CHAPTER 2

# Structure of Bacterial Cells

## CHAPTER CONTENTS



## SHAPE & SIZE OF BACTERIA

Bacteria are classified by shape into three basic groups: **cocci, bacilli,** and **spirochetes** (Figure 2–1). The cocci are round, the bacilli are rods, and the spirochetes are spiral-shaped. Some bacteria are variable in shape and are said to be **pleomorphic** (many-shaped). The shape of a bacterium is determined by its rigid cell wall. The microscopic appearance of a bacterium is one of the most important criteria used in its identification.

In addition to their characteristic shapes, the arrangement of bacteria is important. For example, certain cocci occur in pairs (**diplococci**), some in chains (**streptococci**), and others in grapelike clusters (**staphylococci**). These arrangements are determined by the orientation and degree of attachment of the bacteria at the time of cell division. The arrangement of rods and spirochetes is medically less important and is not described in this introductory chapter.

Bacteria range in size from about 0.2 to 5 µm (Figure 2–2). The smallest bacteria (*Mycoplasma*) are about the same size as the largest viruses (poxviruses) and are the smallest organisms capable of existing outside a host. The longest bacteria rods are the size of some yeasts and human red blood cells (7 µm).

## STRUCTURE OF BACTERIA

The structure of a typical bacterium is illustrated in Figure 2–3, and the important features of each component are presented in Table 2–1.

### Cell Wall

The cell wall is the outermost component common to all bacteria (except *Mycoplasma* species, which are bounded by a cell membrane, not a cell wall). Some bacteria have surface features external to the cell wall, such as a capsule,

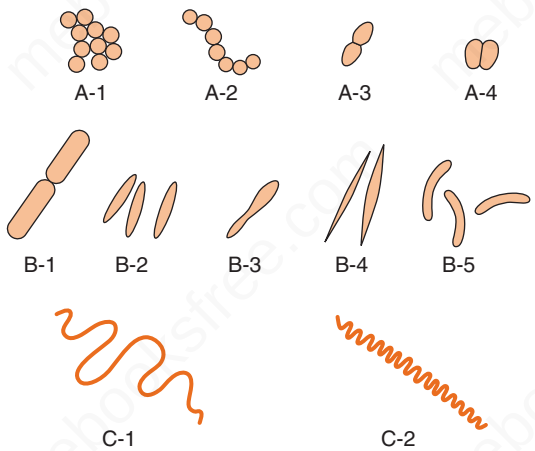


**FIGURE 2–1** Bacterial morphology. **A:** Cocci in clusters (e.g., *Staphylococcus;* A-1); chains (e.g., *Streptococcus;* A-2); in pairs with pointed ends (e.g., *Streptococcus pneumoniae;* A-3); in pairs with kidney bean shape (e.g., *Neisseria;* A-4). **B:** Rods (bacilli): with square ends (e.g., *Bacillus;* B-1); with rounded ends (e.g., *Salmonella;* B-2); club-shaped (e.g., *Corynebacterium;* B-3); fusiform (e.g., *Fusobacterium;* B-4); comma-shaped (e.g., *Vibrio;* B-5). **C:** Spirochetes: relaxed coil (e.g., *Borrelia;* C-1); tightly coiled (e.g., *Treponema;* C-2).

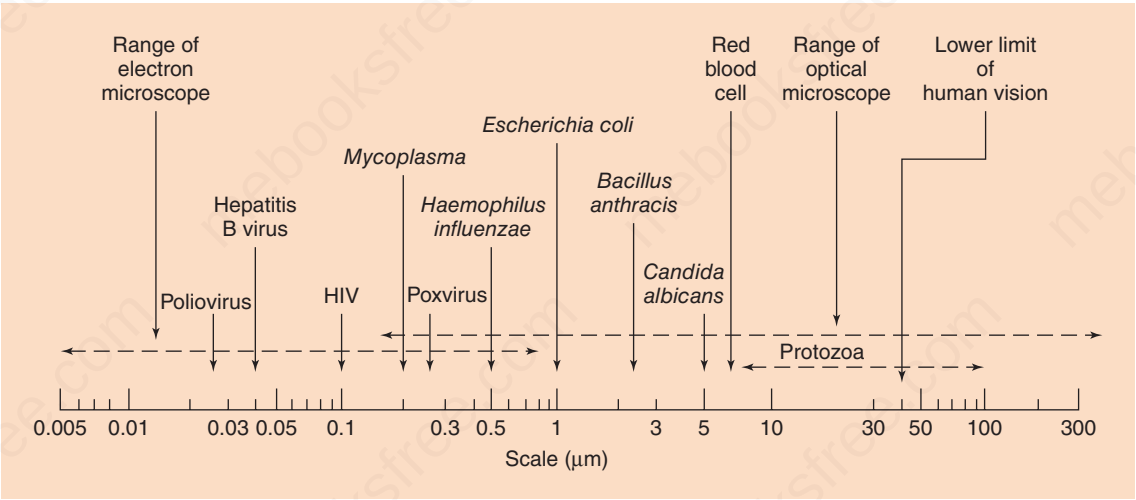


**FIGURE 2–2** Sizes of representative bacteria, viruses, yeasts, protozoa, and human red cells. The bacteria range in size from *Mycoplasma,* the smallest, to *Bacillus anthracis,* one of the largest. The viruses range from poliovirus, one of the smallest, to poxviruses, the largest. Yeasts, such as *Candida albicans,* are generally larger than bacteria. Protozoa have many different forms and a broad size range. HIV, human immunodeficiency virus. (Reproduced with permission from Joklik WK et al. *Zinsser Microbiology*. 20th ed. Originally published by Appleton & Lange. Copyright 1992, McGraw-Hill.)

flagella, and pili, which are less common components and are discussed next.

The cell wall is located external to the cytoplasmic membrane and is composed of **peptidoglycan** (see page 6). The peptidoglycan provides structural support and maintains the characteristic shape of the cell.

### Cell Walls of Gram-Positive and Gram-Negative Bacteria

The structure, chemical composition, and thickness of the cell wall differ in gram-positive and gram-negative bacteria (Table 2–2, Figure 2–4A, and "Gram Stain" box).

(1) The peptidoglycan layer is much thicker in gram-positive than in gram-negative bacteria. Many gram-positive bacteria also have fibers of teichoic acid that protrude outside the peptidoglycan, whereas gram-negative bacteria do not have teichoic acids.

