Survey of Protists

Protozoa and Slime Molds

Learning Objectives

By the end of this exercise you should be able to:

1. Describe the features characterizing each protozoan phylum, including slime molds.
2. List examples, habitats, reproductive methods, and unique features of the protozoan phyla, including slime molds.
3. Become familiar with phylum representatives.

MULTI-MEDIA Please visit **www.mhhe.com/vodopich10e** to review multi-media resources tailored to this lab.

Protozoans (*proto* = first, *zoan* = animal) are among the most versatile of all organisms on earth. Protozoa, however, like algae, is a descriptive term rather than a taxonomic group. Protozoans are eukaryotes with an animal-like, heterotrophic ecology, which means they are active consumers and not photosynthetic. (Remember that heterotrophs get their nutritional energy from organic molecules made by other organisms.) Typically, protozoans have food vacuoles to enclose food particles for digestion and contractile vacuoles to expel excess water. Their single cells employ a variety of features for motility, and they live everywhere from a drop of pond water to the intestines of termites. Review table 25.1 and the supergroups of protists that include common phyla of protozoans. Review table 26.1 for key characteristics of common protozoans.

AMOEBAS (PHYLUM RHIZOPODA)

Amoebas occur throughout the world in marine, freshwater, and terrestrial environments. Their unifying characteristic is the presence of **pseudopods,** movable extensions of cytoplasm used for locomotion and gathering food. Amoebas lack flagella, and most reproduce asexually. Amoebas have been traditionally unified within phylum Rhizopoda. However, further research may show that these protists with pseudopods are polyphyletic.

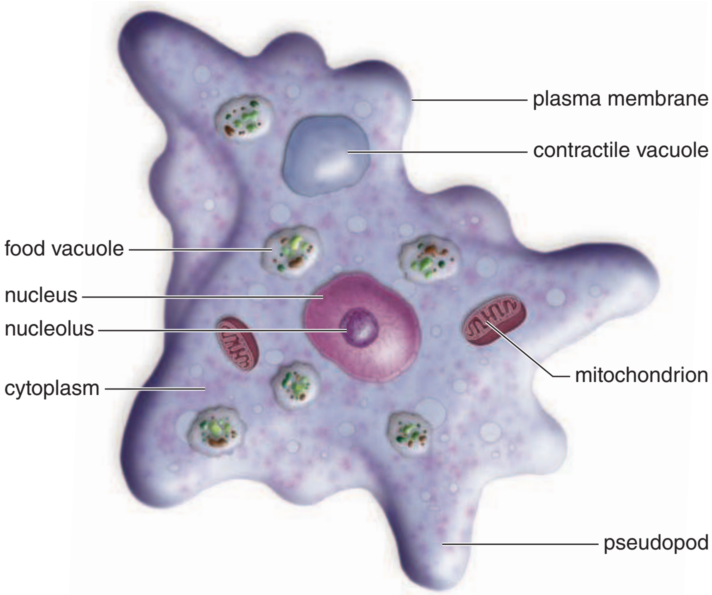


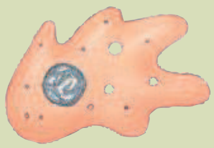
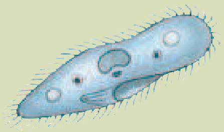
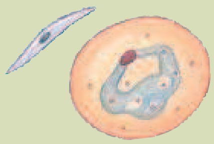
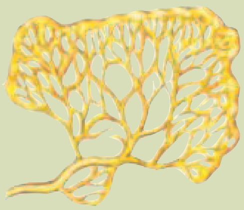
Figure 26.1
Phylum Rhizopoda. (*a*) Light micrograph of *Amoeba proteus* showing blunt pseudopodia (160×). (*b*) Anatomy of *Amoeba.*

Amoeba

Amoeba is a genus among many organisms commonly called amoebas that has a structure and physiology typical of most amoeboid genera (fig. 26.1). *Amoeba* are **phagocytic,** meaning they engulf food particles and form a **food vacuole** surrounded by a membrane. They then secrete enzymes into

Table 26.1

Types of Protists

Group	Phylum	Typical Examples		Key Characteristics
Heterotrophs with no Permanent Locomotor Apparatus				
Amoebas	Rhizopoda	*Amoeba*		Move by pseudopodia
Forams	Foraminifera	Forams		Rigid shells; move by protoplasmic streaming
Heterotrophs with Flagella				
Zoomastigotes	Sarcomastigophora	*Trypanosoma*		Heterotrophic; unicellular
Ciliates	Ciliophora	*Paramecium*		Heterotrophic unicellular protists with cells of fixed shape possessing two nuclei and many cilia; many cells also contain highly complex and specialized organelles
Nonmotile Spore-Formers				
Sporozoans	Apicomplexa	*Plasmodium*		Nonmotile; unicellular; the apical end of the spores contains a complex mass of organelles
Heterotrophs with Restricted Mobility				
Water molds	Oomycota	Water molds, rusts, and mildew		Terrestrial and freshwater
Cellular slime molds	Acrasiomycota	*Dictyostelium*		Colonial aggregations of individual cells; most closely related to amoebas
Plasmodial slime molds	Myxomycota	*Physarum*		Stream along as a multinucleate mass of cytoplasm

