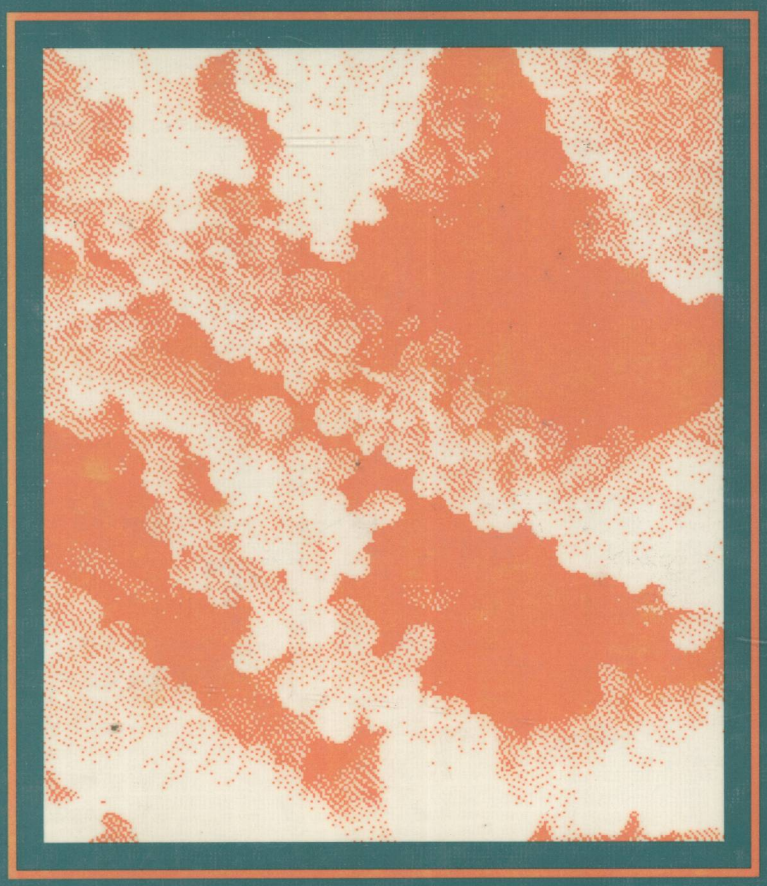
Essential Dental MICROBIOLOGY



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Essential Dental MICROBIOLOGY

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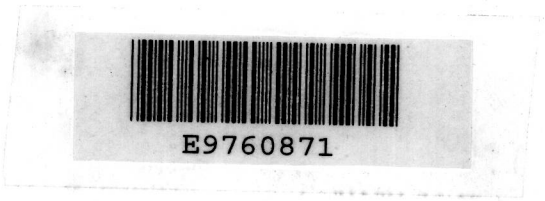

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Preface

The complex interactions of the microbial flora that frequent the body and the impact of these organisms on oral medicine make it imperative that dental practitioners understand the significance of microbiology to themselves and to their patients. It has been the editors' purpose to produce a book that presents this science in a way that will facilitate dental students' mastery of this knowledge and enhance their ability to apply it to dental practice.

Although many factors contributed to the genesis of this text, perhaps the most fundamental was the exponential increase in new information not only in microbiology, but also in other fields such as molecular biology and immunology. Diseases such as AIDS, Lyme disease, and Hepatitis C, previously unknown or undetected, have emerged rapidly. The etiologic agents of some such as human immunodeficiency viruses have had a profound effect on the practice of dentistry. In addition, the application of new technology such as genetic engineering and monoclonal antibodies have contributed to the information explosion in medical and dental microbiology.

It has become apparent that it is no longer practical to present a broad nonselective course, but rather, in order to best use the limited time available to dental students, all instructors must be more discriminating in their coverage of their respective fields; they must concentrate on the essential facts and concepts needed to produce a well-rounded, competent, dental professional. To this end, many of the authors initially collaborated in developing a document describing the essential material for dental school microbiology courses. This was circulated for review by the dental school microbiology community throughout the United States and Canada, and based on the responses, was modified to produce a core curriculum for a dental school microbiology course. Subsequently this document was used by the Microbiology Section of the American Association of Dental Schools as the basis for writing the Curricular Guidelines for Microbiology (*J Dent Ed.* 1984;84:109.). The knowledge and experience gained from these efforts led to this textbook, which parallels these documents in scope, but has been expanded and updated as additional information and concepts critical to the education of future dental practitioners became apparent.

A factor of equal significance to the development of this text was the need to hold the dental students' interest. Therefore, we have included and emphasized oral and/or dental aspects throughout the text, beginning with the basic microbiology chapters and including the immunology and applied medical microbiology chapters. Oral applications are cited throughout each chapter, and most chapters include a special section on oral significance to emphasize or summarize the material of dental interest. The essential material in each chapter is supplemented by suggested contemporary readings for more advanced students or those who wish to pursue a topic in more detail.

The editors wish to express their thanks to the contributing authors, to the various editors at Appleton & Lange who encouraged us in what seemed a never-ending task, and to all of the reviewers who provided constructive and helpful suggestions.

