MEDICAL PROTOZOOLOGY

AND

HELMINTHOLOGY

(REVISED 1965)

U. S. NAVAL MEDICAL SCHOOL
NATIONAL NAVAL MEDICAL CENTER
BETHESDA, MARYLAND

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PREFACE

This manual was prepared and edited and has been continuously revised by the staff of the United States Naval Medical School. It is intended primarily as an instructional guide for clinical laboratory students of the Navy, and as a simplified ready reference for both physicians and technicians in the field of Medical Protozoology and Helminthology.

Many sources have been consulted in the preparation of this manual to accomplish the desired objective. Considerable emphasis has been placed upon illustrations and diagrams to facilitate an understanding of the subject matter. The procedures and techniques herein outlined for detection and identification are proven methods currently in use in the laboratories of the United States Naval Medical School, National Naval Medical Center, Bethesda, Maryland.

For additional information on detailed morphology, epidemiology, symptomatology, etc., the student should consult recognized texts in the fields of parasitology and tropical medicine.

J. H. Stover, Jr.

J. H. STOVER, JR.
CAPT MC USN
Commanding Officer
U. S. Naval Medical School
National Naval Medical Center
Bethesda, Maryland

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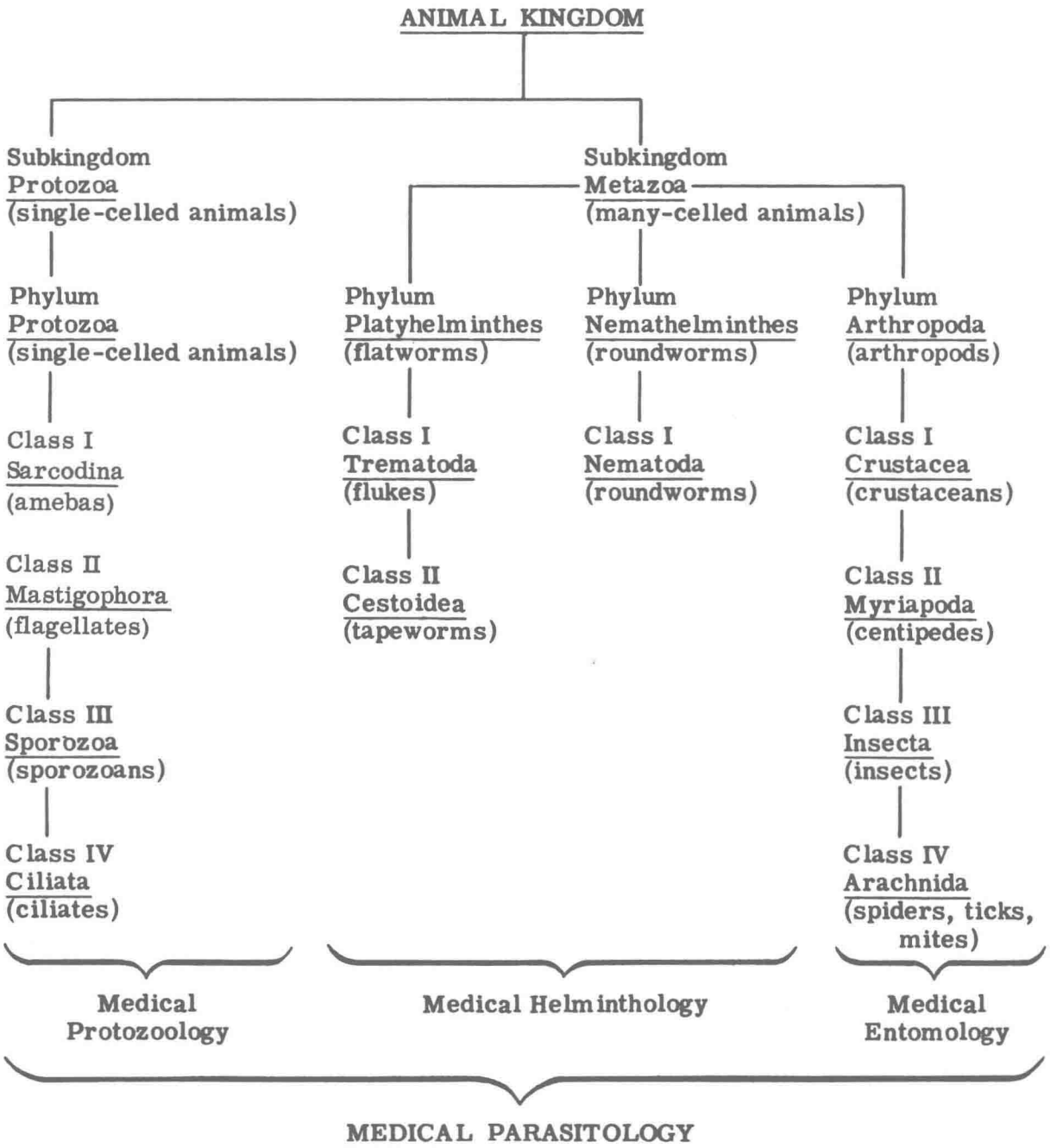
INTRODUCTION

