

BASIC MEDICAL MICROBIOLOGY

SECOND EDITION ROBERT F. BOYD, Ph.D., AND BRYAN G. HOERL, Ph.D.

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Second Edition

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PREFACE

We wrote this book with the purpose of making it particularly useful for students; that is, we not only cite facts, but give the explanation behind the facts. We present a balanced coverage of the important areas of medical microbiology suitable for any student planning a health science career and for liberal arts students with an interest in medical microbiology.

The first chapters cover basic bacteriology: cytology, growth and reproduction, laboratory identification procedures, physiology, and genetics. Although there is a wealth of new information in the field of molecular biology, we have opted not to explain these concepts in biochemical detail. Instead, we discuss the molecular concepts in general terms, emphasizing their application to medical science. The chapters that follow—on immunology, bacterial disease, virology, mycology, and parasitology—do not require a broad background in molecular biology or biochemistry.

Much of medical microbiology depends on the ability to separate and identify microorganisms on the basis of their individual characteristics. These characteristics do not readily emerge from an organ-systems organization. In this text the chapters that deal with infectious agents are organized according to the individual agents rather than according to the organs and systems affected.

Four topics not found in most medical microbiology textbooks are included here. Chapter 17, an introduction to the bacterial diseases, prepares the student to understand why the species of bacteria are described the way they are and to understand the language and the organization of those descriptions. Chapter 32, Oral Microbiology, discusses the current theories of caries and periodontal disease as well as the characteristics of oral microorganisms. It provides the scientific background for the practice of preventive dentistry, particularly as it relates to understanding the nature and control of dental plaque. Chapter 33 describes the reasons for the incidence of hospital-acquired infections, the infective agents involved, and how they can be eliminated or reduced in the hospital setting. The increased use of antibiotics and immunosuppressive drugs, the development of new surgical techniques including transplantation, and the use of more diagnostic procedures in the hospital, have made hospital-acquired infections a constant threat to patients and hospital personnel alike. Chapter 34, on anaerobic infections, discusses the major diseases caused by nonsporeforming anaerobes. The advent of improved isolation techniques has made it possible to detect a much wider prevalence of infections by nonsporeforming anaerobes than had been appreciated previously.

In this second edition, topics have been updated; many new and better quality illustrations have been added; parts of the text have been strengthened through

Preface

rephrasing, expansion, or elimination; the selected readings have been supplemented; and more terms have been added to the glossary. Nearly all chapters have been revised to accommodate new information. Because of the increasing importance of the topics or because of recent research developments the following chapters have been considerably enlarged: Chapter 9, Storage and Transfer of Genetic Information; Chapter 12, Interaction Between Host and Infectious Agents; Chapter 22, The Gram-Negative Enteric Bacilli and Related Organisms; Chapter 28, Viruses; Chapter 29, Selected Viral Diseases; Chapter 30, The Pathogenic Fungi; and Chapter 32, Oral Microbiology.

The users of the first edition have given us helpful comments and suggestions. We hope that in responding to these suggestions and through the changes described above we have retained, indeed have increased, the readability of the book—a quality that was considered a primary strength of the first edition.

Writing and publishing a book involve many people, and it sometimes seems unfair that the authors receive so much credit. We would like to give a special word of appreciation to the authors and publishers who have permitted the use of their illustrations and tables. We acknowledge our indebtedness to the administrators of Marquette University School of Dentistry for their interest and encouragement, to our illustrator Mary Anne Komorowski, to Beverly Boyd, who typed much of the manuscript, and to the staff members of Little, Brown and Company (especially Barbara Ward, Carol Snarey, and Cynthia Baron), for their capable and patient guidance.

R. F. B.

B. G. H.

CONTENTS

PREFACE ix

I. GENERAL BACTERIOLOGY

1. HISTORY, SCOPE, AND CLASSIFICATION OF BACTERIA 3
2. CYTOLOGY 11
3. NUTRITION 29
4. GROWTH AND REPRODUCTION 37
5. LABORATORY CULTIVATION, ISOLATION, AND IDENTIFICATION 49
6. ENZYMES 73
7. ENERGY METABOLISM 81
8. BIOSYNTHESIS AND METABOLIC CONTROL 97
9. STORAGE AND TRANSFER OF GENETIC INFORMATION 109

