Chapter 1

The Scope of Microbiology

Microbiology is the study of living organisms of microscopic size, which include bacteria, fungi, algae, protozoa, and the infectious agents at the borderline of life that are called viruses. It is concerned with their form, structure, reproduction, physiology, metabolism, and classification. It includes the study of their distribution in nature, their relationship to each other and to other living organisms, their effects on human beings and on other animals and plants, their abilities to make physical and chemical changes in our environment, and their reactions to physical and chemical agents.

Microorganisms are closely associated with the health and welfare of human beings; some microorganisms are beneficial and others are detrimental. For example, microorganisms are involved in the making of yogurt, cheese, and wine; in the production of penicillin, interferon, and alcohol; and in the processing of domestic and industrial wastes. Microorganisms can cause disease, spoil food, and deteriorate materials like iron pipes, glass lenses, and wood pilings.

Most microorganisms are unicellular. In unicellular organisms all the life processes are performed by a single cell. In the so-called higher forms of life, organisms are composed of many cells that are arranged in tissues and organs to perform specific functions. Regardless of the complexity of an organism, the cell is the basic structural unit of life. All living cells are fundamentally similar.

The word *cell* was first used more than two centuries ago by an Englishman, Robert Hooke (1635–1703), in his descriptions (1665) of the fine structure of cork and other plant materials. The honeycomblike structure he observed in a thin slice of cork (see Fig. 1-1) was due to the cell walls of cells that were once

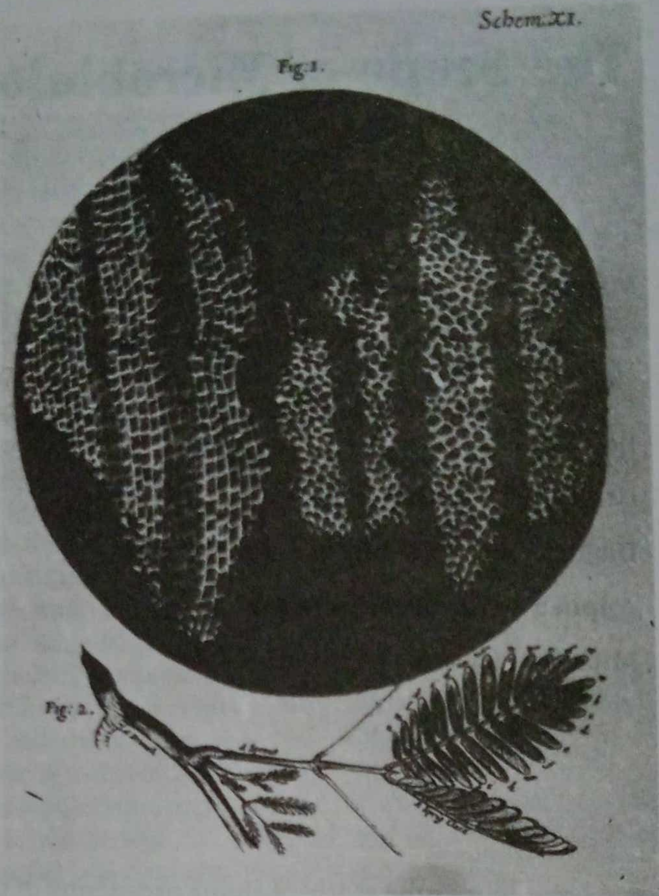
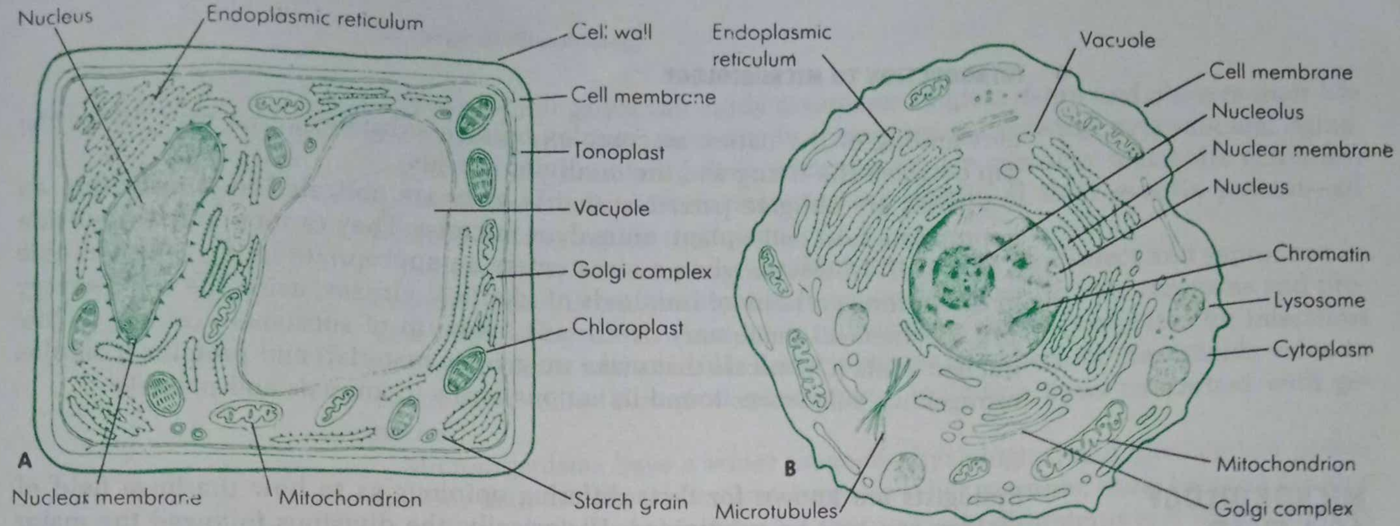


