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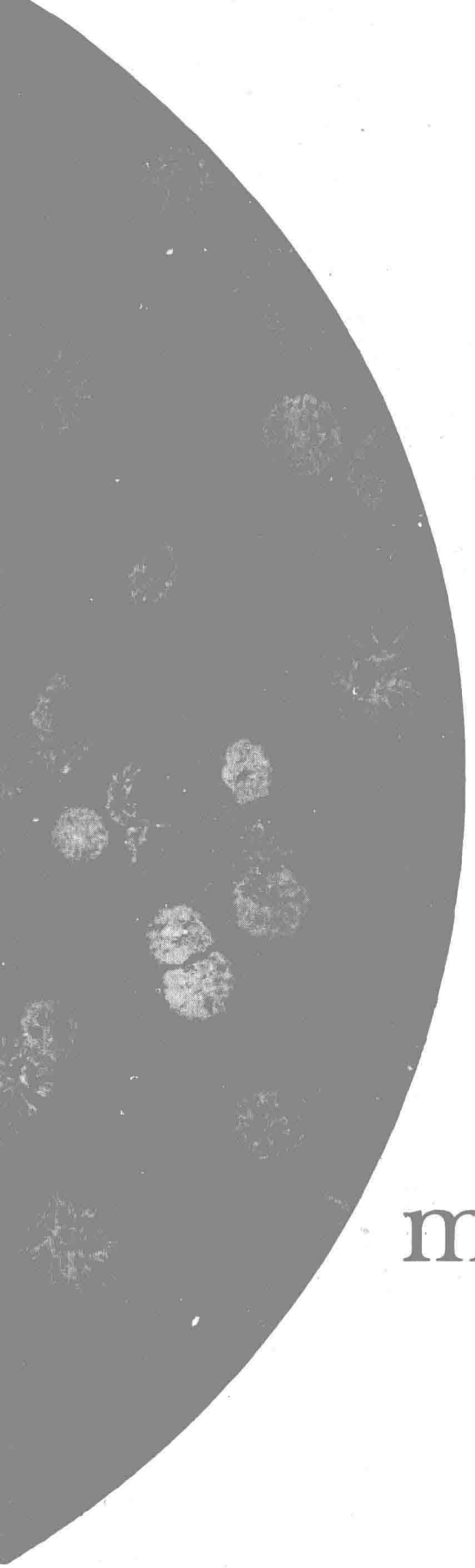

microbiology

MICROBIOLOGY

Library of Congress Catalog Card Number 57–12591

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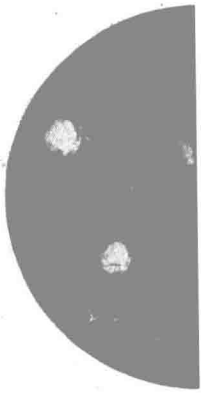

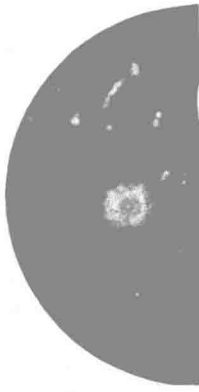

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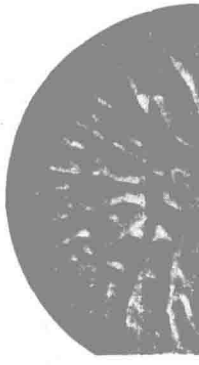

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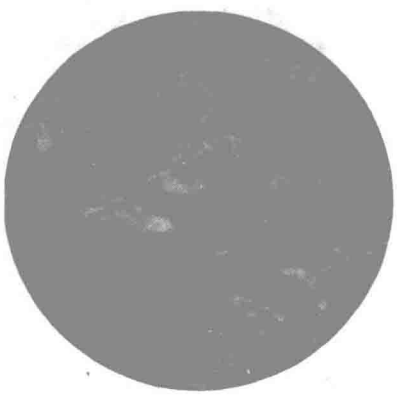
McGRAW-HILL BOOK COMPANY, INC.
New York Toronto London 1958

