

Human Protozoology

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HUMAN PROTOZOOLOGY



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PREFACE

This book represents essentially the subject matter of the course in Protozoology given by the writers in the School of Hygiene and Public Health of the Johns Hopkins University, and has been prepared particularly for the use of students in this course. It is hoped that it will also be of use to students pursuing similar courses in other institutions and to public health officers and physicians in general. We wish in the very beginning to emphasize the fact that a rigid selection has been practiced regarding the material included. This has been necessary because of the limitation of our subject and the great amount of literature available. The PROTOZOA constitute one of the largest groups in the animal kingdom both with respect to number of species and number of individuals. They have attracted the attention of many investigators and have been used extensively for the study of behavior, physiology, genetics, etc.; and their importance as causative agents of disease has made them the objects of research of medical men as well as of biologists. For these and other reasons the published researches on PROTOZOA are extraordinarily numerous and widely scattered in the literature. We have attempted first to set forth the general biology of the PROTOZOA as illustrated by each group studied; then to give typical life-histories of representative species either from man or the lower animals; and finally to present a more detailed account of the species living in man.

What we call the biology of the PROTOZOA includes the study of morphology, taxonomy, habitats, maintenance of individuals and maintenance of races. The study of morphology enables one to identify species and locate them in

their proper systematic position. The subject of habitats involves the study of geographical distribution, modes of existence, and the relations of PROTOZOA to the physical and biological factors of their environment. The individual protozoon is able to exist in its habitat because of certain adjustments and reactions to factors in its environment. These reactions are made possible by the reception of stimuli through its sensitive protoplasm; by movements which enable it to escape its enemies, reach an optimum medium, and find food; by capturing, ingesting and digesting food and egesting unsuitable materials; by carrying on respiratory exchanges with the surrounding medium; and by secreting skeletal substances, digestive fluids, etc.; and by excreting waste products. The races of PROTOZOA are maintained by means of reproduction. This subject involves the study of the nuclei and other organelles that play a part in reproduction, the various methods of asexual and sexual reproduction, heredity, life-cycles, evolution, and the complex relations between parasites and their hosts and intermediate hosts.

It is possible in many cases to become thoroughly familiar with parasites that may be obtained easily from lower animals that are similar to species living in man. These species have for this reason been selected wherever possible for purposes of illustration. The importance of the study of the parasites of lower animals may here be emphasized since much of what we know about the human parasites was first learned from investigations of those of lower animals, and we must even today construct the life-cycles of many human parasites of which our knowledge is meager with the aid of data gained from studies of those in lower animals. Free-living PROTOZOA must also be considered in our account of Human Protozoology since the basis of our knowledge of the taxonomy, morphology, physiology, reproduction, etc., of all PROTOZOA rests upon the investigations of hundreds of students of the free-living species.

There are at present several excellent text-books of Protozoology available; these, however, are too general in their treatment for the audience we hope to reach with the present book, and, moreover, the very valuable data on human PROTOZOA that have been reported since the beginning of the war can now be obtained only in monographs on special groups.

The authors have carried on investigations with many of the PROTOZOA described in this book and have verified as many of the facts as possible regarding others, but the field is so large that many of the statements have necessarily been based on work published in scientific periodicals or in text-books and reference books. The principal books and periodicals consulted are listed in the bibliography at the end of the book.

Because this work is not a monograph but is intended as a text-book for beginning students in Protozoology the authors have considered it best to omit extensive references to the nomenclature of most of the species discussed and also the name of the original designator of the species.

The authors recognize that there is at present no consensus of opinion regarding the classification of the PROTOZOA. They have therefore either adopted the system as used by the authorities on the various groups or have adapted these systems as seemed best in the light of recent knowledge and the purposes for which the book has been written.

In the course in Protozoology as presented to the students at the School of Hygiene and Public Health the senior author gives the work on intestinal MASTIGOPHORA, SPOROZOA, and ectozoic and entozoic INFUSORIA, and the junior author gives the work on free-living PROTOZOA, AMOEBAE and the HæMOFLAGELLATES. For this reason certain chapters in the book (VI-XI, XIII) have been prepared by the senior author and the others (I-V, XII, XIV, XV) by the junior author. While there has been close cooperation between the

authors, nevertheless they are solely responsible for their respective chapters.

Many of the figures in the book have been made by the authors or under their direction from slides in their collections, and a number of them have not been published elsewhere. The authors are greatly indebted to Prof. W. A. Kepner, of the University of Virginia, for certain unpublished figures and observations which he kindly placed at their disposal. They are also indebted to Dr. F. M. Root, Dr. B. D. Reynolds, Mr. F. O. Holmes, and Miss F. A. Coventry, of the Department of Medical Zoology, School of Hygiene and Public Health, for other original unpublished drawings. The two colored plates of the malarial organisms were made by Miss Ethel Norris from specimens selected by the senior author.

The authors also wish to thank Dr. L. R. Cleveland and Dr. E. R. Becker for assistance in preparing certain portions of the text, and Mr. F. O. Holmes and Miss L. R. Deem for aid in preparing the manuscript for the publishers. The junior author is particularly indebted to his wife, Lucy Graves Taliaferro, for assistance in the preparation of those portions of the text for which he is responsible.

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HUMAN PROTOZOOLOGY

CHAPTER I¹

INTRODUCTION TO THE ORGANIZATION OF THE PROTOZOA

The object of the present introductory chapter is not to give a comprehensive treatment of the organization and structure of the protozoa, but rather to serve as a general introduction to the more technical accounts which follow. For more extensive descriptions of the structure of the various groups of the protozoa, the student is referred to the larger general works and to the monographs on special groups which are given in the bibliographies.

A. Classification of the Protozoa

Before describing the chief structural differentiations found in the different groups of the PROTOZOA, it is well to point out that the phylum is generally divided, according to the kind of organs of locomotion (Fig. 1), into four classes. The classes are as follows:

1. CLASS SARCODINA.—In their typical condition these forms are characterized by the use of temporary extensions of the body, or *pseudopodia*, as organs of locomotion and food capture. Correlated with the formation of pseudopodia,

¹By W. H. Taliaferro.

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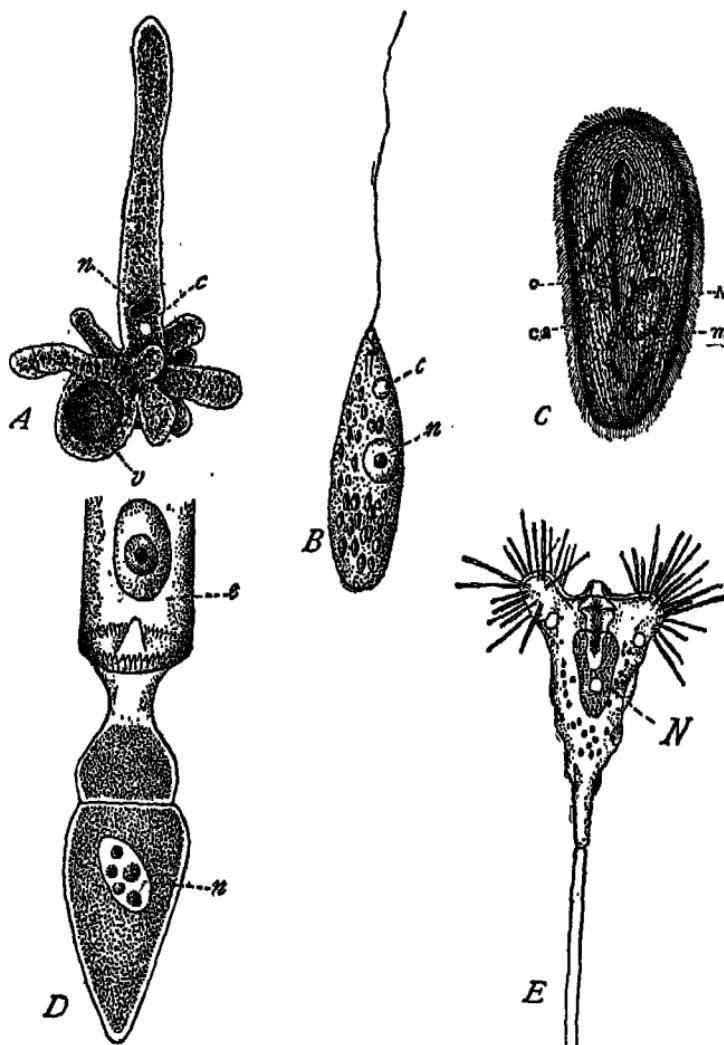


Fig. 1.—Types of Protozoa.

A, *Amœba proteus*, a representative of the SARCODINA. B, *Peranema trichophorum*, a representative of the MASTIGOPHORA. C, *Frontronia leucas*, a ciliate representative of the INFUSORIA. D, *Pyxinia sp.*, a representative of the SPOROZOA. E, *Tokophrya quadripartita*, a suctorian representative of the INFUSORIA. *c*, contractile vacuole; *ca*, canals leading to contractile vacuole; *e*, epithelial host-cell; *m*, micro-nucleus; *N*, macronucleus; *n*, nucleus; *v*, food vacuole. Drawn at various magnifications. (All from Calkins, B and E after Bütschli, C after Schewiakoff, and D after Wasielewsky.)

the body (with a few exceptions, such as the pellicle of *Amaeba verrucosa*) is non-corticate, i.e., it has no tough limiting membrane or cuticle. Many forms are naked; others form shells or skeletons.

2. CLASS MASTIGOPHORA.—In their typical condition these forms are characterized by the possession of filamentous whip-like structures, or *flagella*, as organs of locomotion. The body may be corticate or non-corticate.
3. CLASS SPOROZOA.—As a group these forms are exclusively parasitic and lack definite external organs of locomotion or food capture.
4. CLASS INFUSORIA.—These forms, in some stage of their life-history, are characterized by the possession of numerous vibratile, hair-like filaments, or *cilia*, as organs of locomotion. Cilia are differentiated from flagella chiefly by their smaller size and greater numbers. Typically, the INFUSORIA exhibit a division of the nuclear material—the vegetative chromatin occurring in a macronucleus and the generative chromatin occurring in a micronucleus.

B. The Ectoplasmic Organelles

The protoplasm composing the body of a protozoon is typically differentiated into an outer layer, the *ectoplasm*, and an inner layer, the *endoplasm*. The ectoplasm is the layer which is in the most intimate contact with the environment and is the seat of the organs of locomotion, the sense organs, and the protective mechanisms. In the SARCODINA, as pointed out in the preceding paragraph, locomotion is effected by means of temporary extrusions of the protoplasm, called pseudopodia, which are always formed from the ectoplasm although the endoplasm may flow in later. They are mainly of two types, viz., lobopodia and axopodia, of which the first are simply more or less fluid extrusions of the body,

while the second possess a central supporting structure or axostyle (Fig. 5). In the MASTIGOPHORA the organs of locomotion are vibratile thread-like processes called flagella. These flagella are always comparatively long and few in number, and have probably arisen by a gradual transition from pseudopodia. Flagella have been divided by Lankester into two types: tractella and pulsella. The first type is situated at the end which is anterior when the organism is in motion and drags the body along; the latter is generally situated posteriorly and pushes or propels the body forward. In a number of the parasitic flagellates, there is frequently a thin layer of protoplasm which connects a given flagellum, throughout the greater part of its length, with the body and is known as an undulating membrane. Cilia, which are the organs of locomotion in the class INFUSORIA, are also slender thread-like processes which differ from flagella chiefly in that they are very much smaller in comparison to the body of the protozoon, occur in much greater numbers, and differ in their type of movement. Cilia are generally arranged in rows, and there is a definite coordination between the beat of the individual cilia in each row. Thus each cilium contracts slightly after the one posterior to it and slightly before the one anterior to it. When seen in side view, this produces the illusion of a rotating wheel. When the waves of contraction of a number of parallel bands of cilia are viewed from above, they appear very much like a wheat field in a strong wind. Among the INFUSORIA there are several modifications of the cilia, such as cirri, membranelli, and undulating membranes. These are all produced by the fusion of a number of cilia.

While pseudopodia, flagella, and cilia are primarily organs of locomotion, they are also secondarily concerned in obtaining food. There is considerable evidence that they also serve

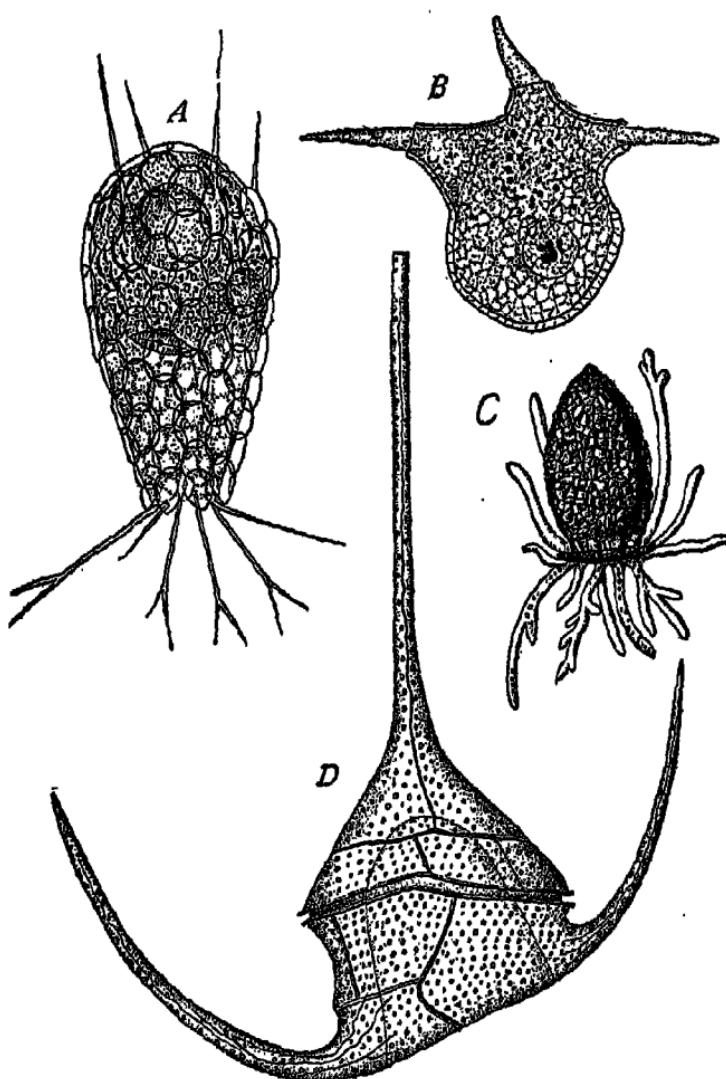


Fig. 2.—Shells and tests.

A, *Euglypha alveolata*: shell of oval siliceous plates glued together by siliceous (?) cement. B, *Cochliopodium digitatum*: test membranous and perforated for pseudopodia. C, *Diffugia urceolata*: shell composed of minute particles of sand. D, *Ceratium tripos*: shell of cellulose plates of diverse size and shape. Drawn at various magnifications. (A, B and D from Calkins; A after Schewiakoff and D after Stein. C after Leidy.)

as tactile sense organs in some forms. Before leaving the organs of locomotion, mention should also be made of certain contractile elements known as myonemes, which are muscle-like and occur in the body of certain of the *MASTIGOPHORA*, *INFUSORIA* and *SPOROZOA*.

The ectoplasm exhibits its protective function in the formation of a protective covering or cuticle over the body. This cuticle may be the modification of the entire ectoplasm, as in the periplast of certain flagellates, or it may involve only the superficial layer, as in the pellicle of the ciliates and certain amoebae, and in the epicyte of gregarines. The ectoplasm may even secrete a shell or "lorica" about itself, or cement together foreign particles, such as sand grains, to form such a shell (Fig. 2). Not only does the ectoplasm exhibit these types of passive protection, but in some species structures are present which are employed primarily, if not solely, as active modes of defense, such as the trichocysts of *INFUSORIA*.

The contractile vacuoles are generally considered ectoplasmic in origin. The exact function of these organelles is unknown. As a rule they are found in fresh-water species and are absent in a great many parasitic and marine forms. Some hold that their function is to regulate the osmotic pressure of the cell by the elimination of a portion of the water which is constantly being taken into the cell by imbibition and osmosis. Others believe that they are chiefly concerned with the elimination of nitrogenous wastes and possibly with the elimination of carbon dioxide (respiration). All three processes may take place simultaneously.

C. The Endoplasmic Organelles

In contrast to the ectoplasm which is chiefly concerned with such functions as locomotion, protection, and material

intake and outgo, the endoplasm is involved in the functions of reproduction and nutrition. The endoplasmic organelle of chief interest is the nucleus which will be considered later in detail. Besides the nucleus, in the holozoic protozoa, food vacuoles are often present which may be termed "temporary stomachs." In these, food particles are suspended and subjected to the digestive ferments of the cell, and, after digestion is completed, the indigestible residue is eliminated in various ways which will be considered later under the physiology of the different groups. In the holophytic protozoa organelles are found similar to those which occur in the green cells of ordinary plants, such as chromatophores or chromoplasts, pyrenoids, etc. In addition to these organelles of nutrition, numerous metaplastic granules occur in both the plant and animal-like protozoa, such as paramylum, glycogen, and fat globules, if they result from anabolic processes; or various types of granules, crystals, and pigment grains, if from catabolic processes.

As stated above, the nucleus is by far the most important of the endoplasmic organelles and undoubtedly plays a dominant rôle in the control of the various morphological and physiological changes which take place in the body of the protozoon. While enucleated fragments of a protozoon can often live for a greater or less period of time, apparently all anabolic processes are interfered with. Furthermore, a large mass of experimental and observational evidence supports the view, which was first advanced by Ernst Haeckel in 1866, that the nucleus is the chief organ of inheritance.

In a short review of this nature we can not do any more than give some of the chief characteristics of the protozoan nucleus. The nuclear structures are generally divided into chromatin, the fundamental nuclear substance, and achromatinic or accessory substances. The latter consist of: (1) nuclear reticulum, (2) a nuclear membrane which separates the nucleus from the surrounding protoplasm and is generally

considered to be a modification of (1), (3) the nuclear sap which fills the entire nuclear cavity, and (4) plastin which occurs in one or more masses in the nuclear cavity. If the latter masses are pure plastin they are called nucleoli, but, if they contain a mixture of chromatin and plastin, they are termed karyosomes.² The chromatin may be present in

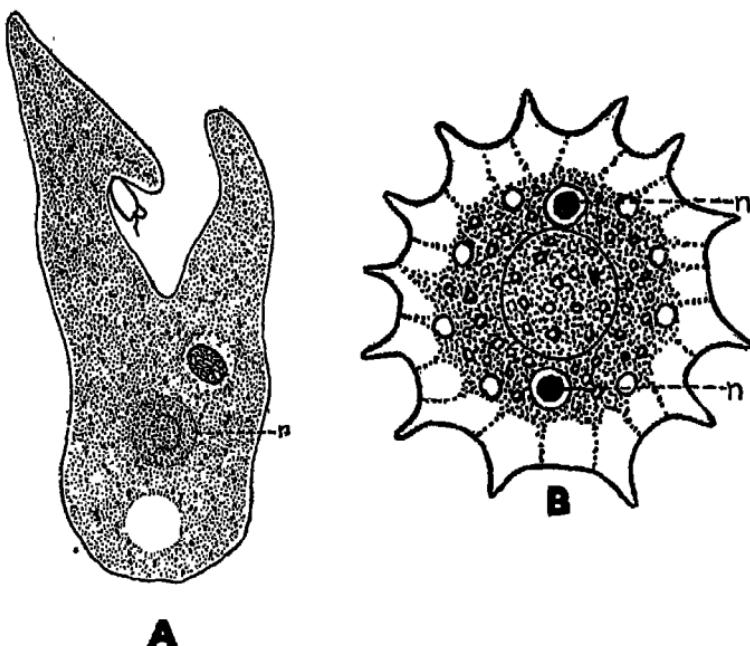


Fig. 3.—A, *Amoeba proteus* showing the granular type of nucleus. B, *Arcella dentata* showing the vesicular type of nucleus. n, nucleus. A \times 166; B \times 310. (A after Kepner and Taliaferro and B after Hegner.)

masses mixed with plastin; it may occur as granules at the nodal points of the reticulum; or it may occur in both locations in the same nucleus. Minchin recognizes two principal types of nuclei, the vesicular type in which the chromatin is

² It is probable that some karyosomes are composed entirely of chromatin. Cf. *Endamoeba histolytica*.

concentrated in a single mass or grain, and the granular type in which the chromatin is distributed in grains throughout the entire nucleus (Fig. 3). While some protozoan nuclei may be described as strictly of the vesicular or granular type, the majority are intermediate in structure and partake of the characters of both types.

Besides the achromatinic structures defined above, there is another organelle which may be intra- or extra-nuclear, i.e., the centrosome. It may lie outside of the nucleus as a minute grain (or pair of grains), as is true in the METAZOA, or it may occur within the nucleus, in which case it generally lies within a karyosome. It is concerned with the kinetic activities of the cell during division, at which time it is more readily visible, and is also associated with the kinetic activities of the organs of locomotion as is exemplified in the "basal granules" of flagella where it is generally designated a blepharoplast. The functions of blepharoplast and centrosome may be performed by the same body, as in *Mastigamoeba setosa*, or by definite separate ones, as in *Spongomonas* (Fig. 4). In the latter case the blepharoplast either arises from the centrosome at each cell division or is believed to have originally arisen from such a centrosome. The close

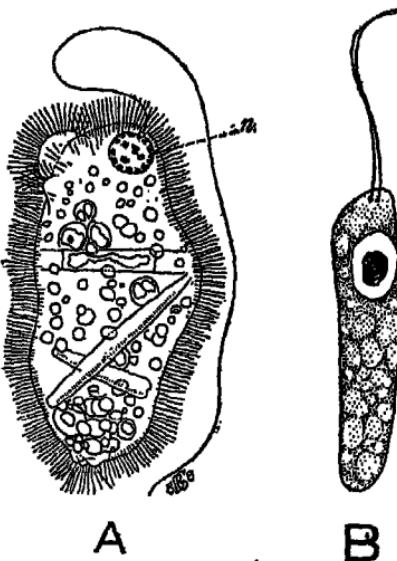


Fig. 4.—Types of flagellar insertion.
 A, *Mastigamoeba setosa*: n, nucleus from which flagellum arises directly.
 B, *Spongomonas splendida*: the two flagella arise from definitive blepharoplasts. A $\times 360$ and B $\times 2400$. (From Minchin; A after Goldschmidt and B after Hartmann and Chagas.)

relation between the nuclear apparatus and the locomotor organs may be seen in certain of the SARCODINA as well as in the MASTIGOPHORA and CILIATA. Thus, among the SARCODINA which possess more or less permanent pseudopodia (Fig. 5), in such forms as *Actinophrys sol*, the axial fila-

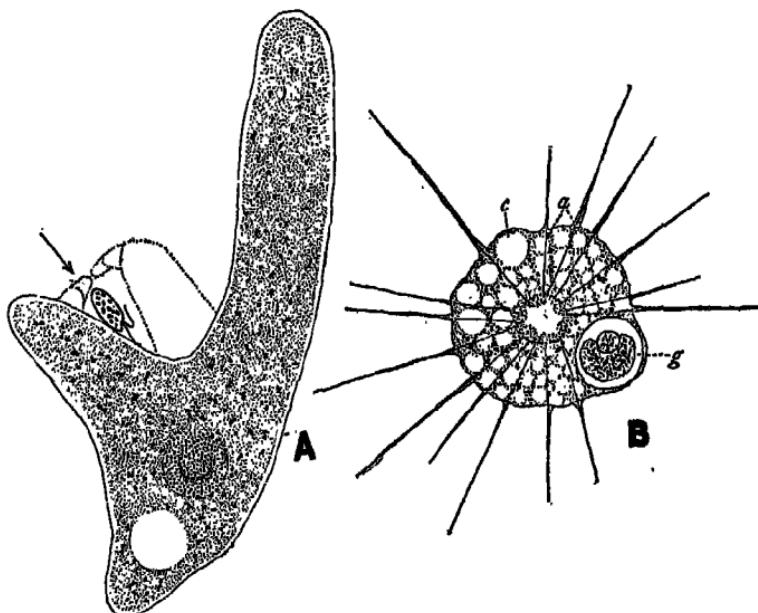


Fig. 5.—Types of pseudopoda.

A, *Amoeba proteus* showing lobopodia. B, *Actinophrys sol* showing axopodia. *a*, axial filaments which radiate from the nucleus; *c*, contractile vacuole; *g*, food vacuole. A $\times 233$; B magnification not stated. (A after Kepner and Taliaferro and B from Calkins after Greenacher.)

ments of the pseudopodia radiate directly from the nucleus (Fig. 5), and, in other forms, such as *Oxnerella maritima*, the axial filaments radiate from the centrosome which is situated outside of the nucleus.

In the forms which have been discussed, all of the chromatin material is located in one nucleus, but where more are

present they are probably of equal potentiality and perform the same functions. In the INFUSORIA, however, there is a qualitative division of the nuclear material into a vegetative nucleus or macronucleus and one or more generative or genetic nuclei or micronuclei.

Some observers believe that in certain flagellates (Hartmann's BINUCLEATA) the nuclear material which is concerned with kinetic activities is located in a separate nucleus or kinetonucleus. The present authors do not agree with this contention, but maintain that the kinetonucleus is homologous with the so-called parabasal body of the intestinal flagellates (see the section on HÆMOFLAGELLATES) and has no nuclear function.

Mention should be made here of chromidia. In a large number of protozoa there is either scattered through the cytoplasm or arranged in definite masses (chromidial net) chromatic particles, known as chromidia (Fig. 16). Some investigators believe that the various forms of chromidia arise by the extrusion of chromatin from the nucleus. Others have described the reverse process, the origin of new nuclei from the chromidia. While the present authors are not prepared to dispute the fact that chromidia may arise from substances extruded from the nucleus, they do not believe that there is sufficient evidence that nuclei ever arise from chromidia. They are in harmony with the contention of Kofoid (1921) that the various descriptions of the *de novo* origin of nuclei and of the origin from chromidia are based upon a misinterpretation of the facts.

D. Reproduction

I. ASEXUAL REPRODUCTION

The common method of asexual reproduction in the protozoa is that of simple or binary fission. During this process first the nucleus, then the cytoplasm divides. In some cases the nucleus undergoes a series of divisions after which the

body divides into as many parts as there are nuclei. Such a process is known as multiple fission. In other cases the division of the cytoplasm only takes place at special periods in the life-cycle so that, as a result, the typical condition of the organism is that of a plasmodium. When division of such a plasmodium does take place, it generally forms two or more multinucleate bodies. Such a process is known as plasmotomy.

By far the most common method of nuclear division is by some form of mitosis which is a complicated but orderly series of changes in which both the various chromatinic and achromatinic structures in the nucleus play a rôle, and in which there is an accurate quantitative and qualitative halving of each chromatin element. This probably is universally true of the free-living species in spite of the fact that a number of cases of direct nuclear division or amitosis have been described in such forms. It seems probable that with finer cytological technique and more careful study these will be found to be cases of mitosis. While a number of parasitic and entozoic forms retain the mitotic method of nuclear division, many of them have apparently adopted, probably as a result of extreme specialization or degeneration, amitosis as the prevailing method of nuclear division. During amitosis the nucleus or its chromatin is simply constricted into two parts without the careful halving of each separate chromatin element. The general sequence of events in both mitosis and amitosis in the Protozoa are similar to those found in the higher forms and are too well known to need extended description here. In consequence, the following discussion is limited to those features which are more or less peculiar to the Protozoa.

a. Mitosis

The fundamental events which take place in mitosis in the Protozoa do not differ from those in metazoan cells. There are, however, many peculiarities in the division of protozoan

nuclei which have led to the classification of mitosis in the protozoa into the three following groups:

(1) PROMITOSIS.—This type of mitosis is characterized by the fact that (1) the entire process takes place within the nuclear membrane, and (2) the daughter karyosomes with their contained centrosomes constrict to form two huge

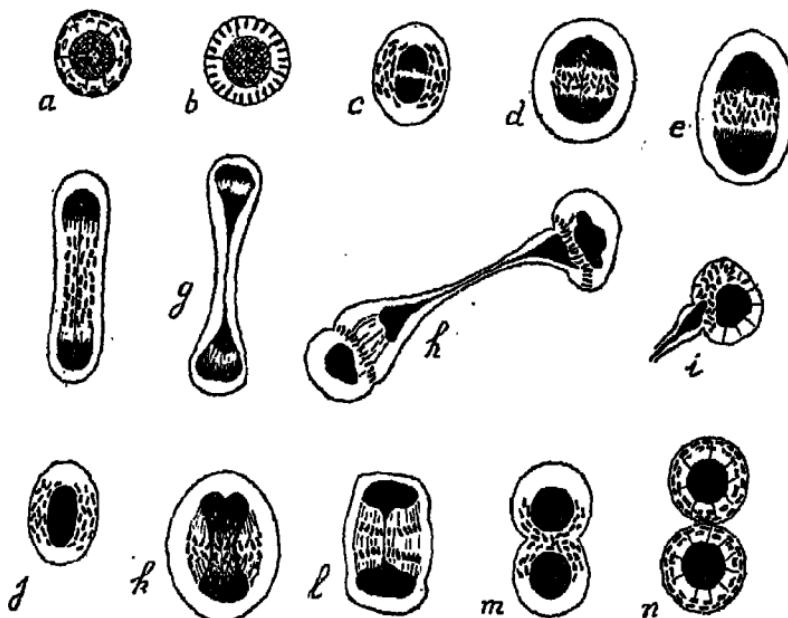


Fig. 6.—Two methods of promitotic division in *Amœba diplomitotica*. a, resting nucleus. b-i, first method. j-n, second method. In i only one of the two halves of the nuclear figure is shown. (From Chatton after Aragão.)

masses known as polar bodies. Promitosis is well illustrated in the division of *Amœba diplomitotica* (Fig. 6). In this form two types of promitosis occur. In the first type the chromosomes do not arrange themselves in a definite equatorial plate but are scattered irregularly on the spindle. In the second type the chromosomes are arranged in a definite equatorial plate and at the metaphase divide to form two

daughter plates. Both of these types of promitosis in *A. diplomototica* illustrate the formation and behavior of the polar caps or polar bodies.

(2) MESOMITOSIS.—This type of mitosis probably originated from the foregoing by a modification of the karyosome. In nuclei which divide by mesomitosis the karyosome has lost most of its plastin and chromatin elements, leaving little

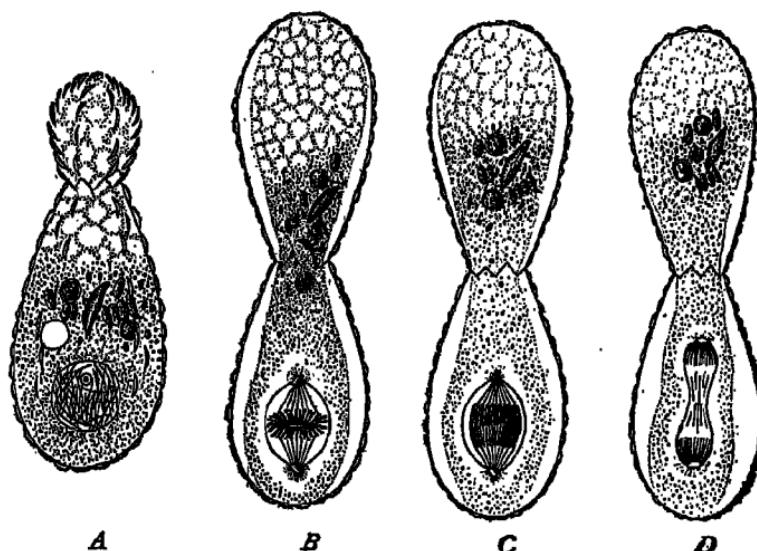


Fig. 7.—Mesomitosis in *Euglypha alveolata*. A-D, different stages in the division of the nucleus and the formation of the second individual. \times about 450. (From Calkins after Schewiakoff.)

more than the centrosome. In this manner the formation of polar caps is eliminated and a more or less typical mitotic figure results, produced, however, entirely within the nuclear membrane. Division of this type occurs in *Euglypha alveolata*, as described by Schewiakoff (1888) (Fig. 7).

(3) METAMITOSIS.—Mitosis of this type is characterized by the collaboration of both cytoplasmic and nuclear elements. In most cases the nuclear membrane disappears during the

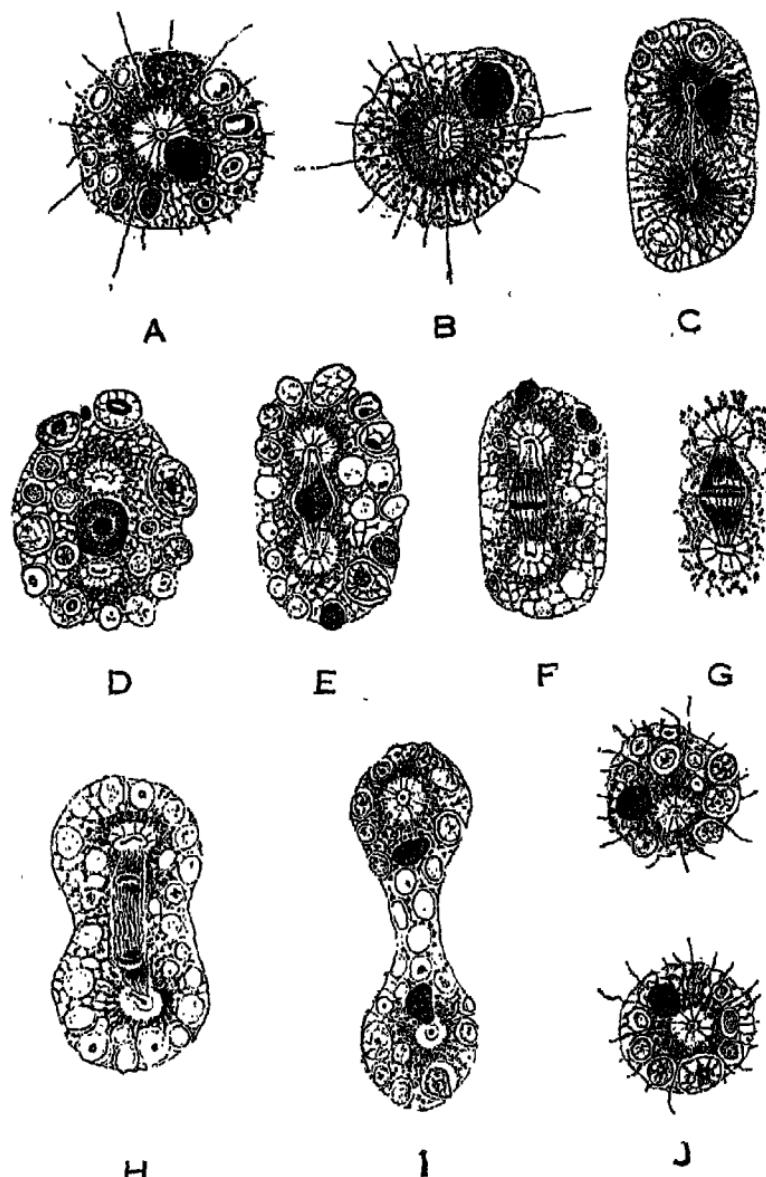


Fig. 8.—Metamitosis in *Ossnerella maritima*. A, resting nucleus; B-J, different stages in the division of the nucleus and the body, $\times 1250$. (After Dobell.)

process, although in some cases a portion of it remains. This type of division differs very little from the usual type found in metazoan cells. It probably arose from the mesomitotic type by the centrosome becoming an extranuclear organelle. It is beautifully represented in the division of some of the HELIOZOA, as, for example, in *Oxnerella maritima*, as described by Dobell (1917) (Fig. 8).

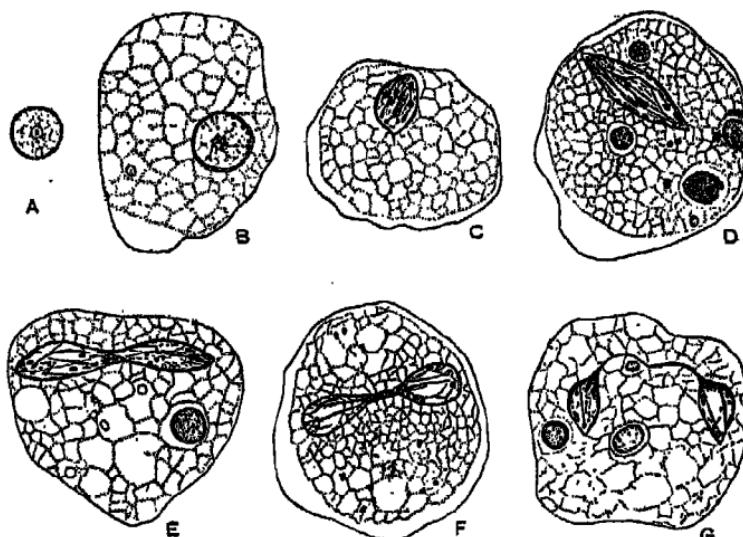


Fig. 9.—Nuclear division in *Endamoeba histolytica*. A, resting nucleus in large trophozoite; B-G, different stages in nuclear division. Note the absence of definite chromosomes and true mitotic figures. $\times 1250$. (After Dobell.)

From the foregoing it must not be concluded that the three types of division form sharply limited classes; they really represent a very gradual series. A number of very peculiar atypical cases of mitosis in the protozoa which do not fit readily into this classification need not be described here. For a much fuller, but very diagrammatic account of mitosis in the protozoa, the reader is referred to Robert (1914).

b. Amitosis

Amitosis differs from mitosis in that the chromatin in the nucleus is simply constricted into two masses without the elaborate quantitative and qualitative division of each chromatin element as is found in mitosis. It is unnecessary to give a detailed account of amitosis in any particular form. Almost every gradation between simple amitosis and true mitosis has been described. In some cases it is very difficult to classify the type of division. The division of *Endamoeba histolytica*, as described by Dobell (1919), is an example of this kind. At first this appears to be a true mitosis, but more careful consideration seems to indicate that the actual division of the chromatin elements takes place irregularly by amitosis. The sequence of events in this division is given in figure 9, which, with its legend, is self-explanatory. A more recent account of the division of *Endamoeba coli* by Swezy (1922) indicates that nuclear division in this member of the genus is mitotic.

In the group INFUSORIA, where there is a separation of the chromatin into a vegetative nucleus or macronucleus and a generative nucleus or micronucleus, the

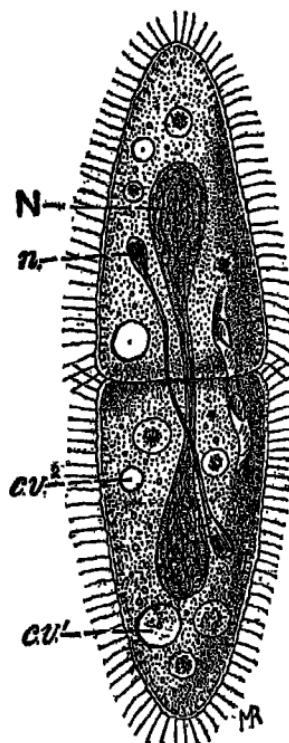


Fig. 10.—Fission in *Paramecium caudatum*.

The macronucleus (*N*) divides by amitosis and the micronucleus (*n*) divides by mesomitosis. *c.v.*¹, original and *c.v.*², new contractile vacuoles in one daughter individual. (From Minchin after Bütschli and Schewiakoff.)

two nuclei do not behave alike in division. The micronucleus always divides by mitosis—generally a mesomitosis, whereas the macronucleus divides by amitosis. This is well shown in figure 10, which represents the division of *Paramecium caudatum*. In the budding of some of the Suctoria the macronucleus divides by a multiple amitosis or budding.

It is very interesting to speculate on the probable evolution of mitosis in the protozoa. Minchin (1912) and others believe that amitosis represents the primitive condition and that the gradations described above between amitosis and mitosis represent the probable evolution of the process. Criticisms of this view are (1) that amitosis is probably degenerative rather than primitive, and (2) that most cases of amitosis and intermediate types occur in entozoic or semi-entozoic species. If these objections are well founded, as the authors believe they are, the typical mode of nuclear division in the protozoa is that of mitosis. Entozoic species, however, exhibit every gradation in the degeneration toward amitosis.

2. SEXUAL REPRODUCTION

In following the life-histories of the forms which are described in the succeeding pages, we will find that many forms have a definite sexual stage in their life-history. The important thing to note is that in these cases the nuclei undergo a true reduction division as in the case of gamete production in the metozoa. Likewise these gametic nuclei, or nuclei with the reduced amount of chromatin, come together and fertilization takes place. An account of this process will be deferred until the INFUSORIA are described, when the process will be considered, not only from a structural but from a physiological and genetical standpoint. At the same time the very interesting question of endomixis will be considered.

CHAPTER II¹

A GENERAL CONSIDERATION OF THE SARCODINA

A. Introduction

1. CHARACTERISTICS OF THE CLASS

The Sarcodina are differentiated from the other classes of the PROTOZOA by the fact that the "adult" organisms are devoid of permanent organs of locomotion—locomotion and food ingestion being effected by more or less temporary protoplasmic processes or pseudopodia. From an evolutionary standpoint the MASTIGOPHORA and not the SARCODINA probably represent the most primitive class of the PROTOZOA. Taken as a whole, however, the SARCODINA present fewer structural differentiations than the MASTIGOPHORA, and for that reason are considered first.

2. *Amœba proteus* AND ALLIED SPECIES AS REPRESENTATIVES OF THE CLASS

The characters of the class are well brought out in the free-living species *Amœba proteus*. This organism (Fig. 11, A) is usually about 600 μ in length during locomotion although it may reach a length of 1,200 μ . The protoplasm of its body consists of an outer layer of clear ectoplasm and an inner layer of granular endoplasm. The former is not smooth as in most species, but is folded so as to form

¹By W. H. Taliaferro.

definite longitudinal ridges along the animal (Fig. 11, A). The latter contains a number of small granules or micromeres and sometimes a number of crystals of the form shown

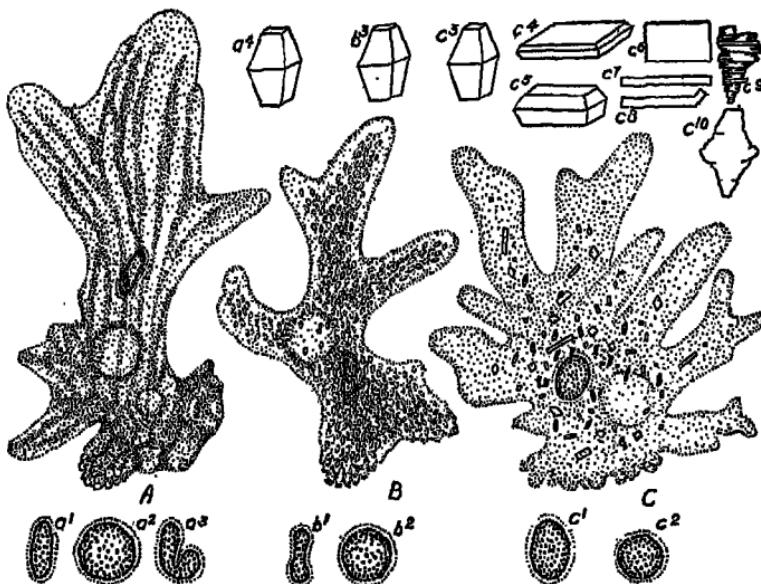


Fig. 11.—A, *Amœba proteus* in locomotion. Note especially the longitudinal ridges. a^1 , equatorial view of the discoid nucleus. a^2 , a polar view of the nucleus. a^3 , equatorial view of a folded or crushed nucleus frequently found in large individuals. a^4 , shape of crystals found in this species. B, *Amœba discooides* in locomotion. b^1 , b^2 , equatorial and polar views of the discoid nucleus. b^3 , shape of the crystals found in the ameba. C, *Amœba dubia* in locomotion. c^1 and c^2 , equatorial and polar views of the ovoid nucleus. c^3 - c^{10} , shapes of crystals found in *A. dubia*. In these drawings only such characters as are of special interest for the purpose of this work are emphasized. Dimensions in microns: A, 600 in length; B, 450 in length; C, 400 in length; a^1 , 46×12 ; b^1 , 40×18 ; c^1 , 40×32 ; a^4 , maximum, 4.5; b^3 , maximum, 2.5; c^3 - c^{10} , maxima, 10 to 30. (Figures and legend after Schaeffer.)

in figure 11, A, a^4 . It contains the nucleus which, in the younger specimens, is a bi-concave disc, but, in the older specimens, is often folded and convoluted, and which, in

both living and stained specimens, can be seen to contain a large number of chromatin granules. The contractile vacuole also lies within the endoplasm. This organelle undergoes a regular diastole and systole, filling itself with a clear fluid from the protoplasm and discharging it to the exterior. Finally, the endoplasm contains food vacuoles which serve as temporary stomachs and which may contain food in all stages of digestion. The animal moves by means of large lobose pseudopodia. These always begin by an extrusion of the ectoplasm into which the endoplasm flows almost immediately. As the animal moves along, there is a decided tendency for the pseudopodia to be formed in succession on alternate sides. As we shall see later, these pseudopodia not only serve as organs of locomotion, but also as organs for food capture and ingestion.

Asexual reproduction is effected by binary fission. The nucleus divides by mitosis and this is followed by the constriction and division of the cytoplasmic body. The complete life-history of *Amœba proteus* is not known at the present time although a number of interesting observations have been made by Scheel (1899), Metcalf (1910), Calkins (1907), and Hausman (1920). In fact, it may be said that the life-history of no amoeba is known completely at the present day. Most species that have been studied undergo encystment and during some stage in their life-cycle may form flagellated gametes. One of the most thorough pieces of work along this line is that of Miss Wilson (1916) on *Nægleria* (= *Dimastigamœba*) *gruberi* to which the reader is referred. Recently Miss Bunting (1922) has described a very interesting life-cycle in a coprozoic amoeba. This work is summarized in the account of coprozoic forms (see p. 506).

Not only is there great confusion regarding the life-history of the amoebæ, but also in reference to their classification. This no doubt results from the fact that there

are so few characteristics in these organisms which can be readily described and recognized. Various investigators have described and classified amœbæ on their cytoplasmic characters, nuclear characters, or details of their life-history. We will probably find in the future, however, that many of the organisms which we now consider distinct species are different stages in the life-history of one organism and that many of our so-called species will have to be broken up into smaller groups. *Amœba proteus* itself furnishes a good example of the confusion that now exists in the classification of this group. Schaeffer (1917a), by careful microscopical observations and appropriate culture methods, has demonstrated that under this name we have been confusing three different species. There is, first, the original form described by Leidy (1879) under this name. As stated above it is characterized by the possession, in the younger forms, of a nucleus in the shape of a bi-concave disc, which may become folded and convoluted in the older forms. It also has definite ectoplasmic ridges which are constant cell structures. There is, second, a form much like the true *Amœba proteus* except that it possesses a disc-like nucleus which never becomes folded and its ectoplasm never shows the ridges constantly present in *Amœba proteus*. Schaeffer has given the name *Amœba discoidea* to this species (Fig. II, B). Finally, there is the form described by Penard (1902) under the name "*Amœba proteus*" which possesses an ovoid nucleus instead of a discoidal one and which does not show any cytoplasmic ridges. Furthermore, it is characterized by a type of locomotion different from that of the previous species. To this form Schaeffer has given the name *Amœba dubia* (Fig. II, C). By means of carefully pedigreed cultures, Schaeffer has been able to show that these three forms breed true and probably represent true species.

B. Classification

The SARCODINA are differentiated for two modes of life. On the one hand the amoeboid type, such as *Amœba proteus*, is characteristic of creeping forms, and on the other hand the radiate type is characteristic of floating forms. This difference in habit is used as a basis for the classification of the group; first, the RHIZOPODA or creeping forms, and second, the ACTINOPODA or floating forms.

The following classification is patterned largely after Minchin (1912):

CLASS SARCODINA

A. Subclass Rhizopoda.—Typically creeping forms with lobose (branched, root-like or finger-like) or reticulose (anastomosing) pseudopodia.

I. ORDER LOBOSA. Amœboid forms of simple structure and lobose pseudopodia; skeleton lacking or in form of a simple shell.

1. SUBORDER GYMNAMOEBA. Without shell or skeleton.
2. SUBORDER THECAMOEBA. With shell.

II. ORDER PROTEOMYXA. With filose or reticulose pseudopodia and without shells. Flagellated and heliozoon-like stages often occur.

III. ORDER FORAMINIFERA. With reticulose pseudopodia and with shells.

IV. ORDER MYCETOZOA. Semi-terrestrial forms with reproduction by resistant spores and formation of plasmodia.

B. Subclass Actinopoda.—Typically floating forms with radiating, unbranched pseudopodia.

V. ORDER HELIOZOA. Principally fresh water, without a "central capsule."

VI. ORDER RADIOLARIA. Exclusively marine, with a "central capsule."

I. LOBOSA

The members of this order are differentiated from the remainder of the RHIZOPODA by the character of their pseudopodia, which are typically lobose or finger-like in shape and are never filose or reticulose. The order is generally sub-divided into the GYMNAMOEAE, which are



Fig. 12.—*Amœba verrucosa*. The body of the amoeba is covered with a delicate pellicle and in consequence movement and the formation of pseudopodia are very sluggish. The nucleus lies within an elongated vacuole. $\times 500$. (Drawn by Dr. W. A. Kepner.)

devoid of shells or tests, and the THECAMOEAE, which possess simple shells or tests.

The genus *Amœba* and allied forms are included under the GYMNAMOEAE. A description of three of the larger free-living species (*A. proteus*, *A. discoides* and *A. dubia*) has already been given. Figure 12 shows another species, *Amœba verrucosa*. The majority of the species in the genus *Amœba* are uninucleate although a few possess a large number of nuclei, such as *Amœba carolinensis* described by Wil-

son (1900) as *Pelomyxa carolinensis* (Fig. 13). All of the species exhibit a sharp differentiation between endoplasm and ectoplasm, but great specific variations exist in the viscosity of the ectoplasm. The ectoplasm of such forms as *A. limicola*, for example, is very fluid in nature whereas that of *A. verrucosa* is modified into a tough pellicle. The allied genus *Pelomyra* includes a number of fresh water amoebæ which possess two or numerous nuclei (Fig. 14). The characteristic feature of this genus is that the cytoplasm contains a number of refringent bodies which are of an albuminous nature and which are associated with a bacterial organism

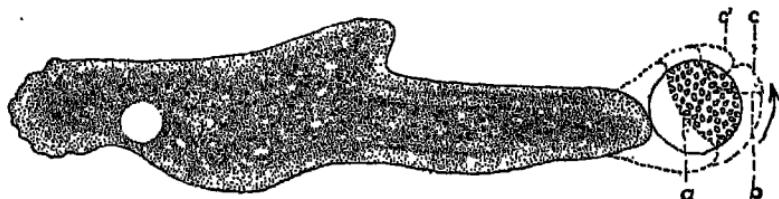


Fig. 13.—*Amoeba carolinensis*. A large multinucleate amoeba. The contractile vacuole is seen in the posterior part of the body. The organism is ingesting a plant; *Eromosphaera*; *a*, *b*, *c*, and *c'* indicate successive positions of the pseudopodia involved in the process. $\times 75$. (After Kepner and Edwards.)

Cladotrichix pelomyxa Vely. Furthermore the organisms generally load their cytoplasm with sand and debris of all kinds in addition to food material. *Pelomyxa* reproduces itself asexually by simple fission and sexually by the formation of gametes. In some forms, the gametes are heliozoon-like with slender radiating pseudopodia which conjugate in pairs. Each resulting organism develops into a young pelomyxa (Bott, 1907).

The suborder GYMNAMOEBA is particularly interesting from a parasitological standpoint because it includes all of the parasitic species of amoebæ. These will be considered in detail in Chapter III.

The suborder THECAMOEBA includes those forms which

have lobose pseudopodia and possess a simple shell, that is, one which consists of but a single chamber with a single opening through which pseudopodia are protruded. In composition the shells vary greatly in different genera. They may be secreted entirely by the animal, as in *Hyalosphenia*, or may consist of foreign particles cemented together as in *Diffugia* (Fig. 15). A majority of the THECAMOEBAE possess

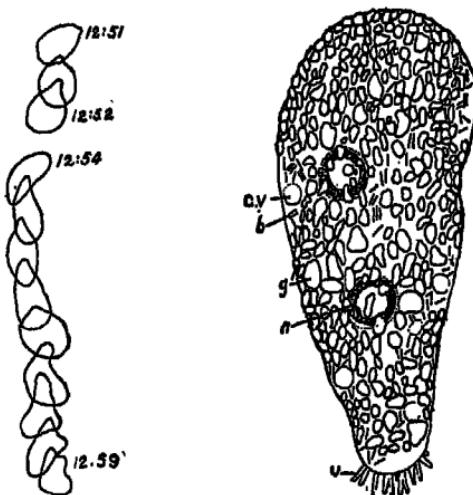


Fig. 14.—*Pelomyxa schiedti*. *b*, bacterial rods characteristic of the genus *Pelomyxa*. *c.v.*, contractile vacuole. *g*, glycogen bodies. *n*, nucleus. *u*, uroidal projections. At the left is shown a series of outlines of a specimen during locomotion. Length, about 75 microns. (After Schaeffer.)

a single nucleus, but a large number possess numerous nuclei. In the genus *Arcella*, for example, *A. dentata* has two nuclei, and *A. polypora* has as many as fifteen. Associated with the nucleus in many forms is a ring of chromidia. The exact function of this chromidial body (Fig. 16), as it is often called, is unknown. It may arise from the nuclear material. Several investigators have even described the origin of primary nuclei from it at certain stages in the life-cycle of

the organism. The work of Hegner (1920) indicates that it does not play the same rôle in vegetative reproduction as do the nuclei, and it is probable that, even if it does arise

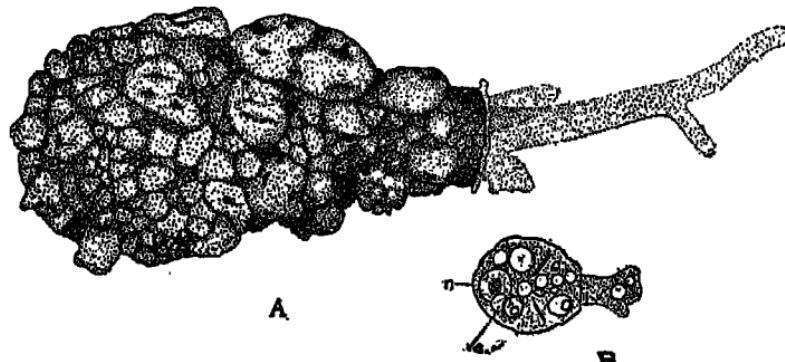


Fig. 15.—Examples of THECAMOEAE. A, *Diffugia pyriformis* in which the shell consists of minute sand grains cemented together. B, *Hyalosphenia* sp. in which the shell consists of plates secreted by the animal. c.v., contractile vacuole; n, nucleus. A $\times 240$; B $\times 600$. (A drawn by Dr. B. D. Reynolds; B drawn by Dr. W. A. Kepner.)

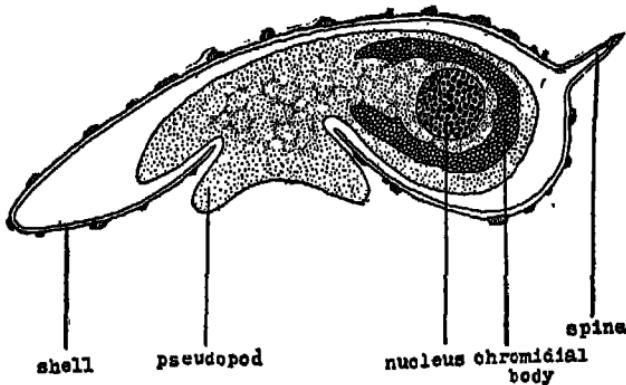


Fig. 16.—*Centropyxis aculeata*. Diagrammatic sagittal section showing nucleus and chromidial body. (After Root.)

from the nucleus, it can never regain its nuclear function. Asexual reproduction takes place by binary fission, just as in the GYMNAMOEAE, but is complicated by the presence of

a shell. In the case of *Diffugia corona* the organism prior to division accumulates a number of sand grains within its interior. At the time of reproduction the protoplasm absorbs water, swells, and projects through the mouth of the shell. The projecting mass finally attains the size and assumes the shape of another diffugia. The sand grains then rise to the surface, spread over it, and become embedded in a chitinous secretion which hardens and completes the formation of a new shell. By the time the new shell is formed, the nucleus has already divided and one of the daughter nuclei migrates into the newly protruded mass of protoplasm. Finally, the protoplasmic masses separate and two diffugiae are formed, one inhabitating the old shell and the other the new shell. (Jennings, 1916.)

2. PROTEOMYXA

This order contains a number of forms of very doubtful relationships, the only characters which they all possess in common being the absence of a shell and the formation of filose or reticulose pseudopodia. Different authors classify this group in different ways. Minchin (1912) combines them with the LOBOSA to form the order AMOEBA. More recently Doflein (1916) places them among the primitive HELIOZOA. They undoubtedly do show many characteristics similar to both the LOBOSA and HELIOZOA. Doflein recognizes three families: (1) the HELIOFLAGELLIDÆ, including flagellated forms and forms with heliozoon-like pseudopodia, which may or may not also possess flagella; (2) the ZOOSPORIDÆ, including forms with heliozoon-like pseudopodia which never possess flagella in the heliozoan stage, reproduction being accompanied by the formation of a cyst which eventually liberates flagellated swarmers; and (3) the VAMPYRELLIDÆ, including forms whose pseudopodia, while they may be filose or reticulose, are not as truly heliozoon-like as are those found in the first two families. In many

ways the last family seems to possess more amoeba-like than heliozoan characteristics. Reproduction, in it, is associated with the formation of cysts which, when ripe, liberate amoebulae and never flagellated swarmers.

An example of the PROTEOMYXA is found in *Pseudospora volvocis* which often parasitizes colonies of *Volvox*. (See Robertson, 1905.) The species is a member of the family ZOOSPORIDÆ and occurs in an amoeboid, flagellated and helio-

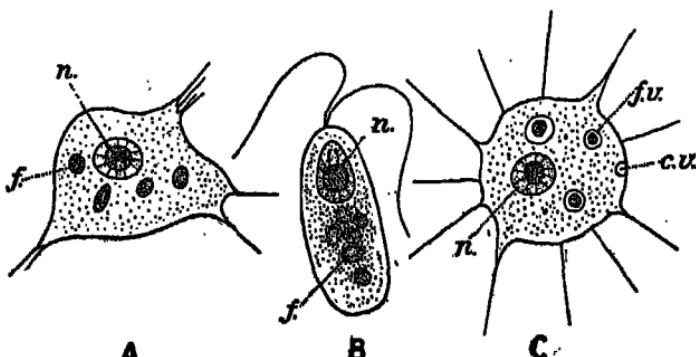


Fig. 17.—*Pseudospora volvocis*. A, amoeboid stage. B, flagellated stage. C, heliozoon-like stage. c.v., contractile vacuole; f and f.c., food vacuoles; n, nucleus. (From Kerr after Robertson.)

zoan stage (Fig. 17). In infected colonies of *Volvox* the amoeboid stages can be seen creeping about and devouring the cell-individuals of its host.

3. FORAMINIFERA

All of the FORAMINIFERA possess shells, but the organisms differ from the THECAMOEBAE in that they form reticulose (rarely, filose) pseudopodia, which are used principally for capturing food as the organisms are typically very slowly creeping forms. Their shells are of various compositions. Those which are secreted by the animal are generally chitinous or calcareous in nature, but a few are siliceous or gelatinous in composition. Some consist of foreign materials

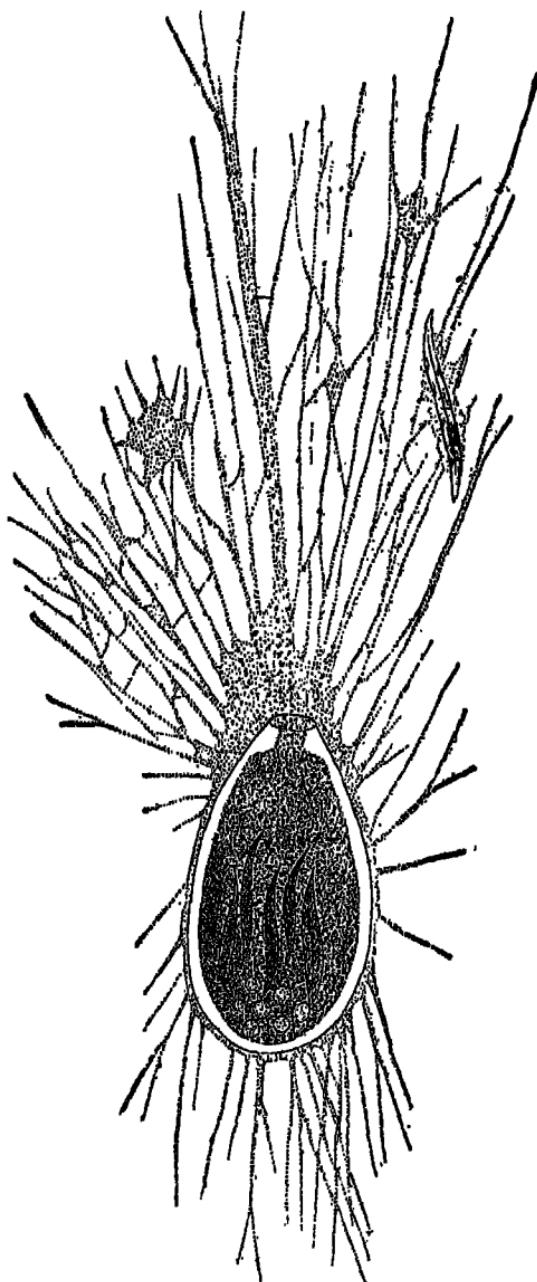


Fig. 18.—*Allogromia ovoidea*: an example of a monothalamic foraminiferan. (From Doflein after Schultze.)

cemented together. Thus, for example, *Haliphysema tumnowiczi* selects sponge spicules and *Technitella thompsoni* selects the calcareous plates of echinoderms. Structurally, their tests are generally divided into imperforate and perforate types, the former possessing a single opening through which the pseudopodia are protruded, just as in the case of the THECAMOEBAE, the latter possessing, in addition, a large

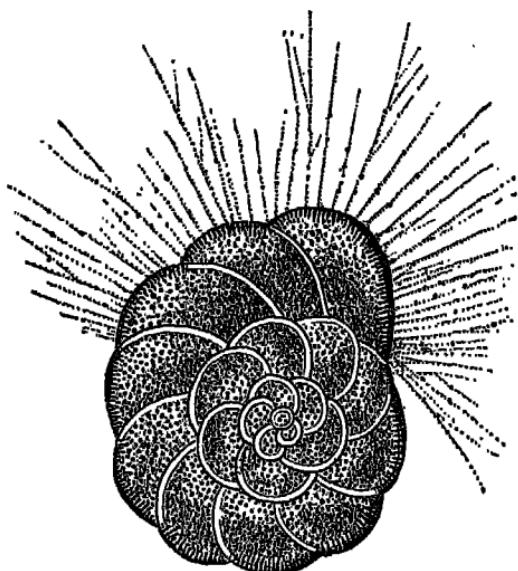


Fig. 19.—*Rotalia freyeri*: an example of a polythalamous foraminiferan. (From Doflein after Schultze.)

number of fine pores through which the network of pseudopodia project. In the simpler FORAMINIFERA the shells contain a simple chamber similar to that of the THECAMOEBAE and are known as monothalamous (Fig. 18). Among these forms reproduction occurs by binary fission just as in the latter group. In the more complex forms, however, at the time of division the organisms do not separate but simply add another chamber to the shell structure. This gives rise to the polythalamous or many chambered shells. In the

formation of such shells, each succeeding chamber is larger than the preceding one (Fig. 19). Such polythalamous species, as far as is known, exhibit a complex life-history in which there is an alternation of generations combined with a dimorphism in the adult condition. The best known life-history is that of *Polystomella crispa* (Lister, 1903-1906). The FORAMINIFERA lack the sharp distinction between ectoplasm and endoplasm that was noted in the amoebae. The cytoplasm often becomes loaded with brown plastids which are apparently of a metabolic nature. Their elimination, which is generally considered to be a type of defecation, takes place periodically, just prior to the formation of a new chamber. By far the majority of the FORAMINIFERA are marine forms, although a few occur in fresh water. It is probable that some of the so-called PROTEOMYXA will have to be classed as naked FORAMINIFERA when their life-history is more clearly understood. Many investigators, the present authors included, consider the XENOPHYOPHORA as an appendix to the FORAMINIFERA. Other authors, notably Minchin, elevate them to a separate order. They are all organisms of a deep sea habit and form a plasmodium containing numerous nuclei and chromidia encased in a network of hollow tubes which is composed of an organic substance apparently related to spongin. In fact, Haeckel originally classified them as sponges. A classification of these organisms can be found in Schultze (1905).

4. MYCETOZOA

The MYCETOZOA are semi-terrestrial forms which live for the most part saprophytically on decaying vegetable matter. The adult plasmodium is generally a sheet or network of protoplasm containing many thousands of nuclei and numerous contractile vacuoles. It sometimes reaches a large size,—several inches in diameter, and is often brightly colored. Movement of the plasmodium is associated with beautiful

streaming movements of the internal protoplasm. The plasmodia react to unfavorable conditions, such as drying, by forming numerous small cysts, each containing about ten to twenty nuclei. When these are moistened, they liberate small plasmodia which coalesce to re-form the original mass. Reproduction is associated with the formation of a complicated capsular structure known as a sporangium, inside of which a large number of uninucleate spores are formed. When the sporangium is ripe, it bursts and, aided by such factors as the wind, distributes the spores. Protecting each spore from drying is a tough envelope which, when moistened, bursts and liberates a uninucleated amoeba, known as a myxamoeba, the structure of which is very similar to that of a true amoeba. It possesses a single contractile vacuole and moves and captures food by means of its pseudopodia. After a time it develops a flagellum, being then known as a myxoflagellate, which may also lead a free existence and multiply by fission, or it may even encyst. The flagellum functions very little as an organ of locomotion, the myxoflagellate not only moving but also capturing food, such as bacteria, by means of its pseudopodia although both this form and the amoeba-like form may obtain a certain amount of food saprophytically. In time the myxoflagellate loses its flagellum and reassumes an amoeboid form which differs from the first in that it tends to clump and later to fuse with other amoeboid forms. The resulting plasmodium feeds and grows by multiplication of its nuclei and increase of its cytoplasm. It is to be noted that in the fusion of the amoeboid aggregation to form the plasmodium, the nuclei do not fuse, but retain their identity.

5. HELIOZOA

As a group the HELIOZOA are characterized by a more or less spherical body with stiff radiating pseudopodia. Although a few attach themselves by a definite stalk, the majority of

forms are floating and, with the exception of a few marine forms, live in fresh water. They can be differentiated from the RADIOLARIA by the absence of a central capsule. As an order, the group is very difficult to limit. In addition to the PROTEOMYXA, which show a great many affinities to the true HELIOZOA, as noted above, there are many other border line groups. For this reason the following discussion will be limited to a few of the characteristics that are undoubtedly

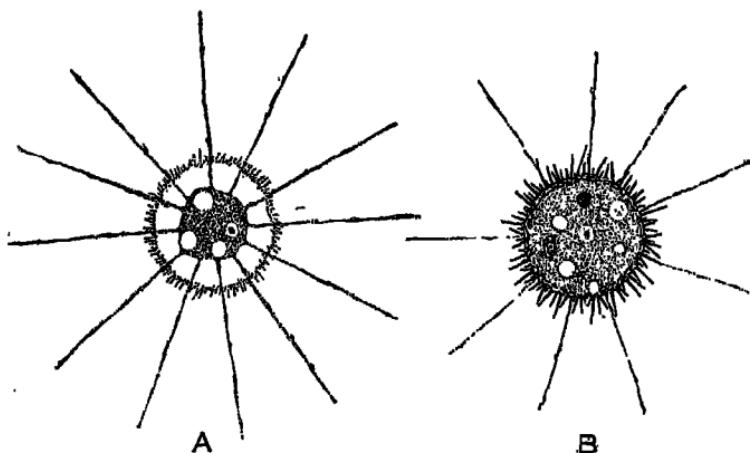


Fig. 20.—HELIozoA.

A, *Heterophrys* sp. B, *Acanthocystis aculeata*. A $\times 1000$; B $\times 500$. (Original.)

heliozoan. The body is generally divided into two regions: (1) a cortical layer, which is alveolar in appearance, contains the contractile vacuole and gives rise to the pseudopodia, and (2) a medullary layer which contains the nuclear apparatus, food vacuoles, and, in some cases, symbiotic algae. The stiff, radiating pseudopodia, in the majority of species, are supported by fine internal rods, called axostyles, which show a very interesting relation to the nucleus. In the uninucleate forms, such as *Actinophrys*, they all radiate from the central nucleus (Fig. 5, B). In other forms, such as *Camptonema*

nutans, in which there is an equal number of nuclei and pseudopodia, they each arise from a single nucleus. Many multi-nucleate forms, such as *Actinosphærium*, have probably risen from the *Actinophrys* type by a multiplication of the nucleus. In other species, such as *Acanthocystis*, the axostyles radiate from a central granule and the nucleus is eccentrically placed. In the sedentary *Wagnerella*, the body is differentiated into a head stalk and basal plate. The former contains a central grain from which the pseudopodia radiate, and the latter contains the nucleus. Many HELIOZOA possess skeletons which may be either simple or complex in structure and may be composed of various materials. Asexual reproduction is effected by either binary fission or gemmation. Sexual phases have also been described in various species, but they are too complicated to be considered here. A few forms, such as *Raphidiophys*, remain loosely connected after binary fission and thus form colonies.

6. RADIOLARIA

The RADIOLARIA possess very much the same form as the HELIOZOA. They are, however, exclusively marine and, except in very rare cases, possess a central capsule which is a membranous structure and divides the protoplasm of the body into an intra- and extra-capsular region (Fig. 21). The extra-capsular region is itself divided into three layers. (1) The assimilative layer contains the food, which is taken in by the pseudopodia, and various metaplastic granules. (2) The calymma, which contains a large number of vacuoles filled with fluid, is supposed to have a hydrostatic function for, when the vacuoles are expelled from the body, the animal sinks to the bottom and, when they are reformed, it again rises. In some species this layer also contains a number of yellow granules or cells which are supposedly symbiotic algae. (3) The external layer surrounds the body and from it the pseudopodia arise. As a rule, these pseudopodia are com-

posed entirely of protoplasm, but, in some species, they are supported by axial rods. The intra-capsular region contains the nuclear apparatus which may consist of one or numerous nuclei. The protoplasm of the intra- and extra-capsular

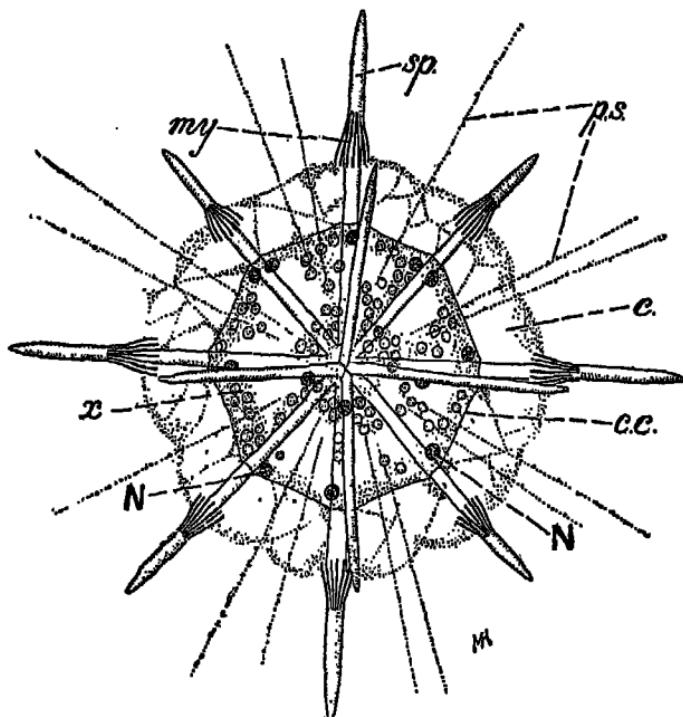


Fig. 21.—*Acanthometra elastica*: a radiolarian. *c.*, calymma, which is vacuolated and is probably hydrostatic in function; *c.c.*, central capsule, which is characteristic of the order; *my*, "myophrisks," which are peculiar rod-like bodies clustered around each spine; *N*, nuclei, which are numerous; *p.s.*, pseudopodia, *sp.*, spines of skeleton of which there are twenty; *x*, yellow cells. (From Minchin after Bütschli, Leuckart and Nitsche's "Zoologische Wandtafeln.")

regions is connected by pores in the central capsule. In a few species in which the skeleton is lacking or in which it consists of a few spicules, reproduction takes place by binary fission. In the majority of species, however, reproduction

is associated with the formation of flagellated swarm spores which rise by a process of multiple fission within the intracapsular region.

C. General Physiology

The fact that the members of the group SARCODINA, especially the various species of amœbæ, are visibly less differentiated than most other PROTOZOA and METAZOA, has led many investigators to hope that the fundamental physiological processes would be exhibited in a less complex manner in these forms than in other animals. While this has led to many interesting and important results, it has not proved to be an easy road to a knowledge of the fundamentals of general physiology. The physiology of the so-called simple amœbæ presents the same problems as are found in other animals. Indeed, the very fact that all of the physiological processes have to be studied in such minute organisms which possess so few structural differentiations tends to make the problems all the more difficult.

I. NUTRITION AND ASSIMILATION

The great majority of the SARCODINA are holozoic in their nutrition. The animals capture, ingest and digest their food, and finally eliminate the waste matter. The SARCODINA ingest their food, essentially, by surrounding it with the more or less fluid protoplasm of the body. Then they pass it into the endoplasm where it lies within a vacuole, known as a food vacuole. Ingestion of food by *Amœba proteus* is shown in figure 5, A and figure 22, by *A. carolinensis* in figure 13, and by *Allogromia ovoidea* in figure 18. The location of the food vacuole is usually constantly changing due to currents in the endoplasm. The different stages in the digestion of food have been studied by a number of observers whose results are not, however, always in agree-

ment and will not, therefore, be taken up in detail in this outline. Briefly, the endoplasm secretes various ferments which are poured into the food vacuoles and finally digest the food particles, the soluble products of digestion being absorbed by the endoplasm. After digestion and absorption are completed, the indigestible residue is eliminated in most

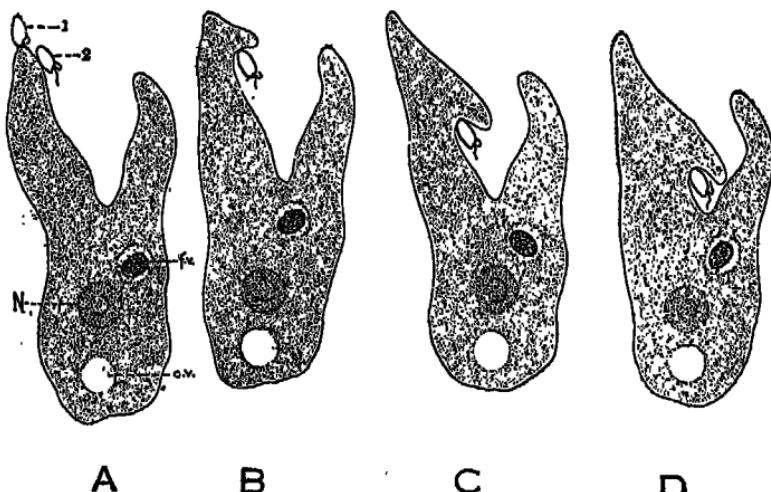


Fig. 22.—*Amœba proteus* ingesting a small flagellate; *Chilomonas paramecium*. A, B, C and D, different stages in the process: at D the protoplasm of the amœba flowed above and below the prey so that it was enclosed within a food vacuole. 1, position of prey when the amœba came in contact with it; 2, position of prey when ingesting reaction started; c.v., contractile vacuole; f.v., food vacuole; N, nucleus. $\times 100$. (After Kepner and Taliaferro.)

cases by simply being passed out of the body. In some forms, such as the FORAMINIFERA, however, there is a great accumulation of fecal material, known as the "stercome," which is eliminated at definite periods. It is interesting to note that the parasitic amœbæ have largely retained this holozoic method of nutrition. Among the human amœbæ, *Endamoeba histolytica* feeds on red cells and various tissue elements while the other species devour almost every type of

intestinal debris, bacteria, and even cysts of other protozoa. These species, especially *Endamoeba histolytica*, however, probably supplement their holozoic nutrition with the direct absorption of material through the body surface.

2. SECRETION

A large number of widely different substances are secreted as a result of the vital activities of the SARCODINA. We have already noted the production of digestive ferments by the endoplasm. Supplementary to the capturing of food other secretions are formed. The FORAMINIFERA and HELIOZOA secrete a substance which kills their prey and makes it stick to a pseudopodium so that it can be drawn into the body. Then, too, there is some evidence that even the amoebæ often paralyze actively moving prey before coming in contact with it. In this connection, it is interesting to note the feeding habits of *Vampyrella*. This organism, which belongs to the order PROTEOMYXA, lives almost entirely on filamentous algae, such as *Spirogyra*. In feeding it secretes an enzyme which dissolves a hole in one of the walls of the filament and then it extracts the living protoplasm through the hole. Among the shelled SARCODINA various substances are secreted in the manufacture of the tests. The pseudopodia of many forms, such as *Diffugia*, are covered with a mucilaginous substance which apparently functions in the adhesion of these organelles to the substratum. *Arcella* has the power of secreting gas bubbles within its endoplasm which probably perform a hydrostatic function, enabling the animal to raise and lower itself through the water. The human parasitic ameba, *Endamoeba histolytica*, apparently secretes a proteolytic enzyme which dissolves the wall of the intestine allowing the animal to make its way through the tissues and to absorb food materials from the histolysed tissue.

3. EXCRETION

Closely allied to the process of secretion is that of excretion. The soluble waste materials are excreted either by diffusion through the general body surface or after accumulation in the contractile vacuole by emptying from it to the exterior. Some investigators believe that the primary function of the contractile vacuole is to eliminate such wastes from the organism. This organelle, however, is comparatively rare in parasitic forms and is often lacking in marine organisms. Other investigators consider it to be primarily concerned in compensating for the tendency of the protozoan body to imbibe water and in regulating the osmotic pressure of the organism. This may explain the slowness of pulsation or the absence of the vacuole in marine or parasitic forms. In any case, however, the contractile vacuole voids fluid which has passed through the body of the protozoon and probably does, therefore, play some rôle in the elimination of soluble wastes, whatever its primary function may be.

Another very effective method of eliminating waste materials from the body is to throw them out of solution by precipitation. Various insoluble wastes of this kind are found in the SARCODINA. The crystals described in *Amœba proteus* are probably of this nature. The pigment in *Actinosphærium* is apparently a waste product formed from the degeneration of the chromatin of the nucleus. Later, we will see that the melanin pigment of certain blood-inhabiting SPOROZOA is probably an insoluble waste formed from the digestion of haemoglobin. Organisms which form excretion crystals often rid themselves of them just prior to encystment.

4. RESPIRATION

Respiration, in its broadest sense, includes any process by which an organism liberates the potential energy represented in the complex chemicals of its body. It may be effected

either aerobically by oxidation or anaerobically by the splitting up of complex chemical substances into simple compounds. The end result in either case is the same. Practically all free-living protozoa live in an environment containing more or less dissolved oxygen. Through the general body surface the animal absorbs the oxygen and eliminates the carbon dioxide and water which result from the oxidation. The fact, as stated above, that the contractile vacuole is continuously discharging fluid to the exterior which is passed through the body of the organism makes it probably that this is a particularly effective method of eliminating these wastes. The experiments of Lynch (1919) and others indicate that respiration takes place in the cytoplasm independently of the nucleus. Many parasitic forms live in environments which are partially or totally lacking in oxygen. Here respiration must be of the anaerobic type.

5. MOVEMENT

The most important transformation of energy is that which is involved in movement. Amœboid movement has always been one of the most fascinating problems of general physiology. Investigators are, however, so far from an agreement as to the mechanism of this phenomenon that it seems useless to go into detail here. Suffice it to say that the old surface tension theory, which assumed that pseudopodia were formed as a result of a local diminution in the surface tension at the surface of the body of the amœba, will not adequately account for the process. Protozoa are a complex series of colloidal substances and the formation of pseudopodia is probably connected with such phenomena as solation and gelation. As such, it can not be due simply to a diminution of surface tension on the *surface* of a more or less homogeneous mass of fluid as is the case in the so-called physical imitations of amœboid movement, although changes in surface tension probably play an important part in the

changes in the different phases within the colloid. The most recent work is that on *Amœba proteus* by Mast (1923), who maintains the following: The body of an amœba is divided into three parts: (1) the plasmasol which is a central elongated fluid portion, (2) the plasmagel which is a solid layer surrounding the plasmasol and which is divided into an outer hyaline and an inner granular portion, and (3) the plasmalemma which is a very thin elastic surface layer or membrane. The plasmasol and the granular portion of the plasmagel constitute the endoplasm and the hyaline portion of the plasmagel and the plasmalemma constitute the ectoplasm. During locomotion the plasmasol continuously flows forward and at the anterior end changes into plasmagel while at the posterior end the reverse process takes place. The plasmalemma, which is a fairly permanent structure, is sufficiently distinct to move freely over the plasmagel and during locomotion slides forward over the plasmagel and turns down at the anterior end where it comes in contact with the substratum. Here it adheres and remains stationary until, due to the forward movement of the amœba, it is located at the posterior end when it moves upward and forward again. The attachment of the plasmalemma to the substratum makes forward movement possible. The energy involved in forward movement is derived from several processes. 1. The plasmasol is hypertonic and the plasmagel and plasmalemma are semi-permeable, both of which result in an excess inflow of water that stretches the two outer layers until their elasticity equals the diffusion pressure. 2. There is a local swelling of the plasmagel accompanied by a decrease in its elasticity at the tip of the forming or advancing pseudopodium. 3. During the last process, there is a contraction of the remainder of the plasmagel, and a liquefaction at the inner surface of the posterior end of the plasmagel giving rise to an increase in volume in the plasmasol at this point; both the contraction and the liquefaction tend to drive the

plasmasol forward. 4. The forward flow of the plasmasol is also aided by the gelation of the plasmasol at the anterior end resulting in a decrease in volume. When the true explanation of amoeboid movement is found, it will probably give a key not only to the formation of pseudopodia but to the movement of flagella, cilia and even muscular contraction. For a discussion of the earlier work see Jennings (1904), Dellinger (1906), Hyman (1917), and Schaeffer (1920).

As has been pointed out in Chapter I, the pseudopodia of the SARCODINA may be divided into two chief types—lobopodia and axopodia. The former are the common type and are the ones found in the amoebæ. They consist of an extrusion of the body with no specific structural differentiation. Axopodia, on the other hand, found in such forms as *Actinophrys* (Fig. 5, B), are capable of swinging or bending movements and possess a secreted axis which is either rigid or elastic in nature. They are interesting as they probably represent a transitional type between true flagella on the one hand and pseudopodia on the other. Besides movement there are many other transformations of energy in PROTOZOA, just as there are in higher animals and plants. These, however, have been investigated to such a slight degree that we need not consider them here.

6. REACTIONS TO STIMULI

In general, it may be said that the reaction of an animal to a stimulus involves three systems: first, a sense receptor; second, a conducting system; and third, an effector. Such structures are not visibly differentiated in forms like *Amœba*, but the portion of the body which receives the stimulus is also the one which reacts so that conduction is probably little more than a general diffusion of the stimulus through a localized portion of the body—or possibly over a localized portion of the surface of the body. This type of reaction can be seen when an actively moving amoeba is touched with

a glass rod (Fig. 23). The portion which is touched stops and contracts. The animal then continues its movement along a line which is slightly at an angle from the former line of movement. Such reactions can probably be explained as a result of the inhibition of pseudopodia formation at the point of stimulation coupled with the general tendency of the animal to move along the original line of movement. As Jennings (1906) points out, in these reactions it is primarily the point that is stimulated that reacts. There seems to be no necessity for assuming a conduction of the stimulus other than a general diffusion. If, in the above case, the mechanical

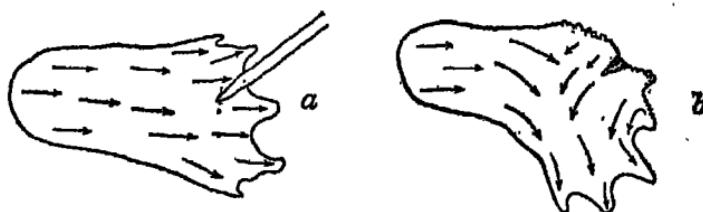


Fig. 23.—Negative reaction to mechanical stimulation in an amoeba. The animal is stimulated with the tip of a glass rod while it is moving as indicated by the arrows in *a*. The resulting negative reaction is shown in *b*. (After Jennings.)

stimulation is made strong enough, the amoeba can be made to reverse its line of direction completely. In such cases we may assume that the effects of the stimulation with its resultant inhibition of pseudopodial formation has diffused over a greater portion of the body.

Many of the reactions of amoebae to external stimuli are similar to their reaction to mechanical stimulation. In the case of the reaction of amoeba to light, for example, we find that the animals are negative, that is, they move away from the source of illumination. Also, if they come in contact with a brightly illuminated area, they will not pass into it but will "avoid" it (Fig. 24). After considering these reactions, Mast (1911) says, "In view of these facts it is probably true

that the orientation of all the RHIZOPODS in light is due to a local response to a local stimulation, a direct inhibition of the movement of the part most highly illuminated. This

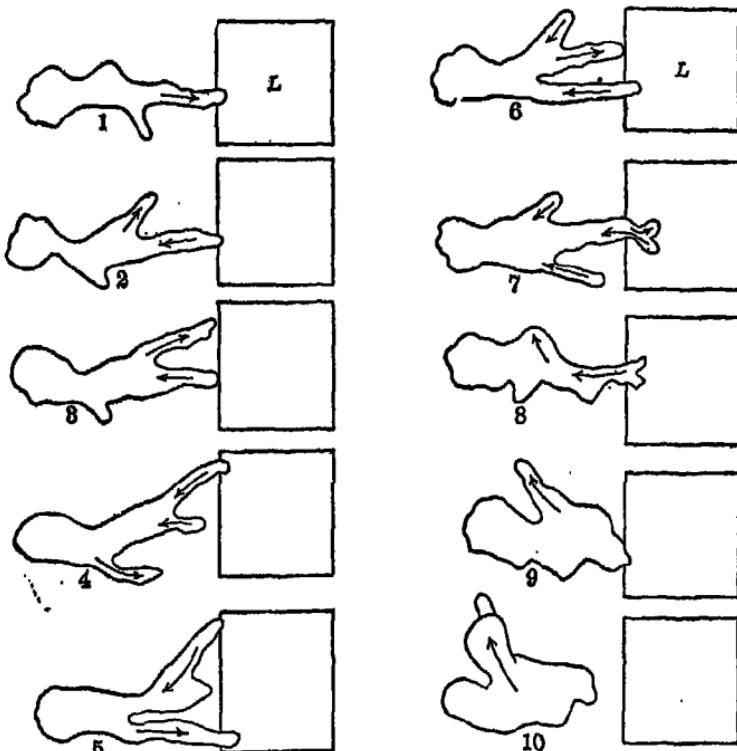


Fig. 24.—Sketches representing the reactions of an amoeba proceeding toward an intense area of light, the rays of which were perpendicular to the slide. L, field of light formed by focusing a section of a Welsbach mantle on the slide. 1-10, successive positions of the amoeba a little less than one-half minute apart. Arrows indicate direction of streaming in pseudopods. (Legend and figure after Mast.)

would, of course, result in the prevention of the formation of pseudopodia on the more highly illuminated side, and the organism would turn until both sides are equally illuminated,

and symmetrically located points on the body equally stimulated."

Many of the most interesting reactions of amoebae are encountered in the capture of food. For a detailed account of some of these reactions the reader is referred to the works of Jennings (1906), Kepner and Taliaferro (1913), Mast and Root (1916), Schaeffer (1917), Kepner and Edwards (1917) and Kepner and Whitlock (1921). (See also figures 5, A; 13; and 22.) Some of the reactions are essentially like those described above in that it is the part stimulated that reacts primarily. On the other hand, some of the food reactions (see especially Kepner and Taliaferro, 1913) indicate that the part stimulated does not necessarily primarily react but that the animal may coordinate its behavior so as to utilize the configuration of the pseudopodia existing just prior to the reaction. Such complicated reactions suggest a means of conducting the stimulus and of coordinating the formation of pseudopodia at different places on the body. In other words, the animal reacts as a whole.

A remarkable reaction has recently been described in *Difflugia* by Kepner and Reynolds (1923) who find that a severed pseudopodial fragment, if separated by a distance not greater than 1.5 mm., is recovered and reappropriated—the fragment entering immediately into the protoplasmic structure of the cell-body. The same phenomenon occurs in *Arcella*, where Reynolds ("Interactions of protoplasmic masses in relation to the study of heredity and environment in *Arcella polypora*," *Bio. Bull.* 1924, in press) has carefully studied the process from a physiological and genetical standpoint.

CHAPTER III¹

THE ECTOZOIC AND ENTOZOIC SARCODINA

A. The Amœbæ Entozoic in Man

i. INTRODUCTION

a. Incidence

During the last few years, chiefly as a result of the interest which grew out of the recent World War, a number of investigators have made studies of the intestinal infections of man. One fact of paramount importance has developed from this work, viz., infections with *Endamoeba histolytica*, the parasite of amœbic dysentery, can no longer be considered tropical but are world wide in their distribution. In fact, we can sum up the geographical distribution of all the entozoic amœbæ of man by saying that they all occur, in different percentages in each species, wherever man occurs.

The incidence of the various intestinal amœbæ is well illustrated in some of the recent surveys in the British Isles and in the United States among those inhabitants who have never been abroad from their respective countries. Recently Dobell (1921) has analyzed the data published by a number of investigators and has found the following incidence among 3,146 individuals who had never left the British Isles during their life time:

¹ By W. H. Taliaferro.

	Number infected	Percentage infected	Estimated percentage incidence
<i>Endamæba histolytica</i>	107	3.4	7-10
<i>Endamæba coli</i>	571	18.1	36-54
<i>Endolimax nana</i>	146	4.6	9-13
<i>Iodamæba williamsi</i>	8	.25	0.5-0.75

In this table the "estimated percentage incidence" probably gives a true picture of the actual incidence of the infections. It is much higher than the percentage of individuals found infected because the majority of the above individuals were examined only once, and the examination of a series of individuals once each has been found to uncover only approximately one-third of the actual infections present. Similarly a series examined thrice each uncovers approximately one-half to two-thirds of the infections present. In the table above Dobell arrived at the "estimated percentage incidence" after analyzing his data and taking into consideration the number of times the individuals in each series were examined. Dobell's compilation can be compared with the following data for *E. histolytica* obtained by Boeck (Boeck and Stiles, 1923) in the examination of 8,029 persons in the United States who are grouped into four general classes as follows: (1) U. S. soldiers designated "Foreign" who were credited with foreign service; (2) U. S. soldiers designated "Home" who were credited with only home service; (3) persons designated "Unknown" whose military service could not be ascertained; and (4) persons designated "None" who had no military service:

War service	Total number examined	Number infected with <i>E. histolytica</i>	Percentage infected	Estimated percentage incidence
Home.....	2,584	93	3.5	8-9
Foreign.....	3,536	100	2.8	7.8-9
None.....	1,547	129	8.3	13-17
Unknown.....	362	11	3.0	7-9
Total...	8,029	333	4.1	8-10

The following table gives the percentages of infections with the commoner intestinal amoebae for all 8,029 persons without regard to their classification. In this series all individuals were examined once and some as many as six times.

	Number infected	Percentage infected	Estimated percentage infected
<i>Endamoeba histolytica</i>	333	4.1	8-10
<i>Endamoeba coli</i>	1,596	19.6	36-46
<i>Endolimax nana</i>	1,060	13.2	25-31
<i>Iodamoeba williamsi</i>	404	5.0	10-15

In the tables above no mention has been made of *Dientamoeba fragilis*. This intestinal amoeba is apparently very rare and has been reported in only 33 cases (see Taliaferro and Becker, 1924). The cases which have been reported, however, are widely distributed over the world. There is also no mention of *Endamoeba gingivalis* in the tables because this form is not an intestinal form. It is, however, an extremely common inhabitant of the human mouth and is world wide in its distribution.

b. Pathogenicity

All of the recent work on the amœbæ of man, which has been accumulating so rapidly, points to the conclusion, which is of paramount interest from the medical standpoint, that all of the amœbæ occurring in man are harmless commensals with the exception of *Endamoeba histolytica*. This same work indicates, just as surely, that *E. histolytica* lives, and can live, only at the expense of the tissues of its host. In spite of the fact, however, that it is a tissue parasite and sometimes produces amœbic dysentery and abscesses of the liver, by far the majority of infected persons never show any clinical symptoms of disease, but simply live along as healthy carriers of the organism. The question of the pathogenicity of *E. histolytica* will be discussed in detail when this organism is described.

c. Historical Survey

An excellent summary of the development of our knowledge of the amœbæ living in man is given by Dobell (1919), from whom most of the following facts are taken, although in most cases we have carefully examined the original articles. The development of our knowledge can be taken up under several heads.

(1) DISCOVERY OF THE ENDAMOEBAE LIVING IN MAN.—A short description of an amœbæ from the tartar of the teeth by Gros (1849) in Russia marks the discovery of *E. gingivalis*, which is apparently not only the first amœba of man that was described, but the first entozoic amœba of any animal to be discovered. The honor of first describing an intestinal amœba in man belongs to Lewis (1870), whose description may be found in a report on Cholera in India. Although this amœba can not be identified with certainty from Lewis' article, a coworker, Cunningham (1871), gave

a much clearer and more careful account of the organism, which makes it possible to identify it as being chiefly, if not entirely, *E. coli*.

E. histolytica was first seen by Loesch in 1873 (published in 1875) in the stools from a young Russian peasant named Markoff who came to Petrograd to look for work, where he contracted dysentery and died. Loesch's description of the parasite is excellent—far better than many of the subsequent accounts. Besides describing the parasite, he attempted to infect 4 dogs by mouth and rectum. In the only dog which developed dysentery, he found amebæ in the ulcers of its intestine. While Loesch's description leaves no doubt that his patient had an infection of *E. histolytica*, we feel, contrary to Dobell (1919), that he also had a concomitant infection of *E. coli*. This is indicated by the fact, as also pointed out by Mesnil (1920), that Loesch in describing the inclusions found in the amebæ, notes that, "Ausser dem Kern, den Vakuolen und den Körnchen beobachtet man in dem Protoplasma nicht selten verschiedene, von aussen aufgenommene geformte Bestandteile, wie dieses schon kurz erwähnt wurde. Am häufigsten trifft man kleine Gebilde an: wie Bakterien, Vibrionen, Mycothrixketten, Mikrokokken; doch findet man ausnahmsweise auch grössere Elemente, wie rote und weisse Blutkörperchen, Kerne zerfallener Zellen, Amylumkörnchen," etc. While some of the bacteria may have been secondary invasions of *E. histolytica*, such a profusion of them strongly indicates the presence of *E. coli*, whereas the occurrence of starch grains makes it almost conclusive, as we do not believe *E. histolytica* would have ingested them. Consequently, we feel that Loesch was dealing with a mixed infection, and for this reason we believe it is permissible to restrict his name *coli* to one of these forms, viz., the harmless commensal *Endamoeba coli*. In this way we escape many of the difficulties discussed by Dobell in regard to the nomenclature of the various species.

(2) DEMONSTRATION THAT AN AMOEBA MAY BE PATHOGENIC.—The demonstration of the etiological rôle of *E. histolytica* in the production of so-called "tropical dysentery" dates from the original observations and animal experiments of Loesch which were noted above. To Koch (in Koch and Gaffky, 1887) belongs the credit of first seeing amoebæ in sections of intestine from patients who had died from dysentery. Koch's work led to the important investigations of Kartulis (1885-1913) who (Kartulis, 1887) noted the presence of amoebæ in the pus from a liver abscess, which confirmed the already suspected connection between dysentery and post dysenteric liver abscess, and, later (Kartulis, 1904) first found the parasite in amoebic abscesses of the brain. In America, *E. histolytica* was first seen by Osler (1890), who confirmed the fact that this organism occurred in liver abscesses. His findings led to the work of Councilman and Lafleur (1891) on the pathology of amoebic dysentery and amoebic abscess of the liver, which still stands as a classic in pathology. By this time all the circumstantial evidence necessary had accumulated to show that the amoebæ associated with dysentery and with liver abscess were the same. It remained, however, for Kruse and Pasquale (1894) to give experimental evidence of this identity by infecting cats with the amoebæ from a liver abscess. Later Marchoux (1899) obtained a liver abscess in a cat which had been experimentally infected with *E. histolytica*. Liver abscess was produced experimentally in the dog by Harris (1901). In addition to the investigations already mentioned, the work of Hlava (1887) and Kovacs (1892) should also be noted because of their contributions on the etiological rôle of *E. histolytica*.

(3) DIFFERENTIATION BETWEEN *E. histolytica* AND *E. coli*.—While a large mass of data indicated that amoebæ might lead to diseased conditions in man, the work of Grassi (1879-1888), Calandruccio (1890), Celli and Fiocca (1894-

1895), and Casagrandi and Barbagallo (1895-1897) seemed to contradict this. This discrepancy was due largely to the fact that it was not known that there were more than one species of amoeba found in the intestine. The possibility that there might be more apparently occurred to Councilman and Lafleur (1891), Kartulis (1891) and Lutz (1891) at about the same time. The truth of this possibility was first demonstrated by Quincke and Roos (1893). (See also Roos, 1894.) These investigators gave an excellent description of the differences in appearance, movement and inclusions in the amoeboid stages of *E. histolytica* and *E. coli*, and also reported the discovery of the cysts of *E. histolytica*, although their descriptions of them were by no means accurate. Huber (1903) first correctly counted the number of nuclei in the cysts of *E. histolytica*, and in the same year Schaudinn did the same for *E. coli*. Finally, the work of Walker (1911) left no doubt that there was a pathogenic amoeba living in man which produces 4-nucleate cysts and a non-pathogenic one which produces 8-nucleate cysts.

(4) LIFE-HISTORY OF INTESTINAL AMOEBAE.—The fact that the cysts of amoebae are infective *per os* apparently was first shown experimentally by Grassi (1888) and Caladruccio (1890), who state that they were able to infect human beings by allowing them to swallow cysts of *E. coli*. Quincke and Roos (1893) and Roos (1894) were able to infect cats by feeding them cysts of *E. histolytica*. (In the earlier work of Loesch dogs were fed amoebae and also given rectal injections—the latter probably accounted for the infection.) From this time on our knowledge of the life-cycle of the amoebae of man should have progressed rapidly. Schaudinn (1903), however, described a most complicated life-history of the intestinal amoebae which was, for the most part, entirely erroneous, but which, due to the weight of his authority, has persisted, even, to some extent, to the present time. Musgrave and Clegg (1904-1906) in the Philippines also

exerted a great deal of influence at about the same time. These investigators not only did not differentiate between the intestinal amœbæ, but confused them with free-living forms, and, as a result, concluded that all amœbæ are or may become pathogenic and that the surface flora of the Philippine Islands carried a large number of these potential parasites. To the investigators, Walker (1911) and Walker and Sellards (1913) is due the credit for putting our knowledge of the intestinal amœbæ back on a firm basis. In speaking of the work of these investigators, Dobell (1919) says: "It confirmed and vastly extended the earlier observations of the Italian workers, of Quincke and Roos, and of others, and placed our knowledge of the subject on a firm foundation of fact which is still unshaken and probably unshakable." Walker (1911), besides conclusively demonstrating the existence of two species in man, definitely showed that the amœbæ which Musgrave and Clegg cultured from feces and which occur in the water supply of Manila were free-living amœbæ, non-pathogenic to man, and furthermore that they could be cultured from feces simply because their cysts had been ingested and had passed through the body unchanged. Walker and Sellards (1913) continued this work by carrying out a carefully planned series of infection experiments on human beings which showed how man acquired his infection and indicated the relation of man to the parasites (carriers of *E. histolytica*, etc.).

(5) RECENT WORK ON AMŒBÆ.—Since the work of Walker it has been demonstrated that three other distinct species of amœbæ, i. e., *Endolimax nana*, *Iodamoeba williamsi*, and *Dientamœba fragilis*, may live in the intestine of man. For a historical account of these forms the reader is referred to Dobell (1919). Of the recent investigators on the amœbæ in man, Darling, Dobell, James, Kofoid and Wenyon deserve particular mention. Dobell, especially, has not only added greatly to our knowledge but, in his work of

1919, has given us a monograph which, from a zoological standpoint, is undoubtedly the best single account of the amoebæ of man that has been written.

Recently there have appeared descriptions of several supposedly new species of amoebæ living in man. Three of these have been carefully examined by Wenyon (1922) who believes that it is probable that *Entamoeba paradysenteria* described by Chatterjee is *E. histolytica*, that *Entamoeba macrohyalina* described by Tibaldi is *E. gingivalis*, and that *Councilmania lafleuri* described by Kofoid and Swezy is *E. coli*. By far the most extensive of these investigations is that of Kofoid and Swezy (1921) on *Councilmania lafleuri*. This, however, as Wenyon maintains, may not be a good species, inasmuch as there are a number of points in Kofoid and Swezy's description which need further investigation. Faust (1923) has just described what he believes to be a new species of amoeba under the name *Caudamoeba sinensis* which produces dysentery and is more susceptible to emetine treatment than *E. histolytica*. If Faust's amoeba is a new species, it will probably have to be placed in the genus *Endamoeba*.

d. Nomenclature

In the following pages the nomenclature of the various species of the amoebæ of man has been adopted from Stiles (Boeck and Stiles, 1923), with the exception that the genus *Iodamoeba* is recognized as distinct from the genus *Endolimax*. This differs somewhat from that given by Dobell (1919) and accepted by the majority of English writers as follows. In the first place, the genus *Endamoeba*, Leidy (1879), type *E. blattæ* by original designation, is used, while Dobell uses *Entamoeba*, Casagrandi and Barbagallo (1895), type *E. coli*. Although the Congress on Zoological Nomenclature has not specifically acted on this case, the authors believe that the spirit of their rulings in other cases makes it best to consider *Entamoeba* a homonym of *Endamoeba* and

hence an invalid name. If the species *gingivalis*, *coli* and *histolytica* are not co-generic with *E. blattæ*, then the next available name for the genus is *Pomeramæba* Luehe (1909). At the present time, however, the authors prefer, provisionally, to consider them co-generic and to place them in the genus *Endamæba*, Leidy, 1879. In the second place, the present authors give the authority for *E. coli* as the name for the harmless commensal to Loesch (1875) on the basis, as pointed out before, that this investigator had a mixed infection of *E. coli* and *E. histolytica*, to either of which it is permissible to restrict his name. While it should have been restricted to the latter, the first reviser, Schaudinn, did the former, and therefore, we have to stand by his decision. In the third place, the proper name for the amoebæ of the iodine cysts is considered to be *Iodamæba williamsi* Prowazek, 1911, and not *I. bütschlii* Prowazek, 1912, as maintained by Dobell. This has been discussed in detail by Taliaferro and Becker (1922). The following is a synopsis of the genera and species living in man:

Genus Endamæba, Leidy, 1879.

Type by original designation: *E. blattæ* Bütschli, 1878.

Species occurring in man:

E. coli (Loesch, 1875), Hickson, 1909.

E. histolytica (Schaudinn, 1903), Hickson, 1909.

E. gingivalis (Gros, 1849), Smith, Middleton and Barrett, 1914.

Genus Endolimax, Kuenen and Swellengrebel, 1917.

Type and only species in man:

E. nana (Wenyon and O'Connor, 1917), Kuenen and Swellengrebel, 1917.

Genus Iodamæba, Dobell, 1919.

Type and only species in man:

I. williamsi (Prowazek, 1911), Taliaferro and Becker, 1922.

Genus Dientamæba, Jepps and Dobell, 1918

Type and only species in man:

D. fragilis, Jepps and Dobell, 1918.*e. Cultivation*

A number of investigators have attempted to cultivate one or more of the entozoic amoebæ *in vitro*. It is unnecessary to review the results of the earlier investigators because their so-called positive results were due to the confusion of free-living forms with entozoic species. It is, for example, comparatively easy to culture amoebæ from fresh human stools, but the amoebæ are in reality free-living forms, which, as cysts, were ingested and passed through the body unchanged. Then, too, it is exceedingly easy to contaminate cultures with cysts of free-living forms contained in the dust of the laboratory. Recently, however, Cutler (1918) maintains that he has been able to cultivate *E. histolytica* on both an egg medium and a blood-clot medium, of which, although both gave equally good growths, the latter was clearer. It consisted of the following:

- (1) Add 500 c. c. of human blood clot to 1,000 c. c. of water.
- (2) Boil one hour.
- (3) Filter. Add 0.5% Na Cl, and 1% peptone.
- (4) Sterilize in tubes by steaming 20 minutes on each of 3 successive days.
- (5) Before inoculation add a few drops of fresh blood to each tube.

The optimum temperature for the cultures was 28°–30° C. Cutler maintains that he successfully cultured amoebæ from six stools, all of which contained blood and mucous. Of these six, one culture ran indefinitely *in vitro*. At first, daily subculturing was tried, but later, when bacterial action did not render the medium acid, subcultures were made every

two or three days. Cyst formation took place readily in the cultures.

An examination of the descriptions and figures in Cutler's papers (1918 and 1919) seems to leave no doubt that he was dealing with *E. histolytica*, but some investigators are not convinced that he actually cultivated the organisms. Dobell and Douglas (see Dobell, 1919, p. 70) were not able to repeat Cutler's work and Dobell suggests that "his [Cutler's] observations may be capable of a different interpretation from that which he has put upon them." Dobell, however, does not state in what respect this interpretation might be different. It certainly is very desirable that other investigators repeat Cutler's work.

Regardless of the status of Cutler's work, it seems to the authors that there can be no doubt that Barret and Smith (1923) have succeeded in cultivating *Endamæba barreti* from the turtle. This work has been checked in this laboratory and the morphology of the forms carefully studied both in the turtle and in cultures by Taliaferro and Holmes (1923). Barret's medium is very simple, consisting of 1 to 10 dilution of inactivated human serum in 0.5% saline. Taliaferro and Holmes have found that inactivated rabbit's serum will also answer the purpose. The cultures are kept in an ordinary refrigerator and subcultures made every one or two weeks. This amœba differs from *E. histolytica* in that it is an entozoon of a cold-blooded animal and in that it is a bacteria feeding commensal instead of a tissue parasite.

2. THE GENUS *Endamæba*

a. *Endamæba histolytica*

(1) AMOEBOID FORMS.—In the active stage *E. histolytica* generally varies in diameter from $20\ \mu$ to $30\ \mu$. The extreme range in size is, however, much greater than this. According to Dobell (1919) it ranges from $18\ \mu$ to $40\ \mu$, and according to Nöller (1922) from $20\ \mu$ to $50\ \mu$, and Kofoid, Korn-

hauser and Swezy (1919) note specimens as small as $7\text{ }\mu$ in diameter. In very fresh preparations, according to Dobell (1919), the organisms move about very rapidly in a slug-like manner with very little differentiation between endoplasm and ectoplasm. Very soon after leaving the body, however, they cease most of their progressive locomotion and throw out, with explosive rapidity, thin, blade-like, hyaline pseudopodia which are composed entirely of ectoplasm which is sharply differentiated from the endoplasm. Although this type of pseudopodia formation may be the result of degeneration, it is undoubtedly very characteristic of the species and serves to distinguish *E. histolytica* from *E. coli*. In the amoebæ, just after they are passed from the body, the endoplasm is homogeneous except for the presence of small endoplasmic granules or micromeres. The only inclusions are the food vacuoles. Bacteria or vacuoles other than the food vacuoles in the endoplasm indicate that the organism is moribund and degenerate. There is at present no conclusive evidence that *E. histolytica* ever ingests bacteria as food. On the other hand, cases are described in which *E. histolytica* is parasitized by a bacterium (Dobell, 1919).

When present, the food vacuoles generally contain red blood cells although they may contain ingested leucocytes and fragments of tissue cells. The number of red cells present may be very large—Dobell (1919) cites an instance in which over 40 were counted in a single specimen. Not only does *E. histolytica* ingest red cells, but the predominance of evidence points to the conclusion that it is the only intestinal amoeba that does. There are occasional accounts of *E. coli* ingesting erythrocytes, but, as these are by no means conclusive, for all practical purposes an amoeba containing red blood cells can be assumed to be *E. histolytica*.

A contractile vacuole is never found in *E. histolytica* or in any of the other entozoic species living in vertebrates.

The nucleus is either inconspicuous or invisible in the

living organism. In fixed and stained specimens, however, its structure is clearly brought out (Fig. 26, A). It is spherical and vesicular, generally ranging in size from 4μ to 7μ . Its general plan of structure is similar to that of the other members of the genus *Endamæba*, except that it is decidedly "poorer" in chromatin. This organelle is surrounded by a delicate achromatinic membrane. Just inside this membrane there is a closely applied peripheral layer of chromatin granules. At the center of the nucleus there is a small deeply staining karyosome which is probably composed entirely of chromatin, and which, in healthy specimens, is rarely, if ever, eccentrically placed, as in *E. coli*. It is immediately surrounded by a delicate achromatic capsule. Between the achromatic capsule and the peripheral layer of chromatin granules there is a delicate linin network which is normally devoid of chromatin.

(2) LIFE-HISTORY.

Primary Site of Infection.—The large ameboid forms of *E. histolytica* occur in the large intestine of man where they are found in the mucosa, the sub-mucosa, and the muscular layers (Fig. 25, A and B). Harris (1898) and Kuenen (1909) have noted cases of infection of the small intestine in man, and Dale and Dobell (1917) of two similar infections of the cat, although such invasions are very rare. The recent experiments of Sellards and Leiva (1923) indicate that, in experimental infections of the cat with *E. histolytica*, stasis is an important factor in the occurrence of the initial lesions in the lower portion of the large bowel; and these investigators believe that it "is probably an important factor in determining the location of the lesions within the large bowel in spontaneous dysentery in man."

The available evidence at present points to the fact that whenever *E. histolytica* occurs in man it lives at the expense of the tissues of the host—penetrating them and giving rise

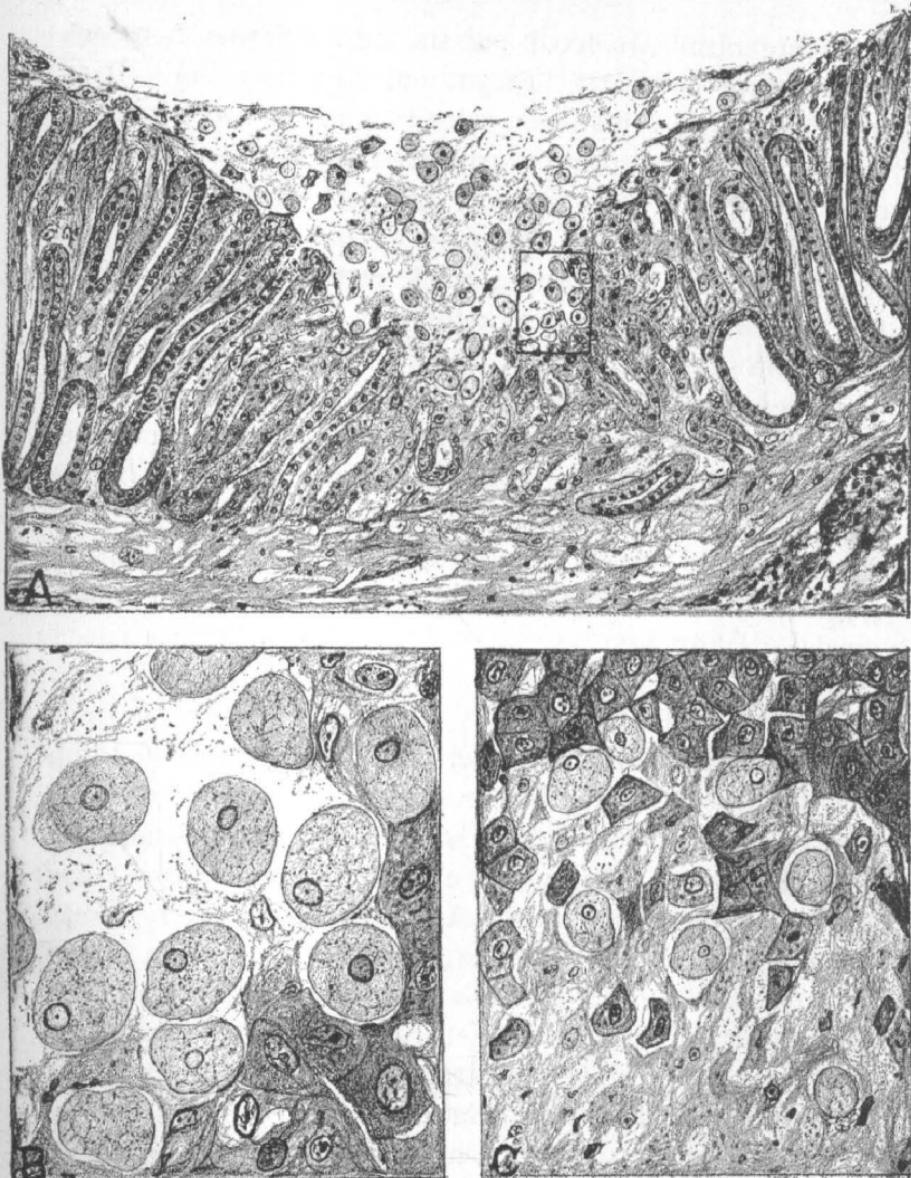


Fig. 25.—Lesions of amoebiasis (infection with *E. histolytica*) in man.
A, section of human intestine showing an early intestinal ulcer, in which the amoebæ have partly destroyed the mucosa. B, a higher magnification of that portion of A which is enclosed in the square and which shows the specimens of *E. histolytica* in close contact with the healthy mucosa. C, section of the periphery of a liver abscess in man which shows necrosed tissue and the amoebæ in contact with the healthy tissue. A $\times 100$; B $\times 500$; C $\times 300$. (Original.)

to typical ulcerations. In penetrating the tissues the amoebæ probably do not force their way mechanically, but secrete a proteolytic enzyme which actually breaks down the tissue. Absorption of the greater part of their food probably takes place then, directly through the general ectoplasm. Of course, this mode of nutrition is supplemented, as noted above, by the ingestion of solid particals (red-blood cells, tissue-fragments and leucocytes) just as in other amoebæ.

