Chapter 16 The World of Bacteria IV: Gram-Positive Filamentous Bacteria of Complex Morphology

OUTLINE ***Bergey's Manual of Systematic Bacteriology*, Volume 4**

Filamentous Bacteria that Divide in More Than One Plane

Filamentous Bacteria That Form True Sporangia

***Streptomyces* and Similar Genera**

Additional Filamentous Bacteria Having Uncertain Taxonomic Placement

Volume 4 of *Bergey's Manual* is devoted entirely to certain aerobic, Gram-positive bacteria which form structures similar to those that are characteristically found in the microscopic eucaryotic fungi. These structures include (1) a **mycelium** composed of branched filamentous hyphae, these generally being of much smaller diameter (approximately 1 μm) than the hyphae formed by microscopic fungi (5 to 10 μm in diameter), and (2) asexual spores, which are termed **conidiospores** (or simply **conidia**) if they are naked, or **sporangiospores** if they are enclosed within a specialized sac (**sporangium**). The spores represent a major means of reproduction, since they are produced in large numbers, each capable of giving rise to a new organism. Although they are not heat-resistant, the spores are resistant to desiccation and aid survival of the species during periods of drought. The Gram-positive filamentous bacteria are mainly harmless soil organisms, although a few are pathogenic for humans, other animals, or plants. In the soil they are saprophytic and chemoorganotrophic, and they have the important function of degrading plant or animal residues; however, some are best known for their ability to produce a wide range of antibiotics useful in treating human disease. One genus fixes N_2 symbiotically in woody plants. In this chapter we shall consider some of the genera included in the fourth volume of *Bergey's Manual*.

BERGEY'S MANUAL OF SYSTEMATIC BACTERIOLOGY, VOLUME 4

Table 16-1 lists the major groups of bacteria included in Volume 4 of the *Manual*. The reader should also note that some bacteria, the nocardioforms, are included in Volume 2 of the *Manual* rather than Volume 4 and have been described previously in this textbook (see Chap. 14). The dividing line between the no-

Table 16-1. Gram-Positive Filamentous Bacteria Included in *Bergey's Manual*, Volume 4

Section	Some Major Characteristics
FILAMENTOUS BACTERIA THAT DIVIDE IN MORE THAN ONE PLANE	The hyphae divide not only transversely but also longitudinally to produce clusters or packets of cells or spores; cell-wall type III*; soil organisms, animal pathogens, and symbiotic nitrogen-fixers are represented
FILAMENTOUS BACTERIA THAT FORM TRUE SPORANGIA	Harmless soil and water organisms whose hyphae divide in a single plane; the spores are formed within special sacs; cell-wall types II or III*
STREPTOMYCES AND SIMILAR GENERA	The hyphae divide in a single plane; long chains of conidiospores are formed at the tips of sporogenic hyphae; the organisms are mainly harmless soil organisms that are noted for production of antibiotics; a few are human or plant pathogens; cell-wall type I*
ADDITIONAL FILAMENTOUS BACTERIA HAVING UNCERTAIN TAXONOMIC PLACEMENT	A heterogeneous collection of organisms whose relationships to the major groups of Gram-positive filamentous bacteria is not yet agreed upon; some have remarkable morphological or physiological properties; a few organisms are pathogenic for humans; the cell-wall types vary

* For cell-wall types, see Table 16-2.

cardioforms and the bacteria described in the present chapter is indistinct and controversial, and is based upon such factors as (1) whether or not the hyphae can reproduce by fragmentation and (2) the chemical composition of the cells, particularly the walls. In general, the organisms described in Volume 4 of the *Manual* do not undergo fragmentation of the mycelium. With regard to chemical composition, Table 16-2 indicates the major patterns that occur among the Gram-positive filamentous bacteria with regard to cell-wall amino acid composition

Table 16-2. Amino Acid and Sugar Patterns of Aerobic, Gram-Positive, Filamentous Bacteria

	Amino Acid Pattern Designations for Cell-Wall Types			
Composition of Peptidoglycan	I	II	III	IV*
Optical isomer of diaminopimelic acid present	LL	*meso*	*meso*	*meso*
Presence of glycine interpeptide bridges linking the tetrapeptides	+	+	−	−
	Sugar Pattern Designations for Cell Types			
Sugars Present in Hydrolysates of Whole Cells	A	B	C	D
Arabinose	+	−	−	+
Galactose	+	−	−	−
Xylose	−	−	−	+
Madurose	−	+	−	−

*Type IV is distinguished from Type III by the occurrence of the sugars arabinose and galactose in the cell walls of Type IV.

and to the sugars present in whole cell hydrolysates; these differences, as well as differences in DNA base composition (mol % G + C values; see Chap. 3) and in the phospholipid composition of the cell membranes, have been used extensively to classify these organisms, in addition to the morphological features. For example, the genus *Streptomyces* has a type I cell-wall amino acid pattern and a type C sugar pattern (i.e., chemotype I, C), whereas *Dermatophilus* has a type III amino acid pattern and a type B sugar pattern (chemotype III, B). The bacteria included in Volume 4 of *Bergey's Manual* are mainly of the following types: I, C; II, D; III, B; or III, C, whereas the nocardioforms included in Volume 2 of the *Manual* are mainly type 4, A. Moreover, the organisms included in Volume 4 do not possess mycolic acids in their cell walls, whereas some nocardioforms do have these lipids.

FILAMENTOUS BACTERIA THAT DIVIDE IN MORE THAN ONE PLANE

The organisms in this group have hyphae that divide not only transversely but also longitudinally to produce clusters or packets of coccoid or cuboid cells or spores. One of the three genera, *Geodermatophilus* (*chemotype III, C*) is a soil organism; the other two genera are quite different, as indicated below.

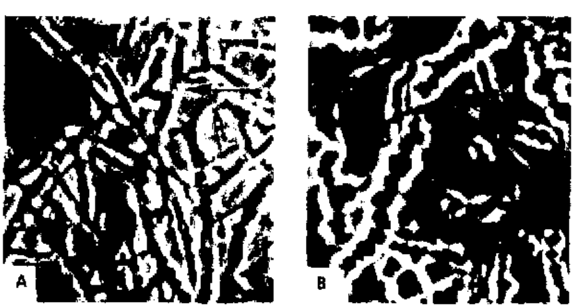
Dermatophilus

The members of this genus belong to *chemotype III, B*. The narrow tapering hyphal filaments develop septa that are formed in transverse and in horizontal and vertical planes; this results in the formation of mulberrylike clusters of cocci (see Fig. 16-1). Each coccus is released as a motile spore bearing a tuft of flagella. The single species of the genus, *D. congolensis*, is not a soil organism; rather, it is a parasite and pathogen of wild and domestic mammals, causing a skin infection (streptothrichosis).

Frankia

The members of this genus belong to *Chemotype III, D*. Like *Rhizobium* species

Figure 16-1. Phase-contrast photomicrographs of *Dermatophilus congolensis* suspended in a 28% albumin solution to reveal cellular detail (X1,100, approx.). (A) Branching hyphae in early stages of division that is mainly transverse, although a few longitudinal septa are already evident (arrow). (B) Mulberrylike clusters of cocci enveloped in mucoid capsular material that appears light against the denser, more refractive, albumin solution. [*Courtesy of D. S. Roberts, in M. P. Starr, H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (eds.), The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria, Springer-Verlag, New York, 1981, p. 2011*.]



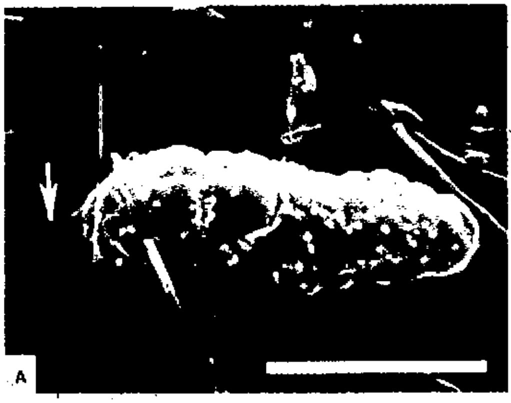


Figure 16-2. (A) Scanning electron micrograph of a large sporogenic body of *Frankia* strain AvcIl from broth culture; thickened spore-bearing hypha (arrow) attaches the spore-bearing body to a vegetative hypha. Bar indicates 10 µm. *(Courtesy of D. Baker and J. G. Torrey, Can J Microbiol* **26***:1066, 1980.)* (B) Scanning electron micrograph illustrating numerous *Frankia* vesicles and hyphae within the infected cell of a root nodule of *Elaeagnus umbellata*. Bar indicates 10 µm. *(Courtesy of D. Baker, W. Newcomb, and J. G. Torrey, Can J Microbiol,* **26***:1072, 1980.)*

(see Chap. 13), these bacteria are highly efficient microaerophilic N_2-fixers that occur within the root nodules of plants. Unlike *Rhizobium* species, however, they infect nonleguminous woody plants, such as alders. Isolation of the symbiont defied all efforts for years, but in 1978–1980 cultures were isolated by means of elaborate procedures such as (1) microdissection of nodules to remove the bacterial clusters, or (2) sucrose gradient centrifugation of nodule suspensions, which allowed separation of the organisms from extraneous material on the basis of differences in density. Both within root nodules and in laboratory culture, *Frankia* strains form branching hyphae from the ends of which are produced *vesicles* (globular structures on short stalks; see Fig. 16-2); circumstantial evidence suggests that these may be the sites of N_2 fixation. Also produced are *sporogenic bodies* (often called sporangia, but whether they are true sporangia is uncertain) which contain masses of spores that are cemented together (Fig. 16-2). The spores arise by hyphal divisions that occur in several planes.

FILAMENTOUS BACTERIA THAT FORM TRUE SPORANGIA

The members of this group form an extensive substrate mycelium; some genera also form an aerial mycelium. In either instance spores are formed within a sac called a *sporangium*, which is formed aerially at the tip of a sporogenic hypha *(sporangiophore)*.

The sporangium may be either of two major kinds, depending on the genus: (1) round, rod-shaped, or irregular, containing *masses of spores*, or (2) fingerlike, club-shaped, or pear-shaped, containing *one to four spores arranged linearly* within the sporangium. The wall of the sporangium is derived from an expansion of the outer layer of the bilayered wall of the hypha, forming a sac. The inner hypha grows into this sac and subsequently undergoes septation to form spores. Some examples of genera forming multispored sporangia are illustrated in Figs. 16-3 and 16-4; in some instances the spores are motile by means of flagella and can swim away from ruptured sporangia (see Fig. 16-5). *Chemotypes of sporangia-forming bacteria are either III, B or II, D.* Sporangia-forming bacteria are widespread in nature, occurring in humus-containing soils, dead plant parts such as pollen and leaves, or on shed animal material such as hair.

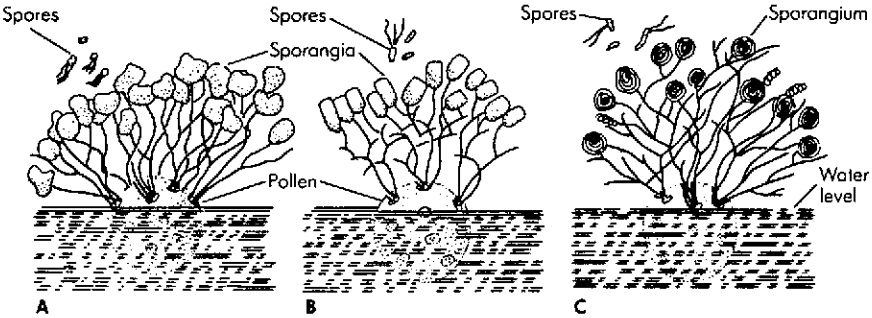


Figure 16-3. Semidiagrammatic drawings of three genera of bacteria that form multispored sporangia. The bacteria are growing on the pollen of *Liquidambar styraciflua.* The pollen floats at the surface of the water, the hyphae and sporangiophores emerging into the air through the small circular pits in the wall of the pollen grains. (A) *Actinoplanes.* (B) *Ampullariella.* (C) *Spirillospora* (X350). *(From McGraw-Hill Encyclopedia of Science and Technology, vol. 1, 1968.)*

Figure 16-4. *Streptosporangium roseum,* showing sporangia, containing nonmotile spores, on aerial hyphae. The sporangia range from 7 to 19 μm in diameter, usually 8 to 9 μm. *(Courtesy of Mary P. Lechevalier.)*

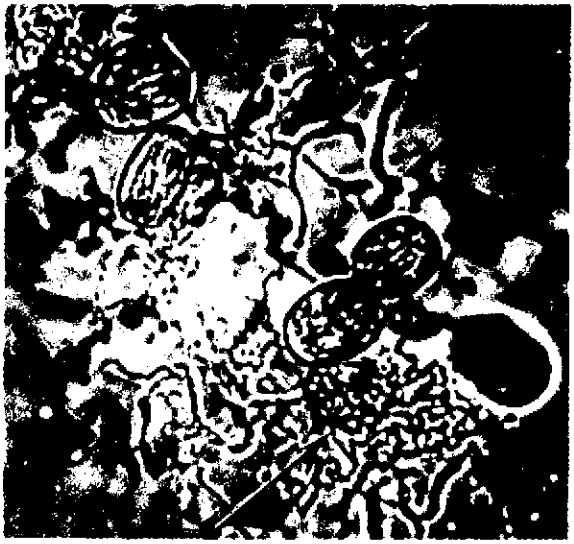


Figure 16-5. Sporangia of *Actinoplanes rectilineatus* in a water mount showing spores in longitudinal rows within the sporangia and one ruptured sporangium (arrow) with spores swimming away (X970). *(Courtesy of M. P. Lechevalier and H. A. Lechevalier, Int J Syst Bacteriol,* 25*:371, 1975.)*

Figure 16-6. *Streptomyces viridochromogenes* showing spiral chains of spores on aerial mycelium. Specimen from culture grown on yeast extract–malt extract medium for 7 days at 25°C (X450). *(Courtesy of Tom Cross.)*

Species of the genus *Actinoplanes* can polymerize amino acids to form polypeptide and depsipeptide antibiotics and can also synthesize polyene-type macrolides, glycolipid, and aromatic polycyclic antibiotics.

STREPTOMYCES AND SIMILAR GENERA

The members of this group belong to *chemotype I, C*. They usually form an abundant aerial mycelium bearing long chains of conidiospores (5 to 50 or more per chain; see Fig. 16-6). Unlike the hyphae of the substrate mycelium (i.e., submerged below the surface of the medium), the aerial hyphae possess an extra cell-wall layer (sheath). The hyphal tip undergoes septation within this sheath to form a chain of conidia (see Fig. 16-7).

Several genera occur within the group, including *Streptoverticillium, Actinopycnidium, Actinosporangium, Chainia, Elytrosporangium, Kitasatoa,* and *Microellobosporia,* but the most familiar genus is *Streptomyces*. Indeed, the other genera—except for *Streptoverticillium*—may deserve to be merged with *Streptomyces* to form a single genus. Species and subspecies are differentiated by the following characteristics:

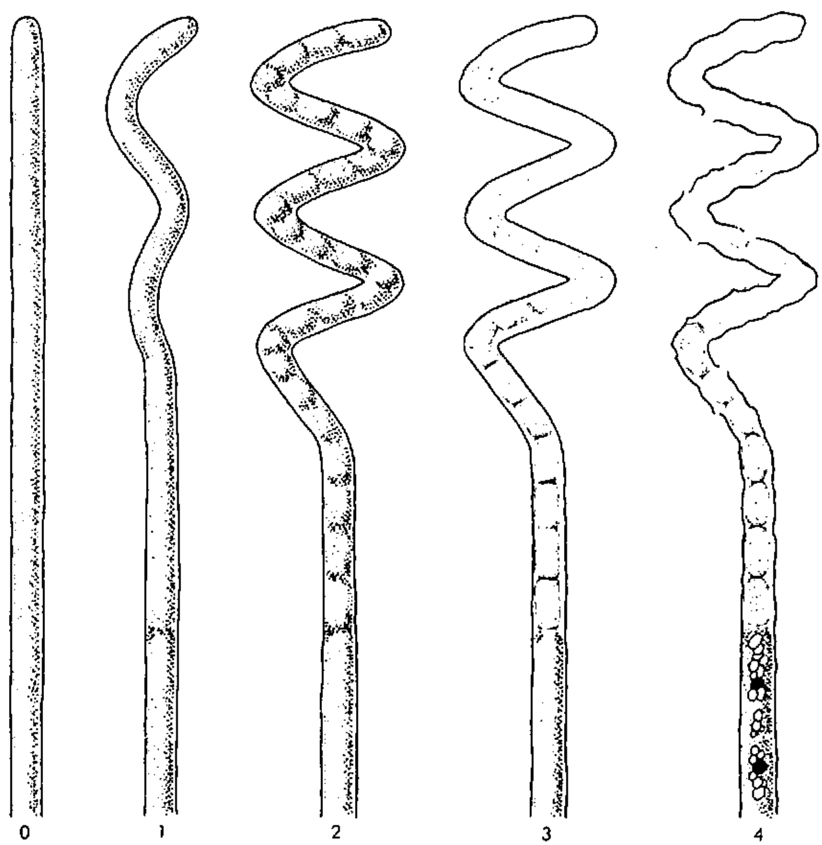


Figure 16-7. Diagram of sporulation stages in *Streptomyces coelicolor*. After a phase of growth (0) the sporulating hyphae are divided into long cells by ordinary cross walls, and the tips begin to coil (1). The apex is then partitioned into spore-sized compartments by sporulation septa (2). The cell walls thicken and constrictions appear between the young spores (3). As spores mature, they round off and separate (4). Some spores begin to germinate immediately after maturation. *(Redrawn from H. Wildermuth and D. A. Hopwood, J Gen Microbiol, 60:51–59, 1970; by permission.)*

1 ***Morphology of spore chain.*** The chains may be straight, flexuous (wavy), or coiled to various degrees. They also vary in the number of spores per chain.
2 ***Spore surface.*** By electron microscopy, the surface of the conidia may be smooth, warty, spiny, or hairy, the texture depending on the kind of adherent sheath material (for example, see Fig. 16-8).
3 ***Color of aerial mycelium.*** The pigmentation of the aerial mycelium falls within any of seven color groups: white, yellow, violet, red, blue, green, or gray. These terms cannot precisely describe the many hues that can occur; therefore, a system of published color standards is used for this purpose.
4 ***Color of substrate mycelium.*** This may differ from that of the aerial mycelium and is determined by observing the reverse side of the growth after removal of most of the agar medium with a razor blade.
5 ***Color of the medium.*** Many streptomycetes may form pigments that are excreted into the medium. These may be water-soluble, or they may precipitate in the medium close to the cells.
6 ***Physiological characteristics.*** This refers to characteristics such as utilization of various carbohydrates and organic acids, nitrate reduction, urea and esculin hydrolysis.

No other genus in bacteriology has been divided into as many species as has *Streptomyces*. The names of nearly 340 species and 39 subspecies are officially recognized, but thousands of additional names have been unofficially assigned (mostly in literature dealing with patents; see below). It is likely that DNA homology and other techniques of molecular biology will eventually indicate that a much smaller number of species exists than is represented by these names. Why then have so many names been used? The main reason rests with the ability of streptomycetes to make a *great number and variety of antibiotics* (see Table 16-3 for a few examples). As a result of the screening of soil samples by pharmaceutical companies to obtain new antibiotics, thousands of new antibiotics have been discovered, and the great majority have been made by streptomycetes. The discoverer of a new antibiotic had to have a name for the organism producing it in order to meet scientific publication or patent requirements. The organism might have been identified as belonging to an established species; however, many isolates failed to agree in all details with the description of any established species. Although some variation in characteristics does occur among the strains of any bacterial species, an easier (but much less satisfactory) solution to such a taxonomic problem was simply to give the isolate a new

Figure 16-8. Hairy conidia of *Streptomyces acrimycini* as seen by (A) transmission electron microscopy and (B) scanning electron microscopy. The bar indicates 1 μm. *(Courtesy of A. Dietz and J. Mathews, Int J Syst Bacteriol, 27:282, 1977.)*

Table 16-3. Some Antibiotics Made by *Streptomyces* Species

Antibiotic*	Species
Amphotericin B	*S. nodosus* and others
Chloramphenicol	*S. venezuelae* and others
Chlortetracycline	*S. aureofaciens* and others
Cycloserine	*S. garyphalus* and others
Erythromycin	*S. erythraeus*
Kanamycin	*S. kanamyceticus*
Neomycin	*S. fradiae* and others
Nystatin	*S. noursei* and others
Oxytetracycline	*S. rimosus* and others
Streptomycin	*S. griseus* and others
Tetracycline	*S. viridifaciens* and others

* The modes of action of these antibiotics are described in Chap. 24.

Table 16-4. Filamentous Bacteria Having Uncertain Taxonomic Placement

Genus	Chemotype	Spores	Major Characteristics
Actinomadura	III, B	Short chains of conidia formed on aerial hyphae	Mainly soil saprophytes, but can cause actinomycetoma in humans
Nocardiopsis	III, C	Long chains of conidia formed on aerial hyphae	As in *Nocardia* (Chap. 14), the substrate mycelia undergo fragmentation; unlike *Nocardia*, mycolic acids are absent and the cell wall chemotype is different; soil saprophytes, but occasionally isolated from human or animal infection
Actinopolyspora	IV, A	Long chains of conidia formed on aerial hyphae.	Extreme halophiles: growth occurs only in the presence of 10–30% NaCl; isolated from dairy salt
Actinosynnema	III; galactose and mannose are present	Chains of rodshaped conidia are formed on aerial hyphae	Form compacted groups of erect hyphae called *synnemata*; the hyphae are often fused to form a stemlike structure which bears chains of conidia; isolated from grass
Thermomonospora	III, B or C	Single conidia formed at the tips of very short sporogenic hyphae	Some grow best at 40–45°C, others at 35–40°C; they occur in high temperature habitats such as composts and can decompose cellulose
Thermoactinomyces	III, B or C	Single spores formed either at the tips of short sporogenic hyphae or are sessile (directly attached to vegetative hyphae)	The spores are endospores and survive 95°C for 10 min, are highly refractile, and contain dipicolinic acid; the organisms grow best at about 50°C; they occur in high-temperature habitats such as damp hay, composts and piles of moist grain
Sporichthya	I	Forms upright aerial chains of conidia	Strikingly different from all other filamentous bacteria in that aerial hyphae are formed but *no substrate hyphae*; a single cell attaches to the agar surface and divides to form an upright chain of conidia, each with a basal, collarlike structure

species name. Thus many species came to be distinguished from one another on the basis of only minor differences.

Most streptomycetes are harmless saprophytes occurring mainly in soils of neutral pH, although some species prefer acidic or alkaline soil. They can grow in soils having less water content than that needed for most other bacteria, and because of their spores they can survive well in dry soil. Many streptomycetes can degrade polymeric organic substances in soil that are refractory to being

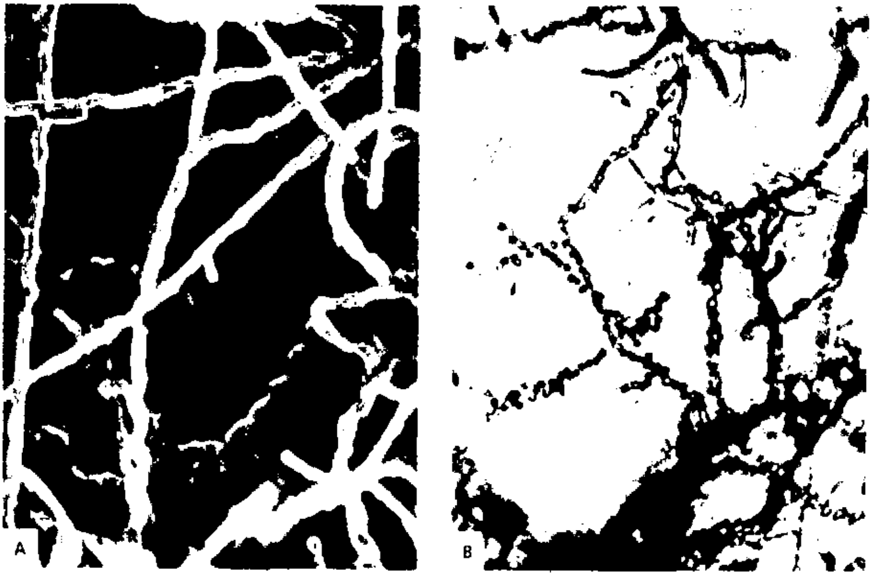


Figure 16-9. (A) Scanning electron micrograph of the extreme halophile, *Actinopolyspora halophila*, grown on a medium containing 20% NaCl, showing aerial hyphae and conidia (X4,600). *(Courtesy of M. B. Gochnauer, G. G. Leppard, P. Komaratat, M. Kates, T. Novitsky, and D. J. Kushner, Can J Microbiol,* **21***:1500–1511, 1975.)* (B) *Thermoactinomyces vulgaris* showing aerial mycelium and single sessile endospores borne along the length of the hyphae. Specimen taken from a culture grown on Czapek's agar plus 0.5% yeast extract which was incubated for 2 days at 55°C (X800). *(Courtesy of Tom Cross.)*

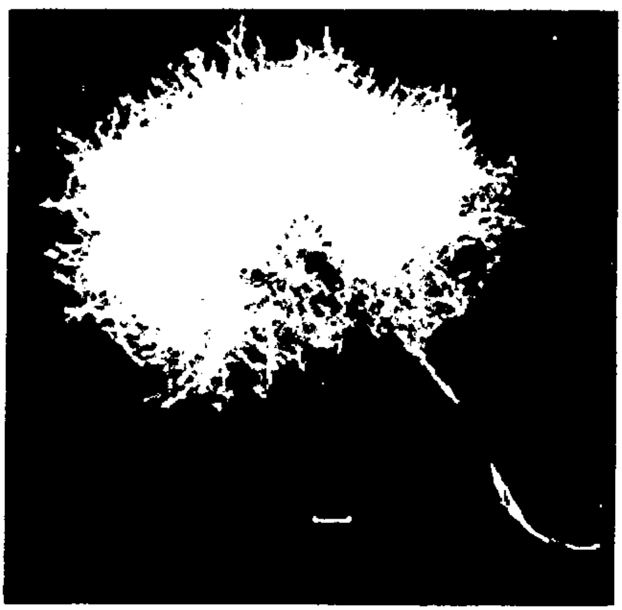


Figure 16-10. Scanning electron micrograph of *Actinosynnema mirum* showing a synnema (compacted group of erect hyphae forming a stemlike structure) at lower right and the aerial mycelium with chains of conidiospores arising from its tip. The bar indicates 10 μm. *(Courtesy of T. Hasegawa, M. P. Lechevalier, and H. A. Lechevalier, Int J Syst Bacteriol,* **28***:304–310, 1978.)*

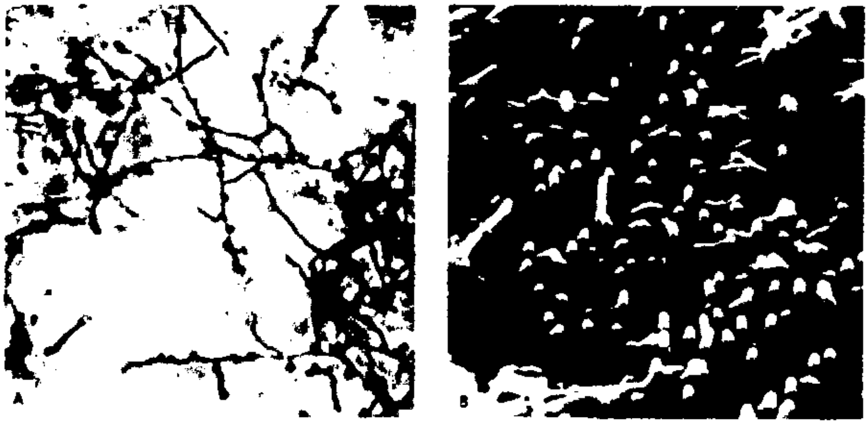


Figure 16-11. (A) *Thermomonospora mesophila*, showing monospores on the aerial hyphae. The spores are about 1 μm in diameter. (*Courtesy of Mary P. Lechevalier.*) (B) Scanning electron micrograph of *Sporichthya polymorpha* showing a general view of developing upright cells. This genus grows only at the surface of the medium and forms no substrate mycelium (X3,900). (*Courtesy of S. T. Williams, J Gen Microbiol, 62:67–73, 1970.*)

decomposed by many other microorganisms, e.g., starch, pectin, and chitin. A few species are plant pathogens, the most important of these being *Streptomyces scabies*, the causative agent of "common scab" in potatoes and sugar beets. One species, *Streptomyces somaliensis*, is pathogenic for humans, causing actinomycetoma. Nonstreptomycetes can also cause actinomycetoma: see the genera *Nocardia* (Chap. 14) and *Actinomadura* (Table 16-4).

ADDITIONAL FILAMENTOUS BACTERIA HAVING UNCERTAIN TAXONOMIC PLACEMENT

The taxonomic placement of the bacteria of this heterogeneous group is not yet agreed upon. Many of the organisms have unusual and striking morphological or physiological characteristics, such as the extreme halophilism exhibited by *Actinopolyspora* (Fig. 16-9A), formation of heat-resistant endospores by *Thermoactinomyces* (Fig. 16-9B), formation of compacted groups of erect hyphae which have a stemlike appearance (*synnemata*; singular, synnema) by *Actinosynnema* (Fig. 16-10), the thermophilic and cellulolytic properties of *Thermomonospora* (Fig. 16-11A), and development of upright cells by *Sporichthya* (Fig. 16-11B). The major characteristics of these and other genera are summarized in Table 16-4.

QUESTIONS

1 Besides *Frankia*, what other genera of symbiotic, nodule-inducing nitrogen-fixing bacteria can you list? Are these genera also Gram-positive? (Refer also to Chap. 13.)
2 Distinguish between *Nocardia*, *Pseudonocardia*, and *Nocardiopsis*. (The first two are described in Chap. 14.)
3 Give the outstanding features of each of the following genera: (*a*) *Thermoactinomyces*, (*b*) *Dermatophilus*, (*c*) *Actinoplanes*, (*d*) *Actinosynnema*, (*e*) *Sporichthya*.
4 Why is it that the genus *Streptomyces* contains so many named species?
5 What is responsible for the surface texture (i.e., smooth, warty, hairy, spiny) of the conidia of streptomycetes?
6 The cell walls of many soil fungi contain chitin. Therefore, in addition to the

production of certain antifungal antibiotics, how might streptomycetes be able to compete successfully with fungi when growing in soil?

7 Despite its extreme halophilic nature, *Actinopolyspora* belongs to the eubacteria and not to the archaeobacteria (in contrast, for example, to *Halobacterium*). What sort of evidence might have led to this conclusion?

8 Define the following terms: synnemata, conidia, sporangiophore, sessile spore, sporangium, chemotype.

REFERENCES

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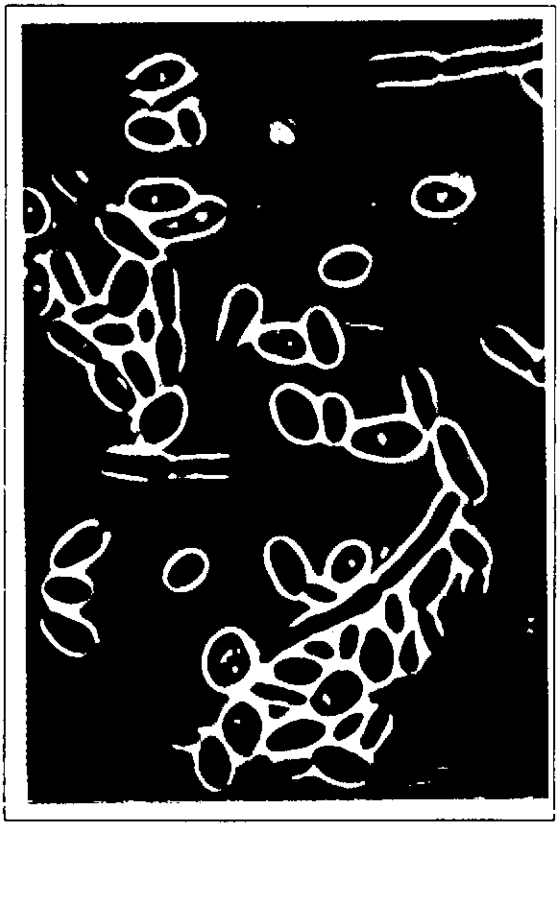
Starr, M. P., H. Stolp, H. G. Trüper, A. Balows, and H. G. Schlegel (eds.): *The Prokaryotes: A Handbook of Habitats, Isolation, and Identification of Bacteria*, Springer-Verlag, New York, 1981. *Volume II of this monumental reference work provides a wealth of descriptive information and illustrations concerning the various genera of Gram-positive filamentous bacteria.*

Williams, S. T. (ed.): *Bergey's Manual of Systematic Bacteriology*, vol. 4, Williams & Wilkins, Baltimore (in preparation). *Volume 4 of this international work is presently being prepared and will provide detailed descriptions of the genera and species of the bacteria discussed in Chap. 16.*

PART FIVE MICROORGANSIMS—FUNGI, ALGAE, PROTOZOA, AND VIRUSES



The many kinds of microbes

Professor John O. Corliss of the University of Maryland is recognized for his scholarly knowledge of the protozoa. His recent contribution entitled "A Puddle of Protists" (in *The Sciences*, New York Acadamy of Science, May–June, 1983) is recommended reading to introduce this part of the text. And so in the discourse that follows, we borrow freely from his article the historical development of human knowledge on the protists and other "wee beasties."

It all began in the nineteenth century when the German evolutionist Ernst Haeckel proposed a third biological kingdom, the Protista, to include all the many microbes, large and small, especially those which could not be neatly classified with either the *plants* or the *animals*. While this was a convenient taxonomic device, it had its own problems because the microbes assembled together included members which were fundamentally dissimilar. For instance, the bacteria, the protozoa, the algae, and the fungi were all lumped together. This is not to say that there was no common thread running through them all: indeed, to be counted as a protist, an organism must be unicellular in at least one stage of its life history and must at no stage develop organized tissues. Nonetheless, Haeckel's revolutionary proposal essentially fell flat and has laid dormant for all these years.

After Haeckel, the next breakthrough in the classification of the largely unicellular forms came a century later. Roger Y. Stanier and his colleagues at the University of California at Berkeley proposed two assemblages of organisms based mainly on the type of nucleus present within their cells. These two kingdoms of organisms are called the *procaryotes* (prenucleated cells) and the *eucaryotes* (cells with a true nucleus). The number of their differential characteristics (some of which we have propounded in the beginning of this text) has grown as biologists probe into the molecular properties of the cells of these two groups. Such findings have strengthened the major dichotomy between them.

Very recently, Carl Woese, of the University of Illinois, and other molecular geneticists working primarily with bacteria, have proposed that the procaryotes themselves should be split into two groups. This split results in three kingdoms of organisms: the **Eucaryota**, the **Archaeobacteria** of Woese, and the rest of the **Procaryota**. The archaeobacteria are distinguished from the other procaryotes by the sequence of bases in their ribosomal RNA, the chemical composition of their lipids, and their cell wall structure. They constitute a group of procaryotes that are found in specialized or extreme habitats—the hot acidic niches of the thermoacidophilic bacteria, the nearly saturated salt solutions of the extreme halophilic bacteria, and the highly anaerobic environment required for the growth of the methane-producing bacteria.

In this part of the text we shall look at the world of the eucaryotic protists—the fungi (molds and yeasts), the algae, the protozoa—as well as that group of taxonomically elusive infectious entities which we call the viruses and which we are hard pressed to know where to place in the evolved classification schemes for all the life forms.

Preceding page. The yeast *Saccharomyces uvarum*. *(Courtesy of I. Benda and Avi Publishing Co., Inc.)*

Chapter 17 Fungi—Molds and Yeasts

OUTLINE

The **fungi** (singular, **fungus**) are a group of eucaryotic organisms that are of great practical and scientific interest to microbiologists. One good reason for this is obviously that many fungi are of microscopic cellular dimensions. The gross appearance of many multicellular fungi is familiar to each and every one of us. We have seen the velvety blue-and-green growth on rotting oranges and lemons as well as on stale cheeses, the whitish-gray furry outgrowths on bread and jam, and the mushrooms in the fields. These are the bodies of various fungi. Thus fungi have a diversity of morphological appearances, depending on the species. Fungi comprise the molds and yeasts. Whereas molds are filamentous and multicellular, yeasts are usually unicellular. Fungi are eucaryotic spore-bearing protists that lack chlorophyll. They generally reproduce both sexually and asexually.

THE IMPORTANCE OF FUNGI

The fungi are heterotrophic organisms—they require organic compounds for nutrition. When they feed on dead organic matter, they are known as **saprophytes**. Saprophytes decompose complex plant and animal remains, breaking them down into simpler chemical substances that are returned to the soil, thereby increasing its fertility. Thus they can be quite beneficial to humans. But they can also be undesirable when they decompose timber, textiles, food, and other materials.

Saprophytic fungi are also important in industrial fermentations: for example, the brewing of beer, the making of wine, and the production of antibiotics such as penicillin. The leavening of dough and the ripening of some cheeses also depend on fungal activity.

As **parasites** (i.e., when living in or on another organism), fungi cause diseases in plants, humans, and other animals. Although fungal diseases are less commonly encountered than bacterial or virus diseases in humans and other animals, they are of great importance in causing diseases of plants.

Quite apart from the applied aspects of the study of fungi, these microorganisms are to be studied in their own right as biological entities. The science or study of fungi is called **mycology**. Fungi have also become tools for the physiologist, biophysicist, geneticist, and biochemist, who find them highly suitable subjects for the study of some biological processes.

DISTINGUISHING CHARACTERISTICS OF FUNGI

Fungi are eucaryotic chemoorganotrophic organisms that have no chlorophyll. The **thallus** (plural, **thalli**), or body of a fungus may consist of a single cell as in the yeasts; more typically the thallus consists of filaments, 5 to 10 μm across, which are commonly branched. The yeast cell or mold filament is surrounded by a true cell wall (the exception being the slime molds, which have a thallus

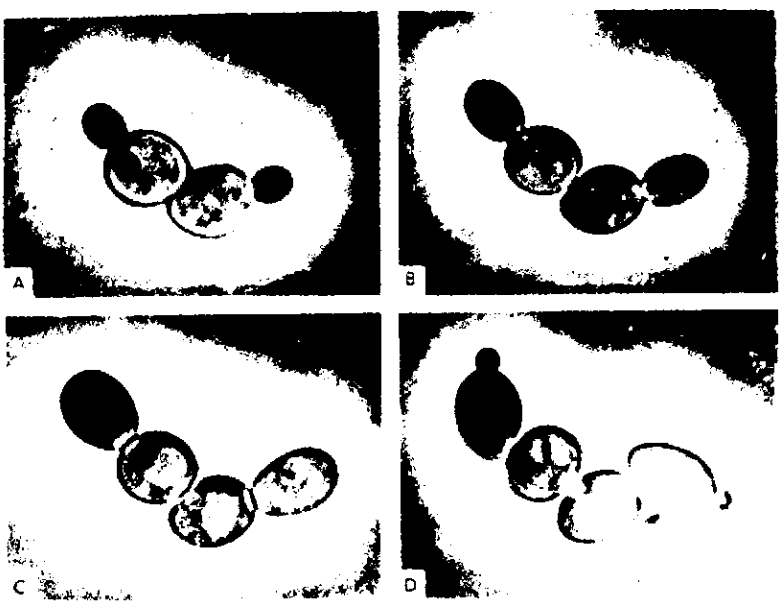


Figure 17-1. Actively budding yeasts produce mature daughter cells in about 30 min. These photographs were taken at 10-min intervals. The typical unicellular nature of yeast cells is shown. *(Courtesy of Carl C. Lindegren.)*

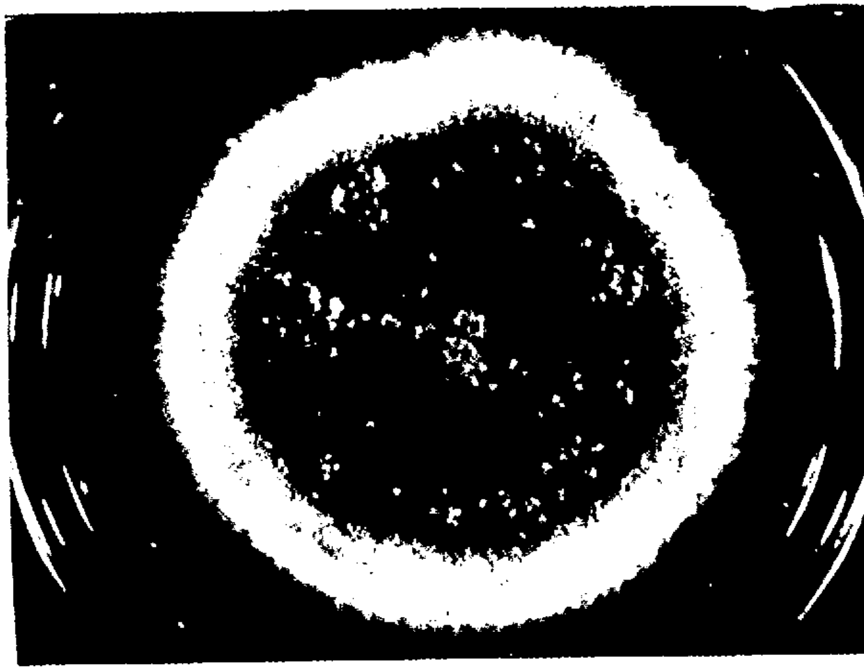


Figure 17-2. A mold colony growing in a Petri dish. Note the filamentous growth of the organism. The powdery appearance is due to the presence of thousands of asexual spores, or conidia. The species shown belongs to the genus *Penicillium*, the same genus of mold that produces the antibiotic penicillin.

consisting of a naked amoeboid mass of protoplasm). Fig. 17-1 shows the typical morphology of a yeast cell.