(2) In contrast, the gram-negative bacteria have a complex outer layer consisting of lipopolysaccharide, lipoprotein, and phospholipid. Lying between the outer-membrane layer and the cytoplasmic membrane in gram-negative bacteria is the **periplasmic space,** which is the site, in some species, of enzymes called β-lactamases that degrade penicillins and other β-lactam drugs.

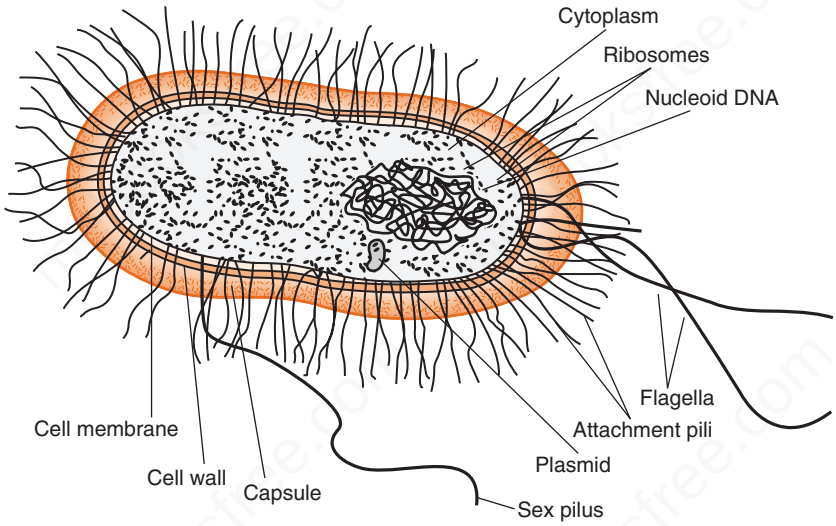


**FIGURE 2–3** Bacterial structure. (Reproduced with permission from Ryan K et al. *Sherris Medical Microbiology*. 4th ed. Copyright 2004, McGraw-Hill.)

**TABLE 2–1 Bacterial Structures**

| Structure | Chemical Composition | Function |
|---|---|---|
| **Essential components** | | |
| Cell wall | | |
| Peptidoglycan | Glycan (sugar) backbone with peptide side chains that are cross-linked | Gives rigid support, protects against osmotic pressure, is the site of action of penicillins and cephalosporins, and is degraded by lysozyme |
| Outer membrane of gram-negative bacteria | Lipid A | Toxic component of endotoxin |
| | Polysaccharide | Major surface antigen used frequently in laboratory diagnosis |
| Surface fibers of gram-positive bacteria | Teichoic acid | Major surface antigen but rarely used in laboratory diagnosis |
| Plasma membrane | Lipoprotein bilayer without sterols | Site of oxidative and transport enzymes |
| Ribosome | RNA and protein in 50S and 30S subunits | Protein synthesis; site of action of aminoglycosides, erythromycin, tetracyclines, and chloramphenicol |
| Nucleoid | DNA | Genetic material |
| Mesosome | Invagination of plasma membrane | Participates in cell division and secretion |
| Periplasm | Space between plasma membrane and outer membrane | Contains many hydrolytic enzymes, including β-lactamases |
| **Nonessential components** | | |
| Capsule | Polysaccharide[1] | Protects against phagocytosis |
| Pilus or fimbria | Glycoprotein | Two types: (1) mediates attachment to cell surfaces; (2) sex pilus mediates attachment of two bacteria during conjugation |
| Flagellum | Protein | Motility |
| Spore | Keratinlike coat, dipicolinic acid | Provides resistance to dehydration, heat, and chemicals |
| Plasmid | DNA | Contains a variety of genes for antibiotic resistance and toxins |
| Granule | Glycogen, lipids, polyphosphates | Site of nutrients in cytoplasm |
| Glycocalyx | Polysaccharide | Mediates adherence to surfaces |

[1]Except in *Bacillus anthracis,* in which it is a polypeptide of D-glutamic acid.

The cell wall has several other important properties:

(1) In gram-negative bacteria, it contains **endotoxin,** a lipopolysaccharide (see pages 9 and 44).

(2) Its polysaccharides and proteins are antigens that are useful in laboratory identification.

(3) Its **porin** proteins play a role in facilitating the passage of small, hydrophilic molecules into the cell. Porin proteins in the outer membrane of gram-negative bacteria act as a channel to allow the entry of essential substances such as sugars, amino acids, vitamins, and metals as well as many antimicrobial drugs such as penicillins.

### Cell Walls of Acid-Fast Bacteria

Mycobacteria (e.g., *Mycobacterium tuberculosis*) have an unusual cell wall, resulting in their inability to be Gram-stained (Figure 2–4B). These bacteria are said to be **acid-fast** because they resist decolorization with acid–alcohol after being stained with carbolfuchsin. This property is related to the high concentration of lipids, called **mycolic acids,** in the cell wall of mycobacteria.

Note that *Nocardia asteroides* is **weakly acid-fast**. The meaning of the term "weakly" is that if the acid-fast staining process uses a weaker solution of hydrochloric acid to decolorize than that used in the stain for Mycobacteria, then *N. asteroides* will *not* decolorize. However, if the regular-strength hydrochloric acid is used, *N. asteroides* will decolorize.

In view of their importance, three components of the cell wall (i.e., peptidoglycan, lipopolysaccharide, and teichoic acid) are discussed in detail here.