Reproduction.—The only known process of reproduction among the large active forms is binary fission which probably takes place within the intestinal ulcers. During the process the nucleus divides by a kind of semi-mitosis, and then the body constricts and divides.

Our knowledge of the details of the nuclear division we owe to Dobell (1919) from whom the following account is taken. When the nucleus begins to divide, it increases in size, the karyosome breaks up into several smaller masses of chromatin and the peripheral layer of chromatin granules begins to migrate toward the center of the nucleus. The nucleus now gradually becomes spindle-shaped and the chromatin is arranged in threads and more or less irregular masses, as is shown in figure 9, C. At first these figures strongly suggest true mitosis, but Dobell, after a very careful study, was unable to resolve the picture into a true mitotic figure with definite chromosomes and spindle fibers. The spindle-shaped nucleus now constricts in the middle and divides (Fig. 9, E-G) apparently without any relation to the individual masses of chromatin. The whole process seems to be more amitotic than mitotic, and is, in all probability, the result of a degeneration from true mitosis.

Precystic Forms.—The large active amoebæ live at the base of the intestinal ulcers where they absorb or ingest their nourishment and reproduce by binary fission. A certain number of the amoebæ, however, are constantly passing out of the ulcers into the lumen of the intestine preparatory to

encystment, where they undergo a marked reduction in size, lose their food inclusions, round up and become very sluggish. These so-called precystic forms are intermediate in size

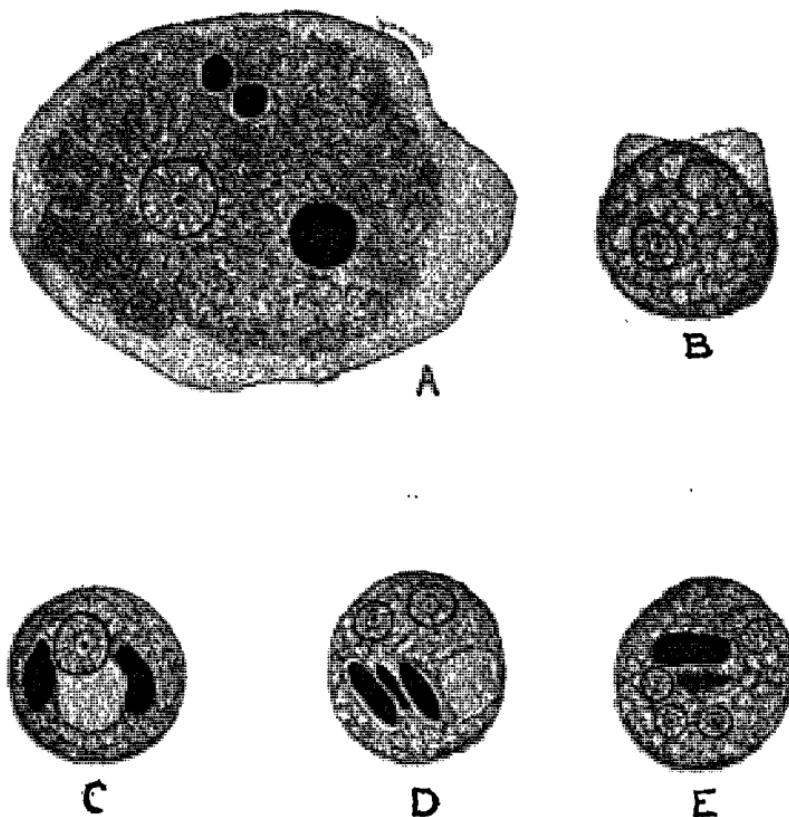


Fig. 26.—*Endamoeba histolytica*.

A, large active form which has ingested three red blood cells. B, precystic amoeba. C, D and E, uninucleate, binucleate and quadrinucleate cysts, respectively. The glycogen masses which were present in C and D have been dissolved, leaving a clear vacuole in each. Note the shape of the chromatoid bodies. $\times 2000$. (After Dobell.)

between the large active forms and the cysts. (Fig. 26, B.) Structurally the peripheral chromatin of the nucleus tends to become slightly thicker and the karyosome slightly larger

and somewhat eccentrically placed. Thus the precystic forms of *E. histolytica* closely approach the precystic forms of *E. coli* in structure making it very difficult to distinguish between the two species at this stage.

Cysts.—As the precystic forms pass down the intestine, they finally cease all pseudopodial formation, secrete a transparent, smooth cyst-wall about themselves of about $0.5\ \mu$ in thickness, and thus form uninucleate cysts of from $5\ \mu$ to $20\ \mu$ in diameter. In their further passage down the intestine, the cysts do not change in size but undergo an internal development which may be considered under three heads (Fig. 26, C-E). First, the nucleus, which is single and possesses a diameter roughly one-third that of the entire cyst, undergoes two consecutive divisions, producing four nuclei in the mature cyst. The structure of each of these is essentially the same as in the large active amœbæ (size about $\frac{1}{8}$ diameter of cyst), although an accumulation of the peripheral ring of chromatin at one pole may occur. Judging from the condition of affairs in other intestinal amœbæ, occasional super-nucleate cysts with eight nuclei may occur, but this has not been clearly demonstrated, and for all practical purposes, may be disregarded. Second, a mass of glycogen (occasionally several) appears in the cytoplasm of the cysts, generally soon after encystment during the uninucleate condition, which first increases in size and then is absorbed so that it disappears either shortly before or shortly after the cyst becomes quadrinucleate. It takes a typical deep mahogany color when observed in iodine. Third, at about the time the glycogen masses appear, certain refractile bodies become visible in the cytoplasm which stain deeply with chromatin stains and have been termed chromatoid bodies by Dobell. When fully developed, their appearance, as long stumpy rods with rounded ends, is rather characteristic and differs markedly from the splinter-like chromatoids found in cysts of *E. coli*. Freshly passed quadrinucleate

cysts usually contain one or several chromatoid bodies, but, if the cysts are kept under observation for several days, these are finally absorbed.

In a later section it will be shown that many species of free-living protozoa have been found not to be homogeneous in regard to size, but to be composed of a large number of stocks or "pure lines" which breed true but which differ among themselves. Similarly Dobell and Jepps (1918) have shown that the species *E. histolytica* is composed of many diverse races which are distinguishable from one another by the dimensions of their cysts.

Mode of Infection.—Active amœbæ, which are passed either in the liquid stools of carriers or in the stools of persons suffering with dysentery, degenerate very quickly after passing from the body and, even if swallowed before degeneration sets in, are killed by the digestive ferments. They do not, therefore, constitute infective forms. The average person infected with *E. histolytica*, who is passing formed stools, passes cysts in various stages of development. According to Dobell (1919) immature cysts never develop any further outside of the body and die much more quickly than do the mature quadrinucleate cysts. The mature cysts live for a considerable time outside of the body, provided they are protected from drying, bacterial action and high temperature (see Boeck, 1921 and 1921 a), and represent the sole infective form. When swallowed, they probably pass unchanged through the stomach and hatch in the small intestine (see Chatton, 1917, and Dobell, 1919). Whether they liberate a single four-nucleate amœba or four small amœbæ is not known. The work of Chatton (1917), as pointed out by Dobell (1919), is by no means conclusive on this point. Eventually the organisms liberated from the cyst set up a primary site of infection in the large intestine, but the sequence of events between hatching and the beginning of the first amœbic ulcer is unknown.

(3) SEXUAL PHENOMENA.—In the outline of the life-history, as we have given it, there has been no mention of any sexual phenomena. There is no evidence of sexual phenomena in the active life of the amoebæ nor in the nuclear phenomena during the development of the cysts. Conjugation may take place sometime between the hatching of the cysts and the beginning of a new infection. This is, however, simply a possibility with no evidence in its favor.

(4) SECONDARY SITES OF INFECTION.—The intestine is always the primary site of infection. In some cases, however, the amoebæ set up secondary sites. As important as these secondary sites are from a medical standpoint, they represent "mistakes" on the part of the parasite. The amoebæ in a secondary site are in a *cul de sac* from which they can not escape and in which they never develop infective forms, i. e., cysts. They migrate from the intestine most commonly to the liver *via* the portal circulation where hepatic abscesses (Fig. 25, C) result. Having once gotten to the liver they occasionally pass on to other organs, especially the lungs and brain (see Dobell, 1919). Recently Warthin (1922) has found amoebæ in the testis. Amoebic abscess of the spleen has been noted and cases of amoebæ in the urine seem to be authentic.²

(5) RELATION OF *E. histolytica* TO ITS HOST.—In a great majority of cases a person parasitized by *E. histolytica* does not exhibit any clinical symptoms. This does not mean that the amoebæ are not living at the expense of the tissues of the intestine, but rather that the ulcers are small and that the inroads of the parasite are constantly being made good by

²Kofoid and Swezy (1922) believe that they have found *E. histolytica* in the bone lesions of Ely's non-bacterial or second type of arthritis, and Kofoid, Boyers and Swezy (1922) also believe that they have demonstrated the amoebæ in the lymphatic glands of Hodgkin's disease.

the host. In such cases an equilibrium seems to be established between host and parasite. On the other hand, in a small percentage of cases this equilibrium is disturbed and the parasite "runs amuck," giving rise to various intestinal disorders, ranging from occasional diarrhoea to typical amœbic dysentery and often other generalized effects. It is to be emphasized that the production of dysentery is "harmful" to the parasite as well as to the host, because in cases of dysentery the amœbæ are swept out of the bowel before they can encyst, and hence before the infective forms are produced. If all amœbic infections resulted invariably in dysentery, the parasite would soon become extinct due to the absence of infective forms.

A person who harbors *E. histolytica* and who exhibits no clinical symptoms but who constantly passes the cysts in his stools is termed a carrier. Carriers of *E. histolytica* have been divided by Walker (1911) and Walker and Sellards (1913) into two types—contact and convalescent. The former have never exhibited any clinical symptoms, whereas the latter have experienced one or more attacks but have recovered clinically without losing the infection. The work of Walker and Sellards (1913) (see Dobell's analysis, 1919, p. 40) indicates that probably not more than 10 per cent of the people infected with *E. histolytica* ever show any marked clinical symptoms. It seems probable that the percentage is higher in the tropics than in the temperate climates. It is to be emphasized, then, that the carrier condition represents the usual and normal type of infection, and that, from a public health standpoint, the carrier is the only person of importance, because he alone can disseminate the infection. When the equilibrium between host and parasite is disturbed and dysentery ensues, the infection can not be disseminated because the amœbæ are washed out of the intestine before they can encyst.

(6) INFECTIVITY OF *E. histolytica* TO LOWER ANIMALS.*Question of Animal Reservoirs of the Parasite.*—

E. histolytica can be experimentally transmitted to dogs and cats, and spontaneous infections of the former have been described (see Kartulis, 1891, 1913; Darling, 1915; and Ware, 1916). Dysentery invariably results, however, and the parasite is apparently never able to encyst. For this reason dogs and cats can never act as reservoirs of the parasite. Brug (1919) gives one experiment which indicates that the rat can be infected and can become a true carrier of the parasite and pass cysts. If this observation be confirmed, the rat may be able to spread the infection. Besides these animals the rabbit and the guinea pig have been infected experimentally.

(7) DISSEMINATION OF THE CYSTS OF *E. histolytica*.—

We know that man acquires his infection with *E. histolytica* by swallowing the cysts of the parasite, probably with contaminated food or drinking water. Drinking water is an ideal medium for the carriage of the cysts as it prevents both desiccation and intense bacterial action. The work of Boeck (1921 a) indicates that cysts when kept in plain water will live for months. Flies may be instrumental in contaminating the food or water. The work of Wenyon and O'Connor (1917) and of Root (1921) indicates that the cysts survive a long time in the fly's intestine and that they can pass through it and be deposited in a living condition in the feces.

b. Endamoeba coli

(1) AMOEBOID FORMS.—The large vegetative forms of *E. coli* closely approach *E. histolytica* in size, ranging in diameter from 18μ to 40μ . The most usual size is from 20μ to 30μ in diameter. While *E. coli* can not be differentiated from *E. histolytica* by size, there are other characteristics in which the two species differ markedly. After leaving the body, *E. coli* is rather sluggish and never exhibits

the explosive extrusion of clear hyaline, blade-like pseudopodia so characteristic of *E. histolytica*. There is no sharp demarcation between ectoplasm and endoplasm as in the latter species and the endoplasm is much more granular in appearance. A study of the numerous food vacuoles observable in most specimens indicates that *E. coli* eats almost anything which occurs in the digestive tract with the exception of red blood cells and tissue elements (Fig. 27, A), such as various intestinal bacteria, starch grains, plant debris and even cysts of other protozoa. This sharp contrast between the food of *E. coli* and *E. histolytica* is an expression of the marked physiological differences of the two organisms, one being a harmless commensal and the other a pernicious tissue parasite. Besides food vacuoles, the endoplasm often contains peculiar vacuoles which look like clefts in the protoplasm. These may possibly be a sign of degeneration, but they are, nevertheless, markedly different from the bubble-like vacuoles which appear in degenerating *E. histolytica*.

Unlike *E. histolytica*, the nucleus of *E. coli* is generally easily visible in the living organism where it appears as a more or less clear area surrounded by a highly refractile beaded ring. Its finer structure is only seen after careful fixation and staining. In size it ranges in diameter from $4\ \mu$ to $8\ \mu$. It is built on the same fundamental plan as other species of the genus *Endamæba*, and is, therefore, similar in general plan to that of *E. histolytica*. It differs from the nucleus of *E. histolytica* in the following points: (1) The chromatin granules which form the peripheral layer just inside of the nuclear membrane are much larger and more closely set together, making a much thicker "ring" of chromatin, and they may show a distinct thickening toward one pole (see Fig. 27, A). (2) The karyosome is larger and is eccentric in position, a very constant characteristic which is quite useful in distinguishing the two species. It is to be remembered, however, that the karyosome in a certain per-

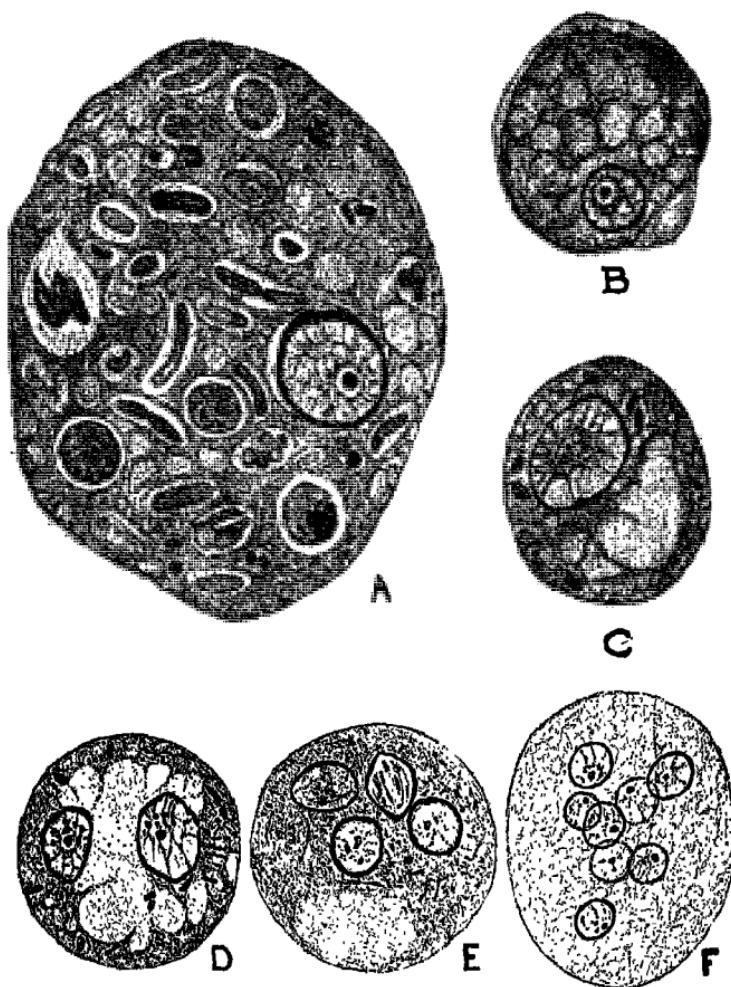


Fig. 27.—*Endamoeba coli*.

A, large active form. B, precystic amoeba. C, D, E, and F, 1-, 2-, 4-, and 8-nucleate cysts, respectively. The glycogen masses in C, D and E have been dissolved, leaving clear vacuoles. $\times 2000$. (After Dobell.)

centage of specimens will appear to be central due to the fact that the nuclei may be observed away from or toward the pole toward which the karyosome is displaced. (3) The achromatic capsule surrounding the karyosome appears to be denser and the network surrounding the capsule is much more pronounced. (4) Generally a few very definite chromatin granules lie in the network between the karyosome and the peripheral layer of chromatin. This is never seen in normal specimens of *E. histolytica*.

(2) PRECYSTIC FORMS.—The incidents leading to encystation in *E. coli* are much like those described for *E. histolytica*. The forms undergo a great reduction in size, lose all food inclusions, round up and become very sluggish. The precystic forms of *E. coli* and *E. histolytica* are very similar, and it is very difficult to distinguish between them (Fig. 27, B). It is true that in *E. coli* the peripheral "ring" of chromatin granules is somewhat thicker, the karyosome slightly larger and more eccentrically placed and the chromatin granules between the karyosome and peripheral "ring" more apt to be present, but, in spite of these facts, a differential diagnosis based on precystic forms alone is very difficult, and a positive diagnosis would be much more conclusive if postponed until either the large vegetative forms or the cysts can be obtained. As one would expect, the precystic forms of *E. coli* range in size from that of the large vegetative forms to that of the cysts. In general they measure 15 μ to 18 μ in diameter.

(3) CYSTS.—In the normal course of events the precystic forms become spheroidal and secrete a cyst wall about themselves. The cysts vary in size from 10 μ to 30 μ or even more in diameter. It is at once apparent that size is not an absolute criterion with which one can distinguish *E. coli* from *E. histolytica*. In practice, however, it is generally safe to assume that a cyst smaller than 10 μ is not *E. coli* and one larger than 20 μ is not *E. histolytica*. Just as in

E. histolytica, there is much evidence to show that the species is composed of a number of races, each race producing cysts of different sizes. This fact is probably true of all of the amoebæ living in man. It is in accord with what every investigator, who has carefully studied the question, has found, viz., that any species of protozoon is composed of races which are *per se* constant but which differ *inter se* in size.

At first the cysts are uninucleate but by three successive divisions become 2-, 4-, and 8-nucleate (Fig. 27, C-F). These divisions are simply straightforward nuclear divisions such as occur when the large vegetative forms divide, and have recently been described by Swezy (1922). According to her work, the nuclei divide by a true mitotic process and not a semi-mitotic one as described by Dobell (1919) for *E. histolytica* in the cat. They do not, however, always divide synchronously, thus there may occasionally be 3-, 5-, or 7-nucleate cysts. Supernucleate cysts occur. These usually contain 16 nuclei, but Dobell (1919) records the finding of one with 18 and another with at least 20. The resting nuclei in the cysts are in every way similar to those found in the large active amoebæ and can be distinguished from *E. histolytica* by means of the same characteristics.

The glycogen vacuoles found in the cysts of *E. coli* are typically larger than those found in *E. histolytica*. They are present not only in the earlier stages of the development of the cysts but may even be present as more or less diffuse masses in the precystic amoebæ. In binucleate cysts they are generally present and very large (Fig. 27, D). In 4-nucleate cysts, they are sometimes found, although much decreased in size, whereas, in the mature 8-nucleate cysts, they are extremely rare.

Although not always present, chromatoid bodies very frequently occur in the cysts, especially in the immature cysts, and less frequently in the mature 8-nucleate cysts just after they are passed. In shape the chromatoid bodies generally

remind one of splintered glass and often assume peculiar filamentous shapes. *E. coli* does not have the rod-like chromatoid bodies with rounded ends which are so typical of *E. histolytica*. This difference is a great aid in diagnosis. (See figure 187, f.)

(4) LIFE-HISTORY.—The large active amoebæ probably live in the liquid contents of the upper colon, where they feed on bacteria and debris and multiply by fission. As they pass down to the lower colon in the more solid feces, encystation occurs, as has already been described. The cysts pass out of the body with the feces, and it is certain from the earlier work of Grassi (1888) and Calandruccio (1890) and the later work of Walker and Sellards (1913) that other human beings become infected by swallowing these cysts. In the new host they probably hatch in the small intestine, liberating either eight small amoebæ or an 8-nucleate amoeba which later divides into eight small amoebæ, which, in turn, pass into the large intestine and establish themselves. There is absolutely no conclusive evidence that a sexual phase takes place in the life-history of *E. coli*, although it may be possible. This amoeba, unlike *E. histolytica*, is confined to the alimentary canal of its host during its whole life-history, and never makes secondary excursions or feeds on tissues.

c. *Endamoeba gingivalis*

(1) DESCRIPTION OF ORGANISM.—*E. gingivalis* exhibits considerable range in size. According to Prowazek (1904), the diameter may vary from 6μ to 32μ , and Smith and Barrett (1915) state that it may even reach 60μ . During locomotion the organism throws out clear hyaline pseudopodia with the ectoplasm sharply differentiated from the endoplasm, resembling, in this respect, *E. histolytica*. The appearance of the endoplasm, on the other hand, more closely resembles that of *E. coli* in that it is generally closely packed with food vacuoles (Fig. 28) containing numerous

bacteria and peculiar inclusions which are apparently the remains of nuclei from ingested cells, such as salivary corpuscles or other leucocytes or cells. Red blood cells may occasionally be ingested but, according to Dobell (1919), not as frequently as some authors suppose.

The nucleus of *E. gingivalis* during life is rather inconspicuous and, according to Dobell (1919), generally ranges in size from 2.5μ to 3μ . Its structure is that of a typical *Endamoeba*, as is shown in Fig. 28. The chromatin granules,

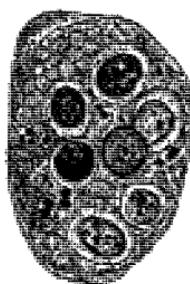
which lie just within the nuclear membrane, are closely packed together to form a very uniform and distinct "ring" of chromatin at the periphery of the nucleus. The karyosome is large and may be either centrally or eccentrically placed with a definite achromatic capsule surrounding it at times. Some authors figure a linin network between the achromatic capsule and the external ring of chromatin, which, in well-fixed specimens,

is entirely devoid of chromatin, just as is the case with *E. histolytica*.

Fig. 28.—*Endamoeba gingivalis*. $\times 2000$. (After Dobell.)

(2) LIFE-HISTORY.—*E. gingivalis* is an extremely common entozoon of man and is

generally found in the tartar of the teeth and in the materia alba around them, and may often be found in many suppurative and inflamed conditions of the mouth and throat. Bass and Johns (1914) and Smith and Barrett (1915) maintained that the organism was the etiological agent of pyorrhoea. There is at present, however, no conclusive evidence to show that *E. gingivalis* is ever instrumental in the production of any diseased condition of the mouth. It is far more likely that the organisms occur in such abundance in suppurative conditions due to the increased food supply of bacteria and broken down body cells than that they have any causative rôle in the production of these conditions. During



the height of the belief that *E. gingivalis* was the causative agent of pyorrhœa, some investigators maintained that emetine acted as a specific against the organism, but later work has shown this hypothesis to be untenable and, at the present time, no substance is known which exerts a specific action on it.

In their various sites of infection the amoebæ probably reproduce by binary fission as do the intestinal forms. Occasional stages in the process of nuclear division have been observed. Multiple fission has been described by Nowlin (1917), but not entirely convincingly.

While several authors have noted, and even figured cysts, it seems very doubtful whether the structures described were actually cysts, but, even if they occur, they must be very rare. The question arises, then, how is the amoeba transferred from man to man? Goodrich and Moseley's (1916) suggestion that it is transmitted in the amoeboid state by direct contact from mouth to mouth seems to be the most plausible hypothesis.

3. THE GENUS *Endolimax*

a. *Endolimax nana*

(1) AMOEBOID FORMS.—This small amoeba, when rounded, ranges in diameter from $6\ \mu$ to $12\ \mu$ with an average diameter of $8\ \mu$ (Dobell, 1919). On leaving the body, progressive locomotion rapidly ceases and pseudopodial formation results merely in changing the shape of the body. Movement is generally rather sluggish and the pseudopodia are always very blunt. The differentiation between ectoplasm and endoplasm varies considerably in different specimens. Like the other commensal amoebæ of the human intestine this form ingests bacteria and other microorganisms which can be seen in food vacuoles within the cytoplasm.

The visibility of the nucleus varies considerably in living specimens. In fixed and stained preparations its structure is

very characteristic. The diameter of the nucleus varies from 1μ to 3μ , and the following structures can be seen (Fig. 29):

1. A delicate achromatic membrane in which deeply staining granules (chromatin?) are sometimes seen.
2. A peculiar karyosome in which most, if not all, of the chromatin is embedded. This karyosome generally consists of one large mass connected by strands with one or several smaller masses. While the general appearance of the karyosome is very characteristic, no two nuclear pictures are exactly alike.
3. Sometimes a few linin fibers which run from the karyosome to the nuclear membrane.

Of the different structures in the nucleus, it is the karyosome which serves to distinguish this form from the other amœbæ of the human intestine and which serves to characterize the genus *Endolimax*.

Just what portion of the intestine constitutes the habitat of this species is unknown. According to Dobell (1919), there are some reasons for assuming that it is the small intestine. Undoubtedly, however, it is confined to the lumen of the intestine during its entire life-history and is always a harmless commensal. The general outline of the life-cycle of *E. nana* is closely similar to that of *E. coli*.

(2) PRECYSTIC FORMS.—As in other entozoic amœbæ, the precystic forms round up, become sluggish and lose their food inclusions. Unlike *E. coli* they are not markedly smaller than the active amœbæ.

(3) CYSTS.—The cysts of *E. nana* are typically oval in outline although they may be spherical or even, though rarely, irregular in shape (Fig. 29, D). The oval cysts generally measure 8μ to 10μ in length and 7μ to 8μ in breadth (Dobell, 1919). At first they are uninucleate, then binucleate and finally the mature cysts contain four, occasionally eight, nuclei. These latter are to be looked upon

as supernucleate cysts, corresponding to 16-nucleate *coli* cysts. The structure of the nuclei in every stage of the development of the cysts is identical with the nuclei found in the active amœboid forms. At no stage in the development of the cysts of *E. nana* are there typical chromatoid bodies. Dobell (1919), however, notes the occasional presence of peculiar inclusions which may be granular, rod-like, or even filamentous in form. Their exact nature is unknown,

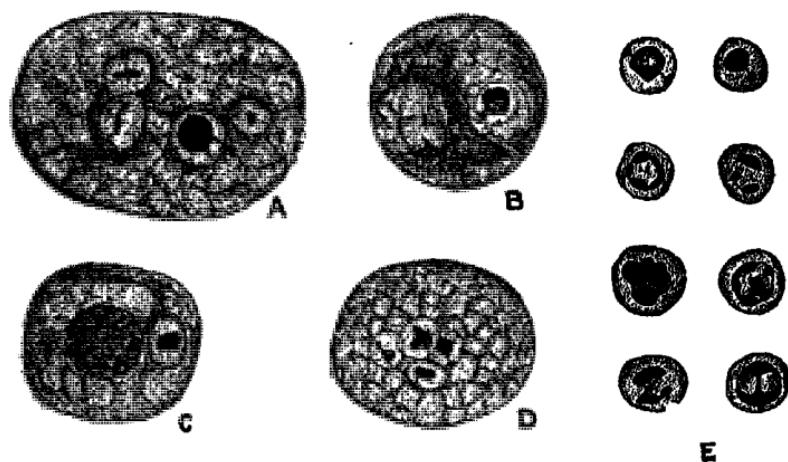


Fig. 29.—*Endolimax nana*.

A, large active amoeba; B, precystic amoeba; C, active amoeba parasitized by *Sphaerita*; D, quadrinucleate cyst; E, eight nuclei, showing some of the variations in the shape of the karyosome. All $\times 3000$. (A, B and C, original; D, after Taliaferro and Becker; E, after Dobell, rearranged.)

although they may be comparable to chromatoid bodies. Diffuse glycogen masses may be seen occasionally in the precystic amoebæ. They are, however, generally formed in the binucleate cysts and may be found thereafter in any of the succeeding stages in the development of the mature cysts. Besides the nuclei, practically all cysts contain a variable number of small refractile granules which are probably volutin.

4. THE GENUS *Iodamæba**a. Iodamæba williamsi*

(1) AMOEBOID FORMS.—The active amoeboid stages of this species are generally from 9μ to 14μ in diameter (Taliaferro and Becker, 1922), although larger and smaller specimens occur. Dobell (1919) notes the finding of forms as small as 5μ and as large as 17μ to 20μ in diameter. Brug (1921) gives the range of size as 7μ to 20μ , and Prowazek (1912 a) in his description of *Entamæba bütschlii*, which is the same species, gives 24μ as the maximum size, but, as Dobell (1919) points out, the latter may have mistaken a small specimen of *E. coli* for the form in question. The movement of the active amoebæ is closely similar to a small *E. coli*. The visibility of the nucleus in the living animal is largely dependent upon the amount of food within the cytoplasm. This species, just as *E. coli*, has the habit of loading its cytoplasm with bacteria and almost every type of intestinal debris. In common with the other non-pathogenic amoebæ of man, it apparently never feeds on red blood cells or tissue elements.

The size of the nucleus varies in different specimens but is roughly about one-quarter of the diameter of the entire animal. In stained specimens its structure not only clearly differentiates this species from the other intestinal amoebæ of man, but also clearly indicates the characters of this genus (Fig. 30, A and B). The nucleus is surrounded by a definite nuclear membrane in which granules are often embedded and within which there is a large spherical, centrally placed karyosome whose diameter is roughly one-third to one-half the diameter of the nucleus. Arranged around the periphery of the karyosome there is a single layer of refractile granules, the so-called "peripheral chromatin." These granules take both nuclear and cytoplasmic stains. They may be overstained with haematoxylin until they are indistinguishable

from the karyosome, or they may be completely decolorized and restained with cytoplasmic stains. Between the karyosome and nuclear membrane there is a clear space which may be traversed by several delicate linin fibers.

(2) Cysts.—As the organisms prepare for encystment they round up and lose their food inclusions and undergo some, but very little, reduction in size. Dobell (1919) maintains that there is no diminution in size before encystment, but this is contrary to our experience. (See Taliaferro and Becker, 1922.) The cysts themselves are remarkable structures. Many of them are practically spherical in shape, but others are extremely irregular in outline. For this reason it is difficult to give a single measure of size. Dobell (1919) has taken the average of the greatest length and the greatest width of a given specimen as the "size." In our experience, using this measure of "size," the majority of the cysts lie between $6.4\ \mu$ and $16.6\ \mu$, with an average of $9.1\ \mu$ (Taliaferro and Becker, 1922). The mature cysts are uninucleate, although supernucleate cysts with two nuclei are occasionally found, in about 0.2 per cent of the cysts, according to Taliaferro and Becker (1922). The nucleus itself shows the same structures as that found in the motile forms, but there is a rearrangement of the karyosome and the granules of "peripheral chromatin." The karyosome migrates to an eccentric position and the granules of "peripheral chromatin," instead of being disposed in a single layer around the karyosome, are massed at one pole (Fig. 30, C and D). These granules, particularly in the nuclei of the cysts, behave very irregularly in haematoxylin stains. They often appear as a crescent-shaped mass or as a number of deeply staining granules (see Fig. 30, E and F). The most noticeable cytoplasmic inclusion in the cyst is the glycogen which is generally present and usually occurs as a single large mass, although two or several masses may be present (Fig. 30,

C and D). No chromatoids have been described in *I. williamsi*, but small refractile granules which are probably volutin occur in the cysts.

Wenyon and O'Connor (1917) and Dobell, Gettings, Jepps, and Stephens (1918) have noted an extremely inter-

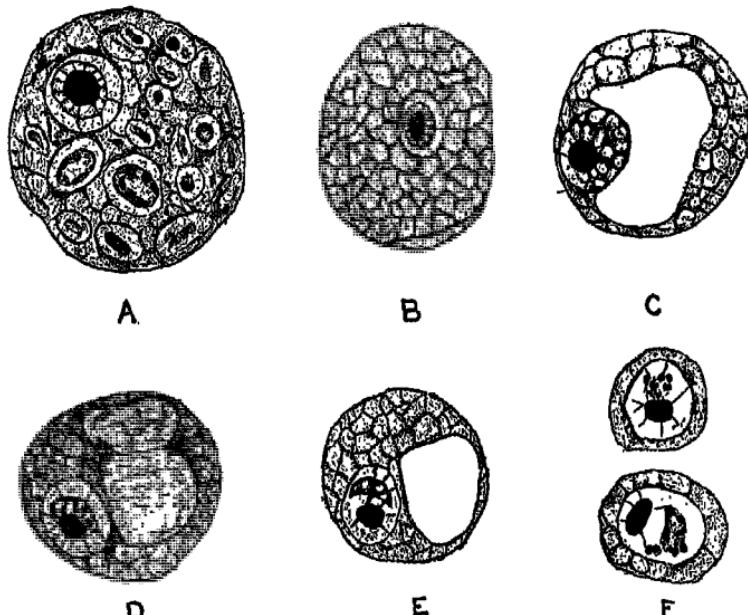


Fig. 30.—*Iodamæba williamsi*.

A, active amoeboid form. B, precystic amoeba. Note the layer of "peripheral chromatin" granules which surrounds the karyosome. C and D, cysts showing the rearrangement of the "peripheral chromatin." The clear spaces represent the position of the glycogen which has been dissolved. E, cyst showing incorrect nuclear picture due to faulty staining. The peripheral chromatin is represented by the crescent-shaped mass. F, two nuclei of similar cysts in which the "peripheral chromatin" is represented by several deeply staining granules beside the karyosome. $\times 3000$. (After Taliaferro and Becker.)

esting fact in regard to *I. williamsi*, viz., that emetine acts on this amoeba just as it does on *E. histolytica*. *I. williamsi* is, then, the only non-pathogenic amoeba of the human intestine which responds to emetine treatment.

5. THE GENUS *Dientamæba*a. *Dientamæba fragilis*

This rare entozoon of man (Fig. 31) has been found in only about 33 cases (Taliaferro and Becker, 1924). It is the most delicate of the amoebæ found in man and can only be studied in very fresh stools since degeneration begins almost immediately after the fecal material leaves the body. The process of degeneration is interesting in that it is initiated by the formation of a central vacuole which grows in size until there is little more than a hull of protoplasm surrounding the vacuole. In this stage the organisms can easily be mistaken for *Blastocystis hominis*. Up to the present time cysts of the species are unknown. This fact, coupled with the fact that the motile amoebæ are so susceptible to environmental changes, raises the question of the mode of infection. Jepps and Dobell (1918) have suggested that the organism may be a natural entozoon of some other host and that man may simply represent a "blind alley" in which the amoeba is unable to complete its life-history. If this be the correct interpretation, *D. fragilis* is much like *E. histolytica* in the cat where the organism can live but can not develop infective forms.

During locomotion *D. fragilis* protrudes leaf-like, hyaline pseudopodia which have dentate margins and are composed almost entirely of ectoplasm sharply marked off from the granular endoplasm. When rounded, it varies from 3.5μ to 12μ in diameter.

The organism is peculiar among the amoebæ of man in that it is normally binucleate. According to Jepps and Dobell (1918), approximately only 20 per cent of the specimens are uninucleate. The latter, they believe, represent a division stage, i. e., when an adult binucleate specimen divides the cytoplasm alone divides forming two uninucleate specimens; the nucleus in each specimen then divides to form an

adult binucleate organism again. The nuclei are rarely visible in the living condition. When stained the following characteristic structures can be seen (Fig. 31):

1. A delicate achromatic membrane surrounding the nucleus.

2. A large central karyosome which consists of a lighter-staining matrix (probably plastin) in which are embedded a number of distinct chromatin granules that represent all of the nuclear chromatin.



Fig. 31.—*Dientamæba fragilis*.

A and B, uninucleate and binucleate specimens, respectively. $\times 3000$. (After Taliaferro and Becker.)

3. A few linin fibers which run from the karyosome to the nuclear membrane.

The food of the amœba consists chiefly, if not entirely, of intestinal bacteria and yeasts.

There is no evidence that it is ever any more than a harmless commensal in the human intestine.

6. DIFFERENTIAL DIAGNOSIS OF THE AMŒBÆ LIVING IN MAN

In the preceding sections the attempt has been made to describe the various species in a fresh healthy condition and, wherever possible, to denote differences. Unfortunately, many diagnoses have to be made on degenerating and otherwise abnormal specimens. Satisfactory results from such diagnoses can only be attained with long practice and care. The beginner in this type of work must always remember that the diagnoses of the various intestinal amœbæ offer some of the greatest, if not the greatest, difficulties in practical protozoology. The general methods of diagnosis and the chief differences between the cysts of the different amœbæ are summarized in Chapter 15.

B. The Amœbæ Ectozoic and Entozoic in Lower Animals

In the preceding section of this chapter we have given a detailed description of all of the amœbæ known to occur in man. In the present section, however, no attempt is made to give an exhaustive description of all of the amœbæ associated with the lower animals. Such a compilation can be found in Nöller (1922). Here only a few of the more representative forms will be discussed.

1. AN ECTOZOIC AMŒBA

Entz (1912) has described a very interesting ectozoic amœba occurring on the fresh water *Hydra*, *Hydra oligactis*, which has also been observed in Baltimore on *H. viridis* by Dr. F. M. Root. Although Entz described this form as *Amœba hydroxena*, it is clearly not a member of this genus and should, therefore, be placed in a new one. The organism is very large, ranging in size from $100\ \mu$ to $368\ \mu$, with one or several contractile vacuoles (Fig. 32). Its nucleus, sometimes there are several, exhibits a very peculiar arrangement of chromatin and achromatinic structures. *Amœba hydroxena* lives on the surface or may even pass into the enteron of *Hydra* where it feeds chiefly on the cells of its host and occasionally on small infusoria. Figure 32, C, drawn from a section loaned by Dr. Root, indicates that the amœba can actually extract the cells from the epithelium although this has not been definitely demonstrated.

2. ENTOZOIC AMŒBÆ

a. The Genus *Endamœba*

This genus includes by far the majority of the entozoic amœbæ known at the present time. The hosts harboring these amœbæ range from insects to every class of the mam-

mals. In the present account we can do little more than note a few.

(1) *E. BLATTÆ*.—Among the insects a most interesting form, *E. blattæ* (Fig. 33), occurs in the cockroach. This

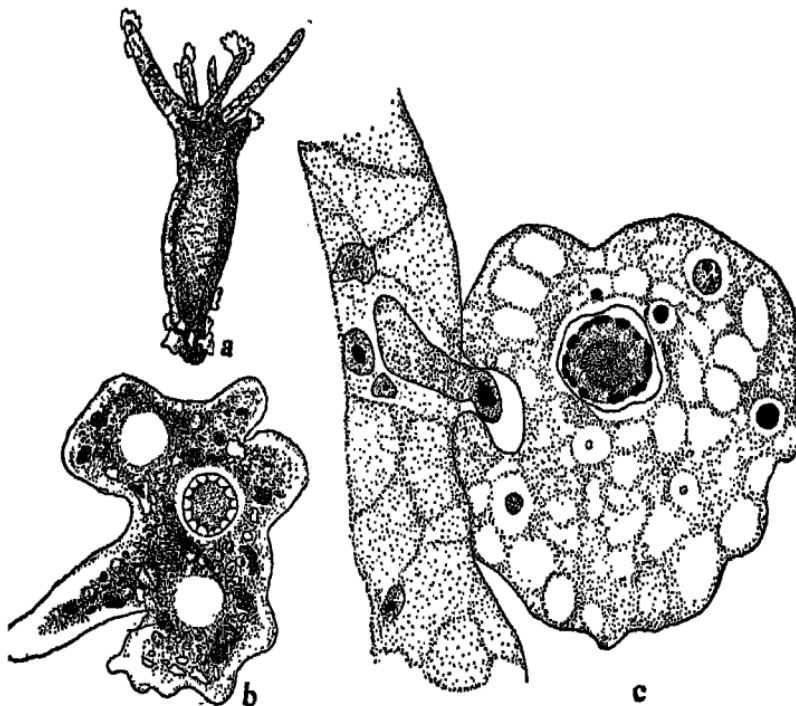


Fig. 32.—*Amoeba hydroxena*.

a, *Hydra* with several specimens of the amoeba on its tentacles and body. b, living specimen of amoeba. c, section of infected *Hydra* showing ingestion of an epithelial cell by an amoeba. a and b, magnification unknown; c \times 1120. (a and b after Entz, c, original from a slide of Dr. F. M. Root.)

amoeba is generally from $12\ \mu$ to $50\ \mu$ in diameter, although forms ranging up to $100\ \mu$ occur. The vegetative specimens are typically uninucleate, although a process of multiple fission has been described in which from 4 to 20 nuclei occur. The cysts are $30\ \mu$ to $70\ \mu$ in diameter and usually contain

12 to 30 nuclei. This form is the type species of the genus *Endamæba*, and, while we consider the *Endamæba* from vertebrates co-generic with it, it must be admitted that the nucleus of the present form, as described by Mercier (1910), does not appear to be very similar to the forms found in vertebrates (Fig. 33, A). On the other hand, a number of the nuclei of the cysts are built on the same fundamental plan as those of *E. coli* and *E. histolytica* (Fig. 33, C and D).

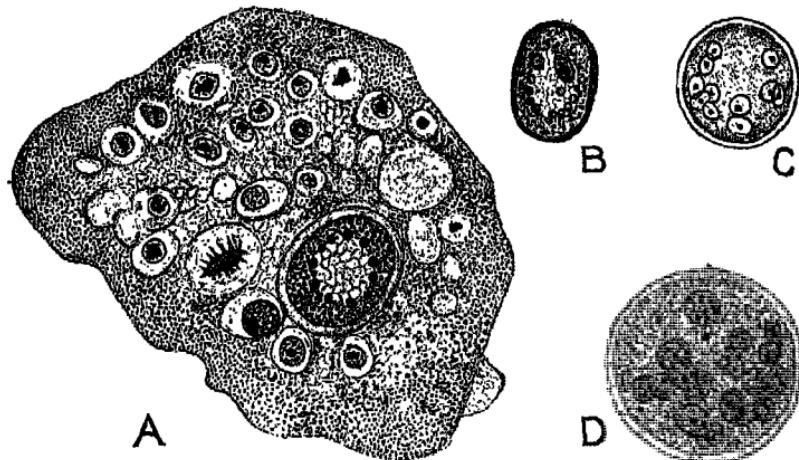


Fig. 33.—*Endamoeba blattæ*.

A, large active form. B, nucleus of large active form. C and D, cysts. All $\times 810$. (A and D after Mercier; B after Schubotz and C drawn by Mr. F. O. Holmes.)

(2) E. BARRETI.³—The amoeba from the turtle, *E. barreti*, is of particular interest (Fig. 34) because it has been successfully cultivated by Barret and Smith (1923) (see also Taliaferro and Holmes, 1923). Except for the work of Cutler on *E. histolytica*, which many investigators do not accept, this is the only entozoic amoeba that has been cultivated.

³The name *E. barreti* is taken from a MS. of Taliaferro and Holmes and is the name proposed for the amoeba which Barret and Smith obtained from the turtle, *Chelydra serpentina*.

(3) E. RANARUM.—*E. ranarum* which lives in the intestine of frogs and tadpoles is remarkable in that it is indistinguishable morphologically from *E. histolytica* of man. According to Dobell (1918), "The active amoebæ can usually be readily distinguished from one another by the inclusions (food bodies) in their protoplasm, but not by their own

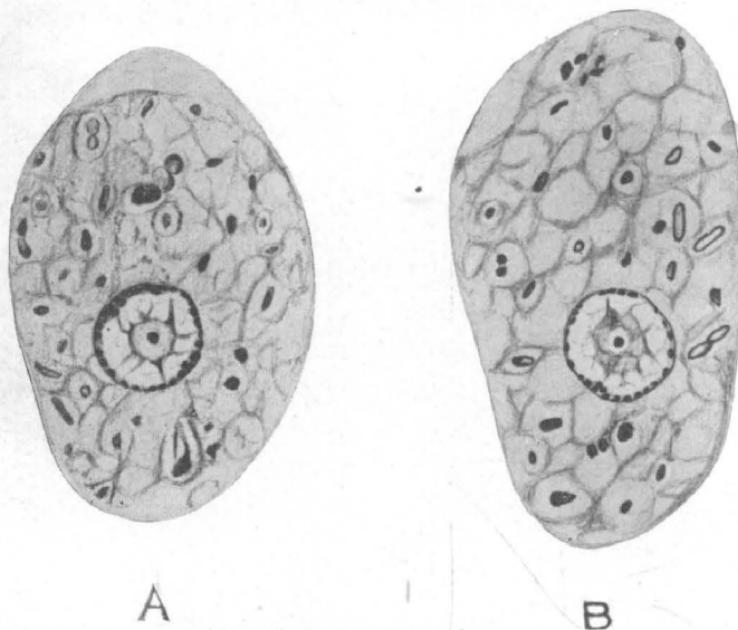


Fig. 34.—*Endamoeba barretti*.

A, specimen from turtle. B, specimen from culture. $\times 2250$. (After Taliaferro and Holmes MS.)

nuclear and cytoplasmic structure; but the precystic amoebæ, devoid of all food bodies, and the cysts, at every stage of development, are so closely alike that preparations of the one could be used as demonstrations of the other." This remarkable similarity led certain investigators to suggest (see Alexeieff, 1914) that the harmless commensal of the frog, when introduced accidentally into man, might become the parasite of amoebic dysentery. Dobell (1918) has, however,

shown by suitable experiments that in all probability the two species are separate and distinct.

(4) E. NUTTALLI AND E. PITHECI.—*Endamæba* have been reported from a number of species of monkeys. A short account of these various investigations is given in Dobell (1919). Monkeys apparently harbor an *Endamæba*, *E. nuttalli* Castellani, 1908, with a 4-nucleate cyst which may be pathogenic, producing dysentery and liver abscess, and

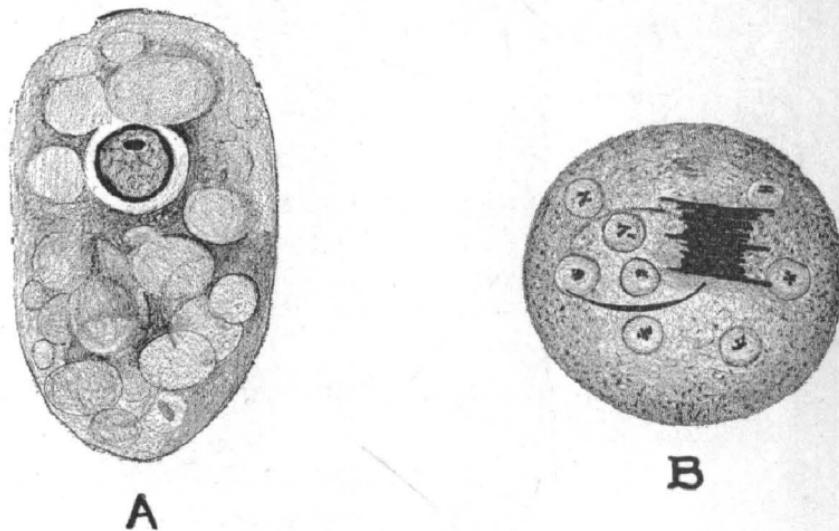


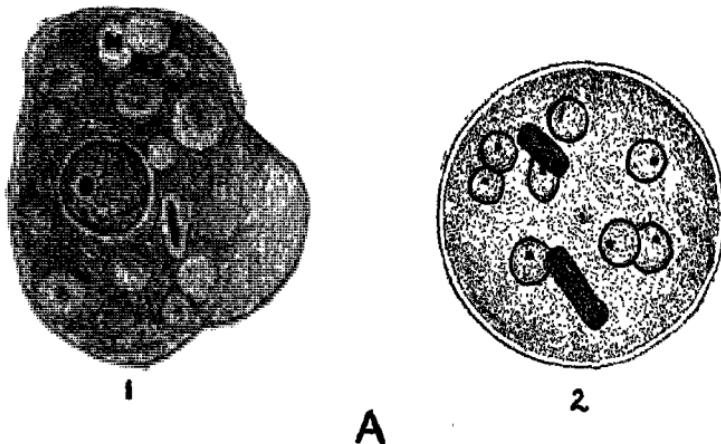
Fig. 35.—*Endamæba pitheci* from the monkey.

A and B, large active form and mature 8-nucleate cyst, respectively.
× 2000. (Drawn by Mr. F. O. Holmes.)

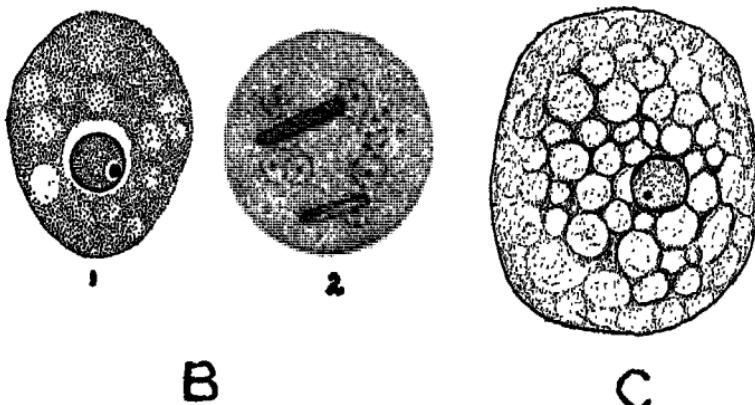
another *Endamæba*, *E. pitheci* Prowazek, 1912, with an 8-nucleate cyst which is non-pathogenic (Fig. 35). At the present time there is no way of distinguishing these species from *E. histolytica* and *E. coli*, respectively. In fact, Dobell (1919) thinks that they may be identical.

(5) OTHER ENDAMÆBÆ.—Among the lower mammals *Endamæba* have been described from mice and rats, guinea pigs, rabbits, pigs and others. Among these, *E. muris* Grassi, 1882, from the mouse *Mus musculus*, and the rat *M. rattus*,

E. cobayæ Walker, 1908, from the guinea pig *Cavia cobaya*, and *E. suis* Hartmann, 1913, from the pig have frequently



A



B

C

Fig. 36.—Representative *Endamoeba* from the lower mammals.

A, *E. cobayæ* from the guinea pig; 1 and 2, large active form and mature 8-nucleate cyst, respectively. B, *E. muriæ* from the rat; 1, and 2, large active form and mature 8-nucleate cyst, respectively. C, *E. suis* from the pig; large active form. $\times 2000$. (Drawn by Mr. F. O. Holmes.)

been encountered in this laboratory and are shown in figure 36.

b. The Genus Endolimax

At the present time it is impossible to say whether or not any of the amoebæ which have been described from the lower animals are co-generic with *Endolimax nana* of man. Nöller (1922) (see page 152) believes that an amoeba described from the frog by Epstein and Ilowaisky (1914) under the name of *Nægleria ranarum* should be placed in the genus *Endolimax*. Another organism, which Minchin (1910) found in the malpighian tubules of the rat-flea, *Ceratophyllus fasciatus*, and which he described under the name of *Malpighiella refringens*, may also be co-generic with *E. nana*. If the latter proves to be the case, the genus *Malpighiella* will have priority over *Endolimax*. *Malpighiellæ* have also been reported from the dog-flea, *Ctenocephalus canis*, by Nöller (1914) and from the vagina of the leech, *Hirudo medicinalis*, by Alexeieff (1913). Descriptions of these amoebæ are given in Nöller (1922).

c. The Genus Iodamæba

O'Connor (1920), Cauchemez (1921) and Nöller (1921 and 1922) report the finding of an *Iodamæba* in the pig which is indistinguishable from *I. williamsi* of man. O'Connor has named this amoeba *I. suis*. Cauchemez and Nöller feel that the two organisms are identical and that the pig represents the original host from which man acquires his infection. This would place *I. williamsi* in much the same position that *Balantidium coli* is supposed to hold. For our part, we feel that it is best to consider them different until more evidence bearing on the question has accumulated. Figure 37, A, shows an amoeboid form and a cyst from a pig slaughtered in Baltimore. Brug (1921 a) has recently described another *Iodamæba* from the monkey, *Macacus cynomolgus*, which he named *I. kueneni*, and maintains that this species can be differentiated from that occurring in man by the possession of a constant darkly staining protoplasmic

area in the cysts. Apparently the same form has been found in this laboratory in a Brazilian monkey known as the varie-

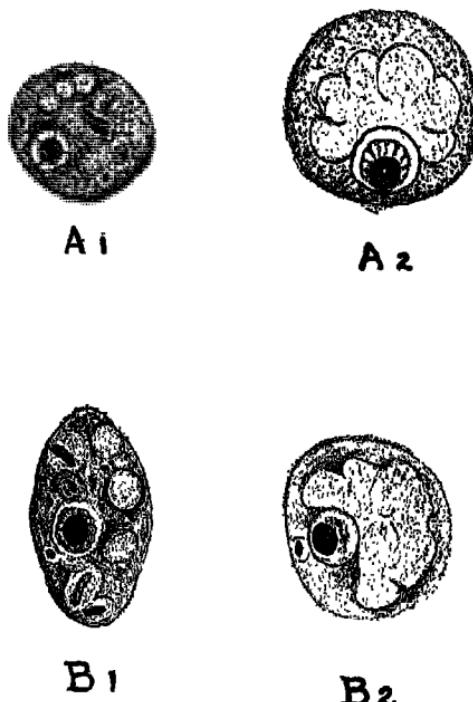


Fig. 37.—*Iodamaba* from lower animals.

A, *I. suis* from the pig; 1 and 2, active amoeboid form and cyst, respectively. B, *I. kuenenii* from the monkey: 1 and 2, active amoeboid form and cyst, respectively. $\times 2000$. (Drawn by Mr. F. O. Holmes.)

gated capuchin monkey, *Cebus variegatus* (Fig. 37, B). We are not certain, however, that Brug's structural differentiation between the cysts from man and the monkey will hold.

CHAPTER IV¹

A GENERAL CONSIDERATION OF THE MASTIGOPHORA

A. Introduction

The MASTIGOPHORA are characterized by the possession of one or more flagella in the "adult stage." This group, however, is difficult to define. As has already been pointed out, many of the SARCODINA are flagellated during some stages of their life-cycles, but, since most of them are undoubtedly amœboid during the greater part of their lives, they are justly included in the SARCODINA. The group RHIZOMASTIGINA is particularly difficult to classify because it partakes of so many characteristics of both classes and contains forms so obviously transitional. It is considered here as belonging to the MASTIGOPHORA since the forms comprising it possess flagella throughout the greater part of their life-cycles. The flagella in some of them, however, are not functional in locomotion but simply drag behind the organism which uses pseudopodia for locomotion and food capture.

The members of the class MASTIGOPHORA show almost every conceivable method of nutrition. Some forms, like *Mastigamæba*, are entirely holozoic, or animal-like in the method of nutrition—others, like *Chlamydomonas* and *Hæmatococcus*, are entirely holophytic or plant-like. Between these two extremes, there is almost every combination of the two methods, and, in addition, most orders contain forms which are completely or partially saprophytic. Such diversity

¹By W. H. Taliaferro.

obviously makes it impossible to pick out any one form as a "typical flagellate," and consequently a short account of the classification of the group will be given followed by a brief description of a few of the typical forms in each order or suborder.

B. Classification

The classification of the flagellates is in rather a chaotic state at the present time. The following one is patterned largely after that of Pascher (1913 and 1914), Lemmermann (1913 and 1914) and Doflein (1916).

CLASS MASTIGOPHORA

A. Subclass *Zoomastigina*.—Animal-like flagellates.

1. ORDER PANTASTOMINA.—Holozoic; no mouth opening; food ingested by means of pseudopodia anywhere on body surface.
 - (1) SUB-ORDER RHIZOMASTIGINA.—Body amœboid; 1-3, seldom 4 flagella.
 - (2) SUB-ORDER HOLOMASTIGINA.—Body only weakly amœboid; numerous flagella radiating from a more or less spherical body.
2. ORDER PROTOMONADINA.—Holozoic, saprophytic or entozoic. In first case food ingested at a definite place on body (generally at base of flagellum) where a permanent mouth opening may be present or absent. Flagella 1-6, rarely 8.
3. ORDER DISTOMATINA.—Mostly entozoic; body bilaterally symmetrical in structure; 4-8 flagella arranged in pairs.
4. ORDER HYPERMASTIGINA.—All entozoic in gut of insects. Flagella very numerous and generally arranged as a thick bunch at anterior end of body.

- B. Subclass Phytomastigina.—Plant-like flagellates.
5. ORDER CHRYSOMONADINA.—Holozoic or holophytic. One or two yellowish chromatophores; 1-2 flagella; with or without stigmata.
 6. ORDER CRYPTOMONADINA.—Holophytic or saprophytic. Two flagella associated with œsophagus-like canal and contractile vacuole; 2 chromatophores may be present.
 7. ORDER EUGLENOIDINA.—Holozoic, holophytic or saprophytic. One or two flagella associated with mouth aperture and œsophagus; complex vacuole system opening into œsophagus; often contains chromatophores, stigmata, etc.
 8. ORDER PHYTOMONADINA.—Exclusively holophytic. Body covered with cellulose wall and without mouth-opening.
 9. ORDER DINOFLAGELLATA.—Holozoic or holophytic. Most forms have a thickened cuticle which forms a *lorica*. Two flagella which usually lie in two grooves in the lorica, the longitudinal one in the longitudinal groove or *sulcus*, and the transverse one in the circular groove or *girdle*.

I. PANTASTOMINA

This order is characterized by the fact that in the adult stage the organisms ingest their food at any point on the body by means of pseudopodia.

In appearance the members of the suborder RHIZOMASTIGINA are amœbæ with well-developed flagella. In fact they exhibit so many transitional characters between the true SARCODINA on the one hand and the MASTIGOPHORA on the other that it is exceedingly difficult to define their exact relationships. Doflein places this suborder as an order of the SARCODINA while Lemmerman places it (the RHIZOMA-

STIGINA) under the MASTIGOPHORA, but includes in it many of the flagellated forms which we have included in the orders

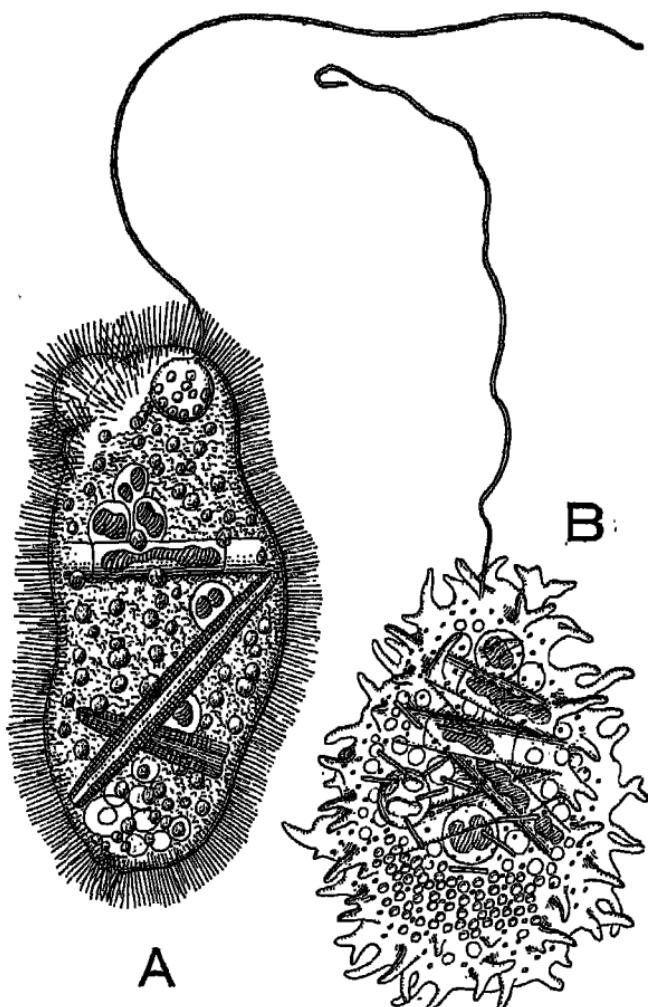


Fig. 38.—Representatives of the suborder RHIZOMASTIGINA.
A, *Mastigamæba setosa*. B, *Mastigella vitrea*. $\times 542$. (From Lemmermann, after Goldschmidt.)

PROTEOMYXA and LOBOSA. The chief genera of this sub-order are *Mastigamæba* and *Mastigella*, both of which possess

a single flagellum (Fig. 38). The genus *Cercomonas*, which possesses two flagella and a representative of which is de-

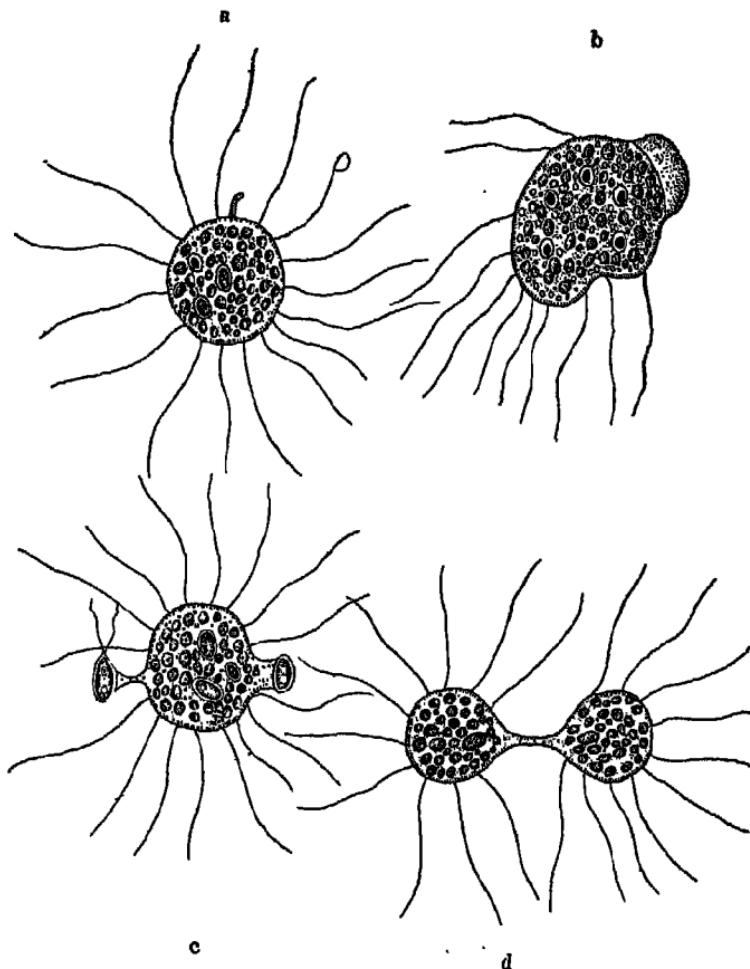


Fig. 39.—*Multicilia lacustris*, a representative of the suborder HOLOMASTIGINA. a, ordinary individual; b, formation of a pseudopodium; c, food capture by means of pseudopodia; and d, division. $\times 500$. (From Doflein after Lauterborn.)

scribed under the section on COPROZOIC PROTOZOA, may belong to this order although certain authors deny this.

Under the suborder HOLOMASTIGINA, there is at present but a single genus—*Multicilia*—which is characterized by the possession of a more or less spherical body from which numerous flagella radiate. One or many nuclei may be present. This suborder is placed in the order PANTASTOMINA because its members ingest food by means of short pseudopodia (Fig. 39, c), although their bodies are otherwise only weakly amoeboid. Reproduction by fission takes place in the motile stage. Cyst formation and sexual phases are, as yet, unknown. The relationship of this group is also greatly in doubt. Penard (1903) believes that they are allied to the HELIOZOA, whereas Doflein (1916) considers them to be Infusoria-like flagellates and to represent the type from which the INFUSORIA arose.

2. PROTOMONADINA¹

This order comprises a vast number of flagellates generally of small or minute size and is particularly interesting in that

¹The older writers generally divided the forms which we have included under the PROTOMONADINA and DISTOMATINA between the orders PROTOMONADINA and POLYMASTIGINA. Under the first order they included only the simpler forms with one, two or three flagella which we have placed in the PROTOMONADINA, such as OICOMONAS and the hæmoflagellates, and in the second they included the rest of the forms (or TETRAMITIDÆ) with 3 or more flagella, more or less equal in size, which we have also placed under the PROTOMONADINA, and the OCTOMITIDÆ with 4 to 8 flagella and a peculiar bilateral symmetry of the body, which we have placed in the DISTOMATINA. (See Minchin, 1912.) Later Hartmann and Chagas (1910) proposed to unite the two orders PROTOMONADINA and POLYMASTIGINA, thus defined, as a single order PROTOMONADINA which they subdivided into two suborders: (1) The MONOZOA, which included the old PROTOMONADINA and the TETRAMITIDÆ (in other words the PROTOMONADINA as defined by the present authors) with the exception of the hæmoflagellates which they placed in their order BINUCLEATA, and (2) the DIPLOZOA, which included the OCTOMITIDÆ, or the DISTOMATINA of our classification.

Doflein (1916) divides the POLYMASTIGINA of the older writers into

it includes the majority of the flagellates living in man. A few entozoic species occurring in termites, which possess as many as 8 flagella, should probably be placed in this order. The members of the group generally possess a very thin cuticle, a weakly amoeboid body and from 1 to 6 flagella arranged in various ways. They may occur singly or in colonies and may form shells about themselves. Contractile vacuoles often occur in the free-living species in which case they empty directly into the surrounding medium. Forms which are holozoic ingest their food at a definite place on the body (generally at the base of the flagellum) where a mouth-opening may be present. In the parasitic forms undulating membranes may be present. Asexual reproduction occurs by longitudinal fission and among the entozoic genera multiple fission undoubtedly occurs. Although sexual reproduction by the conjugation of gametes has been described, its occurrence is exceedingly doubtful because the observations were made on very small entozoic genera where it is extremely difficult to distinguish between true conjugation and division and agglutination phenomena. This question will be taken up again under the life-history of the different entozoic genera.

The simpler free-living PROTOMONADINA are exemplified by the genus *Oikomonas* described by Kent (Fig. 40, A). Many of them, as *Amphimonas* (Fig. 40, B), are sedentary. The so-called choanoflagellates which are sedentary and use their flagella mainly as organs of food capture deserve mention because they have developed a "collar" around the

the POLYMASTIGINA, containing the TETRAMITIDÆ, and the DISTOMATINA, containing the OCTOMITIDÆ, thereby establishing three orders, the PROTOMONADINA, POLYMASTIGINA, and DISTOMATINA. The present authors do not feel that there is any valid reason for separating the TETRAMITIDÆ from the PROTOMONADINA, and for that reason recognize only the two orders PROTOMONADINA and DISTOMATINA which is essentially the classification adopted by Lemmerman (1914).

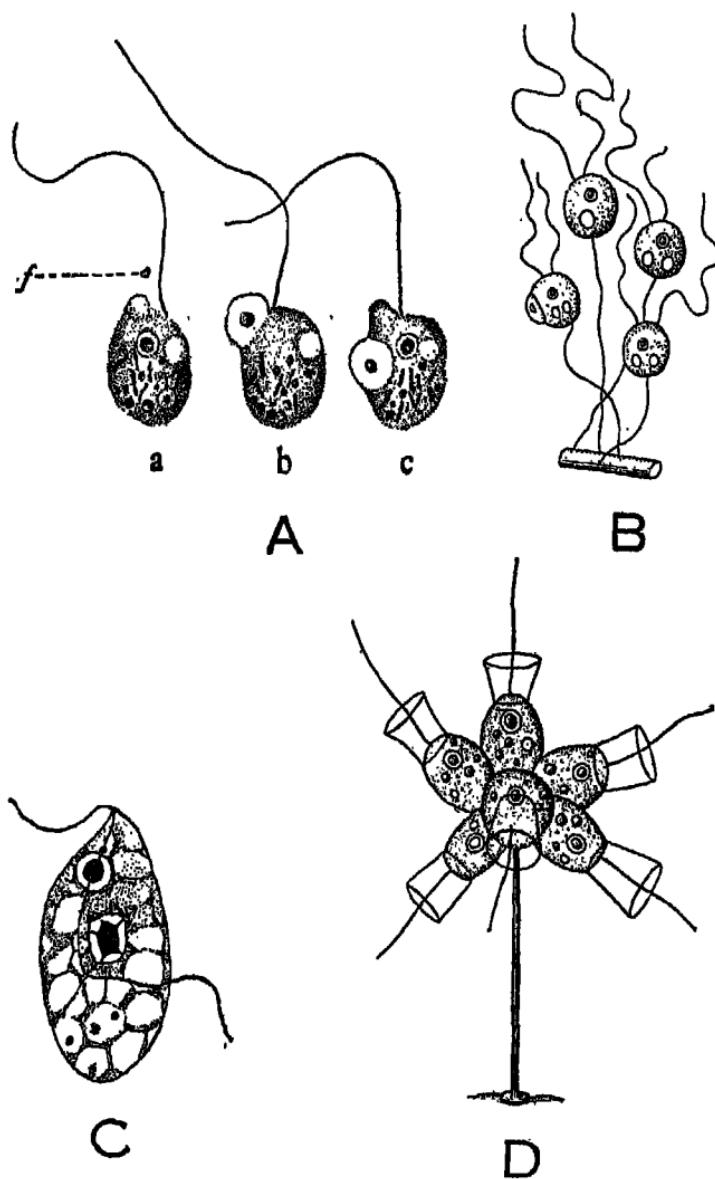


Fig. 40.—Representatives of the order PROTOMONADINA.

A, *Oikomonas termo*, a-c, stages in the process of ingesting food; f, food particle. B, *Amphimonas globosa*. C, *Bodo caudatus*. D, *Codonosiga botrytis*. A $\times 1333$; B $\times 535$; C $\times 3700$; D $\times 590$. (A From Calkins after Bütschli; B and C after Lemmermann; C from Lemmermann after Hartmann and Chagas.)

flagellum which probably plays a rôle in the ingestion of food and which gives them the appearance of the collared endodermal cells found in sponges (Fig. 40, D). Among

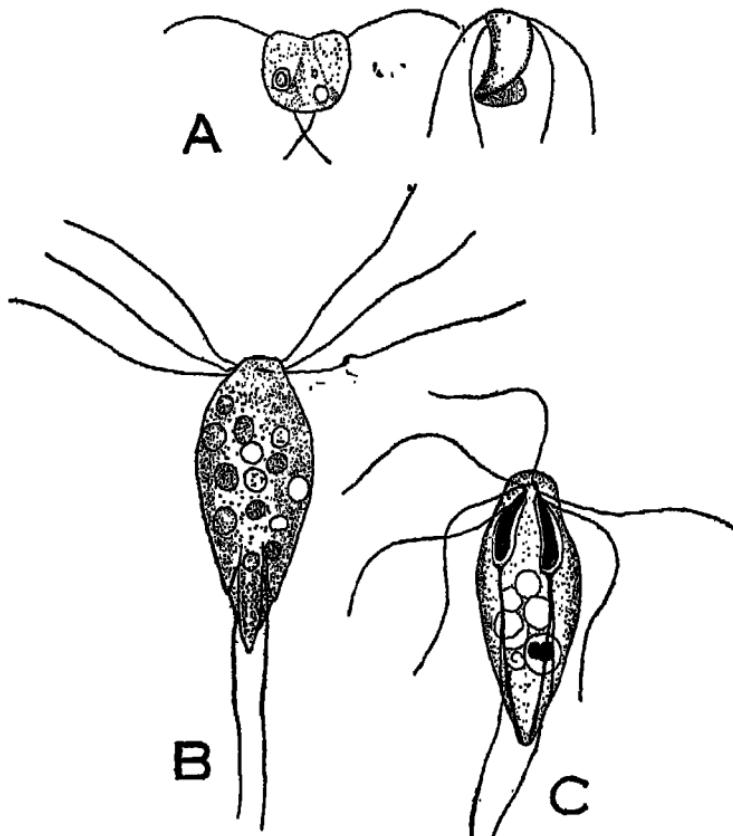


Fig. 41.—Free-living representatives of the order DISTOMATINA.

These figures should be compared with those of *Giardia lamblia*.
A, *Gyromonas ambulans*. B, *Urophagus rostratus*. C, *Hexamitus fissus*. A $\times 900$; B $\times 1500$; C $\times 2250$. (A after Lemmermann; B and C from Lemmermann; B after Klebs and C after Alexeieff.)

the entozoic members of the group are found the haemoflagellates and various intestinal forms such as *Trichomonas*, *Chilomastix*, *Embadomonas* and *Entecromonas*. *Costia necatrix*, an ectoparasite of fishes, also belongs there.

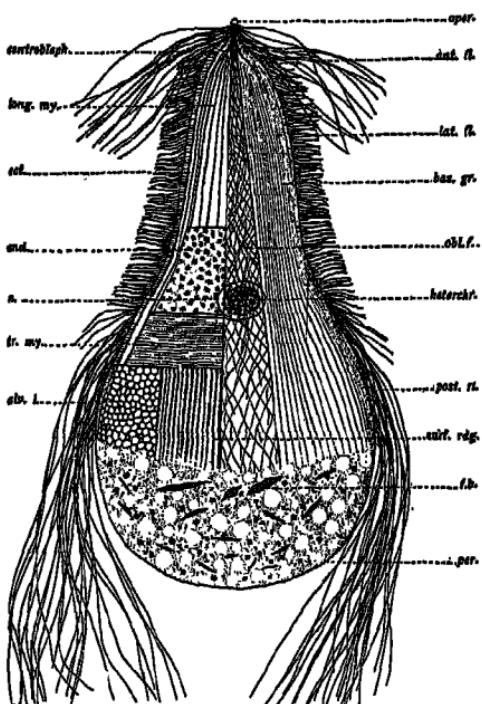


Fig. 42.—Diagrammatic figure of *Trichonympha campanula*.

Sections of the body show the structures found at different levels. Surface ridges form the outer layer with their rows of flagella; beneath are successively the oblique fibers, alveolar layer and transverse myonemes. In the endoplasm are the longitudinal myonemes. Abbreviations: *alv. l.*, alveolar layer; *ant. fl.*, anterior zone of flagella; *bas. gr.*, basal granules; *centrobleph.*, centroblepharoplast; *ect.*, ectoplasm; *end.*, endoplasm; *f. b.*, food bodies; *heterch.*, heterochromosome; *lat. fl.*, lateral zone of flagella; *long. my.*, longitudinal myonemes; *n.*, nucleus; *obl. f.*, oblique fibers; *oper.*, operulum; *per.*, periplast; *post. fl.*, posterior zone of flagella; *surf. rdg.*, surface ridges; *tr. my.*, transverse myonemes. $\times 300$. (Figure and legend after Kofoid and Swezy.)

3. DISTOMATINA

The striking characteristic of the members of this order is the marked bilateral symmetry of the body. Most of the forms are entozoic in habit, but a few are free-living. Among the former are found the genus *Giardia* and some species of *Hexamitus*. *Gyromonas ambulans*, *Urophagus rostratus*, and *Hexamitus fissus* (Fig. 41) are examples of the latter.

Reproduction and the life-history of the entozoic forms will be taken up in detail under the respective genera.

4. HYPERMASTIGINA

This order was founded originally by Grassi to include a number of peculiar flagellates found in the gut of certain in-

sects which, in many ways, possess the most complex structure found among the flagellates. All of the forms possess a large number of flagella which may be arranged in bunches or may be distributed over the entire body. The enormous complexity of structure of the forms is indicated in figure 42, which shows, diagrammatically, the various organelles and structures in *Trichonympha campanula* from the termite *Termopsis angusticollis*.

5. CHRYSOMONADINA

This order includes a number of small forms which typically contain one or two brownish chromatophores and one or two flagella which, in the latter case, may be equal in size or differentiated into a principal and accessory one. The body is often amoeboid and food may even be taken in by pseudopodia. Nutrition is either holophytic or holozoic or both. Asexual reproduction takes place by longitudinal fission. Most species produce resistant stages or spores. Sexual phenomena are unknown. An example of this order is *Coccolithophora* (Fig. 43, C). Colony formation is very frequent and is exemplified in such forms as *Synura uvella* (Fig. 43, A) which causes the "oil odor" in certain drinking waters and is, therefore, of some economic importance.

6. CRYPTOMONADINA

The forms referred to this order are also small, generally measuring 10 μ to 20 μ and in rare cases 40 μ to 80 μ in diameter, but they are much more highly differentiated than those of the last order. The body possesses a firm cuticle, is rarely metabolic and never amoeboid. It is typically differentiated along the lines of a dorso-ventral type of symmetry due to a furrow which runs obliquely across the anterior end of the more flattened "ventral side." The lower CRYPTOMONADINA possess simply such a furrow, but the higher

forms have the furrow deeply sunk into the body at the anterior end along the median line to form a trough, or tube-like cesophagus, at the base of which two flagella are generally inserted. In many species a number of highly refractive rod-like structures are present and are considered by some authors to be trichocyst-like in nature (Pascher,

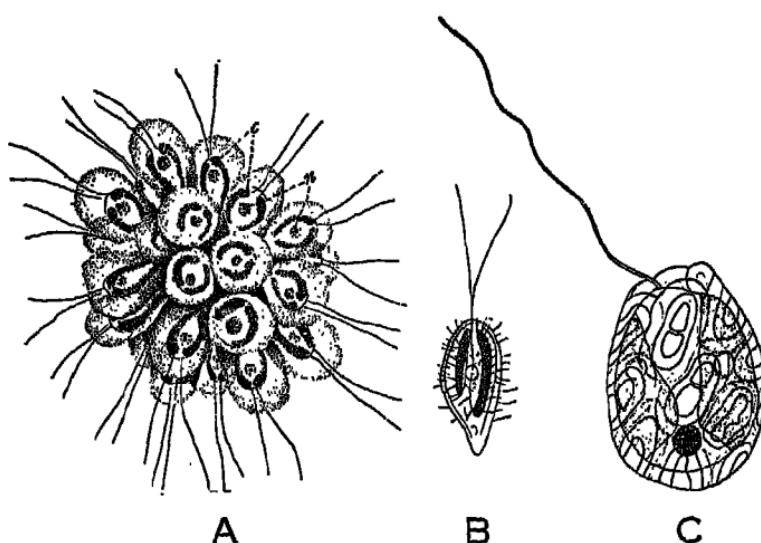


Fig. 43.—Representatives of the order CHRYSMONADINA.

A, *Synura uvella*, a colonial form. B, a single flagellate from a colony of *S. uvella*. C, *Coccolithophora wallichii*. c, chromatophores, of which there are two in each individual; n, nucleus. A, magnification unknown, B \times about 800, C \times about 1400. (A after Calkins, B and C from Jollos, B after Pascher and C after Lohmann.)

1913). The contractile vacuoles, one or several in number, are situated near the anterior end on the dorsal side. The organisms may be colorless or may contain chromatophores, rarely more than two in number, of a yellow, brown, red-brown, red, blue, blue-green or green color which are disc- or band-like, rarely trough-like in shape. The colorless organisms are saprophytic or in a few cases holozoic in their

mode of nutrition. Asexual reproduction takes place by longitudinal fission.

Cysts have been described for some species, but sexual phenomena are, as yet, unknown.

The lower type of organization characterized by the possession of a furrow without oesophagus is exemplified in *Cryptochrysis commutata*, and the higher organization with oesophagus in *Chilomonas paramecium*, and *Cyathomonas truncata* (Fig. 44). Of these forms *C. commutata*, and *C. truncata* possess chromatophores whereas *C. paramecium* is a colorless form, commonly living saprophytically in hay infusions. The symbiotic organisms which are associated with certain marine SARCODINA

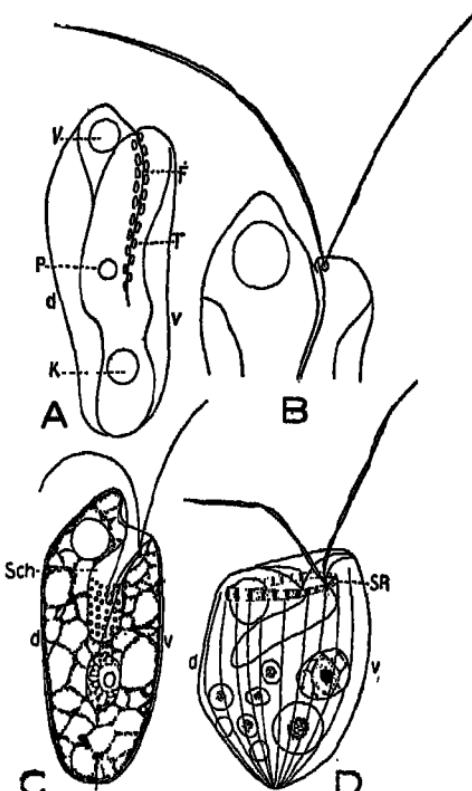


Fig. 44.—The organization of the CRYPTOMONADINA.

A and B, *Cryptochrysis commutata* showing the organization of the lower CRYPTOMONADINA which possess a furrow (*F*) but no oesophagus. C, *Chilomonas paramecium* and D, *Cyathomonas truncata* var. *subrotunda* which show the organization of the higher members of the order and which possess an oesophagus (*Sch.*). *d*, dorsal side; *F*, furrow; *K*, nucleus; *Sch.*, oesophagus; *SR*, cesophageal ring; *T*, trichocyst-like bodies; *V*, vacuole; *v*, ventral side. A and B $\times 2000$; C and D $\times 1000$. (All from Pascher, C and D after Ulehla.)

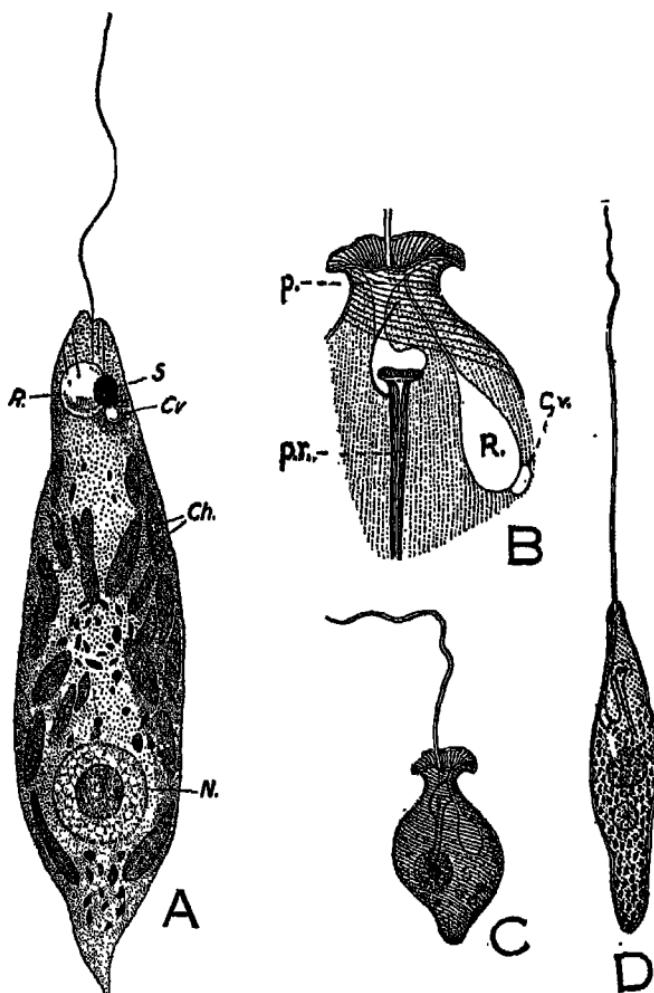


Fig. 45.—Representatives of the EUGLENOIDINA.

A, *Euglena viridis*. B, *Urceolus cyclostomus*, mouth-apparatus and vacuole-system. C, *Urceolus cyclostomus*, entire flagellate. D, *Pera-nema trichophorum*. Cv., contractile vacuole; Ch., chromatophore; N, nucleus; P., pharynx; pr., pharyngeal or cesophageal rods; R., reservoir; S., stigma. A \times about 1500; B \times 1334; C \times 667; D \times 667. (A from Jollos after Doslein; B, C and D after Lemmermann.)

and which are known as zooxanthellæ probably belong to this order.

7. EUGLENOIDINA

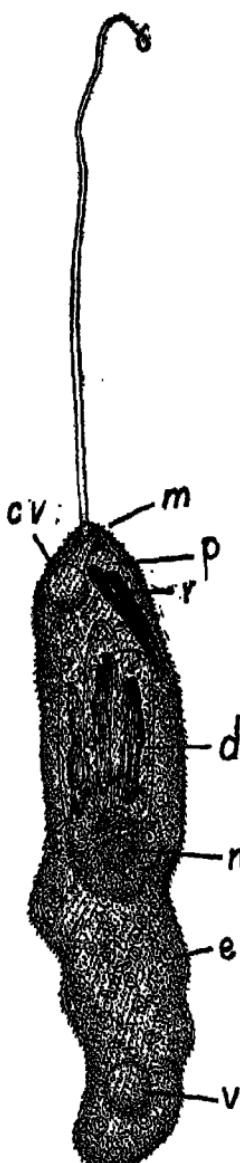
The members of this order are comparatively very large in size and, as far as structural differentiation goes, represent the most highly developed order of the MASTIGOPHORA. The body is covered with a more or less stiff pellicle and is never amoeboid, but quite often exhibits metabolic movements. In this connection it is interesting to note that some of the species (most *Euglenæ*) have the habit at times of losing their flagella and of "crawling" on the substratum by a gliding movement of the body. There is a pit or œsophagus deeply sunken into the anterior end at the base of which the flagellum (rarely two) arises. Associated with the œsophagus is a very characteristic vacuole system which generally consists of a reservoir and one or two accessory contractile vacuoles and is closely correlated with the mode of nutrition. In such holophytic genera as *Euglena*, which possesses green chromatophores, the œsophagus is chiefly a canal through which the contractile vacuole empties to the exterior (Fig. 45, A). In colorless holozoic species, such as *Urceolus cyclostomus* (Fig. 45, B and C) and *Jenningsia diatomophaga* (Fig. 46), the œsophagus is a very highly differentiated structure, supplied with supporting structures known as œsophageal rods (Fig. 46), through which food is ingested and into which a separate canal leads from the contractile vacuole. Besides holophytic and holozoic species there are a number of saprophytic forms, such as *Astasia* and *Peranema* (Fig. 45, D). Their nutrition is considered later in more detail.

The very interesting flagellate, *Euglenamorpha hegneri* (Fig. 47) described by Hegner (1923) and Wenrich (1923) which occurs in the intestine of tadpoles probably belongs to this group, and, if so, is the only representative which

possesses three flagella. It lives between the food mass and the wall of the intestine or rectum of tadpoles and can be

transferred from one tadpole to another of the same or different species either by the association of infected tadpoles with clean tadpoles or by the feeding of clean tadpoles with the infected rectum from infected animals. Toad tadpoles, as well as those of *Rana pipiens* and *R. clamitans*, are susceptible to infection.

Asexual reproduction in the EUGLENOIDINA takes place by means of longitudinal fission. Resistant stages or cysts are known in a number of species. Sexual phenomena have been described in the saprophytic form *Copromonas subtilis*¹ by Dobell (1908). Conjugation begins by the union, at their flagellar ends, of two apparently ordinary "vegetative" individuals. One of the forms loses its



¹ A number of authors have tried to identify this organism with Stein's *Scytonomas pusilla*. For a discussion of this see Dobell and O'Connor (1921), p. 180. After examining Stein's original description we agree with Dobell that it is impossible to identify *C. subtilis* with Stein's organism and therefore we retain Dobell's name.

Fig. 46.—*Jenningsia diatomophaga*, a Diatom-eating member of the EUGLENOIDINA. *c.v.*, contractile vacuole; *d*, ingested diatoms; *e*, excretion bodies; *m*, mouth; *n*, nucleus; *p*, pharynx (or oesophagus); *r*, pharyngeal or oesophageal rods; *v*, vacuole. \times about 438. (After Schaeffer.)

flagellum, and fusion between the two individuals gradually extends posteriorly. During this process, the nucleus of each individual undergoes two reducing divisions. Only one product of each division is preserved, the result being that there are only two nuclei with the reduced amount of chromatin—one from each individual. These two fuse and the organisms then form a zygote with a single flagellum which may do one of two things: (1) it may become an ordinary "vegetative" individual and proceed to reproduce by longitudinal fission or (2) it may encyst. In the latter case the cyst eventually hatches to liberate a single vegetative form. As in many other EUGLENOIDINA, encystment probably also takes place without any preliminary sexual behavior (Fig. 48).

8. PHYTOMONADINA

While the PHYTOMONADINA should be considered as a group of the MASTIGOPHORA, their affinities are undoubtedly closer to the algae than to the PROTOZOA proper. The body is covered with a rigid cellulose membrane (with the exception of one family). Neither amoeboid nor metabolic movement is present. Bi-flagellate forms are typical (four to eight flagella occur), the flagella being inserted through a pore in the cellulose wall. By far the great majority of the species are exclusively holophytic and possess a large cup-shaped green chromatophore. A few colorless forms which live saprophytically are referred to this order, but in no case do any of the forms ingest solid food. A red stigma or eye spot is

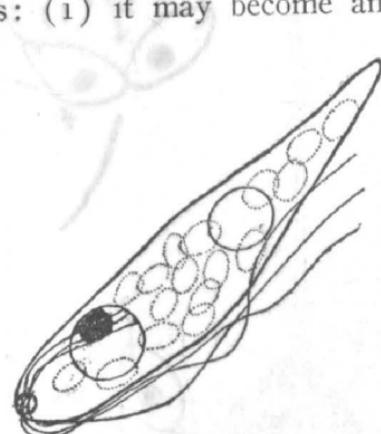


Fig. 47.—*Euglenamorpha hegeri*. An entozoic euglenoid with three flagella living in the intestine and rectum of tadpoles. From a living specimen. $\times 1600$. (After Hegner.)

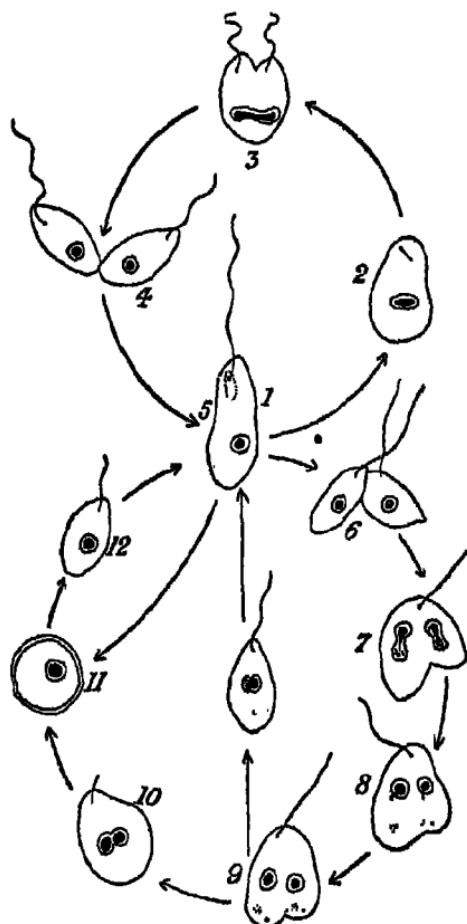


Fig. 48.—Diagrammatic representation of the life-cycle of *Capromonas subtilis*.

1, ordinary "vegetative" individual; 1-5, asexual reproduction; 5-12, syngamy. (From Kerr after Dobell.)

also present (Fig. 49). Asexual reproduction takes place within the cellulose envelope by a series of longitudinal divisions, which give rise to varying numbers of small flagellated swarmers. These eventually burst the old cellulose envelope, swim away and finally grow into adult forms. Sexual phenomena are definitely known for a large

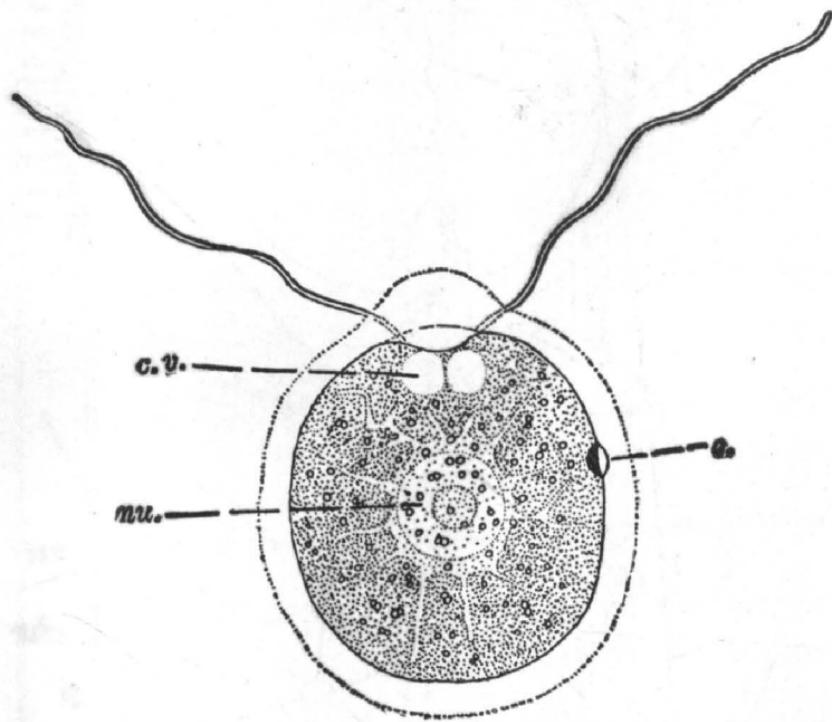


Fig. 49.—*Chlamydomonas reticulata*, a representative of the PHYTOMONADINA. *e*, eye-spot or stigma; *nu*, nucleus; *c.v.*, contractile vacuole. $\times 1000$. (After Dahlgren and Kepner.)

number of species. Gametes much smaller than the asexual swarmers are produced by a series of divisions within the cellulose envelope. Conjugation may be either isogamous or anisogamous. Colony formation is quite frequent in the order, and, in the case of the genus *Volvox* (Fig. 50), it is carried to the highest state of perfection found in living organisms.

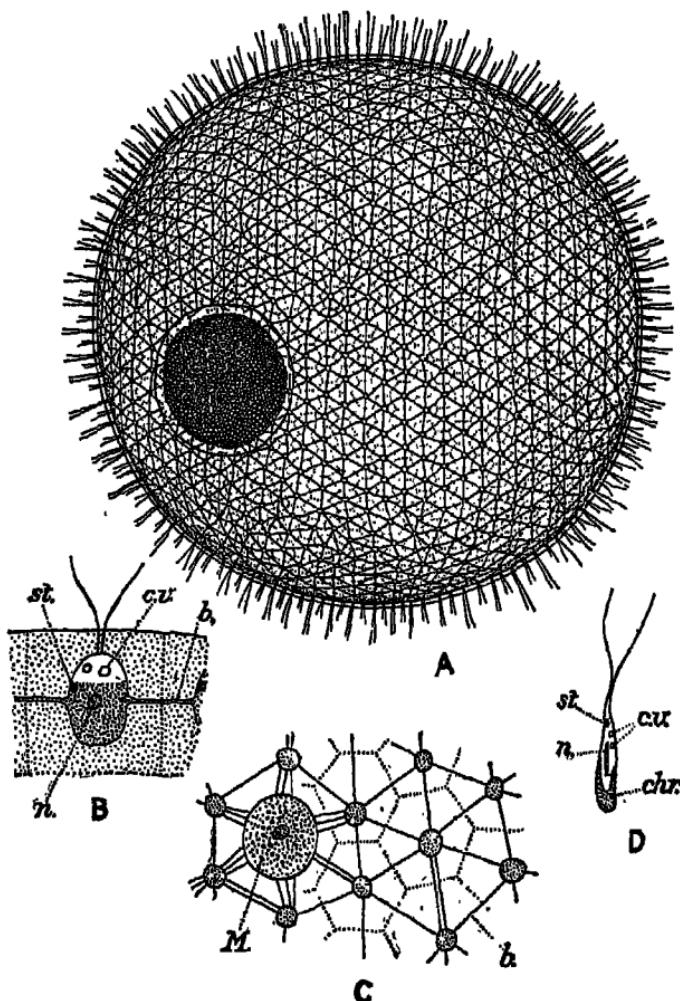


Fig. 50.—*Volvox* sp., a colonial representative of the PHYTOMONADINA. A, entire colony containing a single daughter colony. B, a single individual of the colony highly magnified. C, a surface view of a part of the colony highly magnified. D, a microgamete also highly magnified. *b.*, protoplasmic bridge; *c.v.*, contractile vacuole; *chr.*, chromatophore; *M*, macrogamete; *n.*, nucleus; *st.*, stigma. (After Kerr.)

9. DINOFLAGELLATA¹

As a group the DINOFLAGELLATA are sharply marked off from the other orders of the MASTIGOPHORA. Their most characteristic features are their shell and flagella apparatus. The shell or lorica is a rigid structure, sometimes very bizarre in form, composed of cellulose, or an allied substance, which, with the exception of a few forms, has two grooves, a longitudinal groove or sulcus, and a circular groove or girdle. Two flagella are present which issue from pores in the lorica and lie within these grooves, the longitudinal flagellum lying in the longitudinal groove and the transverse flagellum in the girdle (Fig. 51). The majority of forms are holophytic and possess chromatophores which may be brown, pale green or yellow. A few forms, however, have taken on a holozoic method of nutrition and may even ingest their food by pseudopodia. Frequently a stigma is present. Asexual reproduction takes place by fission with the line of division in the transverse plane. This is really a longitudinal fission of the animal as it is twisted in the shell. Each of the daughter forms receives one-half of the original shell and regenerates the other. In some species, the daughter individuals do not completely separate at the time of division and thus form long chains. Cyst formation has been reported. Reproduction by multiple fission with the formation of swarm spores has been observed in some species, but very little is known of sexual phenomena in this group.

¹ The recent work of Kofoid (1920) indicates that the peculiar pelagic flagellate, *Noctiluca*, should be included in the order DINOFLAGELLATA. Haeckel (1873) created the order CYSTOFAGELLATA to receive this form, and later the genera *Leptodiscus*, *Craspedotella* and *Radiosoum* were added. According to Kofoid the last genus is undoubtedly a skeletonless radiolarian and the others are so little known that their final affinities had best await further investigation. In view of this work we do not recognize the CYSTOFAGELLATA as an order.

Certain members of one group of the dinoflagellates (the BLASTODINIDÆ) lead a parasitic existence, some of them being ectoparasites and others endoparasites. Pouchet (1885) first described one of them, and later Chatton (1906)

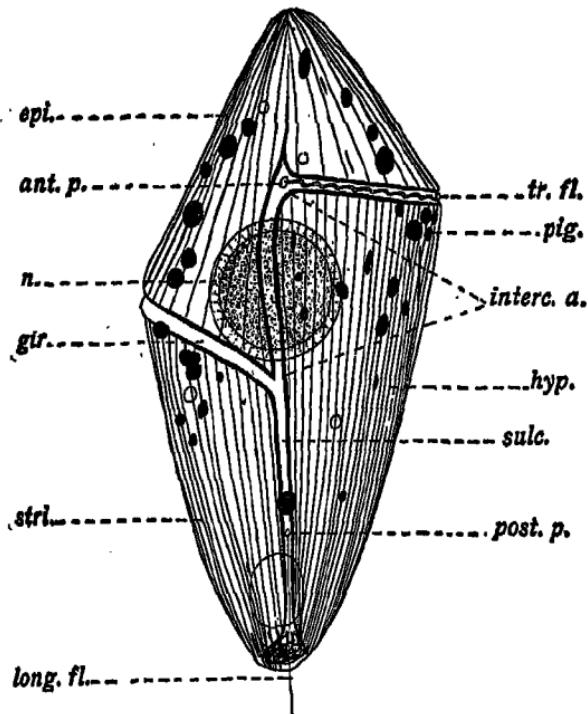


Fig. 51.—*Gyrodinium corallinum*, a dinoflagellate. Ant. p., anterior pore; epi., epicone; gir., girdle; hyp., hypocone; interc. a., intercingular area; long. fl., longitudinal flagellum; n., nucleus; pig., pigment; post. p., posterior pore; pus., pusule; sulc., sulcus; tr. fl., transverse flagellum. $\times 500$. (From Kofoid after Kofoid and Swezy.)

to 1910) and Dogiel (1906) investigated them more extensively, although even yet our knowledge of them is still very fragmentary. *Gymnodinium roscum* is an ectoparasite on the eggs of certain crustacea (Fig. 52). The small ameba-like parasite attaches itself to the outside of the egg and

feeds on it until practically the whole egg is used up. Then sporoblasts are formed which give rise to the infective flagellate stage (Dogiel, 1906; Chatton, 1910). *Oodinium parasiticum* is endoparasitic in the eggs of copepods during part of its life-cycle, the final stage being a small flagellate with the typical flagella and girdle of the DINOFLAGELLATA.

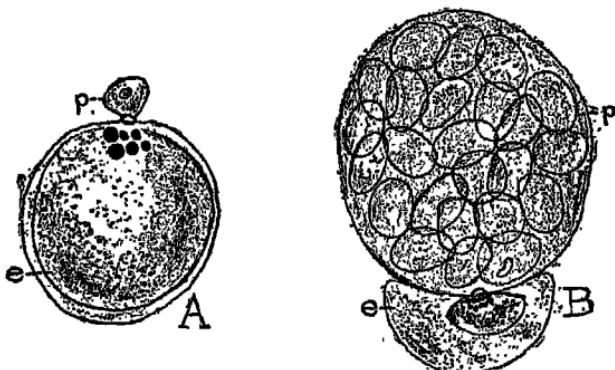


Fig. 52.—*Gymnodinium roseum*, a parasitic dinoflagellate.
A, the parasite, p, attached to the egg, e, of a copepod. B, the parasite has grown at the expense of the egg and undergone reproduction. (After Dogiel.)

C. General Physiology

I. NUTRITION AND ASSIMILATION

The MASTIGOPHORA may be either holophytic, holozoic or saprophytic in their mode of nutrition, or almost any conceivable combination of the three. We have already pointed out that many of the forms included in the PHYTOMASTIGINA possess definite chromatophores which vary greatly in shape but which are more or less characteristic for any given species. These enable the organism to manufacture starch-like substances photosynthetically, just as do higher plants, such as amyllum, or starch, and paramylum, which is allied to starch but which does not give the characteristic blue color which starch does when treated with iodine. Green pigment

predominates in these chromatophores and is apparently in every way identical with the chlorophyll of higher plants, but variously tinted pigments—yellow, brown, blue, green, etc.—occur. The exclusively holophytic forms (notably some of the PHYTOMONADINA) require only the presence of sunlight, certain dissolved minerals, and CO₂ to build up and synthesize their complex food materials. Many of the other chromatophore-bearing species, however, while partially, and in many cases chiefly holophytic, at the same time live and reproduce much more readily in a medium containing some organic matter. Consequently they must, to a certain extent, be saprophytic. Certain forms, such as *Euglena deses*, are generally found and grow best in media heavily laden with organic matter. Thornton and Smith (1914) found that a species of *Euglena* could not be grown in a purely inorganic medium, but would grow if small traces of the amino-acid, tyrosine, were added. The amount of tyrosine necessary was so minute, however, that it probably did not serve as a true food. Many of the pigmented forms apparently tend to take on a saprophytic method of nutrition when opportunity offers. Khawkins (1885), Zumstein (1900), and Ternetz (1912), by growing *Euglena* in an organic medium without sunlight, obtained forms without chromatophores which were apparently entirely saprophytic. The latter observer also obtained *Euglena gracilis* in this manner which persistently lacked its chromatophores and did not regain them even when it was returned to the sunlight.

With the exception of the PHYTOMONADINA, the ingestion of solid food, or holozoic nutrition, is very common in the different orders of the MASTIGOPHORA. It is, of course, the characteristic mode of nutrition in the PANTASTOMINA. As we have already pointed out, many of the RHIZOMASTIGINA are structurally little more than amoebæ supplied with flagella, and their method of food capture is very similar to that observed in small amoebæ (Fig. 53, D). Even the

weakly amoeboid HOLOMASTIGINA ingest food by means of short pseudopodia (Fig. 39). Many of the PROTOMONADINA with no structurally defined mouth, take in food at the base of the flagellum, cf. *Oikomonas* (Fig. 40, A). In the higher flagellates with well-developed mouth-openings, particularly in the holozoic EUGLENOIDINA, the food is actually swallowed. *Jenningsia diatomaphaga*, a member of this order described by Schaeffer (1918), feeds exclusively on diatoms (Fig. 46). "The organism moves along with the

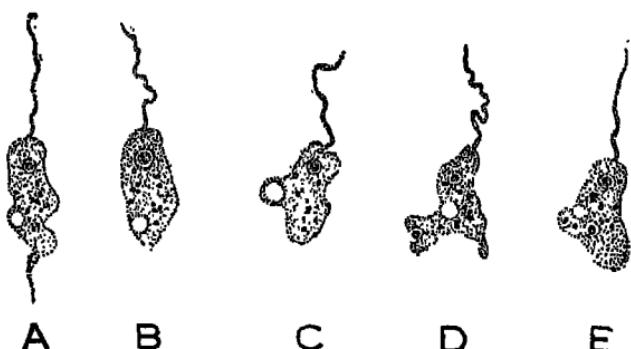


Fig. 53.—*Mastigamoeba invertens*.

A-E, different drawings of the same organism showing amoeboid changes in shape; at D, the organism is shown ingesting a bacterium. $\times 1000$. (Observations and drawings by Dr. W. A. Kepner.)

tip of the flagellum moving about until it comes into contact with a diatom. If hungry, the flagellum is brought into contact with the diatom as much as possible while the animal continues with its forward movement. When the anterior end of the organism comes nearly into contact with the diatom, the posterior end rears up and violent metabolic movements set in. The anterior end is brought over the diatom and is seen to spread out. The basal part of the flagellum is also seen to move about as if it had a part in the actual swallowing of the food particle. Presently the diatom is seen inside the flagellate, which moves away within

a few seconds, the whole process of feeding taking place within about 20 seconds." (Schaeffer, 1918.) Holozoic nutrition also occurs in many of the chromatophore-bearing species, which are largely holophytic. *Cyrtophora* (CHRYSOMONADINA), a sedentary form and to some extent holophytic, attaches bacteria to its tentacle-like pseudopodia, and carries them into its body by its streaming protoplasm, very similar to the method employed in some of the HELIOZOA. The ingestion of solid particles is, however, more often associated with saprophytic than with holophytic nutrition. In fact, many authors (e.g., Pascher, 1914, p. 12) feel that it is very improbable that exclusively holozoic nutrition ever occurs in flagellates, but that all such forms absorb some of their food through the surface of the body and are to that extent saprophytic.

Exclusively saprophytic nutrition occurs in a number of species. It is particularly prevalent in blood parasites, such as the trypanosomes. The intestinal flagellates may be either entirely saprophytic, like *Giardia*, or they may feed upon bacteria, as is the case in *Chilomastix* and *Trichomonas*.

The order EUGLENOIDINA is fundamentally plant-like with holophytic nutrition, but some of the forms have a decided tendency to take on a saprophytic or holozoic method of nutrition. According to Schaeffer (1918) the following series may be recognized: (1) *Euglena*æ, which possess chlorophyll and are truly holophytic; (2) *Astasia*æ, which are saprophytic and ingest decomposing nitrogenous material which is fluid in nature; (3) some *Peranema*æ, which are also saprophytic but ingest solids; (4) other *Peranema*æ, which are holozoic and ingest small masses of bacteria or pieces of animal and vegetal tissues, or living protophyta; and (5) *Jenningsia*, which is not only holozoic but is predacious in that it captures and ingests moving diatoms. Correlated with the development of the holozoic method and the necessity of swallowing larger and larger objects, the cesophagus

is more and more highly developed. According to Schaeffer (1918), in *Euglena*, it is very little more than a tube-like depression which insures the efficient drainage of the contractile vacuole and takes in only small particles. In *Astasia* it is larger and better adapted to take in liquid food. In *Peranema*, not only is it larger, but it forms a kind of suction-apparatus which can open and close due to supplemental rods which act as skeletal structures. Finally, its highest development is found in *Jenningsia*, where large objects are swallowed (see Figs. 45 and 46).

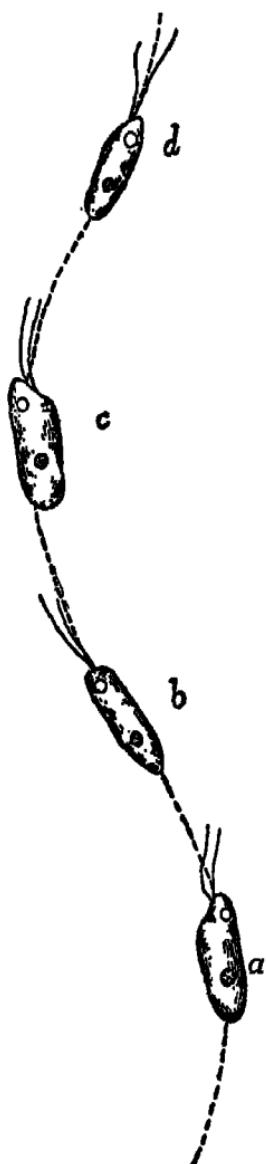
2. SECRETION

The secretion of enzymes and the formation of digestive ferments has already been noted in the SARCODINA. The holozoic flagellates secrete similar enzymes, and, in addition, various kinds of tests, pigments, etc. Of particular interest is the question of toxin production in the parasitic flagellates. Some investigators believe that they have isolated specific poisons from the trypanosomes, which they have termed trypanotoxins. That parasites such as the haemoflagellates produce very harmful effects on the host is certain, but before the idea of their producing specific toxins can be accepted, it will have to be shown that anaphylactic phenomena due to the introduction of foreign proteins (either from destruction of the parasite or the cells of the host) into the blood stream do not account for the apparent poisoning. The intestinal flagellates evidently do not secrete any such harmful substances.

3. TRANSFORMATION OF ENERGY, MOVEMENT, FUNCTIONS OF FLAGELLA

By far the commonest mode of locomotion in the MASTIGOPHORA is by means of flagella. The probable phylogenetic relationship between axopodia, on the one hand,

with their central delicate axial rod, their external layer of protoplasm, and their capability of slow bending movements,



and true flagella, on the other hand, has been pointed out. Fundamentally the structure of a flagellum is very much like that of an axopodium. In fact, Goldschmidt (1907), in one of the *RHIZOMASTIGINA*, has observed the shortening of the flagellum and its final transformation into a pseudopodium which swings back and forth. The flagellum is more or less cylindrical or band-shaped, possesses a central rod-like elastic core and a much more fluid protoplasmic sheath. The elastic core rises from the blepharoplast and seems to be largely a supporting and form-determining structure, while the protoplasmic sheath is probably the contractile element. Movement of the flagellum can be explained as due to the tendency of the external sheath to contract, counterbalanced by the tendency of the elastic core to regain its original shape.

By far the commonest type of flagellum is the tractellum, i. e., a flagellum which drags rather than propels the organism. Sometimes the entire length of the flagellum is thrown into motion and the organism may

Fig. 54.—*Chilomonas paramecium*, showing spiral path of the organism. *a*, *b*, *c*, *d*, successive positions occupied. (After Jennings.)

progress very rapidly, as in the haemoflagellates and their allies. In some of the free-living species, only about a third of the distal end shows any vibratile movements, which often cause the organism to progress in a series of jumps. In other free-living species, like *Peranema*, almost the entire flagellum is held stiff and only a very small part of the distal end shows any movement. An analysis of the path that almost all of the free-living protozoa take reveals the fact that, as they swim through the water, they rotate on their longitudinal axis, at the same time constantly swerving toward a definite side, which results in two things: (1) a spiral course through the water, and (2) a given side of the body always on the outside of the spiral. (See Fig. 54 and Jennings, 1906.) In this way an asymmetrical organism can proceed in straight lines (i. e., the axis of the spiral), whereas it would otherwise swim in circles. Schaeffer (1920) believes that this tendency to pursue a spiral course is inherent in all living organisms — up to and including man.