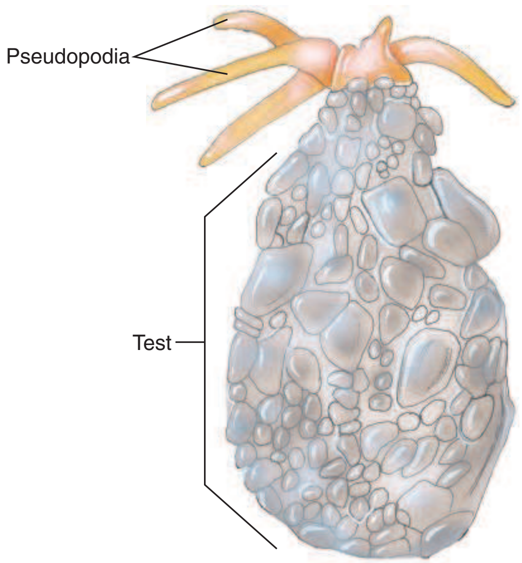


Figure 26.2

Difflugia oblongata, a common freshwater amoeba with a sand-grain case. The case consists of cemented mineral particles collected by the amoeba.

the food vacuole for **intracellular digestion.** A **contractile vacuole** maintains the cell's water balance by accumulating and expelling excess water. Other common amoebas include *Difflugia,* which makes a protective case of sand grains called a **test.** Test is a general term referring to a secreted or partially secreted covering, much like a shell (fig. 26.2). Examine a prepared slide of *Difflugia.* Also examine the amoeba *Entamoeba histolytica,* a parasite that causes dysentery in humans.

Procedure 26.1

Observe *Amoeba* movement and structure

1. Review microscope use and procedures in Exercise 3, as well as the associated videos at the lab manual's website. Use a dissecting microscope (see Exercise 3) to examine a culture of living *Amoeba.* Locate individuals on the bottom of the culture.
2. Prepare a wet mount of living *Amoeba* by using an eyedropper to remove a few drops from the bottom of the culture of organisms.
3. Put the drops in a depression slide if one is available or use a standard slide.

4. Cover the preparation with a coverslip and examine it under low power (10×) (see Exercise 3). Soon the *Amoeba* should move by extending their pseudopods.
5. If nutrient broth is available, add a drop to the preparation and observe the *Amoeba*'s response.
6. Examine a prepared slide of stained *Amoeba,* and locate the structures shown in figure 26.1.
7. Examine any other live amoebas and prepared slides that are available. Draw the basic structure of these organisms.

Question 1

a. Can you detect moving cytoplasm in the extending pseudopods of *Amoeba*?

b. What do you suppose the living *Amoeba* is moving toward or away from?

c. How does the *Amoeba* respond to nutrient broth?

d. About how long would it take an *Amoeba* to move across the field of view on low power?

e. Why is a contractile vacuole of a protozoan often more difficult to see than a food vacuole?

f. Why would excess water tend to accumulate in *Amoeba*?

FORAMINIFERANS (PHYLUM FORAMINIFERA)

These marine organisms are called "shelled amoebas" because they surround themselves with a secreted test and have long, thin, rather stiff pseudopods protruding from their tests (fig. 26.3). The test is made of calcium carbonate and is perforated with pores. The marine fossil record is replete with old and well-preserved tests of forams and is often used by oil companies to locate oil-bearing strata.