**TABLE 2–2 Comparison of Cell Walls of Gram-Positive and Gram-Negative Bacteria**

| Component | Gram-Positive Cells | Gram-Negative Cells |
|---|---|---|
| Peptidoglycan | Thicker; multilayer | Thinner; single layer |
| Teichoic acids | Yes | No |
| Lipopolysaccharide (endotoxin) | No | Yes |

### Peptidoglycan

Peptidoglycan is a complex, interwoven network that surrounds the entire cell and is composed of a single covalently linked macromolecule. It is found *only* in bacterial cell walls. It provides rigid support for the cell, is important in maintaining the characteristic shape of the cell, and allows the cell to withstand media of low osmotic pressure, such as water. A representative segment of the peptidoglycan layer is shown in Figure 2–5. The term **peptidoglycan** is derived from the peptides and the sugars (glycan) that

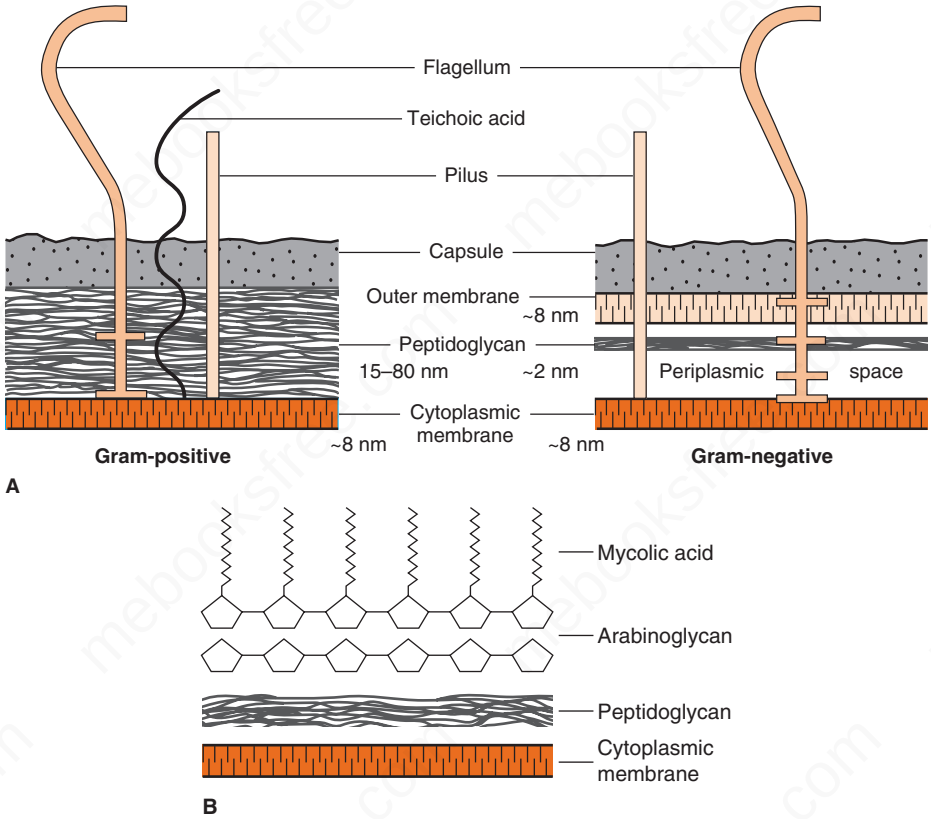


**FIGURE 2–4** **A:** Cell walls of gram-positive and gram-negative bacteria. Note that the peptidoglycan in gram-positive bacteria is much thicker than in gram-negative bacteria. Note also that only gram-negative bacteria have an outer membrane containing endotoxin (lipopolysaccharide [LPS]) and have a periplasmic space where β-lactamases are found. Several important gram-positive bacteria, such as staphylococci and streptococci, have teichoic acids. (Reproduced with permission from Ingraham JL, Maaløe O, Neidhardt FC. *Growth of the Bacterial Cell.* Sinauer Associates; 1983.) **B:** Cell wall of *Mycobacterium tuberculosis*: Note the layers of mycolic acid and arabinoglycan that are present in members of the genus *Mycobacterium* but not in most other genera of bacteria.

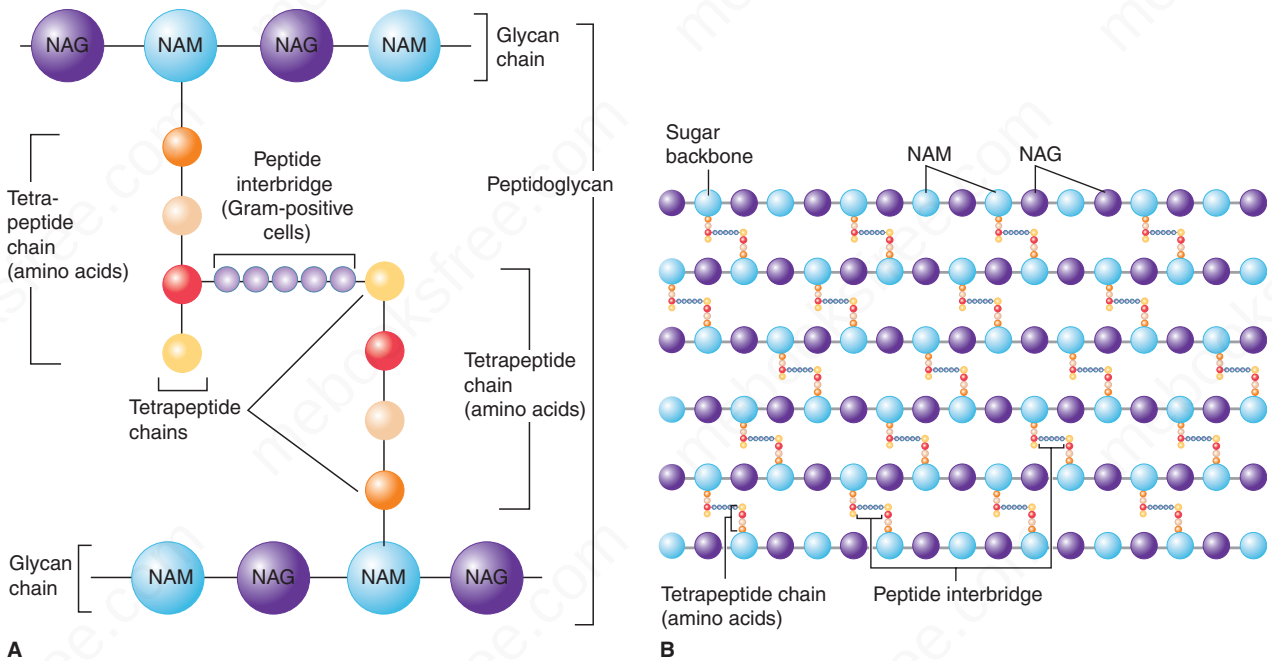


**FIGURE 2–5** Peptidoglycan structure. **A:** Peptidoglycan is composed of a glycan chain (NAM and NAG), a tetrapeptide chain, and a cross-link (peptide interbridge). **B:** In the cell wall, the peptidoglycan forms a multilayered, three-dimensional structure. NAG, *N*-acetylglucosamine; NAM, *N*-acetylmuramic acid. (Reproduced with permission from Nester EW et al. *Microbiology: A Human Perspective*. 6th ed. Copyright 2009, McGraw-Hill.)

