Chapter 5 The Morphology and Fine Structure of Bacteria

OUTLINE

Among the major characteristics of bacterial cells are their size, shape, structure, and arrangement. These characteristics constitute the morphology of the cell. Depending on the species, individual cells are spherical, rodlike, or helical, although many variations of these three basic shapes occur. Furthermore, in certain species of bacteria the cells are arranged in groups, the most common of which are pairs, clusters, chains, trichomes, and filaments. It is important to recognize these patterns of shape and arrangement, since they are often characteristic of a taxonomic group, e.g., a genus. Some bacteria also possess appendages, which can be made visible by special staining techniques or by electron microscopy. All of these morphological features are regard as the gross morphological characteristics of bacterial cells.

The bacterial cell possesses a detailed internal structure. The discovery of this internal structure was made possible by the development of electron-microscope techniques and of instruments for slicing a bacterial cell into extremely thin sections. The terms **microbial cytology** and **bacterial anatomy** have become commonplace in microbiological literature.

The various structures of a bacterial cell differ from one another not only in their physical features but also in their chemical characteristics and in their functions. Thus biologists today seek to integrate the structural, chemical, and

functional properties of the bacterial cell. This area of research studied by biologists is sometimes referred to as biochemical cytology.

THE SIZE, SHAPE, AND ARRANGEMENT OF BACTERIAL CELLS

Size

Bacteria are very small, most being approximately 0.5 to 1.0 μm in diameter. An important consequence of the small size of microorganisms is that the surface area/volume ratio of bacteria is exceedingly high compared to the same ratio for larger organisms of similar shape (Table 5-1). A relatively large surface through which nutrients can enter (or waste products leave) compared to a small volume of cell substance to be nourished accounts for the unusually high rate of growth and metabolism of bacteria. Moreover, because of the high surface area volume ratio, the mass of cell substance to be nourished is very close to the surface; therefore, no circulatory mechanism is needed to distribute the nutrients that are taken in, and there is thought to be little or no cytoplasmic movement within a bacterial cell. Despite these advantages, a high surface area/volume ratio limits the size of bacteria to microscopic dimensions.

Shape and Arrangement

The shape of a bacterium is governed by its rigid cell wall; however, exactly what attribute of this rigid material determines that a cell will have a particular shape is not yet understood. Typical bacterial cells are spherical (cocci: singular, coccus); straight rods (bacilli: singular, bacillus); or rods that are helically curved (spirilla: singular, spirillum) as illustrated in Fig. 5-1. Although most bacterial species have cells that are of a fairly constant and characteristic shape, some have cells that are pleomorphic, i.e., that can exhibit a variety of shapes (Fig. 5-2).

Table 5-1. Comparison of the Surface Area/Volume Ratio of Spherical Organisms of Different Sizes*

Diameter of Sphere, μm	Surface Area, μm^2 ($4\pi r^2$)	Volume, μm^3 ($4\pi r^3/3$)	$\frac{\text{Surface Area}}{\text{Volume}}$, μm^{-1} ($3/r$)
1 μm	3.1	0.52	6
1,000 μm	3.1×10^6	5.2×10^8	0.006
1,000,000 μm	3.1×10^{12}	5.2×10^{17}	0.000006

* For a given volume, the geometrical shape that has the smallest surface area/volume ratio is a sphere; i.e., if two organisms have the same volume, one being spherical and the other cylindrical, the cylindrical organism has the greater surface area/volume ratio.

A *Staphylococcus* B *Lactobacillus* C *Aquaspirillum*

Figure 5-1. Bacteria are generally either (A) spherical (cocci); (B) rodlike (rods or bacilli); or (C) helical (spirilla). However, there are many modifications of these three basic forms. (*Erwin F. Lessel, illustrator.*)

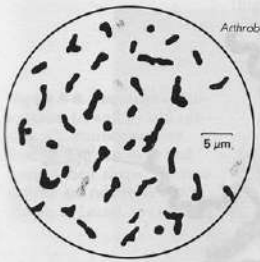

Figure 5-2. Drawing of pleomorphic cells of the genus *Arthrobacter*. (*Erwin F. Lessel, illustrator.*)

Figure 5-4. Photomicrograph of the trichomes of *Saprospira grandis*, composed of individual cylindrical cells that are 1 to 5 μm long and closely attached to one another (X1,650). (*Courtesy of G. J. Hageage, Jr.*)

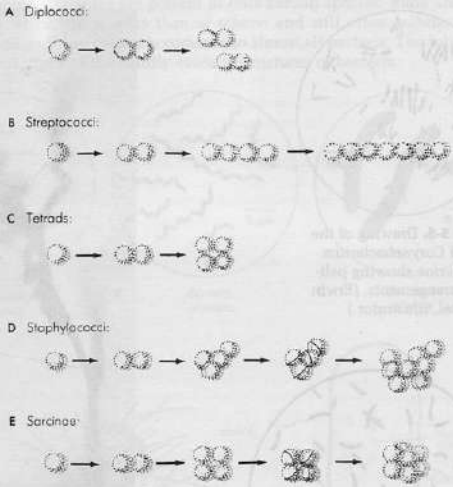

Figure 5-3. Characteristic arrangements of cocci, with schematic illustrations of patterns of multiplication. (A) Diplococci: cells divide in one plane and remain attached predominantly in pairs. (B) Streptococci: cells divide in one plane and remain attached to form chains. (C) Tetracocci: cells divide in two planes and characteristically form groups of four cells. (D) Staphylococci: cells divide in three planes, in an irregular pattern, producing "bunches" of cocci. (E) Sarcinae: cells divide in three planes, in a regular pattern, producing a cuboidal arrangement of cells.

Bacterial cells are usually arranged in a manner characteristic of their particular species. Although it is rare that all the cells of a species are arranged in the same manner, it is the predominant arrangement that is the important feature.

Cocci appear in several characteristic arrangements, depending on the plane of cellular division and whether the daughter cells stay together following division (Fig. 5-3). Bacilli are not arranged in patterns as complex as those of cocci, and most occur singly or in pairs (diplobacilli). But some species, such as *Bacillus subtilis*, form chains (streptobacilli); others, such as *Beggiatoa* and *Saprospira* species, form trichomes, which are similar to chains but have a much larger area of contact between the adjacent cells (Fig. 5-4). In other bacillus species, such as *Corynebacterium diphtheriae*, the cells are lined side by side like matchsticks (palisade arrangement) and at angles to one another (Fig. 5-5).

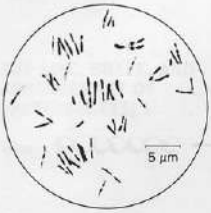

Figure 5-5. Drawing of the cells of *Corynebacterium diphtheriae* showing palisade arrangements. *(Erwin F. Lessel, illustrator.)*

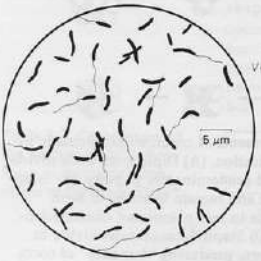

Figure 5-7. Drawing of cells of the genus *Vibrio*, showing the characteristic curved shape and the polar flagella. The flagella are not visible by ordinary staining procedures. *(Erwin F. Lessel, illustrator.)*

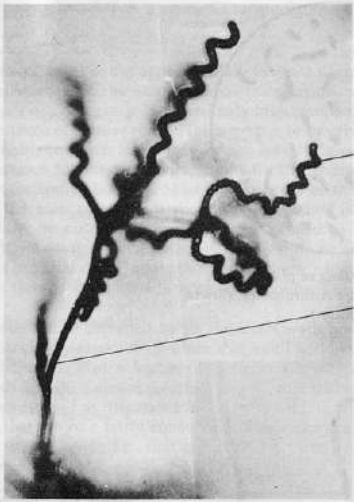

Figure 5-6. Photomicrograph of *Streptomyces viridochromogenes*. This bacterium produces coiled chains of spores (called conidia) which develop at the ends of vegetative filaments called hyphae. *(Courtesy of Mary P. Lechevalier.)*

Still others, such as *Streptomyces* species, form long, branched, multinucleate filaments called **hyphae** (singular, **hypha**) which collectively form a **mycelium** (Fig. 5-6). (Note that the terms *hyphae* and *mycelium* are also commonly applied to the filaments formed by fungi, described in Chap. 17).

Curved bacteria are usually curved with a twist. Bacteria with less than one complete twist or turn have a **vibrioid** shape (Fig. 5-7), whereas those with one or more complete turns have a **helical** shape. Spirilla are rigid helical bacteria, whereas **spirochetes** are highly flexible (Fig. 5-8).