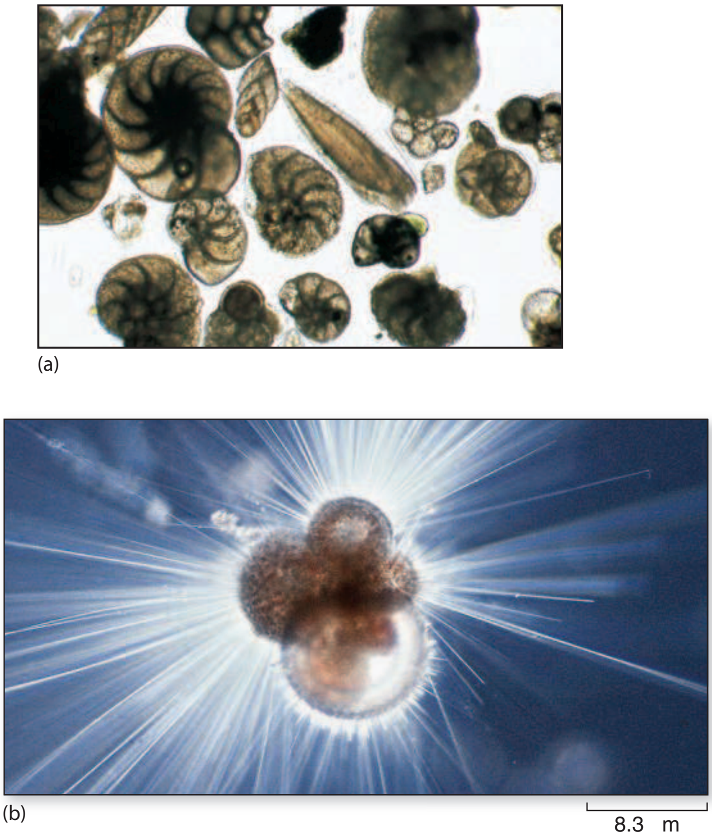


Figure 26.3
Formaminifera. (*a*) The calcereous tests, or shells, of some representative foraminiferans. (*b*) Podia, thin cytoplasmic projections, extend through pores in the calcareous test of this living foram.

Procedure 26.2
Examine foram tests

1. Obtain a prepared slide of foram tests.
2. Search with low magnification the edges of the cover slips for the foraminiferan tests. They are relatively heavy and shift to the side easily.

Question 2
How could fossilized forams in different geological layers of rock or sediment indicate the probability of finding oil?

FLAGELLATES (PHYLUM KINETOPLASTEA)

Flagellates have at least one **flagellum** and are likely the most primitive protozoans. Flagellates are parasitic as well as free-living heterotrophs.

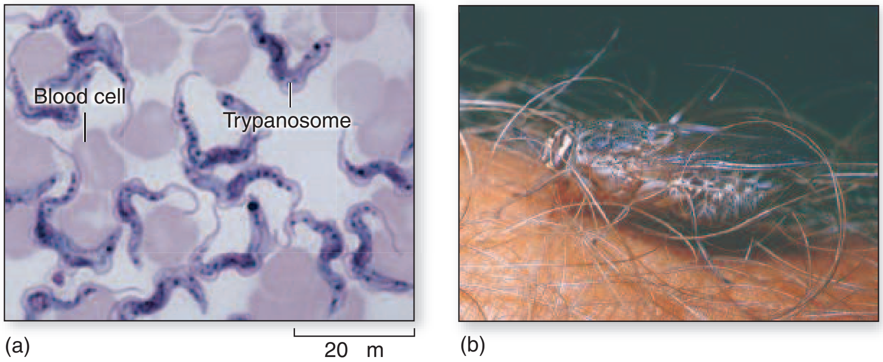


Figure 26.4
A parasite and its vector. (*a*) *Trypanosoma* among red blood cells. The nuclei (dark-staining bodies), anterior flagella, and undulating, changeable shape of the trypanosomes are visible in this photomicrograph. (*b*) The tsetse fly, shown here sucking blood from a human arm, can carry trypanosomes.

Trypanosoma

Among flagellates, trypanosomes (phylum Kinetoplastea) are pathogenic and cause African sleeping sickness and Chagas' disease. Charles Darwin may have died from Chagas' disease, for during his later years he suffered from general fatigue, irregular fever, and heart damage. All of these are symptoms of Chagas' disease transmitted by the bite of an assassin bug, which resembles a common stink-bug. Trypanosomes are common in the tropics and spread by infection from biting insects such as mosquitoes, sand flies, and tsetse flies (fig. 26.4*b*).

Procedure 26.3

Examine a prepared slide of *Trypanosoma* and compare its size to that of *Amoeba*

1. Obtain a prepared slide of *Trypanosoma* among blood cells.
2. Locate the organisms intermingled with the blood cells (fig. 26.4). The organisms are not inside the blood cells; they are in the surrounding plasma.
3. Try to distinguish the flagellum and undulating membrane of an individual. The **undulating membrane** is a thin, flat surface that can be undulated (i.e., waved) for locomotion. A rippling wave travels along the membrane and pushes the organism forward.
4. Trypanosomes are quite small. To estimate its size, first note the magnification of the objective you are using.
5. Refer to your notes from Exercise 3 (The Microscope) concerning the diameter of the field of view associated with the current magnification.
6. Estimate the portion of the diameter of the field of view occupied by a *Trypanosoma.* Calculate the size of a trypanosome.
7. Make a similar estimation of the size of *Amoeba* with a prepared slide at the same magnification.

Question 3

a. How large is a trypanosome relative to *Amoeba*?

b. What alga does a trypanosome superficially resemble?

CILIATES (PHYLUM CILIOPHORA)

More than 8000 species of ciliates have been described, all having characteristically large numbers of **cilia.** Review in your textbook the difference between cilia and flagella. Many ciliates also have two types of nuclei: **micronuclei** and **macronuclei** (fig. 26.5). Macronuclei, which develop from micronuclei, control cellular function, and they divide when ciliates reproduce asexually by mitosis.

