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Jawetz, Melnick, & Adelberg's

Medical Microbiology

LANGE

Geo.F.Brooks
Janet S.Butel
Stephen A. Morse

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twenty second edition

A LANGE Medical Book

Jawetz, Melnick, & Adelberg's Medical Microbiology

Twenty-Second Edition

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Preface

The goals for the twenty-second edition of *Medical Microbiology* remain the same as those of the first edition—to provide a brief, accurate, and up-to-date presentation of those aspects of medical microbiology that are of particular significance in the fields of clinical infections and chemotherapy. The current edition reflects the remarkable advances that have been made in our knowledge of microbes and the molecular mechanisms of microbial disease pathogenesis as well as in the development of modern laboratory and diagnostic technologies.

The basic outline and chapter sequence of the twenty-first edition have been retained for the twenty-second edition. The entire book has been updated based on the development of new information. In Chapter 48, the AIDS case has been brought up to date, taking into account highly active antiretrovirus therapy, and a section on bioterrorism and biologic warfare has been added. The names of pathogens listed on the inside front and back covers have been updated to correspond to the formal and widely accepted names.

Internet access to the medical references and literature has become readily available to most students in the United States and many other countries. This has changed the objective for the references at the end of each chapter. The number of references cited has been decreased with the goal of having a few very recent references, including review articles and book chapters that in themselves contain many citations, providing the reader with an alternative source of reference material.

Dr James Ransom has edited the twenty-second edition as he did the first edition and almost all of the intervening ones. We thank him for helping to maintain the high quality, clarity, and consistency of the text. We also wish to express our appreciation to those whose comments help keep the revisions of this book accurate and current. We especially thank Richard M. Jamison for his help with this edition.

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March 2001

Contents

II. IMMUNOLOGY

III. BACTERIOLOGY

V. MYCOLOGY

VI. PARASITOLOGY

VII. DIAGNOSTIC MEDICAL MICROBIOLOGY & CLINICAL CORRELATION

The Science of Microbiology 1

BIOLOGIC PRINCIPLES ILLUSTRATED BY MICROBIOLOGY

Nowhere is biologic **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 biologic 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, molecular biology,** 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 biologic 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 biologic 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 prokaryotes. 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 biologic 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 biologic 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 vi-

ral nucleic acid 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. Some very small viruses require the assistance of another virus in the host cell for their duplication. The delta agent, also known as hepatitis D virus, is too small to code for even a single capsid protein and needs help from hepatitis B virus for transmission. 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 and one protozoan. 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.

The general properties of animal viruses pathogenic for humans are described in Chapter 29. Bacterial viruses are described in Chapter 7.

PRIONS

A number of remarkable discoveries in the past 3 decades have led to the molecular and genetic characterization of the transmissible agent causing **scrapie,** a degenerative central nervous system disease of sheep. Studies have identified a scrapie-specific protein in preparations from scrapie-infected brains of sheep which is capable of reproducing the symptoms of scrapie in previously uninfected sheep. Attempts to identify additional components, such as nucleic acid, have been unsuccessful. To distinguish this agent from viruses and viroids, the term **prion** was introduced to emphasize its proteinaceous and infectious nature. The prion protein (PrP) is encoded by the host's chromosomal DNA. PrP is a sialoglycoprotein of molecular weight 33,000–35,000 with a high content of α-helical secondary structure that is sensitive to proteases and soluble in detergent. The normal form of the protein (PrP^c) is expressed on the surface of neurons via a glycophosphatidyl inositol anchor in both infected and uninfected brains. An abnormal isoform of this protein (PrP^{Sc}) is the only known component of the prion and is associated with transmissibility. This abnormal isoform differs physically from the normal cellular isoform by its high beta-sheet content, its insolubility in detergents, its propensity to aggregate, and its partial resistance to proteolysis.

