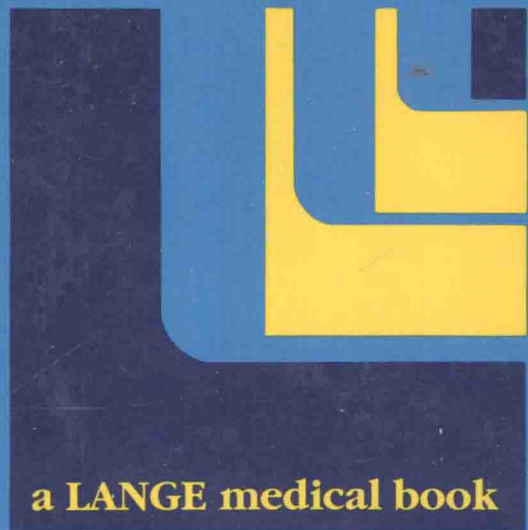


nineteenth edition

Jawetz, Melnick
& Adelberg's
**Medical
Microbiology**

Geo. F. Brooks • Janet S. Butel
L. Nicholas Ornston



a LANGE medical book

Jawetz, Melnick & Adelberg's Medical Microbiology

nineteenth edition

Geo. F. Brooks, MD

Professor of Laboratory Medicine, Medicine, and
Microbiology and Immunology
Chief, Microbiology Section Clinical
Laboratories
University of California
San Francisco

Janet S. Butel, PhD

Professor of Virology & Head, Division of
Molecular Virology
Baylor College of Medicine
Houston

L. Nicholas Ornston, PhD

Professor of Biology
Yale University
New Haven

Ernest Jawetz, MD, PhD

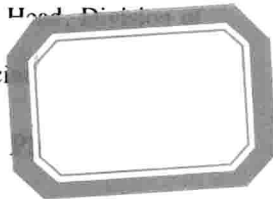
Professor of Microbiology and Medicine,
Emeritus
University of California
San Francisco

Joseph L. Melnick, PhD

Distinguished Service Professor of Virology and
Epidemiology
Baylor College of Medicine
Houston

Edward A. Adelberg, PhD

Professor of Human Genetics
Yale University School of Medicine
New Haven



APPLETON & LANGE
Norwalk, Connecticut/San Mateo, California

0-8385-6241-8

Notice: Our knowledge in clinical sciences is constantly changing. As new information becomes available, changes in treatment and in the use of drugs become necessary. The authors and the publisher of this volume have taken care to make certain that the doses of drugs and schedules of treatment are correct and compatible with the standards generally accepted at the time of publication. The reader is advised to consult carefully the instruction and information material included in the package insert of each drug or therapeutic agent before administration. This advice is especially important when using new or infrequently used drugs.



Copyright © 1991 by Appleton & Lange
A Publishing Division of Prentice Hall
18th Edition © 1989 by Appleton & Lange

All rights reserved. This book, or any parts thereof, may not be used or reproduced in any manner without written permission. For information, address Appleton & Lange, 25 Van Zant Street, East Norwalk, Connecticut 06855.

92 93 94 95 / 10 9 8 7 6 5 4 3 2

Prentice Hall International (UK) Limited, *London*
Prentice Hall of Australia Pty. Limited, *Sydney*
Prentice Hall Canada, Inc., *Toronto*
Prentice Hall Hispanoamericana, S.A., *Mexico*
Prentice Hall of India Private Limited, *New Delhi*
Prentice Hall of Japan, Inc., *Tokyo*
Simon & Schuster Asia Pte. Ltd., *Singapore*
Editora Prentice Hall do Brasil Ltda., *Rio de Janeiro*
Prentice Hall, *Englewood Cliffs, New Jersey*

ISBN: 0-8385-6241-8

ISSN: 1054-2744

Cover Design: Janice Barsevich

PRINTED IN THE UNITED STATES OF AMERICA

Preface

PURPOSE

It is our goal to provide an accurate, up-to-date microbiology text that is comprehensive but not so detailed that it is encyclopedic. Concepts of microbiology essential to understanding clinical infection, disease pathogenesis, prevention, and treatment are stressed; specific details of procedure and technique are purposely omitted. Because of important recent developments in molecular biology, biochemistry, and genetics, relevant information from these areas has been incorporated, extending the book's usefulness to fields other than medicine.

AUDIENCE

This book is principally intended for medical students, but house officers and practicing physicians will find it useful for its current clinical and basic science information. Undergraduate and graduate students in the health sciences will appreciate the book's multi-science perspective. Biochemists and molecular biologists will find it a handy reference text for basic microbiology concepts.

ORGANIZATION

Chapter 1 presents biological principles in the context of microbiology and illustrates how these principles can be used to predict the properties of microorganisms.

Chapters 2 through 8 review the general principles relating to microorganisms and their laboratory observation.

Chapters 9 through 11, which discuss immunology, pathogenesis of bacterial infection, and chemotherapy, review factors that influence the interaction between potentially pathogenic microorganisms and their hosts.

Chapters 12 through 47 review properties of specific groups of pathogens and the diseases with which each

is associated, summarizing the clinical manifestations and current knowledge about laboratory diagnosis, treatment, epidemiology, and control.

Chapter 48 summarizes the principles of diagnostic medical microbiology.

Significant Changes made in this edition include the following:

- The new introductory Chapter 1 defines microbiology as a biological science.
- Chapter 3 has been rewritten to emphasize the functional significance of bacterial classification.
- The bacteriology chapters have been updated, and changes have been made in the chapter on parasitology.
- Many of the virology chapters have been completely reorganized and rewritten to provide a conceptual framework for understanding viruses and viral infections. Chapters have been restructured to emphasize the members of a virus group most important in human disease, and molecular and genetic details have been presented to explain viral pathogenesis or epidemiology.
- The descriptions of pathogenesis of viral diseases in different organ systems are expanded.
- The chapter on herpesviruses is completely rewritten and updated, including new information about the importance of cytomegalovirus in congenital infections and a description of a new virus designated human herpesvirus 6.
- The chapter on AIDS is updated.
- Many new diagrams are presented to illustrate (a) important concepts of viral structure and function, and (b) the clinical course of and immune response to viral infections.