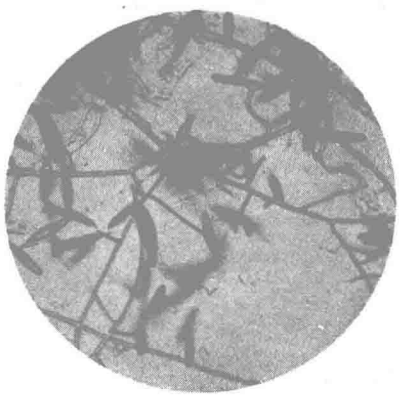
preface

THIS BOOK is intended for the use of students who are having their initial voluntary experience with the world of microbes. Without our knowledge or consent, each of us has spent his entire life in association with these creatures of the invisible world. We are alive today partly because of them and partly in spite of them.

It is hoped that this study will create an interest in microbiology which will stimulate a desire to pursue it further. For those who may not take additional formal training in microbiology, this book should provide a background of knowledge which will make it possible to appreciate and understand the part that microorganisms play in their lives. For these students, we have tried to present the material in such a way that it will be valuable in expanding their cultural background. For the student who will go further with the study of microbiology, the material provides a basic background on which to build a profession based wholly or in part on the activities of microorganisms.

In attempting to provide a panoramic concept of microbiology, it has been necessary to make many generalizations, recognizing, of course, that there are exceptions to all generalizations. In this book the subject is considered under the following heads: (1) a survey of microbial life and the history of microbiology, (2) the characteristics of bacteria as prototypes of all microorganisms, (3) the characteristics and importance of microorganisms other than bacteria, (4) the control of microorganisms, (5) microorganisms and disease, and (6) other aspects of applied microbiology. Considerable effort has been expended to provide devices such as charts, tables, illustrations, review questions, and a glossary which will help the reader to understand and remember the subject matter. In presenting this material, accuracy and simplicity have been our goals. Every attempt has





been made to present the latest facts and concepts in this rapidly growing field. The text has not been documented with extensive references, however. Instead, we have listed a few of the more recent or standard works that may be useful in extending the student's information on several of the subjects included in this book. We have attempted to present the material in a logical sequence, but we are very well aware that the arrangement used will not be universally acceptable. The instructor should feel no compulsion to follow the sequence used in this book, since the material can be presented with equal effectiveness when it is rearranged to fit his individual preference.

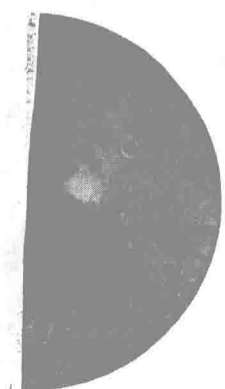
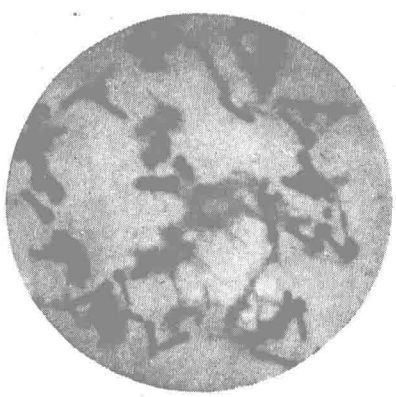
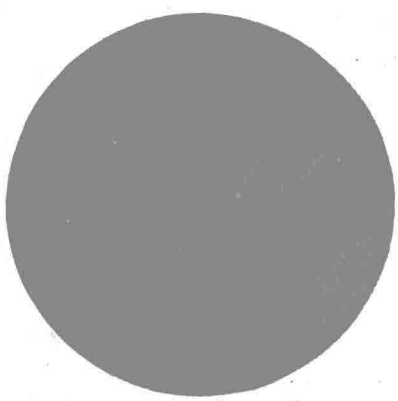
A laboratory manual for use with this text is available. In the manual, the arrangement of experiments closely parallels the order of subject matter in this book, and references are given to appropriate chapters in introductory remarks accompanying each exercise.

