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Microbiology & Immunology

SIXTH EDITION

Louise Hawley
Richard J. Ziegler
Benjamin L. Clarke

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Microbiology and Immunology

SIXTH EDITION

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The authors dedicate this book to their many students who have been a source of stimulation over the years, and to their many colleagues whose research and insight has resulted in the knowledge described herein.

We particularly want to thank Dr. Arthur Johnson, who has retired from both his leadership role as senior author/editor and authorship of the immunology section.



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How to Use this Book

This concise review of microbiology and immunology and its online resources are designed specifically for medical students to successfully prepare for Step 1 of the United States Medical Licensing Examination (USMLE), as well as other examinations. This newest edition remains a succinct description of the most important microbiological and immunological concepts, as well as a review of critical details needed to understand important human infections and the immune system's function and malfunction.

ORGANIZATION

Facilitates Use by Either a Bug Approach or Systems Approach

The book is divided into 12 chapters, starting with basic information and then leading the student quickly to the level of detail and comprehension needed for Step 1. For each major category of microbes (e.g., viruses), there is a fundamental chapter (two for the bacteria) followed by an organ-systems infectious disease approach with critical signs/symptoms, epidemiology, etiology, pathogenesis of infections and immune diseases, and the mechanisms for preventing infection and means of identifying and diagnosing the causative agent. Then an updated Chapter 11 (*Clues for Distinguishing Causative Agents*) presents the diseases a second time, this time utilizing an organ systems-based approach presented by text and great graphic flow-charts starting with symptoms frequently mentioned in case-based questions. Included also are tables listing agents associated with different types of rashes. New to the 6th edition are detailed summary tables of the characteristics and details of the different agents causing meningitis, encephalitis, upper and lower respiratory infections, and pneumonias.

Because many medical schools have switched to a fundamentals block followed by organ system modules, we have created an-online 6th edition *Systems-Based Table of Contents/Guide* which facilitates use in a system-base course by listing both the pages of reading and chapter question-numbers for these courses. This aids faculty using the book in a system-based course and gives the reviewing student options for how they want to organize their review.

The outline format facilitates rapid review of important information. Each chapter is followed by review questions and answers, with explanations that reflect the style and content of the USMLE. These questions are available online as well and can generate systems-based or taxonomic self-quizzes. We have added four separate comprehensive examinations at the end of the book. Each has the same general sub-subject distribution generally found on Step 1 and so may be used as a practice exam and self-assessment tool to help students diagnose their weaknesses prior to, during, and after reviewing microbiology and immunology. The *Comprehensive Exam* questions (accessible online as well) are not mixed with the chapter questions so they can be saved for use after initial study.

Suggestion for increasing your retention: use two cover sheets (one to move down a page and a top one to move left to right) on tables and diagrams to see if you can predict what it is going to say in each section before reading the section.

KEY FEATURES

- Dual approach (bug and system) in one small book along with new online resources allows flexibility in study and self-testing to improve retention.
- An expanded, resource-rich Chapter 11 which has new System Summary Tables at the end.
- Updated four-color tables and figures summarize essential information for quick recall.
- End-of-chapter review tests feature updated USMLE-style questions.
- Four USMLE comprehensive exams with explanations are included in blocks of similar size to USMLE Step 1.
- Updated and current information is provided in all chapters.

We wish you well in your study and exams!

*Louise Hawley, PhD
Richard J. Ziegler, PhD
Benjamin L. Clarke, PhD*

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General Properties of Microorganisms

I. THE MICROBIAL WORLD

A. Microorganisms.

1. Belong to the Protista biologic kingdom.
2. Include some eukaryotes and prokaryotes, viruses, viroids, and prions.
3. Are classified according to their structure, chemical composition, and biosynthetic and genetic organization.