Many of the RHIZOMASTIGINA, although they possess flagella, move by means of pseudopodia, and some of the highly developed EUGLENOIDINA, such as *Euglena*,

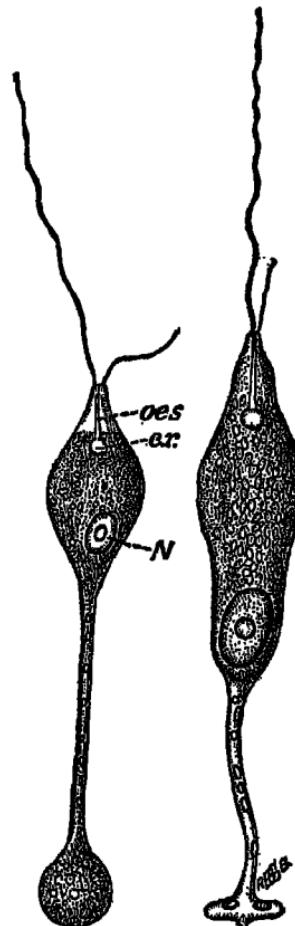


Fig. 55.—*Astasia tenax*, showing the changes in form due to metabolic movement. *oes*, oesophagus; *c.v.*, reservoir of the contractile vacuole; *N*, nucleus. (From Minchin after Stein.)

periodically lose their flagella and move about by crawling along the substratum with a kind of rocking movement. The exact mechanism of this crawling is unknown. According to Schaeffer (1920), it is associated with a motile extracellular protoplasmic film, the presence of which can be demonstrated by observing the movement of particles which become attached to it.

The forms with semi-resistant cuticles, especially the EUGLENOIDINA, often exhibit peristaltic waves of contraction which run down the length of the body. Such movements are termed metabolic (Fig. 55), or euglenoid, as they are especially common in *Euglena*.

In addition to their locomotor capacity, flagella often function in other ways. In sedentary forms, particularly, they serve to set up currents which carry the food to the organism. In many swimming forms, they are used as more or less temporary anchors, and they may even serve as tactile organs (see *Peranema*).

4. REACTIONS TO STIMULI

In the SARCODINA it was seen that, while conduction must be assumed in some of the complex reactions, a large part of the behavior can be explained on the assumption that the stimulus acts directly on the effector. With the exception of the RHIZOMASTIGINA, which closely resemble the amoebæ in their physiology, the flagellates have progressed markedly in the development of a reflex type of behavior (receptor-conductor-effector), due largely to the assumption of definite organs of locomotion and a more or less permanent body form. The development of definite reflexes is well brought out in the following examples.

Reactions to contact stimuli are illustrated in the behavior of *Peranema* as described by Mast (1912). This form either swims or crawls about the substratum; in both cases it is drawn along by a tractellum-like flagellum, only the distal tip

of which exhibits vibratile movements, assisted in crawling, at times, by metabolic contractions of the body. If a crawling specimen touches a solid object with the tip of its flagellum, the following reaction usually follows: "The organism stops, the body bends sharply always toward the larger lip, throwing the anterior end with the flagellum, usually more or less curved and inactive, to one side through an angle of nearly 180 degrees, Figure 1 (our Fig. 56). Then the body gradually straightens again, the anterior end turning back somewhat toward its former position, the flagellum straightens, the tip becomes active and the creature proceeds on its new course, having changed its direction of locomotion approximately 90 degrees." (Mast, 1912, pp. 93-94.) This reaction apparently follows whenever the organism is stimulated, for example, by a glass rod, by certain chemical substances, etc. Two things are to be noted: (1) The organism always turns "towards the larger lip"—it does not simply bend in any direction—or, in other words, the response is a definite motor reaction. (2) This one stereotyped reaction is given for a large number of stimuli, and, as will be shown later in a consideration of the reactions of *Paramecium*, often accounts for both the positive and negative reactions of the organism. The most plausible explanation is that the tip of the flagellum acts as a sense organ along which the stimulus is conducted to a definite part of the body where the bending takes place. The flagellum may, however, simply transmit the stimulus in a mechanical manner, just as a glass rod would do.

In many other cases, however, the receptor-conductor-effector type of system is necessary to explain the behavior. Take, for example, the reactions of *Euglena* to light, the following account of which is taken from Mast (1911). The details of the orientation to light of a crawling specimen, positive to light, are shown in Figure 57. At first the direction of the light rays are indicated by the arrows *oo*.

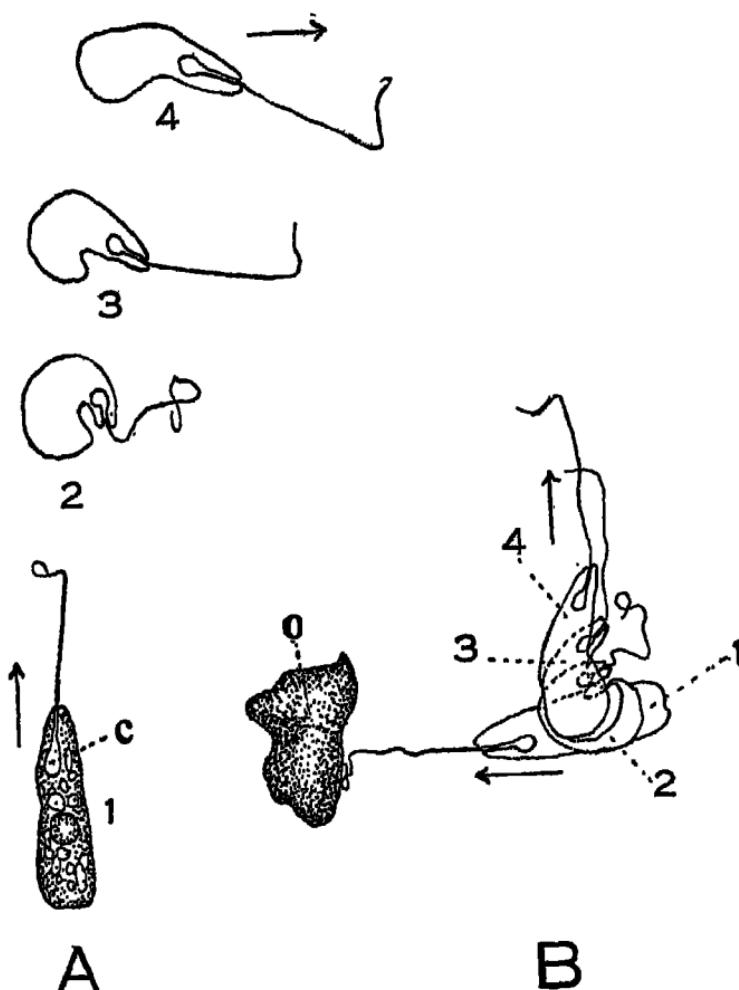


Fig. 56.—*Peranema sp.*, camera lucida outlines representing the reaction to contact stimulation. A, outlines of four individuals which were suddenly killed in different stages of the response. B, the same outlines superimposed to represent the relative positions of a single specimen in different stages of the response. C, reservoir of contractile vacuole; o, solid object; 1, specimen crawling in direction of the arrow, only the bent tip of the flagellum is active; 2, position taken immediately after stimulation, the flagellum is inactive, the turning of the anterior end being due to contraction on one side of the body; 3 and 4, later positions assumed, the body gradually straightens and the tip of the flagellum again becomes active. The response results in a change of approximately ninety degrees in the direction of motion. $\times 530$. (After Mast.)

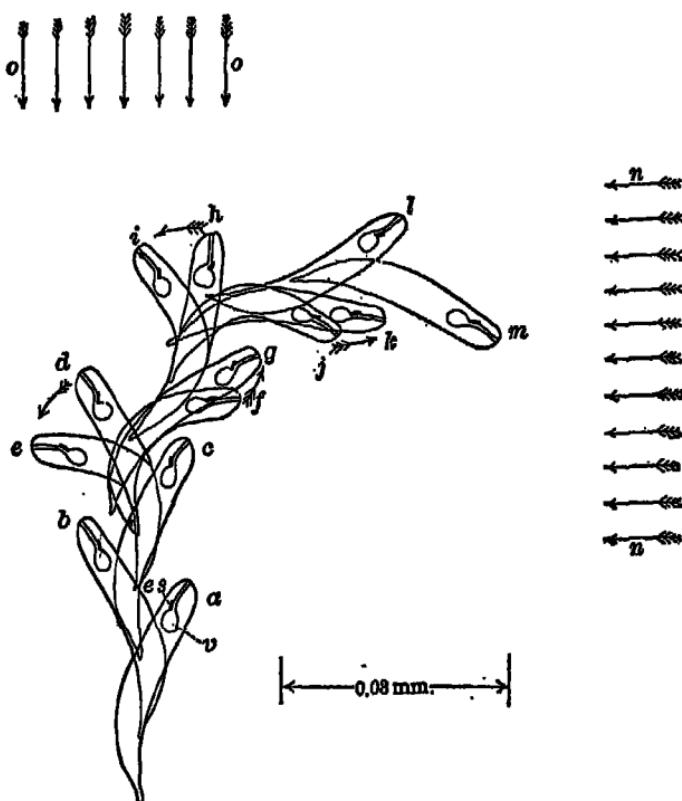


Fig. 57.—*Euglena* sp. in crawling state, showing details in process of orientation; *v*, reservoir of contractile vacuole; *es*, eyespot; *n*, *o*, direction of light; *a-c*, positions of *Euglena* with light from *n* intercepted; *c-m*, positions after light from *n* is turned on and that from *o* cut off so as to change the direction of the rays. If the ray direction is changed when the *Euglena* is in position *c* there is no reaction until it reaches *d*. Then it suddenly reacts by bending away from the source of light to *e*, after which it continues to rotate and reaches position *f*, where it gradually straightens to *g*, and rotates to *h*, when the eye-spot again faces the light and the organism is again stimulated and bends to *i*, from which it proceeds to *j*, etc., to *m*, where it is practically oriented. If the ray direction is changed when the *Euglena* is at *d*, it responds at once and orients as described above. If the intensity from *n* is lower than that from *o* the organism may respond at once when the ray direction is changed no matter in which position it is. (Figure and legend after Mast.)

As the specimen crawls toward the light-source it assumes the positions represented by *a*, *b*, and *c*. If, now, when the specimen reaches *c*, light *oo* is turned off and light *nn* turned on, the specimen shows no reaction until it reaches position *d*, where the "dorsal surface is toward the source of light." When this occurs it bends sharply away from the light, as indicated in *e*. If the light *nn* is not turned on until the specimen reaches *d*, the reaction at *e* follows immediately. This bend in the body is retained until the organism rotates to position *f*. Now, however, it gradually begins to straighten at *g* while it is rotating. This results in the position *h*. At *h* the dorsal side is again exposed to the light and the same type of bending takes place which we described at *d*. The rotating and straightening process is again repeated so that eventually the course of the specimen is directly toward the source of light (*j*, *k*, *l*, and *m*). When the specimens are negative to light the bending reflex takes place in the opposite direction and occurs only when the "ventral" side is illuminated. The mechanism of orientation is the same in both swimming and crawling specimens. The following points are to be noted in these reactions: In the first place, stimulation follows only when a definite side of the organism and only a fairly localized portion of this side (according to further investigations of Mast) is illuminated. We have here, then, a more or less specialized receptor system. In the second place, bending always takes place at the same point on the body. And, in the third place, the receptor and effector are different parts of the body, so that we must assume a conduction of the stimulus. Definite reflexes of this nature are impossible in ameboid organisms. Furthermore, they bespeak a complexity of internal organization which is seldom ascribed to the protozoa.

The question of the sense organelle in the reactions of flagellates to light has always held the attention of students of behavior. Many investigators believe that the stigma is

in some way related to photic stimulation. For a description of its structure see Dahlgren and Kepner (1908) and especially Mast (1916). Mast (1923) has made a very careful study of the structure and function of the eye-spots in *Volvox* and has shown that they function as direction eyes in the orientation of the colonies to light.

5. SYMBIOSIS AND THE RELATIONSHIP BETWEEN TERMITES AND THEIR INTESTINAL FLAGELLATES

The term symbiosis is applied to an association between a host and its entozoa when the association is of benefit to both parties. It is, therefore, distinct from parasitism where the entozoa live at the expense of their host and harm the host more or less, or from commensalism where the entozoa benefit from the association but neither appreciably help nor hinder the host. By far the majority of host-entozoon relationships fall into the last two categories. Thus, for example, *Endamæba histolytica* is a true parasite, whereas all of the other intestinal amœbæ living in man are harmless commensals. While comparatively few cases of true symbiosis are known, some investigators have suspected such a relationship to exist between termites and their intestinal flagellates. Recently, Cleveland (1924) has been able to establish this relationship between the termite *Reticulitermes flavipes* and some of its intestinal flagellates, *Trichonympha* and *Pyrsonympha*. The termite utilizes wood as a food solely by virtue of the cellulose-digesting powers of the protozoa, and the protozoa, in turn, receive from the termite their supply of food and lodging. For a detailed discussion of this very ingenious and careful series of experiments, the reader is referred to Cleveland (1924). (Also, see Cleveland, 1923, for a systematic survey of termites and their protozoa.)

CHAPTER V¹

THE HÆMOFLAGELLATES AND ALLIED FORMS

A. Introduction

The hæmoflagellates and their allies are among the simpler PROTOMONADINA. With the exception of *Cryptobia*, which is not very closely related to the other members of the group, they possess a single flagellum. Their closest relatives among the free-living forms are probably the *Cercomonas* group of flagellates. They all possess a rather simple parabasal body and flagellar apparatus. These and other differentiating features can be understood better after some of the members of the group have been described. The typical habitat is in the blood of a vertebrate or the gut of an invertebrate. The life-history of many species includes an alternation between these two habitats. From a medical standpoint they are of enormous importance, because to this group belong the parasites of the two types of human sleeping sickness, Chagas' disease, and various human leishmanioses. They are also of enormous economic importance because of the losses due to various trypanosomiases of domestic animals.

B. Chief Structural Characteristics of the Various Genera of Hæmoflagellates

I. TRYPANOSOMA.

This genus was created by Gruby (1843) for a parasite in the blood of the frog, now known as *T. rotatorium*. Most

¹By W. H. Taliaferro.

of the members of the genus are characterized by the possession of a more or less spindle-shaped body, a central nucleus, and a spherical or rod-shaped parabasal body,¹ with which is closely associated a small blepharoplast and a flagellum which arises from the blepharoplast and runs anteriorly. The protoplasm of the body unites with the flagellum to form the undulating membrane. The general arrangement of these organelles can be seen by an examination of Figure 58, A. It is to be noted that in routine work where air-dried specimens are used, it is generally impossible to distinguish the blepharoplast—the parabasal body being the only structure visible. A large number of species of this genus occur in the blood of vertebrates and in the gut of invertebrates.

2. CRITHIDIA

The genus *Crithidia* was originated by Leger (1902) for *C. fasciculata* which occurs in the digestive tract of *Anopheles maculipennis*. The structure of the members of this genus is almost identical with that of a trypanosome except that the entire parabasal apparatus has moved anteriorly, as is shown in Figure 58, B. A large number of *Crithidia* are found as gut entozoa in insects. Of these, particularly when they occur in blood-sucking insects, by far the majority are

¹ There is considerable difference of opinion as to the correct name for the organelle which we have designated "parabasal body." The name was originated by Janicki (1911) for the organelle of that name in intestinal flagellates and was first applied to the hæmo-flagellates by Kofoid (1916). In most English works this body is called a kinetonucleus, although recent English workers have used the term kinetoplast which was introduced by Alexeieff (1917). Most German authors follow Schaudinn's terminology and designate it a blepharoplast—in which case they call what we believe to be the true blepharoplast an "end bead" or ignore it altogether. Other workers, particularly the French, follow Laveran's suggestion and call it a centrosome.

simply stages in the life-cycle of vertebrate trypanosomes. Since some, however, occur in non-biting species and are crithidial in structure throughout the greater part of their life-cycle, it is necessary to retain the genus *Crithidia*.

3. HERPETOMONAS

This genus was originated by Kent (1880) for *H. muscae-domesticae* of the house-fly, *Musca domestica*. In the same

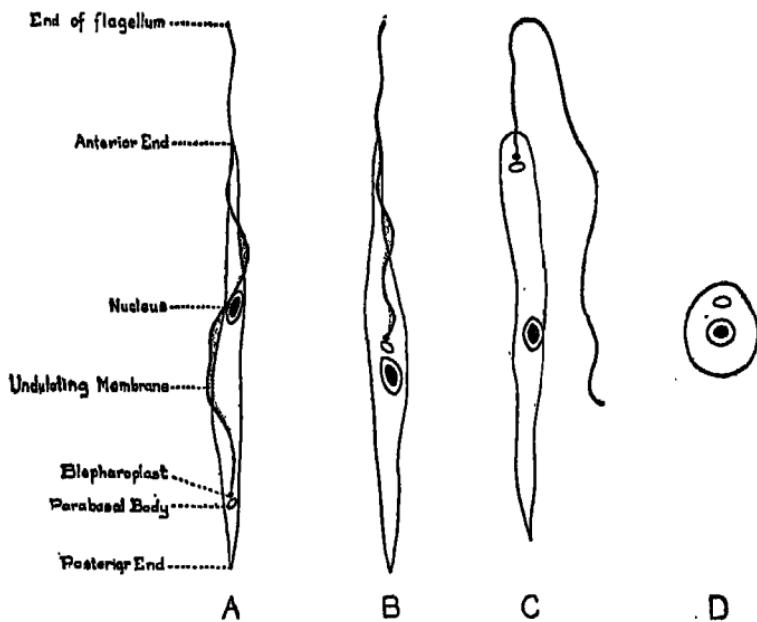


Fig. 58.—Comparative structure of the genera: A, *Trypanosoma* (*T. lewisi*) ; B, *Crithidia*; C, *Herpetomonas*; D, *Leishmania*. (Original.)

work Kent originated another genus, *Leptomonas*, for *L. bütschlii* which he found in a fresh-water nematode *Crilobus gracilis*. While the second species has never been studied since the work of Kent, apparently there is no structural difference between *Herpetomonas* and *Leptomonas*. We accept the name *Herpetomonas* because the first reviser of the

genera, Bütschli (1884), united them and retained the name *Herpetomonas*. This selection was very advisable as the type species, *H. muscae-domesticæ*, is easily obtainable whereas the type species of *Leptomonas* is not. In morphology the changes which are noted between the genus *Trypanosoma* and *Criithidia* have been carried still further in *Herpetomonas* so that the parabasal apparatus is at the anterior end and the undulating membrane has been lost (Fig. 58, C). The members of this genus occur as gut entozoa of many invertebrates. Fantham and Porter (1920) have found a species, *H. denticis*, in the blood of a fish, *Dentex argyrosoma*.

4. LEISHMANIA

Ross (1903) first gave this name to the parasite of human kala-azar. Structurally, it may be considered as having arisen from any one of the preceding genera by the rounding up of the body and the loss of the flagellar apparatus with the exception of the parabasal body which is retained (Fig. 58, D).

5. CRYPTOBIA

This genus was originated by Leidy (1846) for *C. helicis* from the seminal vesicles of several species of snails belonging to the genus *Helix*. *Cryptobia* is very similar in structure to a trypanosome. The chief difference is in the addition of a second flagellum which is free and not attached to the body by an undulating membrane (Fig. 59). The genus occurs as a blood parasite in certain fishes and in the gut of various invertebrates.

The first four genera (*Trypanosoma*, *Criithidia*, *Herpetomonas*, and *Leishmania*) form a closely intergraded series of forms and are apparently very closely related. In studying the life-cycle of various trypanosomes, for example, it is found that some of them may at times assume the structure

of a true *Crithidia*, *Herpetomonas*, or *Leishmania*. Similarly, members of the genus *Herpetomonas* may assume the structural characteristics of the three other genera, and, with the discovery of a trypaniform stage in the life-cycle of *Crithidia gerridis* by Becker (1923), the same may be said of this genus. As far as is known, members of the genus *Leish-*

mania assume only one other type of structure, viz., that of a *Herpetomonas*. The assumption of the structure of another genus by a given form may be looked upon as evidence of a kind of recapitulation. In every case a given form always belongs to a genus because it possesses the typical structure of that genus throughout the major portion of its life-cycle.

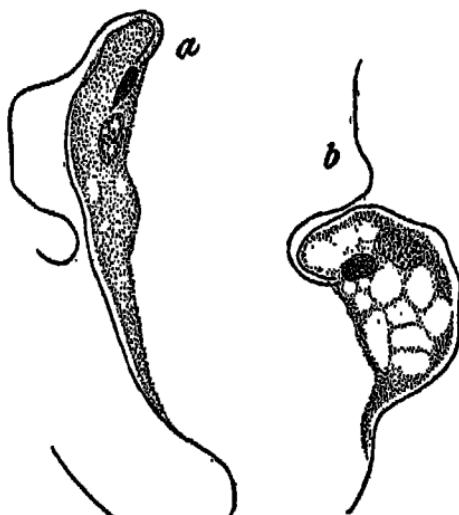


Fig. 59.—*Cryptobia helcis*.

a, typical form; *b*, short, broad form which may be slightly degenerate. $\times 2,467$. (After Crawley.)

It is to be noted that the genus *Cryptobia* does not occur in this series. This genus is ordinarily placed with the haemoflagellates because of the similarity of its structure to that of trypanosomes, but it does not exhibit the usual haemoflagellate development. In the case of *C. cyprini* from the goldfish which is transmitted by a leech, Miss Robertson (1911) could find no evidence that there was any true cycle of development in this form. It remained a *Cryptobia* in structure throughout its sojourn in the invertebrate host.

In fact, the whole development in the intermediate host is probably nothing more than rapid multiplication by asexual reproduction.

The fundamental method of reproduction in the hæmoflagellates is binary longitudinal fission. This process is initiated by the division of the blepharoplast, which, in turn, is followed by a simple constriction and division of the parabasal body and a mitotic division of the nucleus. The flagellum probably does not divide but is retained by one of the daughter blepharoplasts while a new flagellum is developed in conjunction with the other daughter blepharoplast. After the various organelles are duplicated (sometimes before the duplication is completed), division of the body begins at the anterior, i. e., the flagellar end, and proceeds posteriorly. Most, if not all, of the cases of so-called "multiple division," plasmodium and rosette formation are simply modifications of binary division in which the blepharoplasts, flagella, parabasal bodies and nuclei are consecutively duplicated without the complete division of the body. The endogenous budding described in *Critidium* (see p. 176) is peculiar in that the flagellar apparatus in the parent flagellate is discarded to be produced anew in each daughter zooid.

C. The Genus *Trypanosoma*

The genus *Trypanosoma* is one of the most widely distributed parasitic genera known. An enormous number of species are found in the blood of various vertebrates belonging to almost every class. In by far the majority of cases the trypanosomes found in the blood of wild animals apparently do not produce any pathological or clinical symptoms. Even a parasite, such as *T. gambiense* which is so pathogenic to man, seems to occur as a harmless inhabitant of the blood of certain wild game of Africa. Of course, from a practical standpoint, we are chiefly interested in those forms which

live in man and domesticated animals and which are almost universally lethal in their effect.

I. LIFE-CYCLE OF *Trypanosoma* IN GENERAL

The life-cycle of most trypanosomes involves an alternation of hosts. They occur as typical trypanosomes in the blood of some vertebrate and are transmitted to a new host by means of an invertebrate. In the case of terrestrial vertebrates, transmission is effected either by the agency of a blood-sucking insect or some species of ectoparasite. In aquatic vertebrates, the transmission generally takes place by means of leeches. There is at least one trypanosome known in which the invertebrate host has been lost. This is *T. equiperdum* which causes dourine of horses and which is transmitted by coitus. Some observers feel that coitus plays a rôle in the transmission of human trypanosomiasis, but definite proof of this is lacking. Likewise, Lanfranchi (1918) has shown that in mammals trypanosomes can pass from the mother to the offspring through the milk. If this occurs in nature, it must play a very minor rôle in trypanosome diseases. Trypanosomes are apparently unable to pass through the placenta, as hereditary transmission of this character has never been demonstrated. As a rule, the invertebrate host is also unable to transmit the infection to its offspring. A few exceptions to this rule have been found. Brumpt (1907), for example, maintains that *T. inopinatum* is transmitted hereditarily in its invertebrate host, *Helobdella algira*.

There are two methods of transmission from one vertebrate to another by an invertebrate host. The first, and by far the most common, is the indirect or cyclical method. In such cases the trypanosomes are taken into the alimentary canal of the invertebrate host, and there undergo a definite cycle of development. They are not infective to another vertebrate host until this cycle is completed. The second and

rarer is the direct or mechanical method. In such cases the intermediate host fouls its proboscis while it is feeding on an infected animal, and then infects a new host by thrusting its contaminated proboscis into a clean animal.

2. LIFE-CYCLE IN THE VERTEBRATE HOST

It is rather difficult to outline the life-history of a given trypanosome in vertebrate hosts in general because the life-history of a particular species may vary considerably when it lives in different vertebrate hosts. From a physiological standpoint this raises the general question of the adaptation of a parasite to its host. As we have already stated, by far the majority of trypanosomes found in nature do not produce any clinical or pathological symptoms in their host. Many authors look upon such infections as cases of ideal balance or adaptation between host and parasite. According to this view those forms which produce disease are in a state of ill-adjusted balance with their host. The fact that there is a growing body of evidence which indicates that the disease-producing trypanosomes occur as non-pathogenic parasites of wild animals seems to show that the trypanosomes are more pathogenic to the more recently acquired hosts. It would be interesting to trace the life-cycle of a trypanosome, which is non-pathogenic to its natural host but lethal to domestic animals in both types of host, but this, to date, has not been done. We can, however, select the life-histories of a few different species of trypanosomes for purposes of illustration.

a. *T. lewisi*

T. lewisi occurs as a non-pathogenic parasite in the blood of different species of rats all over the world. As we shall see later, it is transmitted from rat to rat by means of the rat-flea. It is one of the best examples of a trypanosome which occurs in the blood, often in enormous numbers, and

yet, which rarely produces any clinical symptoms. The typical shape of the body and its organelles can be seen in figure 58, A. During the "adult" stage of the trypanosome, it generally varies in length from about 27 to 33 microns. It has an unusually large parabasal body and quite a long tip posterior to the parabasal body.

The general course of the life-cycle has been known for some time (see Minchin, 1912, and Minchin and Thomson, 1915) and recently has been the subject of investigation by Taliaferro (1921) and (1923), and Taliaferro and Taliaferro (1922). The course of the infection in the rat can be divided as follows:

1. Multiplicative stage (8 to 10 days; rarely to 32 days).
 - a. Incubation period during which no organisms are in the blood (2 to 4 days; rarely to 8 days).
 - b. Multiplicative period in the blood (4 to 7 days; rarely to 24 days).
2. Stage of "adult" infection (7 to 100 days).

The first period in the above table, namely, the incubation period, is the time which elapses between the introduction of the trypanosomes into the rat and their first appearance in the blood. Its duration varies little whether the infection is received naturally from the invertebrate host or whether it is received by injection of infected blood intraperitoneally, intravenously, or subcutaneously. Very little is known as to just what happens during this period. One thing is certain, however, and that is that the organisms are undergoing rapid multiplication. When the trypanosomes first appear in the blood at the beginning of the multiplicative period in the blood, they are undergoing very rapid multiplication and in consequence exhibit many variations in size and form as is shown in figure 60. Sometimes during the first part of this period, division takes place before the products of preceding divisions have separated. This results in rosette formation

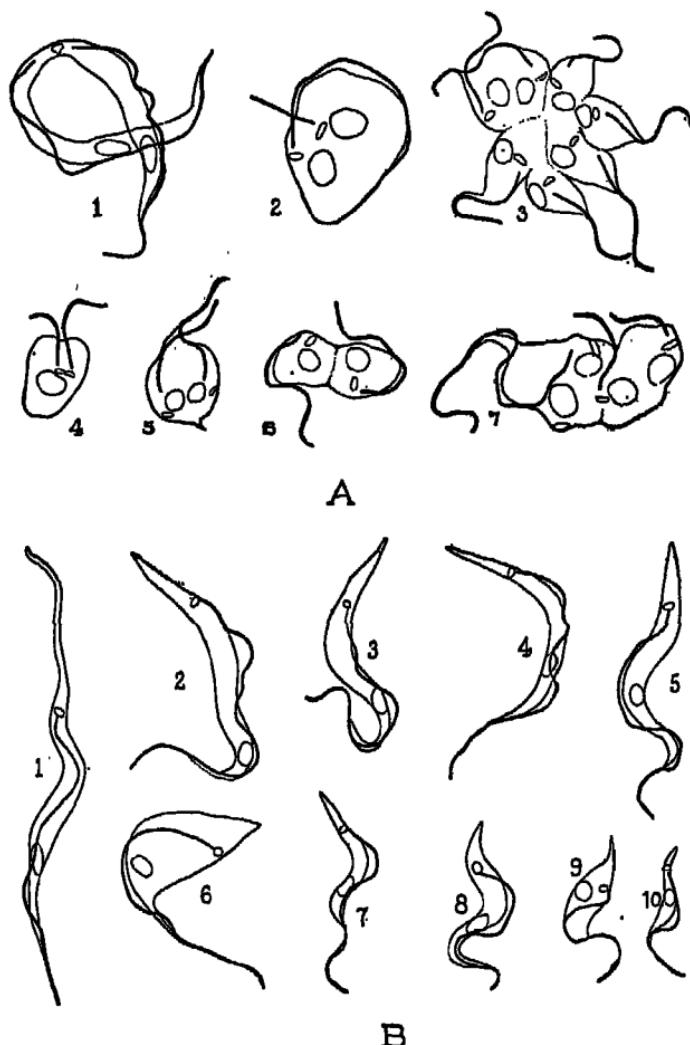


Fig. 60.—*Trypanosoma lewisi* in the multiplicative stage of the infection.

A, rosettes and division stages. B, Organisms which are not actually in the process of division but which exhibit great variability in size and shape as a result of rapid multiplication and consequent growth. $\times 1500$. (A from drawings by Miss F. A. Coventry. B after Taliaferro.)

and in plasmodial forms such as are shown in Figure 60, A. The parabasal body in these forms is often situated anteriorly so that the organisms are crithidiomorphic or even *Herpetomonas*-like in structure. As the multiplicative period progresses, the rate of multiplication becomes slower and slower, and as a result the variability of the organisms be-



Fig. 61.—*Trypanosoma lewisi* in the "adult" stage of the infection. Note the absence of any evidences of division and the uniformity in size and shape as compared with those in figure 60. $\times 1500$. (After Taliaferro.)

comes less and less. The incubation period and the multiplicative period in the blood together represent the only part of the cycle in the rat during which the organisms reproduce. All multiplication ceases by the beginning of the adult stage of the infection and the trypanosomes exist in the blood, sometimes in enormous numbers, without any evidences of division or growth. These "adult" forms are very uniform in size and shape as is shown in figure 61. The

stage of "adult" infection lasts from a few days to several months. At the end of it the trypanosomes disappear from the blood and do not reappear. The rat is then immune to another infection of *T. lewisi* and continues to be for a considerable time; in many cases, throughout its natural life.

A life-history of this nature, involving as it does the gradual retardation and final inhibition of reproduction and growth in the trypanosomes followed by their eventual destruction and the production of immunity in the rat, strongly suggests that the host builds up a very marked resistance against the invading organism. Taliaferro and Taliaferro (1922) have studied the resistance of different hosts to experimental infections including *T. lewisi* in the rat. They point out that from a logical standpoint, such resistance may do one or both of two things: a, it may retard the rate of reproduction of the parasites, or b, it may destroy them after they are formed. A fairly accurate picture of the sum total of these two effects can be obtained by noting the daily fluctuations in the number of trypanosomes per cmm. of blood. If, for example, the number of trypanosomes remains constant or decreases it is known that some type of resistance is operative, but it can not be told whether it is producing its effect by retarding the rate of reproduction or by destroying the trypanosomes after they are formed. The truth of this statement is brought out by the following equation:

Number of parasites per cmm. of blood = number produced by reproduction of parasites - number destroyed in consequence of the resistance.

Furthermore, it can be seen that if the enumerative studies are supplemented with some measure of the rate of reproduction, which is independent of the number of organisms destroyed, the two effects of the resistance noted above can be differentiated.

In their work, these authors differentiated between the

two different types of resistance by, first, making daily counts of the trypanosomes per cmm. of blood, and, second, by making daily determinations of the coefficient of variation for total length. The changes in the coefficient of variation for any factor involving size, such as total length, gives a comparative measure of the rate of reproduction. The rationale of this method is based on the difference in variability in size in the individuals of a sample taken, on the one hand, from a population undergoing rapid multiplication, and, on the other hand, from a population undergoing little or no multiplication. It is evident that the first sample necessarily consists of many young forms in different stages of growth and will exhibit a higher variability in size than a sample of the second type which consists of "adult" forms of more or less "adult" size. Furthermore, the higher the rate of reproduction with its production of young forms the higher will be the coefficient of variation for size. For a more detailed discussion of this measure and its applicability to trypanosomes, the reader is referred to the original papers. Of course, it is only a comparative measure of the rate of reproduction, but an absolute measure could not be obtained unless all the progeny were saved and this is obviously impossible if some are being destroyed in consequence of the resistance. In fact, the great value of the method is that it measures the rate of reproduction irrespective of the number killed.

A description of one experiment will give the general character of the results obtained with *T. lewisi* in the rat. The changes in the trypanosomes per cmm. of blood and the coefficient of variation for total length are given in figure 62. The incubation period lasted four days. When the organisms first appeared in the blood, they showed the unusually high coefficient of variation of 25.32%. By the tenth day it dropped to 3.95%, and remained more or less constant throughout the remainder of the infection. This indicates

that the organisms were undergoing very rapid reproduction when they first appeared in the blood and that the rate of this reproduction steadily decreased, and finally ceased by the tenth day, not to recommence during the remainder of the infection. This conclusion is fully in accord with the description we have already given and with many cytological and observational studies of other investigators. It indi-

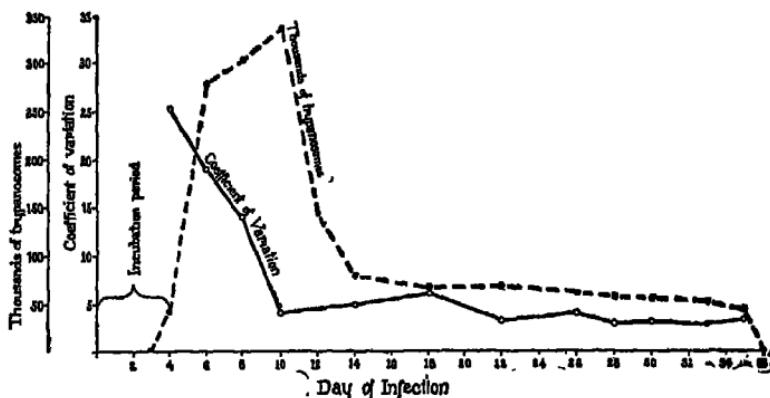


Fig. 62.—*Trypanosoma lewisi* in the rat showing the changes in the number of parasites per cmm. of blood and the coefficient of variation for total length of the parasites. The changes in the coefficient of variation give a comparative measure of the rate of reproduction irrespective of the number of organisms killed in consequence of the resistance of the host. The drop in this constant between the 4th and 10th days indicates the gradual retardation and final inhibition of reproduction. (After Taliaferro and Taliaferro.)

cates that a resistance is built up by the host which steadily retards the rate of reproduction and finally inhibits it by the tenth day. Later experimental work by Taliaferro (1923) demonstrates that this result is due to the formation of a reaction product in the serum of infected rats which inhibits reproduction of the trypanosomes but which does not kill them. Returning to figure 62 it is evident from a consideration of the changes in the number of parasites from day to day that there is a further type of resistance built up: The

number of organisms steadily increased until it reached the high point of 338,000 per cmm. on the tenth day. There was then a sudden decrease until by the fourteenth day there were only 76,000 per cmm. in the blood. This sudden disappearance from the blood can not be explained simply on the cessation of reproductive activity, because had that been the only factor involved the number would have remained constant. We must therefore conclude that a second type of resistance was built up in consequence of which the trypanosomes were actually destroyed. Very little further destructive effect is seen until between the thirty-fifth and thirty-sixth days, when all of the parasites disappear completely and finally from the blood. As yet the fundamental mechanism involved in this first partial and second complete destruction of the trypanosomes has not been experimentally ascertained. Work directed along these lines is, however, in progress in this laboratory.

b. The brucei-Group.

This group of trypanosomes includes among others *T. brucei*, the causative agent of Nagana, a disease of domesticated animals in Africa transmitted chiefly by *Glossina morsitans*; *T. gambiense*, the causative agent of the Gambian human sleeping sickness, which is transmitted by *Glossina palpalis*; and *T. rhodesiense*, the causative agent of the Rhodesian human sleeping sickness, which is transmitted by *Glossina morsitans*.

When the members of the *brucei* group are grown in different laboratory animals, they present different but characteristic pictures which vary with the species of the host rather than with the species of the trypanosome. The work of Pearce and Brown (1918) indicates that the infections, in rats and mice, pursue a continuous course, the organisms steadily increasing in number in the peripheral blood until the host dies. In guinea pigs, the infections more nearly

resemble the relapsing type, periods during which the trypanosomes steadily increase alternate with periods during which they decrease or totally disappear from the blood. In rabbits, the infections are of a very chronic type and extensively involve the tissues as well as the blood.

These different types of infection again raise the question of the resistance which a host builds up against these trypanosomes. Taliaferro and Taliaferro (1922), in a continuation of their investigations described under *T. lewisi*, found that in the continuous infections, such as *T. brucei*, *T. rhodesiense*, and *T. equiperdum* in the rat, and *T. brucei* and *T. rhodesiense* in the mouse, there is no evidence of any resistance being built up either directed toward a retardation of the rate of reproduction or toward a destruction of the parasites after they are formed. The trypanosomes after a longer or shorter incubation period appeared in the blood and reproduced unmolested until the death of the host. In the relapsing infections such as *T. rhodesiense* and *T. brucei* in the dog and guinea pig, it was found that the periodical decreases of the trypanosomes from the blood were solely a result of that type of resistance which is directed toward a destruction of the parasites after they are formed, there being no evidence of any effect on the rate of reproduction as was the case in *T. lewisi* in the rat.

Miss Robertson (1912), using entirely different methods from those of Taliaferro and Taliaferro, has brought forth considerable evidence of the occurrence of an endogenous cycle in *T. gambiense* in the monkey which is characterized by the occurrence of alternate periods of increase and decrease in the number of trypanosomes. Her work indicates that these periods of decrease are associated not only with the destruction of the parasites as in the case of the dog and guinea pig mentioned above, but also with a retardation of the rate of multiplication. *T. gambiense* in the monkey, then, probably encounters much the same type of resistance

as *T. lewisi* in the rat except that, in the latter case, when reproduction once ceases, it never starts again, whereas, in the former case, periods of reproductive activity occur in cycles. The exact condition of affairs in man is not known. Ross and Thomson (1911) have shown, however, that in the case of *T. rhodesiense*, periods of increase of parasites alternate with periods of decrease.

c. *T. cruzi*

T. cruzi presents one of the most peculiar life-histories found among the trypanosomes. In fact, the predominating form of this parasite in the mammal is structurally not a trypanosome but a tissue-inhabiting *Leishmania*. (See Chagas, 1909, 1921, Hartmann, 1910, and Vianna, 1911.)

The trypanosomes which enter the vertebrate host apparently do not divide in the peripheral blood, but enter some tissue cell, round up, lose their flagella, and become *Leishmania*-like forms. Here they multiply by repeated fission and finally produce a large number of *Leishmania*-like bodies. Sooner or later these leishmania forms are retransformed into typical trypanosomes and are liberated into the blood either to infect other tissue cells or to be taken up by the intermediate host. Chagas has described a second type of multiplication of the trypanosomes in the capillaries of the lungs. In this case the original trypanosome which rounds up is supposed to produce eight small merozoites which show a sexual dimorphism. In spite of the evidence brought forth by Chagas (see Chagas, 1921), we do not feel that there has been any adequate demonstration, either of sexual polymorphism or of sexual behavior in trypanosomes. Until further evidence is brought forward, we feel that polymorphism in trypanosomes is a growth phenomenon and not a sexual differentiation.

After the incubation period and during the first part of the course of the infection of *T. cruzi* in man, both typical

trypanosomes in the blood and leishmania forms in the tissues are found. If the patient does not die during this acute stage of the infection, the disease passes into the second or chronic stage which may last for many years until it is terminated by the death of the host. During this part of the infection, no trypanosomes are found in the peripheral blood, but the leishmania forms in the tissues continue to multiply and spread. Chagas has suggested that this may be the result of the formation of an antibody in the blood which reaches such a concentration that by the beginning of the chronic period it kills off the trypanosomes in the blood. The leishmania forms in the tissues, however, are protected and can continue to live and multiply and even probably continue to give off large numbers of trypanosomes which perish shortly after they get into the blood. *T. cruzi* can be grown, and shows its typical life-history in such laboratory animals as the dog, rat, mouse, and guinea pig.

d. Trypanosomes of Cold-blooded Vertebrates

Comparatively little is known concerning the life-history of these forms in their vertebrate hosts. *T. rotatorium* in the frog shows a polymorphism and variation in size which is much greater than is found in *T. lewisi* even at the height of the multiplicative stage. Some very interesting observations have been made on this polymorphism by Machado (1911). In working with *T. diemyctyli* (Fig. 63), Hegner (1921) found that the forms exhibited very little variation in size and his work strongly suggests that he was dealing with infections in the "adult" stage comparable to *T. lewisi* in the rat.

3. LIFE-CYCLE IN THE INVERTEBRATE HOST

a. T. lewisi

Since the classical work of Minchin and Thomson (1915) on the life-history of *T. lewisi* in the rat-flea, this form has

become the starting point for all considerations of the life-cycle of trypanosomes in the invertebrate hosts. The most common ectoparasites found on the rat are: (1) several species of rat-fleas, (2) the rat-louse, *Hæmatopinus spinulosis*, (3) a mite, *Cheops*, and (4) in the laboratory in this latitude, bedbugs. A series of investigators, Nuttall (1908), Baldrey (1909), Breinl and Hindle (1909), Manteufel (1909) and Gonder (1911), have demonstrated that rat-lice

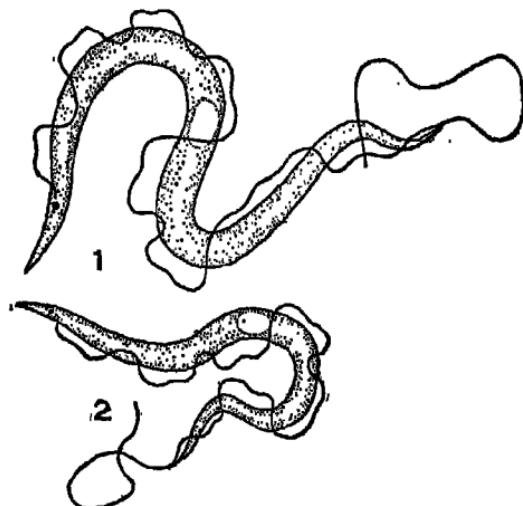


Fig. 63.—*Trypanosoma diemyctyli* from the newt. $\times 1600$. (After Hegner.)

can transmit *T. lewisi* from rat to rat. Transmission by this means, however, is very infrequent and is accomplished only with difficulty. Brumpt (1913) has infected a rat with *T. lewisi* by the intraperitoneal infection of the rectal contents of a bedbug which had previously fed on an infected animal. All of the evidence at present indicates that the natural mode of transmission is by means of the rat-flea,—the species varying with the locality. Rabinowitsch and Kempner (1899) first succeeded in effecting transmission by these

insects. They infected clean rats by the intraperitoneal injection of teased-up fleas which had previously fed on infected rats. These authors did not state the species of flea used. Nöller (1912) and Wenyon (1913) have obtained transmission with the dog-flea, *Ctenocephalus canis*, the human-flea, *Pulex irritans*, and the tropical rat-flea, *Xenopsylla cheopis*. Minchin and Thomson used only the temperate zone rat-flea, *Ceratophyllus fasciatus*.¹

In order to understand the development of *T. lewisi* in the rat-flea as described by Minchin and Thomson (1915), it is necessary to keep in mind the anatomy of the digestive tract of the flea which is shown diagrammatically in figure 64. When a flea feeds on an infected rat, the blood with its contained trypanosomes is passed directly into the stomach. Here, during the first few hours, the trypanosomes exhibit, structurally, very little, if any, differences from the forms which occur in the rat, but, physiologically, they undergo marked changes chief among them being that they are no longer infective to rats and that the type of locomotion is entirely changed, due to a certain rigidity of the posterior third of the body. Sooner or later some of these forms penetrate the epithelial cells lining the stomach of the flea and initiate what Minchin and Thomson designate as the "stomach" phase of development (Fig. 65, 4). Here each of these forms

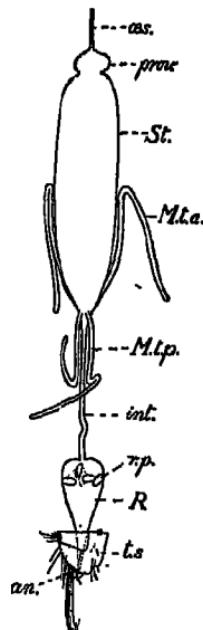


Fig. 64.—Digestive tract of the flea, *Ceratophyllus fasciatus*. *oes.*, œsophagus; *prov.*, proventriculus; *St.*, stomach; *M.t.a.* and *M.t.p.*, malpighian tubule of the anterior and posterior pair respectively; *int.*, intestine; *r.p.*, rectal papillæ; *R*, rectum; *t.s.*, terminal segments; *an.*, anus. $\times 20$. (After Minchin and Thomson.)

¹ See Minchin and Thomson (1915) for the references cited in this paragraph.

rounds up, and undergoes a multiple fission, eventually bursting the cell and liberating a number of daughter trypanosomes (Fig. 65, 5-12). A given daughter trypanosome may then do one of two things: (1) it may infect another epithelial cell and repeat the process, or (2) it may pass down the alimentary canal into the rectum and become attached to the rectal wall by means of its flagellum (Fig. 65, 12-13 and 12, 14a, 14b, 17a and 18), where it constitutes what is probably a continuous source of infection for the remainder of the life of the flea. These rectal forms become crithidial in structure,—the parabasal body migrating anterior to the nucleus, and either continue to reproduce by longitudinal fission, or become retransformed into short, stumpy trypanosomes (Fig. 65, 19 T). These latter forms pass out of the rectum with the feces and constitute the only forms which are able to infect other rats. A secondary migration of the rectal forms may take place in which case as above they reproduce by longitudinal fission, some of the resulting forms being transformed into the typical infective trypanosomes (Fig. 65, 20 T).

Only a comparatively small proportion of the trypanosomes ingested complete the development as we have outlined it. A large number degenerate and eventually perish. These give rise to what Minchin and Thomson designate as the degenerative series. It is unnecessary to take up this series because, although it is of enormous importance to investigators who are trying to differentiate between degeneration and true development, it plays no part in the actual life-history of the trypanosome.

It is well to point out some of the fundamental facts in regard to the development of *T. lewisi* in the rat-flea which have been brought out by the work of Minchin and Thomson.

i. The life-history of *T. lewisi* in the rat-flea is a true cycle of development and is not simply a multiplication of the trypanosomes. This is shown by the fact that, a short

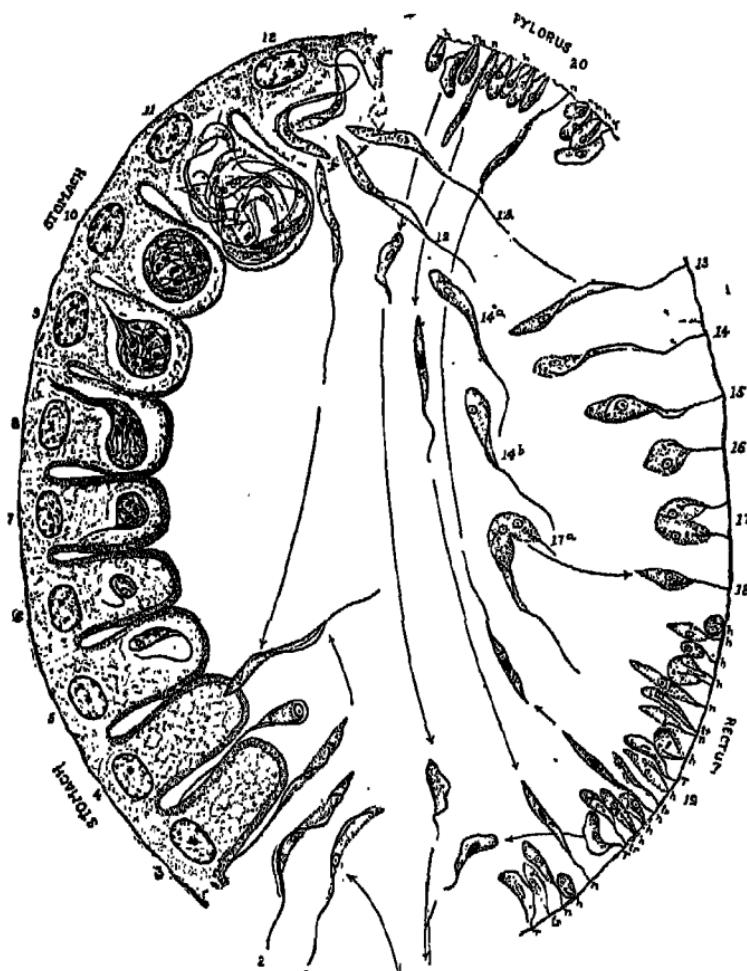


Fig. 65.—Diagrammatic representation of the life-cycle of *Trypanosoma lewisi* in the flea.

The arrows represent the migrations of the trypanosomes. 1, Trypanosome as taken up by flea from the rat; 2, the same slightly modified after a few hours in the flea's stomach; 3-12, the stomach phase of the development; 13-19, the rectal phase; 20, secondary infection of the pyloric region; h, haptomonad or attached form in which binary fission takes place; n, nectomonad or free form; tr, transitional form; T, final infective trypaniform stage. \times about 900. (After Minchin and Thomson.)

time after the trypanosomes are taken up by the flea (probably $\frac{1}{2}$ hour), they are not infective to rats until the short, stumpy infective forms appear in the rectum—or, in other words, until the cycle of development is complete. This was demonstrated by injecting clean rats intraperitoneally with teased-up fleas at different stages in the cycle.

2. The developmental cycle requires a minimum of 5 days for its completion, but, once the infection is established in a flea, the flea can probably infect rats for the remainder of its life.

3. Not a single instance of direct transmission by the fleas was obtained. Infection always took place by the indirect or cyclical method.

4. After the infective forms are developed in the rectum, they pass out with the feces. The rats become infected by ingesting these contaminated feces, either by eating fleas which contain the infective forms in their recta or by licking the moist feces from their fur. The fact that a flea almost invariably defecates during the process of feeding and that the irritation of the bite causes the rat to lick its fur, facilitates the transfer of the infection. *A flea never transmits its infection to a rat by means of its bite*, nor are the trypanosomes ever found in the salivary glands of the flea. The truth of these statements is also shown by the previous work of Nöller (1912) and Wenyon (1913).

5. During the entire development the trypanosomes are restricted to the alimentary canal. They never pass into the body cavity.

6. The authors found no evidence of a sexual phase in the development of *T. lewisi*. At the present time it may be said that there is no conclusive evidence of sexual phenomena in any of the haemoflagellates. In spite of this there is no doubt that the passage of a trypanosome through the invertebrate host exerts a profound effect upon it. Gonder (1911), for example, found that acquired arsenic-resistance

in *T. lewisi* was transmitted from rat to rat, but was lost by passage through the louse. Miss Robertson (1912 a) found that strains of *T. gambiense* showed marked changes in their characteristics after passage through the tsetse fly. This led this author to say, "It seems clear that the cycle in the fly as a whole, whether conjugation occurs or not, has much of the biological significance of the process." According to Taliaferro (1921) the variability in size in a "pure line" of *T. lewisi* increases after passage through the flea. A similar increase occurs in *Paramecium* after conjugation (see Chapter XIV) and may indicate a splitting of the "pure line" into heritably diverse lines as a result of sexual phenomena.

b. T. gambiense and T. rhodesiense

T. gambiense, the causative agent of the Gambian type of human sleeping sickness, is transmitted from man to man by the tsetse fly, *Glossina palpalis*. This fact was first demonstrated by Bruce and Nabarro in 1903, although the same conclusion had previously been reached by Sambon and Brumpt on epidemiological grounds. Our knowledge of the life-cycle of *T. gambiense* in the tsetse fly we owe mostly to Kleine and Taute, Bruce and his collaborators, and Miss Robertson (1913). The following outline of this development is taken chiefly from the last-named author.

The morphological changes which *T. gambiense* undergoes in its development in the tsetse fly are roughly similar to those which we have described for *T. lewisi* in the flea. There are two important respects, however, in which the cycle of *T. gambiense* differs from the latter, viz., (1) there is no intracellular stage during the primary multiplication of the trypanosomes in the anterior part of the alimentary canal, and (2) the crithidial stage of the development takes place in the salivary glands instead of the rectum. The invasion of the salivary glands enables the fly to transfer

the infection from man to man by the bite instead of by fecal contamination.

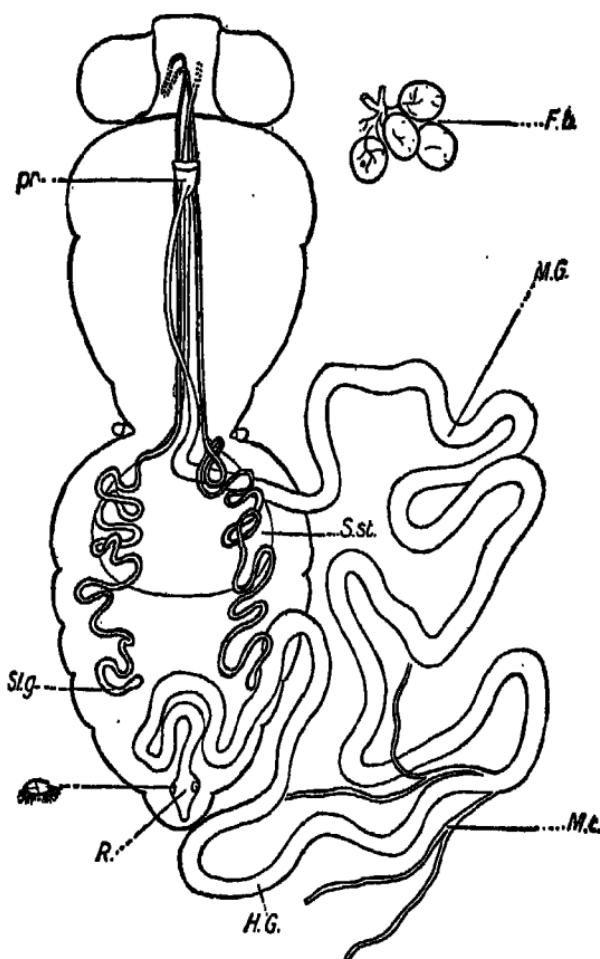


Fig. 66.—Diagrammatic representation of the alimentary canal of *Glossina*. Pr., proventriculus; s.st., sucking stomach; sl.g., salivary gland; M.G., mid-gut; M.t., Malpighian tubes; H.G., hind-gut; R., rectum; F.b., fat bodies. (After Blacklock and Yorke.)

A diagrammatic representation of the intestine of the tsetse fly is shown in figure 66. When a number of flies

ingest a meal of infected blood only a few become infected—the remainder apparently are able to digest the parasites or possibly wash out the incipient infection by the ingestion of a meal of clean blood. If a given fly does become infected, the trypanosomes first become established in the posterior part of the mid-gut where multiplication can begin as early as 36 to 48 hours after ingestion (Fig. 67). From the 10th to the 12th day more and more long, slender forms are noticeable in the gut. These begin to migrate anteriorly and establish themselves in the proventriculus between the 12th and 20th day. The slender proventricular type of trypanosome then migrates to the salivary glands, reaching them *via* the hypopharynx. Here they attach themselves by their free flagella and assume the crithidial arrangement of the parabasal body. These crithidial forms reproduce by longitudinal fission. Some of them, however, are continuously being re-transformed into trypanosomes of a short, stumpy type which represent the only developmental forms capable of infecting man. The minimum time necessary for the development in the salivary glands is from 2 to 5 days. The crithidial stage of development in the salivary glands is strictly homologous with the rectal phase of *T. lewisi* in the flea. Once these crithidial forms are established in the salivary glands they probably form a permanent source for the production of the infective trypanosome forms. The infective trypanosome forms pass down the salivary duct and are introduced into man when the fly bites.

It is to be emphasized that this development is a true cycle, just as is the case with *T. lewisi* in the flea. A short time after a fly ingests the trypanosomes (18 hours according to Bruce) they are not infective if injected into a mammal and this period of non-infectivity lasts until the salivary glands are invaded and the cycle of development is complete. For this reason, barring the chance of direct transfer, the bite of a fly is not infective until from 20 to 30 days after the

infected meal is taken. Once, however, the salivary glands are invaded and the infective type of trypanosome is produced, a given fly can probably continue to infect mammals

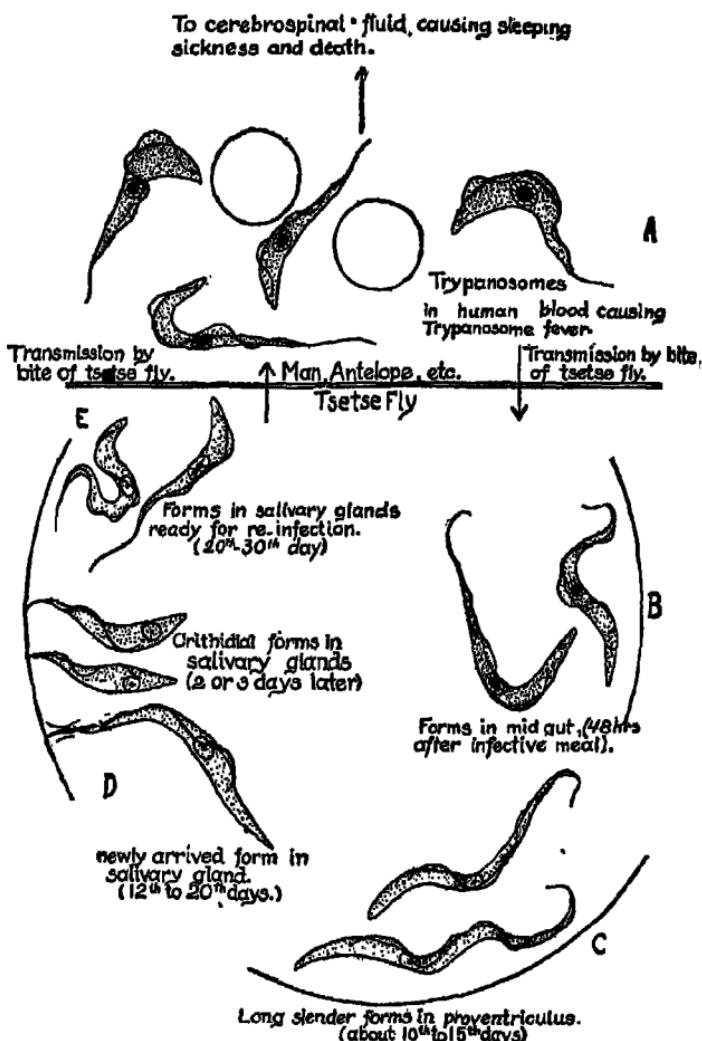


Fig. 67.—Life cycle of *Trypanosoma gambiense*. $\times 1500$. (After Chandler, constructed from figures by Robertson 1913.)

for the remainder of its life. The unusually long time necessary for trypanosomes to complete their cycle of development, coupled with the low percentage of flies which become infected, accounts for the fact that the cycle was missed by so many observers until discovered by Kleine (1909).

In the above account we have dealt only with the developmental series. A large number of the trypanosomes which are taken up by a given fly degenerate and perish. This gives rise to a very confusing series of forms which may be termed the degeneration series.

T. rhodesiense undergoes a similar development in *Glossina morsitans*. Here, however, the cycle of development, according to Kinghorn and Yorke (1912), takes a shorter time—an interval of about 14 days elapsing between the ingestion of the infective meal and the time the fly becomes infective.

c. Trypanosomes of Lower Animals in *Glossina*

In the foregoing account of the development of *T. gambiense* and *T. rhodesiense* in the tsetse fly it was seen that the first part of the development takes place in the intestine whereas the final development, viz., the crithidial stage and production of infective forms, takes place in the salivary glands. A similar life-cycle occurs in *T. brucei*, the causative agent of nagana which is transmitted chiefly by *Glossina morsitans*. In the case of *T. congolense* and *T. simiae*, the first part of the development takes place in the intestine, but the final development occurs in the hypopharynx—the parasites never entering the salivary glands. Finally, in the case of *T. vivax* and *T. uniforme*, the entire development is restricted to the proboscis—the first part of the development taking place in the labral cavity and the final part in the hypopharynx.

d. T. cruzi

There are many points in regard to the development of *T. cruzi* in its invertebrate host, *Triatoma megista*, which are by no means clear at the present time. The development was originally described by Chagas and since then has been studied by several authors. The following is a very brief outline of its main features. After a *Triatoma* has taken a meal of infected blood, the trypanosomes undergo their first change in the mid-gut or stomach of the insect. Here they round up into *Leishmania*-like forms and multiply actively by fission (Fig. 68), after which they increase in size, elongate and become typically *Crithidia*-like in structure (Fig. 68, 11). These crithidia pass into the hind-gut or intestine where they continue to multiply by fission and where some of them are re-transformed into trypanosomes which represent the final infective stage.

It is not definitely known just how the infection is transferred from the *Triatoma* to man. Chagas (1909) and (1921) believes that the trypaniform-type in the hind-gut passes through the general body cavity to the salivary glands of the insect and that such an infective form is injected into the human subject by the bite of the insect, although he admits that the salivary glands are only rarely infected, and that the body cavity of the insect has never been found infected. The best evidence in support of his contentions is in the work of Torres (1913). This investigator, after taking infinite pains to exclude the possibility of fecal contamination, was able to transmit *T. cruzi* to kittens by the bite of infected *Triatomæ*. Brumpt, Mayer, and da Rocha-Lima, however, were unable to obtain transmission by the bite of infected insects, but universally succeeded by injecting the rectal contents of such forms. This has also been the experience of the present author (Taliaferro). Brumpt's experiments led him to suggest that *T. cruzi* was transferred to man by the latter in-

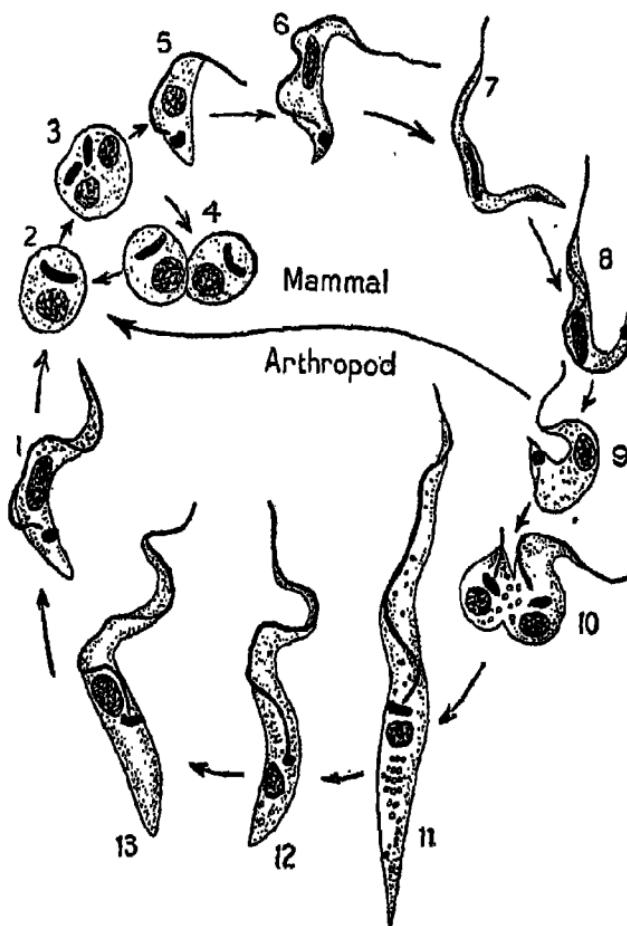


Fig. 68.—Diagrammatic representation of the life-cycle of *Trypanosoma cruzi*. 2-9, cycle in man. 9-13 and 1, cycle in *Triatoma*. In the cycle in man 2-4 represents the leishmania stages in the tissues and 5-9 the trypaniform stages in the blood. In the cycle in the *Triatoma*, 1 represents an infective trypanosome form in the rectum, 9, the forms received in the blood, 10, the division of crithidial forms, 11, a large crithidial form, 12 and 13, transition towards the trypanosome form. (From Blacklock and Yorke after Brumpt.)

gesting the contaminated feces just as is the case with *T. lewisi*. Furthermore, since infection can also take place by depositing the infected feces on the mucosa of the nose and eyes, he points out that such a method of transfer is facilitated by the fact that the *Triatomæ* habitually feed on the face and that infection in man is almost universally contracted during the first few years of life.

To sum up the rather difficult question of the mode of transmission of *T. cruzi* we may say: (1) Infective trypaniform stages do occur in the rectum of the *Triatoma*. These do pass out with the feces as has often been verified by simple microscopical examination in this laboratory (Taliaferro). Such infected feces can produce the infection in susceptible mammals by intraperitoneal, subcutaneous, or intravenous injection or by placing them in the mouth or on the mucosa of the eye or nose. (2) In rare cases Chagas has found trypanosomes in the salivary glands of the *Triatoma* and Torres has been able to infect animals by the bite. The present evidence indicates that invasion of the salivary glands is rare, but this does not preclude the possibility of its being the effective method of transferring the infection to man. In fact, it might explain why children often pass several years of their life free from infection although they are living under conditions in which they are being bitten daily by infected *Triatoma*.

Besides the development as we have outlined it, Chagas (1921) believes that he has been able to observe the first stages in the sexual cycle of the trypanosome in the insect. We feel, however, that considerably more work is necessary to prove that conjugation actually takes place in this species.

In spite of the present confusion in regard to the method of transmission of *T. cruzi* it is easy to see that its general development in the insect is closely comparable to that of *T. lewisi* in the rat-flea. The early multiplication of the

Leishmania-like forms in the mid-gut corresponds to the multiplication of *T. lewisi* in the epithelial cells of the stomach; the crithidial forms in the hind-gut are similar to the crithidial forms of *T. lewisi* in the rectum; and the final infective trypaniform stages correspond in the two species. The development of *T. cruzi* in the insect may be a true cycle of development although no one, as yet, has shown that the developmental forms are non-infective to mammals.

As an intermediate host the *Triatoma* differs from the other insect vectors which have been studied in that it shows almost 100% infection when fed on an infected mammal. This has led Brumpt (1914) to suggest what he terms *xenodiagnosis* in order to detect the organisms in the human blood. If several *Triatoma* are fed on a suspected case of Chagas fever, according to Brumpt, even when the parasites are so scarce in the peripheral blood as to be almost impossible to locate by ordinary microscopical methods, the insects will develop the infection. This method has not been used under practical conditions and would be useless during the chronic stage of the disease.

4. TRYPANOSOMES PARASITIC IN MAN

A detailed discussion of the life-history of various trypanosomes, both in their vertebrate and invertebrate hosts, has been given. It has also been pointed out that there are three species which live in man and which produce very fatal diseases. These are: (1) *Trypanosoma gambiense*, the causative agent of Gambian sleeping sickness; (2) *T. rhodesiense*, of Rhodesian or Nyasaland sleeping sickness; and (3) *T. cruzi*, of South American trypanosomiasis or Chagas' disease. These forms are of such immense medical importance that, even at the expense of some repetition, it is well to outline some of the more important facts in regard to them.

a. Trypanosoma gambiense

(1) HISTORICAL.—We can give here only a few of the more important facts in regard to the history of sleeping sickness and its etiological agent, *T. gambiense*. The first mention of the disease seems to be that of John Atkins (1724) in "The Navy Surgeon," where he describes the sleeping distemper among negroes of the Guinea Coast. An account of the disease was given by Winterbottom in 1803, who notes that slave dealers would not buy negroes with enlarged cervical glands. This is the origin of the so-called "Winterbottom's sign" in the diagnosis of trypanosomiasis. We need not outline here the early theories in regard to the cause of the disease. The question of its etiology was put on a sound basis in 1901 when Forde found actively moving bodies in the blood of a patient from Gambia.¹ These were identified as trypanosomes by J. E. Dutton of the Liverpool School of Tropical Medicine. In 1902 Dutton and Todd examined the blood from 1,043 natives in Africa and found trypanosomes in six individuals. From their investigations they concluded that trypanosome fever was a very mild disease in the African natives, but that these natives might act as a reservoir for the more susceptible white man. In 1902-3 Castellani found trypanosomes in the cerebrospinal fluid, and in 1903 reported these organisms as the etiological cause of the disease. In the same year, Bruce and Nabarro showed that *Glossina palpalis* was the insect vector. This

¹Some authors maintain that trypanosomes were first seen in man by Nepveu (1891), who gave an account of the parasites found in patients from Algeria suffering from malaria. Although Nepveu (1898) later considered that he had actually encountered a trypanosome, his description and figure are so meager that it is impossible to ascertain whether or not the bodies in question were actually trypanosomes. A further fact which throws some doubt on Nepveu's observations is that his patients came from a region in which human trypanosomiasis has never been found.

conclusion had already been reached by Sambon and Brumpt on epidemiological grounds. The later investigations of Bruce and other British workers left no doubt that Dutton's *Trypanosoma gambiense* was the causative agent of sleeping sickness and that the seemingly mild trypanosome fever invariably passed into the deadly sleeping sickness. Thomas (1905) introduced atoxyl for the treatment of experimental trypanosomiasis.

(2) GEOGRAPHICAL DISTRIBUTION.—The so-called Gambian sleeping sickness is now widely distributed in the tropical zone of Africa. Bruce (1915) gives its distribution as follows: "It extends on the north from St. Louis, at the mouth of the river Senegal, to the Bahr-el-Ghagal in the Egyptian Soudan; on the east it reaches to the eastern shore of Victoria Nyanza; and on the south to the southern end of Lake Tanganyika, the river Suapula in Northwest Rhodesia, and Donguela, in Portuguese West Africa." It is probable that its distribution was formerly only along the western portion of this range and that it was carried to the central and eastern portions of tropical Africa in consequence of the opening up of these regions by the white man, with the consequent traveling and introduction of infected humans into new areas. There is very little doubt that Stanley's expedition in 1887 to reach Emin Pasha carried the disease up the Congo region to Lake Albert, where it was contracted by the Sudanese soldiers who were left by Emin Pasha. Then in 1891 these Sudanese introduced the disease into Uganda. The present range of the disease is almost co-extensive with the distribution of its transmitting agent, *Glossina palpalis*.

(3) THE PARASITE.—*T. gambiense*, as found in the blood of man, is a typical example of a so-called polymorphic species—ranging in size from about $10\ \mu$ to $39\ \mu$ (Fig. 69). Minchin (1908) and others have divided the forms into three types: (1) long and slender forms with a long free

whip; (2) short broad forms without free whip; and (3) intermediate forms. These, however, do not represent sharply defined classes, but grade one into another and are probably simply the result of the diversity in size and shape which follows from multiplication and growth. The organisms multiply in blood and lymph spaces by longitudinal fission. We have already noted the periodic fluctuations in the number of organisms in the blood. After the trypanolytic crises which are associated with the disappearance of

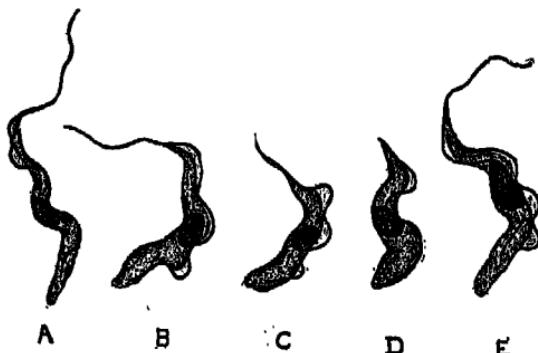


Fig. 69.—*Trypanosoma gambiense* from an experimentally infected monkey. A, long and slender form. B, intermediate form. C and D, short and stumpy forms. E, specimen in process of division. $\times 1500$. (Drawn by Miss F. A. Coventry.)

the organisms, several observers (see Moore and Breinl, 1907, and compare with *Critidium*, page 176) have described the presence of non-flagellated "latent bodies" which occur chiefly in the lungs, spleen and bone marrow and which supposedly can give rise to a new generation of trypanosomes. Further work on the presence and significance of these bodies is badly needed.

(4) CULTIVATION.¹—Long-continued cultivation of *T.*

¹In this connection it is interesting to note that the first haemo-flagellate to be cultivated successfully *in vitro* was *T. lewisi* which was grown on a blood agar (now known as Novy-MacNeal agar) by Novy and MacNeal (1903). Since then a large number of species

gambiense has not been attained. Thomson and Sinton (1912) succeeded in cultivating the organisms on a modified Novy-MacNeal-Nicolle medium for 37 days. The cultural forms closely resembled the developmental forms found in the gut of the tsetse fly. Three days after the cultures were started the trypanosomes apparently lost their power to infect susceptible animals.

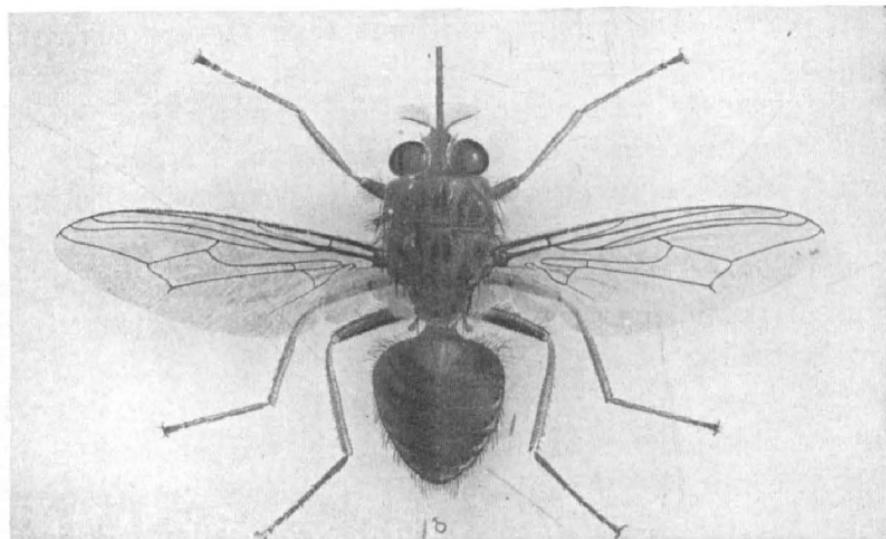


Fig. 70.—*Glossina palpalis*, the insect vector of Gambian sleeping sickness. $\times 4$. (After a painting by Menger.)

(5) TRANSMISSION OF DISEASE AND DEVELOPMENT OF THE VIRUS.—Epidemiological evidence, as well as direct experiment, has demonstrated beyond doubt that *T. gambiense* is transmitted from man to man chiefly by the tsetse fly, *Glossina palpalis* (Fig. 70), although it is probable that

of hæmoflagellates have been grown on artificial media, the majority either on the original medium of these authors or on some modification of it. The Novy-MacNeal-Nicolle medium, or N. N. N. medium which is mentioned in this paragraph and which is also used very extensively in the cultivation of the different species of *Leishmania*, is a simplification of the original formula which was suggested by Nicolle (1908).

under favorable conditions it can develop in other species of *Glossina*. We have already outlined the development in the fly and pointed out that infection in man follows from the bite of the fly. Under ordinary circumstances the bite of the fly is not infective until the trypanosomes have undergone their full cycle of development (20 or more days), but if the fly should bite an uninfected person within two hours after its infective meal, direct mechanical transfer may take place. There is no evidence that *T. gambiense* can develop in any other genus of biting fly. There is a possibility, however, that other biting flies may occasionally disseminate the disease by direct mechanical transfers. The fact that *T. equiperdum* is transmitted from horse to horse by coitus raises the question of a similar method of transfer in the case of sleeping sickness. After reviewing the question in detail, Laveran and Mesnil (1912) come to the conclusion that it is obviously possible if both the man and woman have abrasions on their genital mucosa, but that in any case it is extremely rare and that in no case has a patient contracted the disease in this manner outside of the endemic areas of the disease. In this connection it is interesting to note that hereditary transmission from parent to offspring through the placenta does not take place, and Bruce and his collaborators (1911) failed to get infection by placing infected blood on the healthy mucosa of monkeys.

(6) ANIMAL RESERVOIRS.—Many animals, including different species of wild game in Africa, are susceptible to *T. gambiense* and considerable evidence points to the fact that the latter act as reservoirs of the disease. The fact that a small percentage of *G. palpalis* of certain areas has remained infected for many years after the removal of the population and the fact that hereditary transmission does not take place in the fly necessitate the assumption of some such type of animal reservoir (Duke, 1921). The antelope,

Tragelaphus spekei, seems to be a particularly bad offender in this respect according to the work of Duke (1921).

b. *Trypanosoma rhodesiense*

(1) HISTORICAL.—This parasite is the causative agent of the so-called Nyasaland or Rhodesian sleeping sickness of man. The history of its discovery dates from February, 1910, when Stephens found a certain number of posteriorly nucleated parasites on a blood smear from a rat which was supposedly infected with a strain of *T. gambiense* originally from an Englishman infected in northeast Rhodesia in 1909. Stephens and Fantham (1910) made a careful study of these slides and came to the conclusion that the parasite could not be identified with *T. gambiense* and proposed the name *T. rhodesiense* for it.

Later work indicates that the occurrence of a certain percentage of posteriorly nucleated forms in subnucleated animals is not limited to *T. rhodesiense*. Such forms have been found in *T. pecaudi* by Wenyon (1912), in *T. brucei* by Blacklock (1912), and in *T. equiperdum* by Yorke and Blacklock (1912), but not as yet in *T. gambiense*. In case the occurrence of posteriorly nucleated forms may not be a valid character on which to differentiate *T. gambiense* and *T. rhodesiense*, however, there are other characters, all of which tend to show that the two are separate. (1) *Animal reactions*. *T. rhodesiense* is much more virulent to man and laboratory animals than *T. gambiense*. (2) *Reactions to chemicals*. *T. rhodesiense*, both in man and laboratory animals, is much more difficult to influence therapeutically than *T. gambiense*. In man it is very resistant to atoxyl and other drugs which have been used in the treatment of the Gambian parasite. (3) *Serum reactions*. Mesnil and Ringenbach showed that *T. rhodesiense* was susceptible to baboon serum, while *T. gambiense* was resistant. Similar results were obtained by Laveran and Nattan-Larrier with human serum.

(4) *Insect vector.* In nature *T. rhodesiense* is transmitted almost entirely by *Glossina morsitans*.

(2) GEOGRAPHICAL DISTRIBUTION.—At the present time the more virulent Nyasaland type of sleeping sickness is apparently restricted to Nyasaland, Rhodesia, German and Portuguese East Africa. It is carried by the widely distributed *G. morsitans* and it is entirely possible that it occurs

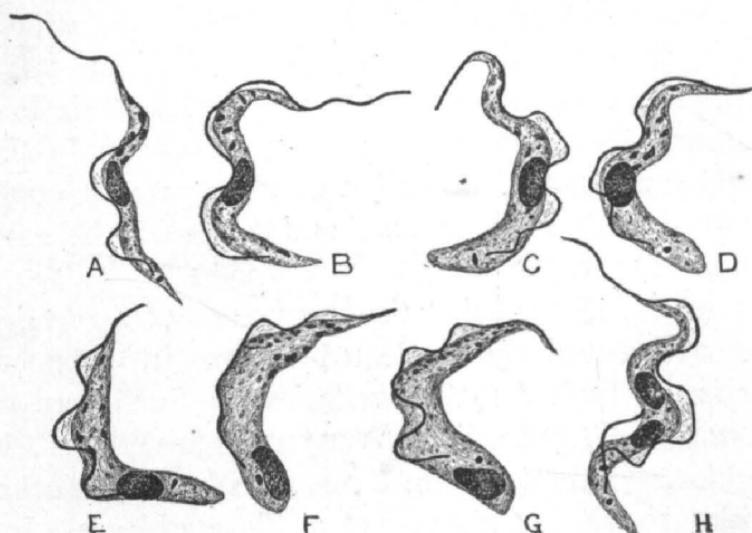


Fig. 71.—*Trypanosoma rhodesiense*
from an experimentally infected rat.

A, long and slender form. B and C, intermediate forms. D, short and stumpy form. E and F, transitional forms approaching the posteriorly nucleated condition. G, posterior-nucleated form. H, a specimen in the process of division. $\times 1500$. (Drawn by Miss F. A. Coventry.)

occasionally in the Gambian sleeping sickness areas but has been diagnosed as the latter disease. Its incidence is always low in comparison with the Gambian type of disease, and, as a corollary, man's natural immunity to the disease is high. Indeed, there is much evidence to support the contention of Bruce (see Bruce, 1915) that *T. rhodesiense* is in reality simply the widely spread *T. brucei* of nagana accidentally adapted to life in human blood.

(3) THE PARASITE.—*T. rhodesiense* in the blood of man is structurally identical with *T. gambiense* and no description is necessary here other than calling attention to Fig. 71. The only morphological difference between the two species is seen if they are subinoculated into rats or other laboratory animals, when *T. rhodesiense* exhibits a certain number of posteriorly nucleated forms (Fig. 71), all of which are short

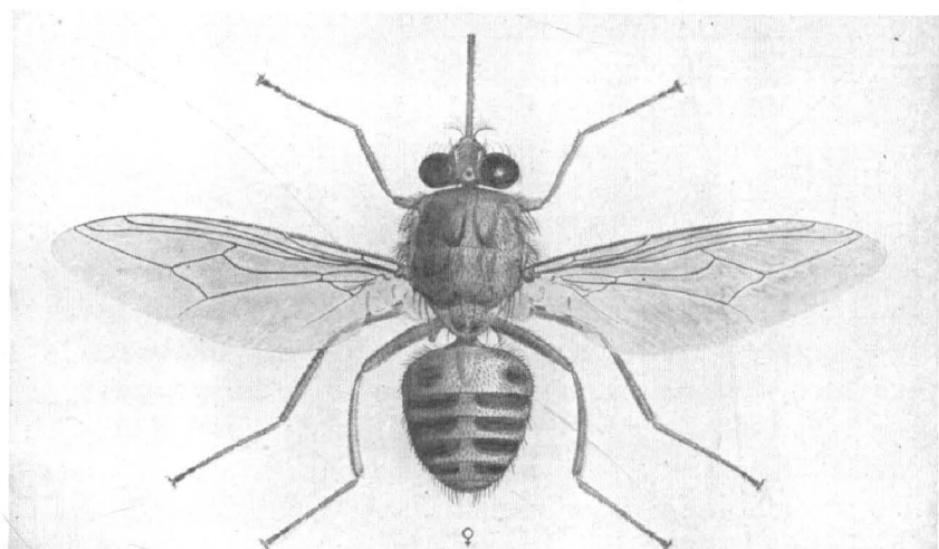


Fig. 72.—*Glossina morsitans*, the insect vector of Rhodesian sleeping sickness. $\times 4$. (After a painting by Menger.)

but never constitute more than five to six per cent of the total number of short forms.

(4) TRANSMISSION OF THE DISEASE AND RESERVOIRS OF THE VIRUS.—*T. rhodesiense* is transmitted from man to man by the bite of the tsetse fly, *Glossina morsitans* (Fig. 72). Its development in the fly is probably similar to that of *T. gambiense*, except that it takes only about 14 days. As with *T. gambiense*, the most usual manner of transfer is by the indirect cyclical method.

Nothing definite can be said in regard to the question of

animal reservoirs of the virus. If Bruce's contention that the organism is identical with *T. brucei* is true, the various animals which suffer from the widely spread nagana are a constant source of the infection.

c. *T. cruzi*: *American Trypanosomiasis (Chagas' Disease)*

(1) HISTORICAL.—The history of American trypanosomiasis is very interesting in that the etiological agent was discovered in the invertebrate host before it was found in man. While carrying on an anti-malarial campaign in the interior of Brazil, Chagas found a large hemipterous insect, *Triatoma megista*, whose gut was heavily infected with a *Crithidia*, infesting the human habitations and attacking the inmates. Consequently, he sent some of these insects to Dr. Oswaldo Cruz, who let them feed on a monkey and later found trypanosomes in the animal's blood. Suspecting that the same trypanosome could be found in the inhabitants of the region, Chagas examined the blood of a large number of people and finally found the organisms in a child. The peculiar life-history of the parasite makes it almost impossible to find it in older patients. In his first account of the disease, published in 1909, Chagas named the parasite *Schistotrypanum cruzi*. The advisability of separating this form generically from other trypanosomes is very doubtful and we have used the nomenclature *Trypanosoma cruzi*. In 1911, Vianna published an account of the pathological anatomy of the disease, which, in addition to its value from the standpoint of pathology, is a notable contribution to the life-history of the parasite.

(2) GEOGRAPHICAL DISTRIBUTION.—Chagas' disease has been reported chiefly from Brazil. It has, however, been found in San Salvador, Venezuela and Peru, and it seems very probable that it has a wide range over the continent of South America.

(3) THE PARASITE.—The trypanosomes which occur in

the peripheral blood show every gradation between very slender forms and thick, broad organisms. Some investigators maintain that these extremes represent a sexual dimorphism, but we believe that they are simply growth stages. In length they average about 20 μ (Fig. 73). The life-cycle of this form has already been discussed (page 142), and it has been pointed out that the trypanosomes do not multiply in the blood but in the tissues. Here they are transformed into *Leishmania*-like organisms (Fig. 74), which are the predominating tissue forms, although foci of flagellated *Trypanosoma*-like forms occur. In any case, all



Fig. 73.—*Trypanosoma cruzi*. Trypaniform stages from the blood of an experimentally infected cat. $\times 1500$. (Drawn by Miss F. A. Coventry.)

of the parasites in a given focus are generally in the same stage of transition. On very rare occasions the leishmania forms are seen in the blood and probably represent the premature rupture of a focus of infection.

(4) TRANSMISSION OF THE DISEASE.—By far the most common insect vector of Chagas' disease is *Triatoma megista* (Fig. 75), although several other species of *Triatoma* can probably transmit the trypanosomes, such as *T. infestans*, *T. sordida*, *T. vitticeps* and *T. dimidiata* var. *maculipennis*. Natural infections have also been found in a closely related species, *Rhodnius prolixus*. It is very interesting to note that Kofoid and McCulloch (1916) have found a trypanosome, *T. triatomæ*, in *Triatoma protracta* from California

which is morphologically indistinguishable from *T. cruzi*. Brumpt has also shown that *T. cruzi* will develop in the common North American Triatoma, *T. sanguisuga*. A de-

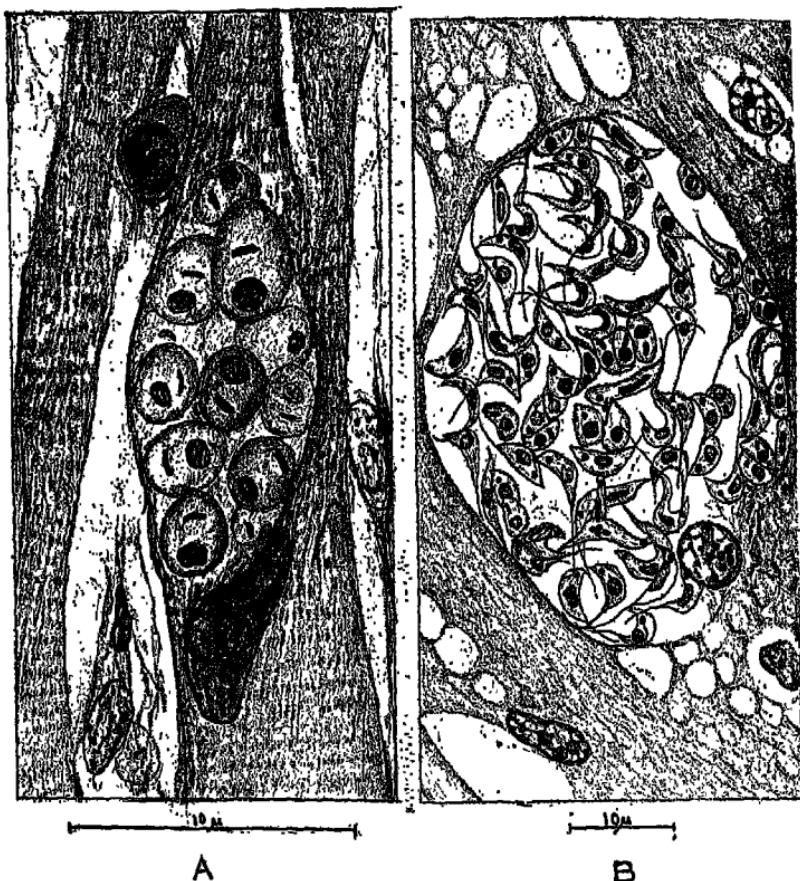


Fig. 74.—*Trypanosoma cruzi* in the tissues.

A, *Leishmania*-forms in human heart muscle. B, flagellated trypaniform stages in human neuroglia cell. Note that the two figures are drawn at different magnifications. (A, original; B, after Vianna.)

scription of the development of *T. cruzi* in *Triatoma* is given on page 154.

(5) PROPHYLAXIS AND TREATMENT.—Treatment of the

disease seems to be hopeless. None of the common trypanocidal drugs exert any marked influence on *T. cruzi*, and this applies even to Bayer 205, which seems so promising in the treatment of other human trypanosomiases. Control of the insect vector seems to afford the only method of controlling the disease, and resolves itself into a rather difficult economic problem. The triatomæ, which, in the endemic regions, are almost universally infected, show a marked preference for the houses of the lower classes, which afford large cracks,

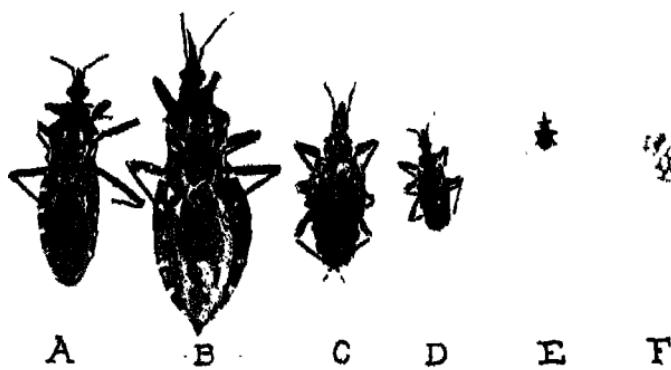


Fig. 75.—*Triatoma megista*, the insect vector of Chagas' disease.
A, adult male. B, adult female. C, D and E, nymphs. F, eggs.
Natural size. (From specimens furnished by Dr. C. Chagas.)

etc., where they may hide during the day and reproduce. From certain experiments carried out in Brazil, it seems probable that if these primitive huts could be destroyed and suitable houses erected, the disease would rapidly decrease in incidence. The cost of such a wholesale procedure would naturally be prohibitive, and, practically, can not be attained until the people are educated and the standard of living raised.

(6) RESERVOIRS.—According to Chagas (1921) and Crowell (1923), *T. cruzi* occurs in the armadillo, *Tatusia*

novemcinata, which is very abundant in the interior of Brazil where Chagas' disease is prevalent. In the armadillo the trypanosome is apparently a non-pathogenic parasite and is transmitted by *Triatoma geniculata*.

d. Methods of Diagnosis for the Human Trypanosomiases

Diagnosis of trypanosomiasis can rest only on the finding of the parasites in man. The differential diagnosis between the different types of the disease is made chiefly on the geographical distribution. In the case of Chagas' disease, the trypanosomes do show certain morphological differences from *T. gambiense* and *T. rhodesiense*, such as the larger parabasal body, etc., but there is no difficulty in its differentiation, as it occurs only in South America. The differentiation between *T. gambiense* and *T. rhodesiense*, on the other hand, is impossible on the basis of structure and can be made only on such points as: (1) the geographical distribution of the two parasites, (2) the greater virulence of *T. rhodesiense*, and (3) the appearance of posteriorly nucleated forms in animals subinoculated with *T. rhodesiense*. The presence of the trypanosomes can be demonstrated in the following ways:

(1) GAMBIAN AND RHODESIAN SLEEPING SICKNESS.

Microscopical Examination of the Blood.—A drop of blood which has been obtained from the ear or finger may be examined for the motile parasites. Examination of the fresh blood is preferable to that of stained smears, because the parasites are usually very scarce and their movement, with its consequent agitation of the red blood cells, aids in their detection. The parasites are generally so scarce that a negative by this method is not conclusive. A much safer method is to draw about 10 c. c. of blood from an arm vein and, after adding about an equal quantity of sodium citrate

solution, to centrifuge. The parasites may then be found in and just above the layer of leucocytes. Some investigators prefer to centrifuge the blood first at low speed until the red cells are thrown down and then to remove and centrifuge the supernatant fluid at high speed. The sediment is then examined for trypanosomes. This method is about as accurate as the one following and can often be used when the latter is impracticable.

Gland Puncture.—This is the method which is usually preferred by the British workers. The glands (especially those of the posterior triangle of the neck) are punctured with a sterile, but dry, hypodermic needle, and a small quantity of gland juice obtained by suction. When examined, this juice almost invariably shows trypanosomes in infected cases. During the later stages of the disease, however, the glands often become sclerosed, in which case an examination of the blood has to be made.

Lumbar Puncture.—During the last, or so-called sleeping sickness, stage of the disease the sediment obtained from centrifuged spinal fluid generally shows trypanosomes. In this connection it is well to note that the cell content of the spinal fluid increases with cerebrospinal involvement. According to some investigators, the cell content actually gives an index to the extent of cerebrospinal involvement.

Inoculation of Susceptible Animals.—Such laboratory animals as the monkey, guinea pig, and rat may be inoculated either subcutaneously or intraperitoneally with blood or gland juice. If these animals become infected it is comparatively simple to demonstrate the trypanosomes in their blood.

Serum Reactions.—Several such tests have been devised, but none of them are accurate enough to rely upon.

Cultivation.—This is entirely too difficult to be of any service.

(2) CHAGAS' DISEASE.—In the acute stages of the dis-

ease, when the trypanosomes are in the peripheral blood, their presence may be demonstrated by the microscopical examination of the blood as outlined above or by its injection into susceptible animals. Gland puncture has not yielded any results with *T. cruzi*. In some cases, showing a cerebro-spinal involvement, the parasites have been recovered from the spinal fluid. During the later stage of the disease, after the trypanosomes have left the peripheral blood, it is practically impossible to make a positive diagnosis. Attempts have been made to puncture various organs and tissues, with the hope of demonstrating the presence of the leishmania forms, but this seems impossible because of the irregular localization of the parasites. A differential diagnosis on clinical symptoms is also practically impossible. In these cases diagnosis is generally made only after an examination of the tissues at autopsy.

5. TRYPANOSOMES PARASITIC IN LOWER ANIMALS

It is impractical to give a detailed account of the trypanosomes found in lower animals. Scores of species have been found in practically every class of the vertebrates, but by far the majority of them do not produce any clinical symptoms of disease. The species in the land vertebrates are generally transmitted by some species of blood-sucking ectoparasites, while those of the aquatic vertebrates are usually transmitted by leeches. When the development of these forms is studied, transmission is almost universally found to be by the indirect or cyclical method. Besides these species of trypanosomes, there are some, however, that have become adapted to life in domesticated animals. This adaptation is probably comparatively recent and the trypanosomes generally produce very lethal diseases. Transmission is effected either by the cyclical method or mechanically. The more important of these parasites are listed in table I.



Fig. 76.—*Trypanosoma brucei* from an experimentally infected rat. A, long and slender form. B and C, intermediate forms. D, short and stumpy form. E and F, specimens in division. $\times 3000$. (Drawn by Miss F. A. Coventry.)

TABLE I—THE TRYPANOSOMES OF THE MORE IMPORTANT ANIMAL TRYPANOSOMIASSES

Method of transmission	Trypanosome	Animals affected	Geographical distribution	Disease
Coitus	<i>T. equiperdum</i>	Horses	Europe, Asia, North Africa and America.	Dourine
Cyclical development in <i>Glossina</i>	<i>T. brucei</i> <i>T. congolense</i> <i>T. vivax</i> <i>T. simiae</i>	Domesticated animals EQUIDÆ and ruminants EQUIDÆ and ruminants Goats	Tropical Africa Tropical Africa Tropical Africa Africa (Nyassaland)	Nagana
Mechanical transfer by biting flies such as <i>Tabanæs</i> , <i>Slo-</i> <i>moxy</i> , etc.	<i>T. evansi</i> <i>T. equinum</i>	Stock and domesticated animals Horses and mules	Asia and North Africa Tropical and subtropical South America	Surra Mal de caderas
Mechanical transfer by non-biting flies such as <i>Musca</i> (?)	<i>T. hippicum</i>	Horses and mules	Central America	Murina

D. The Genus *Crithidia*

Crithidia have been found in the intestines of many insects belonging to widely different orders. A large number of these insects, of course, are the intermediate hosts of vertebrate trypanosomes and the crithidias simply represent developmental stages in the life-history of the trypanosomes. Others, however, cannot show any such relation to vertebrate trypanosomes because they are not blood feeders, and the crithidial forms which they harbor must either belong to the genus *Crithidia* or be some stage in the development of an insect *Herpetomonas*.

a. *C. euryophthalmi*

Miss McCulloch (1917-1919) has described a very interesting *Crithidia* of this type, *C. euryophthalmi*, from the intestine of the hemipterous insect, *Euryophtalmus convivus*, which feeds upon the sap of growing tips of the plant, *Lupinus arboreas*. Although nematodes, yeast-like spores and bacteria are found in this sap, there is no evidence of any flagellate. All available evidence points to the fact that *C. euryophthalmi* is restricted in its life-history to the gut of the insect and passes from one insect to another by the contamination of food by infected feces. Structurally, a form like *C. euryophthalmi*, which belongs to the genus *Crithidia*, can not be distinguished from the crithidial forms of true trypanosomes.

(1) LIFE-CYCLE OF *C. euryophthalmi*.—A diagram of the digestive tract of the host, *Euryophtalmus convivus*, is given in Fig. 77, 1. The life-cycle probably begins with the ingestion of food which has been contaminated with fecal material, including the infective spores. These small oval spores are found in the crop. They possess both a nucleus and a parabasal body and structurally appear very much like a *Leishmania* (Fig. 77, 2). The spores are transformed

into typical crithidial forms by the elongation of the body, outgrowth of the flagella, etc. (Fig. 77, 3-6). Both the developing and mature crithidial forms reproduce by longitudinal fission (Fig. 77, 7). The mature crithidial forms may then either be carried directly to the pyloric expansion by the current of food or they may undergo a process of multiple fission in the crop. The latter may take place either extra- or intracellularly. The first type of extracellular multiple fission is that of somatella formation. This is fundamentally the same type of multiple fission found in the intracellular stage of *T. lewisi* in the flea. The somatella is formed by what may be considered a series of divisions in which the bodies do not separate. Eventually these somatellæ rupture to form a number of small merozoites. The second type of extracellular multiplication is different from any type found in *T. lewisi*, but may be analogous to the production of "latent bodies" in *T. gambiense*, as described by Moore and Breinl (1907). Here a process of endogenous budding takes place (Fig. 77, 12). Then when the parent crithidia breaks, these internal buds are liberated in the form of small zooids. The interesting thing in regard to these zooids is that the original parabasal body and blepharoplast of the parent flagellate do not take part in their formation, as is the case in daughter forms produced by binary fission or in the merozoites described above. The entire flagellar apparatus of the parent organism is discarded (Fig. 77, 11), and each zooid produces this apparatus anew. The exact nature of the intracellular type of multiple fission is not known. It is probable, however, that it is a somatella formation just as is found in *T. lewisi*. The three types of multiple fission, as well as binary fission, result in a swarm of the parasites in the anterior part of the gut which is soon followed by the establishment of the parasites in the pyloric expansion. Here they begin what, in analogy to *T. lewisi*, we may call the rectal phase which leads to the production

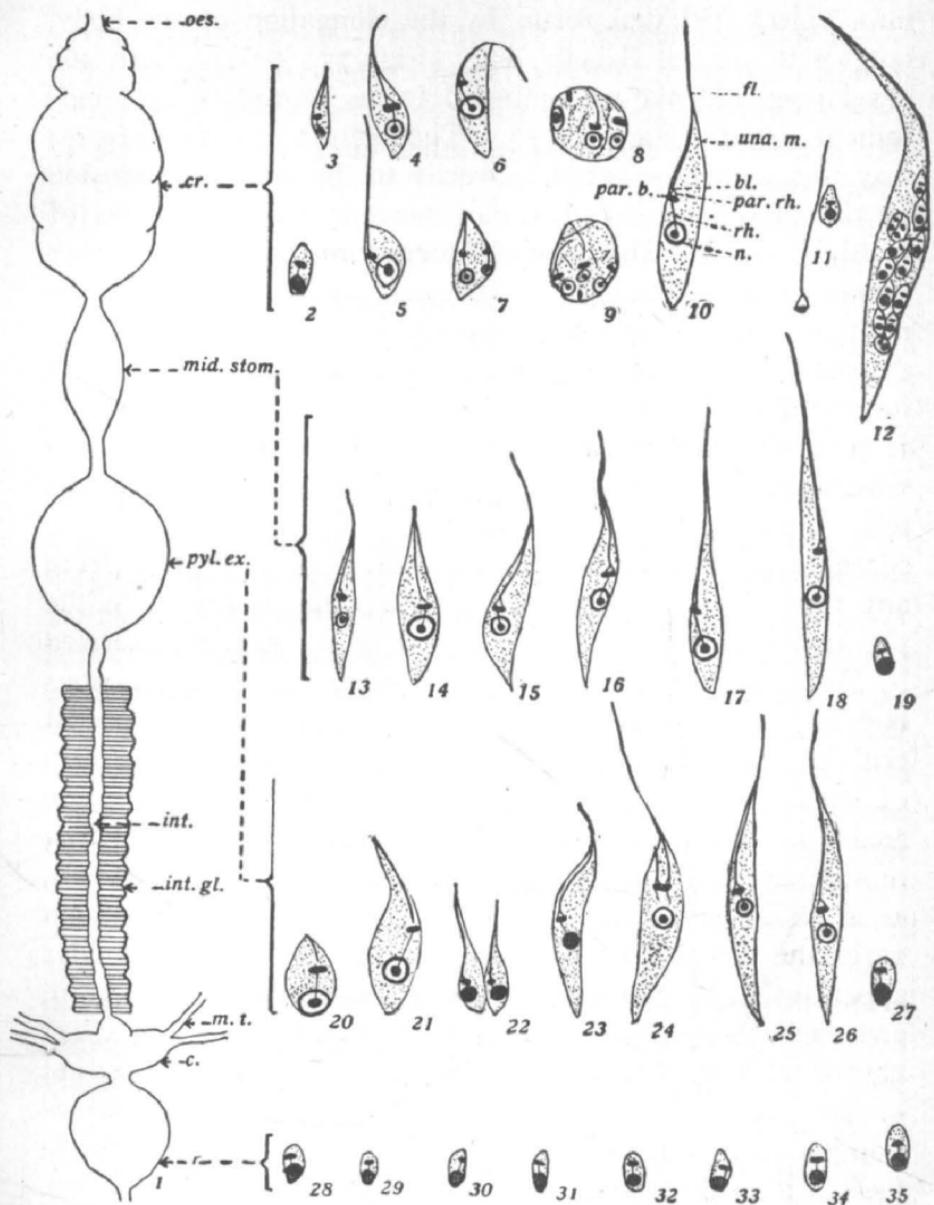


Fig. 78.—*Crithidia euryophtalmi*: 1, diagram of the digestive tract of the host, *Euryophtalmus convivus*, showing the organs between the œsophagus and anus. The remaining figures are outline drawings of the characteristic forms of the flagellate found in the different parts of the digestive tract. *Oes.*, œsophagus; *cr.*, crop; *mid. stom.*, mid-stomach; *pyl. exp.*, pyloric expansion; *int.*, intestine; *int. gl.*, gland of intestine; *m. t.*, malpighian tubules; *c.*, colon; *r.*, rectum. 2-35 \times 1750. (After McCulloch.)

of the infective stages. The infective spores which correspond in structure to those found in the crop, pass into the rectum and leave the body with the feces. Such feces probably contaminate the food of other bugs, and the cycle is started over again.

E. The Genus *Herpetomonas*

The genus *Herpetomonas* is represented by a number of species of flagellates which inhabit the alimentary canal of certain beetles, bugs, lice, cockroaches, fleas and flies.

a. *H. muscæ-domesticæ*

Probably most of the species of North American muscoid flies, green-bottle and blue-bottle flies, etc., are infected to a considerable extent with the type of *Herpetomonas* known as *H. muscæ-domesticæ*. It can be recognized by the almost constant biflagellated appearance of the large flagellated form, which led earlier observers to consider it a biflagellate. We now know that this condition represents merely a division stage, and that, in the resting stage and cyst, the organism shows indications of but one flagellum. The cross-infection experiments of Becker (1923) demonstrate that the parasite, from any one of six species of muscoid flies investigated, is infective to the other five. This fact, coupled with their morphological similarities, makes it extremely probable that the flagellates commonly found in nature in many muscoid flies belong to the same species, *Herpetomonas muscæ-domesticæ*, although other species may occasionally be encountered.

The life-history of *Herpetomonas muscæ-domesticæ*, in so far as it is known, is much simpler than that of *Crithidia euryophtalmi*. In general there are three forms of the organism which may be present in an infection—the "adult" flagellate, the trypaniform stage, and the cyst (Fig. 78). In a fly which is constantly reinfesting itself by ingesting the

excreta of other infected flies, it is possible to find all three stages of the parasite throughout the entire length of the intestine. In heavily infected flies whose food is not so contaminated, however, there is a regional distribution. The fore-gut and anterior part of the mid-gut contain only the actively multiplying flagellated forms which may occur singly

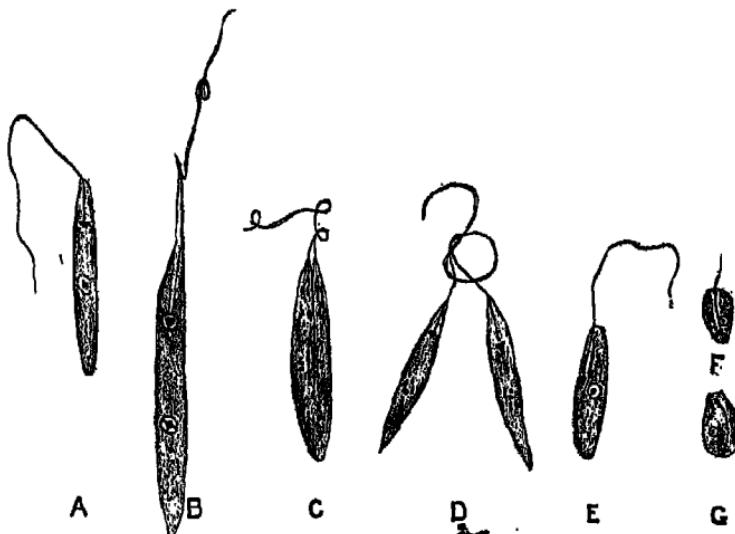


Fig. 78.—*Herpetomonas muscae-domesticae* from the fly,
Phormia regina.

A, uniflagellated form which is comparatively rare. B, biflagellated form which represents an early stage in division but which is the commonest type encountered. C and D, later stage in division. E and F, transitional forms which are to become cysts. F, shows the trypanosome arrangement of the parabasal body. G, cyst with a leishmania-like structure. \times about 1800. (After Becker.)

or in rosettes—large numbers attaching themselves together by their flagella. The posterior part of the mid-gut and the hind-gut contain the trypaniform stages and the cysts, as well as large numbers of the large "adult" flagellated forms. The trypaniform individual represents the transition between the "adult" stage and the cyst and is formed by a simulta-

nieous posterior progression of the parabasal body and a shortening of the organism, which is completed in the cyst. An infected fly may have myriads of these parasites in its intestine, or conditions may be so unfavorable for the multiplication of the parasite that only a few will be present.

The infection is transmitted from fly to fly through the excreta, which contains both flagellated and cyst forms. Infection experiments indicate that the flagellated forms as well as the cysts are infective. Roubaud reports that the cysts are the sub-immediate agents of transmission and that their resistance to drying does not exceed a few days. No investigator has been able to confirm Prowazek's assertion that *Herpetomonas* can be transmitted to the offspring of a fly through the ova.

The only method by which this flagellate is known to multiply is binary fission. It proceeds much the same as in other members of the haemoflagellate group except that in the late stage of nuclear division the chromatin of the nucleus is resolved into a number of discrete granules or chromosomes, presumably four. (See Becker, 1923.) Neither intracellular nor extracellular forms of multiple fission nor sexual phenomena are known definitely to occur in this organism, although such have been described, probably through misinterpretations and confusion of this parasite with other parasites sometimes found in flies.

F. The Genus *Leishmania*

We have already given a diagrammatic description of this genus. Forms with the typical leishmania structure have been noted in the case of *T. cruzi* in man and in the life-history of both *Crithidia* and *Herpetomonas* in the invertebrate. The present genus is characterized by the fact that a typical leishmania occurs in the tissues of its vertebrate host, but develops into a herpetomonas form when grown in culture. It is quite probable that the genus *Leishmania* is

simply an aggregation of insect herpetomonads which have become adapted for life in a mammal. Direct experimental proof of this hypothesis seemed to have been furnished by Laveran and others who maintained that an artificial leishmaniosis could be produced in laboratory animals by the injection of certain insect herpetomonads, but several recent workers (see Becker, 1923 and Shortt, 1923) have not been able to confirm these results. In any case, however, as Minchin (1912) points out, the life-history of a *Leishmania* differs so much from that of a true *Herpetomonas*, which is parasitic only in the digestive tract of insects, that it seems desirable to retain *Leishmania* as a separate genus.

Parasites of the genus *Leishmania* produce two different types of infection—one visceral and the other cutaneous. Kala-azar and a similar disease of dogs (canine leishmaniosis) are visceral in their nature, and tropical sore and South American leishmaniosis are more or less localized skin infections.

I. KALA-AZAR

a. *Historical*

Kala-azar has probably been endemic in parts of India for ages. It first attracted attention when a great outbreak in the Garo Hills of Assam was described by McNaught in 1882 as "malarial cachexia." The outbreak of such a deadly disease led a number of investigators to study its etiology. Giles (1890) came to the conclusion that the disease in Gauhati was in reality ancylostomiasis. In 1897, after a very careful clinical study, Rogers considered it to be a virulent form of malaria, and Ross (1899) studied it in Assam and came to the same conclusion. The first description of the parasites of kala-azar is that of Leishman (1903). Leishman's description was based on a case which he had obtained 2½ years previously. In the spleen from this case he found numerous bodies, which we now recognize as

Leishmania but which he considered to be degenerate trypanosomes. In the same year Donovan reported that he had observed the same bodies in the spleen of a case a short time before Leishman's paper was published. He also noted their presence in fresh splenic blood, and, as they were not flagellated, disproved Leishman's suggestion that they were trypanosomes. Ross (1903) concluded that these bodies represented a new species of sporozoan and proposed the name *Leishmania donovani* for them, but in 1904 Rogers found that, when kept in citrated blood, they multiplied and developed flagella and had, therefore, flagellate, rather than sporozoan affinities.

