BASIC MEDICAL MICROBIOLOGY

FOURTH EDITION

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PREFACE

When we wrote the first edition of *Basic Medical Microbiology*, one of our primary aims was to present a concise and uncluttered view of medical microbiology. In this fourth edition, we have kept in mind these original aims but have made several changes, which we hope will make the text even more useful to students. These changes include the following:

1. We have added many more tables. These not only compare infectious agents but list specific laboratory features and other microbial characteristics.
2. Questions for study at the end of the chapters consist of multiple choice, matching, and/or completion. More than one answer may be appropriate for some questions.
3. Appendices are included. Appendix A provides tables of infectious agents based on the site of infection. Each table includes mode of transmission and method of treatment and/or prevention. In addition, the page number where the infectious disease or agent is discussed is also included. The tables should be helpful for reviewing the text material. Appendix B includes tables of the microbial agents that make up the flora of various body sites and the diseases with which they are associated.
4. The index for the fourth edition was prepared by R.F.B. Specific discussions and terminology that were not included in the third edition are now available for easy location by the student.

In addition, we have made changes in several sections of the book.

Section V, Bacteria That Cause Infectious Disease, is more concise and includes more tables. Diagnostic laboratory techniques have been updated to include more recent diagnostic methods. These chapters also contain updated material on mechanisms of pathogenesis. In addition, we have included discussions of more recently recognized pathogens such as *Helicobacter pylori*, *Ehrlichia* species, and *Chlamydia pneumoniae*.

In Section VI, Virology, Chapter 30, Viruses, was rewritten and condensed, with a discussion of animal viruses appearing first. In addition, there is a section called Diagnostic Virology that discusses the rapid methods now employed in the field. A brief discussion of the rationale for treatment and prevention of viral infections is also discussed. The last section of this chapter is devoted to bacterial viruses. Chapter 31, Viral Diseases, has also undergone revision. The discussion of smallpox has been shortened significantly since this disease has been declared eradicated. Included is a more encompassing discussion of enteroviral diseases. New headings include Hemorrhagic Fever Viruses; The Arboviruses, which now includes a discussion of dengue fever and dengue hemorrhagic fever; and Rotaviruses and Other Agents of Gastrointestinal Disease, which includes a discussion of caliciviruses and astroviruses. Perhaps the most significant change in the chapter is the inclusion of AIDS. Discussion of AIDS appeared originally in the chapter on immunological disorders in the third edition.

Section VII, Medical Mycology, is largely unchanged. However, we have added the pathogen *Pneumocystis carinii* to this section since RNA analysis suggests that it is a fungus and not a protozoan.

In Section VIII, Medical Parasitology, we have added comprehensive tables of all the major animal parasites. This inclusion should be of great help to students.

We would like to thank the reviewers of the third and fourth editions for their invaluable comments. A special thanks goes to the scientists and book and journal publishers who provided photomicrographs and allowed us to use various tables. We would like to thank Ruth Steinberger for the line drawings. We would also like to thank Jon Sarner, without whose efforts this book would have never been published.

R.F.B.
B.G.H.

CONTENTS

I. GENERAL BACTERIOLOGY

1. SCOPE AND HISTORY OF MICROBIOLOGY

OBJECTIVES

To understand the importance of microbiology to medicine as well as to applied areas of science

To describe the contributions of the following scientists to microbiology: Leeuwenhoek, Pasteur, Koch, Lister, Jenner, and Ehrlich

To explain what is meant by the theory of spontaneous generation and the experiments that were performed to refute it

To list the steps that are required to identify the causative agent of an infectious disease

SCOPE

Microbiology is a science that is primarily concerned with the study of microorganisms and viruses. A distinction between microorganisms and viruses is made because viruses do not exhibit cellular characteristics. Viruses are entities that are totally dependent on the presence of a living cell for replication. Microorganisms are for the most part single-celled, but they also include certain multicellular types. When we speak of microorganisms, we must include bacteria, fungi (molds and yeasts), certain algae, and protozoa. Protozoa belong to the group called *animal parasites*, which also includes certain multicellular worms called *helminths*. Algae are not associated with disease in humans and will not be discussed in this text.