Some fungi are **dimorphic**; that is, they exist in two forms. Some pathogenic fungi of humans and other animals have a unicellular and yeastlike form in their host, but when growing saprophytically in soil or on a laboratory medium they have a filamentous mold form. The laboratory identification of such fungal pathogens is often dependent on the demonstration of dimorphism. The opposite dimorphic phenomenon occurs in some plant pathogens. In *Taphrina* (which causes peach leaf curl) or in smuts (which cause diseases of cereal crops), the mycelial form occurs in the host and the unicellular yeastlike form occurs in laboratory culture.

Thus a fungal colony may be a mass of yeast cells (not unlike a bacterial colony except for surface texture), or it may be a filamentous mat of mold as shown in Fig. 17-2.

MORPHOLOGY

In general, yeast cells are larger than most bacteria. Yeasts vary considerably in size, ranging from 1 to 5 μm in width and from 5 to 30 μm or more in length. They are commonly egg-shaped, but some are elongated and some spherical. Each species has a characteristic shape, but even in pure culture there is considerable variation in size and shape of individual cells, depending on age and environment. Yeasts have no flagella or other organelles of locomotion.

The thallus of a mold consists essentially of two parts: the **mycelium** (plural, **mycelia**) and the **spores** (resistant, resting, or dormant cells). The diversity of

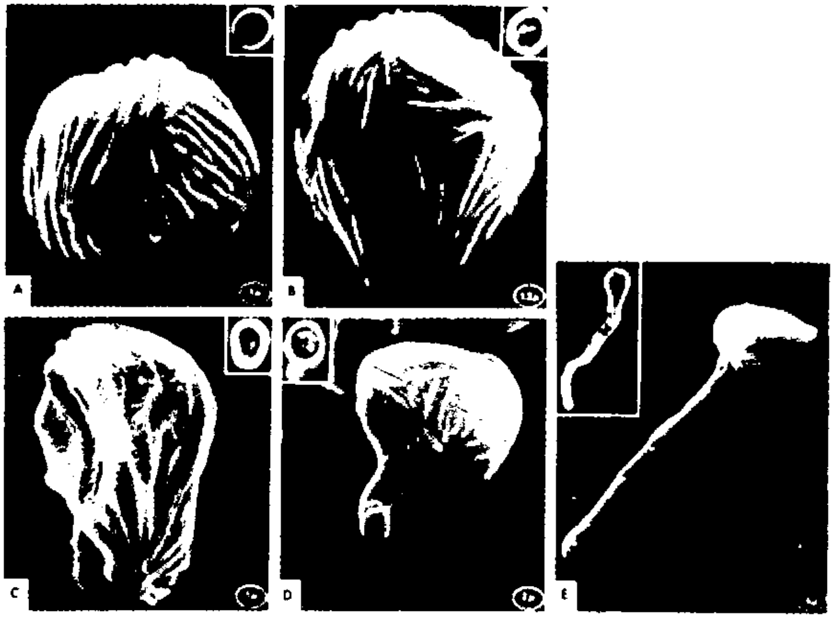


Figure 17-3. Scanning electron micrographs of *Rhizopus stolonifer* spores at sequential stages of germination, with corresponding phase-contrast micrographs (X1,200) inserted. (A) Ungerminated spore; (B) swollen spore; (C) elongated spore; (D) germ-tube emergence; and (E) germ-tube elongation. *(Courtesy of James L. van Etten, Lee A. Bulla, Jr., and Grant St. Julian, "Physiological and Morphological Correlation of Rhizopus stolonifer Spore Germination," J Bacteriol,* **117***:882–887, 1974.)*

these spores will be discussed later. The mycelium is a complex of several filaments called **hyphae** (singular, **hypha**). New hyphae generally arise from a spore which on germination puts out a germ tube or tubes (Fig. 17-3). These germ tubes elongate and branch to form hyphae.

Each hypha is about 5 to 10 μm wide, as compared with a bacterial cell which is usually 1 μm in diameter. Hyphae are composed of an outer tubelike wall surrounding a cavity, the **lumen**, which is filled or lined by protoplasm. Between the protoplasm and the wall is the **plasmalemma**, a double-layered membrane which surrounds the protoplasm. The hyphal wall consists of microfibrils composed for the most part of hemicelluloses or chitin; true cellulose occurs only in the walls of lower fungi. Wall matrix material in which the microfibrils are embedded consists of proteins, lipids, and other substances.

Growth of a hypha is distal, near the tip. The major region of elongation takes place in the region just behind the tip. The young hypha may become divided into cells by crosswalls which are formed by **centripetal invagination** (inward growth) from the existing cell wall. These crosswalls constrict the plasmalemma and grow inward to form generally an incomplete **septum** (plural, **septa**) that has a **central pore** which allows for protoplasmic streaming. Even nuclei may migrate from cell to cell in the hypha.

Hyphae occur in three forms (Fig. 17-4):

1 Nonseptate, or **coenocytic**. Such hyphae have no septa.
2 Septate with uninucleate cells.
3 Septate with multinucleate cells. Each cell has more than one nucleus in each compartment.

Mycelia can be either vegetative or reproductive. Some hyphae of the vege-

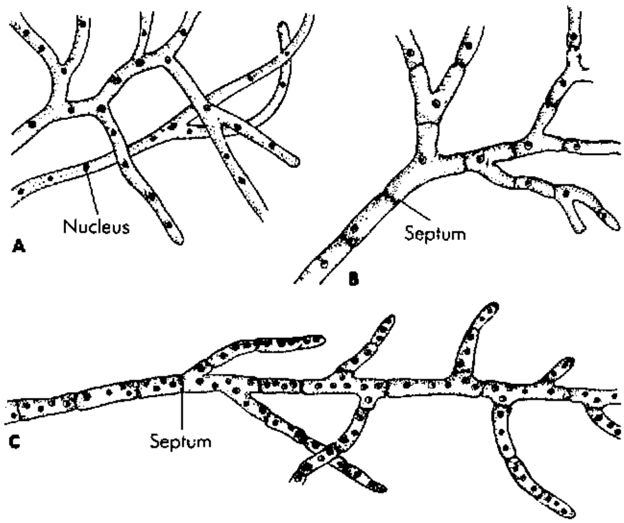


Figure 17-4. Three types of hyphae. (A) Nonseptate (coenocytic), (B) septate with uninucleate cells, (C) septate with multinucleate cells.

tative mycelium penetrate into the medium in order to obtain nutrients; soluble nutrients are absorbed through the walls. (Insoluble nutrients are first digested externally by secreted enzymes.) Reproductive mycelia are responsible for spore production and usually extend from the medium into the air. The mycelium of a mold may be a loosely woven network or it may be an organized, compact structure, as in mushrooms.

REPRODUCTION

Asexual Reproduction

Fungi reproduce naturally by a variety of means. Asexual reproduction (also called somatic or vegetative reproduction) does not involve the union of nuclei, sex cells, or sex organs. It may be accomplished by (1) fission of somatic cells yielding two similar daughter cells; (2) budding of somatic cells or spores, each bud a small outgrowth of the parent cell developing into a new individual; (3) fragmentation or disjointing of the hyphal cells, each fragment becoming a new organism; or (4) spore formation.

Asexual spores, whose function is to disseminate the species, are produced in large numbers. There are many kinds of asexual spores (Fig. 17-5):

1 *Sporangiospores.* These single-celled spores are formed within sacs called sporangia (singular, sporangium) at the end of special hyphae (sporangiophores). Aplanospores are nonmotile sporangiospores. Zoospores are motile sporangiospores, their motility being due to the presence of flagella.
2 *Conidiospores* or conidia (singular, conidium). Small, single-celled conidia are called microconidia. Large, multicelled conidia are called macroconidia. Conidia are formed at the tip or side of a hypha (see Fig. 17-6).
3 *Oidia* (singular, oidium) or arthrospores. These single-celled spores are formed by disjointing of hyphal cells. (See Fig. 17-7.)
4 *Chlamydospores.* These thick-walled, single-celled spores are highly resistant to adverse conditions. They are formed from cells of the vegetative hypha.
5 *Blastospores.* These are spores formed by budding.

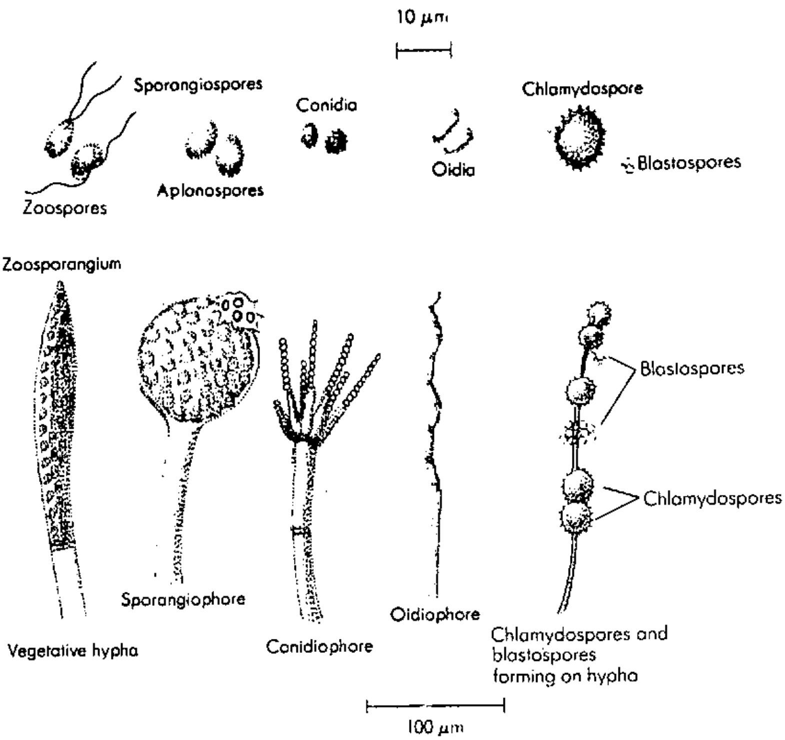


Figure 17-5. Asexual spore types in fungi. *(Redrawn from the McGraw-Hill Encyclopedia of Science and Technology, 1977, vol. 5, p. 117.)*

Figure 17-6. Conidia are produced in large numbers as exemplified here by a species of *Penicillium* (X1,000). *(Courtesy of Douglas F. Lawson.)*

Figure 17-7. Gross appearance of *Mucor rouxii* arthrospores. (A) Light microscopy showing extensive arthrospore formation. (B) High-magnification light microscopy showing variation in arthrospore size and shape. (C) Transmission electron micrograph of arthrospore chain. All bars represent 10 μm. *(Courtesy of C. R. Barrera, New Mexico State University, and the American Society for Microbiology.)*

Sexual Reproduction

Sexual reproduction is carried out by fusion of the compatible nuclei of two parent cells. The process of sexual reproduction begins with the joining of two cells and fusion of their protoplasts (**plasmogamy**), thus enabling the two haploid nuclei of two mating types to fuse together (**karyogamy**) to form a diploid nucleus. This is followed by **meiosis**, which again reduces the number of chromosomes to the haploid number.

The sex organelles of fungi, if they are present, are called **gametangia** (singular, **gametangium**). They may form differentiated *sex cells* (**gametes**) or may contain instead one or more gamete nuclei. If the male and female gametangia are morphologically different, the male gametangium is called the **antheridium** (plural, **antheridia**) and the female gametangium is called the **oogonium** (plural, **oogonia**).

The various methods of sexual reproduction (by which compatible nuclei are brought together in plasmogamy) may be summarized as follows (Fig. 17-8):

1 Gametic copulation. Fusion of naked gametes, one or both of which are motile.
2 Gamete-gametangial copulation. Two gametangia come into contact but do not fuse; the male nucleus migrates through a pore or fertilization tube into the female gametangium.
3 Gametangial copulation. Two gametangia or their protoplasts fuse and give rise to a zygote that develops into a resting spore.
4 Somatic copulation. Fusion of somatic or vegetative cells.

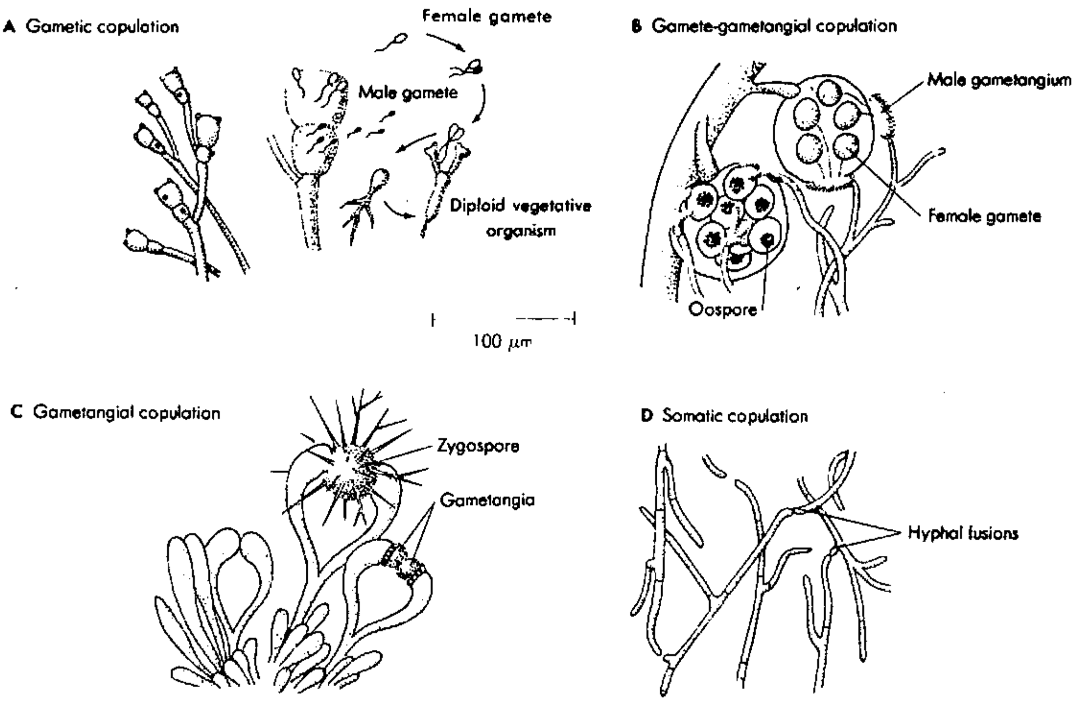


Figure 17-8. Some sexual mechanisms in fungi. (A) Gametic copulation: the fusion in pairs of sexual cells or gametes, formed in specialized sporangialike gametangia; (B) gamete-gametangial copulation: the fusion of a differentiated gamete of one sex with a gametangium of the other sex; (C) gametangial copulation: the direct fusion of gametangia without differentiation of gametes; (D) somatic copulation: the sexual fusion of undifferentiated vegetative cells; (E) spermatization: spermatia uniting with receptive hyphae of the opposite (compatible) strain. *(A–D: From the McGraw-Hill Encyclopedia of Science and Technology, 1971, vol. 5, p. 118.)*

5 Spermatization. Union of a special male structure called a spermatium (plural, spermatia with a female receptive structure. The spermatium empties its contents into the latter during plasmogamy.

Sexual spores, which are produced by the fusion of two nuclei, occur less frequently, later, and in smaller numbers than do asexual spores. There are several types of sexual spores:

1 *Ascospores.* These single-celled spores are produced in a sac called an ascus (plural, asci). There are usually eight ascospores in each ascus (Fig. 17-9).

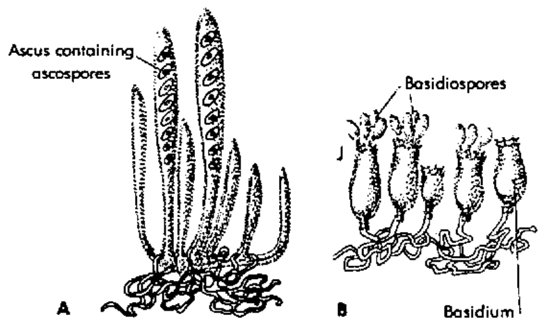


Figure 17-9. Some sexual spore types and the structure of the corresponding reproductive mycelia in fungi. (A) Ascospores. Nuclear fusion takes place in the ascus. The diploid zygote nucleus divides by meiosis almost immediately after fusion, and produces four haploid nuclei. These haploid nuclei divide once more by mitosis, forming the eight ascospores which are typically produced in each ascus. (B) Basidiospores. Nuclear fusion and meiosis take place in the basidium. Basidiospores are then formed exogenously at the tips of the special outgrowths called sterigmata (singular, sterigma). Usually four spores are formed, one at the tip of each sterigma, but if meiosis is followed by a mitotic division then eight basidial nuclei are formed (although rarely do all develop into basidiospores).

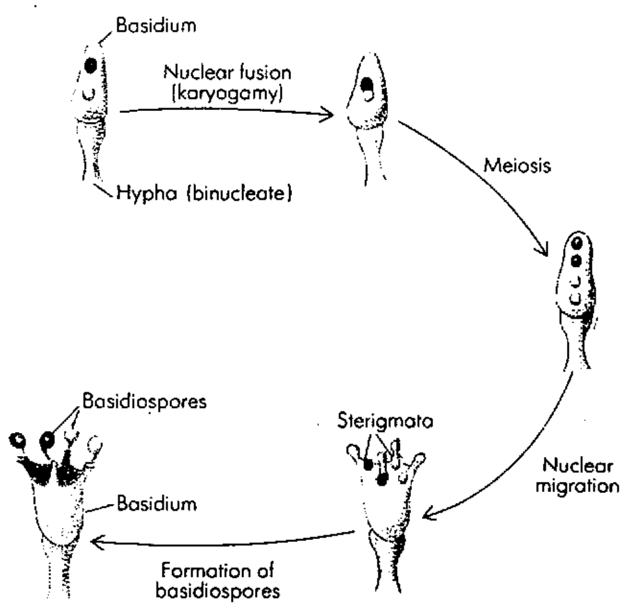


Figure 17-10. The sexual process of basidiospore formation. A basidium begins with one nucleus from each parent. The basidium assumes the shape characteristic of that species and generally produces four tapering projections, the sterigmata. The four nuclei, produced after nuclear fission from meiosis, move toward the sterigmata and form the basidiospores.

2 *Basidiospores.* These single-celled spores are borne on a club-shaped structure called a basidium (plural, basidia) (Fig. 17-9). Their formation is illustrated in Fig. 17-10).

3 *Zygospores.* Zygospores are large, thick-walled spores formed when the tips of

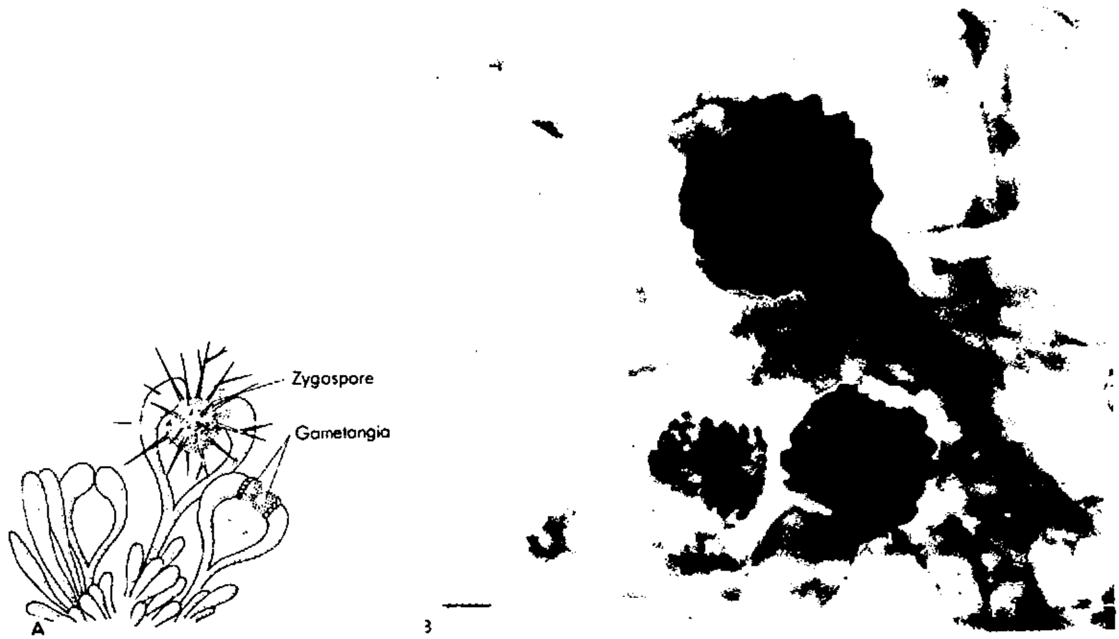


Figure 17-11. (A) The formation of zygospores. *(Redrawn from the McGraw-Hill Encyclopedia of Science and Technology, 1977, vol. 5, p. 118.)* (B) Zygospores in *Mucor hiemalis*. Sexual reproduction in *Mucor hiemalis* occurs when two sexually compatible mating types, + and −, come into contact with each other and produce zygospores. Zygospores of different ages are shown, the oldest one being darkest, largest, and roughest. Bar equals 0.1 mm. *(Courtesy of L. Kapica and E. C. S. Chan, McGill University.)*

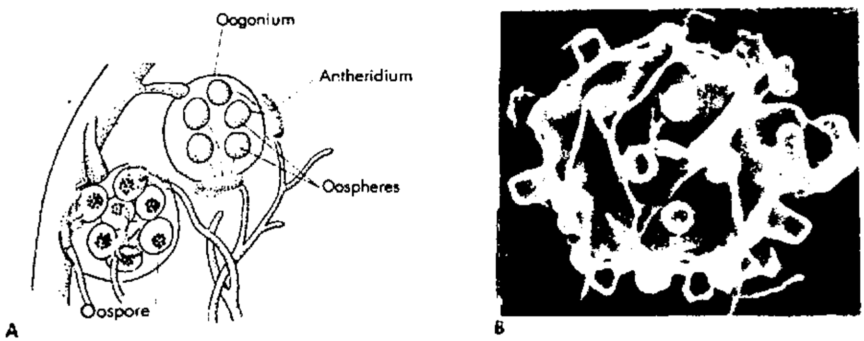
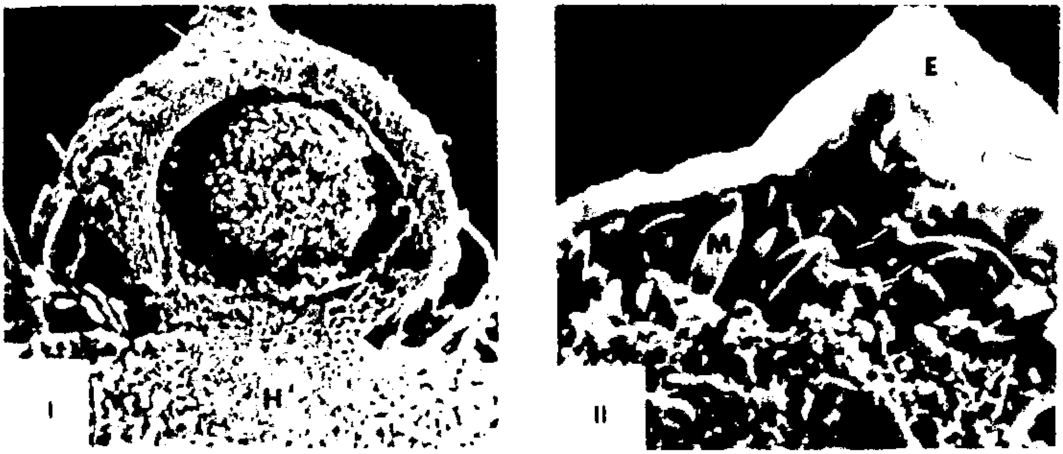


Figure 17-12. (A) The formation of oospores. *(Redrawn from the McGraw-Hill Encyclopedia of Science and Technology, 1977, vol. 5, p. 118.)* (B) Scanning electron micrograph of an oogonium of *Achlya recurva* with appressed antheridia (X780). *(Courtesy of H. E. Huizar, J. T. Ellzey, and W. L. Steffens, The University of Texas at El Paso.)*



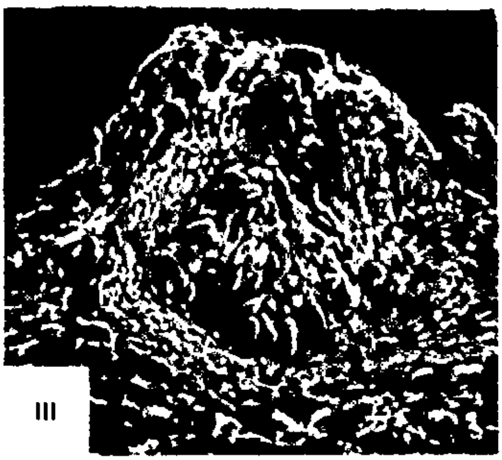


Figure 17-13. Fungal fruiting bodies as seen by scanning electron microscopy. (I) Longitudinal section of a perithecium (ascocarp) of *Ceratocystis fimbriata*. The perithecial wall (W) and hyphae (H) are clearly seen. Other structures seen are ascospores (A) within the perithecial cavity and conidiophores (arrows), which are specialized aerial conidia-bearing hyphae (X250). (II) Cross section of a subepidermal acervulus of *Marsonina juglandis* in black walnut leaf. Mature (M) and immature (arrows) conidia are exhibited. The host epidermis (E) is clearly seen (X1,500). (III) Section of a pycnidium of *Dothiorella ribis* in apple bark tissue, showing conidia compacted in the mucilaginous matrix (X200). *(Courtesy of M. F. Brown and H. G. Brotzman, University of Missouri.)*

two sexually compatible hyphae, or gametangia, of certain fungi fuse together (Fig. 17-11).

4 *Oospores.* These are formed within a special female structure, the **oogonium.** Fertilization of the eggs, or **oospheres,** by male gametes formed in an **antheridium** gives rise to oospores. There are one or more oospheres in each oogonium. This process is illustrated in Fig. 17-12.

Asexual and sexual spores may be surrounded by highly organized protective structures called **fruiting bodies.** Asexual fruiting bodies have names such as **acervulus** and **pycnidium.** Sexual fruiting bodies have names such as **perithecium** and **apothecium.** Some of these structures are shown in Fig. 17-13.

Although a single fungus may produce asexual and sexual spores by several methods at different times and under different conditions, the spores are sufficiently constant in their structures and in the method by which they are produced to be used in identification and classification.

PHYSIOLOGY

Fungi are better able to withstand certain extreme environmental conditions than most other microorganisms. For example, yeasts and molds can grow in a substrate or medium containing concentrations of sugars that inhibit most bacteria; this is why jams and jellies may be spoiled by molds but not by bacteria. Also, yeasts and molds generally can tolerate more acidic conditions than most other microbes.

Some yeasts are facultative; that is, they can grow under both aerobic and anaerobic conditions. Molds and many yeasts are usually aerobic microorganisms. Fungi grow over a wide range of temperature, with the optimum for most saprophytic species from 22 to 30°C; pathogenic species have a higher temperature optimum, generally 30 to 37°C. Some fungi will grow at or near 0°C and thus can cause spoilage of meat and vegetables in cold storage.

Fungi are capable of using a wide variety of materials for nutrition. However, they are heterotrophic. Unlike some bacteria, they cannot use inorganic carbon compounds, such as carbon dioxide, as their sole carbon source. Carbon must come from an organic source, such as glucose. Some species can use inorganic compounds of nitrogen, such as ammonium salts. But all fungi can use organic nitrogen; this is why culture media for fungi usually contain peptone, a hydrolyzed protein product. A summary of the physiological characteristics of fungi in comparison with those of bacteria is found in Table 17-1.

CULTIVATION OF FUNGI

Molds and yeasts can be studied by the same general cultural methods used for bacteria. Nearly all of them grow aerobically on the usual bacteriological culture media at temperatures ranging from 20 to 30°C. Most of them grow more slowly than bacteria, so that media which support bacteria as well as fungi may be overgrown by bacterial contaminants in a mixed inoculum. Where fungi are to be isolated, it is good practice to use a medium that favors their growth but is

Table 17-1. Comparative Physiology of Fungi and Bacteria

Characteristic	Fungi	Bacteria
Cell type	Eucaryotic	Procaryotic
Optimum pH	3.8–5.6	6.5–7.5
Optimum temperature	22–30°C (saprophytes) 30–37°C (parasites)	20–37°C (mesophiles)
Oxygen requirement	Strictly aerobic (molds) Facultative (some yeasts)	Aerobic to anaerobic
Light requirement	None	Some photosynthetic groups occur
Sugar concentration in laboratory media	4–5%	0.5–1%
Carbon requirement	Organic	Inorganic and/or organic
Cell-wall structural components	Chitin, cellulose, or hemicellulose	Peptidoglycan
Antibiotic susceptibility	Resistant to penicillins, tetracyclines, chloramphenicol; sensitive to griseofulvin	Resistant to griseofulvin; sensitive to penicillins, tetracyclines, chloramphenicol

not optimal for the growth of bacteria. Acidic (pH 5.6) media that incorporate a relatively high concentration of sugar are tolerated by molds but are inhibitory to many bacteria.

One of the best-known and oldest media for the growth of fungi was devised by Sabouraud and contains maltose and peptone as its principal ingredients. The most common modification used in America now contains glucose and any one of several specified peptones. This medium is widely used for the isolation of molds and certain yeasts and is especially useful for growing pathogenic fungi from infected body fluids and exudates. Its partial selective action is due to the high sugar concentration and low pH.

Most parts of a mold are potentially capable of growth. Inoculation of a small fragment of mycelium on a medium is sufficient to start a new mold colony. This is done by planting the inoculum on a fresh medium with the aid of a transfer needle, a method similar to that used for bacteria. One difference is that the needle used for molds is stiffer and has a flattened tip for cutting the mycelium. However, an ordinary inoculating loop used for bacteria is suitable for the inoculation of yeasts.

CLASSIFICATION OF FUNGI

The classification of fungi, unlike that of bacteria, is based primarily on the characteristics of the sexual spores and fruiting bodies present during the sexual stages of their life cycles. However, many fungi produce sexual spores and fruiting bodies only under certain environmental conditions, if they are known to produce them at all. Thus the complete or perfect life cycles are as yet unknown for many fungi. Consequently, these imperfectly described fungi must be classified on bases other than the characteristics of their sexual stages. The morphology of their asexual spores and thalli then becomes important—until such time that their sexual traits are observed, which requires reevaluation of their taxonomic status. Thus imperfect higher fungi are provisionally placed in a special class called form-class *Deuteromycetes*. Most of the sexual stages found subsequently among members of this class have been of the ascomycete type. When sexual stages are found, the organisms are reclassified and placed in with the ascomycetes or basidiomycetes (discussed below).

You should be aware that all is not settled in mycological classification and that differences of opinion on classification are very numerous. Divergent views arise because of our incomplete knowledge on the structure, development, and physiology of fungi. Therefore, as our knowledge of fungi increases, our classification schemes are bound to change.

Taxonomy of the fungi follows the recommendations of the Committee on International Rules of Botanical Nomenclature. Accordingly, the various taxa have endings as follows:

Divisions:	*-mycota*
Subdivisions:	*-mycotina*
Classes:	*-mycetes*
Subclasses:	*-mycetidae*
Orders:	*-ales*
Families:	*-aceae*

Genera and species have no standard endings.

In this chapter, the classification scheme proposed by Alexopoulos, the eminent American mycologist, is used. Its outline is shown in Fig. 17-14. A summary of the major organisms in the kingdom *Fungi* is given in Table 17-2.

Table 17-2. Summary of the Major Organisms of the Kingdom *Fungi* (Modified after Alexopoulos, 1979)

Division/Class	Major Distinguishing Characteristics	Representative Organisms
Division *Gymnomycota* (slime molds)	Organisms which ingest particulate nutrients and which lack cell walls in the vegetative stage	
Class *Acrasiomycetes* (cellular slime molds)	Vegetative stage: free-living amoebae which aggregate to form a stalked sorocarp bearing spores in a mucilaginous matrix (Figs. 17-15 and 17-16)	*Dictyostelium discoideum* *Polysphondelium violaceum*
Class *Myxomycetes* (acellular slime molds)	Vegetative stage: a multicellular, wall-less plasmodium, which transforms into highly organized sporangia bearing sporangiospores (Fig 17-17)	*Physarum polycephalum* *Didimium iridis*
Division *Mastigomycota* (flagellated lower fungi)	Aquatic fungi producing motile, flagellated cells	
Class *Chytridiomycetes*	Motile cells bearing a single, posteriorly positioned, whiplash type flagellum	*Allomyces macrogynus*
Class *Hyphochrytridiomycetes*	Motile cells bearing a single, anteriorly positioned, tinsel-type flagellum	*Rhizidiomyces arbuscula* *Hyphochrytrium catenoides*
Class *Plasmodiophoromycetes*	Obligate parasites on higher plants; vegetative stage a plasmodium; motile cells with two unequal anterior whiplash flagella	*Plasmodiophora brassica*
Class *Oomycetes*	Motile cells with two laterally inserted flagella, one tinsel and anteriorly directed, the other whiplash and posteriorly directed	*Saprolegnia ferax*
Division *Amastigomycota* (terrestrial fungi)	Flagella absent	
Class *Zygomycetes*	Sexual reproduction by gametangial fusion; zygote transformed into a thick-walled resting spore, the zygospore; vegetative reproduction by means of mitospores produced within a sporangium (Fig. 17-18)	*Rhizopus stolonifer* *Phycomyces blakesleanus* *Mucor rouxii*
Class *Ascomycetes*	Sexual spores produced endogenously in a saclike ascus typically produced in a well-differentiated ascocarp; vegetative reproduction by conidiospores (Figs. 17-19 and 17-20)	The yeasts; e.g., *Saccharomyces cerevisiae* Morels and truffles *Neurospora crassa*
Class *Basidiomycetes*	Sexual spores produced exogenously on clublike cells called basidia; basidia formed on well-differentiated basidiocarps (Fig. 17-21)	Mushrooms and puffballs Rusts, smuts, and jelly fungi Bracket fungi
Form-Class *Deuteromycetes*	Sexual reproduction absent; vegetative reproduction by means of conidiospores arising from well-defined conidiogenous cells	Molds and mildews Causal agents of ringworm and athlete's foot and many plant diseases *Trichophyton rubra* *Candida albicans* *Alternaria tenuis*

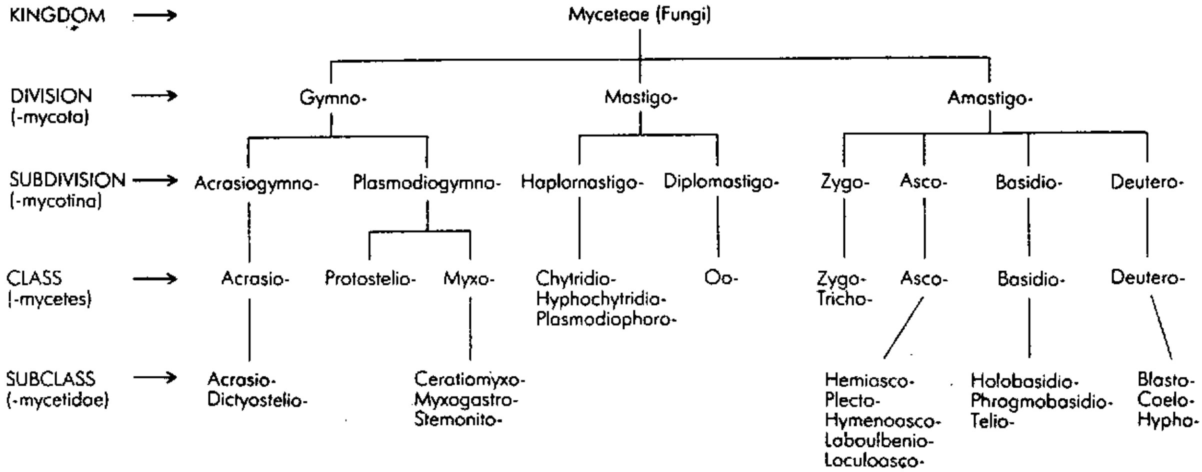


Figure 17-14. Main outline of fungal classification (after Alexopoulos) showing principal taxa. Endings of taxa are shown in brackets (for example, *Gymno-* should be read as *Gymnomycota*).

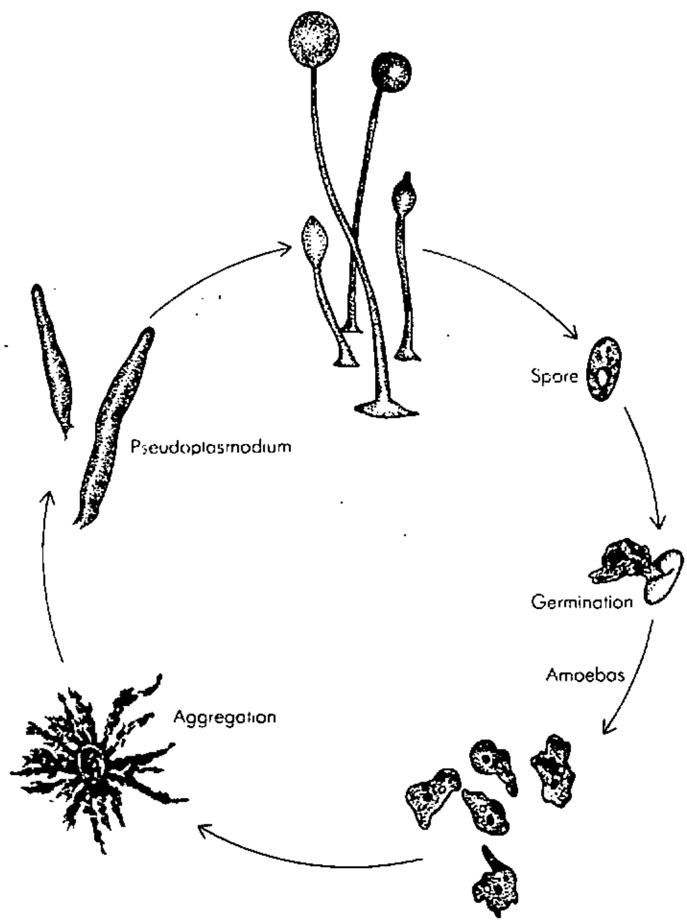


Figure 17-15. The life cycle of the cellular slime mold *Dictyostelium discoideum*. Amoebas migrate into aggregation centers, becoming associated end to end in chains (see Fig. 17-16). They go through several multicellular stages (see Fig. 17-16) to form a fruiting body. The spores disperse to find a suitable environment before they germinate to form amoebas which begin the life cycle anew.

