PROTOZOA

Introduction

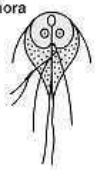
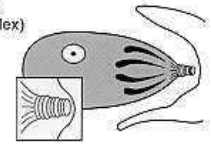
The Protozoa are considered to be a subkingdom of the kingdom Protista, although in the classical system they were placed in the kingdom Animalia. Most of them are free-living organisms; and can be found in almost every possible habitat. Anton van Leeuwenhoek was the first person to see protozoa, using microscopes he constructed with simple lenses, and he described, in addition to free-living protozoa, several parasitic species from animals, and *Giardia lamblia* from his own stools. Virtually all humans have protozoa living in or on their body at some time, and many persons are infected with one or more species throughout their life. Some species are considered commensals, i.e., normally not harmful, whereas others are pathogens and usually produce disease.

Protozoan diseases range from very mild to life-threatening. Individuals whose defenses are able to control but not eliminate a parasitic infection become carriers and constitute a source of infection for others. In geographic areas of high prevalence, well-tolerated infections are often not treated to eradicate the parasite because eradication would lower the individual's immunity to the parasite and result in a high likelihood of reinfection. Many protozoan infections that are not apparent or mild in normal individuals can be life-threatening in immunosuppressed patients, particularly patients with acquired immune deficiency syndrome (AIDS).

Protozoan organisms

Common name of organism or disease	Latin name	Body parts affected
Balantidiasis	*Balantidium coli*	intestinal mucosa
Amoebiasis	*Entamoeba histolytica*	Intestines
Giardia	*Giardia lamblia*	Lumen of the small intestine
Leishmaniasis	*Leishmania*	Cutaneous, mucocutaneous,or visceral
Malaria	*Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale,Plasmodium malariae*	red blood cells
Trichomoniasis	*Trichomonas vaginalis*	female urogenital tract (males asymptomatic)
Sleeping sickness	*Trypanosoma brucei*	blood lymph and central nervous systems
Chagas disease	*Trypanosoma cruzi*	colon, esophagus, heart, nerves, muscle and blood

Table 77-1. Classification of Parasitic Protozoa and Associated Diseases

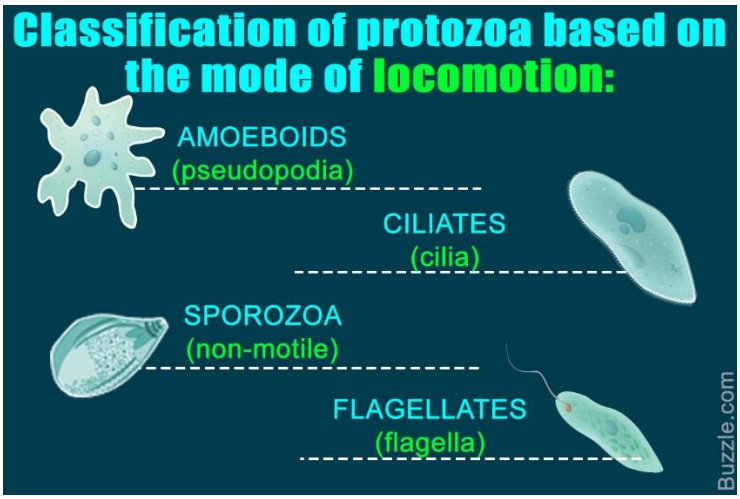
Phylum	Subphylum	Representative Genera	Major Diseases Produced in Human Beings	Chapter
Sarcomastigophora (with flagella, pseudopodia, or both) 	Mastigophora (flagella)	*Leishmania*	Visceral, cutaneous and mucocutaneous infection	82
		Trypanosoma	Sleeping sickness Chagas' disease	
		Giardia	Diarrhea	80
		Trichomonas	Vaginitis	
	Sarcodina (pseudopodia)	*Entamoeba*	Dysentery, liver abscess	79
		Dientamoeba	Colitis	
		Naegleria and *Acanthamoeba*	Central nervous system and corneal ulcers	81
		Babesia	Babesiosis	
Apicomplexa (apical complex) 		*Plasmodium*	Malaria	83
		Isospora	Diarrhea	80
		Sarcocystis	Diarrhea	
		Cryptosporidium	Diarrhea	
		Toxoplasma	Toxoplasmosis	84
Microspora		*Enterocytozoon*	Diarrhea	—
Ciliophora (with cilia) 		*Balantidium*	Dysentery	80
Unclassified	—	*Pneumocystis*	Pneumonia	85