During the years immediately following 1904 human leishmaniosis was described from various parts of the region over which it now occurs. Of particular interest is the fact that Laveran, as early as 1904, found bodies in the spleen of an infant, who had died in Tunis, which were identically like those found in Indian kala-azar. Pianese (1905) described, under the name of infantile anemia, a very deadly disease among young infants in Naples, and probably found leishmania bodies in these patients, although he did not recognize their nature. A great deal of work has been done on this Mediterranean type of disease since 1907 by Nicolle and various collaborators. In 1908 Nicolle proposed to give the disease the name of infantile kala-azar and to designate the parasite, *Leishmania infantum*. At the present time, however, the available evidence indicates that the Indian kala-azar and the Mediterranean or infantile kala-azar are caused by the same parasite which we will designate *Leishmania donovani*.

b. Geographical Distribution

Kala-azar exists in a number of regions in Asia, Africa, and Europe. It is, however, doubtful if it occurs in either of the Americas. Migone (1913) described a case of visceral

leishmaniosis in an Italian who had lived in Paraguay since 1897, but, as this is the only case described from South America and as its origin has not been sufficiently investigated, it is probable that the disease does not occur on this continent. In Asia it is found in India, Ceylon, China, Russia, Turkestan and Asia Minor. In Africa and Europe it occurs in those countries which border on the Mediterranean, including Tunis, Tripoli, Algeria, Egypt, Italy, Sicily, Greece, Malta, Spain and Portugal.

c. *The Parasite*

In advanced stages of kala-azar the *Leishmania* may be found in almost every type of organ, having been described from the spleen, liver, bone marrow, lymph-glands, mesenteric ganglia, endothelial cells of the vessels, intestinal wall, kidney, lungs and testes. In all of these localities the parasites are always intracellular and exhibit a particular preference for the endothelial cells of the blood and lymphatic capillaries and for mono- or polynuclear leucocytes. They exhibit the typical structure of a *Leishmania* and are generally oval or round in outline (Fig. 79). The former vary in size from $2.0 \times 1.0 \mu$ to $4.5 \times 2.5 \mu$ while the latter generally have a diameter of between 1.5μ and 2.5μ . Occasionally larger forms have been seen. For example, Wenyon (1915) found, in the bone marrow of a dog inoculated with the Indian type of virus, large numbers of *Leishmania*, some of which reached the diameter of 8μ to 9μ . In specimens which are well fixed and stained with one of the Romanowsky stains, the cytoplasm appears homogeneous and of a pale blue color, and, besides the nucleus and parabasal body, is generally free from vacuoles or inclusions of any description. The nucleus is either round in outline or slightly oval, and, as a rule, is situated close to the periphery, although it may occur in any relation to the various axes of the body. The parabasal body is much smaller than the nucleus and

shows every gradation in shape from a spherical granule to a baciliform rod. It is generally situated on the side of the nucleus which is farthest away from the periphery of the cell. The parabasal body of some organisms shows a prolongation which Novy (1909) described under the name of

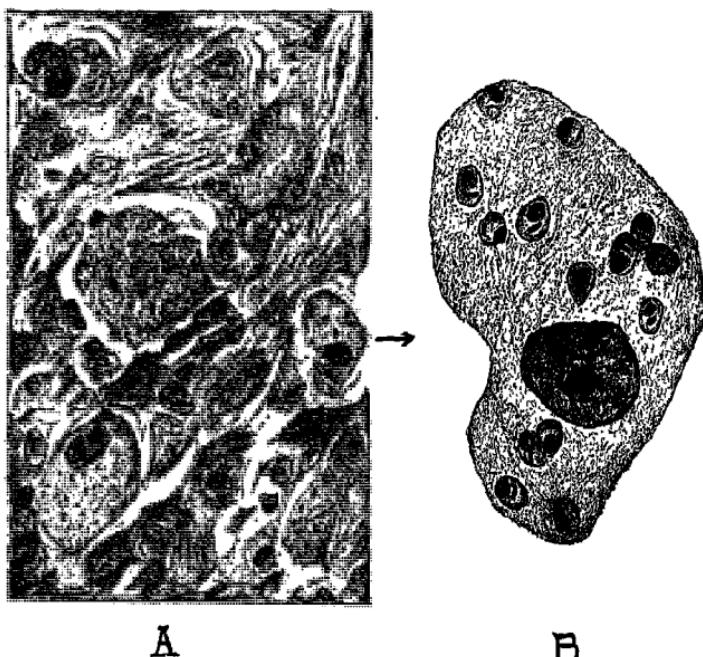


Fig. 79.—*Leishmania donovani* in the human spleen.

A, section of spleen showing five endothelial cells infected with the parasites. B, one cell enlarged to show structure of the parasites. A \times about 550; B \times 2500. (Original.)

rhizoplast and which is the rudiment of a flagellum. This structure has also been described by Gonder (1913).

The parasites multiply by binary fission. According to Laveran this takes place by the division of the nucleus first, the parabasal body next, and finally the cytoplasm. As we have already pointed out in the haemoflagellates, the usual

sequence of events is the initiation of division by the blepharoplast, followed first by the parabasal body, then by the nucleus and finally by the cytoplasm. We believe, therefore, that Laveran is probably mistaken in his description and that the parabasal body will be found to divide before the nucleus. A process of multiple fission has been described by several authors.

It is very interesting to consider how the parasites become distributed over the body and invade the various organs, especially as they never occur in the flagellated condition in the vertebrate and only exhibit the feeblest kind of movement when observed alive on a warm stage. According to Laveran (1917), when the virus is introduced into the blood of a man or an animal, the *L. donovani* are taken up by the leucocytes or endothelial cells, where they multiply until they rupture the containing cell and escape into the blood, whereupon the process is repeated. The entrance of the parasites into other types of cells can hardly be explained except by assuming that they have the power of actively penetrating them. Take, for example, the liver. The infection of this organ is probably initiated by the active ingestion of the parasites by the endothelial cells of the capillaries, in particular the cells of Kupffer. Sooner or later, however, the liver cells themselves must be penetrated, and it seems probable that the parasites assume the aggressive rôle. Then, as each infected liver cell bursts and liberates its contained parasites, the parasites penetrate other cells and in this manner spread the infection throughout the organ.

d. Cultural Forms

Rogers (1904) was the first to observe that, if *L. donovani* is kept in citrated human blood, the parasite would multiply and a large number would develop flagella. Since then various culture media have been used and the flagellated form develops in all of them. If, for example, a tube of

Novy-MacNeal-Nicolle medium is inoculated with the material from a splenic puncture of a case of kala-azar and is kept at from 20° to 22° C., the above transformation takes place quite rapidly. After three days one finds every gradation from true leishmania forms to long herpetomonas forms (Fig. 80). The typical flagellate form measures from $10\ \mu$ to $20\ \mu$ in length and from $1.5\ \mu$ to $4.0\ \mu$ in breadth. In well fixed and stained specimens, the cytoplasm is homogeneous, just as is the case with the leishmania forms found in the

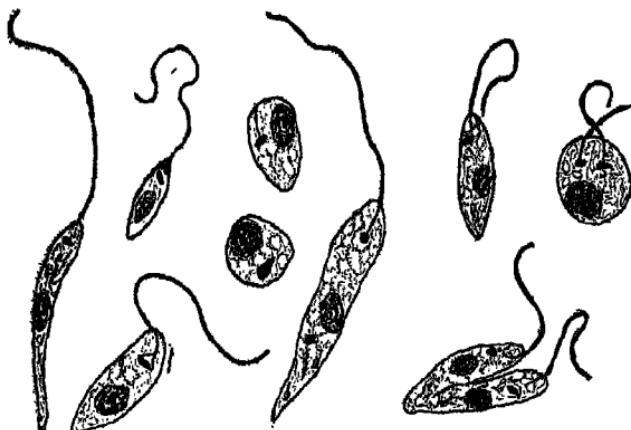


Fig. 80.—*Leishmania donovani* from a culture made from a case of infantile kala-azar, showing typical herpetomonas forms. $\times 2000$. (Original.)

tissues of the body. The nucleus is generally situated near the middle of the body and the parabasal body is situated well toward the anterior end. Associated with, but probably separated from, the parabasal body is a long flagellum which runs anteriorly through the body and projects in a long free whip. Its origin is probably in the blepharoplast, but ordinary methods of staining fail to distinguish this body from the parabasal body. In every way the flagellated cultural forms of *L. donovani* resemble a true *Herpetomonas*, an undulating membrane never being present.

The flagellated forms reproduce by binary fission, the general nature of which is like that described for the genus *Herpetomonas*. In cultures they often show agglutination rosettes. When injected into the blood of a susceptible vertebrate, they are phagocyted, just as are the leishmania forms. Then they quickly lose their flagella, round up and begin their cycle as a true leishmania.

e. Relation of *L. donovani* to Vertebrate Host

In the case of kala-azar, as with many other protozoan diseases, the question arises as to how the parasite produces its effect on the host. Does it lead to the death of the host by the production of specific toxins? We can not go into a detailed discussion of this problem here, but for a review of the literature the reader is referred to the monographic work of Laveran (1917). Briefly, however, it seems certain that, if a toxin is produced, it is an extremely weak one, and we feel that Cornwall and La Frenais (1916) are probably nearer the truth when they suggest that the *Leishmania* produce their effects not by a toxin but by the destruction, with their sheer numbers, of cell after cell of the tissues, the disintegration products of which, when liberated into the blood, probably lead to the clinical manifestations of the disease, such as fever, etc., alterations in the cells of the liver, spleen, and bone marrow, and finally death.

f. Transmission

Since Rogers (1904) showed that *L. donovani* developed into typical herpetomonas forms outside of the human body, there has been no doubt as to the affinities of the organism. As a matter of fact, the genus *Leishmania*, as stated above, probably simply represents insect herpetomonads which have become adapted to life in vertebrates as intracellular parasites, and consequently are probably transmitted from man to man by a blood-sucking insect. Furthermore, the de-

velopment of the parasite in cultures is probably roughly the same as will be found in the invertebrate host. At one time it was supposed that the parasites were present in the peripheral blood only at rare intervals, and for this reason some investigators ruled out the possibility of insect transmission on *a priori* grounds. Now, however, it is fairly certain that small numbers of the parasites are constantly present in the general circulation. In fact, Patton has found the organisms to be particularly numerous in the terminal stages of kala-azar, in subjects suffering from diarrhea or dysentery, which are recognized in India as being particularly contagious (Laveran, 1917). The theory that kala-azar is an insect-borne disease is not, however, the only possibility. Some investigators lean to the theory that man acquires his infections *via* the alimentary canal; others, while admitting that the disease is of insect origin, feel that man represents a "blind alley" for the infection.

Of the blood-sucking insects which prey upon man the bedbug has been most suspected. The chief investigator to uphold the theory of transmission by this insect is Patton, who has published a long series of papers on the subject beginning in 1907. Patton (1912) described the development of *L. donovani* in both *Cimex rotundatus* and *C. lectularius*. In these he found a preflagellate stage (leishmania form) which multiplied by fission within the human leucocytes or macrophages of the ingested blood, a flagellated stage (herpetomonas form) which continued to divide and a post-flagellate stage (leishmania form) in the intestine. This last stage was considered to be the infective form and was supposed to be regurgitated during the process of biting.

Later prolonged researches, a list of which can be found in Laveran (1917), failed to prove that kala-azar is transmitted by the bedbug. Recently, Mrs. Adie (1921) has discovered an intracellular stage in the development of *L. donovani* in the bedbug, but, as this was found only in dead

bugs, it may be simply a secondary invasion and does not add any conclusive evidence that the bedbug is the transmitting agent of kala-azar.

In the Mediterranean region a great deal of attention has been paid to the flea as the possible insect vector of kala-azar, but this work is in much the same condition as that on the bedbug, and need only be mentioned briefly here. In 1908 Nicolle, after he found that the dog was susceptible to the so-called infantile kala-azar and also naturally suffered from a similar disease, suggested that the human disease was of canine origin, and that dog-fleas were the transmitting agents. Basile, beginning in 1910, published a long series of experiments and observations to support this hypothesis. Other investigators, especially da Silva (1913), have been unable to confirm his experiments, and, as is the case with the bedbug, the flea cannot be conclusively incriminated as the transmitting agent of either human or canine leishmaniosis. Experiments have also been carried out with mosquitoes, flies (*Phlebotomus*), ticks and *Triatoma rubrofasciata* without any definite results.

g. Prophylaxis

The work of Rogers and later of Price and Rogers in the tea gardens of the Nowgong district in India (see Rogers, 1919) indicates that the prophylaxis of kala-azar is very simple. They found that, if uninfected coolies were placed in new houses and kept 300 yards from old infected lines, they remained free of the disease. In other words, a distance of 300 yards, which is insufficient to prevent the spread of a mosquito-borne disease, such as malaria, is a barrier to the passage of kala-azar. A summary of the observations of these investigators is given in table II. While segregation of villagers is obviously not as simple as of tea-garden coolies, Young (1914) (see Rogers, 1919) has shown that it can be carried out to great advantage.

HUMAN PROTOZOOLOGY

CHAPTER II.—ERANICATION OF KALA-AZAR FROM ASSAM TEA-GARDENS (FROM PRICE AND ROGERS, 1914)

h. Diagnosis

A positive diagnosis rests on the finding or demonstration of *L. donovani* in the patients. This may be done in several ways.

(1) SPLEEN PUNCTURE.—The examination of stained films of the material obtained from spleen (or liver) puncture is the most reliable means of demonstrating the presence of *L. donovani*. The most successful punctures are those which yield a small amount of splenic juice and in which the syringe does not fill with blood. Smears are made of the juice and are stained in the same manner as blood films—i. e., with such stains as Wright's, Giemsa's, or Leishman's. In such films the parasites are found both intra- and extracellularly. Splenic puncture is most useful in the early doubtful cases, at which time it is least dangerous. It, however, always incurs some danger, and at times it is positively contraindicated. For this reason other methods have been devised which, when successful, make it possible to dispense with spleen puncture.

(2) EXAMINATION OF PERIPHERAL BLOOD.—Some investigators have been very successful in the finding of *L. donovani* in stained films of the peripheral blood. In 84 cases in Madras, Patton (1912) reports that a positive diagnosis in 42 cases was made on the first blood film, 13 on the second, 12 on the third, 5 on the fourth, 2 on the fifth, 4 on the sixth and the remaining after a large number of films were examined. In examining such films it is best to search along the margins of the smears, as the large mononuclear endothelial cells tend to become localized there and in them the parasites are most often found.

(3) CULTURE OF PERIPHERAL BLOOD.—As the parasites can be easily grown on NNN medium, it is possible to cultivate the organisms from the peripheral blood. This method is undoubtedly of great value, but its disadvantage is that when only a few parasites are present several weeks often

elapse before the flagellates can be found in the cultures. (See Young and van Sant, 1923, for a study of the cultivation of *L. donovani* from the peripheral blood.)

(4) SERUM TESTS.—Several serum reactions have been devised for the detection of kala-azar, but none of them are a substitute for finding the parasite. (See Wenyon, 1922.)

2. CANINE LEISHMANIOSIS

Nicolle and Comte (1908) published the first account of a dog naturally infected with *Leishmania*. Since then this subject has created a great deal of interest, because canine leishmaniosis seems to be strictly comparable to human kala-azar and generally occurs in the same locality. It has been described from Asia, Africa and Europe. In the two continents last named it occurs in the countries bordering on the Mediterranean and its distribution corresponds very closely to that of the so-called infantile kala-azar. In Asia it has been found in the Transcaucasus and in Turkestan, but not in the principal Asiatic center of kala-azar, viz., India.

It is unnecessary to give a detailed description of the parasite of canine leishmaniosis, because it closely resembles *L. donovani*. In fact, there are so many things in common between the two organisms that many observers believe them to be identical. The following are some of their points of resemblance: (1) Morphologically they are identical, both in the leishmania and in the flagellated stages. (2) The course of the disease in dogs and its pathology seems to be identical, whether the dogs are inoculated naturally with canine or human virus. (3) The same species of laboratory animals are susceptible to the dog as to the human form. In this connection it is interesting to note that, according to Nicolle and Conor (1914), the dog is actually more susceptible to the human than to the canine virus. (4) With the exception of India, the geographical distribution of the human and canine diseases is remarkably similar.

The question of the method of transmission of any of the leishmanioses is in such a confused state at the present time that it does not seem profitable to discuss the various experiments dealing with the transmission of canine leishmaniosis. It may be noted, however, that in 1908 Nicolle expressed the opinion that it was transmitted from dog to dog through the agency of dog-fleas, but, while Basile and others carried out a series of experiments which seemed to support this hypothesis, since then other investigators have performed experiments which cast a great deal of doubt on Nicolle's hypothesis. Patton (1914) has suggested that canine leishmaniosis is produced by *Herpetomonas ctenocephali*, a common parasite of the digestive tube of the dog-flea. He also holds that the association of the canine and human disease is purely accidental.

3. ORIENTAL SORE

a. Historical

The first mention of oriental sore seems to be that of Russell (1756), who mentioned it in his "Natural History of Aleppo and Parts." Numerous later authors mentioned the condition, calling it by various names, depending on the locality in which it occurred. Willemin (1854) apparently considered it certain that the sore was caused by the water of the river Aleppo, and many other suggestions as to the nature of the disease, of purely historical interest now, were made by various authors. The work of Cunningham (1885), Riehl (1886) and Firth (1891), however, deserves special mention. Cunningham studied pathological material of oriental sore after it had been fixed, sectioned and stained in different ways, and found bodies reproducing by binary fission and spore formation which he believed belonged to the order *Mycetozoa* and caused the disease. The smallest of these bodies measured 6.4μ and the largest from 12.8μ to 25.6μ in diameter. The outline of the body was very

indistinct and there was a variable number of nuclear structures. Firth found Cunningham's bodies and considered them to be sporozoan in nature; he called them *Sporozoa furunculosa*. Riehl, in a case of oriental sore, found bodies measuring from $0.9\ \mu$ to $1.1\ \mu$ in the embryonic tissue cells, in the large epithelial cells and in the polynuclears. It is probable that Cunningham and Firth were both dealing with the protozoan parasites of oriental sore and that Riehl also recognized the parasite but mistook the nucleus for the entire body of the organism. In view of the facts that their description is so imperfect and what they saw considerably doubtful, most authors give the credit of discovering the parasite to Wright (1903) of Boston. Wright's work was done on a single case which occurred in a young girl aged 9 years who was born in Armenia and developed the sore 2 to 3 months following her departure from that country. After making a careful pathological study of this case, Wright published an excellent description of the parasite under the name of *Helcosoma tropicum*, and his data have been confirmed in every case in which oriental sore has been studied. Nicolle and Sicre (1908) succeeded in cultivating the parasite. In their cultures it developed into a typical *Herpetomonas*, thus immediately showing its relation to the parasite of kala-azar and as a consequence it is placed in the same genus under Wright's specific name, i. e., *Leishmania tropica*. Nicolle and his collaborators in 1908 and 1910 opened up a new avenue of experimental attack on the disease by transferring the sore to the monkey and dog.

b. Geographical Distribution

In Asia the disease is scattered over a vast area, including Syria, Palestine, Asia Minor, Caucasus and Transcaucasus, Persia, Mesopotamia, Russia, Turkestan, and India. It is interesting to note that its distribution in the last-named country does not correspond with that of kala-azar. In

Africa it has been found in Algeria, Tunis, Morocco, Egypt, Abyssinia and Tripoli. True oriental sore has not been reported from either North or South America, although a closely related cutaneous leishmaniosis occurs on the latter continent.

c. *The Parasite*

Both intracellular and free parasites are found in material obtained from the puncture of the sores or from the exudate

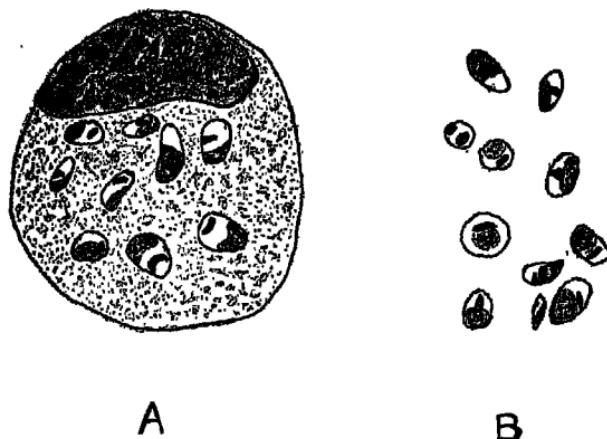


Fig. 81.—*Leishmania tropica* from a case of oriental sore.

A, macrophage containing nine parasites. B, a clump of eleven extracellular parasites taken from the same smear. Note the presence of a nucleus and parabasal body in each parasite. $\times 2000$. (Original.)

of such sores. The intracellular forms are generally found in the mono- and polynuclear leucocytes and in the epithelial cells. In size and structure the parasites are almost identical with *L. donovani* (Fig. 81). The spherical ones are around $2\ \mu$ in diameter and the oval forms range in length from $2\ \mu$ to $5\ \mu$ and from $1\ \mu$ to $2\ \mu$ in breadth. They also show the same type of nucleus and parabasal body as *L. donovani* and reproduce by binary fission.

d. Cultural Forms

The flagellated forms are rarely seen in the sores, although occasionally, in a suppurating sore, one finds a few. When grown on Nicolle-Novy-MacNeal agar at 22° C., the parasites readily develop flagella. Their transformation into herpetomonas forms and their general structure are so similar to the parasite of kala-azar that it is not necessary to describe them here.

e. Transmission

The first striking fact in regard to *L. tropica* is that it can be transmitted by inoculation from man to man, although infection can not take place through the unbroken skin. It can also be passed from man to animals, particularly dogs, monkeys and mice. In endemic areas the dogs suffer from a cutaneous leishmaniosis which is probably the same as the human disease and it is not at all improbable that these animals act as reservoirs of the virus. Not only can it be transmitted from man to man by inoculation, but auto-inoculation takes place—soiled linen serving to transfer the virus—if the virus comes in contact with an accidental lesion in the skin. According to Manson, the Jews of Bagdad for many years have made use of the fact that the disease can be inoculated and furthermore that one attack confers immunity by inoculating their children in some unexposed part so that the sores would not occur on the face. The second important fact to note in the transmission of oriental sore is that under natural conditions it almost invariably occurs on some portion of the body which is not covered by clothing and may, therefore, be transferred by a flying insect. Despite this, however, Patton (1912) has suggested the bedbug as the insect vector. Various other investigators have endeavored, in vain, to incriminate different species of biting and blood-sucking *Diptera*, and still others have suggested the possibility of the house-fly. In endemic regions swarms

of the latter feed on the exudate from the sores and may possibly transmit the disease simply mechanically by transferring the virus from a sore to a lesion of the skin. If this be the actual method of transmission, it is possible that biting flies may play a rôle in making the primary skin lesions. At the present time the theory that is in most favor, especially with the British workers, is that transmission is effected by the bite of the sand fly, *Phlebotomus*. Although the evidence in favor of *Phlebotomus* is slowly accumulating, it is not, as yet, conclusive. (See Wenyon, 1922.)

f. Diagnosis

Positive diagnosis can only be made by the demonstration of *L. tropica* in the sores. This may be done either by the microscopic examination of stained smears prepared from the sores or by the cultivation of the parasites on such a medium as the Nicolle-Novy-MacNeal agar. If the sore has not ulcerated, a pipette may be inserted into the center or margin of the papule and the smears made from the serous fluid or blood which is withdrawn. If ulceration has occurred, it is best to puncture or excise a portion of the unulcerated margin of the sore in order to make the smears. The smears should be stained with one of the modifications of the Romanowsky technique, such as Wright's stain. When present the parasites are found free or intracellularly in the tissue cells or leucocytes. Material for cultivation is obtained in the same manner as for smears, except that precautions are taken against contamination—the skin around the point of puncture being sterilized with iodine. Development on Nicolle-Novy-MacNeal medium is rapid. A good growth of flagellates may be obtained within three or four days at a temperature of 22° C. If very few parasites are inoculated, however, a good growth is often not obtained for several weeks.

4. SOUTH AMERICAN LEISHMANIOSIS

a. *Historical*

For many years ulcerations of the skin and mucosa have been recognized and described from Brazil, Peru and other portions of South America. Undoubtedly a number of these cases were what we now designate South American leishmaniosis. According to Tamayo, leishmaniosis of the skin and mucosa has existed in Peru since prehistoric times and the lesion now known as *uta* is reproduced on the water jars of the Incas. Unfortunately, however, there are other infections which produce much the same type of lesion and the same clinical symptoms and can easily be confused with leishmaniosis. As a consequence it is difficult to ascertain with just what disease each of the various authors before 1909 was dealing. In 1909, Carini and Paranhos described sores which developed on the workers employed in the construction of a railroad in northeast Brazil. They found parasites of the type of *L. tropica* in the lesions and identified the ulcers with tropical sore of the orient. Linden-
burg (1909), working in the same state of Brazil, confirmed these conclusions both in regard to the parasites and the identity of the disease with tropical sore. In the same year, Nattan-Larrier, Touin and Heckenroth gave an account of an ulcer contracted in French Guiana in which small numbers of *Leishmania* were found. Pedroso and da Silva (1910) were the first investigators to cultivate the parasite. They used Novy-MacNeal blood agar. During the next few years a number of articles appeared which showed that true leishmaniosis occurred in many regions of Brazil and other parts of South America. Darling (1910) and Darling and Connor (1911) described a cutaneous leishmaniosis from the Canal Zone, and Seidelin (1912) described a similar malady from Yucatan in Mexico. All of these cases,

in which *Leishmania* were found, probably represent the same disease. During the same period a number of excellent descriptions of the disease appeared. Laveran and Nattan-Larrier (1912) proposed to differentiate the parasite of South American leishmaniosis from that of tropical sore and suggested the name *L. tropica* var. *americana*. These investigators worked with material sent from Peru by Escomel. In 1913, Vianna first used intravenous injections of tartar emetic in treating the disease, and a number of subsequent investigators have confirmed the efficacy of this treatment.

b. Geographical Distribution

The principal endemic centers of South American leishmaniosis seem to be in Brazil and Peru. It is also found, however, in Paraguay, Uruguay, the Guineas and Central America, extending as far north as Yucatan in Mexico.

c. The Parasite

Just as in *L. tropica*, both intracellular and free forms of *Leishmania americana* occur. Its general anatomy, appearance, and structure is so similar to *L. tropica* that it is useless to go into detail here. According to Escomel (1913 and 1914), the flagellated forms occur occasionally in the cutaneous ulcers. As in the other *Leishmania* the flagellated herpetomonad forms are found typically in cultures of the parasite. The ulcers in South American leishmaniosis are frequently super-infected with bacteria or fungi—at times even with fly maggots. Quite often many of the clinical symptoms may be due to these super-infections. That the protozoon is, however, the primary cause of the ulcers is shown by the fact that, even in these cases, they show almost immediate improvement after the administration of tartar emetic.

d. Transmission

The question of the transmitting agent of South American leishmaniosis is in as confused a state as the similar question in the other leishmanioses. In fact some investigators feel that it is in reality an insect herpetomonad which has become adapted to life in man and that each case in the human being probably represents a "blind alley" for the infection. There is little doubt that it can be transmitted from man to man by simple inoculation as in the case of tropical sore. Auto-inoculation from a sore to another part of the body (especially the mucosa) is very frequent. Inoculation into dogs has proved successful in the hands of some investigators. Natural infections of dogs are, however, very rare. The original sores are only contracted on exposed portions of the body and most investigators lean to the theory that the disease is transmitted by some biting insect. Various authors, however, have tried to incriminate different biting and blood-sucking insects without success.

e. Diagnosis

The diagnosis of South American leishmaniosis is identical with that for oriental sore. In long standing cases the parasites are very scarce and exceedingly hard to find. Cultivation is often successful even when direct examination of the smears does not reveal the presence of the parasites.

CHAPTER VI¹

THE INTESTINAL FLAGELLATES

A. Classification of the Intestinal Flagellates

The organisms that we group together under the heading of intestinal flagellates belong to the subclass ZOOMASTIGINA of the class MASTIGOPHORA and to the two orders PROTO-MONADINA and DISTOMATINA.

The intestinal flagellates are difficult to classify because of their great number and the diversity of their habitats; they are more numerous than the rhizopods and ciliates and approach closely the sporozoa in number of entozoic species. Almost every species of vertebrate serves as a habitat for intestinal flagellates. For example, only 2 per cent of 329 vertebrates examined by Kofoid were free from them. Furthermore, one species of vertebrate may harbor many species of intestinal flagellates. Thus, an examination of 25 specimens of one species of salamander, *Diemyctylus torosus*, revealed 14 species belonging to 10 genera (Kofoid, 1917, p. 3). Very few of the intestinal flagellates have been studied in detail, and this fact, together with the great number of species, makes a satisfactory classification into families very difficult at the present time.

B. The Genus *Chilomastix*

I. CHILOMASTIX MESNILI

a. Historical

Although the genus *Chilomastix* was not recognized until 1909 by Alexeieff and the species *Chilomastix mesnili* was

¹ By R. W. Hegner.

not distinguished clearly from other human intestinal flagellates until 1910 by Wenyon, it seems evident from drawings and descriptions contained in the literature that investigators really saw the human species as early as 1854. Davaine (1854, 1860) described, under the name *Cercomonas hominis*, two varieties of flagellates of different sizes; the larger variety (Fig. 82, 1), which possessed at the anterior end a structure resembling an oral cavity, is now considered to have been *Chilomastix mesnili*, and the smaller variety (Fig. 82, 2) in which he noted an undulatory movement, was probably *Trichomonas hominis*. During the succeeding fifty years *Chilomastix mesnili* was described and figured by several investigators but always confused with some other species.



Fig. 82.—Specimens of human intestinal flagellates named by Davaine *Cercomonas hominis* var. 1 and 2. Variety 1 is apparently *Chilomastix mesnili* and var. 2, *Trichomonas hominis*. (After Davaine, 1860.)

In 1909 Alexeieff described a new genus and new species of flagellate (Fig. 85) which he found in tadpoles and in an axolotl, giving it the name *Macrostoma caulleryi*, and when Wenyon, in 1910, noted the peculiarities of *Chilomastix mesnili* he placed it in the genus *Macrostoma*. This generic name, however, had already been given to a group of mollusks by Latreille in 1825, and Alexeieff (1910) therefore substituted the name *Tetramitus* for *Macrostoma*, and suggested that if the parasitic species were different from the free-living species it should be placed in a new genus, *Chilomastix*. *Tetramitus* was a name given by Perty in 1852 to certain free-living flagellates which possess four anterior flagella, and since the human flagellate has only three anterior flagella it must be placed in the genus *Chilomastix* whose

characteristics were well defined by Alexeieff in 1912. The species has thus been described as *Chilomastix mesnili* for several years past. Recently Kofoid (1920) proposed that since Moquin-Tandon in his "Elements de Zoologie Médicale" (1860, p. 398) called the larger forms described by Davaine *Cercomonas davainei*, this specific name should be given priority over Wenyon's name *mesnili* and that the species should therefore be called *Chilomastix davainei*. It seems best at the present time, however, to retain the specific name *mesnili*. Many flagellates have been described and given new generic or specific names that were probably specimens of *C. mesnili*. Among these are *Fanapepea intestinalis* Prowazek, 1911; *Difamus tunensis* Gäbel, 1914; and *Cyathomastix hominis* Prowazek and Werner, 1914.

b. Recognition

Chilomastix has for many years been mistaken for other intestinal flagellates, since it has been reported very frequently from man during the past decade but was unknown as a distinct species previous to 1909 when Wenyon found it in the feces of an inhabitant of the Bahama Islands who was a patient in the seamen's hospital in London. Lack of good optical instruments and of proper methods of revealing the number of flagella and other structures were partly responsible for this confusion.

The only common intestinal protozoon living in man that we are liable to mistake at the present time for *Chilomastix* is *Trichomonas hominis*. The latter, however, has an undulating membrane that can be seen clearly in the living animal (Fig. 86), whereas this structure is absent in *Chilomastix*.

c. Habitat

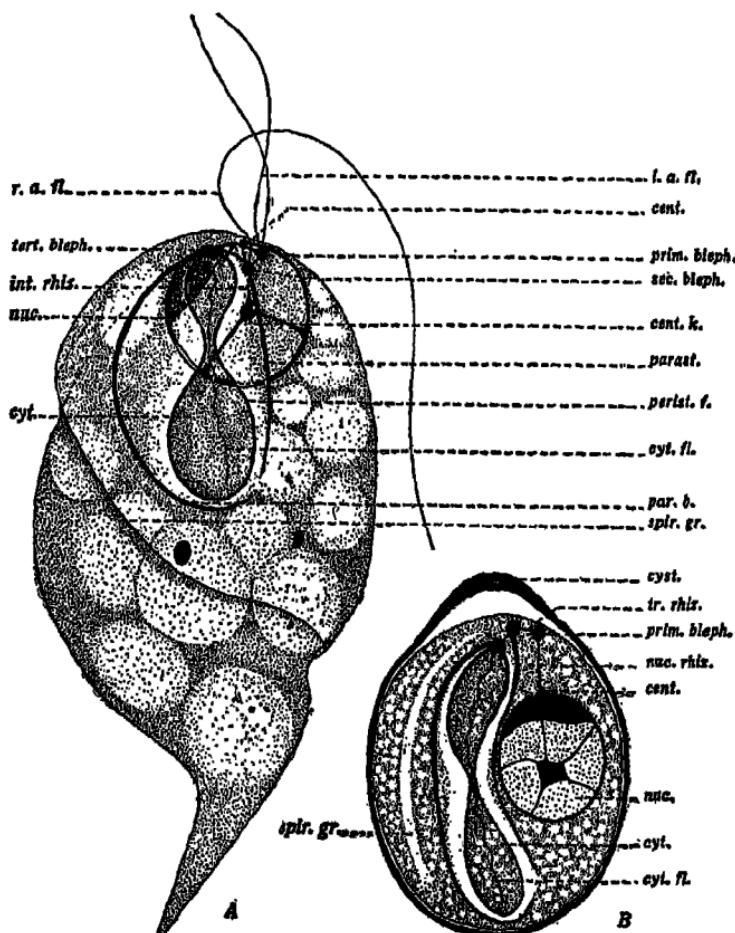
Chilomastix mesnili lives principally in the large intestine of man and appears to be widely distributed. It has been

recorded in man from Europe, Asia, Africa, Oceania, and America and probably occurs in every large land area in the world. Apparently no race of human beings is immune to it since it has been found in Europeans, Americans, Negroes, Arabs, and natives of India and Samoa. Whether the species occurring in man lives also in other animals is not certain.

d. Morphology and Physiology

(1) MOTILE FLAGELLATE (Fig. 83, A).

Living.—*Chilomastix mesnili* is pyriform in shape, rounded at the anterior end and tapering off to a spine-like process at the posterior end. Sometimes the specimens appear to possess a groove extending posteriorly from near the center of the body, and several observers have noticed animals with a distinct twist in this region of the body. The total length of the body varies according to the age and physiological condition of the individual. Average-sized specimens are from 8 to 14 microns long, others are smaller and some reach a length of 24 microns. The width is from one-half to one-fourth the total length. Three flagella extend out from the anterior end; two of these beat back against the body on one side (left) and the third against the body on the other side (right), propelling the animal forward and spirally in a jerky manner. The undulatory motion of the flagella is due to the progress of waves that begin at the point of attachment and proceed back along the flagellum. A fourth flagellum lies within the oral pouch or cytostome where it appears to be attached along the edge of an undulating membrane. The cytostome is about one-half the length of the body and lies on the right side. The mouth opening is situated at the posterior end of it and supported by a loop formed by the fibril on one side of the cytostome. The bacteria which serve as food are brought within reach of the cytostome by currents created by the beating of the flagella.

Fig. 83.—*Chilomastix mesnili*.

A, Motile flagellate illustrating organelles. The spiral groove is not ordinarily so conspicuous as indicated. $\times 6370$.

B, *Chilomastix mesnili*. Cyst. Abbreviations: cent., centrosome; cent. k., central karyosome; cyst., cyst wall; cyt., cytostome; cyt. fl., cystostomal flagellum or undulating membrane; int. rhiz., intranuclear rhizoplast; l. a. fl., left anterior flagella; nuc., nucleus; nuc. rhiz., nuclear rhizoplast; par. b., parabasal body; parasti., parastyle; perist. f., peristomal fiber; prim. bleph., primary blepharoplast; r. a. fl., right anterior flagellum; sec. bleph., secondary blepharoplast; spir. gr., spiral groove; tert. bleph., tertiary blepharoplast; tr. rhiz., transverse rhizoplast. (After Kofoid and Swezy.)

Not all of the bacteria are engulfed by the animal, and, judging from what we know of the free-living PROTOZOA, it is probable that *Chilomastix* is able to select its food. During the feeding process the posterior spine serves to anchor the animal, which then rotates as the bacteria are drawn into the oral pouch. The cytostome is considered by some observers to be an organ of attachment also and both the cytostome and the posterior spine may serve to prevent the organism from being swept out of its intestinal habitat and this consequently may account for the persistence of the infection.

The cytoplasm of *Chilomastix* is modified at the periphery of the body into a firm but elastic pellicle which allows it to change its shape quickly. Thus a specimen varies greatly at different times in length and width and may become rounded, loosing the "spine," which is drawn in. Within the cytoplasm are many vacuoles, some of which contain bacteria in various stages of digestion. Undigested particles are probably thrown off near the posterior end, there being no special organs for this purpose. Excretions due to metabolism no doubt make their way to the outside through the surface of the body and respiration is also carried on through the pellicle. The nucleus and neuromotor apparatus can only be examined in detail in specimens that are properly fixed and stained.

Fixed and Stained.—In specimens fixed in Schaudinn's solution and stained by the iron-hæmatoxylin method (Fig. 83, A) the nucleus appears as a large spheroidal body lying very close to the anterior end on the left side of the body opposite the cytostome. Within the distinct nuclear membrane is a small karyosome and several other masses of chromatin often lying at opposite ends, or the chromatin may be distributed throughout the nucleus in granular form.

According to Kofoid and Swezy (1920), the neuromotor apparatus of *Chilomastix* consists of a centrosome, three blepharoplasts, a nuclear rhizoplast, a transverse rhizoplast,

three anterior flagella, one posterior cytostomal flagellum, a parastyle, a parabasal body and a peristomial fiber. This system serves for locomotion and for obtaining food. Only in the best prepared specimens can all of these structures be made out. As indicated in the figure the centrosome is a small hemisphere flattened against the anterior end of the nucleus. The three blepharoplasts can be made out more easily in the cyst (Fig. 83, B) than in the vegetative form. The primary blepharoplast lies near the anterior end of the nucleus and gives rise to the two flagella that beat back against the left side of the body. It is connected with the centrosome by the nuclear rhizoplast and to the secondary blepharoplast by the transverse rhizoplast. The secondary blepharoplast is situated to the right and from it arises the right flagellum and the parastyle which is a slender slightly curved rod lying in the left wall of the cytostome. The tertiary blepharoplast lies at the anterior end of the cytostome and gives rise to the cytostomal flagellum, the peristomial fiber, which borders the inner free edge of the membranous lip of the cytostome, and the parabasal, a large deeply staining rod, extending along the right side and around the posterior end of the cytostome. According to Kofoid and Swezy, the parabasal is not a motor or contractile organ but is "a reserve body connected with metabolism consequent upon the motor functions of the neuromotor system."

(2) Cyst (Fig. 83, B).

Living.—Cysts are not always present in the feces, their irregular occurrence indicating a possible cyclic process of encystment such as is suspected in certain other flagellates. They may occur alone but are often accompanied by motile forms. The cysts are somewhat lemon-shaped and measure about 7 to 9 microns in length and 4 to 6 microns in width. The transparent wall is thickened at the terminal protuberance where there is a space between the wall and the cytoplasmic contents. The cytoplasm is refractile and no struc-

tures are visible within it except a few bright spots and a brownish area, the nucleus, which is visible only under favorable conditions.

Fixed and Stained.—The nucleus, cytostome, and all of the neuromotor apparatus except the three anterior flagella are retained by the cyst. They are all clearly indicated in the figure and need no further description.

e. Reproduction

(1) VEGETATIVE STAGE.

Binary Fission.—No careful study of the binary fission of the vegetative stage of *Chilomastix mesnili* has yet been made. Living specimens were observed by Boeck (1921) containing two nuclei and two cytostomes. Longitudinal ission beginning at the anterior end separated the two daughter flagellates.

Multiple Fission.—Specimens undergoing multiple fission were also observed by Boeck (1921). They were much enlarged and consisted of a somatella of four zooids, each with a cytostome and a nucleus. Further details of this type of fission in *Chilomastix* are lacking.

(2) CYST.—Mitosis within the cysts of *Chilomastix mesnili* has been described in detail by Kofoid and Swezy. First, the centrosome, blepharoplasts, and rhizoplasts divide and a darkly staining thread, the paradesmose, arises, connecting the daughter centrosomes. The other parts of the neuromotor apparatus do not divide but are duplicated by outgrowths from the blepharoplasts. The old cytostome and its accompanying organelles retain their original position whereas the newly formed structures come to lie on the opposite side of the body. During mitosis the centrosomes separate until they lie on opposite sides of the nucleus. The chromatin forms a spireme, coiled in the axis of the nucleus, which breaks up into five chromosomes, unequal in size.

These divide in the equatorial plate and five pass to either end of the nucleus which has in the meantime become spindle-shaped. The entire nucleus then divides into two equal daughter nuclei and the chromatin becomes distributed within these in the form of a karyosome near the center and other masses on the nuclear membrane. Division of the somatella has not been observed but is probably longitudinal,

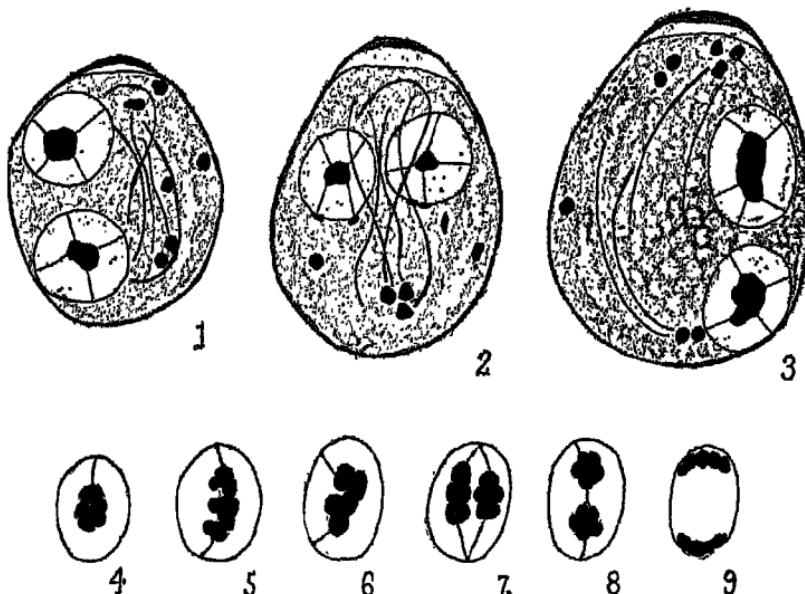


Fig. 84.—*Chilomastix mesnili*. 1-3, cysts containing two nuclei each, several blepharoplast-like bodies and a number of fibrils. 4-9, cyst nuclei in various stages of mitosis. $\times 3500$. (After Hegner.)

judging from the position of the nuclei and neuromotor apparatus. Whether more than one division takes place within the cyst is not known.

Dobell (Dobell and O'Connor, 1921) does not accept the results of Kofoid and Swezy regarding the division of *Chilomastix* within the cyst, but the writer (Hegner, 1923) has found among his preparations cysts containing two nuclei and others in which the nucleus was in various stages of

mitosis (Fig. 84). The complicated series of fibrils could not be traced with such certainty as described by Kofoid and Swezy, but the observations, as far as they were carried, confirmed their work. The chromosomes appeared to be five in number. A stage of nuclear division within the cyst has also been described by Leiva (1921) in *C. intestinalis* from the guinea-pig.

2. SPECIES OF *Chilomastix* IN LOWER ANIMALS

Chilomastix has been recorded from various lower animals and new specific names applied to the specimens from different hosts; for example, *C. caulleryi* (Alexeieff, 1909) from the tadpoles of frogs (Fig. 85),



Fig. 85.—*Chilomastix caulleryi* from tadpoles. $\times 1450$. (After Alexeieff.)

C. motella (Alexeieff, 1912) from fish, *C. intestinalis* (Kuczynski, 1914) from the guinea-pig, *C. bittencourti* (Fonseca, 1915), from the rat, *C. cuniculi* (Fonseca, 1915) from the rabbit, and *C. caprae* (Fonseca, 1915) from the goat. It remains for detailed investigations of these forms to prove that they are really different species. The "species" that has received the most attention is

C. intestinalis Kuczynski from the guinea-pig. This form was briefly described by Kuczynski (1914) and studied more in detail by Leiva (1921). It resembles *C. mesnili* in general structure but both the trophozoite and cyst are larger. The active flagellate is 13 μ to 16 μ long and 7 μ to 9 μ broad and the cyst is 7.9 μ long and 7.2 μ broad. The anterior end of the cyst is broader than that of *C. mesnili* and the whole cyst less nearly pyriform in shape.

Mitosis followed by plasmotomy has been described by Belar (1921) in *C. aulastomi* from the horse leech.

C. The Genus *Trichomonas*

I. INTRODUCTION

Species of the genus *Trichomonas* have been described from the mouth, intestine, and vagina of man. The genus was established by Donné in 1837 with *T. vaginalis* as the type species. Donné was unable to determine accurately the number of anterior flagella, but Künstler (1884) states that there are four, and Lynch (1915) also gives this number as present both in specimens from vaginal mucus and from cultures. Human trichomonads with three and five anterior flagella have also been reported. Whether these should be considered separate species or subspecies or varieties is at present in doubt, and the final decision must depend on the results of genetical studies of specimens grown in culture. The number of flagella in other flagellates is, however, a rather constant character and the evidence at present indicates the ultimate establishment of as many species as there are variations in the number of flagella. If we adopt the flagellar number as a specific criterion the generic name for trichomonads with four flagella is therefore *Trichomonas* since the species described by Donné had four flagella and the generic name *Tetratrichomonas* (Parisi, 1910) must be abandoned. The smaller of the two intestinal forms called *Cercomonas hominis* by Davaine (1860) was no doubt a *Trichomonas* (Fig. 82, 2). After giving the large form of Davaine's *Cercomonas hominis* the name *Chilomastix mesnili*, the smaller variety must receive the specific name *hominis*. Human intestinal trichomonads have been described with three, four, and five flagella. Those with three flagella belong to the genus *Tritrichomonas* (Kofoid, 1920), those with four to the genus *Trichomonas* (Donné, 1837), and those with five to the genus *Pentatrichomonas* (Chatterjee, 1915).

2. TRITRICHOMONAS AUGUSTA

The trichomonads living in man are difficult to prepare so as to bring out their structure in detail. For this reason a description of a species that occurs in the intestine of frogs, toads, and salamanders will first be presented, since the species living in man and in other animals are similar in most respects to that of the frog. *Tritrichomonas augusta* Alexeieff of the frog has been very carefully investigated by Kofoid and Swezy (1915), from whose studies the following account has largely been prepared.

a. Recognition

Tritrichomonas may be distinguished in the living condition (Fig. 86) from other intestinal flagellates by the pres-

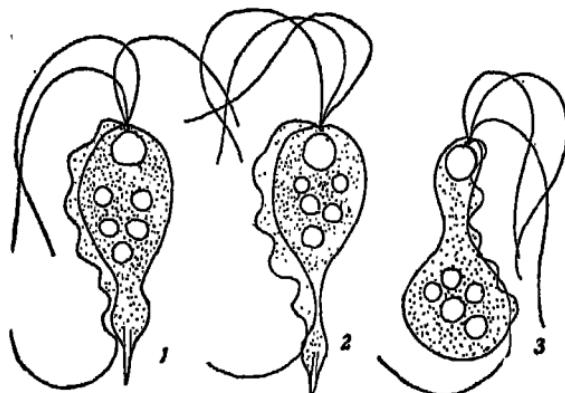


Fig. 86.—*Tritrichomonas augusta*. Camera lucidia drawings, $\times 1500$, showing the changes in shape of a single living specimen. (After Kofoid and Swezy.)

ence of (1) an undulating membrane, (2) the marginal flagellum which becomes free on one side near the posterior end and (3) frequently a portion of the axostyle projecting from or near the posterior end. In preparations properly fixed and stained these structures as well as three anterior flagella can be made out (Fig. 87).

b. Habitat

Tritrichomonas augusta is most abundant at the junction of the large and small intestine and the upper part of the rectum.

The incidence of infection is as high as 88%, at least in certain localities. This species is very resistant and is able to live outside of the host for considerable periods. Apparently normal specimens were found by the writer on three out of five occasions in distilled water in which living frogs had been kept for 18 hours. Others were kept alive in normal saline solution under a sealed cover glass for 70 days and would undoubtedly have lived longer if the preparation had not been disturbed; and Kofoid and Swezy kept specimens in culture for six months.

c. Morphology and Physiology

Cysts of trichomonads have been described by many investigators, but these are now known to be cysts of other protozoa, *Blastocystis*, or other bodies, and true cysts are still unknown.

(1) LIVING FLAGELLATE.—Living specimens are typically pear-shaped but the cytoplasm is extremely mobile and changes in shape are frequent and extensive (Fig. 86). Pseudopodia-like projections are common especially near the posterior end. A thin pellicle is probably present.

Living specimens vary remarkably in size, ranging from 11 μ to 50 μ in length; most specimens are 18 μ to 28 μ long and 8 μ to 15 μ broad. These great variations may be due in part to the presence of races that are heritably different in size; they also undoubtedly represent different stages in growth, the smallest specimens resulting from rapid binary fission or from multiple fission. There is no evidence that specimens of different sizes or shapes are sexually differentiated as males or females.

The anterior flagella are three in number, of equal length

and about as long as the body. They beat backward together, drawing the body forward in a jerky fashion and causing it to rotate as it moves through the medium. The fourth flagellum runs along the edge of the undulating membrane, becoming free near the posterior end of the body, the free part being as long as or longer than the body.

Of particular interest is the undulating membrane. This seems to consist of cytoplasm that is tough but mobile like that of the pellicle, being very constant in extent but easily thrown into waves by the marginal flagellum. No one knows exactly what effect its movement has nor how great its importance is as a locomotor organ.

Tritrichomonas augusta possesses at the anterior end on the ventral surface opposite the undulating membrane a cytostome shaped like a broad comma and extending posteriorly about one-fourth the length of the body. Some species of *Tritrichomonas* have many bacteria within their cytoplasm but only rarely is this true in *T. augusta*. Staining reactions indicate that the vacuoles scattered throughout the cytoplasm have an alkaline content and suggest that some form of alkaline liquid food such as the dissolved proteins in the intestine serves as nourishment in this form. These vacuoles do not move over a definite course within the body as do those of certain free-living PROTOZOA such as *Paramecium* but are forced about by changes in the shape of the organism.

(2) STAINED FLAGELLATE.—Within the body are various structures that can be seen clearly only in specimens properly fixed and stained (Fig. 87). These are the nucleus, axostyle, parabasal body, blepharoplast, rhizoplast, and chromidia.

The nucleus is a spheroidal body situated near the anterior end, often being partly hidden by the anterior enlargement of the axostyle. It contains chromatin usually in the form of a central karyosome and a number of scattered granules.

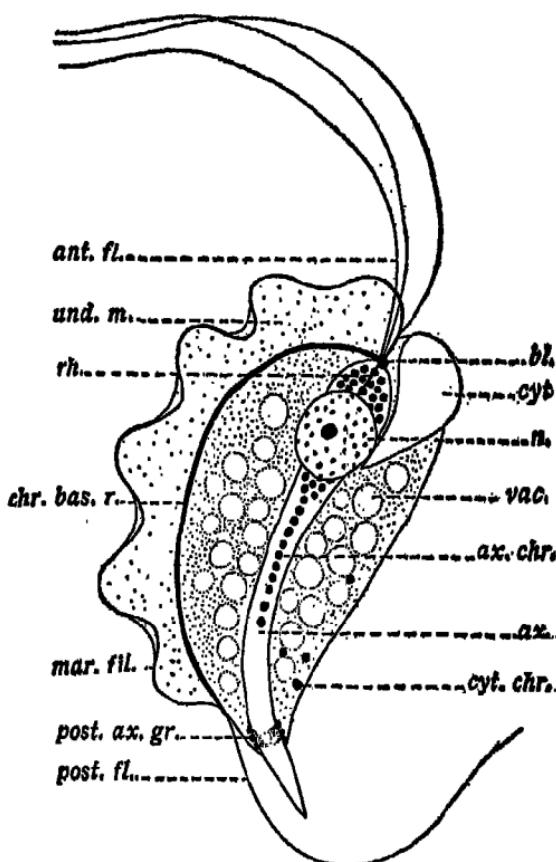


Fig. 87.—*Tritrichomonas augusta*. Diagrammatic figure, $\times 1500$, showing organelles. (After Kofoid and Swezy.)

Ant. fl., anterior flagella; ax., axostyle; ax. chr., axostylar chromidia; bl., blepharoplast; chr. bas. r., chromatic basal rod or parabasal body; cyt., cytostome; cyt. chr., cytoplasmic chromidia; mar. fil., marginal filament; n., nucleus; post. ax. gr., posterior axostylar granules; post. fl., posterior flagellum; rh., rhizoplast connecting blepharoplast and nucleus; und. m., undulating membrane; vac., food vacuole.

The axostyle is a subcylindrical rod of homogeneous hyaline substance extending from the anterior end, where it is enlarged, through the axis of the body and usually projecting a short distance from the posterior end where it terminates in a point. Within the substance of the axostyle are from 15 to nearly 100 chromidial granules, and in many specimens there are from one to three pairs of these granules situated at the periphery of the axostyle at the point where it emerges from the body at the posterior end. The function of the axostyle is problematical. Many investigators consider it simply an axial skeletal support; others attribute to it the rôle of an organ of fixation, having seen the organism attached to the substratum by its posterior end. Kofoid and Swezy (1915), however, consider it "a powerful motor organ which comes into function when the animal is on a substrate, and doubtless plays an important part in the life of the organism in the mucus of the intestinal surface" (p. 311). Its movements can best be seen in specimens in fresh material from the mucous surface of the intestine. In such preparations, when very little fluid is added, the axostyle may be seen to bend and curve like a flagellum as it lashes from side to side. It thus serves as an intracytoplasmic flagellum.

At the anterior end of the axostyle is a spheroidal body about $0.5\ \mu$ in diameter known as the blepharoplast. From this body arise the three anterior flagella, the fourth flagellum that runs along the margin of the undulating membrane, and the chromatic basal rod or parabasal body. It is connected with the nucleus by a rhizoplast.

The parabasal body extends from the blepharoplast along the base of the undulating membrane throughout its entire length. It stains deeply in chromatin dyes and appears to have some function connected with the metabolism and control of the motor activity of the organism.

Within the cytoplasm of many specimens are a variable

number of chromatin granules (*cyt. chr.*) of unknown origin and function.

d. Reproduction

(1) BINARY FISSION (Fig. 88, a, b, c).—Binary fission in trichomonads has been described in great detail by Kofoid and Swezy (1915) in *Tritrichomonas augusta*. This process usually occurs in large specimens. The nucleus begins to divide by a primitive form of mitosis and not amitotically as had been described previously by Martin and Robertson (1911). During division the nuclear membrane remains intact, the chromatin breaks up into a chromidial cloud and then condenses into a ragged chromatin thread. From this thread five chromosomes are formed, one large, two medium and two small. These then split longitudinally into two each. Only a very feebly developed achromatic spindle is developed, but one-half of each of the five daughter chromosomes becomes located at either end of the nucleus which has become spindle-shaped. The nucleus finally constricts into two, in each of which the chromatin becomes rearranged as in the nucleus of the typical flagellate form. During nuclear division the other organelles become duplicated, part of them dividing and others arising from the blepharoplasts. The flagellum along the undulating membrane is the first of these organelles to divide. It begins to split at the anterior end, the split extending gradually posteriorly until it also involves the free part of this flagellum. Soon the undulating membrane divides, beginning at the anterior end; a new parabasal body grows out of the blepharoplast, and a new flagellum also. The blepharoplast then divides, one-half taking with it two of the old flagella, and the other half the third old flagellum and the newly formed one. The daughter blepharoplasts now separate and take up positions at opposite ends of the nucleus and between them appears a deeply staining thread, the paradesmose, which later fades away and

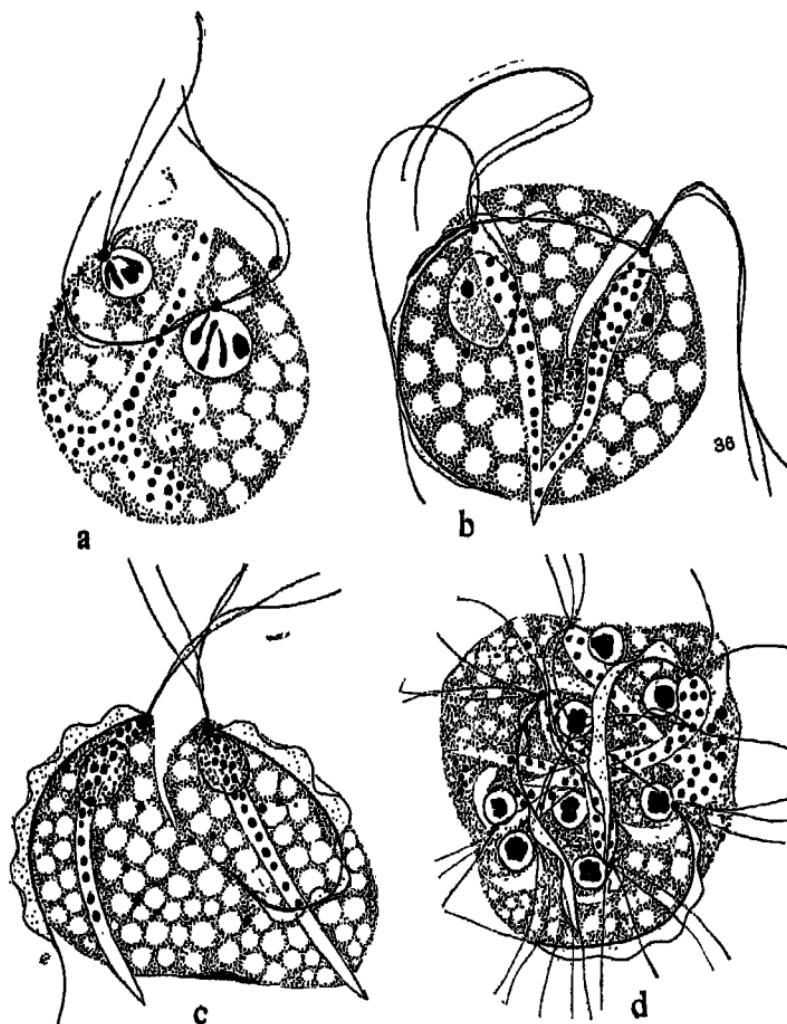


Fig. 88.—*Trichomonas augusta*. Stages in binary and multiple fission. a, Binary division with nucleus divided showing chromosomes, and axostyle beginning to divide. b, Later stage in binary division. c, Two daughter cells about to separate. d, Multiple fission: a somatella with eight nuclei and four axostyles. $\times 2175$. The ends of the flagella in c and d are not shown. (After Kofoid and Swezy.)

disappears. A third flagellum grows out from each blepharoplast, thus completing the definite number. Each blepharoplast then divides into a centrosoine and a basal granule, but neither at this time nor during the division of the original blepharoplast into two is there any evidence of mitosis which would sustain the contention of those who believe that this structure is a second nucleus. The axostyle is the last organelle to begin division. A split appears at the anterior end and proceeds posteriorly until two axostyles are formed. Various positions are assumed by the group of organelles during the process of binary fission and the cell undergoes many twistings and contortions before it finally divides four or five hours later into two. Division is longitudinal as is characteristic of other flagellates. The division of *Tritrichomonas augusta* is mitotic but differs in certain particulars from the usual type of cell division in metazoan cells. The nuclear membrane remains intact, no astral systems of fibers are formed, and the extranuclear apparatus complicates the process; but chromosomes are formed, split longitudinally into halves and migrate to opposite poles of the spindle as they do in the metazoan cell, and this equal division of chromatin is after all the most important result of mitosis.

(2) MULTIPLE FISSION (Fig. 88, d).—The division of a single trichomonad into more than two daughters is now known to be a normal process in the life-history of a number of species. Both binary fission and multiple fission may occur in the same preparation and the first division in the latter is indistinguishable from binary fission. If the specimen is undergoing multiple fission two succeeding divisions follow the first, resulting in a somatella consisting of eight merozoites. Each merozoite includes the various organelles characteristic of a typical flagellate. The axostyle, however, does not divide as rapidly as the other organelles, and somatellæ with four or eight nuclei and only two or four axostyles

are not unusual. After the merozoites are all completely formed they separate from the somatella one by one, this

being aided by twisting and rotating movements. The detached merozoites cannot be distinguished from specimens produced by binary fission.

The identification of the chromatic basal rod as a parabasal body by Kofoid and Swezy has been brought into question by Wenrich (1921) who has made a careful study of the trichomonas of the mouse, *Tritrichomonas muris*. As shown in figure 89, a club-shaped body is considered by Wenrich to be the parabasal body. This body appears in material fixed with osmic or chromic acid, but is absent from specimens fixed in Schaudinn's solu-

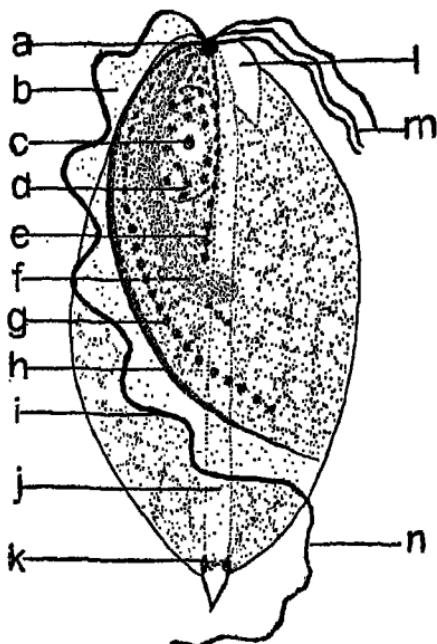


Fig. 89.—*Tritrichomonas muris*. Partly diagrammatic figure showing a, blepharoplast; b, undulating membrane; c, karyosome; d, nucleus; e, inner row of chromatic granules; f, parabasal body; g, outer row of chromatic granules; h, chromatic basal rod; i, posterior flagellum as chromatic margin of undulating membrane; j, axostyle; k, chromatic ring at point of emergence of axostyle; l, cytostome; m, anterior free flagella; n, posterior free flagellum. (After Wenrich.)

sion. Wenrich differs also from Kofoid and Swezy in his description of certain features of the division process. He records six chromosomes in *Tritrichomonas muris* instead of

five as reported by the latter in the same species; and states that the new axostyles grow out from the blepharoplasts instead of resulting from the division of the old axostyle.

3. TRICHOMONAS VAGINALIS

Trichomonas vaginalis (Fig. 90) is the type species of the genus *Trichomonas*. It was described and figured by Donné in 1837 in his "Recherches microscopique sur la nature du mucus," having been found in abundance in vaginal mucus. It has since been recorded from various localities in Europe, in Ceylon, equatorial Africa, America, etc.

Ten per cent of the women examined at a gynecological clinic in Paris were found to be infected (Brumpt, 1913) and careful and extensive studies elsewhere would probably reveal a larger incidence of infection than is usually supposed to exist. *T. vaginalis* has been seen by the writer and has been reported by others in the urine of women and also in the urine of men (Dock, 1891, Fonseca, 1916). Donné saw only a single flagellum. Blochmann, who reexamined this species in 1884, records three anterior flagella, but Künstler in the same year made a more careful study observing four anterior flagella, a blepharoplast, undulating membrane, cytostome, parabasal body, nucleus and axostyle. Bensen (1910) found only three anterior flagella and no axostyle in the specimens he studied. According to him there are present two basal granules (blepharoplasts) from one of which the undulating membrane arises and from the other the three anterior flagella. A rhizoplast connects the nucleus

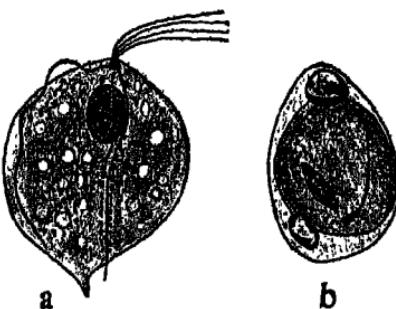


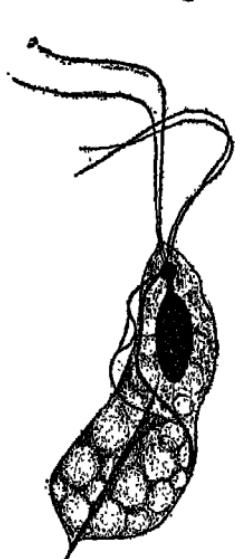
Fig. 90.—*Trichomonas vaginalis*. a, Normal flagellate. b, Flagellate engulfed by a phagocyte. $\times 1330$. (Modified after Brumpt.)

with the flagella-bearing basal granule. Lynch (1915) records four flagella for *T. vaginalis* and considers it the same species as that found in the mouth. Goodey and Wellings (1917), however, think these two species are distinct and it is probably best to accept them as separate species until more is known about them.

Trichomonas vaginalis ranges in size from 12μ to 26μ in length and from 6μ to 18μ in width.

4. TRICHOMONAS BUCCALIS

Trichomonads have been found in the human mouth by various investigators. The earliest record is that of O. F.



Müller (1773) (after Kofoid, 1920), who found in material from the tartar between human teeth small organisms which were probably flagellates belonging to this group. These organisms were rediscovered by Mandl (1838-1845). Since then a number of investigators have found flagellates in the human mouth, all belonging to one species so far as can be determined from the literature. This species has four flagella and may be the same as *Trichomonas vaginalis*, but it seems best at present to consider it provisionally as a separate species. Wenyon and O'Connor (1917) have reported a case in which there were only three anterior flagella present instead of the usual four; the fact that many specimens in this case must have been swallowed without establishing an intestinal infection indicates that this mouth species probably differs from that in the intestine.

Fig. 91.—*Trichomonas buccalis*. $\times 4100$. (After Goodey and Wellings.)

Goodey and Wellings in 1917 gave the name *Tetratricho-*

monas buccalis to the mouth-inhabiting trichomonad. The specific name *buccalis* stands but the generic name *Tetra* must be abandoned; the correct name of the species from the human mouth is *Trichomonas buccalis* Goodey and Wellings, 1917.

Trichomonas buccalis (Fig. 91) has not been studied in detail. It lives in the mucus and tartar between human teeth. In shape it resembles other human species. According to Goodey and Wellings (1917) specimens taken from the mouth and fixed and stained are 7 μ to 12 μ in length. There are four anterior flagella, 14 μ to 16 μ in length; two of these are longer than the other two. An undulating membrane with a marginal flagellum arises from the blepharoplast and ends posteriorly about two-thirds to three-fourths the length of the body. No free posterior flagellum or only a very short one is present. The nucleus is oval or elongated with a distinct karyosome and diffuse chromatin. The nucleus is connected with the blepharoplast by a rhizoplast. The parabasal body is not always as well developed as in the intestinal form. An axostyle is present projecting about 5 μ from the posterior end; this is not clear and refractile as in other species, but stains more deeply than the cytoplasm. Many bacteria are present in the cytoplasm; these are probably engulfed through a cytostome. Ohira and Noguchi (1917) observed both binary longitudinal fission and multiple fission in cultures. Of particular interest is their statement that the blepharoplast divides by mitosis. The trichomonad from the mouth seems to differ from other species in the following respects: there is a very short or no posterior flagellum; the parabasal body is not always well developed; the chromatin in the nucleus is diffuse; and the axostyle stains more deeply than the cytoplasm.

5. TRICHOMONAS HOMINIS

This species (Fig. 92), which is also known by the name *Trichomonas intestinalis*, is probably the organism described

as a small variety of *Cercomonas hominis* by Davaine (Fig. 82, 2). There has been a large number of reports on this species but none of them is sufficiently detailed, probably on account of its small size and the difficulty in obtaining satisfactory preparations. It has been recorded from many countries and is evidently world-wide in its distribution. Much confusion has resulted from the discovery of trichomonads

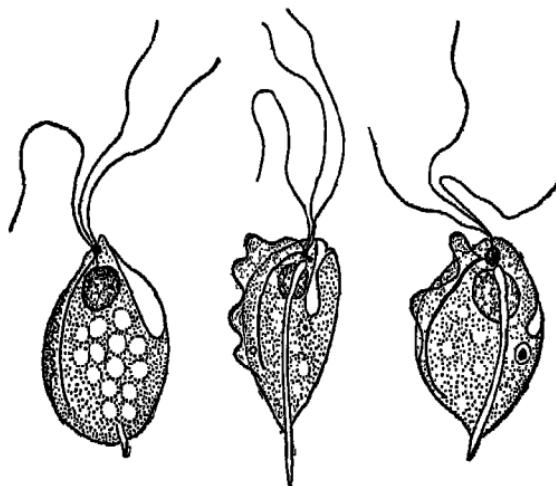


Fig. 92.—*Trichomonas hominis*. The specimens shown here have three anterior flagella and hence belong to the genus *Tri* if the number of flagella is considered of generic value. $\times 2300$. (After Faust.)

in human stools with three, four, and five flagella (see page 211).

Trichomonads have been reported from the intestine of man from practically every region where they have been looked for. In most cases the number of flagella has not been definitely ascertained, and no doubt many of the records do not distinguish between *Trichomonas* and *Chilomastix*. The commonest type noted seems to be that with four flagella, hence belonging to the species *Trichomonas hominis*. This species is smaller than that in the mouth, *T. buccalis*, or that

in the vagina, *T. vaginalis*, and is more difficult to study. It varies considerably in size, usually measuring from $10\ \mu$ to $15\ \mu$ in length and from $3\ \mu$ to $5\ \mu$ in breadth. It resembles the other species in shape and structure, being pyriform in shape and possessing an undulating membrane with a marginal flagellum that becomes free near the posterior end, a nucleus, an axostyle, a blepharoplast, a parabasal body, and a cytostome. No bodies that can with certainty be called cysts of *Trichomonas hominis* have yet been identified. Organisms appear in the stools of infected persons intermittently but may be present daily in one patient for at least three weeks and may be found in the stools of one case for several months or more. Binary fission has been observed, the division beginning at the anterior end, and multiple fission has also been reported.

Human intestinal trichomonads with five anterior flagella have been encountered by only a few investigators. Derrieu and Raynaud reported specimens from Algiers in 1914; Chatterjee (1915, 1917) has recorded this organism in thirty cases of chronic dysentery in Bengal; Wenyon and O'Connor (1917) cite one case in the Near East; and Haughwout and de Leon (1919) found one case of this infection in Manila. *Pentatrichomonas* resembles the other forms in shape and averages from $8\ \mu$ to $15\ \mu$ in length and from $5\ \mu$ to $13\ \mu$ in width. The flagella are from $8\ \mu$ to $17\ \mu$ long. Haughwout and de Leon observed in some of their specimens (Fig. 93) a row of chromatinic granules near and parallel to the parabasal body and noted the absence of such granules from the axostyle. Perhaps the most interesting fact about this pentaflagellate is its habit of ingesting red blood cells, as reported by both Chatterjee and Haughwout and de Leon. Over one hundred specimens were encountered by the latter containing one or more erythrocytes of various sizes, evidently in different stages of digestion; this is thus not adventitious but a normal process in the feeding habits of the organism.

One specimen was seen to devour three erythrocytes within an hour. Whether this characteristic is peculiar to this species and accounts for the dysenteric condition of all of the infected persons thus far examined remains to be determined.

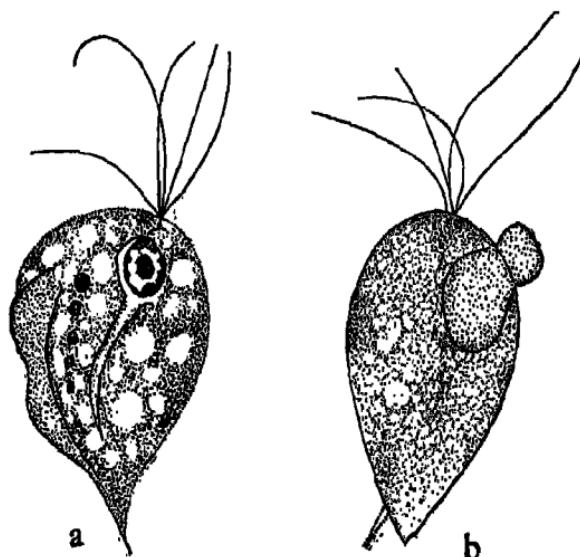


Fig. 93.—*Pentatrichomonas*. a, Specimen showing morphology. b, Specimen ingesting an erythrocyte. (After Haughwout and de Leon).

6. TRICHOMONADS OF LOWER ANIMALS

Flagellates that have been placed in the genus *Trichomonas* have been described from many of the lower animals; their specific names have usually been derived from that of the genus to which the host belongs. For example, in mammals there are *T. muris* from the rat, *T. caviae* from the guinea-pig, and *T. suis* from the pig; in amphibians, *T. augusta* and *T. batrachorum*; in reptiles, *T. lacertæ*; and in birds, *T. columbarium*.

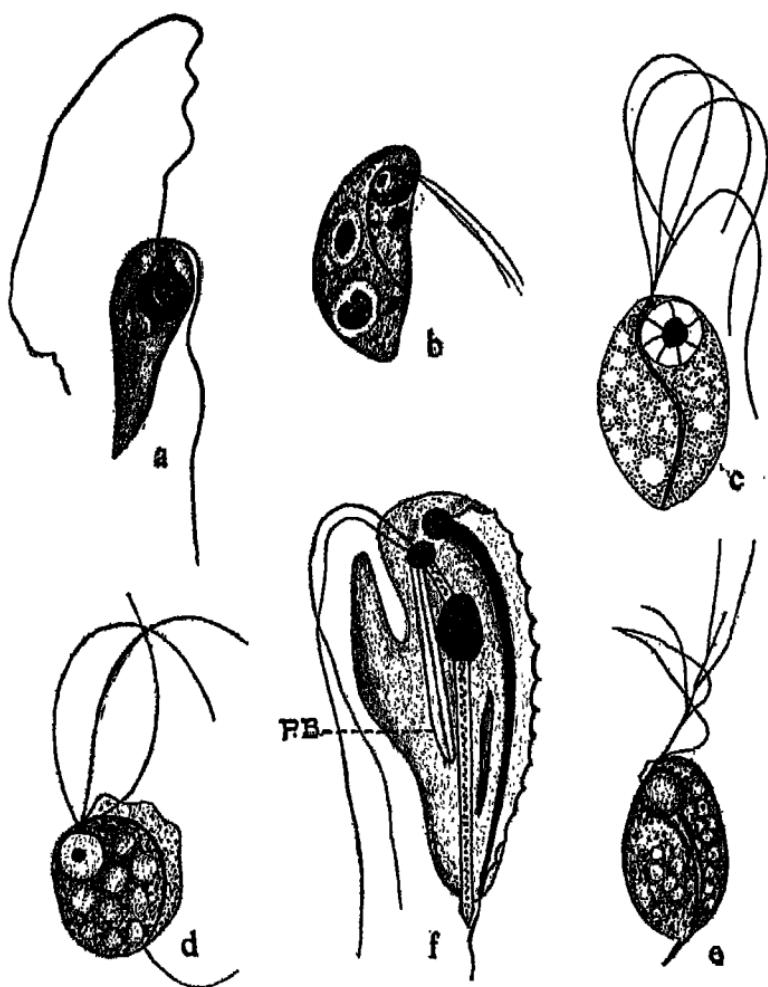


Fig. 94.—Intestinal flagellates of lower animals.

a, *Prowazekella lacerta* from reptiles and Amphibia. b, *Protrichomonas legeri* from a fish. $\times 150$. c, *Monocercomonas melolonthae* from a salamander. $\times 2480$. d, *Trichomititus parvus* from Amphibia. $\times 2583$. e, *Hexamastix batrachorum* from a salamander. $\times 1500$. f, *Ditrichomonas termitis* from termites. (a, after Kuhn; b, e, after Alexeieff; c, d, after Swezy; f, after Cutler.)

Tritrichomonas muris, Hartmann 1910 (Fig. 89), is one of the most easily obtainable trichomonads of lower animals other than *T. augusta* in the frog. It is a species abundant in the cæcum of rats and mice. Some of its characteristics are as follows: it is more rotund than the human and amphibian species; 10 μ to 16 μ in length and 5 μ to 11 μ in breadth; three anterior flagella; undulating membrane wide; axostyle usually curved; no axostylar chromidia; a row of 8-16 large cytoplasmic chromidial granules parallel to the chromatic basal rod. Wenrich (1921) mentions both outer and inner rows of chromatic granules and both a chromatic basal rod and a parabasal body. Both binary and multiple fission have been observed in this species.

Among the flagellates closely allied to *Trichomonas* are certain genera and species that exhibit interesting variations in structure. Several of these are illustrated in figure 94.

Prowazekella lacertæ (Fig. 94, a) occurs in certain reptiles and amphibians; has two flagella, one directed forward, the other backward; a spherical nucleus; two blepharoplasts connected with the nucleus by a rhizoplast; and a parabasal body.

Protrichomonas legeri (Fig. 94, b) inhabits the intestine of a marine fish, *Bor salpa*. Near the anterior end of this species is an ovoid nucleus near which are three blepharoplasts. From these blepharoplasts three free flagella arise and a curved rod resembling somewhat the chromatic basal rod of *Trichomonas*, but which may be an axostyle.

Monocercomonas melolonthæ (Fig. 94, c) has been recorded from various insects and amphibians. It has four free anterior flagella, a vesicular nucleus, and an axostyle.

Trichomitus parvus (Fig. 94, d) is a species that occurs in the intestine of certain amphibians. It has three free anterior flagella that arise from a blepharoplast situated on the surface of a vesicular nucleus; an undulating membrane bordered by a flagellum which becomes free near the

posterior end; a chromatic basal rod at the base of the undulating membrane; but no cytostome nor axostyle are present.

Hexamastix batrachorum (Fig. 94, e) lives in the intestine of salamanders. It possesses an axostyle and six anterior flagella which are more or less fastened to one another near

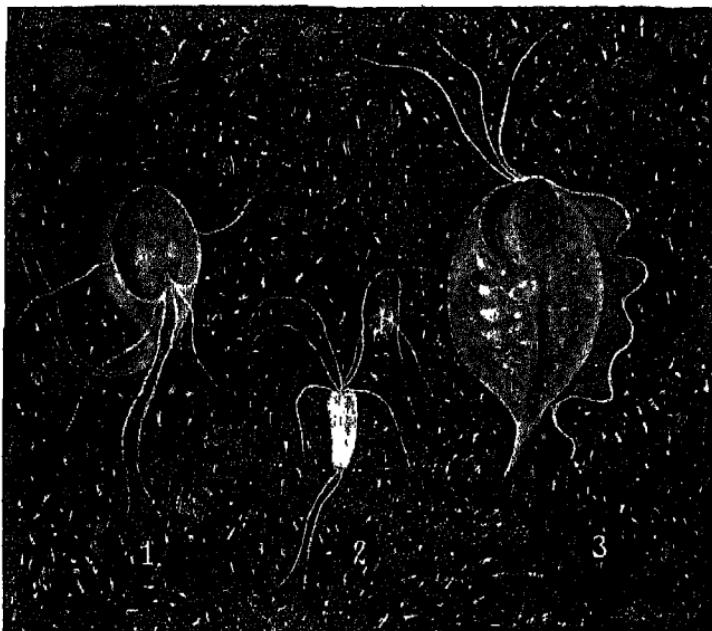


Fig. 95.—Flagellates, from the intestine of the rat, as seen when alive. 1, *Giardia muris*. 2, *Hexamitus muris*. 3, *Tritrichomonas muris*. (After Hegner.)

their proximal ends; one flagellum forms what appears to be a minute undulating membrane.

Ditrichomonas termitis (Fig. 94, f) is a large slender species that lives in the intestine of certain termites. Its average size is 50μ in length and 22μ in breadth. Only two anterior flagella are present. Specimens prepared by certain methods exhibit bodies of a mitochondrial nature

near the axostyle in the neighborhood of the nucleus. The "parabasal body" of Kofoid is a distinct rod called by Cutler (1919) "the chromatic base of the undulating membrane," and the term parabasal is applied by him to another structure. The nucleus is oval in shape and completely filled with chromatin. Binary longitudinal division occurs.

D. The Genus *Embadomonas*

The genus *Embadomonas* was established in 1911 by Mackinnon for a flagellate (*E. agilis*) living in the intestine of certain caddice fly larvae. The following year (Mackinnon, 1912) a second species, *E. alexcieffi*, was found in the intestine of crane-fly larvae. A species inhabiting the human intestine was discovered in 1917 by Wenyon and O'Connor in two persons in Alexandria, Egypt, and named by them *Waskia intestinalis*. It was soon pointed out (Chalmers and Pekkola, 1918) that *Waskia* was a synonym of *Embadomonas* and this human species has since been known as *Embadomonas intestinalis*. This flagellate is apparently of wide distribution but nowhere very abundant. It has been reported from Egypt (Wenyon and O'Connor, 1917), England (Broughton-Alcock and Thomson, 1922) and the United States (Kofoid, Kornhauser, and Plate, 1919; Hogue, 1921). Faust and Wassell (1921) have described from China what they believe to be another species from man, *Embadomonas sinensis*.

Embadomonas intestinalis (Fig. 96) "is a small active oval organism which dances about amongst the faecal matter by means of its two flagella of different strength and action. The long, thin, anterior flagellum lashes about continuously and propels the flagellate through the liquid, while the stouter and shorter flagellum which projects from the cytostome may work either regularly, but at a different rate from the anterior one, or irregularly with periods of rest alternating

with periods of activity. This independent action of the stout cytostome flagellum, especially when its action is intermittent, gives a peculiar jerky movement to the anterior end of the flagellate as it swims forward under the regular action of the long anterior flagellum. In cover-glass preparations the flagellate has a peculiar habit of applying itself to the surface of the cover-glass or slide to which it adheres. In this position the action of the two flagella can be easily studied, and it is in this side view attitude that the flagellate reminds one so forcibly of the body outline of a bird." (Wenyon and O'Connor, 1917, p. 87.)

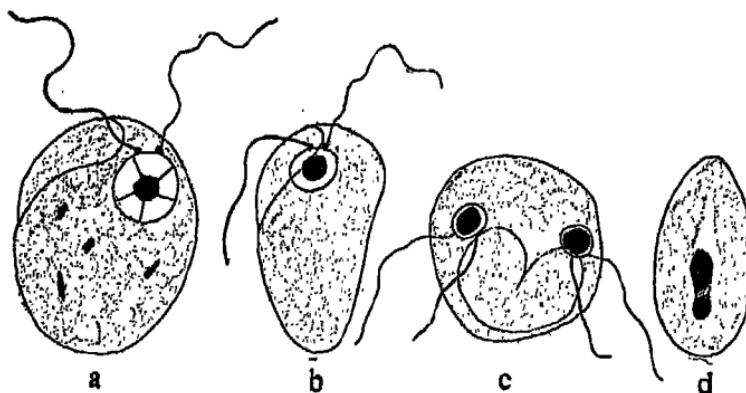


Fig. 96.—*Embadomonas intestinalis*. a and b, Motile flagellates. c, Flagellate undergoing binary fission. d, Cyst. $\times 4800$. (Original. From slides prepared by Dr. M. J. Hogue.)

This species ranges in length from 3.5μ to 9μ , and in breadth from 1.5μ to 6μ . Most of the specimens seem to be from 5μ to 6μ long and from 3μ to 4μ broad. On one side near the anterior end is a large cytostome which in stained specimens (Fig. 96) is seen to be supported by a fiber. The single nucleus situated near the anterior end of the body is spherical and has a distinct membrane and a central karyosome. On the anterior surface of the nucleus are two blepharoplasts from each of which arises a flagellum.

One of these flagella is thin, about as long as the body and directed anteriorly; the other is shorter and stouter and extends out through the cytostome. The food of *E. intestinalis* consists of bacteria, and often many food vacuoles are present in the cytoplasm.

Multiplication of the trophozoite is apparently by longitudinal binary fission (Fig. 96, c). Stages have also been observed that suggest multiple fission (Broughton-Alcock and Thomson, 1922).

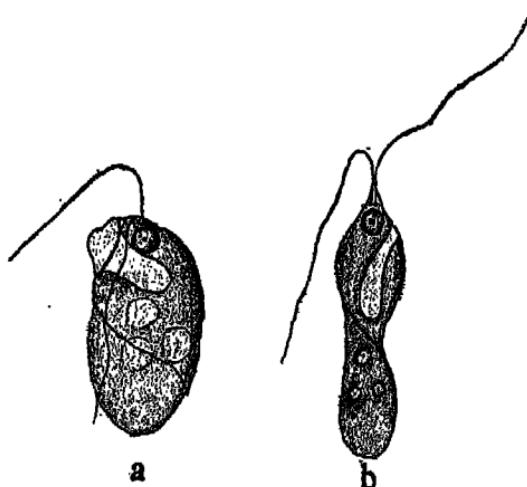


Fig. 97.—*Embadomonas simensis*. a. Quiescent form. b. Actively motile specimen. (After Faust.)

Cysts that they thought were probably of *E. intestinalis* were found by Wenyon and O'Connor in the stools of their two infected patients. That these were really cysts of this species was proved by Hogue (1921), who was the first to cultivate this species of flagellate and who found similar cysts in cultures containing no other protozoon. The cysts (Fig. 96, d) are pear-shaped and range from $4\ \mu$ to $9\ \mu$ in length and from $2.5\ \mu$ to $4.8\ \mu$ in breadth. Within the cyst is a single nucleus and the remains of the fiber that supports the cytostome.

Embadomonas sinensis (Fig. 97) is the name applied to a flagellate observed by Faust and Wassell (1921) in the diarrheic stools of 9 out of 27 Chinese examined in the Church General Hospital, Wuchang, during July, 1921. This is described by Faust (1922) as a very active species, with very plastic protoplasm resulting in great changes in shape. The body may be ovoidal when quiet or elongated and eugleniform when actively progressing. The motile forms (Fig. 97, b) average 14 μ long and 4.2 μ broad; quiescent forms (Fig. 97, a) are 10 μ long and 7 μ broad and attenuated eugleniform specimens may be 20 μ long. The cytostome is broad and pouch-like in the quiescent specimens. The flagella, which are not so markedly different as those of *E. intestinalis*, arise each from a blepharoplast; these, however, are some distance from the nucleus. The nucleus resembles that of *E. intestinalis* but is smaller. Division of the trophozoite is by longitudinal fission, the posterior end of the animal splitting before the anterior organelles are completely divided. The cysts are ovate-elongate and 6 μ long by 3 μ broad.

Several species of the genus *Embadomonas* have been recorded from lower animals. *E. agilis*, the type species (Mackinnon, 1911), lives in the larvæ of caddice flies. This species is small and has a nucleus with a group of chromatin granules in the center. *E. alexeieffi* (Mackinnon, 1912) resembles *E. agilis* but is a larger form and occurs in the larvæ of crane flies. *E. belostomæ* was found by Brug (1922) in the cecal appendage of the bug, *Belostoma indicum*; it also appears from Brug's figures to be a large species measuring from 16 μ to 26 μ long and from 4.5 μ to 6 μ broad. The cysts of all these species resemble those of the human forms. *E. wenyonii* is a species found by Fonseca (1917) in the cecum of a Brazilian monkey, *Cebus caraya*.

E. The Genus *Enteromonas*

The human flagellate, *Enteromonas hominis*, was discovered and named by Fonseca in 1915. It occurred in the stools of a child in Brazil suffering from enteritis. Some confusion exists regarding its exact morphology but this has in part been cleared up by Dobell and O'Connor (1921). At least six reported species of flagellates that have been referred to as many different genera have been grouped together by these authors in the single species *Enteromonas hominis*. These are *Enteromonas hominis* (Fonseca, 1915), *Octomitus hominis* (Chalmers and Pekkola, 1916), *Tricercomonas intestinalis* (Wenyon and O'Connor, 1917), *Trichomastix hominis* (Chatterjee, 1917), *Dicercomonas soudanensis*=*Diplocercomonas soudanensis* (Chalmers and Pekkola, 1919a, 1919b) and *Enteromonas bengalensis* (Chatterjee, 1919). Whether these will eventually be retained as a single species or separated into several species or genera depends on the results of further investigation. At present the information available is too fragmentary to warrant the recognition of the genera and species as reported by various authors and it seems best to group them all together as one species. The various flagellates listed above have been found in Brazil, Egypt, the Soudan, India, Samoa, Germany, England and the United States. Wenyon and O'Connor (1917) found about a dozen cases in the Near East, in which all flagellates disappeared in a few days; Jepps (1921) recorded one case among 917 men examined in England, and O'Connor (1922) observed this species in 13 (5.8%) of 229 persons examined in the Samoan group of islands.

The best available account of this flagellate is that of Wenyon and O'Connor (1917). These authors studied carefully living and stained specimens of both trophozoites and cysts.

The living trophozoites (Fig. 98, a) are very active and dance about rapidly in the medium. They are spherical or ovoidal in shape and capable of rapid changes, being to a certain extent amoeboid. They measure from 4μ to 8μ in length. No cytostome is visible but bacteria in food vacuoles are present within the body, hence solid particles apparently are ingested. The number and location of the flagella vary according to the descriptions of different investigators. Wenyon and O'Connor describe three free anterior flagella and one posteriorly directed flagellum that is attached

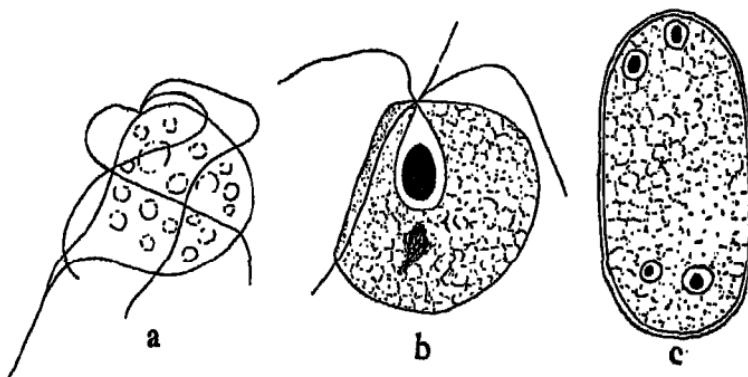


Fig. 98.—*Enteromonas hominis*. a, Motile flagellate as seen when alive. b, Motile flagellate, stained. c, Cyst, stained. (After Wenyon and O'Connor.)

throughout part of its length to a flattened side of the body and extends out freely behind (Fig. 98, b). Fonseca (1920) records only three flagella, two of which are short and anterior, the third longer and directed posteriorly but not attached to the body. The nucleus is near the anterior end and is of the vesicular type with a large central karyosome. Several blepharoplasts are located on the outside of the nuclear membrane near the anterior end. Stages have been observed by several investigators that indicate that both longitudinal fission and multiple fission of the trophozoites

may occur. Cysts (Fig. 98, c) were found by Wenyon and O'Connor in stools containing trophozoites. They were ovoidal, 6 μ to 8 μ long and 3 μ to 4 μ broad, and contained one, two or four nuclei, situated near the ends.

Fonseca (1918) has described a species of *Enteromonas*, *E. intestinalis* from the rabbit and Lynch (1922) another from the guinea pig, *E. caviae*. Lynch believes that he can distinguish between the flagellate described by Fonseca as *Enteromonas hominis* and that described by Wenyon and O'Connor as *Tricercomonas intestinalis*. Specimens of the latter were found by him in the lower intestinal tract of a woman and corresponded in every way to the account given by Wenyon and O'Connor. They were cultivated successfully in artificial media (see page 262). The flagellates found by Lynch in the guinea pig, on the other hand, resembled those he studied in man except as regards the flagella, of which there are only three; two free anterior flagella and one often lying back over the body frequently distinctly unattached. This form thus corresponds to the description of *Enteromonas* given by Fonseca.

F. The Genus *Giardia*

I. INTRODUCTION

Species of the genus *Giardia* are known to occur in Amphibia, birds and mammals. The genus name was introduced by Künstler (1882) in his description of the species, *G. agilis*, that he found in the tadpoles of frogs. Giardias, however, were described from man much earlier than this. To Leeuwenhoek belongs the credit for their discovery, although Lambl is usually referred to as having recorded them for the first time.

Lambl in 1859 noted specimens in the mucous excrement of children. He placed the flagellates he found in the genus *Cercomonas* and proposed the specific name *intestinalis*.

He noted the tadpole-like shape of the body, the presence of a roundish-oval sucker, and two nuclear-like bodies within; and described some of the movements of the body. Post-mortem examinations (Lambl, 1860) revealed the duodenum as the habitat of the organism.

Cunningham, in 1872, described what were evidently giardias from stools of cholera patients in India, and noted the presence of "small cilia" rapidly vibrating on the concave (ventral) surface.

More detailed description of *Giardia* were given by Grassi (1879, 1881), who found specimens not only in human beings but also in mice, rats, and cats.

Perroncito, in 1887, described a number of cases of giardiasis in which there were alternate periods of diarrhoea and constipation. During the latter, cysts were observed and definitely recognized as a stage in the life-history of the organism of value in preserving the race and providing a means for the distribution of the species. Encystment was studied in rats and mice and was found to occur in the large intestine.

The following year appeared the notable work of Grassi and Schewiakoff (1888) on the giardias of laboratory rats and mice containing drawings which from that time to the present day have been common figures in our text and reference books. This work furnished a better idea of the organism than any that preceded it but we now know it to be lacking in exactness.

An excellent study of the giardias in the rabbit was made by Metzner in 1901. He noted the tapering posterior end; the diamond-shaped posterior area between the thicker lateral triangular shields; that the anterolateral and posterolateral flagella have thick intracytoplasmic portions; that the anterolateral flagella form a chiasma near the anterior end of the sucking disc; that certain of the flagella extend out from knobs (basal granules); that the ventral body ("parabasal")

may or may not be present, is variable in size and may consist of nutritive material; and that the nuclei contain nucleoli (karyosomes). Metzner attempts with considerable success to coordinate the functions of the flagella and other structures that are now included by Kofoid under the term neuromotor apparatus.

Wenyon's (1907) work on the giardias of mice cleared up several points that had been represented incorrectly by previous investigators. He showed that no bridge exists between the nuclei such as Grassi and Metzner described, and made more accurate statements regarding the arrangement of the neuromotor apparatus. Binary fission within the cysts was noted but not in great detail and the presence of copulation cysts reported by earlier investigators was denied.

Most of the investigators up to this time used the giardias of lower animals as material. Bohne and Prowazek (1908) and Bensen (1908), however, studied specimens from human beings. Bohne and Prowazek discuss the origin of the flagella and the location of the "parabasal body." They recognize a karyosome in each nucleus connected with a granule ("centrosome") on the nuclear membrane by a rhizoplast; the latter is continued by a rhizoplast which extends from the "centrosome" toward the median axis of the body near the center of the sucking disc. Cysts were noted within which binary fission took place.

Up to this time only one species of *Giardia* (*Lamblia*) was recognized, but Bensen (1908) distinguished three species, *G. cuniculi* from the rabbit, *G. muris* from the mouse, and *G. intestinalis* from man. Opinions have differed regarding the splitting up of the organisms into species in this way, but at present these and species that have been named since 1908 are considered valid.

Multiple division within the cyst of *Giardia* was first described by Noc (1909) but not in great detail.

Rodenwaldt (1912) also studied human giardias. He

gives an interpretation of the origin of the flagella; describes two axostyles, and the peristomal fibers; gives an account of some of the stages in nuclear division; and discusses the structure of, and division within, the cyst.

Of historical interest, as Dobell (1920) has recently pointed out, is the fact that the first parasitic protozoon discovered in man was probably the species we are now considering. Free-living protozoa were discovered by the great Dutch microscopist Leeuwenhoek (1632-1723). This was in 1676. A few years later (1681) Leeuwenhoek announced in a letter to the Royal Society of London the discovery in his own stools of "very prettily moving animalcules, some rather larger, others somewhat smaller than a blood corpuscle, and all of one and the same structure. Their bodies were somewhat longer than broad, and their belly, which was flattened, provided with several feet with which they made such a movement through the clear medium and the globules that we might fancy we saw a pissaed running up against a wall. But although they made a rapid movement with their feet, yet they made but slow progress." Continuing his letter he says, "Of these animalcules I saw at one time but one in a (quantity of) material the size of a grain of sand, but again at other times some four or five, or even six or eight. . . . I have also examined my excrement when it was of ordinary consistency, and also mixed with clean water, but could discover therein no animalcules; but whenever the material was somewhat looser than usual, I have, contrary to my expectation, still seen animalcules in it." Davaine (1860), Stein (1867), Leuckart (1879) and many others have identified the protozoon thus described as *Balantidium coli*. Bütschli (1887), however, thinks that it must have been a flagellate, and Dobell (1920), who has recently reexamined Leeuwenhoek's letter, points out convincingly that it not only was a flagellate but that it belonged to the genus *Giardia*. Leeuwenhoek compared it to a pissa-

bed; this is a local name applied to an arthropod belonging to the ISOPODA and usually known in this country as a sow-bug. The sow-bug lives in damp places under stones, logs, etc., is oval in shape, has a convex dorsal surface, a flat under surface and a number of legs. Leeuwenhoek's protozoon was about the size of a blood corpuscle, i. e., only about $\frac{1}{100}$ the area of *Balantidium coli*, was flattened on one side, and made rapid movement but slow progress. These statements all indicate that the organism was none other than *Giardia lamblia*. It is also clear from Leeuwenhoek's words that he discovered the fact that the vegetative, motile giardias appear only in loose stools, and that the number of specimens in the stools varies from time to time and is no indication of the extent of the infection. The reexamination of Leeuwenhoek's studies leads to the conclusion that he was the first to discover protozoa living in man and that these protozoa belonged to the species *Giardia lamblia*. He was apparently also the first to make critical studies of human feces (Dobell, 1920).

2. *Giardia lamblia* FROM MAN

The most detailed morphological studies of *Giardia lamblia* that have been published are those of Simon (1921). This investigator examined thousands of specimens and accurately measured hundreds of them from many different hosts. The following description is based on his results.

a. *Morphology of Trophozoite* (Fig. 99)

The trophozoite of *G. lamblia* ranges in length, exclusive of the caudal flagella, from $9.25\ \mu$ to $20.25\ \mu$, with an average of $13.70\ \mu$, and in breadth from $5.0\ \mu$ to $10.25\ \mu$, with an average of $6.6\ \mu$. The ratio of body length to body breadth averages 1.84. The body is pear-shaped in dorsal or ventral view with a broad rounded anterior end and a narrow tapering posterior portion. A side view shows the body to be thin

with an arched dorsal surface and a broad but rather shallow ventral concavity, the sucking disc—sometimes erroneously called a cytostome. The body is bilaterally symmetrical with corresponding organelles on either side of the median longi-

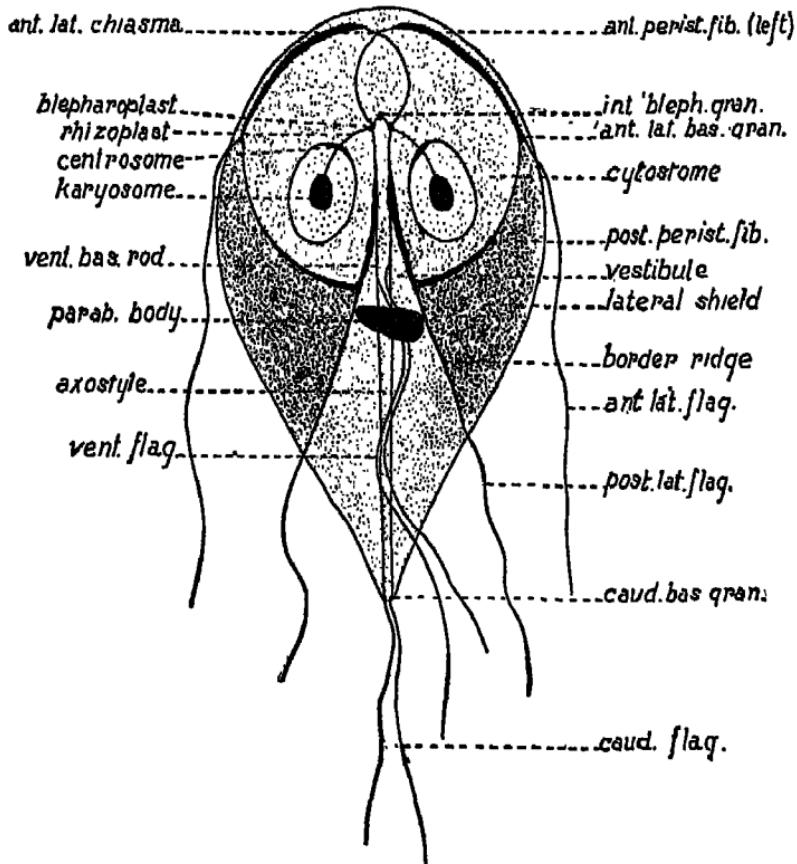


Fig. 99.—*Giardia lamblia*. Motile flagellate showing details of structure. (After Simon.)

tudinal plane. Bordering the posterior margin of the sucking disc on either side is a shield-shaped portion of the body that is thicker than the triangular area situated between; these are the lateral shields. The part of the animal lying behind the lateral shields is often referred to as the "tail."

Giardia does not change its shape to any great extent and evidently has a very firm cuticle. The sucking disc, however, may be expanded and contracted and the tail recurved dorsally over the back. The margin of the sucking disc is raised from the rest of the body and stiffened by definite fibrils; these are the two anterior and two posterior "peristomal" fibers which are separated slightly at the point where the anterolateral flagella pass out of the body. The inner edges of the lateral shields are also raised and supported by fibers that are much thickened anteriorly. Between the lateral shields the body is thinner and a narrow groove extends forward into the sucking disc—this depression has been called by Simon the vestibule.

The four pairs of flagella can be seen in the living animal but their intracytoplasmic portions can be traced only in specimens that have been properly fixed and stained. Each anterior lateral flagellum arises from a blepharoplast at the end of one of the axostyles, curves forward toward the opposite side of the animal, crosses the other flagellum forming a "chiasma" where a granule can sometimes be seen, then follows the anterior lateral margin of the sucking disc posteriorly until it emerges at the side near the anterior end of the body. At the point of emergence it is often possible to distinguish a basal granule. Kofoid (1922) has suggested that a chiasma does not really exist but that the two flagella simply come into contact without crossing. The evidence is very strong, however, that there is a chiasma. The posterior lateral flagella also arise from the blepharoplasts at the anterior ends of the axostyles, pass back along the inner margins of the lateral shields and emerge at the posterior ends of the lateral shields. No basal granules appear where they become free from the body. The caudal flagella seem to arise from two basal granules lying at the extreme posterior end of the body. They may really be extensions of

the axostyles or, in other words, the axostyles may be the intracytoplasmic portions of these flagella. The ventral flagella arise within the vestibule from what seem to be thickenings of the axostyles. They are larger and stronger than the other flagella and usually move together so that in stained specimens they are parallel to each other. They are in active movement when the animal is otherwise at rest, at which time they probably create a current which supplies a constant change in the medium within the vestibule.

The trophozoite when in a "resting" condition possesses two nuclei. These are elliptical bodies situated on either side of the axostyles and dorsal to the sucking disc. They measure 1.5μ to 3.0μ in length with an average of 2.14μ and 1.0μ to 2.0μ in breadth with an average of 1.49μ . The ratio of length to breadth averages 1.36 (Simon, 1921). The chromatin within the resting nucleus is all concentrated in a central karyosome. This is connected by a thin linin fibril with a deeply staining mass on the anterior surface of the nucleus that has been interpreted by Kofoid as a centrosome. A curved rhizoplast extends from this body to the blepharoplast at the end of one of the axostyles. Between the two blepharoplasts is an arch-like fibril in the center of which is an interblepharoplastic granule.

The axostyles are two in number and extend longitudinally in the median line from near the center of the sucking disc to the posterior end. The functions of the axostyles are not definitely known but may be supporting and possibly locomotory under certain conditions. Just posterior to the sucking disc and dorsal to the axostyles often appear one or a pair of deeply staining club-shaped masses that lie in a more or less transverse position. These have been homologized by Kofoid with the "parabasal bodies" that have been reported in certain other flagellates but their true nature has not yet been determined definitely.

b. Cysts (Fig. 100)

In the lower part of the intestine *Giardia* encysts, becoming oval in shape with a wall that is of uniform thickness, dense and highly refractive. The cysts range from 8.0μ to 14.0μ in length with an average of 10.7μ , and from 6.0μ to 10.0μ in breadth with an average of 7.47μ . The ratio of length to breadth averages 1.37 (Simon, 1921).

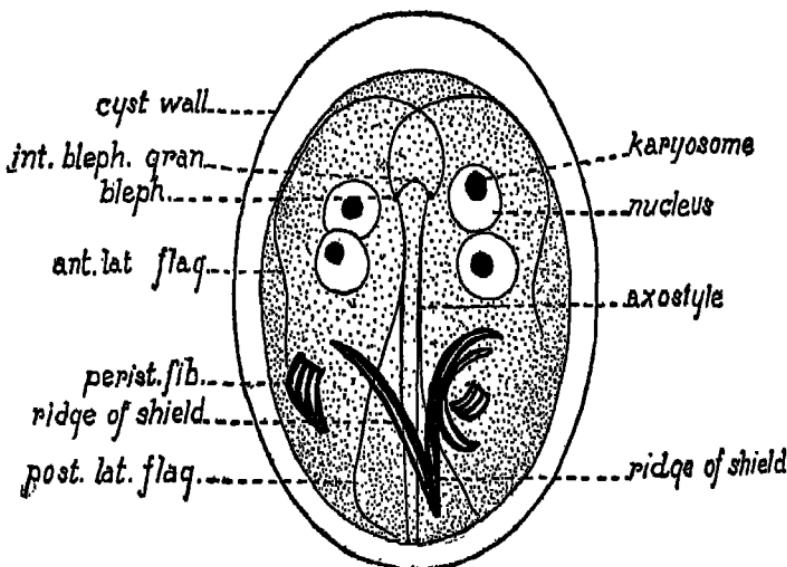


Fig. 100.—*Giardia lamblia*. Cyst. (After Simon.)

Cysts with 2, 4, 8, and 16 nuclei respectively occur, indicating that three successive nuclear divisions may take place after the cysts are formed. These nuclei are spherical, instead of oval as in the trophozoite, and are usually distributed two near one end, four near one end or two near each end, or four near each end. The various fibers present in the trophozoite persist in the cysts as in *Chilomastix* (see page 205). The duplication of these fibers results in the presence of so many structures within the cyst that identification of the various types represented is very difficult. No "parabasal

bodies" are visible in the cysts. Cysts may be very numerous in the feces of carriers, over twenty million per gram having been counted in one case (Kofoid and Swezy, 1922).

c. Multiplication

Kofoid and Swezy (1922) have recently published a partial account of the division of both trophozoites and cysts of *Giardia lamblia*. The trophozoites divide by longitudinal frontal binary fission. Multiple fission has been reported by Noc (1909) but has not been confirmed. Preceding division the nucleus undergoes mitosis without the dissolution of the nuclear membrane. The karyosome breaks up into four chromosomes. The blepharoplasts and "centrosomes" appear to divide; this seems also to be the method of duplication of the axostyles and "parabasal bodies." The flagella, on the other hand, seem to become double in number by the out-growth of a second complete set. Plasmotomy then occurs. The nuclei within the cyst also divide by mitosis and the other organelles within the cytoplasm undergo multiplication, but plasmotomy within the cyst has not yet been observed.

3. GIARDIAS FROM LOWER ANIMALS

The most important morphological characteristics that are of value in distinguishing the various species of *Giardia* are the size and shape of the body, of the nuclei, and of the "parabasal bodies," and the size and internal structure of the cysts. We now have adequate descriptions of the giardias from tadpoles (*G. agilis*), rats and mice (*G. muris*), meadow mice (*G. microti*), rabbits (*G. duodenalis*), dogs (*G. canis*), and guinea-pigs (*G. caviae*). Giardias have been reported also from cats, sheep, and birds. There is apparently great specificity among giardias with respect to their hosts, that is, each host species, except in the case of laboratory and wild rats and the house mouse, seems to be infected with a distinct species of *Giardia*. The specific differences are brought

out in the following paragraphs and in the diagrams presented in figure 101.

Giardia agilis is a long slender species. It ranges in length from 14.4μ to 28.9μ with an average of 22.0μ and in

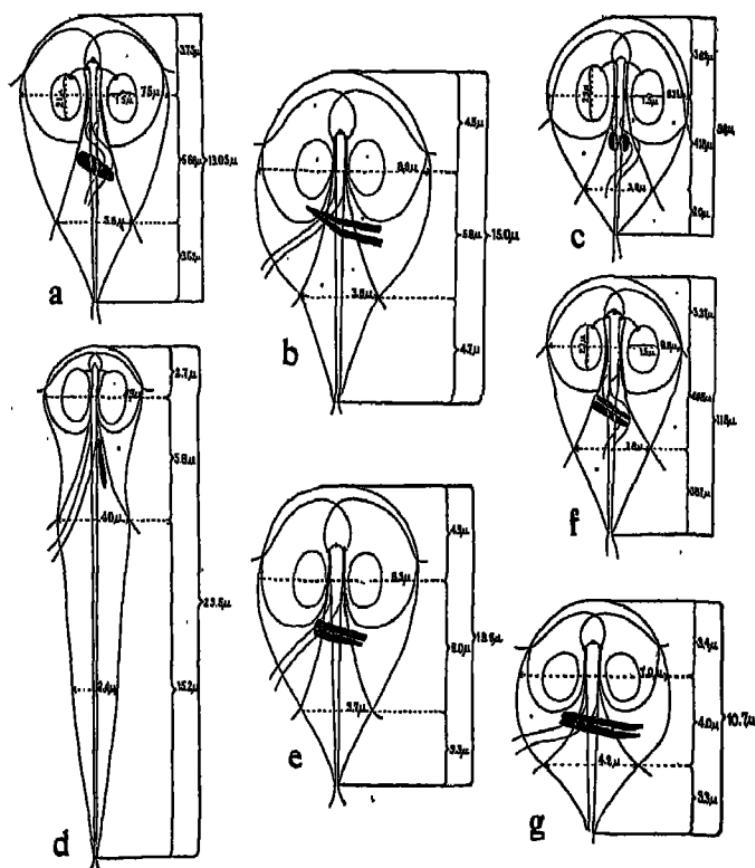


Fig. 101.—Giardias from man and lower animals.
Diagrams showing the specific differences between giardias living in different species of animals.

a, *G. lamblia* from man. b, *G. duodenalis* from the rabbit. c, *G. muris* from rats and mice. d, *G. agilis* from frog tadpoles. e, *G. canis* from the dog. f, *G. microti* from meadow mice. g, *G. carrie* from the guinea-pig. $\times 2800$. (a, c, f, after Simon; b, d, e, g, after Hegner.)

breadth from 2.6μ to 5.1μ with an average of 4.5μ . The tail is extremely long, occupying about two-thirds the entire length of the body; the nuclei are very large; the karyosomes within the nuclei are also very large; only one "parabasal body" is present and this is parallel to the axostyles and rod-shaped; and the cysts are spherical (Alexeieff, 1914).

Giardia duodenalis from the rabbit (Fig. 101, b). This species was first described by Davaine (1875) as *Hexamita duodenalis*. It was recognized by Bensen (1908) as a separate species but only recently has a detailed study been made and its principal specific characters described (Hegner, 1922 b). The trophozoite of *G. duodenalis* ranges from 12.7μ to 18.7μ in length with an average of 15.8μ and in breadth from 7.7μ to 11.0μ with an average of 9.1μ . The ratio of body length to breadth is 1.74. In general it is characterized by a narrowing of the anterior region, as seen in front view, and a broadening across the center of the lateral shields, by a comparatively long "tail" and by two long bent "parabasal bodies." It is both broader and longer than the species, *G. lamblia*, from man, but the difference in the ratio of body length to body breadth shows that the latter is more slender than *G. duodenalis*. The data recorded confirm the opinions expressed by several previous investigators that the rabbit is parasitized by a species distinct from that living in man.

Giardia canis from the dog (Fig. 101, e). The giardia from dogs has been mentioned by several investigators but was not recognized as a separate species until 1922 (Hegner, 1922 b). It ranges in length from 11.9μ to 17.0μ with an average of 13.8μ and in breadth from 7.6μ to 10.2μ with an average of 8.5μ . The ratio of body length to breadth is 1.62. The trophozoite differs from that of *G. lamblia* from man and *G. duodenalis* from the rabbit principally in size, shape, and the form of the "parabasal bodies." The anterior end of the trophozoite in front view is broader than in the

other two species; the diameter across the center of the lateral shields is less than in *G. duodenalis*; the two "parabasal bodies" are longer than the parabasals of *G. lamblia*, but shorter than those of *G. duodenalis*; and the "tail" is shorter than in *G. duodenalis*, but about the same relative length as in *G. lamblia*. The cysts of *G. canis* are of the same shape as those of *G. lamblia*, but slightly larger both in length and breadth.

Giardia muris from rats and mice (Fig. 101, c). This species was first separated by Bensen (1908) from those of the rabbit and man. It ranges from $7.25\ \mu$ to $12.75\ \mu$ in length with an average of $9.75\ \mu$ and from $5.25\ \mu$ to $9.75\ \mu$ in breadth with an average of $7.26\ \mu$. The ratio of body length to breadth is 1.35. The body is thus comparatively short and broad. The "parabasal bodies" are usually two in number and are subellipsoidal masses that are sometimes fused into one.