## GRAM STAIN

This staining procedure, developed in 1884 by the Danish physician Christian Gram, is the most important procedure in microbiology. It separates most bacteria into two groups: the gram-positive bacteria, which stain blue, and the gram-negative bacteria, which stain red. The Gram stain involves the following four-step procedure:

(1) The crystal violet dye stains all cells blue/purple.

(2) The iodine solution (a mordant) is added to form a crystal violet–iodine complex; all cells continue to appear blue.

(3) The organic solvent, such as acetone or ethanol, extracts the blue dye complex from the lipid-rich, thin-walled gram-negative bacteria to a greater degree than from the lipid-poor, thick-walled gram-positive bacteria. The gram-negative organisms appear colorless; the gram-positive bacteria remain blue.

(4) The red dye safranin stains the decolorized gram-negative cells red/pink; the gram-positive bacteria remain blue.

The Gram stain is useful in two ways:

(1) In the identification of many bacteria.

(2) In influencing the choice of antibiotic because, in general, gram-positive bacteria are more susceptible to penicillin G than are gram-negative bacteria.

However, not all bacteria can be seen in the Gram stain. Table 2–3 lists the medically important bacteria that cannot be seen and describes the reason why. The alternative microscopic approach to the Gram stain is also described.
Note that it takes approximately 100,000 bacteria/mL to see 1 bacterium per microscopic field using the oil immersion (100×) lens. So the sensitivity of the Gram stain procedure is low. This explains why a patient's blood is rarely stained immediately but rather is incubated in blood cultures overnight to allow the bacteria to multiply. One important exception to this is meningococcemia in which very high concentrations of Neisseria meningitidis can occur in the blood.

make up the molecule. Synonyms for peptidoglycan are **murein** and **mucopeptide**.

Figure 2–5 illustrates the carbohydrate backbone, which is composed of alternating *N*-acetylmuramic acid and *N*-acetylglucosamine molecules. Attached to each of the muramic acid molecules is a tetrapeptide consisting of both D- and L-amino acids, the precise composition of which differs from one bacterium to another. Two of these amino acids are worthy of special mention: diaminopimelic acid, which is unique to bacterial cell walls, and D-alanine, which is involved in the cross-links between the tetrapeptides and in the action of penicillin. Note that this tetrapeptide contains the rare D-isomers of amino acids; most proteins contain the L-isomer. The other important component in this network is the peptide cross-link between the two tetrapeptides. The cross-links vary among species; in *Staphylococcus aureus,* for example, five glycines link the terminal D-alanine to the penultimate L-lysine.

Because peptidoglycan is present in bacteria but not in human cells, it is a good target for antibacterial drugs. Several of these drugs, such as penicillins, cephalosporins, and vancomycin, inhibit the synthesis of peptidoglycan by inhibiting the transpeptidase that makes the cross-links between the two adjacent tetrapeptides (see Chapter 10).

**Lysozyme,** an enzyme present in human tears, mucus, and saliva, can cleave the peptidoglycan backbone by breaking its glycosyl bonds, thereby contributing to the natural resistance of the host to microbial infection. Lysozyme-treated bacteria may swell and rupture as a result of the entry of water into the cells, which have a high internal osmotic pressure. However, if the lysozyme-treated cells are in a solution with the same osmotic pressure as that of the

**TABLE 2–3 Medically Important Bacteria That Cannot Be Seen in the Gram Stain**

| Name | Reason | Alternative Microscopic Approach |
|---|---|---|
| Mycobacteria, including *M. tuberculosis* | Too much lipid in cell wall so dye cannot penetrate | Acid-fast stain |
| *Treponema pallidum* | Too thin to see | Dark-field microscopy or fluorescent antibody |
| *Mycoplasma pneumoniae* | No cell wall; very small | None |
| *Legionella pneumophila* | Poor uptake of red counterstain | Prolong time of counterstain |
| Chlamydiae, including *C. trachomatis* | Intracellular; very small | Inclusion bodies in cytoplasm |
| Rickettsiae | Intracellular; very small | Giemsa or other tissue stains |

bacterial interior, they will survive as spherical forms, called **protoplasts**, surrounded only by a cytoplasmic membrane.

### Lipopolysaccharide

The lipopolysaccharide (LPS) of the outer membrane of the cell wall of gram-negative bacteria is **endotoxin**. It is responsible for many of the features of disease, such as fever and shock (especially hypotension), caused by these organisms (see page 44). It is called endotoxin because it is an integral part of the cell wall, in contrast to exotoxins, which are actively secreted from the bacteria. The constellation of symptoms caused by the endotoxin of one gram-negative bacterium is similar to another, but the severity of the symptoms can differ greatly. In contrast, the symptoms caused by exotoxins of different bacteria are usually quite different.

The LPS is composed of three distinct units (Figure 2–6):

(1) A phospholipid called lipid A, which is responsible for the toxic effects.

(2) A core polysaccharide of five sugars linked through ketodeoxyoctulonate (KDO) to lipid A.

(3) An outer polysaccharide consisting of up to 25 repeating units of three to five sugars. This outer polymer is the important somatic, or O, antigen of several gram-negative bacteria that is used to identify certain organisms in the clinical laboratory. Some bacteria, notably members of the genus *Neisseria*, have an outer lipooligosaccharide (LOS) containing very few repeating units of sugars.

### Teichoic Acid

Teichoic acids are **fibers located in the outer layer of the gram-positive cell wall** and extend from it. They are composed of polymers of either glycerol phosphate or ribitol phosphate. Some polymers of glycerol teichoic acid penetrate the peptidoglycan layer and are covalently linked to the lipid in the cytoplasmic membrane, in which case they are called **lipoteichoic acid;** others anchor to the muramic acid of the peptidoglycan.

The medical importance of teichoic acids lies in their ability to **induce inflammation and septic shock when caused by certain gram-positive bacteria**; that is, they activate the same pathways as does endotoxin (LPS) in gram-negative bacteria. Teichoic acids also mediate the attachment of staphylococci to mucosal cells. Gram-negative bacteria do not have teichoic acids.

