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fourth edition

Examination & Board Review

Medical Microbiology & Immunology

Warren Levinson
Ernest Jawetz

a LANGE medical book

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Warren Levinson, MD, PhD
Professor of Microbiology
Department of Microbiology and Immunology
University of California, San Francisco

Ernest Jawetz, MD, PhD
Professor of Microbiology and Medicine Emeritus
Department of Microbiology and Immunology
University of California, San Francisco

APPLETON & LANGE
Stamford, Connecticut

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Preface

This book is a concise review of the medically important aspects of microbiology. It covers both the basic and clinical aspects of bacteriology, virology, mycology, parasitology, and immunology. Its 2 major aims are (1) to assist those who are preparing for the USMLE (National Boards) and (2) to provide students who are presently taking medical microbiology courses with a brief and flexible source of information.

These aims are achieved by utilizing several different formats, which should make the book useful to students with varying study objectives and learning styles:

(1) A narrative text for complete information.
(2) Summaries of important microorganisms for rapid review of essentials.
(3) Review questions at the end of each chapter.
(4) Sample questions in the USMLE (National Board) style, with answers provided after each group of questions.
(5) A USMLE (National Board) practice examination consisting of 160 questions with answers provided at the end of the examination.
(6) Clinical case discussions to illustrate the relevance of the material to patient problems.

In addition, the following specific features should be emphasized:

(1) The information is presented succinctly, with stress on making it clear, interesting, and up to date.
(2) There is strong emphasis in the text on the clinical application of microbiology and immunology to infectious diseases.
(3) In the clinical bacteriology and virology sections, the organisms are separated into major and minor pathogens. This allows the student to focus on the clinically most important microorganisms.
(4) Key information is summarized in useful review tables. Important concepts are illustrated by figures in color.
(5) The 535 USMLE (National Board) practice questions cover the important aspects of each of the subdisciplines on the USMLE—Bacteriology, Virology, Mycology, Parasitology, and Immunology. These practice questions are in the two formats used in the current USMLE. A separate section containing *extended* matching questions is included. In view of the emphasis placed on clinical relevance in the USMLE, another section provides questions set in a clinical case context.
(6) Brief summaries of medically important microorganisms are presented together in a separate section to facilitate access to the information and to encourage comparison of one organism with another.
(7) Ten clinical cases are presented as unknowns for the reader to analyze in a realistic, problem-solving way. These cases illustrate the importance of basic science information in clinical decision-making.

After teaching both medical microbiology and clinical infectious disease for many years, we believe that students appreciate a book that presents the essential information in a readable, interesting, and varied format. We hope you find this book meets those criteria.

San Francisco
December 1995

Warren E. Levinson, MD, PhD
Ernest Jawetz, MD, PhD

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W. L. gratefully acknowledges the invaluable assistance of his wife, Barbara, in making this book become a reality.

W. L. dedicates this book to his father and mother, who instilled a love of scholarship, the joy of teaching, and the value of being organized.

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Part 1: Basic Bacteriology

Bacteria Compared With Other Microorganisms 1

AGENTS

The agents of human infectious diseases belong to 5 major groups of organisms: bacteria, fungi, protozoa, helminths, and viruses. The bacteria belong to the prokaryote kingdom, the fungi and protozoa are members of the kingdom of protists, and the helminths (worms) are classified in the animal kingdom (Table 1–1). The protists are distinguished from animals and plants by being either unicellular or relatively simple multicellular organisms. The helminths are complex multicellular organisms that are classified as metazoa within the animal kingdom. Taken together, the helminths and the protozoa are commonly called parasites. Viruses are quite distinct from other organisms. They are not cells but can replicate only within cells.

IMPORTANT FEATURES

Many of the essential characteristics of these organisms are described in Table 1–2. One salient feature is that bacteria, fungi, protozoa, and helminths are cellular, whereas viruses are not. This distinction is based primarily on 3 criteria.

(1) Structure. Cells have a nucleus or nucleoid (see below) containing DNA; this is surrounded by cytoplasm, within which proteins are synthesized and energy is generated. Viruses have an inner core of genetic material (either DNA or RNA) but no cytoplasm, and so they depend on host cells to provide the machinery for protein synthesis and energy generation.