San Francisco, Houston,
and New Haven
April, 1991

Geo. F. Brooks
Janet S. Butel
L. Nicholas Ornston
Ernest Jawetz
Joseph L. Melnick
Edward A. Adelberg

SI units of measurement in the biological range.

Prefix	Abbreviation	Magnitude
kilo-	k	10^3
deci-	d	10^{-1}
centi-	c	10^{-2}
milli-	m	10^{-3}
micro-	μ	10^{-6}
nano-	n	10^{-9}
pico-	p	10^{-12}

These prefixes are applied to metric and other units. For example, a micrometer (μm) is 10^{-6} meter (formerly micron, μ); a nanogram (ng) is 10^{-9} gram (formerly millimicrogram, $\text{m}\mu\text{g}$); and a picogram (pg) is 10^{-12} gram (formerly micromicrogram, $\mu\mu\text{g}$). Any of these prefixes may also be applied to seconds, units, mols, equivalents, osmols, etc. The Angstrom (\AA , 10^{-7}) is now expressed in nanometers (eg, $40 \text{\AA} = 4 \text{ nm}$).

Table of Contents

Preface	ix
SI Units of Measurement in the Biological Range	x
SECTION I: FUNDAMENTALS OF MICROBIOLOGY	
1. The Science of Microbiology	1
Biological Principles Illustrated by	Prokaryotes 2
Microbiology 1	Protists 4
Viruses 1	
2. Cell Structure	7
Optical Methods 7	Staining 28
Eukaryotic Cell Structure 7	Morphological Changes During Growth 29
Prokaryotic Cell Structure 9	
3. The Major Groups of Bacteria	32
Purposes of Classification of Bacteria 32	Descriptions of the Principal Groups of
Criteria for Classification of Bacteria 32	Bacteria 34
Classification Systems 32	
4. The Growth, Survival, & Death of Microorganisms	39
Survival of Microorganisms in the Natural	Synchronous Growth 42
Environment 39	Growth Parameters 42
The Meaning of Growth 39	Definition & Measurement of Death 43
Exponential Growth 40	Antimicrobial Agents 44
The Growth Curve 41	
Maintenance of Cells in the Exponential	
Phase 42	
5. Cultivation of Microorganisms	49
Requirements for Growth 49	Environmental Factors Affecting Growth 52
Sources of Metabolic Energy 49	Cultivation Methods 53
Nutrition 50	
6. Microbial Metabolism	57
Role of Metabolism in Biosynthesis &	Biosynthetic Pathways 70
Growth 57	Patterns of Microbial Energy-Yielding
Focal Metabolites & Their Interconversion 59	Metabolism 73
Assimilatory Pathways 65	Regulation of Metabolic Pathways 78
7. Microbial Genetics	82
Organization of Genes 82	Mutation & Gene Rearrangement 90
Replication 85	Gene Expression 90
Transfer of DNA 87	

8. Genetic Engineering	95
Physical Separation of Differently Sized DNA	Site-Directed Mutagenesis 101
Fragments 95	Analysis With Cloned DNA: Hybridization
Preparation of DNA Fragments With Restriction	Probes 102
Enzymes 95	Manipulation of Cloned DNA 103
Cloning of DNA Restriction Fragments 96	Recombinant Strains in the Environment 103
Characterization of Cloned DNA 100	

SECTION II: IMMUNOLOGY

9. Immunology	105
Immunity & the Immune Response 105	Hypersensitivity 117
Antibodies (Immunoglobulins) 108	In Vitro Antigen-Antibody Reactions 120
Antibody-Mediated (Humoral) Immunity 111	Tolerance & Autoimmune Disease 124
The Complement System 112	Transplantation Immunity 125
Phagocytosis 113	Tumor Immunity 126
Cell-Mediated Immunity 115	Active Immunization in Childhood 126
Natural Killer (NK) Cells 116	Recommended Immunization of Adults for
The Major Histocompatibility Complex 116	Travel 126

SECTION III: BACTERIOLOGY

10. Pathogenesis of Bacterial Infection & Host Resistance to Infection	130
Pathogenesis of Bacterial Infection 130	Attributes of the Host That Determine Resistance
Identifying Bacteria That Cause Disease 130	to Infection 140
The Infectious Process 131	Mechanisms of Nonspecific Host
Bacterial Virulence Factors 131	Resistance 140
	Resistance & Immunity 144
11. Antibacterial & Antifungal Chemotherapy	149
Mechanisms of Action of Clinically Used	Host-Parasite Relationships 157
Antimicrobial Drugs 149	Clinical Use of Antibiotics 157
Resistance to Antimicrobial Drugs 152	Antimicrobial Drugs Used in Combination 158
Origin of Drug Resistance 152	Antimicrobial Chemoprophylaxis 159
Drug Dependence 154	Disinfectants 161
Antimicrobial Activity in Vitro 154	Antimicrobial Drugs for Systemic
Antimicrobial Activity in Vivo 155	Administration 161
Drug-Parasite Relationships 156	
12. Spore-Forming Gram-Positive Bacilli: <i>Bacillus</i> & <i>Clostridium</i> Species	180
<i>Bacillus</i> Species 180	<i>Clostridium</i> Species 182
13. Non-Spore-Forming Gram-Positive Bacilli: <i>Corynebacterium</i>, <i>Propionibacterium</i>, <i>Listeria</i>, & <i>Erysipelothrix</i>	188
<i>Corynebacterium diphtheriae</i> 188	<i>Erysipelothrix rhusiopathiae</i> (<i>Erysipelothrix</i>
Other <i>Corynebacteria</i> (Diphtheroids) &	<i>insidiosus</i>) 192
<i>Propionibacteria</i> 191	<i>Rothia</i> 192
<i>Listeria monocytogenes</i> 191	
14. The Staphylococci	194
15. The Streptococci	200
<i>Streptococcus pneumoniae</i> (Pneumococcus) 208	
16. Enteric Gram-Negative Rods (Enterobacteriaceae)	212
Diseases Caused by Enterobacteriaceae Other	The <i>Salmonella</i> -Arizona Group 218
Than <i>Salmonella</i> & <i>Shigella</i> 215	The <i>Shigellae</i> 221