B. Eukaryotic cells (Table 1.1).

1. Contain organelles and a nucleus bounded by a nuclear membrane.
2. Contain complex phospholipids, sphingolipids, histones, and sterols.
3. Lack a cell wall. (Plant cells and fungi have a cell wall.)
4. Have multiple diploid chromosomes and nucleosomes.
5. Have relatively long-lived mRNA formed from the processing of precursor mRNA, which contains exons and introns.
6. Have 80S ribosomes and uncoupled transcription and translation.
7. Include **protozoa** and **fungi**.
 - a. Organisms in **kingdom Protozoa** are classified into seven phyla; three of these phyla (Sarcostigophora, Apicomplexa, and Ciliophora) contain medically important species that are human parasites.
 - b. Organisms in **kingdom Fungi**:
 - (1) Are **eukaryotic** cells with a complex carbohydrate cell wall.
 - (2) Have ergosterol as the dominant membrane sterol.
 - (3) May be **monomorphic**, existing only as single-celled **yeasts** or multicellular, filamentous **molds**.
 - (4) May be **dimorphic**, existing as **yeasts or molds depending on temperature and nutrition**.
 - (5) May have both asexual and sexual reproduction capabilities. Deuteromycetes, or Fungi Imperfecti, have no known sexual stages.

C. Prokaryotic cells (see Table 1.1).

1. Have no organelles, no membrane-enclosed nucleus, and no histones; in rare cases, they contain complex phospholipids, sphingolipids, and sterols.
2. Have 70S ribosomes composed of 30S and 50S subunits.
3. Have a cell wall composed of peptidoglycan-containing muramic acid.
4. Are haploid with a single chromosome.
5. Have short-lived, unprocessed mRNA.
6. Have coupled transcription and translation.

table 1.1 Components of Microbial Cells

Structure	Composition	Cell Type					
		Fungi	Gram-Positive Bacteria	Gram-Negative Bacteria	Mycoplasmas	Chlamydia*	Rickettsia*
Envelope capsule	Polysaccharide or polypeptide	- or +**	+ or -	+ or -	-	-	-
Wall							
Chitin	Poly-N-acetylglucosamine	+	-	-	-	-	-
Peptidoglycan	Poly-N-acetylglucosamine-N-acetylmuramic acid tetrapeptide	-	+	+	-	-	+
Periplasm	Proteins and oligosaccharides	-	-	+	-	+	+
Lipoprotein	Lipoprotein	-	-	+	-	+	+
Outer membrane	Proteins, phospholipids, and lipopolysaccharide	-	-	+	-	+	+
Appendages							
Pili	Protein	-	+ or -	+ or -	-	-	-
Flagella	Protein	-	+ or -	+ or -	-	-	-
Cell membrane	Proteins and phospholipids	+ (plus ergosterol)	+	+	+	+	+
Cytosol							
Organelles	Protein, phospholipids, and nucleic acids	+	-	-	-	-	-
80S ribosomes	Protein and RNA	+	-	-	-	-	-
70S ribosomes	Protein and RNA	-	+	+	+	+	+
Genetic material							
Nucleus	Protein, phospholipids, and nucleic acids	+	-	-	-	-	-
Nucleoid	Protein and nucleic acids	-	+	+	+	+	+
Plasmids	DNA	+ or -	+ or -	+ or -	+ or -	+ or -	+ or -
Transposons	DNA	+	+	+	-	+	+
Spores							
Reproductive spores	All cellular components	+	-	-	-	-	-
Endospores	All cellular components plus dipicollic acid	-	+ or -	-	-	-	-

*Obligate intracellular pathogens.

***Cryptococcus neoformans* is the only medically important fungus with a capsule.

7. Include **typical bacteria**, **mycoplasmas**, and **obligate intracellular bacteria**.
 - a. **Typical bacteria:**
 - (1) Have a **cell wall**.
 - (2) May be normal flora or may be pathogenic in humans.
 - (3) Do not have a sexual growth cycle; however, some can produce asexual spores.
 - b. **Mycoplasmas:**
 - (1) Are the smallest and simplest of the bacteria that are self-replicating.
 - (2) Lack a cell wall.
 - (3) Are the only prokaryotes that contain **sterols**.
 - c. **Obligate intracellular bacteria** include **Rickettsia** and **Chlamydia**.
 - (1) **Rickettsia** are incapable of self-replication and depend on the host cell for adenosine triphosphate (ATP) production.
 - (2) **Chlamydia** are bacteria-like pathogens with a complex growth cycle involving intracellular and extracellular forms. They depend on the host cell for ATP production.