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Robert R. White, PhD

Samuel Rosen, PhD

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CHAPTER 1

Bacterial Structure

Thomas T. Lillich

INTRODUCTION

BACTERIAL SIZE AND SHAPE

BACTERIOLOGIC METHODS
- **Staining Techniques**
- **Brightfield Microscopy**
- **Darkfield Microscopy**
- **Phase Contrast Microscopy**
- **Electron Microscopy**

BACTERIAL ULTRASTRUCTURE
- **Bacterial Cell Surfaces**
- **Bacterial Cell Envelope**
- **Bacterial Cytoplasm**

ORAL ASPECTS

SUGGESTED READINGS

INTRODUCTION

Bacteria are a ubiquitous, nutritionally and environmentally diverse group of microscopic, unicellular prokaryotic organisms. They are members of the kingdom Protista, which also includes algae, fungi, protozoa, and viruses. There are two major subdivisions of the Protista, prokaryotes and eukaryotes, based primarily on the presence or absence of a nuclear membrane. Bacteria are prokaryotes and do not have a nuclear membrane. The eukaryotes, which include algae, fungi, and protozoa, do have a membrane surrounding their genetic material. Viruses are not included in this classification because they are chemically and structurally different from the other four groups of Protista. Bacteria are also different from some eukaryotes in that they are unicellular. They may be found in groups or clusters, but there is no differentiation within the population—no cellular specialization that is characteristic of plant and animal cells. Each bacterial cell retains its autonomy, that is, its ability to metabolize, grow, and reproduce apart from the other cells. Animal and plant cells, on the other hand, survive only as part of the larger, multicellular organism.

Classification of bacteria makes it possible to identify the etiologic agents of infectious diseases. Taxonomy is the means of classifying microbes. The species is the basic unit of classification. A bacterial species is a group of organisms that share a set of characteristics. Some of the characteristics used to determine relatedness among bacteria are shown in Table 1–1. Phenotypic analysis includes microscopic examination of individual bacteria or their colonies and the study of their metabolic traits. A thorough genetic analysis is essential for accurate classification because the sequence and arrangement of nucleotide base-pairs on the bacterial chromosome determine all other cellular characteristics. Additional information can be obtained from analysis of bacterial metabolic requirements and capabilities and from serologic studies that reveal similarities in the surface structures of bacteria that bind the same immunoglobulin molecules.

The most important aids to bacterial classification are the genetic and biochemical characteristics of the microorganisms. As new techniques and tests become available, the classification of some bacteria changes. For example, when they were first isolated in 1921, all black-pigmented *Bacteroides* were included in the same genus, *Bacteroides melaninogenicus*. Since

TABLE 1–1. BACTERIAL CHARACTERISTICS USED IN TAXONOMY

Phenotype
DNA analysis
Guanine + cytosine content (%)
DNA–DNA hybridization
Chemical analysis
Lipids
Cell wall polymers
Isoprenoid quinones
Cytochromes
Enzymes
Serology

Adapted from Zambon JJ. Bacterial Classification. In: Newman MG, Nisengard R, eds. Oral Microbiology and Immunology. *Philadelphia: WB Saunders; 1988.*

then, more detailed studies using biochemical and molecular biologic techniques have resulted in the division of this genus into six species, several of which are found in the mouth. Numerical taxonomy is an approach to classification that uses information about bacterial phenotype, genotype, metabolism, and serology in a different way. As much information as possible is collected about each organism. Each characteristic is assigned a value, often the same one, and a similarity profile is constructed. Organisms are grouped according to their similarity profiles. Those with 90% or greater similarity in characteristics usually are considered to be the same species.

Bacteria are named according to the binomial system of nomenclature that uses two names with Latin endings to describe a species. The first word indicates the genus, the second the species. Both describe characteristics based on metabolism, shape, color of colony, site from which isolated, or the individual who first described the microbe.

This chapter provides a description of bacteria and those structures that are important in their growth and reproduction. It begins with a general discussion of morphology and cellular arrangement. Brief mention is then made of some of the analytic techniques used to study them. The rest of the chapter is devoted to a description of bacterial structure. Groundwork is laid here for a more detailed treatment of structure–function relationships in subsequent chapters.

BACTERIAL SIZE AND SHAPE

Bacteria are the smallest organisms that have the capability to metabolize, grow, and reproduce independently of other cells. Table 1–2 is a listing of weights and measures used to describe bacteria and their environments.

Bacteria can be divided morphologically into spheres, rods, and spirals (Fig 1–1). Each shape may occur in several variations, as illustrated in Fig 1–2. Spherical bacteria, also called cocci, average about 1.0 μm in diameter, although they can range from 0.4 μm to 2.0 μm. They may exist as single cells or as pairs (diplococci), chains (streptococci), clusters (staphylococci), or tetrads (sarcinae), which result from incomplete separation of daughter cells that arise by division of the mother cell in one or more planes. Bacterial rods, or bacilli, usually have the same diameter as cocci but range in length from 2 μm to 20 μm. Bacilli not much longer than they are wide are called coccobacilli. Those that grow very long are filamentous forms. The ends of bacilli may be either tapered or blunt. Rod-shaped bacteria also may be curved, either gently into a comma or more severely into a helix that appears to have several