17. Pseudomonads, Acinetobacters, & Uncommon Gram-Negative Bacteria	224
The <i>Pseudomonas</i> Group 224	Uncommon Gram-Negative Bacteria 227
<i>Acinetobacter</i> 226	
18. Vibrios, Campylobacters, & Associated Bacteria	230
The Vibrios 230	The Campylobacters 233
<i>Aeromonas</i> 232	<i>Helicobacter pylori</i> 234
<i>Plesiomonas</i> 233	
19. Haemophilus, Bordetella, & Brucella	237
The <i>Haemophilus</i> Species 237	The Brucellae 241
The Bordetellae 239	
20. Yersinia, Francisella, & Pasteurella	245
<i>Yersinia pestis</i> & Plague 245	<i>Francisella tularensis</i> & Tularemia 247
<i>Yersinia enterocolitica</i> & <i>Yersinia pseudotuberculosis</i> 246	The Pasteurellae 248
21. The Neisseriae	250
<i>Neisseria gonorrhoeae</i> (Gonococcus) 250	Other Neisseriae 255
<i>Neisseria meningitidis</i> (Meningococcus) 254	
22. Infections Caused by Anaerobic Bacteria	257
Physiology & Growth Conditions for Anaerobes 257	Immunity in Anaerobic Infections 260
Anaerobic Bacteria Found in Human Infections 258	The Polymicrobial Nature of Anaerobic Infections 261
Pathogenesis of Anaerobic Infections 260	Diagnosis of Anaerobic Infections 261
	Treatment of Anaerobic Infections 261
23. Legionellae & Unusual Bacterial Pathogens	263
<i>Legionella pneumophila</i> & Other Legionellae 263	<i>Bartonella bacilliformis</i> 265
Bacteria That Cause Vaginosis 265	<i>Calymmatobacterium</i> (<i>Donovania</i>) <i>granulomatis</i> 266
<i>Streptobacillus moniliformis</i> 265	Cat-Scratch Disease 266
24. Mycoplasmas & Cell Wall-Defective Bacteria	268
Mycoplasmas 268	Cell Wall-Defective Bacteria 270
25. Mycobacteria	272
<i>Mycobacterium tuberculosis</i> 272	<i>Mycobacterium leprae</i> 278
Other Mycobacteria 277	
26. Spirochetes & Other Spiral Microorganisms	280
<i>Treponema pallidum</i> 280	Leptospirae 285
Diseases Related to Syphilis 283	<i>Spirillum minor</i> (<i>Spirillum morsus muris</i>) 287
Other Spirochetal Organisms 283	Spirochetes of the Normal Mouth & Mucous Membranes 287
<i>Borrelia recurrentis</i> 283	Fusospirochetal Disease 287
<i>Borrelia burgdorferi</i> & Lyme Disease 285	
27. Normal Microbial Flora of the Human Body	289
Role of the Resident Flora 289	Normal Flora of the Intestinal Tract 291
Normal Flora of the Skin 289	Normal Flora of the Urethra 292
Normal Flora of the Mouth & Upper Respiratory Tract 290	Normal Flora of the Vagina 292
	Normal Flora of the Eye (Conjunctiva) 292
28. Rickettsial Diseases	294
29. Chlamydiae	300
Psittacosis (Ornithosis) 302	Respiratory Tract Involvement With <i>Chlamydia trachomatis</i> 306
Ocular, Genital, & Respiratory Infections Due to <i>Chlamydia trachomatis</i> 304	Lymphogranuloma Venereum (LGV) 306
Trachoma 304	Other Agents of the Group 307
Genital Chlamydial Infections & Inclusion Conjunctivitis 305	

SECTION IV: MYCOLOGY

30. Medical Mycology	309
Classification of Fungi 311	Deep Mycoses (Systemic Mycoses) 318
Growth & Isolation of Fungi 312	Opportunistic Mycoses 323
Superficial Mycoses 312	Actinomycetes 327
Cutaneous Mycoses 312	Hypersensitivity to Fungi 330
Subcutaneous Mycoses 316	Mycotoxins 330

SECTION V: PARASITOLOGY

31. Medical Parasitology	332
Classification of Parasites 332	Free-Living Amebas 346
Intestinal Flagellates 333	Blood Sporozoans 347
The Hemoflagellates 335	Other Sporozoans 351
Intestinal Amebas 341	<i>Pneumocystis carinii</i> 355
<i>Balantidium coli</i> 345	Helminths 355

SECTION VI: VIROLOGY

32. General Properties of Viruses	366
Introduction to Viruses 366	Purification & Identification of Viruses 378
Definitions in Virology 366	Reaction to Physical & Chemical Agents 378
Evolutionary Origin of Viruses 366	Replication of Viruses 379
Classification of Viruses 367	Genetics of Animal Viruses 382
Principles of Viral Structure 371	Natural History (Ecology) & Modes of
Chemical Composition of Viruses 373	Transmission of Viruses 387
Cultivation & Assay of Viruses 375	Diagnosis of Viral Infections 388
33. Pathogenesis & Control of Viral Diseases	389
Principles of Viral Diseases 389	Prevention & Treatment of Viral Infections 398
Pathogenesis of Viral Diseases 389	
34. Adenoviruses	408
Properties of Adenoviruses 408	Adenovirus Infections in Humans 413
35. Herpesviruses	418
Properties of Herpesviruses 418	Cytomegalovirus 430
Herpesvirus Infections in Humans 422	Epstein-Barr Virus 434
Herpes Simplex Viruses 422	Human Herpesvirus 6 438
Varicella-Zoster Virus 427	B Virus 438
36. Poxviruses	441
Properties of Poxviruses 441	Orf Virus Infections 449
Poxvirus Infections in Humans: Vaccinia &	Molluscum Contagiosum 449
Variola 443	Tanapox & Yaba Monkey Tumor Poxvirus
Monkeypox Infections 448	Infections 449
Cowpox Infections 448	
37. Hepatitis Viruses	451
Properties of Hepatitis Viruses 451	Hepatitis Virus Infections in Humans 456