Parasitology is the science of parasitism. Parasitism is the relation between parasites and their hosts. A parasite is an organism that lives on, or within, and is metabolically dependent on some other living organism. We are concerned only with parasitism in animals with emphasis placed on those found in, or of greater importance to man.



DEFINITIONS

Parasitism - an association between two specifically distinct organisms in which one, the parasite, is metabolically dependent on the other, the host.

Parasite - an animal or plant that lives in or on another organism, the host, from which it requires something for its existence and development and in which it sometimes causes disease.

Endoparasite - a parasite that lives within the body of the host.

Ectoparasite - a parasite that lives upon the body of the host.

Erratic parasite - a parasite that wanders into an organ in which it does not usually live.

Facultative parasite - an organism that is capable of living either free or as a parasite.

Incidental parasite - a parasite that establishes itself in a host in which it does not usually live.

Obligatory parasite - a parasite which depends for its existence upon its host.

Periodic parasite - a parasite that makes short visits to its host to obtain nourishment or other benefits.

Permanent parasite - an organism that is parasitic throughout its entire life cycle.

Pseudoparasite - an object that is mistaken for a parasite.

Temporary parasite - a parasite that is free-living during a part of its life cycle.

Host - an organism which harbors a parasite.

Definitive host - the host which harbors the adult or sexual stages of the parasite.

Vector host - the host usually responsible for infection of the vertebrate host.

Intermediate host - the host which harbors the larval or asexual stages of the parasite and one in which there may be effective parasite multiplication.

First intermediate host - the first host parasitized by the immature stages of the parasite. Usually this host is an invertebrate.

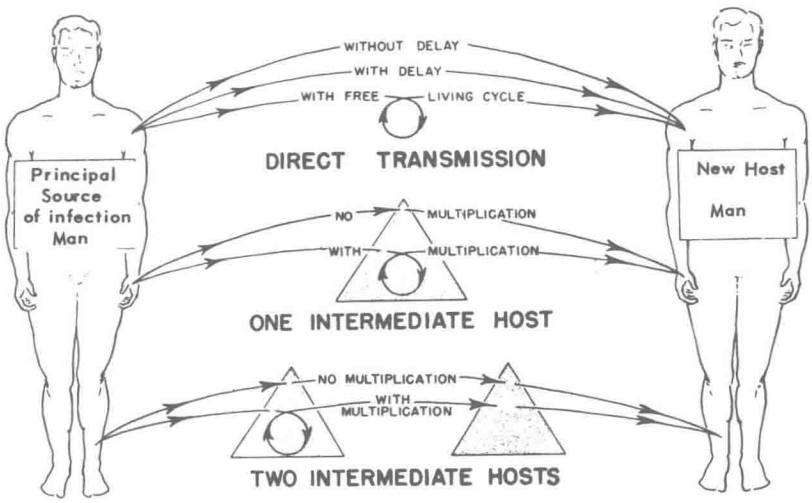
Second intermediate host - a host which harbors an immature stage of a parasite after it has left the first intermediate host. This host may be a vertebrate or an invertebrate.

Reservoir host - a host, usually a vertebrate, other than the host of interest, which is able to take its place in the life cycle of the parasite and may serve as a source of infection.