TABLE 1–2. WEIGHTS AND MEASURES

Unit	Symbol	Conversion				
		nm	μm	mm	cm	m
Nanometer (millimicron)[a]	nm (mμ)	1	10^{-3}	10^{-6}	10^{-7}	10^{-9}
Micrometer (micron)	μm (μ)	10^{2}	1	10^{-3}	10^{-4}	10^{-6}
Millimeter	mm	10^{6}	10^{3}	1	10^{-1}	10^{-3}
Centimeter	cm	10^{7}	10^{4}	10	1	10^{-2}
Meter	m	10^{9}	10^{6}	10^{3}	10^{2}	1
		ng	**μg**	**mg**	**g**	**kg**
Nanogram	ng	1	10^{-3}	10^{-6}	10^{-9}	10^{-12}
Microgram (gamma)	μg (γ)	10^{3}	1	10^{-3}	10^{-6}	10^{-9}
Milligram	mg	10^{6}	10^{3}	1	10^{-3}	10^{-6}
Gram	g	10^{9}	10^{6}	10^{3}	1	10^{-3}
Kilogram	kg	10^{12}	10^{9}	10^{6}	10^{3}	1

[a]Parentheses indicate former designation.

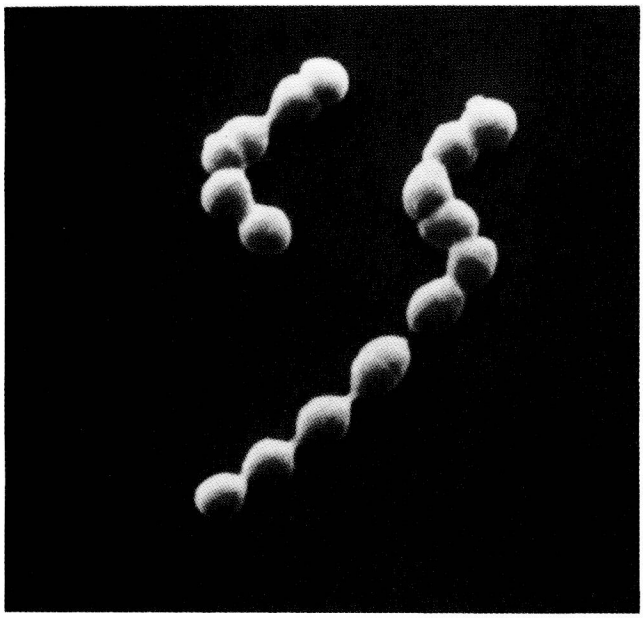
sinusoidal waves when viewed through a microscope. The shape and arrangement of bacteria in clinical specimens are important aids in the diagnosis and treatment of infectious diseases.

BACTERIOLOGIC METHODS

There is a variety of methods used to study bacteria. Although many sophisticated techniques are finding their way into diagnostic laboratories, one of the most useful aids in the diagnosis and treatment of bacterial diseases remains visual observation of living or heat-fixed, stained specimens with a light microscope.

Staining Techniques

One of the most useful techniques was developed by the nineteenth century Danish bacteriologist, Christian Gram. The value of the Gram stain is its ability to differentiate most bacteria into two major groups, gram positive or gram negative, based on their color after staining. This distinction is important diagnostically because gram-positive and gram-negative bacteria differ in their susceptibility to antibiotics. The staining procedure consists of the following steps carried out on a specimen that has been air dried and heat fixed to a clean microscope slide: (1) the specimen is stained with a crystal violet solution, (2) the slide is flooded with Gram's iodine solution, which



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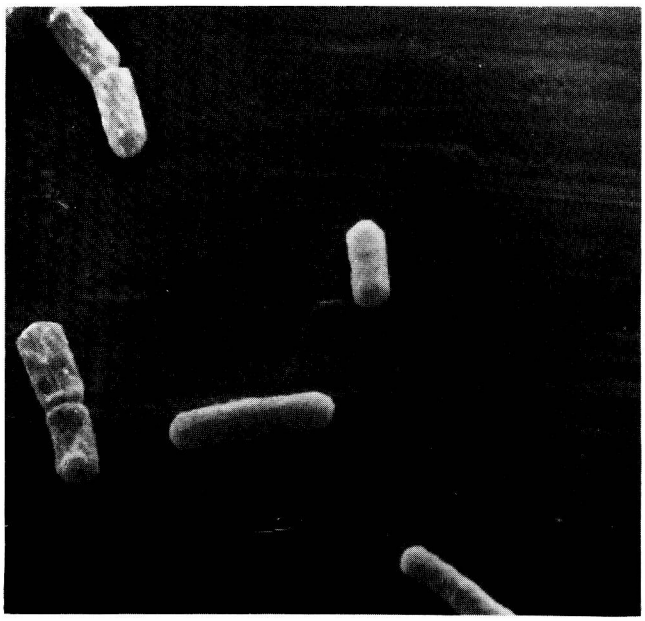