Paramecium

This free-living, freshwater genus is widely studied and easily observed. *Paramecium,* like most ciliates, undergoes a sexual process called **conjugation** (fig. 26.6). During conjugation, individuals from two different strains align longitudinally and exchange nuclear material (fig. 26.6*b*). This exchange seems to stimulate metabolism of the individuals and is usually followed by frequent mitosis. Asexual reproduction is more common than conjugation and includes mitosis of the micronucleus and transverse fission (fig. 26.6*a*) of the macronucleus and cell body.

African Sleeping Sickness

Human African trypanosomiasis, also known as sleeping sickness, is a vector-borne parasitic disease. Parasites of genus *Trypanosoma* are transmitted to humans by the bite of tsetse flies (*Glossina* spp.; fig. 26.4*b*), which acquire their infection from humans or other infected animals (World Health Organization Fact Sheet No. 259). The most vulnerable people are in rural populations dependent on agriculture, fishing, animal husbandry, or hunting. *Trypanosoma brucei gambiense (T.b.g)* accounts for 95% of reported cases of sleeping sickness (fig. 26.A). After continued control efforts, the number of cases reported in 2009 dropped below 10,000 for the first time in 50 years. This trend continued in 2010 with 7139 new cases reported. For unknown reasons, many regions of 36 sub-Saharan affected countries have tsetse flies, but no sleeping sickness. A person can be infected for months or even years without developing the major signs and symptoms of the disease, which include fever, headaches, joint pains, and itching. Advanced stages disrupt the sleep cycle and disrupt coordination.

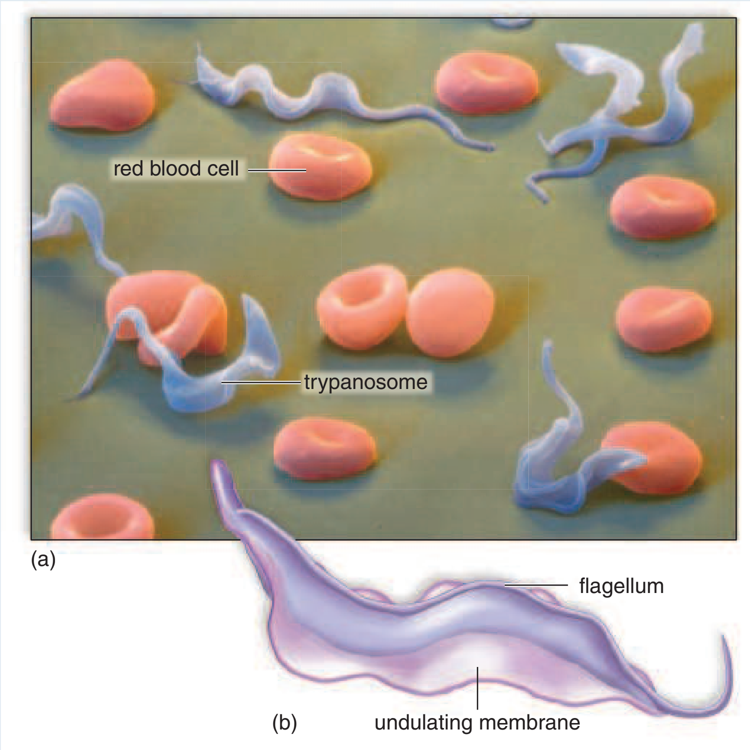


Figure 26.A

Trypanosoma brucei. (*a*) Micrograph of *Trypanosoma brucei*, a causal agent of African sleeping sickness, among red blood cells. (*b*) This drawing shows the general structure of *Trypanosoma brucei.*

Procedure 26.4

Examine conjugating and dividing *Paramecium*

1. Obtain a prepared slide of conjugating *Paramecium* and one of transversely dividing (binary fission) *Paramecium.*
2. Notice the alignment of the conjugating cells. Their nuclei are in close proximity.
3. Notice the plane of division of the transversely dividing cells.

Procedure 26.5

Observe living *Paramecium*

1. Prepare a wet mount from a culture of living organisms.
2. Add a drop of methylcellulose to your wet mount to slow the *Paramecium* and make it easier to examine.
3. Describe aspects of their movement in comparison to *Amoeba.*

4. Identify as many structures as possible in figure 26.5.

Question 4

a. Are cilia visible on living or prepared *Paramecium*?

b. Does *Paramecium* rotate as it moves?