D. Viruses.

1. Are not cells and are not visible with the light microscope.
2. Are **obligate intracellular parasites**.
3. Contain no organelles or biosynthetic machinery, except for a few enzymes.
4. Contain either RNA or DNA as genetic material.
5. Are called **bacteriophages** (or **phages**) if they have a bacterial host.

E. Viroids.

1. Are not cells and are not visible with the light microscope.
2. Are **obligate intracellular parasites**.
3. Are single-stranded, covalently closed, circular RNA molecules that exist as base-paired, rod-like structures.
4. Cause plant diseases but have not been proven to cause human disease, although the RNA of the hepatitis D virus (HDV) is viroid-like.

F. Prions.

1. Are infectious particles associated with subacute progressive, degenerative diseases of the central nervous system (e.g., Creutzfeldt-Jakob disease).
2. Copurify with a specific glycoprotein (PrP) that has a molecular weight of 27 to 30 kDa. They are resistant to nucleases but are inactivated with proteases and other agents that inactivate proteins.
3. Are altered conformations of a normal cellular protein that can autocatalytically form more copies of itself.

II. HOST-PARASITE RELATIONSHIP

- A. **Normal flora** consist mainly of bacteria, but fungi and protozoa may be present in some individuals. They can provide useful nutrients (e.g., vitamin K) and release compounds (e.g., colicins) with antibacterial activity against pathogenic bacteria.
 1. They reside in the skin, mouth, nose, oropharynx, large intestine, urethra, and vagina.
 2. Normal flora may produce disease if they invade normally sterile areas of the body or are not properly controlled by the immune system.
- B. **Microbial pathogenicity** refers to a microbe's ability to cause disease, which depends on genetically determined virulence factors. A microbe's pathogenicity is related to its:
 1. Entry
 2. Colonization
 3. Escape from host defense mechanisms
 4. Multiplication
 5. Damage to host tissues

C. Virulence factors are chromosomal and extrachromosomal (plasmid) gene products that affect aspects related to an organism's:

1. Invasion *properties*
2. Adherence and colonization
3. Tissue damage induced by toxins, immune system reactions, and intracellular growth
4. Eluding host defense mechanisms
5. Antibiotic resistance

III. STERILIZATION AND DISINFECTION

A. Terminology.

1. **Sterility**—total absence of viable microorganisms as assessed by no growth on any medium.
2. **Bactericidal**—kills bacteria.
3. **Bacteriostatic**—inhibits growth of bacteria.
4. **Sterilization**—removal or killing of all microorganisms.
5. **Disinfection**—removal or killing of disease-causing microorganisms.
6. **Sepsis**—infection.
7. **Aseptic**—without infection.
8. **Antisepsis**—any procedure that inhibits the growth and multiplication of microorganisms.

B. Kinetics of killing.

1. Killing is affected by the medium, the concentration of organisms and antimicrobial agents, temperature, pH, and the presence of endospores.
2. It can be exponential (logarithmic); can result in a killing curve that becomes asymptotic, requiring extra considerations in killing final numbers, especially if the population is heterogeneous relative to sensitivity.