NOMENCLATURE

In the classification of animal parasites, numerous subdivisions of the main groups are necessary. The names applied follow the laws laid down in the "International Rules of Zoological Nomenclature* ". The parasites with which we are concerned belong to three phyla of the animal kingdom: Protozoa, Platyhelminthes, and Nemathelminthes. Each phylum is divided first into classes and these into orders, families, genera, and species. Scientific names must be Latin or Latinized; family names are formed by adding -idae to the stem of the name of the type genus; generic names should consist of a single word written with a capital initial letter and italicized; the specific name should always begin with a small letter. The author of a scientific name is the person who first publishes the name with a definition or description of the organism given. The names of the genera and species are underlined in writing them. This serves as an instruction to print them in italics.

*For an explanation of the principles regarding nomenclature refer to: Faust, E.C., and Russell, P.F., 1957. Clinical Parasitology, Lea & Febiger, p. 29, or Faust, E.C., 1955, Animal Agents and Vectors of Human Disease, Lea & Febiger, p. 24.



DIRECT TRANSMISSION
(Intermediate host not required)

Without delay—

parasite immediately infective for man:
- Entamoeba histolytica
- Giardia lamblia
- Trichomonas vaginalis
- Balantidium coli
- Plasmodium species (under special circumstances)*
- Trypanosoma species (under special circumstances)
- Leishmania species (under special circumstances)
- Hymenolepis nana
- Enterobius vermicularis
- Sarcoptes scabiei
- Pediculus humanus
- Phthirus pubis

With delay—

parasite must develop to infective stage:
- Enterobius vermicularis
- Ascaris lumbricoides
- Trichuris trichiura
- Ancylostoma species
- Necator americanus
- Strongyloides stercoralis
- Trichinella spiralis

With free-living cycle—

parasite must develop to infective stage:
- Strongyloides stercoralis (under special circumstances)

ONE INTERMEDIATE HOST
(Obligatory)

No multiplication but parasite undergoes development in intermediate host:
- Wuchereria species
- Onchocerca volvulus
- Loa loa
- Dracunculus medinensis
- Taenia species
- Dipylidium caninum
- Hymenolepis diminuta
- Hymenolepis nana (under special circumstances)

With multiplication and development in intermediate host:
- Schistosoma species
- Trypanosoma species
- Leishmania species
- Plasmodium species*
- Echinococcus granulosus*

TWO INTERMEDIATE HOSTS
(Obligatory)

No multiplication but parasite undergoes development in both intermediate hosts:
- Diphyllobothrium latum

With multiplication in first intermediate host—development in both:
- Clonorchis sinensis and other liver flukes
- Fasciolopsis buski and other intestinal flukes
- Paragonimus westermani

*In these species man is the intermediate host and some other animal the definitive host. The life cycles, however, fall easily into the above scheme.

Fig. 1 - Principles and means of parasite transmission

MORPHOLOGY AND BIOLOGY

The Protozoa are unicellular animals. Like each cell of the Metazoa (many-celled animals), their single cell consists of cytoplasm and a nucleus or nuclei. The cytoplasm is differentiated into an outer layer, the ectoplasm, and an inner mass, the endoplasm.

The ectoplasm is a dense, resilient structure. It performs the functions of the skin (protection), the limbs (locomotion), the mouth (ingestion of food), and the excretory organs of the higher animals. The most obvious of these functions is locomotion. It is accomplished by means of what has been termed ectoplasmic organelles: in the ameba, ectoplasmic protrusions - pseudopodia; in the flagellates, long, thread-like filaments - flagella; and in the ciliates, short, hair-like filaments - cilia. These organelles also serve to procure food. Some species of protozoa encyst; the ectoplasm then is modified into the firmer, more resistant cyst wall.