II. CONTROL OF MICROORGANISMS

10. STERILIZATION AND DISINFECTION 133
11. CHEMOTHERAPY 169

III. HOST-PARASITE INTERACTION

12. INTERACTION BETWEEN HOST AND INFECTIOUS AGENTS 193

IV. IMMUNOLOGY

13. NONSPECIFIC HOST RESISTANCE FACTORS 215
14. ACQUIRED IMMUNITY 227
15. IN VITRO ANTIGEN-ANTIBODY REACTIONS 257
16. IMMUNOLOGICAL DISORDERS 275

V. BACTERIA THAT CAUSE INFECTIOUS DISEASE

17. INTRODUCTION TO THE BACTERIAL DISEASES 309
18. THE GRAM-POSITIVE COCCI 317
19. THE GRAM-NEGATIVE DIPLOCOCCI: THE *NEISSERIA* 345
20. THE GRAM-POSITIVE SPOREFORMERS: THE BACILLI AND CLOSTRIDIA 359
21. *CORYNEBACTERIUM DIPHTHERIAE* 373

Contents

- 22. THE GRAM-NEGATIVE ENTERIC BACILLI AND RELATED ORGANISMS 381
- 23. THE GRAM-NEGATIVE COCCOBACILLARY AEROBIC BACTERIA 411
- 24. MYCOBACTERIA 423
- 25. THE SPIROCHETES 433
- 26. MYCOPLASMAS AND L-FORMS 449
- 27. RICKETTSIAE AND CHLAMYDIAE 457
- ADDENDUM: LEGIONNAIRES' DISEASE 469

VI. VIROLOGY

- 28. VIRUSES 475
- 29. SELECTED VIRAL DISEASES 515

VII. MEDICAL MYCOLOGY

- 30. THE PATHOGENIC FUNGI 581

VIII. MEDICAL PARASITOLOGY

- 31. THE PROTOZOA, HELMINTHS, AND ARTHROPODS 617

IX. SELECTED TOPICS

- 32. ORAL MICROBIOLOGY 661
- 33. HOSPITAL-ACQUIRED INFECTIONS 687
- 34. INFECTIONS CAUSED BY NONSPOREFORMING ANAEROBES 701

GLOSSARY 717

INDEX 735

PART I

CHAPTER 1

GENERAL BACTERIOLOGY

HISTORY, SCOPE, AND CLASSIFICATION OF BACTERIA

DIVISION
PART NUM
FAMILY
GENUS
SPECIES

HISTORY

GERM THEORY OF DISEASE

In some of the ancient civilizations before the birth of Christ disease was believed to be a punishment sent from the gods for certain human indiscretions. 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.

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 man and other animals. Leeuwenhoek was the first to describe microscopic organisms found in pond water and later made observations on bacteria he found in the debris surrounding teeth. It took two centuries, however, for the germ theory of disease to be placed on sound scientific principles. Plenciz, a Viennese physician, in 1762 proposed that infectious disease was spread through the air by "contagious animalcules." Not until 1836 was disease transmission first observed in silkworms. Fungal infections of silkworms could be transmitted from infected worms to healthy ones.

Lack of public lavatories, improper drainage of sewage, and use of streams as sources of drinking water as well as receptacles for refuse were responsible for many epidemics such as cholera and plague. In 1854 John Snow noted during a cholera epidemic that water contaminated with human feces could cause cholera. At about the same time a few physicians in both Europe and America concluded that doctors who handled patients in one ward of the hospital were sources of infection to patients in other wards. It was judged that physicians should wash their hands in chloride of lime before handling patients. The practice was not accepted by the majority of doctors, who refused to believe they were unclean.

In 1857 Louis Pasteur, a French chemist, formulated the theory of fermentation by microorganisms and demonstrated how microorganisms can convert sugar into lactic acid. From these experiments Pasteur theorized that microorganisms could cause disease through similar chemical processes. In England 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. Although this proce-

dures dramatically decreased fatalities from surgical wound infections, Lister's results were not appreciated or accepted.