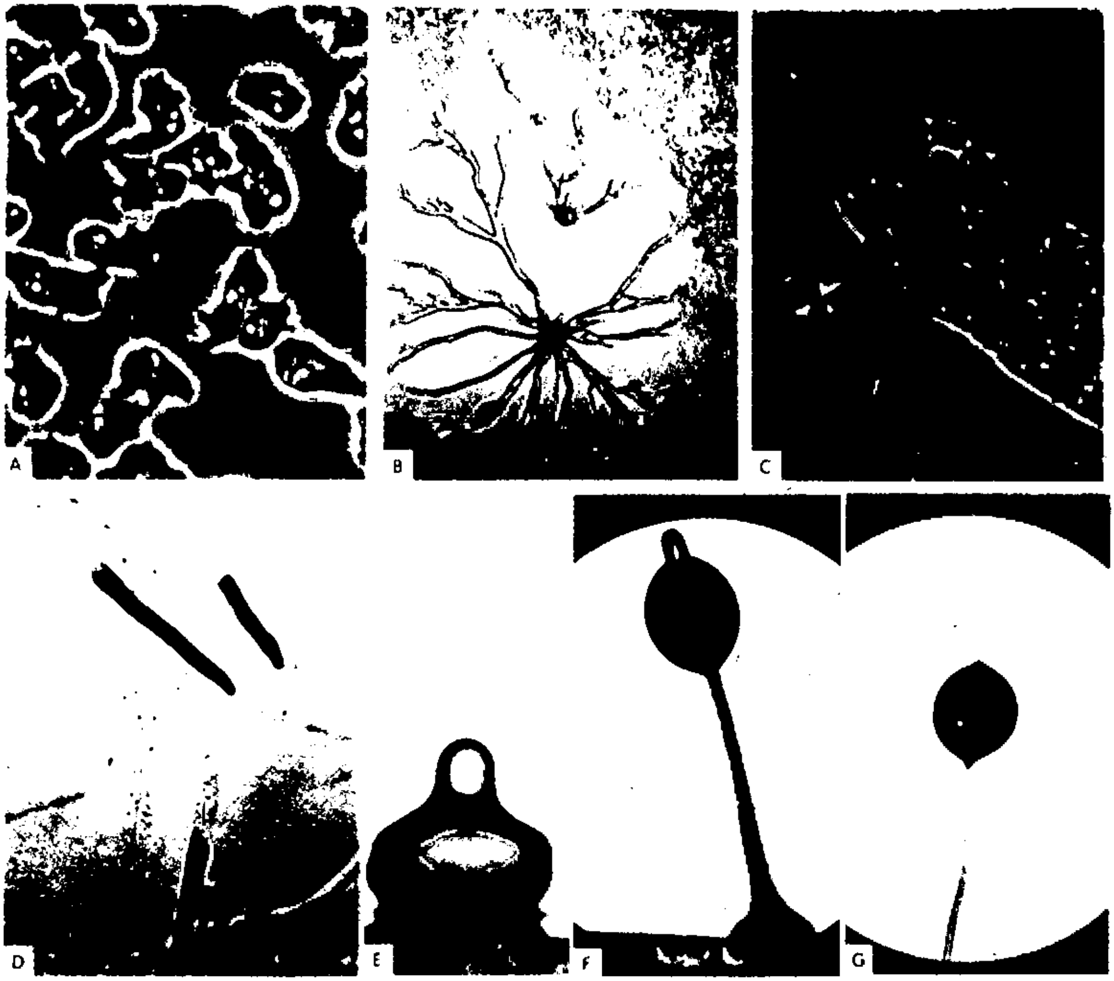


Figure 17-16. Fruiting structures in dictyostelium are formed by an orderly differentiation of myxamoebas, starting with (A) cells which have no regular shape or orientation (phase-contrast microscopy, X442). In (B) (X11) we see a pseudoplasmodium in a well-advanced stage composed of many myxamoebas which are now oriented into the stream of flow (C) (phase-contrast microscopy, X300). As the pseudoplasmodia migrate, they leave trails of slime on the surface of the agar (D) (X12). Fruiting bodies of the slime molds are called sori (masses of spores). An early stage in the formation of a fruiting body in dictyostelium is seen in (E), where the organisms have assumed a vertical orientation (X53). In this figure the stalk is just beginning to form, but (F) shows the entire sorocarp, including the basal disk bearing an immature sorus (X42). A mature sorus (X42) is shown in (G). [*Courtesy of Kenneth B. Raper, Proc Am Phil Soc,* **104**(*6*):*579–604, December 1960.*]

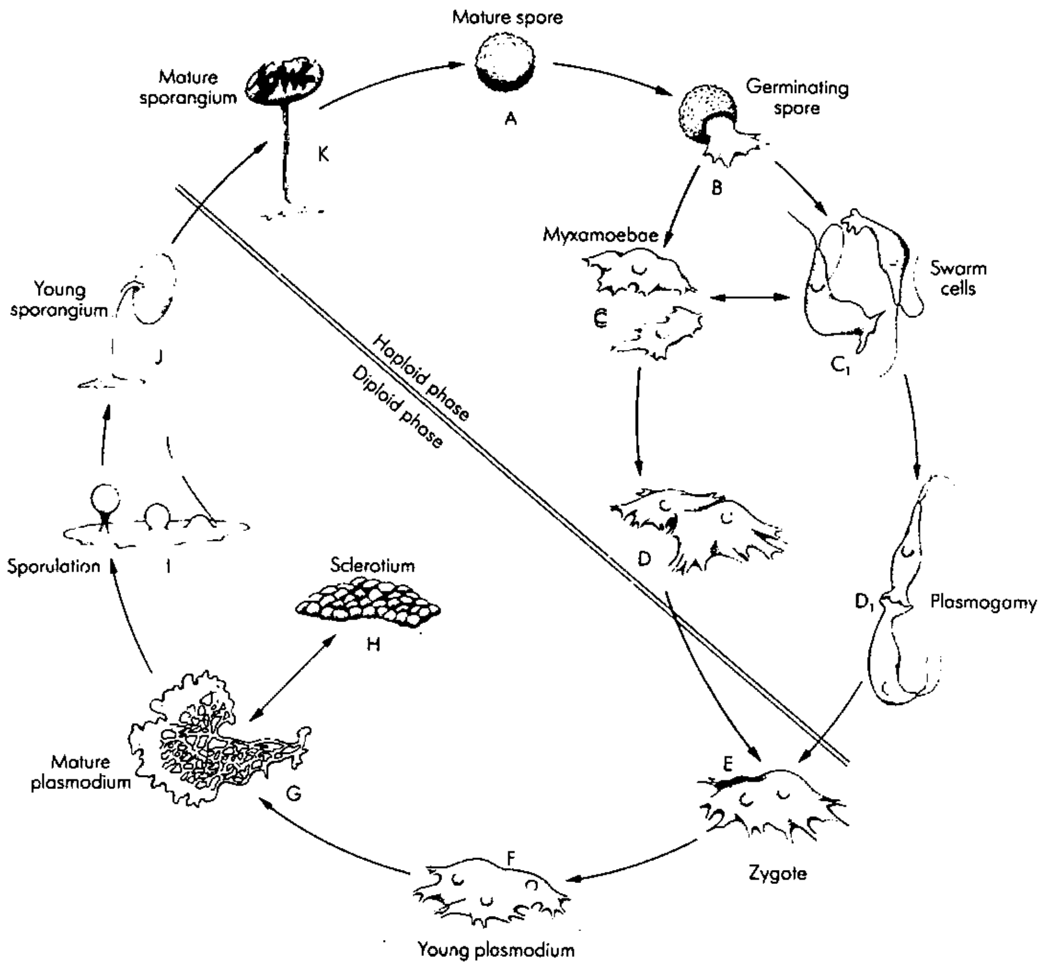


Figure 17-17. Life cycle of a typical myxomycete. (A) Mature haploid spore, (B) germinating spore, (C) myxamoebae, (C_1) swarm cells, (D) fusing myxamoebae, (D_1) fusing swarm cells, (E) young zygote, (F) young plasmodium, (G) mature plasmodium, (H) sclerotium, (I) sporulation—sporangial initials, (J) young premeiotic sporangium with spores, and (K) mature postmeiotic sporangium. *(Erwin F. Lessel, illustrator.)*

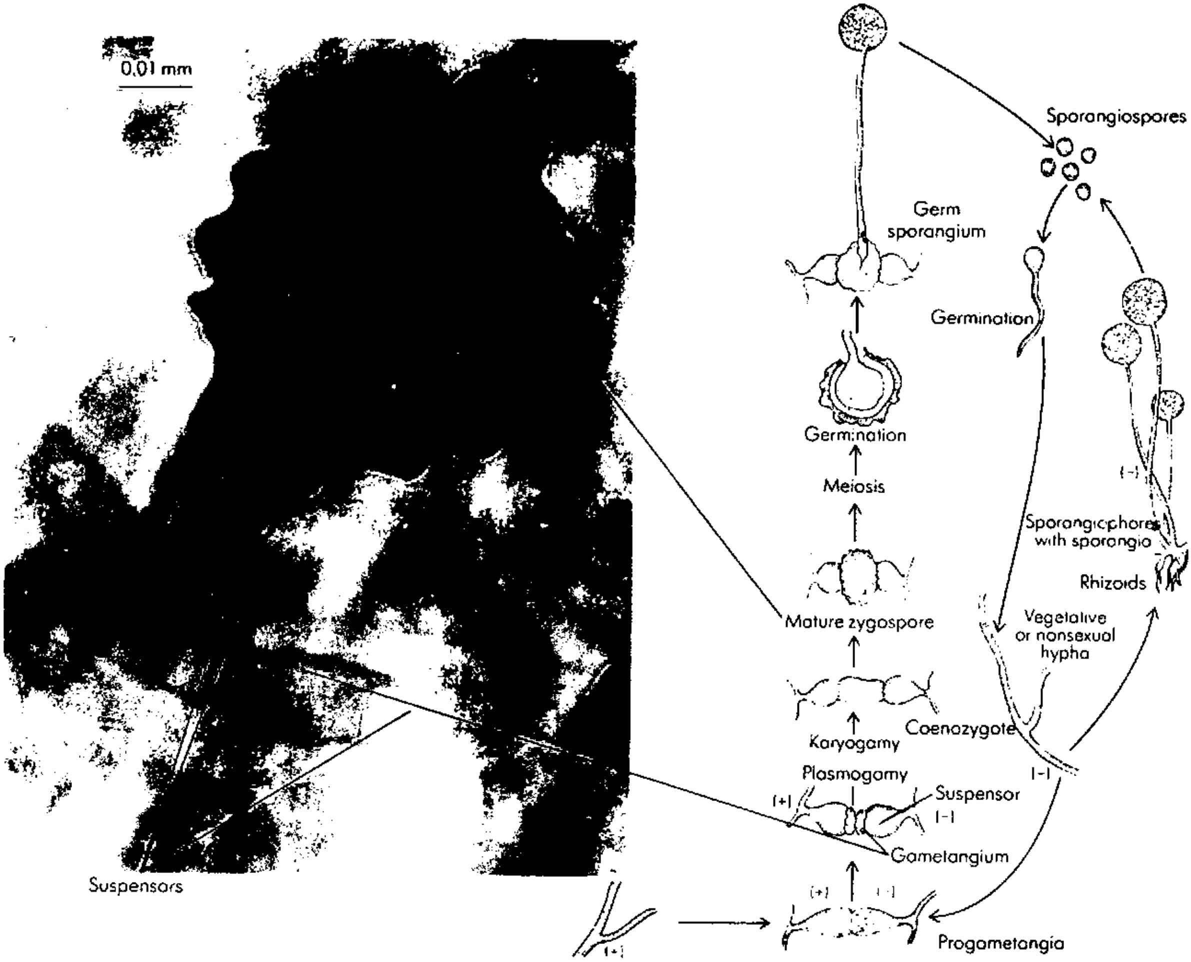


Figure 17-18. Life cycle of *Rhizopus stolonifer.* When the wall of the sporangium disintegrates, sporangiospores are released. A sporangiospore germinates to develop into an organism with many vegetative hyphae. Rhizoids, which penetrate into the medium, are also formed. Directly above the rhizoids, one or more sporangiophores are produced. The top of each sporangiophore develops into a sporangium containing sporangiospores. This completes the asexual portion of the life cycle. Sexual reproduction requires two sexually compatible types (+ and −). When these types come into contact with one another, copulating branches called progametangia are formed. A septum then forms near the tip of each progametangium, separating it into two cells, a terminal gametangium and a suspensor cell. The walls of the two contacting gametangia dissolve at the point of contact, the two protoplasts mix (plasmogamy), and the + and − nuclei fuse (karyogamy) to form many zygote nuclei. The structure which contains them is called the coenozygote. The wall around the coenozygote thickens and its surface becomes black and warty, forming the mature zygospore, which lies dormant for 1 to 3 or more months. The zygospore germinates to form a new organism; meiosis takes place during the germination process. Bar equals 0.01 mm. *(Photomicrograph courtesy of L. Kapica and E. C. S. Chan, McGill University.)*

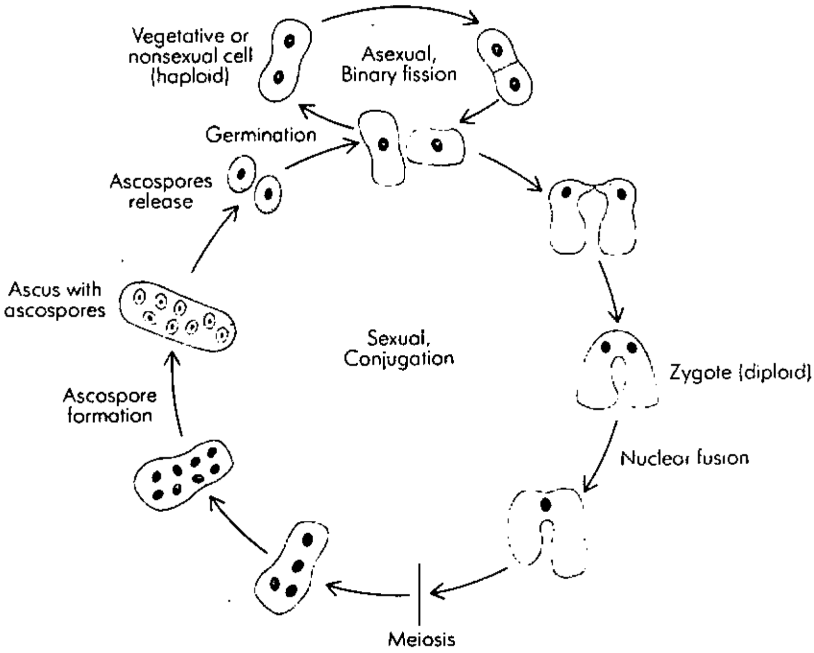


Figure 17-19. Life cycle of the common yeast *Schizosaccharomyces*. Asexual reproduction is by binary fission. Sexual reproduction is by conjugation of compatible cells with the subsequent formation of ascospores. (Haploid: half the number of chromosomes characteristic of a species; diploid: the number of chromosomes characteristic of a species; zygote: a diploid cell resulting from the fusion of two haploid cells.)

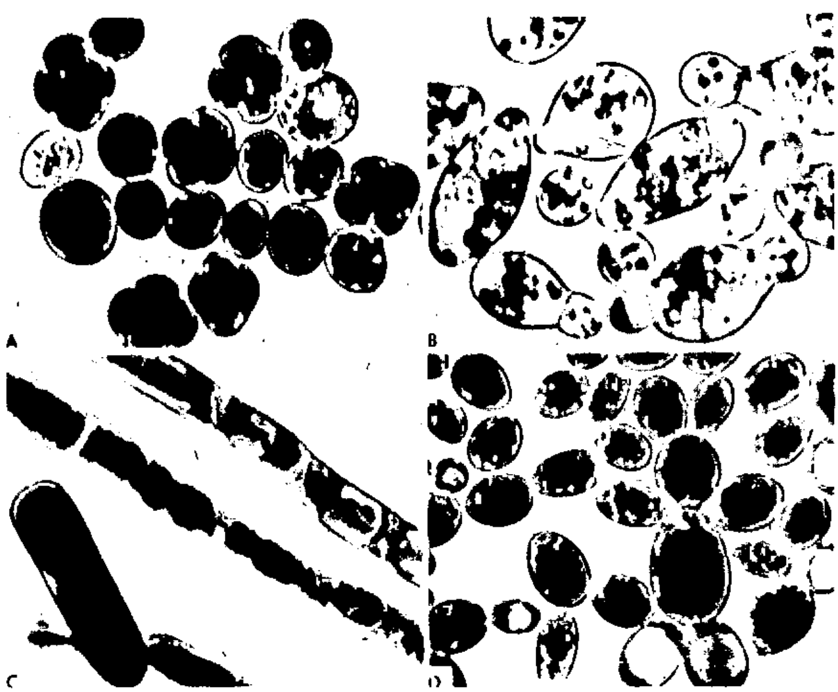


Figure 17-20. The morphology of yeasts varies widely. (A) *Saccharomyces cerevisiae* with cells appearing as vegetative forms, budding cells, and spores (X73). *(Courtesy of George Svihla and with permission of The Microscope and Crystal Front.)* (B) *Saccharomyces ludwigii* (about X95.) *(From George Svihla, Argonne Natl Lab Annu Rep, 1965.)* (C) *Geotrichum candidum* (about X110). (D) *Pichia membranaefaciens* (X88). *[(C) and (D), courtesy of George Svihla.]*

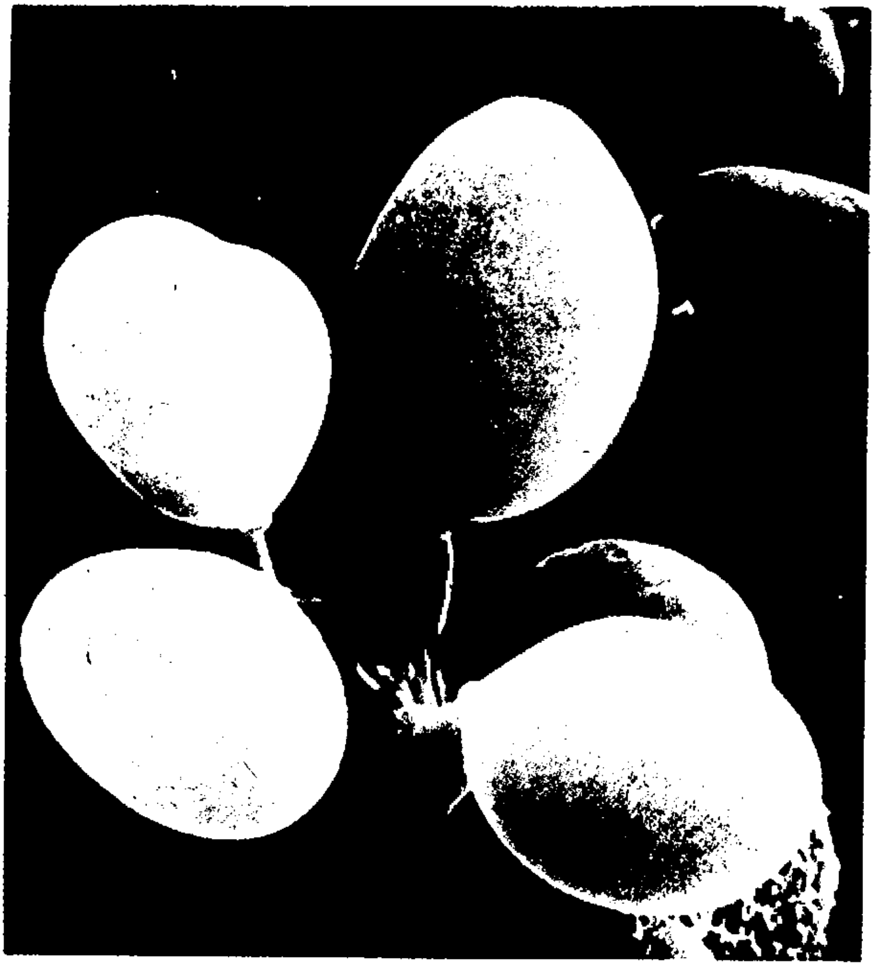


Figure 17-21. Scanning electron micrograph of a basidium bearing four basidiospores (magnification approximately X8,000). *(Courtesy of Stanley F. Flegler, Pesticide Research Center, Michigan State University.)*

SOME FUNGI OF SPECIAL INTEREST

Many of the properties, activities, and characteristics of fungi can best be described by examples. The following are some of the genera most frequently encountered in microbiology. Among them are some of the most interesting to microbiologists because of their unique biological properties or particular economic or medical importance.

Synchytrium (Class *Chytridiomycetes*)

More than 100 species of this genus are parasitic on flowering plants. Many species are not very destructive to the host plant, but they induce the formation of galls on leaves, stems, and fruits. The most serious parasite is *S. endobioticum*, the cause of black wart disease of potato. The dark warts on the potatoes are galls in which the host cells have been stimulated to divide by the fungus. The resting sporangia are released by the decay of the warts and they may remain alive for many years in the soil. The zoospores released from a sporangium are capable of swimming for about two hours in soil water until a new host tuber is found to infect. The zoospores may also fuse to form zygotes which retain their flagella and swim actively for a time. The zygote then encysts on the surface of the host epidermis and penetrates the host cell to infect it. The results of zygote infection differ from infection by zoospores. When infected by a zoospore, the host cell reacts by undergoing **hypertrophy**, i.e., increase in cell volume, and adjacent cells also enlarge to form the characteristic rosette. When infected by a zygote, the host cell undergoes **hyperplasia**, i.e., repeated cell division resulting in the characteristic wart. Control of wart disease is based largely on the breeding of resistant varieties of potatoes. Figure 17-22 shows the life cycle of *Synchytrium*.

Saprolegnia (Class *Oomycetes*)

Species of *Saprolegnia* are common in soil and fresh water; hence they are commonly called water molds. They are saprophytic on plant and animal remains, but a number of species such as *S. ferax* and *S. parasitica* have been

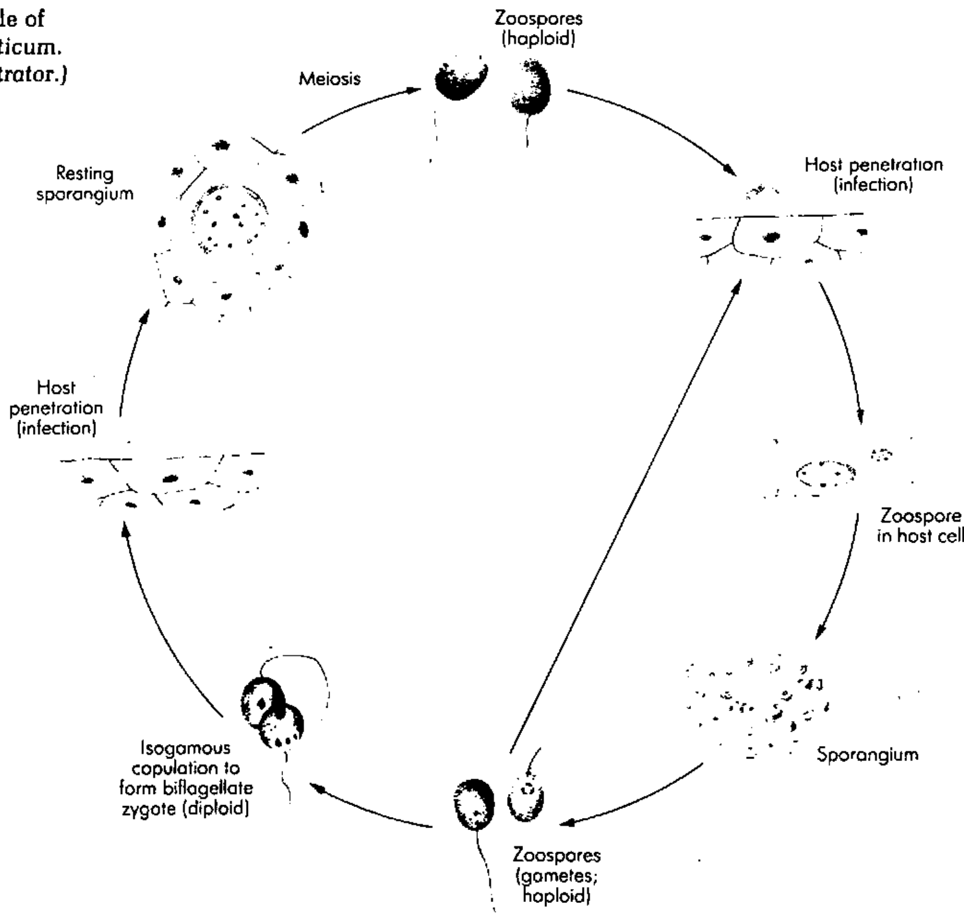
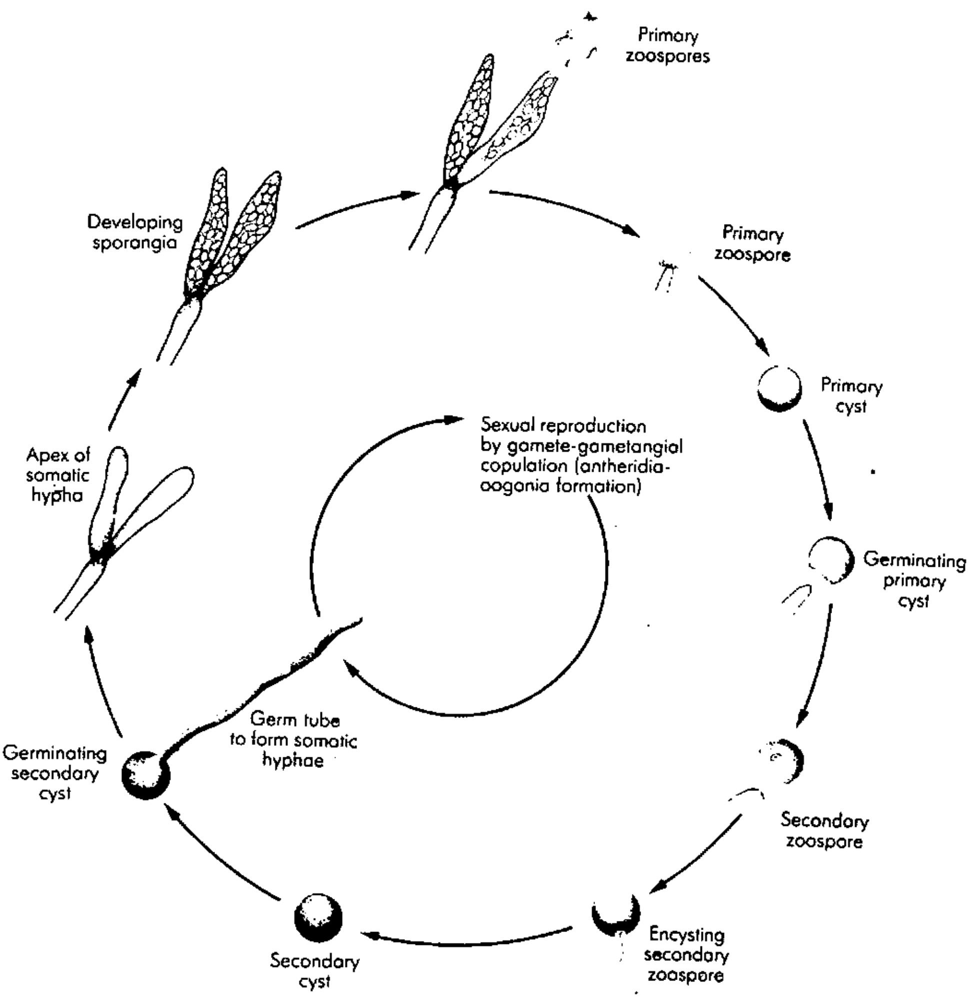


Figure 17-22. Life cycle of *Synchytrium endobioticum*. (*Erwin F. Lessel, illustrator.*)

implicated in diseases of fish and their eggs. *S. parasitica* causes severe epidemics of disease among fish in the natural environment.

The somatic portion of the organism consists of two types of hyphae: the rhizoidal hyphae which enter the substratum and serve to anchor the organism and to absorb nutrients, and the branched hyphae on which the reproductive organs are formed. Elongated, tapering sporangia are formed at the tips of somatic hyphae. The nuclei present differentiate into zoospores. An opening develops at the tip of the sporangium and the pear-shaped primary zoospores escape into the surrounding aqueous environment. They swim for some time (from a minute to over an hour), then withdraw their flagella and encyst. This cyst, after a period of rest (2 to 3 h, depending on the species), germinates to release a further bean-shaped secondary zoospore. The secondary zoospore may

Figure 17-23. Life cycle of *Saprolegnia*. *(Erwin F. Lessel, illustrator.)*



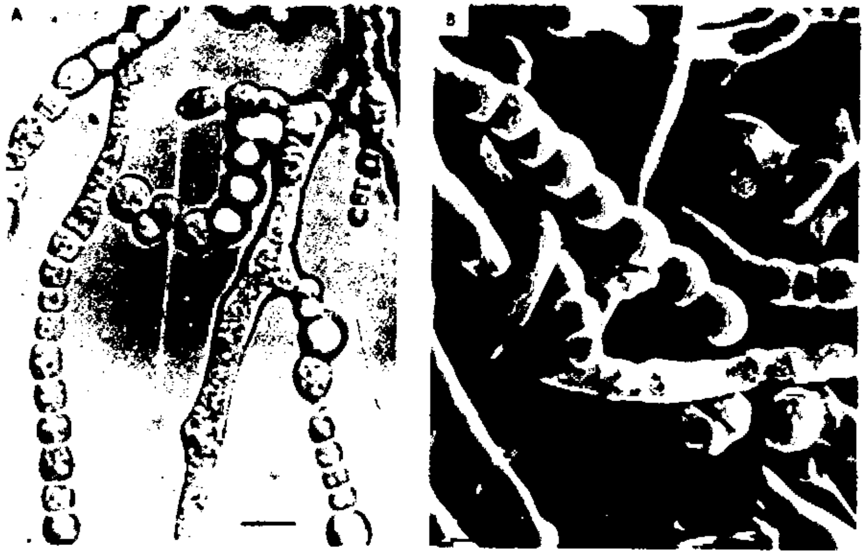


Figure 17-24. Formation of arthrospores (yeastlike forms) by Mucor rouxii. (A) As seen by light microscopy. (B) Scanning electron micrograph. Bars equal 10 µm. *(Courtesy of C. R. Barrera, New Mexico State University, and the American Society for Microbiology.)*

swim vigorously for several hours before encysting again. This encysted spore now germinates by sending a germ tube that develops into a hypha, forming a new colony (see Fig. 17-23). When conditions are favorable for sexual reproduction, the somatic hyphae give rise to oogonia and antheridia.

Mucor (Class *Zygomycetes*)

Members of this genus occur abundantly in soil and manure and on fruits, vegetables, and starchy foods. Some are responsible for food spoilage, but others are used in the manufacture of some cheeses and other foods. Common species are *M. racemosus* and *M. rouxii*. Morphologically, their mycelia are usually white or gray and are nonseptate. Sporangiophores may be branched. The columellae(sterile structures in sporangium) are round, cylindrical, or oval. Spores are black or brown and are smooth in appearance. Zygospores are produced when plus and minus strains (so called because there is no morphological differentiation between the male and female strains even though there is physiological heterothallism) of the organism are both present. Zygospores are also formed in growth on artificial media in the laboratory. No stolons or rhizoids are produced. It is interesting that in the absence of a fermentable carbon source in a medium consisting of yeast extract and peptone supplemented with potassium acetate, *Mucor* may grow in a yeastlike instead of a filamentous form (Fig. 17-24).

Rhizopus (Class *Zygomycetes*)

These are common bread molds (Fig. 17-25), which cause much food spoilage. They grow on bread, vegetables, fruits, and other food products. The most commonly encountered species is *R. stolonifer*. Morphologically, they have nonseptate, cottony mycelia with sporangiophores arising at the nodes where the rhizoids form. Their sporangia are usually quite large and black; their columellae are hemispherical. The base of the sporangium, or **apophysis**, is cupshaped. These molds produce clusters of rootlike holdfasts called **rhizoids**, as

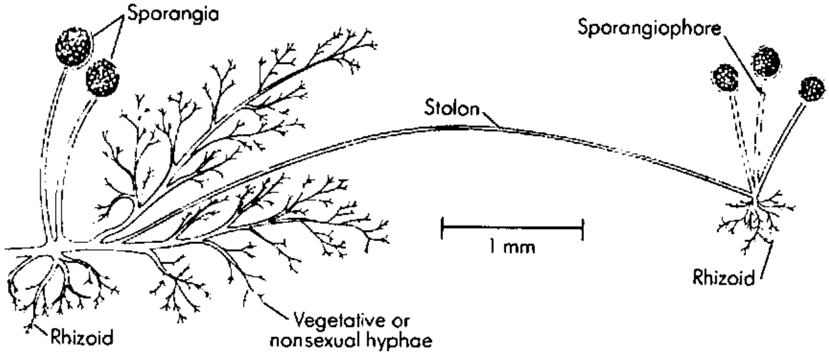


Figure 17-25. *Rhizopus stolonifer*, the common bread mold. This fungus forms rootlike hyphae (rhizoids), vegetative hyphae which penetrate the substrate, and fertile hyphae, which produce sporangia at the tips of sporangiophores (spore-producing hyphae). Stolons are rootlike filaments which connect individual organisms.

well as stolons or "runners" (like those of strawberry plants) capable of taking "root" where they may give rise to new organisms. The life cycle of *R. stolonifer* is given in Fig. 17-18. It may be seen that *R. stolonifer* is heterothallic. Sexual reproduction requires two thalli of different mating types. Since the thalli are indistinguishable morphologically, they are designated plus and minus instead of male and female. (Generally, with gametes that differ morphologically, the larger gamete of a pair is regarded as female, the smaller as male. Also, a gamete that leaves the structure where it was formed and later fuses with a relatively stationary gamete is regarded as male.) When both mating thalli are present, the hyphal tips differentiate into **progametangia** which come into contact and develop into **gametangia** by the formation of septa. The walls between two gametangia then dissolve and their protoplasts coalesce. Nuclei of both mating types fuse in pairs, producing many zygote nuclei. The structure that contains them is then called a **coenozygote**. The wall of the coenozygote soon thickens, turns black, and becomes rough; it develops into the **zygospore**. The resting stage of the zygospore lasts from 1 to 3 months, and sometimes even longer. Upon germination, meiosis takes place, the zygospore breaks open, and a single sporangiophore bearing a **germ sporangium** at its tip emerges. The germ sporangium is similar to an asexually produced sporangium. Some germ sporangia contain spores all of one mating type (either plus or minus), but others contain spores of both mating types in about equal numbers.

Schizosaccharomyces (Class *Ascomycetes*)

All fission yeasts belong to this genus. They reproduce by transverse division as well as by ascospores (see Fig. 17-19 for the life cycle of *Schizosaccharomyces*). The best known species is *S. octosporus*, which has been isolated from currants and honey. Its cells are globose to cylindrical, uninucleate and haploid. *S. pombe* is the fermenting yeast of some kinds of beer (such as African millet beer), and it has been isolated from sugar molasses and from grape juice. *S. versatilis*, isolated from grape juice, grows like a yeast, but it can also form a true mycelium. Fig. 17-26 illustrates *Schizosaccharomyces*.

Saccharomyces (Class *Ascomycetes*)

There are about thirty species of *Saccharomyces*. The best known is *S. cerevisiae*, strains of which are used in the fermentation of beer and wine and in baking. It is found in nature on ripe fruit. Grape wines are often made by spontaneous fermentation by yeasts growing on the surface of the fruit. Thus *S. cerevisiae* is a yeast of great economic importance. Its cells are elliptical, measuring about 6 to 8 by 5 μm. (See Fig. 17-20 for the morphology of these cells.) They multiply asexually by budding. Where a bud has formed on a cell, a raised scar remains (Fig. 17-27). As many as 23 bud scars have been seen on a single cell. During budding, the nucleus divides by constriction and a portion of it enters the bud along with other organelles. The cytoplasmic connection is closed by the laying down of cell-wall material. Under appropriate conditions, *S. cerevisiae* forms asci. The cytoplasm of the cell differentiates into four thick-walled spherical spores, although the number of spores can be fewer (Fig.

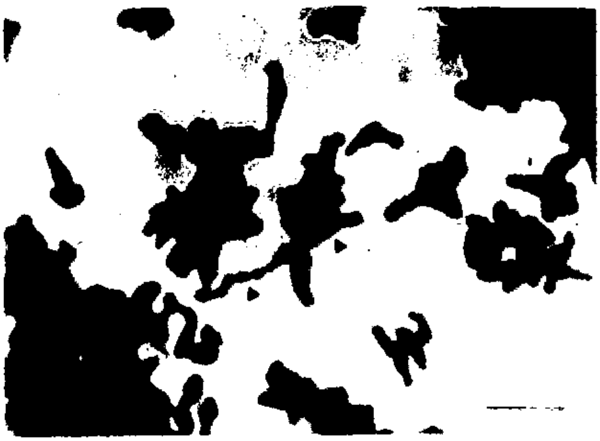


Figure 17-26. The fission yeast *Schizosaccharomyces octosporus*. Vegetative cells as well as ascospores in asci (▶) are clearly seen. Bar equals 10 μm. *(Courtesy of L. Kapica and E. C. S. Chan, McGill University.)*

Figure 17-27. *Saccharomyces cerevisiae* showing bud and bud scars (arrows) (X1,600). *(Courtesy of R. G. Kessel and C. Y. Shih. Scanning Electron Microscopy in Biology, Springer-Verlag, Berlin, 1974.)*

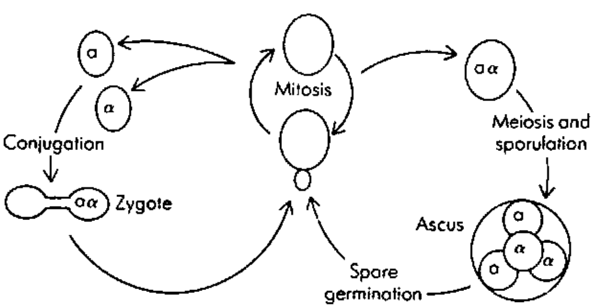


Figure 17-28. The life cycle of *Saccharomyces cerevisiae*; *a* and α refer to mating-type alleles; unlabeled cells may be *a*, α, or *a*α.

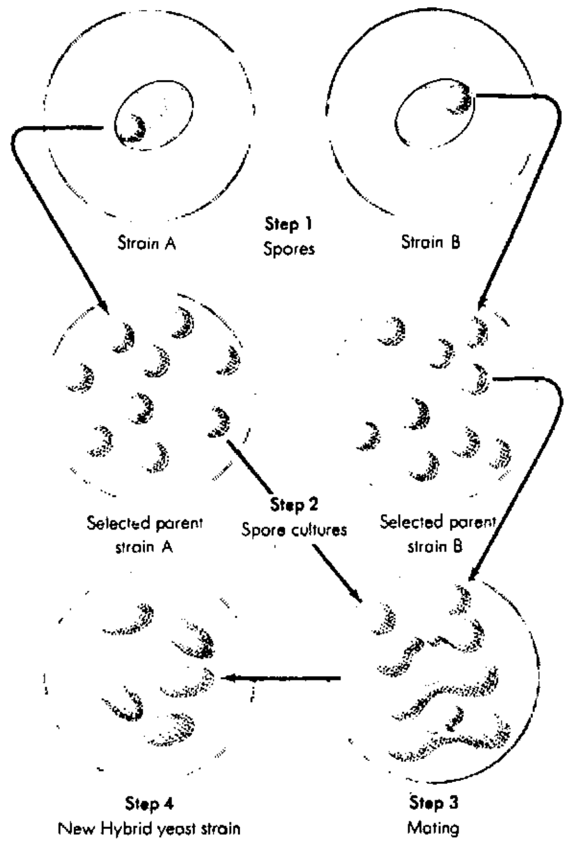


Figure 17-29. The four steps in the process of hybridization. Step 1: Yeast cells from two strains A and B are shown after they have been induced to form spores. The spores are removed by microdissection by means of a micromanipulator. Step 2: Each individual spore is planted in a nutrient medium and allowed to multiply to produce separate spore cultures. After careful testing for the desirable characteristics, two spore cultures of different sexes are selected for mating or crossbreeding. Step 3: When these two spore cultures are brought together, instead of budding they fuse in pairs and produce a completely new yet stable combination of inherited qualities. Step 4: The new hybrid strain shown is a combination of the best qualities of strains A and B. The new hybrid grows and reproduces by budding. As it grows, the new inherited qualities are equally transmitted to all cells reproduced. *(From A. J. Salle, Fundamental Principles of Bacteriology, 7th ed., McGraw-Hill, New York, 1973.)*

17-20A). The cells from which asci develop are diploid and the nuclear divisions which precede spore formation are meiotic. Figure 17-28 shows the life cycle of *S. cerevisiae*. It may be noted that many strains of the yeast are heterothallic, and the ascospores are of two mating types. Mating type is specifically controlled by a single gene which exists in two allelic states *a* and α, and segregation at reduction-division preceding ascospore formation gives rise to two *a* and two α ascospores (Fig. 17-28). Fusion normally occurs only between cells of differing mating types, a process termed **legitimate copulation.** Such fusions result in diploid cells which form asci containing viable ascospores. Many studies have been made of yeast genetics. For instance, by means of **hybridization** (crossing of different yeasts) it is possible to develop strains of yeasts (hybrids) with desirable characteristics from two genetically different strains. This technique is illustrated in Fig. 17-29.

Neurospora (Class *Ascomycetes*)

This genus of *Ascomycetes* is of particular interest to biologists because of its wide use in the study of genetics and metabolic pathways. Two well-known species are *N. crassa* and *N. sitophila*. Some species are responsible for food

Figure 17-30. *Agaricus* sp. *(Courtesy of B. Kendrick, University of Waterloo.)*

spoilage, and some are used in industrial fermentations. Certain species produce ascospores, and some species are heterothallic. Morphologically, *Neurospora* produces a loose network of long strands of septate aerial mycelia. Conidia, usually oval, form branched chains at the tips of the aerial hyphae. Because of the characteristic pink or red color of the conidia and the common occurrence of *Neurospora* species on farinaceous foods, they are often called pink bread molds. The ascospores of neurospora are produced in perithecia.

Agaricus (Class *Basidiomycetes*)

The best known species are *A. campestris*, the field mushroom, and *A. bisporus*, the cultivated mushroom. The pink coloration of the young gills is due to cytoplasmic pigment in the spores. The gills later turn a purplish brown because of the deposition of dark pigments in the spore wall. Most of the larger species of *Agaricus* are edible. (See Fig. 17-30.)

Filobasidiella neoformans (Class *Basidiomycetes*)

Of the approximately 12,000 species of *Basidiomycetes*, none was implicated in human disease until recent times. The perfect stage of *Cryptococcus neoformans* was discovered in 1975; it is now called *Filobasidiella neoformans* (Fig. 17-31). It is an important basidiomycetous pathogen of humans, causing cryptococcosis, a generalized (systemic) mycotic infection involving the bloodstream as well as the lungs, central nervous system, and other organs.

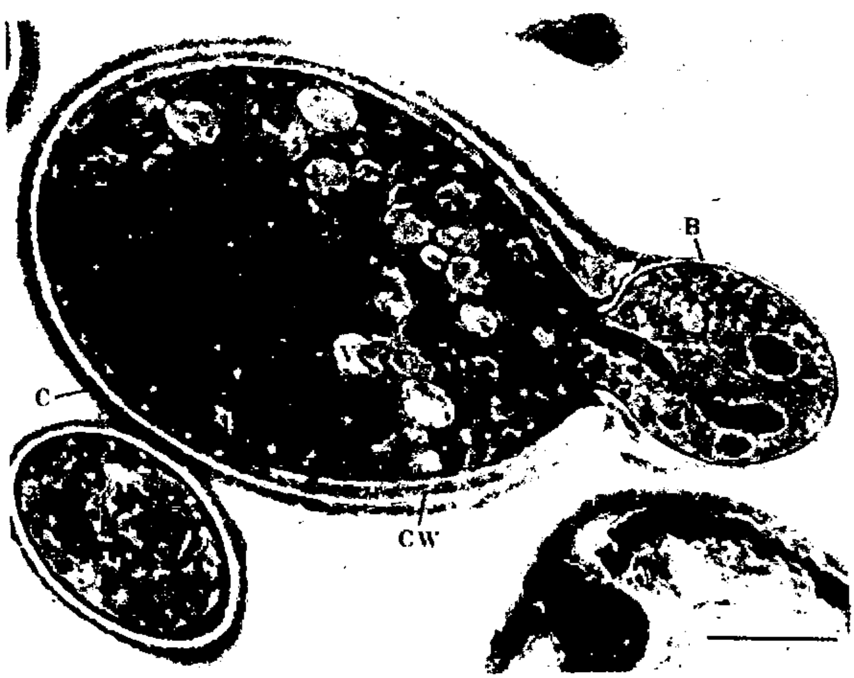


Figure 17-31. *Filobasidiella neoformans* cell. A budding cell is seen emerging from the parental cell. B = bud, C = capsule, CW = cell wall, M = mitochondria, N = nucleus, V = vacuole. The bar indicates 1 μm. *(Courtesy of Phyllis C. Braun, Georgetown University.)*

Aspergillus (Class *Deuteromycetes*)

The aspergilli are widespread in nature, being found on fruits, vegetables, and other substrates which may provide nutriment. Some species are involved in food spoilage. They are important economically because they are used in a number of industrial fermentations, including the production of citric acid and gluconic acid, both produced in abundance by *A. niger*. Morphologically, the aspergilli produce septate, branching mycelia with the vegetative portions submerged in nutrient. The conidiophores, or fertile hyphae, arise from foot cells which may also be submerged. Conidiophores may be septate or nonseptate. At the apex, the conidiophore inflates to form a vesicle. This in turn gives rise to sterigmata, which may be single-layered (primary) or double-layered (primary and secondary). Conidia arise from the sterigmata and are borne in chains. The vesicles vary in size and shape, depending on the species. Conidia are produced within the tubular sterigmata and are extruded to form spore chains (Fig. 17-32). Conidia are of various colors and are quite characteristic of the species; the most common colors are black, brown, and green. Aspergilli grow in high concentrations of sugar and salt, indicating that they can extract water required for their growth from relatively dry substances.