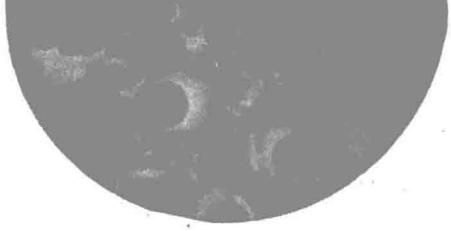
Remarkable advances in many aspects of microbiology have been made in the past decade. However, if any one area of the subject could be singled out as having been most extensively studied during the past ten or twenty years, this area would be metabolism, which has led to an exploitation of the microbe as an experimental tool for the study of biology in general. This, indeed, leads one to propose that microbiology might well be the field through which the student should be introduced to biology, since in microbiology he can learn the basic elements of metabolism, reproduction, nutrition, and genetics. The single cell is simple to use and comparatively easy to understand, and everyone has had some experience with it.

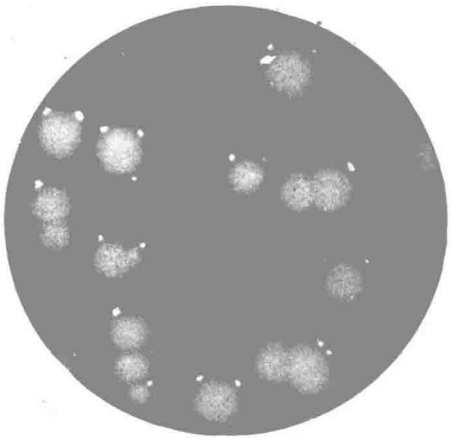
It has been our intent in this book to emphasize modern trends and to point out their implications. We have not, however, felt that we could ignore the past, with its many ingenious explanations; hence, in our presentation of the material, we have tried to achieve an appropriate balance between old and new.

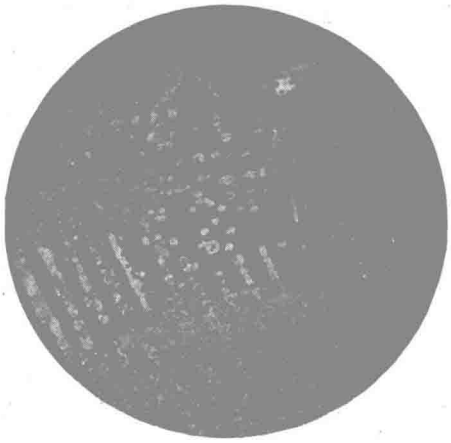
We are indebted to a host of colleagues who have given willingly of their time and expert knowledge in reviewing the manuscript as a whole or those chapters in their special fields. As a result of their help, we feel that the contents are factually correct; whether the facts are adequately interpreted and presented is our own responsibility. We have had the generous and willing cooperation of many individuals and organizations. In the case of illustrative and tabular material, sources are acknowledged in the legends, and in the case of chapter opening photographs, are listed on page 551. We should like to name each of the persons and organizations that have contributed to this book, but this would not be possible, since our gratitude needs to be expressed to so many for so much.

