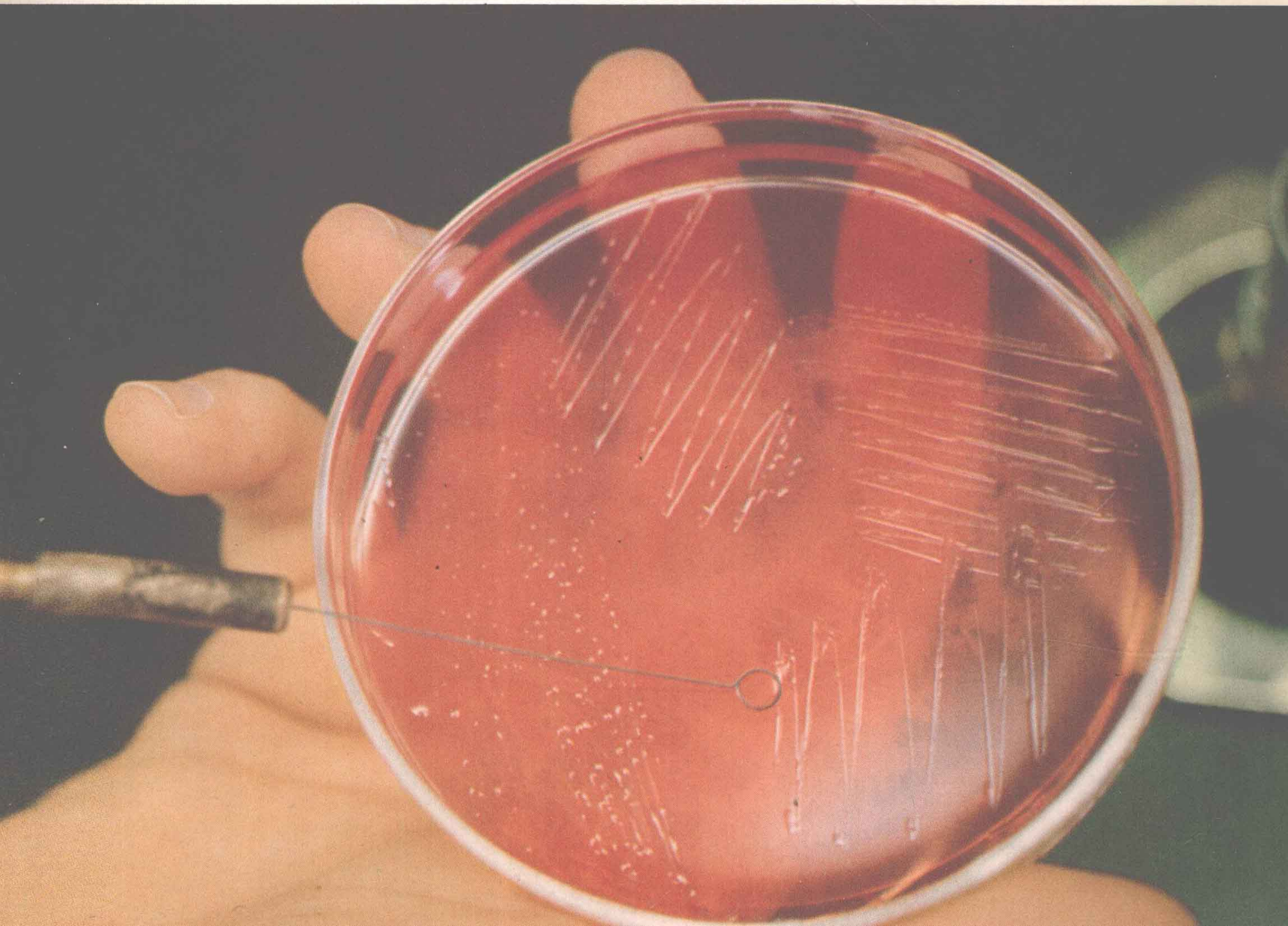
General microbiology

THE STUDENT'S TEXTBOOK

Peter Hunter



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with 440 illustrations

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Preface

This text is designed to introduce a student to the important aspects of the discipline of microbiology in a manner that allows for easy learning. The book does not pretend to examine all facets of the field; this is left to the many reference books available. Instead, important concepts have been selected and presented in an easy-to-read fashion that allows the student to learn with a minimum of difficulty. This is accomplished by sectioning the information into learning blocks that are supported by behavioral objectives (statements indicating what the student should learn). Whether the objectives have been achieved may then be tested through use of the self-evaluation questions at the end of each chapter. These questions allow the student to assess how much has been learned after each block of information is presented. Thus the student may progress independently at a comfortable speed. Since much of the learning of facts takes place outside of the classroom, more classroom time is available for the instructor to supplement the material by citing applications and by open discussion.

The first six chapters of the book contain information that is fundamental to all subdivisions of microbiology. They cover the areas of classification, nomenclature, microscopy, cell structure and function, metabolism, nutrition, cultivation and cellular growth, and the control of microorganisms.

The remaining four chapters are concerned with introducing the student to some of the more important areas of applied microbiology. Information is provided in the disciplines of water and sewage microbiology, microbiology of the air, microbial industrial processes, food and dairy microbiology, medical microbiology, and immunology and serology. All of these chapters are approached with the role of the technologist in mind, and information on laboratory testing is provided whenever possible. These chapters may be used to provide a good overview of microbiological processes, or certain chapters may be selected specifically for students who are entering careers in medical laboratory technology, nursing, environmental studies, food technology, or industrial microbiology.