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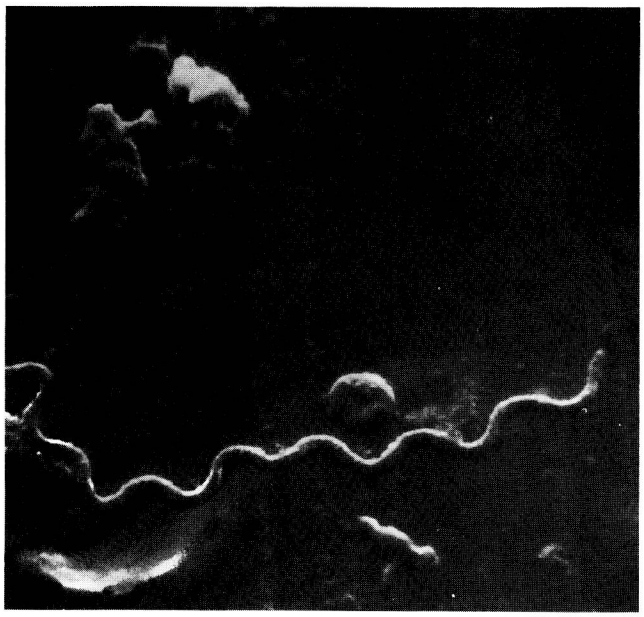

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Figure 1–1. Scanning electron photomicrographs of the three basic morphologic types of bacteria. A. Cocci. B. Bacilli. C. Spirals. *(From Klainer AS, Geis I.* Agents of Bacterial Disease. *Hagerstown, MD: Harper and Row; 1973, with permission.)*

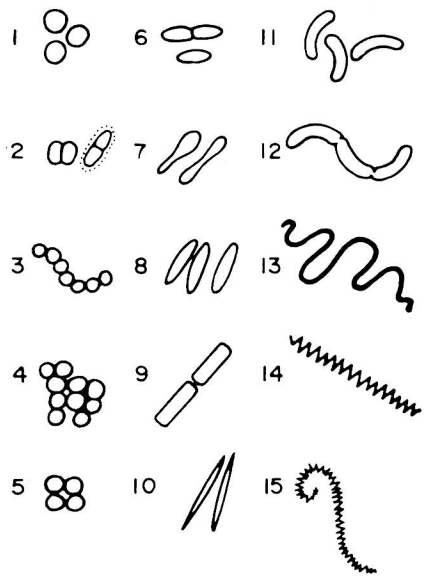

Figure 1–2. Variations in basic bacterial morphology. 1. Single cocci. 2. Pairs of cocci. 3. Chains of cocci. 4. Clusters of cocci. 5. Cocci in tetrads. 6. Coccobacilli. 7. Club-shaped bacilli. 8. Bacilli with rounded ends. 9. Bacilli with square ends. 10. Bacilli with tapered ends (fusiforms). 11. Vibrios. 12. *Spirillum.* 13. *Borrelia.* 14. *Treponema.* 15. *Leptospira. (From Joklik WK, Willett HP, Amos DB, eds.* Zinsser Microbiology, *19th ed. E. Norwalk, CT: Appleton Lange 1984:25.)*

acts as a mordant, (3) the specimen is decolorized with alcohol, and (4) a counterstrain, usually safranin, is applied. In the light microscope, gram-positive cells appear violet, and gram-negative cells are red. The difference in reaction is due to the different permeability of the cell walls to the crystal violet–potassium iodide complex formed during the staining procedure. Gram-positive cell walls are impermeable to the complex and retain the primary crystal violet stain. The complex is readily removed, however, from gram-negative cells by an alcohol wash, rendering them colorless and making possible expression of the counterstain color, in this case the red of safranin.

Acid-fast staining is another common differential staining method. This technique is used to differentiate members of the genera *Mycobacterium* and *Nocardia* from other bacteria. Clinical specimens, often sputum or lung aspirates, are placed on a microscope slide, stained with phenolic carbolfuchsin, decolorized with dilute mineral acid, and counterstained with methylene blue. Bacteria that retain the red primary carbolfuchsin stain after the acid rinse are classified as acid fast. Nonacid-fast organisms are decolorized by acid alcohol and appear blue after counterstaining. Mycobacterial acid fastness is due to the unique cell wall lipid content of members of the genus. The presence of acid-fast organisms in the sputum of an individual is a very important clue in the diagnosis of tuberculosis and other mycobacterial diseases.

There are several other staining techniques used to determine bacterial structure and morphology. Special techniques have been developed to demonstrate bacterial structures, such as flagella, endospores, capsules, and nuclear material.

Brightfield Microscopy

The compound brightfield microscope is a basic tool of microbiology. It is compound because it has three lens systems and brightfield because it uses visible light passing directly through the specimen to the observer's eye. Image magnification up to 1000× is possible using an oil immersion lens to reduce light scatter as it passes from the specimen into the objective lens. Maximum magnification is limited by the ability to distinguish between two objects that are a certain distance apart. This ability is called the resolving power and is a function of the wavelength of light used and the angle at which the light enters the objective lens. Under optimum conditions, using blue light and oil immersion, objects can be resolved if they are no closer than 0.2 μm. Since they are transparent, it is very difficult to observe unstained bacteria with a brightfield microscope because there is so little contrast between the microorganisms and the background. Most specimens are stained before observation using one of the techniques described earlier in this chapter.

Darkfield Microscopy

Because staining a bacterial culture or clinical specimen precludes studying living cells, techniques involving darkfield and phase contrast microscopy have been developed to observe unstained preparations in an aqueous environment. Both techniques are being used by dental practitioners for patient education and to monitor the effectiveness of treatment.