C. Methods of control.

1. **Moist heat** (autoclaving at 121°C/250°F for 15 minutes at a steam pressure of 15 pounds per square inch) kills microorganisms, including endospores.
2. **Dry heat** and **incineration** are both methods that oxidize proteins, killing bacteria.
3. **Ultraviolet radiation** blocks DNA replication.
4. **Chemicals:**
 - a. **Phenol** is used as a disinfectant standard that is expressed as a phenol coefficient, which compares the rate of the minimal sterilizing concentration of phenol to that of the test compound for a particular organism.
 - b. **Chlorhexidine** is a diphenyl cationic analog that is a useful topical disinfectant.
 - c. **Iodine** is bactericidal in a 2% solution of aqueous alcohol containing potassium iodide. It acts as an oxidizing agent and combines irreversibly with proteins. It can cause hypersensitivity reactions.
 - d. **Chlorine** inactivates bacteria and most viruses by oxidizing free sulfhydryl groups.
 - e. **Quaternary ammonium compounds** (e.g., **benzalkonium chloride**) inactivate bacteria by their hydrophobic and lipophilic groups, interacting with the cell membrane to alter metabolic properties and permeability.
 - f. **Ethylene oxide** is an alkylating agent that is especially useful for sterilizing heat-sensitive hospital instruments. It requires exposure times of 4 to 6 hours, followed by aeration to remove absorbed gas.
 - g. **Alcohol** requires concentrations of 70% to 95% to kill bacteria given sufficient time. Isopropyl alcohol (90% to 95%) is the major form in use in hospitals.

Review Test

Directions: Each of the numbered items or incomplete statements in this section is followed by answers or completions of the statement. Select the ONE lettered answer that is BEST in each case.

1. A pharmaceutical company has developed a new compound that is well tolerated by the body and inhibits the sterol ergosterol synthesis. Screening of anti-infectious agent activity should be directed toward
 - (A) Bacteria
 - (B) Chlamydia species
 - (C) Fungi
 - (D) Rickettsia species
 - (E) Viruses
2. 50S ribosomal subunits are found in
 - (A) Bacteria
 - (B) Fungi
 - (C) Prions
 - (D) Protozoa
 - (E) Viruses
3. The normal flora of the large intestine consists mainly of
 - (A) Bacteria
 - (B) Fungi
 - (C) Protozoa
 - (D) Viruses
 - (E) No microbial agents
4. The minimal concentration of alcohol necessary to kill bacteria and enveloped viruses is
 - (A) 30%
 - (B) 40%
 - (C) 50%
 - (D) 60%
 - (E) 70%
5. Human obligate intracellular pathogens that depend on the host cell for ATP production are
 - (A) Bacteriophages
 - (B) Mycoplasma species
 - (C) Prions
 - (D) Rickettsia species
 - (E) Viroids
6. Dimorphism is a characteristic of
 - (A) Bacteria
 - (B) Fungi
 - (C) Prions
 - (D) Rickettsia species
 - (E) Viruses
7. A new infectious agent has been isolated from deer ticks. It lacks a cell wall but has 70S ribosomes. This agent is most likely a
 - (A) Bacterium
 - (B) Chlamydia species
 - (C) Mycoplasma species
 - (D) Rickettsia species
 - (E) Virus
8. The infectious agent associated with Creutzfeldt-Jakob disease is extremely hardy, but can be inactivated by
 - (A) Catalases
 - (B) Hyaluronidases
 - (C) Nucleases
 - (D) Phospholipases
 - (E) Proteases
9. Quaternary ammonium compounds inactivate bacteria because they
 - (A) Alter metabolic properties of membranes
 - (B) Bind irreversibly to DNA
 - (C) Denature proteins
 - (D) Inactivate 50S ribosomes
 - (E) Oxidize free sulfhydryl groups

Answers and Explanations

- 1. The answer is C.** Fungi have ergosterol as their dominant membrane sterol. Mycoplasmas are the only prokaryotes with sterols in their cytoplasmic membrane, but they do not synthesize their own sterols.
- 2. The answer is A.** Bacteria have 70S ribosomes composed of 30S and 50S subunits. Fungi and protozoa have 80S ribosomes, and prions and viruses do not have ribosomes.
- 3. The answer is A.** Bacteria form the majority of the normal flora of the large intestine. Other types of human infectious agents are not usually present except in time of disease.
- 4. The answer is E.** An alcohol concentration of 70% to 95% is necessary to kill bacteria.
- 5. The answer is D.** Chlamydia and rickettsia are obligate intracellular pathogens because they depend on the host cell to provide them with ATP.
- 6. The answer is B.** Certain species of pathogenic fungi are dimorphic (i.e., existing as yeast or mold forms depending on their environment).
- 7. The answer is C.** Mycoplasmas are the only microbes that lack a cell wall, but they do have 70S ribosomes.
- 8. The answer is E.** The infectious agent of Creutzfeldt-Jakob disease is a prion which is inactivated by proteases.
- 9. The answer is A.** The hydrophobic and lipophilic portions of quaternary ammonium compounds react with the lipid components of the bacterial membrane so that it can no longer perform its normal metabolic and permeability functions, thus killing the cell.