This book is dedicated to my mother for always inspiring me to do my best and to my wife and children for their great patience during the book's development. I would like to thank all of my students for their helpful comments on the format of the book. Special thanks go to my typist, Cecilia Rick.

Peter Hunter

Note to the student

HOW TO USE THIS BOOK

Text and learning objectives

The text portion of each chapter contains drawings and photographs to illustrate the basic concepts discussed. After each section of information, complete sets of learning objectives are included to aid you in determining the main points to be learned from the material. You may either turn directly to the objectives before reading each section, or you may read the text section first, coming to the objectives in turn and reviewing the material with them in mind.

In the text itself, key words are set in bold type and defined in context. These words will assist you in locating information in the text as well as in building a vocabulary of microbiological terms.

Self-evaluation questions

A section of self-evaluation questions and answers has been included at the end of each chapter to test your knowledge of the text material. The questions are varied and consist of multiple choice, true-false, fill-in-the-blank, fill-in-table, draw-and-label, and matching questions. The questions will serve not only to test your knowledge but also to reinforce the learning process. They will measure how well you have met the objectives and indicate whether you are ready to be examined on the material.

The self-evaluation questions may be answered immediately after a section of the text is covered, or they may be left until the entire chapter has been studied, whichever technique best reinforces your own learning process. The questions for each section of the text are identified by the numbers in parentheses after each set of learning objectives. This arrangement enables you to progress from section to section, testing each time whether you have met the objectives. Many of the questions test your knowledge of lower level objectives (list, name, cite, define, etc.), but do not forget that these lower level objectives represent the building blocks of higher level objectives (such as the development of principles and concepts and the construction of hypotheses). Your self-evaluation will represent your own appraisal of how well you are meeting the lower level objectives.

Contents

1 Prelude to microbiology

Our lives are influenced tremendously by the existence and activities of microorganisms in our environment. The study of these microorganisms and their activities is the science of microbiology. This chapter is a presentation of some preparatory information that should enhance the student's understanding of the field of microbiology. It begins by outlining the various subdivisions of the field and briefly discusses some historical aspects that slowed the development of the discipline. The chapter includes a section on the nomenclature (naming) and classification of microorganisms and briefly describes the various categories of microorganisms and their relative sizes.

Microbiology involves investigation of microbial **form, structure, reproduction, physiology, metabolism,** and **identification.** All of these areas are covered in this text with a particular emphasis on the beneficial and detrimental effects on people as well as the physical and chemical changes that microorganisms cause in various substances. The topics discussed include medical, food, industrial, and water and sewage microbiology.

■ Define microbiology.

■ List six major factors about microorganisms that may be investigated in the field of microbiology.

■ List the two reasons for the study of microbiology in this text.

(1-3)

SUBDIVISIONS OF MICROBIOLOGY

Microbiology is subdivided according to the particular aspect of microorganisms being studied. There are three main criteria by which the field may be subdivided.

1. Microbiology may be classified according to the type of microorganism under study.

   bacteriology Study of bacteria.
   phycology Study of algae.
   mycology Study of fungi (molds and yeast).
   protozoology Study of protozoa.
   virology Study of viruses.
   rickettsiology Study of rickettsiae.
   microbiology Study of all types of microorganisms.

2. Subdivisions may be distinguished by the habitat in which the microorganisms are found, for example, aquatic or freshwater microbiology, soil microbiology, marine microbiology, and air microbiology.
3. The field may be subdivided according to the application of the knowledge.

   clinical microbiology Study of disease in humans and animals.
   pathogenic microbiology Study of disease in humans, animals, and plants.
   agricultural microbiology Study of microorganisms in relation to agriculture (includes soil and plant microbiology).
   industrial microbiology Study of the microorganisms used to produce industrial products (alcohol, organic acids, vitamins, amino acids, etc.).
   geomicrobiology Study of microorganisms with relation to geology.
   food microbiology Study of microorganisms with relation to spoilage and development of food as well as health hazards associated with foods.
   microbial genetics Study of genetic changes in microorganisms.
   microbial ecology Study of the relationships between microorganisms and their environments.

There is considerable overlap between the various subdivisions of microbiology. For example, a microbiologist doing genetic studies to produce a strain of yeast adapted to an industrial process to produce food for people would fit into the subdivisions of mycology, microbial genetics, industrial microbiology, and food microbiology.

■ List the three criteria used to subdivide the discipline of microbiology and give at least three examples in each subdivision.

■ Given a description of a microbiologist's work, list the subdivisions employed in the field.

(4-5)

FACTORS IN DEVELOPMENT OF THE SCIENCE OF MICROBIOLOGY

This section is not to be a discussion of the ideas of every scientist in the field of microbiology. However, a brief account of the more important contributions that led to the modern study of microbiology is presented to give perspective to current microbiological concepts as described in the rest of the text.

The science of microbiology did not develop to any extent until the latter part of the nineteenth century. Development was inhibited until (1) microscopes were built that could give high optical resolution (ability to see between small distances); (2) the concept of spontaneous generation was discredited; and (3) better techniques were developed to confirm that microorganisms caused disease and could be spread from one to another (the germ theory).

In 1665 **Robert Hooke** was the first to see bacteria and protozoa with the aid of a crude compound microscope. Unfortunately, Hooke did not make accurate drawings or descriptions of these microscopic creatures. It was not until 1673, when **Anton van Leeuwenhoek** described microorganisms in great detail and reported his findings to the scientific world, that great interest in microorganisms was stimulated. This incredible man used a simple hand lens that he ground himself to obtain magnifications of up to 200 times (200×). His hand microscope consisted of a lens mounted in a base and a screw device that allowed extension of a pin to hold the specimen. Fig. 1-1 is a drawing of this primitive but effective microscope.

