

of microbial invasion, and in fact hospital-acquired infections have become so commonplace that they have been given the specific designation of nosocomial infections.

The individual who knows something of the peculiar attributes of each medically important species of microorganism will be able to take advantage of its vulnerabilities. By understanding microorganisms, their anatomy and physiology, something of what they can do, and how they produce disease, we can know much more of how they can be controlled.

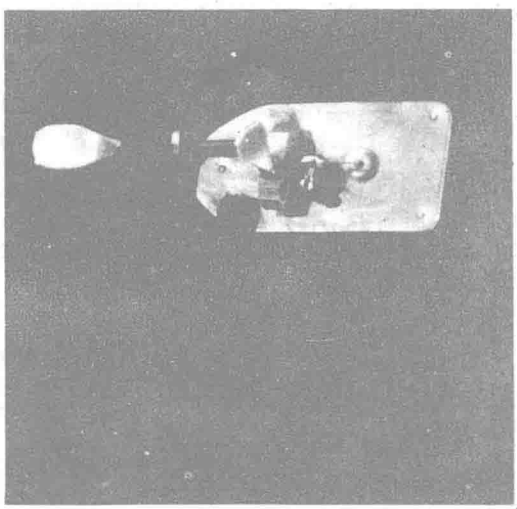
EVOLUTION OF THE STUDY OF MICROORGANISMS

The existence of microorganisms had long been suspected when their presence was verified by microscopic observation in about 1683 through the investigations of the Dutch merchant Antony van Leeuwenhoek (1632–1723). Leeuwenhoek was an amateur scientist who devoted a great deal of time to his hobby of grinding lenses (see Figure 1-1). With his lenses he observed everything he could think of and described microorganisms in rainwater, seawater, scrapings from between the teeth, fermenting mixtures, and many other materials. Many of the minute organisms, including protozoa, yeast, and bacteria, were seen in motion, and he referred to them as "animalcules." His drawings were remarkably accurate, particularly when one realizes that the highest magnification possible with his lenses was about 300 times, in contrast with today's compound microscope which provides a magnification of 1000 times. As judged from his drawings, Leeuwenhoek's lenses were the best of his time, and although he kept his lens grinding techniques secret, he shared his observations in great detail in voluminous letters to the Royal Society of London.

The Theory of Spontaneous Generation

At the same time as Leeuwenhoek's observations, a challenge was beginning to be made to the theory of spontaneous generation. This

Figure 1-1. *a.* Leeuwenhoek's microscope utilized a single biconvex lens to view bacteria suspended in a drop of liquid placed on a moveable pin. *b.* Although his microscope was capable of only 200 to 300-fold magnification, Leeuwenhoek was able to achieve these remarkable drawings submitted to the Royal Society of London.



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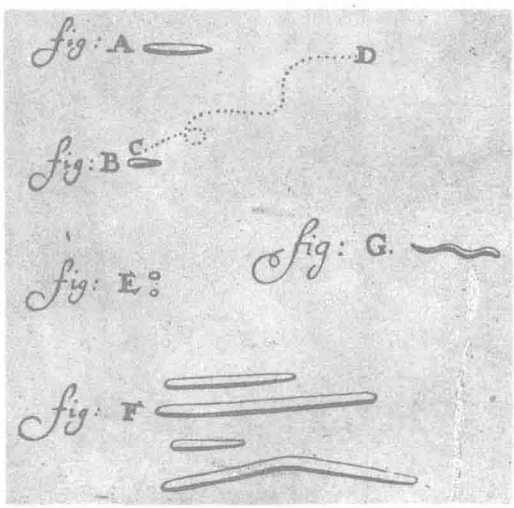