Before proceeding farther into the development of the germ theory of disease, we must point out the significance of Pasteur's early experiments concerning the origin of microscopic life. They were important because the establishment of microbiology as an independent science hinged on their success. Until the time of Pasteur many people believed that microscopic forms of life were generated spontaneously from nonliving organic material. It had been routinely observed by proponents of this theory that broth, if left in an open vessel for 24 hours, soon developed a cloudy appearance, which was found to be caused by microorganisms. Pasteur believed, however, that microorganisms were present on the dust particles in the air. When the microbe-laden particles fell into the broth, they rapidly reproduced, thereby giving rise to the cloudiness. In order to prove his hypothesis, Pasteur designed an experiment in which the broth in the vessel was heated to destroy any living microbes that might be present. The mouth of the vessel was plugged with cotton. Any microbes on the dust particles in the air would be trapped in the cotton plug, leaving the broth free of contamination. After several days at room temperature the broth was observed to be free of contaminating microorganisms. This and other experiments proved beyond all doubt that microorganisms do not arise spontaneously but originate by a process similar to that in other living organisms.

In the late 1800s a German physician named Robert Koch demonstrated the relationship of microorganisms to infectious disease. Koch studied anthrax, a disease of cattle that can secondarily affect man. He isolated the organisms from infected cattle in pure culture; that is, the culture contained only anthrax organisms and no contaminating microbial species. Koch 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 etiology (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 would thus reduce human misery.

CONTROL OF INFECTIOUS AGENTS

Immunity

It had been recognized for more than 2000 years that individuals who recovered from disease 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 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 failed to produce the disease cholera but did protect 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 typhoid.

Chemotherapy

In the late 1800s Paul Ehrlich, a German chemist, noted that dyes were selectively absorbed in some cell types and not in others. His observations led him to believe that certain chemicals taken into the body could selectively destroy bacteria and not affect normal body cells, a process he called "chemotherapy." Ehrlich began a systematic search for a chemical that could be used against the microorganism that caused syphilis. After 606 trials an arsenical compound, which Ehrlich called "606," was found to be effective in the treatment of syphilis. Until the discovery of penicillin, 606, or salvarsan, remained the major chemotherapeutic agent in the treatment of syphilis. Following Ehrlich's success many scientists began to test thousands of chemicals for antibacterial activity. In 1932, after years of research, Gerhard Domagk, a German chemist, prepared and tested a large number of dyes and discovered that the red dye Prontosil was highly effective in the treatment of numerous bacterial diseases. It was later discovered that in the body Prontosil was converted to a colorless derivative, called *sulfanilamide*, that was the active antibacterial component.

It had been known for several years before the turn of the century that certain bacteria and molds were capable of producing substances that inhibited the growth or killed various types of microorganisms. In 1928 Sir Alexander Fleming discovered that a particular culture of *Staphylococcus* on an agar plate had become contaminated with mold from the air. Some of the colonies around one mold had stopped growing. The mold, later found to be *Penicillium notatum*, produced an antibacterial substance which Fleming called *penicillin*. In 1940 Chain and other scientists in Florey's laboratory in Oxford purified penicillin from culture fluids and demonstrated its potency. In 1942 penicillin was ready for injection into human subjects.

Selman Waksman, a Russian immigrant to the United States, during his undergraduate studies was interested in the types of microorganisms found in the soil. His work with a group of soil fungi called *actinomycetes* led to the discovery of the antibiotic actinomycin in 1940 and streptomycin in 1943. Streptomycin was soon shown to destroy bacteria not affected by penicillin, especially the tuberculosis bacillus. The experimentation and discoveries of Fleming and Waksman prompted more extensive search for other antibiotics. After World War II, a wider range of antibiotics was discovered including tetracycline, chloramphenicol, and erythromycin.

SCOPE

Microbiology is a science that deals with the study of microorganisms and viruses. Such study includes an understanding of the structure, physiology,

metabolism, classification, and genetics of microorganisms. In addition, the student of microbiology must have an insight into the relationship of microorganisms to their environment, other microorganisms, and man.

Even though all infectious agents have one thing in common—i.e., being small—there is a tremendous diversity in their individual members. Microorganisms can be divided into representative groups based on differences in biological properties. These groups include the bacteria, yeasts and molds, and protozoa. Viruses, because they are intracellular parasites, are given separate status. The explosion of microbiological information in the past 20 years has been so great that it has resulted in the creation of specialties within each of the representative groups of microorganisms. For example, the bacteriologist may develop special 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 appear in certain environments; there are now divisions of applied microbiology such as space microbiology, soil microbiology, and food microbiology.