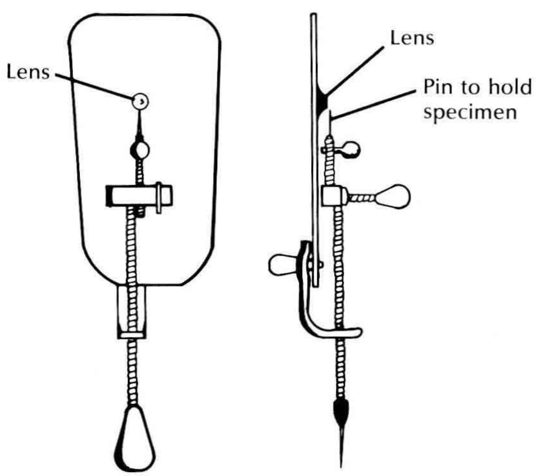


Fig. 1-1. Model of Leeuwenhoek's primitive microscope.

Development of compound microscopes continued from the time of Hooke (when magnifications were 14× to 42×) until the early nineteenth century, when magnifications of 1000× were possible. Most modern light microscopes still only give useful magnifications of 1000×, but they have many improved features for specimen handling and higher resolution. A more extensive study of microscopes is included in Chapter 3.

- List three factors that were necessary for the development of the science of microbiology.
- Discuss the important contribution(s) of Robert Hooke and Anton van Leeuwenhoek to the field of microbiology.
- Compare the compound light microscopes of the early nineteenth century to today's microscopes with respect to magnification, resolution, and ease of handling specimens.

(6-10)

An important factor in the history of microbiology was the controversy surrounding the **spontaneous generation theory.** The spontaneous generation theory stated that living organisms developed spontaneously on dead material left standing. The belief that animals arose spontaneously from the soil, plants, and other animals was originally advocated by **Aristotle** during the third century B.C. This theory was still widely accepted by many seventeenth century scientists, even though they realized that microorganisms existed. It was believed that meat left exposed to the air developed microorganisms spontaneously; the meat (dead material) possessed some mystical power to generate these living entities. However, there were others who believed that the microorganisms did not arise in this spontaneous manner but were present all the time in the dead material or the air and simply increased in numbers when the meat was left standing. Thus the struggle began.

Many scientists believed that exposing meat to warmth and air resulted spontaneously in maggots. **Francesco Redi** (1626-1697) doubted this and set up an experiment to disprove it. He covered meat with gauze and observed that flies laid eggs from which the maggots developed. Thus maggots had a definite parent, and they did not arise from the meat. But the controversy went on, since most spontaneous generation advocates could not comprehend that such small entities as microorganisms could have a parent and therefore believed that they must arise on their own.

In 1749 **John Needham** exposed meat to hot ashes, but when the meat was left alone for a time, bacteria arose in it. He concluded that the bacteria must not have been present previously or they would have been killed and that therefore they originated from the meat. During the same period, **Spallanzani** boiled beef broth for an hour and then sealed the flasks. After several days, the flasks that were sealed showed no growth. Spallanzani concluded that the microorganisms must have been destroyed and therefore did not develop from the meat. But Needham argued that it was merely the lack of air in the flask that kept the bacteria from arising from the meat.

It was not until 65 years later that the answer came through the work of **Theodor Schwann** and **Franz Schulze.** They passed air through strong acids or red-hot tubes into boiled beef extracts. Each time the air was treated, no microorganisms developed in the growth medium. They concluded that the microorganisms were in the air and that the growth medium did not generate them. Fig. 1-2 shows the type of setup used by these workers. However, the extremists were still not convinced, and they believed that the acid or extreme heat had altered the air to inhibit the development of microorganisms.

To further discredit that argument, **Von Dusch** and **Schroeder** in 1850 proved that microorganisms existed in air by simply passing air through cotton fibers into a previously boiled beef broth. The result was no growth; they concluded that the microorganisms were in the air and the cotton stopped them from entering the medium. Their apparatus is shown in Fig. 1-3.

The technique of using cotton to filter microorganisms and yet allow air through is still used widely in microbiology laboratories today. The use of cotton filters was the beginning of the downfall of the spontaneous generation theory, and the work of **Louis Pasteur** ended the controversy in 1861. He designed a flask with a long, curved tube leading to the growth medium. After boiling the medium, he would simply allow the flasks to sit. At no time did microorganisms ever develop in them. Pasteur's gooseneck flask is illustrated in Fig. 1-4. Air passed

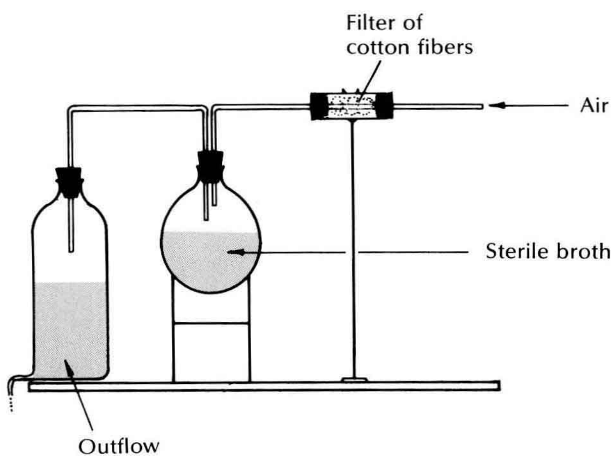


Fig. 1-3. Apparatus by which air was filtered in early experiments aimed at discrediting theory of spontaneous generation.