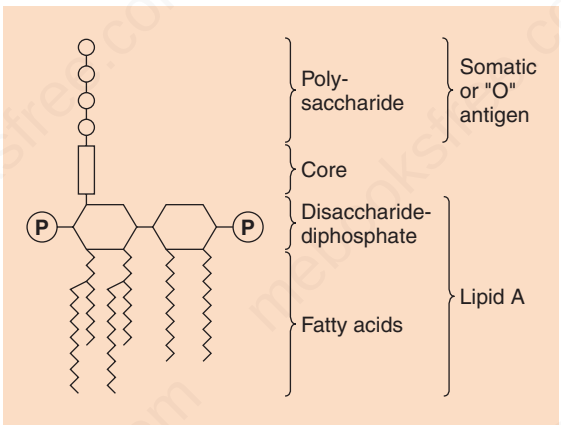


**FIGURE 2–6** Endotoxin (lipopolysaccharide [LPS]) structure. The O-antigen polysaccharide is exposed on the exterior of the cell, whereas the lipid A faces the interior. (Reproduced with permission from Brooks GF et al. *Medical Microbiology.* 19th ed. Originally published by Appleton & Lange. Copyright 1991, McGraw-Hill.)

## Cytoplasmic Membrane

Just inside the peptidoglycan layer of the cell wall lies the cytoplasmic membrane, which is composed of a phospholipid bilayer similar in microscopic appearance to that in eukaryotic cells. They are chemically similar, but eukaryotic membranes contain sterols, whereas prokaryotes generally do not. The only prokaryotes that have sterols in their membranes are members of the genus *Mycoplasma.* The membrane has four important functions: (1) active transport of molecules into the cell, (2) energy generation by oxidative phosphorylation, (3) synthesis of precursors of the cell wall, and (4) secretion of enzymes and toxins.

## Cytoplasm

The cytoplasm has two distinct areas when seen in the electron microscope:

(1) An amorphous matrix that contains ribosomes, nutrient granules, metabolites, and plasmids.

(2) An inner, nucleoid region composed of DNA.

### Ribosomes

Bacterial ribosomes are the site of protein synthesis as in eukaryotic cells, but they differ from eukaryotic ribosomes in size and chemical composition. Bacterial ribosomes are 70S in size, with 50S and 30S subunits, whereas eukaryotic ribosomes are 80S in size, with 60S and 40S subunits. The differences in both the ribosomal RNAs and proteins constitute the basis of the selective action of several antibiotics that inhibit bacterial, but not human, protein synthesis (see Chapter 10).

### Granules

The cytoplasm contains several different types of granules that serve as storage areas for nutrients and stain characteristically with certain dyes. For example, volutin is a reserve of high energy stored in the form of polymerized metaphosphate. It appears as a "metachromatic" granule since it stains red with methylene blue dye instead of blue as one would expect. Metachromatic granules are a characteristic feature of *Corynebacterium diphtheriae,* the cause of diphtheria.

### Nucleoid

The nucleoid is the area of the cytoplasm in which DNA is located. The DNA of prokaryotes is a single, circular molecule that has a molecular weight (MW) of approximately

$2 \times 10^9$ and contains about 2000 genes. (By contrast, human DNA has approximately 100,000 genes.) Because the nucleoid contains no nuclear membrane, no nucleolus, no mitotic spindle, and no histones, there is little resemblance to the eukaryotic nucleus. One major difference between bacterial DNA and eukaryotic DNA is that bacterial DNA has no introns, whereas eukaryotic DNA does.

## Plasmids

Plasmids are extrachromosomal, double-stranded, circular DNA molecules that are capable of replicating independently of the bacterial chromosome. Although plasmids are usually extrachromosomal, they can be integrated into the bacterial chromosome. Plasmids occur in both gram-positive and gram-negative bacteria, and several different types of plasmids can exist in one cell:

(1) **Transmissible** plasmids can be transferred from cell to cell by conjugation (see Chapter 4 for a discussion of conjugation). They are large (MW 40–100 million), since they contain about a dozen genes responsible for synthesis of the sex pilus and for the enzymes required for transfer. They are usually present in a few (1–3) copies per cell.

(2) **Nontransmissible** plasmids are small (MW 3–20 million), since they do not contain the transfer genes; they are frequently present in many (10–60) copies per cell.

Plasmids carry the genes for the following functions and structures of medical importance:

(1) Antibiotic resistance, which is mediated by a variety of enzymes, such as the beta-lactamase of *S. aureus, Escherichia coli,* and *Klebsiella pneumoniae.*

(2) Exotoxins, such as the enterotoxins of *E. coli*, anthrax toxin of *Bacillus anthracis*, exfoliative toxin of *S. aureus* and tetanus toxin of *Clostridium tetani*.

(3) Pili (fimbriae), which mediate the adherence of bacteria to epithelial cells.

(4) Resistance to heavy metals, such as mercury, the active component of some antiseptics (e.g., merthiolate and mercurochrome), and silver, which is mediated by a reductase enzyme.

(5) Resistance to ultraviolet light, which is mediated by DNA repair enzymes.

Other plasmid-encoded products of interest are as follows:

(1) Bacteriocins are toxic proteins produced by certain bacteria that are lethal for other bacteria. Two common mechanisms of action of bacteriocins are (i) degradation of bacterial cell membranes by producing pores in the membrane and (ii) degradation of bacterial DNA by DNAse. Examples of bacteriocins produced by medically important bacteria are colicins made by *E. coli* and pyocins made by *Pseudomonas aeruginosa*. Bacteria that produce bacteriocins have a selective advantage in the competition for food sources over those that do not. However, the medical importance of bacteriocins is that they may be useful in treating infections caused by antibiotic-resistant bacteria.

(2) Nitrogen fixation enzymes in *Rhizobium* in the root nodules of legumes.

(3) Tumors caused by *Agrobacterium* in plants.

(4) Several antibiotics produced by *Streptomyces.*

(5) A variety of degradative enzymes that are produced by *Pseudomonas* and are capable of cleaning up environmental hazards such as oil spills and toxic chemical waste sites.

## Transposons

Transposons are pieces of DNA that move readily from one site to another either within or between the DNAs of bacteria, plasmids, and bacteriophages. Because of their unusual ability to move, they are nicknamed "jumping genes." Some transposons move by replicating their DNA and inserting the new copy into another site (replicative transposition), whereas others are excised from the site without replicating and then inserted into the new site (direct transposition). Transposons can code for drug-resistant enzymes, toxins, or a variety of metabolic enzymes and can either cause mutations in the gene into which they insert or alter the expression of nearby genes.