In addition to the common bacterial shapes, many others also occur: pear-shaped cells (e.g., *Pasteuria*); lobed spheres (e.g., *Sulfolobus*); rods with squared rather than the usual hemispherical ends (e.g., *Bacillus anthracis*); disks arranged like stacks of coins (e.g., *Caryophanon*); rods with helically sculptured surfaces (e.g., *Seliberia*); and many others.

BACTERIAL STRUCTURES

Examination of a bacterial cell reveals various component structures. Some of these are external to the cell wall (Fig. 5-9); others are internal to the cell wall

(Fig. 5-10). Some structures are present in only certain species; some are more characteristic of certain species than of others; and still other cellular parts, such as the cell wall, are naturally common to almost all bacteria. The following are brief descriptions of the readily evident structures of bacteria.

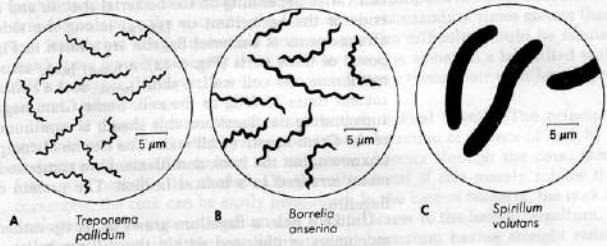

Figure 5-8. Drawings of spirochetes (A and B) and spirilla (C). Spirochetes are flexible and can twist and contort their shape, whereas spirilla are relatively rigid. (*Erwin F. Lessel, illustrator.*)

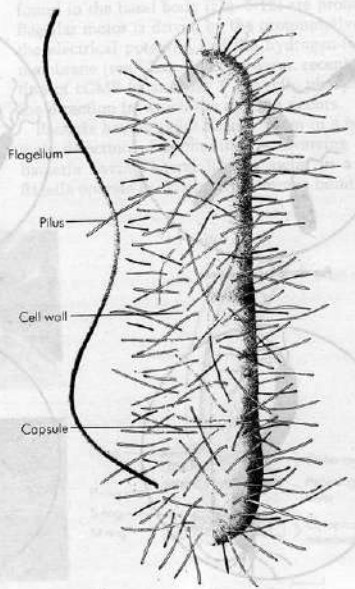

Figure 5-9. Drawing of the major structures external to the bacterial cell wall. Certain structures, e.g., capsules, flagella, and pili, are not common to all bacterial cells. (*Erwin F. Lessel, illustrator.*)

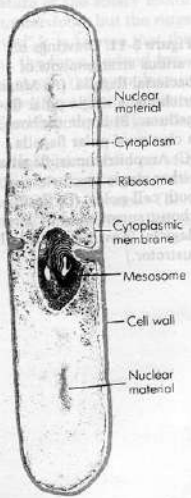

Figure 5-10. Drawing of the major structures which occur within the bacterial cell wall. Certain structures, e.g., mesosomes, are not common to all bacterial cells. (*Erwin F. Lessel, illustrator.*)

STRUCTURES EXTERNAL TO THE CELL WALL

Flagella and Motility

Bacterial flagella (singular, flagellum) are hairlike, helical appendages that protrude through the cell wall and are responsible for swimming motility. They are much thinner than the flagella or cilia of eucaryotes, being 0.01 to 0.02 μm in diameter, and they are also much simpler in structure. Their location on the cell varies depending on the bacterial species and may be polar (at one or both ends of the bacterium) or lateral (along the sides of the bacterium). Some arrangements of bacterial flagella are shown in Fig. 5-11. A flagellum is composed of three parts (Fig. 5-12): a basal body associated with the cytoplasmic membrane and cell wall, a short hook, and a helical filament which is usually several times as long as the cell. Some Gram-negative bacteria have a sheath surrounding the flagellum; this sheath is continuous with the outer membrane of the Gram-negative cell wall. The chemical composition of the basal body is unknown, but the hook and filament are composed of protein subunits (monomers) arranged in a helical fashion. The protein of the filament is known as flagellin.

Unlike a hair, a flagellum grows at its tip rather than at the base. Flagellin monomers synthesized within the cell are believed to pass along the hollow center of the flagellum and are added to the distal end of the filament.

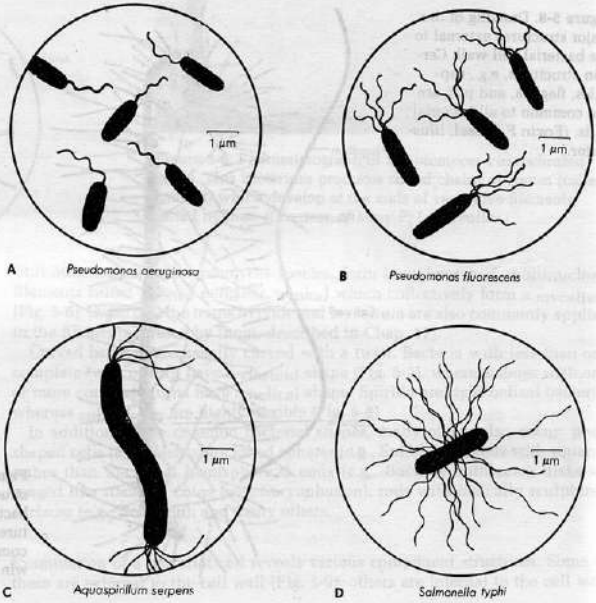

Figure 5-11. Drawings of various arrangements of bacterial flagella. (A) Monotrichous; a single polar flagellum. (B) Lophotrichous; a cluster of polar flagella. (C) Amphitrichous; flagella, either single or clusters, at both cell poles. (D) Peritrichous; surrounded by lateral flagella. *(Erwin F. Lessel, illustrator.)*

Hydrodynamics of Flagella

Large motile bodies such as boats and fish make use of the inertia of water for their propulsion. When pushed against with, for example, an oar, a propeller blade, or fins, the water temporarily acts as a solid, thereby enabling the boat or fish to generate a forward propulsive force. However, the small size of bacteria prohibits their use of the inertia of water to gain propulsive force, because the drag forces due to the viscosity of water become thousands of times greater than any forces that can be generated from inertia. The difficulty would be similar to what we would encounter if we attempted to row a boat on a lake filled with thick molasses. However, bacteria can swim many times their own length per second under analogous conditions!

Bacteria propel themselves by rotating their helical flagella. The principle involved can be illustrated by imagining the penetration of a piece of cork by a corkscrew. If one tries to ram the corkscrew directly through the cork, great force will probably be needed. On the other hand if one merely rotates the corkscrew, the cork can be easily penetrated. In the case of bacteria, the cork is analogous to the viscous medium and the corkscrew to the helical flagellum. It is apparent from this analogy that a mutant bacterium having straight rather than helical flagella would be unable to swim. The nature of the rotary motor that spins each corkscrew-shaped flagellum is still not understood, but the rings found in the basal body (Fig. 5-12) are probably involved. It is known that the flagellar motor is driven by the protonmotive force, i.e., the force derived from the electrical potential and the hydrogen-ion gradient across the cytoplasmic membrane (see Chap. 10). Moreover, recent studies suggest that the concentration of cGMP (guanosine 3′,5′-cyclic phosphoric acid) within the cell governs the direction in which the rotation occurs.

Bacteria having polar flagella swim in a back-and-forth fashion; they reverse their direction of swimming by reversing the direction of flagellar rotation. Bacteria having lateral flagella swim in a more complicated manner. Their flagella operate in synchrony to form a bundle that extends behind the cell (Fig.