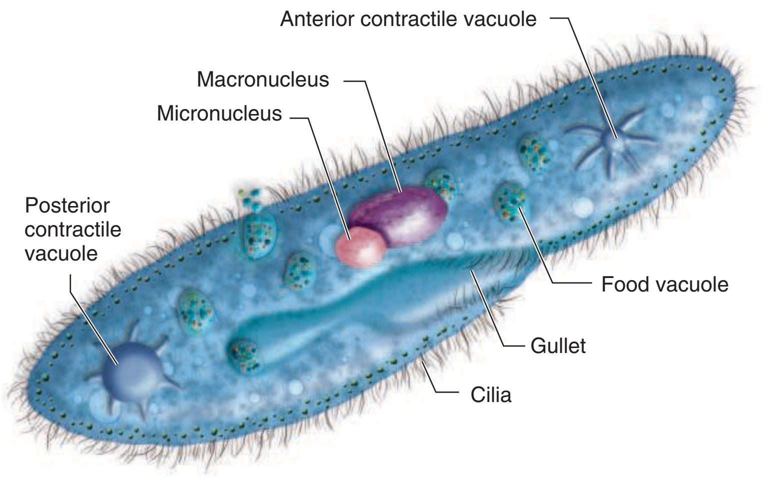


Figure 26.5
Paramecium. The main features of this familiar ciliate include cilia, two nuclei, and numerous specialized organelles.

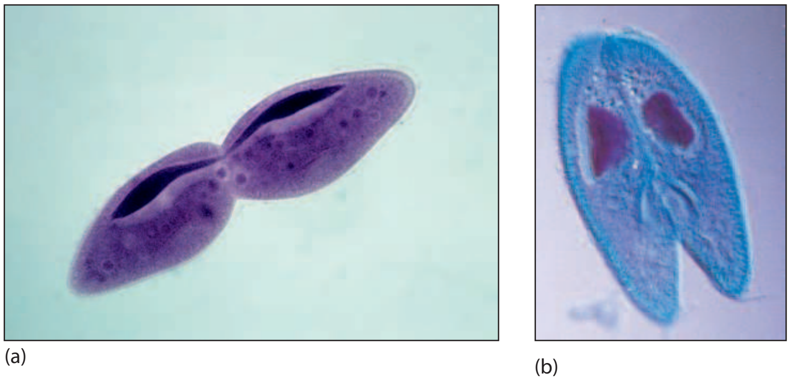


Figure 26.6
Reproduction among paramecia. (*a*) A mature *Paramecium* divides asexually by transverse fission. (*b*) During conjugation, individuals exchange genetic material. Conjugation is a sexual process.

c. How does movement of *Paramecium* compare with that of *Amoeba*? With a flagellated alga?

Question 5

a. Why is the division of *Paramecium* cells called "transverse" fission?

b. Why is transverse fission not a sexual process?

c. What are the advantages and disadvantages of conjugation in *Paramecium*?

Vorticella and Other Ciliates

This freshwater ciliate is sessile (i.e., attached to a substrate) and has two notable features: (1) a contractile stalk that attaches the organism to the substrate, and (2) a cell body with a corona of cilia. To feed, *Vorticella* extends its contractile stalk to push the cell body as far as possible from the substrate and from other individuals. Then it rapidly beats its cilia to capture food particles. This is a type of filter feeding (fig. 26.7).

Procedure 26.6

Examine *Vorticella* and other ciliates

1. Use a dissecting microscope to examine a living colony of *Vorticella,* which often grows on the glass and other hard substrates in stagnant aquaria.
2. Tap the sides of the dish and observe the contraction of each stalk.

3. When the stalks are extended, notice the whirling action of the cilia at the open end of each bell-shaped individual.

4. Examine a prepared and stained slide of *Vorticella* and draw its general shape.

5. Examine other cultures or prepared slides of ciliates available in the lab. Draw the general shape of these organisms and describe the movement of living specimens.

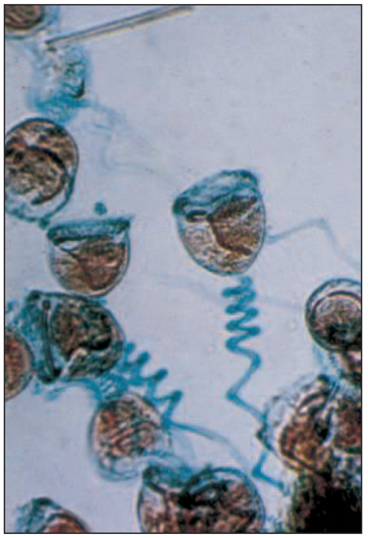


Figure 26.7
Vorticella, phylum Ciliophora, are heterotrophic, feed largely on bacteria, and have retractable stalks.

Question 6

a. What is the value or function of rapid contraction of the stalk of *Vorticella*?

b. What is the probable function of the moving cilia of *Vorticella*?

APICOMPLEXANS (PHYLUM APICOMPLEXA)

Apicomplexans are typically nonmotile parasites of animals. Frequently, these parasites have complex life histories and life stages with various morphologies occurring in multiple hosts.