Transposons typically have four identifiable domains. On each end is a short DNA **sequence of inverted repeats,** which are involved in the integration of the transposon into the recipient DNA. The second domain is the gene for the transposase, which is the enzyme that mediates the excision and integration processes. The third region is the gene for the repressor that regulates the synthesis of both the transposase and the protein encoded by the fourth domain, which, in many cases, is an enzyme mediating antibiotic resistance (Figure 2–7). Note that for simplicity, the repressor gene is not shown in Figure 2–7.

Antibiotic resistance genes are transferred from one bacterium to another primarily by **conjugation** (see Chapter 4). This transfer is mediated primarily by plasmids, but some transposons, called **conjugative transposons,** are capable of transferring antibiotic resistance as well.

In contrast to plasmids or bacterial viruses, transposons are not capable of independent replication; they replicate as part of the DNA in which they are integrated. More than one transposon can be located in the DNA; for example, a plasmid can contain several transposons carrying drug-resistant

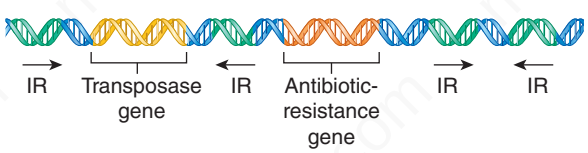


**FIGURE 2–7** Transposon genes. This transposon is carrying a drug-resistance gene. IR, inverted repeat. (Reproduced with permission from Willey JM et al. *Prescott's Principles of Microbiology.* New York: McGraw-Hill, 2009.)

genes. **Insertion sequences** are a type of transposon that has fewer bases (800–1500 base pairs), since they do not code for their own integration enzymes. They can cause mutations at their site of integration and can be found in multiple copies at the ends of larger transposon units.

## Structures Outside the Cell Wall

### Capsule

The capsule is a gelatinous layer covering the entire bacterium. It is composed of polysaccharide, except in the anthrax bacillus, which has a capsule of polymerized D-glutamic acid. The sugar components of the polysaccharide vary from one species of bacteria to another and frequently determine the serologic type (serotype) within a species. For example, there are 84 different serotypes of *Streptococcus pneumoniae,* which are distinguished by the antigenic differences of the sugars in the polysaccharide capsule.

The capsule is important for four reasons:

(1) It is a determinant of virulence of many bacteria since it limits the ability of phagocytes to engulf the bacteria. Negative charges on the capsular polysaccharide repel the negatively charged cell membrane of the neutrophil and prevent it from ingesting the bacteria. Variants of encapsulated bacteria that have lost the ability to produce a capsule are usually nonpathogenic.

(2) Specific identification of an organism can be made by using antiserum against the capsular polysaccharide. In the presence of the homologous antibody, the capsule will swell greatly. This swelling phenomenon, which is used in the clinical laboratory to identify certain organisms, is called the **quellung reaction.**

(3) Capsular polysaccharides are used as the antigens in certain vaccines because they are capable of eliciting protective antibodies. For example, the purified capsular polysaccharides of 23 types of *S. pneumoniae* are present in the current vaccine.

(4) The capsule may play a role in the adherence of bacteria to human tissues, which is an important initial step in causing infection.

### Flagella

Flagella are long, whiplike appendages that move the bacteria toward nutrients and other attractants, a process called **chemotaxis.** The long filament, which acts as a propeller, is composed of many subunits of a single protein, flagellin, arranged in several intertwined chains. The energy for movement, the **proton motive force,** is provided by adenosine triphosphate (ATP), derived from the passage of ions across the membrane.

Flagellated bacteria have a characteristic number and location of flagella: some bacteria have one, and others have many; in some, the flagella are located at one end, and in others, they are all over the outer surface. Only certain bacteria have flagella. Many rods do, but most cocci do not and are therefore nonmotile. Spirochetes move by using a flagellumlike structure called the **axial filament,** which wraps around the spiral-shaped cell to produce an undulating motion.

Flagella are medically important for two reasons:

(1) Some species of motile bacteria (e.g., *E. coli* and *Proteus* species) are common causes of urinary tract infections. Flagella may play a role in pathogenesis by propelling the bacteria up the urethra into the bladder.

(2) Some species of bacteria (e.g., *Salmonella* species) are identified in the clinical laboratory by the use of specific antibodies against flagellar proteins.

### Pili (Fimbriae)

Pili are hairlike filaments that extend from the cell surface. They are shorter and straighter than flagella and are composed of subunits of pilin, a protein arranged in helical strands. They are found mainly on gram-negative organisms.

Pili have two important roles:

(1) They mediate the **attachment** of bacteria to specific receptors on the human cell surface, which is a necessary step in the initiation of infection for some organisms. Mutants of *Neisseria gonorrhoeae* that do not form pili are nonpathogens.

(2) A specialized kind of pilus, the sex pilus, forms the attachment between the male (donor) and the female (recipient) bacteria during conjugation (see Chapter 4).

### Glycocalyx (Slime Layer)

The glycocalyx is a polysaccharide coating that is secreted by many bacteria. It covers surfaces like a film and allows the bacteria to **adhere firmly** to various structures (e.g., skin, heart valves, prosthetic joints, and catheters). The glycocalyx is an important component of biofilms (see page 37). The medical importance of the glycocalyx is illustrated by the finding that it is the glycocalyx-producing strains of *P. aeruginosa* that cause respiratory tract infections in cystic fibrosis patients, and it is the glycocalyx-producing strains of *Staphylococcus epidermidis* and viridans streptococci that cause endocarditis. The glycocalyx also mediates adherence of certain bacteria, such as *Streptococcus mutans,* to the surface of teeth. This plays an important role in the formation of plaque, the precursor of dental caries.