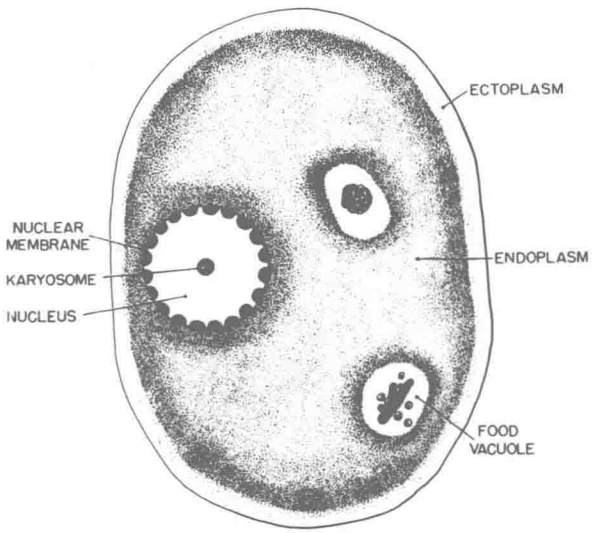
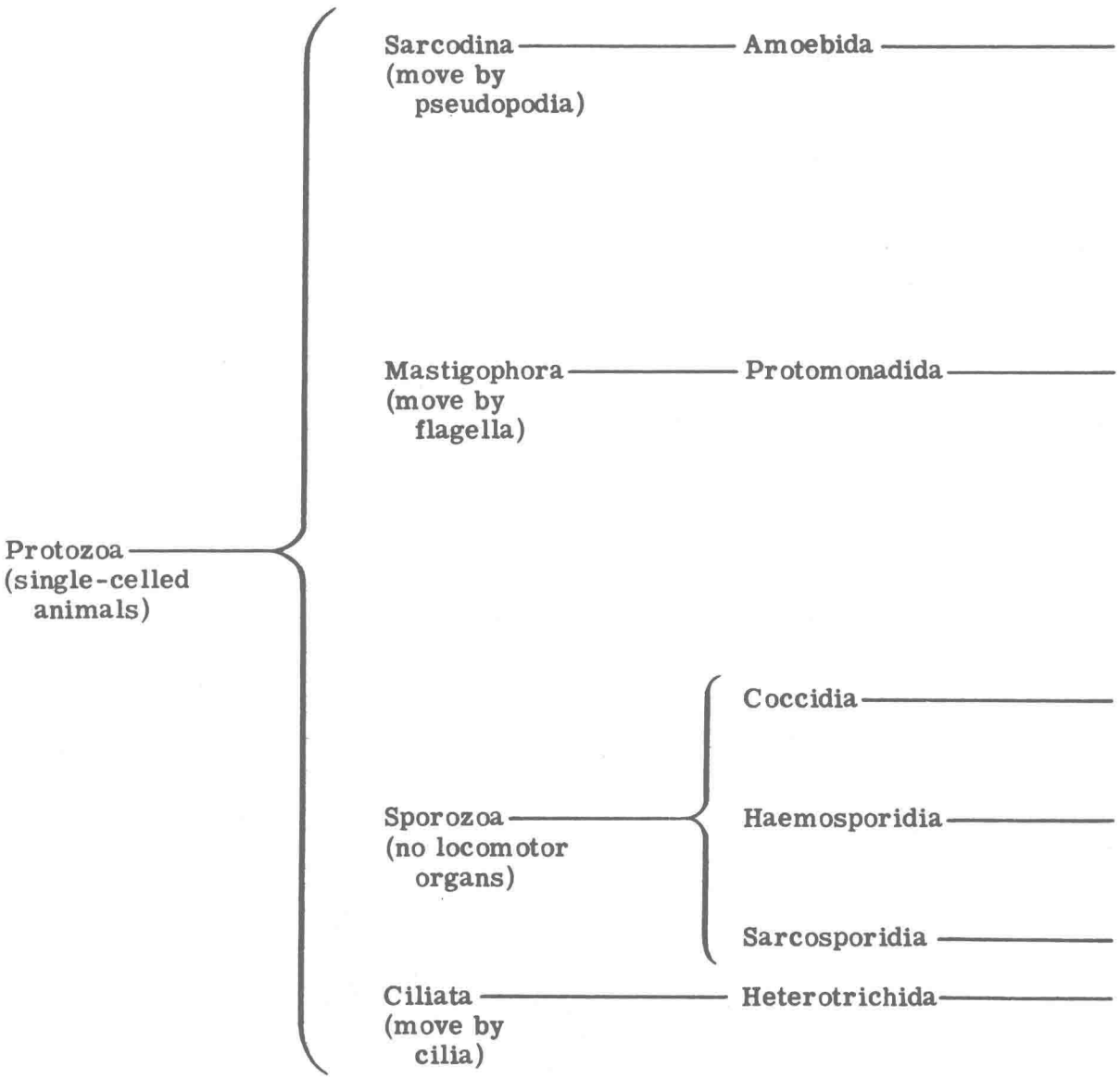