Michael J. Pelczar, Jr.
Roger D. Reid

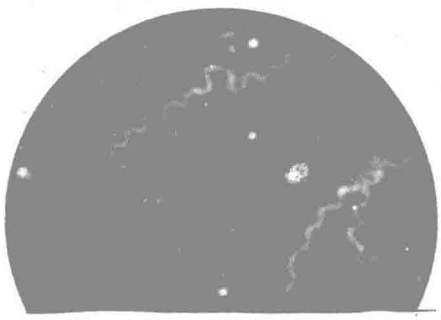










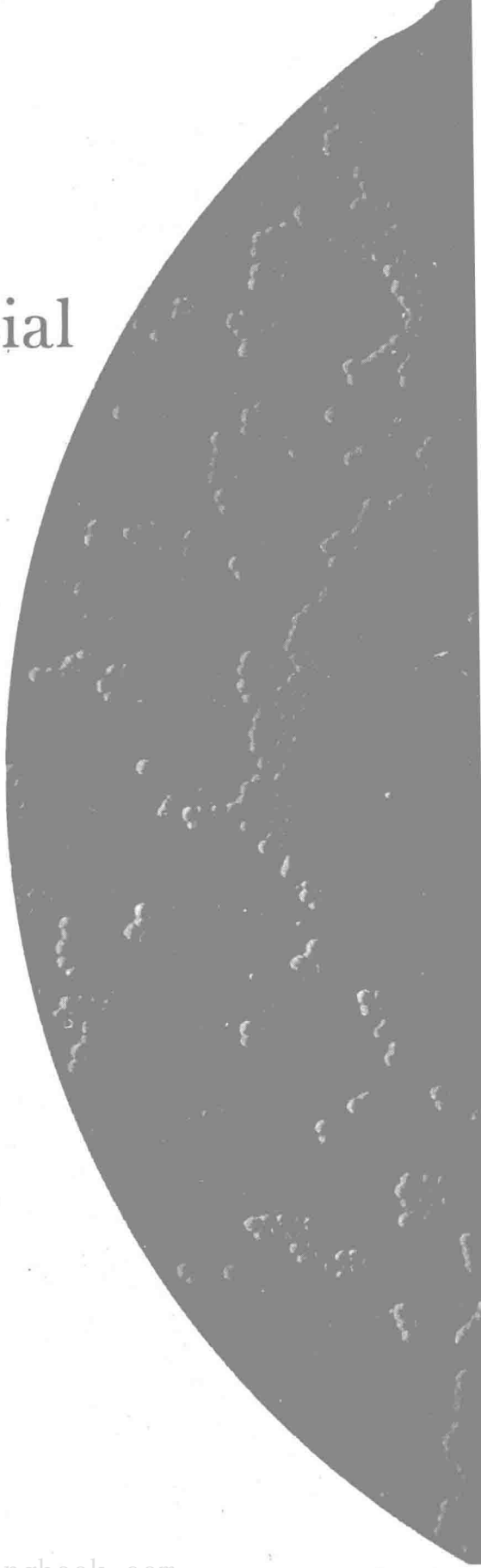
1
introduction to microbiology



contents

1

a survey of microbial life



WHEN MAN learned to grind optical lenses and use them to observe things too small to be seen by the unaided eye, he brought a whole new world into view—a world of plants and animals so small that millions of them can be found in a single drop of water or milk and thousands of them can stand on the head of a pin. It is a world of minute but powerful creatures. These organisms, invisible without the aid of a powerful microscope, can make healthy men sick and strong materials weak; they can make good food unsafe and unsavory; they can destroy buildings, rot tents, and wreck harbors. Some of them manufacture drugs that counteract the harmful effects of sinister microbes; some convert the juices of grapes into wine and sweet apple cider into vinegar; others change the starch and sugar in fruit and grain into alcohol. Some microbes make leather strong, soft, and pliable; others "cure" tobacco and give pickles, kraut, and other foods their characteristic flavors and textures. Although the list of important products resulting from their activity is long and surprising, no one guessed until a hundred years ago how much or in how many ways microorganisms affect our lives.

Microorganisms in the soil dispose of organic material in dead plants and animals by changing the complex proteins and carbohydrates into the simple compounds or elements

of which they are constituted, so that the carbon, nitrogen, and other chemicals can be used again as food for new plants and, subsequently, for animals. Other soil microorganisms "fix" nitrogen, that is, take it out of the air where it abounds in a form not acceptable to higher plants and combine it with other elements to form compounds from which higher plants can obtain nitrogen to build their protein structures.

It is of this world of microorganisms and what they are and do—how they help us and how they can harm us, how they can be made more useful and less harmful—that the science of microbiology deals. It is the science of life in its simplest structural form—the single-celled plants and animals, including that mysterious and fascinating group of organisms called *viruses,* which are so small that the ordinary high-powered microscope fails to reveal them. In our study we shall speculate on the question whether these viruses are truly living creatures or whether they are on the borderline between animate and inanimate things. Indeed, we shall behold a new world opening before us.