38. Picornaviruses (Enterovirus & Rhinovirus Groups)	469
Properties of Picornaviruses 469	Other Enterovirus Types 479
Enterovirus Group 471	Rhinovirus Group 479
Poliomyelitis 471	Foot-and-Mouth Disease (Aphthovirus of Cattle) 480
Coxsackieviruses 475	
Echoviruses 477	
39. Reoviruses & Rotaviruses	482
Properties of Reoviruses 482	Orbiviruses 486
Rotaviruses 483	Other Agents of Viral Gastroenteritis 486
Reoviruses 486	
40. Arthropod-Borne & Rodent-Borne Viral Diseases	488
Human Arboviral Infections 488	Rodent-Borne Hemorrhagic Fevers 502
41. Orthomyxoviruses (Influenza Viruses)	506
Properties of Orthomyxoviruses 506	Influenza Virus Infections in Humans 511
42. Paramyxoviruses & Rubella Virus	517
Properties of Paramyxoviruses 517	Mumps Virus Infections 525
Parainfluenza Virus Infections 521	Measles (Rubeola) Virus Infections 527
Respiratory Syncytial Virus Infections 523	Rubella (German Measles) Virus Infections 531
43. Coronaviruses	536
Properties of Coronaviruses 536	Coronavirus Infections in Humans 537
44. Rabies & Slow Virus Infections	539
Rabies 539	Encephalomyocarditis Virus Infection (Mengo Fever) 545
Aseptic Meningitis 544	Slow & Unconventional Virus Diseases 545
Lymphocytic Choriomeningitis 544	
45. Tumor Viruses & Oncogenes	549
General Features of Viral Carcinogenesis 549	Tumor Suppressor Genes 561
RNA Tumor Viruses (Retroviruses) 552	DNA Tumor Viruses 561
Cellular Oncogenes 559	Viruses & Human Cancer 569
46. AIDS & Lentiviruses	571
Properties of Lentiviruses 571	HIV Infections in Humans 574
47. Emerging Viral Diseases of Humans	583
Parvovirus Diseases 583	Guillain-Barré Syndrome 585
Central Nervous System Degenerative Disorders 584	Diabetes Mellitus 585
Immune Complex Diseases 585	Viral Arthritis 586
	Epidemic Neuromyasthenia 586

SECTION VII: DIAGNOSTIC MEDICAL MICROBIOLOGY

48. Principles of Diagnostic Medical Microbiology	587
Communication Between Physician & Laboratory 587	Diagnosis of Infection by Anatomic Site 598
Diagnosis of Bacterial & Fungal Infections 587	Anaerobic Infections 604
The Importance of Normal Bacterial & Fungal Flora 596	Diagnosis of Chlamydial Infections 605
Laboratory Aids in the Selection of Antimicrobial Therapy 596	Diagnosis of Viral Infections 606
Index	617

Section I. Fundamentals of Microbiology

The Science of Microbiology

1

BIOLOGICAL PRINCIPLES ILLUSTRATED BY MICROBIOLOGY

Nowhere is biological **diversity** demonstrated more dramatically than by microorganisms, creatures that are not directly visible to the unaided eye. In form and function, be it biochemical property or genetic mechanism, analysis of microorganisms takes us to the limits of biological understanding. Thus, the need for **originality**—one test of the merit of a scientific **hypothesis**—can be fully met in microbiology. A useful hypothesis should provide a basis for **generalization**, and microbial diversity provides an arena in which this challenge is ever-present.

Prediction, the practical outgrowth of science, is a product created by a blend of technique and theory. **Biochemistry** and **genetics** provide the tools required for analysis of microorganisms. **Microbiology**, in turn, extends the horizons of these scientific disciplines. A biologist might describe such an exchange as **mutualism**, ie, one that benefits all of the contributing parties. In biology, mutualism is called **symbiosis**, a continuing association of different organisms. Should the exchange operate primarily to the benefit of one party, the association is described as **parasitism**, a relationship in which a **host** provides the primary benefit to the parasite. Isolation and characterization of a parasite—eg, a pathogenic bacterium or virus—often requires effective mimicry in the laboratory of the growth environment provided by host cells. This demand sometimes represents a major challenge to the investigator.

The terms “mutualism,” “symbiosis,” and “parasitism” relate to the science of **ecology**, and the principles of environmental biology are implicit in microbiology. Microorganisms are the products of **evolution**, the biological consequence of **natural selection** operating upon a vast array of genetically diverse organisms. It is useful to keep the complexity of natural history in mind before generalizing about microorganisms, the most heterogeneous subset of all living creatures.

A major biological division separates the eukaryotes, organisms containing a membrane-bound nucleus, from prokaryotes, organisms in which DNA is not physically separated from the cytoplasm. As described below and in Chapter 2, further major distinctions can be made between eukaryotes and pro-

karyotes. Eukaryotes, for example, are distinguished by their relatively large size and by the presence of specialized membrane-bound organelles such as mitochondria.

As described more fully below, microbial eukaryotes are termed **protists**, and within this group the major subdivisions are the **algae**, the **protozoa**, the **fungi**, and the **slime molds**.

Eukaryotes and prokaryotes are organisms because they contain all of the enzymes required for their replication and possess the biological equipment necessary for the production of metabolic energy. Thus, eukaryotes and prokaryotes stand distinguished from **viruses**, which depend upon host cells for these necessary functions.

VIRUSES

The unique properties of viruses set them apart from living creatures. Heterogeneity among viruses is assured by their dependence upon a host for replication. In a sense, a virus can be regarded as a genetic extension of its host. Host-virus interactions tend to be highly specific, and the biological range of viruses mirrors the diversity of potential host cells. Further diversity of viruses is exhibited by their broad array of strategies for replication and survival.