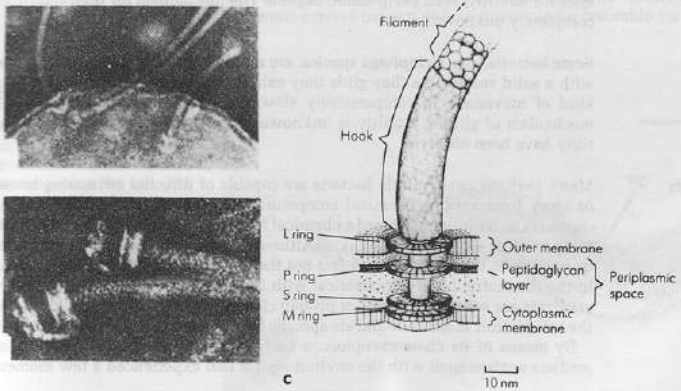

Figure 5-12. The mechanism of attachment of flagella to a Gram-negative bacterial cell (*Pseudomonas aeruginosa*). (A) Prior to electron-microscope examination, the cells were partially lysed and then negatively stained to make the point of flagellar attachment (basal body) more visible (X80,000 approx.). (B) Isolated flagella showing basal body at one end. (C) Model of basal body illustrating its structure and attachment to a Gram-negative bacterium. The flagella of Gram-positive bacteria have only two basal rings. *(Courtesy of T. Iino, University of Tokyo.)*

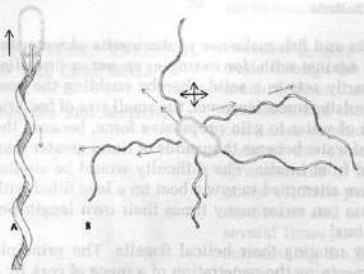

Figure 5-13. Diagram of the configuration and arrangement of peritrichous flagella during swimming and tumbling. The small arrows indicate the direction of propagation of helical waves along the flagella. (A) During swimming the flagella are in the form of left-handed helices and rotate counterclockwise in synchrony to form a bundle. The large arrow indicates the direction of swimming. (B) During tumbling the flagella reverse their rotation, portions of the flagella acquire a short wavelength and right-handed configuration, and the bundle flies apart. The cell cannot swim under these conditions and instead exhibits a chaotic motion, as symbolized by the large crossed arrows. *(Courtesy of R. M. MacNab and M. K. Ornston, J Mol Biol* **112:1**, *1977.)*

5-13). However, when the flagellar motors reverse, conformational changes occur along the flagella, the bundle flies apart, and the cell tumbles wildly. Finally, the flagellar motors resume their normal direction, the flagellar bundle again forms, and the cell begins to swim—but now in a different direction. This sequence of events occurs repeatedly, so that the motility becomes a series of periods of swimming (runs) punctuated by periods of tumbling (twiddles), with a change in direction after each tumble.

Swimming Motility Without Flagella

Certain helical bacteria (spirochetes) exhibit swimming motility, particularly in highly viscous media, yet they lack external flagella. However, they have flagellalike structures located within the cell, just beneath the outer cell envelope (see Fig. 13-1). These are called periplasmic flagella; they have also been termed axial fibrils or endoflagella. They are responsible for the motility of spirochetes, but how they accomplish this is not yet clear. Other helical bacteria called spiroplasmas are able to swim in viscous media, yet lack any apparent organelles for motility, even periplasmic flagella. The mechanism for their motility is completely unknown.

Gliding Motility

Some bacteria, e.g., *Cytophaga* species, are motile only when they are in contact with a solid surface. As they glide they exhibit a sinuous, flexing motion. This kind of movement is comparatively slow, only a few μm per second. The mechanism of gliding motility is unknown; no organelles responsible for motility have been observed.

Bacterial Chemotaxis

Many, perhaps most, motile bacteria are capable of directed swimming toward or away from various chemical compounds—a phenomenon called bacterial chemotaxis. Swimming toward a chemical is termed positive chemotaxis; swimming away is negative chemotaxis. Although chemicals may act as attractants or repellents, the stimulus is in fact not the chemical itself but rather a change in the concentration of the chemical with time, i.e., a temporal gradient. Such gradients are sensed by means of protein chemoreceptors which are located on the cytoplasmic membrane and are specific for various attractants and repellents.

By means of its chemoreceptors, a bacterium continually compares its immediate environment with the environment it had experienced a few moments

earlier. To illustrate this, suppose we are observing the behavior of a bacterium that has peritrichous flagella and for which glucose is an attractant. If the cell is placed in a homogeneous glucose broth, the glucose concentration remains constant regardless of the direction of the bacterium's swimming, and the glucose-specific chemoreceptors can sense no change in glucose concentration. Consequently, the cell exhibits a normal swimming pattern—periods of swimming with intermittent periods of tumbling. Suppose that the cell is now placed in a long capillary tube with a higher concentration of glucose at one end than at the other. If the cell happens to swim toward the higher concentration of glucose (i.e., in the "right" direction), the chemoreceptors sense that the glucose concentration is increasing with time. This results in suppression of normal tumbling, causing the cell to swim smoothly ahead for a long period before it tumbles. On the other hand, if the cell happens to swim toward the end of the tube where there is less glucose (i.e., in the "wrong" direction), the chemoreceptors sense that the glucose concentration is decreasing with time, and no suppression of tumbling occurs. Therefore, the cell soon tumbles, changes direction, and tries again until finally the "right" direction is achieved. (In a gradient of a repellent compound, the right direction would be down the gradient, i.e., toward a *decreasing* concentration, and the wrong direction would be up the gradient.)

Tactic responses are not limited to chemical gradients. For instance, phototrophic bacteria exhibit positive phototaxis toward increasing light intensities and are repelled by decreasing light intensities. Still another type of taxis is exhibited by *Aquaspirillum magnetotacticum*; this organism exhibits directed swimming in response to the earth's magnetic field or to local magnetic fields (magnets placed near the culture). This is attributed to a chain of magnetite inclusions (magnetosomes) within the cell, which allows the cell to become oriented as a magnetic dipole (Fig. 5-14). Because of the downward inclination of the Earth's magnetic field in the regions where these bacteria have been found, magnetotaxis may serve to direct the cells downward in aquatic environments toward oxygen-deficient areas more favorable for growth.

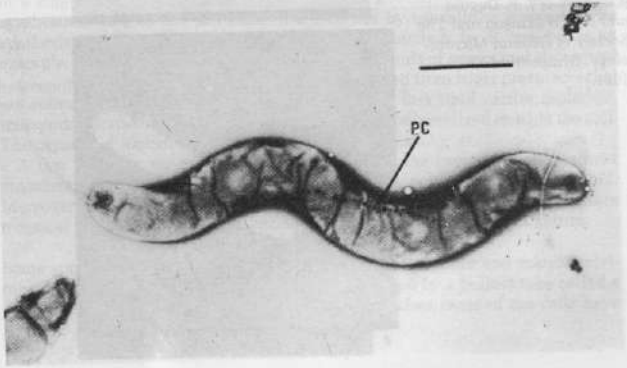

Figure 5-14. Negatively stained cell of *Aquaspirillum magnetotacticum* showing a particle chain (PC) of highly electron-dense magnetite inclusions (magnetosomes) within the cell. The bar represents 1 μm. (Courtesy of D. L. Balkwill, D. Maratea and R. P. Blakemore, *J. Bacteriol* **141**:1399, 1980.)

Pili (Fimbriae)

Pili (singular, pilus) are hollow, nonhelical, filamentous appendages that are thinner, shorter, and more numerous than flagella (Fig. 5-15). They do not function in motility, since they are found on nonmotile as well as motile species. There are, however, several functions associated with different types of pili. One type, known as the F pilus (or sex pilus), serves as the port of entry of genetic material during bacterial mating (see Chap. 12). Some pili play a major role in human infection by allowing pathogenic bacteria to attach to epithelial cells lining the respiratory, intestinal, or genitourinary tracts. This attachment prevents the bacteria from being washed away by the flow of mucous or body fluids and permits the infection to be established.

Capsules

Some bacterial cells are surrounded by a viscous substance forming a covering layer or envelope around the cell wall. If this layer can be visualized by light microscopy using special staining methods, it is termed a capsule. If the layer is too thin to be seen by light microscopy it is termed a microcapsule; if it is so abundant that many cells are embedded in a common matrix, the material is called slime.

By light microscopy, capsules appear to be amorphous gelatinous areas surrounding a cell (Fig. 5-16A); however, special techniques designed to preserve delicate structures for observation by electron microscopy have revealed that capsules consist of a mesh or network of fine strands (Fig. 5-16B).