Figure 1-1. Robert Hooke's drawing of a thin slice of cork as he observed it under the microscope. This drawing was included in a report made to the Royal Society (London) in 1665. He is credited as being the first person to use the word *cell*. *(Courtesy of National Library of Medicine.)*

living. But the concept of the cell as the structural unit of life—the **cell theory**—is credited to two Germans, Matthias Schleiden and Theodor Schwann, who in 1838–1839 described cells as the basic structural and functional units of all organisms. Schleiden and Schwann recognized that all cells, no matter what the organism, are very similar in structure. As the concept of the cell as the basic unit of life gained acceptance, investigators speculated on the nature of the substance contained within the cell. **Protoplasm** (Greek *proto*, "first"; *plasm*, "formed substance", introduced to characterize the living material of a cell), is a colloidal organic complex consisting largely of protein, lipids, and nucleic acids. These substances are enclosed by membranes or cell walls; and the protoplasm always contains nuclei or an equivalent nuclear substance (see Fig. 1-2). Developments in electron-microscope techniques have made it possible to reveal the complex intricacies of intracellular organization (see Fig. 1-3).

All biological systems have the following characteristics in common: (1) the ability to reproduce; (2) the ability to ingest or assimilate food substances and metabolize them for energy and growth; (3) the ability to excrete waste products; (4) the ability to react to changes in their environment—sometimes called **irritability**; and (5) susceptibility to mutation. In the study of microbiology, we encounter "organisms" which may represent the borderline of life. These are the viruses, which are simpler in structure and composition than single cells. Viruses provide an exciting challenge and an opportunity to gain a better un-



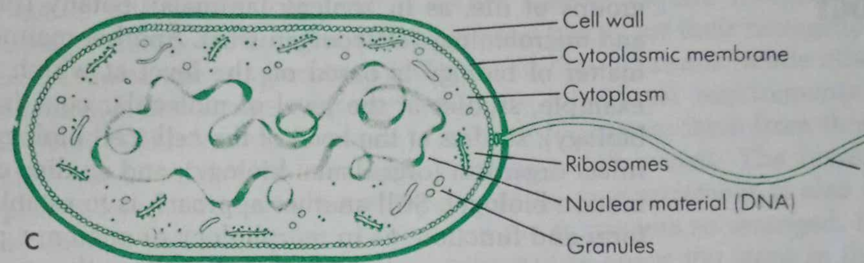


Figure 1-2. Generalized diagram of typical cell structures. (A) Plant cell. (B) Animal cell. (C) Bacterial cell. *(Erwin F. Lessel, illustrator.)*

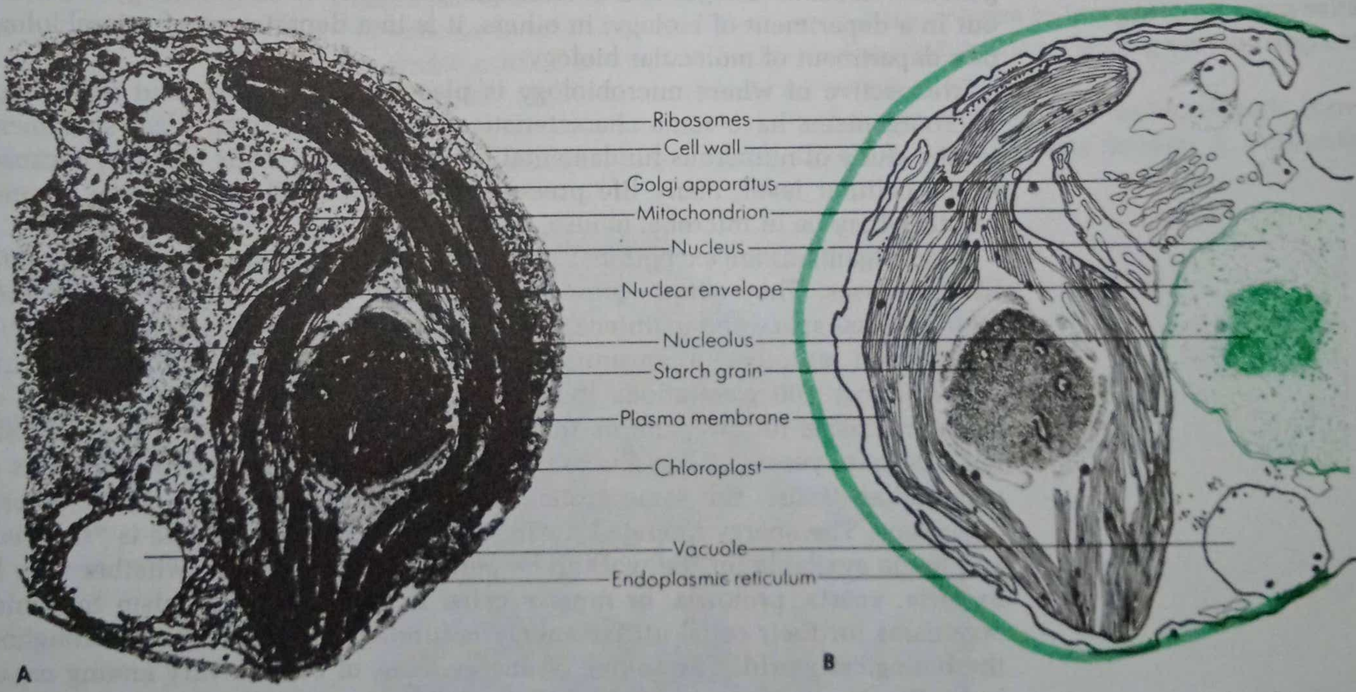


Figure 1-3. (A) Electron micrograph of the alga *Chlamydomonas reinhardii* (X15,000), a eucaryotic cell. *(Courtesy of George E. Palade, The Rockefeller University, by permission of Holt, New York, publishers of Ariel G. Leowy and Philip Seikovitz, Cell Structure and Function, 1969.)* (B) Schematic representation of (A). *(Erwin F. Lessel, illustrator.)*

derstanding of the nature of complex organic substances that may bridge the gap between the living and the nonliving worlds.