Giardia microti from meadow mice (Fig. 101, f). The giardia from meadow mice was first distinguished from the species that occurs in rats and mice by Kofoid and Christian-sen (1915). Since then this species has been noted by Simon (1922) in meadow mice from Nova Scotia and Maryland and probably also by Splendore (1920) in Italy, although the last-named writer described the organism he worked with as a new species, *G. pitymysi*. *G. microti* ranges in length from $8.25\ \mu$ to $13.75\ \mu$ with an average of $11.11\ \mu$, and in breadth from $5.25\ \mu$ to $10.25\ \mu$ with an average of $7.58\ \mu$. The ratio of body length to breadth is 1.49. The body of this species is thus longer and more slender than that of *G. muris*, the greater length being due principally to the greater length of the tail. The "parabasal bodies" are long, parallel, and lie at an angle with the axostyles.

Giardia caviae from the guinea-pig (Fig. 101, g). The guinea-pig giardia has just been recognized as a distinct species (Hegner, 1923). It ranges from $8.0\ \mu$ to $14.0\ \mu$ in

length with an average of $10.7\ \mu$ and from $5.6\ \mu$ to $10.1\ \mu$ in breadth with an average of $7.2\ \mu$. The ratio of body length to breadth is 1.48. It is thus a small and very broad species and, as in *G. duodenalis*, the place of greatest breadth is across the center of the lateral shields. The two "parabasal bodies" are in the form of long slender rods which occupy a position dorsal to the axostyles as in other species, but usually not parallel to the dorsal ventral plane; this peculiar tilting is indicated by the fact that one must focus up and down in order to bring their entire length into view.

Giardias from birds have been reported by several investigators but none of these has been studied carefully. Gonder (1911) claims to have found specimens in the blood of a falcon, *Elanus caeruleus*, but none in the intestine of the same host. To this form he gave the specific name *sanguinis*. It was $12\ \mu$ long and $8\ \mu$ broad, and resembled *G. muris* more than any other species. A second species, *G. ardeæ*, was described by Nöller (1920) from the intestine of the heron, *Ardea cinerea*. Finally Kotlán (1922, 1923) has reported giardias from the intestine of *Ardea cinerea*, *Lanius cœllurio* and several other species. Kotlán considers these to be *G. ardeæ*. They were $10\ \mu$ to $16\ \mu$ long and $9\ \mu$ to $10\ \mu$ broad.

4. INFECTION OF MAN WITH GIARDIAS

Grassi believed that the *Giardia* of man and of rats, mice and cats belonged to one species and that human beings became infected by eating food contaminated by the giardia-containing feces of the lower animals. However, he was unable to infect himself although he swallowed large numbers of specimens (Grassi, 1882, p. 424). Dr. Calandroncis, according to Piccarde (1895), was more successful, since he found motile flagellates in his stools 25 days after swallowing cysts. An attempt by Moritz and Hözl (1882) to infect a human being with cysts from mice was negative.

Several investigators have tried to infect animals. For example, Perroncito claims to have infected two white mice with human cysts, two control mice remaining uninfected. Stiles states that he infected a guinea pig with cysts from man. Negative results were obtained by Bohne and Prowazek in their attempts to infect rabbits, rats and young cats, both *per os* and *per rectum*, with human fecal material containing both motile flagellates and cysts. Fecal emulsion containing giardias was injected by Salomon into the small intestine of a rabbit and two cats. The rabbit was negative but "egg-shaped cysts" were observed in the cats as long as a month later.

The experiments of Fantham and Porter were more thoroughly carried out and seem to prove that laboratory animals may be infected with giardias from man. They examined their experimental animals (8 kittens and 9 mice) for a considerable period before the feeding was begun in order to be certain that they were uninfected. They were then fed washed cysts. Daily fecal examinations were made and at the end the animals were studied *post mortem*.

Six of the 8 kittens were positive; diarrhoea developed usually on the third day; and death resulted in from one to 8 weeks. The cysts fed to one of the kittens that succumbed had been kept previously for 74 days. Six of the 9 mice also became infected, but the disturbances produced were not so severe.

Porter later carried out some experiments on rats, house flies, blow flies, and cockroaches (*Periplaneta orientalis* and *P. americana*) in Johannesburg. She found giardia cysts in samples of water from a supply used by natives and produced giardia diarrhea in clean rats by mixing some of this water with their food. She also reports that giardia cysts may pass unharmed through the flies and cockroaches named above and that these cysts are then infective to white rats.

Simon (1922) has more recently carried out infection experiments in an attempt to establish human giardias in rats. He used five adult laboratory rats and five wild rats. None of these was found to be infected after being fed on food containing living cysts of *G. lamblia*. Further experiments with young laboratory rats proved that cysts of *G. lamblia* do not pass through the stomach of these rodents alive. The conclusion reached by Simon is that human giardiasis is not contracted from *G. muris* of rats and mice but is due to a distinct species.

The infection experiments thus far reported leave the subject of cross infection with giardias of man and the lower animals very much in doubt. Further experiments carried on under better controlled conditions must be performed before any definite conclusions can be reached. In the meantime it seems best to assume that human beings can be infected only by the giardias from man and that the organism is infective only when the cysts are in a certain stage of development. The large number of carriers of giardia cysts among the general population and the lack of adequate sanitary precautions in many localities makes it easily possible for this organism to spread from man to man. Exactly how this takes place is not definitely known but cysts may be distributed in water, milk and other liquids, in foods, by flies and other passive carriers, and by contact.

G. Intestinal Flagellates in General

I. GEOGRAPHICAL DISTRIBUTION

The three species of human intestinal flagellates that are best known, namely *Chilomastix mesnili*, *Trichomonas hominis*, and *Giardia lamblia*, appear to be cosmopolitan in their distribution, having been found among the inhabitants of every region of the earth where careful examinations have been made. A wide distribution also seems probable with

Embadomonas intestinalis and *Enteromonas hominis*, but our data regarding these species are at present inadequate, due largely to the small size of the organisms and their apparent rarity.

We are accustomed to think of intestinal protozoa as being primarily tropical in their distribution. It is true that conditions in the tropics are more favorable for the spread of these organisms than those in temperate countries and that there is consequently a greater incidence the nearer one approaches the equator, but recent surveys, especially in connection with the examination of soldiers, have shown the percentage of infection with intestinal flagellates of persons living in temperate zones to be surprisingly high. We cannot, therefore, consider intestinal disturbances associated with intestinal flagellates as distinctively tropical, but as liable to be present wherever conditions are favorable. These conditions exist in any community without adequate means of fecal disposal, thus leading to frequent contamination and resultant mass infections. Thus one would expect a lower incidence among residents in cities where open plumbing is the rule, a higher incidence in smaller communities where latrines are used, and the highest incidence in those parts of the country where there are no methods of fecal disposal and soil pollution is prevalent.

2. FACTORS OF THE INTESTINAL HABITAT

In studying the intestinal flagellates of man it is interesting to consider their organization and activities in connection with their habitat. The factors in the habitat of free-living flagellates differ widely from those of the intestinal species. (a) The latter live in total darkness and at a constant temperature of 98.6 degrees F. (b) Peristalsis tends to carry the flagellates from the anterior to the posterior portions of the intestine along with the other intestinal contents. This is prevented in *Giardia* by means of the sucking disc,

possibly in *Trichomonas* by the apparently adhesive nature of the extruded portion of the axostyle and by amoeboid processes, and possibly in *Chilomastix* by attachment to the intestinal epithelium by means of the cytostome. (c) They must move about in a comparatively viscous medium. To cope successfully with this viscous medium the flagella must be well developed. The axostyles characteristic of *Trichomonas* and *Giardia*, the undulating membrane of the former, and the intracytoplasmic portions of the flagella of the latter may possibly have evolved under the influence of this environmental factor. The function of the axostyle is problematical. Many investigators consider it simply an axial skeletal support; others attribute to it the rôle of an organ of fixation having seen the organism attached to the substratum by its posterior end. Kofoid and Swezy (1915), however, consider it "a powerful motor organ." Its movements can best be seen in specimens in fresh material from the mucous surface of the intestine. In such preparations when very little fluid is added the axostyle may be seen to bend and curve like a flagellum as it lashes from side to side. It thus serves as an intracytoplasmic flagellum. (d) Intestinal flagellates must cope with chemical conditions quite different from those of their free-living relatives. Their environment contains the digestive juices, the products of digestion, and other biochemical products produced by the host. These chemical substances may pass by absorption into the body of the flagellate and there have a profound influence upon the development of intracytoplasmic bodies and upon metabolism in general. (e) The nature of the food probably differs for the different species as is known to be true among free-living protozoa, and hence food conditions constitute a factor of considerable importance. The character of the food of the host has a direct influence on the chemical nature of the intestinal contents. A holozoic flagellate might also be affected by the type of bacteria available as food.

(f) In the lower divisions of the intestine the products of bacterial decomposition are present in sufficient quantities to materially alter the intestinal contents.

Recent experiments by the writer (Hegner, 1923) indicate the influence of changes in diet on the incidence, distribution, and number of intestinal flagellates. Rats were used as experimental animals and *Giardia muris*, *Hexamitus muris* and *Trichomonas muris* were the flagellates studied (Fig. 95). Control rats fed principally on vegetable proteins and carbohydrates gave the following results: *Giardia muris*: incidence, 95 per cent.; distribution, duodenum, jejunum, and ileum; optimum habitat, duodenum. *Hexamitus muris*: incidence, 90 per cent.; distribution, duodenum, jejunum, and ileum; optimum habitat, ileum. *Trichomonas muris*: incidence, 100 per cent.; distribution, caecum and ileum; optimum habitat, caecum.

Rats fed on a carnivorous diet for 7 or 8 days showed an incidence of infection of 93.3 per cent. with *Giardia muris*, but the number present decreased decidedly especially in the duodenum and jejunum. Similarly the incidence of infection with *Hexamitus muris* was 93.3 per cent., but the number of individuals doubled in the ileum and decreased to almost zero in the jejunum and duodenum. *Trichomonas muris*, which was present in great abundance in control rats, almost disappeared from the caecum of the animals fed on a carnivorous diet, the average number per field in the former being 91.2 and in the latter 1.73. The rapid change from a preponderance of acidophilus bacteria in the controls to a preponderance of putrefactive bacteria in the rats fed on a carnivorous diet may account for the changes noted. The suggestion is offered that human patients suffering from the so-called flagellate diarrhoeas may be benefited by partaking of a carnivorous diet.

The parasitic existence also profoundly modifies the life-history of the organism since a resistant stage, the cyst,

is usually necessary to make possible the transference of the organisms from one host to another, and since in intestinal flagellates as well as in parasites in general, vast numbers of these resistant stages must be produced because of the very slight chance that any of them will gain entrance to a new host. Perhaps multiple fission, which occurs in *Trichomonas* and the cysts of *Giardia*, is correlated with the necessity for a more rapid method of multiplication thus increasing the number of individuals and the chances of further infection.

3. EVOLUTION

The entozoic flagellates are not confined to one subclass or order but several orders contain entozoic species. These species are often clearly modified from nearly related free living species and we have forced upon us the conclusion that the entozoic mode of existence has arisen separately in each order or family or genus. Thus among the PROTO-MONADINA are *Chilomastix* and *Trichomonas*; among the DISTOMATINA, *Giardia*; among the EUGLENOIDINA, *Euglenamorpha hegneri*; among the HYPERMASTIGINA, *Lophomonas*; and among the DINOFLAGELLATA, *Blastodinium*.

The origin of the undulating membrane so characteristic of certain flagellates is a subject about which it is interesting to speculate. It involves two problems: (1) what factors led to its formation, and (2) how did it arise? The type of undulating membrane that prevails among the ciliates, e. g., that in the cytostome of *Paramecium*, is formed simply by the fusion of a row of cilia. In flagellates the undulating membrane is an ectoplasmic sheet with a flagellum running along its outer margin. Undulating membranes of this type are especially prevalent among entozoic flagellates and thus seem to be related to some environmental factor encountered in this habitat. Perhaps the density of the medium, i. e., the intestinal chyme or the blood stream, requires a structure

more powerful than flagella to enable the animal to swim through it. It has been suggested (Minchin, p. 57) that the movements of the undulating membrane may serve to renew the fluids that bathe the organism while at rest. This might also require more force because of the density of the fluids than that provided by the flagella. The undulating membrane may have arisen in several different ways. For example, a trailing flagellum such as is present in *Enteromonas* (Fig. 98) may have become attached to the side of the body and have drawn out a thin layer of the ectoplasm into a membrane; or the point of origin of a flagellum may have shifted backward from the anterior end as in the developmental stages of *Trypanosoma lewisi* and have drawn out a membrane between it and the body proper.

Of special interest from an evolutionary viewpoint is the condition in *Giardia*. In this genus duplication of the nuclei and most of the structures included in the neuromotor apparatus has taken place. Such a duplication may have arisen by the division of these structures in a primitive genus resembling *Chilomastix* which later failed to undergo cell division. A shifting of the organelles thus duplicated might result in a binucleate, bilateral organism like *Giardia* (Kofoid and Swezy, 1922).

4. EXTENT OF INFECTION

Hegner and Payne (1920) reviewed over 35 reports published by American, English and French investigators during the years 1916-1919 describing the results of protozoan surveys that had been made by them. These reports are based on studies made on all fronts during the war, on examinations of soldiers invalidated home for various causes, and on material obtained from various classes of men, women, and children who had never been out of their native land. Most of the latter were either recruits or were confined in insane hospitals or other institutions. These sur-

veys were carried out in many cases under unfavorable conditions and by inexperienced persons but they probably indicate a lower rate of infection than really existed. The results are quite surprising. Of approximately 20,000 persons examined about 12% were infected with *Giardia*, 3.5% with *Chilomastix*, and 3% with *Trichomonas*. A large number of apparently healthy persons were infected. Thus Baylis (1919) reports among 400 healthy recruits 5.5% infected with *Giardia*; and Kofoid, Kornhauser and Plate (1919) found among U. S. home service troops 5% infected with *Giardia*.

Chilomastix mesnili was recorded in twenty-three of thirty-five reports mentioned above. The incidence of infection averaged about 3.5% with a maximum of 11.7%. Among the reports from Americans are those made by Kofoid and his associates at New York and Berkeley, California. At New York 2300 men from overseas gave an incidence of 4.2%, and 576 home service men an incidence of 3.5%. At Berkeley the 534 persons examined gave 5.3% of infection. We may therefore consider *Chilomastix* a common intestinal flagellate of man.

The relation between infection and the age, sex, race, and occupation of the person examined is practically unknown. There is considerable evidence, however, that the incidence of infection, at least with giardias, is higher in children than in adults. The following data brought together by Dobell (1921) seem quite conclusive.

The youngest children found infected were reported by Miss Nutt; they were 3 weeks, 3 months, 9 months, 11 months, and 12 months old, respectively. That conditions are similar in the United States is indicated by the work of Maxcy (1921) who found 17 per cent of infection with giardias in children in the group from one to five years, and almost 40 per cent among children from 6 to 12 years of age. It may be true, as Dobell suggests, that the higher

Investigator	Place	Percent <i>G. lamblia</i>	
		Adults	Children
Matthews and Smith.....	Liverpool.....	7.0	14.1
Campbell.....	Bristol.....	3.9	16.3
Miss Nutt.....	Leeds.....	3.8	39.8
" "	Sheffield.....	7.2	15.8
McLean.....	Reading.....	9.3	17.4

incidence of infection among children is only apparent, being due to the greater difficulty of finding the organisms in the stools of adults, but this still remains to be determined.

Multiple infections with intestinal protozoa are quite common, that is, one person may be infected at the same time with several different species. Practically all possible combinations of infections have been noted such as *Chilomastix mesnili* and *Giardia lamblia*; *C. mesnili*, *Trichomonas hominis*, and *Endamæba coli*; *Endamæba histolytica*, and *G. lamblia*, etc.

5. METHODS OF INFECTION

We think we know in a general way how intestinal flagellates are transferred from one host to another. In the first place, since the organism must pass a certain amount of time outside of the body under diverse and often disadvantageous circumstances, it must be resistant to environmental factors. The cyst stage in the life-cycle of most of the intestinal flagellates meets these conditions. Passage must also be accomplished through the anterior portion of the alimentary tract where various agents such as gastric juice are encountered. Cysts are able to withstand these conditions making it possible for the organisms to reach the intestine. Cysts have

been described for most of the species but none are yet known with certainty to belong to *Trichomonas hominis*. The cysts pass out of the alimentary canal in the feces. How do they obtain entrance into a new host? We suppose that this occurs by the contamination of water, milk and other foods but have very little evidence to prove it. We know that there are plenty of carriers of the intestinal flagellates, that is, apparently healthy persons who are infected and are passing cysts almost continuously in their stools. We also know that cysts will live in a moist situation for a considerable period outside of the body. Surveys of intestinal protozoa indicate that infections are more numerous in localities where sanitation is inadequate; infections are more prevalent in country districts than in cities and in the southern than in the northern United States.

The relations between flies and the distribution of the cysts of intestinal protozoa have been studied by Stiles and Keister (1913), Wenyon and O'Connor (1917) and by Root (1921). These studies prove that flies can and do carry cysts and that they may deposit them alive in water and milk and on other foods. How frequently human beings are infected by cysts spread by flies cannot be stated. Stiles and Keister recovered giardia cysts from flies that had been fed on fecal matter containing cysts of this flagellate. Wenyon and O'Connor using *Musca domestica*, *Calliphora* and *Lucilia* found that 5 out of 8 flies fed on infected feces contained living cysts of *Giardia lamblia* 24 hours later; that droppings of flies fed on infected feces contained living cysts of *G. lamblia* 40 minutes after feeding; that droppings from one out of 229 wild flies contained cysts of *G. lamblia* and that living motile specimens of *Trichomonas* were present in droppings of flies 5 minutes after being fed on infected feces.

Root found that motile stages of *Chilomastix mesnili* were killed within an hour in the intestine of the fly (*Musca*

domestica) but might pass through within 7 minutes apparently unharmed; that cysts of *G. lamblia* could live in the intestine of the fly for 16 hours; that cysts of *Chilomastix mesnili* could live in the intestine of the fly for 80 hours; and that cysts of *G. lamblia* could live in the intestine of drowned flies for 4 days. Whether living cysts may be liberated from drowned flies and thus become infective to man is not known.

The method of infection with *Trichomonas hominis* is more difficult to explain than that with the protozoa that produce cysts. It seems probable that the motile flagellate is the infective stage. This, as Hegner and Becker (1922) have shown, lives for a remarkably long time in fecal material outside of the host. Living specimens of *Trichomonas* were obtained from a stool $37\frac{1}{2}$ hours after it was passed and specimens were obtained in cultures inoculated with fecal material 79 hours old. The great viability shown by the trophozoites of this species may enable it to gain access to new hosts without the aid of cysts and indicates that there may be a correlation between this fact and the absence of cysts.

6. VIABILITY OF CYSTS

The degree of success of an intestinal flagellate in infecting new hosts depends largely on the viability of the cysts. This differs according to the species of flagellate and the nature of the environment. Boeck (1921) has carried out a series of careful experiments with respect to the degree of temperature that these cysts can withstand. It was found that cysts of *Giardia lamblia* are killed at a temperature of 64 degrees C. and those of *Chilomastix mesnili* at 72 degrees C. Under ordinary circumstances therefore these cysts will not be killed by high temperatures. In the experiments performed by Boeck death was determined by the "eosin test," that is, a drop of 1 per cent eosin solution was added to a

drop containing washed cysts; the eosin stains the dead cysts but does not affect those that are alive.

The distribution of infective cysts depends largely on their viability. It has been shown for various intestinal protozoa that bacteria and the products of putrefaction lower the vitality of cysts and the latter die sooner and hence do not have as great an opportunity to reach the intestine of a new host. Specimens of cysts when left in fecal specimens seldom remain alive more than 10 days. Washed cysts, on the other hand, remain alive for a much longer period. Boeck (1921) found that washed cysts of *Giardia lamblia*, when kept at room temperature, were still viable at the end of 32 days and those of *Chilomastix mesnili* at the end of 187 days when 88 per cent. were still alive. Other specimens kept on slides and sealed under covers with vaseline lived in the case of *G. lamblia* for at least 66 days and of *C. mesnili* for at least 232 days.

7. CULTIVATION OF INTESTINAL FLAGELLATES

Chilomastix mesnili. Boeck (1921) was the first to report the cultivation of *Chilomastix mesnili* in artificial media. The medium that gave the best results consisted of a mixture of four parts of Locke's solution and one part of human blood serum. Test tubes containing 5 c.c. of this mixture were inoculated and incubated at 37 degrees C. The life of these cultures ranged from two to ten days with an average of about seven days. The culture flagellates were smaller than those from the human intestine, probably because of rapid multiplication since more small specimens were present during the first three or four days when the number in the culture was increasing rapidly than during the succeeding days. Boeck thinks that the decrease in the number of flagellates present in a culture and death were probably due to the poisonous action of the chemical decomposition products of bacterial metabolism. In the human

intestine these products are carried to the outside and hence the flagellates are able to persist. The periodic evacuation of these poisonous products may account for the irregular appearance of the flagellates in human infections.

A more easily prepared and better medium for the cultivation of *Chilomastix mesnili* and other intestinal flagellates is the ovomucoid medium as used by Hogue (1921).

Trichomonas buccalis. Several investigators have cultivated *T. buccalis* in artificial media. Lynch (1915) used acidified bouillon kept at a temperature of 30 degrees C. He cultivated both the trichomonad from the mouth and from the vagina. Ohira and Noguchi (1917) found equal parts of ascitic fluid and Ringer's solution better as a medium since bacterial multiplication is not so rapid and the flagellates do not disappear so soon. In such a medium the organisms live at the bottom of the tubes with bacterial sediment. Transfers were made every day when incubated at 37 degrees C. or every other day at 23-37 degrees C. Over twenty generations were obtained by these methods. The organisms in the cultures seemed to be larger than those from the mouth being 10 μ to 26 μ in length and 4 μ to 22 μ in breadth.

Trichomonas vaginalis. This species has been cultivated by Lynch (1915) in the same artificial media used by him for the culture of *T. buccalis*, and also by Barlow (1916).

Trichomonas hominis. A number of investigators have succeeded in cultivating flagellates of this species. Lynch (1915) used as a culture medium, bouillon with 0.05% acetic acid and incubated at 30 degrees C. These cultures lasted for about four days and during this time an increase both in number and size was noted. Subcultures were made every three days. Boyd (1919) cultivated *Trichomonas* in saline solution; found motile flagellates in his original culture fifty-nine days after inoculation; and made six transfers. He reports what he considers to be cysts of *Trichomonas* in his

cultures but these were probably trophozoites that had become spherical in shape and inactive. Kofoid and Swezy (1921) used as culture media (1) a sterile cereal-lettuce infusion and (2) human ascitic fluid diluted with 10 vols. of physiological salt solution. Organisms were maintained for six weeks by subculturing at 3 or 4 day intervals.

The simplest medium on which to grow this organism is the ovomucoid mixture used by Hogue (1921). This is prepared as follows: "The whites of six hen's eggs are thoroughly shaken up with glass beads. This is added to 600 c.c. of 0.7 per cent sodium chlorid solution. The mixture is cooked for 20 to 30 minutes over a boiling water bath, and is constantly agitated while cooking. The coarsest of the coagulated albumin is strained out by first passing through coarse cheese cloth. This is followed by filtering through cotton with a suction pump. The filtrate is then still quite opalescent. By means of a large pipette about 5 c.c. of this filtrate is put into each test tube, and the tubes are stoppered with cotton plugs. These are autoclaved at 15 pounds pressure for 20 minutes."

According to Hogue (1922) trichomonads persist for a longer period in sodium chloride serum water medium. This is prepared by adding 10 c.c.-15 c.c. of sterile sheep serum water to a flask containing 100 c.c. of 0.85 per cent sodium chloride solution that has been sterilized in the autoclave. The serum water consists of one part of sheep serum diluted with three parts of distilled water and is heated to 100° C. for one hour on three successive days. Fifteen c.c. of the medium is placed in each test-tube, inoculated at the bottom with the organisms, covered with paraffin oil to prevent evaporation and incubated at 36° C. Hogue made up media with hydrogen ion concentrations of from 6.8 to 8.4 and found that *Trichomonas hominis* multiplied most rapidly in a medium with a pH 8 but lived longest in a medium with a pH 7.2-7.4.

Embadomonas intestinalis. Hogue (1921) was the first to cultivate this species using the ovomucoid medium described above and also a Locke-egg medium and an ox bile salt medium. Cysts appeared in tubes that contained no other protozoon; this is apparently the first authentic record of the formation of cysts by any intestinal flagellate in cultures.

Giardia lamblia has not been cultivated.

The culture method has been shown by Hegner and Becker (1922) to be both possible and practicable for diagnostic purposes. Tubes of the ovomucoid medium are used. It is convenient to transfer the sample of feces to be tested for flagellates to the medium by means of a toothpick. Material is collected on the toothpick by taking a minute amount at random from different parts of the stool until an amount somewhat greater than the size of an apple seed is obtained. The toothpick is then dropped into a test tube of the medium by means of a pair of forceps, and the tube containing both fecal sample and toothpick is incubated at about 36° C.

The medium should be examined for flagellates about 24 hours after inoculation. If present they will be most numerous near the surface of the medium.

In order to increase the certainty of the test for *Chilomastix*, it is best to pipette off the upper one-fourth of the apparently negative cultures and transfer this to a fresh tube of the medium. If *Chilomastix* is present it will then multiply very rapidly.

The culture method can be carried out on a large scale with very little difficulty and has been found to be more reliable than the usual smear method. For example, only two fecal specimens from 110 individuals were found to be positive by the routine examination of smears, whereas positive cultures were obtained from eight of them. The culture method is also more valuable than the smear method when the fecal samples are not fresh. For example, *Chilo-*

mastix was not detected in smears from stools more than 12½ hours after defecation, but appeared in cultures made from stools at least 19 hours old. Positive smears of *Trichomonas* were obtained 37½ hours after a stool was passed but were obtained in cultures from a stool 79 hours old. This method is of particular value for the diagnosis of a form like *Trichomonas* which does not produce cysts and which becomes quiescent in fecal material, and hence difficult to find, a long time before it dies.

CHAPTER VII¹

A GENERAL CONSIDERATION OF THE SPOROZOA

A. Introduction

The SPOROZOA are all parasitic PROTOZOA which usually pass from one host to another in the spore stage. The spore is generally a seed-like body with a covering called the sporocyst. In species whose spores are subjected to air, water or other agents in their passage from one host to another these bodies are very resistant, but in other species which are propagated from one host to another directly, either by being inoculated into the new host by a blood-sucking animal or by being eaten by the new host, the spores do not always have such a resistant covering.

SPOROZOA are among the most widely distributed of all parasitic animals; members of almost every large group of animals in the animal kingdom are parasitized by one or more species. Infection is very common among the vertebrates (mammals, birds, reptiles, amphibia and fishes), arthropods (insects, crustacea, spiders, centipedes, etc.), mollusks (snails, mussels, squids, etc.) and worms, and less common among echinoderms (starfishes, etc.), coelenterates (hydroids, sea anemones, etc.) and PROTOZOA. The most important animal parasite of man, the malarial parasite, is a sporozoon. Comparatively few species are lethal and only a small number seem to be very harmful to their hosts.

¹ By R. W. Hegner.

The life-cycles of the SPOROZOA are often very complicated. Frequently there is an alternation of hosts, certain stages being passed in a vertebrate and other stages in an invertebrate which serves as a transmitting agent from one vertebrate host to another. Two types of reproduction are characteristic of the life-cycles of most species, (1) *multiplicative reproduction* or *schizogony* during which many organisms are formed in a single host, and (2) *propagative reproduction* involving sexual processes and ending in the formation of spores which are usually transferred to another host.

As compared with the other three classes of PROTOZOA, the SPOROZOA are greatly modified by their parasitic existence. These modifications are represented by the absence of locomotor organs, a mouth, anal opening, excretory pore and vacuoles. There is usually a single nucleus present. Nutrition is by absorption. Many organs of the host may be parasitized, especially the alimentary tract, kidneys, blood, muscle and connective tissues. When the parasites live inside of cells they are said to be cytozoic; when among the cells, histozoic; and when in cavities, cælozoic.

The production of spores is not limited to members of the class SPOROZOA but also occurs in species belonging to other classes of PROTOZOA. Furthermore the various groups of SPOROZOA do not seem to be very closely related. They are all placed together in one class, therefore, largely for the sake of convenience and can only be classified successfully after more knowledge has been gained regarding their life-histories. For the present we may separate the class into subclasses and orders as follows. A more detailed classification of each order will be given when these are separately considered.

B. Classification of the Sporozoa

All parasitic; no organs for locomotion or for ingestion of food in adult; reproduction typically by spores.

CLASS SPOROZOA

SUBCLASS 1. TELOSPORIDIA. Formation of spores by adults, ending life of the parent.

ORDER 1. GREGARINÆ. Cœlozoic; reproduction usually by sporogony only. Ex. *Monocystis agilis*; *Gregarina*.

ORDER 2. COCCIDIA. Cytozoic; alternation of schizogony and sporogony. Ex. *Eimeria stiedæ*; *Isospora hominis*.

ORDER 3. HÆMOSPORIDIA. Cytozoic in blood cells of vertebrates; alternation of schizogony and sporogony in alternate hosts; usually no resistant spores. Ex. *Plasmodium vivax*; *Hæmoproteus columbe*; *Hæmogregarina muris*; *Piroplasma bigemina*.

SUBCLASS 2. NEOSPORIDIA. Formation of spores during growth of parent.

ORDER 1. MYXOSPORIDIA. Large multinucleate plasmodium; large spores with usually two polar capsules. Ex. *Myxidium lieberkühni*; *Leptotheca ranarum*.

ORDER 2. MICROSPORIDIA. Spores very small with usually one polar capsule. Ex. *Nosema apis*; *Glugea anomala*.

ORDER 3. SARCOSPORIDIA. Parasites in muscle cells of vertebrates forming sack-like spore cases (Miescher's tubules). Ex. *Sarcocystis muris*.

ORDER 4. HAPLOSPORIDIA. Spores, simple cells without polar capsules. Parasites mostly of fish and invertebrates. Ex. *Ichthyosporidium*; *Rhinosporidium seeberi*.

The life-cycles of the SPOROZOA are so varied that it seems worth while to outline briefly an example that will serve as an introduction to this phase of our study.

C. Stages that May Occur in the Life-Cycle of a Sporozoan

- | | | | |
|--|---|------------|---|
| Stages within the
vertebrate host | { | Schizogony | 1. Sporozoite—stage of entrance into vertebrate host. |
| | | | 2. Trophozoite—young vegetative stage developed from the sporozoite. |
| | | | 3. Schizont—older vegetative stage that will divide into merozoites. |
| | | | 4. Merozoite—one of the small infective forms resulting from the division of the schizont. |
| | | | 5. Macrogametocyte—stage that develops from a trophozoite and produces one or more female gametes. |
| | | | 6. Microgametocyte—stage that develops from a trophozoite and produces a number of male gametes. |
| Stages within the
invertebrate host | { | Sporogony | 7. Gametogenesis—formation of male gametes (microgametes) and female gametes (macrogametes) by the gametocytes. |
| | | | 8. Zygote—stage produced by the fusion of one microgamete with one macrogamete. |
| | | | 9. Oökinete—active vermiform stage developed from the zygote. |
| | | | 10. Oöcyst—passive spherical body into which the oökinete changes in the host. |
| | | | 11. Sporoblasts—cellular divisions of the protoplasm within the oöcyst. Each becomes surrounded by a wall, the sporocyst, and is then known as a spore. |
| | | | 12. Sporozoites—infective stages formed within the spores. |

CHAPTER VIII¹

THE GREGARINES AND COCCILIA

A. Order Gregarinæ

I. INTRODUCTION

The gregarines are common parasites of insects, especially in the digestive tract and body cavity, and less common in other groups of vertebrates and invertebrates. They are at first intracellular but later often become free in cavities of the body. Here they sometimes grow to a comparatively enormous size. Gregarines may be separated into two sub-orders and these again into two groups each, as follows:

EUGREGARINÆ. Without schizogony.

ACEPHALINA. Without an epimerite or divided body; usually inhabiting the coelom. Ex. *Monocystis*.

CEPHALINA. With an epimerite and usually a protomerite and deutomerite. Ex. *Gregarina*.

SCHIZOGREGARINÆ. With schizogony.

MONOSPORA. Zygote forms a single spore. Ex. *Ophryocystis*.

POLYSPORA. Zygote forms many spores. Ex. *Schizocystis*.

Specimens of *Monocystis* and *Gregarina* are easily obtainable from earthworms and insects; examples of the schizogregarines are not usually available for study. In the following pages the life-histories of one type from each of the four groups will be described and then a general account of the entire order will be presented.

¹ By R. W. Hegner.

2. MONOCYSTIS

Monocystis is a eugregarine of the group ACEPHALINA. It lives in the seminal vesicles of the common earthworm and the percentage of infection in these animals is very high. The infective stage is the spore which contains eight sporozoites (Fig. 102, K. A. *sps*). Each sporozoite penetrates a bundle of sperm mother cells (B, *sp*) of the earthworm, and is then termed a trophozoite (B, *tr*). Here it lives at the expense of the cells among which it lies. The spermatozoa of the earthworm, which are deprived of nourishment by the parasite, slowly shrivel up (C), finally becoming tiny filaments on the surface of the trophozoite (D).

When this stage is reached, two trophozoites come together (E) and are surrounded by a common two-layered cyst wall (F, *ep, en*). Each then divides, producing a number of small cells called gametes (G). The gametes unite in pairs (H) to form zygotes (*zy*). It is probable that the gametes produced by one of the trophozoites do not fuse with each other, but with gametes produced by the other trophozoite enclosed in the cyst. Each zygote becomes lemon-shaped, is now known as a sporoblast (I), and secretes a thin hard wall, the sporocyst, about itself, thus becoming a spore. The nucleus of the spore divides successively into two, four, and finally eight daughter nuclei (J); each of these, together with a portion of the cytoplasm, becomes a sporozoite (K, A, *sps*).

Usually the spores of the gregarines make their way to the outside in the feces of the host but when contained in internal organs they must escape in some other way. Two methods of escape may exist in the case of *Monocystis*, (1) the spores may pass down the vas deferens and out through the genital pore either being scattered broadcast or, if copulation is taking place, being transferred directly to the seminal vesicles of another worm; or (2) when worms are

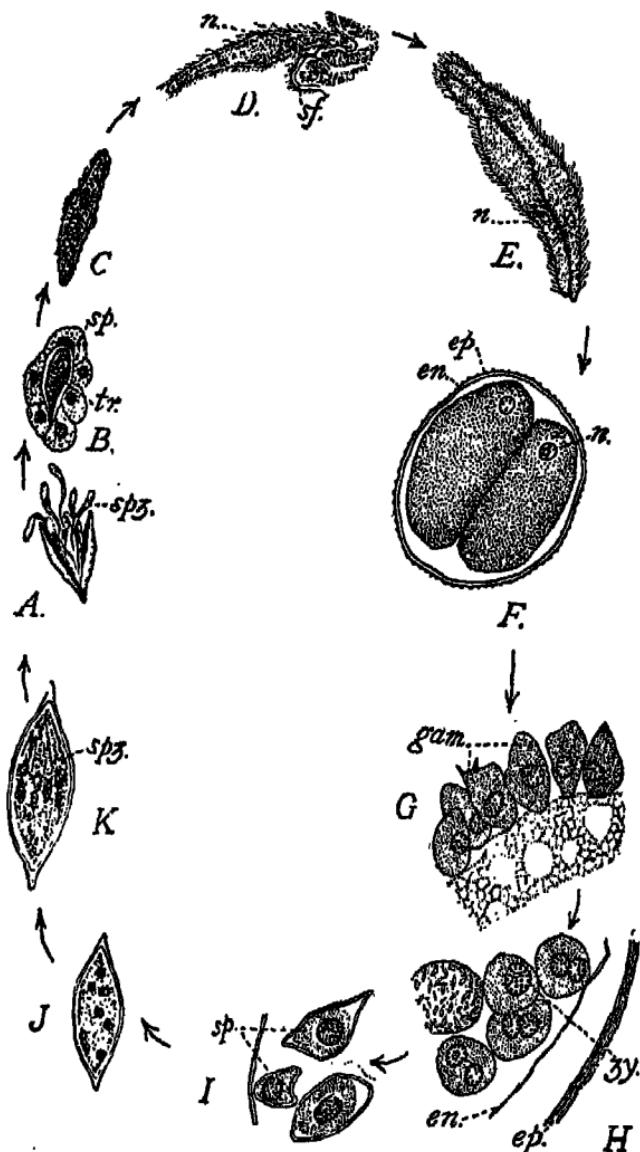


Fig. 102.—*Monocystis*, an acellular gregarine parasitic in the seminal vesicle of the earthworm. A, the eight sporozoites (*sps.*) escaping from the sporocyst. B, a young trophozoite (*tr.*) among the sperm-mother cells (*sp.*) of the earthworm. C, a free individual with a few withered sperm cells adhering to it. D, a mature individual

eaten by birds the spores may pass unharmed through the digestive tract and being cast out in the birds' feces be taken in by uninfected worms with their food.

3. GREGARINA

The cephaline eugregarines are especially abundant in insects, myriapods and crustacea and less numerous in annelid worms, flatworms, mollusks, arachnids, tunicates, and ONYCOPHORA.

The spores of *Gregarina* (Fig. 103, L) are swallowed by the hosts with their food, and the sporozoites are liberated by the action of the digestive juices of the host (A). The falciform sporozoites attach themselves to the intestinal epithelium and bore their way partly into the cell by amoeboid movements, either puncturing the cell wall or weakening it by the secretion of an enzyme. The parasite, which is now known as a trophozoite (B, *tr*), grows at the expense of the cells to which it is attached and of neighboring cells and soon becomes visibly modified into three regions (C), (1) the epimerite (*ep*) which is fastened to the cell, (2) the protomerite (*pr*) which is separated by a septum from (3) the deutomerite (*deu*). The young gregarine is now a cephalont (C). The ectoplasm is differentiated to form an outer cuticle or epicyst, a middle layer, the sarcocyte, and an inner layer, the myocyte. The epimerite is derived from the epicyst, the septum from the sarcocyte, and the contractile fibrils called myonemes, from the myocyte. The endoplasm is very granular and is characterized by the presence of

attached to the sperm-funnel (*sf*) of the earthworm. E, two mature individuals joined side by side. F, two individuals have formed a cyst; *en*, endocyst; *ep*, epicyst; *n*, nucleus. G, gametes (*gam*) formed by one individual within the cyst. H, conjugation of gametes to form zygotes (*sy*). I, zygotes that have secreted spore coats or sporocysts and have become sporoblasts (*sp*). J, a single spore in which the nucleus has divided, forming eight daughter nuclei. K, a fully developed spore containing eight sporozoites (*sps*). (From Hegner, after Cuénod and Bourne.)

great numbers of paraglycogen food bodies. Within the endoplasm, usually near the anterior end of the deutomerite, lies the nucleus, which is large and spherical and contains

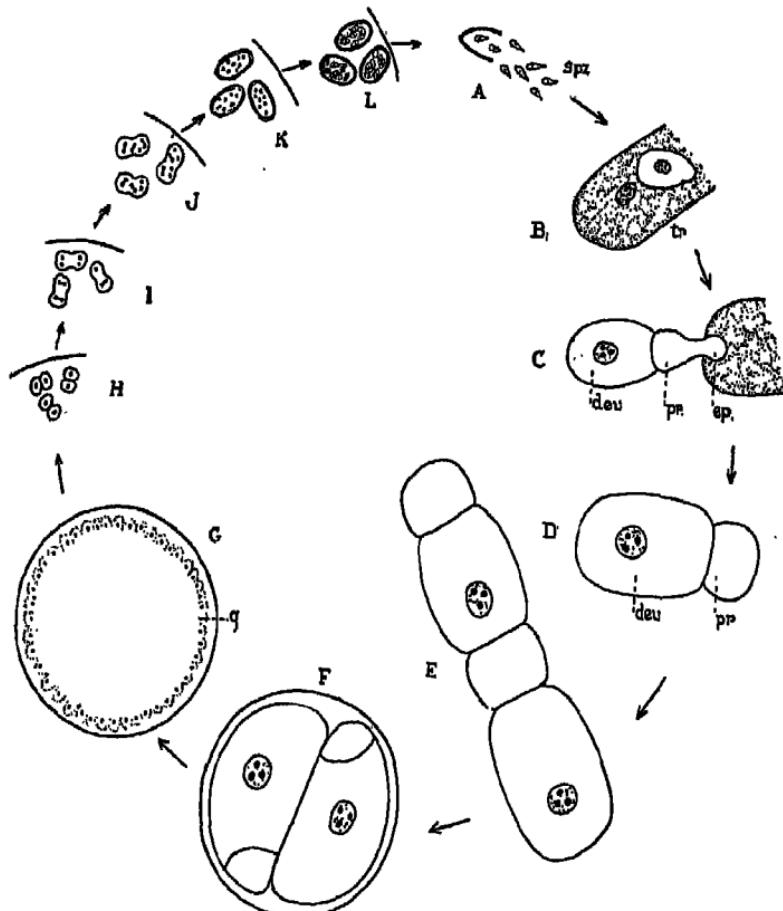


Fig. 103.—*Gregarina*. Diagrams illustrating the life-cycle of a cephaline gregarine.

A, Sporozoites (*spz*) escaping from spore. B, Trophozoite in epithelial cell of host. C, Cephalont attached to host cell. *ep*=epimerite, *pr*=protomerite, *deu*=deutomerite. D, Sporont. E, Two sporonts united end to end in syzygy. F, Cyst containing two sporonts. G, Gametogenesis, *g*=gamete. H, Fusion of gametes in pairs. I, J, Division of nuclei within zygote. K, Young spore with 8 nuclei. L, Spores, containing 8 sporozoites each. (Original.)

one or more large karyosomes often plainly visible in the living state. From 4 to 8 chromosomes have been reported for various species.

As growth continues the epimerite is lost and the parasite becomes free in the lumen of the digestive tract, now being known as a sporont (Fig. 103, D). The sporonts are capable of locomotion but the method by which this is accomplished is still in doubt. According to one theory (Schewiakoff, 1894) the sporont pushes itself forward by secreting a hollow gelatinous stalk at the posterior end; but most observers agree that it "moves forward by imperceptible vertical movements in the myonemes on that side of the body which happens to be ventral at the time, friction being produced with the under surface by the exudation of mucus from the body." (Watson, 1916, p. 24.) This mucus comes from minute pores situated between the longitudinal ridges on the surface of the body. There is no backward movement, but bending and twisting movements are common being also due to the contractility of the myonemes. The sporonts of the genus *Gregarina* have a very peculiar habit of becoming associated end to end in pairs, a condition known as syzygy (Fig. 103, E). This frequently occurs when they are still young. Later, when the sporonts are fully grown, the members of each pair begin to rotate about each other forming a spherical mass which secretes a thick gelatinous wall thus forming a cyst (F). The cyst passes out of the host in its feces and, if subjected to moisture, begins to develop in about 48 hours. The nucleus of each sporont within the cyst undergoes many successive divisions, the daughter nuclei finally being cut off with a small amount of cytoplasm by cell walls. The small cells thus formed are gametes (G, g). These gametes fuse in pairs (fertilization) to form zygotes (H). The zygotes are sporoblasts; they acquire a resistant covering, the sporocyst, and become spores (I-L). Within each spore 8 sporozoites are formed

(K, L). In certain species the spores are liberated from the cysts through spore ducts in from 42 to 60 hours. The spores are at first in long chains but later become separated; they are scattered widely over the ground by wind and rain and when accidentally devoured by new hosts with their food a new infection is brought about.

The life-cycle of a typical cephaline gregarine (Fig. 103) thus includes the following stages: spore-sporozoite-trophozoite-cephalon-t-sporont-syzygy-gametogenesis-fertilization-zygote-sporoblast-sporocyst-spore.

4. OPHRYOCYSTIS

Ophryocystis belongs to the group MONOSPORA of the sub-order SCHIZOGREGARINA. It is characterized by the presence of a multiplicative asexual process (schizogony) in its life-history and by the production of a single spore by each pair of gametocytes.

This genus contains a number of species occurring in the malpighian tubules of beetles. It was first described by Schneider in 1883 and put into a group by itself called the AMBROSPIRIDA. Léger (1907) has worked out its developmental cycle as shown in the accompanying diagram (Fig. 104). The sporozoite (A) attaches itself to the epithelial cells of the malpighian tubules by rigid prolongations (B, C); it then becomes a trophozoite which undergoes a number of nuclear divisions without the formation of cell walls (C, D, E); this is the mycetoid schizont (D). Cell walls then appear cutting up the mass into uninuclear merozoites. These attack new cells (F) and form a new generation of trophozoites, each of which grows, undergoes several nuclear divisions and forms a gregarinoid schizont (G, H). This schizont divides into unicellular sporonts (I, J). The sporonts conjugate in pairs (K) and a cyst forms around each pair (L, M). This is followed by the division of the sporont nucleus into three (L, M); each sporont then

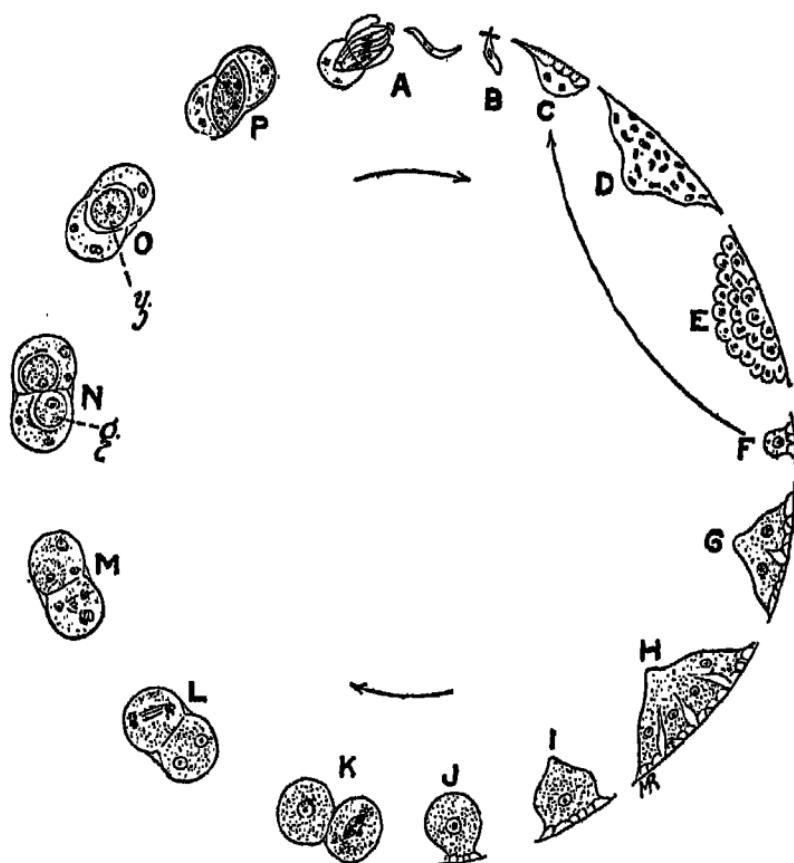


Fig. 104.—*Ophryocystis*. Diagrams illustrating the life-cycle of a monosporous schizogregarine.

A, Spore setting free sporozoites. B, Sporozoite attached to epithelium of host. C, Multiplication of nucleus of trophozoite. D, Multinucleate or "mycetoid" schizont. E, Division of multinucleate schizont into merozoites. F, Each merozoite may become a multinucleate schizont again, or G, H, may become a paucinucleate or "gregarinoid" schizont. H, division of paucinucleate schizont to form young sporonts (I, J). K, Association of two sporonts. L, Formation of common cyst round the associated sporonts, and division of their nuclei. M, Formation of three nuclei in each sporont. N, Separation of a gamete (*g.*) within body of each sporont, while rest of body, with two nuclei, becomes an envelope-cell. O, Two gametes have fused to form the zygote (*z.*) or sporoblast. P, Sporoblast has assumed the form of the spore, and its nuclei have divided into four; ultimately eight nuclei and as many sporozoites are formed. (From Minchin after Léger.)

divides into two cells (N), a small uninuclear cell which is a gamete (g), and a larger binuclear cell which forms a protecting envelope. The two gametes fuse to form a zygote (O), and the zygote produces 8 sporozoites as usual (P, A). This life-cycle is of particular interest since there are two types of schizogony, and a peculiar process of gametogenesis.

5. SCHIZOCYSTIS

Schizocystis belongs to the schizogregarines, and to the group POLYSPORA. It undergoes schizogony and one pair of gametocytes gives rise to many spores. Our knowledge of the life-cycle of *Schizocystis* we owe also to Léger (1900, 1909). This form occurs in the intestine of dipterous larvæ. The sporozoite (Fig. 105, A) attaches itself to an epithelial cell and grows into a trophozoite that becomes a multinucleate schizont, either long and slender (D, E, a) or else shorter and oval in shape (D, E, b). Cell walls divide up the schizont into many uninucleate merozoites (F) which may attack new cells (G^1 , G^2) or may become sporonts (G^3). The sporonts or gametocytes unite in pairs (H, I) and form cysts (J). Each member of the pair produces a number of gametes (K, L), those from one being macrogametes and those from the other, microgametes. These conjugate to form zygotes (M, N) and each zygote after changing into a typical spore, gives rise to 8 sporozoites (A).

6. GREGARINES IN GENERAL

a. Growth of Our Knowledge of the Gregarines

The first description of a gregarine is usually attributed to Redi (1708). Eighty years later, Cavolini (1787) described and figured an organism which he considered a sort of tapeworm but which was undoubtedly a gregarine. The term "gregarine" we owe to Dufour (1828) who found many of them in insects and supposed them to be allied to the

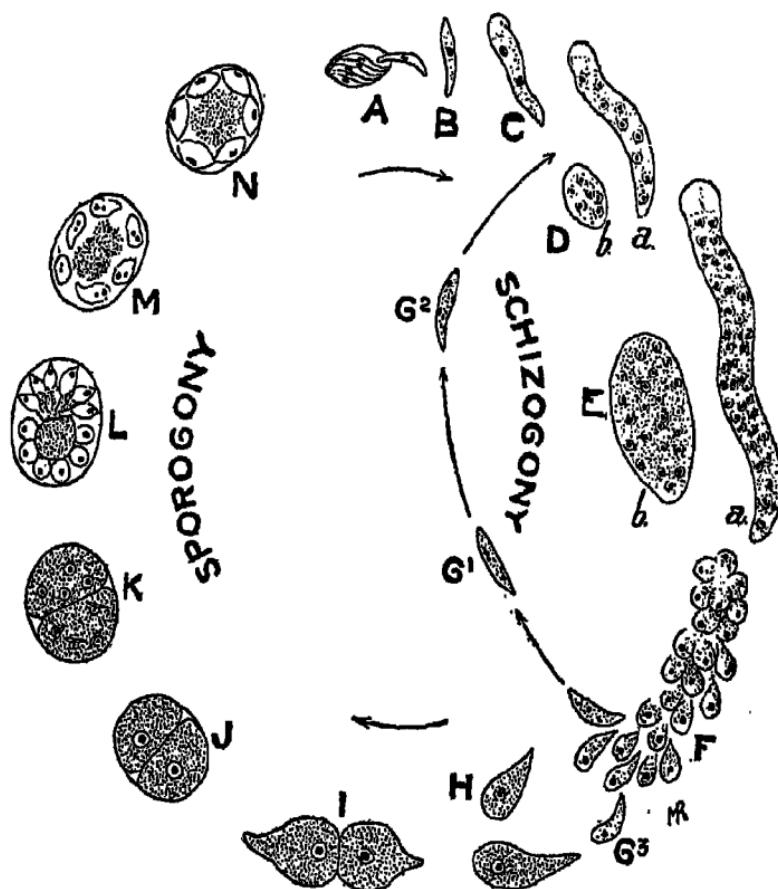


Fig. 105.—*Schizocystis*. Diagrams illustrating the life-cycle of a polysporous schizogregarine.

A, Sporozoite escaping from the spores. B, C, D, E, Growth of sporozoite into multinucleate schizont, of which there are two types: the vermiciform schizont (a), which attaches itself to the epithelium by its anterior end, and the massive schizont (b), which lies free in the gut of the host. F, Division of the schizont into merozoites, which may either grow into schizonts again (G^1 , G^2), or may grow into sporonts (G^3). H, Young sporonts. I, Association of two full-grown sporonts. J, Formation of a common cyst by two associated sporonts. K, Division of nuclei in sporonts. L, Formation of gametes by sporonts. M, Copulation of gametes. N, Each zygote becomes a sporoblast and forms a spore. (From Minchin after Léger.)

trematodes. The nucleus was first accurately described by Siebold (1839), who also observed cysts and spores but failed to establish their relation to the protozoon. Kölliker (1845-1848) contributed greatly to our knowledge of gregarines and announced their unicellular nature although this fact was not immediately accepted, many biologists believing that they were the embryonic stages of worms. To Stein (1848) we owe the demonstration of the connection between gregarines and spores. Leidy (1851-1890) in the United States and Lankester (1863-1882) in England published a number of papers on gregarines. Later contributors were A. Schneider (1873-1892), Cuénnot (1891-1901), Léger (1892-1906), and Léger and Dubosq (1899-1909). The state of our knowledge of the gregarines has been summarized at various times by Lankester (1863), Bütschli (1882), Léger (1892) and Minchin (1903). Much work has more recently been devoted to systemic studies with which in this country the names of Crawley, Hall, Ellis, and Watson are associated.

b. Characteristics of the Gregarines

Vegetative stage at first usually intracellular in epithelial cells, later extracellular in cavities in the body; the adults often become united in syzygy; pairs of adults after encystment form one to many gametes; these may be similar (isogametes) or of two types (anisogametes) which conjugate in pairs; the zygotes produce one spore each, which usually divides to form eight sporozoites.

c. Variations in the Life-Cycles of Gregarines

Many variations in the morphology, activities and life-cycles have been recorded among gregarines. If many of these variations are disregarded, the stages in the life-cycles of gregarines in general may be summarized as follows:

(1) *Sporozoite*.—The sporozoites are usually falciform bodies that are liberated by digestive juices from spores

taken into the digestive tract of the host. In the ACEPHALINA and in some of the CEPHALINA, the parasites become intracellular. The sporozoites probably weaken the resistance of the epithelial cells of the intestine, and penetrate these cells by means of amoeboid activities. In the rest of the CEPHALINA the sporozoites become attached to epithelial cells by means of a rostrum which develops into an organ of attachment called the epimerite.

(2) *Trophozoite or Cephalont*.—Within the cell the parasite becomes more or less oval in shape and takes nourishment by absorption from the surrounding protoplasm. It is now called a trophozoite or cephalont. The nucleus of the host cell breaks up and the cytoplasm undergoes chemical changes as indicated by staining reactions. In certain species it has been shown that many cells take part in the nourishment of the parasite and that all of these are ultimately destroyed. (Watson, 1918.) The CEPHALINA that are extracellular probably absorb food through the epimerite.

(3) *Sporont*.—When the intracellular form breaks out into the digestive cavity (usually), body cavity or blood vessels and when the extracellular forms become free from the epithelial cells to which they were attached the parasites are known as sporonts. The sporonts are uninuclear but may be septate or polycystid, i.e., the body may be divided into two or more parts by septa. Usually only one septum is present. The anterior part of the organism is then known as the protomerite and the posterior nucleated portion as the deutomerite.

(4) *Syzygy*.—This term has been applied to the temporary association of two sporonts by attachment end to end. Generally only two individuals become associated in this way, the anterior partner is the prime, the posterior the satellite. The tendency of gregarines to form groups suggested the name *Gregarina* for these animals.

(5) *Schizogony*.—Certain stages of the schizogregarines

undergo a multiplicative period without conjugation. These stages are called schizonts and the process is known as schizogony. Examples of schizogony have been described on the preceding pages for *Ophryocystis* and *Schizocystis* and illustrated in Figs. 104 and 105.

(6) *Conjugation*.—This is the permanent association of two sporonts. Such sporonts are sometimes called gamonts or gametocytes. The two partners are usually indistinguishable but a sexual difference may be present. In certain cases this type of association may occur when the sporonts are young—a condition known as neogamy. After union a cyst is formed around the conjugating pair.

(7) *Gametogenesis*.—By a succession of nuclear divisions, each conjugant within the cyst becomes provided with many nuclei. These migrate to the surface where they are cut off by cell walls to form a peripheral layer of small cells which are the gametes. Much of the cytoplasm of the gametocyte is left behind with some of the nuclei as residual protoplasm. The gametes of the two conjugants may be similar in their characteristics (isogamy) or may differ more or less (anisogamy).

(8) *Fertilization*.—The gametes within the cyst unite in pairs to form zygotes. When the gametes are visibly different dissimilar gametes always unite together, i.e., the gametes derived from one conjugating sporont always unite with those derived from the other. It is probable that this is also the case when the gametes produced by the two sporonts within a cyst are visibly alike. Reduction in the chromosomes has been described by several investigators. In a gregarine of the cockroach (*Diplocystis schneideri*) the conjugating gametes each possess three chromosomes, two spherical and equal in size and one ovoid and larger. These six chromosomes become arranged as three homologous pairs on the first division spindle of the zygote. Entire chromosomes are separated and reduction occurs at this division

in the zygote and not, as in the case of METAZOA, before fertilization. The chromosomes divide during the two succeeding divisions, and the nuclei of the eight sporozoites are thus produced each with the reduced number (3) of chromosomes. (Dobell and Jameson, 1915.)

(9) *Zygote*.—The zygote is oval or spindle-shaped. It is surrounded by a membrane, the sporocyst, composed generally of an outer layer, the episore, and an inner layer, the endospore. The entire zygote is now a spore and the contents, the sporoblast. Within the sporoblast the nucleus undergoes three successive divisions and the cytoplasm divides, usually longitudinally, in such a way as to form eight sporozoites, each with one nucleus. A residuum of cytoplasm is left behind.

(10) *Spore*.—The spore with its contained sporozoites usually passes out of the body in the faeces, and new hosts are usually infected by contamination of their food and water supply.

B. Order Coccidia

1. INTRODUCTION

The coccidia are common parasites of vertebrates, myriapods, and mollusks, and are less common in insects, annelids, and flat worms. Two suborders may be recognized as follows:

EIMERIDEA. Gametocytes do not conjugate; many microgametes formed. Ex. *Eimeria stiedæ*; *Isospora hominis*.

ADELEIDEA. Gametocytes conjugate; 4 microgametes. Ex. *Adelea ovata*.

2. EIMERIA STIEDÆ

One of the best known of the coccidia is *Eimeria stiedæ* (*Coccidium oviforme* or *C. cuniculi*) a species that occurs in the liver and intestine of the rabbit (Fig. 106). This species

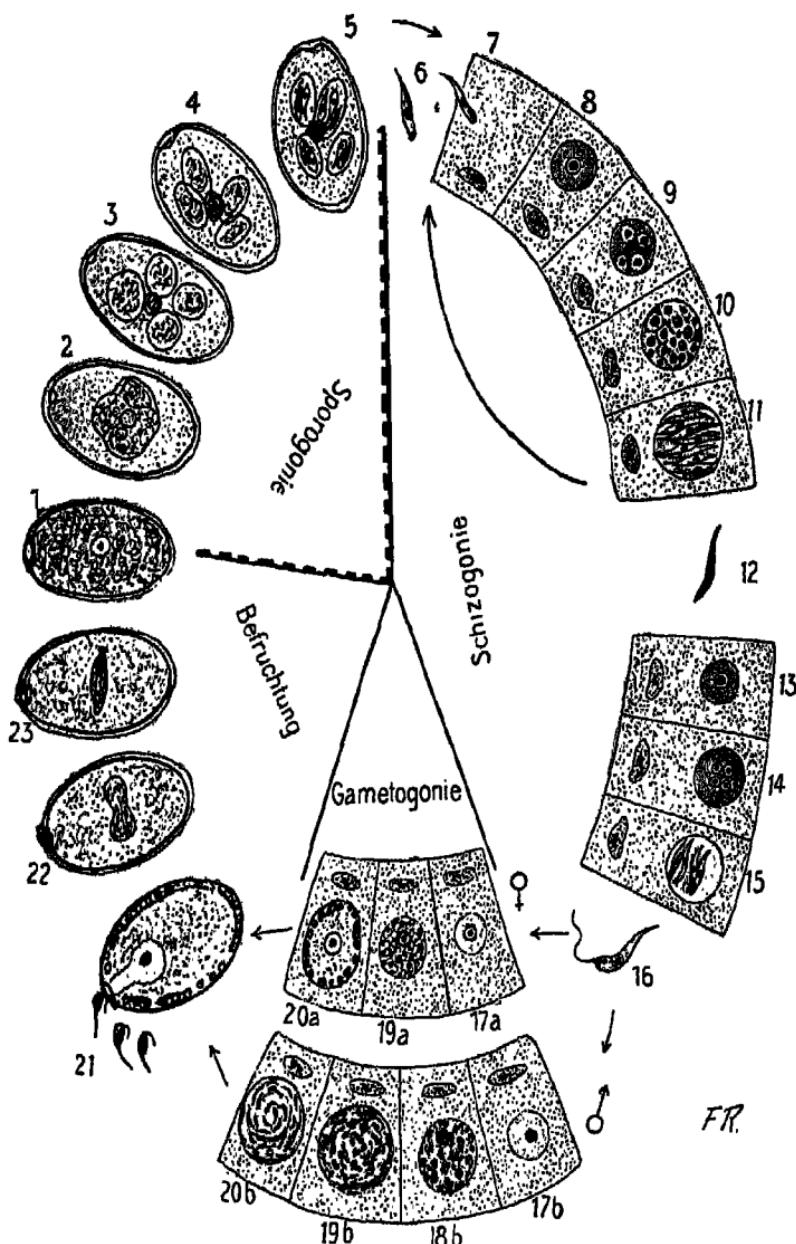


Fig. 106.—*Eimeria stiedae*. Diagrams illustrating the life-cycle of a coccidium. 1-5, Sporogony. 6-15, Schizogony. 17-20, Gametogenesis. 21-23, Fertilization. (After Reich.)

has a life-cycle that is probably similar to that of *Eimeria wenyonii* that has been found living in man.

The sporozoites (Fig. 106, 6) escape through the micropyle of the spores in the intestine of the rabbit. They glide along somewhat like a minute gregarine and bore their way into the epithelial cells (7), where they become trophozoites (8).

The trophozoite feeds at the expense of the cytoplasm in the host cell and grows into a large spherical schizont with a vesicular nucleus containing a large karyosome (8).

The nucleus of the fully grown schizont undergoes successive divisions until there are 16 or 32 daughter nuclei present (9, 10). Cell walls then appear cutting up the schizont into an equal number of uninucleate merozoites (11). The merozoites may attack new epithelial cells and inaugurate another similar multiplicative process (schizogony) or may enter epithelial cells (12) and produce one set of only 4 merozoites (13-15) which differ from the others in possessing a basal granule to which is attached a flagellum (16).

These flagellated merozoites penetrate other epithelial cells and become either macrogametocytes (17, a) or microgametocytes (17, b). The macrogametocyte grows into a large oval body containing many plastinoid and chromatoid granules which wander to the periphery where they form a sort of membrane (17, a, 19, a, 20, a). It develops into the macrogamete (21). The microgametocyte becomes large and spherical and within it are formed a large number of microgametes (17, b-20, b). These are biflagellated, one flagellum extending forward from the anterior end and the other trailing backward (21).

Fertilization (21) takes place either in the epithelial cell or in the lumen of the intestine. One microgamete enters the micropyle of each macrogamete and the nuclei of the two gametes fuse.

The fertilized egg or zygote (22, 23) then passes out of the intestine as an oöcyst in the feces of the rabbit (1). Sporogony occurs outside of the host. The protoplasm within the oöcysts rounds up into a spherical mass (2); then divides into four spherical sporoblasts (3) which later become ovoidal (4), secrete a sporocyst and becomes spores. Part of the protoplasm is not used in the formation of the spores but remains behind as a residuum. Within each spore, two sporozoites develop (5); in this process also a small amount of protoplasm forms a residuum. New infections result from the contamination of the food of the rabbit with the spores containing sporozoites.

3. OTHER COCCIDIA

The life-cycles of other coccidia differ in many details from that of *Eimeria stiedæ*, a recital of which would be confusing. A few variations, however, in habitat and sporogony are indicated by the following selected species.

Cryptosporidium muris occurs in the gastric glands of the mouse; the trophozoites are free as in gregarines and a single tetrazoic spore (with four sporozoites) is formed by the zygote.

Cyclospora caryolytica parasitizes the nuclei of the intestinal epithelium of the mole; polyspermy occurs (the entrance of a number of microgametes into a single egg) but the nucleus of only one fuses with that of the macrogamete; two dizoic spores (each with two sporozoites) are produced by the zygote.

Caryospora simplex lives in the intestinal epithelium of the snake, *Vipera aspis*; one octozoic spore (with 8 sporozoites) is formed by the zygote.

Caryotropha mesnili is a parasite in the body cavity of a marine annelid; about 20 spores are formed in each oöcyst and each spore produces 12 sporozoites.

Adelca ovata, a representative of the suborder Adeleidea,

occurs in the myriapod, *Lithobius*; the nuclei of the schizont divide by multiple as well as by binary division; the gametocytes, which are of different sizes, associate in pairs at the time of gametogenesis, as they do in the gregarines; 4 non-flagellated microgametes are formed; many dizoic spores are produced in the oöcyst.

Among the pathogenic Coccidia should be mentioned *Eimeria avium* which has been extensively studied by Fantham. This species which occurs in birds has a life-cycle very similar to that of *Eimeria stiedæ*.

Isospora bigemina, a coccidium inhabiting the intestinal epithelium of dogs and cats, has been considered by many to be the same as that recorded for man. Its oöcyst, however, is much larger than that of *Isospora hominis* and it undoubtedly is a separate species.

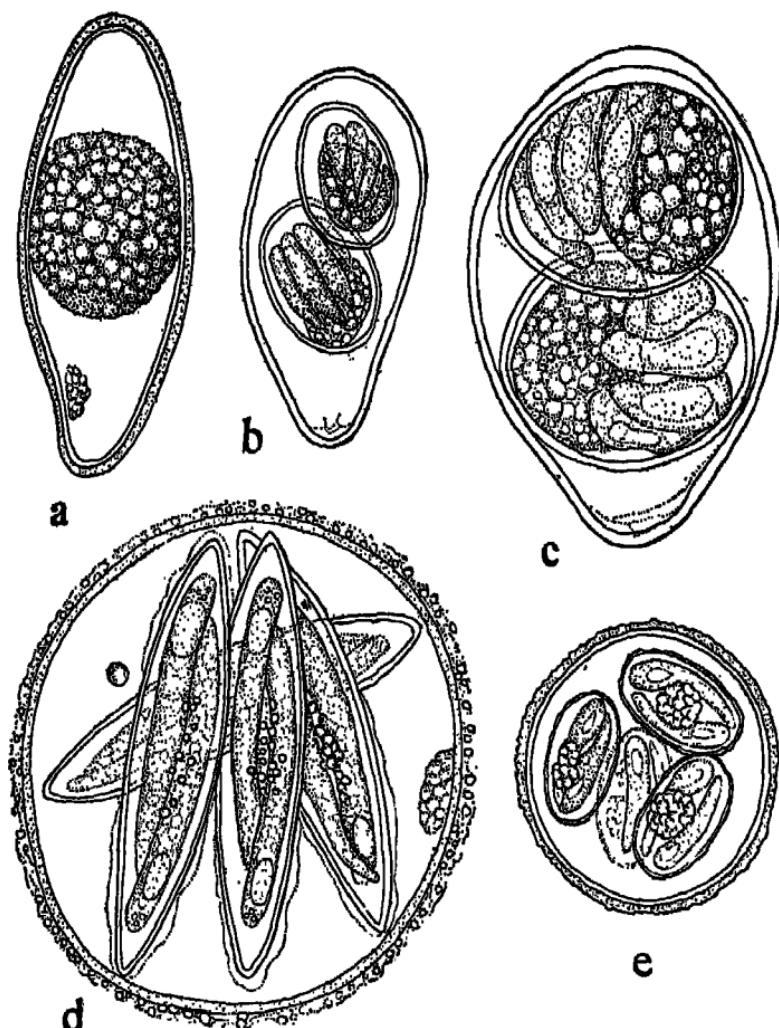
Eimeria zürni is the etiological factor of red diarrhoea in cattle and occurs especially in Switzerland, Sweden and Denmark. From 2% to 4% of the infected animals die within two days after becoming sick.

4. COCCIDIA PARASITIC IN MAN

The situation as regards the Coccidia reported from man has been admirably cleared up in a report by Dobell (1919). This author, who has personally carried on investigations on the Coccidia, was able to obtain and to read almost all of the literature on human coccidiosis and was thus in a position to make a satisfactory revision of the subject. Prior to the year 1915 a number of cases of human coccidiosis were described—often in language difficult to understand and inadequate both with regard to facts and to figures. The first case was recorded by Gubler (1858); if the bodies found by Gubler were really Coccidia the case was probably one of hepatic coccidiosis. The first case of intestinal coccidiosis in man is that of Kjellberg (Virchow, 1860), who found in the small intestine a species probably belonging to the genus

Isospora. Besides these, 8 other cases of what appear to have been human coccidiosis were reported up to 1915. Of the entire number, 5 were in the liver, 2 in the intestine and one in the feces.

A review of the writings of parasitologists indicates that the consensus of opinion is that the parasite of human coccidiosis is the same as that found in the liver and intestine of rabbits, cats and dogs, and that man is only a casual or accidental host. Several investigators, however, have more recently added so greatly to our knowledge of the COCCIDIA of man that this opinion seems to be erroneous, and there seems to be no doubt but that human coccidiosis is caused by distinctive species of COCCIDIA and that man is a true host. The best known species is *Isospora hominis*, first described by Virchow in 1860 and given the specific name *hominis* by Rivolta in 1878. This species was found by Wenyon (1915) and others in the feces of soldiers suffering from dysentery and enteritis and invalidated to England from Gallipoli; it has been noted in over 150 cases. All the stages of sporulation were observed by Wenyon and oöcysts containing two tetrazoic spores were demonstrated. The stages of development in the intestine are still unknown. The oöcysts (Fig. 107, *a, b*) are elongate ovoid in form, 25 to 33 microns in length and 12.5 to 16 microns in width. In the feces the contents of the oöcyst divides into 2 sporoblasts in each of which four vermiform sporozoites are formed. *Isospora hominis* has also been recorded from men who had been in Egypt, Salonika and Mesopotamia. The infections recorded have mostly been small and transitory although several heavy infections were found and oöcysts were passed by one patient for three weeks. No pathogenic effects can be attributed to the presence of this coccidium, the intestinal ailments of the patients in which the infections were found being probably due to other causes. No successful treatment has been devised for human coccidiosis although emetine

Fig. 107.—Oöcysts of COCCIDIA. $\times 1600$.

a, *Isospora hominis* from man, before spore formation. b, *I. hominis*, containing two spores with four sporozoites each. c, *I. bigemina*, from the cat, containing two spores with four sporozoites each. d, *Eimeria oxyspora* from man containing four spores, each with two sporozoites. e, *E. wenyoni* from man containing four spores, each with two sporozoites. (a-d, after Dobell; e, after Wenyon.)

hydrochloride has been tried, and no evidence is available that lower animals can be parasitized by the human species of coccidia.

Eimeria wenyonii (Fig. 107, e) is a species discovered by Wenyon in 1915 and named by Dobell (1919). Only its oöcysts have been observed. These are spherical, about 20 microns in diameter and with an outer rough surface. There are four spores in the oöcyst each of which contains two sporozoites; these are already differentiated when the oöcysts are passed by the patient. Only four cases of this species have been recorded to date.

Eimeria oxyphora (Fig. 107, d) is known from three cases (Dobell, 1919, Broughton-Alcock and Thomson, 1922, Thomson and Robertson, 1923). When passed in the feces the four dizoic spores are completely differentiated. The oöcyst is spherical and from $36\ \mu$ to $52\ \mu$ in diameter. *Eimeria* (?) sp., the hepatic coccidium of man, although the first human coccidium to be discovered, is one of the least known, there being but five cases recorded and these records are inadequate.

Eimeria snijdersi (Dobell, 1921) is the most recently described species of human coccidium. It was discovered by Snijders in Sumatra. The oöcyst is spherical averaging about 45 microns in diameter, and contains four spindle-shaped spores. It is not yet certain that *E. oxyphora* and *E. snijdersi* are not one and the same species. (See Broughton-Alcock and Thomson, 1922.)

The genera and species of the coccidial parasites of man are as follows:¹

¹ Wenyon (1923) has just published an extensive piece of work on coccidiosis of cats and dogs and the status of the *Isospora* of man in which he concludes, (1) that there are three species of coccidia of the genus *Isospora* occurring in dogs and cats, *I. felis*, n. sp., *I. rivolta* (Grassi, 1879) and *I. bigemina* (Stiles, 1891), (2) that the small *Isospora* of man is *I. hominis* (Railliet and Lucet, 1891), (3) and

1. *Isospora hominis* Rivolta..... 1878
2. *Eimeria wenyonii* Dobell..... 1919
3. *Eimeria oxyphora* Dobell..... 1919
4. *Eimeria (?) sp.* Dobell..... 1919
5. *Eimeria snijdersi* Dobell..... 1921

5. GROWTH OF OUR KNOWLEDGE OF THE COCCIDIA

The discovery of the coccidia is usually attributed to Hake who in 1839 recorded "pus corpuscles" in liver nodules of the rabbit, but Dobell (1922) has found a reference to oval corpuscles in the bite of rabbits in a letter of Leeuwenhoek dated October 19, 1674. These were probably oöcysts of *Eimeria stiedæ* and hence to Leeuwenhoek must be given the credit for their discovery although he did not know what they were. Coccidia were found by Remak (1845) in the small intestine of the rabbit and their similarity to the psorosporia of the MYXOSPORIDIA was pointed out; and later Lieberkühn (1854) definitely announced them to be psorospers. Stieda (1865) studied especially sporogony in the rabbit and Eimer (1870) discovered trophozoites in epithelial cells and established their pathogenicity. To Leuckart (1879) we owe the name COCCIDIA and their position in the class SPOROZOA. Fertilization in the COCCIDIA was established by Schaudinn and Siedlechi (1897) and three years later Schaudinn (1900) published the first complete account of the life-cycle of any species, that of *Coccidium schubergi* parasitic in the myriapod, *Lithobius*.

Among the more recent investigators who have added to our knowledge of this group are Dobell, Hadley, Jollos, Leger, Duboscq, Reich, Schellack and Tyzzer.

that a "larger form discovered in the faeces of man during the war, and regarded by Dobell as identical with the small form described by Virchow" is a distinct species for which the name *Isospora belli* is proposed.

6. A COMPARISON OF GREGARINES AND COCCIDIA

The life-cycles of gregarines and coccidia differ in the following characteristics.

(1) The trophozoite in the coccidia is intracellular; in gregarines it is often extracellular.

(2) The gametocytes of coccidia usually do not conjugate; in gregarines they do.

(3) The macrogametocyte in the coccidia produces one macrogamete; in the gregarines it gives rise to many macrogametes.

(4) Only one zygote is formed in a cyst in the coccidia; many zygotes occupy one cyst in the gregarines.

(5) Many spores result from each zygote in the coccidia; only one is derived from each zygote in the gregarines.

The characteristics of the coccidia may thus be stated as follows:

Trophozoite intracellular; schizogony always forms part of the life-cycle; macrogametocytes and microgametocytes usually do not conjugate; the macrogametes are large and single; the microgametes are usually small and numerous; each zygote produces many spores.

CHAPTER IX¹

THE ORDER HÆMOSPORIDIA EXCLUSIVE OF THE MALARIAL PARASITES

A. Introduction

HÆMOSPORIDIA are intracellular parasites of the blood cells of vertebrates. Among their number are included some of the most destructive of all animal parasites, such as the malarial organism, and the parasite that causes Texas fever in cattle. One of their characteristics is an alternation of hosts in the life-cycle, an *asexual multiplicative phase* (*schizogony*) taking place in a vertebrate host and a *sexual propagative stage* (*sporogony*) in an invertebrate host (arthropod or leech). The families listed below are placed in this order for the sake of convenience and with the idea that further knowledge of these groups may enable us to assign them to a more definite position in the protozoan series than is possible at the present time.

FAMILY 1. HÆMOGREGARINIDÆ. Unpigmented parasites of red and white cells in peripheral blood. Ex. *Hæmogregarina muris*, *H. hominis*.

FAMILY 2. TOXOPLASMIDÆ. Unpigmented parasites in white cells in internal organs. Ex. *Toxoplasma pyrogenes*.

FAMILY 3. PIROPLASMIDÆ. Unpigmented parasites in red cells in peripheral blood. Ex. *Piroplasma canis*, *P. bigemina*.

FAMILY 4. PLASMODIDÆ. Pigmented parasites. Ex. *Plasmodium vivax*, *Hæmocystidium simondi*, *Hæmoproteus columbae*.

¹ By R. W. Hegner.

B. Hæmogregarines**I. HÆMOGREGARINA MURIS**

The first hæmogregarine was found in the frog by Chausat in 1850. Since then various species have been reported from mammals, birds, reptiles, amphibians and fishes. The life-cycle that is best known is that of *Hæmogregarina muris* (*Hepatozoon perniciosum*) which was worked out by Miller (1908) at the Hygienic Laboratory in Washington. This species undergoes schizogony in the rat and sporogony in a mite, *Lelaps echidninus*, which feeds on the blood of the rat. The mites are eaten by the rats and spores of *H. muris* contained in them are liberated in the rat's intestine (Fig. 108, 22). The sporozoites that escape penetrate the intestinal villi (Fig. 108, 1), enter the blood stream (2), and are carried to the liver, where they penetrate the liver cells (3). Here the trophozoites grow at the expense of the liver cells of the host into schizonts (4) in which develop from 12 to 20 merozoites (5-8). Certain of the merozoites, when they break out of the host cell, enter fresh liver cells and pass through another asexual cycle (schizogony); others make their way into mononuclear leucocytes in the blood, where they develop into gametocytes (8).

If these parasitized leucocytes are taken with the blood into the digestive tract of the mite, the gametocytes are freed by the digestive juices (Fig. 108, 9) and proceed to copulate in pairs (10). One of each pair becomes a large, granular macrogamete which encircles the other, which is a smaller, spherical, less granular microgamete (11). Complete fusion now takes place (12); the zygotes thus formed change into oökinetes which make their way through the intestinal wall (13) into the body cavity (14) and become encysted oöcysts in the body tissues (15). From 50 to 100 sporoblasts are formed within the greatly enlarged oöcyst (17, 18); these

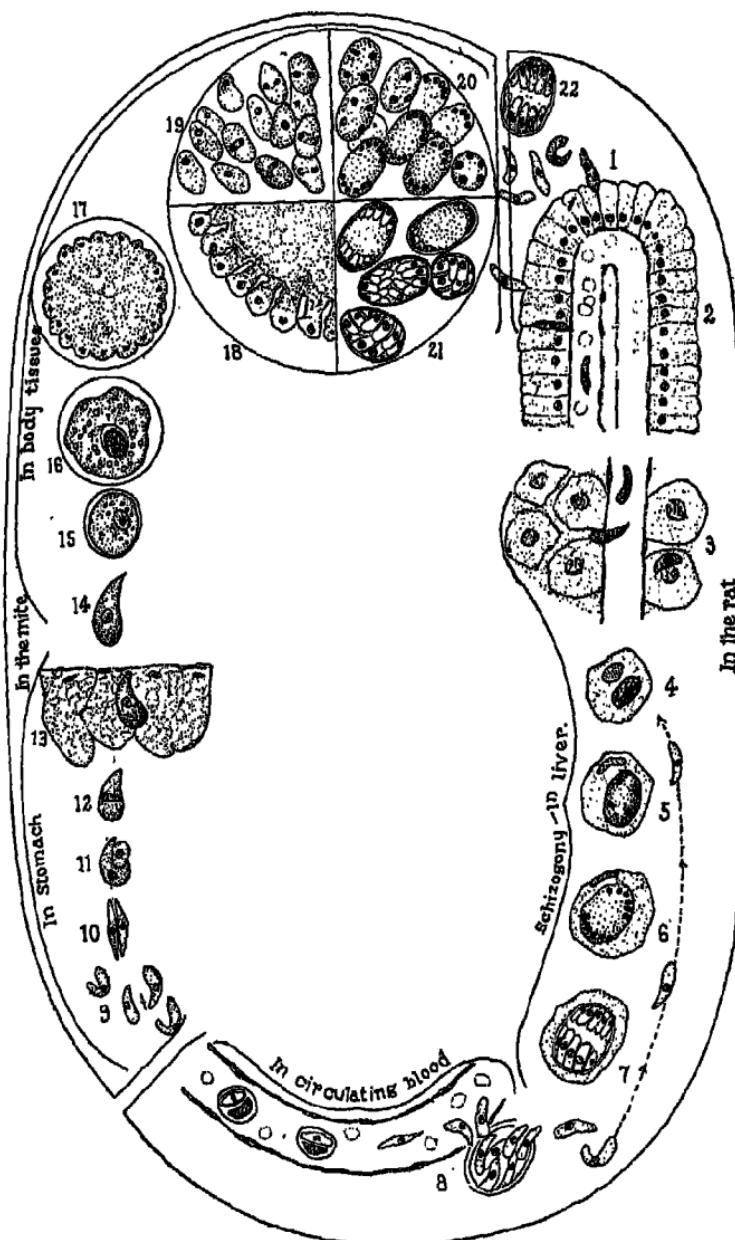


Fig. 108.—*Hemogregarina muris*. Diagrams illustrating the life cycle in the rat and the mite. For description see text, page 294. (After Miller.)

secrete sporocysts, thus becoming spores, and each breaks up into about 16 sporozoites (19-21). The mite, which has now reached the infective stage, transfers the parasite to any rat that chances to eat it. The distribution of the parasites is made easy by the habit of the mites of feeding on the rats at night and leaving them in the day time.

2. HÆMOGREGARINES OF OTHER ANIMALS

The hæmogregarines of the frog are the easiest to obtain for study in most localities. When only a few are present concentration by centrifuging the blood is effective. These parasites are sausage-shaped with an oval nucleus and many metachromatinc granules but no pigment (Fig. 109). When

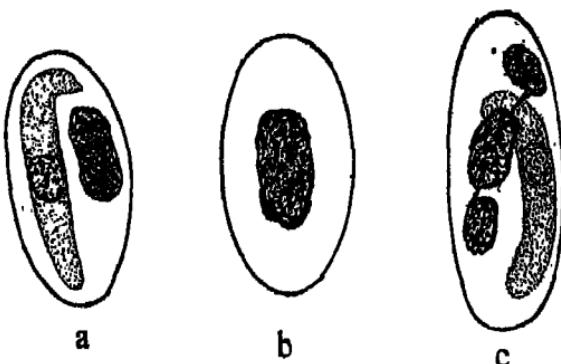


Fig. 109.—Hæmogregarines in the blood of a frog.

a, *Hæmogregarina*. b, Normal red cell. c, *Karyolysis*. $\times 550$.
(Original.)

free from the corpuscle the organism glides along like a gregarine. Red blood corpuscles have been seen by Nere-sheimer (1909) to throw out processes toward an approaching hæmogregarine and apparently to aid in their own penetration. Lankester rediscovered the hæmogregarines of the frog in 1871, and connected them with the life-cycle of *Trypanosoma rotatorium*. Gaule in 1880 claimed them to be normal cell inclusions. The best of the earlier work was

done by Danilewsky (1885) and Labb   (1894). At the present time the life-cycles of these species in amphibia are not fully known. Various generic names have been applied to them, such as *Drepanidium*, *Laverania*, *Lankestercella*, *Karyolysus* and *H  mogregarina*. It seems best to adopt the genus name *H  mogregarina*. Certain species act on the nucleus of the parasitized blood cells in such a way as to break it up or karyolyze it and are known as *Karyolysus* (Fig. 109, c); *K. lacertarum* occurs in the blood of lizards as well as in that of frogs.

The life-cycle of *H  mogregarina stepanowi*, which passes through schizogony in European water tortoises and sporogony in leeches, has been thoroughly worked out (Reichenow, 1911). One striking difference between it and the life-cycle of *H. muris* described above is the occurrence of two types of schizogony. The sporozoites develop in the blood cells into macroschizonts which produce by schizogony macromerozoites. These may repeat this cycle or may enter blood cells and develop into microschizonts which produce micromerozoites by microschizogony. The micromerozoites may repeat the microschizogony or in other red cells develop into sexually differentiated sporonts (gametocytes). The gametocytes copulate in pairs if taken into the digestive tract of the leech and sporogony follows.

H  mogregarines have been reported from many species of mammals. *H. canis* is a parasite of the dog and the tick; *H. bovis* occurs in cattle; *H. jaculi* in the jerboa in Egypt, and *H. felis* in cats.

Snakes and lizards are parasitized by *Karyolysus* and *H. stepanowi* already mentioned. The python harbors *H. pythonis*, the cobra, *H. naja  *, the crocodile, *H. hankini*, etc.

The h  mogregarines of fish include *H. simondi* of the sole and *H. anarrhichadis* of the catfish.

Arag  o has described a number of species in birds.

3. "HÆMOGREGARINES" OF MAN

Five species of "hæmogregarines" have been described in man.

1. *Hæmogregarina hominis* (Fig. 110). This species was found by Krumpf (1917) in China in the spleen of a man

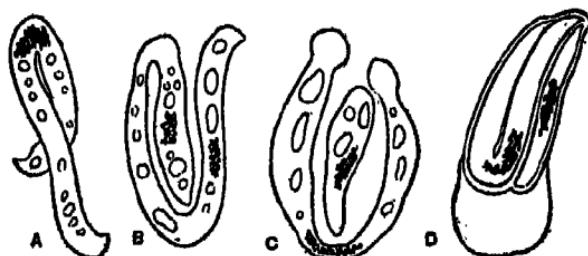


Fig. 110.—*"Hæmogregarina hominis"* from man. A, B, C, Free parasites. D, Parasite within blood cell. (From Brumpt, after Krumpf.)

suffering from splenomegaly. It lives in the red cells and being about 20 microns in length is bent or twisted as indicated in the figure. A nucleus is visible and chromatoid bodies. Specimens were also found free in the blood. Nothing was learned regarding its life-cycle.



Fig. 111.—*"Hæmogregarina inexpectata"* from man. A to F, Parasites within blood cells, G, I, J, Free parasites. H, Normal red cell. (From Brumpt, after Roubaud.)

2. *Hæmogregarina inexpectata* (Fig. 111). Roubaud (1919) records this species from a Belgian girl who had lived for two years (1912-1914) in the Belgian Congo. Parasites were found 4 years later in the peripheral blood. Fully grown specimens lie bent upon themselves in the red

blood cells and resemble somewhat the crescents of the parasite of æstivo-autumnal malaria. They are from 9 to 11 microns long and 3 microns in diameter. Two nuclei were observed in some of the specimens and two specimens were sometimes found in a single cell; these conditions may be due to binary division. Free young forms occurred in the blood; these were about 5 microns long, often binucleate, and may be merozoites. Stages resembling schizogony were also present in the peripheral blood. A parasite similar to *H.*

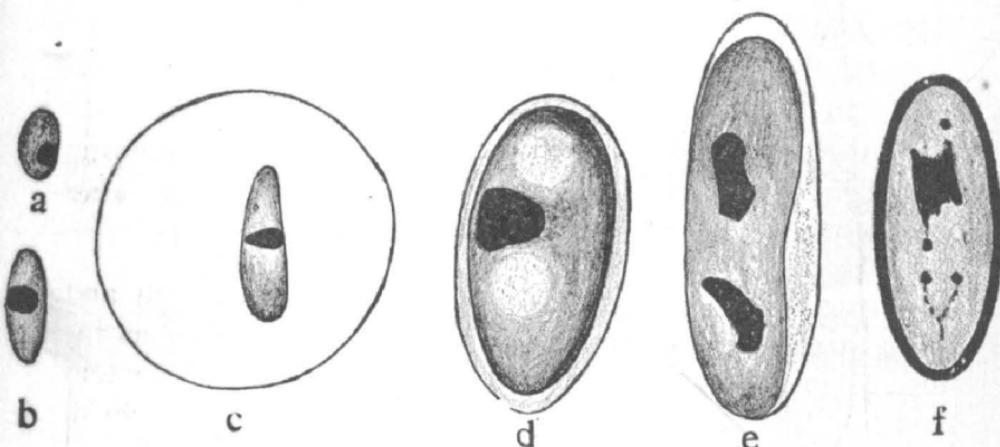


Fig. 112.—“*Hæmogregarina elliptica*” from man. a, b, Small free parasites. c, Young parasite within blood cell. d, Encapsulated parasite. e, Parasite in division. f, Cyst. (After Edm. and Et. Sergent and Parrot.)

inexpectata was noted by Lebœuf (1921) in a young girl suffering from splenomegaly at Brazzaville in the French Congo.

3. *Hæmogregarina elliptica* (Fig. 112). Parasites found in the blood of a girl three years old living in Corsica are considered by Edm. and Et. Sergent and Parrot (1922) to be a new species of hæmogregarine. Both intra- and extra-cellular forms were noted. Their characteristic elliptical shape is indicated in the figures.

4. *Hæmogregarina gallica*. This parasite is described by

Noc (1922) as differing from the three species previously recorded. It is crescent-shaped and one end is bent back on the body for about $5.4\ \mu$. The total length of the organism is $19.8\ \mu$. The finely alveolar cytoplasm is surrounded by a simple membrane. Two nuclei are present; one submedian, the other near one end. Merozoites from $5\ \mu$ to $6\ \mu$ in length and $1.5\ \mu$ in breadth were observed.

5. *Hæmogregarina equatorialis*. Another new species has been described by Nattan-Larrier (1922, 1923) having been found by him in 1906 in a man aged 31 years who had lived in the French Congo. The most frequent forms were lenticular in shape with blunt ends, $6\ \mu$ to $7.5\ \mu$ long and $1.6\ \mu$ to $2.4\ \mu$ broad. The cytoplasm contained many chromatin granules and fine grains that were perhaps pigment. A single, small rounded or oval nucleus was present. Encysted forms surrounded by a refringent membrane were observed.

These five "species" of "hæmogregarines" cannot be accepted as good species until further and more extensive investigations have been carried out.¹

C. Toxoplasmidæ

The genus name *Toxoplasma* was first employed by Nicolle and Manceaux in 1908 for certain parasites they found in the leucocytes of the gondi (*Ctenodactylus gondii*) in Tunisia. Since then many other species have been described including *T. cuniculi*, Carini, from Brazilian rabbits; *T. canis*, Mello, from dogs in Italy and Brazil, *T. talpæ*, Prowazek, from moles in Japan, *T. columbae*, from pigeons in

¹ Wenyon (1923) has just published a critical review of these "hæmogregarines" in which he expresses the belief that none of the bodies described are really hæmogregarines but are better explained on the assumption that they are vegetable cells present because of contamination.

Brazil, *T. pyrogenes* Castellani, from man, and *T. sp.* Plimmer, from reptiles. The family Toxoplasmidæ was established by Franca in 1917 for "Oval or reniform hæmocytzoa, without pigment, inhabiting the leucocytes in the internal organs and occasionally in the circulating blood. Without two nuclei. Schizogony within the cells."

Toxoplasma gondii averages from 5 to 5.5 microns in length and from 3 to 4 microns in breadth. The nucleus contains a distinct karyosome and none or very little peripheral chromatin. Intracellular parasites sometimes divide rapidly giving rise to small forms by a sort of pseudo-schizogony; these often produce spherical masses containing



Fig. 113.—"*Toxoplasma pyrogenes*." Parasites from the spleen of man. Normal red cell at the right. (From Brumpt, after Castellani.)

20 or more parasites. Multiplication is by binary fission, usually longitudinal. The karyosome becomes dumb-bell shaped and divides into two; the nucleus then divides into two; and finally the cytoplasmic body divides. The parasite probably causes the liquefaction of the cytoplasm of the host cell immediately surrounding it since it usually appears to lie in a sort of vacuole. When many parasites are present the host cell undergoes necrosis.

"*Toxoplasma pyrogenes*" was found by Castellani in 1913 in a case of splenomegaly in the tropics. Fedorovitch (1916) reported parasites from the peripheral blood of a case of splenomegaly in a child on the coast of the Black Sea and in the blood of a dog from the same region, that may belong to this species, or may belong to the hæmogregarines (Sergent

and Parrot, 1922). Chalmers and Kamar (1920) have also reported what they believed to be specimens of this parasite that were found in a splenic film from a fever patient in the Sudan. Castellani and Chalmers (1919) describe "*Toxoplasma pyrogenes*" as "Roundish oval or crescentic bodies 2.5-6.0 microns in diameter, with blue staining cytoplasm, and with one large roundish mass of chromatin at one pole or in the centre. In one instance the faintest appearance of a flagellum seemed to be present. Occasionally the bodies were larger, roundish or pear-shaped, and possessed two chromatin masses, one at each pole or close together. The bodies were generally free, and only in one specimen were a few found in a leucocyte. While in the spleen numerous bodies of this description were found in this case, in the peripheral blood they were absent."

It does not seem best to accept "*Toxoplasma pyrogenes*" as a species of protozoon parasitic in man on the basis of the data at present available. It has been included here in order to call attention to what may possibly be an etiological factor in tropical splenomegaly.

De Raadt (1913) has reported bodies that may be parasitic in nature in a case of splenomegaly in Borneo to which he has given the scientific name *Ovaplasma anucleatum*. These have been placed provisionally in the family Toxoplasmidæ. They are ring-like or pyriform; usually located in leucocytes, although also present rarely in red cells; and possess a large vacuole but no visible nucleus. Reproduction is by binary fission and budding.

D. Piroplasmidæ

The piroplasmas are parasites of the red blood cells of vertebrates during part of their life-cycle and of the bodies of ticks, which act as intermediate hosts during the rest of their life cycle. They are unpigmented, and multiply within the red blood cells by binary or multiple fission or by bud-

ding. Many gaps still exist in our knowledge of the life-histories of the members of the family and very little is known concerning sexual phases and sporogony. There appear to be no human parasites included in the family although one form, *Bartonella bacilliformis*, the parasite of Oroya fever in man, is tentatively placed in the group. Among the genera that include species pathogenic to animals

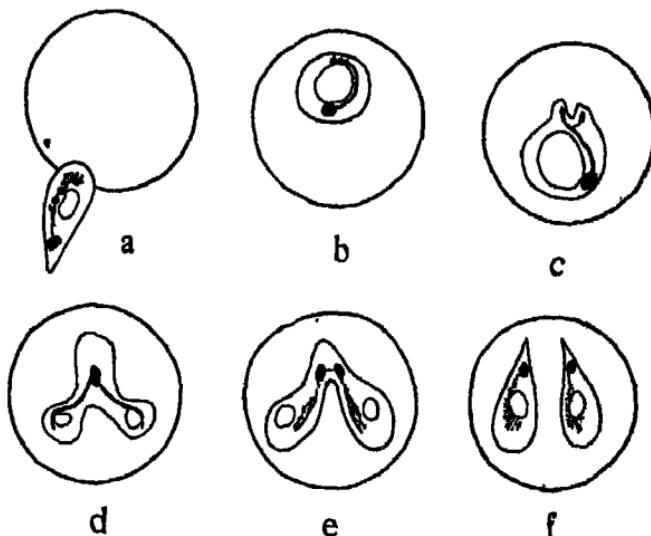


Fig. 114.—*Piroplasma canis*. Stages in division in the circulating blood of the dog. a, Free parasite entering red cell. b, Within red cell. c, Early stage in division. d, e, Later stages in division. f, Two daughter parasites ready to break out of red cell. (After Nuttall.)

are *Piroplasma* (*Babesia*), *Theileria*, *Nuttallia*, *Anaplasma*, *Rangelia*, and *Bartonella*. The most important genus is *Piroplasma*; it includes species parasitic in cattle, sheep, horses, dogs, rodents and monkeys. The species whose life-cycle is best known is *P. canis* of dogs.

Piroplasma canis in dogs. This species was discovered by Piana and Galli Valerio (1895) in dogs suffering from fever and jaundice. The disease of which it is the etiological

agent is known as canine piroplasmosis or malignant jaundice or bilious fever. It occurs in various parts of Europe, Asia and Africa. The invertebrate hosts are the ticks, *Rhipicephalus sanguineus* and *Hyalomma leachi*. Stages in the penetration and division of the organism in the red blood cells of the dog are shown in Fig. 114. The usual result is division by a sort of budding process into two pyriform bodies. Sometimes 4, 8, 16 or more occur in one cell. Several investigators have described flagellated stages free in the blood, but their relation to the life-cycle has not been definitely determined. Organisms taken into the body of the tick are stimulated to further development, but more investigations are necessary before the details of the cycle in the intermediate host can be given in full. Of particular interest is the fact, first discovered by Smith and Kilborne (1893) in the case of *P. bigemina* in cattle, that "hereditary" transmission occurs in the tick. The eggs laid by an infected tick carry the piroplasmas within them, and the ticks that develop from these eggs are also infected and capable of transmitting the infection to the vertebrate host.

Other genera and species of the family PIROPLASMIDÆ that may be mentioned are as follows:

(1) *Piroplasma bigemina*, Smith and Kilborne, 1893. This is the parasite of Texas fever in cattle. It has been recorded from various parts of the world and is transmitted usually by ticks of the genus *Boophilus*. Texas fever was the first protozoan disease known to be transmitted by an arthropod and this discovery by Smith and Kilborne in 1893 was of very great importance in the growth of our knowledge of the relation between insects and disease. The parasites are present in the red blood cells characteristically as a pair of pyriform bodies about 4 microns long.

(2) *Piroplasma bovis* Babes, 1888, is a similar parasite that causes red water or haemoglobinuric fever in European cattle. Its intermediate host is *Ixodes ricinus*. This species

often lies on the surface of the red blood cells, and frequently occurs in pairs that are slender, pyriform in shape, and meet at a rather wide angle.

(3) *Piroplasma ovis* Babes, 1892, occurs in sheep in various parts of the world, causing anaemia and haemoglobinuria, and is transmitted by *Rhipicephalus bursa*. Mortality among sheep due to its presence is high.

(4) *Piroplasma pitheci* was discovered by P. H. Ross in 1905 in monkeys of the genus *Cercopithecus* from Uganda. The parasites were present in the red cells as ovoid or pyriform bodies about 2.5 microns long. The intermediate host was not determined.

(5) *Theileria parva*, Theiler, 1904, is the cause of East Coast fever or Rhodesian red-water fever in cattle. It occurs



Fig. 115.—*Anaplasma marginale* in the red cells of cattle. $\times 2200$.
(Original from slides prepared by Brumpt.)

in Transcaucasia, Macedonia and India as well as in Africa. The parasites may be very abundant in the peripheral blood, 80 to 90 per cent of the red cells being infected and each cell often bearing 5 to 8 parasites. The appearance of the parasites within the cells varies, some being rod-shaped, others ring-formed or cruciform. Most of the parasite seems to consist of chromatin, being very poor in cytoplasm. Various species of *Rhipicephalus* act as intermediate hosts in various regions.

(6) *Anaplasma marginale*, Theiler, 1910, exists as a spherical body of chromatin less than 0.5 microns in diameter situated at or near the edge of the red cells (Fig. 115). It causes a red-water fever in several parts of the world. As many as 50 per cent of the red cells may be infected and each cell may contain 3 to 5 organisms. The ticks that act as

intermediate hosts are probably *Boophilus decoloratus* and *Rhipicephalus simus*.

(7) *Nuttalia equi* Laveran, 1899, occurs in horses, mules, donkeys and zebras in Africa, Germany, Italy and Venezuela, causing haemoglobinuria. The parasites in the red cells are spherical, pyriform or rod-shaped, sometimes appearing in rosettes of four.

(8) *Bartonella bacilliformis* is the term applied by Strong, Tyzzer, Brues, Sellards, and Gastiaburu in 1915 to the sup-

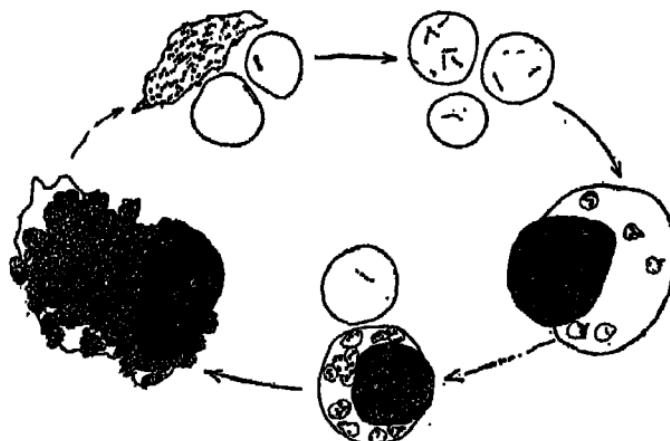


Fig. 116.—*Bartonella bacilliformis*, the supposed parasite of Oroya fever in man. For description see text. (From Castellani and Chalmers after Strong, Tyzzer, Brues, Sellards and Gastiaburu.)

posed parasite of Oroya fever (Fig. 116). These parasites were discovered by Barton in 1905 at Lima, Peru, and more thoroughly studied by Strong and his colleagues (1915). They occur as rods or more rarely spherical bodies that are capable of moving about within the red blood cells; the rods measure from 1.5 to 2.5 microns in length and the spherical forms from 0.5 to 1 micron in diameter. In severe cases from 20 to 30 per cent of the red cells are parasitized and one red cell may contain many parasites.

Multiplication takes place in the endothelial cells in the

lymph glands and spleen. Here small spherical bodies occur containing a varied number of granules; each body breaks up into as many parts as there are granules and each of these becomes rod-shaped with the granule at one end; these rods are supposed to break out of the endothelial cell and infect fresh corpuscles. The transmitting agent of the parasite is unknown and its systematic position is still in doubt. It was considered a protozoon by Barton and seems to resemble the piroplasmas in certain respects, hence it is generally included tentatively in this family.

E. Plasmodidæ Exclusive of the Malarial Parasites

I. THE GENUS *Hæmoproteus*

The members of this genus are pigmented parasites and appear in the shape of a halter in the red blood cells of birds, hence the generic term *Halteridium* applied to them by Labbé in 1894. Unlike the malarial organism, *Hæmoproteus* does not force the nucleus of the parasitized cell out of place. Schaudinn published in 1904 a detailed life-cycle of *Hæmoproteus noctuae* in the little owl, *Athene noctua*, but he was apparently dealing with *Hæmoproteus*, a spirochete and a trypanosome combining the three in one life-cycle. Many species of *Hæmoproteus* have been described, usually being named after the host in which they were found. The best account available at present of the life-cycle of a member of this genus is that of Aragão (1908) of *Hæmoproteus columbae* in the pigeon. According to this investigator macrogametocytes (Fig. 117, 1b-5b) and microgametocytes (Fig. 117, 1a-5a) are sucked from the blood of the bird into the stomach of flies of the genus *Lynchia*. Here developmental stages occur similar to those that are passed through by the malarial parasites in the stomach of mosquitoes, that is, reduction, the formation of microgametes (5a), fertilization (6), and the transformation of the zygote into an

oökinete (7). The later history of the parasite in the fly is unknown. The stage (oökinete?) that is inoculated into the bird when the infected fly bites gives rise to small parasites

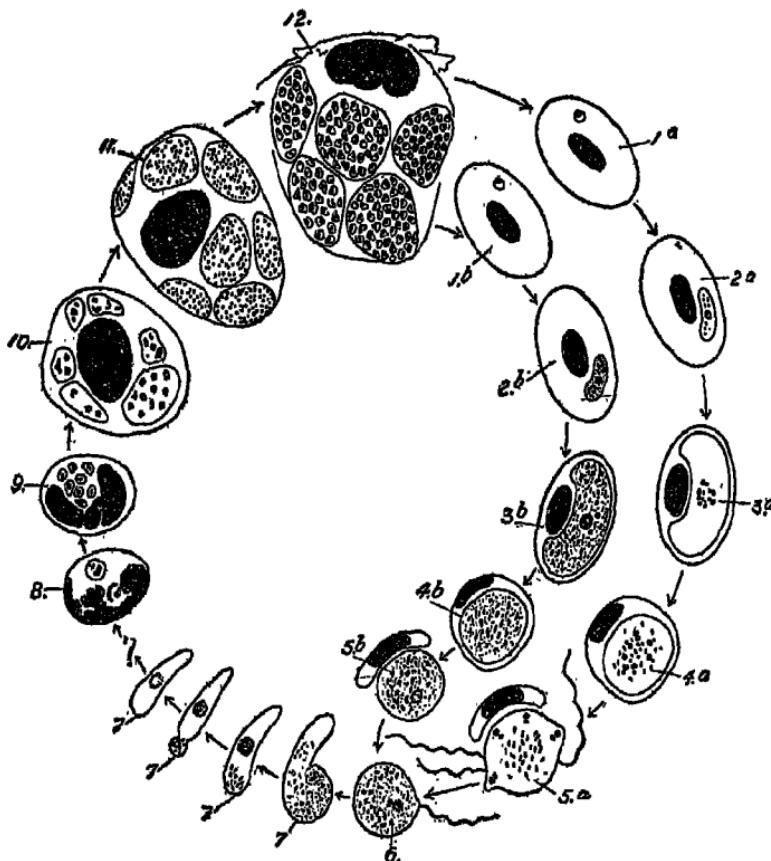


Fig. 117.—*Hæmoproteus columbae*. Stages in the life cycle. For explanation see text. (From Castellani and Chalmers after Aragão.)

within leucocytes in the lungs (Fig. 117, 8-12). These divide into a number of uninucleate forms each of which grows enormously in size. Many merozoites are produced by each of these and are liberated into the blood stream,

where they attack the red corpuscles. The trophozoites that develop from them become the halter-like gametocytes. It is of interest to note that MacCallum (1898) discovered the true significance of the "exflagellation" process of the malarial parasites of man shortly after observing the formation of microgametes and the process of fertilization in the halteridia of birds. Opie had previously noticed two types of fully grown halteridium parasites, one being granular and the other hyaline in appearance. It was while comparing the behavior of these two types that MacCallum made his great discovery. He describes his observations as follows:

"I decided, however, on the impulse of another idea, to observe carefully in the same field a granular form and a hyaline form from the time of extrusion from the corpuscle to the beginning of the motile stage, and, having found such a field, the following picture presented itself:—The two forms lay at some distance from one another, separated by the plasma and a few corpuscles. The granular form happened to escape from the corpuscle first and lay perfectly quiet beside the free nucleus and the shadow of the corpuscle. Soon the hyaline body, becoming greatly agitated, burst from the corpuscle and threw out active flagella, which beat about for a few moments and finally tore themselves loose. Then came the acme of the process. One of the four flagella passed out of the field, but the remaining three proceeded directly toward the granular form, lying quietly across the field, and surrounded it, wriggling about actively. One of the flagella, concentrating its protoplasm at one end, dashed into the granular sphere, which seemed to put out a process to meet it, and buried its head, finally wriggling its whole body into the organism, which again became perfectly round. The remaining flagella, seeking to repeat this process, were evidently repulsed, and soon became inactive and degenerated. Immediately on the entrance of the flagel-

lum the pigment of the organism was violently agitated, without, however, any disturbance of the outline of the organism. Soon all became quiet again and the period of quiescence lasted for about fifteen minutes, when a conical process began to appear at one margin of the organism, which, increasing in size, drew into itself most of the protoplasm, the pigment to a certain extent being gathered into the remainder. Finally most of the pigment was concentrated into a small round appendage, which remained attached to the end of what now had become an elongated fusiform body, which soon swam away with a gliding motion.

"Have we not here, without much doubt, a sexual process in the organisms, the result of which is the motive vermiculus? This is a process which we might have expected and which I am confident will be found to occur in the case of the human malarial parasites, although on account of the small number of organisms to be found in each slide in the latter case, the completion of the process will rarely be observed."

2. THE GENUS *Leucocytozoon*

The leucocytozoa (Fig. 118), like halteridium, are parasitic only in birds, not, however, within the red blood cells,

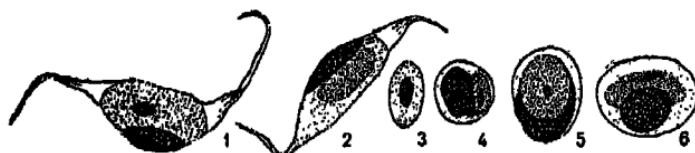


Fig. 118.—*Leucocytozoon sabraesi* from the domestic fowl. 1, Macrogametocyte. 2, Microgametocyte. 3, Normal red cell. 4-6, Young forms. \times about 750. (From Brumpt, after Mathis and Léger.)

but, as their name implies, in leucocytes. The forms present in the blood are unpigmented gametocytes, and macrogametocytes and microgametocytes can be distinguished

from each other. In either case the host cell becomes long and spindle-shaped. The cytoplasm of the macrogametocyte takes the stain more readily than that of the microgametocyte; its nucleus is smaller and more compact and is provided with a karyosome. If blood containing these gametocytes is drawn from the body and examined on a slide, the macrogametocytes may be seen to become spherical and escape from their leucocytic hosts, whereas the microgametocyte passes through the stage of exflagellation, producing about eight thread-like microgametes. As in the malarial parasite,

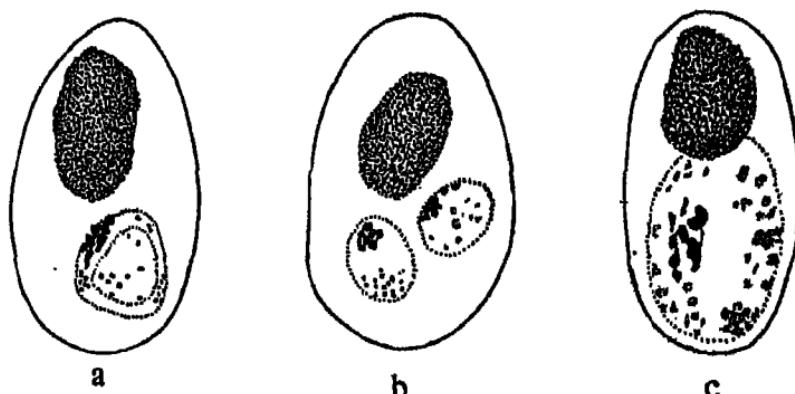


Fig. 119.—*Hæmocystidium simondi* from the gecko. a, Signet ring stage. b, Two merozoites. c, Microgametocyte. \times about 2000. (After Dobell.)

each macrogamete is fertilized by a single microgamete and the zygote transforms into an oökinete. All the stages in the life-cycle of the genus *Leucocytozoon* have not been worked out. The method of transmission is unknown. The presence of these parasites has been observed in many species of birds belonging to various families.

3. THE GENUS *Hæmocystidium*

To this genus belong a number of pigmented parasites of the red blood cells of reptiles. Dobell (1910) has de-

scribed part of the life-cycle of a species, *H. simondi* (Fig. 119), that occurs in the gecko of Ceylon. Schizogony takes place in the peripheral blood. In many respects the schizonts resemble those of the malarial parasites, but they are not amœboid and give rise to only two, or rarely four, merozoites. Large parasites that almost completely fill the corpuscle have been identified as macrogametocytes; the development of these gametocytes has not been observed. *Hæmocystidium* may be a synonym of *Hæmoproteus* according to more recent investigations (Shortt, 1922).

CHAPTER X¹

THE MALARIAL PARASITES

A. Introduction

The family PLASMODIDÆ of the order HÆMOSPORIDIA contains the genus *Plasmodium* to which the malarial parasites of man belong, and several other genera that are parasitic in lower animals only. Because of their very great human significance the malarial parasites of man will be considered in detail, followed by a shorter discussion of the other genera.

Three species of the genus *Plasmodium* are parasites of human malaria. These are *P. vivax*, the organism of tertian malaria; *P. malariae*, the organism of quartan malaria, and *P. falciparum*, the organism of estivo-autumnal malaria. The term "malaria" was first used by Torti in 1753 and was derived from two Italian words, *mal*-bad and *aria*-air, because of the belief existing at that time that the disease was due to bad air arising from marshes. Malarial fevers are characterized by periodicity, the deposition of pigment in the tissues of the body, anaemia, and enlargement of the spleen. Various synonyms of the term malaria exist in English and other languages, such as ague, marsh fever, and intermittent fever in English, paludisme and fievre palustre in French, paludismo in Italian, and Wechselfieber in German. Various terms are also applied to the fevers due to the three different species of malarial parasites. The fever

¹By R. W. Hegner.

produced by *Plasmodium vivax* is usually called tertian or benign tertian, by *P. malariae*, quartan, and by *P. falciparum*, estivo-autumnal, malignant tertian or subtertian. There is a difference of opinion as to whether the organism of estivo-autumnal malaria belongs to the genus *Plasmodium* or should be placed in a separate genus *Laverania*. If the latter, its correct scientific name is *Laverania malariae* Grassi and Feletti.