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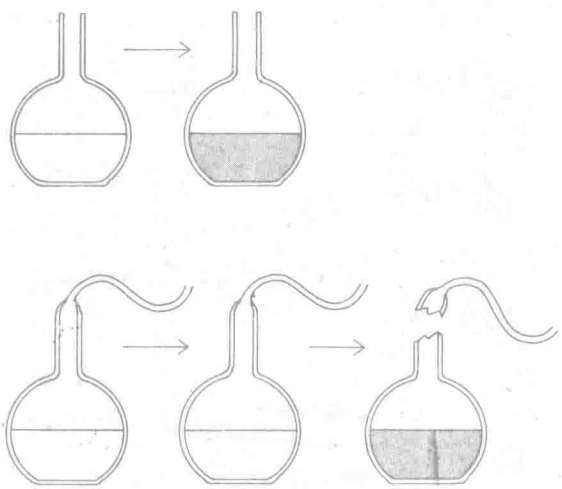
theory proposed the spontaneous origin of living organisms, particularly from decaying organic matter. Over time the theory was not too difficult to disprove for large multicellular organisms such as mice, snakes, and flies; by the middle of the eighteenth century the concept of spontaneous generation of visible and complex forms of life had been largely laid to rest. But it was still widely believed that microorganisms arose spontaneously. John Needham, an English biologist and priest (1713–1781), published a paper in 1749 in support of spontaneous generation in which he claimed that microorganisms arose in his infusions, or broths, whether he boiled them, covered them, or took any other precautions. The controversy was defined when an Italian naturalist and priest, Lazzaro Spallanzani (1729–1799), claimed that Needham had not taken sufficient precautions to prevent microorganisms in the air from entering heated infusions after they had cooled. However, many of Spallanzani's contemporaries found it difficult to accept his totally new concept that putrefaction or decay was initiated by microorganisms floating on dust particles in the air, and so his arguments were widely ignored. These critics felt that by sealing the solutions so completely, Spallanzani was eliminating material necessary to life. The controversy concerning the spontaneous generation of microorganisms continued until the middle of the nineteenth century.

The experiments of two men, Louis Pasteur, a French chemist, crystallographer, and "father of modern microbiology" and the English physicist John Tyndall, provided the final disproof of spontaneous generation. Pasteur poured meat infusions into flasks and then drew the top of each flask into a long, curved neck that would admit air but not dust (see Figure 1-2*a*). He found that after the infusions were heated they would remain sterile indefinitely unless he broke the neck of the flask, thus allowing dust to enter the infusion. He further demonstrated that if he placed a series of these flasks along a dusty road, opened them, and then resealed them a few minutes later, microorganisms would grow in nearly all flasks. On the other hand,

Figure 1-2. *a.* Pasteur's swan-necked flasks remained sterile because the bend in the neck excluded dust particles. *b.* Similarly, broth remained sterile in Tyndall's dust-free incubation chamber. In both cases the broth was exposed to air, but dust was excluded.



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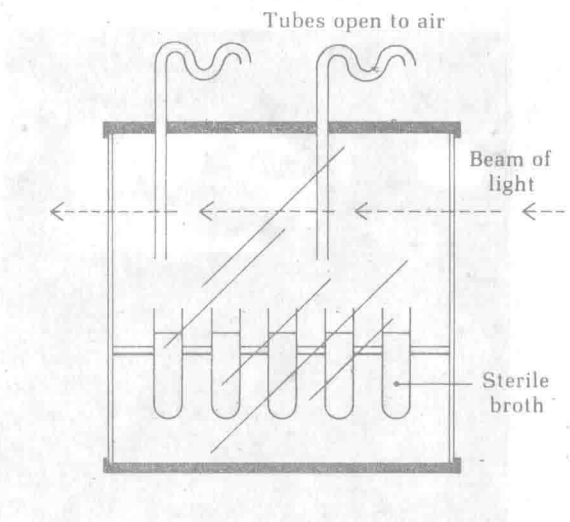

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if he performed the same experiment on the top of a mountain where there was little dust, practically none of his flasks became contaminated.

Pasteur's experiments appeared to end forever the controversy of spontaneous generation, but the idea was not laid to rest until the work of John Tyndall became known. Tyndall had observed that the pathway of a bright beam of light through air is visible because it is refracted by dust particles in the air. When, however, the air is completely free of dust, the beam cannot be seen. Tyndall constructed a specially designed box (Figure 1-2*b*), and after the dust in the box had settled (verified by the observation that he could no longer see a beam of light pass through the air) he carefully placed tubes of sterile infusions into the box. As long as the air was not disturbed, the infusions remained sterile even though open to the air, again demonstrating that microorganisms exist on dust particles in the air and that they are not spontaneously generated.