There are additional prion diseases of importance. Kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Straussler-Scheinker disease, and fatal familial insomnia affect humans. Bovine spongiform encephalopathy (BSE), which is thought to result from the ingestion of feeds and bone meal prepared from rendered sheep offal, has been responsible for the deaths of more than 170,000 cattle in Great Britain since its discovery in 1985. A new variant CJD has been associated with human exposure to BSE in the UK and France.

Human prion diseases are unique in that they manifest as sporadic, genetic, and infectious diseases The study of prion biology is an important emerging area of biomedical investigation, and much remains to be learned.

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. The specialized region of the cell containing DNA is termed the **nucleoid** and can be visualized by electron microscopy. 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. Recent data based on genome sequencing indicate that the number of genes within a prokaryote may vary from 468 in *Mycoplasma genitalium* to 4288 in *Escherichia coli,* and many of these genes must be dedicated to essential functions such as energy gen-

eration, macromolecular synthesis, and cellular replication. Any one prokaryote carries relatively few genes that allow physiologic 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 physiologic 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 physiologic 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.

Many bacteria exploit a cell-cell communication device called **quorum sensing** to regulate the transcription of genes involved in diverse physiologic processes, including bioluminescence, plasmid conjugal transfer, and the production of virulence determinants. Quorum sensing depends on the production of one or more diffusible signal molecules termed **autoinducers** or **pheromones** which enable a bacterium to monitor its own cell population density. It is an example of multicellular behavior in prokaryotes.

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. This loss of function is reflected by the presence of a smaller genome with fewer genes.

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, physiologic, 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 cytochrome 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 & Archaebacteria: 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 archaebacteria, has received relatively little attention, in part because many of its representatives are difficult to study in the laboratory. Some archaebacteria, 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 archaebacteria 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 archaebacteria 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 archaebacteria 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 archaebacterial 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 physiologic 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 chromosomes 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.** This term can be applied only metaphorically to the prokaryotes, which exchange fragments of DNA through recombination. Taxonomic groupings of eukaryotes frequently are based on shared **morphologic 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 physiologic 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 sporozoons, 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 45.

Slime Molds

These organisms are characterized by the presence, as a stage in their life cycle, of an ameboid multinucleate mass of cytoplasm call a **plasmodium.** The plasmodium of a slime mold is analogous to the mycelium of a true fungus. Both are coenocytic. 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 physiologic responses that may contribute to survival.

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Cell Structure

2

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 μm, the smallest separable diameters are thus about 0.2 μm. 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 μm 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 μm, thus allowing resolution of particles with diameters of 0.1 μm. Such microscopes, employing quartz lenses and photographic systems, are too expensive and complicated for general use.

The Electron Microscope

The high resolving power of the electron microscope has enabled scientists to observe the detailed structures of prokaryotic and eukaryotic cells. The superior resolution of the electron microscope is due to the fact that electrons have a much shorter wavelength than the photons of white light.

There are two types of electron microscopes in general use: the transmission electron microscope (TEM), which has many features in common with the light microscope; and the scanning electron microscope (SEM). The TEM was the first to be developed and employs a beam of electrons projected from an electron gun and directed or focused by an electromagnetic condenser lens onto a thin specimen. As the electrons strike the specimen, they are differentially scattered by the number and mass of atoms in the specimen; some electrons pass through the specimen and are gathered and focused by an electromagnetic objective lens, which presents an image of the specimen to the projector lens system for further enlargement. The image is visualized by allowing it to impinge on a screen that fluoresces when struck with the electrons. The image can be recorded on photographic film. TEM can resolve particles 0.001 μm apart. Viruses, with diameters of 0.01–0.2 μm, can be easily resolved.

The SEM generally has a lower resolving power than the TEM; however, it is particularly useful for providing three-dimensional images of the surface of microscopic objects. Electrons are focused by means of lenses into a very fine point. The interaction of electrons with the specimen results in the release of different forms of radiation (eg, secondary electrons) from the surface of the material, which can be captured by an appropriate detector, amplified, and then imaged on a television screen.