(2) Method of replication. Cells replicate either by binary fission or by mitosis, during which one parent cell divides to make 2 progeny cells while retaining its cellular structure. Prokaryotic cells, eg, bacteria, replicate by binary fission, whereas eukaryotic cells replicate by mitosis. In contrast, viruses disassemble, produce many copies of their nucleic acid and protein, and then reassemble into multiple progeny viruses. Furthermore, viruses must replicate within host cells, because, as mentioned above, they lack protein-synthesizing and energy-generating systems. With the exception of rickettsiae and chlamydiae, which are bacteria that also require living host cells for growth, bacteria can replicate extracellularly.

Table 1–1. Biologic relationships of pathogenic microorganisms.

Kingdom	Pathogenic Microorganisms	Type of Cells
Animal	Helminths	Eukaryotic
Plant	None	Eukaryotic
Protist	Protozoa Fungi	Eukaryotic Eukaryotic
Prokaryote	Bacteria	Prokaryotic
	Viruses	Noncellular

Table 1–2. Comparison of medically important organisms.

Characteristic	Viruses	Bacteria	Fungi	Protozoa and Helminths
Cells	No	Yes	Yes	Yes
Approximate diameter (μm)[1]	0.02–0.2	1–5	3–10 (yeasts)	15–25 (trophozoites)
Nucleic acid	Either DNA or RNA	Both DNA and RNA	Both DNA and RNA	Both DNA and RNA
Type of nucleus	None	Prokaryotic	Eukaryotic	Eukaryotic
Ribosomes	Absent	70S	80S	80S
Mitochondria	Absent	Absent	Present	Present
Nature of outer surface	Protein capsid and lipoprotein envelope	Rigid wall containing peptidoglycan	Rigid wall containing chitin	Flexible membrane
Motility	None	Some	None	Most
Method of replication	Not binary fission	Binary fission	Budding or mitosis[2]	Mitosis

[1] For comparison, a human red blood cell has a diameter of 7 μm.
[2] Yeasts divide by budding, whereas molds divide by mitosis.
[3] Helminth cells divide by mitosis, but the organism reproduces itself by complex, sexual life cycles.

(3) Nature of the nucleic acid. Cells contain both DNA and RNA, whereas viruses contain either DNA or RNA but not both.

EUKARYOTES & PROKARYOTES

Cells have evolved into 2 fundamentally different types, **eukaryotic** and **prokaryotic,** which can be distinguished on the basis of their structure and the complexity of their organization. Fungi and protozoa are eukaryotic, whereas bacteria are prokaryotic.

(1) The eukaryotic cell has a true **nucleus** with multiple chromosomes surrounded by a nuclear membrane and uses a mitotic apparatus to ensure equal allocation of the chromosomes to progeny cells.

(2) The **nucleoid** of a prokaryotic cell consists of a single circular molecule of loosely organized DNA lacking a nuclear membrane and mitotic apparatus (Table 1–3).

In addition to the different types of nuclei, the 2 classes of cells are distinguished by several other criteria.

(1) Eukaryotic cells contain **organelles,** such as mitochondria and lysosomes, and larger (80S) ribosomes, whereas prokaryotes contain no organelles and smaller (70S) ribosomes.

Table 1–3. Characteristics of prokaryotic and eukaryotic cells.

Characteristic	Prokaryotic Bacterial Cells	Eukaryotic Human Cells
DNA within a nuclear membrane	No	Yes
Mitotic division	No	Yes
DNA associated with histones	No	Yes
Chromosome number	One	More than one
Membrane-bound organelles such as mitochondria and lysosomes	No	Yes
Size of ribosome	70S	80S
Cell wall containing peptidoglycan	Yes	No

(2) Most prokaryotes have a rigid external cell wall that contains **peptidoglycan,** a polymer of amino acids and sugars, as its unique structural component. Eukaryotes, on the other hand, do not contain peptidoglycan. Either they are bound by a flexible cell membrane or, in the case of fungi, they have a rigid cell wall with chitin, a homopolymer of N-acetylglucosamine, typically forming the framework.

(3) The eukaryotic cell membrane contains **sterols,** whereas no prokaryote, except the wall-less *Mycoplasma,* has sterols in its membranes.