Viruses are obligate parasites; that is, they are obligated to grow within an appropriate host cell—plant, animal, or microbe. They cannot multiply outside a host cell. However, when a virus enters an appropriate living cell, it is able to direct the synthesis of hundreds of identical viruses, using the cell's energy and biochemical machinery. A virus is made up of substances unique to life: **nucleic acids** (chemicals that make up genetic material) and **proteins** (complex nitrogenous substances found in various forms in animals and plants).

MICROBIOLOGY AS A FIELD OF BIOLOGY

Biologists are known for their differing opinions as to how the huge field of biology can best be subdivided. Historically, the divisions followed the major groups of life, as in zoology (animals), botany (plants), entomology (insects), and microbiology (microorganisms). Another manner of subdividing the subject matter of biology is based on the level at which the study is conducted: for example, studies at the level of molecular constituents of the cell (molecular biology); studies at the level of the cell (cell biology); studies at the level of the intact organism (organismal biology); and studies of groups of organisms (population biology). Still another approach is to establish divisions on the basis of form and function, as in morphology or anatomy, physiology, metabolism, and genetics. In some colleges and universities, the study of microbiology is carried out in a department of biology; in others, it is in a department of microbiology or a department of molecular biology.

Irrespective of where microbiology is placed in the broad field of biology, microorganisms have some characteristics which make them ideal specimens for the study of numerous fundamental life processes. This is possible because, at the cellular level, many life processes are performed in the same manner whether they be in microbe, mouse, or human.

Microorganisms are exceptionally attractive models for studying fundamental life processes. They can be grown conveniently in test tubes or flasks, thus requiring less space and maintenance than larger plants and animals. They grow rapidly and reproduce at an unusually high rate; some species of bacteria undergo almost 100 generations in a 24-h period. The metabolic processes of microorganisms follow patterns that occur among higher plants and animals. For example, yeasts utilize glucose in essentially the same manner as cells of mammalian tissue; the same system of enzymes is present in these diverse organisms. The energy liberated during the breakdown of glucose is "trapped" and made available for the work to be performed by the cells, whether they be bacteria, yeasts, protozoa, or muscle cells. In fact, the mechanism by which organisms (or their cells) utilize energy is fundamentally the same throughout the biological world. The source of energy does, of course, vary among organisms. Plants are characterized by their ability to use radiant energy, whereas animals require chemical substances as their fuel. In this respect some microorganisms are like plants, others like animals; and some have the unique ability of using either radiant energy or chemical energy and thus are like both plants and animals. Furthermore, some microorganisms, the bacteria in particular, are able to utilize a great variety of chemical substances as their energy source—ranging from simple inorganic substances to complex organic substances.

In microbiology we can study organisms in great detail and observe their life processes while they are actively metabolizing, growing, reproducing, aging, and dying. By modifying their environment we can alter metabolic activities, regulate growth, and even change some details of their genetic pattern—all without destroying the organisms.

For example, **bacteriophages**, which are viruses that infect and reproduce in bacteria, demonstrate the complete sequence of host-parasite reactions and provide a model by which virus-host cell reactions can be postulated for infections in higher plants and animals. Bacteriophages have been of inestimable value in elucidating many biological phenomena, including those concerned with genetics.

Microorganisms have a wider range of physiological and biochemical potentialities than all other organisms combined. For example, some bacteria are able to utilize atmospheric nitrogen for the synthesis of proteins and other complex organic nitrogenous compounds. Other species require inorganic or organic nitrogen compounds as the initial building blocks for their nitrogenous constituents. Some microorganisms synthesize all their vitamins, while others need to be furnished vitamins. By reviewing the nutritional requirements of a large collection of microorganisms, it is possible to arrange them from those with the simplest to those with the most complex requirements. The increasing complexity of nutritional requirements in such an arrangement is also a reflection of the decreasing synthetic capacity of the organisms so arranged. In addition, this kind of arrangement provides information about the steps in the synthesis of various metabolites, e.g., from atmospheric oxygen to inorganic nitrogen salts to amino acids. The biochemist has used microorganisms having varying degrees of synthetic ability to investigate pathways of synthesis.

In his presidential address to the Society of American Bacteriologists (now The American Society for Microbiology) in 1942, the late Selman A. Waksman (Fig. 1-4) observed:

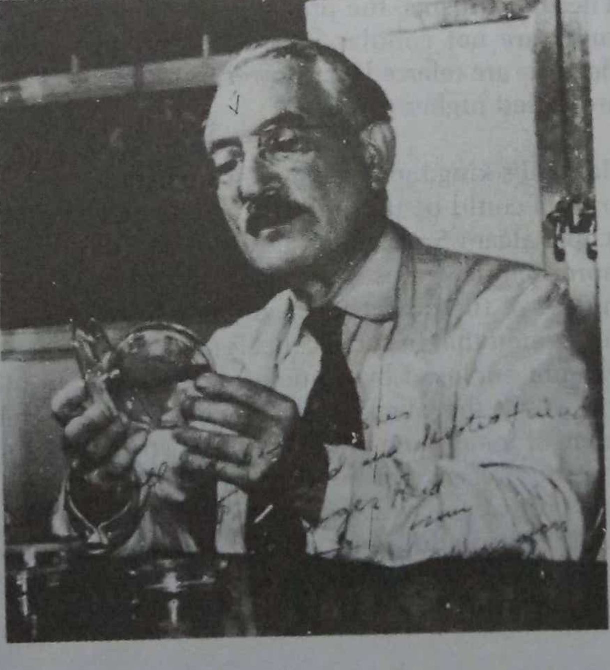


Figure 1-4. Selman A. Waksman (1888–1973), world's foremost authority on soil microbiology and codiscoverer of the antibiotic streptomycin.