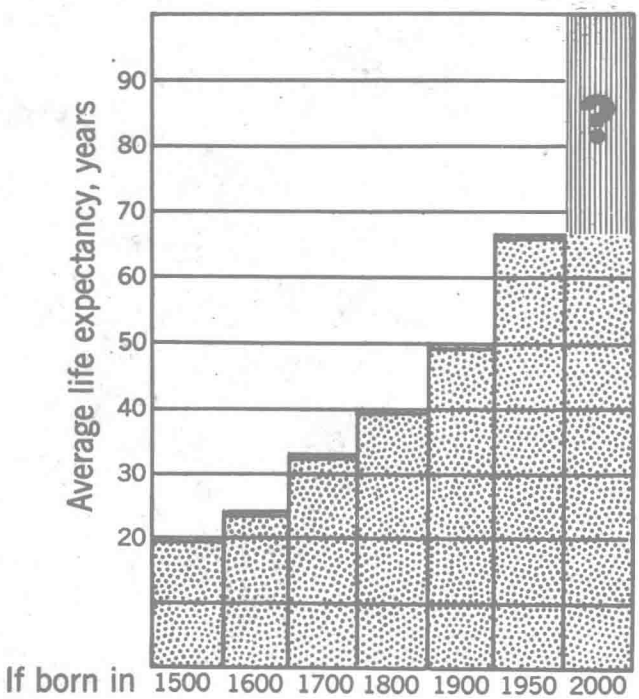


Fig. 1-1. **Average life expectancy in the United States since 1500. One of the most important factors contributing to increased longevity in the United States has been improved methods of prevention and control of infectious diseases.**

The microbes that benefit mankind must be encouraged, domesticated, and put to use, just as many higher plants and animals are used by man. The microbes that cause suffering, economic loss, and death must be controlled and denied entry into our environment. We have expended much more effort in protecting ourselves from harmful bacteria than in learning how to use the helpful kinds. The increase in man's life expectancy over the centuries, or even in the past fifty years, is proof of his success in controlling many disease-producing microbes (Fig. 1-1). At the turn of this century, the leading causes of death were the diseases caused by bacteria, viruses, and protozoa. Today the leading causes of death in the United States are organic diseases, such as heart and kidney diseases and malignancies, and accidents. Even the causes of war casualties have changed. Up to and including World War I, the majority of deaths in wars came from epidemic diseases of the men in the armed forces. This was not true in World War II and the Korean War.

Man has learned to defend himself against his invisible enemies among the microorganisms by using antibiotics and other chemotherapeutic agents, vaccines, antitoxins, and many other weapons. He has learned to preserve foods by refrigeration, heat-sterilization, and pasteurization. He has learned to protect the part of his home that can be destroyed by microbial action with paint and chemicals. By selective breeding he has developed infection-resistant plants.

Without certain kinds of microbes, milk and meat would be in very short supply. Bacteria living in the rumen (first stomach) of cattle, sheep, deer, and other ruminants syn-

thesize amino acids (the building blocks for proteins) and vitamins. Others degrade cellulose, which is the carbohydrate material that gives plants their rigidity.

Our appreciation of these microscopic creatures will grow as we become better acquainted with them.

Divisions of Microbiology

Microbiologists specialize in the study of certain groups of microorganisms. Strictly speaking, *bacteriology* is the study of bacteria, a type of microbe, but often the term is used as a synonym for microbiology. The two terms will be used interchangeably in this text. *Protozoology* is the study of protozoa; a special branch of protozoology called *parasitology* deals exclusively with the parasitic or disease-producing protozoa. *Mycology* is a branch of microbiology which deals with fungi such as yeasts and molds, and *virology* is the division that treats of viruses, usually including the rickettsiae.

Other divisions of the science of microbiology are based on its applications to our daily lives. *Medical microbiology* is the branch that deals with infectious diseases of man and other animals. *Agricultural microbiology* is concerned with the microorganisms that affect various phases of agriculture, including soil fertility. *Dairy microbiology* deals with the use of microorganisms in the dairy industry, and *plant pathology* with those that cause diseases of plants. *Industrial microbiology* utilizes microorganisms to convert raw materials found in nature into products of industrial importance. These chemical reactions, commonly referred to as *fermentations,* are employed in the production of vinegar, beer, alcohol, wine, and antibiotics (e.g., penicillin). Other industrial applications of microbial activity are made in the manufacture of leather and hemp. Industrial microbiology is also concerned with the control of the activities of microorganisms that are detrimental and cause spoilage or deterioration of raw materials and finished products in storage.