Darkfield microscopes use a method of illumination that makes possible the observation of bacteria and bacterial structures that are too thin to be seen with a brightfield microscope but long enough to have a characteristic shape. This technique is particularly useful for observing spirochetes that have a diameter of 0.1 to 0.15 μm and flagella that are invisible by more conventional brightfield techniques. Darkfield illumination uses a substage condenser that produces an inverted, hollow cone of light that does not pass directly into the objective lens. Only light that is dif-

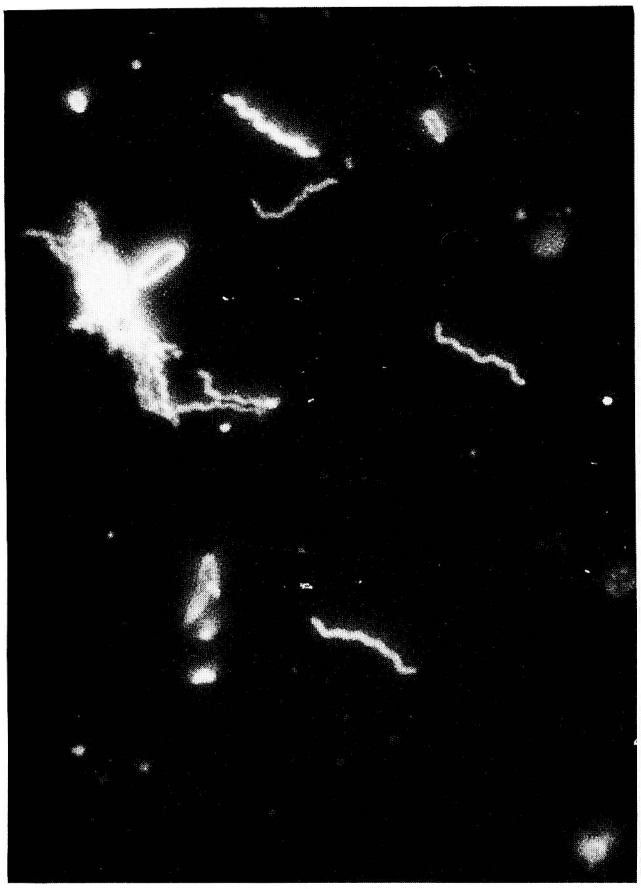

Figure 1–3. Darkfield photomicrograph of gingival debris showing several curved bacteria. ×1000. *(Courtesy of Dr. Max A. Listgarten, University of Pennsylvania School of Dental Medicine.)*

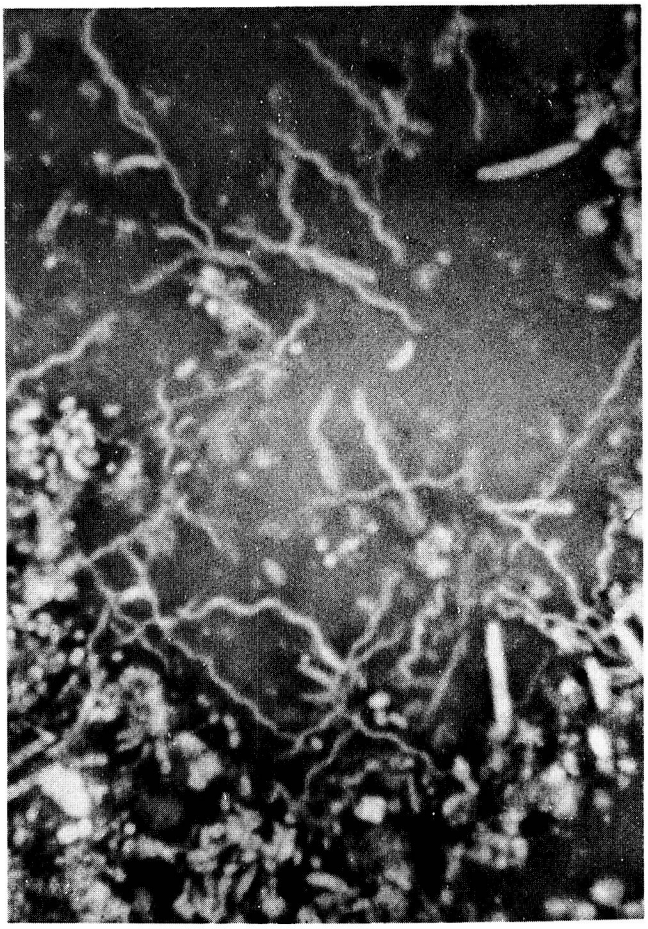

Figure 1–4. Phase contrast photomicrograph showing mixed gingival crevice microflora. ×1000. *(Courtesy of Dr. William Briner, The Procter and Gamble Company.)*

fracted while passing through the specimen reaches the objective. Consequently, bacteria or other particles that deflect the light rays appear as bright white or silver objects against a black background (Fig 1–3). Darkfield microscopy is a rapid, noninvasive method of evaluating the bacteriologic status of oral tissues, particularly the periodontium, where this technique allows visualization of the spirochetes and other delicate gram-negative bacteria that comprise a significant proportion of the flora, especially in diseased sites.

Phase Contrast Microscopy

Another technique by which microorganisms can be observed in unstained preparations is phase contrast microscopy. This method also can be used to observe intracytoplasmic organelles. The technique uses special microscope condensers and objectives to increase the contrast between cells and their environment or between cytoplasmic organelles by accentuating the differences in densities (Fig 1–4). Microorganisms can appear either darker or lighter than the background depending on the equipment used.