An important technique in electron microscopy is the use of "shadowing." This involves depositing a thin layer of heavy metal (such as platinum) on the specimen by placing it in the path of a beam of metal ions in a vacuum. The beam is directed at a low angle to the specimen, so that it 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 is made from the "negative" image, a three-dimensional effect is achieved (eg, see Figure 2–26).

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 or uranyl salts (eg, Figure 42–1). Without these heavy metal salts, there would not be enough contrast to detect the details of the specimen.

Darkfield Illumination

Darkfield microscopy is frequently performed on the same microscope on which brightfield microscopy is performed. Illumination for darkfield microscopy is obtained using a special condenser that both blocks direct light rays and deflects light off

a mirror on the side of the condenser at an oblique angle. This creates a "dark field" that contrasts against the highlighted edge of the specimens and results when the oblique rays are reflected from the edge of the specimen upward into the objective of the microscope. This technique is particularly valuable for observing organisms such as *Treponema pallidum,* a spirochete which is less than 0.2 μm in diameter and therefore cannot be observed with direct light (Figure 2–1).

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 though 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.

A confocal microscope uses intense laser light beams and computer-assisted image enhancement to provide a nearly three-dimensional image from thick fluorescent specimens. Confocal microscopy has made significant contributions to the field of cell biology.

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 cell. The procedure, called autoradiography, has been particularly useful in following the replication of DNA, using tritium-labeled thymidine as a specific tracer. A variation of this method that employs labeled nucleic acid probes is called **in situ hybridization** and has been used to detect the presence of viral, bacterial, and fungal nucleic acid in cells and tissues.

Figure 2–1. Positive darkfield examination. Treponemes are recognized by their characteristic corkscrew-shaped and deliberate forward and backward movement with rotation about the longitudinal axis. (Reproduced, with permission, from Morse SA, Moreland AA, Thompson SE [editors]: *Atlas of Sexually Transmitted Disease.* Gower, 1990.)

EUKARYOTIC CELL STRUCTURE

The Nucleus

The nucleus is bounded by a membrane that is continuous with the endoplasmic reticulum. The nuclear membrane exhibits selective permeability due to pores that permit the exchange of large molecules such as proteins and mRNAs, into and out of the nucleus; small molecules can diffuse freely in and out of the nucleus. The chromosomes of eukaryotic cells contain linear DNA macromolecules arranged as a double helix. They are only visible with a light microscope when the cell is undergoing division and the DNA is in a highly condensed form; at other times, the chromosomes are not condensed and appear as in Figure 2–2. Eukaryotic DNA macromolecules are associated with basic proteins called histones that bind to the DNA by ionic interactions.

Cytoplasmic Structures

The cytoplasm of eukaryotic cells is characterized by the presence of an endoplasmic reticulum, vacuoles, self-reproducing plastids, and an elaborate cytoskeleton composed of microtubules, microfilaments, and intermediate filaments.

The **endoplasmic reticulum** is a network of membrane-bounded channels. In some regions of the endoplasmic reticulum, the membranes are coated with ribosomes; proteins synthesized on these ribosomes pass through the membrane into the channels of the endoplasmic reticulum, through which they can be transported to other parts of the cell. A related structure, the **Golgi apparatus,** pinches off vesicles that can fuse with the cell membrane, releasing the enclosed proteins into the surrounding medium.

The plastids include **mitochondria,** which contain in their membranes the respiratory electron transport system, and **chloroplasts** (in photosynthetic organisms). The plastids contain their own DNA, which codes for some (but not all) of their constituent proteins and transfer RNAs.

Anaerobic eukaryotes are amitochondriate, but some (eg, the microaerobic parasitic flagellate *Trichomonas vaginalis*) possess a membrane-bound organelle, the hydrogenosome. The hydrogenosome is defined by its unusual function: under anaerobic conditions it produces hydrogen gas from the oxidation of pyruvate or malate.

The cytoskeleton includes arrays of actin **micro-**