Acanthamoeba species are free-living amoebas that inhabit soil and water. Cyst stages can be airborne. Serious eye-threatening corneal ulcers due to *Acanthamoeba* species are being reported in individuals who use contact lenses. The parasites presumably are transmitted in contaminated lens-cleaning solution. Amoebas of the genus *Naegleria*, which inhabit bodies of fresh water, are responsible for almost all cases of the usually fatal disease primary amoebic meningoencephalitis. The amoebas are thought to enter the body from water that is splashed onto the upper nasal tract during swimming or diving. The lack of effective vaccines, the paucity of reliable drugs, and other problems, including difficulties of vector control, prompted the World Health Organization to target six diseases for increased research and training. Three of these were protozoan infections; Malaria, Trypanosomiasis, and Leishmaniasis.

Structure

Most parasitic protozoa in humans are less than 50 μm in size. The smallest (mainly intracellular forms) are 1 to 10 μm long, but *Balantidium coli* may measure 150 μm. Protozoa are unicellular eukaryotes. As in all eukaryotes, the nucleus is enclosed in a membrane. The organelles of protozoa have functions similar to the organs of higher animals. The plasma membrane enclosing the cytoplasm also covers the projecting locomotory structures such as pseudopodia, cilia, and flagella. The outer surface layer of some protozoa, termed a pellicle, is sufficiently rigid to maintain a distinctive shape, as in the trypanosomes and Giardia. In most protozoa the cytoplasm is differentiated into ectoplasm (the outer, transparent layer) and endoplasm (the inner layer containing organelles); the structure of the cytoplasm is most easily seen in species with projecting pseudopodia, such as the amoebas. Some protozoa have a cytosome or cell “mouth” for ingesting fluids or solid particles. Contractile vacuoles for osmoregulation occur in some,

such as *Naegleria* and *Balantidium*. Many protozoa have subpellicular microtubules; in the Apicomplexa, which have no external organelles for locomotion, these provide a means for slow movement. Many other structures occur in parasitic protozoa, including the Golgi apparatus, mitochondria, lysosomes, food vacuoles, conoids in the Apicomplexa, and other specialized structures.



[List and Images of Free living and parasitic protozoans]

Classification

In 1985 the Society of Protozoologists published a taxonomic scheme that distributed the Protozoa into six phyla. Two of these phyla; the Sarcomastigophora and the Apicomplexa, contain the most important species causing human disease. This scheme is based on morphology as revealed by light, electron, and scanning microscopy. *Dientamoeba fragilis*, for example, had been thought to be an amoeba and placed in the family Entamoebidae. However, internal structures seen by electron microscopy showed that it is properly placed in the order *Trichomonadida* of flagellate protozoa. In some instances, organisms that appear identical under the microscope have been assigned different species names on the basis of such criteria as geographic distribution and clinical manifestations; a good example is the genus *Leishmania*, for which subspecies names are often used. Biochemical methods have been employed on strains and species to determine isoenzyme patterns or to identify relevant nucleotide sequences in RNA, DNA, or both. Extensive studies have been made on the kinetoplast, a unique mitochondrion found in the hemoflagellates and other members of the order *Kinetoplastida*. The DNA associated with this organelle is of great interest. Cloning is widely used in taxonomic studies, for example to study differences in virulence or disease manifestations in isolates of a single species obtained from different hosts or geographic regions. Antibodies (particularly monoclonal antibodies) to known species or to specific antigens from a species are being employed to identify unknown isolates. Eventually, molecular taxonomy may prove to be a more reliable basis than morphology for protozoan taxonomy, but the microscope is still the most practical tool for identifying a protozoan parasite.