Penicillium (Class *Deuteromycetes*)

Members of this group (Fig. 17-6), also *Deuteromycetes*, occur widely in nature. Some species cause rot or other spoilage of fruits, vegetables, preserves, grains, and grasses. Others are used in the ripening of cheese, e.g., Roquefort, blue, and Camembert. Some are used in industrial fermentations, and one of the best-known antibiotics (penicillin) is produced by *P. notatum* and *P. chrysogenum*. These molds are closely related to the aspergilli, and some reproduce sexually by ascospore formation. Ascus formation is observed in the penicillia less commonly than in aspergilli. The conidial stages of *Penicillium* and *Aspergillus* are so distinct and well known that they are discussed on that basis rather than on their perfect stages; see Fig. 17-33.

Penicillia have septate vegetative mycelia which penetrate the substrate and then produce aerial hyphae on which conidiophores develop. Conidiophores may be branched and have brushlike heads bearing spores. Clusters of sterigmata are usually in one place, and from each is formed a chain of conidia. The color of the mature plants is useful in helping to identify species. They grow best at temperatures ranging from 15 to 30°C.

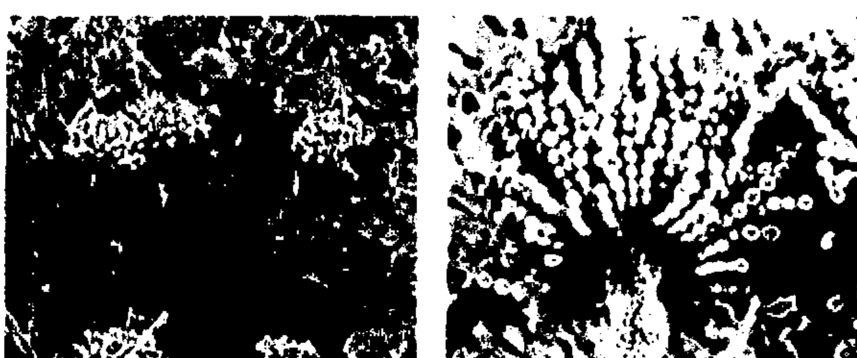


Figure 17-32. *Aspergillus flavus* as seen by scanning electron microscopy. Left: Conidiophores bearing large heads of conidia (X150). Right: Conidial head of the mold (X1,000). *(Courtesy of M. F. Brown, University of Missouri. From E. J. King and M. F. Brown, Can J Microbiol, 29:653–658, 1983.)*

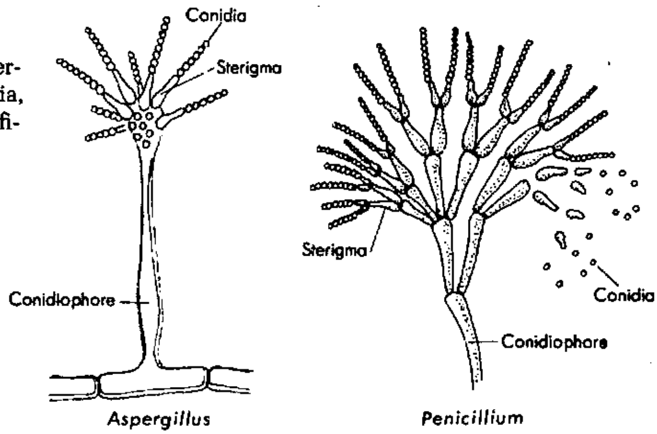


Figure 17-33. Conidial heads of *Aspergillus* and *Penicillium*. Note the different arrangements of conidia, which are useful in identification.

Candida (Class *Deuteromycetes*)

Candida albicans is often isolated from warm-blooded animals, including humans, where it exists as part of the normal microbiota of mucous membranes. Sometimes this fungus may beome pathogenic, causing candidiasis, a disease of the mucous membranes of the mouth (thrush), vagina, and alimentary tract. More serious infections can involve the heart (endocarditis), the blood (septicemia), and the brain (meningitis). It seems that predisposition factors such as other diseases, physiological disorders, obesity, alcoholism, and prolonged use of broad-spectrum antibiotics and steroids can create conditions in which *C. albicans* can cause disease. This makes the fungus an opportunistic pathogen. Fig. 17-34 shows the morphology of *C. albicans*.

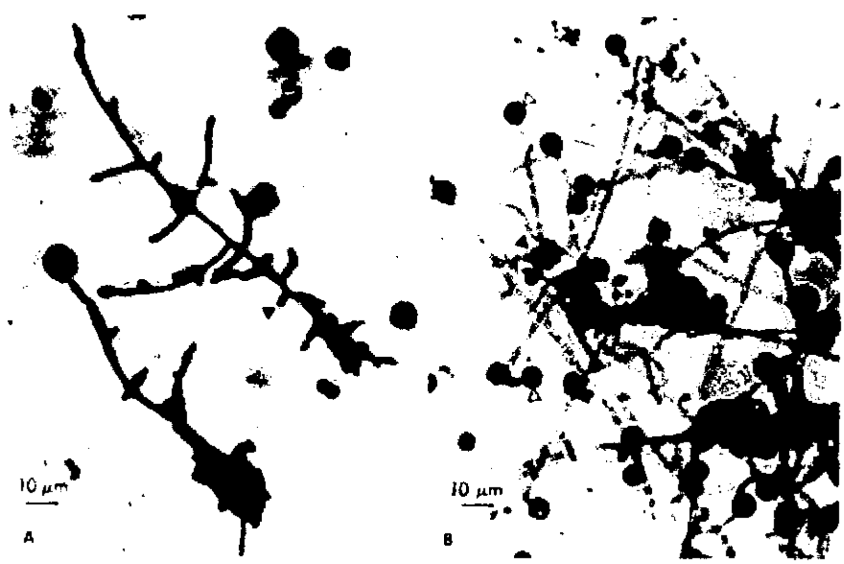


Figure 17-34. *Candida albicans*, a yeast pathogenic for humans. (A) Note pseudomycelia and blastospores (▶) in a urine sample from an infected patient. (B) The yeast also forms chlamydospores (▷) as well as pseudomycelia and blastospores (▶), when grown on a special medium in the laboratory. *(Courtesy of L. Kapica and E. C. S. Chan, McGill University.)*

MOLDS AND THEIR ASSOCIATIONS WITH OTHER ORGANISMS

There are some interesting partnerships in nature involving a mold and some other organism. In some of these associations the partners are dependent on each other and cannot survive alone. In others, the individuals can survive independently.

Lichens

Lichens are composite organisms composed of fungi and algae, each contributing to the benefit of both. The algae synthesize carbohydrates by photosynthesis and obtain other nutrients from the fungi; the fungi depend on the algae for organic carbon. Lichens are discussed in greater detail in Chap. 18.

Fungi and Nematodes

Nematodes are small roundworms predominantly pathogenic for plants; some may be parasitic in humans and other animals, and some are saprophytes. Plant-pathogenic nematodes live in the soil, where they destroy roots and other underground parts of plants. One of their greatest natural enemies is certain fungi that thrive on a diet of nematodes. These fungi use interesting techniques to capture and overcome the much larger nematodes. One of the most unusual techniques employs a loop of fungal hyphae. Upon contact by the nematode, the loop constricts around the body of the worm and holds it firmly while penetrating the prey with haustoria (special hyphal branches that penetrate a host cell) which will digest the nematode's body (Fig. 17-35). Other trapping devices used by fungi to capture nematodes, rotifers, and protozoa in the soil involve an adhesive on the mycelium. Still another fungal snare is a complex network of mycelia which grasps the worm as it tries to penetrate the fungal barrier.

Fungi as Parasites of Insects

Not all associations of fungi with higher organisms are beneficial to the host. Common among the detrimental associations are fungal infections of insects, of which more than 50 are known. At times, some appear as epidemics, when they

Figure 17-35. A nematode snared by hyphae of *Arthrobotrys conoides* (X400). *(Courtesy of David Pramer and Shimpei Kuyama, Bacteriol Rev,* **27**:*282–292, 1963.)*

destroy large numbers of insect hosts. The fungus *Entomophthora muscae* has been the cause of mild epidemics among common houseflies, crickets, and grasshoppers. Such epidemics are self-limiting and can only rarely be used effectively to destroy insect pests; e.g., pathogenic fungi which attack aphid infestations of citrus orchards have been used in attempts to develop biological control methods for insect pests.

Mycorrhizas

A mycorrhiza is an infected root system arising from the rootlets of a seed plant. The word *mycorrhiza* is derived from the Greek, meaning "fungus root." These associations are usually beneficial to the host plant as well as the symbiont, and sometimes the host cannot thrive without the benefits derived from the fungus. Mycorrhizas enhance mineral absorption by the green plants. Host plants usually have a built-in check on the fungus to prevent it from causing injury to the rootlets. Truffles are subterranean fruiting bodies of certain *Ascomycetes* which grow in association with some trees, oak and beech being common symbionts in this mycorrhizal partnership. The fungus provides certain nutrients to the tree, which in turn provides essential growth substances to the fungus. Such relationships are quite common. Truffles consist of a mass of ascospores and mycelia, covered with a thick knobby rind of mycelium. They have a pleasant odor, flavor, and texture which make them highly prized by gourmets.

In plants that do not have root hairs, e.g., orchids, the fungi absorb nutrients and water for their hosts. Under natural conditions, orchids rarely grow without fungal partners. Experimentally, orchids will grow alone if appropriate nutrients are provided.

Another type of mycorrhiza is found in *Monotropa uniflora* (Indian pipe), which has no chlorophyll but grows well in the heavy shade of the forest floor as long as the fungi are on its roots to gather food for both host and parasite.

QUESTIONS

1. Yeasts, like molds, are fungi. How are they morphologically different from molds? How do they resemble bacteria?
2. In what ways are fungi important to humans?
3. Describe the phenomenon of dimorphism as it exists in certain pathogenic fungi.
4. Draw a diagram of a hypha and label it with the following: wall, lumen, plasmalemma, matrix, nucleus, septum, microfibrils, and central pore.
5. Describe the different ways by which fungi reproduce asexually.
6. Describe sexual reproduction as it occurs in fungi.
7. Explain the difference between sexual spores and asexual spores, with particular reference to their formation.
8. Compare the physiology of fungi with that of bacteria. Which physiological features are used to advantage in the preparation (formulation) of selective media for fungi?
9. What is unique about the class *Deuteromycetes*, making it different from other classes of fungi?
10. Compare the criteria used to identify molds with those used to identify bacteria.

11 What are the unique features of the slime molds that distinguish them from the true fungi?
12 Describe the life cycle of the cellular slime mold *Dictyostelium discoideum.*
13 Describe how a typical myxomycete reproduces sexually.
14 What main features distinguish the members of the division *Mastigomycota* from the members of the division *Gymnomycota*?
15 Compare the sexual and asexual reproduction of *Saccharomyces* and *Schizosaccharomyces.*
16 Define these terms: basidiocarp, sterigma, and basidium.
17 Describe how *Synchytrium endobioticum* infects a new host.
18 Describe the process of sexual reproduction in the mold *Saprolegnia.*
19 Describe the following:
  (*a*) Dimorphism in *Mucor*
  (*b*) Plus and minus strains in *Rhizopus stolonifer*
  (*c*) Asexual reproduction of *Schizosaccharomyces*
  (*d*) Budding in *Saccharomyces*
  (*e*) Yeast hybridization
20 Why are the following fungi important to our economy or health: (*a*) *Aspergillus niger,* (*b*) *Penicillium notatum,* (*c*) *Neurospora sitophila,* (*d*) *Agaricus bisporus,* (*e*) *Candida albicans,* and (*f*) *Filobasidiella neoformans*?
21 In what manner are the following associated with fungi: (*a*) lichens, (*b*) nematodes, and (*c*) orchids?

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Chapter 18 Algae

OUTLINE

This chapter introduces the **algae** (singular, **alga**), many of which are unicellular microorganisms. These organisms are ubiquitous; many live in aquatic environments but many also thrive as terrestrial and subterranean algae. Algae contain chlorophyll and are photosynthetic. They differ from other green plants in having simple reproductive structures for sexual reproduction. For example, the unicellular algae themselves may function as gametes. In their asexual reproduction, many algae produce flagellated spores and/or nonmotile spores in sporangia.

Algae are of great general interest to all biologists because single algal cells are complete organisms capable of photosynthesis and the synthesis of a multitude of other compounds which constitute the cell. Geneticists and evolutionists find algae interesting and useful for study because of their conspicuous pattern of evolutionary specialization. The convenient techniques used for the study of bacteria and molds are applicable to microscopic algae discussed in this chapter.

In size, habitat, and reproductive processes algae are a heterogeneous group. They range from microscopic unicellular forms comparable in size to bacteria to seaweeds that may grow to many feet in length. The study of this unique group of organisms is called **phycology**.

OCCURRENCE OF ALGAE

Many thousands of algal species occur in nature. There are few places on earth where algae of some kind cannot be found. They occur in great abundance in the oceans, seas, salt lakes, freshwater lakes, ponds, and streams. Many are found in damp soil, on rocks, stones, and tree bark, and on other plants and animals.

Small aquatic forms make up a large part of the free-floating microscopic life in water called plankton, which is the principal food for aquatic animals, including such large ones as whales. (Plankton is generally considered to be composed of both algae and microscopic animal forms. Phytoplankton is made up of plants, i.e., algal forms, and zooplankton is composed of animal organisms.) Algae are found where there are sufficient light, moisture, and simple nutrients to sustain them.

Some species of algae grow on the snow and ice of polar regions and mountain peaks, sometimes occurring in such abundance that the landscape becomes colored by the red pigments in their cells. At the other extreme, some algae grow in hot springs at temperatures as high as 55°C. Some freshwater algae have adapted their metabolism to the high salt concentrations found in the brine lakes of the arid southwestern United States. The salinity of marine environments varies from place to place, but marine algae adapt to the variations in salt concentration. Others adjust to air and to drying when they are exposed at low tide. Marine algae are not normally found in northern waters of the ocean at depths greater than 150 to 180 ft. In the clearer, warmer tropical waters, however, where the sunlight is more direct and has a longer daily period, they may be found at depths as great as 600 ft. These and other factors are responsible for the zonation phenomenon, which is the stratification of certain kinds of algae at certain depths and locations in the ocean.

Some algae are adapted to moist soil, the bark of trees, and the surface of rocks, which the algae degrade. The decomposition products are made available for soil building and enrichment.

Algae are often a problem in water supplies because they produce undesirable tastes and odors. An especially notorious offender is a flagellated brown alga belonging to the genus *Synura*. Heavy algal growths may form blankets or mats which interfere with the use of some natural waters for recreational purposes. These algal mats may act as barriers to the penetration of oxygen into the water; they prevent photosynthesis by excluding light from deeper water and thus may cause fish and other marine animals to suffocate. Algae may even increase the corrosive quality of water and cause the disintegration of concrete.

On the other hand, when dispersed in natural waters algae increase the oxygen concentration through photosynthesis. Heavy growth of some algae reduces hardness of water and removes salts which are the cause of brackishness. An installation using this principle on a large-scale experimental basis has been built in South Dakota in an area where the natural water contains a high concentration of organic salts.

Some algae are endophytic; that is, they are not free-living but live in other organisms. Such algae are widespread in protozoa, molluscs, sponges, and corals.

THE BIOLCGICAL AND ECONOMIC IMPORTANCE OF ALGAE

Algae as Primary Producers

Most algae are aquatic organisms. Since 70 percent of the earth's surface is covered with water, it is probable that as much carbon is fixed (captured as carbon dioxide and changed to organic carbon compounds such as sugars) through photosynthesis by algae as is fixed by all land flora. As mentioned before, tiny floating algae constitute the phytoplankton of the sea and serve as an important food source for other organisms. These algae form the base or beginning of most aquatic food chains because of their photosynthetic activities and are therefore called primary producers of organic matter.

Algae are also present in soil even if their presence is not so obvious. They are probably important in stabilizing and improving the physical properties of soil by aggregating particles and adding organic matter. In addition, in many countries where the large red and brown seaweeds are plentiful, they are used as fertilizer.

Commercial Products from Algae

Many products of economic value are derived from algal cell walls. Three of these, agar, alginic acid, and carrageenan, are extracted from the walls of algae. Another, diatomaceous earth, is composed of millions upon millions of diatom glass walls deposited over time in either freshwater or the ocean.

Agar and carrageenan are polymers of galactose, or galactose-containing compounds, with sulfate groups. Some of the sulfate groups are involved in the bonds between individual sugar residues. Agar and carrageenan are both called sulfated galactans, with carrageenan the more sulfated compound. Alginic acid consists of uronic acid residues. All three compounds are used either to make gels or to make solutions viscous.

Carrageenan is extracted from the walls of several red algae. Species of *Chondrus*, *Gigartina*, and *Eucheuma* are most frequently used. Carrageenan has been used as a stabilizer or emulsifier in foods such as ice cream and other milk products. It is also used as a binder in toothpaste or in pharmaceutical products, as well as an agent in ulcer therapy. Carrageenan is also useful as a finishing compound in the textile and paper industries, as a thickening agent in shaving creams and lotions, and in the soap industry.

Agar is well known as a solidifying agent in the preparation of microbiological media. It is obtained from red algae. Species of *Gelidium* and *Gracilaria* are used extensively. It is also important in the food industry where it is valuable in the manufacture of processed cheese, mayonnaise, puddings, jellies, baking products, and canned goods. In the pharmaceutical industry agar can be used as a carrier for a drug. Lotions and ointments can contain some agar.

Alginic acid and its salts are obtained from the walls of brown algae, where they may represent as much as 25 percent of the dry weight. Species of brown algae producing this compound include *Macrocystis*, *Agarum*, *Laminaria*, *Fucus*, and *Ascophyllum*. About 50 percent of the ice cream in the U.S. contains alginates, which provide a smooth consistency and eliminate ice crystal formation. Alginates are also incorporated into cheeses and bakery products, especially frostings. Other industrial applications include paper manufacturing, the printing of fabrics, and paint thickening.

An alginate material is used by dentists for making impressions of the teeth for crowns, etc. The stipe (stemlike part) of the brown alga *Laminaria japonica* may be used by physicians for cervical dilatation and/or softening of the cervix (for example, in performing an abortion or placing a radium implant). The stipe is cut into sections, dried, and sterilized by ethylene oxide gas; such prepared stipes are available commercially. The section is put into place and swells gradually, rendering symmetrical dilatation of the cervical canal and softening of cervical tissue. Japanese physicians have used this method for more than a century.

Diatomaceous earth is used primarily for filters or filter aids. It is especially suitable because it is not chemically reactive, is not readily compacted or compressed during use, and is available in many grades. The material is so finely divided that one gram of diatomaceous earth has 120 square meters of surface area, and yet in use up to 90 percent of the volume of the filter cake is open space. Diatomaceous earth is also used for polishing delicate surfaces because the diatom walls are so lightly silicified that they collapse under pressure and do not damage the surfaces.

Algae as Food

Many species of algae (mostly red and brown algae) are used as food in the Far East. Of the red algae one of the most important is *Porphyra*; it is used as a food in Japan, where it is called "nori" and is usually processed into dried sheets. The algae are collected and washed in fresh water to remove debris, then chopped and spread on frames to dry into thin sheets. Nori is commonly toasted over a flame and sprinkled in soup or rice, or it is rolled around flavored rice with fish or vegetables to make a popular luncheon snack called sushi.

Although several species are still collected wild, *Porphyra* has been cultivated since 1570. Today Japan's seaweed cultivation is the most advanced in the world. The nori industry is based mainly on *P. tenera* and *P. yezoensis* (Fig. 18-1A), although up to seven other species have been cultivated in Tokyo Bay in the past.

Other red algae, such as *Chondrus*, *Acanthopeltis*, *Nemalion*, and *Eucheuma*,

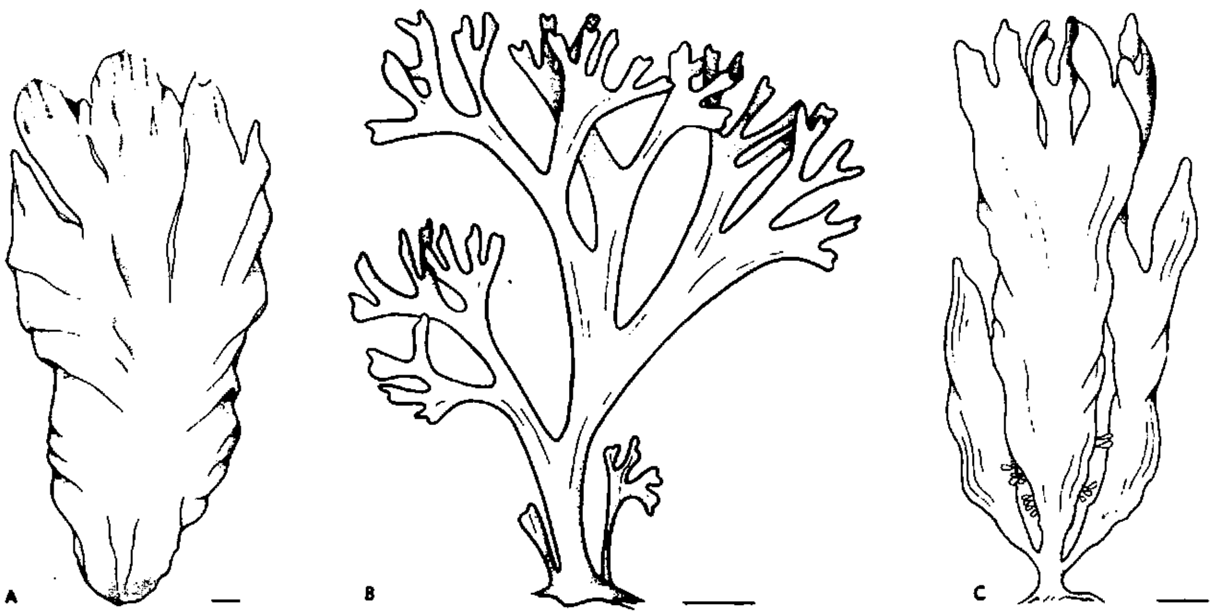


Figure 18-1. Some algae used as food. (A) *Porphyra yezoensis* Ueda. (B) *Chondrus crispus* Stackhouse. (C) *Palmaria palmata* (L.) O. Küntze. Bar = 1 cm. *(Erwin F. Lessel, illustrator)*

are locally collected and prepared. Most of them are eaten as vegetables or in soups or prepared as sweetened jellies.

Red algae continue to be a significant food in China. Such algae have been a food staple and delicacy for the Chinese for thousands of years. For example, *Gracilaria* is mentioned in Chinese *materia medica* dating back to 600 B.C. In the past two decades, China has developed an impressive seaweed cultivation program. The species farmed are the edible brown alga *L. japonica* as well as *Porphyra* and *Gracilaria*. *Porphyra* and other red seaweeds are expected to be increasingly important in the Chinese diet.

In contrast to the diversity of species eaten by Asian and Polynesian peoples, red algal food usage in Europe and North America has centered around three genera: *Porphyra* or laver, *Chondrus* (Fig. 18-1B) or Irish moss, and *Palmaria* or dulse (Fig. 18-1C).

Laver is used extensively in the British Isles. The miners of southern Wales are the major laver consumers. About 40 to 50 tons of dried dulse are produced each year in Canada. The alga is collected in the Bay of Fundy and on the shores of Nova Scotia. Dulse is commonly eaten as a snack in taverns.

There is increasing interest in the use of the smaller forms of algae, especially *Chlorella*, as food for humans and domestic animals. When these organisms are grown under suitable conditions, they provide a rich source of protein comprising all the amino acids essential for animal growth. They are also a good source of carbohydrates and fats. The nutritive value of the microscopic algae has been demonstrated in tests with rats and chicks. Methods for mass cultivation of these plants, using waste products and sewage for their nutrition, have been developed. After algae have been grown on waste products, the residues can be disposed of in streams and lakes without causing pollution that would destroy aquatic animals. Although much can be said in favor of using algae in place of higher plants for human food, general acceptance of their use in this country is not to be expected until food from other sources is in short supply. In the meantime algae will doubtless find wide application as animal feeds or feed supplements.

Algae and Diseases

Although few algae are pathogenic, one, *Prototheca*, has been found to be a probable pathogen of humans. It has been found in systemic and subcutaneous infections, as well as in bursitis (an inflammation of the joints). *Prototheca* is a colorless *Chlorella*-like organism which superficially resembles yeasts (Fig. 18-2).

Several species are parasitic on higher plants; e.g., the green alga *Cephaleuros* attacks leaves of tea, coffee, pepper, and other tropical plants, causing considerable damage. Some algae live in the roots and fleshy parts of higher plants; liverworts, duckweeds, and other hosts to such algae do not seem to be harmed by their presence.

Some of the extracellular inhibitors produced by algae have been shown by chemical analysis to be simple chemical substances; e.g., acrylic acid is produced by a unicellular alga in plankton. It is quite possible that as we learn more about algae and their extracellular secretions, their usefulness will become more apparent.

Some planktonic algae produce toxins which are lethal to fish and other

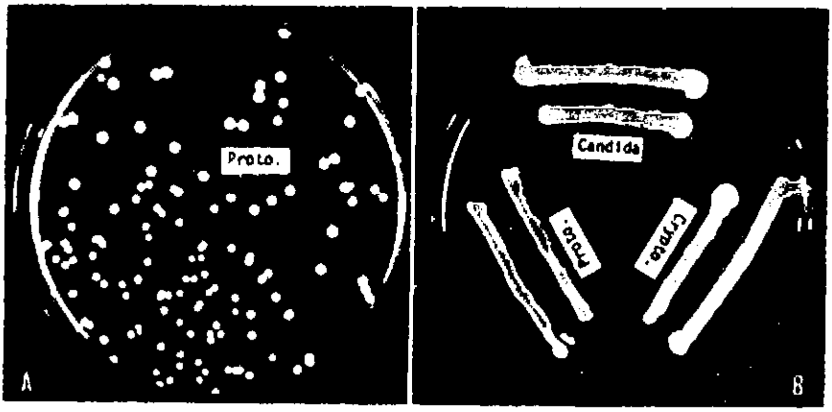


Figure 18-2. Prototheca cells are colorless and do not carry out photosynthesis. However, the presence of starch-containing inclusions consitutes significant evidence for their algal nature. They resemble yeast cells in their gross growth characteristics on media, which can lead to errors in laboratory diagnosis. (A) Colonies of *P. wickerhamii* on Sabouraud agar. (B) Streaked cultures of *P. wickerhamii* (Proto.), *Candida albicans* (Candida), and *Filobasidiella* (*Cryptococcus*) *neoformans* (Crypto.). The latter two species are yeasts. (*Courtesy of L. Kapica, McGill University.*)

animals. These toxins are extracellular, or they are liberated from the alga by bacterial decomposition of water blooms. It is known, however, that certain marine dinoflagellates belonging to the genera *Gymnodinium* and *Gonyaulax* cause death of aquatic animals by producing a high molecular weight neurotoxin. Those few dinoflagellate toxins which have been identified are among the strongest toxins known—50 times stronger than curare, a poison with which certain primitive peoples tipped their darts.

Shellfish poisoning occurs along the northeastern coast of North America as well as in the North Pacific. The organisms are *Gonyaulax catenella* on the west coast and *Gonyaulax excavata* on the east coast. Yearly outbreaks occur around Nova Scotia. The bloom of these dinoflagellates usually lasts just a few weeks, and often it is safe to eat shellfish about two weeks after the end of the bloom. The poisoning to humans comes from eating filter feeders, i.e., clams, scallops, or mussels, which filter the plankton from seawater as a source of food and accumulate the poison (toxin).

After ingesting sufficient toxin, the victim first experiences a numbing of the lips, tongue, and fingertips, usually within 30 min of eating shellfish. The diaphragm is soon affected and in serious cases respiratory failure can result.

CHARACTERISTICS OF ALGAE

Morphology

Algae have a wide range of sizes and shapes (Fig. 18-3). Many species occur as single cells that may be spherical, rod-shaped, club-shaped, or spindle-shaped. Others are multicellular and appear in every conceivable form, shape, and degree of complexity, including membranous colonies, filaments grouped singly or in clusters with individual strands that may be branched or unbranched, and tubes (which may or may not be divided by cell walls). Some colonies are simply aggregations of single, "identical" cells that cling together after division; others are composed of different kinds of cells specializing in particular functions. These colonies become quite complex and superficially resemble higher plants in structure.

Algal cells are eucaryotic. In most species the cell wall is thin and rigid. Cell walls of diatoms are impregnated with silica, making them thick and very rigid; they are often delicately sculptured with intricate designs characteristic of the

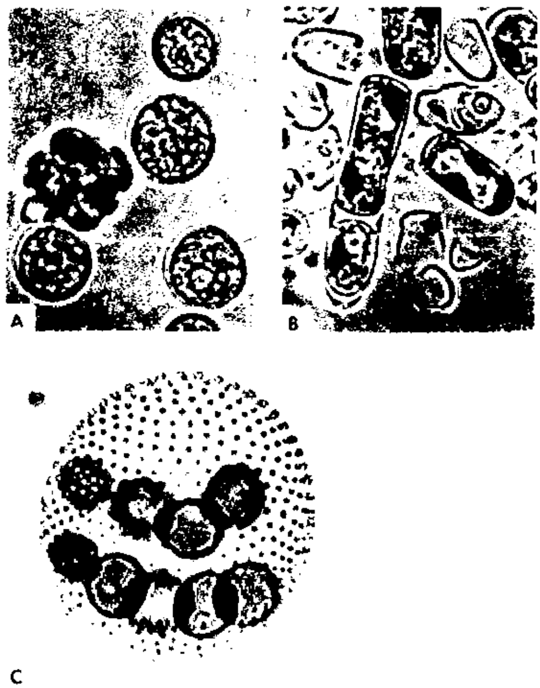


Figure 18-3. Algae occur in a wide variety of sizes, shapes, and arrangements. (A) The green alga *Chlorococcum scabellum*. This photomicrograph shows vegetative cells and a cluster of aplanospores (X760). (B) Another alga, *Pseudobumilleriopsis* sp. (X912). [*(A) and (B) courtesy of Harold C. Bold.*] (C) *Volvox spermatosphaera*, illustrating parental colony with developing daughter colonies. (*Courtesy of Richard C. Starr.*)

species or variety. The motile algae such as *Euglena* have flexible cell membranes called periplasts. The cell walls of many algae are surrounded by a flexible, gelatinous outer matrix secreted through the cell wall, reminiscent of bacterial capsules. As the cells age, the outer matrix often becomes pigmented and stratified.

Algae contain a discrete nucleus. Other inclusions are starch grains, oil droplets, and vacuoles. Chlorophyll and other pigments are found in membrane-bound organelles known as chloroplasts. These chloroplasts may be massive structures situated near the wall (parietal) or embedded in the midst of the cytoplasm (asteroidal). They may occur as one, two, or many per cell; they may be ribbonlike, barlike, netlike, or in the form of discrete disks, as in green plants. Within the plastid (chloroplast) matrix or stroma are found flattened membranous vesicles called thylakoids. The fine structure of a eucaryotic algal cell is shown in Fig. 18-4.

Algal Pigments

The chloroplasts of different divisions of algae containing similar pigments appear to have similar thylakoid arrangements. Chloroplast ultrastructure and pigment chemistry have been used as markers for algal phylogeny. Table 18-1 shows the divisions (primary taxa in algal classification) of algae, with some of their photosynthetic pigments.

It should be pointed out that several divisions of algae include colorless members, e.g., certain species and genera in the *Euglenophycophyta*, the *Pyrrophycophyta*, and the *Chlorophycophyta*. These are sometimes considered

Figure 18-4. The fine structure of an algal cell is revealed by this near-median longitudinal section of a cell of *Ochromonas danica*. The single Golgi body (g) lies anterior to the nucleus (n). The endoplasmic reticulum (ER) is visible on the right side of the section. A single chloroplast (C) is shown, but it appears to be two chloroplasts because of the plane of sectioning. A large starch-containing vacuole (v) occupies almost half of the cell. Numerous mitochondria (m) are present in the peripheral cytoplasm of the cell (X12,120). (*Courtesy of Sarah P. Gibbs, McGill University.*)

protozoa. Nevertheless, some colorless flagellates have been shown to possess chloroplasts. There are three kinds of photosynthetic pigments in algae: chlorophylls, carotenoids, and biloproteins (also called phycobilins). Their distribution is shown in Table 18-1.

Chlorophylls

There are five chlorophylls: *a*, *b*, *c*, *d*, and *e*. Chlorophyll *a* is present in all algae, as it is in all photosynthetic organisms other than anoxygenic photosynthetic bacteria. Chlorophyll *b* is found in the *Euglenophycophyta* and *Chlorophycophyta* and in no other algal division. Chlorophyll *c* is more widespread and is present in members of *Xanthophycophyta*, *Bacillariophycophyta*, *Chrysophycophyta*, *Pyrrophycophyta*, *Cryptophycophyta*, and *Phaeophycophyta*. Chlorophyll *d* appears to be present only in the *Rhodophycophyta*. Chlorophyll *e* is rare and has been identified in only two genera of *Xanthophycophyta*, namely, *Triboneara* and the zoospores of *Vaucheria*.

Carotenoids

There are two kinds of carotenoids: carotenes and xanthophylls. Carotenes are linear, unsaturated hydrocarbons, and xanthophylls are oxygenated derivatives

Table 18-1. Some Properties of Major Algal Taxonomic Groups (Divisions)

Taxonomic Group (Division)	Chlorophyll	Carotenoids*	Biloproteins	Storage‡ Products	Flagellation and Details of Cell Structure
Rhodophycophyta† (red algae)	*a*, rarely *d*	β-carotene, zeaxanthine ±α-carotene	Phycoerythrin, phycocyanin	Floridean starch, oils	Flagella absent
Xanthophycophyta† (yellow-green algae)	*a*, *c*, rarely *e*	β-carotene, diadinoxanthin, heteroxanthin, vaucheriaxanthin ester		Chrysolaminarin, oils	2 unequal, apical flagella
Chrysophycophyta (golden algae)	*a*, *c*	β-carotene, fucoxanthin		Chrysolaminarin, oils	1 or 2 equal or unequal, apical flagella; in some, cell surface covered by characteristic scales
Phaeophycophyta (brown algae)	*a*, *c*	β-carotene ±α-carotene, rarely ϵ-carotene, fucoxanthin, violaxanthin		Laminarin, soluble carbohydrates, oils	2 lateral flagella
Bacillariophycophyta (diatoms)	*a*, *c*	β-carotene ±α- carotene, rarely ϵ-carotene, fucoxanthin		Chrysolaminarin, oils	1 apical flagellum in male gametes; cell in two halves; the walls silicified with elaborate markings
Euglenophycophyta (euglenoids)	*a*, *b*	β-carotene ±γ-carotene diadinoxanthin		Paramylon, oils	1, 2, or 3 equal, slightly apical flagella; gullet present
Chlorophycophyta (green algae)	*a*, *b*	β-carotene ±α-carotene, rarely γ-carotene and lycopene, lutein		Starch, oils	1, 2, 4 to many, equal, apical or subapical flagella
Cryptophycophyta (cryptomonads)	*a*, *c*	α-carotene ±β-carotene, rarely ϵ-carotene, alloxanthin	Phycoerythrin, phycocyanin	Starch, oils	2 lateral flagella; gullet present in some species
Pyrrophycophyta (dinoflagellates)	*a*, *c*	β-carotene, peridinin, neoperidinin, dinoxanthin, neodinoxanthin		Starch, oils	2 lateral, 1 trailing, 1 girdling flagellum; in most, there is a longitudinal and transverse furrow and angular plates

* Only predominant xanthophylls are included.
† Some workers have recently separated a new division, *Eustimatophycophyta*, from these.
‡ These may be polymers of glucose molecules with variations in chemical linkages, oils, or cyclic alcohols.

of these. The predominant carotenes and xanthophylls present in the various algal divisions are shown in Table 18-1.

Biloproteins (Phycobilins)

These are water-soluble pigments, whereas chlorophylls and carotenoids are lipid-soluble. Phycobilins are pigment-protein complexes and are present in only two algal divisions: the *Rhodophycophyta* and *Cryptophycophyta*. There are two kinds of phycobilins: phycocyanin and phycoerythrin. The proportion of one kind of pigment to another can vary considerably with changes in environmental conditions. Pigment quantitation is therefore not too reliable for use as a taxonomic feature.

Motility

The motile algae, also called the swimming algae, have flagella occurring singly, in pairs, or in clusters at the anterior or posterior ends of the cell. Since the advent of electron microscopy, considerable variation of taxonomic significance has been found in algal flagella. It will suffice for this discussion to mention three types: whiplash (cylindrical and smooth); tinsel (cylindrical and with hairlike appendages); and ribbon, or straplike. Some algae have no means of locomotion and are carried about by tides, waves, and currents. Others move about by means other than flagella. In some forms only the zoospores, the asexual reproductive cells, are motile. Some attach themselves to the substrate in the body of water where they are living and are occasionally broken loose by currents, which move them to new locations.

A small red or orange body, the eyespot, is often present near the anterior end of motile algae. Other structures occurring in certain algae include spines or knobs on their exteriors and gelatinous stalks by which they may be anchored to some object.

Reproduction

Algae may reproduce either asexually or sexually. Some species are limited to one of these processes, but many have complicated life cycles employing both means of propagation.

Asexual reproductive processes in algae include the purely vegetative type of cell division by which bacteria reproduce. A new algal colony or filament may even start from a fragment of an old multicellular type from which it has broken. However, most asexual reproduction in algae is more complex than this and involves the production of unicellular spores, many of which, especially in the aquatic forms, have flagella and are motile; these are called zoospores. The nonmotile spores, or aplanospores, are more likely to be formed by the terrestrial types of algae. However, some aplanospores can develop into zoospores.

All forms of sexual reproduction are found among the algae. In these processes

Figure 18-5. *Polysiphonia lanosa*, a species of red alga, found in the littoral and sublittoral zones in eastern Canada. It is an erect plant, growing attached to other vegetation and other algae (as shown here growing attached to the brown alga *Ascophyllum nodosum*) as well as shells, stones, and woodwork. The bushy filamentous thallus is *P. lanosa* (▶). (*Courtesy of E. C. S. Chan, McGill University.*)

there is a fusion (conjugation) of sex cells, called **gametes**, to form a union in which "blending" of nuclear material occurs before new generations are formed. The union of gametes forms a **zygote**. If the gametes are "identical," i.e., if there is no visible sex differentiation, the fusion process is **isogamous**. If the two gametes are unlike, differing in size (male and female), the process is **heterogamous**. As we proceed to the higher, though not necessarily larger, forms of algae, the sexual cells become more characteristically male and female. The ovum (female egg cell) is large and nonmotile, and the male gamete (sperm cell) is small and actively motile. This type of sexual process is termed **oogamy**. Exclusively male or exclusively female thalli also exist. Although these thalli may look alike, they are of opposite sex types, since one produces male gametes and the other ova. Such plants are called **unisexual** or **dioecious**. Plants in which gametes from the same individual can unite are said to be **bisexual** or **monoecious**.

CLASSIFICATION

Although specialists do not agree on the details of algal classification, algae are generally classified on the basis of the following characteristics:

1 Nature and properties of pigments
2 Chemistry of reserve food products or assimilatory products of photosynthesis
3 Type and number, insertion (point of attachment), and morphology of flagella
4 Chemistry and physical features of cell walls
5 Morphological characteristics of cells and thalli
6 Life history, reproductive structures, and methods of reproduction

The major divisions of algae (Table 18-1) will be discussed briefly. Only a few of the outstanding characteristics of each group will be mentioned.

Rhodophycophyta—The Red Algae

The *Rhodophycophyta*, or red algae, are marine forms found in the warmer seas and oceans, but some of them grow in colder water as well as in freshwater. Most red algae grow in the subtidal (submerged) zone, only a few being able to survive desiccation or exposure. *Rhodophycophyta* are smaller than most *Phaeophycophyta*, rarely becoming more than 2 or 4 ft long. Their reproductive process is highly specialized. Asexual reproduction is accomplished by nonmotile spores. Sexually, however, they reproduce heterogamously by the union of well-differentiated nonmotile male and female germ cells, the **spermatia** and **carpogonia** (female sex organs), respectively. Some species deposit upon their surfaces lime from seawater; ultimately this results in deposition of lime in the ocean and plays a part in the formation of algal reefs. Several red algae are of economic importance, particularly *Gelidium*, from which agar is made. Irish moss (*Chondrus crispus*), the source of carrageenan, is another species of *Rhodophycophyta*. A genus (*Polysiphonia*) that is commonly found in marine waters is illustrated in Fig. 18-5.