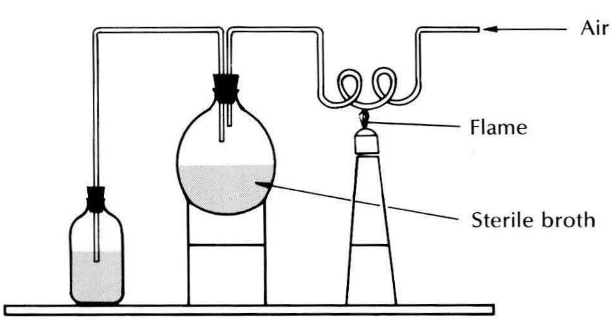


Fig. 1-2. Apparatus by which air was sterilized in early experiments aimed at discrediting theory of spontaneous generation.

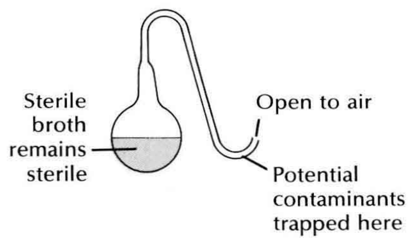


Fig. 1-4. Pasteur's gooseneck flask that allowed only air but no dust particles to enter sterile broth. The experiments effectively disproved the theory of spontaneous generation. (From Lane, T. R., editor: Life: the individual, the species, St. Louis, 1976, The C. V. Mosby Co.)

freely through the tube, but dust particles were trapped in the curved portion. With this simple experiment, Pasteur proved that microorganisms entered substrates on dust particles in the air and did not evolve spontaneously from the growth material.

Finally, **John Tyndall** designed an apparatus to prove that dust carried microorganisms. The apparatus consisted essentially of a box that allowed a beam of light to pass through, making it possible to observe dust particles. Tyndall introduced dust into the box and watched it dropping into the medium below. This resulted in growth of microorganisms in the medium. If no dust was seen, the medium remained free of microorganisms. Fig. 1-5 is a drawing of an apparatus such as Tyndall used.

After many years of argument, scientists finally discarded the theory of spontaneous generation and accepted the fact that microorganisms entered substrates via dust and other means of transfer. But the overall effect of this controversy was the slow advancement of the field of microbiology.

■ Describe the spontaneous generation theory and how it slowed the advancement of the field of microbiology.

■ Describe briefly the outstanding contribution(s) of the following with respect to the spontaneous generation controversy: Aristotle, Redi, Needham, Spallanzani, Schwann and Schulze, Von Dusch and Schroeder, Pasteur, and Tyndall.

(11-12)

The **germ theory** states that microorganisms cause disease and can be spread from one host to another, thus transmitting the disease. As early as 1762 **Anton von Plenciz** stated that living agents were the cause of disease and that different germs were responsible for different diseases.

In the 1800s **Oliver Holmes** insisted that puerperal fever (childbirth fever) was contagious and that it probably was transmitted from mother to mother via midwives and physicians. **Semmelweis** pioneered the concept of utilizing antiseptics in obstetrical practice to avoid infection. However, most physicians ignored both him and the concept.

In 1890 **Lister**, who was interested in Pasteur's concept of boiling surgical bandages and instruments to prevent infection, began using a spray of carbolic acid over the region where surgery was being done. He also soaked surgical bandages and instruments in the antiseptic. This greatly reduced the

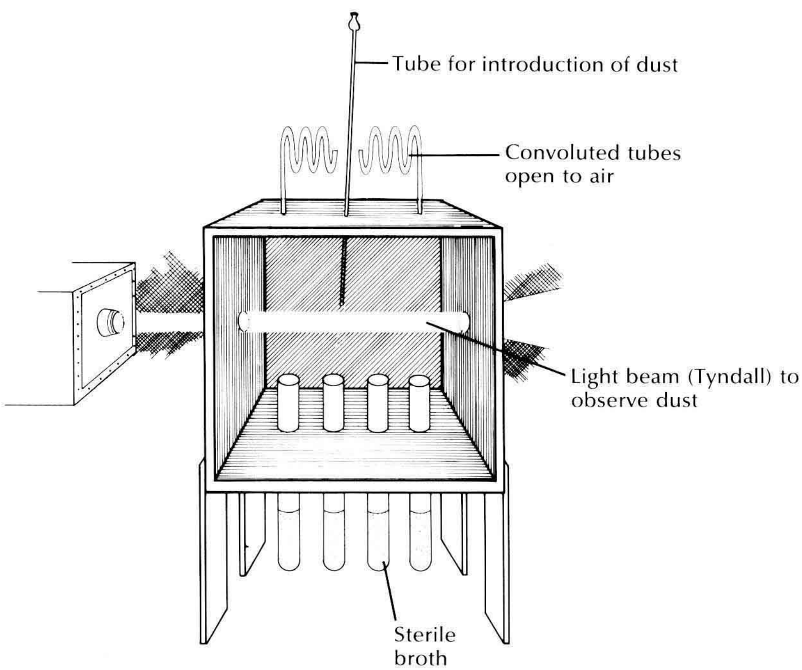


Fig. 1-5. Box designed by Tyndall for observing dust particles, used in proving that microorganisms are carried by dust particles in the air.

incidence of infection after surgery, and his procedures were the forerunners of present-day aseptic techniques.