Medical microbiology, which is the subject of this book, explores the detrimental effects of microorganisms on people and animals in the disease process. Since various representative types of microorganisms can be pathogenic to humans, the medical microbiologist must have a basic knowledge of the chemical and physical properties of all pathogenic microbial agents—bacteria, viruses, yeasts, molds, and protozoa. The primary responsibility of the medical microbiologist is the understanding of the etiology (causation), pathogenicity (disease manifestation), laboratory diagnosis, and treatment of infectious agents. His obligations may also include determining the epidemiology of disease and developing measures for the control and prevention of infections in the community. The fact that many major pestilences like 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. Human resistance to disease or immunity to infection 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 provided the microbiologist with new laboratory diagnostic tools. Thus 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 caused the microorganism to become a useful model for the study of biological properties, particularly in the areas of genetics and metabolism. The studies were 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. 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 DNA (cell free) was capable of transforming 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 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. The analysis of the chemical makeup of biological material and its relationship to genetic information is the foundation of the science of molecular biology. With the aid of microorganisms as experimental "guinea pigs" a broader understanding of all life processes is being developed.

CLASSIFICATION

Until the latter part of the nineteenth century the biological world was classified into two kingdoms: plant and animal. With the advent of microbiology as a science, there has been an ever-increasing accumulation of information concerning the morphological and physiological properties of microorganisms. It was soon realized that most microorganisms did not fit into either the plant or the animal kingdom. Many microorganisms were endowed with properties that were not present in either plant or animal cells whereas others resembled either one or both cell types. In the late 1900s a third kingdom was proposed to include all the microorganisms—protozoa, fungi, algae, and bacteria. The kingdom was called the *Protista*. Further probing into the microbiological world revealed that even among the protists there existed two basic cell types: the eukaryotic and prokaryotic cells. Eukaryotic organisms include the unicellular algae, protozoa, and fungi as well as all higher plants and animals while the prokaryotic cells include the bacteria and blue-green algae. Some of the principal differences between these two groups are given in Table 1-1.

Although prokaryotes and eukaryotes are believed to have descended from some common ancestral form of life, recent evidence by Carl Woese and George Fox indicates that a third group may have branched off independently from the two major divisions. Called the *methanogens*, members of this group inhabit the intestinal tract of humans and animals and are commonly found in water sediments. Evidence for their possible independent branching is suggested by the unrelatedness of certain bacterial cell wall components as well as the existence of enzymes not found in other microorganisms.

Bacteria are named according to the binomial system of nomenclature devised by Linnaeus in 1753. This system uses the genus and species as the major taxonomic groups. Each distinct kind of bacterium is recognized as a species. Following the binomial system of classification each species is therefore given a two-word name. The first word is the *genus*; the second, the *species*. Only the genus name is capitalized. Often the genus and species names tell a great deal about the organism. For example, with the species *Staphylococcus aureus*, "staphylo" describes the morphology of the cell (i.e., cocci in clusters) and "aureus" indicates that the organism produces a gold pigment. In most texts the

Table 1-1. Differences Between Prokaryotic and Eukaryotic Cells

Cellular Characteristic	Prokaryotic Cell	Eukaryotic Cell
Nuclear membrane	Absent	Present
Chromosome number	1	More than 1
Mitotic division	Absent	Present
Mitochondria	Absent	Present
Golgi apparatus	Absent	Present
Nature of cytoplasmic ribosomes	70S ^a	80S
Ameboid movement	Absent	Present or absent
Vacuoles for storage of macromolecules	Absent	Present

^a S refers to Svedberg units, which are relative indicators of the rate of sedimentation of cellular components or molecules during centrifugation.

scientific name of the organism (set off by italics or underlining) is initially spelled out and later abbreviated; for example, *Staphylococcus aureus* becomes *S. aureus*.

The taxonomic approach to bacterial classification is illustrated in *Bergey's Manual of Determinative Bacteriology*. In earlier editions a bacterium was classified according to kingdom, phylum, class, order, family, genus, and species, just as is done for plants and animals. In the recent eighth edition of *Bergey's Manual* the authors propose that many of the categories in such a classification scheme are cumbersome and of little value to the student trying to identify a microorganism. They now recognize the kingdom Prokaryotae, which has two divisions: the Cyanobacteria (blue-green bacteria and algae) and the Bacteria. Microorganisms are now placed into descriptive groups—gram-positive cocci, gram-negative anaerobic cocci, gram-negative facultative rods, etc. Within these groups are families, genera, and species. Genus and species are still considered the most important taxonomic groups. Each genus and species has several characteristics that provide for its separation from other genera and species. Some of the more definitive characteristics that provide a means of identification and separation of bacteria are morphology, Gram-staining reactions, spore formation, flagellation, and a variety of biochemical and physiological properties. Table 1-2 presents the new and old systems of classification of *Streptococcus pyogenes*.