Another criterion by which these organisms can be contrasted is **motility.** Most protozoa and some bacteria are motile, whereas fungi and viruses are nonmotile. The protozoa are a heterogeneous group that possess 3 different organs of locomotion: flagella, cilia, and pseudopods. The motile bacteria move only by means of flagella.

Review Questions

1. What are the differences between bacteria and viruses?
2. Are bacteria prokaryotic or eukaryotic? What are the differences between prokaryotic and eukaryotic cells?
3. What are the similarities and the differences among bacteria, fungi, and protozoa?

Structure of Bacterial Cells 2

SHAPE & SIZE

Bacteria are classified by shape into 3 basic groups: **cocci, bacilli,** and **spirochetes** (Fig 2–1). 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.

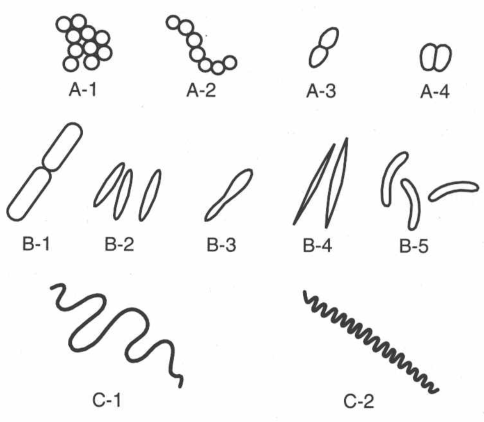

Figure 2–1. Bacterial morphology. *A:* Cocci: in clusters, eg, *Staphylococcus* (A-1); chains, eg, *Streptococcus* (A-2); in pairs with pointed ends, eg, *Streptococcus pneumoniae* (A-3); in pairs with kidney bean shape, eg, *Neisseria* (A-4). ***B:*** Rods: with square ends, eg, *Bacillus* (B-1); with rounded ends, eg, *Salmonella* (B-2); club-shaped, eg, *Corynebacterium* (B-3); fusiform, eg, *Fusobacterium* (B-4); comma-shaped, eg, *Vibrio* (B-5). ***C:*** Spirochetes: relaxed coil, eg, *Borrelia* (C-1); tightly coiled, eg, *Treponema* (C-2). (Modified and reproduced, with permission, from Joklik WK et al: *Zinsser Microbiology,* 20th ed. Appleton & Lange, 1992.)

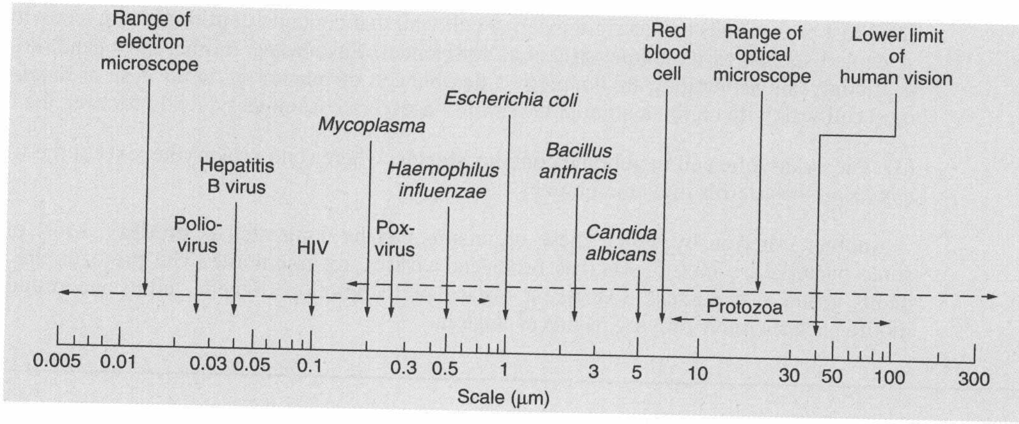

Figure 2–2. Sizes of representative bacteria, viruses, yeasts, protozoa, and human red cells. The bacteria range in size from *Mycoplasma*, the smallest, to *Bacillus anthracis*, one of the largest. The viruses range from poliovirus, one of the smallest, to poxviruses, the largest. Yeasts, such as *C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

Bacteria range in size from about 0.2 to 5 μm (Fig 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 the host. The longest bacteria rods approach the size of some yeasts and human red blood cells (7 μm).