Xanthophycophyta—The Yellow-Green Algae

These yellow-green algae were once classified with the green algae. However, their pale green or yellow-green coloration indicates that they have a unique group of pigments. They are found more frequently in temperate regions in freshwater and marine habitats, as well as on and in soil. But they are not dominant organisms in any environment.

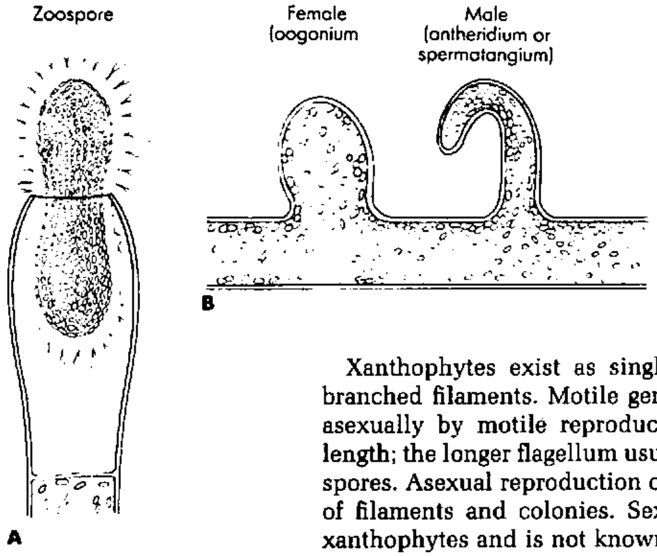


Figure 18-6. Asexual and sexual reproduction in *Vaucheria*. (A) Release of a single zoospore from a zoosporangium. The zoospore is multinucleated and has many pairs of slightly unequal flagella covering its surface. (B) Female (left) and male (right) reproductive structures are shown. These structures are macroscopic but the reproductive cells (sperms and eggs) are microscopic. Numerous disclike plastids are also shown. *(Erwin F. Lessel, illustrator)*

Xanthophytes exist as single cells, colonies, and both branched and unbranched filaments. Motile genera are not common, although some reproduce asexually by motile reproductive cells (zoospores). Flagella are of unequal length; the longer flagellum usually has hairs in two rows. Some have nonmotile spores. Asexual reproduction can also occur by cell division and fragmentation of filaments and colonies. Sexual reproduction is rarely observed in certain xanthophytes and is not known for others.

The xanthophyte walls are typically of cellulose and pectin. The cellular storage product is an oil or (a branched glucan) chrysolaminarin.

Vaucheria, the water felt, is a well-known member of this division and is widely distributed on moist soil and in both quiet and rapidly flowing water. Both freshwater and marine species are known. *Vaucheria* has a very large macroscopic filamentous form. The coenocytic filaments form a felty mass. Septa are laid down only when gametangia and zoosporangia are formed. In addition to the many nuclei in the filaments, there are thousands of ellipsoidal chromatophores or plastids.

Zoospores are formed singly in terminal sporangia in asexual reproduction. Their flagella are inserted in pairs all over the zoospore surface. Sex organs are large and develop as side branches (Fig. 18-6). Sperms are formed in elongate, curved spermatangia. They have a pair of flagella which are inserted laterally. The egg, formed singly within an erect oogonium, is uninucleate and sperms get within the wall through a pore.

Chrysophycophyta—The Golden Algae

Species of *Chrysophycophyta* are predominantly flagellates; some are amoeboid, with pseudopodial extensions of the protoplasm. The naked amoeboid forms can ingest particulate food with the pseudopodia. Nonmotile coccoid and filamentous forms are also included in the division.

The *Chrysophycophyta* differ from the green algae in the nature of their pigments, in storing reserve food as oil or chrysolaminarin rather than as starch, and in their frequent incorporation of silica. Most forms are unicellular, but some form colonies. Their characteristic color is due to the masking of their chlorophyll by brown pigments. Reproduction is commonly asexual (binary fission) but occasionally isogamous.

Ochromonas is an interesting unicellular genus with unequal flagellation. One species is remarkably versatile in nutrition in that it may grow photoautotrophically, heterotrophically, or phagotrophically. Although many chryso-

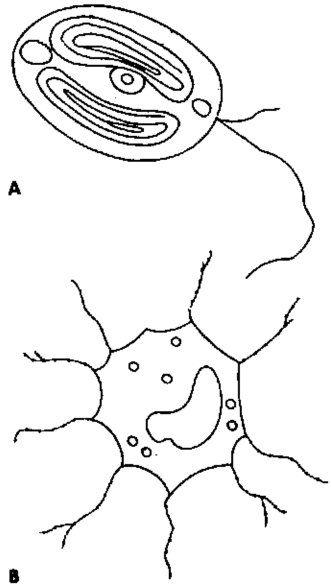


Figure 18-7. Algae of *Chrysophycophyta*. (A) *Ochromonas* sp. A commonly occurring unicellular flagellate with flagella markedly unequal in length. There are two distinctive plastids in the photosynthetic forms. (B) *Chrysamoeba* sp. It is an amoeboid cell with a number of long rhizopodia radiating from the central mass of protoplasm.

Figure 18-8. Sargassum is a type of brown alga which gets its name from the Sargasso Sea, where it is found in great abundance. The ball-like structures are air bladders. X1. (*Courtesy of General Biological Supply House.*)

phytes are to some degree amoeboid, *Chrysamoeba* is a strongly amoeboid genus with flagella (see Fig. 18-7).

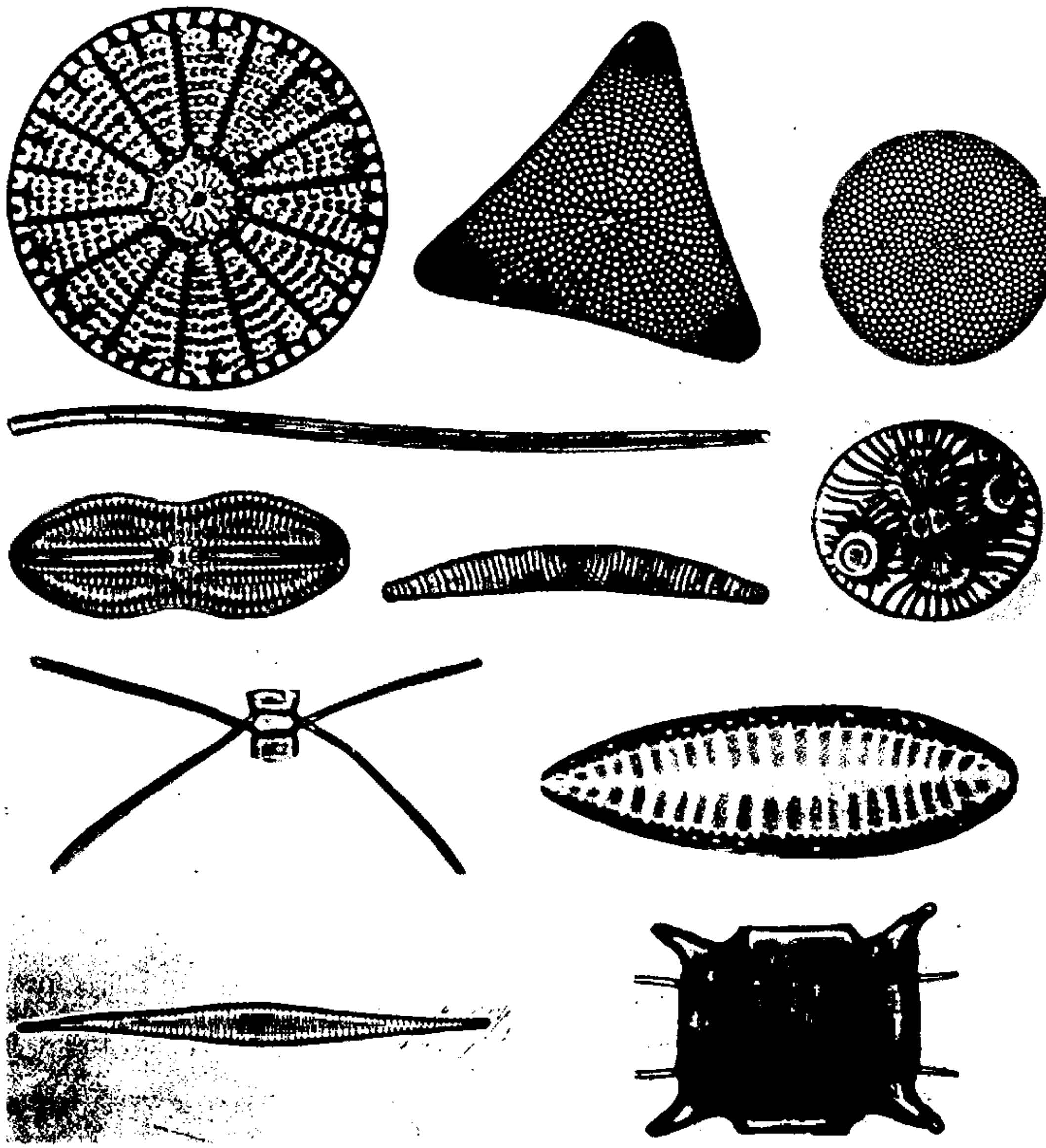
Phaeophycophyta—The Brown Algae

These algae are multicellular and contain a brown pigment which gives them their characteristic color and common name of **brown algae**, or brown seaweeds. Nearly all are marine and most frequently found in the cool ocean waters. They are structurally quite complex, and some—the kelps—are large, the individual plants reaching a length of several hundred feet. Many have holdfasts; and some have air bladders, which give them buoyancy. The brown algae reproduce asexually, by zoospores, and sexually, both isogamously and heterogamously. This group includes algae used in commerce, such as the many varieties of kelp. They are used as food for humans, other animals, and fish; in medicinal preparations; in fertilizers; and as a source of iodine and mineral salts. Others, such as *Sargassum natans*, occur as a great floating population in that part of the Atlantic Ocean known as the Sargasso Sea (Fig. 18-8)

Bacillariophycophyta—The Diatoms

Members of this group are the diatoms, found in both fresh and salt water and in moist soil. Abundant in cold waters, diatoms are the most plentiful form of plankton in the Arctic. The thousands of species of diatoms provide an ever-present and abundant food supply for aquatic animals. Diatoms are either unicellular, colonial, or filamentous and occur in a wide variety of shapes (Fig. 18-9). Each cell has a single prominent nucleus and massive ribbonlike, or smaller lenslike, plastids. They produce shells (cell walls) containing silica, some of which are very beautiful. Shells of diatoms are called **frustules**. Deposits of these shells resulting from centuries of growth are called **diatomite** or **diatomaceous earth**. Although diatomite from prehistoric times is found in Oregon, Nevada, Washington, Florida, and New Jersey, the world's largest and most productive commercial source is at Lompoc, California. It is used in insulating materials; as a filter for clarifying fruit juices, cane sugar, wine, beverages, and swimming pool water; in cosmetic bases; and as polishing material.

Figure 18-9. Diatoms are unicellular algae found abundantly in fresh and salt water. Their hard silica-containing walls consist of two halves which fit together like a Petri dish and its cover. They occur in myriads of shapes, many with beautiful surface designs. Magnifications range from X400 to X800. (*Courtesy of Johns-Manville Research Center.*)



Euglenophycophyta— The Euglenoids

These unicellular organisms are actively motile by means of flagella, and they reproduce by cell division. Of particular interest is the genus *Euglena*, which is representative of a group designated as animals by some zoologists but as plants by many botanists. There are sound arguments to support either side. H. C. Bold brings them together by saying that *Euglena* may be regarded "as an organism descended from animallike ancestors and developing in the direction of the photosynthetic self-sufficiency of plants." (See Fig. 18-10.) *Euglena* is widely distributed and occurs in soil as well as in water, where it often forms a velvety film or bloom.

The *Euglena* cell is not rigid; it is pliable. There is no cell wall containing cellulose. The outer membrane is an organized periplast. An anterior "gullet" (Fig. 18-10) is present even though no food is ingested through it. Certain species develop a prominent stigma or red eyespot. Contractile vacuoles and fibrils are also present in the cell. All these are animal attributes. On the other hand, the organism carries out photosynthesis in chloroplasts and is facultatively autotrophic; these are plant attributes. The majority can assimilate organic substances during photosynthesis. A few types can even ingest particulate food through transient openings adjacent to the gullet.

Reproduction is by longitudinal binary fission. Dormant cysts are formed by all types. (Cysts are dormant stages whose walls are physically and sometimes chemically distinct from the wall of the vegetative cells from which they are derived.)

Chlorophycophyta— The Green Algae

Members of this large and diverse group of organisms, called **green algae**, are principally freshwater species. They are also found in seawater, and many of them are terrestrial. The cells of the *Chlorophycophyta* have a well-defined nucleus and, usually, a cell wall, and the chlorophyll and other pigments are in chloroplasts, as in higher plants. The majority of green algae contain one chloroplast per cell. It may be laminate, cup-shaped, or reticulate. The chloroplasts also often contain dense regions called **pyrenoids**, on which surface starch granules are formed. Food reserves are stored as starch, a product of photosynthesis.

There are many single-celled forms and many colonial types of green algae. Many unicellular green algae are motile by flagella action. Colonial types occur as spheres, filaments, or plates. Some species have special structures called holdfasts, which anchor them to submerged objects or aquatic plants.

Chlorophycophyta reproduce by zoospores, fission, and other asexual methods or by isogamous and heterogamous sexual means. From the evolutionary standpoint, they represent not only the primitive plants but intermediate forms progressing to those which reproduce by advanced heterogamy involving the union of differentiated sex cells, eggs, and sperm.

Chlamydomonas is considered a typical green alga (Fig. 18-11). It is a typical unicellular, motile, green alga and is widely distributed in soils and freshwater. Cell organization is shown in Fig. 18-12. It varies from 3 to 29 μm in common forms and is motile except during cell division. Motility is by means of two

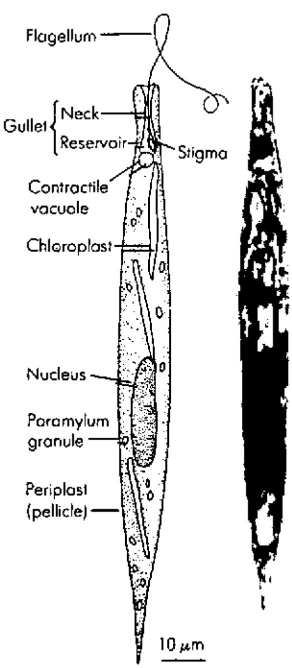


Figure 18-10. Left: Schematic representation of a euglenoid; right: *Euglena acus*. (*Courtesy of Carolina Biological Supply Company.*)

Figure 18-11. *Chlamydomonas* in vegetative and palmelloid state. Usually the cells in the palmelloid state are nonflagellated and are embedded in a gelatinous matrix. Flagella reappear and the cells swim away when favorable conditions return (X1,500). (*Courtesy of R. G. Kessel and C. Y. Shih, Scanning Electron Microscopy in Biology, Springer-Verlag, Berlin, 1974.*)

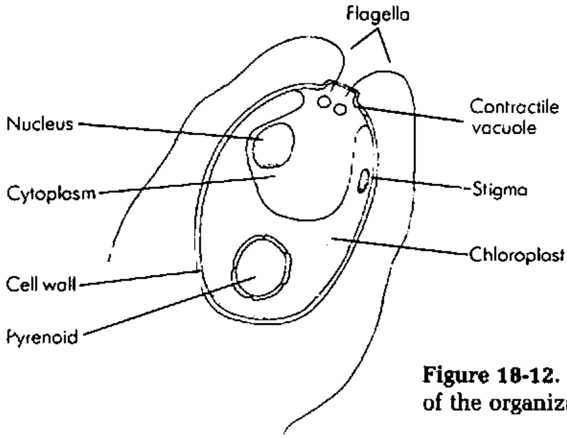


Figure 18-12. Diagrammatic representation of the organization of *Chlamydomonas* sp.

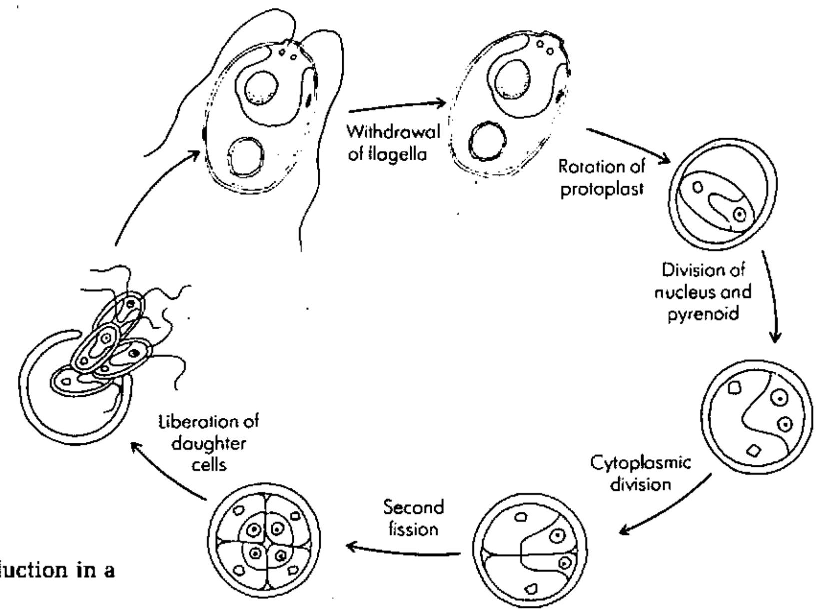


Figure 18-13. Asexual reproduction in a species of *Chlamydomonas*.

flagella. Each cell has one nucleus and a single large chloroplast that in most species is cup-shaped, although in some it may be star-shaped or layered. The cell wall contains cellulose; in many species an external gelatinous layer is also present. There is some evidence that the red eyespot or stigma in the chloroplast is the site of light perception.

In asexual reproduction the free-swimming individual becomes nonmotile by withdrawing its flagella and undergoes longitudinal fission of the protoplast to form two, four, or eight daughter protoplasts. The daughter cells develop two flagella each and construct new cell walls. They are then liberated from the parent cell wall. This cycle may be repeated indefinitely in laboratory culture or in nature (see Fig. 18-13).

In some cases, the daughter cells do not develop flagella and escape. Instead, they keep on multiplying within a more or less gelatinized matrix. The masses of cells so formed are called **palmelloid** stages (Fig. 18-11). The formation of such stages is determined by environmental conditions which are generally favorable to growth but not to motility. Any individual cell, however, can develop flagella and escape from the mass. Palmelloid stages occur in many eucaryotic algae as predominant or as occasional phases of development.

Under certain conditions, sexual reproduction occurs in many species of *Chlamydomonas*, by isogamy, heterogamy, or oogamy. The first is by far the most common. In each type the diploid zygote remains dormant for some time. Meiosis occurs as the zygote germinates. Typically, four or eight motile haploid cells are released. One of the conditions for sexual reproduction is the compatibility of mating types. When cells of compatible-mating types are present, they aggregate. From these aggregates, pairs of gametes emerge and unite to form zygotes.

In addition to motile unicellular algae like *Chlamydomonas*, other, nonmotile unicellular green algae are widely distributed. One of the most important of these is *Chlorella*, which has served as a useful tool in many investigations on photosynthesis and supplemental food supply.

Volvox is a colonial green alga which may form water blooms. Its colonies are visible to the naked eye. Each colony contains from 500 to thousands of cells arranged at the surface of a watery colloidal matrix. The individual cells are biflagellate and are morphologically similar to that of *Chlamydomonas* (see Fig. 18-3C).

Figure 18-14. *Acetabularia* sp., the mermaid's wine goblet (X0.75). (*Courtesy of General Biological Supply House.*)

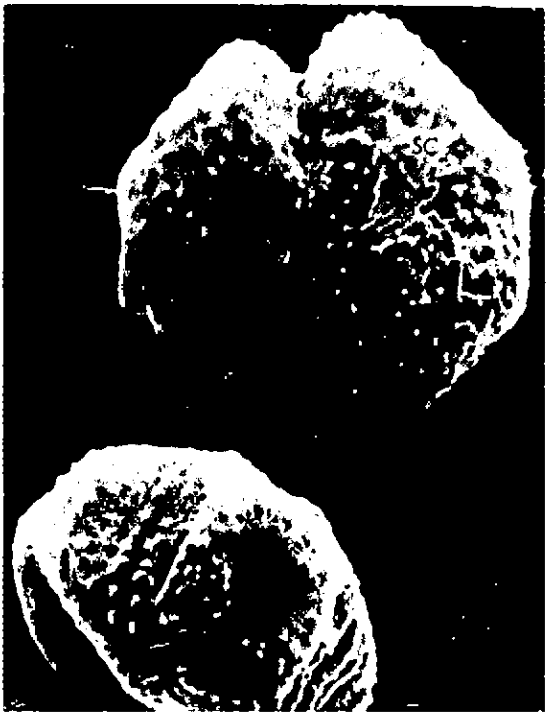


Figure 18-15. Scanning electron micrograph of a desmid, *Cosmarium* sp. The cells are uninucleate and composed of two halves with a constriction in the middle of cells. A single nucleus lies embedded in the middle of the cytoplasm that separates the two chloroplasts into semicells (SC) (X800). (*Courtesy of R. G. Kessel and C. Y. Shih, Scanning Electron Microscopy in Biology, Springer-Verlag, Berlin, 1974.*)

Acetabularia is one of the most interesting radially symmetrical forms. Figure 18-14 shows why it is called the mermaid's wine goblet.

Desmids (Fig. 18-15) are interesting green algae found in a wide variety of attractive shapes and designs. Each cell is made up of two symmetrical halves (semicells) containing one or more chloroplasts. In some, the semicells are joined by an isthmus where the nucleus lies.

Ulothrix is a filamentous form (Fig. 18-16) found in flowing streams, attached to twigs or stones by holdfasts at the bases of the filaments. It reproduces asexually by means of flagellated zoospores, which are produced in pairs or in multiples of 2 up to 16 per cell. Each zoospore may become attached to a solid object in the water and develop into a filament. Sexual reproduction may also occur, in which 32 or 64 biflagellate (with two flagella) isogametes are produced.

Figure 18-16. *Ulothrix*. Segments of filaments are shown. (*Courtesy of Carolina Biological Supply Company.*)

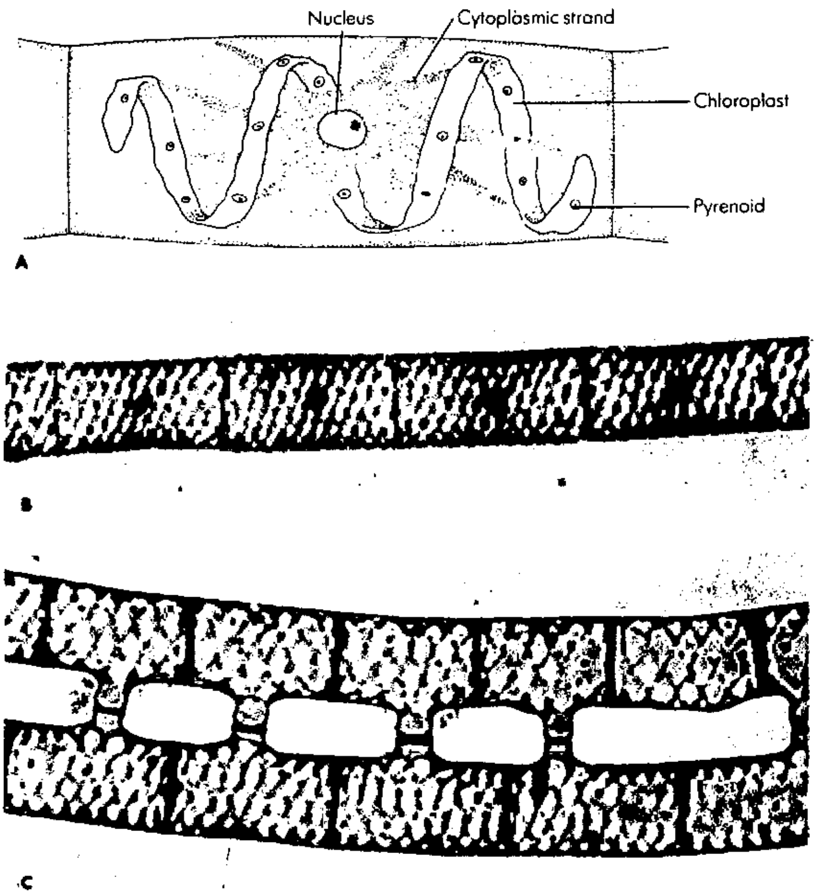


Figure 18-17. *Spirogyra* (A) Diagram of a vegetative cell. (B) A vegetative filament. (C) Conjugation. Formation of fertilization tubes between conjugating filaments. (*Courtesy of Carolina Biological Supply Company.*)

By fusion of a pair of gametes a zygote is formed, which germinates to form four zoospores; these then attach to a stone or another object in the water and develop into filaments.

A very common green alga is *Spirogyra* (Fig. 18-17), a filamentous form seen in the scums that cover ponds and slow-moving water. It is of interest because of its common occurrence and its possession of unusual chloroplasts, which are arranged spirally.

Cryptophycophyta— The Cryptomonads

The cryptomonads are a small group of biflagellate organisms. They have two unequal flagella, which arise from the base of a groove; both are of the tinsel type, with stiff hairs. The cells are slipper-shaped, are flattened into a dorsal-ventral plane, and occur singly. Some forms have a cellulose wall while others are naked, being surrounded only by a plasmalemma with a thin granular material on the outside. There are one or two plastids, with or without pyrenoids, per cell. Food reserve is stored as a true starch as well as oil. Reproduction is either by means of longitudinal cell division or the formation of zoospores or cysts. Sexual reproduction has been confirmed in the genus *Cryptomonas*. A

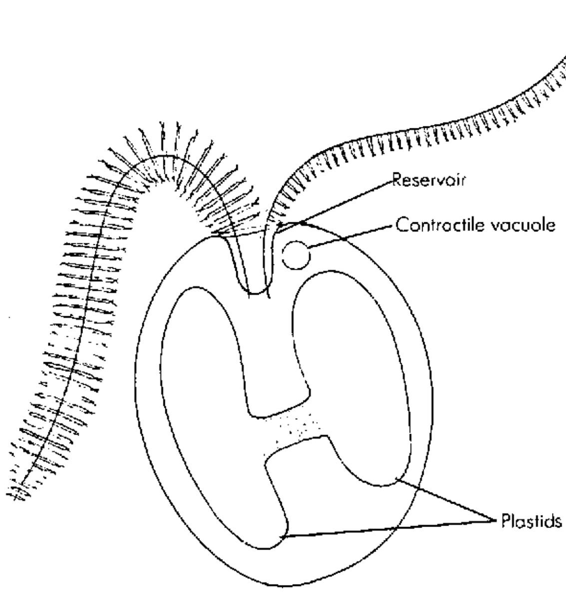


Figure 18-18. *Cryptomonas* cell. It has a flattened oval shape with two flagella coming from the anterior reservoir. *(Erwin F. Lessel, illustrator)*

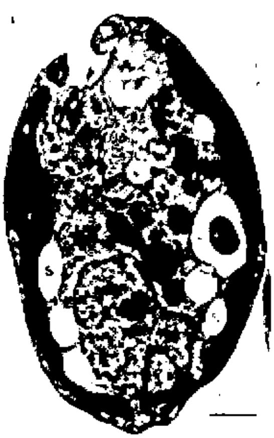


Figure 18-19. Longitudinal section of a cell of *Cryptomonas*. The periplastidal compartment which surrounds the chloroplast (C) contains starch (S), ribosomes, dense globules (arrows), and a nucleomorph (Nm) or nucleus. The chloroplast endoplasmic reticulum (CER) is continuous at places with the nuclear envelope (stars). Other structures seen are the Golgi (Go), pyrenoid (Py), and basal bodies (Bb). Bar represents 1 um. *(Courtesy of Sarah P. Gibbs, McGill University. From L. McKerracher and Sarah P. Gibbs, "Cell and Nucleomorph Division in the Alga Cryptomonas," Can J Botany, 60:2440–2452, 1982.)*

Cryptomonas cell is shown in Fig. 18-18. The fine structure of a cryptomonad cell is shown in Fig. 18-19.

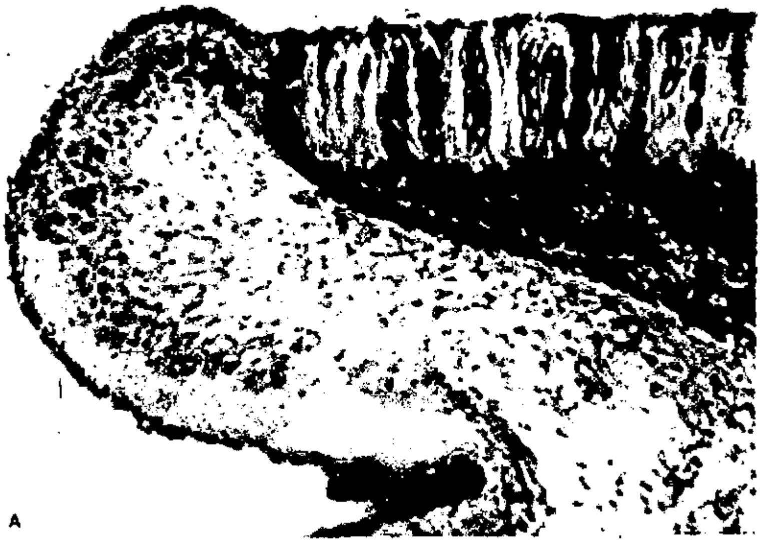
Pyrrophycophyta—The Dinoflagellates

This division includes the dinoflagellates, a diverse group of biflagellated unicellular organisms. The dinoflagellates are so named because of their twirling motion rather than their morphology. These organisms constitute an important component of marine, brackish, and fresh bodies of water. This is another group of organisms that has both plantlike and animallike characteristics. The cells are typically flattened and have a transverse constriction, the girdle, usually around the cell equator. Distinctive features of dinoflagellates are that the flagella are inserted in the girdle and that the flagella are arranged with one encircling the cell and one trailing. Hairs project from the flagellar surface. Dinoflagellates can move a distance 100 times their own length each second! Many dinoflagellates are covered only by a plasmalemma (e.g., *Gymnodinium* is a naked organism). In some forms there is a wall made of cellulose. Still others have a series of cellulose plates within the plasmalemma. These are termed thecal plates and dinoflagellates with them are said to be armored. Dinoflagellates are an important constituent of plankton. They are best known as the organisms that produce "red tides," or blooms in which the concentration of cells may be so great as to color large areas of the ocean red, brown, or yellow. Such an organism is *Gonyaulax*. Other marine dinoflagellates such as *Noctiluca* are luminescent. Asexual reproduction takes place through division of the cell. Sexual reproduction is occasionally observed. For example, a single *Noctiluca* cell reportedly can form uniflagellate isogametes that can fuse to form a zygote, which develops directly into a *Noctiluca*.

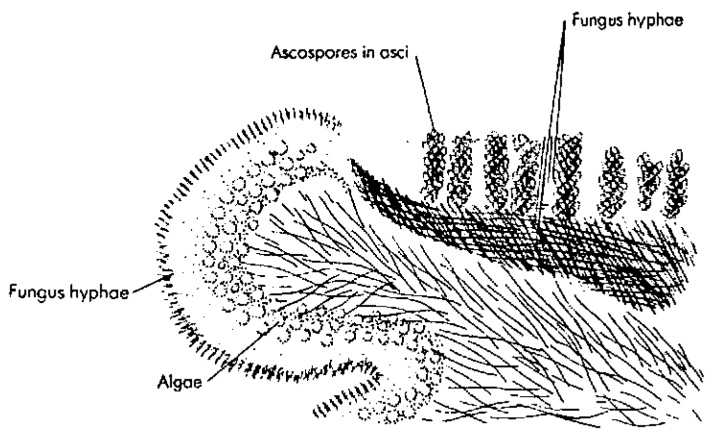
LICHENS

Lichens are composite organisms consisting of algae or cyanobacteria living in association with fungi. The name derives from the Greek meaning "scaly or leprous," which aptly describes the appearance of many species in nature. They grow on rocks (Fig. 18-20), tree bark, and other substrates generally unsuitable for the growth of other plants. Many lichens are able to grow at the low temperatures found at high altitudes and in polar environments. Reindeer mosses

Figure 18-20. A crustose (flat, appressed) lichen, *Lasillia papulosa*, growing on a rock. (*Courtesy of Alvin R. Grove.*)



A



B

Figure 18-21. The structure of lichens is suggested by the two parts of this illustration. (A) A portion of a vertical section through a foliose lichen thallus, *Physica* sp. (X150). (From *General Biological Supply House.*) (B) Detail of algal and fungal relationships in a foliose lichen.

are lichens which furnish forage and fodder for herbivorous animals in the Arctic regions; they may cover many kilometers of land with ankle-deep growth. The colors of lichens range from white to black through shades of red, orange, yellow, and green.

Morphology

Morphologically, a simple lichen is made up of a top layer of tightly woven fungal mycelia, below this a layer of photosynthetic cells, and below that another layer of fungus (Fig. 18-21). The bottom layer may attach to the substrate directly or by means of short twisted strands of hyphae, called rhizines, which serve as anchors. Some lichens are more complex than this. For example, in some a midlayer of fungus directly below the alga appears to be a reservoir for stored food.

Not all species of algae (or cyanobacteria) or all species of fungi can enter into a lichenlike relationship. Most lichen fungi are *Ascomycetes*, although a few are *Basidiomycetes*. The algae belong to division *Chlorophycophyta* (green algae). Lichen fungi are unique in their preference for growth in association with algae or cyanobacteria. Typically, each lichen thallus consists of a single species of fungus associated with a single species of alga or cyanobacterium. About 30 different genera of algae and cyanobacteria have been found in lichens. The commonest is the unicellular green alga *Trebouxia*. The other two most common genera are *Trentepohlia* (a filamentous alga) and *Nostoc* (a cyanobacterium). These three genera account for the photosynthetic partner in over 90 percent of all lichen species. However, each lichen species does not necessarily have a specific photosynthetic partner. For example, one *Calothrix* species is found in *Lichina confinis* in Norway or Spain, but another photosynthetic species is found in *L. confinis* in Sweden.

The assignment of a single Latin binomial to an "organism" consisting of two different microorganisms raises obvious difficulties. Consequently, virtually all the morphological characteristics now used in lichen classification are fungal; and it is acceptable to speak of a "lichenized fungus," with the Latin name referring to the fungus. About 18,000 species of lichens have been described.

There are three kinds of lichens: **crustose** (flat, appressed); **foliose** (leaflike); and **fruticose** (shrublike). Crustose lichens grow closely appressed to the substrate (rock, wood, etc.), or even within its surface. Foliose lichens are flattened like leaves but may not be connected to the substrate at all points. Fruticose lichens have an erect shrublike or filamentous morphology and can be about 10 cm high. (See Figs. 18-20 and 18-22.)

Reproduction

Lichens reproduce predominantly by vegetative processes. Propagation by fragmentation occurs when bits of the thallus are broken off from the parent plant and fall on a suitable substrate. Lichens may produce asexual "reproductive bodies" called **soredia** which are knots of hyphae containing a few algal cells. During sexual reproduction, the components of lichens reproduce independently of each other. The fungal components produce ascospores, and when they germinate they must come in contact with algal cells or they will not be able to survive. The algal partner reproduces by cell division or occasionally by spores. Lichens grow slowly because of their low metabolic rate. Many species grow less than a centimeter per year. Some lichens have therefore reached great age in nature, for example, 4,500 years for some species in the Arctic regions.

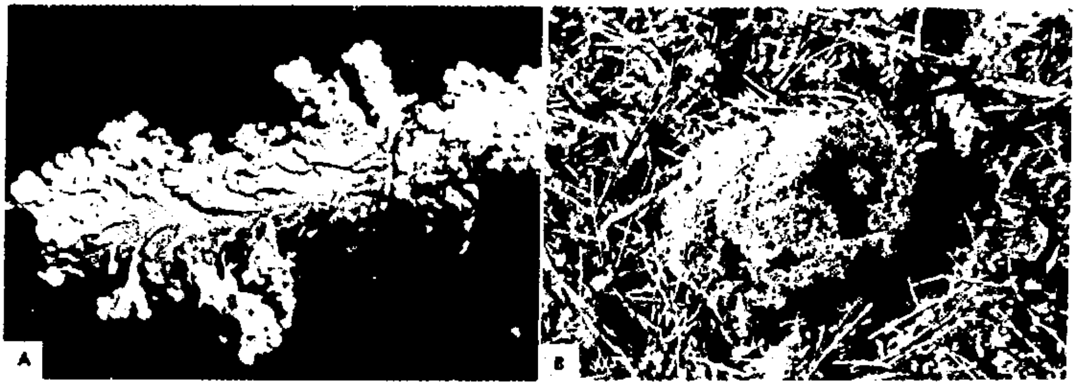


Figure 18-22. Examples of two types of lichens. (A) foliose lichen; (B) fruticose lichen. (*Courtesy of Carolina Biological Supply Company.*)

Such longevity suggests a very well-balanced association between the symbionts. They are also very resistant to heat and desiccation.

Symbiotic Nature

It is generally agreed that lichens are the product of a mutualistic symbiosis, in which each partner of the association derives something essential or useful for its survival. Apparently the alga or cyanobacterium provides the fungus with food, particularly carbohydrates produced by photosynthesis, and possibly with vitamins. The fungus probably absorbs, stores, and supplies water and minerals required by the alga or cyanobacterium, as well as providing carbon dioxide (a needed growth factor) and a moist, sheltered supporting framework for the photosynthetic partner. The fungus is able to obtain nourishment from its partner by means of tiny rootlike projections called **haustoria** (singular, **haustorium**) which penetrate the photosynthetic cell.

Chemical Interaction

The chemical interaction of lichens is interesting because many of their organic products ("lichen substances") are unique. Unusual fats and phenolic compounds make up from 2 to 20 percent of the dry weight of lichen bodies. Litmus, the well-known pigment indicator, is obtained from lichens. Essential oils from some species are used in perfumes. Lichen pigments are used in England to color many of the woolen fabrics of Harris tweeds. Interest has grown in lichens as a source of new chemical substances that show promise for a variety of applications.

QUESTIONS

1 How are algae similar to and different from higher green plants?
2 How would you distinguish unicellular algae from photosynthetic bacteria?
3 Where are algae found?
4 Why are algae important in the food chain of aquatic environments?
5 Discuss the various uses of algae that make them commercially important.
6 Describe some of the algae used as food for human consumption.
7 Explain the etiology of shellfish poisoning.
8 Discuss the characteristics of algae that are used as a basis for algal classification.
9 What is diatomaceous earth? Why is it used commercially?
10 What are the attributes *Euglena* shares with plants and with animals?
11 Describe briefly the life history of *Chlamydomonas*.
12 Why are dinoflagellates of medical concern?
13 Describe the microbial association that exists in a lichen.

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Chapter 19 Protozoa

Protozoa (singular, **protozoan**), from the Greek *protos* and *zoon*, meaning "first animal," are eucaryotic protists. They occur generally as single cells and may be distinguished from other eucaryotic protists by their ability to move at some stage of their life cycle and by their lack of cell walls. Protozoa are predominantly microscopic in size. The majority are between 5 and 250 μm in diameter. Colonies of protozoa also occur. In a protozoan colony, the individual cells are joined by cytoplasmic threads or are embedded in a common matrix. Thus colonies of protozoa are essentially aggregates of independent cells. The study of these eucaryotic protists is called **protozoology.**

By a conservative estimate, there are more than 65,000 described species of protozoa distributed among seven named phyla. (In 1964, there were about 48,000 members of what was then known as a single phylum *Protozoa*.) Slightly more than 50 percent of the species are fossil forms. Of the remaining 50 percent, some 22,000 are free-living species while 10,000 species are parasitic. Of the latter, only a few species cause disease in humans, but these few inflict much misery and death on millions of people. Even though the present species count appears staggering, there are hundreds of thousands of species yet to be described (even in 1983, an average of two new protozoan species were discovered daily).

OCCURRENCE OF PROTOZOA

Protozoa are found in all moist habitats. They are common in the sea, in soil, and in freshwater. Free-living protozoa have even been found in the polar regions and at very high altitudes. Parasitic protozoa may be found in association with most animal groups. Many protozoa survive dry conditions by the formation of a resistant **cyst**, or dormant stage. For example, the soil amoeba *Naegleria* is a resistant cyst in dry weather, is a naked amoeba in moist soil, and becomes flagellated when flooded with water. Parasitic protozoa can modify their morphology and physiology to cope with a change in host. For example, the malarial parasite *Plasmodium* produces male gametes in response to a drop in temperature on transfer from a warm-blooded mammalian host to a mosquito.

The distribution of **trophic** (vegetative) forms of protozoa in the sea and freshwater and of cyst forms in the atmosphere has resulted in the spread of free-living species throughout the world.

ECOLOGY OF PROTOZOA

From the ecological standpoint, protozoa may be divided into free-living forms and those living on or in other organisms. The latter group is referred to as the **symbiotic** protozoa. Some of the symbiotic ones are **parasitic** and may cause disease. Others such as those found in the gut of the termite are beneficial to the host (live in a **mutualistic** association).

Free-Living Protozoa

Free-living protozoa are found in a variety of habitats. The factors which influence the distribution and number of free-living protozoa in a habitat are moisture, temperature, light, available nutrients, and other physical and chemical conditions. The vegetative, or trophic stages of free-living protozoa occur in every type of salt water, freshwater, sand, soil, and decaying organic matter.

Light

Obviously, for those protozoa which bear chromatophores (these protozoa are considered algae by phycologists) and carry out photosynthesis, sunlight is essential. It follows also that those protozoa which feed on photosynthetic microorganisms also require sunlight, albeit indirectly. On the other hand, some protozoa avoid light and thrive in a environment where it is absent.