I. BACTERIAL STRUCTURE

A. Shape. Along with other properties, shape is used to identify bacteria. It is determined by the mechanism of cell wall assembly.

1. Bacterial shape usually can be determined with appropriate staining and a light microscope.
2. **Types:**
 - a. **Round** (coccus)
 - b. **Rod-like** (bacillus)
 - c. **Spiral**
3. Cocci and bacilli often grow in doublets (diplococci) or chains (streptococci). Cocci that grow in clusters are called staphylococci.
4. Some bacterial species are **pleomorphic**, such as *Bacteroides*.
5. Antibiotics that affect cell wall biosynthesis (e.g., penicillin) may alter a bacteria's shape.

B. Nucleus. In bacteria, the nucleus generally is called a **nucleoid** or **nuclear body**.

1. The bacterial nucleus is not surrounded by a nuclear membrane, nor does it contain a mitotic apparatus.
2. **Composition.** The nucleus consists of polyamine and magnesium ions bound to negatively charged, circular, supercoiled, double-stranded DNA; small amounts of RNA; RNA polymerase; and other proteins.

C. Cytoplasm.

1. Bacterial cytoplasm contains ribosomes and various types of nutritional storage granules.
2. It contains **no organelles**.

D. Ribosomes. Bacterial ribosomes contain proteins and RNAs that differ from those of their eukaryotic counterparts.

1. **Types.** Bacterial ribosomes have a sedimentation coefficient of 70S and are composed of 30S and 50S subunits containing 16S, and 23S and 5S RNA, respectively.
2. Ribosomes engaged in protein biosynthesis are membrane bound.
3. Many antibiotics target ribosomes, inhibiting protein biosynthesis. Some antibiotics selectively target the 70S ribosomes (e.g., erythromycin), but not 80S ribosomes.

E. Cell (cytoplasmic) membrane.

1. **Structure.** The cell membrane is a typical phospholipid bilayer that contains the following constituents:
 - a. Cytochromes and enzymes involved in electron transport and oxidative phosphorylation.
 - b. Carrier lipids, enzymes, and **penicillin-binding proteins (PBP)** involved in cell wall biosynthesis.
 - c. Enzymes involved in phospholipid synthesis and DNA replication.
 - d. Chemoreceptors.
2. **Functions:**
 - a. Selective permeability and active transport facilitated by membrane-bound permeases, binding proteins, and various transport systems.
 - b. Site of action of certain antibiotics such as polymyxin.

F. **Mesosomes** are controversial structures that are **convoluted invaginations** of the plasma membrane.

1. **Septal mesosomes** occur at the septum (cross-wall); **lateral mesosomes** are nonseptal.
2. **Function:** participate in DNA replication, cell division, and secretion.

G. Plasmids.

1. Plasmids are small, circular, nonchromosomal, double-stranded DNA molecules that are:
 - a. Capable of self-replication.
 - b. Most frequently extrachromosomal but may become integrated into bacterial DNA.
2. **Function:** contain genes that confer protective properties such as antibiotic resistance, virulence factors, or their own transmissibility to other bacteria.