STRUCTURE

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

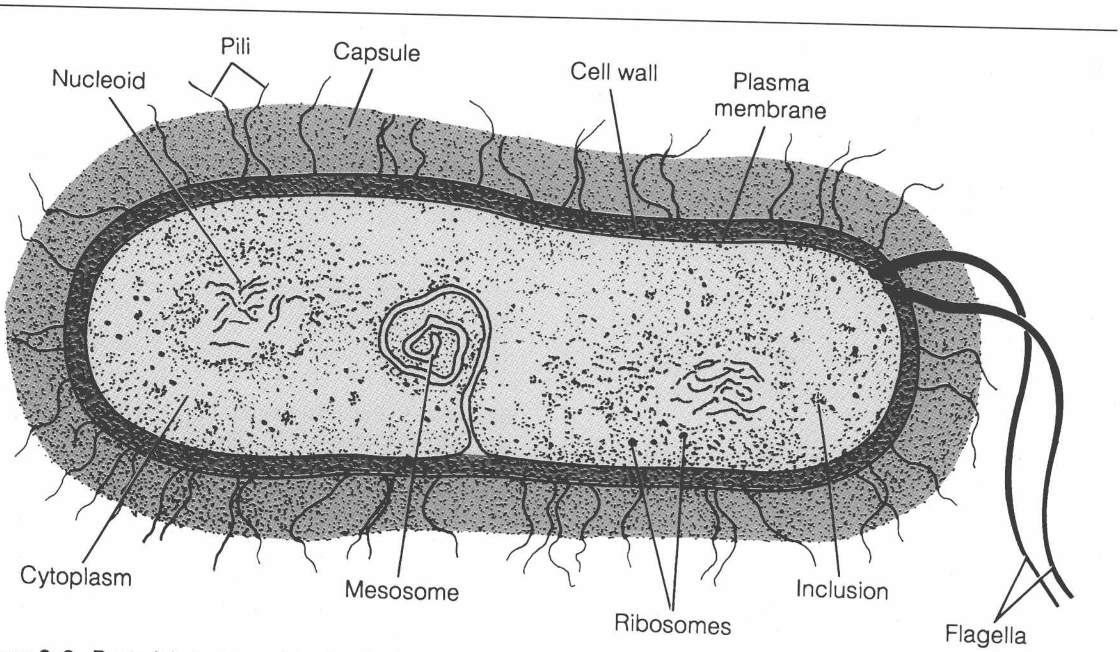

Figure 2–3. Bacterial structure. (Modified and reproduced, with permission, from Tortora G, Funk B, Case C: *Microbiology: An Introduction,* 2nd ed. Benjamin/Cummings, 1986.)

Table 2–1. Bacterial structures.

Structure	Chemical Composition	Function
Essential components		
Cell wall		
Peptidoglycan	Sugar backbone with peptide side chains that are crosslinked	Gives rigid support, protects against osmotic pressure; is the site of action of penicillins and cephalosporins and is degraded by lysozyme.
Outer membrane (gram-positive organisms)	Teichoic acid	Major surface antigen but rarely used in laboratory diagnosis.
Outer membrane (gram-negative organisms)	Lipid A	Toxic component of endotoxin.
	Polysaccharide	Major surface antigen used frequently in laboratory diagnosis.
Cytoplasmic 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, enzymes, and toxins.
Granule	Glycogen, lipids, polyphosphates	Storage sites of food.
Glycocalyx	Polysaccharide	Mediates adherence to surfaces.

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

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

The cell wall is a multilayered structure located external to the cytoplasmic membrane. It is composed of an inner layer of **peptidoglycan** (see p 6) surrounded by an outer membrane that varies in thickness and chemical composition depending upon the bacterial type (Fig 2–4). The peptidoglycan provides structural support and maintains the characteristic shape of the cell.