Hydrogen-Ion Concentration

Some protozoa can tolerate a wide range of pH, for example, pH 3.2 to 8.7. However, for the majority a pH range of 6.0 to 8.0 is optimal for maximum metabolic activity.

Nutrients

The protozoan population in an aquatic environment is influenced by the chemical constituents of the water. Some protozoa thrive in water rich in oxygen but low in organic matter (mountain springs, brooks, or ponds); others require water rich in minerals. Some grow in water where there is active oxidation and degradation of organic matter (the majority of freshwater protozoa, such as the ciliates). Still others prefer water with little oxygen but many decomposition products (e.g., black bottom slime and sewage). Some species have been found to live in both salt water and freshwater.

The nutrient supply in a habitat is a major determining factor in the distribution and number of protozoa within it. Species of *Paramecium* and other **holozoic** protozoa (protozoa that eat other organisms) must have a supply of

Figure 19-1. A scanning electron micrograph of a paramecium (P) being ingested by a *Didinium* (D). Note that the cilia on the paramecium are being lysed as the paramecium enters the cytopharynx of the predator. The paramecium is about twice the size of the *Didinium*. But it is gulped down as shown, a situation comparable to a human grabbing a cow and swallowing it whole! The bar represents 10 μm. (Courtesy *of Eugene B. Small and Donald S. Marszalek, Science,* 63:1064–1065, 1969. *Copyright 1969 by the American Association for the Advancement of Science.*)

bacteria or other protozoa (Fig. 19-1). As a general rule, holozoic protozoa which feed on a variety of organisms are widely distributed; those that are more selective and feed only a few species are limited in their distribution.

Temperature

Most protozoa have an optimum temperature of between 16 and 25°C; the maximum is between 36 and 40°C. The minimum temperature is less detrimental. The temperature tolerance varies with different environmental conditions. Even warm waters (30 to 56°C) of hot springs have been known to contain protozoa. The so-called red snow of high altitudes is due to the presence of several hematochrome-bearing flagellates (considered algae by some biologists). In the **encysted** stage (a thick-walled structure in an inactive stage), protozoa can withstand a far greater temperature variation than in the trophic stage.

Symbiotic Protozoa

The association between these protozoa and their hosts or other organisms can differ in various ways. The term *symbiotic* describes any type of coexistence between different organisms.

In *commensalism* the host is neither injured nor benefited, but the commensal is benefited. **Ectocommensalism** is often represented by protozoa which attach themselves to a host's body. **Endocommensalism** is the association when the protozoan is inside the host's body, e.g., the protozoa which live in the lumen of the alimentary tract.

Mutualism occurs between some protozoa and their hosts. For example, certain flagellates are present in the gut of termites and digest the woody material eaten by the termite to a glycogenous substance which can be used by the host cells. If deprived of these flagellates, the termite dies; if the flagellates are removed from the termite gut, they too perish.

In parasitism, one organism—the parasite—lives at the expence of the other. The parasite feeds on the host cells or cell fragments by pseudopodia or cytostome (an opening for ingestion of food; see Fig. 19-4), or enters the host tissues and cells, living upon the cytoplasm and even the nuclei. As a result, the host may develop pathological conditions. The sporozoa are strictly parasitic and are among the most important of the disease-producing protozoa.

Some parasitic protozoa parasitize other protozoan or **metazoan** (animals whose bodies consist of many cells) parasites. Such an association is termed **hyperparasitism**.

THE IMPORTANCE OF PROTOZOA

Protozoa serve as an important link in the **food chain** of communities in aquatic environments (see Chap. 26). For example, in marine waters. zooplankton (animallike organisms) are protozoa that feed on the photosynthetic phytoplankton (plantlike organisms). They in turn become food for larger marine organisms. This food chain can be represented as follows:

Light energy → Phytoplankton (Primary producers) → Zooplankton (Primary consumers) → Carnivores (Secondary consumers)

Also of particular importance in the ecological balance of many communities, in wetlands as well as aquatic environments, are the saprophytic and bacteria-feeding protozoa. They make use of the substances produced and organisms involved in the final decomposition stage of organic matter. This can be represented by the following sequence:

Dead bodies of producers and consumers, and their excretion products, including feces → Decomposition by fungi and bacteria → Ingestion of bacteria by protozoa

It follows that microorganisms are important in the degradation of sewage. Although bacteria participate in a prominent way in the process, the role of protozoa is becoming more completely understood and appreciated. Biological sewage treatment involves both anaerobic digestion and/or aeration (see Chap. 27). Anaerobic protozoa such as species of *Metopus*, *Saprodinium*, and *Epalxis* are active in the anaerobic steps, while those treatment steps requiring aeration and flocculation include the aerobic protozoa such as *Bodo*, *Paramecium*, *Aspidisca*, and *Vorticella*.

In the treatment of industrial wastes, where there is an accumulation of nitrates and phosphates, the settling tanks are illuminated to promote the growth of algae and protozoa. These protists remove the inorganic material from the water for their own synthesis. Water quality is improved, and the autotrophs are skimmed from the water surface, dried, and used as fertilizer.

Some protozoa cause disease in animals, including humans. They have caused untold misery. Such parasitic protozoa multiply within the host much as bacteria do. Some live only as **obligate parasites** and may produce chronic or acute diseases in humans. Some well-known protozoan diseases in humans are intestinal amoebiasis, African sleeping sickness, and malaria (see Chap. 38).

Protozoa have also become important research organisms for biologists and biochemists for the following reasons. Many protozoa are easily cultured and maintained in the laboratory. Their capacity to reproduce asexually enables **clones** to be established with the same genetic makeup.

Studies of mating types and killer particles in *Paramecium* have shown a relationship between genotype and the maintenance of cytoplasmic inclusions and endosymbionts. *Tetrahymena, Euplotes,* and *Paramecium* species have been used to study cell cycles and nucleic acid biosynthesis during cell division.

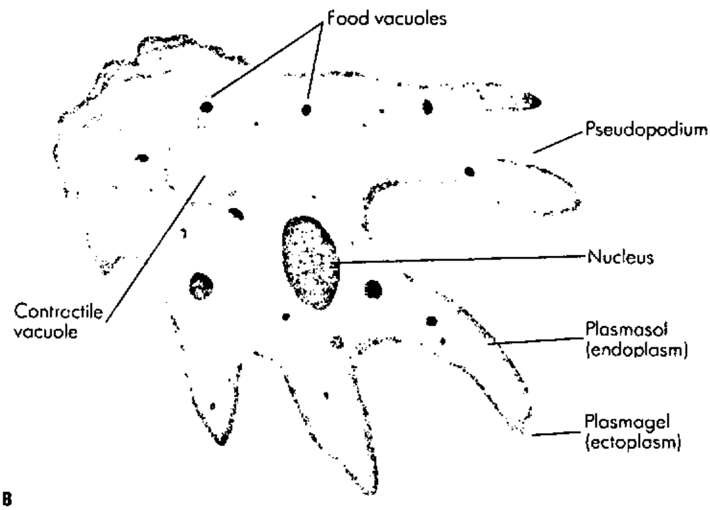
MORPHOLOGY OF PROTOZOA

The size and shape of these organisms show considerable variation. For example, *Leishmania donovani*, the cause of the human disease kala azar, measures 1 to 4 μm in length. *Amoeba proteus* measures 600 μm or more (Fig. 19-2). Certain common ciliates reach 2,000 μm, or 2 mm, and the **tests** (a kind of protective envelope) of some extinct (fossilized) members of *Foraminiferida*, the nummulites, measure up to 15 cm in diameter.

Intracellular Structures

Like all eucaryotic cells, the protozoan cell also consists of cytoplasm, separated

A



B

Figure 19.2. (A) *Amoeba proteus* photographed with a scanning electron microscope. By this technique the pseudopodia and other structures of the cell are remarkably clear. The bar represents 10 μm. (*Courtesy of Eugene B. Small and Donald S. Marszalek, Science,* **63**:*1064–1065, 1969. Copyright 1969 by the American Association for the Advancement of Science. By permission.*) (B) Anatomy of *Amoeba proteus*. (*Erwin F. Lessel, illustrator.*)

from the surrounding medium by a special cell envelope, and the nucleus or nuclei.

Cytoplasm

The cytoplasm is a more or less homogeneous substance consisting of globular protein molecules loosely linked together to form a three-dimensional molecular framework. Embedded within it are the various structures that give protozoan cells their characteristic appearance.

Submicroscopic protein fibrils (**fibrillar bundles, myonemes,** and **microtubules**) are groups of parallel fibrils in the cytoplasm. Protozoan contractility is probably due to these fibrils.

In several forms of protozoa, pigments are diffused throughout the cytoplasm. The hues are numerous; e.g., they can be green, brown, blue, purple, or rose.

In the majority of protozoa, the cytoplasm is differentiated into the **ectoplasm** and the **endoplasm**. The ectoplasm is more gel-like and the endoplasm is more voluminous and fluid, but the change from one layer to another is gradual. Structures are predominantly found in the endoplasm (see Fig. 19-2).

Like other eucaryotic cells, protozoa have membrane systems in the cytoplasm. They form a more or less continuous network of canals and lacunae giving rise to the **endoplasmic reticulum** of the cell. Other structures in the cytoplasm include ribosomes, Golgi complexes or **dictyosomes** (piles of membranous sacs), mitochrondria, **kinetosomes** or **blepharoplasts** (intracytoplasmic basal bodies of cilia or flagella), food vacuoles, contractile vacuoles, and nuclei (Fig. 19-2).

Nucleus

The protozoan cell has at least one eucaryotic nucleus (Fig. 19-3). Many protozoa, however, have multiple nuclei (e.g., almost all ciliates) throughout the greater part of the life cycle. The protozoan nuclei are of various forms, sizes, and structures. In several species, each individual organism has two similar nuclei. In the ciliates, two dissimilar nuclei, one large (macronucleus) and one small (micronucleus), are present (Fig. 19-4). The macronucleus controls the metabolic activities and regeneration processes; the micronucleus is concerned with reproductive activity.

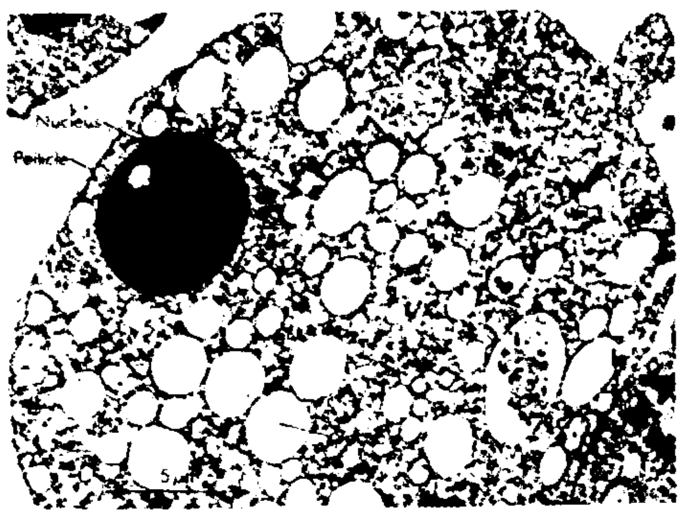
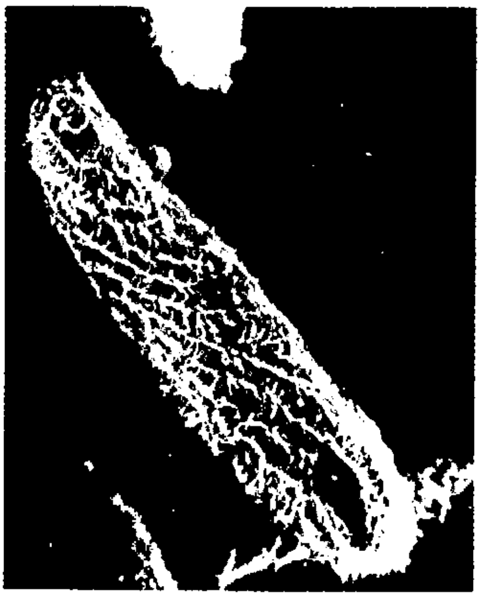


Figure 19-3. Section of the amoeba *Entamoeba histolytica.* (*Courtesy of Z. Ali-Khan and Margaret Gomersall, McGill University.*)



A

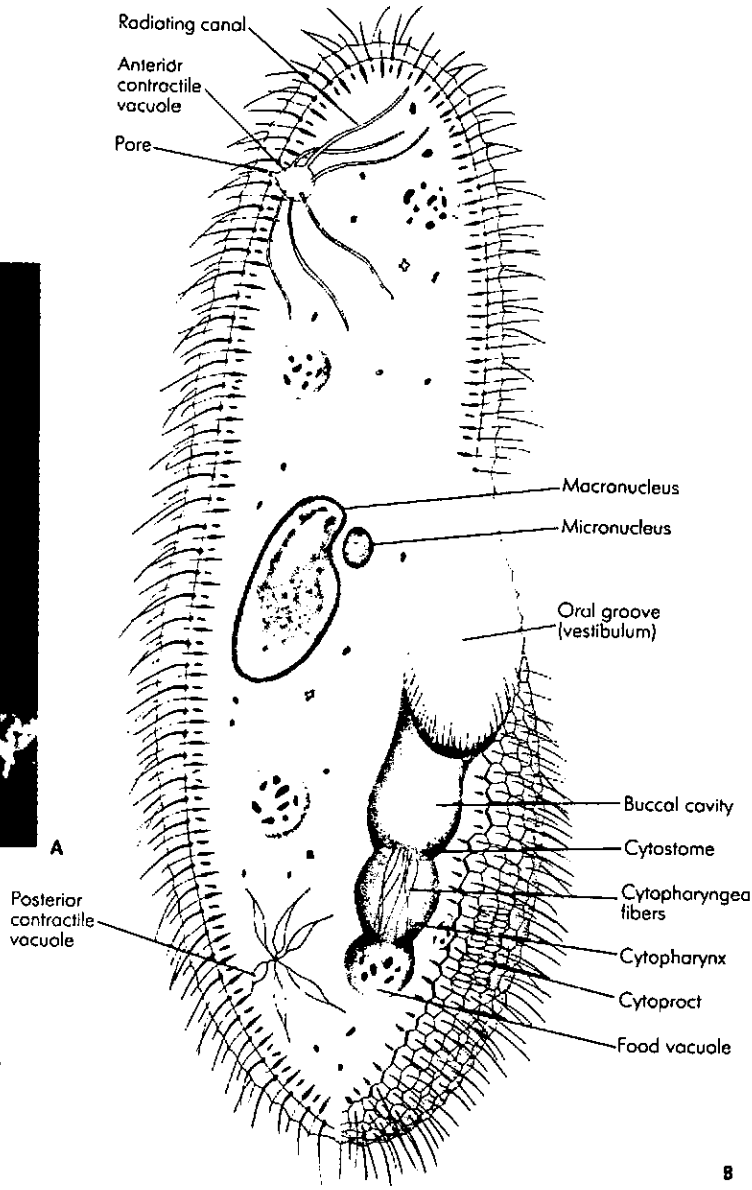


Figure 19-4. (A) Ciliary beat of *Paramecium bursaria*. The ciliary rows on the dorsal surface are illustrated. Note that by special techniques the pattern of ciliary coordination and the form of ciliary beat have been preserved for scanning electron microscopy (X457). (*Courtesy of R. G. Kessel and C. Y. Shih, Scanning Electron Microscopy in Biology, Springer-Verlag, Berlin, 1974.*) (B) Anatomy of *Paramecium*. (*Erwin F. Lessel, illustrator.*)

The essential structural elements of the nucleus are the chromosomes, the nucleolar substance, the nuclear membrane, and the karyoplasm (nucleoplasm). It has been shown that the number of chromosomes is constant for a particular species of protozoan. For example, *Spirotrichonympha polygyra* has 2 haploid chromosomes; *Spirotrichosoma magnum* has 60. Some protozoa divide only asexually (by mitosis). Others may divide either asexually or sexually (by meiosis).

Plasmalemma and Other Cell Coverings

The cytoplasm with its various structures is separated from the external environment by a cell unit membrane (**plasmalemma**). The plasmalemma not only provides protection but also controls exchange of substances (semipermeable); it is the site of perception of chemical and mechanical stimuli as well as the establishment of contact with other cells (cell sensitivity to external factors). Although all protozoa possess a cell membrane, many protozoa have compound coverings of membranes modified for protection, support, and movement. Such combinations of membranes are referred to as a **pellicle.** In *Euglena* the pellicle is organized to ensure flexibility; in *Paramecium* it is quite rigid.

Actually, in its simplest form, the pellicle is the plasmalemma itself, e.g., amoebas are surrounded by a plasmalemma only. However, even in these, some species (e.g., *A. proteus*) have a diffuse layer of mucopolysaccharides over the plasmalemma. This layer is thought to play an important role in **pinocytosis** (uptake of fluids and soluble nutrients through small invaginations in the cell membrane that subsequently form intracellular vesicles) or in adhesion of the cell to the substratum.

The pellicle of a ciliate is thick and often variously ridged and sculptured. There may even be rows of elevated platelets and nodular thickenings. For example, the pellicle of *Paramecium* consists of three membranes, the outer one sculptured in a series of evenly distributed polygons. The contact between adjacent polygons results in a series of ridges, giving a latticed pattern.

Additional protective coverings external to the pellicle have also evolved for some protozoa. This results in the great diversity of forms exhibited by protozoa. These coverings are known variously as **thecae, shells, tests,** or **loricae** and occur in almost all major groups of protozoa. The theca is a secreted layer directly apposed to the cell surface. Tests, shells, and lorica are coverings that are loose-fitting. Special openings provide the connection with the environment. The coverings consist of very different materials. In general, they have an organic matrix reinforced by incrustation of inorganic substances such as calcium carbonate or silica.

We are familiar with mountain ranges or geological deposits of limestone, fusuline chalk, and green sandstone. These were formed by continuous sinking of calcareous shells and silicon skeletons of planktonic amoebas and other protozoa to the bottom of ancient oceans. For example, the white cliffs of Dover are made up of billions of scales of the phytoflagellates called coccolithopharids plus the shells of millions of foraminiferans.

Feeding Structures

Food-gathering structures in the protozoa are diverse and range from the pseudopodia of amoebas through the tentacular feeding tubes of suctorians to the well-developed "mouths" of many ciliates. Amoebas gather food by means of pseudopodial engulfment. In ciliates the **cytostome** is the actual opening through which food is ingested (Fig. 19-5). It ranges from a simple round opening to a slitlike structure surrounded by feeding membranelles. It is usually found anteriorly and remains open all the time in some groups; in other species it can be opened and closed.

An **oral groove** (Fig. 19-4B) is an indentation in the pellicle of certain ciliates. It guides food toward the cytostome and acts as a concentrating device. The addition of membranelles to the oral groove makes it a **peristome** (Fig. 19-5).

Figure 19-5. Ventral surface of a *Euplotes aediculatus* cell. (A) Cirri. Each cirrus is composed of 80 to 100 individual cilia that are not fused but beat as a functional unit in locomotion. (B) Cilia. Two to three long rows of cilia that function in locomotion as well as in food collection. (C) Peristome (buccal cavity). (D) Cytostome (cell "mouth") (X512). (*Courtesy of John A. Kloetzel, University of Maryland, Baltimore County.*)

On its edges are located cilia that function to facilitate feeding. The **cytopharynx** is a region through which nutrients must pass to be enclosed in a food vacuole.

Cysts

Many protozoa form resistant cysts at certain times of their life cycle. As indicated before, these cysts are able to survive adverse environmental conditions such as desiccation, low nutrient supply, and even anaerobiosis. In parasitic protozoa, the developmental stages are often transmitted from host to host within a cyst. Other kinds of cysts (e.g., reproductive) are also known.

Intestinal protozoa comprise about 50 percent of the parasitic species. In most instances they enter the alimentary tract as resting cysts, hatch in a suitable region, and then leave the host again as dormant cysts. By this means they can survive for long periods outside the host. Asexual reproduction in some ciliates and flagellates is associated with cyst formation. Sexual reproduction of sporozoa invariably results in a cyst.

The cyst wall is secreted as a closely fitting extracellular coat or structure. The cytoplasm is commonly attached to the cyst wall at one or several points; it is reduced in size and dormant.

Other Protective Structures

Some protozoa protect themselves with structures other than external coverings. These include various structures or materials formed within membrane-bound vesicles. For example, certain ciliates secrete a mucilage from subpellicular vesicles called **mucocysts**. Several protozoa probably defend themselves by the expulsion of harpoonlike **trichocysts**, although their function has not been actually proved. Other protozoa, such as *Didinium* sp. (Fig. 19-1), have
These have a threadlike tubular structure with an occlusion at the distal end which may contain toxin. When the toxicyst is discharged the toxin is distributed along the surface of the thread. Toxicysts are used to paralyze and capture prey; the toxin causes paralysis and cytolysis when it contacts protozoan prey.

Figure 19-6. *Crithidia* sp., a flagellate, at two magnifications. Bar represents 10 μm. (*Courtesy of Z. Ali-Khan and E. C. S. Chan, McGill University.*)

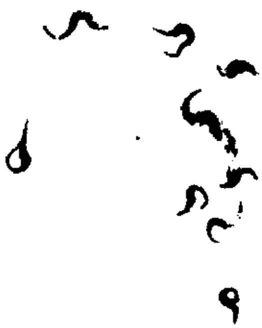


Figure 19-7. *Trypanosoma brucei* exhibiting a flagellum with undulating membrane on each cell. Giemsa stain (X1,560). (*Courtesy of P. Gardiner, International Laboratory for Research on Animal Diseases, Nairobi, Kenya, and the Society of Protozoologists. From P. R. Gardiner, L. C. Lamont, T. W. Jones, and I. Cunningham, J Protozool, 27:182–185, 1980.*)

In a similar manner, haplocysts occur in the tentacles of suctorian protozoa and are used to contact and immobilize prey.

Locomotor Organelles

Protozoa may move by three types of specialized organelles: pseudopodia, flagella, and cilia. In addition, a few protozoa without such organelles can carry out a gliding movement by body flexion.

Pseudopodia

A pseudopodium is a temporary projection of part of the cytoplasm of those protozoa which do not have a rigid pellicle (Fig. 19-2). Pseudopodia are therefore characteristic of the amoebas (*Sarcodina*). These organelles are also used for capturing food substances.

Flagella and Cilia

The flagellum is an extremely fine filamentous extension of the cell (Fig. 19-6). As a rule, the number of flagella present in an individual protozoan varies from one to eight; one or two is the most frequent number. A flagellum is composed of two parts: an elastic filament called an axoneme and the contractile cytoplasmic sheath that surrounds the axoneme.

In certain parasitic *Mastigophora*, such as *Trypanosoma* (Fig. 19-7), there is

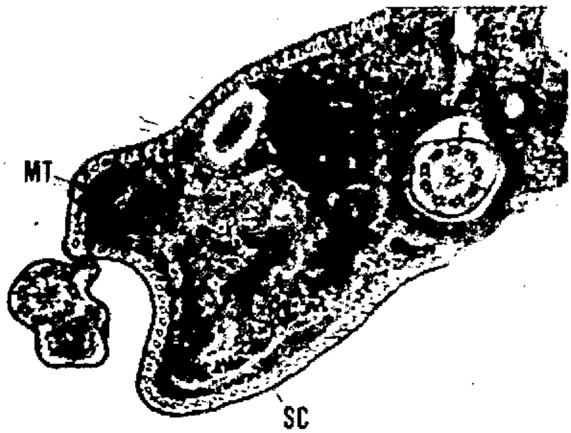


Figure 19-8. Transverse section of an infective form of *Trypanosoma brucei* showing the uniform, electron-dense surface coat covering the cell body and the flagellum (with the typical "9 + 2" structure). Surface coat or pellicle = SC; F = flagellum; MT = microtubules. (X92,500). (*Courtesy of P. Gardiner, International Laboratory for Research on Animal Diseases, Nairobi, Kenya, and the Society of Protozoologists. From P. R. Gardiner, L. C. Lamont, T. W. Jones, and I. Cunningham, J Protozool,* **27:***182–185, 1980.*)

a very delicate membrane that extends out from the side of the body with a flagellum bordering its outer margin. When the membrane vibrates, it shows a characteristic undulating movement; thus it is called the **undulating membrane.**

Cilia, in addition to their locomotor function, also aid in the ingestion of food and serve often as a tactile organelle. They are fine and short threadlike extensions from the cell (Figs. 19-4 and 19-5). They may be uniform in length or may be of different lengths depending on their location. Generally, cilia are arranged in longitudinal, oblique, or spiral rows, inserted either on the ridges or in the furrows.

Electron microscopy has shown that the fine structure of the flagella and cilia of all eucaryotes follows the same basic design. Sections show two central and nine double peripheral microtubules ("9 + 2" structure) along most of the length of the shaft, which is enveloped by a membrane continuous with the pellicle (Fig. 19-8).

REPRODUCTION OF PROTOZOA

As a general rule, protozoa multiply by asexual reproduction; the majority of higher animals reproduce by sexual means. This is not to say that sexual processes are absent in the protozoa. Indeed, many protozoa are able to carry out both asexual and sexual processes. Some parasitic forms may have an asexual phase in one host and a sexual phase in another host

Asexual Reproduction

Asexual reproduction occurs by simple cell division, which can be **equal** or **unequal**—the daughter cells are of equal or unequal sizes, respectively. If two daughter cells are formed, then the process is called **binary fission;** if many daughter cells are formed, it is **multiple fission. Budding** is a variation of unequal cell division.

Binary Fission

The simplest form of binary fission is found in the amoebas (Fig. 19-9). The pseudopodia are withdrawn before the nucleus divides. After the nucleus divides, the organism elongates and constricts in the center in order to form two daughter cells.

Amoebas with special protective coverings are more complex in their manner

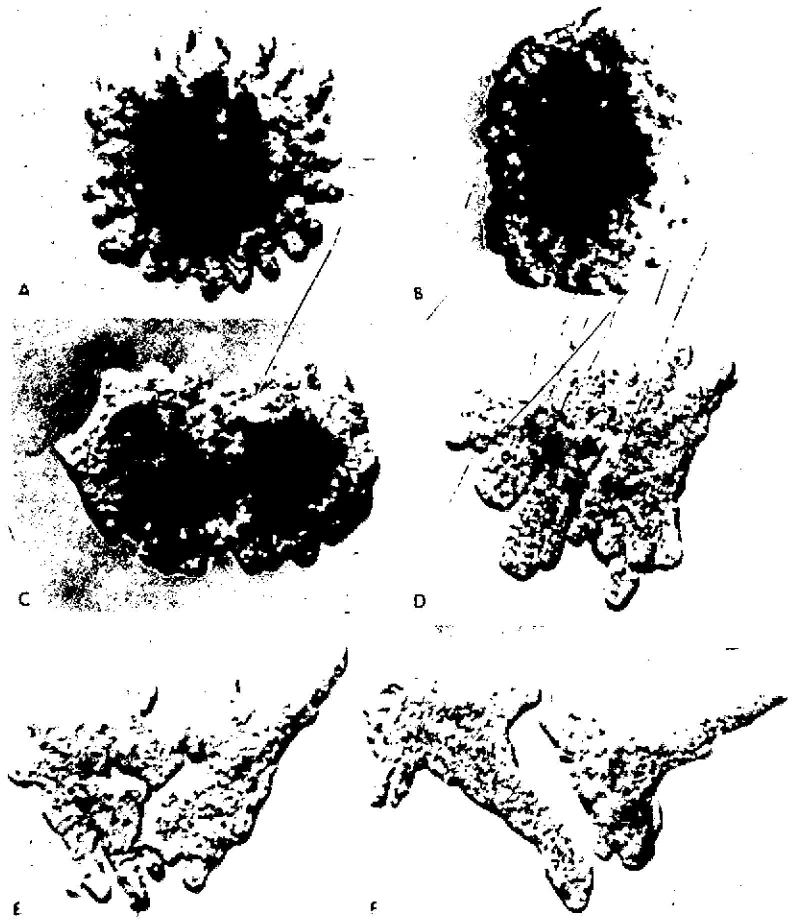


Figure 19-9. Amoebas reproduce by fission as shown in this series of photomicrographs of the dividing amoeba. Total elapsed time from (A) through (F) is 21 min. Intermediate stages photographed at (B) 6 min, (C) 8 min, (D) 15 min, (E) 18 min, and (F) 21 min. Reproductive material is concentrated in a band at the equator of the nucleus; the chromosomes divide, half of each going to one of the two new nuclei. As the cell divides one of the newly formed nuclei goes to each daughter cell; reproduction is complete when the cells are completely separated. *(Courtesy of Carolina Biological Supply Company.)*

of binary fission, which is directly related to the type of covering they possess. In those with soft coverings, the division plane is longitudinal along the body axis and the covering constricts into two halves. In those with more rigid coverings, part of the cytoplasm protrudes from the aperture (opening in covering) to secrete a new covering over its surface. Only after the formation of the new covering does nuclear division proceed, and binary fission is completed by cytoplasmic division.

In flagellates, with the exception of the dinoflagellates, fission is longitudinal along the major body axis. Since the flagella themselves are incapable of division, they must be regenerated from basal bodies (the **blepharoplasts** which arise in the vicinity of the old basal bodies. Thus multiplication of basal bodies usually precedes cell division. In dinoflagellates, division is at right angles to the cell axis because the flagella which determine the plane of division are not at the front end but at the side of the cell.

Transverse fission is characteristic for ciliates. Fission occurs at a right angle

Figure 19-10. Binary fission in *Paramecium multimicronucleatum*. Note the transverse constriction furrow which extends inward from the equator so that one organism is eventually divided into two daughter organisms, each of which eventually achieves the volume of the parent (X550). (*Courtesy of R. G. Kessel and C. Y. Shih, Scanning Electron Microscopy in Biology, Springer-Verlag, Berlin, 1974.*)

to the long axis of the cell (Fig. 19-10). In the *simplest* case of transverse fission, an equatorial furrow appears first which separates the surface cell layer into an anterior and a posterior half. A constriction follows, leading to the separation of two daughter cells. Their form and structure usually indicate from which half of the mother cell they developed.

The presence of cilia and other complex organelles has a profound influence on cell division in the ciliates. For example, ciliates have two types of nuclei—the macronucleus, which determines vegetative processes, and the micronucleus, which is involved in sexual processes. During asexual binary fission the diploid micronucleus divides normally by mitotic division. The macronucleus undergoes DNA synthesis and divides into two portions without the regular reduplication of the chromosomes (amitosis). However, each daughter macronucleus contains the full complement of genes (in fact, in multiple sets—polyploidy). In some primitive ciliates found in marine sands of the intertidal zone, the macronucleus is incapable of division; after fission of the organism a new one is formed from the micronucleus. In general, with regard to the other organelles, those originally present in each half are retained, and those which are lost during division are regenerated. Depending on the extent of differentiation of the species, divisions may involve extensive reorganizations, including transformation of preexisting structures and formation of new ones.

Multiple Fission

In multiple fission, a single mother (parental) cell divides to form many daughter (filial) cells. Division is usually preceded by formation of multiple nuclei within the mother cell, which then cleaves rapidly to form a corresponding number of daughter cells. Multiple fission is not as widespread as binary fission but it often takes place in addition to the latter process. In ciliates and flagellates, this type of fission is found in relatively few species. Multiple fission occurs commonly in the foraminifera, the radiolaria, and the heliozoa. Perhaps the best-known examples of multiple fission are found in the sporozoa, e.g., in the malarial parasite *Plasmodium* where it is known as schizogony and serves to spread the parasite quickly in the host.

Budding

The term *budding* is not used in the same sense as mycologists use it in describing the asexual reproductive process in the yeast *Saccharomyces*. Instead, in protozoology it is often used to describe the varied processes by which sessile protozoa produce motile offspring. That is, the mother cell remains sessile and releases one or more swarming daughter cells. The swarmer differs from the parent cell not only in a lower degree of differentiation but also in the possession of special locomotor organelles. Some form of budding is found in all sessile ciliates and is used to disseminate the species while the mother cell remains in situ.

Budding can be **exogenous** or **endogenous.** The former involves formation and separation of the bud toward the outside. Definite exogenous budding is seen in suctorians when a portion or portions of an adult sessile suctorian bud from the parent, develop cilia, and swim away. In endogenous budding, which also occurs in many suctorian species, the swarmer is formed inside the mother cell (see Fig. 19-17F).

Sexual Reproduction

Various types of sexual reproduction have been observed among protozoa. Sexual fusion of two gametes (**syngamy** or **gametogamy**) occurs in various groups of protozoa. Conjugation, which is generally a temporary union of two individuals for the purpose of exchanging nuclear material, is a sexual process found exclusively in the ciliates (see Fig. 19-11). After exchange of nuclei, the conjugants separate and each of them gives rise to its respective progeny by fission or budding. However, some ciliates show "total conjugation," with complete fusion of the two organisms.

When the gametes (which develop from trophozoites) are morphologically alike, they are called **isogametes.** When they are unlike in morphology (as well as physiology), they are **anisogametes** and can be either **microgametes** or **macrogametes.** That is, they are like the spermatozoa and the ova of metazoa, respectively. Thus microgametes are motile, relatively small, and usually numerous in comparison to macrogametes. Anisogametes are common among the sporozoa. For example, in *Plasmodium vivax* (a sporozoan that causes a type of malaria), anisogamy results in the formation of **ookinetes** or motile zygotes which give rise to a large number of **sporozoites** (long, slender bodies with an oval nucleus and firm cuticle, capable of producing new infection).

Regeneration

The capacity to regenerate lost parts is characteristic of all protozoa, from simple forms to those with highly complex structures. When a protozoan is cut in two,

Figure 19-11. Pair of *Euplotes aediculatus* cells during conjugation. They are united by their apposed ventral surfaces (X710). (*Courtesy of John A. Kloetzel, University of Maryland, Baltimore County.*)

the nucleated portion regenerates but the anucleated portion degenerates. In general, the nucleus is necessary for regeneration. In ciliates, the macronucleus alone (or even just a portion of it) is sufficient for this process.

CLASSIFICATION OF PROTOZOA

The myriad forms of protozoa have been grouped together. not because they are all related in an evolutionary way, but simply as a matter of convenience. The classification scheme of the protozoa now in common use was developed by the Committee on Systematics and Evolution of the Society of Protozoologists and published in 1980 (see reference at the end of the chapter). Electron microscopy has played a significant role in the revision of this classification scheme. The old classification schemes of protozoa were based primarily on organelles of locomotion.

The protozoa might be considered a subkingdom of the kingdom *Protista*. The major groups are called **phyla** (singular, **phylum**). An abbreviated classification scheme of these organisms is shown in Table 19-1.

CHARACTERISTICS OF THE MAJOR GROUPS OF PROTOZOA

The Flagellates (Subphylum *Mastigophora*)

These protozoa are conventionally divided into two groups: the plantlike forms (class *Phytomastigophorea*, the phytoflagellates) and the animallike forms (class *Zoomastigophorea*, the zooflagellates). Plantlike protozoa usually contain green or yellow chloroplasts as well as flagella and are photosynthetic. Many are colonial in nature. These organisms (considered as algae by some biologists) are discussed in Chap. 18. The zooflagellates have no chlorophyll and must obtain nutrition heterotrophically. All members of this group have one or more flagella; some members are capable of forming pseudopodia.

Asexual reproduction in the zooflagellates occurs by longitudinal binary fission. A form of multiple fission takes place in some organisms. Encystment is common. Sexual reproduction is not common.

Table 19-1. Abbreviated Classification of the Subkingdom *Protozoa*, Describing the Major Taxa and Their Characteristics

Taxonomic Group		Characteristics
Phylum I.	*Sarcomastigophora*	Single type of nucleus; sexuality, when present, essentially syngamy (union of gametes); flagella, pseudopodia, or both types of locomotor organelles
	Subphylum *Mastigophora*	One or more flagella typically present in trophozoites; division by longitudinal binary fission; sexual reproduction known in some groups
	Class *Phytomastigophorea*	Plantlike flagellates typically with chromatophores; amoeboid forms in some groups; sexuality well studied in some groups; mainly free-living; e.g., *Euglena*, *Chlamydomonas*
	Class *Zoomastigophorea*	Animallike flagellates; chromatophores absent; one to many flagella; amoeboid forms, with or without flagella, in some groups; mainly parasitic; e.g., *Leishmania*, *Trypanosoma*, *Giardia*, *Trichomonas*
	Subphylum *Opalinata*	Binary fission occurs between rows of flagella which cover the entire body surface; known life cycles involve syngamy with anisogamous flagellated gametes; all parasitic; e.g., *Opalina*

Table 19-1. (*continued*)

Taxonomic Group		Characteristics
	Subphylum *Sarcodina*	Pseudopodia, or locomotive protoplasmic flow without discrete pseudopodia; flagella restricted to developmental stages when present; asexual reproduction by fission; sexuality, if present, associated with flagellate or, more rarely, amoeboid gametes; mostly free-living species
	Superclass *Rhizopoda*	Locomotion by pseudopodia; includes the naked (*Amoeba*) and testate amoebae (*Arcella*) and the foraminifera (*Allogromia*)
	Superclass *Actinopoda*	Often spherical; usually planktonic; pseudopodia delicate, with axopodia (i.e., with a supportive axial filament); some naked, others with tests of chitin, silica, or strontium sulfate; reproduction asexual and/or sexual; trophic cells rarely flagellated; includes the classes *Acantharea* and *Heliozoea*
Phylum II.	*Labyrinthomorpha*	Spindle-shaped or spherical vegetative cells capable of producing a network of mucus tracks; parasitic on marine plants; e.g., *Labyrinthula*
Phylum III.	*Apicomplexa*	The apicomplexans may be regarded as the sporozoa (organisms with a spore-forming stage in their life history); organisms are grouped together by the possession of an apical complex—the total combination of anteriorly located organelles in the sporozoan; these include the polar ring (within which is a conoid—an electron-dense conical structure composed of coiled filaments); rhoptries (paired tubular organelles enlarged at the posterior end); and micronemes (elongate, electron-dense organelles); (all these organelles are shown in Fig. 19-12); micropores generally present at some stage; cilia absent; sexuality by syngamy; all species parasitic
	Class *Sporozoea*	Reproduction generally both sexual and asexual; oocysts generally containing infective sporozoites which result from sporogony; locomotion of mature organisms by body flexion, gliding, or undulation of longitudinal ridges; flagella present only in microgametes of some groups
	Subclass *Gregarinia*	Trophozoites large and extracellular; parasites of digestive tracts and body cavities of invertebrates; some glide by body flexion
	Subclass *Coccidia*	Trophozoites small and typically intracellular; e.g., *Toxoplasma*, *Plasmodium*
	Subclass *Piroplasmia*	Small, piriform, round, rod-shaped, or amoeboid parasites of vertebrate blood cells, with ticks as vectors; locomotion by body flexion or gliding; e.g., *Babesia*
Phylum IV.	*Microspora*	Intracellular parasites of invertebrates, particularly arthropods; invaded host cells are hypertrophied; spores are small, up to 6 μm, and some have a coiled filament; single sporoplasm with simple or complex extrusion apparatus; spores have polar tube and cap but none have polar capsules (see Fig. 19-13); e.g., *Nosema*
Phylum V.	*Acetospora*	Spores with one or more sporoplasms; without polar capsules or polar filaments; all parasitic; e.g., *Haplosporidium*
Phylum VI.	*Myxozoa*	Spores of multicellular origin, with one or more polar capsules and sporoplasms (Fig. 19-13); all species parasitic; cysts develop in infected internal organs of vertebrates, particularly fish; e.g., *Ceratomyxa*
Phylum VII.	*Ciliophora*	Organisms possess cilia or compound ciliary organelles in at least one stage of the life cycle; two types of nuclei are present—typically, a large macronucleus and a smaller micronucleus; reproduction is by asexual fission (binary transverse); the sexual process of conjugation is also exhibited; most are free-living, though many are commensalistic and a few parasitic

Table 19-1. *(continued)*

Taxonomic Group	Characteristics
Class *Kinetofragminophorea*	Oral infraciliature (linear orientation of subpellicular basal granules and associated subpellicular tubular fibrils) only slightly distinct from somatic infraciliature; cytostome often apical (or subapical) or midventral, on surface of body; cytopharyngeal apparatus commonly prominent; compound ciliature, oral or somatic, typically absent; e.g., *Acineta, Didinium, Balantidinium*
Class *Oligohymenophorea*	Oral apparatus generally well defined; oral ciliature clearly distinct from somatic ciliature; cytostome usually ventral and/or near anterior end; cysts common; colony formation in some groups; e.g., *Tetrahymena, Paramecium, Vorticella*
Class *Polymenophorea*	Well-developed adoral zone of membranelles (these are oriented to the left of the oral cavity and beat in a circular and clockwise manner, driving food toward the cytostome); one or several lines of "paroral" ciliature; somatic ciliature complete or reduced or appearing as cirri (see Fig. 19-5); cytostome at bottom of buccal cavity; cysts very common in some groups; often large and free-living forms in a great variety of habitats; e.g., *Stentor, Euplotes*

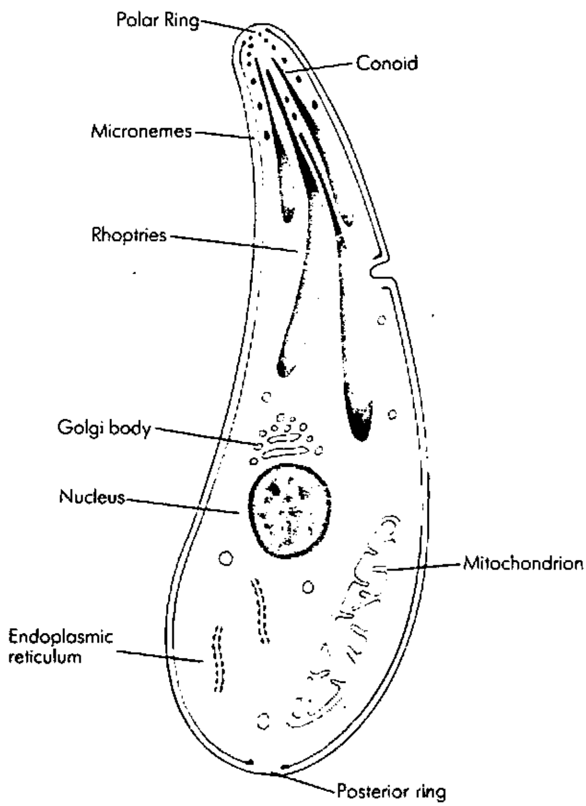


Figure 19-12. Diagram of a merozoite or sporozoite of an apicomplexan showing the apical complex and other organelles. *(Erwin F. Lessel, illustrator)*

The Zooflagellates (Class *Zoomastigophorea*)

The choanoflagellates (order *Choanoflagellida*) are distinctive in that they are either stalked or embedded in jelly, and each cell has a thin transparent collar

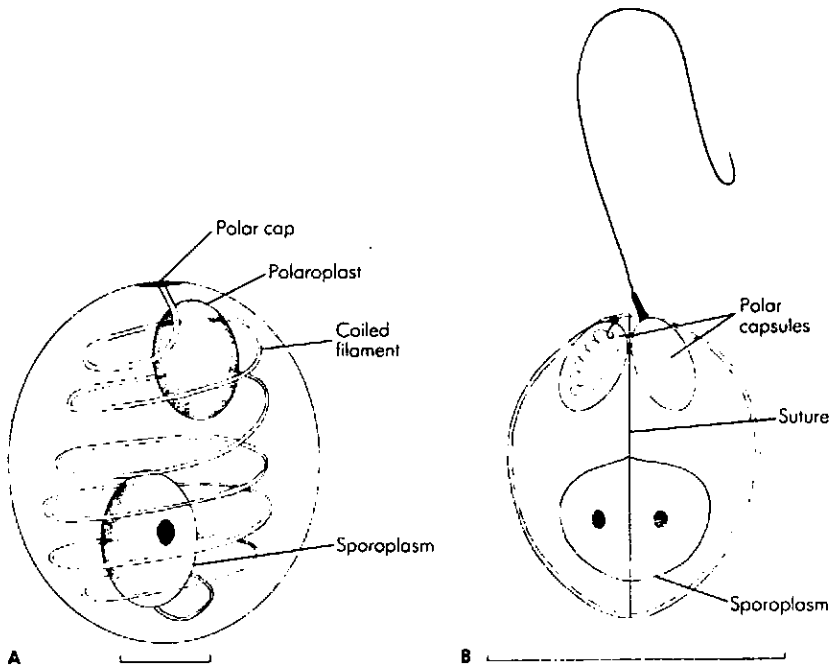


Figure 19-13. (A) Diagram of a microsporidian spore in which there are no polar capsules. (Scale = 1 μm.) (B) Diagram of a typical myxosporidian spore showing two polar capsules: one contains a coiled polar filament; the other, an extruded filament. A longitudinal suture is present, as is a binucleate sporoplasm. (Scale = 10 μm.) *(Erwin F. Lessel, illustrator)*

that encircles a single flagellum (Fig. 19-14). The collar functions as a food-catching device.