Fig. 2 - A protozoan

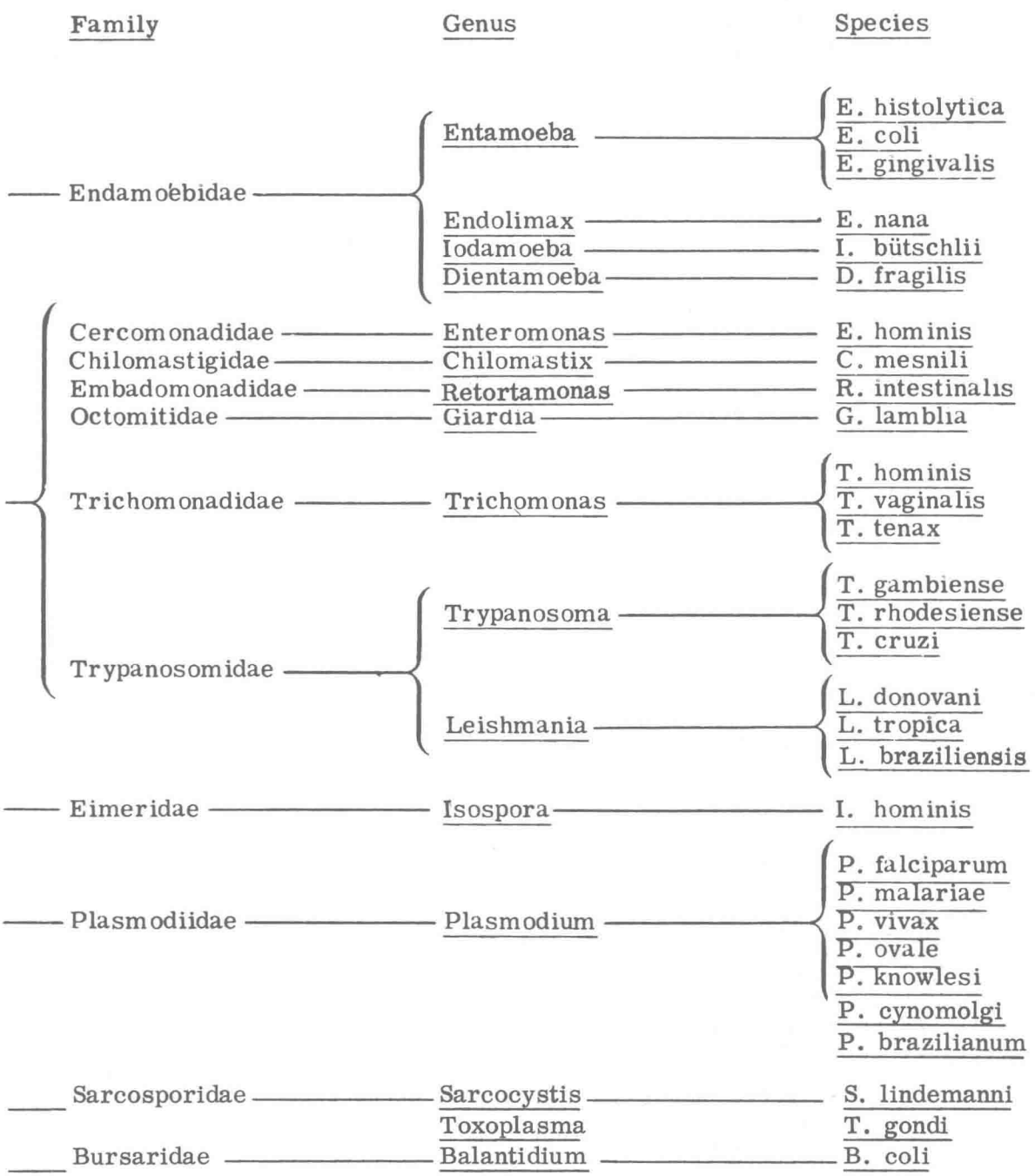
The endoplasm is a thinner substance, flowing like water or a syrupy solution within the ectoplasm. It contains various inclusions, i.e., mitochondria, Gelgi bodies, etc. It is the "viscera" concerned mainly with nutrition. It usually contains ingested material in various stages of digestion, such as bacteria, vegetable cells, tissue cells, and starch granules. Some of these may be within vacuoles, so-called food vacuoles. In some protozoa there are also contractile and hydrostatic vacuoles in the endoplasm which have to do with the elimination of waste products and with fluid balance.