The explosion of microbiological information in recent years has been so great that it has resulted in the creation of specialties concerned with each of the representative groups of microorganisms being studied. For example, the bacteriologist may develop skills in various aspects of bacteriology such as bacterial physiology, bacterial genetics, or bacterial cytology. The microbiologist may specialize in the study of microorganisms as they relate to applied and environmental fields. There are now divisions of microbiology such as space microbiology, soil microbiology, aquatic microbiology, food microbiology, and industrial microbiology.

Medical microbiology, which deals primarily with microorganisms that cause infectious disease, is the branch of microbiology with which the student reading this book is primarily concerned. Since various representative types of microorganisms can cause disease in humans, the medical microbiologist must have a basic knowledge of the chemical and physical properties of the potentially harmful microbial agents—bacteria, viruses, yeasts, molds, and protozoa. The primary responsibility of the medical microbiologist is to understand the etiology (causation), disease manifestations, laboratory diagnosis, and treatment of infectious disease. The microbiologist's obligations may also include determining the epidemiology of disease (e.g., how diseases are transmitted) and developing measures for the control and prevention of diseases in the community. The fact that many major pestilences such as smallpox, diphtheria, and plague no longer decimate populations as they once did is testimony to the advances made in epidemiology, control, and prevention of infectious disease. Resistance or immunity to disease has been recognized for years. This area of study is referred to as *immunology* and deals specifically with the relationship of antigens or foreign substances to antibody production in the host. Because of its relationship to disease, immunology is also considered an important part of the medical microbiologist's background. The development of new concepts in molecular biology and cellular immunity has not only expanded our knowledge of the immune process but has also provided the microbiologist with new laboratory diagnostic tools. The medical microbiologist can now identify and classify microorganisms using immunological techniques.

In its early history, microbiology as a science was concerned with the identification and control of microorganisms. Major advances in microscopy and biochemical techniques from 1940 to the present have enabled scientists to use microorganisms as a model for the study of biological properties, particularly in the areas of genetics and metabolism. These studies are aided by the fact that microorganisms divide very rapidly and are easily cultivated and maintained in the laboratory—properties not common to higher forms of life.

During the development of microbiology, it was soon realized that many of the metabolic processes occurring in microbial systems were similar if not identical to those of cells in higher systems, including the human. In 1944 Avery, MacLeod, and McCarty discovered that isolated (cell-free) DNA was capable of transforming (producing a genetic change in) certain intact bacterial cells. This was one of several experiments proving that DNA was the hereditary material of the cell. The discovery of the structure of DNA by Watson and Crick in 1953 and the results obtained from experiments in microbial genetics provided the basic clues to genetic mechanisms not only in bacteria but in higher forms of life as well. Scientists are now able to manipulate genes and to transfer genes from one species of organisms into the DNA of totally unrelated microbial species. Such techniques referred to as *genetic engineering* are revolutionizing microbiology and related sciences. This technique, along with our greater understanding of molecular biology and biochemistry, is advancing us near the brink of conquering, or at the very least tempering, many of the major diseases, both infectious and noninfectious, that now afflict humankind. In addition, many biological properties unavailable to some organisms, such as plants' ability to fix nitrogen from the air or the capacity to produce large quantities of a single protein to be used as food, may become realities in the very near future. The future for microbiology, both medical and nonmedical, is so bright and promising that we can hardly wait for each day's new discoveries to be revealed. We hope that the importance of these discoveries will be imparted to you as you read each chapter. But keep in mind that you can fully appreciate these discoveries only if you understand the basic biological properties of microorganisms.

HISTORY

EARLY CONCEPTS OF DISEASE

Microbiology had its origins in the concepts that were first formulated to explain disease. In some ancient civilizations disease was believed to be a punishment sent from the gods for human wrong-doings. Many of the philosophers during these early periods in history, however, were of the belief that disease was transmitted by invisible "animals." Since the animals could not be seen, the theory remained just that, a theory. The Italian physician Fracastorius (1485–1553) later postulated that disease was transmitted by invisible particles or seeds from one person to another or from contact with the clothing or utensils of the infected. Two hundred years elapsed before a detailed description of microorganisms was made.