The complete life-cycle of the malarial parasites consists of an asexual cycle, involving schizogony, in man, and a sexual cycle, including sporogony, in the mosquito. Only female mosquitoes belonging to certain species of the genus *Anopheles* are parasitized by the malarial parasite and are able to transfer these parasites to man. In all three types of malaria the stages are in general alike: (1) sporozoites are inoculated into man by infected mosquitoes; (2) asexual multiplication by schizogony occurs in the blood of man; (3) sexual stages, gametocytes, are also formed in the blood of man; (4) the gametocytes can develop further only in the stomach of certain mosquitoes, where they first produce male gametes and female gametes; (5) these undergo fertilization, thus forming zygotes, which change to worm-like oökinetes, and become oöcysts in the wall of the mosquito's stomach; (6) within the oöcysts many sporozoites are formed, some of which become located in the salivary glands of the mosquito, which is then infective to man. There are thus a number of links in the chain of events that are necessary before malaria can exist in any locality: (1) there must be human beings containing a sufficient number of male and female gametocytes in their blood to infect mosquitoes; (2) species of mosquitoes susceptible to infection must have access to these human carriers; (3) environmental conditions must be favorable for the existence of the mosquitoes and for the development of the parasites within the

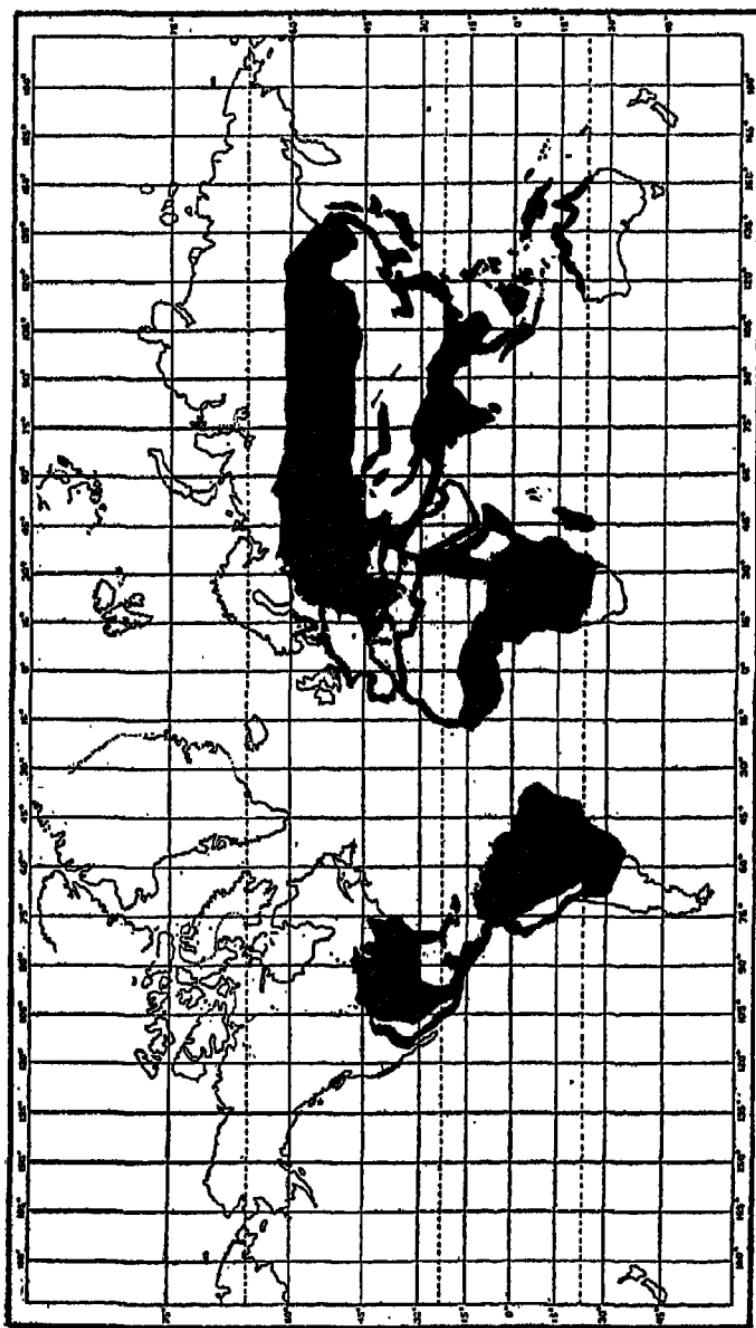


Fig. 120.—Present distribution of malaria in the world. (After James.)

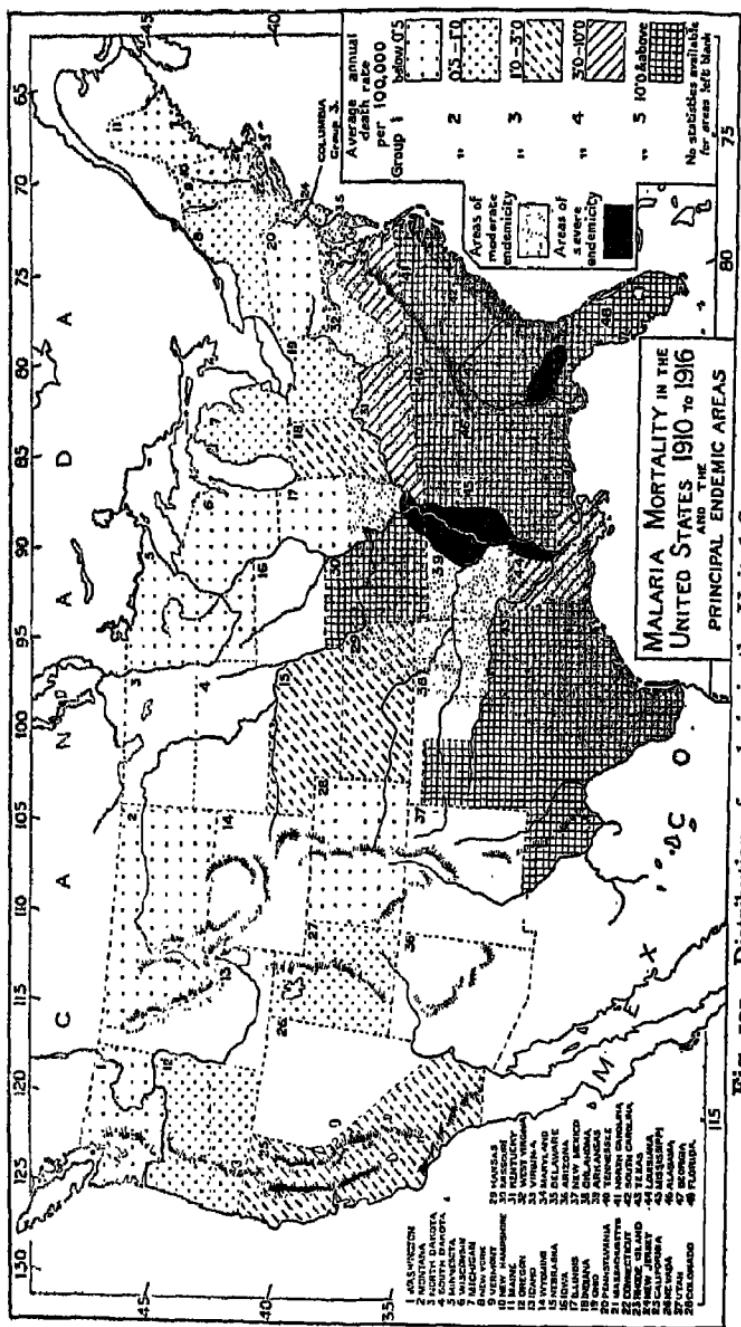


Fig. 1a.—Distribution of malaria in the United States. (After James.)

mosquitoes; and (4) other human beings susceptible to infection must be available for the mosquitoes to bite.

As the accompanying maps show (Figs. 120 and 121), malaria is a disease that is widespread in tropical and sub-tropical countries. In these localities it is not only the most important disease due to animal parasites, but is often the cause of more sickness and death than any other disease. Malaria results in the physical and mental inefficiency of millions of people and is largely responsible for the slow development of many very fertile tropical countries.

B. Stages in the Life-Cycles of the Malarial Parasites in Man

I. PLASMODIUM VIVAX

The stages in the life-cycle of the three species of malarial parasites are similar in general features and in certain details, but differ in others. The plan adopted below is to present in considerable detail the stages of the tertian parasite in man and then to direct attention to the differences between this and the other two species. It is customary to study these parasites in blood smears stained by the Romanowsky method. This stains the cytoplasm blue and the chromatin red and leaves the pigment unstained. The figures in Plates I and II were made from preparations stained in this way. In Table III, page 331, the characteristics of the three species are contrasted.

a. Sporozoite

The infective stage in the mosquito is known as a sporozoite, a minute spindle-shaped cell 10 to 12 microns in length and 1 to 2 microns in width. These sporozoites are stored in the salivary glands of the insect. When the mosquito "bites" it pierces the skin with its proboscis and a salivary

secretion containing the parasites then passes along a groove and into the wound.

The sporozoite possesses a firm and elastic but non-resistant cuticle which maintains its definite shape. Near the center is an oval nucleus. Within the blood stream it is capable of a gregarine-like gliding movement and also of peristaltic (euglenoid) contractions and lateral flexions. The red cells of the blood seem to be entered by boring movements of the sporozoite—a process that requires from about 40 to 60 minutes in drawn blood (Fig. 122). Probably not all of the sporozoites that are inoculated by the mosquito are able to infect blood cells, since many are no

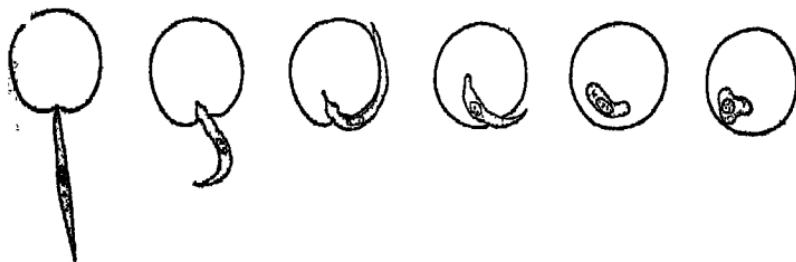
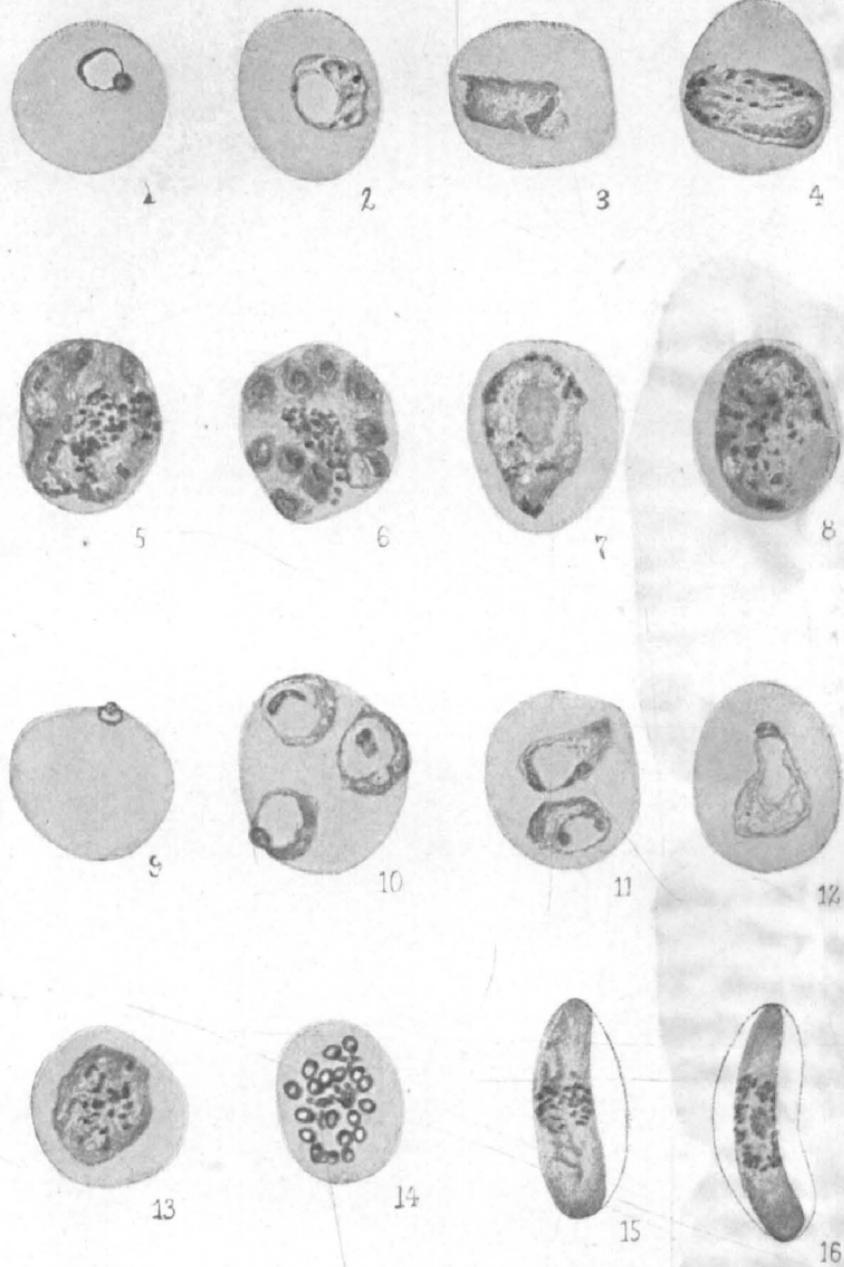


Fig. 122.—Sporozoite of a malarial parasite penetrating a red blood cell. (After Schaudinn.)

doubt engulfed by phagocytes and others may not succeed in penetrating the corpuscles. The number of sporozoites injected depends on the number present in the mosquito and the number of times the mosquito bites. There are perhaps not more than 10,000 sporozoites in the salivary glands of a single mosquito and usually less than this number. That several thousand may enter the blood from a single mosquito seems quite reasonable. Sometimes the blood cells are doubly infected—that is, more than one malarial parasite is present in a single corpuscle. This is especially characteristic of the estivo-autumnal species; it probably occurs only after several asexual cycles have caused a considerable increase in the number of parasites in the blood.



b. Trophozoite

The sporozoite changes by contraction into an amoeboid form after it enters the blood cell, being about 2 microns in diameter (Fig. 122). It is a hyaline body now known as a trophozoite (Plate I, 1). In living blood the trophozoite may be seen actively throwing out and withdrawing pseudopodia within the corpuscle. The trophozoite of tertian malaria is especially active—a fact that suggested its specific name "*vivax*." The protoplasm of the blood cell is used as food by the trophozoite and nutriment is probably also obtained from the blood stream. As growth proceeds the corpuscle becomes enlarged and about 6 to 8 hours after being infected fine, reddish-brown granules begin to appear within the parasite (Plate I, 3). These are pigment granules (also called melanin or haemozoin), and probably represent chiefly the by-products of the digestion of hemoglobin. They may be seen to move about in the parasite due to streaming movements of the cytoplasm. In preparations stained by the Romanowsky method the young trophozoite appears ring-

Plate I.—Stages in the life cycle of the organism of tertian malaria, *Plasmodium vivax*, in the blood of man. All drawn under the writer's direction by Miss Ethel Norris from original preparations at a magnification of 2200 diameters (stained with Wright's stain).

1, Trophozoite in ring stage. 2, Slightly older trophozoite; note amoeboid shape, enlargement of red cell and Schüffner's dots. 3, Older amoeboid stage of schizont containing pigment granules. 4, Infection of a single red cell with two trophozoites in the amoeboid stage. 5, Well grown schizont containing pigment granules; note Schüffner's dots and enlargement of red cell. 6, Early stage in schizogony (segmentation); the 4 chromatin masses are in division. 7, Later stage in schizogony; the chromatin masses are still dividing. 8, Formation of merozoites just before breaking out into the blood; the pigment granules are massed near the center *outside* of the merozoites. 9, Another parasite undergoing schizogony showing merozoites in process of formation. 10, Macrogametocyte; note large size, dark color, and smaller chromatin mass at one side; Schüffner's dots are present. 11, Microgametocyte; note smaller size, lighter color, and larger chromatin mass.

shaped with a minute red mass of chromatin on one side, a central vacuole-like area, and an outer border of blue-colored cytoplasm which is thicker usually opposite the chromatin-mass (Plate I, 1). Older trophozoites change from the ring stage to an amoeboid shape as indicated in Plate I, 2-4, and by the end of 40 hours almost entirely fill the blood corpuscle (Plate I, 5). The corpuscle gradually increases in size during the growth of the trophozoite—a characteristic peculiar to the tertian infection. Granules that stain a bright pink color may also appear in the part of the red cell not occupied by the parasite (Plate I, 2, 5).

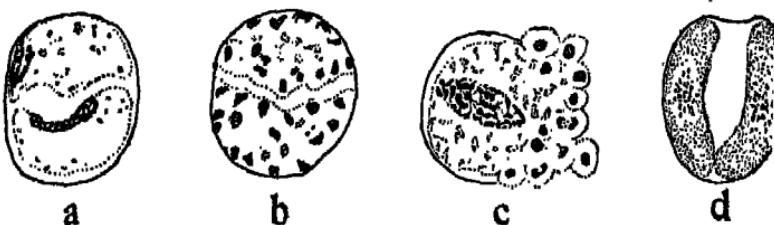
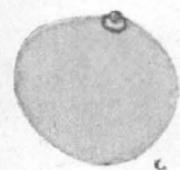


Fig. 123.—Red cells each infected by two malarial parasites. a, Two macrogametocytes. b, Two schizonts ready to segment. c, A segmenting schizont and a gametocyte. d, Two gametocytes (crescents). (a, b, After Thomson and Woodcock; c, After Schaudinn; d, After Manson-Bahr.)

These are probably degenerations of the protoplasm of the parasitized cell and are termed Schüffner's dots. They are characteristic of tertian infections and thus of diagnostic value. Schüffner's dots, however, are not always evident in stained specimens. They may appear when the parasite is in the ring stage but usually become visible later. At first they are minute in size but grow larger with age. The presence or absence of these dots seems to be due to the character of the stain used, its strength, and the length of the staining process. Sometimes a single red cell may be parasitized by two or more malarial organisms (Plate I, 4). This, however, occurs more frequently in infections with *P. falciparum* than with *P. vivax*, and usually only after the



parasites become very numerous in the blood (Plate II, 10, 11). If a red cell contains two parasites, these may both be schizonts and produce merozoites (Fig. 123, b), or one may be a schizont and the other a gametocyte of either sex (Fig. 123, c), or both may be gametocytes (Fig. 123, a, d).

c. Schizogony

Trophozoites that undergo multiple division are called schizonts (Plate I, 5) and the process is known as schizogony. The fully grown schizont at the end of 40 hours is from 8 to 10 microns in diameter. Growth now ceases and the chromatin mass by successive divisions forms from about 15 to 24 smaller masses, usually 18 to 20 (Plate I, 6-9). The details of the dividing nuclei are not very clear in mate-

Plate II.—Figures 1-8. Stages in the life cycle of the organism of quartan malaria, *Plasmodium malariae*, in the blood of man. Figures 9-16. Stages in the life cycle of the organism of astivo-autumnal malaria, *Plasmodium falciparum*. All drawn under the writer's direction by Miss Ethel Norris from original preparations at a magnification of 2200 diameters (stained with Wright's stain).

P. malariae.—1, Trophozoite in the ring stage. 2, Older trophozoite. 3, Schizont approximately rectangular in shape; note failure of red cell to become enlarged and absence of Schüffner's dots. 4, Older schizont. 5, Presegmentation stage; ten merozoites are being formed; the pigment is aggregated in the center. 6, Merozoites completely formed. 7, Microgametocyte; note pale color, and chromatin mass near center. 8, Macrogametocyte; note darker color and chromatin mass near one side.

P. falciparum.—9, Trophozoite in ring stage; note small size, and position at edge with chromatin dot extended over border. 10, Triple infection of one red cell with three young schizonts. 11, Double infection of one cell with older schizonts. 12, Old schizont just before disappearance from peripheral blood; note small size and failure of red cell to become enlarged. 13, Presegmentation stage; note chromatin in separate masses and pigment aggregated near center. 14, Merozoites fully formed; note small size, large number, and irregular distribution of merozoites. 15, Microgametocyte; note crescent shape, comparative thickness, blunt ends, wide distribution of chromatin and pigment granules. 16, Macrogametocyte; note comparative thinness, end not so blunt and chromatin in center surrounded by wreath of pigment granules.

rial prepared in the usual way, but the process seems to be a sort of primitive mitosis. Several stages in this process are shown in Fig. 124. While this is taking place, the pigment granules aggregate in the center of the schizont. Then each chromatin mass with a small amount of the surrounding cytoplasm is cut off from the rest of the body (Plate I, 8). This process of schizogony or "segmentation" requires about 6 or 8 hours and results in the formation of from 15 to 24 minute cells called merozoites. The merozoites are liberated from the blood cells about 48 hours after infection. With them are also liberated into the blood, the pigment, the remains of the blood cells, and probably toxins produced by the parasite.



Fig. 124.—Nuclear division in the schizonts of the tertian malarial parasite, *Plasmodium vivax*. (After Schaudinn.)

The pigment granules are taken up by leucocytes and other phagocytic cells and deposited in the spleen, liver, brain, bone marrow, etc. Meckel, in 1847, recognized that the dark color of the organs in persons who had died of malaria was due to the accumulation in them of pigment, and Virchow, in the following year, noted pigment in the blood cells. In 1875, Kelsch pointed out the frequency of melaniferous leucocytes in the blood of patients suffering from intermittent fever and five years later concluded that this characteristic could be used for diagnosing malaria.

The merozoites when freed from the parasitized cells attach themselves to fresh red corpuscles (Fig. 125), become trophozoites and repeat the asexual cycle. Many of the

merozoites are probably destroyed by phagocytes or do not succeed in entering fresh cells, but it seems probable that the number of parasitized red cells is increased about tenfold at the end of each asexual period of 48 hours.

Ross (1911) has made some interesting calculations regarding the number of parasites that may be present in a malarial patient and the number necessary to produce clinical symptoms. Assuming that 1,000 sporozoites inoculated by a mosquito succeed in developing into schizonts and that 10 of the 15 to 24 merozoites produced by each schizont

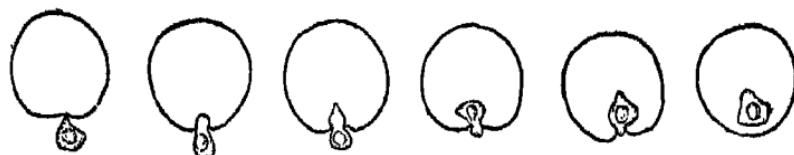


Fig. 125.—Merozoite of a malarial parasite penetrating a red blood cell. (After Schaudinn.)

succeed in infecting fresh cells, the number of parasitized cells would be as follows:

No. of days	0	2	4	6
No. of parasites	1,000	10,000	100,000	1,000,000
No. of days	8	10	12	
No. of parasites	10,000,000	100,000,000	1,000,000,000	

If we further assume that 1 c. mm. of blood contains 5,000,000 red cells and that an average man contains 3,000,000 c. mm. of blood, the number of red blood cells in an average man would equal 15,000,000,000,000. Assuming still further that 50 parasites must be present in each c. mm. of blood before clinical symptoms appear, the conclusion is reached that an average man must contain about 150,000,000 parasitized blood cells before he experiences clinical symp-

toms. To find a parasite in a film made from the blood of such a patient would probably require on the average from 10 to 15 minutes. It is therefore not surprising that parasites are difficult to find during the periods between relapses when clinical symptoms are absent.

d. Period of Incubation

The interval between the inoculation of parasites by the mosquito and the appearance of clinical symptoms is called the period of incubation. Clinical symptoms are due to the breaking down of the red cells and the liberation of merozoites, pigment granules, remains of the red cells and probably toxic substances. They do not appear until a sufficiently large number of parasites undergo schizogony at one time—a number estimated by Ross as 150,000,000 in an average man. Ordinarily the period of incubation is from 14 to 18 days in tertian malaria, 18 to 21 days in quartan malaria, and 9 to 12 days in estivo-autumnal malaria. These differences are due to the rate of reproduction and probably also to the virulence of the parasite. The tertian parasite undergoes schizogony every 48 hours and each schizont produces from 15 to 24 merozoites, whereas the quartan parasite requires 72 hours for the asexual cycle and each schizont gives rise to only 6 to 12 merozoites; the former therefore increases more rapidly and requires a shorter period in which to multiply sufficiently to cause clinical symptoms. The estivo-autumnal parasite undergoes asexual reproduction more quickly than the quartan parasite and is also apparently more virulent, hence the shorter period of incubation. The asexual cycle is repeated again and again in the blood of man and probably may continue for months or years, that is, as long as a person remains infected.

During the early stages of a primary infection with tertian malaria schizogony takes place in all of the parasites at about

the same time, but, after a number of asexual generations certain schizonts may undergo segmentation before the others. The result is that schizonts not yet fully grown, schizonts undergoing segmentation, and cells containing ring stages may all be present at the same time in the blood of a single patient.

e. *Gametocytes*

After several generations of merozoites have been produced, a new stage of the parasite begins to appear in the blood. This is the gametocyte or sexual stage. Schizogony continues to occur as before, but some reaction between the parasite and the host probably brings about a change in certain of the schizonts or in the merozoites they produce. The gametocytes develop from merozoites, but no one knows why certain merozoites produce schizonts and others gametocytes. According to certain authorities, the gametocytes can be distinguished when trophozoites, there being no ring stage on account of the absence of the vacuole-like structure. The characteristics of gametocytes and schizonts may be contrasted as follows (Craig, 1909, pp. 45-46) :

	<i>Gametocytes</i>	<i>Schizonts</i>
Size.....	Larger.....	Smaller
Shape.....	Less amoeboid, circular..	More amoeboid, irregular
Vacuole.....	Absent, no ring stage....	Present, ring stage
Melanin.....	More, earlier.....	Less, later
Development....	60 hours.....	30 hours

Two kinds of gametocytes are formed, female or macrogametocytes (Plate I, 10) and male or microgametocytes (Plate I, 11). These can be distinguished in the peripheral blood of man by means of the following characteristics:

	<i>Macrogametocytes</i>	<i>Microgametocytes</i>
Size.....	13-16 microns.....	9-11 microns
Cytoplasm, stained	Darker blue.....	Lighter blue
Chromatin mass.	Small, compact, eccentric	Large, diffuse, central
Melanin.....	Long rods.....	Small rods

Apparently the early stages in the growth of the gametocytes are passed through in the internal organs and are hence uncommon in the peripheral blood. Macrogametocytes usually seem to be more numerous than microgametocytes. No further development of the gametocytes occurs in the blood of man and after a few days they probably disintegrate unless taken into the stomach of certain mosquitoes.

f. Double Infections

These result from the presence of two groups of parasites that undergo schizogony on alternate days. Such a double infection may arise by the inoculation of sporozoites by mosquitoes on two successive nights. The parasites of each group grow and segment independently of the other, hence the patient suffers a paroxysm every twenty-four instead of every forty-eight hours.

2. PLASMODIUM MALARIAE

Quartan malaria is caused by *Plasmodium malariae*, the species originally discovered by Laveran in 1880 and named by him in 1881. It is not necessary to give the life-cycle of this species in as great detail as that of *P. vivax* and only the more striking differences between the two species will be noted. These differences are illustrated in Plates I and II, and are also indicated in the table on page 331.

The period of growth and of schizogony of *P. malariae*

is longer than that of *P. vivax*, i. e., 72 hours instead of 48 hours. The trophozoites are more delicate and much less active (Plate II, 1, 2). The parasitized red cell is no larger and may even be smaller than non-parasitized cells and may also be darker in color. The pigment granules are darker brown and are present in the form of large, irregular, slightly motile grains. In stained specimens no Schüffner's dots appear in the red cell. The schizonts require about 60 hours to reach their full size; they are very often band-shaped or quadrilateral in the early stages (Plate II, 3, 4). The fully grown schizonts are more circular in outline and much smaller than those of *P. vivax*. Schizogony (Plate II, 5, 6) results in the production of from 6 to 12 merozoites that are arranged in a single ring or rosette. The gametocytes (Plate II, 7, 8) are oval in outline and considerably smaller than those of *P. vivax*. The distinguishing characteristics of macrogametocytes and microgametocytes are similar to those of tertian malaria.

Double and triple infections with *P. malariae* sometimes occur. In these cases there are two or three groups of parasites that undergo the asexual cycle independently and at intervals of about 24 hours. This is due to inoculations by mosquitoes on different days. Clinical symptoms appear in cases of single infection every 72 hours, in cases of double infection on two successive days and then again on two successive days after an interval of 24 hours, and in cases of triple infection every 24 hours. Mixed infections with *P. malariae* and *P. vivax* may occur at the same time in a single patient.

3. PLASMODIUM FALCIPARUM

Estivo-autumnal, tropical, quotidian, subtertian, or malignant tertian malaria is caused by *P. falciparum*. This species exhibits the following characteristics. The asexual cycle is not so definite in length as that of the other two species

requiring from 24 to 48 hours. Schizogony very rarely occurs in the peripheral blood. The trophozoite is smaller than that of the other two species, with a thinner band of cytoplasm in the ring stage, and is actively amoeboid. The rings are frequently located at the periphery of the red cell, the chromatin dot often projecting over the edge (Plate II, 9). The chromatin mass is often divided into two dots which may be situated side by side or on opposite sides of the ring. One red cell is often infected by two parasites and less often by three or more (Plate II, 10); and when a severe infection occurs there may be more parasites present than red cells. The schizont is small, being only about 5 microns in diameter (Plate II, 11, 12). It undergoes division in the internal organs especially the spleen, bone-marrow and capillaries of the brain, giving rise to 8 to 10 or more merozoites which may be arranged in a rosette or irregularly (Plate II, 13-14). As many as 32 merozoites have been recorded from a single schizont. The parasitized red cells are usually not enlarged; they may be partly decolorized, and sometimes spots, called Maurer's dots, may be present. These are fewer in number, larger and less regular in shape than Schüffner's dots and usually stain brick red instead of bright pink. They do not occur so frequently as do Schüffner's dots in tertian malaria. Pigment appears in the schizonts generally as a single dark mass. Schizogony seems to take place at less regular intervals among the members of a group of estivo-autumnal parasites than in the other two species, hence ring stages and growing schizonts are most always present at the same time.

The gametocytes of *P. falciparum* are so different from those of the other two species that this type has by some been referred to a separate genus and called *Laverania malariae*. Both macrogametocytes and microgametocytes are crescentic in shape, being from 9 microns to 14 microns long and 2 or 3 microns broad. The macrogametocytes (Plate II, 16) are slender; stain a deeper blue; and possess

a single mass of chromatin in the center surrounded by a compact circle of pigment. The microgametocytes (Plate II, 15) are broader; stain a lighter blue; and possess chromatin and pigment granules scattered irregularly throughout the middle third of the crescent.

The growing gametocytes are characterized by their elongate, angular appearance, chromatin often in the form of a long streak on one side and pigment widely scattered. As the crescentic form develops, a distinct membrane is formed, apparently maintaining the sausage-like shape of the gametocytes, and the material of the red cell is used up, leaving only the cell wall surrounding them like a shadow.

As in the other two types of malaria, a patient may be infected with more than one group of estivo-autumnal parasites and more or less continuous schizogony may be going on accompanied by frequent paroxysms.

Infections with *P. falciparum* and *P. vivax* or with *P. falciparum* and *P. malariae* or with all three may occur in one patient at the same time. These mixed infections are more difficult to diagnose than infections with only one species of parasite and one should not make a report as soon as one type has been found but should continue his examination until he is convinced that he is or is not dealing with a mixed infection.

4. DISTINGUISHING FEATURES OF THE THREE SPECIES OF *Plasmodium* OCCURRING IN MAN

The characteristics of the three species described above that are of most importance in diagnosis may be stated briefly as follows:

1. *P. vivax*. The rings are comparatively large, not as a rule at the edge of the red cell, and not usually more than one in each cell. The older trophozoites are conspicuously anæboid. Growth is accompanied by a distinct enlargement of the red cell and frequently by the appearance of Schüff-

ner's dots in the cell that stain a bright pink. Schizogony occurs in the peripheral blood and from 15 to 24 merozoites are formed. The gametocytes are large and oval or circular in outline.

2. *P. vivax*. The rings are generally not so massive as in *P. vivax*, are usually not at the edge of the cell and not more than one in each cell. The older trophozoites are frequently rectangular in outline. No enlargement of the parasitized red cell occurs and no Schüffner's dots appear. The schizont is small and oval; and segments in the peripheral blood into from 6 to 12 merozoites that are often regularly arranged like a rosette. The gametocytes are similar to those of *P. vivax* but much smaller.

3. *P. falciparum*. The rings are small, compact, frequently at the edge of the cell, and often two or more occur in a single cell. The chromatin material in the ring is frequently divided into two dots. The trophozoites do not grow very large in the peripheral blood, schizogony taking place usually in the internal organs. The gametocytes are sausage-shaped "crescents."

C. Stages in the Life-Cycles of the Malarial Parasites in the Mosquito

Complete accounts of the stages in the life-cycles of the separate species of malarial parasites in the mosquito are not available, hence it is necessary to piece together the results of investigations of all three parasites. The gametocytes that are formed in the blood of man do not undergo further development in the human host, but probably disintegrate in the course of a week or two in the peripheral blood. If, however, fresh blood containing "ripe" gametocytes is placed on a slide, maturation and gamete-formation is initiated and developmental stages occur that are similar to some of those that take place in gametocytes in the stomach of the mosquito.

THE MALARIAL PARASITES

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TABLE III. DISTINGUISHING FEATURES OF THE THREE SPECIES OF PLASMODIUM OCCURRING IN MAN

	<i>P. vivax</i>	<i>P. malariae</i>	<i>P. falciparum</i>
1. Type of fever.....	Tertian (benign tertian) simple or double	Quartan; simple, double or triple	Estivo-autumnal; quotidian; subterian; malignant tertian
2. Length of asexual cycle.	48 hours.....	72 hours.....	24-48 hours.....
3. Stages in peripheral blood	All.....	All.....	No segmenting stages
4. Parasites in infected red cells	Usually one in each.....	Usually one in each.....	Often two or more in one cell
5. Movement of young trophozoite	Active amoeboid.....	Very slow amoeboid.....	Active amoeboid
6. Size of infected red cells	Larger than normal.....	About normal.....	About normal
7. Color of infected red cell.	Nearly normal.....	Darker than normal.....	Partially decolorized
8. Granules in infected red cells	Schiffner's dots.....	None.....	Maurer's dots
9. Pigment.....	Short rods, actively motile	Grains, large, irregular, very slightly motile	Few, small, irregular grains, feebly motile
10. Shape of schizont.....	Circular.....	Quadrilateral.....	Circular
11. Number of merozoites.....	15-24.....	6-12.....	8-10 or more
12. Arrangement of merozoites	Irregular or 2 rings.....	One ring, a rosette.....	Irregular or rosette
13. Gametocytes.....	Spherical or ovoid.....	Spherical or ovoid.....	Crescentic

a. Maturation

The processes of maturation have not been worked out very carefully. As in higher organisms, the nucleus of both types of gametocytes divides and part of the chromatin is cast out in the form of polar bodies (Fig. 126). In *P. falciparum* the mature macrogamete escapes from the membrane of the crescent through an opening or rupture about the middle of its length and then assumes a spherical form. The microgametocyte, after polar body formation, also escapes from its limiting membrane, but the latter "gives way evenly all over, so that the male gametocyte assumes a

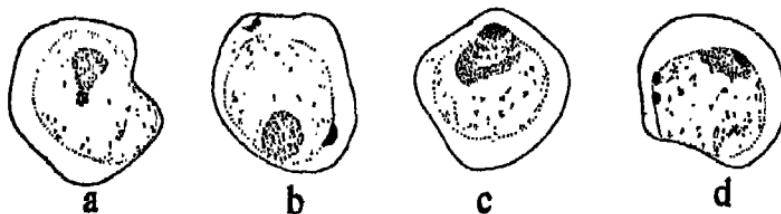


Fig. 126.—Maturation of gametocytes of *Plasmodium vivax*. a, Chromatin granules separating from nucleus. b, c, d, Chromatin in the form of "polar bodies" being extruded from surface. (After Thomson and Woodcock.)

spherical form more readily and more directly than does the female" (Thomson and Woodcock).

b. Formation of Microgametes

The next stage in the development of the microgametocyte was for a long time known as "exflagellation." The nucleus, after casting out part of its chromatin, undergoes several successive divisions, then after a period of internal activity of the protoplasm, from 6 to 8 whip-like processes burst out of the microgametocyte, each consisting apparently of a fine thread of chromatin with a very thin covering of cytoplasm; these are the microgametes. They lash about until they break away from the residual protoplasm and then swim about in the blood.

c. Fertilization

Microgamete stages were seen by Laveran and caused him to place the parasite in a genus of algæ, *Oscillaria*. Many of the earlier students considered this stage a distinct genus to which they gave the name *Polymitus*. Manson, as late as 1894, proposed the hypothesis that the "flagellating bodies" set free in the stomach of the mosquito developed further in water when the mosquito died. It remained for MacCallum at the Johns Hopkins University to clear up the situation by the discovery in 1898 of the true nature of this process. After noting microgamete formation and fertilization in *Halteridium*, as described on page 309, MacCallum looked for and discovered a similar process in a case of estivo-autumnal malaria. This he described in the following words:

"Since the preparation of this article for the press I have examined the blood of a woman suffering from an infection with the estivo-autumnal type of organism, in which a great number of crescents were to be seen. These in a freshly made slide of blood, with very few exceptions, retained their crescentic shape for only a few minutes. They soon drew themselves up, thus straightening out the curve of the crescent while shortening themselves into the well-known ovoid form. After the lapse of 10 to 12 minutes most of them were quite round and extra-corpuscular, the 'bib' lying beside them as a delicate circle or 'shadow' of the red corpuscle.

"After 20 to 25 minutes certain ones of these spherical forms became flagellated; others, and especially those in which the pigment formed a definite ring and was not diffused throughout the organism, remained quiet and did not become flagellated. In a field where an example of each form could be watched, the flagella broke from the flagellated form and struggled about among the corpuscles, finally approaching the quiet spherical form; one of them entered, agitating the pigment greatly, sometimes spinning the ring

about. The rest were refused admission, but swarmed about, beating their heads against the wall of the organism. This occurred after 35 to 45 minutes.

"After the entrance of the flagellum the organism again became quiet and rather swollen, but although in the two instances in which this process was traced the fertilized form was watched for a long time, no form analogous to the 'vermiculus' was seen."

As in most higher animals the macrogamete (Fig. 127, 3b) is large and passive and the microgamete (3a) small and active. One microgamete fuses with one macrogamete (4a, 4b), their cytoplasm becoming mixed together and their chromatin-masses combining to form a single mass. Thus is formed the zygote (Fig. 127, 5). All stages in the life-cycle of the parasite except "ripe" gametocytes are destroyed in the stomach of the mosquito. The length of time required for gametocytes in the human blood to reach the stage at which they are able to undergo further development in the stomach of the mosquito is not known definitely, but is probably about one week. It is thus obvious that mosquitoes do not become infected unless the malarial patient whose blood they ingest contains ripe gametocytes. It is also clear that both macrogametocytes and microgametocytes must be present in order that both types of gametes can be formed and fertilization take place. Malaria carriers have been reported in whose blood there were no microgametocytes. Mosquitoes fed on this blood were not infective to man. The gametocytes must also be ingested by certain species of mosquitoes before they are able to continue their development, since apparently gametocytes as well as the other stages that occur in the blood of man are destroyed in the stomach of mosquitoes other than those belonging to the genus *Anopheles*. There may even be species of anopheline mosquitoes that cannot be infected; at least, we know that some species are more easily infected than others. How

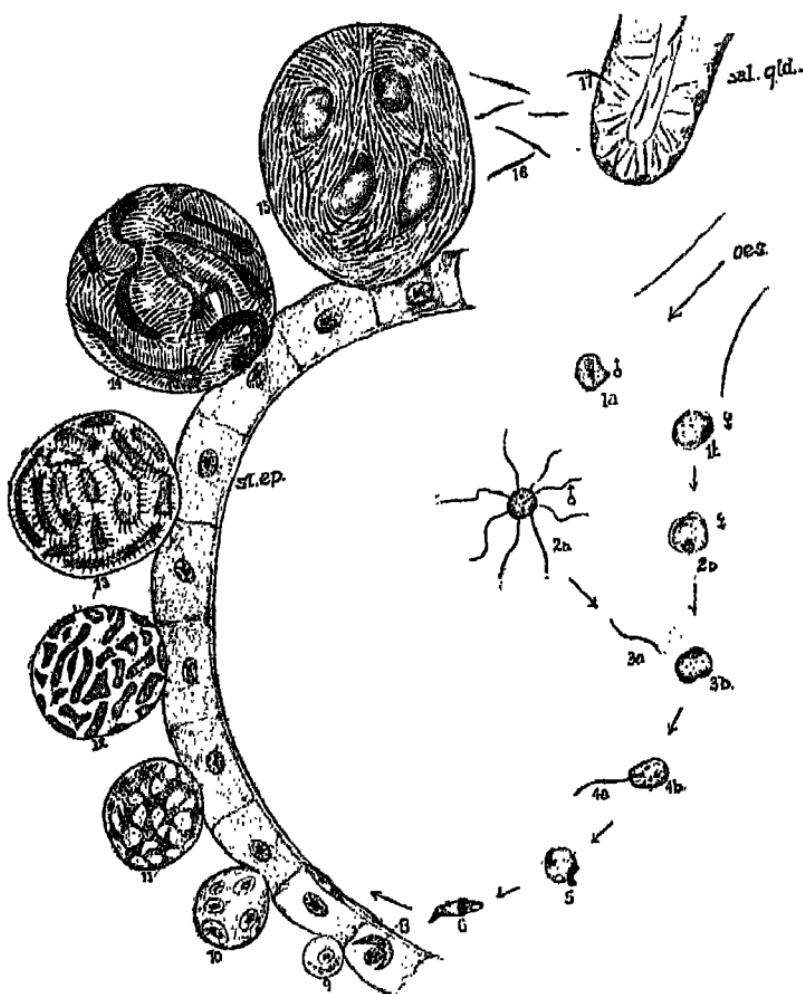


Fig. 127.—Cycle of the malarial parasite of man within the body of a mosquito.

1a, Maturation of microgametocyte. 1b, 2b, 3b, Maturation of macrogametocyte. 2a, Formation of microgametes—"exflagellation." 3a, Microgamete. 4a, 4b, Fertilization. 5, Zygote. 6, Oökinete. 8, Penetration of stomach wall of mosquito (*st. ep.*) by oökinete. 9, Young oöcyst. 10, 11, Formation of sporoblasts in oöcyst. 12, 13, 14, Formation of sporozoites by sporoblasts. 15, Oöcyst full of ripe sporozoites. 16, Sporozoites free in body cavity of mosquito. 17, Sporozoites entering salivary gland (*sal. gld.*), *ws* = *oesophagus*. (After Grassi.)

many ripe gametocytes that are ingested by the mosquito succeed in undergoing maturation and their gametes subsequent fertilization is not known. Darling's (1910) experiments on this subject indicate that at least 12 gametocytes per cubic millimeter of blood must be present in order that a mosquito may become infected. It follows from this observation that human carriers are not very dangerous if their blood contains a small number of gametocytes and that a carrier with many gametocytes is of considerable importance. What factors are necessary to bring about maturation and the formation of microgametes are not known definitely, but they may include access to the air, an alteration in the density of the blood and lowering of the temperature.

d. Oökinete

The zygote is at first spherical and passive (Fig. 127, 5) but soon becomes elongated and active (6), being capable of gliding movements resembling those of the gregarines. The transformation of the zygote requires from 12 to 24 hours; it is now called an oökinete or sometimes a vermicule. No further stages occur in drawn blood but in the stomach of the mosquito the oökinete moves about actively, finally boring its way into the stomach wall (8) and establishing itself between the outer epithelial layer and the muscular layers as a spherical body called the oöcyst (9).

e. Oöcyst

Fertilization, the change to the oökinete form, and the formation of an oöcyst occupy about 40 hours at ordinary summer temperature. The oöcyst is surrounded by a membrane probably produced by the tissues among which it lies. It lives at the expense of the surrounding cells, growing enormously in size and eventually projecting like a knob into the body cavity (Fig. 127, 9-15). At first the oöcysts measure

about 12 to 14 microns in diameter but when fully developed are usually from 50 to 60 microns in diameter. Infected mosquitoes can be recognized by the presence in the stomach wall of from one to 500 of these conspicuous oöcysts (Fig. 128).

f. Sporogony

Inside of the oöcyst the nucleus divides, producing from 20 to 30 daughter nuclei. An equal number of uninucleate irregular cells are then formed; these are the sporoblasts (Fig. 127, 10-11). A residuum of protoplasm containing pigment granules and waste products is not included in the sporoblasts. The sporoblasts continue to grow and the nucleus of each now multiplies by successive divisions; the daughter nuclei migrate to the periphery (12), and into minute projections that grow out from the sporoblasts (13). These projections, each with a single nucleus, become spindle-shaped sporozoites (14) and break away from the remains of the sporoblast (15). The process of sporogony, that is, the formation of sporozoites within the oöcysts, occupies a period of about 4 or 5 days. The oöcysts of the different species of malarial parasites probably differ from one another in various ways, but very little is known about them. The oöcysts of *P. vivax* (Fig. 129, a) are said to be dull-looking

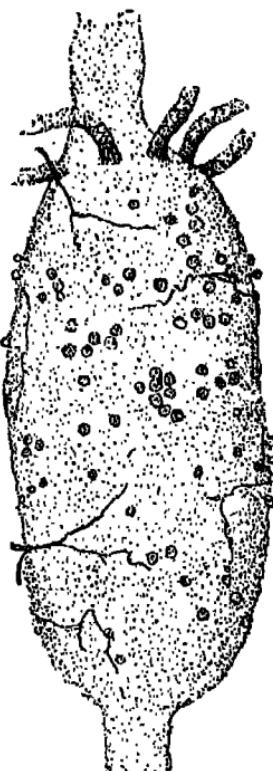


Fig. 128.—Oöcysts of *Plasmodium vivax* about seven days old projecting from the stomach wall of a mosquito, *Anopheles crucians*. (Original.)

and feebly refringent, whereas those of *P. falciparum* (Fig. 129, b) are highly refringent. Pigment in the former appears as indistinct chains, in *P. falciparum* as rather large irregular granules, and in *P. malariae* as a clump of coarse grains.

The length of time required for the entire cycle of development within the mosquito varies according to the species

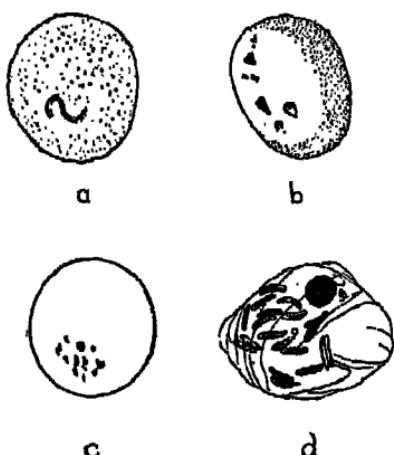


Fig. 129.—Oöcysts of malarial parasites.

a, *Plasmodium vivax*. b, *P. falciparum*. c, *P. malariae*. d, "Black spore." (a, Original. b, After Roubaud. c, After Daniels. d, After Grassi.)

of parasite and the temperature. The optimum temperature for *P. vivax* is from 25° to 30° C., at which temperature it requires about 8 or 9 days. This time is lengthened by lowering the temperature—10 to 12 days at 24° C., 18 or 19 days between 18° and 22° C., and no development at all or abnormal growth below 16° C. A high temperature (35° C.) also seems to produce abnormal development. The optimum temperature for

P. malariae is apparently 22° C. and development requires from 18 to 21 days; and that for *P. falciparum* 30° C., development requiring 10 to 11 days. The minimum temperature at which oöcysts will develop in *P. vivax* is said to be lower than that for *P. falciparum*. This may have a bearing on the geographical distribution of these two species, *P. vivax* being able to complete its development in the mosquito in regions where temperatures prevail that are too low for *P. falciparum*.

The temperature seems to be a factor in determining the length of time a mosquito may remain infective. Most of the observations of investigators who have studied this point seem to indicate that cold weather causes the degeneration of oöcysts and that mosquitoes that have hibernated through the winter are not infective in the spring. The oöcysts known as "black spores" (Fig. 129, *d*) were for a long time supposed to be formed as the result of low temperatures; but they apparently are oöcysts parasitized by MICROSPORIDIA of the genus *Nosema*.

The number of sporozoites produced by each oöcyst varies considerably; in some there are only a few hundred, whereas in others there may be as many as 10,000 (Grassi).

The oöcyst bursts shortly after the sporozoites are fully formed and these bodies escape into the body cavity of the mosquito. This cavity in the mosquito differs from the body cavity in higher animals, being a hæmocœl containing blood. The blood circulates through this cavity and bathes the various internal organs. The sporozoites are carried to all parts of the body in the blood and bore their way into various organs. Mühlens (1921) reports them in the muscles of the thorax, wings, legs, neck and head, in the fat body, etc., as well as in the salivary glands (Fig. 130).

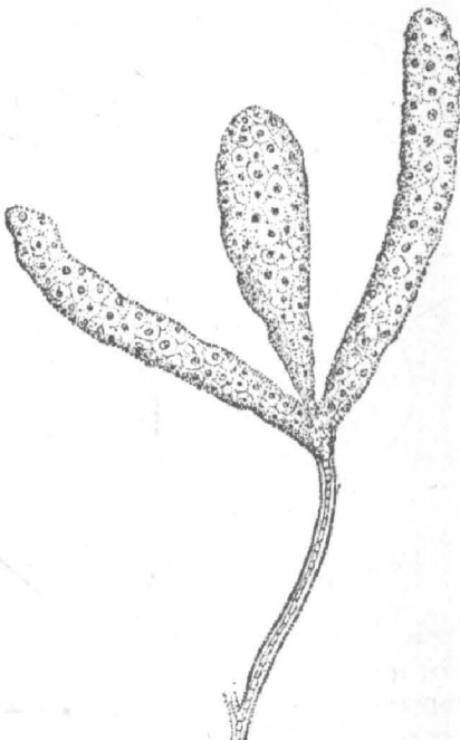


Fig. 130.—Salivary gland of a mosquito. (After Patton and Cragg.)

g. Hereditary Transmission

It has been suggested that some of the sporozoites may penetrate the growing eggs of the mosquito, remain there until the eggs develop, and then make their way into the salivary glands of the new generation. "Hereditary" transmission of this kind exists in the case of *Nosema bombycis*, a protozoan parasite that causes the silkworm disease known as "pebrine" (see page 370), and of *Piroplasma bigemina*, the sporozoan organism of Texas fever in cattle (see page 304). Schaudinn's observation of sporozoites of *Plasmodium vivax* in the ovaries has not been confirmed; in fact, Mühlens (1921) reports sporozoites in practically every part of the body of the mosquito except the ovaries. It seems probable, therefore, that sporozoites are not transmitted to a new generation of mosquitoes through the eggs.

D. The History of our Knowledge of the Malarial Parasites

The true parasite of malarial fever was discovered in 1880 by Alphonse Laveran, a French army surgeon. In 1878 he began his studies of malaria at Bône in Algeria, and announced his discovery in a note that was read by M. Leon Collin before the French Academy of Medicine in Paris on November 23, 1880.

'On December 24, 1880, Laveran reported the results of studies of 44 cases of malaria, in 26 of which he found what he believed to be parasites. He concluded that "there exist in the blood of patients with malarial fever, parasitic elements which have heretofore been confounded with melaniferous leucocytes; the presence of these parasites in the blood is probably the principal cause of the manifestations of paludism."

In his monograph of 1881, Laveran suggested the name *Oscillaria malariæ* for his parasite, largely on the basis of the

presence of the active flagella-like bodies (microgametes) which he thought of as being contained in the spherical bodies and as representing the fully developed or adult stage of the organism. Later in the same year he described smaller elements varying in size from $\frac{1}{6}$ that of a red cell up to that of an entire red cell. "These bodies," he says, "sometimes isolated, sometimes as many as four together, sometimes free in the blood, sometimes attached to the red corpuscles or leucocytes, seem to represent simply one of the phases of development of the parasitic bodies above described." These were undoubtedly schizonts, merozoites, and gametocytes.

Laveran, in the introduction to his treatise on malarial fevers, published in 1884 (introduction pp. V-VI) relates the story of his discovery as follows:

"This work is the result of five years of research pursued in Algeria without relaxation. In writing it, the principal aim has been to make known the parasites which I have had the honor of discovering in the blood of patients afflicted with malarial fever.

"On my arrival at Bône in the month of September, 1878, I did not nourish the hope of discovering the cause of malaria and my first efforts were not made in this direction. I occupied myself entirely at first with the clinical side and pathological anatomy of malarial fevers, striving to free myself from all preconceived ideas, having made a clean sweep of the classical theories.

"The analysis of anatomical lesions observed on subjects dead of pernicious malaria or of malarial cachexia made me realize that the characteristic lesion, the only constant one in malaria, consisted in the presence of pigmented elements in the blood. These pigmented elements were already known, having been in particular very well described by Frerichs; but their nature and their origin were still obscure.

"It was in studying the mode of formation of these pig-

mented elements in the blood that I was brought to recognize their parasitic nature.

"On the 6th of November, 1880, I examined the blood of a patient who was being treated for intermittent fever at the Military Hospital at Constantine. Here I observed for the first time the existence of mobile filaments which adhered to the pigmented bodies, and the living nature of which was undoubted. I had at this moment, even, the intuition that I was in the presence of the true microbe of malaria and all the facts that I have observed since then have only confirmed this first impression.

"I have observed the existence of microbes of malaria in 432 patients suffering from different forms of this disease and I have never encountered these microbes in subjects suffering from other diseases. All the army physicians, who at Constantine have interested themselves in my investigations and who have done me the honor to frequent my laboratory, have been able to recognize the existence of these malarial microbes and to verify the facts which I have reported."

Richard (1882) was the first to confirm Laveran's discovery on the basis of investigations carried on at Phillippeville in Algeria, but it was several years before the scientific world in general realized the significance of Laveran's findings. This was due in part to the fact that attention was at that time so largely directed toward the *Bacillus malariae* of Klebs and Tomassi-Crudeli. As late as 1884 Sternberg described Laveran's organism among the possible etiological factors but concluded that the real cause of malaria was still unknown. The Italian investigators were at first sceptical but later were among the most active investigators of the true malarial organisms. Golgi was the first to distinguish between the different types of malarial parasites. In 1885 and 1886 he described most of the characteristics of the quartan parasite in the blood of man that are used today in

diagnosing this species, and in 1886 and 1889 he performed the same service for the tertian parasite. Golgi was also the first to associate a third type of malaria, namely, estivo-autumnal malaria, with crescents in the blood.

It took many years to locate the malarial parasites in their proper place in the zoological series. Laveran in 1881, as noted above, suggested the name *Oscillaria malariæ* because of the resemblance of the microgametes to filamentous algae. The quartan parasite was named in 1885 by Marchiafava and Celli, *Plasmodium malariæ*. In 1887 Metchnikoff placed these organisms among the COCCIDIA in the class SPOROZOA, but Danilewsky later created a new order, HÆMOSPORIDIA, for the malarial parasites and similar organisms. Grassi and Feletti (1890) recognized five species of human malarial organisms which they placed in two genera, *Hæmamæba* and *Laverania*. The genus name *Plasmodium* has priority over *Hæmamæba*, but their specific name, *vivax*, for the tertian parasite, still stands, and their scientific name for the estivo-autumnal parasite, *Laverania malariæ*, is valid provided we recognize this form as belonging to a genus different from the others. The species name, *falciparum*, that is widely used for the parasite of estivo-autumnal malaria, was proposed by Welch in 1897. There has been from the very beginning considerable discussion as to whether this type of malaria is due to one species or two species of parasites, one causing tertian and the other quotidian fever; a final decision has not yet been reached although most writers recognize only a single species. This question has recently been considered by Craig (1921) and Darling (1921).

Several species of human malarial parasites other than those usually recognized have been described. Theos Emin (1914) reported a variety of the tertian parasite which he called *P. vivax* var. *minuta*, from patients on an island in the Red Sea. Similar parasites had been seen by Craig in the blood of soldiers in the Philippine Islands. Another form

was reported by Stephens (1914) as *Plasmodium tenue*, but probably belonged to the quartan type. That *Plasmodium tenue* is really a distinct species has recently been claimed by Sinton (1922). There are many students of malaria who believe that only one species of malarial organism exists in man. Laveran was always of this opinion. The principal arguments in favor of the plurality of species are (1) differences in the life-cycles of the types usually recognized (see table p. 331), (2) differences in the clinical effects of the three species and (3) inoculation experiments which result in the appearance of the same type of parasite in the patient as that present in the blood of the donor. The arguments brought forward by those in favor of the theory that only one polymorphic species of human malarial organism exists are largely based on clinical observations, latency and relapse. Some of the phenomena to be explained are as follows: (1) the appearance of tertian malaria in the spring in patients that suffered from estivo-autumnal malaria the preceding autumn, (2) the appearance of tertian malaria in patients who had been treated with quinine for estivo-autumnal malaria, (3) changes from the estivo-autumnal type of malaria to the tertian type in patients who by travel are subjected to a change in climate, and (4) the presence frequently of all three types of parasites during epidemics of malaria.

E. Transmission of Malaria by Blood Inoculation

Between the years 1884 and 1889 positive results were obtained by various investigators who carried on inoculation experiments with all three types of human malarial organisms. Gerhardt (1884) inoculated blood from 4 patients with quotidian paroxysms into healthy persons who exhibited quotidian paroxysms after incubation periods of 16 and 6 days respectively. He did not see the organisms. Parasites were observed by Machiafava and Celli (1885) in several

positive cases they obtained by inoculating healthy persons with the blood of malarial patients, but the species had not yet been distinguished and hence were not recorded. Ross (1911) has listed the inoculation experiments that had been reported up to 1905 of which 51 were positive and 6 negative. A few of the more interesting results are as follows: Gualdi and Antolisei (1889) noted quartan parasites in a person who had been inoculated with blood from a quartan patient twelve days earlier. In the same year Antolisei and Angelini (1889) succeeded in transmitting tertian parasites by blood inoculation. Di Mattei in 1891 produced a mixed infection by inoculating quartan parasites into a patient suffering from estivo-autumnal malaria. That the inoculation of a single drop of blood from a malarial patient is sufficient to transmit the infection was proved by Bignami (1898) with blood from a case of estivo-autumnal malaria injected subcutaneously. Thayer (1898) and Elting (1899) obtained negative results with blood containing crescents only, thus showing that crescents are not sufficient to start a new infection. Experiments by Rosenau, Parker, Francis and Beyer (1904) are of interest because they prove that the incubation period is shortened if enough parasites are injected. When 2 c.c. of blood was drawn from a patient suffering from a heavy double infection with tertian parasites typical paroxysms were noted and parasites recovered as soon as 4 days later. It has recently been suggested by Grassi (1921) that mosquitoes might transmit malaria mechanically directly by first biting a patient with infective stages in his blood and immediately afterward biting a healthy person into whose blood a few parasites might find their way from the proboscis of the mosquito.

F. Cultivation of Malarial Parasites in Vitro

The first published report of the actual cultivation in vitro of the malarial parasites is that of Bass (1911). The follow-

ing year Bass and Johns (1912) undertook a detailed study of this subject while in the Panama Canal Zone. The best results were obtained by the following method. Blood is collected, with the least possible exposure to the air, in tubes one-half inch in diameter in which enough of a 50 per cent aqueous solution of dextrose had been previously placed to produce a mixture of 0.5 per cent dextrose. The mixture is defibrinated while the tube is plugged. A column is thus obtained, after the cells have settled, of about $1\frac{1}{2}$ inches of serum. When kept at a temperature of about 40° C. the parasites live and develop in the corpuscles near the surface of the precipitated cells. If more than one generation is to be reared the tube is centrifuged and the leucocytes are drawn off from the top of the cellular precipitate. This prevents the phagocytosis of the merozoites by leucocytes.

Three asexual generations were obtained in leucocyte-free cultures of this kind. Transfers are easily made by adding parasitized cells from one culture to a recently prepared tube containing fresh serum and cells. This should be done about the time of segmentation. One culture of estivo-autumnal parasites was carried through 4 generations in this way. One or more generations were cultivated from 29 different patients suffering from estivo-autumnal malaria, and from 6 cases of tertian malaria. Several interesting observations were made during the course of this work.

Estivo-autumnal parasites appeared to be more resistant than the other species to variations in the culture medium. The tertian gametocytes obtained seemed more resistant than the asexual stages. Stages that might have been gametocytes in the process of parthenogenesis and zygotes were observed, indicating the possibility of cultivating the sexual cycle. A carbohydrate was found to be a necessary element of the culture medium, dextrose being the best, and maltose next best. Parasites can develop only in the presence of and in red cells. Leucocytes phagocytize only parasites outside of

red cells. It is suggested that the appearance and disappearance of parasites in the blood of patients may be due to variations in the dextrose content. The optimum temperature appeared to be 40° C. to 41° C. although slower development occurred at 37° C. and one culture of estivo-autumnal parasites segmented in 4 days at room temperature. At 41° C. estivo-autumnal parasites passed through their asexual cycle in 30 hours, whereas they normally require about 48 hours in man. *P. vivax* and *P. falciparum* remain distinct when grown in culture and variations of the latter indicate that different varieties of the estivo-autumnal parasite may exist in nature. Sexual parasites are more resistant than asexual stages, often living several days after the latter have died out.

J. G. and David Thomson (1913) were among the first to confirm Bass's work on the cultivation of malarial parasites. These investigators cultivated estivo-autumnal parasites on 12 occasions, and tertian parasites on 3. Their method is slightly less complicated than Bass'. The optimum temperature was found to be 38° C. It appears to be unnecessary to remove the leucocytes, and 4 generations were reared in one tube. The morphology of the parasites in the cultures was found to be the same as that in the human host. A tendency for the larger parasites to clump together was observed in the case of the estivo-autumnal parasites, and serves to explain why only young forms of this species are to be found in the peripheral blood, the older forms probably being arrested in the capillaries and being responsible for the pernicious symptoms of estivo-autumnal malaria. The normal number of merozoites in tertian parasites seems to be 16, due to 3 successive divisions and in estivo-autumnal parasites 32, due to 4 successive divisions.

A number of other investigators have successfully used Bass' method for the cultivation *in vitro* of the malarial parasites but no one has obtained stages in the sexual cycle except Perekropoff (1914), and his work needs confirmation.

Sinton (1922) has reported a simplified method of cultivating *Plasmodium falciparum* which enables one to obtain cultures from only 5 to 10 large drops of blood. A specially designed culture tube is required. Small ring forms developed up to segmentation in such a tube. Sinton believes that this method can be made of practical use especially in diagnosis in cases where very few parasites are present in the blood, since the number of parasites increases in the culture tube and hence gives the microscopist a better opportunity to find specimens.

G. The Mechanism of Relapse in Malaria

Among the most interesting problems in malaria still to be solved are those concerned with latency, relapse, immunity and carriers.

a. Latency

What we call the normal incubation period of the malarial parasite is for tertian malaria, 14 to 18 days; for quartan malaria, 18 to 21 days; and for estivo-autumnal malaria, 9 to 12 days. There are many cases on record, however, in which infection and the appearance of symptoms were separated by much longer intervals. The length of this interval depends on many factors, some of which are known whereas others are still unknown. Among these factors are the number of sporozoites inoculated by the mosquito, the virulence of the strain injected, and the immunity of the person parasitized. Systematic malarial surveys have demonstrated that many people have malarial parasites in their blood who have never suffered from clinical symptoms; and frequently malarial symptoms are experienced and parasites found a long time after the patient could have been parasitized. Thus James (1920) describes the case of a man who spent two years in Salonika, Egypt and Palestine without being sick, but exhibited clinical malaria 4 months after his return to

England. This case is similar to many others in which the parasites remained "latent" in the body. Treadgold (1918) found parasites in the blood of 5 out of 63 men who had been in Macedonia for almost a year and who gave no history of malaria. A case of the appearance of malaria due to a surgical operation was reported by Ochlecker (1918). The donor in an operation involving blood transfusion had been in Liberia for 6 years where he took quinine prophylactically. For two and one-half years before the operation he had taken no quinine. He gave no history of malaria. Soon after the transfusion (36 hours) he experienced a chill. The recipient became febrile on the fourteenth day after the operation and tertian parasites were found in the blood. These parasites evidently originated in the blood of the donor. True latency is the condition in which neither clinical symptoms are evident nor parasites are present in the blood, but the term latency is often employed for all cases in which parasites are known to be either in the blood or internal organs without causing clinical symptoms. Two types of latency may be distinguished, (1) primary latency involving a long incubation period, and (2) secondary latency, which is the presence of parasites during long intervals between attacks. The attacks in these cases are often brought on as the result of exposure to cold, sun, alcohol, errors of diet, surgical operations, child-birth, etc.

There are other attacks of malaria that produce mild ambulatory cases, the infection being so light that the patient is very little inconvenienced by it. Blood examination is seldom positive in these cases, but severe symptoms often appear eventually and parasites can then be found in the blood.

b. Relapse

We may distinguish two types of relapse in malaria, (1) true relapse, which is the reappearance of clinical symptoms

after a short interval, and (2) recurrence, which involves an attack after a long interval. We do not know how relapses and recurrences are brought about, but a number of suggestions have been made, one or more of which may eventually be found to represent the facts.

(1) The gametocytes may be stimulated in some way to reproduce in the blood of man. This view has been held by Grassi (1901), Schaudinn (1903), Neeb (1910), Rowley-Lawson (1911), Biedl (1917), and Pontano (1920). Grassi was of the opinion that gametocytes could reproduce asexually. Schaudinn described parthenogenesis in macrogametocytes of the tertian parasite and subsequent schizogony, and Neeb found a type of estivo-autumnal parasite which he considered to be undergoing parthenogenesis. Fertilization, such as normally occurs in the stomach of the mosquito, has been described by Rowley-Lawson and Biedl; this was followed by sporulation and schizogony within the blood cells. Finally Pontano found parasites of tertian malaria which because of peculiarities in their morphology he considered to be gametocytes undergoing reproduction in the peripheral blood. None of these findings are very convincing (see Thomson, J. D., 1917).

(2) Resistant forms may be present in the internal organs where they remain until suitable stimuli bring them out into the circulation. Marchiafava and Bignami were perhaps the first to suggest this. Later theories of this sort have been proposed by Craig, Celli, and James. Craig's hypothesis required the conjugation of two young hyaline rings within a corpuscle. The resultant "zygote" grows until it fills the red cell, then breaks out into the blood stream and is carried into the spleen and bone marrow. Here it remains until the proper stimuli cause it to form and liberate spores which begin a new asexual cycle. The resistant forms described by James are not gametocytes but asexual forms that do not pass through schizogony immediately but remain quiescent

until favorable conditions cause them to become active, when they produce merozoites and bring on a relapse. These hypotheses not only lack convincing evidence in their favor but many arguments against them might be cited both from observational and experimental studies.

(3) There may be a continuous asexual cycle during the intervals between relapses or recurrences, but the number of parasites may be so few that no clinical symptoms are caused and very little chance exists of finding specimens in the blood. This theory is held by Ross and others and seems to have the greatest evidence in its favor. Especially interesting are the studies of Whitmore and Ben-Harel on bird malaria. Clean canaries, when infected with parasite-containing blood from another bird, show parasites in their blood in about two weeks. The number of parasites then decreases rapidly until none appear to be present. Such birds, however, remain infective for at least 29 months and perhaps for the rest of their lives, and fresh birds will become infected by inoculations of blood from them at any time. This proves that infective stages of the parasite are present in the peripheral blood, either in a stage not yet recognized or else in such few numbers that they are practically impossible to find. Ben-Harel, while working under the writer's direction, found parasites, especially in the spleen and bone marrow, many weeks after they had disappeared from the blood. Stages in the process of merozoite formation were noted which proves that asexual reproduction was proceeding at a time when no parasites were to be found in the peripheral blood after an extended examination.

c. Provocatives

Relapses, or at least the reappearance of parasites in the blood, may be brought about by the subcutaneous or intramuscular injection of various substances such as normal serum, adrenalin, ergotin, salvarsan, and strychnin. This

can also be accomplished by radiation with ultraviolet light or with the mercury arc. Adrenalin seems to be especially effective for this purpose. A subcutaneous inoculation of one mg. of this substance is said to cause a temporary but immediate contraction of the spleen, forcing any parasites that may be lodged there into the peripheral blood where they may appear within twenty minutes. This procedure has been recommended for diagnosing supposed cases of latency and by giving a dose of quinine at the same time, for actually destroying the parasites that would otherwise remain unharmed in the internal organs. There is general agreement, however, that provocative agents should not be used except in special cases.

d. Carriers

An active malarial carrier is a person whose blood contains gametocytes by means of which mosquitoes may become infected and thus transfer the infection to healthy individuals. If stages other than gametocytes are present in the blood or in the internal organs the person is a "potential" carrier, since activities resulting in the formation of gametocytes might occur at any time. If all carriers could be eliminated, human malaria would soon die out, since the malarial parasite is not passed on from one generation of infected mosquitoes to another, and man is apparently the only reservoir. That human carriers exist in considerable numbers has been proved by many extensive surveys. In Bolivar County, Mississippi, during 1916-1917, Bass found 6,664 out of 31,459 persons examined to be infected. Of those infected 44.91 per cent were without symptoms when examined and had had no symptoms during the previous year. Later, 19,000 persons were examined in Sunflower County with the result that 16 per cent were found to carry parasites in their blood. Not all of these were active carriers, since gametocytes were not always present.

The effectiveness of a carrier depends on the number of gametocytes present in the blood. Mosquitoes do not seem to become infected unless there are twelve gametocytes (estivoautumnal) presents c.mm. of blood. Therefore a carrier who has only a few gametocytes in his blood is not very dangerous, but one who has many gametocytes may infect more mosquitoes and thereby cause the spread of more malaria than a large number of carriers with a few gametocytes. The age and sex of the gametocytes in the blood of carriers also have a bearing upon the spread of malaria. Since parthenogenesis of the macrogametocyte probably does not occur in the mosquito, both macrogametocytes and microgametocytes must be present in the blood, or fertilization cannot take place in the mosquito's stomach and the mosquito will remain uninfected. The ratio of the two types of gametocytes in the blood varies and sometimes macrogametocytes only have been found. Mosquitoes fed on such patients, as Grassi and others have shown, cannot convey malaria. Age also has an influence on the infectiveness of gametocytes, since several days must elapse between their first appearance in the blood and their ingestion by mosquitoes before development into oöcysts is possible.

H. Malarial Surveys and Malarial Control

The presence of malaria in any locality depends on a number of interdependent factors—the removal of any one of which would effectively eliminate the disease. (1) Since the only natural method of transmission of the malarial parasites from man to man is by the bites of certain mosquitoes of the genus *Anopheles*, the destruction of these mosquitoes would be followed by the disappearance of malaria. (2) Since man is the only reservoir from which mosquitoes can become infected, malaria would cease to exist even where mosquitoes were abundant if human carriers could be eliminated. (3) It is also obvious that malaria would soon die out if mos-

quitoes were prevented by screening or otherwise (a) from biting infected persons and thus becoming infective, or (b) from biting susceptible persons and thus starting new infections.

The control of malaria at the present time is directed toward the elimination of one or all of these factors. Various methods of control have been successfully applied in different parts of the world, but although the general principles of control by mosquito reduction, screening, and the sterilization of carriers are well known, no detailed plan can be prepared for any locality, since novel conditions are encountered in almost every area. Before control measures can be successfully applied, therefore, it is necessary to undertake a survey of each particular area extending over at least one year and preferably two or three before definite control measures are inaugurated.

The methods of carrying on a malaria survey may vary somewhat but always involve (1) the cooperation of the health authorities and physicians of the community, (2) field and laboratory investigations, and (3) the elaboration of a plan for control measures that will not meet with the opposition of the general population and will not require a larger expenditure of funds than the inhabitants are able and willing to pay.

a. Preliminary Arrangements

In areas where there are health officers the first step should be to obtain their cooperation. Through the health officer the physicians in the area can be associated with the project. Experience has shown that most of the expenses of a campaign for malarial control should be borne by the people to be benefited, hence financial assistance should be assured if possible before a survey is begun. This can be accomplished through the town council, chamber of com-

merce, or prominent citizens. The number of men required to carry on a survey depends on the size and population of the area, the thoroughness of the proposed campaign, and the character of the topography. At least one of the participants should be a man with medical training who has had previous experience in malarial control. The staff should also include a trained entomologist, a sanitary engineer, and several assistants to act as clerks and microscopists. At some convenient place rooms should be fitted up for office and laboratory purposes.

b. Field and Laboratory Investigations

These are of two sorts: (1) investigations that must be made in any locality before control measures can be begun and (2) investigations made necessary by peculiar local conditions. The first thing to be done is to make a map of the area. On this map should be located all roads, streams, bodies of water, homes, etc. At the same time physical features of the area should be recorded, such as the nature of the soil and subsoil; ground water level, drainage conditions, high and low water levels in streams and bodies of water, tidal conditions if near the sea shore, character and distribution of vegetation, land under cultivation and character of crops. Visits should then be made to every house in the area and a census obtained. For every inhabitant a card should be filled out including such data as name, age, sex, color, place of birth, address, occupation, length of residence, former residence, clinical history of malaria and size of spleen; and blood smears prepared.

The blood smears may be examined by the laboratory assistants and the results recorded as positive or negative and the species of parasite noted in positive cases. Many methods of making and staining blood smears have been proposed and are in actual use in control campaigns. A practical pro-

cedure is to make a thick smear and a thin smear from each person; the former being used to determine the presence of parasites and the latter to determine, in certain cases, the species. Examinations of blood preparations reveal only part of the positive cases since many infected persons have so few parasites in their peripheral blood that the chances of finding them are slight. The number of positive cases found depends on the ability of the microscopist and the number of fields examined. If 200 fields are examined about 50 per cent more positive cases will be found than if only 100 fields are examined. As the number of fields is increased the percentage of increase of positives decreases. For practical purposes a fairly reliable estimate of the total number of positive cases can be obtained by a method of examination that does not actually reveal them all. These data, together with the "history" reports and results of spleen examinations, indicate approximately the amount of malaria in the region and the proportion of each type.

The average prevalence of malaria can be determined by obtaining the spleen rate in children between the ages of two and ten. A very constant result of malaria is the enlargement of the spleen, especially in children. It is customary to record the spleen as extending from one to four finger-breadths or more beyond the costal margin. In most communities the easiest way to get these data is to obtain permission to examine the children at school. Darling (1923) has recently pointed out that by plotting the distribution of these children on a map it is possible to determine local foci of infection as well as of mosquito breeding.

With the map of the area as a guide a mosquito survey should be made. Every stream and body of water should be examined for larvae and the species determined by breeding out adults. Adult mosquitoes should be collected especially in and about the habitations of those who give positive histories, have enlarged spleens or carry parasites in their

blood. Observations and experiments may be made to determine the habits of the different species of mosquitoes; variations in their numbers and activities at various seasons, and the relative importance of the different species as intermediate hosts.

Other information that is of value in preparing a plan for a campaign includes data regarding rainfall, temperature, living conditions, industrial developments and numbers of different kinds of live stock.

The character of the campaign for malarial control can be based on the results obtained from such a survey as outlined above. It will be found that mosquito control is more feasible in certain localities, the sterilization of carriers in others, or both of these together, with or without screening. Mosquito control is an entomological and engineering project, but the sterilization of carriers is a direct attack upon the parasites themselves and may appropriately be discussed in a book on human protozoology.

The first attempt to control malaria by the sterilization of carriers was made by Robert Koch in 1900 in a small community of 734 persons at Stephansort, German New Guinea. The measures used were successful in reducing the malarial rate. The treatment of carriers has since then been recognized as a means of controlling malaria but in no campaign have the results and cost been adequately determined. Perhaps the most carefully conducted investigations along these lines were those carried out in Mississippi during the years 1916 to 1918 by the state board of health in cooperation with the International Health Board of the Rockefeller Foundation. The results of this work have been published by Bass (1919-1920) and Rose (1919).

A survey was made during 1916 and 1917 of an area of 328 square miles in Bolivar County, Mississippi. The population averaged 90 per square mile, 17.8 per cent of whom were white and 82.2 per cent colored. Thick films were

made and examined and histories obtained from 31,459 persons. Of these, 40.30 per cent gave a history of having had one or more attacks of malaria during the previous twelve months and 28.96 per cent of these were found positive by blood examination. Those who gave a negative history were found to be 15.93 per cent positive on blood examination. The combined percentage of those who gave positive histories and those who were found positive by blood examination was 49.81. Approximately half of the persons found infected were under 20 years of age and negroes were found to have 36.61 per cent more infection than whites. The treatment being prescribed by the local physicians was sufficient to stop clinical symptoms but not such as to eliminate the parasites, hence infected persons were still subject to relapse and possible sources of infection for mosquitoes. As a result of tests a "standard treatment" was devised as follows: "For the acute attack, 10 grains of quinine sulphate by mouth three times a day for a period of at least three or four days, to be followed by 10 grains every night before retiring for a period of eight weeks. For infected persons not having acute symptoms at the time, only the eight weeks' treatment is required.

The proportionate doses for children are: Under 1 year, one-half grain; 1 year, 1 grain; 2 years, 2 grains; 3 and 4 years, 3 grains; 5, 6, and 7 years, 4 grains; 8, 9, and 10 years, 6 grains; 11, 12, 13 and 14 years, 8 grains; 15 years or older, 10 grains."

This Standard Quinine Treatment is recommended by the National Malaria Committee and approved by the United States Public Health Service (Bass, 1921).

In 1918 a test demonstration of malarial control by sterilizing carriers was undertaken in Sunflower County, Mississippi, using the results of the survey in Bolivar County as a basis. The area selected contains about 100 square miles and a population of about 9,000, about 1,000 living in the

town of Ruleville and the rest on cotton plantations. About 75 per cent of the inhabitants were negroes. Malaria was the cause of about 70 per cent of the sickness disability and from one-third to three-fourths of all the persons living on the plantations had one or more attacks of malaria each year. A survey showed that 40 per cent of the rural population had had clinical malaria within twelve months and of the remaining 60 per cent who gave negative histories, 22 per cent had parasites in their blood. An educational campaign was begun and standard treatment was given to all positive cases. The following is an example of the results obtained.

Community No. 1, consisting of 9 square miles, was surveyed and treatment begun on February 7, 1918. This area was resurveyed March 3 to 11, 1919. At this time 440 persons were visited; 208 of these had malaria in 1917; 21 of them were positive since their treatment in 1918; 11 of these were positive on the first survey and their subsequent symptoms were probably due to relapse. The reduction noted was nearly 90 per cent.

Similar results were obtained in other communities. Some of the positive cases found in 1919 were probably due to the fact that some of the persons did not take their quinine and others to the failure of the standard treatment to prevent relapses in all cases. The parasites involved were about 90 per cent *P. vivax*, and 10 per cent *P. falciparum*; quartan parasites were very rare.

I. The Malarial Parasites of Primates

Although examinations of both domestic and wild animals have failed to reveal any malarial parasites that could be considered with certainty as belonging to species occurring in man, and animal inoculations have all been negative (Bass, 1922), recent reports by Reichenow (1920), Blacklock and Adler (1922) and Adler (1923) indicate that certain of the higher apes may be parasitized by the organisms of human

malaria. Reichenow detected parasites in gorillas and chimpanzees from the Cameroons that could not be distinguished from the three human species. Blacklock and Adler also found parasites in a chimpanzee resembling those of the three human species but noted only the crescent form of gametocyte. Two more chimpanzees were later reported by Adler infected with parasites indistinguishable from *P. falciparum* and containing crescents. The evidence that the parasites recorded from chimpanzees are of the human type is not yet complete since (1) subcutaneous and intravenous injections of heavily infected blood from a chimpanzee failed to infect two Europeans; (2) human blood heavily infected with *P. falciparum* failed to infect a chimpanzee; and (3) *Anopheles costalis* did not become infected by feeding on chimpanzee blood containing crescents (Adler, 1923).

CHAPTER XI¹

THE NEOSPORIDIA

A. Introduction

The NEOSPORIDIA are SPOROZOA that give rise to spores during the trophic stage of the life-cycle. From the spores emerge amoebulae and not gregarinulae as in the TELOSPORIDIA. Flagellated stages are entirely lacking in their life-cycle. None of the members of the group are important human parasites, but species from two orders (SARCOSPORIDIA and HAPLOSPORIDIA) have been recorded from man. Many of them, however, are serious parasites of lower animals and of great economic importance, such as the myxosporidian parasites of fish, the microsporidian parasites of silkworms and bees, and the sarcosporidian parasites of domestic animals.

B. The Myxosporidia

The MYXOSPORIDIA are principally parasites of fish. Of the 237 species listed by Kudo (1919), 223 are fish parasites, 8 inhabit AMPHIBIA, 4 reptiles, 1 insects, and 1 annelids. Those of fish are almost equally distributed between fresh-water and marine species, and certain species of parasites occur in fish that inhabit both salt and fresh water. The MYXOSPORIDIA have been found wherever looked for and are thus probably world wide in their distribution. The life-cycle is comparatively simple, since there is no inter-

¹By R. W. Hegner.

mediate host. The principal organs of the host invaded by the parasite are the gall-bladder, gills, kidney, urinary bladder, muscle, integument, connective tissue, spleen, and ovary.

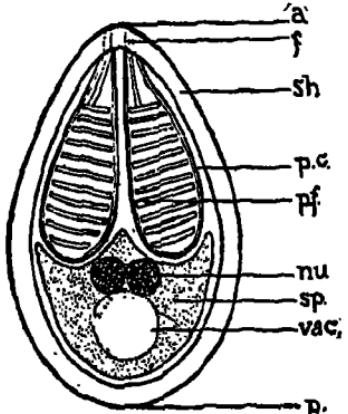
The spore may be selected as the first stage in the life-cycle of the **MYXOSPORIDIA**. This is a complicated structure,

as indicated in the diagram of one from a common genus (*Myxobolus*) shown in figure 131. The spore has a thick outer protective covering, the sporocyst, which consists of two valves; these have a smooth or marked surface, according to the species. Within the sporocysts are two polar capsules (*p. c.*) (sometimes 4, rarely 1), each containing a coiled polar filament (*p. f.*), which is probably usually a hollow thread. This filament may be extruded through a minute pore (foramen) in the anterior end of the polar capsule (*f.*) when stimulated by the digestive juices of the host or by certain chemicals. The rest of the sporocyst is filled with granular sporoplasm (*sp.*) embedded in which are two nuclei (*nu.*) and a vacuole (*vac.*) that is probably of glycogenous nature and stains deeply with iodin. The spores of different species of **MYXOSPORIDIA** differ greatly in shape.

Fig. 131.—Myxosporidian spore. Diagram of a spore of *Myxobolus*. *a.*, Anterior end; *f.*, foramen of polar capsule; *nu.*, nuclei of sporoplasm; *p.*, posterior end; *p.c.*, polar capsule; *p.f.*, coiled polar filament; *sh.*, shell; *sp.*, sporoplasm; *vac.*, iordinophilous vacuole. (After Kudo.)

Some of those of the better known species are shown in figure 132.

New hosts are infected by taking spores into their digestive tracts. Here they are acted on by digestive juices and the polar filaments are extruded, a process that probably



fixes the spore to the intestinal wall. The valves of the sporocyst then open and the sporoplasm, in the form of an amoebula or sporozoite, creeps out. The two nuclei in the sporoplasm fuse either before or after the amoebula emerges.

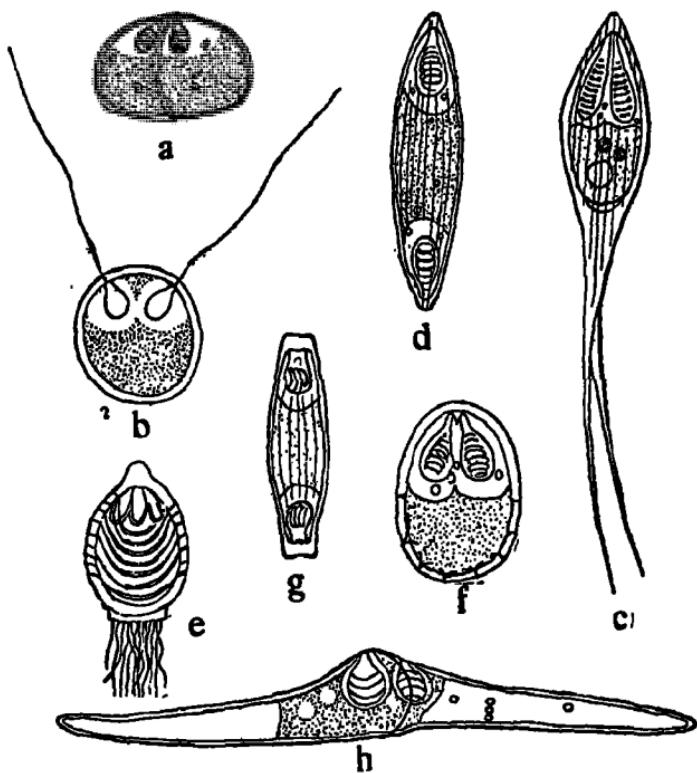


Fig. 132.—Myxosporidian spores.

a, *Leptotheca agilis*. b, *Sphaerospora divergens*. c, *Henneguya gasterostei*. d, *Myxidium lieberkühni*. e, *Chloromyxum leydigii*. f, *Myxobolus pfeifferi*. g, *Sphaeromyxa balbianii*. h, *Ceratomyxa mesospora*. (From Kudo after various authors.)

The amoebula then makes its way to the organ of the host inhabited by the species to which it belongs, by penetrating the intestinal wall and being carried to its definitive habitat by the blood stream or lymph.

The trophic stage or trophozoite into which the amoebula

develops usually becomes massive. The original nucleus of the amoebula multiplies as growth proceeds until a large multinucleate plasmodium is formed. This has more or less permanent pseudopodia that are often localized and are used to a small extent for locomotion, but principally for fixation, and never for capturing food, since nutrition takes place by absorption. Within the trophozoite are distinguished a finely granular ectoplasm and a coarsely granular endoplasm which

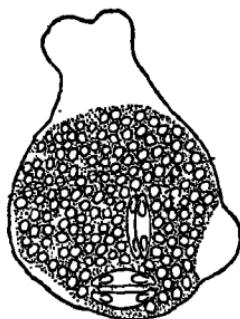
contains, besides many nuclei, fat globules, pigment granules, crystals, etc., and, at certain times, spores in various stages of formation. The trophic stage is spent in tissues or on the walls of cavities of the body. Reproduction is principally by spore formation, but division of the trophozoite (plasmotomy) may also occur. This results in the formation of many trophozoites and consequently increases enormously the number of spores produced. Warm weather seems to favor the vegetative stages, and as in the common parasite of the pike, *Myxidium lieberkühni*, plasmotomy may take place only during

Fig. 133.—*Myxidium lieberkühni*. Plasmodial stage containing two pansporoblasts each with two spores. $\times 440$. (After Mavor.)

the summer, whereas spore formation (Fig. 133) is induced by cold weather and hence occurs in the winter.

The process of spore formation varies widely in different genera and is rather complex. It begins when the trophozoite is young and continues throughout the life of the individual.

Spore formation in the life-cycle of *Myxidium gadi*, which occurs in the pollach (Gadus pollachia), has recently been described by Georgévitch (1919) and is shown in outline in figure 134. As indicated in stage 1, first the oval binucleated sporoplasm issues from the spore; this becomes spheri-



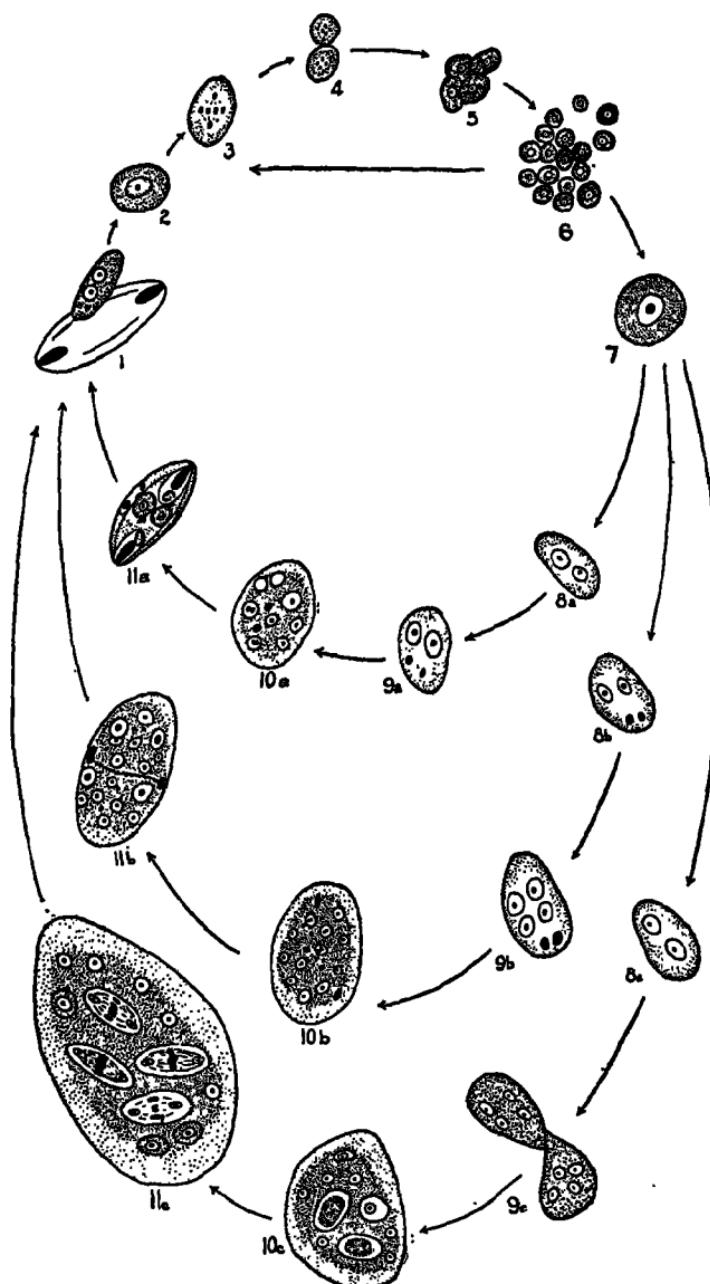


Fig. 134.—*Myxidium gadi*. Stages in the life-cycle. For explanation see text. (After Georgévitch.)

cal and the nuclei within it unite, thus forming a zygote (stage 2). The nucleus of the zygote undergoes division during which a centriole separates from the karyosome, and four chromosomes are formed (3). After mitosis is completed the zygote divides into two schizonts of equal size (4). Each of these undergoes equal division (5), and multiplication continues until many schizonts are produced (6). After schizogony has proceeded for a number of generations, sporogony is inaugurated. Three types of sporogony may take place.

In monosporogony the schizont becomes large (7); its nucleus divides into two daughter nuclei equal in size (8a); these divide again producing two large nuclei and two small vegetative nuclei (9a); the two large nuclei divide again; two of the four then undergo another division resulting in six large and two small nuclei; two of the six large nuclei undergo a reduction in chromosome number from 4 to 2 by casting out a small mass of chromatin into the cytoplasm (10a); the spore is then formed with these two nuclei with the reduced number of two chromosomes as the gamete nuclei (11a); these gamete nuclei fuse later to form the single zygote nucleus which thus contains four chromosomes. Similar phenomena occur when a sporont undergoes disporogony (8b-11b). In this case there are produced two vegetative nuclei and 12 large nuclei; four of the latter undergo reduction and become the gamete nuclei of the two spores formed by each sporont. Polysporogony (8c-11c) also occurs, the sporont becoming a very large plasmodium which may undergo plasmotomy (9c) and in which spore formation proceeds over a considerable period and not synchronously.

In spite of the apparent completeness of the above life cycle, whether sexual processes occur during the life-cycle of *MYXOSPORIDIA*, and, if so, at what place and in what way, are questions that are still so much in doubt that no definite

statements can be made at this time. It seems best, therefore, to refer the student to current literature on this subject (Erdmann, 1917).

C. The Microsporidia

The MICROSPORIDIA are principally parasites of insects but have also been reported from arachnida, crustacea, bryozoa, worms, fish, amphibia, and reptiles. None have been found in warm-blooded vertebrates. In fish they sometimes form cysts or tumors that are visible to the naked eye and resemble in appearance tumors produced by MYXOSPORIDIA. Certain species are pathogenic to animals of value to man and are thus of economic importance; these include *Nosema bombycis*, that causes silkworm disease, *N. apis*, that is responsible for a disease of bees, and *Thelohania contejeani*, a species that has been accused of killing crayfishes in France.

Nosema apis may be selected as a type of the group. It causes an important bee disease known as nosema disease. The life-cycle of this species has been worked out in considerable detail by Fantham and Porter (1912). Bees become infected by ingesting spores that pass out in the feces of diseased bees. These spores are oval in shape and, as the name MICROSPORIDIA suggests, are extremely small, measuring from 4μ to 6μ in length and from 2μ to 4μ in width. No differentiation of the contents is visible in fresh spores, but properly prepared specimens reveal a single polar capsule at the anterior end (Fig. 135, p. c.) containing

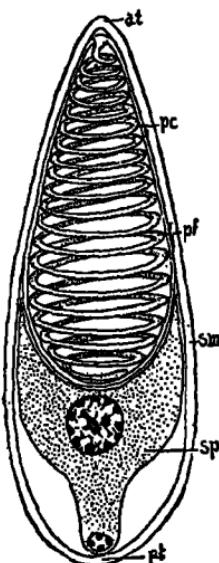


Fig. 135.—Microsporidian spore. *at*, Anterior end; *pc*, polar capsule; *pf*, polar filament; *sm*, spore wall; *sp*, sporoplasm. (After Kudo.)

a coiled polar filament (*p. f.*), and a mass of cytoplasm, the sporoplasm (*sp.*) containing two nuclei. Surrounding and protecting the contents is a thick-walled sporocyst, consisting of a single piece and not of two valves, as in the *Myxosporidia*.

The spore apparently becomes attached to the wall of the bee's digestive tract by the polar filament. This is about $250\ \mu$ long (Fig. 136) and is probably extruded by mechanical or chemical stimuli, since both mechanical pressure and chemical stimulation (perhydrol) have been found to cause its extrusion under experimental conditions (Kudo, 1913, 1918). The sporocyst becomes soft in the bee's chyle-stomach

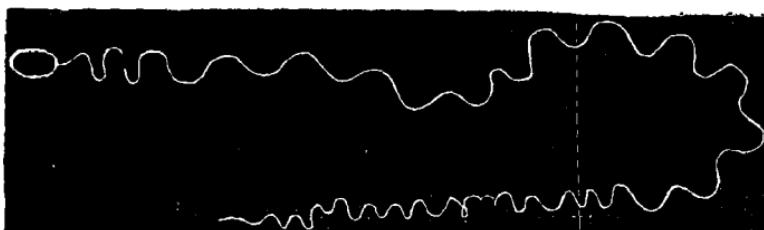


Fig. 136.—Microsporidian spore with polar filament extruded.
 $\times 1200$. (After Kudo.)

and an amoebula or sporozoite, formed by the sporoplasm, creeps out. This amoebula is capable of amoeboid movement and of division, and after a period of wandering its progeny penetrate the epithelial cells, where they become rounded or oval trophozoites from $0.75\ \mu$ to $2.5\ \mu$ in diameter. The trophozoites are nourished by absorption, grow at the expense of the cytoplasm in the host cells, and multiply by division. The daughters may be called schizonts; they divide once or twice and then are known as sporonts or pansporoblasts. These become sporoblasts, each of which forms a single spore. In this way "nests" of spores are produced in the epithelial cells lining the bee's intestinal tract (Fig. 137). During the formation of the spore from the spor-

blast the single nucleus is increased to five; these exhibit division of labor. One of the daughter nuclei, after the first division, divides into two, which control the formation of the sporocyst; the other daughter nucleus buds off a smaller nucleus, which controls the development of the polar capsule, and then divides into two equal nuclei which remain in the sporoplasm. The spores are liberated into the digestive tract by the rupture of the host cell. Some of them

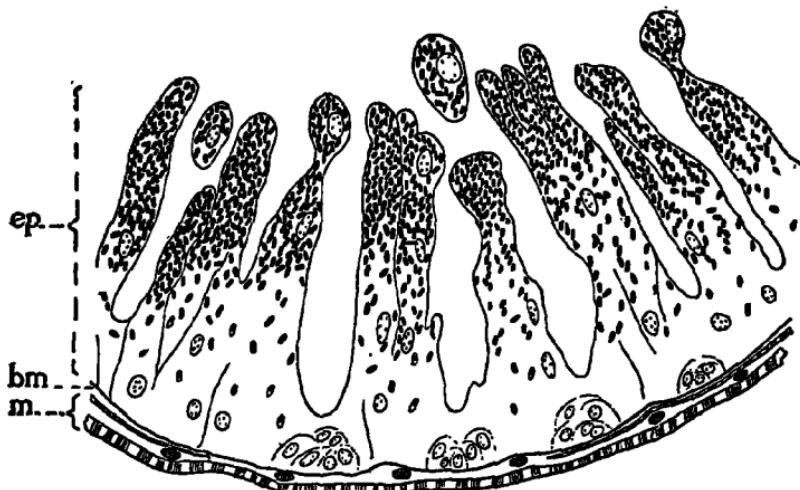


Fig. 137.—*Nosema apis*. Section of honeybee's stomach showing epithelial cells (*ep*) filled with spores; *bm*, basement membrane; *m*, muscular layer. (After White.)

may reinfect cells of the same host, but most of them pass out with the feces. The spores are spread by diseased bees within the hive as well as on the vegetation outside and in the water where the bees go to drink. Infected hives are weakened by the disease and become the prey of robber bees, which thus become infected and carry the infection to other hives. Other animals, including man, and the wind may aid in the distribution of the spores. It seems also that certain bees may be infected by the parasites, but, like human

carriers, are not much inconvenienced by their presence (Fantham and Porter, 1914); they are, however, instrumental in spreading infection.

Another microsporidium of economic importance is *Nosema bombycis*, which is pathogenic to silkworms and causes the disease known as pébrine in France and gattina in Italy. This is the disease which once threatened the silk industry of France and was controlled by the help of Pasteur's investigations. *Nosema bombycis* invades not only the digestive tract, as does *N. apis*, but also other parts of the insect's body, including the ovaries. Spores formed in the ovarian eggs germinate in the larvæ that hatch from the eggs and in this way pass from one generation to the next. Pasteur learned to recognize infected eggs by microscopical examination and could thus eliminate diseased stock.

Other MICROSPORIDIA differ from the species described in various ways—the number of spores formed by the sporont may be 1, 2, 4, 8, 16, or many; the trophozoite may be a multinucleate plasmodium as in *Glugea anomala* which is a species pathogenic to stickleback fish; and other stages in the life-history may vary considerably. A case of hyperparasitism by a microsporidium has been reported as follows: the species *Nosema ichneumonis* parasitizes the ichneumon fly, *Stenichneumon trilineatus*, which is a parasite of the caterpillar of the gooseberry moth, *Abraxas grossulariata* (Fantham and Porter, 1914).

D. The Sarcosporidia

The SARCOSPORIDIA are parasites of vertebrates, especially mammals. They have been recorded from reptiles and birds but never from invertebrates. Tissues, particularly striped muscle, are invaded, where long-rod-like masses of spores are produced that can easily be seen with the naked eye in many cases. These are often called Miescher's tubes after their discoveror (Miescher, 1843). The muscles most fre-

quently parasitized in mammals are those of the oesophagus, larynx, diaphragm, and body-wall. Our knowledge of this

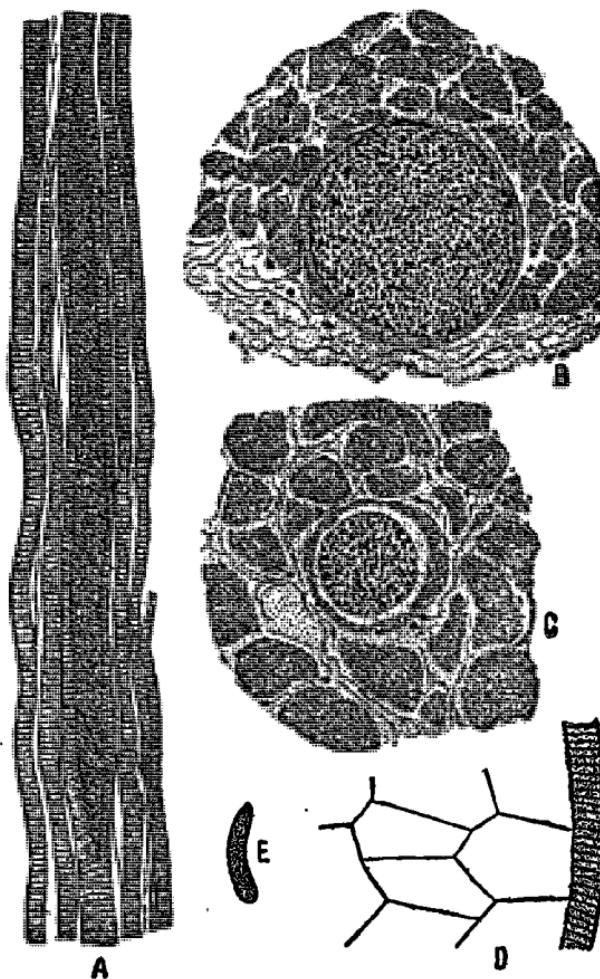


Fig. 138.—*SARCOSPORIDIA* in the vocal cords of man. A, Longitudinal section showing tubule filled with spores in a muscle fiber. B, C, Transverse sections. D, Outer wall of parasite. E, Spore. (From Brumpt, after Baraban and Saint-Remy.)

group of parasites is still very incomplete—opinions differ regarding the structure of the spores; only fragments of the

life-cycles have been described; and the methods of infection are not known. Most of the species cause very little trouble to their hosts but several are quite pathogenic, probably because of their ability to spread throughout the body as is true of *Sarcocystis muris* in the mouse. We know more about this species than any other. In 1901 it was discovered (Smith, 1901) that mice become infected if they are fed on tissues from diseased mice. The spores that are freed in the

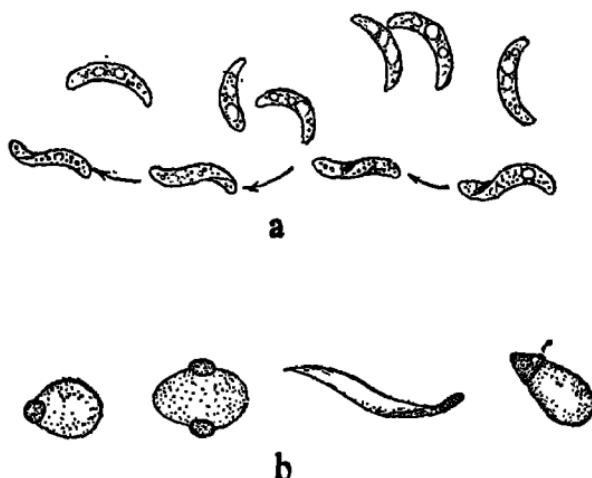


Fig. 139.—Sarcosporidian spores. a, *Sarcocystis muris*. One of the spores is shown in four successive positions. b, *Sarcocystis* sp. from man. (a, After Koch; b, After Darling.)

intestine are capable of movement (Fig. 139, a) and probably bore their way through the epithelium and into the lymph spaces aided by a toxic substance called sarcocystin. This toxin was first discovered by Pfeiffer (1891) and later experimented with by Laveran and Mesnil (1909), who obtained it from *Sarcocystis tenella* of the sheep and found it to be toxic to rabbits. The spores of *S. muris*, which are sickle-shaped, penetrate the epithelial cells within $1\frac{3}{4}$ hours after being ingested by the mouse (Marullaz, 1920). Here they become oval in shape and begin to divide by mitosis. Within

about 24 hours they reach the lymphatic spaces and from here migrate to the muscles where later Miescher's tubes are found. These look like white threads lying parallel to the muscle fibers. They are up to 20 mm. in length and are divided into chambers from 1 to 2 mm. long by 0.25 mm. broad. The spores within these tubes are rounded, elongate or crescent-shaped and variable in size, measuring up to 16 μ in length and up to 9 μ in width.

Guinea pigs have been infected with *S. muris* by feeding them parasitized tissue from mice (Negri, 1908, Darling, 1910) and mice have been infected with *S. tenella* by feeding them tissue from sheep containing this species (Erdmann, 1910). Mice have also been infected by feeding them feces from other mice that had been fed on infected tissue. Cysts may have been present in the feces (Negri, 1910). Herbivorous animals are not, of course, infected by eating diseased tissue, but how they become parasitized is still a mystery. The spores are very delicate and are not able to withstand the conditions outside of the host. They have been reported from the blood and it is possible that they may be carried from one host to another by blood-sucking arthropods.

Some of the species of *Sarcocystis* are as follows:

- S. miescheriana*, Kuhn, 1865, in pigs.
- S. tenella*, Railliet, 1886, in sheep.
- S. blanchardi*, Doflein, 1901, in cattle.
- S. muris*, Blanchard, 1885, in mice.
- S. darlingi*, Brumpt, 1913, in the opossum.
- S. kortei*, Castellani and Chalmers, 1909, in the monkey.
- S. rileyi*, Stiles, 1893, in ducks.

Sarcosporidiosis has been reported in man by various investigators and probably occurs more often than is generally supposed, being discovered only in case an autopsy is made.

(1) In 1868 Lindemann discovered masses in the myocardium of a person who had died of dropsy that may have been SARCOSPORIDIA and that were named by Rivolta (1878)

Sarcocystis lindemannii. (2) In 1893, Kartulis found Miescher's tubes in a Sudanese. (3) Baraban and St. Remy (1894) observed SARCOSPORIDIA in the laryngeal muscles of a man who had been executed (Fig. 138).

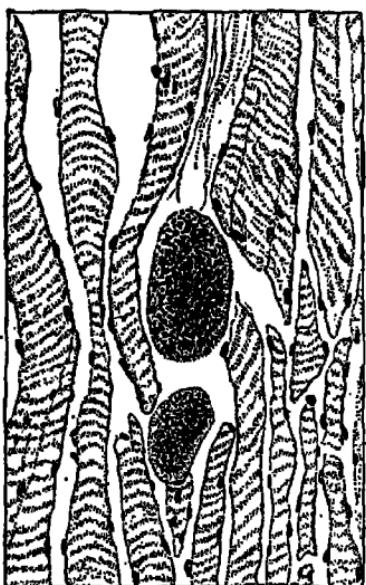


Fig. 140.—Sarcosporidia in the muscles of man. (After Darling.)

How man becomes infected is unknown. It has been suggested by Darling that sarcocystis in vertebrates may represent "side-tracked varieties of parasites of invertebrate animals," and this seems to be the generally accepted opinion at the present time.

E. The Haplosporidia

The HAPLOSPORIDIA are mostly parasites of annelid worms; several species that are pathogenic to fish have been placed in the order (*Ichthyosporidium giganteum*); and one species, *Rhinosporidium seeberi*, has been recorded from man. The members of the order are characterized (1) by the presence of large spores in each of which a single voluminous

were from 150 μ to 1,600 μ long and from 77 μ to 168 μ thick. (4) Vuillemin (1902) recorded what he supposed to be *S. tenella* from a man who died in Nancy from tuberculosis. (5) Darling (1909) observed SARCOSPORIDIA in the biceps of a negro from Barbados (Fig. 139, b), and (6) later (1919) reported a second case in an East Indian in the Federated Malay States (Fig. 140). (7) Recently Cone (1921) claims to have found certain bone lesions in a child due to SARCOSPORIDIA.

nucleus is visible, (2) by the absence of polar capsules, and (3) by a simple type of development. The young parasite is an amoeba which at first multiplies by fission. Later the nucleus of each daughter cell undergoes successive divisions without the intervention of cell walls, the result being a multinucleate plasmodium. This plasmodium may divide (plasmotomy) or produce merozoites (schizogony) or form spores. The spores arise either from sporoblasts, each of which produces a single spore, or pansporoblasts, each of which gives rise to a number of spores. These are the infective stages from which are derived the amoebulae that start a new generation.

Rhinosporidium seeberi.—This species has been reported as a human parasite in a few cases from Argentina, India, Ceylon, and Tennessee. It was first discovered by Seeber in Buenos Ayres in 1896 in a nasal polypus and described by him in 1900. Wernicke in the same year named it *Coccidium seeberi*. The next record of this parasite is that of Kinealy who in 1903 described a nasal polypus in an Indian in Calcutta. This polypus was carefully studied by Minchin and Fantham (1905), who placed the parasite causing it in the order HAPLOSPORIDIA and named it *Rhinosporidium kinealyi*. Several other cases of human infection with this parasite were found by Nair in 1905 in Madras in natives who came from the State of Cochin on the west coast of India and described by Beattie in 1906. Castellani and Chalmers have also found polypi containing this species in Ceylon.

These polypi when cut open (Fig. 141) are found to have cyst-like bodies embedded in them. The largest of these cysts are visible to the naked eye. Inside of the cyst, just within the wall, pansporoblasts are formed by the division of the nuclei in the undifferentiated cytoplasm. These pansporoblasts increase in size toward the center of the cyst and within them are formed from 9 to 16 spores. The

cysts burst when ripe and the spores are scattered. How new infections are brought about is not known.

Ashworth (1923) has made a careful study of this parasite in a native of India and considers it to belong not to the SPOROZOA but to the lower fungi (PHYCOMYCETES) where he has placed it in the suborder CHYTRIDINEÆ and provisionally near the OLPIIDIACEÆ. He describes the

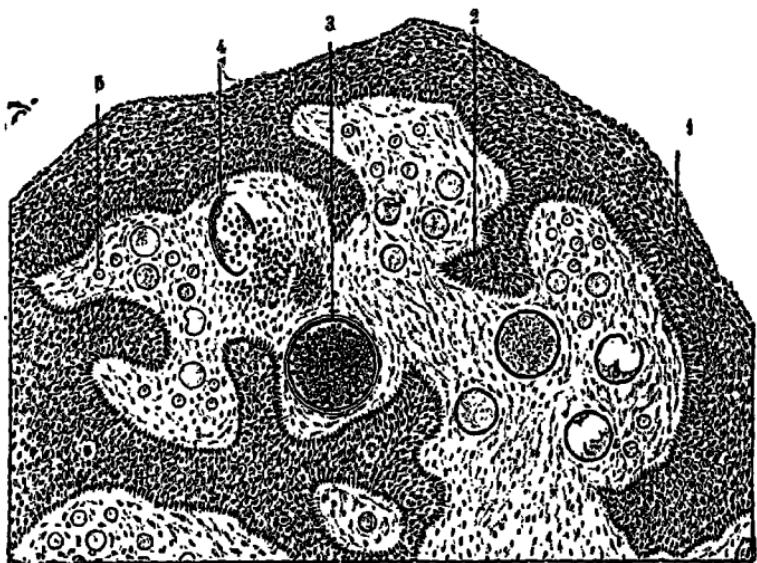


Fig. 141.—*Rhinosporidium seeberi*. Section of a tumor, showing proliferation of epithelial tissue (1, 2), a mature cyst (3), a cyst that has broken down and liberated its pansporoblasts (4), and young cysts (5). (From Brumpt, after Seeber.)

organism as a "spherical or oval non-motile Protistan, found in man in polypoid growths in which it lies usually between the connective tissue cells; recorded from the nose, nasopharynx, uvula, conjunctiva, lacrimal sac, ear, and penis of man. The earliest stages are about 6μ in diameter, with chitinoid envelope, vacuolated cytoplasm, and a vesicular nucleus containing a karyosome. As growth proceeds granules of protein and fat globules appear in the cytoplasm,

and increase in number and in size. When the cell reaches a diameter of about $50\ \mu$ to $60\ \mu$ the nucleus undergoes mitosis; there are four chromosomes. Other nuclear divisions follow, so that 4, 8, 16, 32, 64 nuclei are formed; at these and subsequent divisions all the nuclei (with few exceptions) are in synchronous mitosis. About the time of, or shortly after, the next (seventh) division, when the parasite is about $100\ \mu$ in diameter and has about 128 nuclei, the envelope, hitherto chitinous, becomes much thickened by the deposition of cellulose on the inner surface—except at one point where the future pore will be formed. The nuclear divisions continue, and after the twelfth (i. e., when about 4,000 nuclei are present) division of the cytoplasm occurs, and rounded cells are formed which divide twice to form the young spores (about 16,000). These may all be transformed into fully equipped spores, but more usually some of them—up to about a third—remain almost unchanged; hence in most mature sporangia there are either fully formed spores in the centre and small ones at the periphery, or fully formed spores at one pole and small cells at the other, with intermediate stages in the intervening area in each case. The fully formed spore has a chitinoid envelope, a vesicular nucleus with karyosome, and cytoplasm in the vacuoles of which are refringent spherules, ten to sixteen in number, and each about $1.5\ \mu$ to $2\ \mu$ in diameter. The spores are spherical or oval and $7\ \mu$ to $9\ \mu$ in diameter. They are discharged from the sporangia through a pore formed by rupture of the sporangium wall at the area where little or no cellulose was deposited. Ripe sporangia are whitish and usually .25 to .3 mm. in diameter, but may be larger. Spores are spread in the connective tissue by the tracts of lymph exudate; the spherules disappear, and the trophic stage begins again, the parasite feeding on the fluid in the interstices of the connective tissue; and the cycle is repeated."