Organisms in the order *Kinetoplastida* are grouped together because of the presence of a **kinetoplast** (an extranuclear region of DNA associated with the mitochondrion). The single mitochondrion itself is extensive, traversing the length of the body as single tube, hoop, or network of branching tubes. One or two flagella may be present; if there are two, one is either trailing free or attached to the body, with undulating membranes occurring in some cases. Reproduction is by longitudinal binary fission. Both free-living and parasitic forms are included in the group. Parasitic forms are found in plants and in invertebrate and vertebrate hosts. Some of these are of great economic importance because of their disease potential in domestic animals and humans. Species in this group include *Leishmania* and *Trypanosoma*.

Other groups of zooflagellates are characterized by variations in morphology. For example, members of order *Retortamonadida* have 2 to 4 flagella; those of order *Diplomonadida* have bilateral symmetry and 6 to 8 flagella; and those of order *Trichomonadida* have 2 to 8 flagella and an undulating membrane.

Importance of Some Zooflagellates

Trichonympha campanula (Fig. 19-14G) is interesting because of its habitat, its complex structure, and its unusual ability to digest cellulose. These tiny creatures live in the intestines of termites, which by themselves are unable to digest cellulose, the principal constituent of wood. *T. campanula*, however, ingests wood particles and enzymatically converts the cellulose into soluble carbohydrates easily digested by the insect.

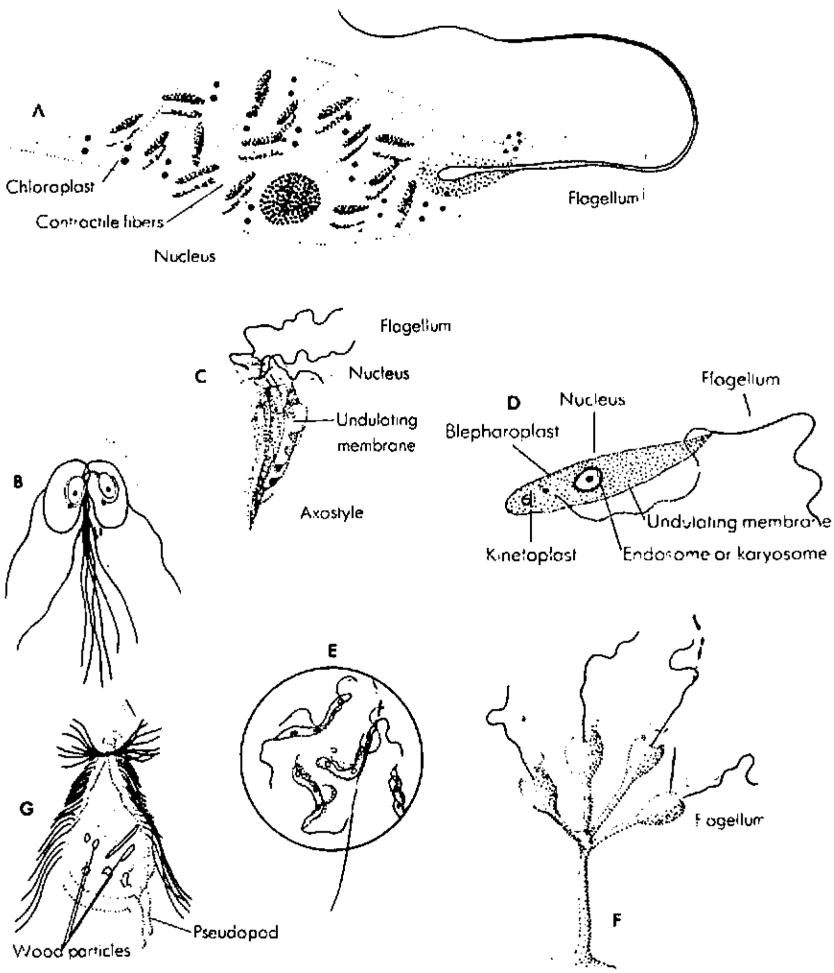


Figure 19-14. Flagellated protozoa. (A) *Euglena gracilis* are solitary, free-living flagellates, with chlorophyll. (B) *Giardia intestinalis*, a parasite found in the human intestine, where it may cause dysentery. (C) *Trichomonas hominis*, also found in the human intestine. Its role in the causation of disease has not been established. (D) *Trypanosoma rhodesiense*, which causes African sleeping sickness. (E) *Trypanosoma cruzi*, the causative agent of Chagas' disease, or South American trypanosomiasis. (F) *Codosiga*, a colonial flagellate with a transparent protoplasmic collar into which food particles are whipped by the action of the flagellum. (G) *Trichonympha*, a complex protozoan that inhabits the intestines of termites, where it converts wood cellulose into soluble carbohydrates that can be utilized by the female. (*Redrawn after Ralph Buchsbaum, Animals Without Backbones, rev. ed., The University of Chicago Press, Chicago, 1948.*)

Several of the flagellated protozoa are responsible for disease in humans. *Giardia intestinalis* (formerly called *G. lamblia*) (Fig. 19-14B) is associated with diarrhea in children and, infrequently, in adults. Its feeding (trophozoite) form has eight flagella and a ventral sucker with which it attaches to the intestinal mucosa. Trichomonads are found in the mouth, where they may contribute to gingivitis; in the intestine, where they may be associated with diarrheal conditions; and in the urethra and vagina, where they cause an inflammation and purulent discharge. Although these organisms are morphologically similar, they are designated as three distinct species on the basis of habitat. Those found in the mouth are called *Trichomonas buccalis*, those in the intestine are *T. hominis* (Fig. 19-14C), and those in the urogenital tract are *T. vaginalis*. Several species of trypanosomes are responsible for disease in humans. They are characterized by a narrow, elongated body with an undulating membrane and a flagellum that

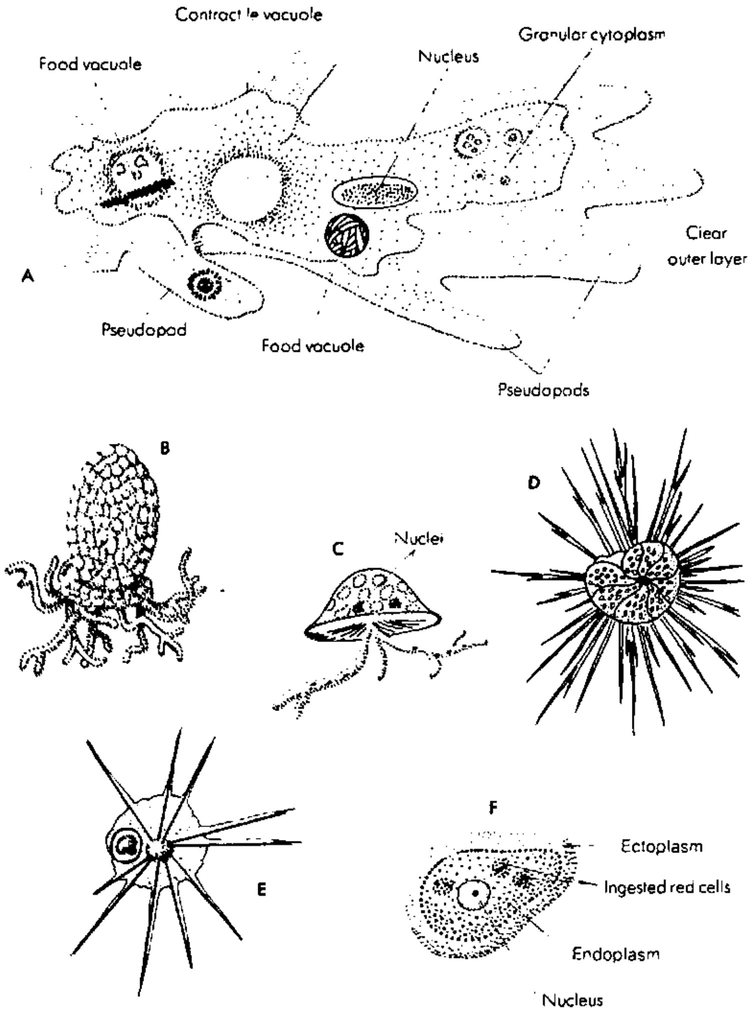


Figure 19-15. Amoeboid protozoa. (A) Diagrammatic sketch showing principal structures of an amoeba. (B) *Difflugia* lives in freshwater and builds a shell of sandgrains and a cement secreted by the cell. (C) *Arcella* is similar to *Difflugia* except that the shell is made of a chitinlike material secreted by the amoeba. It has two nuclei. (D) Foraminifera are saltwater types that build chalky shells with several chambers. They resemble snail shells. Pseudopodia are extended through pores in the shell wall. (E) *Heliozoans* may produce skeletons of silica covering the whole cell, or the body may be covered with a gelatinlike material with rigid pseudopodia made of silica. (F) *Entamoeba histolytica*, a pathogenic amoeba which causes dysentery in humans.

extends the length of the cell and beyond (Fig. 19-14D). They have a single nucleus and reproduce asexually. Some species pass through a complex life cycle, part of which is spent in a bloodsucking insect that transmits the parasite to humans and vertebrate animals. Important species of this group include *Trypanosoma gambiense* and *T. rhodesiense* (Fig. 19-14D), which are transmitted by the tsetse fly and cause African sleeping sickness; and *T. cruzi* (Fig. 19-14E), which is carried to humans by biting insects (e.g., *Triatoma*, or kissing bug) and causes a condition known as Chagas' disease, endemic in South America. Other species are pathogenic for a limited host range. For example *T. equiperdum* causes infections in equines only; transmission is by sexual means.

The genus *Leishmania* includes species which have both nonmotile and motile stages in their life cycles. Bloodsucking insects transmit the motile forms to humans; nonmotile forms lacking flagella are produced inside the cells of the spleen and other organs of the body and sometimes inside white blood corpuscles. *L. donovani* causes a tropical disease known as kala azar, and *L. tropica* causes a skin disease known as oriental sore. *L. brasiliensis*, common in South America, causes a disease characterized by ulceration of the mucous membranes of the mouth and nose.

The Amoebas (Subphylum *Sarcodina*)

Amoebas get their name from the Greek word *amoibe*, meaning "change," because their shapes are constantly changing. A typical example is *A. proteus*.

Morphology

Amoebas are composed of protoplasm differentiated into a cell membrane, cytoplasm, and a nucleus (Figs. 19-2 and 19-15). The cytoplasm shows granules as well as vacuoles containing food, wastes, water, and possibly gases. The outer membrane is selective and permits the passage of certain soluble nutrients into the cell and waste material out of the cell. Solid food is ingested with the help of pseudopods (see below). The nucleus functions in reproduction, metabolism, and the transmission of hereditary characteristics. Amoebas react to various physical and chemical stimuli in their surroundings. This is an **irritability response** which is at least superficially analogous to responses of higher organisms to their environment.

Amoebas are almost constantly in motion. They move by sending out portions of their bodies in one direction so that the whole cell moves into the location of the *pseudopodium*, or false foot, as the projection is called. Several pseudopodia may be sent out at one time from a single cell.

Nutrition and Excretion

Amoebas also use pseudopodia to capture food. The projections surround the food particles, which become enclosed in food vacuoles inside the cytoplasm

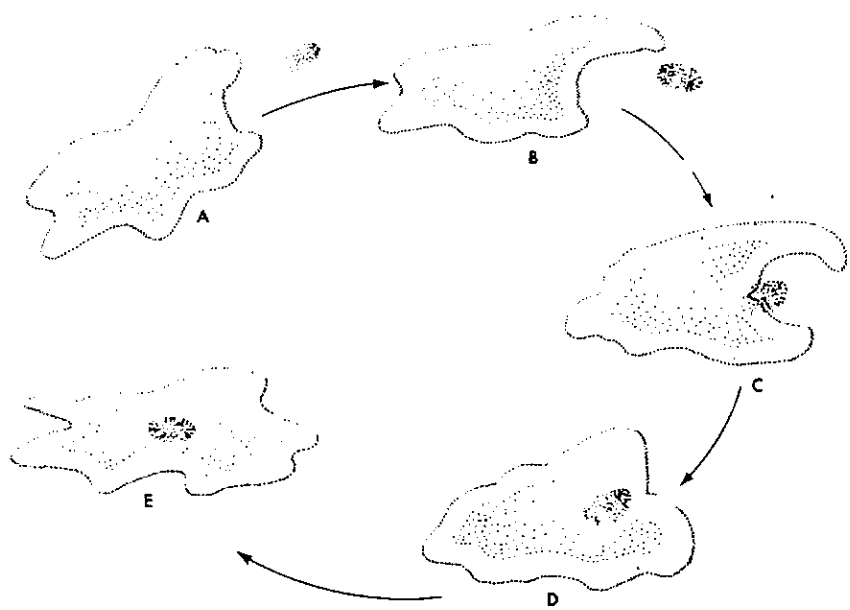


Figure 19-16. Amoeba ingesting food. As the amoeba encounters a food particle (A), a flagellate, pseudopodia form (B) and close in on the particle (C). An opening in the cell membrane allows the food particle to pass into the cell (D and E), where it is digested in a food vacuole or, if not accepted, expelled. (*Redrawn after Ralph Buchsbaum, Animals Without Backbones, rev. ed., The University of Chicago Press, Chicago, 1948.*)

(Fig. 19-16). As the enzymes (and possibly hydrochloric acid) from the cell enter the vacuole, the food is digested and assimilated. Wastes are disposed of through the cell membrane into the surrounding medium. It is possible that amoebas carry on a special kind of respiration in the form of an exchange of gases between the cell and the surrounding fluid, for at rather regular intervals vacuoles apparently containing some carbon dioxide in solution are expelled from the cell through an opening that develops in the cell membrane. Oxygen in the medium is absorbed through the surface of the cell.

Reproduction

Reproduction in amoebas takes places by the simple asexual process of binary fission (Fig. 19-9). For protection during periods that are unfavorable for normal growth, some amoebas have the capacity of encysting. In this condition their metabolic activities are minimized, and the cyst form enables them to survive until conditions again become favorable for growth.

Other Amoeboid Protozoa

Some interesting free-living amoeboid protozoa (Fig. 19-15) are the foraminiferans, many of which produce a chalky shell with numerous chambers. These organisms obtain their food by the action of pseudopodia that extend through pores in the tiny shells. As mentioned before, the famous white (chalk) cliffs of Dover, England and the chalk beds in Mississippi and Georgia contain deposits of these shells. Fossils of extinct foraminiferans in rocks are of value to geologists (because they indicate oil-bearing deposits) and paleontologists. Radiolarians, like foraminiferans, are marine forms, but most of them construct shells of silica. Deposits of their skeletons are incorporated in rocks formed in areas where they have occurred in abundance: Indiana limestone, for example, contains many of them. Freshwater counterparts of the radiolarians are the heliozoida, which are sometimes covered by a gummy substance or needles of silica rather than a skeleton. The genera *Arcella* and *Difflugia* contain species that make shells they can withdraw into for protection.

Species of the genus *Entamoeba* inhabit the intestinal tract of vertebrates. Many of them, such as *E. gingivalis*, which lives in the human mouth, and *E. coli*, which inhabits the human intestine, are harmless. However, one species, *E. histolytica*, is the cause of amoebic dysentery in human beings.

The Sporozoa (Phylum *Apicomplexa*)

All sporozoa are parasitic for one or more animal species. Adult forms have no organs of motility but all are probably motile by gliding at one stage of their life cycle. They cannot engulf solid particles but feed on the host's cells or body fluids.

Many have complicated life cycles, certain stages of which may occur in one host and other stages in a different host. They all produce spores at some time in their life history. Their life cycles exhibit an alternation of generations of sexual and asexual forms, such that the intermediate host usually harbors the asexual forms and the final host the sexual forms. Sometimes humans serve as hosts to both forms.

Toxoplasmosis and malaria are the major human diseases caused by sporozoa. *Toxoplasma gondii* is the etiologic agent of toxoplasmosis. The symptoms of this disease vary greatly depending on the location of the parasites in the body. They can mimic, for example, the symptoms of meningitis and hepatitis. *T.*

gondii is the most widespread of the parasites that infect vertebrates. More than 50 percent of adults in the United States have been infected at some time, but the disease in humans is usually mild and asymptomatic. Spontaneous recovery usually follows. However, transplacental infection, that is, infection of the human embryo, may occur with serious consequences. The result may be a stillborn child or a child with mental retardation and other disorders. It is of interest that the parasite will undergo its sexual reproductive cycle only in the intestinal cells of members of the cat family, including the domestic cat.

The most important sporozoa are those that cause malaria (see also Chap. 38). Malaria is a mosquito-borne disease of humans caused by sporozoa belonging to the genus *Plasmodium* which infect the liver and red blood cells. The final host for the parasite is the female anopheline mosquito; sexual reproduction of the parasite occurs in this host. Malaria has been one of the greatest killers of humans through the ages. At the present time, it has been conservatively estimated that 300 million people in the world have the disease and that about 3 million of them will die of it.

The Ciliates (Phylum *Ciliophora*)

Common examples of the ciliated protozoa are included in the genus *Paramecium*, found in freshwater ponds and lakes where adequate food supplies exist.

Morphology and Structure

Like most amoebas, paramecia are microscopic; some, however, are just barely visible to the unaided eye. The outer layer of the cell is less flexible than the outer membrane of the amoeba, and the interior is composed of semifluid, granular protoplasm containing nuclei and vacuoles of several kinds. Paramecia are easily distinguished by their characteristic shape, which has been likened to that of a slipper (Figs. 19-4 and 19-17). The anterior (front) end of the cell is rounded, and the posterior (rear) end is slightly pointed. The entire cell is covered with hundreds of short hairlike projections called cilia, which are the organs of locomotion and also serve to direct food into the cytostome.

Motility

Paramecia move very rapidly by a rhythmic beating of the cilia. Since cilia can beat in either direction, reverse as well as forward motion is possible and the organism can turn in any direction. The motion of cilia has been compared to that of the arms of a swimmer doing the crawl stroke.

Nutrition and Excretion

Paramecia take in food through a fixed cytostome at the base of the gullet. The food particles are directed by cilia through the oral groove into the gullet and are collected in a food vacuole inside the cell at the end of the cytopharynx, where they are digested by enzymes, as in the amoeba. Undigested particles are eliminated from the cell through the cytoproct.

As in amoebas and other single-celled microorganisms, oxygen enters the cell through the cell membrane and carbon dioxide diffuses out. Waste fluids are collected in the contractile vacuoles, which have fixed positions in paramecia instead of appearing anywhere in the cell, as in amoebas.

There are two types of nuclei in each paramecium, a large macronucleus and one or more small micronuclei. The former regulates the ordinary activities of the cell's metabolism, and the latter is/are associated with reproduction.

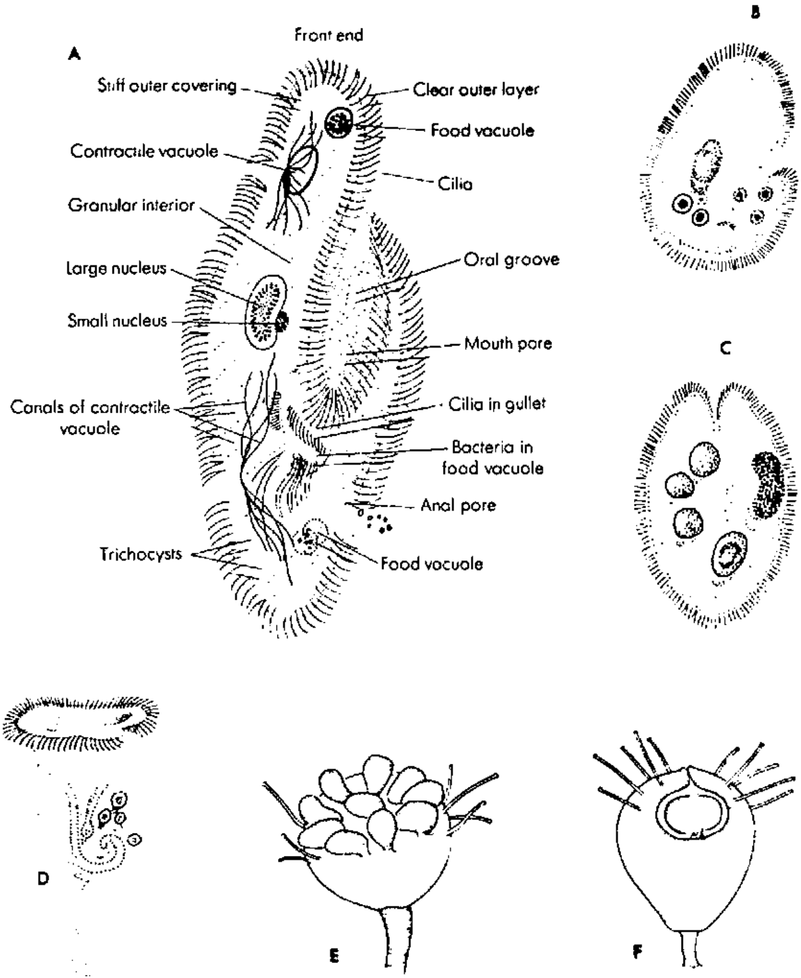


Figure 19-17. Ciliated protozoa. (A) Diagrammatic sketch showing principal structures of a paramecium. (B) *Colpoda*, a common freshwater protozoan. (C) *Balantidium coli*, the only disease-producing parasitic ciliate found in humans. (D) *Vorticella* has a bell-shaped body attached to a contractile stem. Cilia on the outer edge of the bell sweep bacteria and other food particles into the gullet by setting a miniature whirlpool in motion. (E) A protozoan of the subclass *Suctoria*, *Ephelota*, showing external or exogenous budding. (F) Another member of subclass *Suctoria*, *Tokophyra*, showing endogenous budding with larval form free within brood pouch. [*(E) and (F) redrawn after those of John O. Corliss, University of Maryland at College Park, The Ciliated Protozoa: Characterization, Classification, and Guide to the Literature, 2d ed., Pergamon, New York, 1979.*]

Reproduction

Paramecia reproduce asexually by binary fission, in which each of the nuclei elongates and divides into halves (Fig. 19-10). The cell itself constricts across the midline, producing two daughter cells, each of which receives a pair of nuclei. In this type of division, the rear daughter cell receives the gullet of the parent, and a new gullet is formed for the other new cell. Other parts are formed as required. Under favorable conditions, one or more divisions may take place within a 24-h period.

Conjugation may occur when unlike mating strains of paramecia happen to come into contact with one another. Although these cells are not differentiated as male and female, under certain circumstances two individuals come together and unite along their oral grooves. While they are joined in this fashion, their nuclei undergo divisions; then the cells exchange haploid nuclei derived from their micronuclei. In each conjugant, the two haploid nuclei fuse to form a diploid nucleus. The ciliates then separate, and nuclear divisions and fissions result in the asexual form.

Other Ciliated Protozoa

The ciliated protozoa (Fig. 19-17) are represented by many forms other than the paramecia. *Colpoda* is a common freshwater genus. The genus *Didinium* lives on a diet of paramecia, which are captured by a special structure and swallowed whole (Fig. 19-1). The genus *Stentor* comprises large cone-shaped protozoa that move about freely but attach to some object by a tapered lower end while feeding. The rapid movement of the cilia at *Stentor's* upper end sweeps smaller protozoa and bacteria into its gullet. *Vorticella* is bell-shaped. Cilia ring the outer edge of the bell and wash food, principally bacteria, into its gullet. Related colonial ciliates occur in groups attached to a long fibrous stalk with the ability to recoil when disturbed. Clusters of the organisms are comprised of many daughter cells. The only human ciliate parasite is *Balantidium coli*. It causes a type of dysentery.

QUESTIONS

1 What is the derivation of the name protozoa? How are protozoa distinguished from other eucaryotic protists?
2 What is meant by the statement that protozoa have attributes of higher organisms?
3 Give two specific examples of how protozoa adapt or respond to changing environmental conditions.
4 Define the following terms: parasitism, hyperparasitism, ectocommensalism, and endocommensalism.
5 Describe the roles played by protozoa in the natural environment.
6 Describe the roles played by protozoa in the treatment of industrial and domestic wastes.
7 Briefly describe the different organelles found in the cytoplasm of a protozoan such as *Amoeba proteus* or *Paramecium bursaria*.
8 What is a pellicle? Is it the same as the plasmalemma? Explain.
9 What protective coverings, other than the pellicle, do some protozoa possess?
10 Describe the food-gathering structures found in the ciliated protozoa.

11 What are the collective and individual functions of mucocysts, trichocysts, toxicysts, and haptocysts?

12 Describe the various ways by which protozoa move.

13 Compare and contrast asexual reproduction in the protozoa and in the bacteria.

14 Describe the process of nuclear division in the ciliates during binary fission.

15 What is multiple fission? Cite an example of its occurrence in protozoa.

16 How does budding in the protozoa differ from budding in yeasts?

17 Define the following terms: syngamy, isogametes, anisogametes, microgametes, macrogametes.

18 How many phyla are there in the new scheme of protozoan classification (1980)? Do they reflect any evolutionary relationship?

19 In what taxa are the following protozoa found: (*a*) flagellates, (*b*) ciliates, (*c*) amoebas, and (*d*) sporozoa?

20 What is a zooflagellate? Name several zooflagellates responsible for disease in humans.

21 How do the digestive and reproductive processes of a paramecium compare with those of an amoeba?

22 Why is *Trichonympha campanula* an interesting proozoan?

23 Explain with specific examples why the sporozoa are of importance to humans.

24 Write a short description of a common ciliated protozoan.

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Chapter 20 Viruses of Bacteria

Viruses are infectious agents so small that they can only be seen at magnifications provided by the electron microscope. They are 10 to 100 times smaller than most bacteria, with an approximate size range of 20 to 300 nm. Thus they pass through the pores of filters which do not permit the passage of most bacteria.

Viruses are incapable of independent growth in artificial media. They can grow only in animal or plant cells or in microorganisms. They reproduce in these cells by **replication** (a process in which many copies or replicas are made of each viral component and are then assembled to produce progeny virus). Thus viruses are referred to as **obligate intracellular parasites**. (If the least requirement for life is that an organism duplicate itself, then viruses may be viewed as microorganisms.)

Viruses largely lack metabolic machinery of their own to generate energy or to synthesize proteins. They depend on the host cells to carry out these vital functions. However, like the host cells, viruses have the genetic information for replication: and viruses have information in their genes for usurping the host cell's energy-generating and protein-synthesizing systems.

Actually, viruses in transit from one host cell to another are small packets of genes. The viral genetic material is either DNA or RNA, but the virus does not have both. (Host cells have both DNA and RNA.) The nucleic acid is enclosed in a highly specialized protein coat of varying design. The coat protects the genetic material when the virus is outside of any host cell and serves as a vehicle for entry into another specific host cell. The structurally complete mature and infectious virus is called the **virion**.

During reproduction in the host cells viruses may cause disease. In fact, viruses incite the most common acute infectious diseases of humans (like the "cold" or "flu"), and there is growing evidence that they may cause many chronic diseases as well. Significantly, all viruses are generally insensitive to the broad range of available antibiotics such as penicillin, streptomycin, and others.

From the above discussion of what does or does not constitute a virus, we may now attempt a definition for this group of infectious agents. We can define viruses as *noncellular infectious entities whose genomes are a nucleic acid, either DNA or RNA; which reproduce only in living cells; and which use the cells' biosynthetic machinery to direct the synthesis of specialized particles (virions), which contain the viral genomes and transfer them efficiently to other cells.*

Bacterial viruses, or **bacteriophages** (or simply **phages**) have provided the microbiologist with a model for **virology** (the study of viruses) and **molecular biology** (a discipline which examines the structure, function, and organization of the macromolecules in which biological specificity is encoded). We shall discuss the phages in this chapter and follow with a discussion of the viruses that infect animals and plants in Chapter 21.

BACTERIOPHAGES: DISCOVERY AND SIGNIFICANCE

Bacteriophages, viruses that infect bacteria, were discovered independently by Frederick W. Twort in England in 1915 and by Felix d'Herelle at the Pasteur Institute in Paris in 1917. Twort observed that bacterial colonies sometimes underwent **lysis** (dissolved and disappeared) and that this lytic effect could be transmitted from colony to colony. Even high dilutions of material from a lysed colony that had been passed through a bacterial filter could transmit the lytic effect. However, heating the filtrate destroyed its lytic property. From these observations Twort cautiously suggested that the lytic agent might be a virus. D'Herelle rediscovered this phenomenon in 1917 (hence the term *Twort-d'Herelle phenomenon*) and coined the word **bacteriophage**, which means "bacteria eater." He considered the filtrable agent to be an invisible microbe—for example, a virus—that was parasitic for bacteria.

Since the bacterial hosts of phages are easily cultivated under controlled conditions, demanding relatively little in terms of time, labor, and space compared with the maintenance of plant and animal hosts, bacteriophages have received considerable attention in viral research. Furthermore, since bacteriophages are the smallest and simplest biological entities known which are capable of **self-replication** (making copies of themselves), they have been used widely in genetic research. Of importance too have been studies on the bacterium-bacteriophage interaction. Much has been learned about host-parasite relationships from these studies, which have provided a better understanding of plant and animal infections with viral pathogens. Thus the bacterium-bacteriophage interaction has become the model system for the study of viral pathogenicity.

GENERAL CHARACTERISTICS

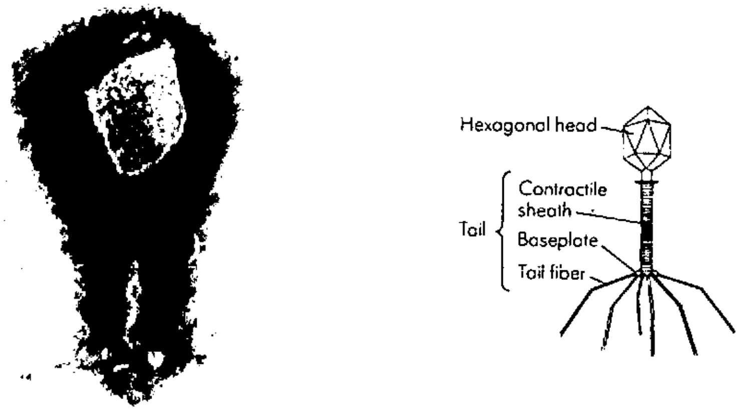
Bacterial viruses are widely distributed in nature. Phages exist for most, if not all, bacteria. With the proper techniques, these phages can be isolated quite easily in the laboratory.

Bacteriophages, like all viruses, are composed of a nucleic acid core surrounded by a protein coat. Bacterial viruses occur in different shapes, although many have a tail through which they inoculate the host cell with viral nucleic acid (Figs. 20-1 and 20-2).

There are two main types of bacterial viruses: **lytic,** or **virulent,** and **temperate** or **avirulent.** When lytic phages infect cells, the cells respond by producing large numbers of new viruses. That is, at the end of the incubation period the host cell bursts or lyses, releasing new phages to infect other host cells. This is called a **lytic cycle.** In the temperate type of infection, the result is not so readily apparent. The viral nucleic acid is carried and replicated in the host bacterial

Figure 20-1. A bacteriophage of *Bacillus thuringiensis* (X297,000). (Courtesy of H.-W. Ackermann, Laval University.)

Figure 20-2. The unique morphology of many bacteriophages is exemplified by this phage of *Salmonella newport*. Each phage has a tail through which it inoculates the host cell with viral nucleic acid. Note that some tails have broken off from the phage heads in this preparation (X59,400). (Courtesy of H.-W. Ackermann, Laval University.)



A B

Figure 20-3. (A) Electron micrograph and (B) diagrammatic representation of the coliphage T2 virion. Magnification in micrograph X189,000. (*Courtesy of H.-W. Ackermann, Laval University.*)

cells from one generation to another without any cell lysis. However, temperate phages may spontaneously become virulent at some subsequent generation and lyse the host cells. In addition, there are some filamentous phages which simply "leak" out of cells without killing them.

MORPHOLOGY AND STRUCTURE

Morphological Groups of Phages

The electron microscope has made it possible to determine the structural characteristics of bacterial viruses. All phages have a nucleic acid core covered by a protein coat, or capsid. The capsid is made up of morphological subunits (as seen under the electron microscope) called capsomeres. The capsomeres consist of a number of protein subunits or molecules called protomers. Figure 20-3 shows the fine structure and anatomy of a common morphological form of the bacteriophage, one with a head and a tail.

Bacterial viruses may be grouped into six morphological types (Fig. 20-4).

A This most complex type has a hexagonal head, a rigid tail with a contractile sheath, and tail fibers.
B Similar to A, this type has a hexagonal head. However, it lacks a contractile sheath, its tail is flexible, and it may or may not have tail fibers.
C This type is characterized by a hexagonal head and a tail shorter than the head. The tail has no contractile sheath and may or may not have tail fibers.
D This type has a head made up of large capsomeres, but has no tail.
E This type has a head made up of small capsomeres, but has no tail.
F This type is filamentous.

Types A, B, and C show a morphology unique to bacteriophages. The morphological types in groups D and E are found in plant and animal (including insect) viruses as well. The filamentous form of group F is found in some plant viruses.

Pleomorphic viruses were recently discovered to have a lipid-containing envelope, have no detectable capsid, and possess double-stranded DNA (ds-DNA). The representative phage is MV-L2.

Phage Structure

Most phages occur in one of two structural forms, having either **cubic** or **helical** symmetry. In overall appearance, cubic phages are regular solids or, more specifically, **polyhedra** (singular, **polyhedron**); helical phages are rod-shaped.

Polyhedral phages are **icosahedral** in shape. (The **icosahedron** is a regular polyhedron with 20 triangular facets and 12 vertices.) This means that the capsid has 20 facets, each of which is an equilateral triangle; these facets come together to form the 12 corners. In the simplest capsid, there is a capsomere at each of the 12 vertices; this capsomere, which is surrounded by five other capsomeres, is termed a **penton**. (See Fig. 20-5A.) For example, the phage ϕX174 exhibits the simplest capsid. In larger and more complex capsids, the triangular facets are subdivided into a progressively larger number of equilateral triangles. Thus a capsid may be composed of hundreds of capsomeres but it is still based on the simple icosahedron model.

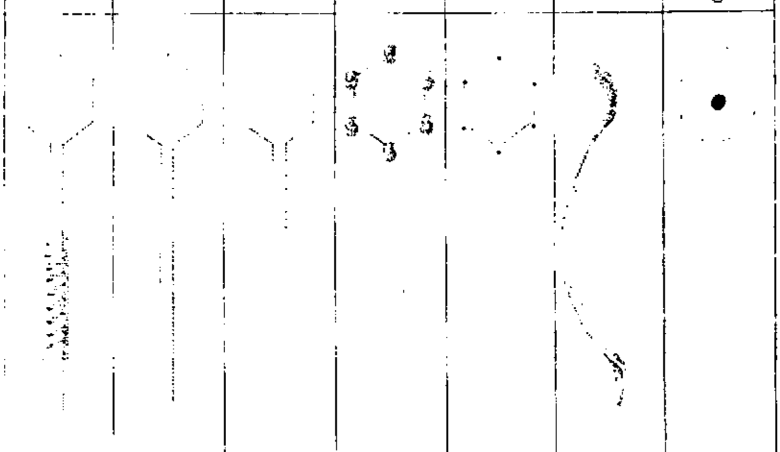
The elongated heads of some tailed phages are derivatives of the icosahedron. For example the head of the T2 and T4 phages is an icosahedron elongated by one or two extra bands of hexons.

Rod-shaped viruses have their capsomeres arranged helically and not in stacked rings (Fig. 20-5B). An example is the bacteriophage M13.

Some bacteriophages, such as the T-even coliphages (T2, T4, and T6), have very complex structures, including a head and a tail. They are said to have **binal symmetry** because each virion has both an icosahedral head and a hollow helical tail (Fig. 20-6).

Phage Nucleic Acids

Different morphological types of phages are also characterized by having different nucleic acid types (Fig. 20-4). All tailed phages contain double-stranded



Type	A	B	C	D	E	F	G
Morphology							
Nucleic acid type and number of strands	DNA, 2	DNA, 2	DNA, 2	DNA, 1	RNA, 1	DNA, 1	DNA, 2
Examples of phages	Coliphages T2, T4, T6	Coliphages T1, T5	Coliphages T3, T7	Coliphage ϕ x 174, S13	Coliphages f2, MS2	Coliphages fd, f1	MV-L2

Figure 20-4. Schematic representation of morphological types of bacteriophages. [*As described by D. E. Bradley, "Ultrastructures of Bacteriophages and Bacteriocins," Bacteriol Rev*, **31**:*230–314 (1967).*] A further group, G, may be added; these phages have a lipid envelope and are pleomorphic.

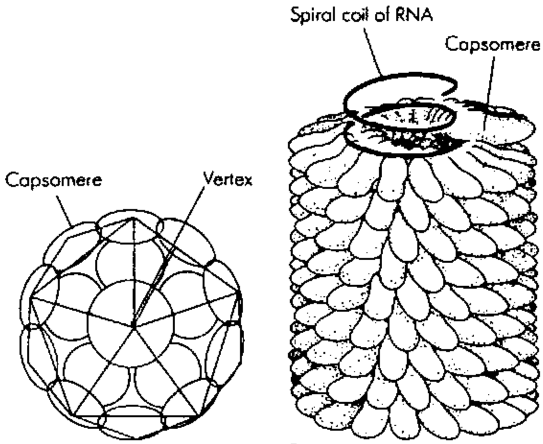


Figure 20-5. (A) Diagram of the simplest icosahedral capsid. The colored triangular outlines delineate the icosahedral symmetry. The circles represent capsomeres. (B) Diagram of a rod-shaped virus with helical symmetry. The capsomeres are arranged helically around a hollow core containing a spiral coil of RNA.

Figure 20-6. The morphology of phage lambda as seen by electron microscopy (X297,000). (Courtesy of *H.-W. Ackermann, Laval University.*)

Table 20-1. Molecular Weights and Type of Nucleic Acid of Commonly Studied Phages

Phage	Host	DNA Type	RNA Type	MW*
φX174	*Escherichia coli*	single-stranded (ss), circular (circ.)		1.7
fd	*E. coli*	ss, circ.		1.7–2.0
M13	*E. coli*	ss, circ.		2.4
T1	*E. coli*	double-stranded (ds)		30
T3	*E. coli*	ds		24
T5	*E. coli*	ds		75
T7	*E. coli*	ds		38
λ	*E. coli*	ds		30
T2	*E. coli*	ds		120
T4	*E. coli*	ds		110
T6	*E. coli*	ds		120
Mu	*E. coli*	ds		25
P22	*Salmonella*	ds		26
SPO1	*Bacillus subtilis*	ds		100
SPO2	*B. subtilis*	ds		25
PM2	*Pseudomonas*	ds, circ.		6
N1	Cyanobacteria	ds		43
f2	*E. coli*		ss	1.2
R17	*E. coli*		ss	1.2
QB	*E. coli*		ss	1.2

* MW = molecular weight, expressed in 10^6 daltons.