Table A.1: Classification of Protozoa

	PHYLUM PROTOZOA						
SUBPHYLUM:	PLASMODROMA	SARCOMASTIGOPHORA			SPOROZOA		CILIOPHORA
CLASS:	RHIZOPODA	MASTIGOPHORA			TELOSPOREA		CILIATEA
SUBCLASS:					COCCIDIA		
ORDER:	Amoebida	Protomonadida		Diplomonadida	Eucoccidiida		
SUBORDER:		Intestinal/ Oral cavity	In blood and tissues	In intestine	Eimerinae	Haemosporina	
GENUS:	*Entamoeba*	*Chilomastix*	*Trypanosoma*	*Giardia*	*Toxoplasma*	*Plasmodium*	*Balantidium*
SPECIES:	*E. histolytica*	*C. mesnili*	*T. Brucei* subgroup	*G. intestinalis*	*T. gondii*	*P. vivax*	*Bal. coli*
"	E. coli	Genus: *Trichomonas*				*P. falciparum*	
"	E. gingivalis	*Tr. hominis* (Syn. Tr. intestinalis)	*T. cruzi*			*P. malariae*	
	GENUS: Endolimax	Tr. vaginalis					
"	*E. nana*	*Tr. tenax*				*P. ovale*	
	Iodamoeba	GENUS: *Enteromonas*	GENUS: *Leishmania*				
	I. butschlii	*E. hominis*	*L. donovani*				
GENUS: *Dientamoeba*		GENUS: *Embadomonas*	*L. tropica*				
D. fragilis		*G. intestinalis*	*L. braziliensis*				

CLASSIFICATION OF MEDICALLY IMPORTANT PARASITES

PROTOZOA

Sarcodina (Amoebae)

(a) Genus, *Entameba* E.g. *Entameba histolytica*
(b) Genus *Endolimax* E.g. *Endolimax nana*
(c) Genus *Iodameba* E.g. *Iodameba butchlii*
(d) Genus *Dientmeba* E.g. *Dientameba fragilis*

Mastigophora (Flagellates)

(a) Genus *Giardia* E.g. *G. lamblia*
(b) Genus *Trichomonas* E.g. *T. vaginalis*
(c) Genus *Trypanosoma* E.g. *T. brucci*
(d) Genus *Leishmania* E.g. *L. Donovani*

Sporozoa

(1) Genus *Plasmodium* E.g. *P. falciparum*
(2) Genus *Toxoplasma* E.g. *T. gondi*
(3) Genus *Cryptosporidum* E.g. *C. parvum*
(4) Genus *Isospora* E.g. *I. Beli*

Ciliates

(a) Genus *Balantidium* E.g. *B. coli*

The basic generally accepted practical classification of the medically important parasitic protozoa