CHAPTER XII¹

A GENERAL CONSIDERATION OF THE INFUSORIA

A. Introduction

1. CHARACTERISTICS OF THE CLASS

From an evolutionary standpoint the INFUSORIA represent the most highly specialized of the protozoa. As a group they are characterized by the possession of cilia during a part or whole of their life-cycle and by the differentiation of their nuclear apparatus into a vegetative macronucleus and a generative micronucleus. In a few of the INFUSORIA the nuclear apparatus is represented by a single type of nucleus (see *Opalina*), but, even here, at the time of division, the chromosomes which represent the vegetative nucleus and those which represent the generative nucleus can be differentiated. The INFUSORIA are divided into two groups: (1) the CILIATA in which cilia are retained throughout the life-cycle and (2) the ACINETARIA or SUCTORIA in which cilia are retained only during the larval stages of the life-cycle, the adult organism losing its cilia and being sedentary.

2. *Paramecium caudatum* AS A REPRESENTATIVE OF THE CLASS

P. caudatum is one of the commonest free-living ciliates and generally appears in most fresh-water infusions. The animal is shaped somewhat like a slipper with a blunt

¹ By W. H. Taliaferro.

anterior end and a more pointed posterior end (Fig. 142). It is difficult to state the average size of the species due to the fact that the species, in common with other species that have been sufficiently studied, consists of a large number of races which breed true and which are *in se* different but *per se* constant in size (cp. pp. 464-466). In general most specimens are 120 μ to 325 μ in length.

A groove-like depression, the oral groove or peristome, extends from near the anterior end obliquely backward and toward the right ending near the middle of the body. At

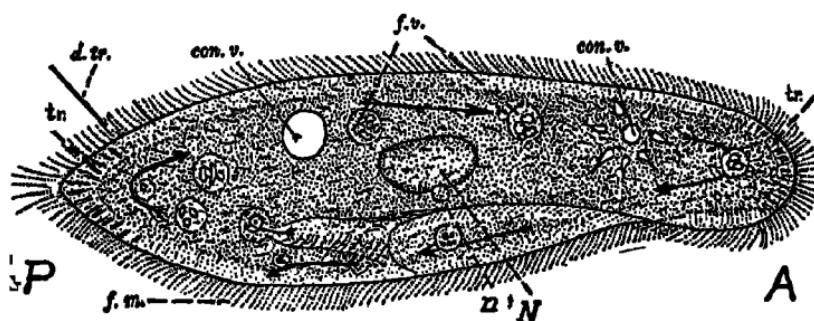


Fig. 142.—*Paramecium caudatum*.

Arrows indicate the direction and course of the food vacuoles within the endoplasm. *A*, anterior end; *con. v.*, contractile vacuoles, the anterior one showing the feeding canals; *d. tr.*, discharged trichocyst; *f.v.*, food vacuoles; *f.m.*, fecal matter which is discharged at a definite spot indicated by arrow; *N*, macronucleus; *n*, micro-nucleus; *P*, posterior end; *tr.*, trichocyst before discharge. $\times 375$. (After Dahlgren and Kepner.)

the posterior end of this groove is the cytostome or mouth which opens into a funnel-shaped cavity, the cytopharynx or gullet. The entire body is covered with cilia, those at the posterior end and in the oral groove being slightly longer than elsewhere. The cilia are arranged in rows corresponding to the striations on the cuticle or pellicle, which is the delicate elastic outer membrane of the animal. Just beneath the cuticle is a thin layer of rather dense non-granular

cytoplasm, the ectoplasm, from which the cilia arise and in which are embedded large numbers of spindle-shaped structures about 4 microns in length called trichocysts. When properly stimulated the trichocysts are discharged and probably serve as weapons of defense. The central part of the body is occupied by the more fluid, granular endoplasm within which are embedded a macronucleus, a micronucleus, two contractile vacuoles, and a variable number of food vacuoles.

The two contractile vacuoles are situated close to the dorsal surface, one near each end of the body. They communicate with a large portion of the protoplasm by means of six to ten radiating canals. Liquid from the protoplasm collects in these canals and is carried into the vacuoles from which, at more or less regular intervals, it is discharged to the outside. The functions of the contractile vacuoles has already been discussed. (See Taylor, 1923.)

The bacteria, other protozoa, and organic particles that serve as food for *Paramecium* are drawn into the oral groove by the beating of the cilia; swept into the cytostome and carried down to the end of the cytopharynx with the aid of an undulating membrane that is attached to its wall. Here a food vacuole forms, consisting of a small quantity of water containing food particles; separates from the cytopharynx; and is swept into the body by the rotary streaming movement of the endoplasm known as cyclosis. Food vacuoles are carried through the endoplasm in a definite course, as indicated in figure 142. Digestive fluids are secreted into them and the products of digestion are absorbed by the surrounding protoplasm. Any undigested matter is thrown out near the posterior end of the body at an anal spot which is visible only at the time of discharge.

Reproduction in *Paramecium* takes place only by binary division although this is interrupted from time to time by conjugation and endomixis (see pp. 449-461). During

binary division the micronucleus divides by a kind of mesomitosis. One daughter micronucleus becomes located near each end of the body, the macronucleus elongates and divides transversely, then the body constricts near the middle and finally pinches in two. The gullet in the meantime produces a bud which becomes the new gullet of the posterior daughter; and a new contractile vacuole arises in each daughter. Various other processes of reconstruction take place before the production of the two daughters which structurally resemble the parent.

B. Classification

The character and distribution of the cilia constitute the principal criteria for the division of the class into subclasses, orders, etc. The following classification is taken, with a few modifications, practically *verbatim* from Minchin (1912).

CLASS INFUSORIA

A. Subclass Ciliata.—Cilia throughout life.

I. Section Aspirigera.—Without a spiral zone of adoral cilia or membranellæ.

i. ORDER HOLOTRICHA.—Cilia approximately equal in length all over the body, forming a continuous, evenly distributed coat, in more primitive forms; arranged in bands or restricted to special regions, in more specialized forms.

(i) SUB-ORDER GYMNOSTOMATA.—Mouth a simple pore, near or at the anterior pole of the body leading into a simple, usually straight cytopharynx without cilia or undulating membranes, but often with a rod-apparatus by which

the mouth is closed and opened for food ingestion.

- (2) SUB-ORDER HYMENOSTOMATA.—Mouth usually at the side of the body and at the bottom of a peristomial depression, leading into a short cytopharynx never supported by a rod-apparatus, but containing an undulating membrane; consequently mouth not capable of being closed, but permanently open.

II. Section Spirigera.—With a conspicuous spiral zone of large cilia or vibratile membranes leading to the mouth; cytopharynx as in *Hymenostomata*.

2. ORDER HETEROTRICHA.—Generally of swimming habit, sometimes sedentary.

(1) SUB-ORDER POLYTRICHA.—Body-cilia forming an even coat.

(2) SUB-ORDER OLIGOTRICHA.—Body-cilia greatly reduced or absent.

3. ORDER HYPOTRICHA.—Typically of creeping habit; the body flattened, with dorsal and ventral surfaces, the ciliation modified and specialized, usually with cirri on ventral surface.

4. ORDER PERITRICHA.—Typically of sedentary habit, the locomotor cilia reduced to a single ring or absent temporarily or permanently; the adoral spiral runs down into a deep depression, the vestibule, into which open the anal pore and contractile vacuoles, and at the base of which is the mouth leading into the cytopharynx.

B. Subclass Acinetaria.—Adult organisms of sedentary habit and devoid of cilia. Larval stages free-swim-

ming and ciliated. Food is captured and ingested by means of special organs or tentacles which are peculiar to the subclass.

I. HOLOTRICHA

a. Suborder *Gymnostomata*

(1) ASTOMATOUS FORMS.—There are a few entozoic genera, such as *Opalina*, whose exact systematic position is difficult to define, but which we tentatively place in the sub-order **GYMNOSTOMATA**. Minchin (1912) places these with certain other entozoic genera, all of which either lack a mouth or possess a very rudimentary one, in a suborder **ASTOMATA**, but, as he points out, this group is a very artificial one whose characteristics are due to convergent adaptation rather than to phylogenetic relationships. Doflein (1916) considers *Opalina* together with *Ichthyophthirius* as primitive **HOLOTRICHÆ**. Metcalf (see Metcalf, 1923) divides the **CILIATA** into the **PROTOCILIATA** and **EUCILIATA** and places the *Opalinidæ* under the **PROTOSCIATI**.

A detailed description of one of these genera, *Opalina*, is given on page 410. One point of particular interest is that *Opalina* possesses only one type of nucleus with no division into macro- and micronuclei as in other ciliates. Even here, however, the work of Metcalf (see Metcalf, 1923) indicates that there are two types of chromosomes, one type containing vegetative chromatin and corresponding to a macronucleus, the other type containing generative chromatin and corresponding to a micronucleus. The nuclei of gametes apparently arise only from generative chromatin. The absence of the two types of nuclei is also known to be characteristic of the parasitic genus, *Ichthyophthirius* and the free-living genus *Trachelocerca*, but, in these genera, the nuclei also occur separately in some stages of the life-history.

(2) TYPICAL FORMS.—These forms possess the simplest

type of mouth found in the ciliates. It is generally a simple pore near the anterior end of the body and leads into a straight cytopharynx which is never supplied with cilia or undulating membranes, but may have a rod-apparatus with which the mouth can be opened or closed. The character-

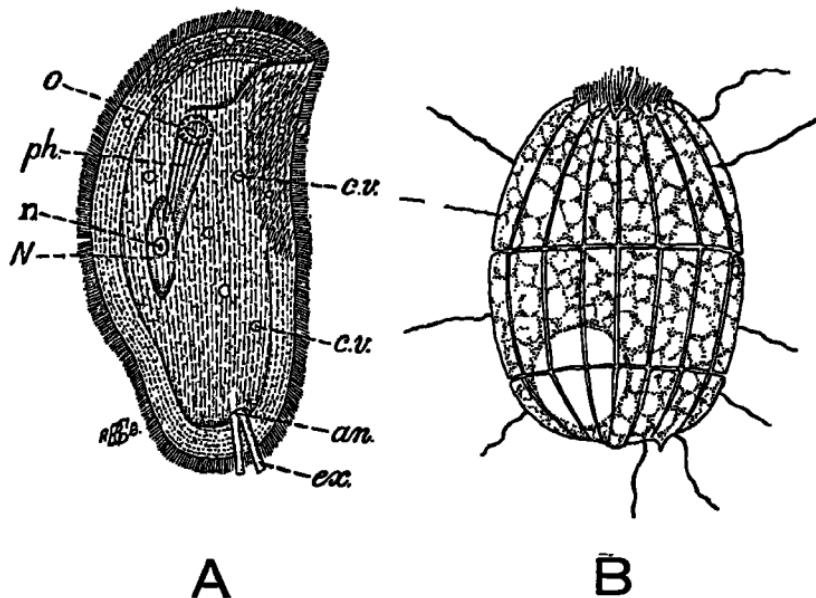


Fig. 143.—Representative of the order HOLOTRICHA, suborder GYMNSTOMATA.

A, *Chilodon cucullulus*. B, *Coleps hirtus*. o, cytostome; ph., cytopharynx surrounded by a supporting apparatus of rods; N, macro-nucleus; n, micronucleus; c.v., contractile vacuole; an., anal pore, temporarily visible during the extrusion of fecal matter (ex.). (A, from Minchin, after Stein. B, drawn by Dr. W. A. Kepner.)

istics of the suborder are well shown in *Chilodon cucullulus* (Fig. 143, A). In *Didinium nasutum* (Fig. 152, I) the body-cilia are restricted to definite bands on the body, and, associated with the cytopharynx, is a peculiar organ known as the "tongue" (Fig. 152) which is used in grasping prey. (For a description see page 397.) In *Coleps hirtus* (Fig.

143, B) the pellicle is thickened to form a lorica composed of many plates.

b. Suborder Hymenostomata

The HYMENOSTOMATA have apparently been derived from the GYMNSTOMATA by the development of a peristomial depression at the base of which the mouth is placed. It is to be noted, however, that the cilia, which are found in the peristomial depression, are differentiated very little, if any, from those of the general body surface, a fact, of course, in marked contrast to the condition found in the three higher orders. The cytopharynx is supplied with an undulating membrane, but never with a rod-apparatus, and, in consequence, the mouth is permanently open. A typical example of this order is *Paramecium caudatum* which we have already described. Other examples are *Paramecium calkinsi* and *Frontonia acuminata* (Fig. 144). *Isotricha prostoma*, an entozoic form in the rumen of ruminants, is also referred to this suborder.

2. HETEROTRICHA

a. Suborder Polytricha

The POLYTRICHA, which form the first suborder of the HETEROTRICHA, resemble the HYMENOSTOMATA in that their bodies are covered with an even coat of cilia and their mouths and cytopharynges are structurally the same. Their most important distinguishing characteristic is the peristomial groove which is much more definitely specialized for obtaining food. The peristome exists in a more or less spiral shape, forming a left-handed spiral around the body in this order, and the peristomial cilia are markedly longer than those on the body surface. The smaller cilia which cover the general body surface are generally arranged in rows. One of the common examples of this suborder is *Spiros-*

tonium ambiguum (Fig. 145) which is one of the largest of the ciliates, sometimes reaching a length of 3 mm. Its most interesting modifications are the greatly elongated bead-

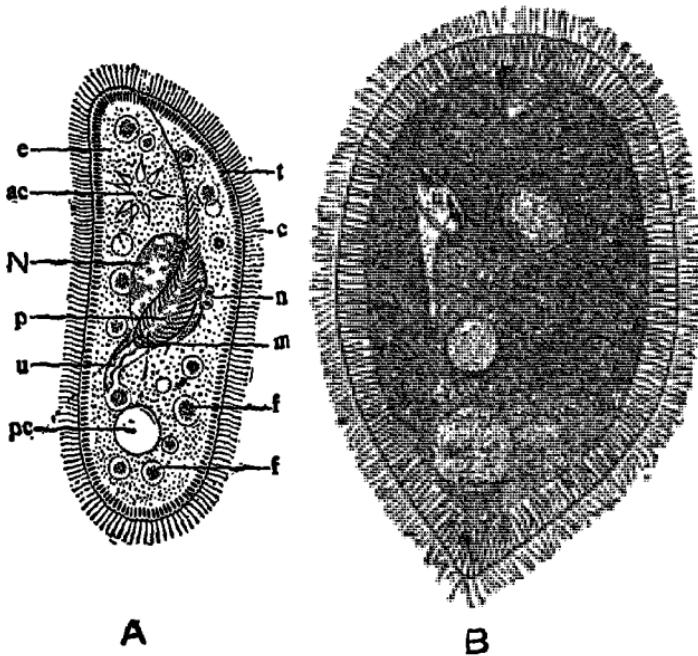


Fig. 144.—Representative of the order HOLOTRICHA, suborder HYMENOSTOMATA.

A, *Paramecium calkinsi*. B, *Frontonia acuminata*. ac, anterior contractile vacuole, surrounded by radiating feeding canals; c, cilia; e, endoplasm; f, food vacuole; m, cytostome or mouth; N, macro-nucleus; n, micronuclei; p, peristome and peristomial cilia; p.c., posterior contractile vacuole; t, trichocysts; u, undulating membrane within cytopharynx. A should be compared with figure 142. The peristome is not clearly visible in B, but the cytostome and cytopharynx with its undulating membrane (u) is clearly indicated. A \times about 400; B \times 500. (A, after Woodruff; B, drawn by Dr. W. A. Kepner.)

like macronucleus and the long feeding canal which supplies the contractile vacuole. Other examples are *Bursaria* and *Stentor*. The suborder is also interesting because it includes

the entozoic genera *Nyctotherus* and *Balantidium*, both of which have representatives entozoic in man.

b. Suborder Oligotricha

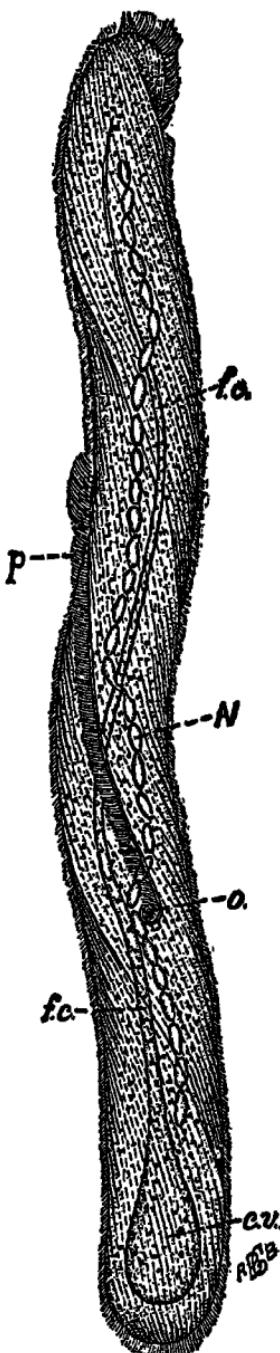
This suborder contains heterotrichous forms which differ from the POLYTRICHA in that the bodycilia are either reduced to special areas on the body or are absent altogether. Among the free-living species is *Halteria grandinella* (Fig. 146). By far the greatest number of species have been placed in the family OPHRYOSCOLECIDÆ which contains many forms entozoic in ruminants and horses including such genera as *Entodinium*, *Ophryoscolex*, and *Diplodinium*. In many of these species, the body is drawn out into spine-like processes and presents a very bizarre appearance.

3. HYPOTRICHIA

In common with the other orders comprising the section SPIRI-

Fig. 145.—*Spirostomum ambiguum*, a representative of the order HETEROTRICHIA, suborder POLYTRICHA.

This is one of the largest ciliates known, sometimes reaching the length of 3 mm. *N*, greatly elongated macronucleus; *o*, cytostome which is placed at the posterior end of the peristome (*p*) ; *c.v.*, contractile vacuole which is supplied by a long feeding-canal (*f.c.*). (From Minchin, after Stein.)



GERA, the peristomial groove is very highly developed and possesses a very strikingly differentiated adoral zone of large cilia. The mouth is essentially the same as in the last order. The cytopharynx is supplied with a very highly differentiated undulating membrane. The forms are typically modified for a creeping mode of life and are strikingly dorso-ventrally flattened with the ventral surface

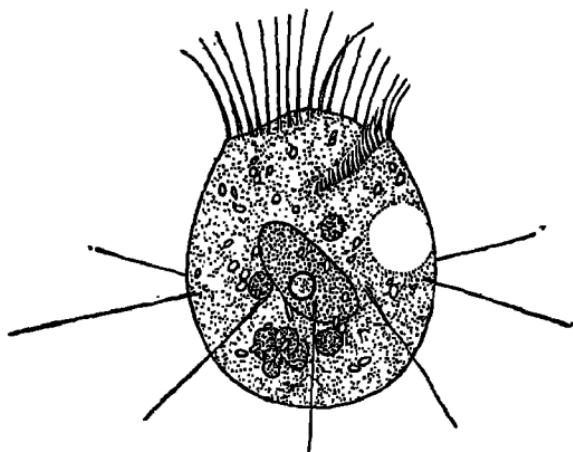


Fig. 146.—*Halteria grandinella*, a representative of the order HETEROTRICHA, suborder OLIGOTRICHA.

Note the almost complete absence of body-cilia. The cilia of the peristome, the macronucleus and micronucleus and the contractile vacuole are clearly shown. $\times 1000$. (Drawn by Dr. W. A. Kepner.)

usually supplied with cirri—groups of cilia fused together—which are used as “legs” for creeping over the substratum.

Examples of this group are *Uroleptus*, *Stylonychia*, *Oxytricha* and *Euplates* (Fig. 147). *Kerona pediculus* is an ectozoic species living on fresh water hydra.

4. PERITRICHA

With the exception of a few species, this order comprises forms highly specialized for a sedentary habit. Except in rare cases, the body-cilia are completely absent but when

present are localized in a definite ring. The feeding apparatus shows the highest specialization of any of the orders of the CILIATA. The adoral spiral zone is wound around the

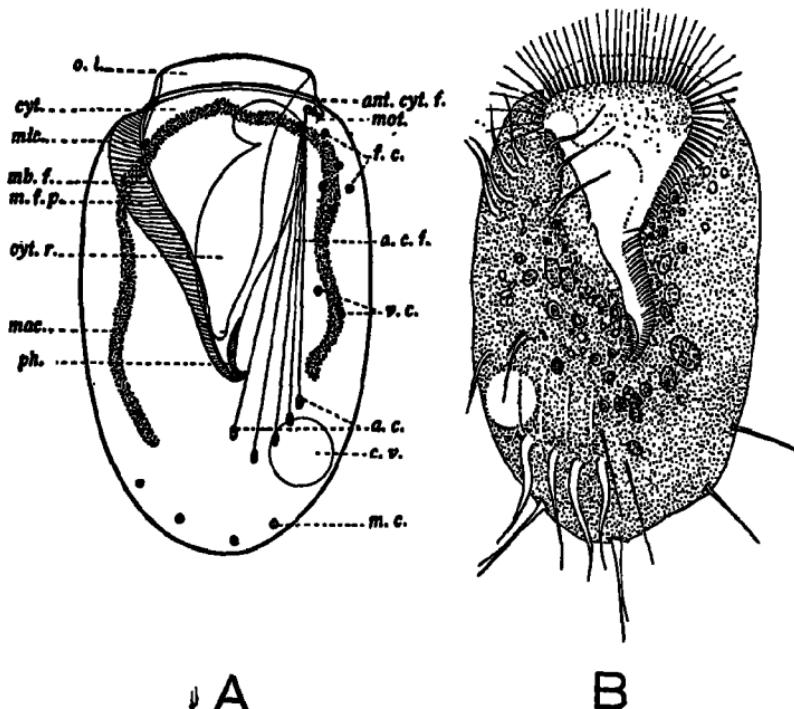


Fig. 147.—*Euploites patella*, a representative of the HYPOTRICHA.

A, Semi-diagrammatic representation of organism from the dorsal side showing the various organelles. B, Living specimen as seen from the ventral side. *ac.*, anal cirrus; *a.c.f.*, anal cirri fiber; *ant. cyt. f.* and *mb. f.*, membranelle fibers; *c.v.*, contractile vacuole; *f.c.*, frontal cirri; *m.c.*, marginal cirri; *mac.*, macronucleus; *m.f.p.*, membranelle fiber plate; *mic.*, micronucleus; *mot.*, motorium; *o.l.*, oral lip; *ph.*, pharynx. A $\times 560$; B $\times 350$. (A after Taylor, slightly modified. B drawn by Dr. W. A. Kepner.)

so-called peristomial disc (see Fig. 148) which can be completely contracted over the mouth by means of myonemes on the margin of the disc. These myonemes are strands of cytoplasm specialized as contractile organelles. The adoral

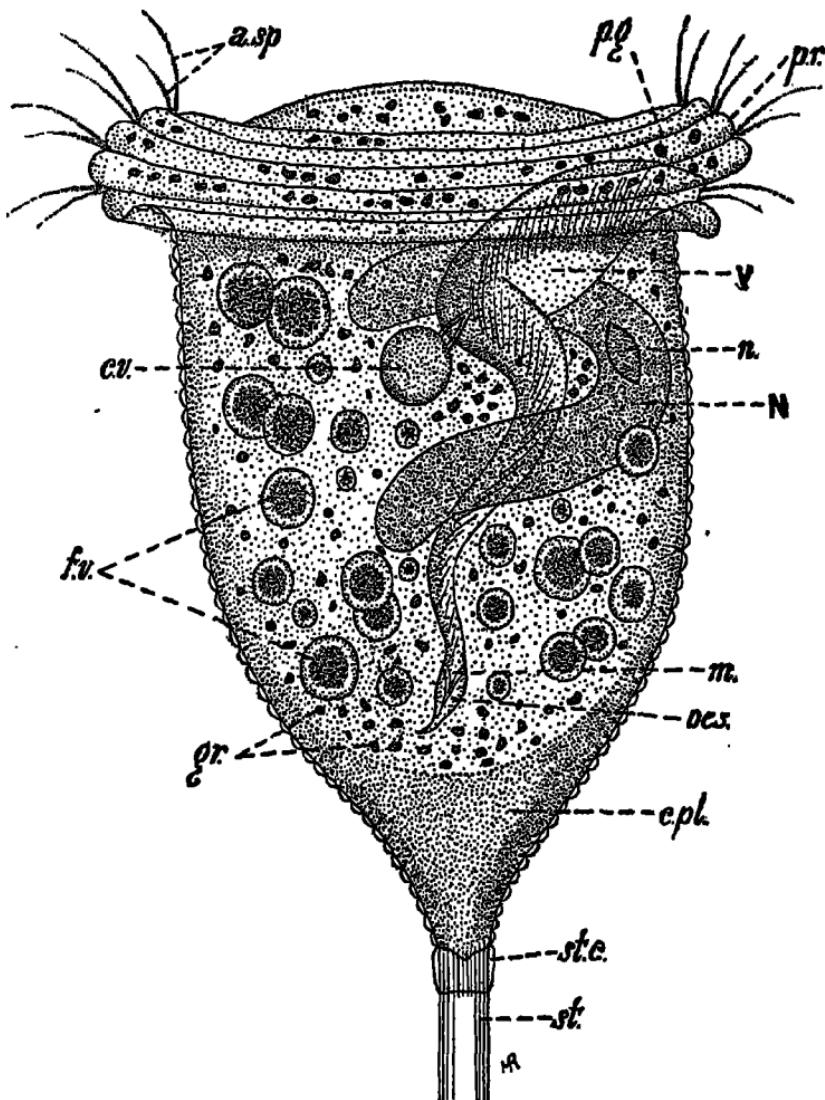


Fig. 148.—*Campanella umbellaria*, a representative of the PERITRICHA.
 a.sp., two undulating membranes of the peristome; c.pl., cortical ectoplasm; c.v., contractile vacuole, which empties by two canals into the vestibule; f.v., food vacuoles; gr., endoplasmic granules; m. cytostome or mouth; N, macronucleus; n, micronucleus; oes., cytopharynx; pg., peristomial groove, which makes $4\frac{1}{2}$ turns; pr., peristomial ridge, which separates the peristomial grooves; st., stalk; st.c., collar of stalk; V, vestibule. $\times 750$. (From Minchin, after Schröder.)

cilia are generally modified to form two undulating membranes which run parallel to each other. Instead of the adoral zone leading directly to the mouth, as in the HETEROTRICHA and HYPOTRICHA, it leads into a vestibule which is supplied with an undulating membrane and into which the anal pore and contractile vacuole as well as the mouth open. (See Fig. 148.) The PERITRICHA may be attached to some object either permanently or temporarily. In *Trichodina*, an ectozoan living on fresh-water *Hydra* and tadpoles, attachment is secured by a sucker-like adhesive organ which is surrounded by a ring of cilia. Similar organs of attachment occur in other ectozoic genera, such as *Lionophrora* and *Cyclochæta*. Forms such as *Campanella* are attached by a long stalk (Fig. 148). In *Carchesium* and *Vorticella*, these stalks are contractile due to the presence of myonemes.

5. ACINETARIA

The ACINETARIA or SUCTORIA are typically sedentary forms which (1) are devoid of any ciliary mechanism in the "adult" stage, (2) have no mouth but capture and ingest food by means of characteristic tentacles, and (3) possess a very simplified structure of the general protoplasm as compared to the CILIATA. They reveal their relationship to the CILIATA, however, (1) by the production of free-swimming ciliated larvae, and (2) by the possession of a typical infusorian nuclear apparatus consisting of separate macro- and micronuclei. The ectoplasm is very feebly developed and the general cytoplasm lacks the structural differentiation found in the CILIATA. All species possess capitate or suctorial tentacles (Fig. 149, B) each of which, as a rule, ends in a sucker-like knob. When a small animal, upon which the suctorian feeds, strikes the end of the tentacle, it is held fast by this sucker-like organ, and the general body-substance of the prey is sucked down the tentacle into the body of

the suctorian. Some species, in addition to suctorial or capitate tentacles, have styliform tentacles. These are used to hold the prey but cannot be used to suck out their contents. They are often spoken of as piercing tentacles, although as a matter of fact they never impale the prey but rather bend around and hold it by virtue of the adhesiveness of the spiral bands of viscous protoplasm with which they are generally supplied (Fig. 149, A). The nuclear apparatus of the ACINETARIA is in general like that of other INFUSORIA.

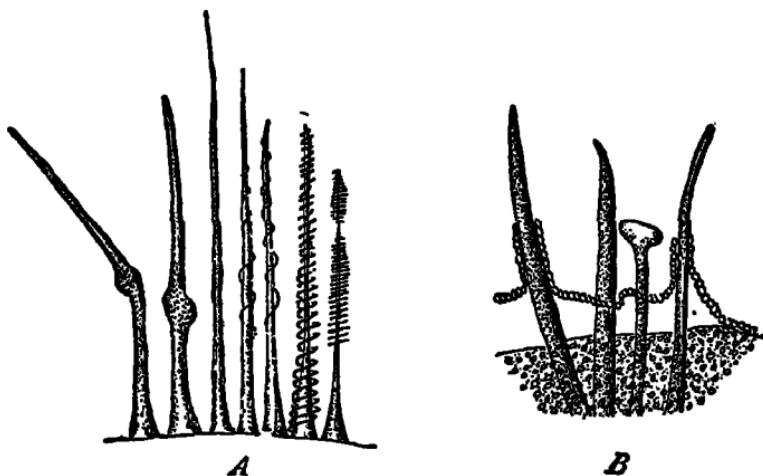


Fig. 149.—Tentacles of ACINETARIA.

A, Different types of styliform tentacles. B, one capitate and three styliform tentacles. (From Calkins, after R. Hertwig.)

Reproduction takes place typically by the formation of ciliated embryos which are formed from buds or processes constricted off from the parent suctorian. These buds may be formed in several ways. (1) In external budding the body of the suctorian divides into two or more parts. One of these parts remains attached to the original stalk while the others develop into ciliated embryos. (2) In internal budding the surface of the suctorian is invaginated to form a cavity, or so-called brood-pouch. One or more buds

develop on the wall of this cavity; are eventually constricted off and develop into ciliated embryos, which swim about in the brood-pouch for a while and eventually escape through a pore. (3) Various methods of budding intermediate between true internal and true external budding occur. In some forms, for example, an invagination of the surface takes place, as in internal budding, but the cavity thus formed remains widely open to the exterior. Its inner surface becomes ciliated and is then evaginated. The evaginated portion is finally constricted off as an embryo. No matter how the embryos are formed, they receive nuclear material from the parent suctorian. After swimming around for a while they settle down, become attached, and are transformed into typical suctarians.

An example of the ACINETARIA is the American species *Podophrya collini*, the morphology and life-history of which was first described by Root (1914). The general shape of the body, which measures about $50\ \mu$ in length and $40\ \mu$ in breadth, is shown in figure 150. It contains from 30 to 60 capitate tentacles, a large macronucleus and 3 to 12 small spherical micronuclei. Under adverse conditions, such as a shortage of food, this species often encysts *in situ*, the gelatinous sheath of the body becoming much thicker and the tentacles degenerating to form peculiar knob-like structures. The formation of embryos takes place by a budding which is of an intermediate type, a description of which can be found in Root (1914). Under certain conditions, such as the entanglement of the tentacles by trichocysts from prey such as *Paramecium*, an adult form will transform itself into a single embryo, which, after swimming about actively for a while, will cease swimming, settle down and become transformed into an adult *Podophrya*.

Conjugation in the ACINETARIA is essentially similar, in its cytological details, to that found in the CILIATA. It may

occur either between two sedentary individuals which happen to be close together or it may occur between a sedentary form and a ciliated form.

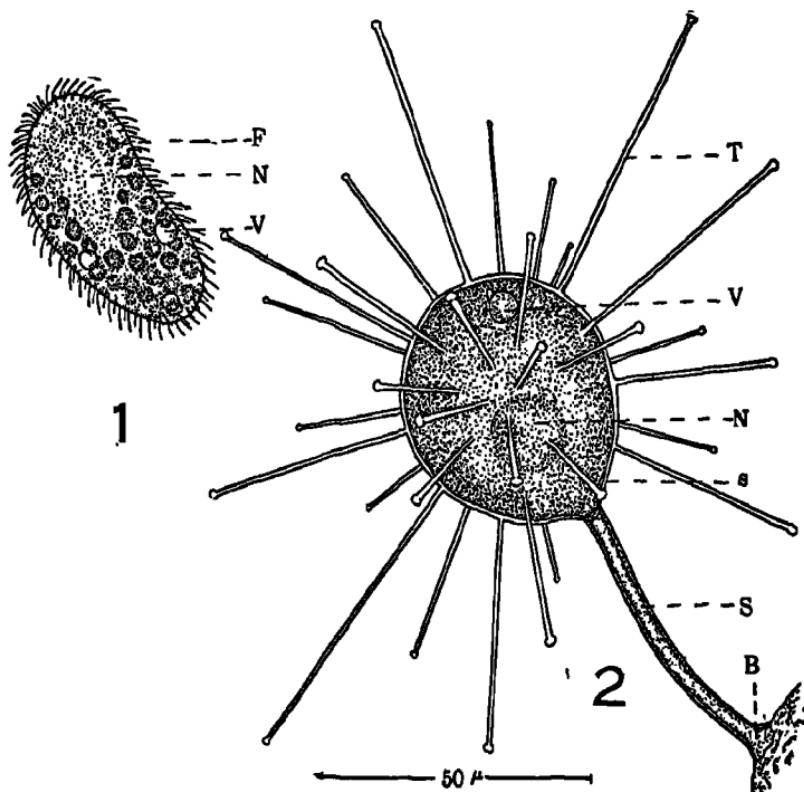


Fig. 150.—*Podophrya collini*, a representative of the ACINETARIA.
1, free-swimming embryo. 2, sedentary adult. *B*, basal disc of stalk; *F*, food vacuoles of embryo; *N*, macronucleus of adult and embryo; *S*, stalk of adult; *s*, gelatinous sheath of adult; *T*, tentacle; *V*, contractile vacuoles of adult and embryo. (After Root.)

The larvae of some ACINETARIA are parasitic within the body of ciliates. At times they even increase by binary fission during their parasitic existence. Sooner or later, however, they escape and eventually become transformed into

the sedentary adult form. Some of the adult forms are ectoparasitic on certain animals. *Ophryodendron*, for example, attaches itself to colonial hydroids, and preys upon the individual zooids.

C. General Physiology

I. NUTRITION AND ASSIMILATION

With the exception of a few parasitic forms, such as *Opalina*, which absorb their nutriment in a saprophytic manner, the members of the class INFUSORIA are holozoic in their nutrition, and are provided with well-developed mouths and cytopharynges. In addition, the orders which fall in the section SPIRIGERA possess a specialized series of adoral cilia which serve to direct the food into the mouth. Most of the HYMENOSTOMATA and the more highly developed orders live on bacteria and other small food particles which are drawn toward the mouth by the cilia of the peristomial groove or the adoral cilia or membranellæ (Fig. 151), and are then forced down the cytopharynx which ends blindly in the endoplasm. As they collect with a certain amount of water at the end of the cytopharynx, they form a vacuole in the endoplasm (Fig. 151) which gradually grows in size and is finally pinched off into the endoplasm (Fig. 151). According to Nirenstein (1905) the food vacuoles which are thus formed in *Paramecium* are at first spindle-shaped and later become spherical. In the endoplasm, they are continuously carried around by cyclosis, and ferments which digest the food particles are secreted into the vacuoles. The soluble products of digestion are then absorbed into and assimilated by the surrounding protoplasm and eventually the indigestible residue is eliminated from the body through a definite pore (see Fig. 143, A, of *Chilodon*) which is termed the anal pore. The behavior of the food vacuoles within the body of the ciliates has been studied by Green-

wood (1894), Nirenstein (1905 and 1909) and Metalnikov (1916 and 1916 a). According to the last author the time

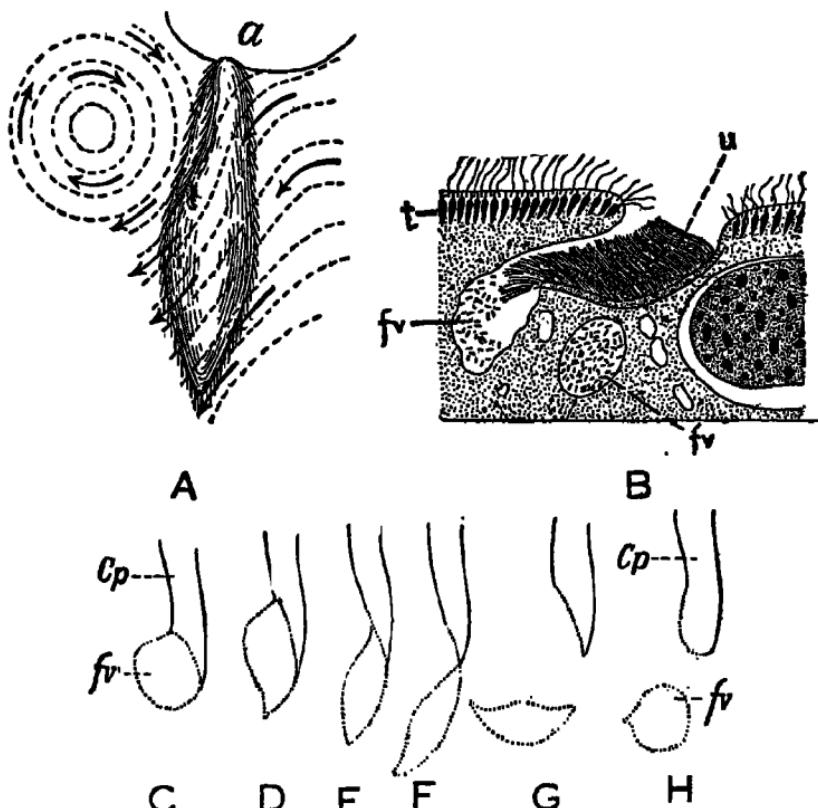


Fig. 151.—The formation of food vacuoles in *Paramecium caudatum*.

A, a specimen at rest against a mass of bacteria (*a*), showing currents produced by the cilia. Note the currents produced by the peristomial cilia. B, section through the cytopharynx, showing the undulating membrane and the manner in which bacteria are swept into the forming food vacuole. C-H, successive steps in the formation of a food vacuole and its liberation into the cytoplasm. *a*, mass of bacteria; *cp.*, cytopharynx; *f.v.*, food vacuole; *t.*, trichocysts; *u*, undulating membrane of cytopharynx. (A, after Jennings; B, from Minchin, after Maier; C-H, after Nirenstein.)

which a given vacuole will circulate in the body of *Paramecium* varies considerably and depends (1) on the char-

acter of the food substance, (2) the character of the external medium, and (3) the internal or physiological state of the organism.

In many of the **GYMNOSTOMATA**, where there is no efficient ciliary mechanism to draw bacteria or other small particles toward the mouth, the organisms swallow very large prey, often other protozoa. The act of swallowing is apparently aided by the body protoplasm at the base of the mouth which produces a kind of sucking action and draws the prey into the body. In *Didinium nasutum* the cytopharynx is supplied with a seizing organ. According to Mast (1909), as a *Didinium* swims around, this seizing organ, when extended, strikes various organisms at random. Those which adhere to it are captured and swallowed. The remarkable process of swallowing is shown in figure 152. As a rule, *Didinium* feeds on *Podophrya*, *Paramecium*, *Colpidium*, *Colpoda*, etc. The predaceous habit, however, is not restricted to the **GYMNOSTOMATA**. Goldsmith (1922) reports that the holotrich, *Frontonia*, feeds chiefly on desmids, diatoms, *Euglena*, *Oscillatoria* and other microscopic plants.

It has already been noted that the **ACINETARIA** capture their prey by means of tentacles. This is well illustrated in the case of *Podophrya collini* which feeds largely on *Paramecium*. Its food reactions have been carefully described by Root (1914) from whose report the following description is taken. A *Paramecium* in swimming about comes in contact with the tips of the tentacles of *Podophrya*, where it always sticks fast. Attempts on the part of the *Paramecium* to free itself only serve to bring it in contact with more of the tentacles, which bend over and attach themselves to the prey. After the prey is quiet, the attached tentacles contract and draw it closer. Then the feeding process begins during which the protoplasm of the *Para-*

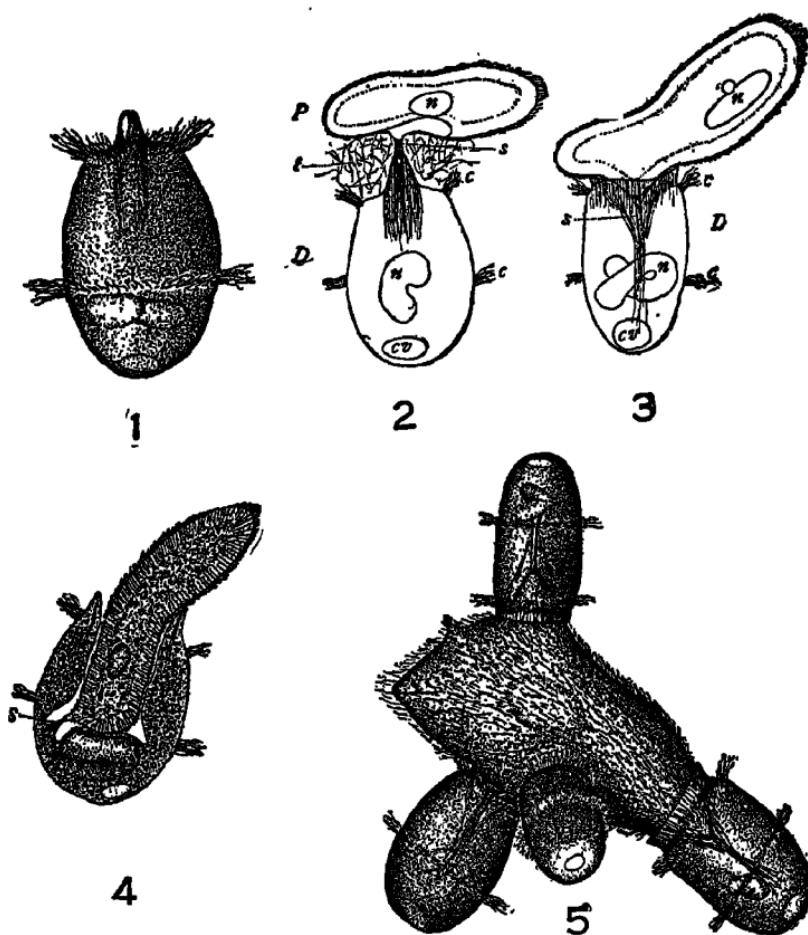


Fig. 152.—The ingestion of food by *Didinium nasutum*.

1, a large specimen of *D. nasutum*. 2, a large *Didinium* immediately after having seized a small *Paramecium*. 3, a specimen showing the seizing organ withdrawn and the beginning of the process of swallowing. 4, a specimen in which the seizing organ, *S*, can be clearly seen attached to the prey after it had been nearly half swallowed. 5, a large *Paramecium* attacked by four small *Didinium*. Under such conditions the *Paramecium* is usually torn in pieces and each *Didinium* gets a portion. Sometimes, however, one *Didinium* gets the entire *Paramecium*, forcing the others off during the process of swallowing. *D*, *Didinium*; *P*, *Paramecium*; *n*, macronucleus; *c*, bands of cilia; *c.v.*, contractile vacuole; *s*, seizing organ; *t*, discharged trichocysts. $\times 240$. (After Mast.)

mecium can be seen flowing down each tentacle into the suctorian where it collects in large food vacuoles.

Various investigators who have studied the food reactions of the INFUSORIA have found that a given species apparently exhibits quite a definite selection of food. Space will not allow an extended account of the findings of these investigators. A few specific instances will suffice. Mast (1909) maintains that in *Didinium nasutum* the selection of food is largely a question of what organisms stick to the seizing organ. It is very much easier, however, to conceive of food selection in forms like *Didinium* than in those ciliates which are provided with a peristome and apparently sweep every particle within a given radius into the cytopharynx. Selection does, however, occur in these forms. Schaeffer (1910), for example, has carried out an exceedingly painstaking investigation of food selection in the heterotrich, *Stentor caeruleus*, and has demonstrated that this species can distinguish between organisms, such as *Phacus* and *Euglena*, and indigestible particles, such as carmine, glass, sulphur, etc., with a very high degree of accuracy, and also, to a certain extent, can distinguish between different species of organisms. It does not, however, seem to be able to discriminate between living organisms and those killed with heat or various solutions. The selection or rejection of materials is effected by changes in the effective beat of the cilia on the pouch and funnel. Metalnikov's (1907, 1912) work indicates that a similar power of discrimination occurs in *Paramecium*. Many of his results have been confirmed by Lund (1914) who worked with *Bursaria truncatella*.

2. SECRETION, EXCRETION AND RESPIRATION

The general discussion of these subjects, which has been given under the SARCODINA, applies almost equally to the INFUSORIA with the exception of the method of eliminating

masses of indigestible wastes from the organism. In the INFUSORIA, with the development of a definite form and so many body differentiations, an anal pore has been developed which functions as a true anus.

3. MOVEMENT

In the motile forms or motile stages of the INFUSORIA, locomotion is effected by means of cilia. Fundamentally, cilia are not only built on the same pattern as flagella, with an inner elastic supporting structure and an outer more fluid sheath, which is probably contractile, but they also probably move in the same manner. As a rule, cilia are situated in rows, and their movement is concerted. Each



Fig. 153.—Schematic representation of the beating of a row of cilia.
(After Verworn.)

cilium contracts a short interval after the one in front of it and before the one behind it. Thus, viewed from the side, a moving row of cilia looks as if successive waves were passing over it (Fig. 153). Furthermore, adjacent cilia of different rows beat in unison. When seen from above a ciliated surface has the appearance of a wheat field in the wind, i. e., successive waves follow one another across the ciliated surface.

The characteristic path of swimming forms is a spiral course, which allows asymmetrical organisms to swim in straight lines, a fact already noted in the *Mastigophora*. Jennings (1906) has carefully analyzed this path (Fig. 154) in *Paramecium* and attributes it to three factors: (1) the effective beat of the body-cilia is chiefly backward so that the organism is driven forward, (2) the effective beat of the body-cilia is also somewhat oblique so that the animal

rotates on its longitudinal axis, and (3) the peristomial cilia beat more effectively than those on the general body surface so that the organism constantly swerves away from the oral side. It can easily be seen that, if the body-cilia did not beat obliquely and hence rotate the body, that the stronger beat of the peristomial cilia would cause the animal to swim in circles. The path as given in figure 154 can be modified under certain conditions but this need not be considered here.

The HYPOTRICHA are organisms that are capable of swimming, but spend most of their time creeping around on the substratum by means of cirri on the ventral surface which represent bunches of fused cilia and act practically as legs. Taylor (1920) notes that, in *Euplotes*, the cirri are aided in creeping by the peristomial membranelles.

4 REACTIONS TO STIMULI

The reflex type of reaction, which we noted in the MASTIGOPHORA, is developed to a much higher state in the CILIATA. The reactions of *Paramecium* to various stimuli have been carefully worked out by Jennings and others, and are summarized in Jen-

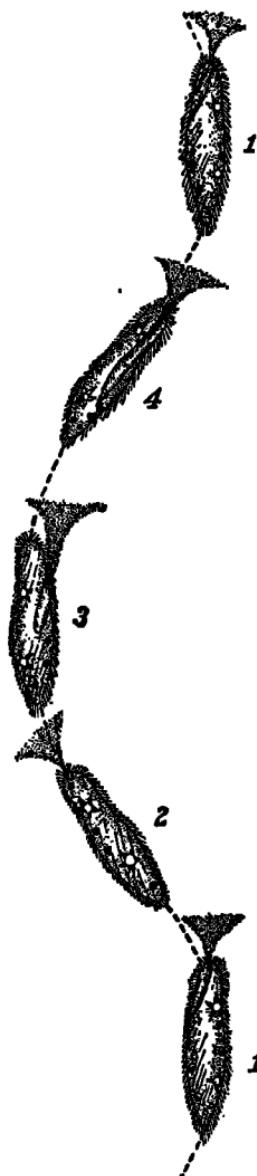


Fig. 154.—Spiral path of *Paramecium*. 1, 2, 3, 4, successive positions occupied. The dotted areas with small arrows show the currents of water drawn from in front. (After Jennings.)

nings (1906). If a specimen is swimming through the water in the typical spiral path and comes in contact with a solid, certain chemical solutions, a hot area, or in fact, any stimulating factor to which it is negative, it gives a very definite motor response. In the words of Jennings (1906), "*Paramecium* has a simple reaction method for meeting all such conditions. It first swims backward, at the same time necessarily reversing the ciliary current. It thus gets rid of the

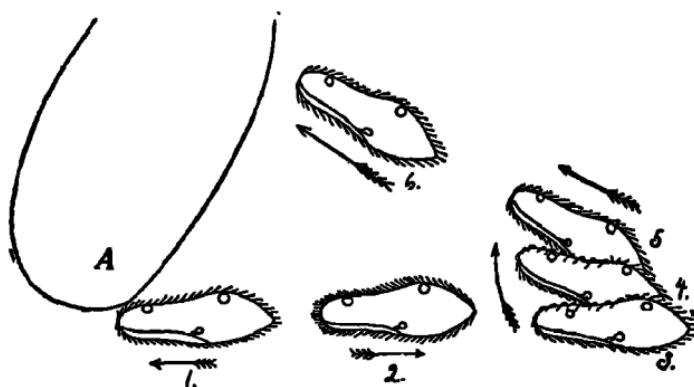


Fig. 155.—Schematic representation of the avoiding reaction in *Paramecium*.

A, solid object or other source of stimulation. 1-6, successive positions occupied by the animal. (The rotation on the long axis is not shown.) (After Jennings.)

stimulating agent,—itself backing out of the region where this agent is found, while it drives away the stimulus in its reversed ciliary current. It then turns to one side and swims forward in a new direction. The reaction is illustrated in Fig. 36 [our figure 155]. The animal may thus avoid the stimulating agent. If, however, the new path leads again toward the region from which the stimulus comes, the animal reacts in the same way as at first, till it finally becomes directed elsewhere. We may for convenience call this reaction, by which the animal avoids all sorts of agents, the 'avoiding reaction.' The different phases

of this avoiding reaction are evidently due to modifications of the three factors in the spiral course. The swimming backward is due, of course, to a reversal of the forward stroke of the cilia. The turning toward aboral side is an accentuation of the swerving that takes place always; it is due to the fact that the cilia at the left side of the body strike during the reaction toward the oral groove instead of away from it. Thus the cilia of both right and left sides now tend to turn the animal toward the aboral side. Finally, the decrease or cessation in the revolution on the long axis is due to the same factor as the increase in swerving toward the aboral side. During the reaction the cilia of the left side oppose the usual revolution on the long axis to the left through the same change which causes them to assist in turning the body toward the aboral side." Jennings finds that the intensity of the avoiding reaction is often modified according to the nature of the stimulus. In all cases, however, its characteristic features are maintained.

It is a simple matter to see how all of the so-called negative reactions of *Paramecium* can be explained on the basis of this one motor response. Strange to say, however, many of the so-called positive reactions are also accounted for by the same reaction. If, for example, a drop of 1/50 per cent acetic acid is introduced on a slide which contains water through which *Paramecia* are uniformly distributed, in time the organisms will collect in the drop of acid (Fig. 156, A). The mechanism of this collection is due to the fact that each *Paramecium*, as it swims around "at random," sooner or later swims into the drop of acid. The animal gives no reaction as it swims into the acid but every time it attempts to swim out of the drop it gives an avoiding reaction. This, of course, keeps the organism within the drop, as is shown in figure 156, B. We thus see that this one definite response enables *Paramecium* to react negatively to adverse stimulation and at the same time to collect in

regions of optimum conditions. There are other definite reactions of *Paramecium*, such as its reactions to gravity and the discharge of its trichocysts, but these play a comparatively minor rôle in its general behavior compared with the avoiding reaction.

The avoiding reaction is probably of a reflex nature. It is the result of a reversal in the effective beat of certain definite cilia, and is very stereotyped and constant. In the reflex the cilia represent the effectors and, although the

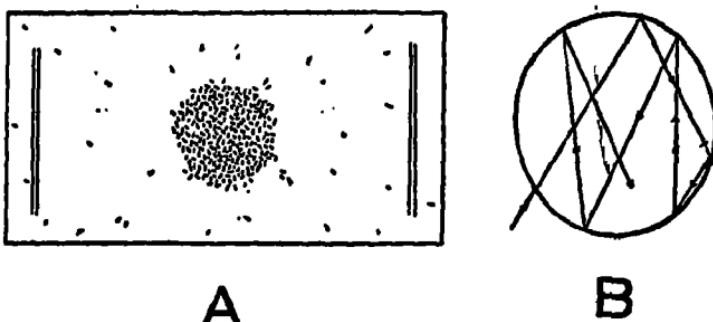


Fig. 156.—The positive reaction of *Paramecium* to acids.

A, collection of *Paramecia* in a drop of 1/50 per cent acetic acid. B, path followed by a single *Paramecium* in a drop of acid showing mechanism of the collection shown in A. Each time the organism comes to the boundary of the acid it gives an avoiding reaction. (After Jennings.)

observations reported above do not prove it, other regions of the body probably receive the stimulation (see especially the reaction to contact stimuli). If this be true, it necessitates, as in the reactions of *Euglena* to light, the assumption of definite conduction paths. Such a receptor-conductor-effector type of reaction is very plainly indicated in the reactions of *Stentor cæruleus* to light. The following account of these reactions is taken from Mast (1911).

When *Stentor* is swimming, it pursues a spiral course with the aboral zone directed toward the outside of the

spiral. It orients negatively to light as can be seen by the examination of figure 157. If, as the animal is swimming away from the light *m*, the light *m* is turned off and light *n* turned on, there is usually no response until the oral side is directed toward the light as is the case at *c*. When this occurs, it may do one of two things: (1) it may stop, back, and orient directly by turning toward the aboral side, as is indicated¹ by the dotted lines, or (2) it may give a much less intense reaction, by simply swerving toward the aboral side without stopping, as is indicated by *c*, *d*, and *e*. In the latter case the oral side is again exposed to the light at *e* and the reaction is repeated. All of the evidence points to the conclusion that there is a highly sensitive region in the anterior portion of the body near the oral side and that this is the only region which is sensitive to light. Even in the exceptional case in which Mast, using high intensities of light, obtained reactions in the position *b*, it is probable that the oral region was stimulated. In *Stentor*, therefore, there is fairly clear evidence that one portion of the body receives the stimulus, that this is transmitted to definite cilia, which in turn produce a definite motor response.

A number of investigators have endeavored to discover a definite fibrillar conduction-system in ciliates, and also, to a certain extent, in flagellates. The most promising attempts to find such an anatomical basis for conduction in ciliates have come from the work of Professor Kofoid's students at the University of California. Sharp (1914) described a so-called neuromotor apparatus in *Diplodinium ecaudatum* which he believed, on purely morphological grounds, to be a conduction-system (see page 440). This was followed by the description of a similar system in *Euplotes patella* by Yocom (1918) and Taylor (1920) and in *Paramecium caudatum* by Rees (1922). The neuromotor apparatus described by these investigators would have remained an interesting morphological detail except for the fact that

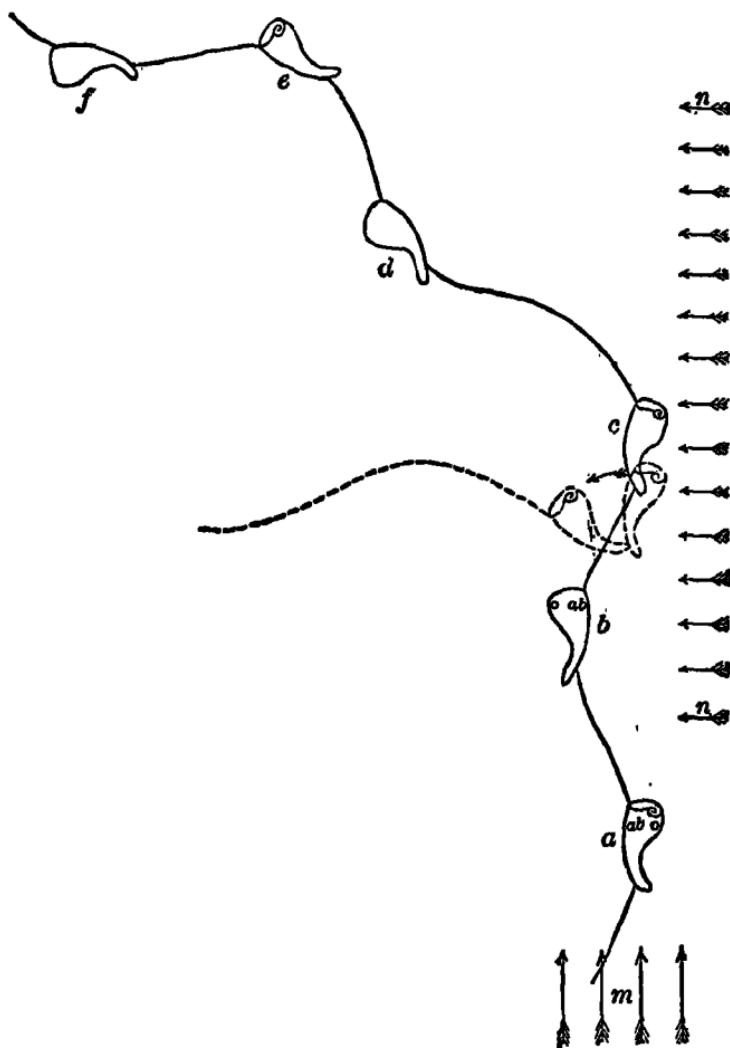


Fig. 157.—Diagrammatic representation of the process of orientation to a horizontal beam of light in *Stentor caruleus*.

The curved line represents the spiral course; the directions of light from two sources; *a-f*, different positions of *Stentor* on its course; *o*, the oral surface; *ab*, the aboral surface. At *a* the *Stentor* is oriented in light from *m*, *n* being shaded. If *n* is exposed and *m* shaded simultaneously when the *Stentor* is in position *b*, there is usually no reaction, if the intensity has not been changed, until it reaches *c* and the oral side faces the light; then the

Taylor (1920) by means of a micro-dissection apparatus obtained considerable direct evidence that the organelles in question did actually function in the coordination of movement of the various ciliary mechanisms of *Euplates*. The following description and account of experiments is taken from his work. The general arrangement of the membranelleæ and various cirri on the ventral surface may be seen in the diagram in figure 147, A. The neuromotor apparatus is a fibrillar system which connects these various mechanisms. It consists essentially (see Fig. 147, A) of a membranelle fiber, *mb.f.*, which connects the row of membranelle fiber plates, a series of five anal cirri fibers, *a.c.f.*, each of which connects with an anal fiber plate, *a.c.*, and finally a central mass or motorium which is connected on the one hand with the membranelle fiber and on the other with the five anal cirri fibers. An incision which severed the membranelle fiber resulted in marked differences in the behavior of the membranelles on the two sides of the incision, whereas other incisions which did not sever this fiber did not result in these differences. Severing the anal cirri fibers resulted in marked disturbances in the normal locomotion whereas normal locomotion was not impaired by other incisions provided they did not sever either the anal cirri fibers or the

organism may respond by suddenly stopping, backing and turning sharply toward the aboral side, as indicated by the dotted outline, and become oriented at once; or it may merely swerve more or less toward the aboral side without stopping. At *e* the oral side is again exposed and the organism is again stimulated and it again swerves from the source of light. This process is continued until the oral side is approximately equally exposed to the light in all positions on the spiral course. If the *Stentor* is at *c* when *n* is exposed it responds at once and orients as described above. If the light from *n* is more intense than that from *m*, or if the organism is very sensitive when *n* is exposed and *m* shaded, it responds at once no matter in which position it is. If it is at *b* it turns toward the source of light, but now repeats the reaction, successively turning in various directions until it becomes oriented. (Figure and legend after Mast.)

membranelle fiber. Finally, cutting the fibers which connected with the motorium resulted in a loss of coordination between the movements of the membranelles and of the anal cirri. These results indicate, although they do not absolutely demonstrate, that the ciliates, with their complex motor responses, are provided with a conduction system which effects a coordination between the different motor organs, and again bring into prominence the very complex organization of some of the protozoa.

CHAPTER XIII¹

ECTOZOIC AND ENTOZOIC INFUSORIA

A. Introduction

Every order of the class INFUSORIA contains ectozoic and entozoic as well as free-living species. The principal human parasite in this class is *Balantidium coli*. Several other species, however, have been reported from man, and many of those that are parasitic in lower animals are of considerable interest since the nature of their environment and the necessity for transmission from one host to another has resulted in structural and physiological modifications and in the evolution of complex life-cycles. Some of the better known species are described below, the emphasis being placed on those that have been reported from man.

The first order, HOLOTRICHA, contains a number of parasites without peristome, cytostome or cytopharynx, such as *Opalina* in the rectum of the frog. These forms are often placed together in a group called the ASTOMATA. The sub-order GYMNOSTOMATA numbers among its parasitic members *Ichthyophthirius* from the skin of fishes, several inhabitants of the stomach of cattle, such as *Bütschlia parva*, as well as several species that have been reported from man. The suborder HYMENOSTOMATA likewise contains parasites that live in the stomach of cattle (ISOTRICHIDÆ). Both sub-orders of the HETEROTRICHA include parasitic genera among which may be mentioned *Balantidium* and *Nyctotherus*.

¹By R. W. Hegner.

that have been reported from man, and *Ophryoscolex* and *Diplodinium* that live in the stomach of cattle. The order HYPOTRICHIA contains an ectozoic form (*Kerona*) that lives on the fresh-water polyp, *Hydra*. In the order PERITRICHIA are several interesting ectozoic genera—*Trichodina* and *Cyclochæta*.

B. *Opalina*, an Astatomatous Holotrich

Species of the genus *Opalina* are common inhabitants of the rectum of frogs and toads. More is known about the life-history of these species than of most other parasitic ciliates due to the researches of Metcalf 1909-1923, Nere-sheimer, 1907, Konsuloff, 1922, and others. One of the commonest species is *Opalina ranarum* which occurs in the large intestine or rectum of the frog. This is a true parasite and exhibits many modifications correlated with its parasitic habits. As shown in figure 158 it is very much flattened, narrow in front and rounded posteriorly. During the summer the vegetative forms measure from 500 μ to 800 μ in diameter. *Opalina* is free-swimming throughout most of its life-cycle. Cilia are present all over the body, being arranged in long spiral rows. Only one type of nucleus is present; this type probably contains both the vegetative and reproductive chromatin that are separated into macronuclei and micronuclei respectively in most other ciliates.¹ Food is absorbed through the surface of the body, no mouth nor anal pore are present, and contractile vacuoles are very primitive in nature.

The life-cycle of *Opalina ranarum* is illustrated in outline in figure 158. During the summer and autumn vegetative reproduction occurs by longitudinal or transverse fission (*a*, *b*, *c*, *d*). Many nuclei are present (*a*) and the number of these is probably closely correlated with the size of the specimen, that is, the larger the animal the greater the number of nuclei. In the spring another type of reproduction is

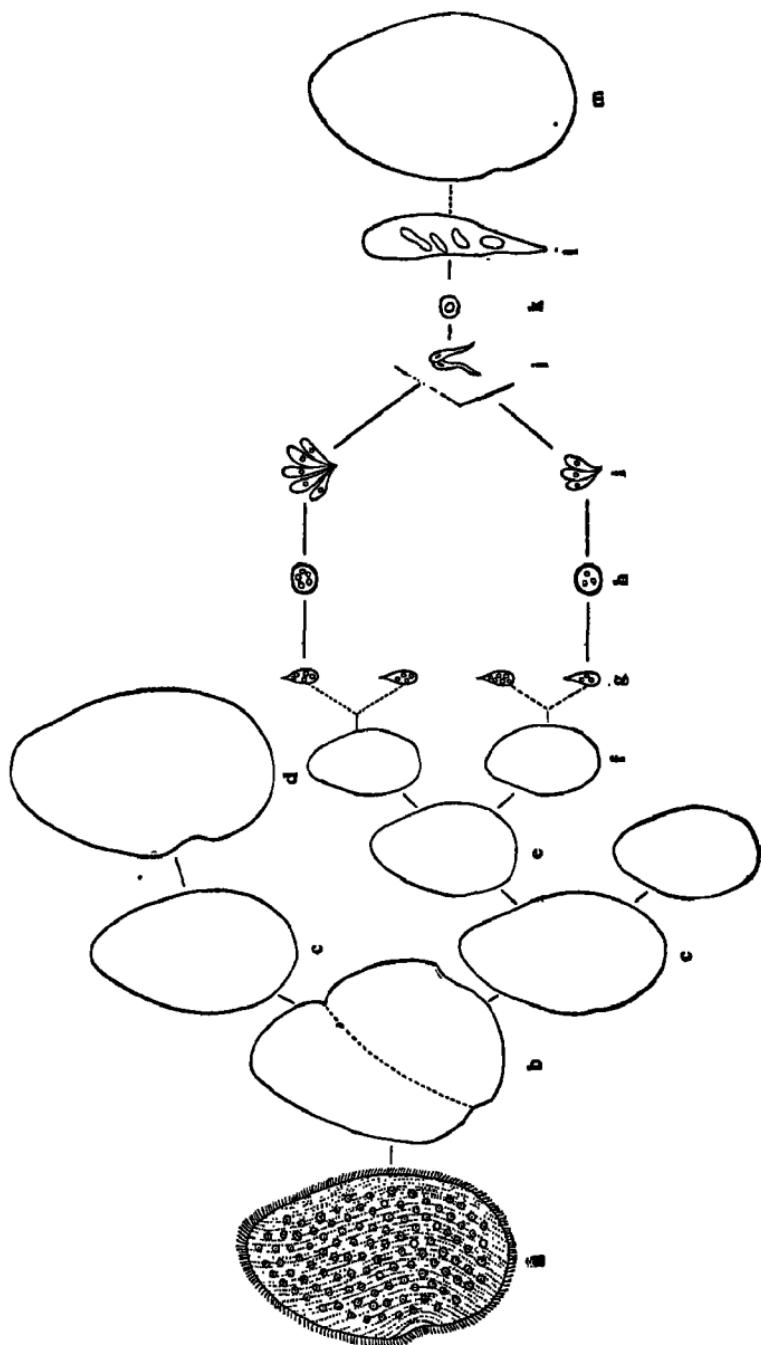


Fig. 158.—*Ophelina ranarum*. Diagram illustrating its life-cycle. For explanation see text. (Original.)

initiated by some unknown stimuli. Some of the large vegetative forms grow and divide as before (*b, c, d*), thus continuing the infection in the host, but in others division is more rapid and no appreciable growth occurs between successive divisions (*b, c, e, f, g*). The result is the production of many small specimens (*f*). Certain investigators claim that in these the nuclei discharge chromatin granules (chromidia) into the cytoplasm and then what remains of the nuclei is absorbed. These chromidia are said to form secondary nuclei which multiply by mitosis. Further cell division results in very small specimens with from three to six secondary nuclei (*g*). From these, infection cysts (*h*) are formed which pass out in the feces of the frog and may then be devoured by tadpoles. In the intestine of the latter the organisms escape from the infection cysts and divide into as many cells as there are nuclei thus forming uninucleate specimens which represent gametes (*i*). The gametes may be of two different sizes and when conjugation (*j*) occurs a small gamete fuses with one of the larger gametes to form a zygote (*k*). The zygotes then become copulation cysts. A young *Opalina* develops from each copulation cyst and as growth proceeds the nuclei increase (*l*), nuclear number and cytoplasmic mass being here very closely correlated (see Hegner and Wu, 1921), until the typical vegetative condition is attained (*m*).

The phases in this life-cycle that are of special interest are those that are obviously related to the transmission of the ciliate to new hosts. This involves rapid multiplication just before the resistant infection cysts are formed which insures a large number of cysts, and the sexual processes that occur in the new host.

The nuclear conditions in the various species of opalinias are so remarkable as to deserve special mention. Largely on the basis of nuclear characteristics Metcalf (1918, 1923) has

divided the ciliates into two divisions (1) PROTOCILIATA, including the OPALINIDÆ, and (2) EUCILIATA, including the rest of the ciliates. Four genera are recognized in the OPALINIDÆ, (1) *Protoopalina*, *Zelleriella*, *Cepedea*, and *Opalina*. The species of *Protoopalina* possess one or more often two nuclei. Each nucleus when in mitosis may be seen to contain two types of chromosomes (Fig. 159); one type lies near the surface of the spindle, is large, flat and constant in number; the other type is the same in number, central in position, small, slender, and in the form of linear granules. One-half of each chromosome of each type passes to each daughter nucleus during mitosis.

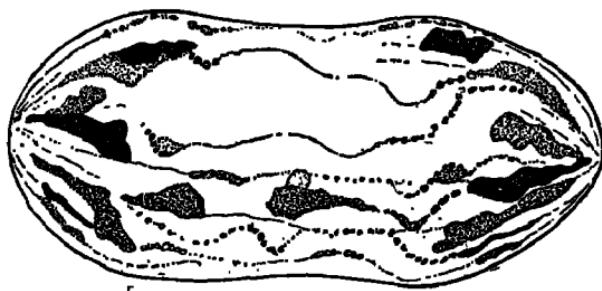


Fig. 159.—*Opalina antilliensis*. A nucleus in anaphase stage of mitosis showing large, flat, "trophic" chromosomes and small, linear, "reproductive" chromosomes. $\times 1780$. (After Metcalf.)

In *Opalina* there are from 4 to several thousand nuclei. Each nucleus contains a few large, flat chromosomes that vary in number in the nuclei of a single animal and many of the granular type that are probably constant in number. During mitosis the large, flat chromosomes divide irregularly or not at all, whereas the granular type appear to be equally divided. The massive chromosomes are presumably trophic in function and correspond to the chromatin of the macro-nucleus in other ciliates. The granular chromosomes are supposed to be reproductive in function and resemble the micronucleus of other ciliates in this respect. Mitosis in cer-

tain species of PROTOCILIATA is very slow, requiring from a few hours to several days for the process. The nuclear membrane persists during mitosis and no centrosome is visible.

Among the various nuclear conditions that occur among the OPALINIDÆ may be mentioned species with one nucleus (1) in early mitosis, (2) in anaphase and (3) in telophase, species with two nuclei (1) resting, (2) in early mitosis, (3) in metaphase, (4) in anaphase, or (5) in telophase, and species with (1) 4 resting nuclei, (2) 5 to 12 resting nuclei, or (3) 100 to several thousand resting nuclei.

C. *Ichthyophthirius*, a Gymnostomatous Holotrich

Another holotrichous ciliate whose life-cycle has been investigated rather fully is *Ichthyophthirius multifilius*. This

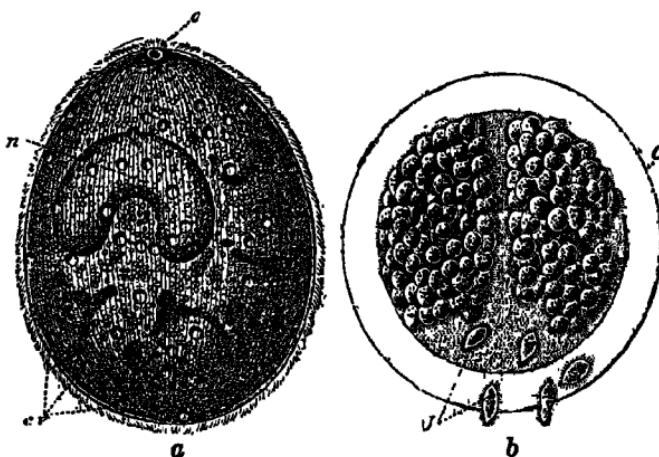


Fig. 160.—*Ichthyophthirius multifilius*. a, Vegetative stage. *a*, oral aperture; *c.v.*, contractile vacuoles; *n*, nucleus. b, Cyst (*c*) from which uninucleate spores (*j*) are escaping. $\times 75$. (After Bütschli.)

species is parasitic in the skin of fishes; often occurs in aquaria; and may be so numerous as to cause the death of its hosts. In the vegetative stage (Fig. 160, *a*) it is about $800\ \mu$ in diameter and lives in a cavity in the epidermis of the

fish. When it escapes from this cavity it sinks to the bottom where it usually becomes encysted and undergoes 8 successive divisions resulting in the production of 256 minute uninucleated ciliates (Fig. 160, b). The sexual phenomena that take place within the cyst are not accurately known, but the ciliates, when they break out, attack new fish hosts into whose skin they bore their way and develop to their full size.

In this species, as in *Opalina ranarum*, peculiar nuclear phenomena occur. During the growth of the ciliate in the epidermis of the fish the micronucleus enters the macronucleus where it appears as a nucleolus-like body. It is extruded again into the cytoplasm when division takes place within the cyst; here it multiplies by mitosis and finally each young parasite is provided with a micronucleus and a macronucleus. The details of this process are still in doubt.

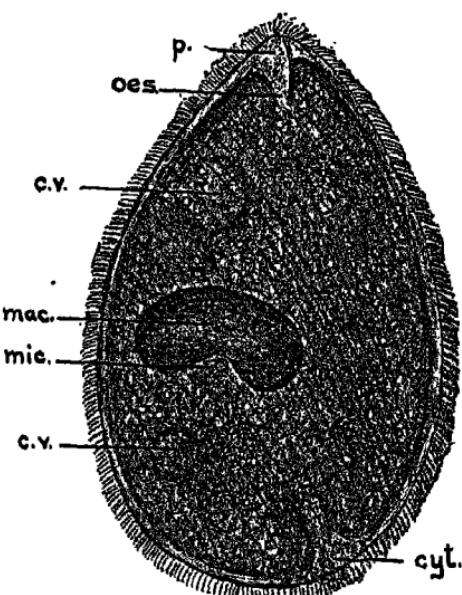


Fig. 161.—*Balantidium coli* from man.
c. v., contractile vacuole; cyt., cytophyge;
mac., macronucleus; mic., micronucleus;
oes., oesophagus; p., peristome. (Original.)

D. The Genus *Balantidium*

Balantidium coli (Fig. 161) seems to be a rather constant and non-pathogenic inhabitant of the intestine of the pig. It was discovered in this animal by Leuckart (1863) soon after it was reported by Malmsten (1857) in man, and was

considered by him as the same species. We are not positive that the species in the pig and man are the same but they have not yet been successfully separated on the basis of morphological differences.

I. *Balantidium coli* AND *B. suis*

The most recent and best morphological account of *Balantidium* in the pig is that of McDonald (1922). This inves-

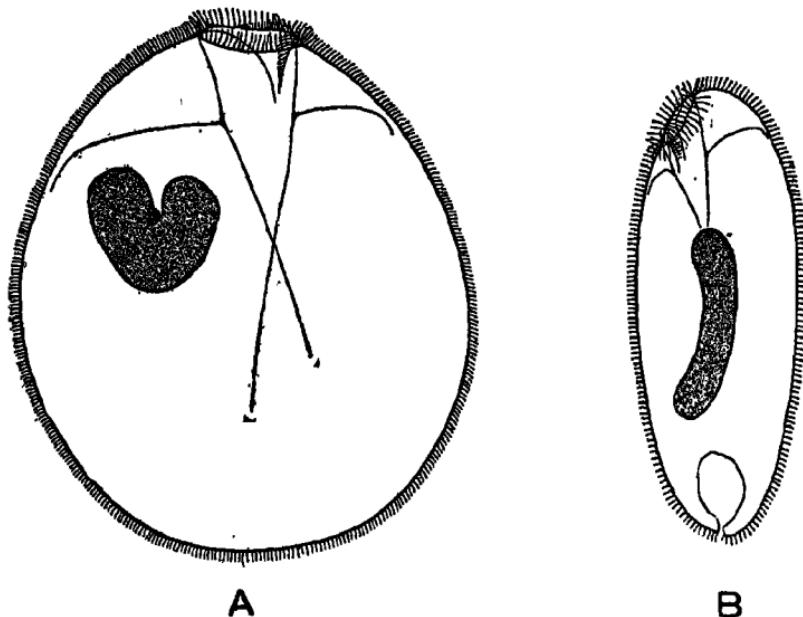


Fig. 162.—A, *Balantidium coli*, and B, *Balantidium suis* from the pig.
× 640. (From Hegner and Holmes, after McDonald.)

tigator examined material from 200 pigs, 68% of which were infected. The parasites were found to be most numerous and active in the cæcum and first three or four feet of the colon; posteriorly they were in progressive stages of encystment; most of those in the rectum were encysted; none were found more than three feet above the ileocæcal valve; and in only two or three instances were any observed in the

small intestine. The living organisms are very sensitive to light and changes in temperature. They avoid the light that is usually used for illuminating purposes. When the temperature is lowered they become less active, their movements being mostly rotary and not progressive; tend to become spherical; and at room temperature die in from six to eight hours. If collected in a vacuum bottle and kept in an incubator at 37.5° they will live for seventy-two hours.

McDonald measured 400 carefully prepared specimens and on the basis of size as well as other morphological characters decided that there are really two species of the genus *Balantidium* in the pig (Fig. 162). Measurements of 200 specimens when plotted produced a bimodal curve; one mode corresponded to animals 1.2 times as long as broad, the other to animals from 1.6 to 1.8 times as long as broad. One hundred specimens from each of two pigs that seemed to contain only one type of *Balantidium* were also measured. In one pig the specimens averaged 86 μ in length and 66 μ in breadth, being 1.3 times as long as broad; in the other the specimens averaged 86 μ in length and about 43 μ in breadth, being 1.99 times as long as broad. These measurements indicate the presence of two species. The larger species seems to correspond in size and other characteristics to the *Balan-*
tidia described by previous investigators and is considered by McDonald to be *Balantidium coli* (Fig. 162, A). The narrower form is regarded as a distinct species and is called *Balantidium suis* (Fig. 162, B). The distinguishing characteristics of the two species are shown on page 418.

An examination by McDonald of 100 specimens obtained from Dr. Walker (Walker, 1913) proved them to be of the *B. coli* type, as was true also of specimens from monkeys that had been infected by material from the pig. The conclusion is reached that *B. suis* probably does not occur in man but that no morphological differences have been shown to exist between *B. coli* from the pig and from man.

Character	<i>B. coli</i>	<i>B. suis</i>
Size:		
range.....	30 μ to 200 μ long x 20 μ to 70 μ broad	35 μ to 120 μ long x 20 μ to 60 μ broad
average.....	86 μ long x 66 μ broad	86 μ long x 43 μ broad
Shape.....	Posterior end rounded; anterior end pointed; greatest diameter posterior to center	Posterior end as pointed as anterior end; greatest diameter at or anterior to center
Cytostome....	Almost but not quite terminal	One-fifth of way posteriorly along ventral surface
Macronucleus.	Bean-shaped: about one-third length of entire animal; width about one-half of length	Rbd or sausage-shaped; at least one-half length of entire animal; width about one-fourth of length

2. MORPHOLOGY OF *Balantidium coli* FROM THE PIG

The morphological characteristics of *B. coli* of the pig are shown in figure 163. The slight eccentricity of the peristome (*per.*) makes it possible to recognize the side on which this region lies as the ventral side, and the opposite surface as the dorsal surface. A thin transparent pellicle (*pel.*) covers the entire surface and extends into the cytopharynx or oesophagus (*œs.*). It is tenacious and flexible, resisting pressure and mechanical change, and hence protective and retentive rather than supportive. The organism plasmolyses quickly in normal salt solution and stains rapidly with intra-vitam stain, which proves that the pellicle is not impervious. Just beneath the pellicle is the ectoplasm (*ect.*)—a layer about 2 μ

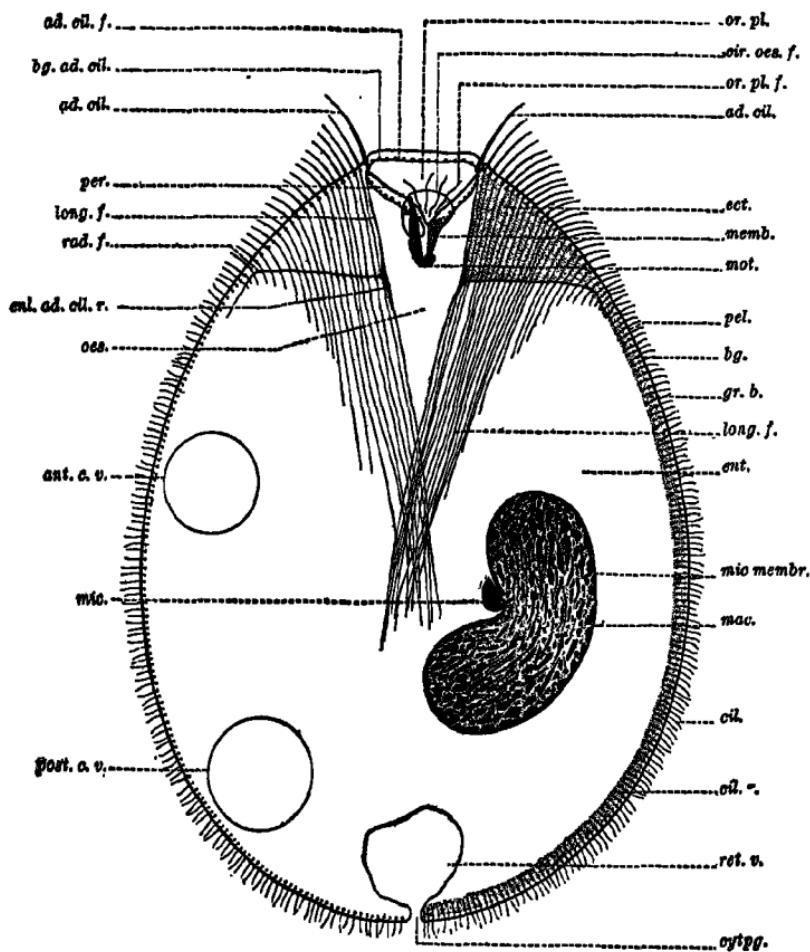


Fig. 163.—*Balantidium coli* from the pig. Diagram showing morphological details.

ad. cil., adoral cilia; *ad. cil. f.*, adoral ciliary fiber; *ad. memb.*, adoral membranelles; *ant. c. v.*, anterior contractile vacuole; *bg.*, basal granule; *bg. ad. cil.*, basal granules of adoral cilia; *cil.*, cilia; *cil. r.*, ciliary rootlet; *cir. oes. f.*, circumoesophageal fiber; *cytpg.*, cytopype; *ect.*, ectoplasm; *enl. ad. cil. r.*, enlargement of adoral ciliary rootlet; *end.*, endoplasm; *gr. b.*, granular band of ectoplasm; *long. f.*, longitudinal fibers; *mac.*, macronucleus; *mic.*, micronucleus; *mot.*, motarium; *nuc. membr.*, nuclear membrane; *oes.*, cesophagus; *or. pl.*, oral plug; *or. pl. f.*, oral plug fibers; *pel.*, pellicle; *per.*, margin of peristome; *post. c. v.*, posterior contractile vacuole; *rad. f.*, radial fiber; *ret. v.*, rectal vacuole. $\times 1250$. (After McDonald.)

thick except at the anterior end where it extends back about one-sixth the length of the animal. In this portion is situated the cytostome and a large part of the neuromotor apparatus. The ectoplasm contains longitudinal bands of differentiated protoplasm similar to those reported in *Stentor* and other ciliates. These consist of hyaline bands in which the cilia are embedded alternating with granular ridges. Cilia (*cil.*) cover the entire surface of the body except the oral plug (*or. pl.*) They are of two sizes. An adoral circlet (*ad. cil.*) passes around the peristome and extends into the cytostome and half way down into the cytopharynx; these are about $8\ \mu$ to $12\ \mu$ long. The body cilia are from $4\ \mu$ to $6\ \mu$ long; they are arranged in long slightly spiral rows from 60 to 120 in number according to the size of the animal, and twist to the left about 120° . The adoral cilia aid in the ingestion of food, whereas the body cilia are locomotor organs. The latter beat at an angle of 20° from right to left, thus rotating the organism from left to right. The path of the animal is straight and not spiral as in *Paramecium*. When an obstacle is encountered an avoiding reaction usually does not result as in *Paramecium*, but an attempt is made to penetrate by boring into small openings with a rotary movement of the body and very mobile changes in shape. Thus a positive reaction toward a solid (thigmotropism) combined with boring movements and a plastic body enable the organism to pass through spaces only one-half as large as the diameter of the body. These characteristics are correlated with the ability to penetrate and may account in part for the pathogenicity of the species in man. The cilia are attached to a row of basal granules that lie in the hyaline bands of ectoplasm. From these basal granules ciliary rootlets (*cil. r.*) extend into the neighboring granular bands where they enlarge into secondary basal granules.

The peristome comprises everything that is situated within the adoral circlet of cilia. It is normally pear-shaped with

the stem extending posteriorly. At its ventral end lies the cytostome. This can be closed by a very mobile nonciliated oral plug. A short cytopharynx extends from the cytostome into the body, where it ends blindly. Food is driven into the cytostome by the synchronous beating of the adoral cilia which creates an eddy with the vortex within the peristome. The oral plug may at certain times close the cytostome. Food vacuoles are formed at the end of the oesophagus and pass into the endoplasm, where they are circulated about very much as are food vacuoles in *Paramecium*. They are usually less than 5 μ in diameter and contain starch granules, bacteria and undigested particles of the food of the host. Cyclosis within the fluid endoplasm proceeds from the inner end of the cytopharynx along the ventral surface, dorsalward near the posterior end, anteriorly along the dorsal surface, then near the apical cone, through the center of the body to the inner end of the cytopharynx again. Digestion takes place within the food vacuoles, the contents being at first acid and later alkaline. Large numbers of paramylum bodies were noted by McDonald within the endoplasm, but no blood corpuscles, as have been reported from cases of balantidial dysentery in man. Cannibalism is suggested by the discovery of partially digested small specimens within the bodies of several large individuals. Undigested particles are collected in a rectal vacuole (*ret. v.*) and cast out through a cytopyghe (*cytpg.*) at the posterior end of the body. This is open only when waste is discharged. The macronucleus (*mac.*) possesses a nuclear membrane and contains chromatin in irregular masses. It is not constant in position. The micro-nucleus (*mic.*) is not over 5 μ in diameter, subspherical in shape, and either flattened against the side of the macronucleus or in a depression in the macronucleus.

Neuromotor apparatus. The neuromotor apparatus of *Balantidium coli*, according to McDonald, is very similar to those described by Sharp (1914) in *Diplodinium* and Yocom

(1918) and Taylor (1920) in *Euplates*. It consists of five principal parts whose positions are shown in figure 163. These constitute an integrated system of fibers with a co-ordinating center and with the power of conducting nervous impulses and of coordinating the motor organelles of the organism. (1) The motorium (*mot.*) is supposed to act as a center of coordination; (2) the circumoesophageal fiber (*cir. oes. f.*) seems to correlate the movements of the oral plug with those of the adoral cilia in feeding and in avoiding reactions; (3) the ciliary rootlets (*cil. r.*); (4) radial fibers (*rad. f.*), and (5) adoral ciliary fiber (*ad. cil. f.*) coordinate the activities of the swimming and feeding organelles.

3. *Balantidium coli* IN MAN

The most important ciliate parasitic in man is *Balantidium coli*. This large heterotrichous protozoon was reported by Malmsten in 1857 in a man 35 years of age who was suffering from diarrhoea. Leeuwenhoek has been credited with its discovery in 1681, but it has recently been shown (Dobell, 1920) that the protozoon he observed in his own stools was *Giardia lamblia*. Since 1857, *B. coli* has been found in man in almost every part of the world, as well as in pigs and primates.

Morphology (Fig. 161).—The shape of *B. coli* is oval, slightly broader at the posterior end and narrowed and pointed at the anterior end. Its size varies according to its stage of growth and reproduction, and the nutritive conditions within its habitat, ranging from 30 μ to 200 μ or more in length and from 20 μ to 70 μ in breadth. The usual range in size is 50 μ to 70 μ long by 40 μ to 60 μ wide. Cilia cover the entire body, being arranged in parallel longitudinal rows. A funnel-shaped peristome that can be expanded and contracted is situated near the anterior end. Into

it solid particles of food are driven by the surrounding cilia which are larger than those on the rest of the body. At the posterior end is a more or less distinct excretory pore, the cytopyge. A clear layer of ectoplasm and a more granular central endoplasm are distinguishable. Near the center of the body is a large kidney-shaped macronucleus and closely associated with it a small spherical micronucleus. Two contractile vacuoles are usually present and a number of food vacuoles in various stages. This species thus resembles in many ways such a free-living form as *Paramecium*. It is a sort of scavenger living in the human intestine but a preference for red blood cells has been reported (Carnes, 1909). The feeding process is probably similar to that described by de Leon (1919) in a species (*B. haughwouti*) parasitic in a snail. Lashing movements of the cilia and membranelles surrounding the cytostome create a current in the fluid medium directed toward the mouth opening. Apparently no selection of food particles is exercised, all particles below a certain size are engulfed and the larger particles escape in the side-flow.

Reproduction (Fig. 164).—*Balantidium coli* reproduces by transverse fission. The macronucleus becomes dumbbell-shaped and apparently undergoes a mass division into two equal parts. The micronucleus divides by a sort of mitosis within the nuclear membrane. One-half of each nucleus migrates toward either end of the body, and the cell then becomes constricted at the center into two daughter cells. The peristome persists in the anterior daughter, whereas a new peristome develops at the anterior end of the posterior daughter. Under certain conditions binary divisions follow one another rapidly without any appreciable growth during the intervals between successive divisions. This results in the formation of "nests" of the parasites in the tissues. Sporulation has also been described (Walker, 1909) but has not been satisfactorily confirmed.

Cysts.—Cysts of two types have been reported in the life-cycle of *B. coli*. A single individual in the intestine may become almost spherical and surrounded by a wall which it secretes. This wall is double, with an outer thicker layer and an inner thinner layer, is very tough, and almost colorless.

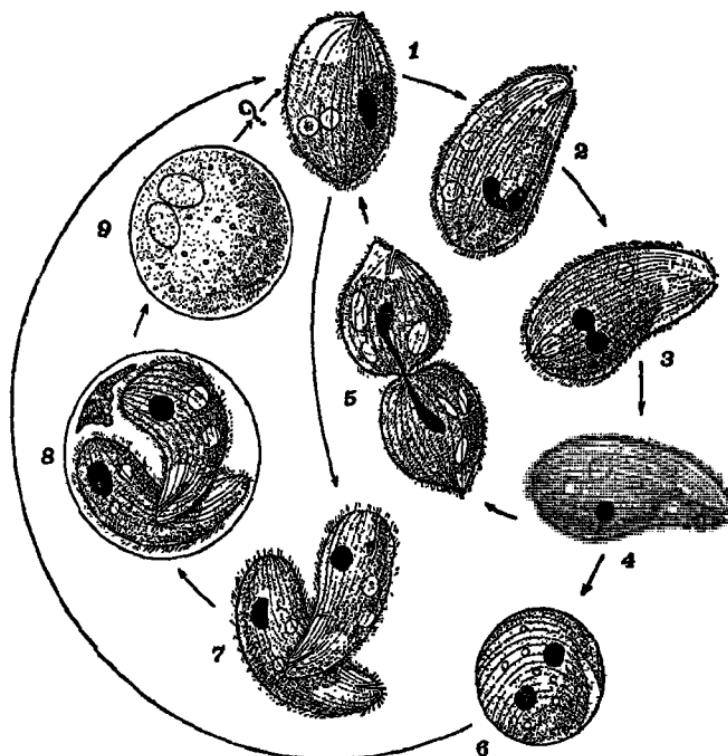


Fig. 164.—*Balantidium coli*. Diagrams showing supposed life-cycle. 1-5, Asexual reproduction by transverse division. 6, Resistant cyst, produced by a single specimen. 7-9, Conjugation and formation of a fusion cyst. (After Brumpt.)

These cysts are able to withstand conditions that would kill the motile forms and hence insure the dissemination of the species. Conjugation cysts have also been described (Gurwitsch, 1896; Brumpt, 1913). After rapid division resulting in a decrease in size to forms only $30\ \mu$ in length conjugation

may occur. Two specimens secrete a cyst wall about themselves, throw out part of their substance, and then fuse into one. The further history of these conjugation cysts is unknown. Encystation seems to be common in pigs but rare in man (Brug, 1919).

Budding has been reported by various authors but has not been definitely established.

Cultivation in Artificial Media.—Reports have appeared indicating that *Balantidium coli* may exist outside of the body in the active motile stage. Kleine (1896) claims to have found it regularly in the diluted sewage from St. Bartholomew's Hospital, London. It seems probable, however, that he saw a free-living species of some other genus. Conn (1905) figures a ciliate from the fresh waters of Connecticut which he refers provisionally to the genus *Balantidium*. The species described by Leon (1919) from the intestinal tract of Philippine snails disappeared from the aquaria in which it was kept in four or five days. Motile specimens of *B. coli* may live for a number of hours after they are passed in the feces of infected persons (30 hours according to Prowazek, 1920). The cysts presumably live for a longer period.

Various investigators have attempted to cultivate species of *Balantidium* on artificial media. Walker (1909) used pure mixed cultures, that is, a pure culture of ciliates plus a pure culture of bacteria, on a medium of two parts of agar, 100 parts of distilled water, and one part of a normal solution of sodium hydrate, for the cultivation of *B. falciformis* from the large intestine of the frog, *Rana palustris*. In such cultures sporulation was found to occur rather frequently, taking place in the non-encysted animals without the intervention of sexual processes. The spores at first were ovoidal, 2.3μ to 3.8μ in diameter, non-motile, and had a "distributed nucleus"; they developed directly into adult ciliates. The sporulation process as thus described lacks confirmation. Marshall (1911), Hinkelmann (1919 a, 1919 b) and Barret

and Yarbrough (1921) have reported the cultivation of *B. coli* from man. The ciliates that Marshall obtained from a culture from the spleen of a patient who died of kala-azar, and that he believed to be balantidia, were probably free-living species, and those that Hinkelmann described as *Balantidium*, from the peripheral blood of man, and cultivated in a medium of blood and water, were probably also free-living specimens with which his media became contaminated. Barret and Yarbrough, however, appear to have really cultivated *B. coli* from man. The medium used consisted of sixteen parts of 0.5 per cent salt solution plus one part of inactivated human blood serum. Tubes containing about 8 cc. of this medium were inoculated at the bottom with about 0.1 cc. of undiluted feces containing balantidia and incubated at 37° C. Division was observed and an increase in numbers occurred. Maximum growth took place in from 48 to 72 hours and subcultures were made every second day as a rule. In this way eleven transfers were obtained in 32 days. Encysted forms were observed in some of the cultures and one case of apparent conjugation was encountered.

4. INFECTION EXPERIMENTS WITH *Balantidium coli*

Besides the morphological evidence already given indicating that the balantidia found in man and the pig are the same species, must be considered the results of a number of infection experiments. Walker (1913) has proved that balantidia from both the pig and man will infect monkeys. He succeeded in infecting 2 out of 4 monkeys by injecting active ciliates from human stools into the rectum, and in infecting 12 out of 13 monkeys by feeding them cysts from pig feces. These data were strengthened by Brumpt (1919), who infected a monkey with the *Balantidium* from the pig, and young pigs with balantidia from the monkey. One of the pigs developed lesions similar to those in man. No one

has yet infected man experimentally with *Balantidium* from either the pig or monkey, although Grassi (1888) and Calandruccio attempted to do this *per os* with cysts from the pig. If balantidiosis in man is brought about, as is generally supposed, by the ingestion of cysts from pig feces, it is obvious that the infection of human beings with cysts from the pig must occur in nature.

Mode of Infection of Human Beings with Balantidium coli.—How man becomes infected with *B. coli* is not known definitely, but the infection experiments cited above furnish almost certain proof that it is brought about by the ingestion of resistant cysts. Such cysts are known to occur both in man and the pig. It is thus probable that cysts from one human being may infect another human being who chances to swallow them, perhaps in contaminated water, milk or other food. The fact that very few cysts are produced in man may account in part for the very small incidence of infection among human beings. There is a general belief that man may become infected also by the ingestion of cysts from the pig, and while certain evidence indicates that this may be true, there is no definite experimental proof.

5. BALANTIDIOSIS

Balantidium coli is comparatively rare in man, especially in the colder regions of the earth. In the tropics, however, the incidence of infection is much higher. For example, Pinto (1919) records 41 positive cases out of 3,917 examined in Southern Brazil (Parana) and Nedergaard (1921) 24 positive cases out of 3,945 examined in Eastern Cuba. When *Balantidium coli* becomes pathogenic it produces a disease known as balantidiosis. As in the case of *Endamoeba histolytica*, only a small proportion of human beings harboring *B. coli* show symptoms, the rest are carriers. About 20 per cent of the cases observed by Walker (1913) showed intestinal symptoms. Whether the parasite attacks the tissues of

the carrier but not extensively enough to cause symptoms, or simply lives in the lumen of the intestine, is not known. The lesions caused by the parasite take the form of ulcers in the walls of the large intestine. The parasites penetrate the mucus, probably by means of burrowing movements, and form small colonies in the mucous and submucous tissues. Here, apparently, the tissues are dissolved by ferments secreted by the parasites. Glaessner (1908) has isolated a diastase and a hemolysin but no proteolytic ferment from the parasite. Descriptions of the pathology of balantidiosis have been provided by Strong (1904), Walker (1913), and Manlove (1917). Many investigators have attempted to discover some specific treatment for balantidiosis, but thus far without success. For a brief survey of this subject the reader is referred to Dobell and O'Connor, 1921, pp. 162-163.

6. DOUBTFUL SPECIES OF *Balantidium* IN MAN

B. minutum (Fig. 165) was first described by Schaudinn (1899) from a patient suffering from diarrhoea. *Balan-*
tidium minutum is a much smaller species than *B. coli*, measuring from 20 μ to 30 μ in length, and from 14 μ to 20 μ in breadth. The peristome is long, reaching to the center of the body; the single contractile vacuole is large and located posteriorly; and the macronucleus is spherical, as is also the micronucleus which lies near it. Encysted specimens were also found. This species appears to be very rare, since it has been reported only a few times. Brooks (1903) records it on Dr. Russell's authority from Porto Rico, and Pinto (1919) lists it from Brazil as present in 5 out of 3,917 fecal samples examined. In spite of these apparent confirmations of Schaudinn's discovery it must be admitted that this species is listed as an entozoic protozoon of man on very meager evidence.

A species resembling *B. minutum*, and named *B. minutum*

sp. *italicum*, has been described by Sangiorgi and Ugdulena (1917) and Sangiorgi (1919) from human feces. This form has an eccentrically placed macronucleus and a micronucleus that varies in position with respect to the macronucleus. It was cultivated in peptone water and agar-agar media in which it measured from 31.5μ to 35μ in length and from 14μ to 25μ in width. Motile forms disappeared from the cultures after a time and cysts became numerous; these produced new vegetative forms after a few days. Encystment was induced by placing motile forms on agar-agar. The cysts that formed on the surface of this medium developed into motile forms when placed in peptone water. This work needs to be repeated.

A careful study of *B. minutum* is very desirable since Schaudinn's observations have never been properly confirmed and the other data contained in the literature are fragmentary and of doubtful value.

Several investigators have described ciliates from the feces of man that they have placed in the genus *Balantidium*. Thus, Krause (1906) found a very large form in the feces of a young woman in Germany, for which, on account of its large size, he proposed the name *Balantidium coli giganteum*. Whether this form was a real inhabitant of the intestine or simply a contamination, and its exact systematic position are difficult to determine. Another *Balantidium* is reported by Barlow (1915) in human feces in Spanish Honduras and called by him *Balantidium coli* variety *Hondurensis*. This must also be considered a doubtful

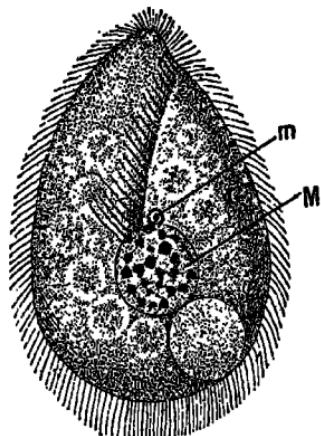


Fig. 165.—*Balantidium minutum* from man. M, macronucleus. m, micronucleus. $\times 1500$. (From Brumpt, after Schaudinn.)

species as well as that described by Sangiorgi (1919) from Albania as *Balantidium coli* sp. *Albanense*.

7. SPECIES OF *Balantidium* IN LOWER ANIMALS

Various species of *Balantidium* have been recorded from many lower animals besides the pig. Those in frogs and salamanders seem to be the most abundant. These include *B. entozoon*, *B. elongatum*, *B. duodenii*, *B. giganteum*, *B. gracilis*, *B. helenae*, *B. rotundum*, and *B. falcifarum*. Species have also been found in fish (*B. piscicola*), coelenterates (*B. hydræ* and *B. medusarum*), flat worms (*B. amphictenides*), sand fleas (*B. orchestium*), cockroaches (*B. blattarum*), snails (*B. haughwouti*), and in horses (da Cunha, 1917). These species can be distinguished from one another morphologically on the basis of the shape of the body, length of the peristome, shape of the nucleus, and number of contractile vacuoles. *B. coli* has been found in the monkey, *Macacus cynomolgus*, by Noc (1908) and Brumpt (1909). It was noted by Joyeux (1913) in a baboon in French Guinea and successfully inoculated into another baboon. It has also been inoculated successfully into monkeys with cysts from man and pig by Walker (1913). Brooks (1903) has also reported this species as the cause of fatal dysentery in orangutans in the New York Zoological Gardens. Hegner and Holmes (1923) have furnished accurate measurements of a balantidium (Fig. 166, B) from a Brazilian monkey, *Cebus variegatus*, and have compared it with the balantidia of man and the pig. This monkey form was found to differ from the others in certain respects. (1) *Size*. The specimens from the monkey averaged $44\text{ }\mu$ in length and $25\text{ }\mu$ in breadth, which is much smaller than *B. coli* and *B. suis* from the pig which average $86\text{ }\mu \times 66\text{ }\mu$ and $86\text{ }\mu \times 43\text{ }\mu$ respectively. (2) *Shape*. The ratio of length to breadth in *B. coli* is 1.30; in *B. suis*, 1.99; and in the monkey balantidium, 1.76. (3) The

cytostome of *B. coli* is almost terminal; of *B. suis*, ventral; and of the monkey balantidium, intermediate in position.

(4) The line of demarcation between ectoplasm and endoplasm in *B. coli* is at right angles to the longitudinal axis of the body; in *B. suis* at a considerable angle; and in the

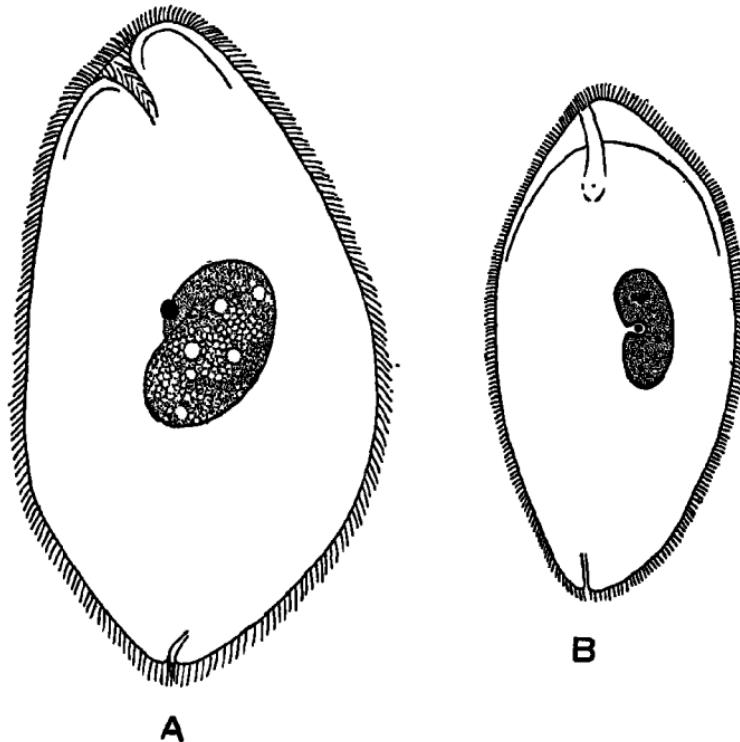


Fig. 166.—A, *Balantidium coli* from the pig. B, *Balantidium* sp. from a Brazilian monkey, *Cebus variegatus*. $\times 1200$. (A, From Hegner and Holmes, after Dobell; B, After Hegner and Holmes.)

monkey balantidium at a slight angle to this axis. (5) The macronucleus of *B. coli* is massive; that of *B. suis* more slender; and that of the monkey balantidium similar to the macronucleus of *B. coli*.

Whether or not these differences between the monkey balantidium and *B. coli* and *B. suis* are of specific signifi-

cance, represent fluctuating variations, or are the result of the presence of heritably diverse races is uncertain. A thorough study of the genus seems necessary before a decision can be reached.

Peculiar nuclear conditions were also encountered by Hegner and Holmes in the balantidium from the Brazilian

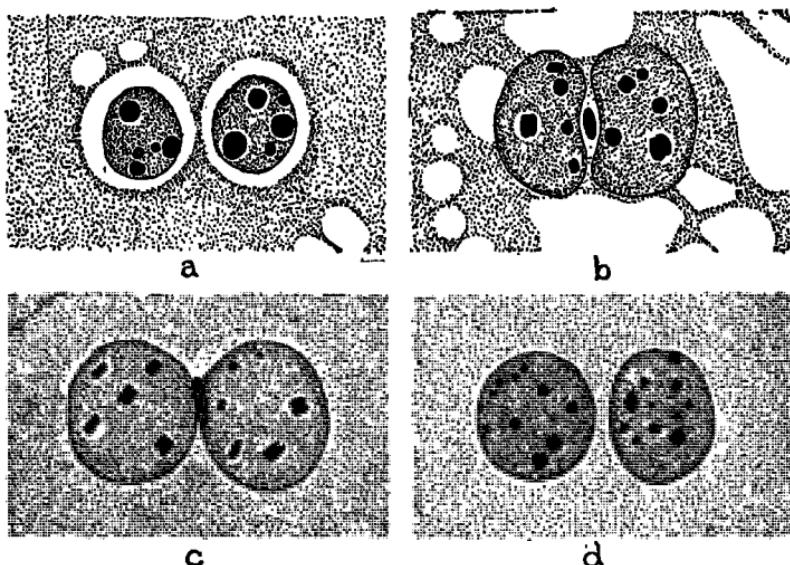


Fig. 167.—Macronuclei of balantidia from a Brazilian monkey showing chromosome-like masses.

a, Three large and 2 small masses in each of two daughter nuclei.
b, Same number of masses, but several of these consisting of paired bodies. c, Five paired masses in each nucleus. d, Ten paired masses in each nucleus. $\times 2500$. (After Hegner and Holmes.)

monkey. Chromosome-like masses of chromatin (Fig. 167) were noted in the macronucleus that appear before division and are rather constant in number (5) and size (3 large and 2 small) in the daughter nuclei, following division. They often occur in pairs, which apparently represent a single mass that has divided into two equal parts, rather than the conjugation of two equal masses. What becomes of these

masses was not determined, but they seem to decrease in size by division and when the nucleus undergoes reconstruction may break down into granules indistinguishable from other chromatin granules in the nucleus.

These chromosome-like bodies in the macronucleus may be trophic chromosomes analogous to the massive, so-called trophic chromosomes that appear during mitosis in certain species of *Opalina* (Fig. 159). No mechanism was discovered to account for their equal distribution to daughter nuclei, but this may be brought about by protoplasmic streaming, such as occurs when multinucleate arcellas divide.

E. The Genus *Nyctotherus* in Man and Lower Animals

Nyctotherus faba (Fig. 168) was described in man by Schaudinn (1899), who found it associated with *Balantidium minutum* in a single patient. The body of this species is bean-shaped and is from $26\ \mu$ to $28\ \mu$ long, from $16\ \mu$ to $18\ \mu$ wide, and $10\ \mu$ to $12\ \mu$ thick. The cytostome extends to the center of the body where it merges into a long, curved cytopharynx. Within the body are a large contractile vacuole at the posterior end, a large macronucleus containing chromatin in four or five large clumps, and a small micronucleus. The oval cysts of this species were also found. Sangiorgi and Ugdulena (1917) claim to have cultivated this species in peptone water and on agar-agar media. Encystment occurred at the surface of the latter; the cysts produced motile forms when placed in peptone water. *N. faba* has also been reported from Brazil (Pinto, 1919), but

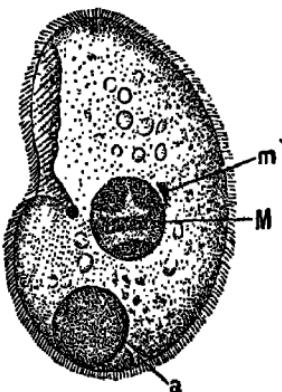


Fig. 168.—*Nyctotherus faba* from man. M, macronucleus. m, micronucleus. a, anal aperture. $\times 1500$. (From Brumpt, after Schaudinn.)

further study is necessary before this species can be accepted definitely as an inhabitant of man.

Two other species of *Nyctotherus* have been described from man. *N. giganteus* (*Balantidium giganteum*), by

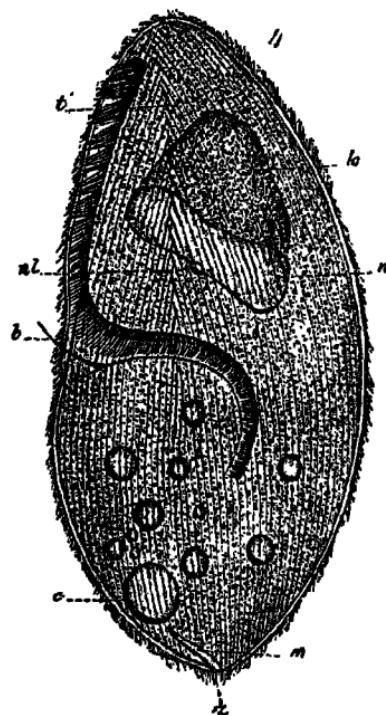


Fig. 169.—*Nyctotherus cordiformis* from the frog. *b*, opening into cytopharynx; *c*, vacuole; *n*, macronucleus; *nl*, micronucleus; *s*, anal aperture. (After Stein.)

Krause (1906), which differs from *N. faba* principally in size, being from 90 μ to 400 μ long and from 60 μ to 150 μ wide, and *N. africanus* by Castellani (1905). The latter has a peculiar shape, is 40 μ to 50 μ long and 35 μ to 40 μ wide; and has the chromatin of the macronucleus in clumps as in *N. faba*. The evidence we now have is insufficient to justify the conclusion that the three species of *Nyctotherus* that have been reported from man are really human entozoic protozoa.

Specimens of the genus *Nyctotherus* may easily be obtained for study from cockroaches and frogs. *N. ovalis* was first discovered in the intestine of the cockroach, *Blatta orientalis*, by

Leidy in 1850, and has recently been studied in detail by Zulereta (1916). The most common species in frogs is *N. cordiformis*. This species has a long peristome extending from the anterior end to the center of the body and leading into a long curved pharynx as indicated in figure 169. It divides by transverse fission; forms cysts very much like

those of *Balantidium coli* (Collin, 1913) ; undergoes sporulation (Walker, 1909) ; and can be cultivated in artificial media (Walker, 1909; Aragao, 1912). Other species occur in the frog (*N. amaniensis*, *N. macropharingeus*, *N. magnus*), in myriapods (*N. velox*), in water beetles (*N. györyanus*), in crustacea (*N. hæmatobius*), in crinoids (*N. comatulæ*), and in fish (*N. piscicola*) (Entz, 1913). Among the infusoria that resemble *Nyctotherus* in being of doubtful occurrence in man are species of the genera *Chilodon*, *Uronema*, and *Colpoda*.

Chilodon dentatus was the name given by Guiart (1903) to a ciliate present in large numbers in the feces of a dysentery patient in Paris, a woman 58 years old. The circumstances under which this ciliate was found suggest that its presence may have been due to contamination after the stools were passed, or else resistant cysts that had passed through the alimentary canal developed in the feces. Manson and Sambon (1909) found many specimens of a "pseudo-parasite," which they named *C. uncinatus*, in the mucus of the stools of a patient who had been in India and Africa, and was also parasitized by *Schistosoma mansoni*. They believe the ciliates described by Guiart belonged to this species also and not to *C. dentatus*. Forms that have been referred to the genus *Chilodon* have also been reported by Seleneu (1910) from the prostate secretions of four patients suffering from gonorrhea. It seems certain that these species, which are normal inhabitants of fresh water, are all either accidental invaders of the alimentary canal of man or else contaminations, but certain species of *Chilodon* are true ectoparasites on the skin of fish. The best known of these is *C. cyprini* (Moroff, 1902; André, 1912), a species 50 μ to 70 μ long, and 30 μ to 40 μ broad. This parasite infests goldfish and other cyprinid fishes and may cause their death.

Another holotrichous ciliate was reported by Martini (1910) in the bloody stools of four dysentery patients in

Tsingtau and named by him *Uronema caudatum*. They measured 30 μ to 43 μ in length, and 11 μ to 15 μ in width; possessed a slit-like peristome, a posteriorly situated contractile vacuole, and one or more long caudal cilia. No dysentery bacteria or amoebae were present and hence these infusoria were accused of causing the dysentery. Martini was able to cultivate this species for several weeks at room temperature in physiological salt solution containing intestinal mucus from the patients. Fischer (1914) thinks he has confirmed these observations at Shanghai, but since this species occurs commonly in fresh water infusions it seems best to regard it as an accidental invader of the stools.