FIRST OBSERVATIONS OF MICROORGANISMS

Anton van Leeuwenhoek (c. 1685), who took up microbiology as a hobby, was an amateur microscope builder. Using a very primitive microscope, he described in some detail the structure of the red blood cells of humans and other animals. Leeuwenhoek was the first to describe microscopic organisms found in pond water and later made observations of bacteria he found in the debris surrounding teeth (Fig. 1-1). Leeu-

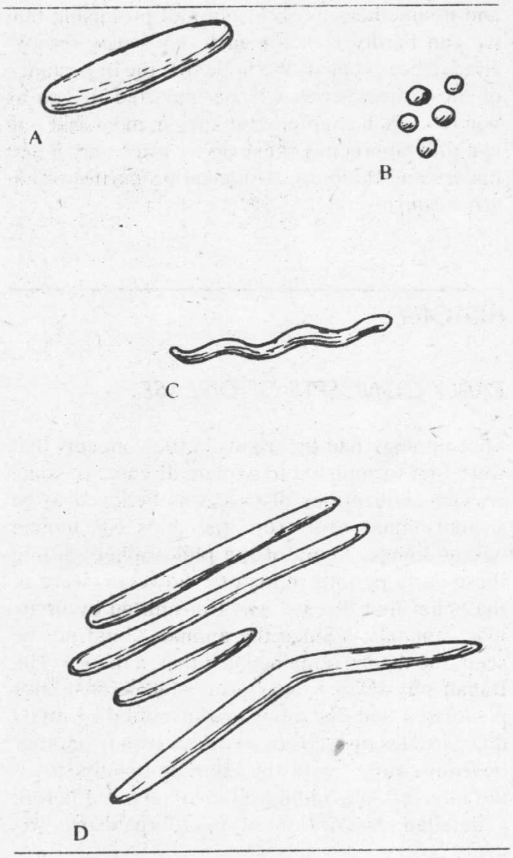

Fig. 1-1. Shapes of microorganisms observed by Leeuwenhoek in samples taken from the human mouth. A. Rod-shaped (bacillus). B. Spherical (cocci). C. Spiral-shaped (spirochete). D. Cigar-shaped rods.

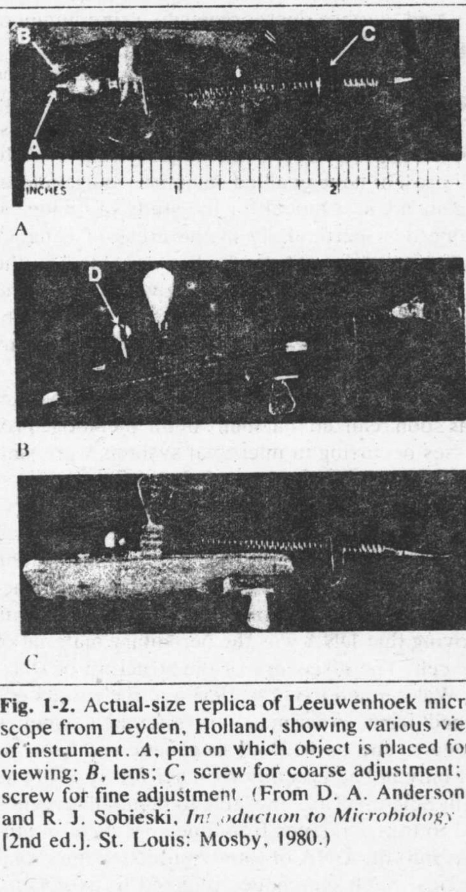

Fig. 1-2. Actual-size replica of Leeuwenhoek microscope from Leyden, Holland, showing various views of instrument. *A*, pin on which object is placed for viewing; *B*, lens; *C*, screw for coarse adjustment; *D*, screw for fine adjustment. (From D. A. Anderson and R. J. Sobieski, *Introduction to Microbiology* [2nd ed.]. St. Louis: Mosby, 1980.)

wenhoek's microscope was a little over 2 inches in length and capable of magnifications approaching 160 to 200 times (Fig. 1-2).