In many instances capsular material is not highly water-soluble and therefore does not readily diffuse away from the cells that produce it. In other instances

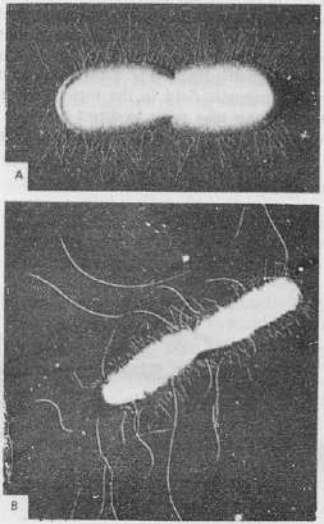

Figure 5-15. Fimbriated bacteria. (A) *Shigella flexneri*: dividing bacilli with numerous fimbriae surrounding the cells (X20,000). (B) *Salmonella typhi*: dividing bacilli with numerous fimbriae and a few flagella (the very long appendages) (X12,500). *(Courtesy of J. P. Duguid and J. F. Wilkinson and The Society of General Microbiology: Symposium XI. 1961.)*

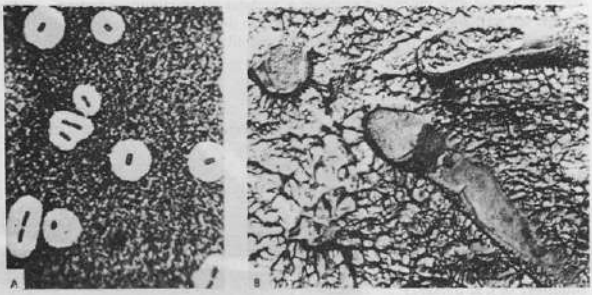

Figure 5-16. Bacterial capsules as seen by light microscopy (A) and electron microscopy (B). (A) India-ink preparation of a capsulated bacterium isolated from a paper-mill operation. The particles of carbon in the ink cannot penetrate the capsules (white areas around the cells). *Courtesy of P. M. Borick, Wallace and Tiernan, Inc.)* (B) Freeze-etch preparation of Gram-positive rod-shaped bacteria isolated from acid mine water, showing a fibrillar polymer network surrounding the cells. The freeze-fracture process has also revealed various internal and surface structures of the cells. *(Courtesy of P. R. Dugan, C. B. MacMillan, and R. M. Pfister, J. Bacteriol* **101**:982, 1970.)

the material is highly water-soluble and dissolves in the medium, sometimes dramatically increasing the viscosity of the broth in which the organisms are cultured.

Capsules can serve a number of functions, depending on the bacterial species. (1) They may provide protection against temporary drying by binding water molecules. (2) They may block attachment of bacteriophages. (3) They may be antiphagocytic; i.e., they inhibit the engulfment of pathogenic bacteria by white blood cells and thus contribute to invasive or infective ability (virulence). (4) They may promote attachment of bacteria to surfaces; for example, *Streptococcus mutans*, a bacterium associated with producing dental caries, firmly adheres to the smooth surfaces of teeth because of its secretion of a water-insoluble capsular glucan. (5) If capsules are composed of compounds having an electrical charge, such as sugar–uronic acids, they may promote the stability of bacterial suspension by preventing the cells from aggregating and settling out, because cells bearing similarly charged surfaces tend to repel one another.

Most bacterial capsules are composed of polysaccharides. Capsules composed of a single kind of sugar are termed **homopolysaccharides**; are usually synthesized outside the cell from disaccharides by exocellular enzymes. The synthesis of glucan (a polymer of glucose) from sucrose by *S. mutans* is an example. Other capsules are composed of several kinds of sugars and are termed **heteropolysaccharides**; these are usually synthesized from sugar precursors that are activated (energized) within the cell, attached to a lipid carrier molecule, transported across the cytoplasmic membrane, and polymerized outside the cell. The capsule of *Klebsiella pneumoniae* is an example.

A few capsules are polypeptides. For example, the capsule of the anthrax organism, *B. anthracis*, is composed entirely of a polymer of glutamic acid. Moreover, this peptide is an unusual one because the glutamic acid is the rare D optical isomer rather than the usual L isomer commonly found in nature.

Sheaths

Some species of bacteria, particularly those from freshwater and marine environments, form chains or trichomes that are enclosed by a hollow tube called a This structure is most readily visualized when some of the cells have

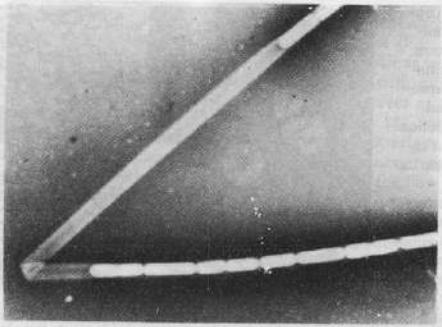

Figure 5-17. Sheathed bacteria. Sheath and cells of *Sphaerotilus natans* stained with nigrosin. Dimensions of individual cells are 1 μm by 2 to 6 μm, and the sheaths may reach a length of several millimeters. *(Courtesy of J. L. Stokes, J Bacteriol* 67:279, *1954.)*

migrated from it (Fig. 5-17). Sheaths may sometimes become impregnated with ferric or manganese hydroxides, which strengthen them.

Prosthecae and Stalks

Prosthecae (singular, prostheca) are semirigid extensions of the cell wall and cytoplasmic membrane and have a diameter that is always less than that of the cell. They are characteristic of a number of aerobic bacteria from freshwater and marine environments. Some bacterial genera such as *Caulobacter* have a single prostheca; others such as *Stella* and *Ancalomicrobium* have several (Fig. 5-18). Prosthecae increase the surface area of the cells for nutrient absorption, which is advantageous in dilute environments. Some prosthecate bacteria may form a new cell (bud) at the end of a prostheca; others have an adhesive substance at the end of a prostheca that aids in attachment to surfaces.

Although the term stalk is sometimes used interchangeably with the terms *prostheca* or *hypha*, it is perhaps better to restrict its use to certain nonliving ribbonlike or tubular appendages that are excreted by the cell, such as those found in the genera *Gallionella* or *Planctomyces* (see Chap. 15). These stalks aid in attachment of the cells to surfaces.

THE CELL WALL

Beneath such external structures as capsules, sheaths, and flagella and external to the cytoplasmic membrane is the cell wall, a very rigid structure that gives shape to the cell. Its main function is to prevent the cell from expanding and eventually bursting because of uptake of water, since most bacteria live in hypotonic environments (i.e., environments having a lower osmotic pressure than exists within the bacterial cells). The rigidity of the wall can be readily demonstrated by subjecting bacteria to very high pressures or other severe physical conditions: most bacterial cells retain their original shapes during and after such treatments. To obtain isolated cell walls for analysis, bacteria usually must be mechanically disintegrated by drastic means, as by sonic or ultrasonic treatment or by exposure to extremely high pressures with subsequent sudden

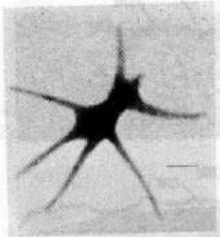

Figure 5-18. *Ancalomicrobium adetum*, a budding bacterium with several prosthecae per cell. Electron micrograph of whole cell, negatively stained. The bar represents 1.0 μm. (Courtesy of J. T. Staley, J Bacteriol **95**:1921, 1968.)

release of pressure. The broken cell walls are then separated from the rest of the components of the disintegrated cells by differential centrifugation. Isolated cell walls, devoid of other cellular constituents, retain the original contour of the cells from which they were derived.

Among the ordinary or typical bacteria (which are sometimes called eubacteria to distinguish them from the phylogenetically distinct group known as the archaeobacteria, discussed in Chap. 3), the walls of Gram-negative species are generally thinner (10 to 15 nm) than those of Gram-positive species (20 to 25 nm). The walls of Gram-negative archaeobacteria are also thinner than those of Gram-positive archaeobacteria. Since the chemical composition of the walls of archaeobacteria is quite different from that of eubacteria, wall thickness rather than chemical composition may be the major factor in the Gram reaction.

The cell wall constitutes a significant portion of the dry weight of the cell; depending on the species and culture conditions, it may account for as much as 10 to 40 percent. Bacterial cell walls are usually essential for bacterial growth and division. Cells whose walls have been completely removed (i.e., protoplasts) are incapable of normal growth and division.

Structure and Chemical Composition

Peptidoglycan

For eubacteria, the shape-determining part of the cell wall is largely peptidoglycan (sometimes called murein), an insoluble, porous, cross-linked polymer of enormous strength and rigidity. Peptidoglycan is found only in procaryotes; it occurs in the form of a "bag-shaped macromolecule" surrounding the cytoplasmic membrane. Peptidoglycan differs somewhat in composition and structure from one species to another, but it is basically a polymer of *N*-acetylglucosamine, *N*-acetylmuramic acid, L-alanine, D-alanine, D-glutamate, and a diamino acid (LL- or meso-diaminopimelic acid, L-lysine, L-ornithine, or L-diaminobutyric acid). The structure of this polymer is depicted in Figs. 11-6 and 11-7. It is important to realize that as tough as peptidoglycan is, it is also in a dynamic state. That is, in order for the cell to grow and divide, portions of the peptidoglycan must continually be degraded by wall-associated hydrolytic enzymes so that new polymer can be added.