There is no field of human endeavor, whether it be in industry or agriculture, or in the preparation of food or in connection with problems of shelter or clothing, or in the conservation of human or animal health and the combating of disease, where the microbe does not play an important and often dominant role.

Waksman, longtime professor of microbiology at Rutgers University, in 1952 was awarded the Nobel prize in physiology or medicine for the part he played in the discovery of the antibiotic streptomycin, which is produced by a soil bacterium.

THE PLACE OF MICROORGANISMS IN THE LIVING WORLD

In biology as in any other field, **classification** means the orderly arrangement of units under study into groups of larger units. Present-day classification in biology was established by the work of Carolus Linnaeus (1707–1778), a Swedish botanist. His books on the classification of plants and animals are considered to be the beginning of modern botanical and zoological **nomenclature**, a system of naming plants and animals. Nomenclature in microbiology, which came much later, was based on the principles established for the plant and animal kingdoms.

Until the eighteenth century, the classification of living organisms placed all organisms into one of two kingdoms, plant and animal. As previously stated, in microbiology we study some organisms that are predominantly plantlike, others that are animallike, and some that share characteristics common to both plants and animals. Since there are organisms that do not fall naturally into either the plant or the animal kingdom, it was proposed that new kingdoms be established to include those organisms which typically are neither plants nor animals.

Haeckel's Kingdom *Protista*

One of the earliest of these proposals was made in 1866 by a German zoologist, E. H. Haeckel. He suggested that a third kingdom, *Protista*, be formed to include those unicellular microorganisms that are typically neither plants nor animals. These organisms, the protists, include bacteria, algae, fungi, and protozoa. (Viruses are not cellular organisms and therefore are not classified as protists.) Bacteria are referred to as lower protists; the others—algae, fungi, and protozoa—are called higher protists.

Procaryotic and Eucaryotic Protists

Haeckel's kingdom *Protista* left some questions unanswered. For example, what criteria could be used to distinguish a bacterium from a yeast or certain microscopic algae? Satisfactory criteria were unavailable until late in the 1940s when more definitive observation of internal cell structure was made possible with the aid of the powerful magnification provided by electron microscopy. It was discovered that in some cells, for example typical bacteria, the nuclear substance was not enclosed by a nuclear membrane. In other cells, such as typical algae and fungi, the nucleus was enclosed in a membrane. This discovery—the absence of membrane-bound internal structures in one group of protists (bacteria) and the presence of membrane-bound structures in all the others (fungi, algae, and protozoa)—was a discovery of fundamental significance. Further research has revealed additional differences in the internal structure of these cells.

These two cell types, as characterized in Table 1-1, have been designated

Table 1-1. Features Distinguishing Procaryotic from Eucaryotic Cells

Feature	Procaryotic Cells	Eucaryotic Cells
Groups where found as unit of structure	Bacteria	Algae, fungi, protozoa, plants, and animals
Size range of organism	1–2 by 1–4 μm or less	Greater than 5 μm in width or diameter
Genetic system		
Location	Nucleoid, chromatin body, or nuclear material	Nucleus, mitochondria, chloroplasts
Structure of nucleus	Not bounded by nuclear membrane; one circular chromosome	Bounded by nuclear membrane; more than one chromosome
	Chromosome does not contain histones; no mitotic division	Chromosomes have histones; mitotic nuclear division
	Nucleolus absent; functionally related genes may be clustered	Nucleolus present; functionally related genes not clustered
Sexuality	Zygote nature is merozygotic (partial diploid)	Zygote is diploid
Cytoplasmic nature and structures		
Cytoplasmic streaming	Absent	Present
Pinocytosis	Absent	Present
Gas vacuoles	Can be present	Absent
Mesosome	Present	Absent
Ribosomes	70S,* distributed in the cytoplasm	80S arrayed on membranes as in endoplasmic reticulum; 70S in mitochondria and chloroplasts
Mitochondria	Absent	Present
Chloroplasts	Absent	May be present
Golgi structures	Absent	Present
Endoplasmic reticulum	Absent	Present
Membrane-bound (true) vacuoles	Absent	Present
Outer cell structures		
Cytoplasmic membranes	Generally do not contain sterols; contain part of respiratory and, in some, photosynthetic machinery	Sterols present; do not carry out respiration and photosynthesis
Cell wall	Peptidoglycan (murein or mucopeptide) as component	Absence of peptidoglycan
Locomotor organelles	Simple fibril	Multifibrilled with "9 + 2" microtubules

Table 1-1. (continued)

Feature	Procaryotic Cells	Eucaryotic Cells
Pseudopodia	Absent	Present in some
Metabolic mechanisms	Wide variety, particularly that of anaerobic energy-yielding reactions; some fix nitrogen gas; some accumulate poly-β-hydroxybutyrate as reserve material	Glycolysis is pathway for anaerobic energy-yielding mechanism
DNA base ratios as moles % of guanine + cytosine (G + C %)	28 to 73	About 40

* S refers to the Svedberg unit, the sedimentation coefficient of a particle in the ultracentrifuge.

NOTE: Definitions of technical words are provided in the glossary at the back of the book.

procaryotic and eucaryotic: organisms of each cell type are called procaryotes and eucaryotes, respectively.

Bacteria are procaryotic microorganisms. The eucaryotic microorganisms in-

Figure 1-5. The bacterium *Escherichia coli*, a typical procaryotic cell. Note the absence of any discrete intracellular organelle structures. The light area represents nuclear material; the dark area is ribosomal material. (*Courtesy I. D. J. Burdett and R. G. E. Murray, J. Bacteriol* **119**:*1039, 1974.*) Inset: *E. coli* cells as seen by light microscopy.