Even though the contributions of these workers were great, the most credit must be given to **Louis Pasteur** and **Robert Koch.** Pasteur studied the fermentation process and a communicable disease that occurred in silkworms. From his data he formulated the germ theory of disease. He believed that, just as wines were turned sour by contaminating bacteria, silkworms had disease-causing microorganisms that could be transmitted from one worm to another. He concluded that disease-producing microorganisms may be transmitted from man to man or animal to animal, resulting in the spread of disease. Therefore he advocated the use of clean bandages and boiled instruments in hospitals to prevent the spread of disease. Pasteur was also the first to prepare vaccines to prevent anthrax, cholera, and rabies. Many more contributions were made by Pasteur, and it is readily apparent why he is called the **father of microbiology.**

While Pasteur was working on preventing anthrax in animals, Robert Koch was isolating in pure culture (only one type of microorganism) the bacterium that caused it. He pioneered methods for smear preparation, staining, and utilization of solid culture media. In his famous **four postulates,** Koch summarized the evidence that microorganisms caused disease and could be spread. These four postulates follow:

1. In all observed cases of a given disease the microorganisms must be found in pathological relationship to the symptoms and lesions.
2. The microorganism must be isolated (grown and separated) in pure culture from the victims for study in the laboratory.
3. This same microorganism, when inoculated into a susceptible host, must reproduce the disease symptoms originally noted.
4. The same microorganism must again be isolated in pure culture from the experimentally inoculated host.

Koch isolated many pathogenic microorganisms, including the tubercle bacillus and cholera bacillus. Today we still practice and verify his postulates, and they are the essence of the modern field of medical microbiology.

There have been many great advances since the time of Pasteur and Koch, but it is beyond the scope of this text to describe the events and persons responsible for them.

■ Define the germ theory of disease.

■ Describe briefly the contribution(s) of the following with respect to the germ theory: Anton von Plenciz, Holmes, Semmelweis, Lister, Pasteur, and Koch.

■ List and discuss Koch's four postulates on the germ theory.

(13-15)

NOMENCLATURE, CLASSIFICATION, AND CATEGORIES OF MICROORGANISMS

Microorganisms are living entities that are individually too small to see with the naked eye; they can be distinguished only with a magnifying device (a microscope). The bacteria, viruses, rickettsiae, fungi (molds and yeast), some algae, and the protozoa are all considered microorganisms.

■ Define microorganisms.

■ List the six categories of microorganisms.

(16-17)

For many years, biologists have categorized all the forms of life into two kingdoms: the animal kingdom and the plant kingdom. This type of system is very effective in the case of macroscopic creatures (ones that can be seen with the naked eye), since it is not difficult to distinguish between, for example, a tree and a horse. However, the task is not as simple for microscopic creatures. To demonstrate this point, refer to the box on p. 6. Note that the animal and plant kingdoms are differentiated by a number of characteristics. However, microorganisms can share characteristics of both kingdoms. (For example, plants are definitely photosynthetic and animals are not, but microorganisms may be either.)

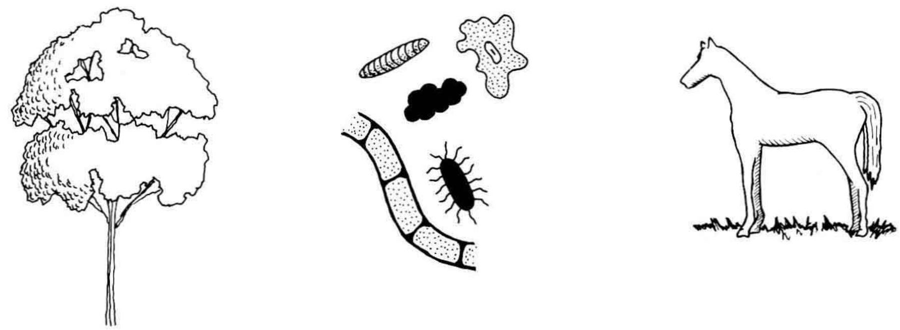
■ Compare the plant and animal kingdoms under the headings used in the box on p. 6, and then compare microorganisms under the same headings to determine which kingdom a particular microorganism belongs to.

(18-24)

Nomenclature

Our current system of nomenclature (naming) is based on the eighteenth century work of a Swedish botanist, **Carl von Linné,** and is called a **binomial**

COMPARISON OF MICROORGANISMS WITH THE PLANT AND ANIMAL KINGDOMS



Characteristic	Plants	Microorganisms	Animals
Cell nucleus	Well defined	May or may not be well defined	Well defined
Cell wall or membrane	Wall rigid	Both rigid wall and flexible membrane examples	Membrane flexible
Stored food	Principally starch	Various types and starch or fat in some	Principally glycogen and fat
Cell pigments	Contain chlorophyll	Mainly no chlorophyll; some contain chlorophyll-like compounds	No chlorophyll
Energy source	Photosynthesis	Organic and inorganic compounds, some photosynthetic	Organic materials
Motility	Nonmotile	Some motile, some nonmotile cells	Motile

system. Each organism is given a two-part name, the **genus** and the **species.** The binomial system of naming microorganisms is indicated in the following examples.