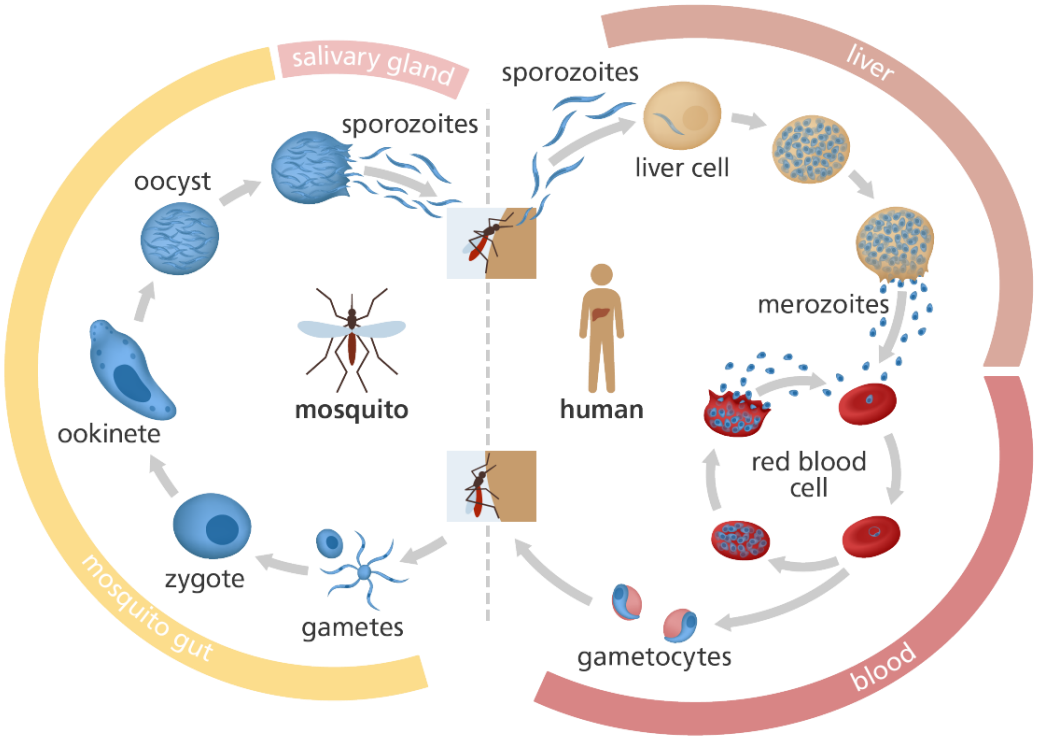
- **Amoebae**
  - *Entamoeba histolytica*
  - *Acanthamoeba species*
  - *Naegleria species*
- **Flagellates**
  - *Giardia lamblia*
  - *Trichomonas vaginalis*
  - *Trypanosoma species*
  - *Leishmania species*
- **Ciliates**
  - *Balantidium coli*
- **Coccidia**
  - Blood and tissue coccidia:
    - *Plasmodium species*
    - *Toxoplasma gondii*
  - Intestinal coccidia:
    - *Isospora belli*
    - *Cryptosporidium parvum*
    - *Cyclospora cayetanensis*
- **Microsporidia**
  - *Encephalitozoon species*
  - *Enterocytozoon species*

Table 5.5 : A classification scheme of protozoa based on r-RNA homology and mitochondria

Phyla	Major Characteristics	Examples
Archaezoa	Protozoal cells without mitochondria. Flagella two or more emerging from the anterior end. Some are parasitic. Others live symbiotically in the digestive tracts of animals. Cysts may be present.	*Trichomonas Giardia*
Microsporidia	Cells without mitochondria and microtubules. Obligate intracellular parasites, including some human pathogenic forms causing diarrhoea and keratoconjunctivitis.	*Nosema*
Rhizopoda	Protozoa exhibiting amoeboid movement with the help of indefinite number of pseudopodia. Phagotrophic. Mostly free-living. The only human pathogenic forms are species of *Entamoeba* which thrive in the intestines and mouth. Some amoebae have chalky or siliceous shells having pores through which the pseudopodia are projected. These amoebae are mostly marine. Cyst formation is known in certain amoebae. Cells reproduce asexually by binary fission.	*Amoeba Entamoeba Foraminifera Radiolaria Heliozoa Arcella*
Apicomplexan	Protozoa have an apical complex of some special organelles. All are obligate parasites. Commonly known as sporozoa, because they form spores. The organisms are without flagella or cilia in their mature stage, but may form motile gametes. They are unable to engulf solid food. The protozoa reproduce both asexually and sexually. Some, malarial like parasite, have a complicated life-cycle, requiring two hosts to complete life-cycle.	*Plasmodium Toxoplasma Babesia*
Ciliophora	Cells with numerous cilia arranged in precise rows and two types of nuclei, sexual reproduction is by conjugation. Cells divide by transverse binary fission. Majority are free-living feeding on smaller microorganisms including protozoa by phagotrophy. Food particles are ingested through a specialized structure, called cytostome. Cyst formation occurs in some. Only human pathogenic ciliate in *Balantidium coli*.	*Paramecium Tetrahymena Balantidium Colpoda Vorticella Stentor*
Euglenozoa	This phylum includes two different groups of flagellated protozoa. They are the *Euglena*-like photosynthetic flagellates and the hemoflagellates, like *Trypanosoma* which are blood parasites. The justifications for including two apparently dissimilar groups in this phylum are similarity in r-RNA sequences and the presence of discoid mitochondria in both.	*Euglena Trypanosoma*