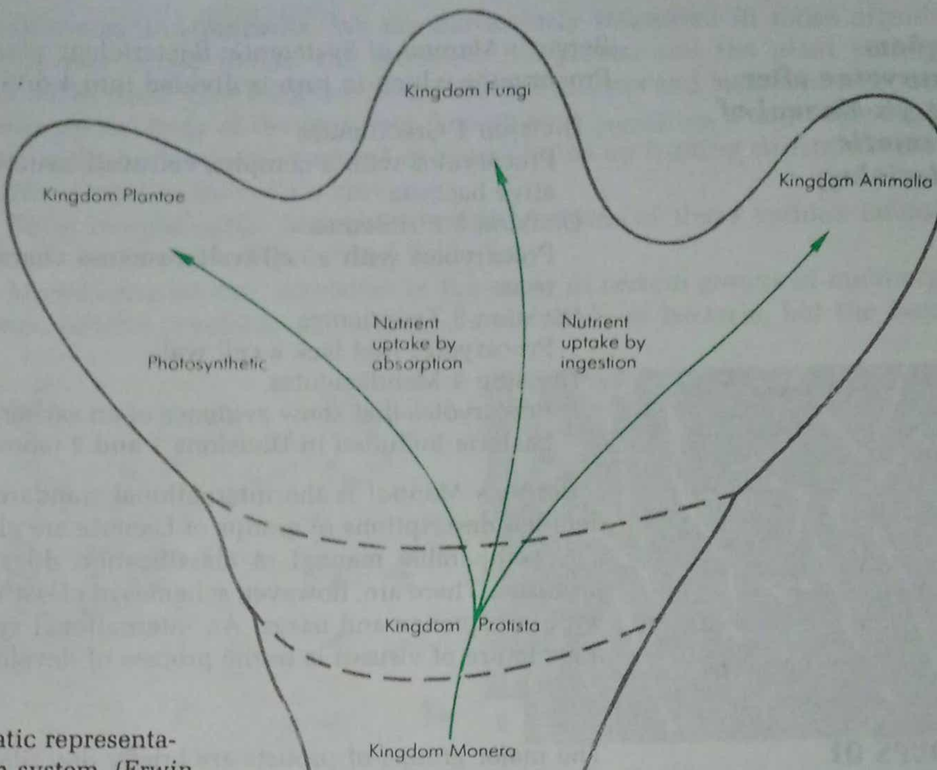


Figure 1-6. A simplified schematic representation of Whittaker's five-kingdom system. *(Erwin F. Lessel, illustrator.)*

clude the protozoa, fungi, and algae. (Plant and animal cells are also eucaryotic.) Viruses are left out of this scheme of classification. Examples of typical procaryotic and eucaryotic cells are shown in Figs. 1-2, 1-3, and 1-5.

Whittaker's Five-Kingdom Concept

A more recent and comprehensive system of classification, the five-kingdom system, was proposed by R. H. Whittaker (1969). This system of classification, shown in Fig. 1-6, is based on three levels of cellular organization which evolved to accommodate three principal modes of nutrition: photosynthesis, absorption, and ingestion. The procaryotes are included in the kingdom *Monera*; they lack the ingestive mode of nutrition. Unicellular eucaryotic microorganisms are placed in the kingdom *Protista*; all three nutritional types are represented here. In fact, as shown in Fig. 1-6, the nutritional modes are continuous: the mode of nutrition of the microalgae is photosynthetic; the mode of nutrition of the protozoa is ingestive; and the mode of nutrition in some other protists is absorptive, with some overlap to the photosynthetic and ingestive modes. The multicellular and multinucleate eucaryotic organisms are found in the kingdoms *Plantae* (multicellular green plants and higher algae), *Animalia* (multicellular animals), and *Fungi* (multinucleate higher fungi). Their diversified nutritional modes lead to a more diversified cellular organization. Microorganisms are found in three of the five kingdoms: *Monera* (bacteria and cyanobacteria), *Protista* (microalgae and protozoa), and *Fungi* (yeasts and molds).

Kingdom *Procaryotae* after *Bergey's Manual of Systematic Bacteriology*

Bergey's Manual of Systematic Bacteriology places all bacteria in the kingdom *Procaryotae* which in turn is divided into 4 divisions as follows:

Division 1 Gracilicutes
Procaryotes with a complex cell-wall structure characteristic of Gram-negative bacteria
Division 2 Firmicutes
Procaryotes with a cell-wall structure characteristic of Gram-positive bacteria
Division 3 Tenericutes
Procaryotes that lack a cell wall
Division 4 Mendosicutes
Procaryotes that show evidence of an earlier phylogenetic origin than those bacteria included in Divisions 1 and 2 (above)

Bergey's Manual is the international standard for bacterial taxonomy. More detailed descriptions of groups of bacteria are given in Part Four of this book.

A comparable manual of classification does not exist for fungi, algae, or protozoa. There are, however, schemes of classification for each group that have wide acceptance and usage. An international system for classification and nomenclature of viruses is in the process of development.

GROUPS OF MICROORGANISMS

The major groups of protists are briefly described below. Although viruses are not protists or cellular organisms, they are included for two reasons: (1) the techniques used to study viruses are microbiological in nature; and (2) viruses are causative agents of diseases, hence, diagnostic procedures for their identification are employed in the clinical microbiological laboratory as well as the plant pathology laboratory.

Algae are relatively simple organisms. The most primitive types are unicellular. Others are aggregations of similar cells with little or no differentiation in structure or function. Still other algae, such as the large brown kelp, have a complex structure with cell types specialized for particular functions. Regardless of size or complexity, all algal cells contain chlorophyll and are capable of photosynthesis. Algae are found most commonly in aquatic environments or in damp soil.

Viruses are very small noncellular parasites or pathogens of plants, animals, and bacteria as well as other protists. They are so small that they can be visualized only by the electron microscope. Viruses can be cultivated only in living cells.