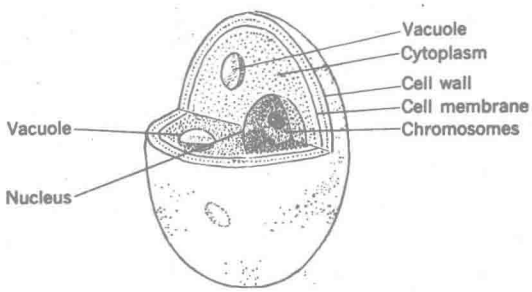


Fig. 1-2. **A typical cell, showing some essential parts. Although some are more complex than others, all cells contain essentially the same parts. The entire body of a unicellular organism is a single cell, whereas multicellular plants and animals are composed of many cells, some of which are especially adapted to carry on specific functions.**

Classification of Microorganisms

The plants and animals with which we are most familiar are living things made up of thousands of cells, each with a special function, organized to carry on the work of the whole organism. Microorganisms are also living things but of exceedingly minute dimensions,[1] the entire organism in many cases being but a single cell with the ability to perform all the life processes of reproduction, metabolism, and growth. Microorganisms can be seen only with the aid of a microscope and are measured in *microns* (a micron is 1/1,000 of a millimeter, or approximately 1/25,000 of an inch).

In spite of great differences in size, shape, and complexity, there is a basic similarity in the structure of all living cells (Fig. 1-2). All are composed of a remarkable substance called *protoplasm* (from the Greek for "first-formed substance"), a colloidal organic complex consisting largely of protein; all have limiting membranes, or cell walls; and all contain

[1] The typhoid bacterium, which is an "average-sized" microorganism, is about 1/15,000 of an inch long. As for weight, it would require about two trillion to weigh one gram and thirty times as many to weigh one ounce.

nuclei. Cells have the three characteristics common to all living things: (1) the ability to reproduce, (2) the ability to take in food substances for growth and energy, and (3) the ability to react to changes in the environment.

All living things are classified as plants or animals, and all microorganisms except protozoa are considered to be plants. Like higher plants, they absorb their food in solution through their cell walls, and some contain the green pigment characteristic of higher plants. Protozoa ingest solid food particles and digest them inside the cell. Higher animals have specialized organs, the stomach and the intestines, where digestion takes place prior to the assimilation of food.

The plant kingdom has five divisions according to reproductive structures and other anatomical (morphological) characteristics. In "Bergey's Manual of Determinative Bacteriology" (7th ed., 1957) the divisions are:

Division I	*Protophyta*	Primitive plants, e.g., bacteria and viruses
Division II	*Thallophyta*	Molds and yeasts
Division III	*Bryophyta*	Mosses and liverworts
Division IV	*Pteridophyta*	Ferns and club mosses
Division V	*Spermatophyta*	Seed-bearing plants

In the study of microbiology, our attention will be directed largely toward organisms belonging to two divisions of the plant kingdom, Division I, *Protophyta,* and Division II, *Thallophyta.* These two divisions include organisms bearing such common names as bacteria, yeasts, molds, algae, viruses, and rickettsiae, as shown in Fig. 1-3. Protozoa, the single-celled *animals,* are also included in the study of microbiology. Their classification will be discussed in Chap. 17.