Life Cycle Stages

During its life cycle, a protozoan generally passes through several stages that differ in structure and activity. Trophozoite (Greek for "animal that feeds") is a general term for the active, feeding, multiplying stage of most protozoa. In parasitic species this is the stage usually associated with pathogenesis. In the hemoflagellates the terms amastigote, promastigote, epimastigote, and trypomastigote designate trophozoite stages that differ in the absence or presence of a flagellum and in the position of the kinetoplast associated with the flagellum. A variety of terms are employed for stages in the Apicomplexa, such as tachyzoite and bradyzoite for *Toxoplasma gondii*. Other stages in the complex asexual and sexual life cycles seen in this phylum are the merozoite (the form resulting from fission of a multinucleate schizont) and sexual stages such as gametocytes and gametes. Some protozoa form cysts that contain one or more infective forms. Multiplication occurs in the cysts of some species so that excystation releases more than one organism. For example, when the trophozoite of *Entamoeba histolytica* first forms a cyst, it has a single nucleus. As the cyst matures nuclear division produces four nuclei and during excystation four uninucleate metacystic amebas appear. Similarly, a freshly encysted *Giardia lamblia* has the same number of internal structures (organelles) as the trophozoite. However, as the cyst matures the organelles double and two trophozoites are formed. Cysts passed in stools have a protective wall, enabling the parasite to survive in the outside environment for a period ranging from days to a year, depending on the species and environmental conditions. Cysts formed in tissues do not usually have a heavy protective wall and rely upon carnivorism for transmission. Oocysts are stages resulting from sexual reproduction in the Apicomplexa. Some apicomplexan oocysts are passed in the feces of the host, but the oocysts of Plasmodium, the agent of malaria, develop in the body cavity of the mosquito vector.