Plasmodium

This pathogen is the best-known apicomplexan and has been the most common killer of humans in history. *Plasmodium* cause malaria, and mosquitoes of the genus *Anopheles* transmit *Plasmodium* from human to human (fig. 26.8). These malarial parasites infect and rupture red blood cells, causing cycles of fever and chills.

Procedure 26.7

Examine a blood smear from a victim of malaria

1. Obtain a prepared slide with *Plasmodium.*

2. Locate the infected blood cells. The organisms are inside the infected blood cells, not in the surrounding plasma as are trypanosomes.

3. Locate and compare infected and uninfected blood cells. The infected cells will exhibit only faint signs of the circular ring stage of the parasite. Sketch what you see (fig. 26.9).

4. Review in your textbook the life cycle of *Plasmodium* (fig. 26.8).

SLIME MOLDS (PHYLUM DICTYOSTELIA)

Slime molds have often been classified in kingdom Fungi, but they have amoeboid characteristics such as phagocytic

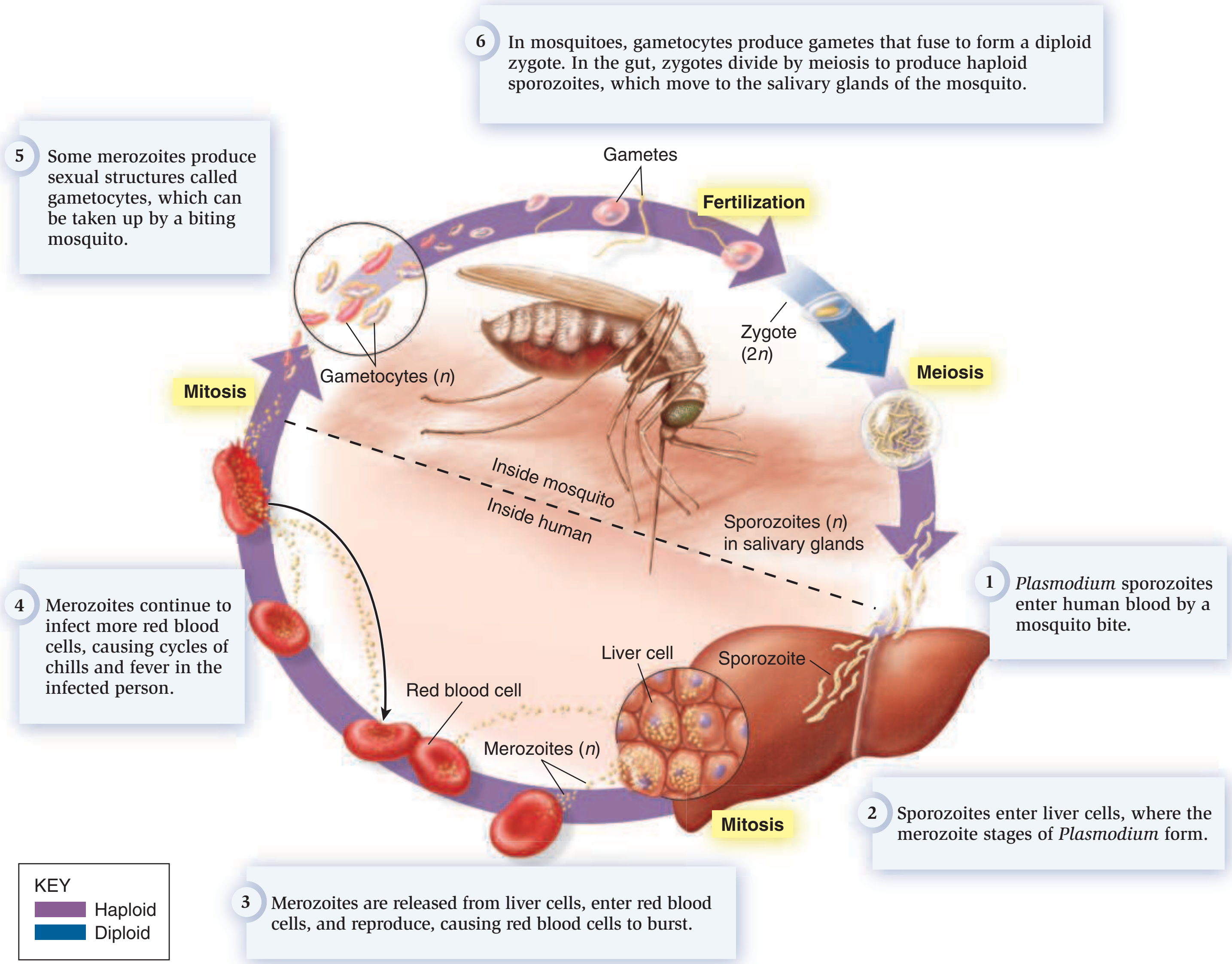


Figure 26.8

Diagram of the life cycle of *Plasmodium falciparum*, the agent of malaria. This life cycle requires two alternate hosts, humans and *Anopheles* mosquitoes.

nutrition and unique unicellular forms and assemblages. They also lack the prominent hyphae of fungi. Slime molds also do not contain chitin in their cell walls as do fungi. Hence, we will classify slime molds as protists. In this exercise you will examine *Physarum* as a representative slime mold.