SPONTANEOUS GENERATION

Even though his was the first description of microorganisms, most of Leeuwenhoek's peers either ignored or denied the existence of these newly discovered organisms. Instead, most scientists accepted the theory of *spontaneous generation*, that is, that life can arise from dead organic matter—a theory first proposed by Aristotle in 384 B.C. For example, the scientific community was convinced that blowflies could arise spontaneously from rotted meat. Scientists such as Francisco Redi (1668) and Spallanzani (1776) performed experiments that showed that organic matter, if protected from contamination by boiling and preventing exposure to the air, did not give rise to new life. The spontaneous generation proponents refuted these experiments by suggesting that a *vital force*, such as air, had been destroyed by boiling.

Franz Schulze and Theodore Schwann in 1836 independently demonstrated that air was not the vital force. Schulze passed heated air into flasks of nutrient broth and showed that heating sterilized the air and prevented growth in the flasks. Schwann passed air through chemical solutions before it entered the flasks containing nutrient media and obtained the same results. The proponents of spontaneous generation were unswayed by these experiments. They countered that the treated air had been stripped of any life-generating forces. Despite these and other experiments that disproved spontaneous generation, the proponents of this theory were still unconvinced.

Only the experiments of Pasteur (Fig. 1-3) would finally put an end to the theory of spontaneous generation. Using swan-necked flasks (Fig. 1-4). Pasteur boiled organic solutions to destroy any "seeds" (microorganisms) that might be present. There was no barrier to the passage of air in these flasks, and they could sit for several days with no visible turbidity, which would indicate the presence of life (seeds or microorganisms). In other words, air outside the flask could diffuse into the organic broth, but any microorganism carried in the air could go no farther than the walls of the flask's neck, where it would settle out. If the liquid in the flask was allowed to come in contact with the organisms in the neck, by tilting the flask and then returning the contaminated fluid by tilting the flask back again, the broth became turbid after 24 hours. Pasteur made many other important contributions to microbiology and related sciences (Table 1-1) and for this reason has been called the Father of Microbiology.

Fig. 1-3. Louis Pasteur (1822–1895). (From *Microbiology*, Fourth Edition by Philip L. Carpenter. Copyright © 1977 by W. B. Saunders Company. Reprinted by permission of CBS College Publishing.)

Table 1-1. Contributions of Louis Pasteur to Microbiology and Related Sciences

Development of attenuated vaccines for anthrax and chicken cholera
Immunization against rabies
Relationship of crystal structure to optical rotation
Study of diseases of swine and silkworms
Discovery of technique for selective destruction of microorganisms by heat (pasteurization)
Refutation of theory of spontaneous generation
Discovery of microorganisms that live in the absence of air (anaerobes)
Contributions to understanding the causes of fermentation

GERM THEORY OF DISEASE

The theoretical explanations of infectious disease as proposed by Fracastorius in 1546 were not supported by experimental proof until 200 years later. Physicians such as Semmelweis in Austria and Oliver Wendell Holmes in the United States implored their physician colleagues to wash their hands before examining pregnant women. Both of these physicians had demonstrated that the