Nomarski microscopy is based on the same principles as phase contrast microscopy but uses polarized light. Contrast is greatest in parts of the specimen where there are large changes in light absorption over a large area. Consequently, Normarski optics are useful to visualize the outlines of cells or intracytoplasmic organelles, such as the nucleus.

Electron Microscopy

The development of the electron microscope has made possible a much more detailed look at the subcellular structure of fixed, stained bacteria. With these instruments, it is possible to increase resolution from 2×10^{-1} to 5×10^{-4} m because electrons are used instead of light waves to create an image of the specimen on a fluorescent screen. Direct magnifications of up to 1×10^{6} are possible. There are two kinds of electron microscopes, transmission electron microscopes (TEM) and scanning electron microscopes (SEM). TEMs are used to study whole cells that have been either stained, fixed, and cut into very thin sections or frozen, freeze-fractured, and stained. These instruments make possible the detailed study of in-

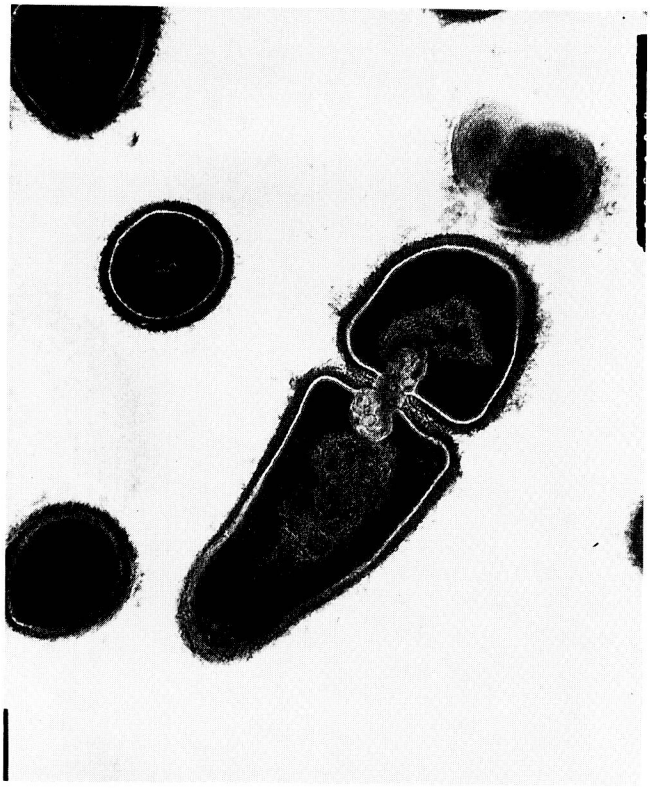

Figure 1–5. Electron photomicrograph of *Leptotrichia dentium*, showing septal mesosomes. *(Courtesy of Dr. M.A. Listgarten, University of Pennsylvania School of Dental Medicine.)*

Figure 1–6. Scanning electron photomicrograph of 48-hour plaque from the gingival area. Note the three-dimensional appearance of the specimen and the presence of rods, filaments, cocci, and several corncobs. ×4000. *(From Lie T.* J Periodont Res. *1977;12:85, with permission.)*

tracellular and cell surface organelles and have become valuable tools in examining the relationships between bacterial structure and function. Figure 1–5 is a TEM of an oral spirochete *Leptotrichia dentium*, showing some internal structures.

SEMs provide a three-dimensional image of the specimen as opposed to the two-dimensional view from a transmission microscope. Specimens are coated with heavy metal and then scanned with an electron beam. SEM is particularly useful to study bacterial surface structures and the interrelationships between organisms in complex, heterogeneous microenvironments, such as dental plaque. Figure 1–6 illustrates the image created by SEM of dental plaque grown in vitro on hydroxyapatite.

BACTERIAL ULTRASTRUCTURE

Because of their size and light refractive properties, information gained from routine light microscopic examination of bacteria is limited to gross morphology and differential staining characteristics. Although such knowledge is helpful in the diagnosis of infectious diseases, little information is gained about cellular structure and organization. Such information is necessary to understand fully how bacteria colonize host tissue and how the resulting association between host and parasite ultimately may cause disease. Detailed studies of bacterial ultrastructure have been undertaken during the past 35 years aided by increasingly sophisticated electron microscopic and immunochemical techniques. Figure 1–7 is a composite drawing of an idealized bacterium, showing the more common, although not universal, structures. Note that the left side depicts a gram-positive bacterium, and the right side shows a gram-negative cell. The following sections discuss bacterial ultrastructure and the role of these structures in disease.