Fermentation

Although Pasteur's early training was as a chemist and crystallographer, his efforts to disprove the theory of spontaneous generation stimulated his interest in the biological activities of microorganisms. One of his first tasks as a microbiological troubleshooter was to find out why the production of wine would occasionally result in the formation of large amounts of lactic acid. He soon observed that more than one type of microorganism was involved. The undesirable lactic acid fermentation resulted from contamination with rod-shaped bacteria; the ethanol production resulted from the activity of yeast cells. This observation was followed by others in which Pasteur proved that each type of bacterium is able to carry out the conversion of glucose or other carbohydrates to specific end products. Thus, one type of bacterium forms lactic acid from sugar, another forms butyric acid, and so on. Pasteur observed that these fermentation processes took place in the absence of air, and he coined the terms aerobic and anaerobic to describe respectively those organisms requiring air and those unable to grow in the presence of air. Pasteur's discoveries were soon utilized in industrial fermentation, but the idea that microorganisms could affect humans and animals was less readily accepted.

Germ Theory of Disease

The contagious nature of certain diseases has been recognized since Biblical times. However, one of the first specific cases of the association of a microorganism with disease was made in 1834 when the Italian Agostino Bassi proved that a disease in silkworms was the result of a fungus infection. In a similar problem Pasteur was called upon in 1865 to study a silkworm disease that was destroying the silk industry in France. He established criteria to identify infected silkworm moths microscopically, and by using female silkworms free of infection, the disease could be eliminated. Joseph Lister (1827–1912), an English physician, soon put to practical use the emerging concept that disease and infection were the result of invading microorganisms. He is credited with the first attempt to prevent infection following surgery by using an antiseptic technique; he used dilute phenol for cleaning hands and instruments, wound dressings, and as an aerosol during surgical procedures. Crude as this practice may have been, it marked the beginning of our effort to control infectious microorganisms.

Once the microbial etiology or cause of infectious diseases was accepted, research activity was directed toward the isolation and identification of the causative agents of the many severe diseases of the day. Thus, the last quarter of the nineteenth century was a time of great activity resulting in tremendous, exciting discoveries. A German physician, Robert Koch (1843–1910), introduced a scientific approach to the field of medical microbiology. He established certain rules

(now known as Koch's postulates) that must be followed to establish a cause-and-effect relationship between a microorganism and a disease. We shall discuss these postulates in detail in Chapter 11; working from these postulates, Koch was able to isolate and grow the etiological agents of such diseases as anthrax, cholera, and tuberculosis.

Another important contribution from Koch's laboratory was the use of agar (a complex polysaccharide isolated from seaweed) to solidify culture media. Agar is valuable to the microbiologist because it will melt at about the temperature of boiling water and once melted will not resolidify until it is cooled to approximately 43°C. Thus, if 2% agar is added to a liquid medium and the mixture is then heated to melt the agar and sterilize the medium, it can be dispensed in tubes or petri dishes where it will solidify when cooled. A solid surface is essential for separating mixtures of bacteria to obtain pure cultures; Koch's use of agar for this purpose proved to be a major advance in bacteriological technique.

Many other scientists earned recognition in the history of microbiology—in fact, some died after being infected by the organisms they were studying (for example, Howard Taylor Ricketts and Stanislas von Prowazek from typhus). Today, a little over a hundred years after Pasteur, research in microbiology is aimed at understanding many complex problems, including the etiology and control of cancer, the genetic control of biochemical syntheses, and the potential use of viruses for the correction of genetic defects. We shall begin by discussing the structure of microorganisms and how this structure may influence the properties of the cell.