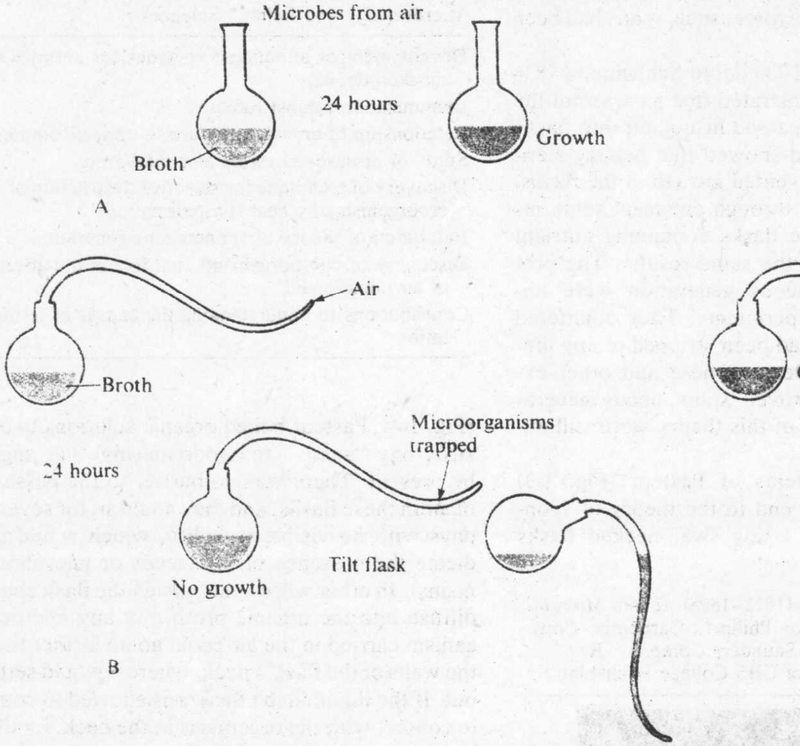

Fig. 1-4. Swan-necked flasks were used by Pasteur to disprove spontaneous generation. Flask A, which is not swan-necked, contains sterile broth that is directly exposed to the air and becomes turbid after 16 to 24 hours. Microorganisms in the air settle into the broth, where they reproduce and make the broth turbid. Flask B, which is swan-necked, also contains sterile broth. Because of its shape air, but not microorganisms, may enter the space above the broth. Microorganisms that are present in the air settle out by gravity in the curved neck of the flask. The broth in the swan-necked flask shows no turbidity even several days after exposure to air unless the flask is tilted and the broth makes contact with microorganisms.

agent of puerperal (childbed) fever was transmitted from an infected patient to an uninfected one by physicians who did not wash their hands. Their pleas were ignored by the majority of physicians who refused to believe they were unclean.

In 1857 Louis Pasteur formulated the theory of fermentation by microorganisms by demonstrating how microorganisms can convert sugar to lactic acid. From these experiments Pasteur theorized that microorganisms could cause disease through similar chemical processes. In England at this time Joseph Lister, a surgeon, recognized the importance of Pasteur's experiments and proposed that infections of open wounds were due to microbes found in the air around the patient. It soon became Lister's policy to spray the air around the patient with phenol before surgical operations. The procedure of destroying or removing viable microorganisms from an environment is called *aseptic technique*. Although Lister's procedures dramatically decreased fatalities from surgical wound infections, his results were not totally accepted in the scientific community.

In the late 1800s a German physician named Robert Koch (Fig. 1-5) demonstrated the relationship between microorganisms and infectious disease. Koch studied anthrax, a disease of cattle that can secondarily affect humans. He isolated the organisms from infected cattle in *pure culture* (the cultivation of a single species of microorganism) and then injected an aliquot of the pure culture into healthy animals, which subsequently became infected. The infectious agent was later isolated from these infected animals. This sequence of isolation, reinfection, and recovery of the infective agent is called *Koch's postulates*. By following Koch's postulates it was now possible to establish the causative agent of many infectious diseases. Although numerous scientists at that time were busily engaged in isolating and characterizing microorganisms, men like Koch and Pasteur were also interested in developing techniques that would be effective in destroying bacteria and thus reduce human misery.

Fig. 1-5. Robert Koch (1843–1910). (From K. L. Burdon and R. P. Williams, *Microbiology* [6th ed.]. New York: Macmillan, 1968. Copyright © 1968 by Macmillan Publishing Company.)