Walls of Archaeobacteria

Although most archaeobacteria possess cell walls, these do not contain peptidoglycan, and their cell-wall fine structure and chemical composition is very different from that of eubacteria. Their walls are usually composed of proteins, glycoproteins, or polysaccharides. A few genera, such as *Methanobacterium*, have walls composed of pseudomurein, a polymer whose structure superficially resembles eubacterial peptidoglycan but which differs markedly in chemical composition (see Chap. 15).

Walls of Gram-Positive Eubacteria

Gram-positive bacteria usually have a much greater amount of peptidoglycan in their cell walls than do Gram-negative bacteria; it may account for 50 percent or more of the dry weight of the wall of some Gram-positive species, but only

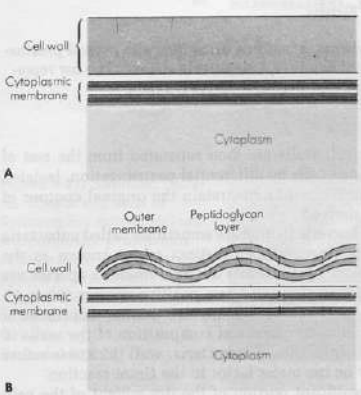

Figure 5-19. Schematic interpretation of cell walls of eubacteria from electron-microscope observations. (A) Gram-positive bacteria, showing thick wall consisting mainly of peptidoglycan. Although the wall is often homogeneous in appearance, in some bacteria it may consist of several layers. (B) Gram-negative bacteria, showing outer membrane and thin peptidoglycan layer. *(Courtesy of A. I. Laskin and H. A. Lechevalier (eds.), Handbook of Microbiology, CRC Press, Inc., Cleveland, 1974.)*

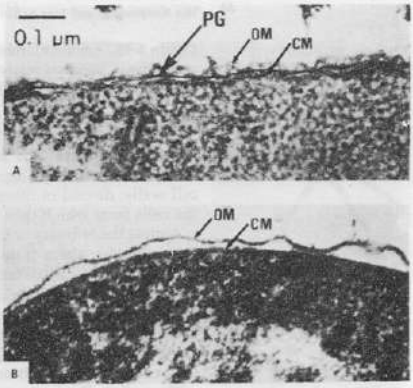

Figure 5-20. (A) Thin section of *Aquaspirillum serpens* showing the wavy outer membrane (OM), the peptidoglycan layer (PG), and the cytoplasmic membrane (CM). (B) Companion preparation of a spheroplast formed by treatment of the cells with a chelating agent and lysozyme. The peptidoglycan layer is missing. *(From R. G. E. Murray, P. Steed and H. E. Elson, Can J Microbiol* 11:547, 1965.)

about 10 percent of the wall of Gram-negative bacteria. Other substances may occur in addition to peptidoglycan. For instance, the walls of *Streptococcus pyogenes* contain polysaccharides that are covalently linked to the peptidoglycan and which can be extracted with hot dilute hydrochloric acid. The walls of *Staphylococcus aureus* and *Streptococcus faecalis* contain teichoic acids—acidic polymers of ribitol phosphate or glycerol phosphate—which are covalently linked to peptidoglycan and which can be extracted with cold dilute acid. Teichoic acids bind magnesium ions, and there is some evidence that they help to protect bacteria from thermal injury by providing an accessible pool of these cations for stabilization of the cytoplasmic membrane. The walls of most Gram-positive bacteria contain very little lipid, but those of *Mycobacterium*, *Corynebacterium*, and certain other genera are exceptions, being rich in lipids called These compounds have the following general structure:

$$R_1\text{—}\underset{\displaystyle OH}{\underset{|}{CH}}\text{—}\underset{\displaystyle R_2}{\underset{|}{CH}}\text{—COOH}$$

where R_1 and R_2 are long hydrocarbon chains. The ability of mycobacteria to exhibit acid-fast staining (i.e., when stained, the cells cannot be decolorized

easily despite treatment with dilute acids) is correlated with the presence of cell wall mycolic acids. A mycolic acid derivative called **cord factor** (trehalose dimycolate) is toxic and plays an important role in the diseases caused by *C. diphtheriae* and *M. tuberculosis*, described in Chap. 36.

Walls of Gram-Negative Eubacteria

The walls of Gram-negative bacteria are more complex than those of Gram-positive bacteria. The most interesting difference is the presence of an outer membrane that surrounds a thin underlying layer of peptidoglycan (Figs. 5-19 and 5-20). Because of this membrane, the walls of Gram-negative bacteria are rich in lipids (11 to 22 percent of the dry weight of the wall), in contrast to those of Gram-positive bacteria. This outer membrane serves as an impermeable barrier to prevent the escape of important enzymes, such as those involved in cell wall growth, from the space between the cytoplasmic membrane and the outer membrane (periplasmic space). The outer membrane also serves as a barrier to various external chemicals and enzymes that could damage the cell. For example, the walls of many Gram-positive bacteria can be easily destroyed by treatment with an enzyme called **lysozyme**, which selectively dissolves peptidoglycan; however, Gram-negative bacteria are refractory to this enzyme because large protein molecules cannot penetrate the outer membrane. Only if the outer membrane is first damaged, as by removal of stabilizing magnesium ions by a chelating agent, can the enzyme penetrate and attack the underlying peptidoglycan layer (see Fig. 5-20B).

The outer membrane of the Gram-negative cell wall is anchored to the underlying peptidoglycan by means of **Braun's lipoprotein** (Fig. 5-21). The membrane is a bilayered structure consisting mainly of **phospholipids, proteins, and lipopolysaccharide (LPS)**. The LPS has toxic properties and is also known as **endotoxin**. It occurs only in the outer layer of the membrane (Fig. 5-21) and is composed of three covalently linked parts: (1) **Lipid A**, firmly embedded in the membrane; (2) **core polysaccharide**, located at the membrane surface; and (3) polysaccharide **O antigens**, which extend like whiskers from the membrane surface into the surrounding medium (Fig. 5-21). Many of the serological prop-

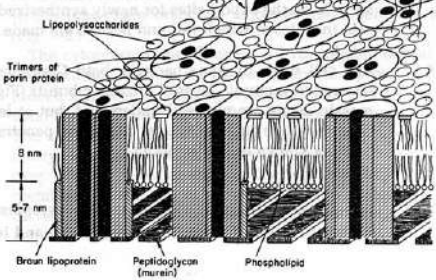

Figure 5-21. Tentative model of the cell wall of a Gram-negative bacterium like *Escherichia coli* or *Salmonella typhimurium*. Not shown is the cytoplasmic membrane, which is located below the peptidoglycan layer. The 8-nm-thick outer membrane of the cell wall is separated from the peptidoglycan layer by a 5 to 7 nm space. Molecules of Braun's lipoprotein extend across this space and anchor the outer membrane to the peptidoglycan. Porins extend from the external surface of the outer membrane down to the peptidoglycan layer. (*Courtesy of H. Nikaido and T. Nakae, Adv Microbial Physiol* **20**:163, 1979.)

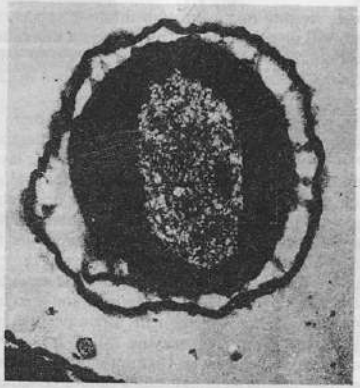

Figure 5-22. Thin section of an *Escherichia coli* cell that was plasmolyzed in a 20% sucrose solution, causing the protoplast to contract. Numerous adhesions are evident between the cytoplasmic membrane and the outer membrane of the cell wall. The light, fibrillar area in the center of the cell is the nuclear material. The bar represents 0.1 μm. (*Courtesy of M. E. Bayer, J Gen Microbiol* 53:395, 1968.)

erties of Gram-negative bacteria are attributable to O antigens; they can also serve as receptors for bacteriophage attachment.

Although impermeable to large molecules such as proteins, the outer membrane can allow smaller molecules, such as nucleosides, oligosaccharides, monosaccharides, peptides, and amino acids, to pass across. This is accomplished by means of channels in special proteins called **porins**, which span the membrane (Fig. 5-21). The various porins are specific for different kinds or classes of small molecules, and some can even allow certain essential large molecules to penetrate, such as vitamin B_{12}. Many porins also serve as receptors for attachment of bacteriophages and bacteriocins.