Distribution of Microorganisms

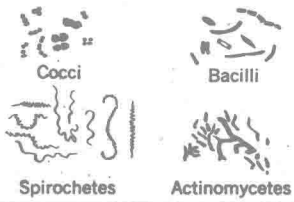
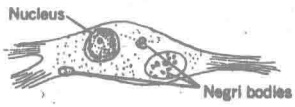
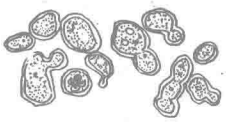
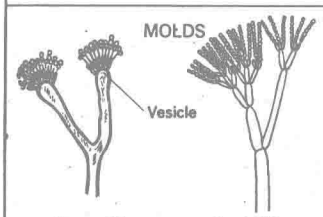
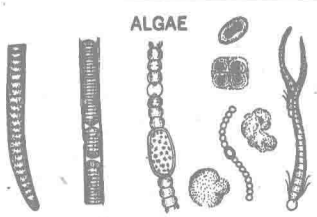
Microorganisms occur nearly everywhere in nature. They are carried by air currents from the earth's surface to the upper atmosphere. Even those indigenous to the ocean may be found many miles away on mountain heights. They are found in sediments in the bottom of the ocean at its greatest depths. The soil is filled with them; there are as many as 50 million in a gram of soil from a fertile field! 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. They occur most abundantly where they find food, moisture, and a temperature suitable for their growth and multiplication. The conditions that favor the survival and growth of many microorganisms are those under which man normally lives, so it is inevitable that we live among a multitude of microbes. They are in the air we breathe and the food we eat. They can be found on the surfaces of our bodies, in our intestines, and in our mouths, noses, and other body openings. It is certainly fortunate that most microorganisms are harmless to us and that we have ways of resisting invasion by those that are potentially harmful.

Microorganisms may be *parasites* or *saprophytes.* Parasites get their food from living organic matter, often from the cells and tissues of our bodies or those of other animals and plants. Among the parasitic microorganisms are those that cause typhoid fever, poliomyelitis, wheat rust, and malaria. Saprophytes feed on dead plants and animals; they greatly outnumber the parasites.

The Study of Microorganisms

Because they are individually so small that we cannot see them without the aid of a microscope, it is not practical to work with single microorganisms. For this reason we study *cultures* containing hundreds or thousands of microorganisms. A culture that consists of a *single kind* of microorganism, regardless of the number of individuals, is called a *pure culture.* If two or more kinds grow together,

Fig. 1-3. **A comparison of some types of microorganisms with regard to morphology, size, and other characteristics.**

MORPHOLOGY	SIZE	IMPORTANT CHARACTERISTICS	PRACTICAL SIGNIFICANCE
BACTERIA 	0.5 to 50.0 μ	Many are motile. All are unicellular. They grow on artificial media. Reproduction is predominantly asexual by binary fission.	Some cause disease. Some are useful in industry. Some improve soil fertility. Some produce antibiotics.
VIRUSES  Inclusion bodies of rabies virus in a nerve cell. The virus is invisible.	0.01 to 0.3 μ	Many are filter passing. They grow only in living cells. Are obligate, intracellular parasites. Some form inclusion bodies.	Viruses cause disease in man, animals, and plants. Virus infections of bacteria are due to bacteriophage.
RICKETTSIAE 	0.3 to 0.6 μ	Arthropod-borne pathogens. They grow only in living tissue.	Cause disease in man. They spend some part of their life in an arthropod host-vector.
YEASTS 	5.0 to 10.0 μ	Grow on artificial media. Reproduce by asexual budding or by sexual processes.	Yeasts are used in the production of alcohol and other organic chemicals. Some cause disease.
MOLDS 	5.0 μ or much larger	Grow on artificial media. They are multicellular and present many morphological features. Reproduction may be by sexual or asexual means.	Are used extensively in fermentation industries, and in food manufacture. Some cause deterioration of wood, cloth, etc. They enrich the soil. Some produce antibiotics. Some cause disease.
PROTOZOA 	2.0 to 20.0 μ	Unicellular animals. Many are motile, some are intracellular parasites. Most protozoa will grow on cellfree media. Reproduction may be sexual or asexual.	Many protozoa are food for aquatic animals. Some cause disease.
ALGAE 	1.0 μ to many feet	Algae cells contain chlorophyll. Many are multicellular. Most are aquatic. May reproduce sexually or by a variety of asexual means.	Are a source of food for aquatic animals and man. An important source of vitamins and chemicals.

as in nature, they form a *mixed culture.* The material on which microorganisms are grown is called a *culture medium.* Media consist of food materials compounded to favor the growth of microorganisms. When a liquid kept in a flask or tube is used as a medium, it is called a *broth* or *fluid medium.* Microorganisms sometimes multiply in such large numbers that an initially clear medium becomes cloudy or turbid. Some microorganisms form a pellicle over the surface of the broth, and others form a sediment at the bottom.