A viral particle consists of a nucleic acid molecule, either DNA or RNA, enclosed in a protein coat or capsid. Proteins—frequently glycoproteins—in the capsid determine the specificity of interaction of a virus with its host cell. The capsid protects the nucleic acid and facilitates attachment and penetration of the host cell by the virus. Inside the cell, viral nucleic acid redirects the host's enzymatic machinery to functions associated with replication of the virus. In some cases, genetic information from the virus can be incorporated as DNA into a host chromosome. In other instances, the viral genetic information can serve as a basis for cellular manufacture and release of copies of the virus. This process calls for replication of the viral DNA and production of specific viral proteins. Maturation consists of assembling newly synthesized nucleic acid and protein subunits into mature viral particles which are then liberated into the extracellular environment. Different viruses are known to infect a wide variety of specific plant and animal hosts as well as prokaryotes

and at least one eukaryotic alga. Virus-like particles that seem to lack an infectious extracellular phase have been found in fungi as well as in several genera of algae.

A number of transmissible plant diseases are caused by **viroids**—small, single-stranded, covalently closed circular RNA molecules existing as highly base-paired rod-like structures; they do not possess capsids. Their molecular weights are estimated to fall in the range of 75,000–100,000. It is not known whether they are translated in the host into polypeptides or whether they interfere with host functions directly (as RNA); if the former is true, the largest viroid could only be translated into the equivalent of a single polypeptide containing about 55 amino acids. Viroid RNA is replicated by the DNA-dependent RNA polymerase of the plant host; preemption of this enzyme may contribute to viroid pathogenicity.

The RNAs of viroids have been shown to contain inverted repeated base sequences at their termini, a characteristic of transposable elements and retroviruses (see Chapter 7). Thus, it is likely that they have evolved from transposable elements or retroviruses by the deletion of internal sequences.

Scrapie, a degenerative central nervous system disease of sheep, is caused by a filterable agent less than 50 nm in diameter. It is resistant to nucleases and other agents that inactivate nucleic acids but is inactivated by proteases and other agents that react with proteins. The infectious particle has been called a prion; it copurifies with a specific protein, but the presence of nucleic acid within the particle has not been ruled out.

By use of recombinant DNA techniques, the gene encoding the major prion protein has been cloned from hamster brain. The gene—and its corresponding mRNA—is present (and thus expressed) in both normal and scrapie-infected brain tissue. Three competing models exist: (1) Scrapie is a conventional virus with an extremely small nucleic acid genome that has escaped detection; (2) the infectious agent is a small, noncoding RNA molecule that binds to prion protein with high affinity, changing the prion's conformation in a self-propagating manner to a pathological form; and (3) the prion protein is itself the infectious agent, inducing the synthesis of posttranslational modifying enzymes that convert a normal protein to the pathological prion form. These models may also apply to the agents of Creutzfeldt-Jakob disease and kuru, which produce very similar diseases in humans.

The general properties of animal viruses pathogenic for humans are described in Chapter 32. Bacterial viruses are described in Chapter 7.

PROKARYOTES

The primary distinguishing characteristics of the prokaryotes are their relatively small size, usually on the order of 1 μm in diameter, and the absence of a

nuclear membrane. The DNA of almost all bacteria is a circle with a length of about 1 mm; this is the prokaryotic chromosome. The chromosomal DNA must be folded more than a thousandfold just to fit within the prokaryotic cell membrane. Substantial evidence suggests that the folding may be orderly and may bring specified regions of the DNA into proximity. Thus, it would be a mistake to conclude that subcellular differentiation, clearly demarcated by membranes in eukaryotes, is lacking in prokaryotes. Indeed, some prokaryotes form membrane-bound subcellular structures with specialized function such as the chromatophores of photosynthetic bacteria. Such prokaryotic structures differ from eukaryotic counterparts in that the membranes surrounding the specialized region are extensions of the cell membrane.

Prokaryotic Diversity

The small size of the prokaryotic chromosome limits the amount of genetic information it can contain. Reasonable estimates of the number of genes within a typical prokaryote are on the order of 1000, and many of these genes must be dedicated to essential functions such as energy generation, macromolecular synthesis, and cellular replication. Any one prokaryote carries relatively few genes that allow physiological accommodation of the organism to its environment. The range of potential prokaryotic environments is unimaginably broad, and it follows that the prokaryotic group encompasses a heterogeneous range of specialists, each adapted to a fairly narrowly circumscribed niche.

The range of prokaryotic niches is illustrated by consideration of strategies used for generation of metabolic energy. Light from the sun is the chief source of energy for life. Some prokaryotes such as the purple bacteria convert light energy to metabolic energy in the absence of oxygen production. Other prokaryotes, exemplified by the blue-green bacteria (**cyanobacteria**), produce oxygen that can provide energy through respiration in the absence of light. **Aerobic organisms** depend upon respiration with oxygen for their energy. Some **anaerobic organisms** can use electron acceptors other than oxygen in respiration. Many anaerobes carry out **fermentations** in which energy is derived by metabolic rearrangement of chemical growth substrates. The tremendous chemical range of potential growth substrates for aerobic or anaerobic growth is mirrored in the diversity of prokaryotes that have adapted to their utilization.

Prokaryotic Communities

A useful survival strategy for specialists is to enter into **consortia**, arrangements in which the physiological characteristics of different organisms contribute to survival of the group as a whole. If the organisms within a physically interconnected community are directly derived from a single cell, the community is a **clone** that may contain up to 10^8 cells. The biology of

such a community differs substantially from that of a single cell. For example, the high cell number virtually assures the presence within the clone of at least one cell carrying a variant of any gene on the chromosome. Thus, genetic variability—the wellspring of the evolutionary process called natural selection—is assured within a clone. The high number of cells within clones also is likely to provide physiological protection to at least some members of the group. Extracellular polysaccharides, for example, may afford protection against potentially lethal agents such as antibiotics or heavy metal ions. Large amounts of polysaccharides produced by the high number of cells within a clone may allow cells within the interior to survive exposure to a lethal agent at a concentration that might kill single cells.

A distinguishing characteristic of prokaryotes is their capacity to exchange small packets of genetic information. This information may be carried on **plasmids**, small and specialized genetic elements that are capable of replication within at least one prokaryotic cell line. In some cases, plasmids may be transferred from one cell to another and thus may carry sets of specialized genetic information through a population. Some plasmids possess a **broad host range** that allows them to convey sets of genes to diverse organisms. Of particular concern are **drug resistance plasmids** that may render diverse bacteria resistant to antibiotic treatment.