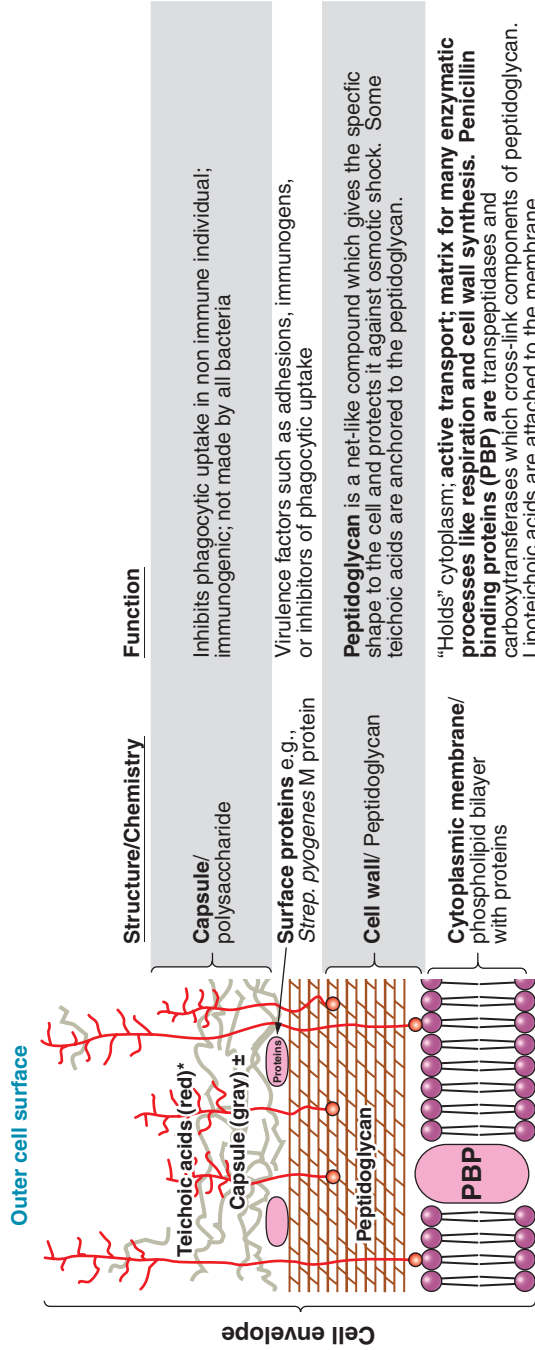
H. Transposons.

1. Transposons are small pieces of DNA that move between the DNA of bacteria and plasmids; they do not self-replicate.
2. **Functions:**
 - a. Code for antibiotic resistance enzymes, metabolic enzymes, or toxins.
 - b. May alter expression of neighboring genes or cause mutations to genes into which they are inserted.

I. Cell envelope (Figs. 2.1 and 2.2).

1. **General structure.** The cell envelope is composed of the macromolecular layers that surround the bacterium. It includes:
 - a. A cell membrane and a peptidoglycan layer except for mycoplasma.
 - b. An outer membrane layer in Gram-negative bacteria.
 - c. A capsule, a glycocalyx layer, or both (sometimes).
 - d. Antigens that frequently induce a specific antibody response.
2. **Cell wall:**
 - a. The cell wall refers to that portion of the cell envelope that is **external to the cytoplasmic membrane** and **internal to the capsule or glycocalyx**.
 - b. It confers osmotic protection and Gram-staining characteristics.
 - c. In **Gram-positive bacteria** it is composed of:
 - (1) Peptidoglycan
 - (2) Teichoic and teichuronic acids
 - (3) Polysaccharides
 - d. In **Gram-negative bacteria**, it is composed of:
 - (1) Peptidoglycan
 - (2) Lipoprotein
 - (3) An outer phospholipid membrane that contains lipopolysaccharide

Gram-Positive Cell Envelope



Cytoplasm

* Teichoic acids (shown in red) are found only in Gram-positive cells.

FIGURE 2.1. Gram-positive cell envelope showing structures and describing their chemistry and function. (Updated from Hawley LB. *High-Yield Microbiology and Infectious Diseases*. 2nd ed. Baltimore, MD: Lippincott Williams & Wilkins; 2007.)