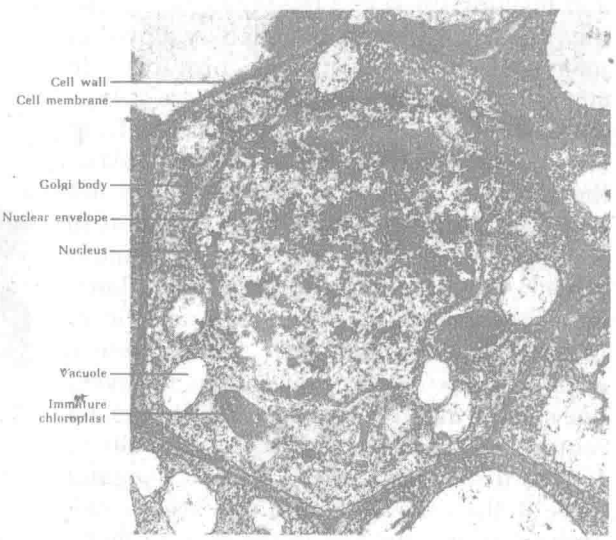
PROCARYOTIC AND EUCARYOTIC CELLS

If the biochemical activities of cells derived from such diverse sources as bacteria, spinach, and rat liver were compared, one would find amazing similarities. All the cells would be found to have their heritable characteristics coded in deoxyribonucleic acid (DNA); all would utilize one general mechanism for the storage of energy; all would have essentially identical methods of protein synthesis, nucleic acid synthesis, and polysaccharide synthesis. This incredible biochemical unity exists throughout the living world, with only minor variations on a major theme. When we examine cells morphologically, we find two distinct types, termed eucaryotic and procaryotic.

The eucaryotic cell, found in most animals and plants, is surrounded by a plasma membrane that regulates the movement of substances into and out of the cell, and it possesses a true nucleus that is separated from the cytoplasm of the cell by a well-defined, two-layered nuclear membrane, or, better, nuclear envelope. Within this nucleus, the DNA along with several kinds of proteins is organized into linear strands called chromosomes. The number of chromosomes in a eucaryotic nucleus is fixed for a given species. For certain fungi it may be 1 or 2; it is 46 for humans, and is other numbers for other plants and animals. When a eucaryotic cell divides, it goes through a rather elaborate process to provide each daughter cell with a full set of chromosomes. During this process, called mitosis, the strands of DNA replicate and the chromosomes condense by supercoiling. The replication of DNA provides each chromosome with two identical sets of genetic information visible as two sets of arms or chromatids on the chromosome. The nuclear envelope disintegrates and a spindle forms with the chromosomes on a plane halfway between the poles of the spindle. Fibers of the spindle attach to the chromosomes and the chromatids are pulled apart, each chromatid now becoming a new chromosome and being pulled to one pole or the other of the spindle, where a new nucleus forms. This nuclear division, or karyokinesis, is usually followed by cytokinesis, a division of the material outside the nucleus (the cytoplasm)

somewhere between the two new nuclei. Mitosis results in two daughter cells with chromosomal complements and, therefore, genetic information identical to the parent cell.

Eucaryotic cells also contain within their cytoplasm organelles that carry out metabolic activities to provide energy for the cell. These structures are called mitochondria, and their principal function is the generation of adenosine triphosphate (ATP). In plant cells involved in photosynthesis, the light-trapping pigment, chlorophyll, is contained in an organelle called a chloroplast. In general, the process of photosynthesis converts light energy into chemical-bond energy. Many other organelles, most defined by membranes, take part in metabolic activi-



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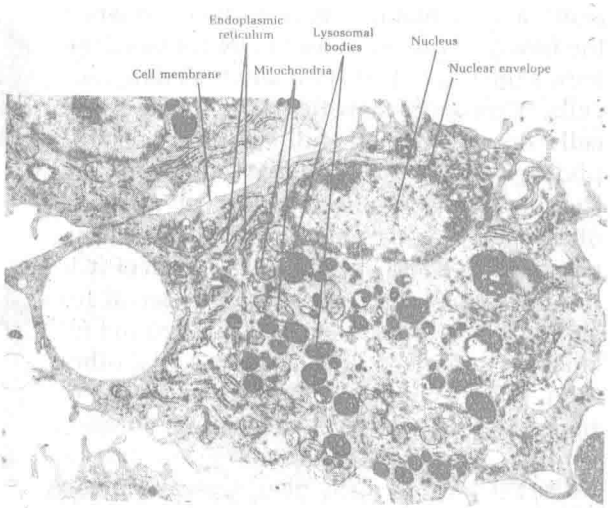