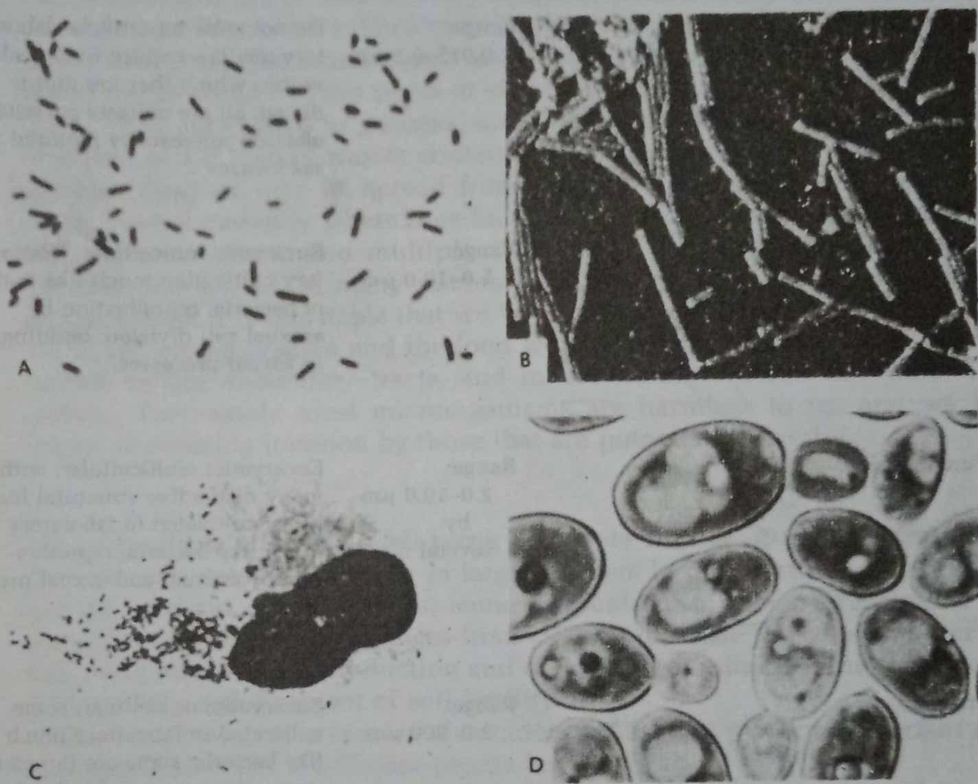
Bacteria are unicellular procaryotic organisms or simple associations of similar cells. Cell multiplication is usually by binary fission.

Protozoa are unicellular eucaryotic organisms. They are differentiated on the basis of morphological, nutritional, and physiological characteristics. Their role in nature is varied, but the best-known protozoa are the few that cause disease in human beings and animals.

Fungi are eucaryotic lower plants devoid of chlorophyll. They are usually multicellular but are not differentiated into roots, stems, and leaves. They range in size and shape from single-celled microscopic yeasts to giant multicellular mushrooms and puffballs. We are particularly interested in those organisms commonly called molds, the mildews, the yeasts, and the plant pathogens known as rusts. True fungi are composed of filaments and masses of cells which make up the body of the organism, known as a mycelium. Fungi reproduce by fission, by budding, or by means of spores borne on fruiting structures that are quite distinctive for certain species.

Some morphological and characteristic features of these various microbial groups are shown in Fig. 1-7 and Table 1-2.

Microbiologists may specialize in the study of certain groups of microorganisms. Strictly speaking, bacteriology is the study of bacteria, but the term is



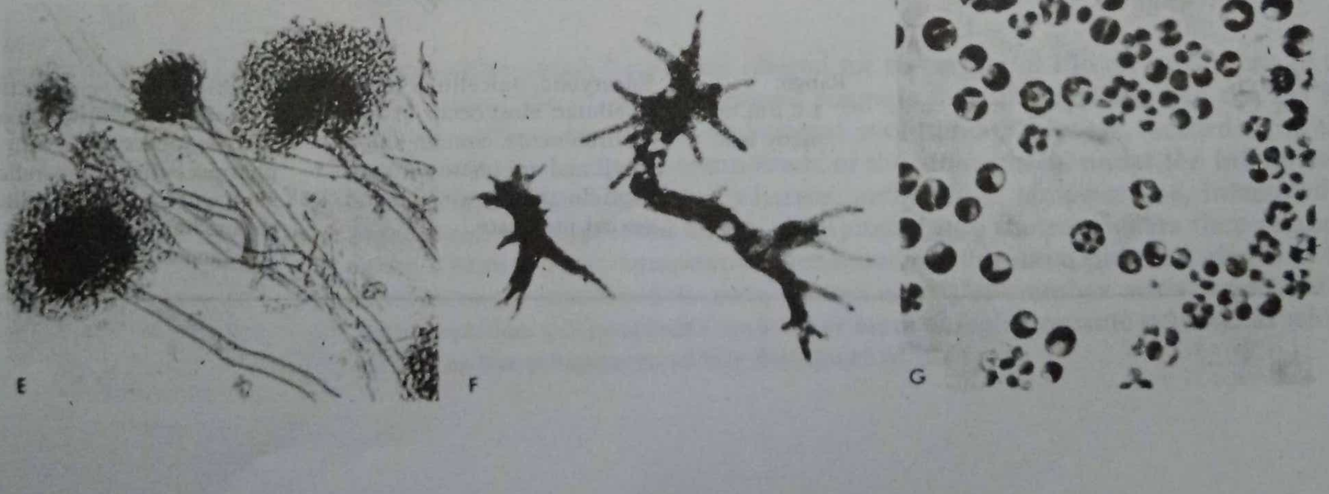
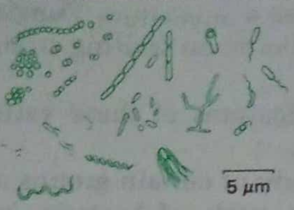
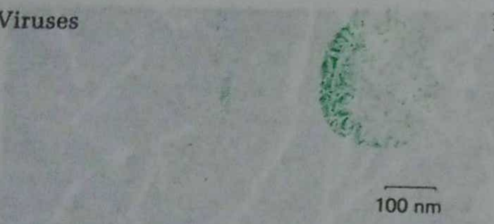
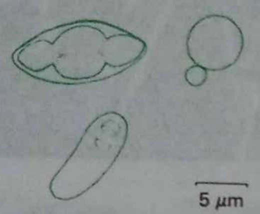
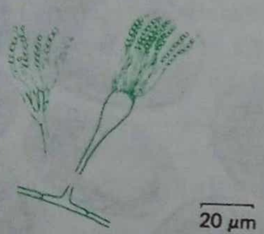
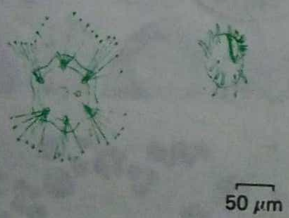
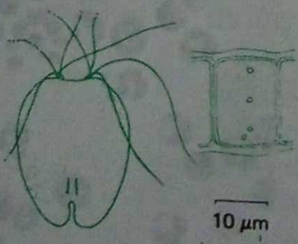