Bizarre plasmodial slime molds such as *Physarum* stream along the damp forest floor in a mass of brightly colored protoplasm called a **plasmodium** in which individual cells are indistinguishable. Plasmodia are coenocytic (multinucleate) because their nuclei are not separated by cell walls, and they resemble a moving mass of slime. The plasmodium of a slime mold should not be confused with the sporozoan genus with the same name.

Often plasmodia of slime molds live beneath detached bark on decomposing tree trunks. Slime mold plasmodia occasionally occur on lawns or on mulch beneath shrubs. The moving protoplasm of the plasmodia conspicuously pulsates back and forth as they engulf and digest bacteria, yeasts, and other organic particles.

Procedure 26.8

Examine a culture of *Physarum*

1. Obtain a dissecting microscope and a petri plate containing a culture of *Physarum* growing on oatmeal flakes.
2. Examine the yellowish trails of the colony under the highest magnification of the microscope. Look for cytoplasmic movement.

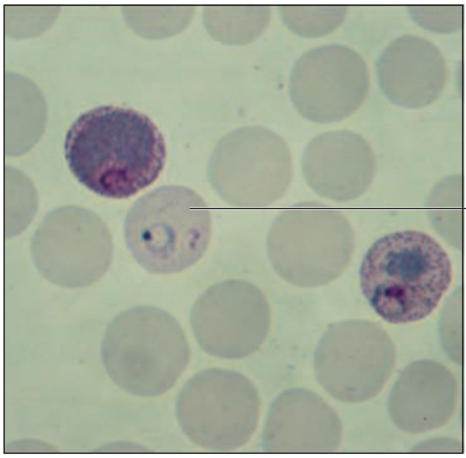


Figure 26.9
Malarial parasite, *Plasmodium*, within a red blood cell (500×).

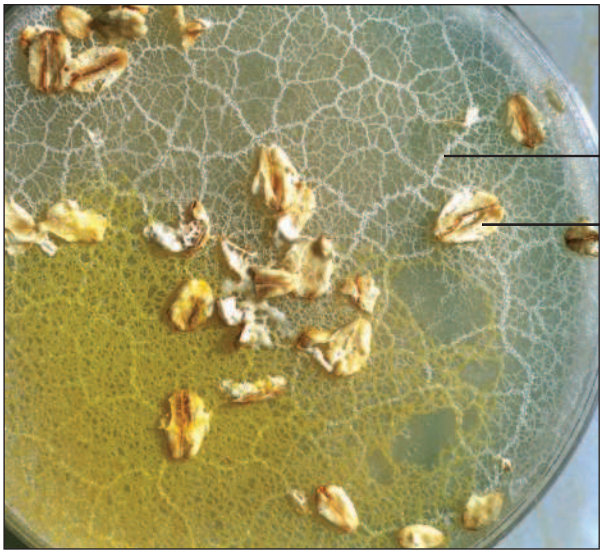


Figure 26.10
Slime mold (*Plasmodium*). Slime molds (division Myxomycota) share characteristics with fungi but also with protistans. Their life cycle includes a plasmodial stage, which is a thin, multinucleate mass of streaming protoplasm with amoeboid or flagellated cells.

3. Look for signs of the organism beginning to condense into darkly tipped sporangia. Sporangia are easily visible without a microscope.

Question 7

a. Is cytoplasmic movement of *Physarum* apparent?

b. Is the movement in a particular direction?

c. What is a possible function of cytoplasmic movement in *Physarum*?

The *Physarum* on demonstration is in the vegetative plasmodial stage and feeds on the oatmeal (fig. 26.10). However, if environmental conditions become less than optimal (e.g., if food or moisture decreases), the plasmodium may dry into a hard resistant structure called a **sclerotium** and remain dormant until conditions improve. Or, if light is available, the diploid plasmodium will move to the illuminated area and coalesce. The condensed structure will grow sporangia, and meiosis will produce spores for dispersal (fig. 26.11). Light is associated with an open environment that allows successful reproductive dispersal; dispersal under or within a tree trunk would be ineffective. Haploid spores produced by meiosis in the sporangia germinate as amoeboid or flagellated organisms. These haploid stages may later fuse as gametes and grow into a new plasmodium.