The principal basis for determining species among most organisms is the ability to interbreed. Most microorganisms do not interbreed, and often they share a number of characteristics. It is therefore more difficult to determine species and their degree of relatedness to other species. For this reason alternative methods—genetic classification, numerical taxonomy—and other techniques are being explored.

GENETIC CLASSIFICATION

Recent advances in molecular biology have provided bacteriologists with information useful in revealing new relationships between microorganisms. For example, attempts are now being made to determine the relatedness between

Table 1-2. Comparison of New and Old Classification of a Bacterial Species: *Streptococcus pyogenes*

Old Classification		New Classification	
Kingdom	Protista	Kingdom	Prokaryotae
Phylum	Protophyta	Division	Bacteria
Class	Schizomycetes	Part number	14. Gram-positive cocci
Order	Eubacteriales	Family	Streptococcaceae
Family	Lactobacillaceae	Genus	<i>Streptococcus</i>
Tribe	Streptococceae	Species	<i>pyogenes</i>
Genus	<i>Streptococcus</i>		
Species	<i>pyogenes</i>		

microorganisms based on similarities in their genetic material. One type of experiment compares the relative percentage of guanine and cytosine bases in the DNA of two species. The base composition of a single species theoretically is an invariable, fixed property and thus affords a measure of species relatedness. A second type of genetic experiment involves the procedure referred to as *molecular hybridization*. The experiment is based on the premise that if two species are similar or related a major portion of the nucleotide sequences in their DNA will also be similar. The experiment measures the ability of the DNA strands from one organism to hybridize (i.e., bind through complementary base pairing) with the DNA strands of the second microorganism. The degree of hybridization is therefore a relative measure of the degree of relatedness between the two microorganisms.

NUMERICAL TAXONOMY

Electronic computers are now being used to analyze all the chemical and physical properties of microorganisms. This approach, called the *adansonian analysis*, is more commonly referred to as *numerical taxonomy*. Based on the known information of a large number of microbial properties, it divides microorganisms into groups. The percentage of similarities for selected properties as well as dissimilarities is calculated. The percentage of similarities is then used to determine the relatedness of various species. Morphology, staining, flagellation, and biochemical properties, for instance, are assigned numerical values. As many as 100 different microbial features can be evaluated and fed into the computer. This approach has proved tedious, and its use is limited because of the lack of uniform "weighting" of the characteristics.

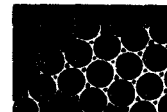
OTHER CLASSIFICATION TECHNIQUES

As technology continues to improve, particularly in microscopy and biochemical techniques, new approaches to species classification are being explored. Ribosomal RNA, for example, has been shown to be a relatively stable molecule with a high resistance to mutation. Thus base sequencing of this molecule has been used for establishing species relatedness. Several proteins such as cytochromes are common to microbial species and they too are being examined to determine similarities between species. The latter approach, however, is still

in its infancy since accurate and rapid techniques for amino acid sequencing have not yet been developed.

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Considering the extremes of environmental stresses that are placed upon the living cell, such as temperature, radiation, and pressure, one wonders what keeps an organism alive. Why can one bacterial cell survive extremes of temperature while another dies if the temperature varies slightly? Why can one group of bacteria multiply in the presence of an antibiotic while another lyses rapidly? Many of the answers lie in the molecular and structural architecture of the various components of the bacterial cell. In this chapter we discuss the structures of the bacterial cell and what makes them so extraordinary in terms of their function, protective value, and relationship to disease.

EUKARYOTIC VS. PROKARYOTIC CELLS

As stated in Chapter 1, the original kingdom, Protista, later called Prokaryotae, was divided into two subgroups based on structural differences: eukaryotic and prokaryotic cells. These structural differences were described in Table 1-1 and are illustrated in Figure 2-1.