Figure 1-7. Morphological features of various groups of microorganisms. (Note that this illustration is only intended to convey the impression of morphological diversity. No size relationship between groups can be obtained from it. The wide range in microbial sizes does not permit both constancy in magnification and showing of meaningful morphological details at the same time.) (A) *Escherichia coli* (X1,000). (B) Tobacco mosaic virus (X100,000). *(Hitachi, Ltd., Tokyo.)* (C) *Rickettsia tsutsugamushi* in cytoplasm of infected cell (X940). *(N.J. Kramis and The Rocky Mountain Laboratory, U.S. Public Health Service.)* (D) *Candida utilis* (X2,000 approx.). *(Courtesy of G. Svihla, J. L. Dainko, and F. Schlenk, J Bacteriol,* **85***:399, 1963.)* (E) *Aspergillus* sp. *(Courtesy of Douglas F. Lawson.)* (F) Amoeba. *(Carolina Biological Supply Co.)* (G) *Chlorella infusionum* (X1,000). *(Courtesy of Robert W. Krauss.)*

Table 1-2. Some Characteristics of Major Groups of Microorganisms *(Erwin F. Lessel, illustrator)*

Group	Morphology	Size	Important Characteristics	Practical Significance
Bacteria		Typical: 0.5–1.5 µm by 1.0–3.0 µm Range: 0.2 by 100 µm	Procaryotic; unicellular, simple internal structure; grow on artificial laboratory media; reproduction asexual, characteristically by simple cell division	Some cause disease; some perform important role in natural cycling of elements which contributes to soil fertility; useful in industry for manufacture of valuable compounds; some spoil foods and some make foods
Viruses		Range: 0.015–0.2 µm	Do not grow on artificial laboratory media—require living cells within which they are reproduced; all are obligate parasites; electron microscopy required to see viruses	Cause diseases in humans, other animals, and plants; also infect microorganisms
Fungi: Yeasts		Range: 5.0–10.0 µm	Eucaryotic; unicellular; laboratory cultivation much like that of bacteria; reproduction by asexual cell division, budding, or sexual processes	Production of alcoholic beverages; also used as food supplement; some cause disease
Fungi: Molds		Range: 2.0–10.0 µm by several mm	Eucaryotic; multicellular, with many distinctive structural features; cultivated in laboratory much like bacteria; reproduction by asexual and sexual processes	Responsible for decomposition (deterioration) of many materials; useful for industrial production of many chemicals, including penicillin; cause diseases of humans, other animals, and plants
Protozoa		Range: 2.0–200 µm	Eucaryotic; unicellular; some cultivated in laboratory much like bacteria; some are intracellular parasites; reproduction by asexual and sexual processes	Food for aquatic animals; some cause disease
Algae		Range: 1.0 µm to many feet	Eucaryotic; unicellular and multicellular; most occur in aquatic environments; contain chlorophyll and are photosynthetic; reproduction by asexual and sexual processes	Important to the production of food in aquatic environments; used as food supplement and in pharmaceutical preparations; source of agar for microbiological media; some produce toxic substances

often used as a synonym for microbiology. Protozoology is the study of protozoa; a special branch of protozoology called parasitology deals exclusively with the parasitic or disease-producing protozoa and other parasitic micro- and macroorganisms. Mycology is the study of fungi such as yeasts and molds. Virology is the science that deals with viruses. Phycology is the study of algae. Further specialization in some aspect of the biology of a particular group of organisms is not uncommon; e.g., bacterial genetics, algal physiology, and bacterial cytology.

DISTRIBUTION OF MICROORGANISMS IN NATURE

Microorganisms occur nearly everywhere in nature. They are carried by air currents from the earth's surface to the upper atmosphere. Even those indigenous to the ocean may be found many miles away on mountaintops. Microbes are found in the bottom of the ocean at its greatest depths. Fertile soil teems with them. They are carried by streams and rivers into lakes and other large bodies of water; and if human wastes containing harmful bacteria are discharged into streams, diseases may be spread from one place to another. Microorganisms occur most abundantly where they find food, moisture, and a temperature suitable for their growth and multiplication. Since the conditions that favor the survival and growth of many microorganisms are those under which people normally live, it is inevitable that we live among a multitude of microbes. They are in the air we breathe and the food we eat. They are on the surfaces of our bodies, in our alimentary tracts, and in our mouths, noses, and other body orifices. Fortunately most microorganisms are harmless to us; and we have means of resisting invasion by those that are potentially harmful.

APPLIED AREAS OF MICROBIOLOGY

Microorganisms affect the well-being of people in a great many ways. As we have already stated, they occur in large numbers in most natural environments and bring about many changes, some desirable and others undesirable. The diversity of their activities ranges from causing diseases in humans, other animals, and plants to the production and deposition of minerals, the formation of coal, and the enhancement of soil fertility.