Bacterial Cell Surfaces

Capsule and Slime Layer or Glycocalyx. The outermost layers of bacteria are important for survival and as virulence factors because it is through these that the microbe interacts with its environment. Most bacteria synthesize polymers that are associated with the cell envelope. If this material has a well-defined outer limit and adheres firmly to the cell wall, it is called a capsule. If its outer limit is less well defined, or if it is easily separated from the cell wall, it is called a slime layer or glycocalyx. These structures usually are carbohydrate heteropolymers or homopolymers. They are immunogenic, and antigenic differences exist in cap-

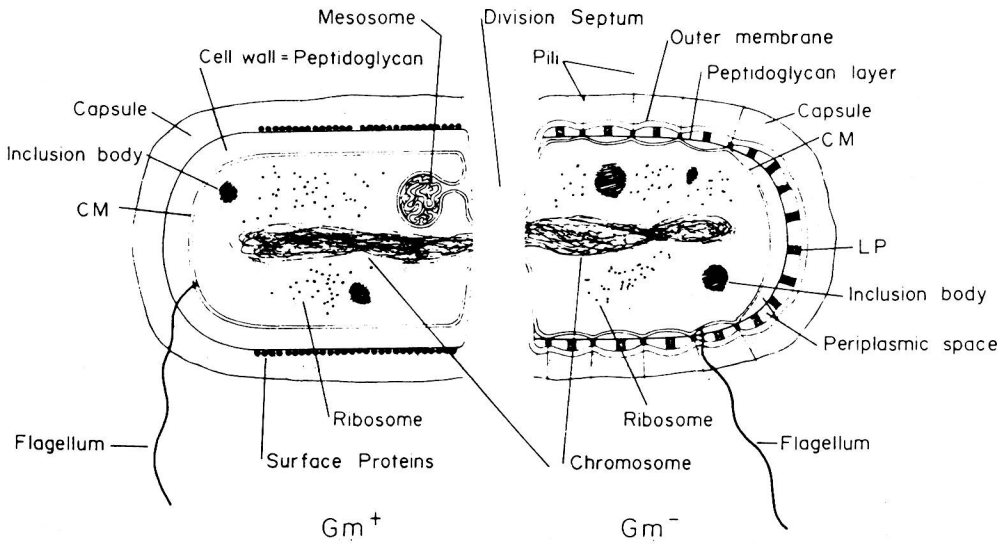

Figure 1–7. Drawing of idealized gram-positive (Gm+) and gram-negative (Gm−) bacteria showing structural similarities and differences. *(From Joklik WK, Willett HP, Amos DB, eds.* Zinsser Microbiology, *18th ed. E. Norwalk, CT: Appleton Lange 1984:24.)*

sules within a species. There are more than 80 serotypes of *Streptococcus pneumoniae,* for example, based on differences in capsular sugars. These differences are useful in identifying the strain causing a particular infection. *Streptococcus mutans,* one of the normal oral microflora, is another bacterium that produces extracellular polysaccharide capsules. It produces polymers of glucose (glucan) and fructose (fructan) from sucrose. These polymers are important in the accumulation of dental plaque and are discussed in detail in Chapter 33. Extracellular polymers may also be composed of protein. Strains of *Bacillus anthracis,* the etiologic agent of anthrax, have polypeptide capsules of D-glutamic acid polymers.

Extracellular polymers are not obligatory for bacterial survival but are important virulence factors because they enable organisms to stick to surfaces and because they reduce the efficiency of phagocytosis, one of the most important internal host defense mechanisms. Encapsulated bacteria are usually extracellular parasites. They owe their pathogenicity to the ability to grow in tissue spaces outside host cells. Capsules, by reducing the efficiency of phagocytosis, enable the bacteria to remain in tissue spaces, increase in numbers, and cause disease.

Flagella. Flagella are long, threadlike structures that are the organs of motility in bacilli, most vibrios, and spirilla. They are composites of several strands of a globular, contractile protein called flagellin, which can extend out from the cell from 3 μm to 12 μm. Flagellin is immunogenic; it is called the H antigen. The diameter of a typical flagellum is 0.01 μm. They can be seen with an electron microscope or with a darkfield microscope but not in a heat-fixed, stained preparation. The proximal end of each flagellum is anchored in the bacterial cell wall and membrane by specialized structures called a hook and a basal body (Fig 1–8). Motion is due to rotation of the flagella around the fixed anchorpoint in the cell wall. Because of their tertiary protein structure, flagella have regular sinusoidal curves with a wavelength that is a species characteristic.

Because bacterial flagella are arranged in several genetically stable patterns, their location can help distinguish between the two bacterial orders, Eubacteriales and Pseudomonadales. Eubacteriales have peritrichous flagella that can occur anywhere on the cell. Pseudomonadales have flagella at the cell poles. If a pseudomonad has a single polar flagellum, it is monotrichous. If there is one at each pole, it is amphitrichous. Some pseudomonads have tufts of lophotrichous flagella at one or both cell poles. Recently, periodontal microbiologists proposed another

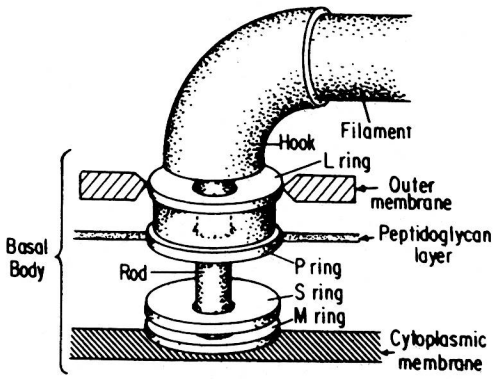

Figure 1–8. Drawing of the structures that anchor the flagellum to the bacterial cell wall and membrane. *(Modified from DePamphlis ML, Adler,* J Bacteriol. *1971;105:396.)*

category of flagellar arrangement, called helicotrichous. This classification is based on the isolation of gram-negative, anaerobic rods from patients with periodontitis. These bacteria, named *Centipeda periodontii*, are long, serpentine cells with a linear zone of flagella that form a helical spiral around the cell body, giving them a centipedelike appearance.