To obtain a pure culture from one that is mixed, it is usually convenient to grow the organisms on or in a *solid medium,* which can be made by adding a solidifying substance such as *agar* to any liquid medium in a flat, round glass container called a *petri dish.* On this medium the organisms grow in aggregates or clumps, each originating from an organism or group of organisms. Such clumps are made up of the progeny of a single organism or of groups of organisms of a single type and are termed *colonies.*

In order to obtain pure cultures in an environment teeming with microorganisms, the culture medium and the equipment must be freed from *contaminants,* as unwanted microorganisms are called. This is done by sterilizing to kill the unwanted organisms that may be present before the medium is *inoculated* with the microorganisms to be cultivated in pure culture. To prevent contaminants from getting in the medium during *incubation* (the period of growth), test tubes and flasks are stoppered with nonabsorbent cotton plugs or covered with a metal, glass, or plastic cap. Petri dishes are made in pairs, so that a cover fits snugly over the dish that contains the medium and culture. Sterilization may be accomplished by heat, filtration, radiation, or chemical means. The method most common in the microbiology laboratory is heat sterilization, which may be carried out in a hot-air oven, by boiling, or by steam under pressure in an apparatus called an *autoclave. Inoculating needles* used to *transfer* a culture from one flask to another are sterilized in the open flame of a *bunsen burner.*

Microscopy. Colonies of microorganisms growing on various types of nutrient culture media can be seen without magnification, but to see the individual cells we must use a high-powered microscope. Even with the aid of a microscope it is difficult to see the individual organisms because of their small size and transparency. To overcome this difficulty a variety of aids to microscopy have been developed, the most common of which is *staining.* Microorganisms are easier to examine when they are spread on a glass slide to make a "smear," passed through an open flame to kill and "fix" them to the slide, and then covered with a solution of dye called a *stain.* From such stained smears it is possible to observe morphological characteristics of individual cells.

Microbiology is a relatively new branch of science, but its importance to many other sciences is now recognized. Biochemists and physiologists are learning about metabolism of higher plants and animals through the study of microorganisms; geneticists have found that their knowledge of inheritance can be advanced rapidly by experimenting with microorganisms, which pass through more generations in a week than man has had since the dawn of history. Microbiology offers an interesting opportunity to study life in a fascinating form.

QUESTIONS

1. Cite the five main divisions of microbiology.
2. List the types of microorganisms studied in microbiology, with a special characteristic of each.
3. Define the term *micron.*
4. Name and define the five principal divisions of plants. In which divisions are microorganisms found?
5. Define the following as they apply to microbiology: pure culture, culture medium, contaminant, colony, and stain.

2

the history of microbiology

History is the story of the achievements of men, but it records only a few outstanding names and events. In reading the history of microbiology, we must remember that many important contributions were also made by men whose names have been forgotten and whose accomplishments have been lost from view in the longer and deeper shadows cast by those who caught the fancy of the chroniclers. It has been said that in science the credit goes to the man who convinces the world, not the man to whom the idea first occurred. So, in the development of bacteriology, the outstanding names are often those of men who convinced the world—who developed a technique or tool that was generally adopted or explained their findings so clearly or dramatically that the science grew and prospered.

Leeuwenhoek's lucid reports on the ubiquity of microbes, for example, enabled Louis Pasteur to discover the involvement of these creatures in fermentation reactions and Koch, Smith, Pasteur, and many others to discover their association with disease. Koch is remembered for his painstaking search for the microorganisms that cause anthrax and tuberculosis and for his insistence that rigid criteria be met before a specific bacterium is held to be the cause of a disease. The building of the Panama Canal dramatized Walter Reed's studies on the epidemiology of yellow fever, but historians remember that Theobald