## Bacterial Spores

These highly resistant structures are formed in response to adverse conditions by two genera of medically important gram-positive rods: the genus *Bacillus,* which includes the agent of anthrax, and the genus *Clostridium,* which includes the agents of tetanus and botulism. Spore formation (sporulation) occurs when nutrients, such as sources of carbon and nitrogen, are depleted (Figure 2–8). The spore forms

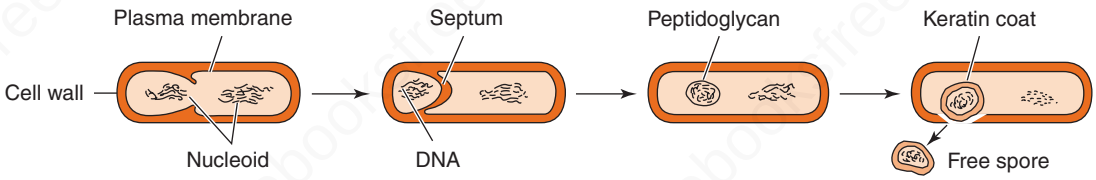


**FIGURE 2–8** Bacterial spores. The spore contains the entire DNA genome of the bacterium surrounded by a thick, resistant coat.

inside the cell and contains bacterial DNA, a small amount of cytoplasm, cell membrane, peptidoglycan, very little water, and most importantly, a thick, keratinlike coat that is responsible for the remarkable resistance of the spore to heat, dehydration, radiation, and chemicals. This resistance may be mediated by **dipicolinic acid,** a calcium ion chelator found only in spores.

Once formed, the spore has no metabolic activity and can remain dormant for many years. Upon exposure to water and the appropriate nutrients, specific enzymes degrade the coat, water and nutrients enter, and germination into a potentially pathogenic bacterial cell occurs. Note that this differentiation process is *not* a means of reproduction since one cell produces one spore that germinates into one cell.

The medical importance of spores lies in their **extraordinary resistance to heat** and chemicals. As a result of their resistance to heat, sterilization cannot be achieved by boiling. Steam heating under pressure (autoclaving) at 121°C, for at least 15 minutes, is required to ensure the sterility of products for medical use. Spores are often not seen in clinical specimens recovered from patients infected by spore-forming organisms because the supply of nutrients is adequate.

Table 2–4 describes the medically important features of bacterial spores.

**TABLE 2–4 Important Features of Spores and Their Medical Implications**

| Important Features of Spores | Medical Implications |
|---|---|
| Highly resistant to heating; spores are not killed by boiling (100°C), but are killed at 121°C. | Medical supplies must be heated to 121°C for at least 15 minutes to be sterilized. |
| Highly resistant to many chemicals, including most disinfectants, due to the thick, keratinlike coat of the spore. | Only solutions designated as sporicidal will kill spores. |
| They can survive for many years, especially in the soil. | Wounds contaminated with soil can be infected with spores and cause diseases such as tetanus (*C. tetani*) and gas gangrene (*C. perfringens*). |
| They exhibit no measurable metabolic activity. | Antibiotics are ineffective against spores because antibiotics act by inhibiting certain metabolic pathways of bacteria. Also, spore coat is impermeable to antibiotics. |
| Spores form when nutrients are insufficient but then germinate to form bacteria when nutrients become available. | Spores are not often found at the site of infections because nutrients are not limiting. Bacteria rather than spores are usually seen in Gram-stained smears. |
| Spores are produced by members of only two genera of bacteria of medical importance, *Bacillus* and *Clostridium*, both of which are gram-positive rods. | Infections transmitted by spores are caused by species of either *Bacillus or Clostridium*. |

## PEARLS

### *Shape & Size*

- Bacteria have three shapes: **cocci** (spheres), **bacilli** (rods), and **spirochetes** (spirals).
- Cocci are arranged in three patterns: pairs (diplococci), chains (streptococci), and clusters (staphylococci).
- The size of most bacteria ranges from 1 to 3 µm. *Mycoplasma,* the smallest bacteria (and therefore the **smallest cells**), are 0.2 µm. Some bacteria, such as *Borrelia*, are as long as 10 µm; that is, they are longer than a human red blood cell, which is 7 µm in diameter.

### *Bacterial Cell Wall*

- All bacteria have a cell wall composed of **peptidoglycan** except *Mycoplasma,* which are surrounded *only* by a cell membrane.
- Gram-negative bacteria have a **thin** peptidoglycan covered by an outer lipid-containing membrane, whereas gram-positive bacteria have a **thick** peptidoglycan and no outer membrane. These differences explain why gram-negative bacteria lose the stain when exposed to a lipid solvent in the Gram stain process, whereas gram-positive bacteria retain the stain and remain purple.

- The outer membrane of gram-negative bacteria contains **endotoxin** (**lipopolysaccharide, LPS**), the main inducer of septic shock. Endotoxin consists of **lipid A**, which causes the fever and hypotension seen in septic shock, and a polysaccharide called **O antigen**, which is useful in laboratory identification.
- Between the inner cell membrane and the outer membrane of gram-negative bacteria lies the **periplasmic space**, which is the location of **β-lactamases**—the enzymes that degrade β-lactam antibiotics, such as penicillins and cephalosporins.
- Peptidoglycan is found *only* in bacterial cells. It is a network that covers the entire bacterium and gives the organism its shape. It is composed of a sugar backbone (**glycan**) and peptide side chains (**peptido**). The side chains are cross-linked by **transpeptidase**—the enzyme that is inhibited by penicillins and cephalosporins.
- The cell wall of mycobacteria (e.g., *M. tuberculosis*) has **more lipid** than either the gram-positive or gram-negative bacteria. As a result, the dyes used in the Gram stain do not penetrate into (do not stain) mycobacteria. The **acid-fast stain** does stain mycobacteria, and these bacteria are often called acid-fast bacilli (acid-fast rods).
- **Lysozymes** kill bacteria by cleaving the glycan backbone of peptidoglycan.
- The cytoplasmic membrane of bacteria consists of a phospholipid bilayer (without sterols) located just inside the peptidoglycan. It regulates active transport of nutrients into the cell and the secretion of toxins out of the cell.

### *Gram Stain*

- **Gram stain** is the most important staining procedure. Gram-positive bacteria stain *purple,* whereas gram-negative bacteria stain *pink*. This difference is due to the ability of gram-positive bacteria to *retain the crystal violet–iodine complex in the presence of a lipid solvent,* usually acetone–alcohol. Gram-negative bacteria, because they have an outer lipid-containing membrane and thin peptidoglycan, lose the purple dye when treated with acetone–alcohol. They become colorless and then stain pink when exposed to a red dye such as safranin.
- Not all bacteria can be visualized using Gram stain. Some important human pathogens, such as the bacteria that cause tuberculosis and syphilis, cannot be seen using this stain.