Genus	Species
First word of the name	Second word of the name
Capitalized and italicized (or underlined)	**Not** capitalized, but italicized (or underlined)
Derivation:	Derivation:
1. Greek or Latin in origin *Micrococcus:* Gr., small grain *Bacillus:* L., small rod	1. An adjective describing the genus *Bacillus albus:* small white rod
2. A new word from Latin or Greek roots *Mycobacterium:* Gr. *mykes,* fungus; Gr. *bacterion,* small rod	2. A noun indicating possession *Salmonella pullorum:* salmonella of chicks
3. The latinized name of a person *Pasteurella:* after Louis Pasteur	3. A person's name *Salmonella senftenberg:* after the person who isolated this bacterium
	4. An explanatory noun *Bacillus radicicola:* root-dwelling bacillus

Occasionally, it is desirable to subdivide a species further to **variety,** because certain lines of the species may differ with respect to some relatively minor characteristic. (For example, *Bacillus cereus* variety *mycoides* is similar to *Bacillus cereus* in all respects except that this strain [variety] tends to spread in a curly growth over the surface of a solid growth medium.)

At times the microbiologist also uses a **common name** to indicate familiar microorganisms, just as we use common names for plants and animals.

Genus and species	Common name
Neisseria gonorrhoeae	Gonococcus
Neisseria meningiditis	Meningococcus
Salmonella typhi	Typhoid bacillus
Quercus rubra	Red oak tree
Homo sapiens	Modern man

The scientific study of microorganisms requires a relatively consistent nomenclature system. In 1948 **The International Congress of the International Society of Microbiology** (founded in 1930) adopted an international code and officially published *The International Code of Nomenclature of Bacteria and Viruses.* This volume has been updated from year to year. The code is based on certain principles; some important ones are the following:

1. Each distinct kind of microorganism is designated as a species.
2. The binomial system of naming is used.
3. The application of names is regulated.
4. A law of priority ensures the use of the oldest available legitimate name.
5. Designation of categories is required for classification of microorganisms (that is, one must try to fit a microorganism into the existing categories rather than increase the number of categories; if the organism does not fit any known category, then a new category may be assigned).
6. Requirements are given for effective publication of new specific names as well as guidance in coining new names.

Ultimately, the function of this nomenclature code is to provide microbiologists in all parts of the world with a common basis for the naming of microorganisms.

■ Define nomenclature and the binomial system of naming.

■ Identify the common name and the genus, species, and variety portions of some microbial names.

■ Name the international nomenclature organization and its code.

■ Discuss the role of this organization.

■ List six important principles followed by this organization.

(25-35)

To facilitate classification, microorganisms must be collected into small groups in which the members of one group resemble each other more than they resemble the members of other groups. A sequence of these groupings, which are called **taxonomic categories,** is used to group related organisms at various levels of similarity. Therefore a scheme of taxa (singular, taxon) was designed. Tables 1-1 and 1-2 outline this scheme in general and specific terms. It may be seen that, in moving from species to kingdom, the number of individuals in each taxon increases, but the basis of selecting the taxon becomes more general (less specific).

The student should also realize that the classification system is very unstable from year to year, and the number of members of each taxon will vary, particularly at the genus and species levels. For

Table 1-1. General scheme of taxonomic categories

Category	Description
Species	Organisms of one kind
Genus	Group of related species
Tribe	Group of related genera
Family	Group of related tribes or genera
Order	Group of related families
Class	Group of related orders
Phylum (division)	Group of related classes
Kingdom	Group of related phyla

Table 1-2. Classification scheme for *Escherichia coli,* a bacterium

Category*	Characteristics
Species: *coli*	Bacterium Lives in colon of man and animals
Genus: *Escherichia*	Ferments lactose with production of acid and gas Does not produce acetyl methyl carbinol Methyl red positive Produces CO_2 and H_2 in equal amounts from glucose Cannot utilize citrate as a sole source of carbon
Family: Enterobacteriaceae	Gram-negative rods commonly found in intestine of animals (either normally or as pathogens) Ferment glucose with or without gas production Twelve genera
Order: Eubacteriales	Cells with rigid cell walls Spherical or straight, rod-shaped cells Nonmotile or motile by peritrichous flagella Not acid fast No trichomes Fourteen families
Class: Schizomycetes	Include the bacteria (most without chlorophyll) Divide by binary fission Ten orders
Phylum (division): Protophyta	Bacteria, blue-green algae, rickettsiae, and viruses Primitive plantlike microorganisms Three classes
Kingdom: Plant	Five phyla (divisions)

*Note the characteristic endings of the taxa family, order, and phylum.

example, in 1923 there were 75 species of the genus *Bacillus*, but today there are only 48 species recorded. In 1957, 149 species of *Pseudomonas* were reported, but today only 29 species are accepted. *Pasteurella pestis*, *P. tularensis*, and *P. pseudotuberculosis* have been reclassified and renamed *Yersinia pestis*, *Francisella tularensis*, and *Yersinia pseudotuberculosis*, respectively.

The dynamic state of the nomenclature system is a result of learning more about each member through research and experience as well as through the addition of newly found members.

■ Define taxon, taxonomic scheme, species, genus, tribe, family, order, class, phylum, and kingdom.

■ Given a number of statements that identify a microorganism, be able to determine which taxon (as in Table 1-2) would be appropriate to use in a taxonomic scheme. (To do this, assess the degree of specificity of each statement given.)