The nucleus is concerned chiefly with reproduction and control of cell functions, Its structure varies greatly. In some species it appears to be merely a mass of chromatin, while in others it has a definite and complex organization. These variations are used in many instances in the identification of protozoa. The details of nuclear morphology and methods of reproduction will be discussed in the description of the various genera and species.

CLASSIFICATION



This chart of classification is used as a means for indicating general relationships among the protozoa studied. It should be realized that a given taxonomic scheme is not necessarily accepted by all authors or workers in this field of study.


Family
Genus
Species
Endamoebidae
Entamoeba
E. histolytica
E. coli
E. gingivalis
Endolimax
E. nana
Iodamoeba
I. bütschlii
Dientamoeba
D. fragilis
Cercomonadidae
Enteromonas
E. hominis
Chilomastigidae
Chilomastix
C. mesnili
Embadomonadidae
Retortamonas
R. intestinalis
Octomitidae
Giardia
G. lamblia
Trichomonadidae
Trichomonas
T. hominis
T. vaginalis
T. tenax
Trypanosomidae
Trypanosoma
T. gambiense
T. rhodesiense
T. cruzi
Leishmania
L. donovani
L. tropica
L. braziliensis
Eimeridae
Isospora
I. hominis
Plasmodiidae
Plasmodium
P. falciparum
P. malariae
P. vivax
P. ovale
P. knowlesi
P. cynomolgi
P. brazilianum
Sarcosporidae
Sarcocystis
S. lindemanni
Toxoplasma
T. gondi
Bursaridae
Balantidium
B. coli

Sarcodina (Flesh-like) or Rhizopoda (Root-footed) Fig. 3

This class contains one family of medical importance, commonly called the amebas, which move by means of pseudopodia - root-like extensions of the cytoplasm. These pseudopodia also serve as structures for obtaining food. Ectoplasm, or both ectoplasm and endoplasm, may take part in making up the pseudopodia. These organelles vary in shape and characteristic activity in the different species.

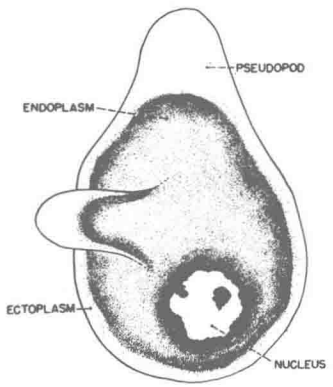


Fig. 3 - An ameba

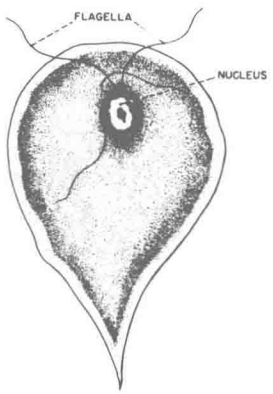


Fig. 4 - A flagellate

Mastigophora (Whip-bearing) Fig. 4

This class contains several families of human protozoa, all belonging to the same order. Several important diseases, such as African sleeping sickness and kala-azar are caused by representatives of this group. The motile forms of this class have thread- or whip-like processes, flagella, for the purpose of locomotion