### *Bacterial DNA*

- The bacterial genome consists of a **single chromosome of circular DNA** located in the nucleoid.
- **Plasmids** are extrachromosomal pieces of circular DNA that encode both exotoxins and many enzymes that cause antibiotic resistance.
- **Transposons** are small pieces of DNA that move frequently between chromosomal DNA and plasmid DNA. They carry antibiotic-resistant genes.

### *Structures External to the Cell Wall*

- **Capsules** are antiphagocytic; that is, they limit the ability of neutrophils to engulf the bacteria. Almost all capsules are composed of *polysaccharide;* the polypeptide capsule of anthrax bacillus is the only exception. Capsules are also the antigens in several vaccines, such as the pneumococcal vaccine. Antibodies against the capsule neutralize the antiphagocytic effect and allow the bacteria to be engulfed by neutrophils. **Opsonization** is the process by which antibodies enhance the phagocytosis of bacteria.
- **Pili** are filaments of protein that extend from the bacterial surface and mediate **attachment** of bacteria to the surface of human cells. A different kind of pilus, the sex pilus, functions in conjugation (see Chapter 4).
- The **glycocalyx** is a polysaccharide "slime layer" secreted by certain bacteria. It **attaches bacteria firmly** to the surface of human cells and to the surface of catheters, prosthetic heart valves, and prosthetic hip joints.

### *Bacterial Spores*

- **Spores** are medically important because they are **highly heat resistant** and are not killed by many disinfectants. Boiling will *not* kill spores. They are formed by certain gram-positive rods, especially *Bacillus* and *Clostridium* species.
- Spores have a thick, keratinlike coat that allows them to survive for many years, especially in the soil. Spores are formed when nutrients are in short supply, but when nutrients are restored, spores germinate to form bacteria that can cause disease. Spores are *metabolically inactive* but contain DNA, ribosomes, and other essential components.

## SELF-ASSESSMENT QUESTIONS

**1.** The initial step in the process of many bacterial infections is adherence of the organism to mucous membranes. The bacterial component that mediates adherence is the:

**(A)** lipid A
**(B)** nucleoid
**(C)** peptidoglycan
**(D)** pilus
**(E)** plasmid

**2.** In the Gram stain procedure, bacteria are exposed to 95% alcohol or to an acetone/alcohol mixture. The purpose of this step is:

**(A)** to adhere the cells to the slide
**(B)** to retain the purple dye within all the bacteria
**(C)** to disrupt the outer cell membrane so the purple dye can leave the bacteria
**(D)** to facilitate the entry of the purple dye into the gram-negative cells
**(E)** to form a complex with the iodine solution

3. In the process of studying how bacteria cause disease, it was found that a rare mutant of a pathogenic strain failed to form a capsule. Which one of the following statements is the most accurate in regard to this unencapsulated mutant strain?
   **(A)** It was nonpathogenic primarily because it was easily phagocytized.
   **(B)** It was nonpathogenic primarily because it could not invade tissue.
   **(C)** It was nonpathogenic primarily because it could only grow anaerobically.
   **(D)** It was highly pathogenic because it could secrete larger amounts of exotoxin.
   **(E)** It was highly pathogenic because it could secrete larger amounts of endotoxin.
4. *Mycobacterium tuberculosis* stains well with the acid-fast stain, but not with the Gram stain. Which one of the following is the most likely reason for this observation?
   **(A)** It has a large number of pili that absorb the purple dye.
   **(B)** It has a large amount of lipid that prevents entry of the purple dye.
   **(C)** It has a very thin cell wall that does not retain the purple dye.
   **(D)** It is too thin to be seen in the Gram stain.
   **(E)** It has histones that are highly negatively charged.
5. Of the following bacterial components, which one exhibits the most antigenic variation?
   **(A)** Capsule
   **(B)** Lipid A of endotoxin
   **(C)** Peptidoglycan
   **(D)** Ribosome
   **(E)** Spore
6. β-Lactamases are an important cause of antibiotic resistance. Which one of the following is the most common site where β-lactamases are located?
   **(A)** Attached to DNA in the nucleoid
   **(B)** Attached to pili on the bacterial surface
   **(C)** Free in the cytoplasm
   **(D)** Within the capsule
   **(E)** Within the periplasmic space
7. Which one of the following is the most accurate description of the structural differences between gram-positive bacteria and gram-negative bacteria?
   **(A)** Gram-positive bacteria have a thick peptidoglycan layer, whereas gram-negative bacteria have a thin layer.
   **(B)** Gram-positive bacteria have an outer lipid-rich membrane, whereas gram-negative bacteria do not.
   **(C)** Gram-positive bacteria form a sex pilus that mediates conjugation, whereas gram-negative bacteria do not.
   **(D)** Gram-positive bacteria have plasmids, whereas gram-negative bacteria do not.
   **(E)** Gram-positive bacteria have capsules, whereas gram-negative bacteria do not.
8. Bacteria that cause nosocomial (hospital-acquired) infections often produce extracellular substances that allow them to stick firmly to medical devices, such as intravenous catheters. Which one of the following is the name of this extracellular substance?
   **(A)** Axial filament
   **(B)** Endotoxin
   **(C)** Flagella
   **(D)** Glycocalyx
   **(E)** Porin
9. Lysozyme in tears is an effective mechanism for preventing bacterial conjunctivitis. Which one of the following bacterial structures does lysozyme degrade?
   **(A)** Endotoxin
   **(B)** Nucleoid DNA
   **(C)** Peptidoglycan
   **(D)** Pilus
   **(E)** Plasmid DNA
10. Several bacteria that form spores are important human pathogens. Which one of the following is the most accurate statement about bacterial spores?
    **(A)** They are killed by boiling for 15 minutes.
    **(B)** They are produced primarily by gram-negative cocci.
    **(C)** They are formed primarily when the bacterium is exposed to antibiotics.
    **(D)** They are produced by anaerobes only in the presence of oxygen.
    **(E)** They are metabolically inactive yet can survive for years in that inactive state.

## ANSWERS

(1) **(D)**
(2) **(C)**
(3) **(A)**
(4) **(B)**
(5) **(A)**
(6) **(E)**
(7) **(A)**
(8) **(D)**
(9) **(C)**
(10) **(E)**

## PRACTICE QUESTIONS: USMLE & COURSE EXAMINATIONS

Questions on the topics discussed in this chapter can be found in the Basic Bacteriology section of Part XIII: USMLE (National Board) Practice Questions starting on page 709. Also see Part XIV: USMLE (National Board) Practice Examination starting on page 751.