A. 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 (box and Table 2–2).

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

(2) In contrast, the gram-negative organisms have a complex outer layer consisting of lipopolysaccharide, lipoprotein, and phospholipid. Lying between the outer-membrane layer and the cytoplas-

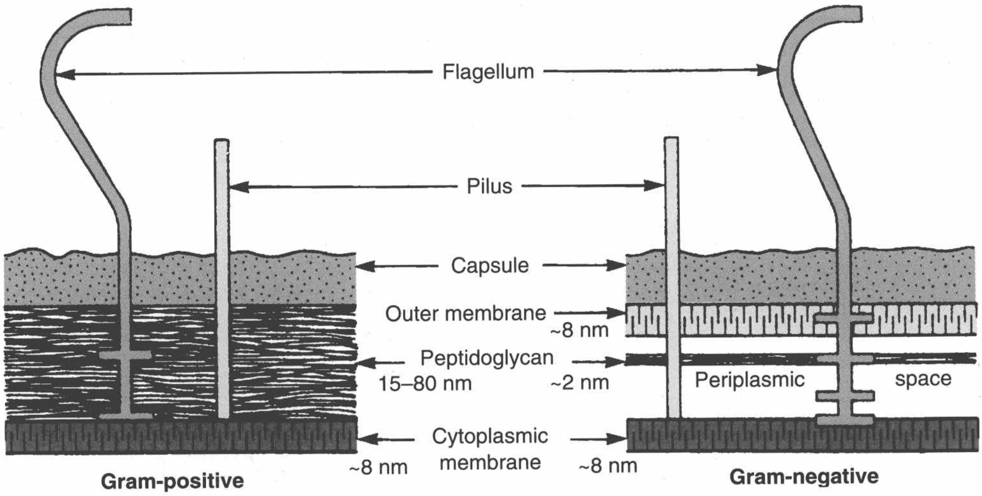

Figure 2–4. Cell walls of gram-positive and gram-negative bacteria. Note that the peptidoglycan in gram-positive bacteria is much thicker than in gram-negative bacteria. (Reproduced, with permission, from Ingraham JL, Maaløe O, Neidhardt FC: *Growth of the Bacterial Cell,* Sinauer Associates, 1983.)

mic membrane in gram-negative bacteria is the **periplasmic space,** which is the site, in some species, of enzymes called beta-lactamases that degrade penicillins and other β-lactam drugs.

The cell wall has several other important properties:

(1) in gram-negative organisms, it contains **endotoxin,** a lipopolysaccharide (see p 31);

(2) its polysaccharides and proteins are antigens that are useful in laboratory identification; and

(3) its **porin** proteins play a role in regulating the passage of small, hydrophilic molecules into the cell. Porin proteins in the outer membrane form a trimer that acts, usually nonspecifically, 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.

B. Cell Walls of Acid-Fast Bacteria: Mycobacteria, eg, *Mycobacterium tuberculosis,* have an unusual cell wall, resulting in their inability to be Gram-stained. These bacteria are said to be **"acid-fast,"** since they resist decolorization with acid-alcohol after being stained with carbolfuchsin. This property is related to the high concentration in the cell wall of lipids called mycolic acids.

In view of their importance, 3 components of the cell wall, ie, peptidoglycan, lipopolysaccharide, and teichoic acid, will be discussed in detail.

C. 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

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
Lipoprotein and phospholipid	No	Yes

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 2 groups: the gram-positive bacteria, which stain blue, and the gram-negative bacteria, which stain red. The Gram stain involves the following 4-step procedure.

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

(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; the gram-positive bacteria remain blue.

The Gram stain is useful in 2 ways:

(1) in the identification of many bacteria, and

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

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 Fig 2–5. The term "peptidoglycan" is derived from the peptides and the sugars (glycan) that make up the molecule. Synonyms for peptidoglycan are murein and mucopeptide.

Fig 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 2 tetrapeptides. The cross-links vary among species; in *Staphylococcus aureus,* for example, 5 glycines link the terminal D-alanine to the penultimate L-lysine.

Since peptidoglycan is present in bacteria but not in human cells, it is a good target for antibacterial drugs. Several of these drugs, such as the penicillins and cephalosporins, inhibit its synthesis (see Chapter 10).

The enzyme **lysozyme,** which is 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 entrance 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 bacterial interior, they will survive as spherical forms, called protoplasts, surrounded only by a cytoplasmic membrane.

D. Lipopolysaccharide (LPS): The LPS of the outer layer 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, caused by these organisms. It is called endotoxin because it is an integral part of the cell wall, in contrast to exotoxins, which are freely released from the bacteria. The pathologic effects of endotoxin are similar irrespective of the organism from which it is derived.