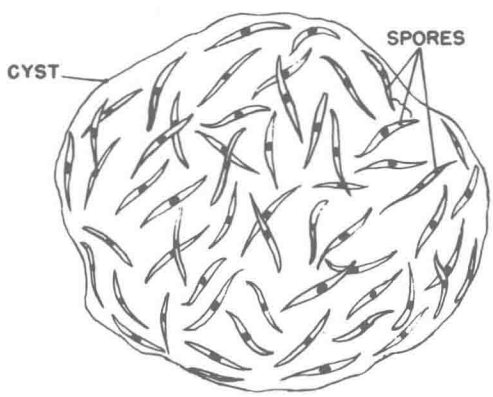


Fig. 5 - A sporozoan

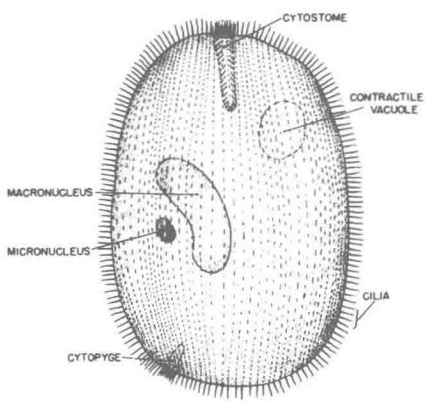


Fig. 6 - A ciliate

and obtaining food. Some species have an undulating membrane in addition to the flagellum.

Sporozoa (Spore-animal) Fig. 5.

This class is identified by the method of reproduction and by the absence of locomotor organelles. Reproduction may be asexual (schizogony) or sexual - after union of male and female gametes and the production of spores (sporogony). All the Sporozoa of man are parasitic within cells, tissues, or body cavities. This class includes the important malaria parasites.

Ciliata

Only one species of medical importance is included in this class. These animals are characterized by a covering of hair-like cilia which serve for locomotion and for direction of food particles into the mouth; the organelles may be evenly distributed over the entire animal or more prominent in certain regions. The body is enveloped in a cuticle which may have only one opening, the cytostome (mouth), or a second opening, the cytopyge (anus). The ciliates usually have both a large macronucleus and a small micronucleus.

By comparing the two tables on pages 7 and 10, it is immediately apparent that not all of the human protozoa cause disease. Some are pathogenic, while others do not seem to be. Since the nonpathogenic forms may be confused with the specific etiologic agents of certain diseases, Medical Protozoology must include a study of all the protozoa to be found in man.

The human protozoa of major importance readily fall into two groups based upon their habitat in the body - the blood protozoa and the intestinal protozoa; a third group of less importance is made up of those inhabiting other parts of the body. These groups provide a better classification for clinical and laboratory study than their zoological relationships. For this reason, the material pertaining to Medical Protozoology will be presented under the headings of blood protozoa and intestinal protozoa; the other protozoa are included with related organisms of the latter group.

PROTOZOA CAUSING SPECIFIC HUMAN DISEASES

Parasites (Diseases)	Vertebrate Hosts	Vector Hosts	Important Reservoir Hosts	Transmission to Man
Entamoeba histolytica (Amebiasis)	Man	None	None	By ingestion (mature cyst)
Balantidium coli (Balantidiasis)	Hogs, Man	None	Hogs	By ingestion (mature cyst)
Giardia lamblia (Giardiasis)	Man	None	None	By ingestion (mature cyst)
Trichomonas vaginalis (Trichomonad vaginitis)	Man	None	None	By contact (flagellate)
Trypanosoma gambiense Trypanosoma rhodesiense (African sleeping sickness)	Man, Animals	Tsetse flies (Glossina species)	Various animals	By inoculation (bite of fly)
Trypanosoma cruzi (Chagas' disease)	Animals, Man	Reduviid bugs, (Triatoma species)	Armadillos, Opossums Man	By contamination of bite by infective feces of bug
Leishmania donovani (Kala-azar) Leishmania tropica (Oriental sore) Leishmania braziliensis* (Espundia)	Man, Dogs	Sand flies (Phlebotomus species)	Dogs	By inoculation (bite of fly; direct transmission possible)
Plasmodium vivax Plasmodium falciparum Plasmodium malariae Plasmodium ovale Plasmodium cynomolgi (Malaria)	Man, monkeys (for P. cynomolgi)	Female anopheline mosquitoes	None	By inoculation (bite of mosquito; also by transfer of infected blood)

*Dogs and other animals have been implicated in the life cycles of Leishmania donovani and L. tropica but the part played by hosts other than man in the case of L. braziliensis is yet questionable. Naturally infected dogs have been found in South America.