A. *Giardia* species	1. Protozoan parasite residing in reproductive tract of cattle
B. *Entamoeba histolytica*	2. Equine piroplasm – pear-shaped organisms that may join, giving the effect of a Maltese cross
C. *Balantidium coli*	3. "Onion skin" tissue cysts found in skeletal muscle of dogs transmitted by ingestion of tick, *Amblyomma americanum*
D. *Cystoisospora* species in dogs and cats	4. Intestinal protozoan of cats (only definitive host) – zoonotic parasite.
E. *Toxoplasma gondii*	5. Coccidiosis in cattle
F. *Trypanosoma cruzi*	6. Infectious enterohepatitis – "blackhead" in turkeys – intermediate host is *Heterakis gallinarum*
G. *Babesia canis*	7. Greatly distorts the shape of the avian WBC
H. *Cytauxzoon felis*	8. Canine piroplasm – basophilic, pear-shaped organism within canine RBCs
I. *Hepatozoon americanum*	9. Amebic dysentery in humans
J. Rumen ciliates	10. Unique oocysts that are the largest of the coccidians
K. *Eimeria bovis, Eimeria zuernii*	11. Very large ciliated protozoan found in the large intestine of swine
L. *Cryptosporidium* species	12. Colorless sporulated oocysts – 3 to 5 μm in diameter on fecal flotation (zoonotic parasite).
M. *Babesia bigemina*	13. Bluish, sausage-shaped body within the cytoplasm of the avian RBC
N. *Tritrichomonas foetus*	14. Cecal ciliate of guinea pig – usually nonpathogenic, but may be observed in diarrheic conditions
O. Cecal ciliates of horses	15. Equine protozoal myeloencephalitis (EPM)
P. *Eimeria leuckarti*	16. Most commonly diagnosed clinical condition in puppies and kittens (coccidiosis).
Q. *Babesia equi*	17. Oval cysts with a refractile wall and two to four nuclei – may be distorted to semilunar appearance on standard fecal flotation
R. *Klossiella equi*	18. Coccidian that parasitizes the small intestine of swine, especially young piglets
S. *Sarcocystis neurona*	19. Coccidiosis within the bile ducts of the liver of rabbits
T. *Klossiella muris*	20. Intracellular flagellate (amastigote) found within reticuloendothelial cells of capillaries, spleen, etc. – transmitted by phlebotomine sand flies – oonotic parasite
U. *Cystoisospora suis*	21. "Mutuals" of horses
V. *Histomonas meleagridis*	22. Piroplasm of cattle transmitted by tick, *Boophilus annulatus*
W. *Trichomonas gallinae*	23. Feline piroplasm – "bejeweled ring" within stained RBCs of cats
X. *Haemoproteus* species	24. Nonpathogenic coccidian found in kidneys of wild and laboratory rats
Y. *Plasmodium* species of birds	25. Ciliate infecting the skin, gills, fins, and eyes of freshwater tropical and ornamental fish in home aquaria
Z. *Leukocytozoon* species	26. "Mutuals" of cattle
AA. *Eimeria stiedai*	27. Oocysts found on histopathologic examination of the horse kidney and in urine sediment
BB. *Tritrichomonas caviae*	28. Found within crop washes and crop swabs of pigeons, doves, and poultry
CC. *Ichthyophthirius multifiliis*	29. Hemoprotozoan of Central and South America with two forms: trypomastigote and amastigote (zoonotic parasite)
DD. *Leishmania* species	30. Avian malaria

Reproduction

Reproduction in the Protozoa may be asexual, as in the amoebas and flagellates that infect humans, or both asexual and sexual, as in the Apicomplexa of medical importance. The most common type of asexual multiplication is binary fission, in which the organelles are duplicated and the protozoan then divides into two complete organisms. Division is longitudinal in the flagellates and transverse in the ciliates; amoebas have no apparent anterior-posterior axis. Endodyogeny is a form of asexual division seen in *Toxoplasma* and some related organisms. Two daughter cells form within the parent cell, which then ruptures, releasing the smaller progeny which grow to full size before repeating the process. In schizogony, a common form of asexual division in the Apicomplexa, the nucleus divides a number of times, and then the cytoplasm divides into smaller uninucleate merozoites. In *Plasmodium*, *Toxoplasma*, and other apicomplexans, the sexual cycle involves the production of gametes (gamogony), fertilization to form the zygote, encystation of the zygote to form an oocyst, and the formation of infective sporozoites (sporogony) within the oocyst.

Some protozoa have complex life cycles requiring two different host species; others require only a single host to complete the life cycle. A single infective protozoan entering a susceptible host has the potential to produce an immense population. However, reproduction is limited by events such as death of the host or by the host's defense mechanisms, which may either eliminate the parasite or balance parasite reproduction to yield a chronic infection. For example, malaria can result when only a few sporozoites of *Plasmodium falciparum*, perhaps ten or fewer in rare instances are introduced by a feeding Anopheles mosquito into a person with no immunity.