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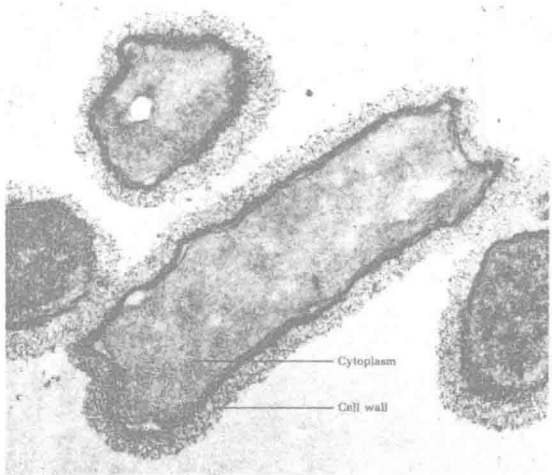
Figure 1-3. *a*. Section of cell from stem of a young pea plant, *Pisum sativum* (×9,945). *b*. Section of an animal cell, in this case a macrophage from a mouse (×6,240).

ties, motility, and other functions of eucaryotic cells. Some eucaryotic organelles may be seen in the micrographs of plant and animal cells in Figure 1-3; however, a complete review is better left to textbooks of cell biology.

The distinctions between eucaryotic and procaryotic cells probably represent the only major example of dichotomy in the evolution of the cell. The type of cell represented by the bacteria, including actinomycetes, spirochetes, rickettsiae, chlamydiae, and mycoplasmas, and the blue-green algae, the procaryotic cell, is bounded by a plasma membrane (cytoplasmic or protoplasmic membrane), but it does not possess a true nucleus, since its DNA is not separated from the cytoplasm by a nuclear envelope. Also, the DNA of the procaryotic cell does not exist in multiple distinct chromosomes as in the eucaryotic cell but in a single continuous thread; however, as we shall learn later, many procaryotic cells possess small pieces of extrachromosomal DNA which control some of the activities of the cell. In any event one can see that the procaryotic cell does not require a mechanism as elaborate as mitosis for the distribution of its DNA to daughter cells. Rather, the DNA of a procaryotic cell appears to be attached to the plasma membrane which is just inside the cell wall, and as the cell divides into two cells (binary fission), a new membrane is formed between the newly divided copies of DNA, extending across the cell to form two identical daughter cells. Unlike eucaryotic cells, procaryotic cells do not possess mitochondria and, if photosynthetic, do not possess chloroplasts. Cytoplasmic streaming, a flow of contents often seen in eucaryotic cells, is not seen in procaryotic cells, but one might expect it is not necessary in a cell sufficiently small for simple diffusion to move material around inside the cytoplasm. Procaryotic cells, other than the mycoplasmas and extreme halophils, possess a cell wall containing muramic acid, a compound not found in eucaryotic cells. Procaryotic cells also possess smaller ribosomes (70S; S represents Svedberg units, a unit of measurement for the rate at which a particle will sediment during high speed centrifugation) than found in the cytoplasm of eucaryotic cells (80S); however, this difference does not apply to the mitochrondrial ribosomes of eucaryotes, since they also are 70S. And, although many eucaryotic cells

Figure 1-4. *a.* Section of bacterium *Klebsiella aerogenes* (×20,730). *b.* Bacterium *Bacillus mucroides* (×26,000).



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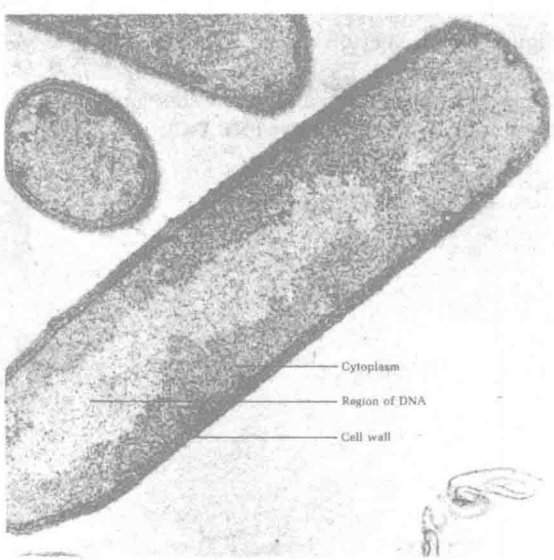

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can engulf particulate material by a process called phagocytosis, procaryotic organisms are not able to take any material inside their cells unless it is first solubilized. Figure 1-4 shows two procaryotic cells with prominent features labeled.

CLASSIFICATION OF MICROORGANISMS

Exactly where and how do microorganisms fit into the hierarchy of living things? We can find the answer to this question by reviewing some elementary principles of biological classification.