One of the questions posed by the structure of Gram-negative cell walls is: How can water-insoluble, lipophilic substances such as LPS pass from their place of synthesis within the cytoplasm and cytoplasmic membrane across a watery periplasmic space to be inserted into the outer membrane? A likely explanation has been provided by the discovery of numerous **adhesions**, or points of direct contact between the two membranes (Fig. 5-22). These adhesions seem to be the export sites for newly synthesized LPS and porins, and they are also the sites at which pili and flagella are made.

Macromolecular Surface Arrays

The cell walls of some bacteria, both Gram-negative and Gram-positive, are covered by a mosaic layer of protein subunits (Fig. 5-23). The functions of these mosaic layers are not well understood, but at least one function is to protect Gram-negative bacteria against attack and penetration by other small, predatory bacteria known as bdellovibrios.

STRUCTURES INTERNAL TO THE CELL WALL

Immediately beneath the cell wall is the **cytoplasmic membrane**. This structure is approximately 7.5 nm (0.0075 μm) thick and is composed primarily of phos-

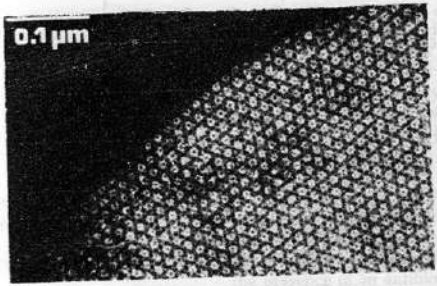

Figure 5-23. Electron micrograph showing a macromolecular surface array of protein subunits of the outer surface of a cell-wall fragment from *Aquaspirillum serpens*. (Courtesy of R. G. E. Murray. From N. R. Krieg, Bacteriol Rev **40**:55, 1976.)

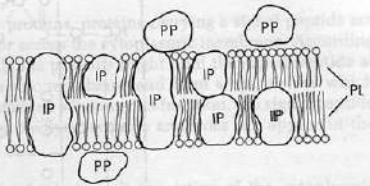

Figure 5-24. Schematic interpretation of the structure of the cytoplasmic membrane. Phospholipids (PL) are arranged in a bilayer such that the polar portions (circles) face outward and the nonpolar portions (filaments) face inward. IP = integral protein; PP = peripheral protein. Note that some integral proteins, such as transport proteins, are believed to span the membrane.

The Cytoplasmic Membrane

pholipids (about 20 to 30 percent) and proteins (about 60 to 70 percent). The phospholipids form a bilayer in which most of the proteins are tenaciously held (integral proteins) (Fig. 5-24); these proteins can be removed only by destruction of the membrane, as with treatment by detergents. Other proteins are only loosely attached (peripheral proteins) and can be removed by mild treatments such as osmotic shock. The lipid matrix of the membrane has fluidity, allowing the components to move around laterally. This fluidity appears to be essential for various membrane functions and is dependent on factors such as temperature and on the proportion of unsaturated fatty acids to saturated fatty acids present in the phospholipids.

A significant difference exists between the phospholipids of eubacteria and those of archaeobacteria. In eubacteria the phospholipids are phosphoglycerides, in which straight-chain fatty acids are ester-linked to glycerol (Fig. 5-25). In archaeobacteria, the lipids are polyisoprenoid branched-chain lipids, in which long-chain branched alcohols (phytanols) are ether-linked to glycerol (Fig. 5-25).

The cytoplasmic membrane is a hydrophobic barrier to penetration by most water-soluble molecules. However, specific proteins in the membrane allow, indeed facilitate, the passage of small molecules (i.e., nutrients and waste products) across the membrane; these transport systems are discussed in Chap. 11. The cytoplasmic membrane also contains various enzymes involved in respiratory metabolism and in synthesis of capsular and cell-wall components; moreover, because of its impermeability to protons (hydrogen ions), the cytoplasmic membrane is the site of generation of the protonmotive force—the force that drives ATP synthesis in many organisms, certain nutrient transport systems, and flagellar motility (see Chaps. 10 and 11). Consequently the cytoplasmic

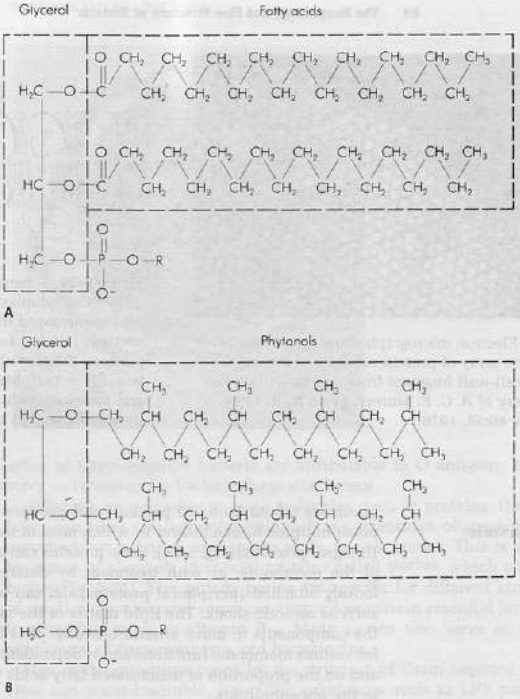

Figure 5-25. (A) Example of a eubacterial phospholipid, showing two unbranched, long-chain fatty acids ester-linked to glycerol. (B) Example of an archaeobacterial phospholipid, showing two branched phytanol chains that are ether-linked to glycerol. (R is any of several compounds such as ethanolamine, choline, serine, inositol, or glycerol.)

membrane is an extremely important functional structure, and damage to it by physical or chemical agents can result in the death of the cell.

Proteins are synthesized within the cell, but some can pass across the cytoplasmic membrane barrier to the outside; examples of such exported molecules are the protein components of cell walls (e.g., porins or lipoproteins) or the exocellular enzymes that are secreted by many bacteria into their culture medium, such as penicillinases, proteinases and amylases. Other proteins made within the cell may pass into the cytoplasmic membrane and remain there (e.g., enzymes such as cytochromes and membrane-bound dehydrogenases). The mechanism by which transport of these proteins occurs into or across the cytoplasmic membrane is unknown. A related question is: How does a cell "know" which of the many kinds of proteins within the cell to transport out of the cell? This question has been partially answered: The genes that code for these proteins carry a message that results in the addition of a sequence of about 20 extra amino acids (the **signal peptide**) to the proteins during their synthesis

within the cell. Unlike ordinary proteins, proteins carrying a signal peptide are destined to be transported into or across the cytoplasmic membrane. According to one hypothesis, special membrane proteins might bind the signal peptide at the inner surface of the cytoplasmic membrane and form a channel by which the protein can traverse the membrane. Whatever its function, the signal peptide is subsequently removed by a proteolytic enzyme and does not appear in the final, transported protein.

Protoplasts, Spheroplasts

Protoplasts

A protoplast is that portion of a bacterial cell consisting of the cytoplasmic membrane and the cell material bounded by it. Protoplasts can be prepared from Gram-positive bacteria by treating the cells with an enzyme such as lysozyme, which selectively dissolves the cell wall, or by culturing the bacteria in the presence of an antibiotic such as penicillin, which prevents the formation of the cell wall. In either case, the osmotic pressure of the medium must be sufficiently high to protect the organisms from bursting. Bacteria normally occur in hypotonic environments (i.e., environments having a lower osmotic pressure than that within the bacterial cells) and they continually take up water by osmosis; thus, they tend to expand, pressing the cytoplasmic membrane tightly against the rigid cell wall. In the absence of a rigid cell wall, there is nothing to prevent the continued expansion and eventual bursting of a protoplast. This bursting can be prevented by preparing protoplasts in an isotonic medium, i.e., in a medium that has an osmotic pressure similar to that of the protoplast. Such osmotically protected protoplasts are soft and fragile and are spherical, regardless of the original shape of the cell.

Spheroplasts

Round, osmotically fragile forms of Gram-negative bacteria can be prepared by procedures similar to those used for the protoplasts of Gram-positive bacteria. However, the cell walls of Gram-negative bacteria differ from those of Gram-positive bacteria by possessing an outer membrane. Although the peptidoglycan of the cell wall may be destroyed by lysozyme or its synthesis inhibited by antibiotics, the flexible outer membrane of the cell wall remains (Fig. 5-20B). Because the treated cell has two membranes, the cytoplasmic membrane of the protoplast plus the outer membrane of the cell wall, the cell is called a spheroplast rather than a protoplast.

Some bacteria, the mycoplasmas, never have cell walls and are bounded by only a cytoplasmic membrane; therefore, they have many of the properties of protoplasts, yet they manage to thrive nonetheless. Most mycoplasmas are parasites of animals, plants, or arthropods, and therefore live in osmotically favorable or isotonic environments. Some are able to attain a degree of rigidity by incorporating cholesterol into their cytoplasmic membranes. Most mycoplasmas have a more or less spherical shape, but one genus, *Spiroplasma*, consists of helical cells. How such cells are able to maintain this shape in the absence of a cell wall is unknown.