Repeated cycles of schizogony in the bloodstream can result in the infection of 10 percent or more of the erythrocytes; about 400 million parasites per milliliter of blood.

a) Hologamy: The two ordinary mature protozoan individuals do not form gametes but themselves behave as gametes and fuse together to form zygote.

e.g. *Copromonas*

b) Isogamy: When two fusing gametes are similar in size and shape but differ in behaviour, they are called isogametes and their union, isogamy. Isogametes are generally produced by multiple fission

e.g. *Chlamydomonas*

c) Anisogamy: When two fusing gametes, differ morphologically as well as in behaviour, they are called anisogametes and the fusion of these dissimilar gametes is called anisogamy. Small and motile gametes are the male or microgametes and large non-motile ones are the female or macrogametes.

e.g. *Plasmodium, Volvox*

d) Autogamy: It is the fusion of gametes derived from the same parent cell. Pseudopodia are withdrawn and a cyst is formed. Now meiotic division takes place and two daughter nuclei with half number of chromosomes are formed. After sometime, gametic nuclei fuse to form a zygote nucleus.

e.g. *Actinophrys*

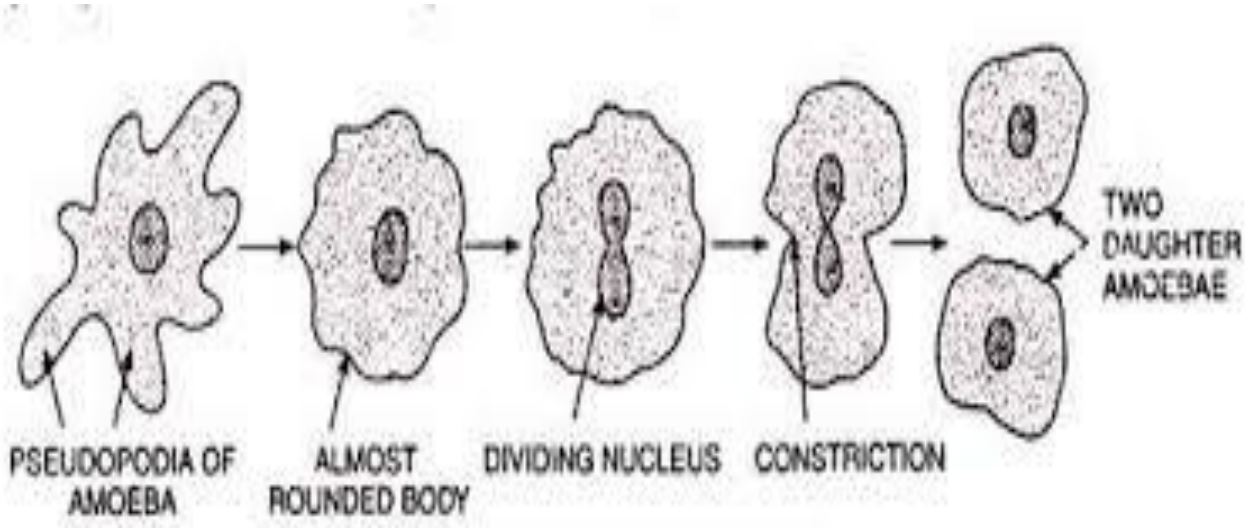
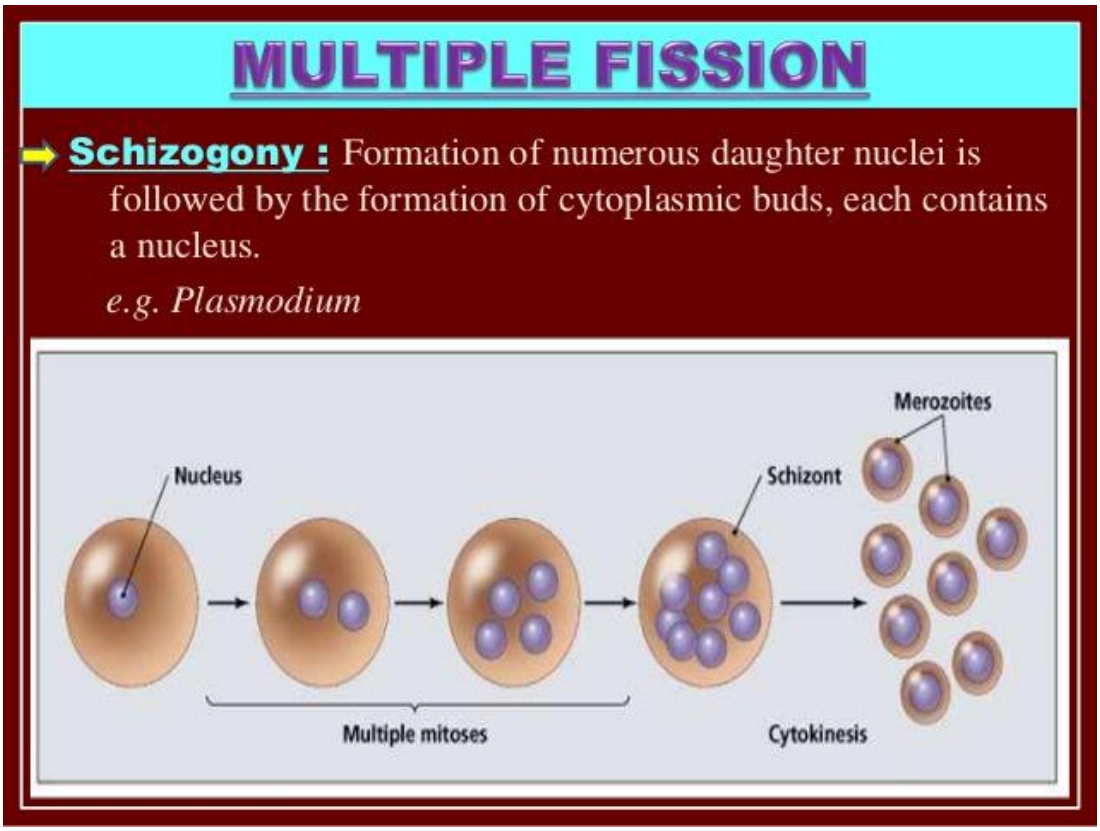