(36-39)

Classification

As described earlier in this chapter, it is difficult at times to classify microorganisms into either the plant or the animal kingdom. To overcome this problem, in 1866 **E. H. Haeckel** advocated the development of a new kingdom, the **Protista.** The kingdom Protista includes all microorganisms, regardless of whether they are plantlike or animal-like. Protists are characterized by their lack of definite cellular arrangement as well as a lack of differentiation of cells for specific metabolic functions. In other words, protists are usually unicellular (some may be multicellular), and there is no formation of cells into tissue for specific functions (kidney cells for excretion, nerve cells for impulses, etc.). This kingdom is further subdivided into two categories, the **Procaryota** and the **Eucaryota.**

The Procaryota, or lower protists, have a primitive type of nucleus, which is usually a loose network of DNA with no clearly defined membrane. Also, their cell division process is not as complex as **mitosis** (which occurs in higher forms). This group includes the bacteria, blue-green algae, and rickettsiae (the viruses may also be included).

The Eucaryota, or higher protists, possess a well-defined nucleus with a definite nuclear membrane. Nuclear division is a more complex process, mitosis (with the presence of chromosomes). This group includes the protozoa, fungi (molds and yeast), and algae (except blue-green algae).

This system in effect eliminates the problem of deciding whether a microorganism is a plant or an animal, and for this reason it is more appealing to both the learner and the professional. In study of the morphology and cell structure of various microorganisms this system is easier to interpret. Further differences between Procaryota and Eucaryota will be discussed in Chapter 2.

One of the most widely accepted and utilized classification systems for bacteria (including rickettsiae) is outlined by *Bergey's Manual of Determinative Bacteriology* (published by The Williams & Wilkins Co.). This manual was published in 1923 and since then has been revised seven times; the eighth edition was published in 1974. Since the seventh edition (1957) of this manual is still in general use and much of the current literature is based on it, the following is a brief description of the classification system used in that manual. This will be followed by a description of the system used in the eighth edition (1974).

The seventh edition describes all species of bacteria (including rickettsiae) in their proper taxonomic position from kingdom through species as outlined in Table 1-2. It holds to the traditional concept that most microorganisms fit into the plant kingdom and recognizes five divisions, or phyla, of the plant kingdom.

Division I. Protophyta—primitive plants (bacteria, rickettsiae, and viruses)
Division II. Thallophyta—molds, yeast, and algae
Division III. Bryophyta—mosses and liverworts
Division IV. Pteridophyta—ferns and club mosses
Division V. Spermatophyta—seed-bearing plants

Divisions I and II contain the microorganisms and may be further expanded as follows:

Division Protophyta
- **Class Schizophyceae**
  - Blue-green algae
  - Contain plant chlorophyll and a blue pigment called phycocyanin
- **Class Schizomycetes**
  - Bacteria—most do not contain chlorophyll
  - Ten orders
- **Class Microtatobiotes**
  - Two orders: Rickettsiales (rickettsiae) and Virales (viruses)
  - Cells very small obligate intracellular parasites
  - Most viruses pass through bacteriological filters

Division Thallophyta
- **Subdivision Mycota (fungi)**
  - Do not contain chlorophyll
  - No roots, stems, or leaves
  - Include the molds, yeast and slime molds, water molds, and lichens
- **Subdivision Chlorophycophyta (algae)**
  - Contain chlorophyll
  - No roots, stems, or leaves

The various microbial groups in the plant kingdom are outlined in the tree diagram in Fig. 1-6. *Bergey's Manual* further divides the class Schizomycetes into 10 orders and then identifies each family, genus, and species. Each species is described in detail with respect to its identifying characteristics.

The system just described is considered an **artificial scheme;** that is, researchers have determined various characteristics (morphological, physiological, biochemical, ecological, etc.) of the microorganisms under study and used them as the basis of differentiation. Such a scheme is in contrast to a **natural or phylogenetic scheme,** in which microorganisms are grouped on the basis of evolution or natural relationships and a kind of **family tree** is constructed. Phylogenetic classification is relatively easy for the higher plants and animals, but it is very difficult to set up for microorganisms. This is particularly true for the lower protists, whose characteristics may be changed rapidly by **genetic mutation.**

After an extensive investigation of all the possible characteristics of a group of microorganisms, certain features may be selected as more significant distinguishing features to reduce the amount of testing necessary to identify each organism. For example, *Salmonella typhi* and *Proteus vulgaris* are bacterial strains with similar morphology; they are both motile and at times found in the intestine of man. However, *Salmonella* does not hydrolize urea, whereas *Proteus* splits urea rapidly. In this case the features of morphology, motility, and source play a secondary role only; hydrolysis of urea is **weighted** more heavily and becomes the distinguishing feature between the two genera.

This reduction in necessary testing is particularly valuable to the diagnostic technologist in a hospital laboratory, since speed of identification is very important. The time required for the complete classification of an unknown disease-producing microorganism is seldom available. Hence shortcuts using only the most important tests (the weighted ones) are essential to speedy identification. An excellent example of this situation is given in Table 1-3; a tech-