The survival strategy of a single prokaryotic cell line may lead to a range of interactions with other organisms. These may include symbiotic relationships illustrated by complex nutritional exchanges among organisms within the human gut. These exchanges benefit both the microorganisms and their human host. Parasitic interactions can be quite deleterious to the host. Advanced symbiosis or parasitism can lead to loss of functions that would allow growth of the symbiont or parasite independent of its host.

The **mycoplasmas**, for example, are parasitic prokaryotes that have lost the ability to form a cell wall. Adaptation of these organisms to their parasitic environment has resulted in incorporation of a substantial quantity of cholesterol into their cell membranes. Cholesterol, not found in other prokaryotes, is assimilated from the metabolic environment provided by the host. Loss of function is exemplified also by obligate intracellular parasites, the **chlamydiae** and **rickettsiae**. These bacteria are extremely small (0.2–0.5 μm in diameter) and depend upon the host cell for many essential metabolites and coenzymes. Some evidence suggests that the host cell may even provide energy in the form of ATP to these bacteria.

The most widely distributed examples of bacterial symbionts appear to be chloroplasts and mitochondria, the energy-yielding organelles of eukaryotes. A substantial body of evidence points to the conclusion that ancestors of these organelles were **endosymbionts**, prokaryotes that established symbiosis within the cell

membrane of the ancestral eukaryotic host. The presence of multiple copies of the organelles may have contributed to the relatively large size of eukaryotic cells and to their capacity for specialization, a trait ultimately reflected in the evolution of differentiated multicellular organisms.

Classification of the Prokaryotes

An understanding of any group of organisms requires their **classification**. An appropriate classification system allows a scientist to choose characteristics that allow swift and accurate categorization of a newly encountered organism. The categorization allows prediction of many additional traits shared by other members of the category. In a hospital setting, successful classification of a pathogenic organism may provide the most direct route to its elimination. Classification may also provide a broad understanding of relationships among different organisms, and such information may have great practical value. For example, elimination of a pathogenic organism will be relatively long-lasting if its habitat is occupied by a nonpathogenic variant.

The principles of prokaryotic classification are discussed in Chapter 3. At the outset it should be recognized that any prokaryotic characteristic might serve as a potential criterion for classification. Not all criteria are equally effective in grouping organisms. Possession of DNA, for example, is a useless criterion for distinguishing organisms because all cells contain DNA. The presence of a broad host range plasmid is not a useful criterion because such plasmids may be found in diverse hosts and need not be present all of the time. Useful criteria may be structural, physiological, biochemical, or genetic. **Spores**—specialized cell structures that may allow survival in extreme environments—are useful structural criteria for classification because well-characterized subsets of bacteria form spores. Some bacterial groups can be effectively subdivided on the basis of their ability to ferment specified carbohydrates. Such criteria may be ineffective when applied to other bacterial groups that may lack any fermentative capability. A biochemical test, the **Gram stain**, is an effective criterion for classification because response to the stain reflects fundamental and complex differences in the bacterial cell surface that divide bacteria into two major groups.

Genetic criteria are increasingly employed in bacterial classification, and many of these advances are made possible by the development of recombinant DNA technology. It is now possible to design DNA probes that swiftly identify organisms carrying specified genetic regions with common ancestry. Comparison of DNA sequences for some genes led to the elucidation of **phylogenetic relationships** among prokaryotes. Ancestral cell lines can be traced, and organisms can be grouped on the basis of their evolutionary affinities. These investigations have led to some striking conclusions. For example, comparison of cyto-

chrome c sequences suggests that all eukaryotes, including humans, arose from one of three different groups of purple photosynthetic bacteria. This conclusion in part explains the evolutionary origin of eukaryotes, but it does not fully take into account the generally accepted view that the eukaryotic cell was derived from the evolutionary merger of different prokaryotic cell lines.

Bacteria & Archaeobacteria: the Major Subdivision Within the Prokaryotes

A major success in molecular phylogeny has been the demonstration that prokaryotes fall into two major groups. Most investigations have been directed to one group, the bacteria. The other group, the archaeobacteria, has received relatively little attention, in part because many of its representatives are difficult to study in the laboratory. Some archaeobacteria, for example, are killed by contact with oxygen, and others grow at temperatures exceeding that of boiling water. Before molecular evidence became available, the major subgroupings of archaeobacteria seemed disparate. The methanogens carry out an anaerobic respiration that gives rise to methane; the halophiles demand extremely high salt concentrations for growth; and the thermoacidophiles require high temperature or acidity (or both). It has now been established that these prokaryotes share biochemical traits such as cell wall or membrane components that set the group entirely apart from all other living organisms. An intriguing trait shared by archaeobacteria and eukaryotes is the presence of **introns** within genes. The function of introns—segments of DNA that interrupt informational DNA within genes—is not established. What is known is that introns represent a fundamental characteristic shared by the DNA of archaeobacteria and eukaryotes. This common trait has led to the suggestion that—just as mitochondria and chloroplasts appear to be evolutionary derivatives of the bacteria—the eukaryotic nucleus may have arisen from an archaeobacterial ancestor.

PROTISTS

The “true nucleus” of eukaryotes (from Gr *karyon* “nucleus”) is only one of their distinguishing features. The membrane-bound organelles, the microtubules, and the microfilaments of eukaryotes form a complex intracellular structure unlike that found in prokaryotes. The agents of motility for eukaryotic cells are flagella or cilia—complex multistranded structures that do not resemble the flagella of prokaryotes. Gene expression in eukaryotes takes place through a series of events achieving physiological integration of the nucleus with the endoplasmic reticulum, a structure that has no counterpart in prokaryotes. Eukaryotes are set apart by the organization of their cellular DNA in chromo-

somes separated by a distinctive mitotic apparatus during cell division.