There are many more species of microorganisms that perform important roles in nature than there are disease-producing species.

A summary of the major fields of applied microbiology appears in Table 1-3.

MICROBIOLOGY AND THE ORIGIN OF LIFE

Many explanations have been offered for the origin of life on earth. One of the more acceptable of these proposals suggests that life originated in the sea following millions of years of a chemical evolutionary process. According to this hypothesis the inorganic compounds of the atmosphere, under the influence of ultraviolet light, electrical discharges, and/or high temperatures, interacted to form organic compounds which precipitated into the sea, where they accumulated. These organic compounds, subjected to additional physical effects of the environment, combined to form amino acids. The amino acids interacted to form peptides, polypeptides, and other more complex organic substances which served as the precursors of the first form of life.

Table 1-3. Major Fields of Applied Microbiology

Field	Some Applied Areas
Medical microbiology	Causative agents of disease; diagnostic procedures; diagnostic procedures for identification of causative agents; preventive measures
Aquatic microbiology	Water purification; microbiological examination; biological degradation of waste; ecology
Aeromicrobiology	Contamination and spoilage; dissemination of diseases
Food microbiology	Food preservation and preparation; foodborne diseases and their prevention
Agricultural microbiology	Soil fertility; plant and animal diseases
Industrial microbiology	Production of medicinal products such as antibiotics and vaccines; fermented beverages, industrial chemicals; production of proteins and hormones by genetically engineered microorganisms
Exomicrobiology	Exploration for life in outer space
Geochemical microbiology	Coal, mineral and gas formation; prospecting for deposits of coal, oil, and gas; recovery of minerals from low-grade ores

The time scale of chemical evolution, biological evolution, and the emergence of microbial life is shown in Fig. 1-8.

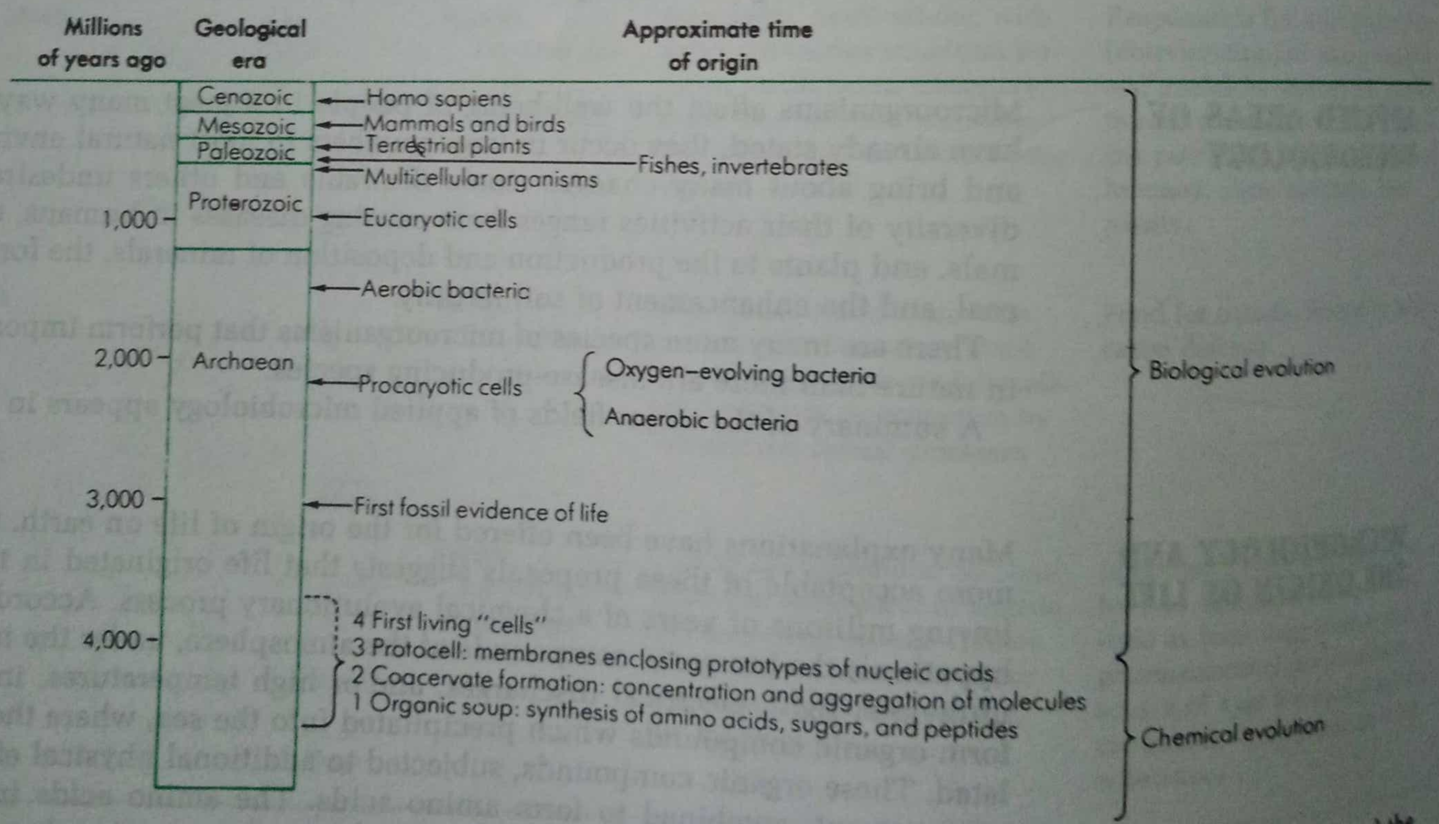


Figure 1-8. Time scale of the chemical evolution, the biological evolution, and the occurrence of microbial life.