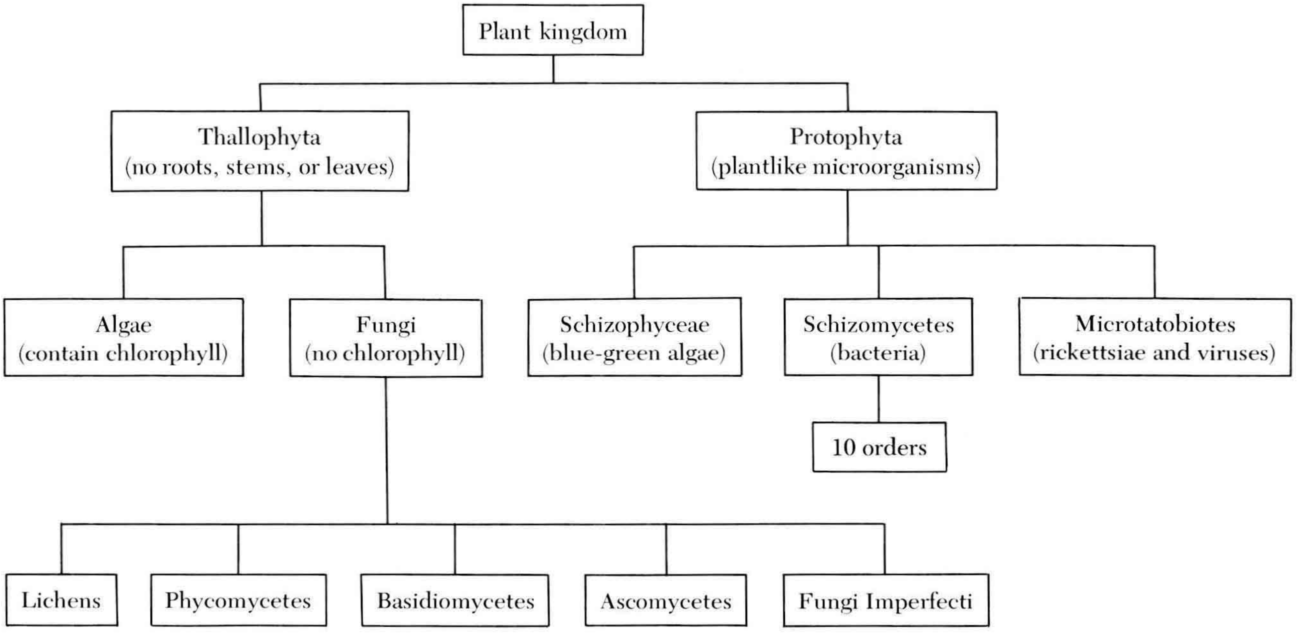


Fig. 1-6. Evolutionary relationships between groups of microorganisms in the plant kingdom.

Table 1-3. Biochemical tests important in identification of *Corynebacterium* species

Species	Tests for utilization of: Starch	Glucose	Sucrose
C. diphtheriae var. *mitis*	−	+	−
C. diphtheriae var. *gravis*	+	+	−
C. pseudodiphtheriticum	−	−	−
C. xerosis	−	+	+

nologist has identified an unknown bacterium as a member of the genus *Corynebacterium* and now wishes to identify the species name. From past research the worker knows that the tests of starch utilization and fermentation of glucose and sucrose are important for identification of species among this genus. Therefore it is possible to further identify the *Corynebacterium* from the results of these three tests. For example, negative results on all three tests would identify *C. pseudodiphtheriticum* as the species in question.

■ Describe the two characteristics that would place a microbe in the kingdom Protista.

■ Describe three cellular differences between eucaryotic and procaryotic cells.

■ Describe the seventh edition of *Bergey's Manual* in regard to the layout for identification of microorganisms.

■ Draw and label a tree diagram illustrating the position of microorganisms in the plant kingdom (as in Fig. 1-6).

■ Compare Bergey's system (in the seventh edition) to Haeckel's system with respect to differences in principle.

■ Define artificial scheme of classification and discuss the role of such a scheme in the diagnostic identification of microorganisms.

■ Describe what is meant by the weighting of characteristics of microorganisms.

■ Given the characteristics of several species of bacteria, design a functional system of tests that will provide a means for the most rapid identification of these species.

(40-54)

If a worker is not in a diagnostic laboratory, it is possible to classify an unknown microorganism by using another taxonomic tool called the **keying out system.** A key is simply a series of yes-or-no questions leading to the correct microorganism. If the answer to a question is no, one is directed to another question, until the description fits and the answer is yes. This leads the investigator to the name of the microorganism. The questions represent the characteristics of all the microorganisms known to date. Again, some weighting has been done to save time.

One such key to identifying bacteria is a manual written by **V. B. Skerman,** called *A Guide to the Identification of the Genera of Bacteria* (second edition published in 1967 by The Williams & Wilkins Co.). This manual keys out all the genera of bacteria and then directs the reader to *Bergey's Manual* for further identification at the species level. Some parts of *Bergey's Manual* are also written in the form of a key to allow for more rapid identification. Such a key is shown on p. 11. This excerpt from *Bergey's Manual* (seventh edition) keys out the 10 orders of bacteria in the class Schizomycetes (the bacteria).

This key starts with the roman numeral *I*. If the first statement is correct (that is, if it fits the description of the bacteria under study), then you advance to *A*. If the answer to *A* is yes, then the organism is from the order Pseudomonadales. However, if the answer is no, then you proceed to *B* and answer either *1* or *2* to identify the order. If your answer for *I* was not correct (that is, if it does not fit the description), you are then directed to *II*, *A*. If *A* is correct, you progress to *1* and *2*. If *A* is not correct, then you advance to *B*. If *B*, *1* is correct, the order is Actinomycetales. If *1* is not correct, you progress to *2*, *a*. If *a* is correct, then the order is Beggiatoales. If *a* is the incorrect description, you move to *aa*, *b*. If answer *b* is correct, then either *c* or *cc* is the correct order. However, if *b* does not describe the bacterium, you progress to *bb;* the order Mycoplasmatales must be correct. If the description of the final order does not fit, you have made an error and must go through the complete key again. The validity of this key is only as good as the testing or observations of the microorganism. If you made a mistake during your study, you will probably get an erroneous answer—or you may never get one.

■ Describe the function of a key used to identify microorganisms.

■ List at least two manuals that use the key system for identification.