THE PROKARYOTIC CELL (BACTERIA) SIZE, SHAPE, AND CHEMICAL COMPOSITION

Bacteria range in length from 0.5 to 40 μm (1 μm = 10^{-6} meters). For most bacteria the average length is between 1 and 5 μm . The length of a bacterial cell can vary considerably depending on the stage of growth and the medium used to cultivate the organism. Size, therefore, is not a useful parameter for the identification of similar groups of microorganisms.

Bacteria can be grouped according to three basic shapes (Fig. 2-2A): spherical (coccus), rod (bacillus), and helical (spirillum). After cell division many bacterial cells do not completely separate from each other and give rise to a number of arrangements. This is especially the case in the cocci and less so in the bacilli. Depending on the plane of division, five types of coccal arrangements can be produced: diplococci, streptococci, staphylococci, tetrad, and sarcinae (Fig. 2-2B).

Some bacilli are so small that they appear coccal in shape. They are referred to as *coccobacillary forms*. In addition, many bacillary forms are *pleomorphic*; i.e., they can exist in different morphological forms. For example, when microscopically examining a smear of *Hemophilus influenzae*, one notes that the typical coccobacillary form predominates, but filamentous forms occasionally exist. The reason for these variations is not understood. Sometimes pleomorphism is useful in the identification of a microorganism.

The chemical composition of bacterial cells is influenced by the state of growth and the environmental conditions under which the cells are cultured. For example, cells during optimal growth may have a protein content reaching

Figure 2-1. The structural differences between eukaryotic and prokaryotic cells.

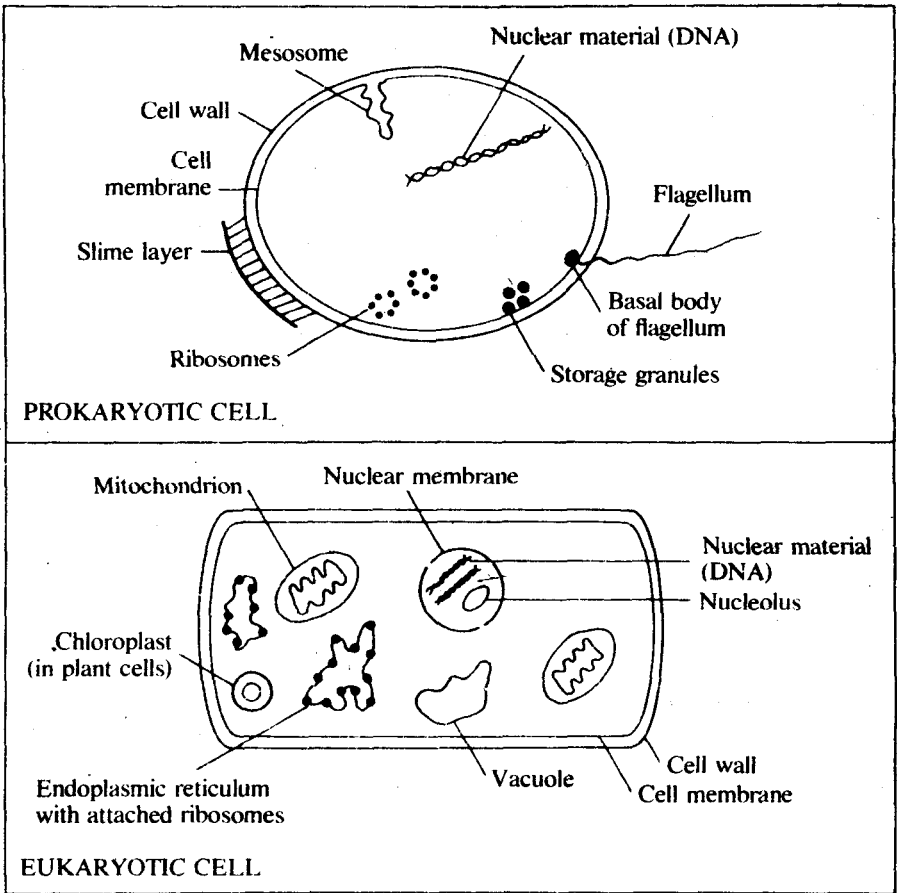


Figure 2-2. Bacterial morphology. A. Various shapes of bacteria. B. Various arrangements of the cocci.