For many years most biologists assigned every form of life to one of two great kingdoms: the animal kingdom and the plant kingdom. The members of each kingdom were then arranged in order of their complexity using a phylogenetic, or natural, classification. Such a classification requires knowledge of fossil forms for the purpose of grouping organisms into an evolutionary tree composed of phyla, classes, orders, tribes, families, genera, and species (plus numerous intermediate levels in some schemes). However, not only were fossils of microorganisms not available until very recent times, but it was not even possible to decide into which kingdom they should be placed. Some possess certain characteristics commonly associated with animals, as the ability to move. Others have chlorophyll and obtain their energy from photosynthesis, as do the green plants. Still others possess characteristics of both plants and animals. Thus, it was necessary to make some rather arbitrary decisions on how microorganisms should be classified, and early schemes placed all microorganisms, except those complex microbes called protozoa, in the plant kingdom.

As more and more was learned about microorganisms, many biologists believed that they should be placed in a separate kingdom. As a result, a third kingdom, called the Protista, was proposed by Haeckel in 1866 to include the bacteria, fungi, algae, and protozoa. Subsequently, this kingdom was divided into two large divisions in which the eucaryotic Protista were considered as higher protists and the procaryotic Protista as lower protists. Within these divisions, microorganisms were assigned to classes, orders, tribes, families, genera, species, and subspecies. However, insofar as procaryotic cells are concerned, there is no known evolutionary relationship upon which to base such a system of classification, and the schemes in use were really keys attempting to group similar organisms together. It is not surprising, therefore, that the classification of microorganisms is constantly changing, as illustrated by a recent suggestion for a five-kingdom scheme. Because of the great difference between procaryotes and eucaryotes, this recent proposal places all procaryotes in a separate kingdom, the Monera. Unicellular eucaryotes, algae and protozoa, are considered Protista, and fungi receive their own kingdom (Fungi). Plants and animals are, of course, still around as plants and animals. Thus, classification of microorganisms is artificial, and one can only wonder how many kingdoms the future will bring. On the other hand, these schemes are useful in our attempt to give some semblance of order to the endless array of living forms involved in disease.

The Classification of Bacteria

The system for classification of bacteria routinely used in the United States is outlined in *Bergey's Manual of Determinative Bacteriology*. This manual was first published in 1923, and has undergone periodic revisions, with the eighth edition published in 1974.

Bergey's Manual is concerned only with the bacteria, and the current edition has deviated considerably from its predecessors in its approach to classification. The editors of this edition have placed all of the procaryotic organisms into a new kingdom called the Procaryotae. However, the authors of this new classification have felt no compulsion to place microorganisms into orders, families,

Table 1-1. Key to the Bacteria

*Identifying feature**	*Classification*
I. Phototrophic	Part 1
II. Chemotrophic	
A. Chemolithotrophic (chemoautotrophic)	
1. Derive energy from the oxidation of nitrogen, sulfur or iron compounds, do not produce methane from carbon dioxide	
a. Cells glide	Part 2
aa. Cells do not glide	
b. Cells ensheathed	Part 3
bb. Cells not ensheathed	Part 12
2. Do not oxidize nitrogen, sulfur or iron compounds, produce methane from carbon dioxide	Part 13
B. Chemoorganotrophic (chemoheterotrophic)	
1. Cells glide	Part 2
2. Cells do not glide (exceptions in Part 19)	
a. Cells filamentous and ensheathed	Part 3
aa. Cells not filamentous and ensheathed	
b. Products of binary fission not equivalent (have appendages other than flagella and pili or reproduce by budding).	Part 4
bb. Not as above	
c. Cells not rigidly bound	
d. Cells spiral-shaped, have cell wall	Part 5
dd. Cells not spiral-shaped, no cell wall	Part 19
cc. Cells rigidly bound	
d. Gram negative	
e. Obligate intracellular parasites	Part 18
ee. Not as above	
f. Curved rods	Part 6
ff. Not curved rods	
g. Rods	
h. Aerobic	Part 7
hh. Facultatively anaerobic	Part 8
hhh. Anaerobic	Part 9
gg. Cocci or coccobacilli	
h. Aerobic	Part 10
	Part 7
hh. Anaerobic	Part 11
dd. Gram positive	
e. Cocci	