As an alternative to flagellar motility, some gram-negative bacteria, most notably spirochetes, have from 2 to more than 10 fibers, called axial filaments, attached at each pole and wound tightly around the entire length of the cell between the outer and cytoplasmic membranes (Fig 1–9). Movement is due to the alternate contraction and relaxation of these filaments, causing the cell to bend and then straighten.

Other gram-negative bacteria (eg, *Capnocytophaga ochraceus*) exhibit motility by gliding on a solid surface, such as agar. This type of motility seems to be associated with the presence of proteinaceous rotary assemblies in the cell envelope, although the actual mechanism of locomotion remains unknown.

The in vivo role of motility in the pathogenesis of infectious disease is unclear. Intuitively, it is reasonable to assume that the mechanisms described, in conjunction with chemotaxis, would enable bacteria to gain access to anatomic sites conducive to their growth and reproduction and may therefore serve as virulence factors. Whether or not the environmental conditions necessary for motility exist in many body sites is moot. The oral cavity is one anatomic site that is aqueous enough for some form of motility to play a role in the spread of bacteria. It has been suggested that many of the organisms found in the unattached dental plaque of periodontal pockets gain entrance to this site either by their own motility or by being transported passively while attached to species that are motile.

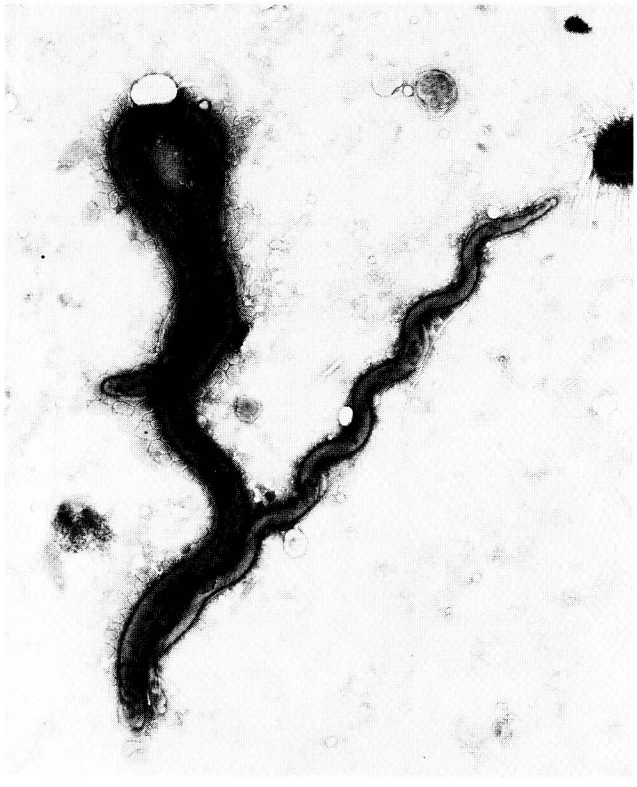

Figure 1–9. Axial filaments of oral spirochetes. *(From Listgarten MA, Socransky SS.* J Bacteriol. *1964;88:1087, with permission.)*

Pili. These fine, hairlike, proteinaceous appendages, also called fimbriae, are most often found on gram-negative bacteria. They are shorter, thinner, and straighter than flagella and have no role in motility. There are two types, each with its own function. Conjugal, or sex, pili are found on the male cell of conjugating bacterial pairs. These structures mediate the transfer of genetic material between cells by conjugation and episome transfer. The other type, somatic pili, mediate bacterial adsorption to host tissues.

Pili increase the pathogenic potential of bacteria. Conjugal pili facilitate the transfer of genetic material between members of the same or closely related species. A consequence of this promiscuity is the spread of antibiotic resistance among closely related bacteria, with the associated treatment complications. Somatic pili also give bacteria an ecologic advantage by enabling them to adsorb to host tissues. Adsorbed organisms subsequently may colonize the tissue if environmental conditions are suitable for their growth and reproduction. Furthermore, somatic pili have been associated with virulence in many bacteria, most notably *Neisseria gonorrhoeae* and *Actinomyces viscosus*, a periodontal pathogen. Gnotobiotic rats monoinfected with an *A. viscosus* strain with numerous pili suffered severe alveolar bone loss, whereas animals infected with a strain with far fewer pili did not (Fig 1–10).

Bacterial Cell Envelope

The bacterial cell envelope is composed of the cell wall and the cytoplasmic membrane. The structure and chemical composition of the cell envelope vary from one genus to another. Several general comments can be made about these variations that explain some of the differences observed among bacteria. First, variation in the chemical composition and consequent permeability of the cell envelope, particularly the cell wall, allow separation of bacteria into gram-positive and gram-negative cells. Closer examination of the cell envelopes of these two major bacterial subgroups reveals some important chemical and structural differences (Fig. 1–11). Gram-negative envelopes are generally more chemically and structurally complex than are those of gram-positive cells. They are multilayer structures consisting of a cytoplasmic membrane surrounded by a relatively thin peptidoglycan layer en-