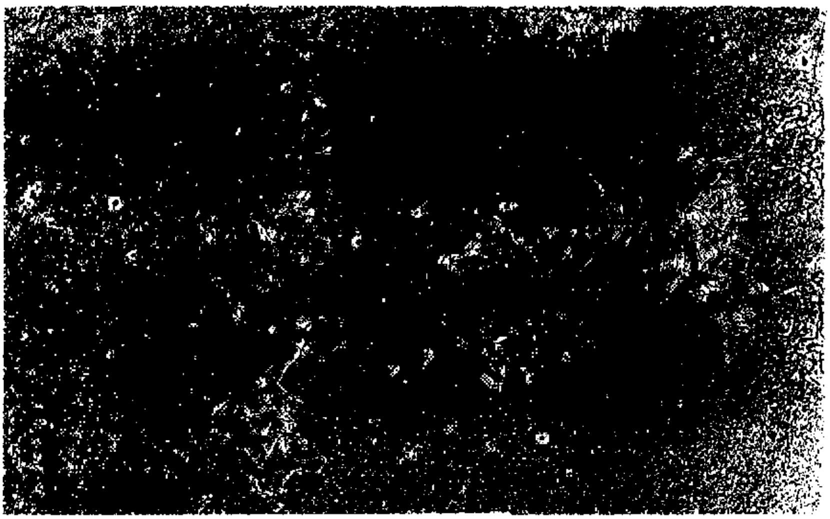


Figure 20-7. A DNA molecule isolated from *Bacillus subtilis* bacteriophage SP 50 appears in this electron micrograph as a tangled thread (X90,000). (*Courtesy of William S. Reznikoff and C. A. Thomas, Jr.*)

DNA. The phages with large capsomeres (group D) and the filamentous ones (group F) have single-stranded DNA. Group E phages have single-stranded RNA. The DNAs of some phages are circular under certain conditions. (Circular simply means a *closed loop*; the molecule is in the form of a loose folded coil packed inside the capsid.) The DNA of phage ϕX174 is circular both in the virion and in the host cell. The DNA of phage **lambda** (λ) is linear in the virion, but on entering the host cell the cohesive ends join to form a circle. The molecular weights and nucleic acid types of commonly studied bacteriophages are shown in Table 20-1. Figure 20-7 shows a DNA molecule isolated from a phage.

THE CLASSIFICATION AND NOMENCLATURE OF BACTERIOPHAGES

It may be apparent by now that the common names of bacteriophages do not follow particular guidelines. They are simply designations or code symbols assigned by investigators. Although serving the practical needs of the laboratories, this is a haphazard way of naming a group of microorganisms.

Consequently, the International Committee on Taxonomy of Viruses (ICTV) has a Bacterial Virus Subcommittee working on the classification and nomenclature of bacteriophages. However, taxonomic development within the bacterial viruses remains slow and difficult for two main reasons. First, some 1,900 descriptions of bacterial virus isolates of known morphology have been published. About 150 new descriptions are published each year. Many of these descriptions give a characterization of the virus that is quite inadequate for establishing its relationships with other phages. (This difficulty may be surmounted in the near future since guidelines have now been established for the characterization of a new phage isolate.) Second, a substantial proportion of the scientists who work with bacterial viruses are molecular biologists rather than virologists. They work with a relatively small selection of extremely well characterized viruses and are quite satisfied with simple code designations for these

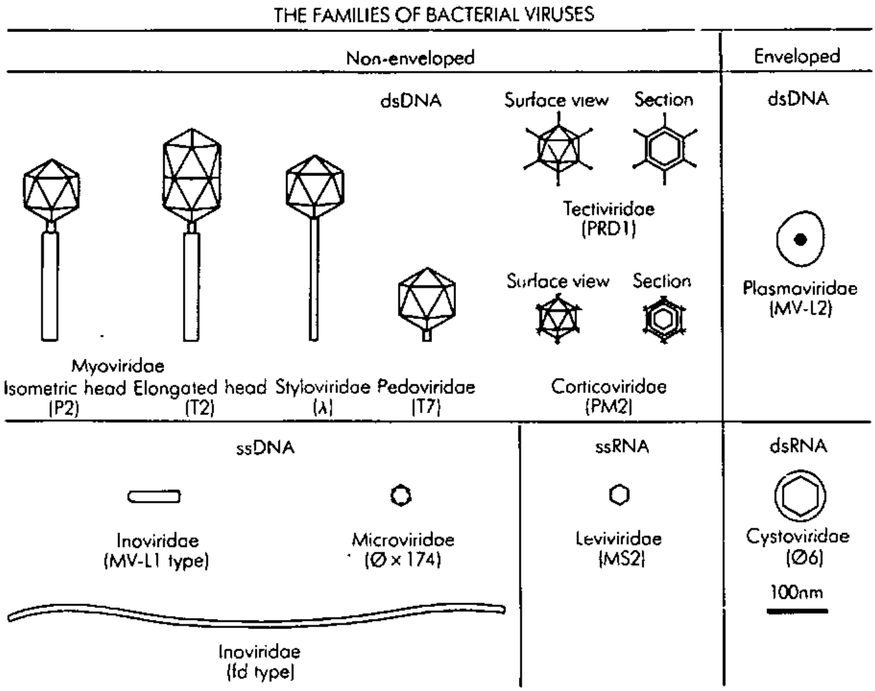


Figure 20-8. Schematic representation of the families of bacterial viruses. Note that all the diagrams have been drawn to the same scale and provide a good indication of the shapes and relative sizes of the virions. To aid recognition, a well-known member of the family is given in parentheses, but the dimensions and shape used for the drawing may not be exactly those of the virus example given. (*Drawings by H.-W. Ackermann, Laval University. Reproduced from R. E. F. Matthews, "Classification and Nomenclature of Viruses,"* Intervirology, **17**:1–199, 1982. *By permission from S. Karger AG, Basel, Switzerland.*)

viruses because these biologists have little need for viral classification, nomenclature, or natural relationships.

The Bacterial Virus Subcommittee has now recommended names for families of phages (names ending in *-viridae*). These families are illustrated in Fig. 20-8.

SOME BACTERIOPHAGES OF *Escherichia coli*

The most extensively studied group of bacteriophages are the coliphages, so called since they infect the nonmotile strain B of *Escherichia coli.* They are designated T1 to T7. All of these phages are composed almost exclusively of DNA and protein in approximately equal amounts. Except for T3 and T7, all have tadpole shapes, with polyhedral heads and long tails. The tails of T3 and T7 are very short (see Fig. 20-4). The T phages range from about 65 to 200 nm in length and 50 to 80 nm in width. The continuous, or circular, molecule of double-stranded DNA (about 50 μm long, or about 1,000 times as long as the phage itself) is tightly packed in the protein head.

There are other bacteriophages for *E. coli* whose morphology and chemical composition are very different from those of the T phages. The f2 phage, for example, is much smaller than the T phages and has a single-stranded linear molecule of RNA, rather than DNA. It has no visible tail.

There are also coliphages that possess single-stranded DNA. Morphologically they can be either icosahedral (cubic symmetry) or filamentous (helical symmetry). An icosahedral phage with circular single-stranded DNA is ϕX174.

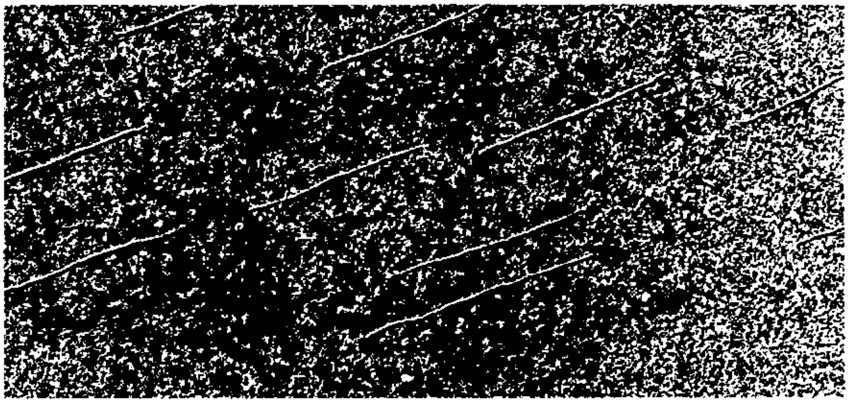


Figure 20-9. Electron micrograph of If1 phage that infects *Escherichia coli*. The phage absorbs specifically to the I pilus, a type of surface appendage of cells (X24,750). (*Courtesy of R. L. Wiseman, The Public Health Research Institute of the City of New York, Inc.*)

Filamentous coliphages (Fig. 20-9) were discovered long after the tadpole-shaped phages were known. Filamentous phages are continuously produced by viable and reproducing bacteria (and without the host-cell lysis characteristic of the virulent icosahedral phages). Such filamentous phages for *E. coli* include the fd and f1 bacteriophages (Fig. 20-4). They all have circular single-stranded DNA.

REPLICATION OF BACTERIAL VIRUSES

Much of what is known about bacteriophage replication has come from studies of the virulent even-numbered T phages (T2, T4, T6) of *E. coli*. However, it is apparent that the basic sequence of events during phage replication is similar for most phages. Variation occurs with respect to the number of phage proteins made and the degree of usurping of host functions. We shall use the T-even phages as a model for discussing phage replication.

Adsorption

The first step in infection of a host bacterial cell by a phage is adsorption (Fig. 20-10). The tip of the virus tail becomes attached to the cell via specific receptor sites on the cell surface. Attachment is specific in that certain viruses and susceptible bacteria have complementary molecular configurations at their opposing receptor sites. In some cases, the specific receptor of the bacterium is part of the bacterial lipopolysaccharide, although any surface structure can function as a specific phage receptor, including flagella. pili, and carbohydrates and proteins in the membrane or cell wall. Fig. 20-11 shows the adsorption of phage T4 to its host cell by means of the interaction of the tip of the phage core (tail tube within the sheath) and the cytoplasmic membrane of the host spheroplast.

It should be noted that infection of a host bacterial cell cannot occur without adsorption. Some bacterial mutants have lost the ability to synthesize specific receptors; they also become resistant to infection by the specific phage.

Initial adsorption of the phage to the receptor is reversible (i.e., the phage can be washed away) when only the tips of the tail fibers attach first to the cell surface. But this soon becomes irreversible when the tail pins attach; this is shown in Fig. 20-12. (Six tail pins protrude from the base of the tail.)

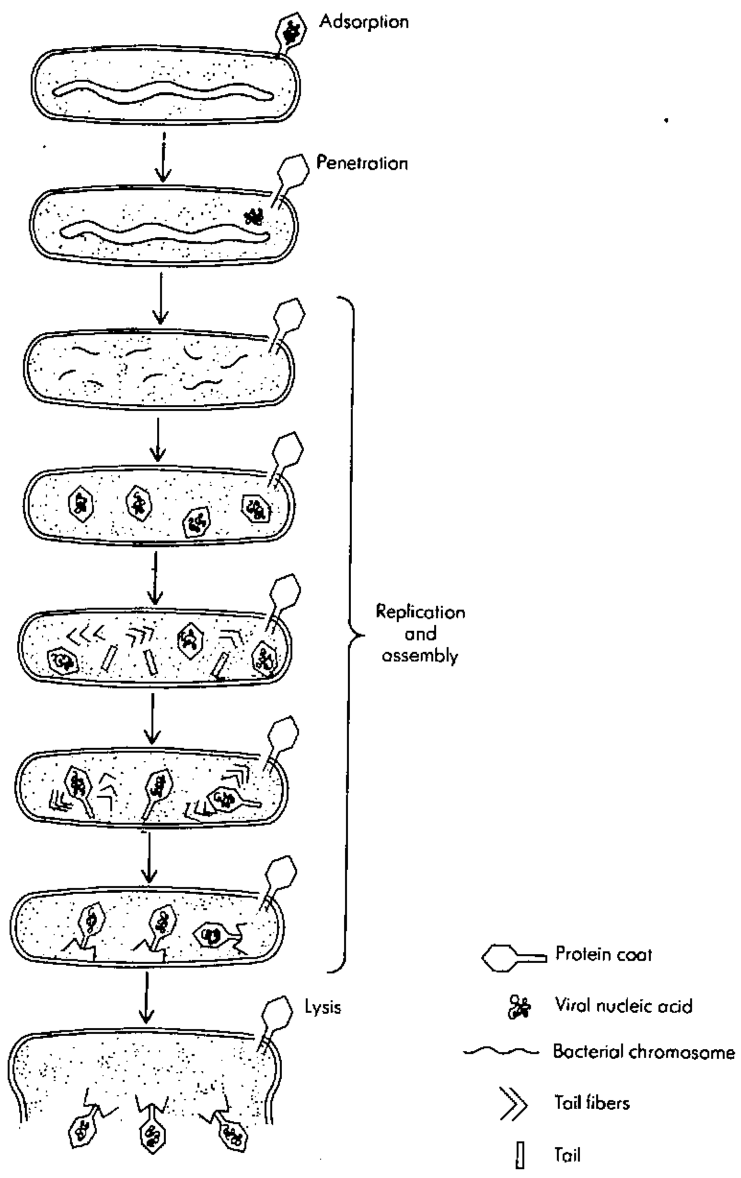


Figure 20-10. Viruses infect bacteria by injecting the contents of the virus head (viral nucleic acid) through a puncture hole in the cell wall. The viral nucleic acid then takes control of the cell metabolism and "directs" the bacterium in the synthesis of more viral nucleic acid and other materials needed for making complete virus particles. In a short time the newly formed virus particles are released by a sudden rupture of the cell wall (lysis) and are free to infect other susceptible bacteria.

Figure 20-11. Phage T4 adsorption and DNA injection into *Escherichia coli* spheroplasts. The phage particles were incubated with spheroplasts at 37°C for 10 min, directly negatively stained, and examined under an electron microscope. Left: X63,500; right: X150,000. (*Courtesy of S. Mizushima, Nagoya University. From H. Furukawa, T. Kuroiwa, and S. Mizushima, "DNA Injection During Bacteriophage T4 Infection of Escherichia coli," J Bacteriol,* **154***:938–945, 1983.*)

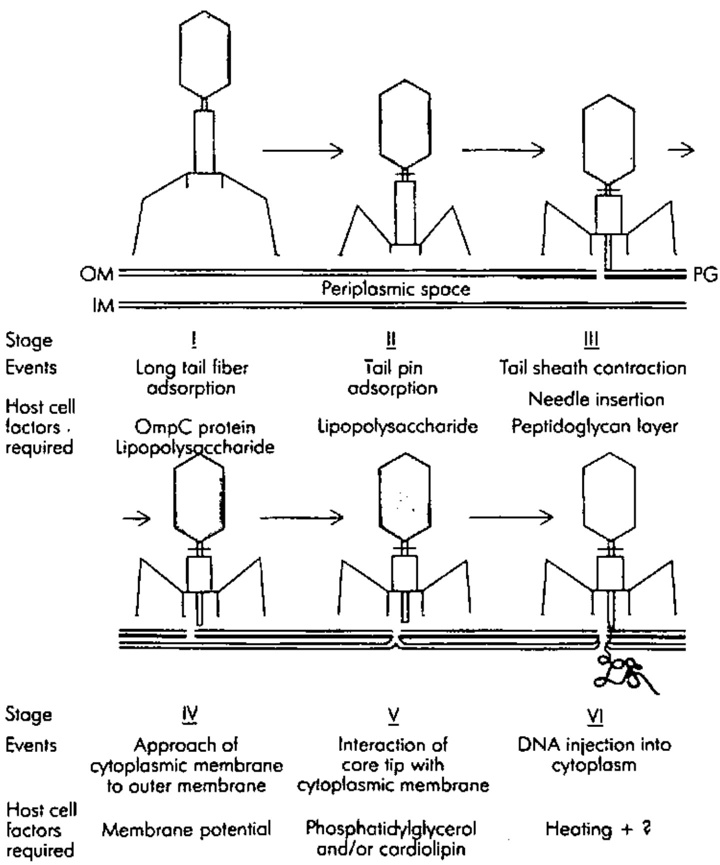


Figure 20-12. Summary of process of phage T4 infection. OM, outer membrane; IM, inner membrane; PG, peptidoglycan layer. (Reproduced *from H. Furukawa, T. Kuroiwa, and S. Mizushima, "DNA Injection During Bacteriophage T4 Infection of Escherichia coli," J Bacteriol,* **154***:938–945, 1983. Courtesy of S. Mizushima, Nagoya University.*)

Penetration

If too many phages are attached to the bacterium and penetrate it, there may be premature lysis (lysis from without), which is not accompanied by the production of new virus.

The actual penetration of phage into the host cell is mechanical. But it may be facilitated by localized digestion of certain cell surface structures either by phage enzymes (e.g., lysozyme) carried on the tail of the phage or by viral activation of host degradative enzymes.

In the T-even phages, penetration is achieved when:

1 The tail fibers of the virus attach to the cell and hold the tail firmly against the cell wall;
2 The sheath contracts, driving the tail core into the cell through the cell wall and membrane; and
3 The virus injects its DNA the way a syringe injects a vaccine.

The protein coat, which forms the phage head, and the tail structure of the virus remain outside the cell. The processes of adsorption and penetration are shown in Fig. 20-12.

Phages, such as T1 and T5, that do not have a contractile sheath also inject their nucleic acid through the cell envelope, possibly at adhesion sites between the inner and outer membranes. That is, sheath contraction is not a prerequisite for phage infection. The filamentous, rod-shaped DNA phages (such as fd and M13), like animal viruses, enter the bacterial cell as discrete virions prior to the liberation of the DNA from the phage capsid.

Transcription

In the case of phage T4, transcription occurs in several stages leading to the formation of immediate early, delayed early, and late gene products, so named on the basis of their time of appearance. (The sequence of transcription events of phage DNA in other bacteriophages may vary from that of the T4 model discussed here.) In brief, bacterial mRNA and bacterial proteins stop being synthesized within a few minutes after entry of phage DNA. Bacterial DNA is quickly degraded to small fragments and the nucleoid region of the bacterium becomes dispersed. Some phage mRNA is made immediately after infection. The amount of phage DNA increases after a brief delay. Virion-specific proteins appear somewhat later, followed by appearance of organized capsid precursors and resulting in the formation of mature infectious capsids.

Immediate early phage genes are transcribed using the existing bacterial RNA polymerase. For the most part, these genes code for nucleases that break down host DNA (rendering its nucleotides available for phage DNA synthesis) and for enzymes that alter the bacterial RNA polymerase so that it will preferentially transcribe delayed early phage genes.

Delayed early genes code for phage enzymes which produce unique phage DNA constituents such as 5-hydroxymethylcytosines (which replace cytosine in the bacterial DNA); which glucosylate these nucleotides; or which destroy precursors of cytosine deoxynucleotides so that no bacterial cytosine will be incorporated into phage DNA. These alterations enable the phage to survive because bacterial restriction enzymes (nucleases) are unable to degrade phage DNA modified by the substitution of 5-hydroxymethylcytosine for cytosine and by glucosylation of this substituted base. Further, a phage nuclease will destroy any DNA that has unsubstituted cytosine. Delayed early genes also code for polymerases and ligases that play specific roles in phage DNA replication and recombination and for a second altered RNA polymerase that will transcribe the late genes.

Late gene products include the structural components of new phage particles (e.g., heads, tails, and fibers). They also include a phage lysozyme (an endolysin) which will lyse the bacterial cell, releasing the mature virions.

Assembly and Release

Only after the synthesis of both structural proteins and nucleic acid is well under way do the phage components begin to assemble into mature phages. About 25 min after initial infection, some 200 new bacteriophages have been assembled (see Fig. 20-13), and the bacterial cell bursts, releasing the new phages

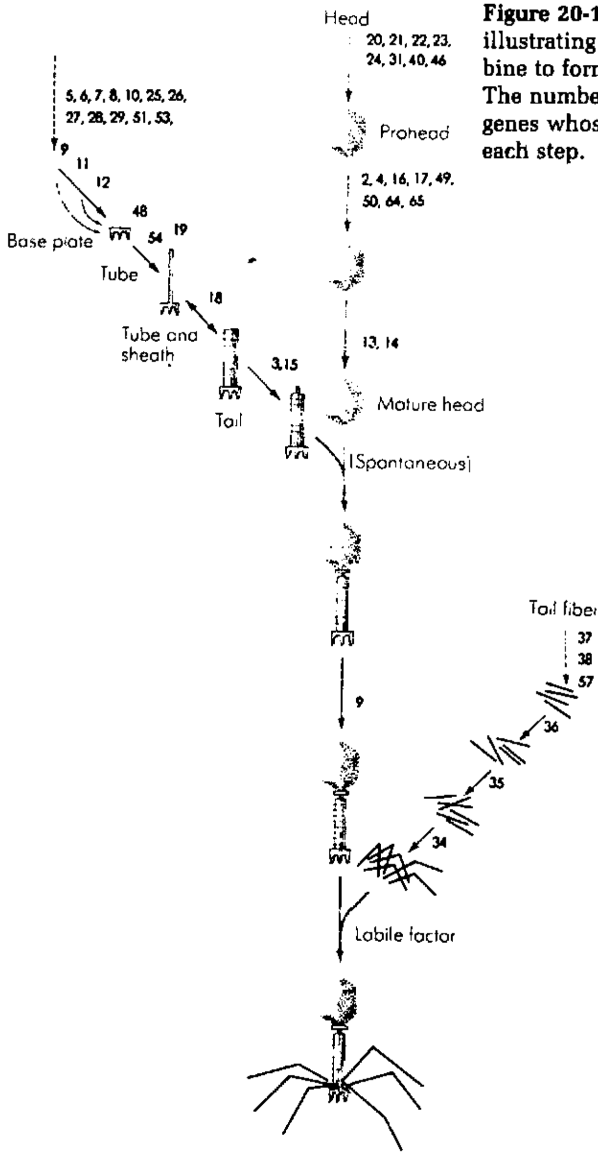


Figure 20-14. Morphogenetic pathway illustrating the branches which combine to form complete virus particles. The numbers refer to the T-even phage genes whose products are involved at each step.

Figure 20-13. The intracellular location of a phage is shown. This cell of *Pseudomonas cepacia* 383 was sampled 5 min prior to culture lysis. Electron-dense particles (arrows) occupy the ribosome-rich regions of the cell. Note the hexagonal shape of some particles. (*Courtesy of T. G. Lessie, University of Massachusetts.*)

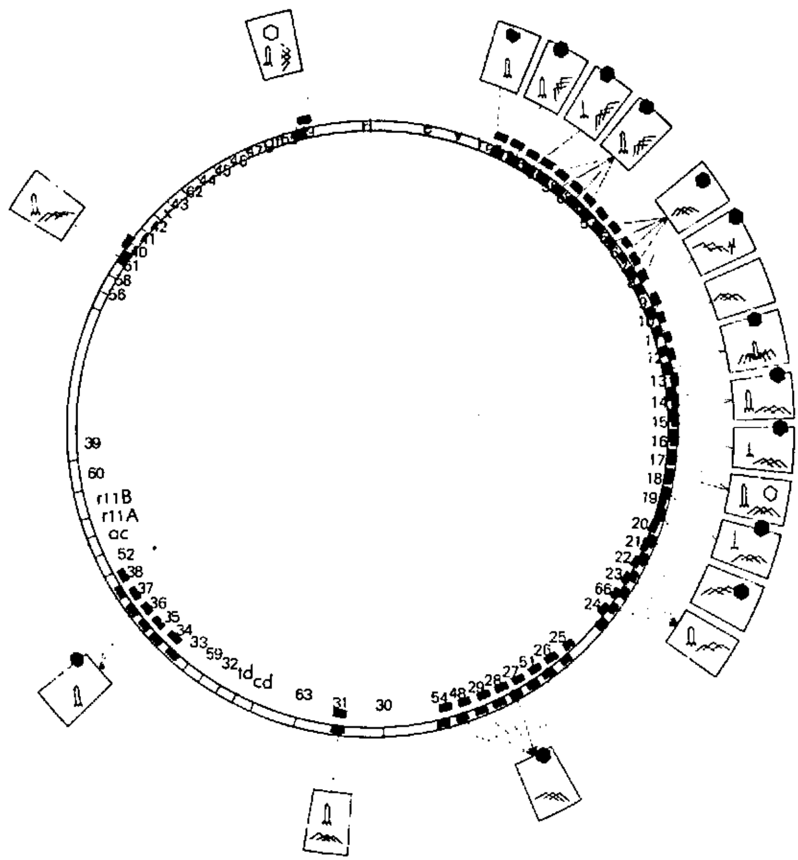


Figure 20-15. A simplified genetic map of the T4 virus. The squares show the morphological element whose production is governed by a particular gene. (*Based on data from W. B. Wood and H. R. Revel, Bacteriol Rev,* **40**:*847, 1976.*)

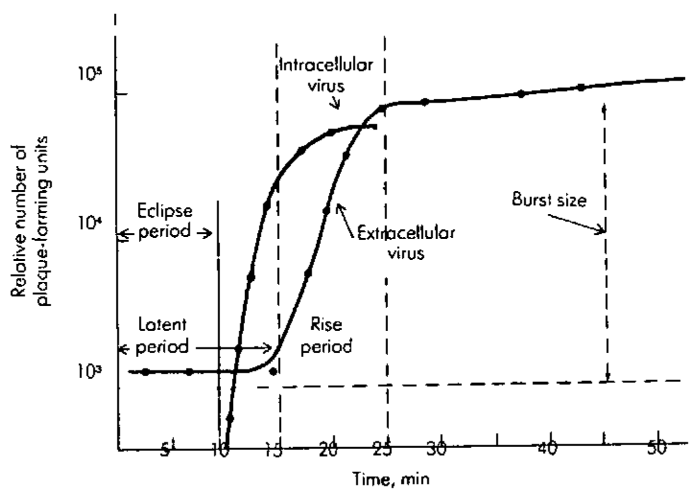


Figure 20-16. One-step growth curve of plaque-forming units. In a one-step growth experiment, after adsorption of the virus on the host, the suspension is diluted to such an extent that virus particles released after the first round of replication cannot attach to uninfected cells; thus only one round of replication can occur. Each plaque-forming unit is equal to one phage particle in the original suspension.

to infect other bacteria and begin the cycle over again. The overall process of phage infection and replication is shown in Fig. 20-10. The assembly of phage T4 is illustrated in Fig. 20-14. As may be seen from this figure, three different pathways are involved in the formation of a head, tail, and tail fibers, each being genetically controlled. Fig. 20-15 is a simplified genetic map of the T4 virus showing the 50 or so "architectural" genes which control the synthesis and assembly of new virus.

THE VIRAL MULTIPLICATION (REPLICATION) CYCLE

The sequence of events initiated by the injection of the phage nucleic acid and culminating in the release of newly synthesized virions is termed the **viral multiplication** or **replication cycle**. It can be plotted as a one-step multiplication cycle as shown in Fig. 20-16, which describes the production of progeny virions by cells as a function of time after infection under **one-step conditions** (i.e., the cells are infected simultaneously and secondary infection by progeny virus is eliminated by dilution).

Specifically, during the first 10 minutes or so after injection of phage DNA, no phage can be recovered by disrupting the infected bacterium. This is termed the **eclipse period**. At the end of this period, mature phages begin to accumulate intracellularly (Fig. 20-13) until they are released by cell lysis. No newly released extracellular phages can be seen until lysis begins; the time from infection until lysis is the **latent period** (see Fig. 20-16). The extracellular phage number increases until it reaches a constant **titer** (number assayed) at the end of the multiplication cycle; this time interval is termed the **rise period** (Fig. 20-16). The yield of phage per bacterium is called the **burst size** and is shown also in Fig. 20-16. This procedure affords observation of a single cycle of bacteriophage growth.

It may be mentioned that there are other phages which can replicate without drastic interruption of host cell physiology. For example, the filamentous single-stranded DNA phage fd can replicate within a host cell and is released from it

Medical Use of Virulent Phages

Since virulent or lytic phages can destroy their host bacterial cells, it was logical to think that inoculation of such phages into a bacteria-infected individual would result in elimination of the pathogens. However, after numerous studies, there is no evidence to show that phages can be used therapeutically to destroy bacterial pathogens in the human body, principally because phages do not persist in the body. Consequently, the primary uses of bacteriophages are in the identification of bacterial strains and as genetic models in molecular biology.

Thus lytic phages have been used in the detection and identification of pathogenic bacteria. Strains of bacteria (of a single species) may be characterized by their resistance or susceptibility to lysis by specific virulent phages. The resulting pattern of lysis (from visible plaques on a lawn of bacterial growth) of a bacterial strain by different phage types gives an indication of the identity of the bacterium. This laboratory procedure is termed **bacteriophage-typing** and is used routinely for the identification of certain strains of bacterial pathogens such as the staphylococci and the typhoid bacilli. In this way phages serve as a tool for medical diagnosis and for tracing of the source of a disease spreading in a community.

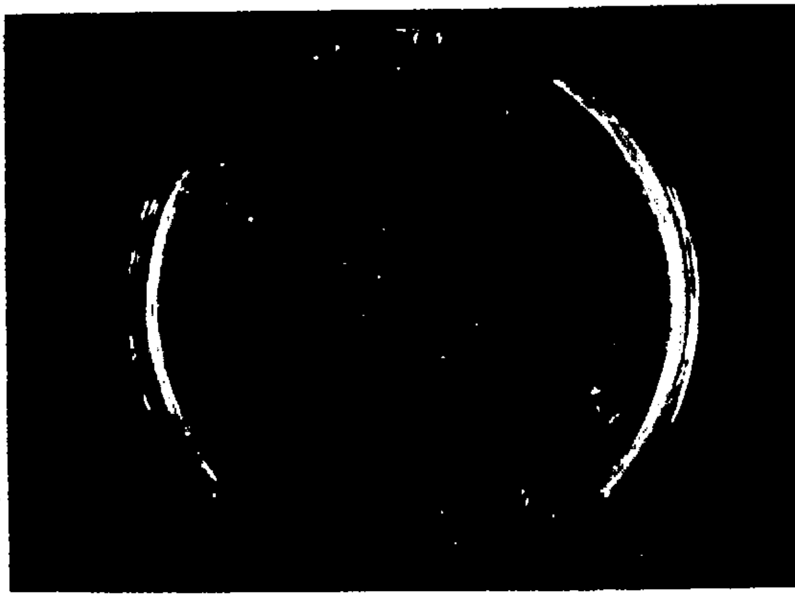


Figure 20-17. Plaques (clear zones) are formed when a bacterial growth in a Petri dish is lysed by a bacteriophage. (*Courtesy of C. Alfieri and E. C. S. Chan, McGill University.*)

Isolation and Cultivation of Bacterial Viruses

Bacterial viruses are easily isolated and cultivated in young, actively growing cultures of bacteria in broth or on agar plates. In liquid cultures, lysing of the bacteria may cause a cloudy culture to become clear, whereas in agar-plate cultures, clear zones, or **plaques**, become visible to the unaided eye (Fig. 20-17).

The principal requirement for isolation and cultivation of phages is that optimal conditions for growth of the host organisms be provided. The best and most usual source of bacteriophages is the host habitat. For example, coliphages or other phages pathogenic for other bacteria found in the intestinal tract can best be isolated from sewage or manure. This is done by centrifugation or filtration of the source material and addition of chloroform to kill the bacterial cells. A small amount (such as 0.1 ml) of this preparation is mixed with the host organism and spread on an agar medium. Growth of phage is indicated by the appearance of plaques in the otherwise opaque growth of the host bacterium as shown in Fig. 20-17.

as mature virions without accompanying cell lysis or death. That is, the infected cells continue to reproduce themselves as well as the virus, and the mature virions are extruded fom the cell surface continuously over a long period of time. This type of release mechanism is called a **productive infection.**

LYSOGENY

Not all infections of bacterial cells by phages proceed as described above to produce more viral particles and terminate in lysis. An entirely different relationship, known as **lysogeny,** may develop between the virus and its bacterial host (Fig. 20-18). In lysogeny the viral DNA of the temperate phage, instead of taking over the functions of the cell's genes, is incorporated into the host DNA and becomes a prophage in the bacterial chromosome, acting as a gene. In this situation the bacterium metabolizes and reproduces normally, the viral DNA being transmitted to each daughter cell through all successive generations. (Thus prophages behave as plasmids and are in fact considered as such by most molecular biologists.) Sometimes, however, for reasons unknown, the viral DNA is removed from the host's chromosome and the lytic cycle occurs. This process is called spontaneous induction. Infection of a bacterium with a temperate phage can be detected by the observations that the bacterium is resistant to infection by the same or related phages and that it can be induced to produce phage particles. A change from lysogeny to lysis can sometimes be induced by irradiation with ultraviolet light or by exposure to some chemical.

Mechanism of Lysogeny

A good part of our knowledge on lysogeny comes from studies on the coliphage lambda (λ). It is generally considered to be typical of the temperate phages.

When a sensitive bacterium is infected by a temperate phage, two things may happen. In some of the infected cells, multiplication of the phage occurs and a lytic cycle takes place. In the other infected cells (ranging from a few to 100 percent, depending on both the host and the phage), the multiplication of the phage is repressed (because late genes required for phage multiplication and host lysis are switched off) and lysogenization occurs. Specifically, the temperate phage possesses a gene that codes for a repressor protein which makes the cell resistant to lysis initiated either by the prophage or by lytic infection by other viruses. (Radiation or chemicals may induce release of the prophage from the host genome so that a lytic cycle can ensue.)

The repressor protein (also called immunity repressor since the cell is resistant to lysis from externally infecting phage) from λ phage has been isolated and purified. It is an acidic protein with a molecular weight of 26,000. It reacts with two different operator sites on the λ phage genome to prevent the expression of phage lytic functions and the formation of mature phage particles. Thus the repression of phage genes is very much like the repression of bacterial operons (discussed in Chap. 12).

More specifically, the lysogenic state is governed by the activity of the regulatory region of the λ phage genome, which both bestows immunity to externally infecting phages and causes integration of the phage genome into cellular DNA. This region is termed the immunity operon. Upon infection by λ phage, the phage *cro* gene is transcribed, resulting in the synthesis of a protein repressor that *inhibits* the synthesis of the immunity repressor. Thus the basic mechanism in the production and maintenance of the lysogenic state is the antagonism of two repressors—the immunity repressor and the *cro* repressor, which prevents immunity.

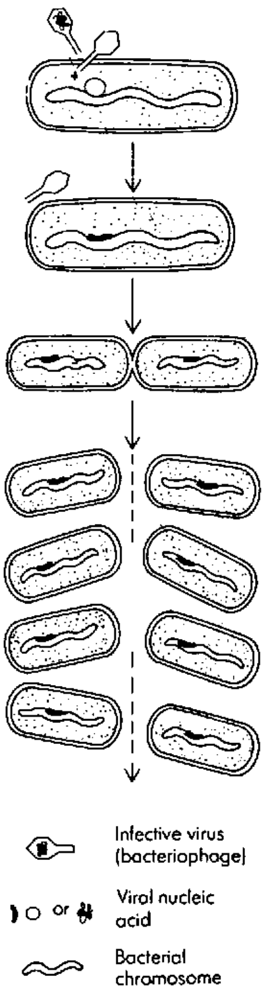


Figure 20-18. Lysogeny is a process in which the viral nucleic acid does not usurp the functions of the host bacterium's synthetic processes but becomes an integral part of the bacterial chromosome. As the bacterium reproduces, viral nucleic acid is transmitted to the daughter cells at each cell division. In the lysogenic state the virus is simply one of the bacterial genes. Under certain natural conditions or artificial stimuli (such as exposure to ultraviolet light), the synthesis of virus may take over, and lysis occurs.

As previously mentioned, the lytic cycle of bacteriophage λ can be induced by radiation, e.g., ultraviolet light. At the molecular level, this induces the synthesis of a host cell protein encoded in the *recA* gene of *E. coli*. This protein has proteolytic activity; once induced to accumulate, it cleaves the immunity repressor, preventing the latter from binding to the λ prophage. It is suggested that spontaneous induction of lysis may involve the same mechanism.

No RNA phages have yet been shown to be temperate. It is possible that temperate RNA phages exist; the phage could form a DNA copy of the RNA genome, which can then be integrated into the bacterial chromosome.

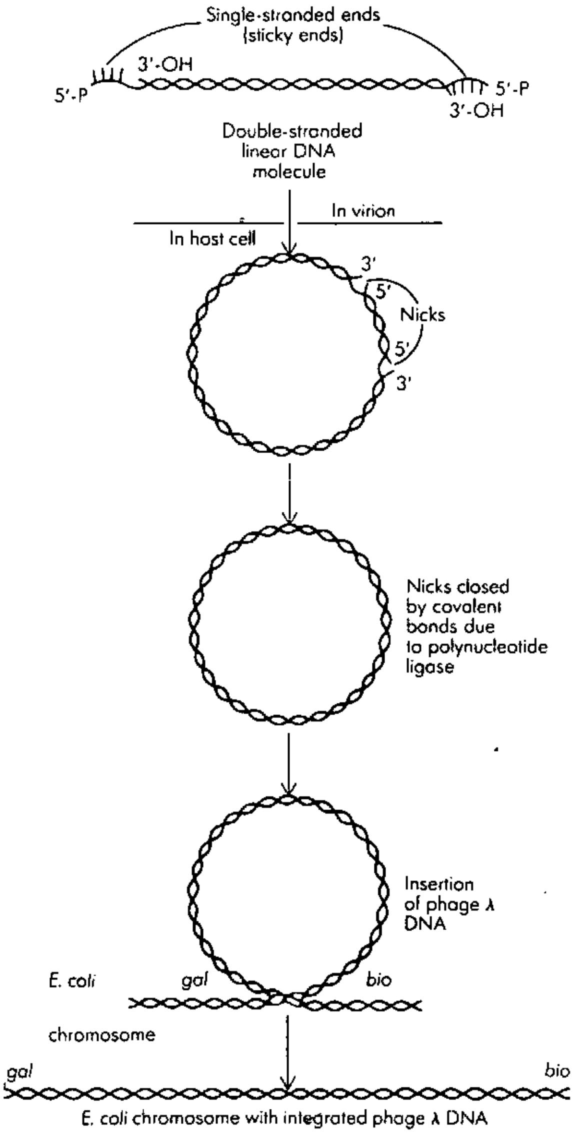


Figure 20-19. Conversion of phage λ linear DNA molecule into covalently closed ring and its insertion into the *Escherichia coli* chromosome.

Medical Aspects of Lysogeny

Diphtheria is caused by the bacterial pathogen *Corynebacterium diphtheriae*. Its capacity to cause disease is directly related to its ability to produce a toxin. It can only produce toxin when it carries a temperate phage. In the same way, only those streptococci which carry a temperate phage can produce the erythrogenic (rash-producing) toxin of scarlet fever. In another known instance, some types of botulism toxin are produced by *Clostridium botulinum* as a result of lysogeny. This phenomenon, in which a prophage is able to make changes in the properties of a host bacterium in lysogeny, is termed **lysogenic conversion**. Its occurrence is based on the fact that not all phage genes in lysogeny are blocked by the immunity repressor. Part of the phage DNA can be transcribed to form new proteins.

Bacterial lysogeny is a good conceptual model for the study of oncogenic, or cancer-producing viruses since these viruses also have the capacity of perpetuating their genomes in infected cells.

Prophage DNA Replication

A mechanism exists to allow the prophage DNA to replicate in step with the host cell DNA. When phage λ DNA is injected from the capsid (in which it exists as a linear double-stranded form), it rapidly forms into a closed circular molecule (Fig. 20-19). A λ phage enzyme, coded by the *int* gene, directs the insertion of the phage DNA into the bacterial chromosome. This enzyme recognizes a specific site on both the phage DNA and the bacterial chromosome and catalyzes a single site-specific recombination event, resulting in the insertion of the phage DNA into the cell genome (between the *gal* and *bio* genes) as shown in Fig. 20-19. It should be noted that different temperate phages have their own specific sites of integration on the bacterial chromosome. However, some temperate phages, such as phage Mu, have no site specificity for insertion and may even be able to insert multiple copies of their DNA into a single bacterial chromosome. Wherever insertion occurs, inactivation of the specific bacterial gene gives rise to a *mutant*; hence the name *phage Mu* was derived.

QUESTIONS

1 Describe the nature of a bacterial virus with respect to the following: (*a*) size, (*b*) growth, (*c*) metabolism, and (*d*) genome.
2 Define a virus and explain why it is defined that way.
3 Who coined the word *bacteriophage* and what does it mean?
4 Sketch seven morphological groups of phages and give an example of a phage for each group.
5 Why are bacteriophages suitable subjects for research in genetics?
6 What features distinguish a bacteriophage from a bacterium?
7 Define the following terms: lysogeny, lysis, temperate, and virulent.
8 Distinguish between the two structural forms of phages.
9 Explain why the icosahedron is considered the basic structure for all cubic viruses.
10 How would you describe the structure of the heads of the T2 and T4 phages? Why do we say that these phages have binal symmetry?

11 Give the names of five commonly studied phages and the nature of their nucleic acids.
12 Give some reasons for the slow and difficult development of phage taxonomy.
13 List the family names for the following viruses: T2, λ, T7, fd, and ϕX174.
14 Describe the differences between the filamentous coliphages and the tadpole-shaped ones.
15 Describe the technique by which phages are isolated.
16 What is the T series of bacteriophages?
17 Explain the process and application of phage-typing.
18 What are some unique features of phage adsorption on a host cell surface?
19 Describe the sequence of events that occurs when T-even phages penetrate an *E. coli* cell.
20 Describe the process of transcription in phage T4 infection with respect to immediate early, delayed early, and late gene products.
21 Explain the meaning of the following terms with reference to the viral multiplication cycle: eclipse period, latent period, rise period, and burst size.
22 In what way does the immunity operon of phage λ regulate lysogeny?
23 Explain the mechanism that allows prophage DNA to replicate in step with host DNA.
24 What is the relationship of temperate phages to the disease-producing ability of some bacteria? Why does this occur?

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