Membranous Intrusions and Intracellular Membrane Systems

Bacterial cells do not contain membrane-enclosed organelles corresponding to the mitochondria and chloroplasts of eucaryotic cells. However, bacteria may have specialized invaginations of the cytoplasmic membrane that can increase their surface area for certain functions.

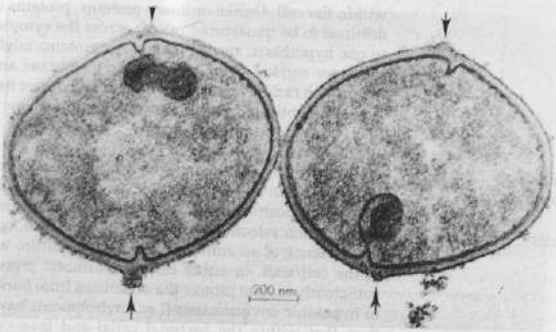

Figure 5-26. Thin section of the Gram-positive bacterium *Streptococcus faecalis*, showing the beginning stages of cell division occurring beneath a thickened equatorial ridge of the cell wall (arrows). A central mesosome (m) is present in each cell and is seen to be a complex invagination of the cytoplasmic membrane. Nuclear material (n) appears as a light, fibrillar area. *(Courtesy of J. M. Garland, A. R. Archibald and J. M. Baddiley, J Gen Microbiol* 89:73, *1975.)*

Many bacteria, especially Gram-positive bacteria, possess membrane invaginations in the form of systems of convoluted tubules and vesicles termed mesosomes. Those known as central mesosomes penetrate deeply into the cytoplasm, are located near the middle of the cell, and seem to be attached to the cell's nuclear material; they are thought to be involved in DNA replication and cell division (Fig. 5-26). In contrast, peripheral mesosomes show only a shallow penetration into the cytoplasm, are not restricted to a central location, and are not associated with nuclear material; they seem to be involved in export of exocellular enzymes such as penicillinase.

Extensive intracellular membrane systems occur in methane-oxidizing bacteria, in certain chemoautotrophic bacteria (Fig. 5-27), and in nearly all phototrophic bacteria. They serve to increase surface area for various metabolic activities. For example, in phototrophic bacteria they are the site of the photosynthetic apparatus of the cell; the infoldings provide a large surface area to accommodate a high content of light-absorbing pigments. In the phototrophs known as cyanobacteria, special intracellular membranes (thylakoids) occur that seem to be separate from the cytoplasmic membrane.

The Cytoplasm

The cell material bounded by the cytoplasmic membrane may be divided into (1) the cytoplasmic area, granular in appearance and rich in the macromolecular RNA-protein bodies known as ribosomes, on which proteins are synthesized; (2) the chromatinic area, rich in DNA; and (3) the fluid portion with dissolved substances. Unlike animal or plant cells, there is no endoplasmic reticulum to which ribosomes are bound; some ribosomes are free in the cytoplasm, and others, especially those involved in the synthesis of proteins to be transported out of the cell, are associated with the inner surface of the cytoplasmic membrane. When the ribosomes of procaryotes undergo sedimentation in a centrifuge, they have a sedimentation coefficient of 70 Svedberg units (70S) and are composed of two subunits, a 50S and a 30S subunit. This is in contrast to the

ribosomes of eucaryotic organisms, which have a sedimentation coefficient of 80S and are composed of a 60S and a 40S subunit.

Cytoplasmic Inclusions and Vacuoles

Concentrated deposits of certain substances are detectable in the cytoplasm of some bacteria. Volutin granules, also known as metachromatic granules, are composed of polyphosphate. They stain an intense reddish-purple color with dilute methylene blue and can be observed by light microscopy. By electron microscopy they appear as round, dark areas (Fig. 5-28). Volutin serves as a reserve source of phosphate. Another polymer often found in aerobic bacteria, especially under high-carbon, low-nitrogen culture conditions, is a chloroform-soluble, lipidlike material, poly-β-hydroxybutyrate (PHB), which can serve as a reserve carbon and energy source. PHB granules can be stained with lipid-soluble dyes such as Nile blue. By electron microscopy they appear as clear round areas (Fig. 5-28). Polysaccharide granules, i.e., glycogen, can be stained brown with iodine. By electron microscopy they appear as dark granules (Fig. 5-28). Another type of inclusion is represented by the intracellular globules of elemental sulfur that may accumulate in certain bacteria growing in environments rich in hydrogen sulfide.

Some bacteria that live in aquatic habitats form gas vacuoles that provide buoyancy. By light microscopy these are bright, refractile bodies; by electron microscopy they are seen to have a regular shape: hollow, rigid cylinders with more or less conical ends and having a striated protein boundary. This boundary is impermeable to water, but the various dissolved gases in the culture medium can penetrate it to fill the cavity. The identifying feature of gas vacuoles is that they can be made to collapse under pressure and thereby lose their refractility.

Nuclear Material

In contrast to eucaryotic cells, bacterial cells contain neither a distinct membrane-enclosed nucleus nor a mitotic apparatus. However, they do contain an

Figure 5-27. Electron micrograph of a thin section of a chemoautotrophic bacterium, Nitrosococcus oceanus, showing an extensive intracellular membrane system. (*Courtesy of S. W. Watson.*)

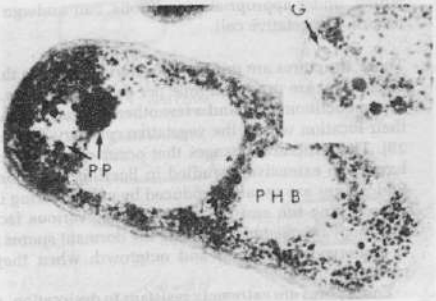

Figure 5-28. Thin section of *Pseudomonas pseudoflava* showing polyphosphate (volutin) granules (PP), poly-β-hydroxybutyrate granules (PHB), and glycogenlike granules (G). (*Courtesy of G. Auling, M. Reh and H. G. Schlegel, Int J Syst bacteriol* **28**:82, 1978.)

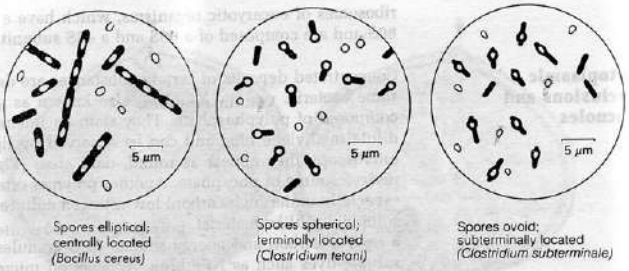

Figure 5-29. Drawings showing the location, size, and shape of endospores in cells of various species of *Bacillus* and *Clostridium* (Erwin F. Lessel, illustrator.)

area near the center of the cell that is regarded as a nuclear structure, and the DNA of the cell is confined to this area. Because it is not a discrete nucleus, this nebulous structure has been designated by such terms as the nucleoid; the chromatin body; the nuclear equivalent; and even the bacterial chromosome, since it consists of a single, circular DNA molecule in which all the genes are linked. The nucleoid can be made visible under the light microscope by Feulgen staining, which is specific for DNA. By electron microscopy it appears as a light area with a delicate fibrillar structure (for example, see Figs. 5-22 and 5-26). The behavior of the nucleoid in growing, dividing bacteria has been observed by use of phase-contrast microscopy with a medium having a high refractive index.

SPORES AND CYSTS

Certain species of bacteria produce spores, either within the cell (endospores) or external to the cell (exospores). The spore is a metabolically dormant form which, under appropriate conditions, can undergo germination and outgrowth to form a vegetative cell.

Endospores

These structures are unique to bacteria. They are thick-walled, highly refractile bodies that are produced (one per cell) by *Bacillus*, *Clostridium*, *Sporosarcina*, *Thermoactinomyces*, and a few other genera. The shapes of endospores and also their location within the vegetative cell vary depending on the species (Fig. 5-29). The structural changes that occur during the development of endospores have been extensively studied in *Bacillus* and *Clostridium* species (Fig. 5-30). Endospores are usually produced by cells growing in rich media but which are approaching the end of active growth. Various factors such as aging or heat treatment are needed to activate the dormant spores (i.e., permit them to be able to undergo germination and outgrowth when they are placed in a suitable medium).