THE GOLDEN AGE OF MICROBIOLOGY (1870–1890)

Isolation Techniques

It was in the Koch-Pasteur era (1870–1890) that tremendous strides were made in microbiology. It was during this era, called the *Golden Age of Microbiology*, that the microbial agents responsible for so many of the fatal diseases of that time were isolated (Table 1-2). As you can see from Table 1-2, all of these diseases are caused by bacterial agents. During this period viruses had not been discovered and little was known about fungi or animal parasites as infectious agents. Perhaps one of the most important technical advances during this period was the discovery that *agar-agar* could be used with microbiological media for isolating microorganisms in pure culture. Agar-agar is a carbohydrate isolated from seaweed that is stable to heat, enzyme-stable, solid, transparent, and easily sterilized. Frau Hesse, the wife of a physician interested in the bacteriology of air, had been using agar-agar in her kitchen to prepare fruits and jellies. She suggested that agar-agar might be used in culture media. Dr. Hesse mentioned his wife's suggestion to Robert Koch, who adapted the new medium in his laboratory. The use of agar-agar in media made possible the isolation of single colonies on a solid medium, a technique that could not be accomplished with liquid media. This discovery, coupled with the design of the *Petri dish* (Fig. 1-6) in 1887 by J. R. Petri as a container for media, provided bacteriologists with a new tool for isolating bacteria in pure culture. Soon other microbiologists, using Koch's postulates and aided by these new isolation techniques, would

Table 1-2. Disease-producing Bacteria Discovered During the Golden Age of Microbiology (1870–1890)

Year	Disease	Causative agent	Researcher(s)
1872	Anthrax	*Bacillus anthracis*	Rayer and Devaine, Pasteur and Koch
1873	Relapsing fever	*Borrelia recurrentis*	Obermeier
1874	Leprosy	*Mycobacterium leprae*	Hansen
1879	Gonorrhea	*Neisseria gonorrhoeae*	Neisser
1880	Pneumonia	*Diplococcus pneumoniae*	Pasteur, Sternberg
1880	Abscesses	*Staphylococcus aureus*	Pasteur, Ogston, Rosenbach
1882	Tuberculosis	*Mycobacterium tuberculosis*	Koch
1883	Cholera	*Vibrio cholerae*	Koch
1884	Diphtheria	*Corynebacterium diphtheriae*	Klebs, Loeffler
1884	Tetanus	*Clostridium tetani*	Nicolaier, Kitasato
1885	Food-borne illness, paratyphoid	*Salmonella choleraesuis* and related species	Salmon and Smith, Gärtner, Schottmüller
1887	Epidemic meningitis	*Neisseria meningitidis*	Weichselbaum
1887	Brucellosis	*Brucella melitensis* and other species	Bruce, Bang

make major contributions to an understanding of the microbial agents causing disease.

Staining

Visualization of microorganisms was enhanced by the discovery of various staining agents. Weigert in 1878 was the first to stain bacteria using various aniline dyes. Further refinement in staining led to Gram's stain (1884), which can be used for most bacterial species, and the Ziehl-Neelsen stain, which is used for staining the organism causing tuberculosis. Loeffler utilized methylene blue stain to identify the organism causing diphtheria.

Immunology

It had been recognized for more than 2000 years that individuals who recovered from some diseases could not "catch" the disease a second time. In 1796 Edward Jenner discovered that milkmaids infected with the mild variety of pox called *cowpox* were immune to the more severe form of the disease, *smallpox*. Jenner inoculated fluid from a cowpox pustule into a healthy boy and later infected the same boy with smallpox fluid. The boy did not contract smallpox.

In 1879 Pasteur, while studying cholera in chickens, noted that if chicken cholera bacteria were left on laboratory media for extended periods of time, they lost their virulence (they became *attenuated*). The attenuated bacteria, when injected into healthy chickens, not only failed to cause cholera but protected them from infection by fresh virulent strains. These experiments eventually led to our present-day vaccination techniques and methods of immunization. Pasteur later developed immunization procedures in the treatment of anthrax in animals and rabies in humans. Today all of us are aware of vaccination procedures used against such diseases as tetanus, diphtheria, polio, and whooping cough.

Elie Metchnikoff, working in Pasteur's laboratory, observed that certain white blood cells could ingest microorganisms or other small foreign matter. Metchnikoff firmly believed that immunity to infection was totally dependent on the special white blood cells (phagocytic cells) that digested other cells. Paul Ehrlich, an associate of Robert Koch, was also interested in resistance to infection. He believed that immunity to infection was due to certain soluble substances in the