In general, genetic transfer among eukaryotes depends upon fusion of **haploid gametes** to form a **diploid** cell containing a full set of genes derived from each gamete. The life cycle of many eukaryotes is almost entirely in the diploid state, a form not encountered in prokaryotes. Fusion of gametes to form reproductive progeny is a highly specific event and establishes the basis for eukaryotic **species**, a term that can be applied only metaphorically to the prokaryotes. Taxonomic groupings of eukaryotes frequently are based on shared **morphological properties**, and it is noteworthy that many taxonomically useful determinants are those associated with reproduction. Almost all successful eukaryotic species are those in which closely related cells, members of the same species, can recombine to form viable offspring. Structures that contribute directly or indirectly to the reproductive event tend to be highly developed and, with minor modifications among closely related species, extensively conserved.

Microbial eukaryotes—**protists**—are members of the four following major groups: algae, protozoa, fungi, and slime molds. It should be noted that these groupings are not necessarily phylogenetic: Closely related organisms may have been categorized separately because underlying biochemical and genetic similarities may not have been recognized.

Algae

The term “algae” has long been used to denote all organisms that produce O_2 as a product of photosynthesis. One major subgroup of these organisms—the blue-green bacteria, or cyanobacteria—are prokaryotic and no longer are termed algae. This classification is reserved exclusively for photosynthetic eukaryotic organisms. All algae contain chlorophyll in the photosynthetic membrane of their subcellular chloroplasts. Many algal species are unicellular microorganisms. Other algae may form extremely large multicellular structures. Kelps of brown algae sometimes are several hundred meters in length. A full description of the algae can be found in Bold HC, Wynne MJ: *Introduction to the Algae: Structure and Reproduction*. Prentice-Hall, 1978. A highly readable account of the properties of algae and other protists is presented in Sagan D, Margulis L: *Garden of Microbial Delights: A Practical Guide to the Subdivisible World*. Harcourt Brace Jovanovich, 1988.

Protozoa

Protozoa are unicellular nonphotosynthetic protists. The most primitive protozoa appear to be flagellated forms that in many respects resemble representatives of the algae. It seems likely that the ancestors of these protozoa were algae that became **heterotrophs**: the nutritional requirements of such organisms are met by organic compounds. Adaptation to a heterotrophic

mode of life was sometimes accompanied by loss of chloroplasts, and algae thus gave rise to the closely related protozoa. Similar events have been observed in the laboratory as either mutation or physiological adaptation has given rise to colorless descendants of algal cells.

From flagellated protozoa appear to have evolved the ameboid and the ciliated types; intermediate forms are known that have flagella at one stage in the life cycle and pseudopodia (characteristic of the ameba) at another stage. A fourth major group of protozoa consists of the sporozoans, parasites with complex life cycles that include a resting or spore stage.

Fungi

The fungi are nonphotosynthetic protists growing as a mass of branching, interlacing filaments ("hyphae") known as a mycelium. Although the hyphae exhibit cross-walls, the cross-walls are perforated and allow free passage of nuclei and cytoplasm. The entire organism is thus a coenocyte (a multinucleated mass of continuous cytoplasm) confined within a series of branching tubes. These tubes, made of polysaccharides such as chitin, are homologous with cell walls. The mycelial forms are called **molds**; a few types, **yeasts**, do not form a mycelium but are easily recognized as fungi by the nature of their sexual reproductive processes and by the presence of transitional forms.

The fungi probably represent an evolutionary offshoot of the protozoa; they are unrelated to the actinomycetes, mycelial bacteria that they superficially resemble. Fungi are subdivided as follows: *Zygomycotina* (the phycomycetes), *Ascomycotina* (the ascomycetes), *Basidiomycotina* (the basidiomycetes), and *Deuteromycotina* (the imperfect fungi).

The evolution of the ascomycetes from the phycomycetes is seen in a transitional group, members of which form a zygote but then transform this directly into an ascus. The basidiomycetes are believed to have evolved in turn from the ascomycetes. The classification of fungi is discussed further in Chapter 30.

Slime Molds

These organisms are characterized by the presence, as a stage in their life cycle, of an ameboid multinucleate mass of cytoplasm called a **plasmodium**. The plasmodium of a slime mold is analogous to the mycelium of a true fungus. Both are coenocytes. In the latter, cytoplasmic flow is confined to the branching network of chitinous tubes, whereas in the former the cytoplasm can flow in all directions. This flow causes the plasmodium to migrate in the direction of its food source, frequently bacteria. In response to a chemical signal, 3',5'-cyclic AMP (see Chapter 7), the plasmodium, which reaches macroscopic size, differentiates into a stalked body that can produce individual motile cells. These cells, flagellated or ameboid, initiate a new round in the life cycle of the slime mold. The cycle frequently is initiated by sexual fusion of single cells.

The life cycle of the slime molds illustrates a central theme of this chapter: the interdependency of living forms. The growth of slime molds depends upon nutrients provided by bacterial or, in some cases, plant cells. Reproduction of the slime molds via plasmodia can depend upon intercellular recognition and fusion of cells from the same species. Full understanding of a microorganism requires both knowledge of the other organisms with which it coevolved and an appreciation of the range of physiological responses that may contribute to survival.

REFERENCES

Books

- Ainsworth GC, Sussman AS, Sparrow FK (editors): *Fungi: An Advanced Treatise*. 4 vols. Academic Press, 1973.
- Barnett JA, Payne RW, Yarrow D (editors): *Yeasts: Characteristics and Identification*. Cambridge Univ Press, 1984.
- Bold HC, Wynne MJ: *Introduction to the Algae: Structure and Reproduction*. Prentice-Hall, 1978.
- Carlile MJ, Shekel JJ (editors): *Evolution in the Microbial World*. Cambridge Univ Press, 1974.
- Diener TO: *Viroids and Viroid Diseases*. Krieger, 1979.
- Laskin AI, Lechevalier HA (editors): *CRC Handbook of Microbiology*, 2nd ed. Vol 1: *Bacteria*, 1977; Vol 2: *Fungi, Algae, Protozoa and Viruses*, 1979. CRC Press.
- Levandowsky M, Hutner SH (editors): *Biochemistry and Physiology of Protozoa*, 2nd ed. Academic Press, 1979.
- Luria SE et al: *General Virology*, 3rd ed. Wiley, 1978.
- Margulis L: *Symbiosis in Cell Evolution: Life and Its Environment on the Early Earth*. Freeman, 1981.
- Ragan MA, Chapman DJ: *Biochemical Phylogeny of the Protists*. Academic Press, 1977.
- Sagan D, Margulis L: *Garden of Microbial Delights: A Practical Guide to the Subdivisible World*. Harcourt Brace Jovanovich, 1988.
- Sleigh MA: *The Biology of Protozoa*. University Park Press, 1975.
- Stanier RY, Adelberg EA, Ingraham J: *The Microbial World*, 4th ed. Prentice-Hall, 1976.
- Woese CR, Wolfe RS (editors): *The Bacteria: A Treatise on Structure and Function*. Vol 8. Academic Press, 1985.