INTESTINAL PROTOZOA

DEFINITIONS

Trophozoite - the motile form which feeds, multiplies, and maintains the colony in the host. (These are also called "vegetative forms" and "trophic forms." The term "trophozoite," although strictly belonging to the Sporozoa, is more convenient.)

Cyst - the immotile form protected by a cyst wall and designed for transmission to new hosts.

Encystation - the transformation of a trophozoite into a cyst.

Precystic form - a rounded trophozoite just before encystation.

Excystation - hatching of the cyst with the liberation of a motile metacystic trophozoite.

Chromatin - the portion of the nucleus which is readily stained.

Chromatoid - the material staining like chromatin, found in the cytoplasm and not part of the nucleus.

Volutin - a chromatoid substance occurring as granules in the cytoplasm.

Karyosome - nucleolus, a dark-staining body in the chromatin network of the nucleus.

Blepharoplast - a small dark-staining mass forming the base of a flagellum, acting as a center for movement of the organism.

CLASSIFICATION

The following protozoa have been found to inhabit the human intestinal tract:

Five species of SARCODINA
- Entamoeba histolytica
- Entamoeba coli
- Endolimax nana
- Iodamoeba bütschlii
- Dientamoeba fragilis

Five species of MASTIGOPHORA
- Giardia lamblia
- Chilomastix mesnili
- Trichomonas hominis
- Retortamonas intestinalis
- Enteromonas hominis

One species of CILIATA
- Balantidium coli

Two species of SPOROZOA
- Isospora hominis
- Isospora belli

*For taxonomic relationships of these protozoa, see pages 6 and 7.

PATHOGENICITY

Entamoeba histolytica is by far the most pathogenic of the intestinal protozoa. Two strains, i.e. the large and small types, are recognized. The former apparently is the cause of amebic dysentery and of various less severe intestinal disturbances as well as of amebic abscess of the liver and other organs. The other amebas are usually considered harmless parasites, living in the lumen of the bowel. It is possible that Dientamoeba fragilis may at times be pathogenic, but sufficient evidence for its incrimination is not available. Giardia lamblia is the only flagellate which has been definitely shown to cause intestinal disturbances. The ciliate, Balantidium coli, may in some instances cause ulcerative colitis and chronic dysentery.

Clinicians might be justified in limiting their interest to the three protozoa which are known to cause disease. Such limitation is not possible, however, in the clinical laboratory, mainly on account of Entamoeba histolytica. This ameba may resemble not only the other four species of ameba but also the flagellates. It is, therefore, necessary to know and learn to recognize some distinguishing characteristics of each of the intestinal amebas and flagellates in order to differentiate them from E. histolytica. This task is undoubtedly the most difficult in the laboratory diagnosis of parasitic diseases, and few workers find time and opportunity to master it. Differentiation of the smaller and larger strains of E. histolytica may be important in certain cases of infection. Reliable differentiation depends upon accurate observation and measurement of trophozoites and cysts.

LIFE CYCLES

In order to understand the appearance in feces of the various forms of the intestinal protozoa, it is necessary to know the essential features of their life cycles. The life cycle of Entamoeba histolytica will be given first and in some detail; then the life cycles of the others may be understood by merely referring to similarities and differences.

Entamoeba histolytica (Fig. 7)

The preferred habitat of E. histolytica is the colon. Here it usually lives in the lumen, causing its host no annoyance. Occasionally it invades the bowel wall, producing ulcerative lesions and dysentery. Sometimes the invading trophozoites are transported in the blood vessels to the liver and occasionally to other organs where they multiply and cause abscesses. E. histolytica feeds on blood and tissue elements when available; otherwise it apparently exists on undigested food particles in the fecal stream. It multiplies by binary fission and thereby maintains a colony against a daily loss of millions in the feces. Under certain conditions some of the lumen trophozoites (not those within the host tissues) become encysted. The