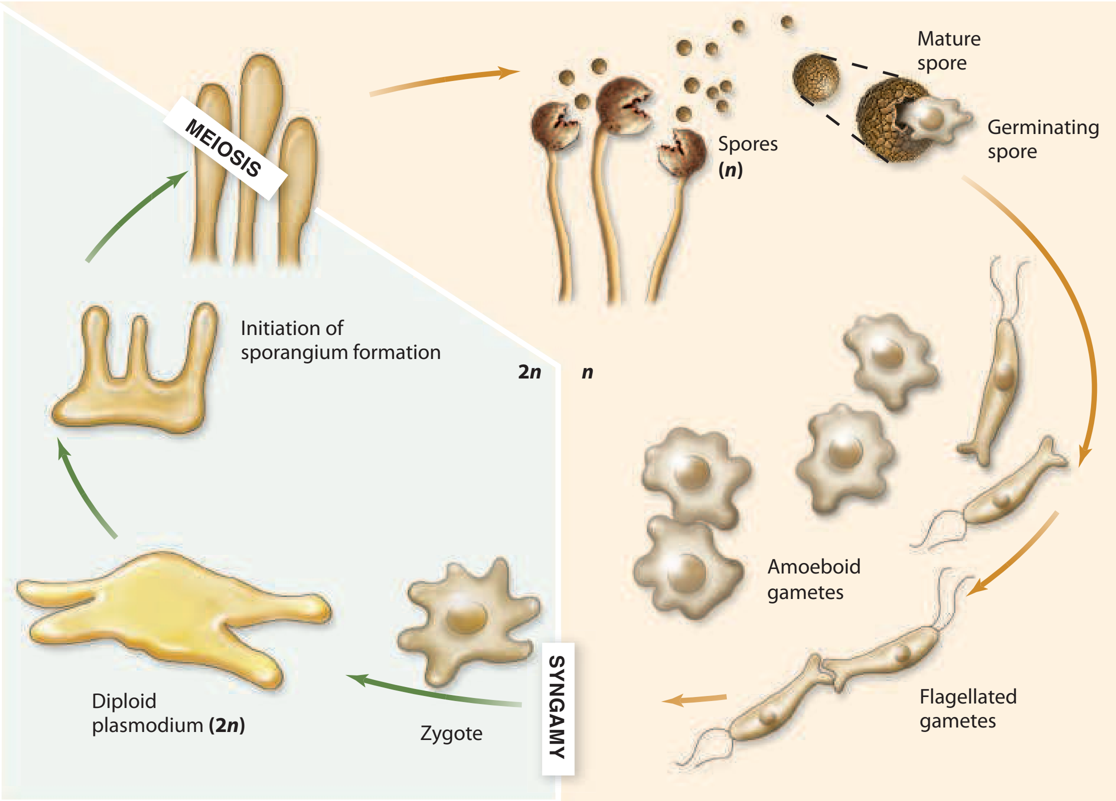


Figure 26.11

Life cycle of a plasmodial slime mold. When food or moisture is scarce, a diploid plasmodium stops moving and forms sporangia. Haploid spores form by meiosis. The spores wait until conditions are favorable to germinate. Spores can give rise to flagellated or amoeboid gametes; the two forms convert from one to the other readily. Fusion of the gametes (syngamy) forms the diploid zygote, which gives rise to the mobile, feeding plasmodium by mitosis.

INVESTIGATION

The Sensitivity of Protozoa to Nutrients

Observations: Protozoa are particularly sensitive to changes in their aquatic world, including changes in the amount and type of nutrients in the water. Nutrient broth elicits a response from protozoans seeking food. Nutrients include a variety of organic compounds, including fats, carbohydrates, and proteins.

Question: What kinds of nutrients elicit the strongest response from protozoa?

a. Establish a working lab group and obtain Investigation Worksheet 26 from your instructor.

b. Discuss with your group ways to measure protozoan response to introduced solutes. Pose a well-defined question relevant to the preceding observation and question. Record your question on Worksheet 26.

c. Translate your question into a testable hypothesis and record it.

d. Outline on Worksheet 26 your experimental design and supplies needed to test your hypothesis. Ask your instructor to review your proposed investigation.

e. Conduct your procedures, record your data, answer your question, and make relevant comments.

f. Discuss with your instructor any revisions to your questions, hypotheses, or procedures. Repeat your work as needed.

Questions for Further Thought and Study

1. What requirements might make culturing parasitic zoomastigotes difficult in the lab?

2. Why do some scientists call conjugation "sexual reproduction" and others do not?

3. Is the cell the fundamental unit of life in plasmodial slime molds? Or is the "whole organism," regardless of cellular composition, the fundamental unit? Explain your answer.

4. What functions do cilia, flagella, and pseudopods have in common?

5. What factors may account for the ubiquitous occurrence and great structural diversity of unicellular organisms?

6. In what sense are protists "primitive" and in what sense are they "advanced"?

7. Why are unicellular organisms that reproduce by mitosis considered immortal?