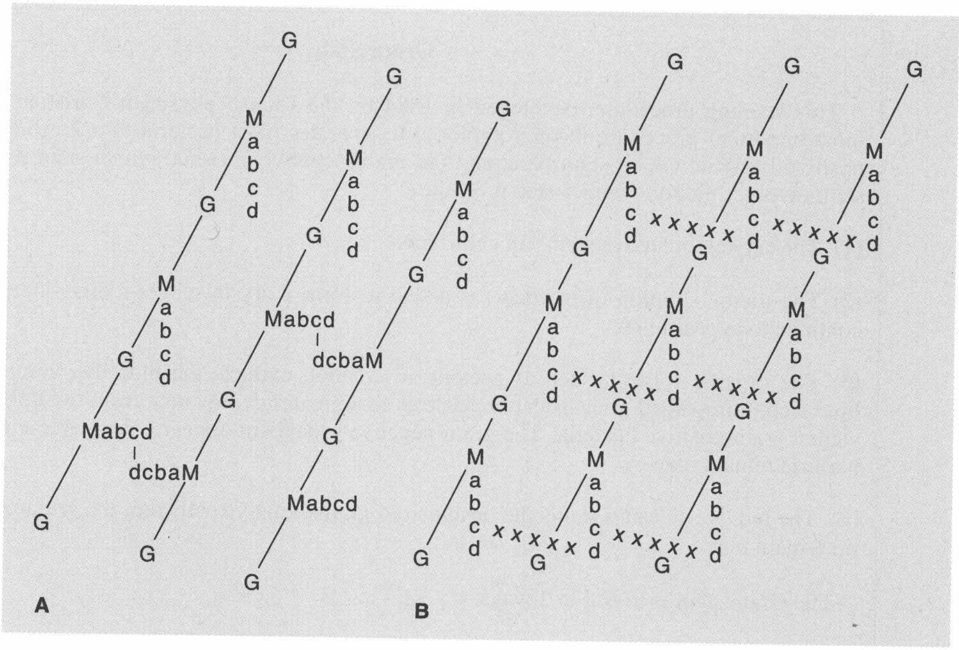

Figure 2–5. Peptidoglycan structure: *Escherichia coli* ***(A)*** has a different cross-link from that of *Staphylococcus aureus* ***(B)***. In *E coli,* c is cross-linked directly to d, whereas in *S aureus,* c and d are cross-linked by five glycines. However, in both organisms the terminal D-alanine is part of the linkage. M, muramic acid; G, glucosamine; a, L-alanine; b, D-glutamic acid; c, diaminopimelic acid ***(A)*** or L-lysine ***(B)***; d, D-alanine; x, pentaglycine bridge. (Modified and reproduced, with permission, from Joklik WK et al: *Zinsser Microbiology,* 20th ed. Appleton & Lange, 1992.)

The LPS is composed of 3 distinct units (Fig 2–6):

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

(2) a core polysaccharide of 5 sugars linked through ketodeoxyoctulonate (KDO) to lipid A; and

(3) an outer polysaccharide consisting of up to 25 repeating units of 3–5 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.

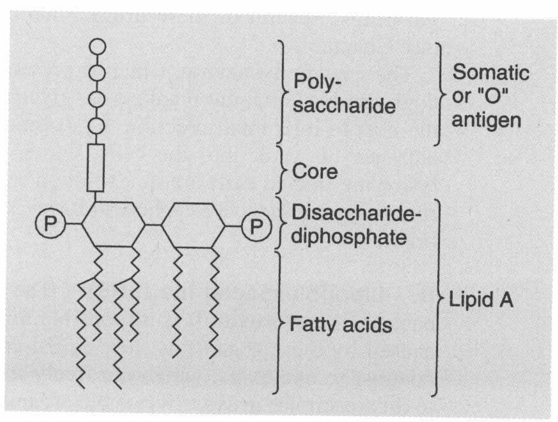

Figure 2–6. Endotoxin (LPS) structure. The O antigen polysaccharide is exposed on the exterior of the cell, whereas the lipid A faces the interior. (Modified and reproduced, with permission, from Brooks GF et al: *Medical Microbiology,* 19th ed. Appleton & Lange, 1991.)