FIG. 10.3. Binary fission in *Amoeba*.



Nutrition

The nutrition of all protozoa is holozoic; that is, they require organic materials, which may be particulate or in solution. Amoebas engulf particulate food or droplets through a sort of temporary mouth, perform digestion and absorption in a food vacuole, and eject the waste substances. Many protozoa have a permanent mouth, the cytosome or micropore, through which ingested food passes to become enclosed in food vacuoles. Pinocytosis is a method of ingesting nutrient materials whereby fluid is drawn through small, temporary openings in the body wall. The ingested material becomes enclosed within a membrane to form a food vacuole.

Protozoa have metabolic pathways similar to those of higher animals and require the same types of organic and inorganic compounds. In recent years, significant advances have been made in devising chemically defined media for the in vitro cultivation of parasitic protozoa. Research on the metabolism of parasites is of immediate interest because pathways that are essential for the parasite but not the host are potential targets for antiprotozoal compounds that would block that pathway but be safe for humans. Many antiprotozoal drugs were used empirically long before their mechanism of action was known. The sulfa drugs, which block folate synthesis in malaria parasites, are one example.

The rapid multiplication rate of many parasites increases the chances for mutation; hence, changes in virulence, drug susceptibility, and other characteristics may take place. Chloroquine resistance in *Plasmodium falciparum* and arsenic resistance in *Trypanosoma rhodesiense* are two examples.

Competition for nutrients is not usually an important factor in pathogenesis because the amounts utilized by parasitic protozoa are relatively small. Some parasites that inhabit the small intestine can significantly interfere with digestion and absorption and affect the nutritional status of the host; *Giardia* and *Cryptosporidium* are examples. The destruction of the host's cells and tissues as a result of the parasites' metabolic activities increases the host's nutritional needs. This may be a major factor in the outcome of an infection in a malnourished individual. Finally, extracellular or intracellular parasites that destroy cells while feeding can lead to organ dysfunction and serious or life-threatening consequences.