Articles & Reviews

- Bruenn JA: Viruslike particles of yeast. *Annu Rev Microbiol* 1980;34:49.
- Cloud P: Evolution of ecosystems. *Am Sci* 1974;64:54.

- Diener TO: Viroids: Structure and functions. *Science* 1979;205:859.
- Fox GE et al: The phylogeny of prokaryotes. *Science* 1980;209:457.
- Girard M: The Pasteur Institute's contribution to the field of virology. *Annu Rev Microbiol* 1988;42:745.
- Knoll AH, Barghoorn ES: Precambrian eukaryotic organisms: A reassessment of the evidence. *Science* 1975; 190:52.
- Kolenbrander PE: Intergeneric coaggregation among human oral bacteria and ecology of dental plaque. *Annu Rev Microbiol* 1989;43:622.
- Lake JA et al: Eubacteria, halobacteria, and the origin of photosynthesis: The photocytes. *Proc Natl Acad Sci USA* 1985;82:3716.
- Lemke PA: Viruses of eukaryotic microorganisms. *Annu Rev Microbiol* 1976;30:105.
- Prusiner SB: Scrapie prions. *Annu Rev Microbiol* 1989; 43:345.
- Raff RA, Mahler HR: The nonsymbiotic origin of mitochondria. *Science* 1972;177:575.
- Robertson HD, Branch AD, Dahlberg JE: Focusing on the nature of the scrapie agent. *Cell* 1985;40:725.
- Van Valen LM, Maiorana VC: The archaeobacteria and eukaryotic origins. *Nature* 1980;287:248.
- Wallace DC: Structure and evolution of organelle genomes. *Microbiol Rev* 1982;46:208.
- Woese CR, Magrum LJ, Fox GE: Archaeobacteria. *J Mol Evol* 1978;11:245.

OPTICAL METHODS

The Light Microscope

The resolving power of the light microscope under ideal conditions is about half the wavelength of the light being used. (Resolving power is the distance that must separate two point sources of light if they are to be seen as two distinct images.) With yellow light of a wavelength of $0.4\text{ }\mu\text{m}$, the smallest separable diameters are thus about $0.2\text{ }\mu\text{m}$. The **useful magnification** of a microscope is the magnification that makes visible the smallest resolvable particles. Microscopes used in bacteriology generally employ a 90-power objective lens with a 10-power ocular lens, thus magnifying the specimen 900 times. Particles $0.2\text{ }\mu\text{m}$ in diameter are therefore magnified to about 0.2 mm and so become clearly visible. Further magnification would give no greater resolution of detail and would reduce the visible area (field).

Further improvement in resolving power can be accomplished only by the use of light of shorter wavelengths of about $0.2\text{ }\mu\text{m}$, thus allowing resolution of particles with diameters of $0.1\text{ }\mu\text{m}$. Such microscopes, employing quartz lenses and photographic systems, are too expensive and complicated for general use.

The Electron Microscope

Using a beam of electrons focused by magnets, the electron microscope can resolve particles $0.001\text{ }\mu\text{m}$ apart. Viruses, with diameters of $0.01\text{--}0.2\text{ }\mu\text{m}$, can be easily resolved.

An important technique in electron microscopy is the use of "shadowing." This involves depositing a thin layer of metal (such as platinum) on the object by placing it in the path of a beam of metal ions in a vacuum. The beam is directed obliquely, so that the object acquires a "shadow" in the form of an uncoated area on the other side. When an electron beam is then passed through the coated preparation in the electron microscope and a positive print made from the "negative" image, a three-dimensional effect is achieved (eg, see Figs 2-21, 2-22, and 2-23).

Other important techniques in electron microscopy include the use of ultrathin sections of embedded material; a method of freeze-drying specimens, which prevents the distortion caused by conventional drying procedures; and the use of negative staining with an electron-dense material such as phosphotungstic acid (eg, see Fig 44-1).

The **scanning electron microscope** provides 3-dimensional images of the surfaces of microscopic objects (eg, see Fig 3-1). The object is first coated with a thin film of a heavy metal and then scanned by a downward-directed electron beam. Electrons scattered by the heavy metal are collected and focused to form the final image.

Darkfield Illumination

If the condenser lens system is arranged so that no light reaches the eye unless reflected from an object on the microscope stage, structures that provide insufficient contrast with the surrounding medium can be made visible. This technique is particularly valuable for observing organisms such as the spirochetes, which are difficult to observe by transmitted light.

Phase Microscopy

The phase microscope takes advantage of the fact that light waves passing through transparent objects, such as cells, emerge in different phases depending on the properties of the materials through which they pass. A special optical system converts difference in phase into difference in intensity, so that some structures appear darker than others. An important feature is that internal structures are thus differentiated in living cells; with ordinary microscopes, killed and stained preparations must be used.

Autoradiography

If cells that have incorporated radioactive atoms are fixed on a slide, covered with a photographic emulsion, and stored in the dark for a suitable period of time, tracks appear in the developed film emanating from the sites of radioactive disintegration. If the cells are labeled with a weak emitter such as tritium, the tracks are sufficiently short to reveal the position of the radioactive label in the cell. This procedure, called autoradiography, has been particularly useful in following the replication of DNA, using tritium-labeled thymidine as a specific tracer.

EUKARYOTIC CELL STRUCTURE

The principal features of the eukaryotic cell are shown in the electron micrograph in Fig 2-1. Note the following structures.