Endospores are extremely resistant to desiccation, staining, disinfecting chemicals, radiation, and heat. For example, the endospores of *Clostridium botulinum* type A have been reported to resist boiling for several hours. The degree of heat resistance of endospores varies with the bacterial species, but most can resist treatment at 80°C for at least 10 minutes. What causes this heat resistance has

been a subject of intense study, but the explanation is still not clear. During sporulation, a dehydration process occurs in which most of the water in the developing spore is expelled; the resulting dehydrated state may be an important factor for heat resistance.

All endospores contain large amounts of dipicolinic acid (DPA), a unique compound that is undetectable in the vegetative cells yet can account for 10 to 15 percent of the spore's dry weight. It occurs in combination with large amounts of calcium and is probably located in the core, i.e., in the central part of the spore. The calcium-DPA complex may possibly play a role in the heat resistance of endospores. Synthesis of DPA and the uptake of calcium occur during advanced stages of sporulation.

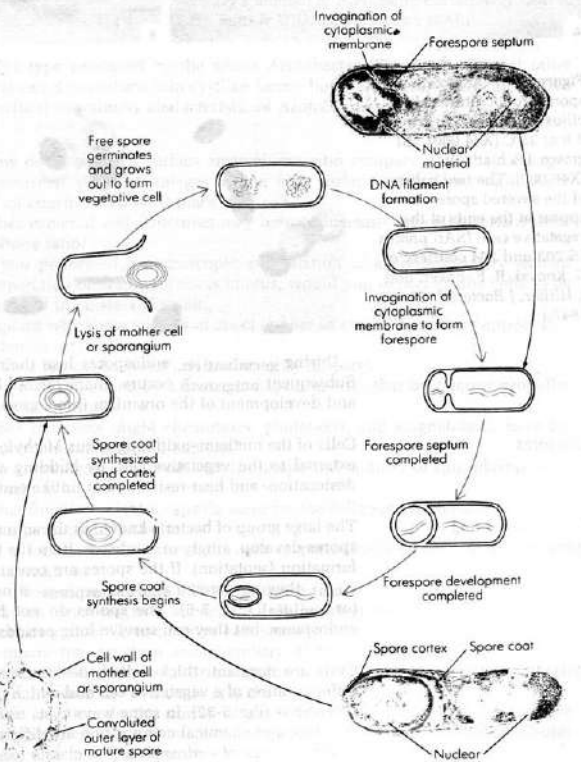

Figure 5-30. Structural changes in the bacterial cell during sporulation. (*Erwin F. Lessel, illustrator; redrawn, with modifications from L. E. Hawker and A. H. Linton, Microorganisms—Function, Form and Environment, 2d ed., University Park Press, Baltimore, 1979.*)

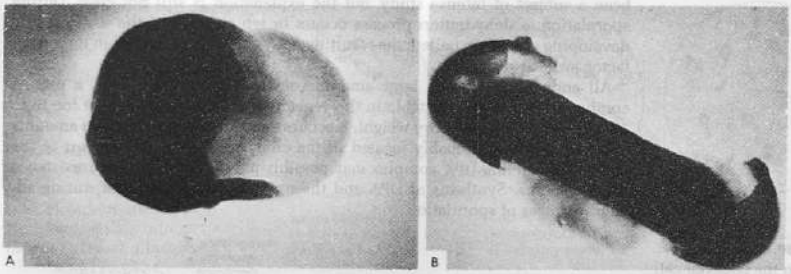

Figure 5-31. Outgrowth of spores from cultures of *Bacillus mycoides*: (A) grown 2 h at 35°C (X44,000), (B) grown 1¾ h at 35°C (X46,000). The two halves of the severed spore coat appear at the ends of the vegetative cell. (*SAB photos LS 203 and 204 courtesy of G. Knaysi, R. F. Baker, and J. Hillier, J Bacteriol,* **53**:525, 1947.)

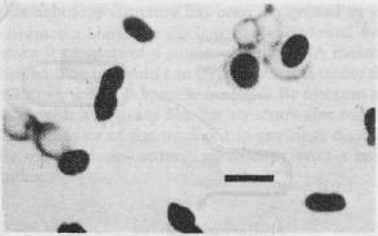

Figure 5-32. Outgrowth of cysts from cultures of an *Azotobacter* strain. Vegetative cells are also evident. [*Courtesy of Y.-T. Tchan and P. B. New, from N. R. Krieg (ed.), Bergey's Manual of Systematic Bacteriology, vol. 1, Williams & Wilkins, Baltimore, 1984.*]

During germination, endospores lose their resistance to heat and staining. Subsequent outgrowth occurs, characterized by synthesis of new cell material and development of the organism into a growing cell (Fig. 5-31).

Exospores

Cells of the methane-oxidizing genus *Methylosinus* form exospores, i.e., spores external to the vegetative cell, by budding at one end of the cell. These are desiccation- and heat-resistant, but unlike endospores they do not contain DPA.

Conidiospores and Sporangiospores

The large group of bacteria known as the actinomycetes form branching hyphae; spores develop, singly or in chains, from the tips of these hyphae by crosswall formation (septation). If the spores are contained in an enclosing sac (sporangium), they are termed sporangiospores; if not, they are called conidiospores (or conidia) (Fig. 5-6). The spores do not have the high heat resistance of endospores, but they can survive long periods of drying.

Cysts

Cysts are dormant, thick-walled, desiccation-resistant forms that develop by differentiation of a vegetative cell and which can later germinate under suitable conditions (Fig. 5-32). In some ways cysts resemble endospores; however, their structure and chemical composition are different and they do not have the high heat resistance of endospores. The classic example of a cyst is the structurally

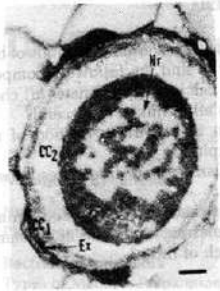

Figure 5-33. Fine structure of an *Azotobacter* cyst. The exosporium (Ex) and the two layers of exine (CC_1 and CC_2) are visible. A nuclear region (Nr) and a cytoplasmic region containing ribosomes are observable within the central body. [*Courtesy of Y.-T. Tchan and P. B. New, from N. R. Krieg (ed.), Bergey's Manual of Systematic Bacteriology, vol. 1, Williams & Wilkins, Baltimore, 1984.*]

complex type produced by the genus *Azotobacter* (Fig. 5-33). Several other bacteria can differentiate into cystlike forms, but these seem to lack the degree of structural complexity characteristic of *Azotobacter* cysts.

QUESTIONS

1 How does the cell's surface area/volume ratio compare with that of larger organisms? What advantages does a high surface area/volume ratio offer? What constraints does it place on a cell?
2 What bacterial cell structures may help to increase the cell's surface area/volume ratio?
3 If you performed a microscopic examination of an appropriately stained preparation of *Staphylococcus aureus*, would you expect all the cells to be arranged in clusters? Explain.
4 Explain why some species of cocci appear as chains but others appear in a cuboidal arrangement.
5 Draw a typical bacterial cell and identify all parts.
6 Contrast propulsion by a bacterial flagellum with that by a screw propeller on a submarine.
7 What functions might chemotaxis, phototaxis, and magnetotaxis have for bacteria in their natural habitats?
8 What problems associated with the shape and motility of spiroplasmas still remain to be solved?
9 What function might a capsule serve for the following bacteria?
   (*a*) a pathogenic bacterium
   (*b*) a soil bacterium where the soil is periodically subjected to drought conditions
   (*c*) a bacterium living in a flowing stream
10 Why are Gram-negative eubacteria usually much easier to disrupt by sonic oscillation than Gram-positive eubacteria?
11 Compare the structure and chemistry of the cell walls of Gram-positive eubacteria versus those of Gram-negative eubacteria. List some major differences between the cell walls of archaeobacteria versus those of eubacteria.
12 What function do the porins of the outer membrane of a Gram-negative eubacterial cell wall serve? What functions do cytoplasmic membrane/outer membrane adhesions serve?

13 In what kinds of bacteria and in what kinds of bacterial cell structures would we be most likely to find the following compounds: (*a*) peptidoglycan, (*b* teichoic acids, (*c*) calcium dipicolinate, (*d*) cholesterol, (*e*) lipopolysaccharide, (*f*) phytanols ether-linked to glycerol?

14 Is spore formation in bacteria a method of reproduction or a means of multiplication? Explain.

15 What are the similarities and differences between protoplasts and spheroplasts?

16 Is it proper to refer to bacterial cells as containing a typical nucleus? Explain.

17 Name several cytoplasmic inclusions or substances. What function might be associated with each of these?

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