Another species belonging to this order that has been recorded from man is *Colpoda cucullus*. This form was reported in human feces by Schulz (1899), but its presence was probably due to contamination after the feces were passed.

F. Entozoic Ciliates of Primates

Because the primates are so closely related to man their parasites are of particular interest. However, these animals do not seem to be badly infested with parasitic ciliates. Brumpt and Joyeux (1912) examined many monkeys in several localities, notably French Guinea, but found ciliates in only one chimpanzee from the Congo. This they placed in the family OPHRYOSCOLECIDÆ and in a new genus, calling it *Troglodytella abrassarti*. Attempts to grow it in cats and other monkeys failed. Reports of the discovery of *Balantidium coli* in primates have already been mentioned (p. 430). Recently a variety of the ciliate of the chimpanzee, *Troglodytella abrassarti acuminata*, has been reported by Reichenow (1920) from a chimpanzee living in the Kamerun and another new species was found by the same investigator in a gorilla suffering from dysentery and named by him *T.*

gorillæ (Fig. 170). All of these ciliates are large, measuring about $200\ \mu$ in length and over $100\ \mu$ in breadth, and appear to be pathogenic to their hosts.

G. Ciliates Living in the Digestive Tract of Mammals

The digestive tract of some of the larger mammals is abundantly supplied with ciliates, their descriptions dating back to 1858. Twenty-four species belonging to 6 genera have been recorded from the stomach of ruminants (deer, cattle, etc.) and twenty-one species belonging to 10 genera from the cæcum of the horse. Some of these are among the most complex of all protozoa.

Sharp (1914) has given a detailed account of a ciliate that lives in the stomach of cattle. This species, *Diplodinium ecaudatum* (Fig. 171) may be free from spines or bear 1, 2, 3, 4, or 5 spines at the posterior end. Apparently when division occurs now)

the offspring possess the same number of spines as the parent; this indicates that these are probably heritably diverse lines of one species but names have been given to the different types as follows:

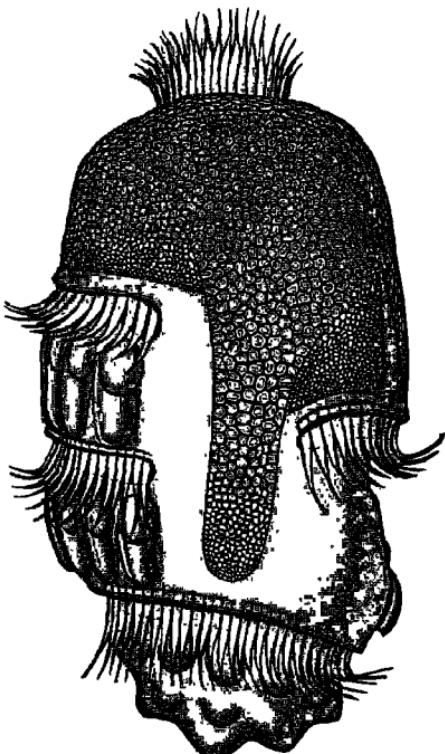
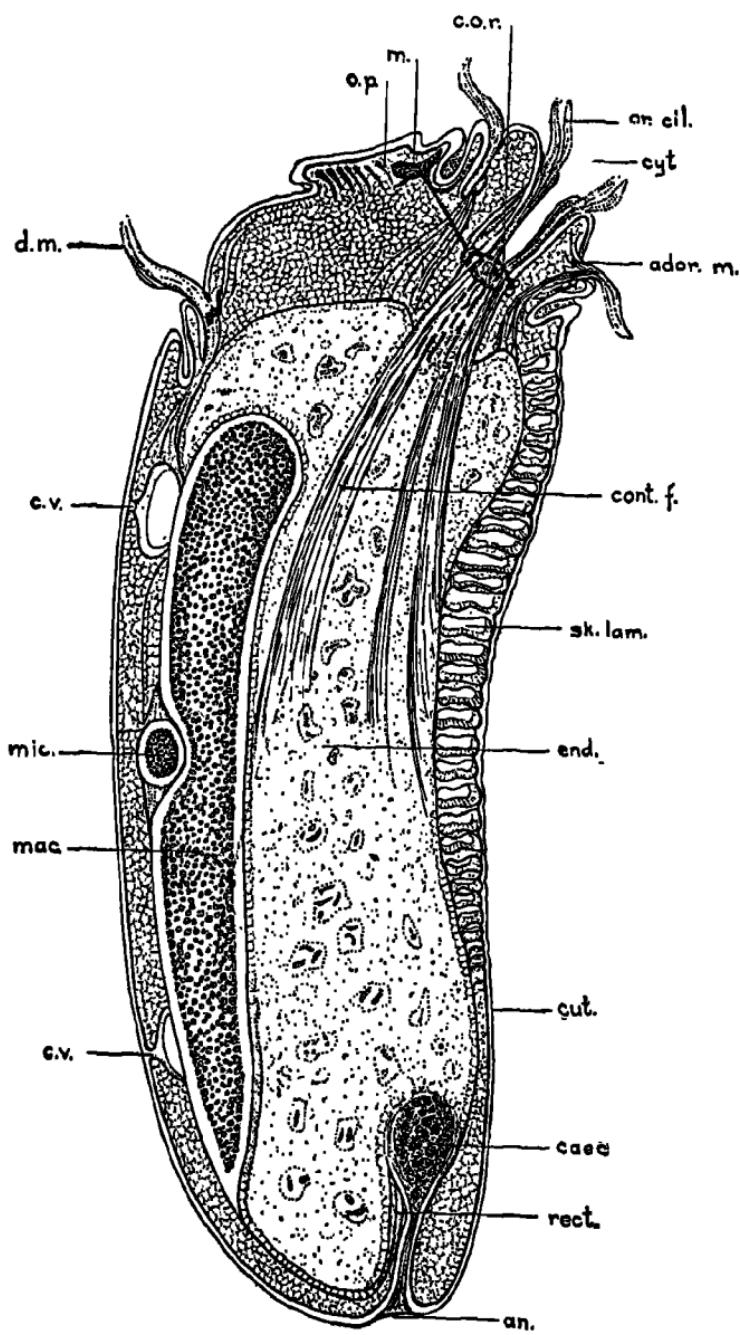


Fig. 170.—*Troglodytella gorillæ* from the gorilla. $\times 300$. (After Reichenbach now.)



Diplodinium ecaudatum ecaudatum without spines; *D. e. caudatum*, with one spine; *D. e. bicaudatum*, with two spines; *D. e. tricaudatum*, with three spines; *D. e. quadricaudatum*, with four spines; and *D. e. cattanei*, with five spines.

The most interesting thing about *Diplodinium* is its great complexity, and an examination of its structure will very quickly convince one of the fallacy of defining protozoa like this one as simple unicellular organisms. Reference to the figure reveals the presence of an external resistant cuticular covering (*cut.*), probably of value in protecting the animals from mechanical injury and from certain fluids in the intestine. Certain areas of the cuticle are related to skeletal structures underneath and are therefore called left, ventral and right skeletal areas (*sk. lam.*). Surrounding the mouth or cytostome (*cyt.*) are oral cilia (*or. cil.*) which serve to convey food particles into the mouth. A short distance away from the mouth is a row of adoral membranelles (*ador. m.*) which circle about the adoral region. Beneath the cuticle is a layer of ectoplasm modified in three places to form areas of skeletal laminæ (*sk. lam.*). Embedded in the dorsal surface of the ectoplasmic layer are two contractile vacuoles (*c. v.*), one anterior, the other posterior, a small spherical micronucleus (*mic.*) and a very long macronucleus (*mac.*) shaped like the blade of a knife. At the posterior end is an invagination of the ectoplasm which serves as an anal opening (*an.*) and is connected with a tube, the rectum (*rect.*), which is surrounded by longitudinal rectal fibres, and opens into a pear-shaped cavity, the cæcum (*cæc.*). The mouth opening is at one side of the anterior end; it leads into

Fig. 171.—*Diplodinium ecaudatum* from cattle.

an., anal opening; *ador. m.*, adoral membranelles; *cæc.*, cæcum; *cont. f.*, contractile fibers; *c.o.r.*, circumoesophageal ring; *cut.*, cuticle; *c.v.*, contractile vacuole; *cyt.*, cytostome; *d.m.*, dorsal membranelles; *end.*, endoplasm; *m.*, motorium; *mac.*, macronucleus; *mic.*, micronucleus; *o.p.*, operculum; *or. cil.*, oral cilia; *rect.*, rectum; *sk. lam.*, skeletal laminæ. $\times 1100$. (After Sharp.)

the œsophagus, which opens into the central mass of endoplasm (*end.*). Surrounding the œsophagus and extending about half-way back through the ectoplasm is a broad layer of contractile fibers (*con. f.*) which serve to draw in the adoral region when they contract. Most interesting of all, perhaps, are the nerve rings and fibers. These consist of a central motorium (*m.*) from which extend out the following strands: (1) one to the roots of the dorsal membranelles (*d. m.*), (2) one to the roots of the adoral membranelles (*ador. m.*), (3) one to the circumœsophageal ring strand (*c. o. r.*), and (4) several into the ectoplasm of the operculum (*op.*).

Sharp concludes that this complicated system of fibers is probably nervous in function and constitutes a true neuromotor apparatus.

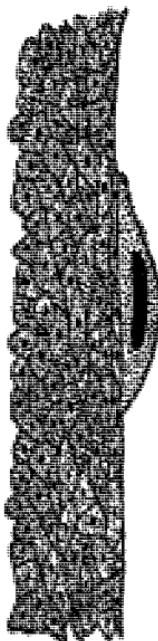


Fig. 172.—
Cyclochæta domerguei, a
peritrichous
ciliate ectopar-
asite on fish.
(After Dof-
lein.)

H. Ectozoic Peritrichous and Hypotrichous Ciliates

Certain members of the order PERITRICHA exhibit in a remarkable fashion various degrees of dependence on other animals leading to the parasitic habit. The tendency exhibited by free-swimming ciliates to come to rest against some other animal or solid object seems to have evolved into a sedentary habit which has brought about the formation of various organs of attachment. Stages in this process are described and figured by Faure-Fremiet (1905). Thus the common vorticellids (Fig. 148) are attached by a long stalk containing a muscular cord that by contradiction may draw the body away from danger and by extending in another direction enables it to enter a slightly different environment. Both temporarily and permanently fixed species occur in the order. The latter when attached to animals obtain trans-

portation but as a rule do no particular harm to their host. Among the species temporarily fixed, however, are parasites that may be pathogenic. For example, *Cyclochæta domerguei* (Fig. 172), an ectoparasite on the skin of fishes, may cause the death of very young hosts.

The ectozoic ciliates most easily obtainable belong to the genus *Trichodina* (Fig. 173). These are short and hour-

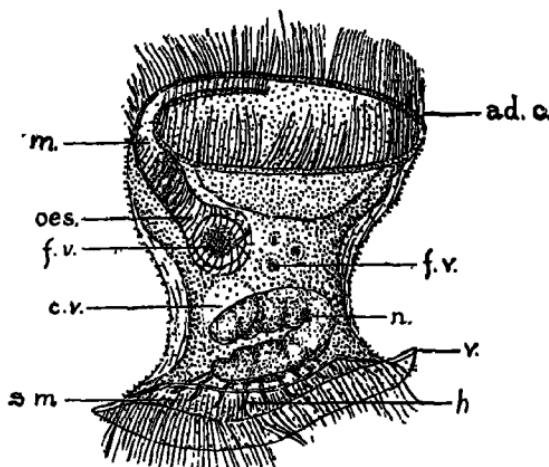


Fig. 173.—*Trichodina pediculus*, a peritrichous ciliate ectozoic on fish.

ad. c., adoral cilia; *c.v.*, contractile vacuole; *f.v.*, food vacuole; *h.*, hooks; *m.*, mouth; *n.*, macronucleus; *o.es.*, oesophagus; *s.m.*, striated membrane; *v.*, velum. (After Clark.)

glass-shaped, with a spiral band of adoral cilia (*ad. c.*) leading into a cytopharynx or oesophagus (*o.es.*), a ribbon-shaped nucleus (*n.*), and one contractile vacuole (*c. v.*). The aboral surface is modified into a disc-like organ of attachment consisting of an inner ring of radially arranged teeth, an outer band of cilia, and a peripheral fold, the velum (*v.*). Our most common species is *Trichodina pediculus*; it may be found gliding along on the surface of the body of the fresh-water *Hydra*, on tadpoles and other aquatic animals. Other species are ectozoic on the fresh-water flatworms of the

genus *Planaria* and on the gills of certain fishes and molluscs. Caullery and Mesnil (1915) have described a case of symbiosis involving *Trichodina patella*, which lives on the gills of the mollusc, *Patella*, and algae of the genus *Zooxanthella*. Each ciliate contained more than sixty of the algae. The condition observed by these investigators is indicated in the section shown in figure 174, b. The shape and position of the macronucleus and micronucleus are shown in figure 174, a.

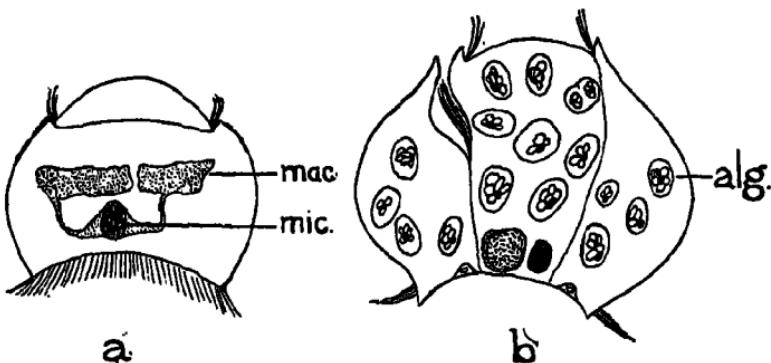


Fig. 174.—*Trichodina patella*.

a, Section showing the shape and position of the macronucleus (*mac.*) and micronucleus (*mic.*). b, Section showing symbiotic algae (*alg.*). (After Caullery and Mesnil.)

A good example of a hypotrichous ectoparasite is *Kerona pediculus*. This species, like *Trichodina pediculus*, also lives on the fresh-water polyp, *Hydra*, creeping about on the surface with untiring activity. It is dependent upon its host for food, but besides the cells of the host will ingest other protozoa, especially euglenoids; the latter, however, are not ingested if the *Kerona* is detached from its host. The morphology of the organism is shown in figure 175.

I. Parasitic Suctoria

The sedentary and more or less parasitic habits of the SUCTORIA are correlated with modifications that tend toward

simplicity of body structure and complexity of reproductive activities and life-cycles. Many SUCTORIA do no particular harm to the organisms to which they are attached but some of them are true parasites. The life-cycle of one of these, *Ophryodendron abietinum*, has been worked out by Martin (1909). This is a marine species that is dimorphic in the adult stage exhibiting two forms, (1) proboscidiform individuals (Fig. 176, a, b, c), and (2) vermiform individuals (d); the former were once described by Robin (1879) as nematodes, and the latter by Wright (1861) as gregarines. The proboscidiform adult (Fig. 176, a) has a pyriform shape, no stalk and a long contractile proboscis with many tentacles at the end. The vermiform individuals (Fig. 176, d) are long and cylindrical with a solid stalk passing into the posterior end, but without a proboscis. Ciliated embryos (Fig. 176, e) are also produced by this type (Fig. 176, d) and probably develop into proboscidiform individuals.

Root (1922) has recently described a new parasitic spe-

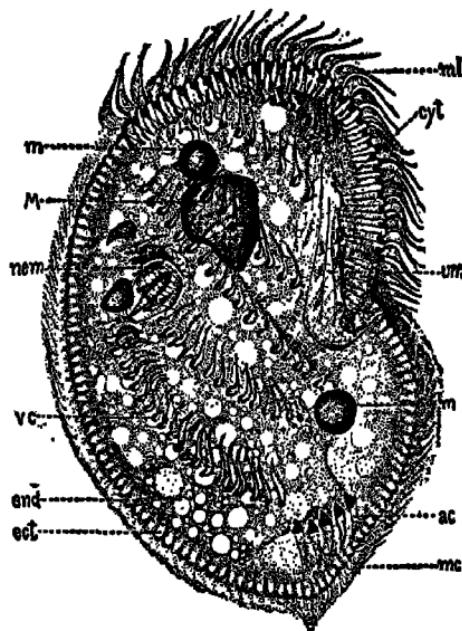


Fig. 175.—*Kerona pediculus*. Dorsal view showing (ac) anal cirri, (cyt) cytostome, (ect) ectoderm, (end) endoderm, (m) micronucleus, (M) macronucleus, (mc) marginal cirri, (ml) membranelles, (nem) nematocyst, (vc) ventral cirri, (um) undulating membrane. (After Uhlemeyer.)

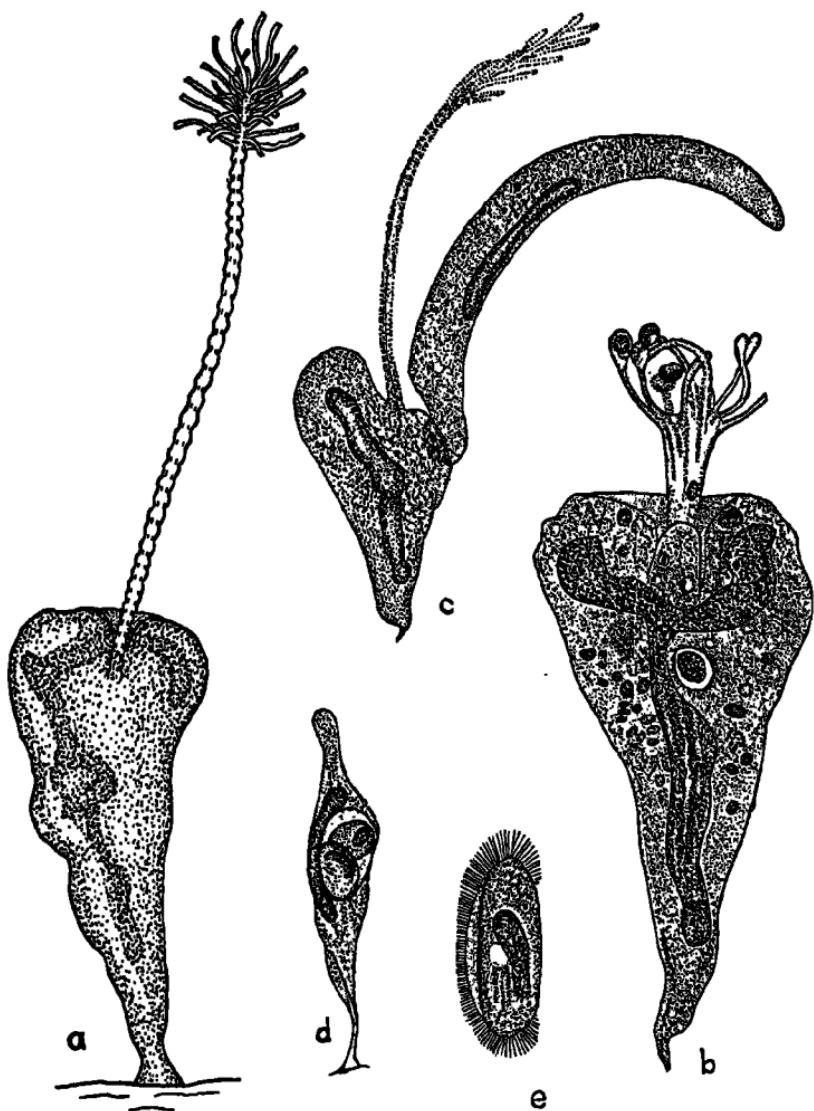


Fig. 176.—*Ophryodendron abietinum*.

a, Proboscidiform individual. b, Proboscidiform individual feeding, with nematoblasts at end of tentacles. c, Proboscidiform individual with full-grown vermiform bud on right and beginning of another on left. d, Vermiform individual containing ciliated embryos. e, A ciliated embryo containing macronucleus and micronucleus. (a, Drawn by Dr. F. M. Root; b-e, After Martin.)

cies, *Acinetopsis tentaculata*, which feeds on another suctorian, *Ephelota coronata*. The parasite seizes and draws its victim within reach of its tentacles by means of its proboscis. Free-swimming ciliated embryos are formed by internal budding; these settle down and grow into the adult stage.

Collin (1912) recognizes two types of commensalism and two types of parasitism among the SUCTORIA. Ecto-com-

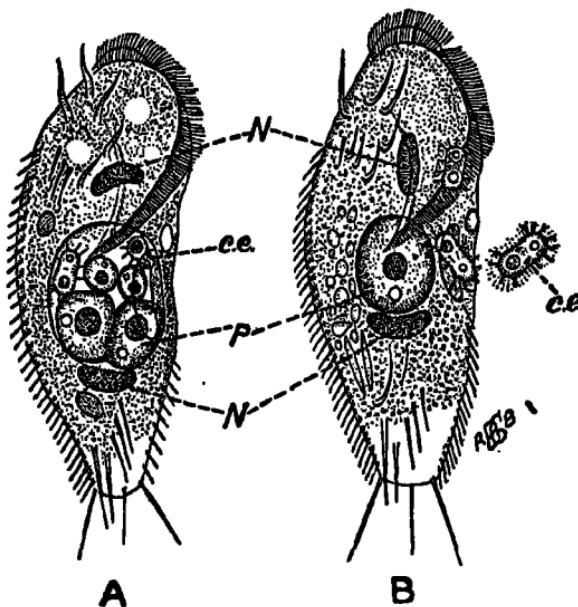


Fig. 177.—*Sphaerophrya*, a suctorian endoparasitic in *Styloynchia mytilus*. N, Macronuclei of *Styloynchia*. P, Parasitic suctorian. c.e., Ciliated embryo. (From Minchin, after Stein.)

mensals are species that attach themselves more or less regularly to some species of aquatic animal. For example, *Discophrya elongata* prefers the shells of gastropods, whereas *D. steinii* is rather strictly limited to the water beetle, *Dytiscus marginalis*. Endo-commensals live in natural cavities in their hosts. Thus *Trichophrya salparum* lives in the pharyngeal cavities of certain tunicates.

The true parasites also live both on the surface and in the interior of their hosts. *Ophryodendron abietinum*, described above, is an example of a true ectoparasite. *Sphaerophrya* (Fig. 177, P) is a genus of endoparasites whose species live within the bodies of ciliates, such as *Stentor*, *Paramecium*, and *Stylonychia*. A sort of brood pouch is formed within the host in which the "embryo" suctorian lives with tentacles retracted. Ciliated larvae (Fig. 177, c.e) are formed in the brood pouch, separate from the host, and develop into free-living individuals.

J. General Remarks on Ectozoic and Entozoic Infusoria

As the preceding pages indicate, every large group of INFUSORIA contains ectozoic and entozoic species. These differ from their free-living relatives principally in habits, form, and life-cycle. Many types of parasitism are exhibited by the various species and it is not very difficult to arrange series of forms ranging from entirely free-living species to those that are entirely parasitic.

The ectozoic parasites may creep about over the body of the host, like *Trichodina*, or may be sedentary, like *Ophryodendron*. The creepers usually possess a flattened body, cilia restricted and modified for locomotion on the ventral surface and often for tactile purposes on the dorsal surface, and more or less specialized organs of temporary attachment to their hosts. The sedentary ectoparasites are more often long and attached to their hosts by means of an aboral stalk. The cilia in these is frequently restricted to the oral end and in the SUCTORIA are replaced by tentacles. The various organelles are similar to those present in free-living species but often modified as described above. The entozoic INFUSORIA range from the simple scavenger type, like *Nyctotherus*, to the true parasitic type, like *Opalina*. The former resembles what we believe to be near the primitive ciliate condition, being ovoid in shape, with a mouth,

with cilia nearly of equal length all over the body, with holozoic nutrition, and with one macronucleus, one micronucleus, and one or two contractile vacuoles. In *Opalina* and other astomatous forms no mouth occurs, nutrition taking place by absorption. The apparent simplicity of *Opalina* as regards structure may not be a sign of primitiveness but may be due to changes brought about by its parasitic habits.

The life-cycles of the parasitic ciliates are complicated because of the necessity for transmission of individuals to new hosts. This involves the production of large numbers of the infective stage—a stage that must undergo the various vicissitudes encountered during the passage from one host to another. The organisms at this stage are usually protected by a cyst wall, and since the chances of establishing themselves in new hosts are very small, their formation must be preceded by rapid multiplication, such as occurs in *Opalina*.

CHAPTER XIV¹

GENETICS AND PHYSIOLOGY OF REPRODUCTION IN THE PROTOZOA

The study of genetics seems, at first, to be very remote from the interests of parasitologists. This, however, is far from being the case. Many of the most interesting problems of entozoa and their relations to their hosts are bound up in an understanding of the fundamental principles of genetics. Among the genetical questions which are especially interesting may be mentioned the following: Are the individuals comprising a species uniform in their structural and physiological characteristics and in their effects on their hosts, or, are they an assemblage of many heritably diverse stocks which differ among themselves but are *per se* constant? How constant is each strain of parasites from generation to generation? Is such a strain susceptible to environmental effects, and if so, how long do these effects last? Do permanent strains with new characteristics ever arise because of changes in the environment? Are there other ways in which new strains may arise?

These questions thus far have been studied with the help of morphological and physiological characteristics of free-living species. Nevertheless it is to be hoped that they

¹By W. H. Taliaferro. The author is much indebted to Professor H. S. Jennings for many helpful suggestions in the preparation of this chapter.

will eventually be attacked in various parasitic species and in order to understand the problems it is absolutely necessary to build on the facts already established for the free-living forms. A great deal, if not the most, of the genetical work on the protozoa has been done with ciliates. For this reason it is well to review some of the results obtained with these forms, and then consider from a comparative standpoint some of the results obtained with other classes. A review of the work done on the genetics of the ciliates up to 1914 was made by Dobell (1914) and an extremely thorough review of the genetics of the protozoa as a whole is given in the recent book of Jennings (1920).

A. Life-Cycle of the Ciliates

I. BINARY FISSION AND CONJUGATION

In considering the life-cycle of the ciliates it seems best to consider first the species *Paramecium aurelia* which has been used so extensively in this type of work and which is very similar to *P. caudatum* (page 378) except that it possesses two micronuclei. Such a form generally reproduces for long periods asexually by binary fission. During this process the micronuclei divide by a true mitosis of the "meso" type while the macronucleus simply constricts into two parts amitotically; and then the body divides transversely, each half receiving one macronucleus and two micronuclei. An outline of this process is given in figure 178. Sooner or later asexual reproduction ceases and a sexual phase of the life-history begins. Two individuals unite along their oral surfaces. The macronucleus of each con-

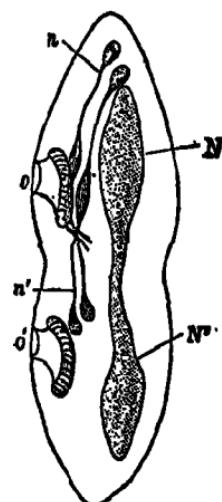


Fig. 178.—Binary fission in *Paramecium aurelia*. N, N', macronucleus; n, n', micronucleus; o, o' mouth. (After Hertwig.)

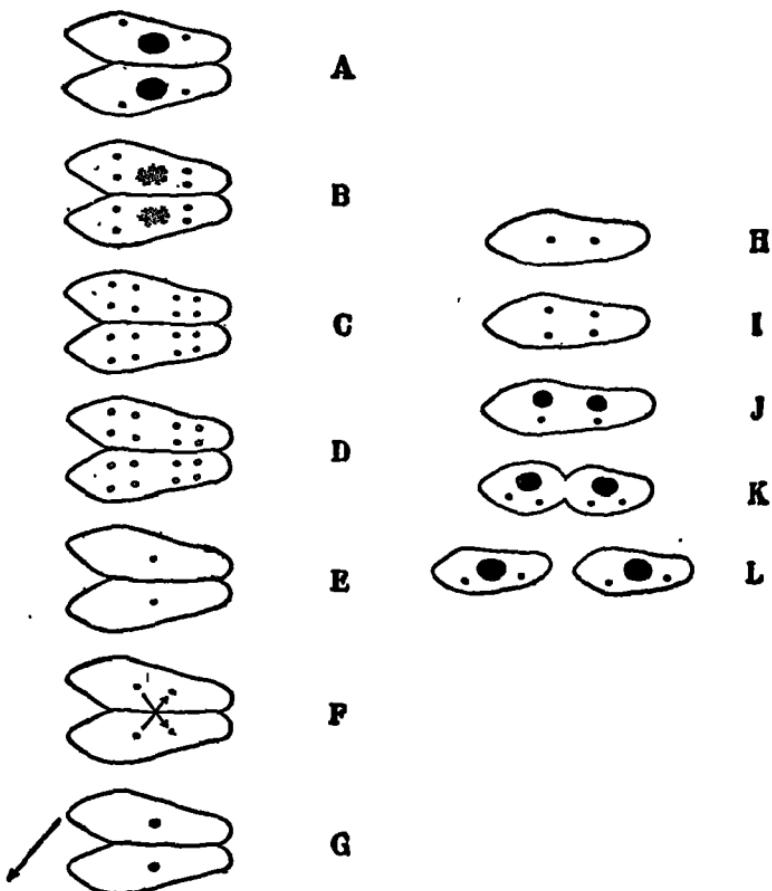


Fig. 179.—Diagram of the nuclear changes during conjugation in *Paramecium aurelia*.

A, union of two individuals along the peristomial region; B, degeneration of macronucleus and first division of the micronuclei; C, second division of micronuclei; D, seven of the eight micronuclei in each conjugant degenerate (indicated by circles) and disappear; E, each conjugant with a single remaining micronucleus; F, this nucleus divides into a stationary micronucleus and a migratory micronucleus. The migratory micronuclei are exchanged by the conjugants and fuse with the respective stationary micronuclei to form the synkarya. This is reciprocal fertilization. G, conjugants, with synkarya, separate (only one is followed from this point); H, first division of synkaryon to form two micronuclei; I, second reconstruction division; J, transformation of two micronuclei into macronuclei; K, division of micronuclei accompanied by cell division; L, typical nuclear condition restored. (After Woodruff.)

jugant begins to disintegrate and eventually disappears, while at the same time, the micronucleus of each conjugant undergoes two successive mitotic divisions, thus giving rise to eight micronuclei in each organism, of which seven disintegrate (Fig. 179, A-D). The one remaining undergoes a third mitotic division so that each conjugant has two micronuclei, of which one—the stationary micronucleus—remains in the organism, and the other—the migratory micronucleus—migrates into the other conjugant (Fig. 179, F). As a consequence each conjugant has both a stationary micronucleus that arose from its original micronucleus and a migratory micronucleus that arose from the micronucleus of its mate; which fuse to form a single micronucleus (*synkaryon*) (Fig. 179, G). The two conjugants now separate and the nucleus of each undergoes two successive divisions to form four nuclei (Fig. 179, I). Of these four nuclei two become macronuclei and two micronuclei (Fig. 179, J). The two micronuclei divide and at the same time the body constricts and divides so that eventually there are formed from each exconjugant two organisms each with a single macronucleus and two micronuclei (Fig. 179, K and L).

Conjugation is a process of reciprocal fertilization. In the preceding paragraph those two divisions which follow immediately on the fusion of the two conjugants (Fig. 179, B, C) were spoken of as mitotic divisions. In reality they are maturation divisions comparable to similar divisions in the maturation of spermatozoa and ova in the metozoa. As a result of these maturation divisions the single micronucleus which gives rise to the stationary and migratory micronuclei possesses only half the chromosome number present in the ordinary asexual individual and, in consequence, both the stationary and migratory micronuclei are true pronuclei with the gametic or reduced number of chro-

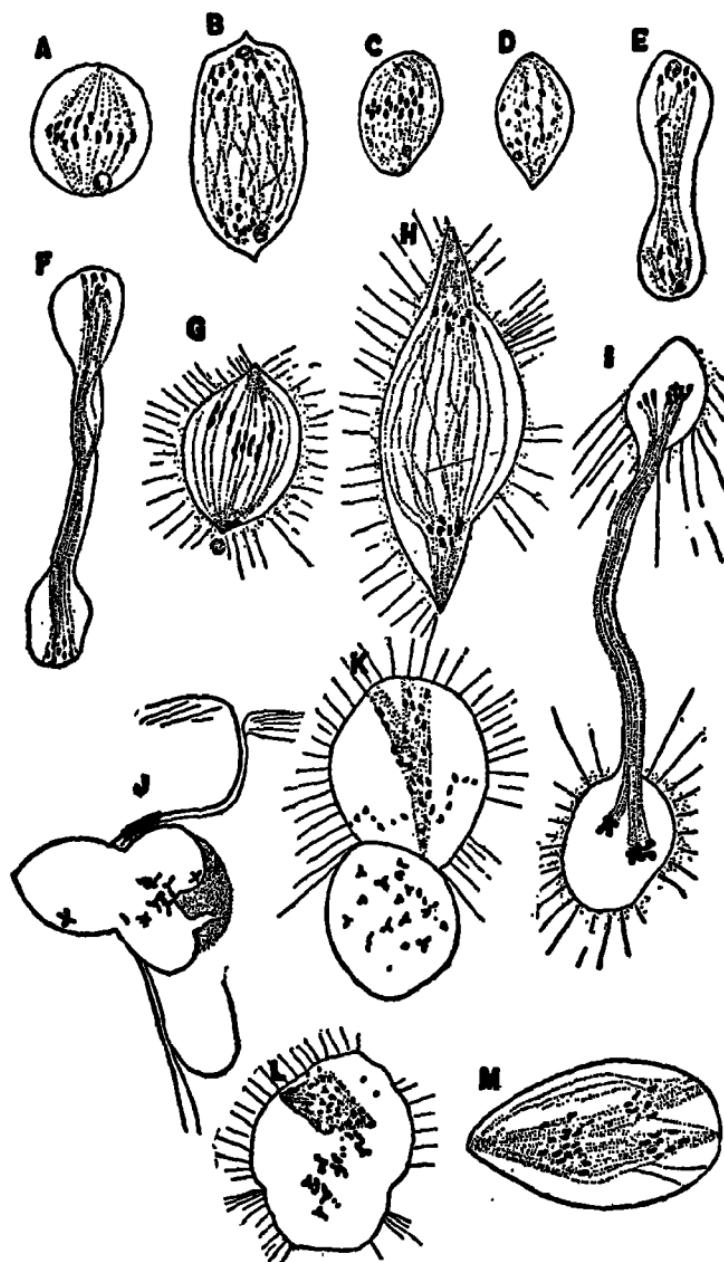


Fig. 180.—Reduction and fertilization in *Didinium nasutum*.
A and B, the first of the three divisions of the micronuclei, corresponding to fig. 179, B; also known as "first maturation" division.

mosomes.¹ In *Paramecium* the chromosomes are so numerous that it is impossible to tell just where reduction takes

¹ In the present discussion of micronuclear division and conjugation, the interpretation of Calkins and Cull (1907) has been assumed to be correct. Some doubt as to the validity of their work has been raised by Dehorne (1920), whose observations indicate that the chromatin in the nucleus of *Paramecium* is arranged in a thread rather than in separate chromosomes, and that the third micronuclear division, i.e., the one that produces the stationary and migratory micronuclei, is a reducing or differentiating division. These results are, however, so incompatible with and differ so markedly from the findings not only of Calkins and Cull on *Paramecium* but also of other investigators on other ciliates that we prefer to await their confirmation before accepting them. A very full summary of the work on nuclear division and changes in chromosome number during conjugation may be found in Calkins (1919).

In A a spindle has formed with 16 chromosomes; in B, each chromosome has divided so that each of the resulting micronuclei will receive 16 chromosomes.

C, D, E and F, the second or true reducing division of the micronuclei corresponding to fig. 179, C. C, spindle with 16 chromosomes; D, the chromosomes beginning to segregate into two groups; E, nucleus beginning to constrict and each daughter nucleus receiving only 8 chromosomes; F, latter stage in division.

G, H and I, the third micronuclear division which forms from a single nucleus, the migratory and stationary nucleus corresponding to fig. 179, F (before migration). G, each of the 8 chromosomes dividing; H and I, division of the nucleus to form two micronuclei each with 8 chromosomes.

J, the migratory nucleus passing through the membrane that separates the two conjugants, into the other individual, corresponding to fig. 179, F.

K, union of the migratory and stationary nuclei. The latter (above) is larger than the former.

L, the two nuclei almost completely united.

M, the first division of the nucleus (synkaryon) formed by the union of the migratory and stationary nuclei, corresponding to fig. 179, H.

The union is not quite complete so that at the right end two spindles can be seen. Each of the 16 chromosomes has divided so that each daughter nucleus will receive 16 chromosomes. (From Jennings, after Prandtl.)

place, although Calkins and Cull (1907) believe that it occurs at the first micronuclear division. In other forms, however, it has been observed and generally occurs at the second micronuclear division. In *Didinium nasutum*, according to Prandtl (1906), there are 16 chromosomes which are reduced to 8 at the second micronuclear division, and are again increased to 16 when the migratory and stationary nuclei fuse (Fig. 180). (See Gregory, 1923, for an account of reduction and fertilization in *Oxytricha fallax*.)

The process of conjugation, as has been outlined for *Paramecium*, will serve as a general statement of the process in the CILIATA, although there are many variations in the sequence of events. In many sedentary forms, like *Vorticella*, conjugation occurs between a large sessile form and a small motile form. During the process the latter is almost completely absorbed (all except the enveloping membrane, which drops off) by the sessile form, so that only one exconjugant results. In *Opalina* conjugation occurs between ciliated gametes. For a detailed description of these forms the reader is referred to more extensive works on the subject.

It has been previously noted in describing the INFUSORIA that the macronucleus represents the vegetative chromatin and is the nuclear material which takes part in the physiological processes of the cell. The micronucleus, on the other hand, represents the generative chromatin and is apparently a reserve of nuclear material from which the macronucleus is periodically replenished and also is eventually concerned with all genetical characters. In conjugation two things occur: (1) a replenishment of the macronucleus from a micronucleus and (2) a mixing of the chromatin of two different individuals. These two components most authors have either confused or failed to distinguish in discussing the effects of conjugation. Before taking up this question, however, it is necessary to state the conclusions reached by

Maupas (1888 and 1889) as a result of his long series of observations because our present mass of data has accumulated largely as a result of his classical works.

2. LIFE-CYCLE OF CILIATES AS MAINTAINED BY MAUPAS

Maupas observed that, if ciliates were kept for a long time under laboratory conditions without conjugation, they weakened, developed abnormalities and finally died. He believed that this was due to the fact that they were not allowed to conjugate and he maintained that their lives ran in definite cycles, the length of which was measured by the number of asexual divisions, and was different for different species but the same for different individuals of the same species. Beginning from the time a given ex-conjugant separated from its mate, this cycle was divided into three periods: (1) the agamic period or period of immaturity, (2) the eugamic period or period of sexual maturity, lasting until the beginning of senescence, and (3) the period of senescence which lasted until death. Maupas believed that the individuals which resulted from the divisions in the agamic period did not and could not conjugate because they were sexually immature. Any time during the eugamic period, however, conjugation could occur, and, if it did, the conjugants were reorganized or rejuvenated so that when they came out of the process of conjugation they were immature and began the agamic period again. If conjugation was prevented during this period, they inevitably began the period of senescence which led to death. The following is the life-history of *Stylonychia pustulata* according to Maupas as tabulated by Dobell (1914), from whom it is quoted.

Number of asexual divisions.

1.—Exconjugant

.

.

.

.

} Period of immaturity (agamic period)

130—Age of puberty

.

.

.

.

} Eugamic period

170—

.

.

.

.

} Period of senescence

316—Death

3. PHYSIOLOGICAL EFFECTS OF CONJUGATION

From this short review of Maupas' work and conclusions, it is evident that his idea of conjugation was that it had a physiological significance; that without it the protoplasm of a ciliate became old and finally died, and that with it the organism became "karyogamically rejuvenated." Maupas' work and theoretical conclusions have led directly to a very large amount of exceedingly valuable work—notably that

of Calkins, Enriques, R. Hertwig, Jennings and Woodruff. A more or less detailed summary of the results of these investigators may be found in Dobell (1914) and Jennings (1920). Suffice it to say that later work has not confirmed Maupas' original contention that without conjugation a line of asexually reproducing ciliates undergoes senescence and final death. As various workers perfected their technique, they found that they could keep cultures of a given species of ciliate for more and more generations without conjugation. What may be considered as a demonstration that ciliates may reproduce indefinitely without conjugation is afforded by the exceedingly painstaking experiment of Woodruff (see Woodruff, 1921). Woodruff began his work on *Paramecium aurelia* in 1907 and it is still in progress. At the time of his last report (Woodruff, 1921), he had maintained three races of *P. aurelia*, all of which had been derived from a single specimen and had undergone approximately 12,000 asexual generations without conjugation. Certainly no better experimental proof could be adduced that conjugation is unnecessary for continued asexual reproduction. Nor can we raise the objection, as some authors have done, that Professor Woodruff was dealing with a non-conjugating strain. Although he has prevented conjugation among the individuals of his main lines he has obtained epidemics of conjugation three times among side lines, i. e., lines derived from an individual which resulted from the division of one of the specimens on the main or parent line.

If conjugation is not necessary for continued asexual reproduction as postulated by Maupas, what then is its effect on the organisms? The key to this problem is probably found in the fact which has been noted above and which was early pointed out by Jennings, viz., that conjugation consists of two essentially different processes: (1) a mixing of the chromatin of two different individuals, and (2) the production of a new vegetative or "active" macronucleus. In order

to ascertain the effect of conjugation as a whole, these two processes must be separated and studied one at a time. The discovery of endomixis has made this possible.

4. ENDOMIXIS

Endomixis may have been discovered by Fermor (1913) but it is to the investigations of Woodruff and Erdmann that we owe most of our present knowledge of the process (see Woodruff and Erdmann, 1914, Erdmann and Woodruff, 1916, and Woodruff, 1917). In the same line of *Paramecium aurelia* that is now apparently living indefinitely without conjugation, Woodruff noted a definite rhythm in the division rate, and he and Miss Erdmann found that, correlated with this rhythm, the organisms regularly every 40 or 50 generations replaced their old macronucleus with a new one derived from the micronucleus. In other words, the "active" vegetative macronucleus was regularly replaced by chromatin from the reserve micronucleus just as in conjugation except that there was no admixture of any foreign chromatin. The details of this process in *Paramecium aurelia* which the authors termed *endomixis* is as follows: The nuclear apparatus of *Paramecium aurelia*, as has already been noted, consists of one macronucleus and two micronuclei (Fig. 181, A). During endomixis the macronucleus begins to disintegrate and finally disappears (Fig. 181, B). The micronuclei undergo two successive mitotic divisions producing eight micronuclei (Fig. 181, C) of which six disintegrate (Fig. 181, D). At this point the *Paramecium* divides so that each of the progeny contains a single micronucleus (Fig. 181, E). This single nucleus divides twice mitotically, producing four nuclei, two of which begin to become differentiated as macronuclei (Fig. 181, G and H) and the remaining two divide again to form four micronuclei (Fig. 181, I). At this stage in the process the organism contains two macronuclei and four micronuclei. A division

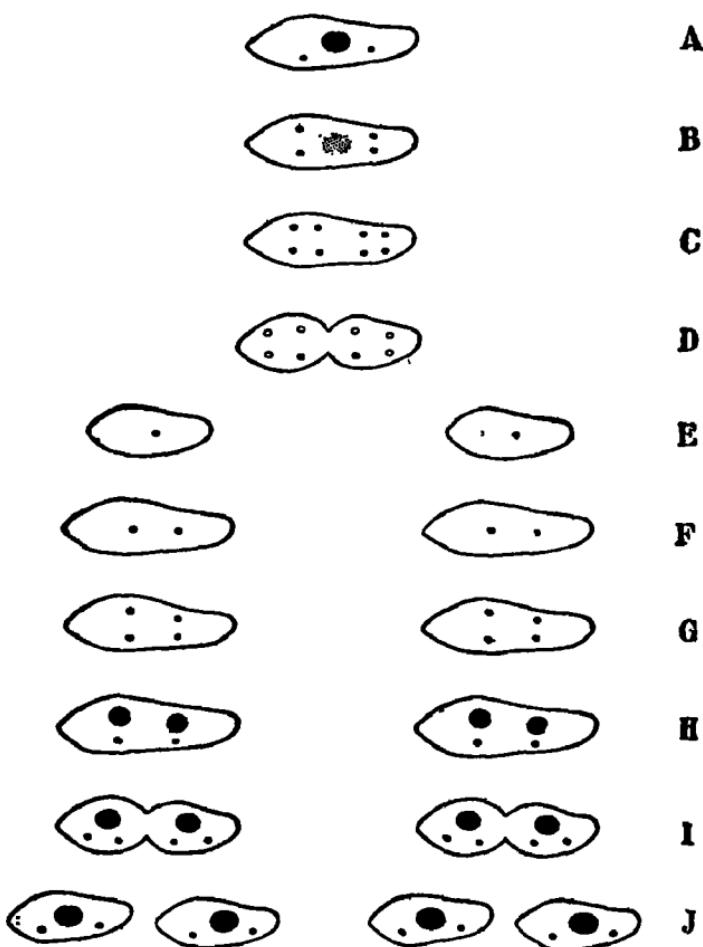


Fig. 181.—Diagram of the nuclear changes during endomixis in *Paramecium aurelia*.

A, typical nuclear condition; B, degeneration of macronucleus and first division of micronuclei; C, second division of micronuclei; D, degeneration of six of the eight micronuclei; E, division of the cell; F, first reconstruction micronuclear division; G, second reconstruction micropnuclear division; H, transformation of two micronuclei into macronuclei; I, micronuclear and cell division; J, typical nuclear condition restored. (After Woodruff.)

of the body ensues so that each of the progeny has a single macronucleus and two micronuclei which was the condition at the beginning of the process (Fig. 181, J). A similar process has been described by Erdmann and Woodruff (1916) in *Paramecium caudatum*, by Calkins (1915) in *Didinium* and in *Uroleptus* (Calkins, 1919). It is, therefore, probably a general feature of the life-history of the CILIATA.

What are the effects of endomixis? There is in the process of endomixis a rejuvenescence in the sense that the old macronucleus is "rejuvenated" or replaced just as in conjugation. Is it not possible that there is some truth in Maupas' idea of rejuvenescence? It may be that a ciliate is unable to reproduce indefinitely unless the old macronucleus is replaced by some of the reserve chromatin of the micronucleus. This can take place during either conjugation or endomixis. If this be true, it must be noted, however, that senescence is not the result of the prevention of conjugation *per se* but is the result of the failure to renew the macronucleus. According to this idea Woodruff's line of *Paramecium* continues to reproduce asexually without conjugation because its members periodically undergo endomixis. What would happen if endomixis as well as conjugation was prevented? An answer to this question is apparently given by the recent investigations of Calkins (1919, 1920, and 1921) on *Uroleptus mobilis*.¹ This ciliate does not undergo endomixis except during the encysted state. In this form, therefore, conjugation can be prevented by separating the pairs soon after each division and endomixis is known not to occur as long as the organisms do not en-

¹ Similar results as these outlined for *Uroleptus* have been obtained by Woodruff and Spencer (1921), and Moore (1922) in *Spathidium spathula*.

cyst. Calkins found that when conjugation and encystment were prevented the animals did not continue to reproduce indefinitely but became weak and eventually died. This senescence and death could be prevented either by encystment, which involved endomixis, or by conjugation. It seems, therefore, apparently impossible for a ciliate to reproduce indefinitely unless the old macronucleus is replaced periodically with some of the reserve or generative chromatin.¹ This replacement may occur during asexual reproduction by endomixis or during conjugation.² The next question which arises is what is the *peculiar* effect of conjugation? The replacement of the macronucleus cannot be considered as peculiar to conjugation because it also occurs during endomixis. The peculiar effect of conjugation must, therefore, be a result of the admixture of the chromatin of two different individuals because this can only occur as a result of conjugation. The effect of this admixture of the chromatin of two individuals—or fertilization—is fundamentally of a genetical nature and therefore it is necessary to go back and consider the genetics of these organisms.

¹ During the last few years several investigators have discovered and studied pedigree cultures of races of INFUSORIA which possess no definitive micronucleus. The individuals of these amicronucleate races never undergo visible endomixis and attempts to conjugate in most races are abortive. The question immediately arises: How can these races live indefinitely without any replacement of the macronucleus? Although cytological data is too meager to hazard an explanation at this time, the apparent macronucleus may represent actually a physical union of both macro- and micronuclear material. For a review of this work see Woodruff (1921) page 559.

² It is interesting to note that Calkins (1921) found that, if pairs of *Uroleptus* which had begun to conjugate were separated by cutting, the two organisms reorganized themselves and were "rejuvenated" although they were cut apart before reciprocal fertilization took place. Here it seems probable that the reorganization involved the replacement of the macronucleus just as in endomixis.

B. Genetics of the Ciliates

I. ASEXUAL REPRODUCTION

Many investigators suppose that the inheritance of diversities in organisms like the ciliates is a very simple phenomenon. It is generally assumed that the animal divides into two more or less equal parts and that each part receives part of the highly differentiated ciliary mechanisms which the original animal possessed, and also any teratological variations or malformations that it may have inherited or received during its life. This, however, is far from being the true state of affairs. When a ciliate divides to form two individuals, most, if not all, of its highly differentiated ciliated structures and highly specialized protoplasmic organs undergo a dedifferentiation and are absorbed to be redifferentiated in each of the offspring. The two offspring do, of course, receive certain things directly from the "parent," viz., their nuclei and one-half of the general cytoplasmic mass (also certain inclusions, such as symbiotic algae and food vacuoles), but, out of these substances, each has to produce anew most of its cell organelles and specializations.

To those who have assumed that inheritance in the ciliates is a mere mechanical "handing on" of the differentiations of the parent, it seems clear that acquired characteristics such as mutilations must be inherited. This, however, is not the case. If, for example, the anterior end is cut off of a *Paramecium*, at the first subsequent division the anterior daughter-individual shows the mutilation but by the third division the mutilation has been completely lost in the "making over" process which accompanies each division (from Jennings, 1920). Various teratological variations are often observed in cultures of ciliates, such as the projection on the aboral surface of *Paramecium* shown in figure 182. Such a malformation is often passed on mechanically to one of the daughter-individuals at the time of division, but it

never develops in the one which does not receive it mechanically nor does it appear in its progeny. For this reason in a long series of divisions with the consequent production of a large number of progeny, only a single organism will possess this malformation (Fig. 182), and often, after a few divisions, the single animal may lose the malformation due to the "making over" process just as was the case with a mutilation.

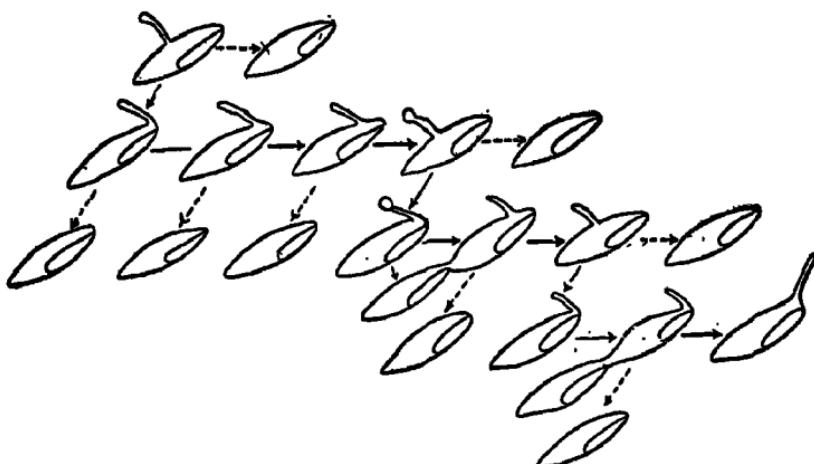


Fig. 182.—Diagram showing asexual reproduction and inheritance in a *Paramecium caudatum* bearing a large projection on its aboral surface. At each division only one individual received the projection; all other offspring and descendants were normal. The inheritance of this abnormality was followed by Jennings (1908) for 22 generations. (After Jennings.)

The conclusion reached, then, is "that the progeny do not receive their organs ready made from the parent; that on the contrary they start from just the same condition the parents did—an undifferentiated condition without organs, on the whole—and produce their characteristics anew. The ground for their producing the same characteristics as the parents lies precisely in this fact, that they start just as the parents did." (Quoted from Jennings, 1920, p. 49.)

Jennings (1908 a, 1909, 1911) first demonstrated that a given species of ciliate is not homogeneous but is composed of an assemblage of diverse stocks or races¹ which differ among themselves but which are *per se* constant. This is homologous to the conditions which obtain in the metozoa and higher plants (the "little species" of Jordan and the "pure lines" of Johannsen). The existence of these diverse stocks is well illustrated in *Paramecium*. Figure 183 shows the variations in size for eight stocks or pure lines of this ciliate. A consideration of the figure shows that while there are many variations in the size of different individuals of each pure line, the average or mean size of each pure line is different. Jennings has demonstrated, beyond doubt, that the mean size of a given pure line is inherited. Thus, if we select the largest and the smallest individual of a given family or pure line and let them reproduce by asexual division until there are several hundred progeny, it will be found that the progeny of the large individual and of the small one both exhibit *the same mean size*. In fact, Jennings (1909) attempted to modify the mean size of a given family by selecting, at each division, either the largest or the smallest individual, but he found that he could not alter the mean size by such procedure. The fact that a given species is composed of a large number of diverse stocks which differ among themselves but which breed true and are very constant *per se*, has been found to hold true for many different physiological and morphological characters in many different protozoa.

These data on the general process of inheritance during

¹ Throughout the present chapter the terms stock, family, race and pure line are used interchangeably to denote a number of organisms all derived by asexual reproduction from a single parent. Their present connotation is, therefore, slightly different from that of the same terms when applied to the higher plants and animals.

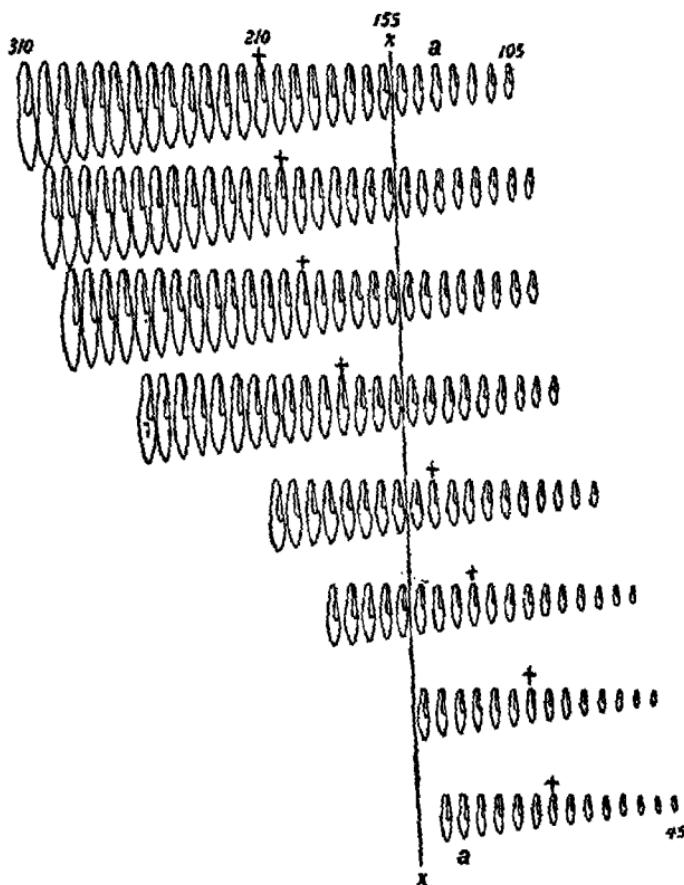


Fig. 183.—Eight diverse families of *Paramecium*, showing variations. Each row represents a single family, showing the maximum, minimum and intermediate sizes of individuals of the given family. The differences in size within the family are due to differences in growth and environment. The differences in average size between the families are hereditary. The numbers show the length in microns. The mean length, for the entire set together, is given by the perpendicular line at 155 microns. The mean size for each family is that of the individual above which is placed a + sign. (After Jennings.)

asexual reproduction and the fact that the descendants of a given organism breed true to a remarkable extent, make the genetical significance of conjugation less difficult to understand.

2. SEXUAL REPRODUCTION

As pointed out above, the process of conjugation consists of two processes, (1) a replacement of the old macronucleus by chromatin from the micronucleus, and (2) a mixing of the chromatin from two different individuals. The first process occurs also during endomixis and seems to result in a kind of physiological "rejuvenescence." At any rate, apparently a ciliate seems unable to reproduce continuously without a periodic replacement of the macronucleus either by endomixis or conjugation. It is, however, the second process which is peculiar to conjugation. What, then, is its effect on the organisms? In a long series of investigations Jennings (see Jennings, 1911 a, 1913; Jennings and Lashley, 1913, 1913 a; and for a general discussion see Jennings, 1920) has come to the conclusion that this mixing of the chromatin of two different individuals produces two results, (1) biparental inheritance and (2) the production of diverse stocks.

The demonstration of biparental inheritance is very difficult and is necessarily somewhat indirect. In *Paramecium* it is indicated by the following type of results: The two sets of progeny resulting from two individuals which have conjugated tend to be more alike after conjugation than before (Jennings and Lashley, 1913 a). Similar conditions are found in regard to rate of fission, vitality (Jennings and Lashley, 1913) and certain structural abnormalities (Stocking, 1915). The tendency of the two individuals from a conjugating pair to produce sets of progeny which are alike is just what is to be expected since,

as a result of conjugation, each possesses the same nuclear constitution.¹

Besides these data, which show that the two sets of progeny arising from a single pair tend to be alike, other data indicate that if conjugation occurs between the individuals of a given pure line the different pairs (*not the different individuals of a single pair*) tend to be different. The demonstration of this second result, viz., the production of diverse stocks, is due chiefly to the work of Jennings and may be exemplified by the following experiment taken from Jennings (1920) (see also Jennings, 1913). "We begin with a set of individuals all alike, all derived by fission from a single parent. We study their rate of fission; taking 174 separate lines of descent we find it to be extremely uniform. For periods of 21 days we find the number of fissions of the first 24 lines to be those given under "Non-conjugants," *a* and *b*, in the following table. (The two individuals, *a* and *b* of the non-conjugants, had begun to mate, but were separated before the mating process occurred.) In these 24 lines the number of fissions in 21 days varied only from 21 to 26; it is on the whole very uniform. One finds little or no indication of inherited differences between the families; they run very evenly.

"Now we allow a large number of the individuals to con-

¹The term "biparental inheritance" is applied to this phenomena by Jennings because it results from the fact that each exconjugant receives its ultimate nuclear organization from *both* itself and its mate.

Our statement that the two exconjugants derived from a single pair possess the same nuclear constitution depends upon the correctness of the view that the third micronuclear division which produces the stationary and migratory nuclei is a straightforward mitosis and not a differential or reducing division. Dehorne (1920) denies this in his work on *Paramecium*, but it is supported by the work of other investigators on other ciliates and is in line with the experimental results obtained by Jennings on *Paramecium*. (See footnote at bottom of page 453.)

jugate, then follow the rate of fission for 88 lines derived from these mates. We find that there are now great differences between the separate families with respect to fission rate. In the table the two last columns, headed "Conjugants," give the number of fissions for 24 lines descended from pairs of conjugants, for the same 21 days as for those that have not conjugated. The two sets (Non-conjugants and Conjugants) were kept throughout under the same conditions.

"In the descendants of those that have conjugated the number of fissions in 21 days varies from 8 to 30, as compared with 21 to 26 in the others. There are now strongly marked differences between the different families that have descended from those that have conjugated."

Comparative fission numbers in 21 days of Conjugants and Non-conjugants derived from a single race E (from Tables 34 and 35; Jennings, 1913). In the non-conjugants, a and b represent two individuals that had begun to mate, but were separated before mating was accomplished. In the conjugants, a and b are the two mates from one pair.

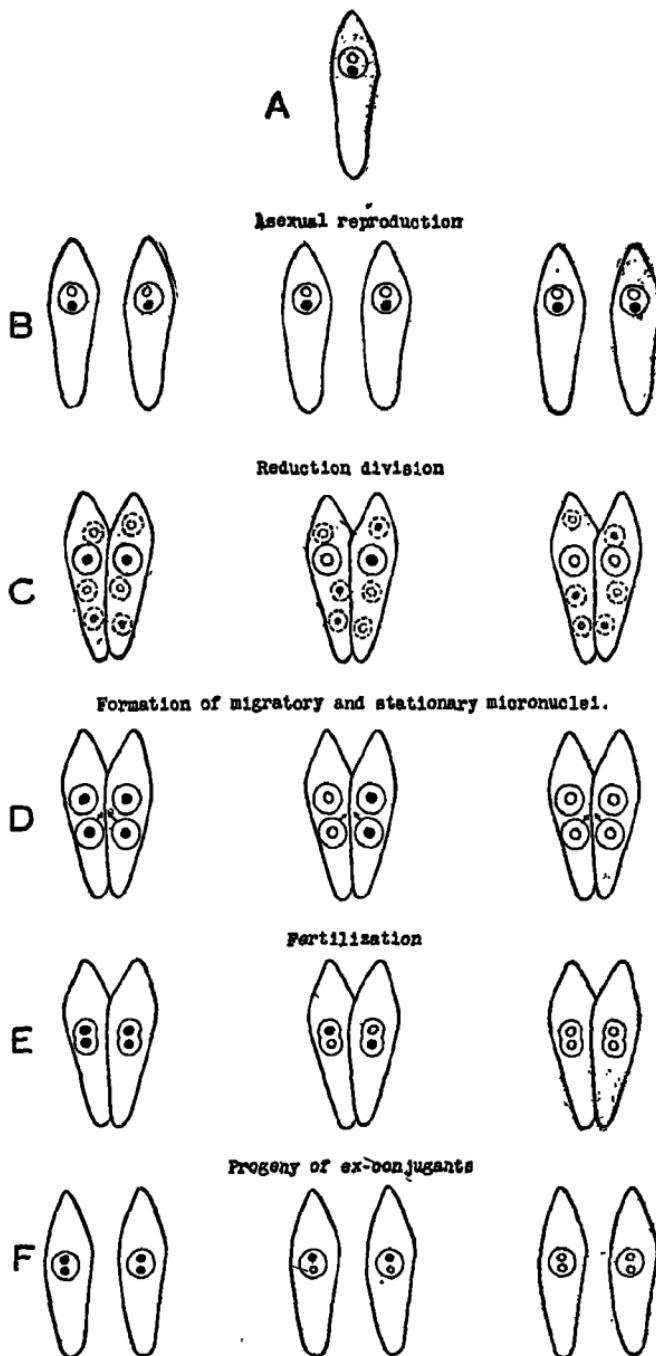
Non-conjugants			Conjugants	
Pair	a	b	a	b
1	25	23	30	29
2	24	23	25	24
3	24	24	29	28
4	24	25	10	9
5	23	24	8	10
6	22	25	24	24
7	22	26	29	28
8	21	25	25	24
9	24	25	30	27
10	22	26	26	26
11	23	24	28	27
12	25	24	11	9

Thus conjugation results on the one hand in the production of similarities between the progeny of the two different members of a single pair and on the other hand in the pro-

duction of dissimilarities between the progeny of different pairs.

It may be at first difficult to see how conjugation can result in a homogeneous pure line splitting up into heritably diverse lines. This result is probably produced by the segregation of nuclear materials at the time of the reduction division of the micronucleus and their recombination at the time of the fusion of the two micronuclei.¹ The manner in which this may bring about the production of new and diverse lines is illustrated in the diagram in figure 184. For the sake of clearness *Paramecium* is assumed to have a single micronucleus with a single pair of diverse chromosomes, which are represented one as solid and the other in outline (Fig. 184, A). All of the progeny which arise from this individual by asexual reproduction—the race, family or pure line—will possess a similar pair of diverse chromosomes (Fig. 184, B) due to the fact that the nuclei in all such individuals arise by mitotic division in which each parent chromosome is accurately halved. At one of the two maturation divisions which follow immediately after the fusion of the conjugants, however, the chromosomes are not halved but each daughter nucleus receives only one of the pair. As a result there are formed four nuclei each with only one chromosome, two having a solid and two an outline chromosome (Fig. 184, C). Then a step of greatest importance takes place, viz., three

¹ Miss Erdmann (1920) and (1922), as a result of her work on *Paramecium aurelia*, maintains that a pure line splits up into heritably diverse lines as a result of endomixis as well as conjugation. As endomixis apparently involves only straightforward mitotic divisions of the micronucleus with no reduction of the chromosomes and subsequent fertilization it is difficult to see how it can produce any such genetical result. If Miss Erdmann is correct in the interpretation of her results, it would indicate that endomixis involves some process for producing new combinations—such as autogamy, or possibly the unequal distribution of the chromatin elements, such as non-disjunction—rather than a simple reorganization.



micronuclei in each conjugant are discarded (Fig. 184, C). The three possibilities that may result are shown in figure 184, C. Both conjugants may retain a micronucleus with a solid chromosome, or both may retain one with an outline chromosome, or one may retain one type and the other the other type. The formation of the migratory and stationary micronuclei is a straightforward mitotic division so that they are simply duplicates of the micronucleus which is retained (Fig. 184, D). At the time of fertilization a migratory and stationary micronucleus fuse and bring the chromosome number back to two (Fig. 184, E). The possible combinations which may result are shown in figure 184, E. In other words, by beginning with two diverse chromosomes some ex-conjugants are obtained with the same diverse pair, some with a pair of one kind of chromosome, and some with a pair of the other kind, or, in other words, two entirely new combinations. In *Paramecium* where the reduced chromosome number is approximately 165 (Calkins and Cull, 1907) the number of possible combinations is exceedingly large. It is to be noted, of course, that if the members of a given pure line are completely homozygous, i.e., if each pair of chromosomes consists of members exactly alike, conjugation cannot result in the origin of new combinations. Such a

Fig. 184.—Diagram showing how conjugation may produce its genetical effects.

A represents a hypothetical ciliate with a micronucleus containing a single pair of diverse chromosomes. B indicates that all of the asexual progeny of A will contain this same chromosomal complex. C indicates the effect of reduction prior to fertilization. The one micronucleus which survives after reduction will contain one of the original pair of chromosomes—it being a matter of chance which one. The three possible combinations in the conjugants are shown. D indicates the fact that the stationary and migratory nuclei of each individual have the same chromosomal complex as the reduced nucleus shown in C. E shows the combinations which result from the reciprocal fertilizations indicated in D. F shows the type of progeny from each ex-conjugant. Note that the members of a single pair are alike but the progeny of different pairs may be diverse. (Original.)

condition could readily occur in such a hypothetical case as that given in figure 184 where there is only one pair, but it is almost inconceivable in a form like *Paramecium* where there are approximately 165 pairs.

In this connection it is well to note that some authors have felt that there is an incongruity in assuming that conjugation tends to make the two sets of progeny from a given pair more alike after conjugation than before and at the same time tends to produce within the same pure line diverse strains which are heritably unlike. A consideration of the facts which we have just presented shows, however, that there need be no contradiction in these processes. The reduction of chromosomes at the beginning of the conjugation and the subsequent recombination at the time of fertilization accounts for the fact that different sets of conjugants may come out of conjugation with entirely different combinations of chromosomes although they began with the same. At the same time, however, the two members of any given pair will always have the same combination (Fig. 184, E and F).

C. Genetics of the Protozoa in General with Especial Reference to Entozoic Forms

The above discussion of some of the fundamental questions of inheritance and the physiological effects of conjugation in the ciliates makes it possible to take up a more general consideration of genetical problems. At the very beginning it is well to point out that in the other protozoa—the MASTIGOPHORA, SARCODINA and SPOROZOA—endomixis is unknown and conjugation is much simpler than in the INFUSORIA. Both of these facts are probably correlated with the fact that there is no such segregation of the nuclear material into a macro- and micronucleus as is the case in the INFUSORIA. There is no evidence at all that protozoa belonging to the MASTIGOPHORA, SARCODINA and SPOROZOA cannot multiply

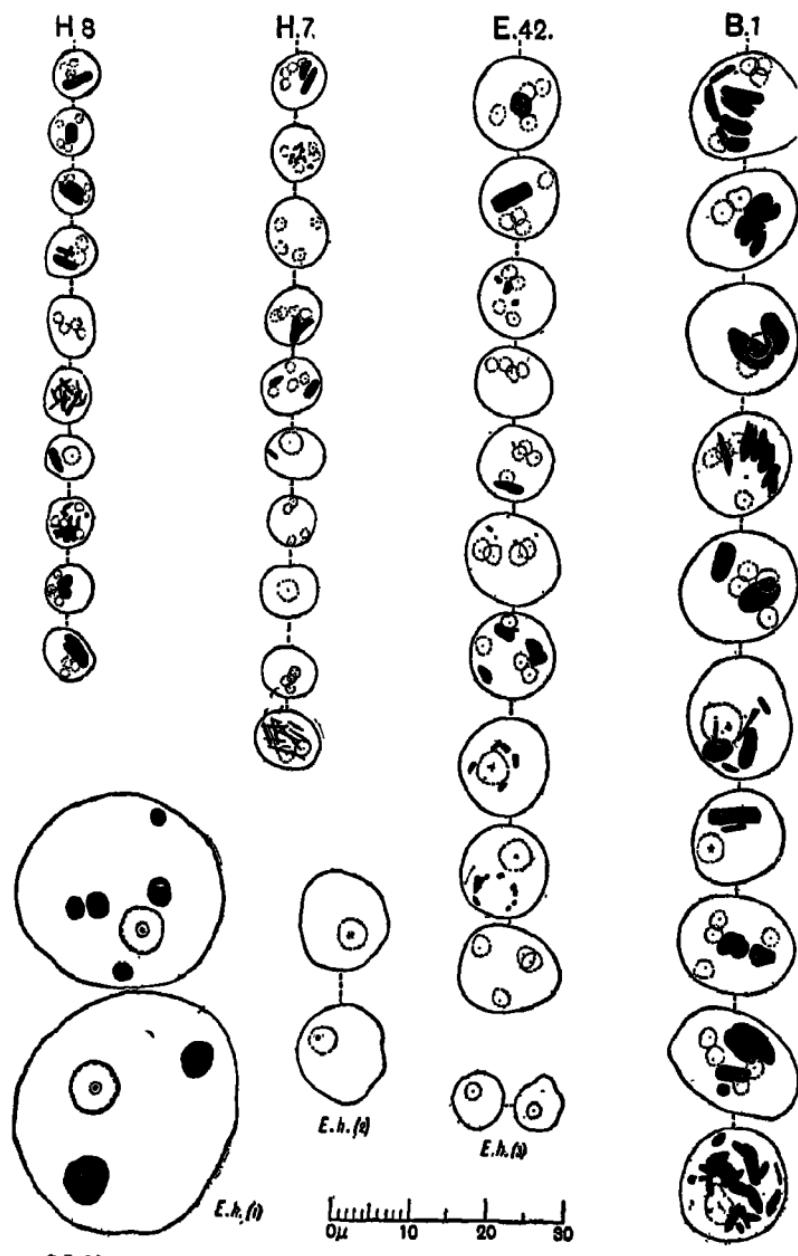
indefinitely without the intervention of conjugation provided they are supplied with suitable cultural conditions.

The general nature of the process of inheritance is the same in the other groups of protozoa as it is in the ciliates. There is very little mechanical "passing on" of differentiations from parent to offspring. All species that have been studied have proved to consist of a large number of diverse stocks which have proved to be very resistant to any attempt to modify them permanently. Conjugation has likewise proved to be the means of increasing the diversity of stocks (for a detailed discussion of these facts the reader is referred to Jennings, 1920).

I. HERITABLE VARIATIONS AND THE PRODUCTION OF NEW STRAINS OF PROTOZOA

It is, of course, perfectly obvious that, to the evolutionist, the question of the origin and production of heritable variations is of first importance because, if he can find inherited variations, he can explain evolution and adaptation by means of natural selection. The question is also of prime importance to the parasitologist. A great deal of work has been done in regard to the increase or decrease of virulence following passage through animals, changed laboratory conditions, etc., the production of poison resistant strains, the origin of strains of pathogenic organisms possessing new characteristics, etc. All of these very important phenomena are related to this problem of the origin or production of heritable variations and can best be attacked from a genetical standpoint.

It has already been emphasized that each species is composed of many diverse stocks or pure lines but that each of these stocks breeds true and is extremely constant in its average characteristics. This composite character of species has been found to hold true for many free-living forms



C. D. del.
Fig. 185.—Outline drawings of cysts of *E. histolytica*, showing the difference in dimensions of ten cysts taken at random from each of

(see the work of Jennings and his co-workers, Jollos, 1921, etc.). The same holds true for the entozoic forms that have been studied. Dobell and Jepps (1918) have shown that the species *Endamoeba histolytica* is composed of a number of distinct races which are distinguishable by the size of the cysts that they produce (Fig. 185). These different strains were first recorded by Wenyon and O'Connor (1917) and their existence is indicated by the work of several other investigators. There can be little doubt that a similar condition holds for *E. coli* (see Dobell, 1919) and probably for all of the entozoic amoebæ of man which produce cysts. The similar existence of diverse races in regard to size is indicated in *Trypanosoma lewisi*, *T. diemyctyli* and *Giardia agilis* by the works of Taliaferro (1921), Hegner (1921) and Hegner (1922) respectively. The most notable characteristic of these races, strains, or pure lines is their constancy. It is true that the individuals of a given stock may show many variations due to age, environment, etc., but these variations are purely temporary and are not inherited. As long as the pure line is reproducing by asexual division without the admixture of foreign chromatin through conjugation its average characteristics are exceedingly constant. At first all attempts to alter the characters of protozoa and other organisms reproducing by uniparental reproduction were unsuccessful. Thus Johannsen (1903) working with self-fertilizing beans, Barber (1903) with asexually reproducing bacteria and yeasts, Jennings with *Paramecium*, Agar (1914) with certain parthenogenetic crustacea, and Lashley

four different cases of infection (*H.8.*, *H.7.*, *E.42*, and *B.1*). Compare with figure 183. This indicates that the species *E. histolytica* is composed of a number of distinct races distinguishable from one another by the size of the cysts which they produce. *E. h. (1)*, two large vegetative forms from a strain forming cysts similar in size to those from case *E.42*; *E. h. (2)*, two precystic forms from a similar strain; *E. h. (3)* two precystic forms from a strain forming cysts similar in size to those of case *H.8.* (After Dobell and Jepps.)

(1915) with *Hydra*, which reproduces asexually by bud formation, and many others were unable to change the character of pure strains by selection.¹

The sum total of this work as Professor Jennings (1922) said in a recent address, "was astonishingly simple and clear. As to the origin of hereditary variations, it resembled the famous chapter on the Snakes of Ireland. It summed itself, in effect, in the succinct, sufficient, exhaustive proposition that there is no inherited variation; hence no origin of such variation." All laboratory experiences, however, point to the conclusion that pathogenic microorganisms do apparently change in their characteristics. Some of the ways in which these organisms may apparently change their hereditary characters without any actual changes, and a review of some of the work, which tends to show that, after all, some permanent hereditary changes may take place in them, follow.

a. Selection Within a Population of Mixed Stocks

The fact that a species is composed of an assemblage of diverse stocks accounts for most of the supposedly heritable alterations of the hereditary characteristics of organisms. If a given laboratory strain of organisms is composed of different stocks, some of these will be more fitted to survive under some conditions and others under other conditions. If the environment is radically changed, it will tend to kill off some stocks while others will tend to survive. This will apparently change the character of the laboratory strain. As a matter of fact, however, these changes in environment have not changed the hereditary characters of a single stock or race, but have simply changed the proportion of certain races in

¹ We mention here higher organisms reproducing by asexual fission like *Hydra*, or by self-fertilization like the bean, or by parthenogenesis like certain crustacea, because the offspring receive their hereditary constitution from a single parent and hence are analogous to uniparental reproduction in the protozoa.

relation to others. This will undoubtedly be found to be at the bottom of many of the effects of animal passage, different media, etc., on laboratory strains of protozoa and other pathogenic microorganisms. Were the laboratory strains derived from a single ancestor, i. e., a pure line, they probably would not show any of these quick changes that are apparently inherited. Of course, even the pure line will change as a result of environmental action, but, in this case, it will quickly revert to its original condition when returned to its original environment—in other words, the environmental effects are not permanent but temporary responses to the environment.

b. *Conjugation*

The fact has already been emphasized that conjugation causes the appearance of new strains of organisms. These new strains are not the result of any change of the hereditary characteristics but are simply the results of new combinations resulting from biparental inheritance. They resemble the ever-changing characteristics which result from the combinations and recombinations in sexual reproduction of higher organisms. Where conjugation occurs in pathogenic organisms, strains with new or different combinations of characters probably occur in this manner. The results of Taliaferro (1921) indicate that some such process may occur when *Trypanosoma lewisi* develops in its invertebrate host, the flea.

c. "*Dauermodifikationen*"

Under this term Jollos (1921) has included a number of persistent modifications which last throughout many generations, but which, according to him, are not permanent in the same sense as mutations. By subjecting pure lines or races of *Paramecium* to arsenious acid and other chemicals over periods lasting for years, Jollos (1921) found it possible to raise the races resistance to these substances. This increased

resistance is transmitted through a number of asexual generations after the organisms are put back into their original cultural medium (and hence periods of endomixis), but is generally lost immediately after conjugation. In the case of certain calcium salts, the increased resistance may even survive one or two conjugation periods. As pointed out above, Jollos does not think these persistent modifications are permanent heritable variations in the sense of mutations. It is, however, interesting to note that the longer a race is subjected to a given substance, the longer the resistance is transmitted after the race is returned to the original cultural fluid. This leaves open the possibility that permanent hereditary variations may be produced in this manner.

To this category probably belong the phenomena of poison resistance which has been noted in various pathogenic organisms. Ehrlich (see Ehrlich, 1909) was the first to point out the significance of these phenomena in trypanosomes. If a host which is infected with these organisms is treated with a dose of an arsenical which does not completely cure the infection, the trypanosomes which repopulate the blood stream possess a higher resistance to arsenic than they did at the beginning. If the process is repeated often enough, the trypanosomes become so resistant that they are unaffected by doses which are sublethal to the host. Similar results have been obtained with dyes and other chemicals. The practical importance of this modification of the pathogenic organisms is self-evident. Some investigators have held that these results are due to the fact that an infection is probably a mixture of stocks and that the less resistant stocks are annihilated by the poison. The modification seems, however, to be an actual change in the organisms because Oehler (1913) has succeeded in obtaining the phenomenon in a pure line infection. Other investigators have maintained that it was a permanent change in the same sense that a mutation is permanent. In support of their contention they have

pointed out that the resistance survives many passages of the virus through untreated animals. Later work has shown, however, that it is not permanent but that in time the resistance of the strain reverts to normal. Nevertheless, it is remarkable how long the resistance lasts, sometimes surviving 400 passages of the trypanosomes through untreated animals—which means countless millions of asexual generations. (See Laveran and Mesnil, 1912.) The resistance is also lost if the species of laboratory animal in which the virus is living is changed (for a summary of this work see Dobell, 1912), and, according to Gonder (1911), it is lost if the trypanosomes are passed through their invertebrate host. All of these facts tend to place the phenomenon of poison resistance or fastness in the category of "Dauermodifikation."

Before leaving the question of poison resistance it is interesting to note that the recent work of Neuschlosz (1919-1920) on *Paramecium* indicates that the physiological basis of this resistance lies in the fact that resistant strains acquire the capability to destroy the poison to which they become resistant. The results of Neuschlosz on *Paramecium* also indicate that when a strain becomes resistant to quinine this resistance can be broken by the addition to the medium of an arsenical compound. This is in line with the experience of certain workers in the treatment of certain strains of malaria which had become resistant to quinine. It is, however, the only known case in which the resistance to one chemical can be broken by subjecting the organisms to another chemical.

d. Hereditary Variations

Comparatively few cases of actual permanent hereditary variations have been observed in the protozoa and kindred organisms. It has been shown, however, that sometimes a pure line of organisms will produce a sport or mutant which will breed true and thus give rise to a heritably new race. Mutations

tions of this character have been observed in various free-living protozoa and some bacteria. For a review of this phenomenon the reader is referred to Jennings (1920). It is not surprising that such occasional large variations are observable in the protozoa because they are well known in all of the higher metazoa and plants which have been sufficiently studied. Are there not also minute hereditary variations, which, under suitable conditions of selection, would cause a gradual separation of a single race into diverse races? The previous work on selection, which was noted above, answered this question definitely in the negative. After his first unsuccessful attempt to obtain any effects of selection in *Paramecium*, however, Jennings (1916) worked with the rhizopod *Diffugia*, and, in an exceedingly long and painstaking investigation on this form, he found that a pure stock or race could be split up into several diverse races by selection. Similar results were obtained by his co-workers: Middleton (1915) with *Styloynchia*, Root (1918) with *Centropyxis*, and Hegner (1918, 1919, 1919 a) with *Arcella*. As Jennings (1920) sums up this work: "Inheritance is very exact, but when we study a family for many generations we find that it is not absolutely precise, for minute hereditary variations gradually appear, and the single race separates into many hereditarily diverse races."

The bearing of the occurrence of both large mutations and minute variations on the origin of new strains of parasitic organisms is obvious. Before leaving this subject it is but fair to point out that Jollos (1921) has suggested that these minute variations, such as Jennings found in *Diffugia*, are probably "Dauermodifikationen"; that Miss Erdmann believes that they are essentially of the same nature as those which she supposes (see footnote, page 469) to occur in endomixis; and that other investigators feel that they are not common in higher organisms and only exist in the protozoa because of the peculiar nuclear organization of the latter.

CHAPTER XV¹

THE DIAGNOSIS OF THE INTESTINAL PROTOZOA

A. Methods of Fecal Diagnosis

A detailed description of the different protozoa which live in man has been given in the preceding sections of this book. A certain number of these organisms produce definite diseases in man and a knowledge of most of the remainder is necessary in order to distinguish them from their disease-producing relatives. The diagnosis of any disease which is produced by a protozoon rests fundamentally on the finding and recognition of the specific protozoon. The method by which this is done varies chiefly with the locality in the body in which the parasite is found and for that reason has, in most cases, been considered under each species or group of species. Although the intestine of man is inhabited by representatives from all four of the classes of PROTOZOA, the diagnosis of all of them requires essentially the same procedure. For this reason a general account of fecal diagnosis has been deferred until all of the intestinal forms have been described. The diagnosis of the various species of intestinal protozoa, in particular the amebæ, is exceedingly difficult and can only be done quickly and accurately after long practice. In addition, the beginner is confronted with the fact that feces contain many confusing elements of a non-protozoan character, and after they have left the body form a

¹ By W. H. Taliaferro.

very favorable medium for the development of free-living coprozoic protozoa. It is with the hope of helping the beginner in overcoming some of these difficulties, as well as outlining the general methods of fecal diagnosis, that the present chapter is prepared.

I. COLLECTION OF FECAL SAMPLES

The prime prerequisite of fecal samples is that they be as fresh as possible. The active stages of the intestinal protozoa (with the exception of *Trichomonas*) die so quickly, generally in a few hours after they are passed from the body, that it is best to consider any motile protozoa which occur in a stool over a day old as being probably coprozoic rather than entozoic. Cysts, on the other hand, while they are easier to diagnose immediately after they have left the body, survive much longer and can be found in stools sometimes after several weeks. In field work and fecal surveys in which it is often impossible to obtain fresh samples, practically all diagnoses are made from cysts.

A number of the older writers maintained that active protozoa could encyst outside of the body and some even advised that stools containing motile forms be kept for some time to allow the motile forms to encyst so that a diagnosis could be made on them. Recent work, on the other hand, indicates that encystation never occurs outside the body, and that even immature cysts when passed probably show no further development. In regard to *E. histolytica*, Dobell, 1919, states, "Those [i. e., cysts] containing less than 4 nuclei never develop to maturity outside of the body, and usually die much sooner than the mature cysts. Even cysts with dividing nuclei do not complete their nuclear divisions. Spindle-figures and other stages arrested in division can be seen to remain unchanged within the cysts until degeneration takes place." Obviously, then, unnecessary postponement of the examination of the stools is not only unnecessary but

also disadvantageous in that it favors the degeneration of the amebæ and the development and increase of coprozoic forms.

When possible, it is always desirable to obtain whole stools as samples so that a thorough macroscopic examination can be made. If specimens have to be shipped through the mail or where it is only feasible to obtain small samples, it is imperative to make the sample representative of the whole stool. From a homogeneous stool a sample may be taken from any part, but otherwise a sample should be made up of bits of each of the different parts of the stool so that microscopic preparations can be made from different areas.

The actual type of container is unimportant provided it is convenient to use and does not leak. Two types are in use in this laboratory. For entire stools or large samples, large-mouthed containers of paraffined-pasteboard (Fig. 186, A) are easily obtained in varying sizes (the $\frac{1}{2}$ pint is the most useful) as they are commonly used at soda fountains as ice-cream containers. Furthermore, their cheapness makes it possible to throw them away after the sample has been examined. For smaller samples, varying sizes of tin pill-boxes are used. If the sample has to be shipped by mail it is essential that the material be in a double container and consequently the fecal sample is generally put in an ordinary tin mailing case which, in turn, is placed in a wooden or pasteboard mailing case.

In the actual collection of the feces it is essential both that the container be clean and dry and that the stool be kept from contact with injurious substances. Wherever possible, the feces should be passed directly into the container. In hospitals it is necessary to see that the receiving receptacle be free from antiseptics, etc. A common practice of defecating in the water closet and then collecting the feces in a container is particularly bad because contact with urine tends to kill

the entozoic forms and wetting favors the development and increase of coprozoic protozoa.

The consensus of opinion is that a naturally passed stool is preferable to one obtained with purgatives. If a purge is

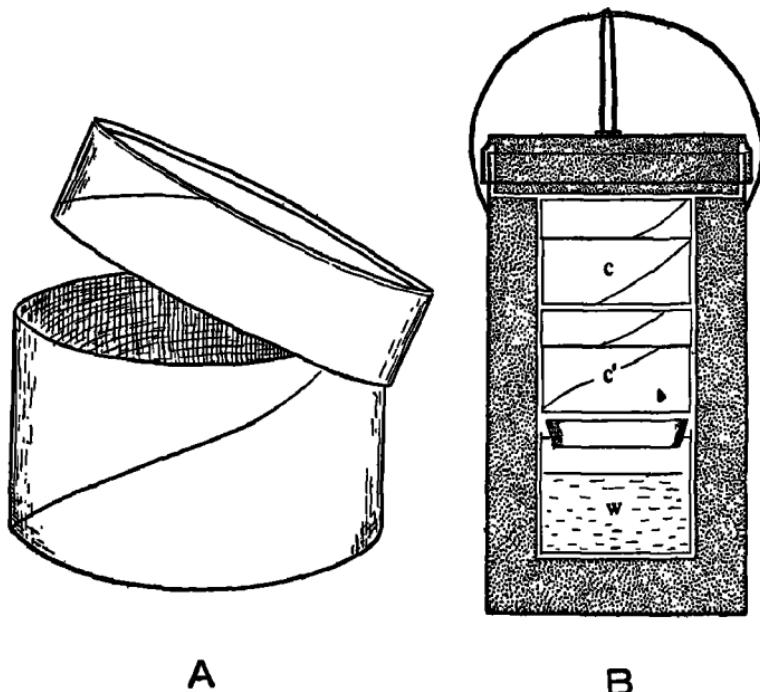


Fig. 186.—A, one-half pint container made of paraffined pasteboard which is supplied to the trade as an ice-cream container and which admirably serves as a fecal container. B, an improvised incubator which keeps two fecal samples at approximately body temperature during transport. The large container or "Thermopak" is an insulated receptacle made to receive the type of container shown in A and is supplied to the trade for the transportation of small amounts of ice-cream. *w*, small can of hot water; *c* and *c'*, two half-pint containers. A $\times \frac{1}{2}$ and B $\times \frac{3}{4}$. (Original.)

necessary when, for example, the large active forms are desired from a person passing formed stools, a saline should be used. Any oils fill the stool with oil droplets which at best are annoying and may be confusing.

For the study of the motile stages, fecal specimens must either be examined immediately or be kept warm in an incubator until they can be examined. In this laboratory we either make arrangements for the patient to defecate in the laboratory or fix an improvised incubator which may be easily carried by hand. A so-called Thermopak,¹ which is simply an insulated receptacle in which the ice cream containers described above can be conveniently carried to keep ice cream from melting, is ideal for this purpose. If a small can of hot water be placed in it along with one or two of the $\frac{1}{2}$ pint containers, the feces will be kept at approximately body temperature for many hours. (Fig. 186, B.)

In the routine examination of motile specimens, it is generally sufficient to keep the stool warm up to the time of examination. It is unnecessary to take precautions to warm the slide during examination. In careful work on these forms, however, it is essential to keep them at approximately body temperature during the time they are studied. (This is, of course, unnecessary for cysts.) In many laboratories various types of warm stages are available for this purpose or, in lieu of these, a fairly efficient makeshift can be made either by placing a heated coin on one end of the slide or by warming the slide at intervals over an electric lamp or radiator. In the latter case, care should be taken not to overheat the slide.

2. SMEAR METHOD OF DIAGNOSIS

By far the commonest and most useful method of diagnosis of intestinal protozoa is the smear method. Smears are made by emulsifying a small bit of feces in a small drop of saline or staining solution on a slide. A wooden toothpick is very convenient for picking up and emulsifying the feces and can be thrown away after the smear is made. In formed stools

¹ Manufactured by the Thermopak Co., New York.

a smear should be made from both the interior and exterior of the fecal mass. The latter procedure is desirable since formed stools are encased in a thin coat of mucus in which the protozoa and their cysts are often entrapped and carried out of the body (Boeck in Boeck and Stiles, 1923). In stools, not homogeneous, successive smears should be made from its different parts. Then a cover glass (preferably a large No. I square) is placed over the drop of emulsion and the mount is ready for microscopic examination. Only experience will show exactly how thick to make the fecal emulsion. One too thick or too dense obscures the protozoa, whereas one too thin causes a waste of effort in searching for the protozoa. The common tendency is to make the mount too thick. Ordinary newspaper print should be clearly discernible through it. In making any preparation of any intestinal protozoa for study or diagnosis, it is absolutely essential that the material never be dried at any point in the technique. Techniques used in the study of the malaria organisms or of trypanosomes which require drying of the specimens at some stage are never applicable in the study of the intestinal forms. The examination of the mount may be systematized by the use of some type of mechanical stage. All cysts and active protozoa can be clearly seen with a 4 mm. objective and No. 5 ocular, although in finer details an oil immersion and a higher ocular are necessary.

Before any smears are made, the stool should be examined macroscopically and note taken of its consistency and color and the presence or absence of mucus and blood. If either mucus or blood should be present, they should be examined especially.

It is very essential that the protozoa be observed alive before they are stained, since many mistakes may thus be eliminated, especially in diagnosing the motile stages. Furthermore, after practice, a number of species can be identified in this way alone. Such an examination may be carried out

by making a smear, as described above, with physiological saline. For ordinary work a warm stage is unnecessary. Some investigators (see especially the recent work of Boeck in Boeck and Stiles, 1923) advocate the use of a neutral red or eosin in the saline to aid in finding the living specimens, and especially to ascertain whether a given sample is negative or positive for protozoa. In a concentration of 1:10,000, the active protozoa are not killed and may even be stained; and, besides, all the fecal debris is stained while the cysts are not, so that the latter stand out as bright refractile spherules on a pink background.

After a preliminary study is made of a smear in saline or in neutral red or eosin solution, a second smear made by emulsifying a bit of feces in iodin solution should be studied. This kills all the protozoa. (The two smears may both be prepared before the microscopic examination of either is made.) The basis of this iodin solution is a saturated solution of iodin in 5% aqueous potassium iodide. In the solution itself, different investigators use different concentrations. We prefer 1 part of the above to 1 part of distilled water. Such a solution loses its efficiency in time, however, and should, therefore, be made up fresh every week or ten days. An iodin mount aids in diagnosis (1) by making it possible to ascertain much of the structure of the nuclei and to count them, (2) by staining the glycogen deep brown, and (3) by facilitating the counting and observation of flagella and certain other cytoplasmic organelles.

Donaldson (1917) (see also Kofoid, Kornhauser and Swezy, 1919, a) recommends a combination of the eosin and iodin solutions whereby the cysts are stained yellow on a pink background. After a long trial of this method we are of the opinion that it really has no advantage over the use of iodin alone, and in no instance does it eliminate the necessity for studying the living protozoa in saline or eosin.

After ascertaining the presence of protozoa in a stool and

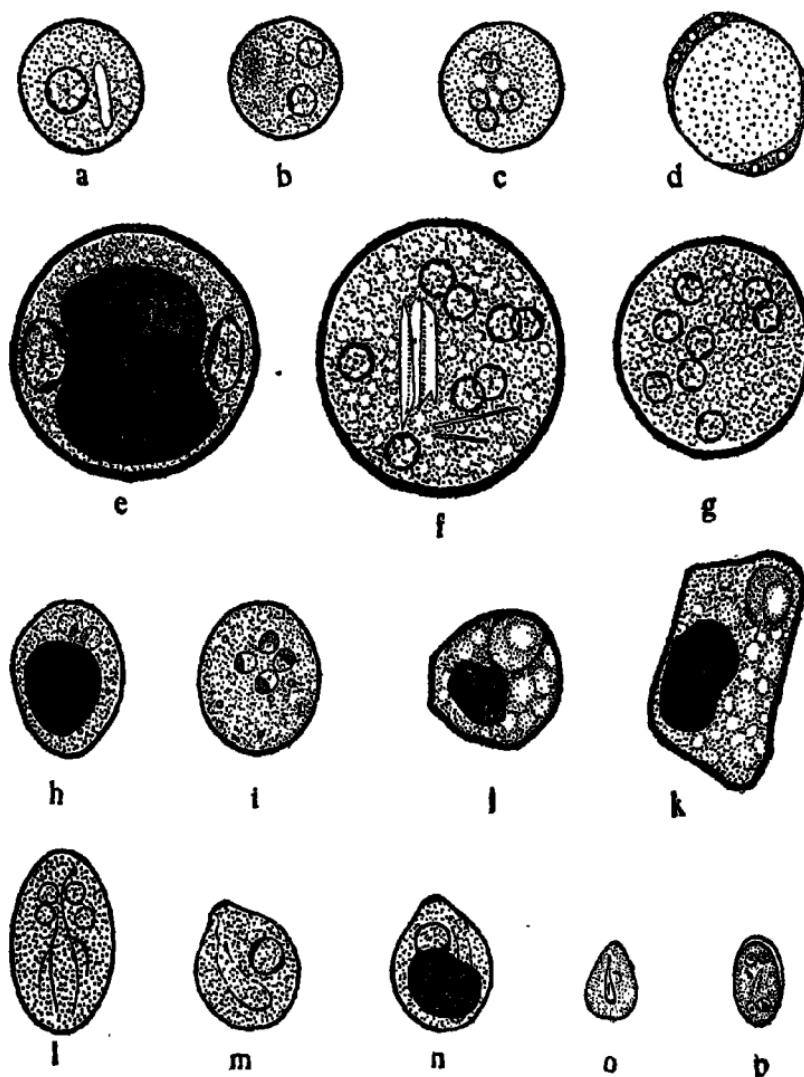


Fig. 187.—Appearance of different intestinal cysts in iodin (with the exception of *o* and *p*, which are stained with iron haematoxylin).
a, b and c, *Endamoeba histolytica*; *d*, *Blastocystis hominis*; *e, f* and *g*, *Endamoeba coli*; *h* and *i*, *Endolimax nana*; *j* and *k*, *Iodamoeba williamsi*; *l*, *Giardia lamblia*; *m* and *n*, *Chilomastix mesnili*; *o*, *Embadomonas intestinalis*; *p*, *Entercromonas hominis*. Chromatoid bodies which are shown in *a* and *f* are represented as clear areas. Glycogen masses which are shown in *e*, *h*, *j*, *k* and *n* are represented as black areas. All $\times 2000$. (*j* and *k*, original; *o* after Broughton-Alcock; *p* after Dobell and O'Connor; the remainder after Matthews.)

after examining them both while living and stained in iodin, it is generally possible to recognize the species to which they belong. Figure 187 shows diagrammatically the appearance of the different cysts in iodin. (It is to be noted that with the exception of *o* and *p* this figure does not attempt to show the finer details of structure that are discernible after staining with iron haematoxylin.) In the table (page 490) there is a brief summary of the differences between the cysts of the four common species of amoebæ. The cyst of the two common intestinal flagellates, *Giardia lamblia* and *Chilomastix mesnili*, are not included here because the characteristics of the cysts of these two species (the cysts of *G. lamblia* with their filamentar inclusions and the lemon-shaped cysts of *C. mesnili* with their buccal fibrils) make it fairly easy to distinguish between them and to recognize their affinities. In no case, however, is this table and figure to take the place of the minute descriptions already given. They are simply intended as short summaries.

3. CONCENTRATION METHODS OF DIAGNOSIS

The smear method of diagnosis is undoubtedly the most satisfactory method for the routine examination of stools. There are times, however, when it is desirable to concentrate the cysts in a given sample. For this purpose several concentration methods have been devised, some of which rely on mechanical means and some on chemical. Of the former, the centrifuge is the most common. Of the latter, various salts with a higher specific gravity than that of the protozoan cysts are used. While these methods are undoubtedly a help in certain types of work and may become even essential in certain research problems, they have their drawbacks, chief among which are: (1) In many cases the time spent is not commensurate with the very small percentage of added positives over the smear method, (2) unless they are used very carefully they often result in the distortion or injury of

TABLE IV.—DISTINGUISHING FEATURES OF THE CYSTS OF THE COMMONER INTESTINAL AMEBAE OF MAN

Characteristic	Condition of cysts*	<i>Endamoeba histolytica</i>	<i>Endamoeba coli</i>	<i>Endamoeba nana</i>	<i>Iodamoeba williamsi</i>
Shape.....	L I S	Spheroidal.....	Spheroidal.....	Ellipsoidal.....	Spheroidal or irregular.
Size (diameter). .	L I S	5 μ -20 μ	10 μ -30 μ	8 μ -10 μ in length x 7 μ -8 μ in breadth	8 μ -12 μ .
Nuclei number.....		4 when mature.....	8 when mature.....	4 when mature.....	1 when mature.
Nuclear structure,	L	Invisible or faintly visible.	Visible and generally easily counted.	Almost always invisible..	Invisible or faintly visible.
	I**	Clearly visible. In 4-nucleate cysts about 1/6 diameter of cyst. In 1-nucleate cysts about 1/3 diameter of cyst.	Clearly visible. In 8-nucleate cysts about 1/5 to 1/6 diameter of cyst. In 1-nucleate cysts about 1/3 diameter of cyst.	Visible. In 4-nucleate cysts about 1/7-1/8 length of cyst. In 1-nucleate cysts about 1/3-1/4 length of cyst.	Clearly visible. Diameter about 1/3-1/5 diameter of cyst.
	S	Karyosome small, centrally located; thin peripheral layer of chromatin; in well-fixed specimens no chromatin between karyosome and peripheral layer.	Karyosome large, eccentric; heavy peripheral layer of chromatin. Chromatin often present between karyosome and peripheral layer. Nuclei often seen with fragmented karyosome or in some stage of division.	Karyosome consists of one, two or several masses of chromatin connected by strands; no peripheral layer of chromatin.	Karyosome very large. Located on one side of nucleus; a mass of granules ("peripheral chromatin") on other side.

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Glycogen.....	Present in young cysts; absent in older cysts. Often appears more or less diffuse.	Present in young cysts, especially in 2-nucleate condition. Usually larger and better defined than in <i>E. histolytica</i> . Same as <i>E. histolytica</i>	Occasionally present, especially in 2-nucleate cysts.	Almost universally present in a well-defined mass, unless cysts have been kept for some time.
L	Appears as a dull inclusion.	Same as <i>E. histolytica</i>	Same as <i>E. histolytica</i>	Same as <i>E. histolytica</i> .
I	Stains dark mahogany brown.	Same as <i>E. histolytica</i>	Same as <i>E. histolytica</i>	Same as <i>E. histolytica</i> .
S	Dissolved out, leaving clear vacuole.	Same as <i>E. histolytica</i>	Same as <i>E. histolytica</i>	Same as <i>E. histolytica</i> .
Chromatoid bodies	Often present, especially in immature cysts. Generally rod-like with round ends.	Present less often than in <i>E. histolytica</i> . Generally resemble splintered glass in form. Same as <i>E. histolytica</i> . Same as <i>E. histolytica</i> . Same as <i>E. histolytica</i> .	None.....	None.
L	Highly refractive inclusion
I	Faintly visible
S	Stains black.
Volutin granules	None.....	None.....	A few granules always present.	Present. Individual granules generally larger than in <i>E. nassa</i> .
L	Highly refractile granules.	Same as <i>E. nassa</i> .
I	Barely visible.....	Same as <i>E. nassa</i> .
S	Black or very dark grey.....	Same as <i>E. nassa</i> .

* Under the "Condition of Cysts" L signifies *living and unstained*; I signifies that they are in *iodin solution*; S signifies that they are *fixed and stained with iron hematoxylin*.

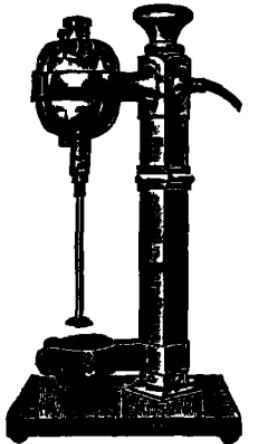
** The character of the nuclei of the various species can be seen more or less clearly when stained with iodin. Usually the bromatin masses are distinguishable, enabling one, with practice, to determine whether the specimen belongs to the genus *Endamoeba*, *Endolimax*, or *Iodamoeba*.

the cysts, and (3) they are inapplicable to the motile forms, with the possible exception of that method given under "Washing."

a. Washing

This simplest of concentration methods is the only one which can be used, although not with quite as satisfactory results, for motile (flagellates) as well as encysted protozoa. By this method, the stool is first mixed in normal saline solution or distilled water, either by hand or with some mechanical device, such as a soda fountain drink mixer (Fig. 188); then strained through two layers of cheesecloth. The suspension is centrifugalized at a speed of 1,200 to 1,600 revolutions per minute after which the colored supernatant fluid is drawn off. More distilled water or normal saline solution is added and the centrifugalization repeated until the supernatant fluid drawn off is clear. The cysts may easily be found at the bottom of the centrifuge tubes.

Fig. 188.—An electrically driven drink mixer which is manufactured for the mixing of soda fountain drinks and which is very satisfactory for the thorough emulsification of stools in saline. \times about $\frac{1}{4}$. (Courtesy of the manufacturers, Hamilton Beach Manufacturing Company, Racine, Wis.)



A modification of this method (applicable only to cysts), which is often used in this laboratory and which was suggested to us by Dr. W. C. Boeck, is as follows: The fecal material after emulsification is strained through two layers of cheesecloth into a tall 2-liter graduate which is almost filled with distilled water. This is allowed to stand over night (in fact it may stand for several days as the cysts will live for many days after the material is diluted

with so much distilled water). Then a small portion of the material at the bottom of the graduate is removed with a long pipette and examined.

b. Ether Concentration Method

Cropper and Row (1917) were the first to devise a method of concentration which is superior to any of the washing methods in that it decreases the specific gravity of the small fecal particles, thus causing them to float in the fecal emulsion and later be withdrawn. The protozoan cysts can be found and easily detected among the coarser lumps.

The following description is a modification of this method worked out by Boeck (1917), which aims to decrease not only the time needed in emulsifying the stool by the use of a soda fountain mixer (Fig. 188), but also the time required to examine the final preparation by the addition of a differential stain.

At least 1 gram of the stool to be examined is placed in 30 cc. of normal saline solution in a mixing glass and stirred with the soda fountain mixer for from 2 to 10 minutes, depending on the consistency of the stool. To facilitate mixing the hard stools, it is advisable to use a wire looped back and forth upon a single plane and so adjusted to the mixing glass that the loops project out toward the center of the glass. When the stool appears to be mixed uniformly, 5 cc. of ether are added, and the resulting mixture stirred for an additional 2 to 3 minutes. The addition of the ether tends to get rid of the foam of the emulsion although, if the stirring is continued very long, the foam re-forms. Then the emulsion should be quickly placed in a separatory funnel and allowed to stand for from 5 to 7 minutes, during which time the cysts will settle to the bottom in the saline solution and the finer debris will float in the layer of water just below the ether. The ether destroys the odor of the stool and dissolves all fats. About 15 cc. of the material, after the

required time, is then drawn from the bottom of the separatory funnel into a 15 cc. centrifuge tube and centrifugalized for 3 minutes at 1,600 revolutions per minute. After the supernatant fluid is withdrawn, a wet mount is made from the residue to which a drop of differential stain, either neutral red (1:10,000) or eosin (1:1,000), is added to aid in the detection of the cysts. When cysts are found, an iodin-stained preparation is made to facilitate a diagnosis.

4. CULTIVATION METHODS OF DIAGNOSIS

The cultivation of the intestinal protozoa of man has been effected in so few species that, so far, no cultivation method is of any wide applicability in the diagnosis of these organisms. The fact, however, that the flagellates *Chilomastix mesnili*, *Trichomonas hominis*, *Enbadomonas intestinalis* and *Enteromonas hominis* may be grown on simple media (see sections on these various species) led Hegner and Becker (1922) to ascertain the comparative efficiency of the routine smear method as compared to making cultures. Their results were limited to *Trichomonas hominis* and *Chilomastix mesnili*. By using a simple ovo-mucoid medium, consisting of a mixture of white of egg and 0.7 per cent normal saline solution, they were able to detect a much higher per cent of positives than with the simple smear method. Their results at the present time, while only preliminary, suggest that the routine examination of stools by the cultural method is not only practical but more efficient than the smear methods. It is, of course, obvious, however, that this method is at present limited to only a few of the species living in the human intestine.

5. PREPARATION OF PERMANENT SLIDES

It is sometimes impossible to ascertain the species of a given protozoon by means of iodin preparations. In such cases and, where a careful study of the structure of the

organisms is desired, it is necessary to make permanent preparations. By far the most valuable and generally applicable stain for these preparations is iron haematoxylin. Smears may be made on either cover glasses or slides, but the former are preferable. The following is a short outline of the steps in the preparation and staining of smears by the Heidenhain iron-haematoxylin process.

1. A small bit of feces is smeared on a cover glass with a glass rod so as to form a thin moist film.
2. Drop immediately face downward into warm (heated only to point where steam rises) Schaudinn's fluid which is composed of the following:

Distilled water saturated with mercuric chloride $HgCl_2$ 65 pts.
95% Alcohol 33 pts.
Glacial acetic acid..... 2 to 5 pts.

The film will float on the surface at first. After a few seconds it can be completely immersed and left in the fluid for from 10 to 20 minutes.

3. Wash and harden by the following steps:
 - a. Wash in 50% alcohol for a few minutes.
 - b. Transfer to 70% alcohol to which has been added a few drops of alcoholic iodin solution (to remove sublimate) for at least 10 minutes.
 - c. Harden in 95% alcohol. When speed is essential this hardening may be shortened to a few minutes, but it is best to leave the film in the alcohol for an hour and even a few days does not injure it.
 - d. Hydrate by successive immersions for a few minutes in 70% and 50% alcohol and distilled water.
4. Mordant by placing in a 2% aqueous iron alum (ammonium-ferric sulphate) 30 minutes or longer

(over night answers as well). While 30 minutes will suffice it is best to mordant for at least 2 hours.

5. Wash in distilled water for a few seconds.
6. Stain in 0.5% aqueous haematoxylin solution. This should be made up several weeks previous to its use and allowed to "ripen." Several hours is sufficient for staining, but a longer time, viz., over night, is better.
7. Wash in distilled water.
8. Differentiate in 1-2% aqueous iron-alum solution. This is by far the most difficult part of the process and can be done accurately only after practice. The overstained films are placed in the iron-alum solution and at intervals taken out, rinsed in distilled water and examined under the microscope. As each film is destained enough, it is washed in distilled water and then placed in running tap water for at least 20 minutes. It is often an advantage to start the differentiation with 2% iron-alum solution, and as the films become destained to dilute the solution to about 0.5% so that the end point is reached more slowly.
9. Wash in distilled water, as noted in (8).
10. Dehydrate by immersing successively for about five minutes each in 50%, 70%, 95% and absolute alcohol (2 changes).
11. Clear by immersing in two changes of xylol (5 minutes each).
12. Mount by lowering the smear face-downward over a drop of Canada balsam on a slide and pressing it down gently.

The foregoing method is tedious, but undoubtedly gives the best results for careful work. It may be greatly shortened by the use of alcoholic solutions (Dobell's method, see Lee, 1921), but often does not give as satisfactory results.

Where protozoa have to be stained quickly for diagnostic work, and where it is impractical to carry out iron-hæmatoxylin staining, Mayer's hæmalum gives good results. Smears are carried through the first three steps outlined above and are taken from distilled water and placed in Mayer's hæmalum, which is made as follows: hæmatoxylin 1 gram, water 1 liter; dissolve and add 0.2 grams of sodium iodate (NaIO_3) and 50 grams of potash alum; dissolve and filter. This is ready for immediate use but does not keep indefinitely. Films are stained for from 10 to 20 minutes, are washed in running tap water until blue and are then ready for dehydration and mounting (steps 10-12 above). If for any reason the films are overstained, they can be differentiated as in the Heidenhain process.

Besides hæmatoxylin there are many other stains which may be used for staining protozoa. One of the most useful of these for amoebæ and their cysts is Dobell's modification of Mann's methyl blue, eosin stain (see Lee, 1921, or Dobell, 1919, or Dobell and O'Connor, 1921).

B. Vegetable Organisms Sometimes Confused with Cysts

I. "HOMOGENEOUS CYSTS"

These structures have been described by Kofoid, Kornhauser and Swezy (1919 and 1919 a) and have been identified by them with the spherical cysts which Wenyon and O'Connor (1917) encountered in one or two cases and which they thought were probably iodin cysts (*Iodamæba williamsi*), devoid of glycogen. Kofoid, Kornhauser and Swezy state that "The nature of their occurrence [they found them in 190 cases out of 2,865 stools examined] is such that they appear to represent a widespread but individually sparse infection of the human digestive tract. The possibility that they are undigested spores of adventitious origin from con-

taminated food or water is not precluded." The following description is taken from these authors. The cysts are spherical with an extreme range in size of from 6μ to 30μ in diameter.¹ The cyst wall is smooth and very similar to the cyst of *E. coli* (Wenyon and O'Connor at first thought these cysts were *E. coli*). At times a break may occur in the wall through which a small mass of protoplasm protrudes (Fig. 189). Within the cysts the protoplasm is remarkable for its

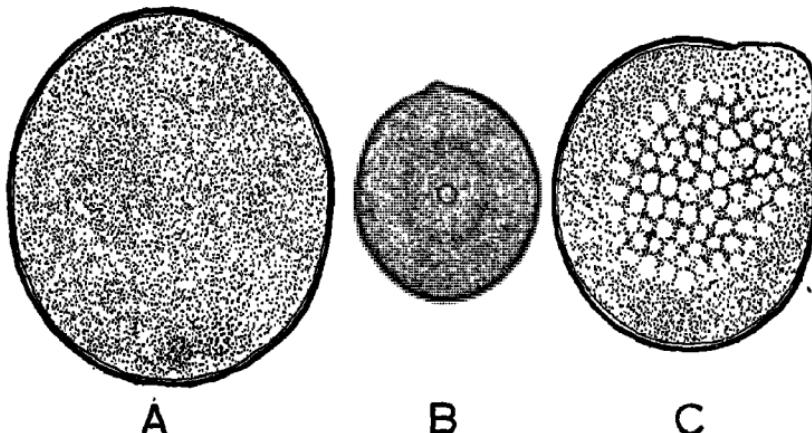


Fig. 189.—"Homogeneous cysts" from human feces, stained with iodin-eosin.

B shows a central vacuole which simulates a nucleus in appearance. C shows the vacuolization of the cytoplasm and the opening in the cyst wall which are observable in some cysts. A and B $\times 2000$; C \times about 1800. (After Kofoid, Kornhauser and Swezy.)

homogeneity, although in a few cases the central part may appear alveolar, or may occasionally contain a small central vacuole such as described by Wenyon and O'Connor. A definite nucleus is never visible.

The systematic relationship of this organism is unknown.

¹ In one paper (1919) the size is stated as usually ranging from 10μ to 30μ , with the occasional occurrence of 7μ . In 20 cysts from 19 cases the range was 10μ to 24μ with an average of 15.5μ . In the other paper (1919a) the size is given as 6μ to 20μ , rarely 30μ .

Kofoid, Kornhauser and Swezy suggest that it may be a chlamydospore of a phycomycete. Its range of size and appearance makes it easily confused with amœbic cysts, but its homogeneity of structure and absence of nuclei when observed in iodin should prevent any such error.

2. BLASTOCYSTIS HOMINIS

This vegetable organism occurs in the intestine of almost every individual and, since it is very often mistaken for amœbic cysts, should be carefully studied by everyone attempting fecal diagnosis. It is a spherical organism and,

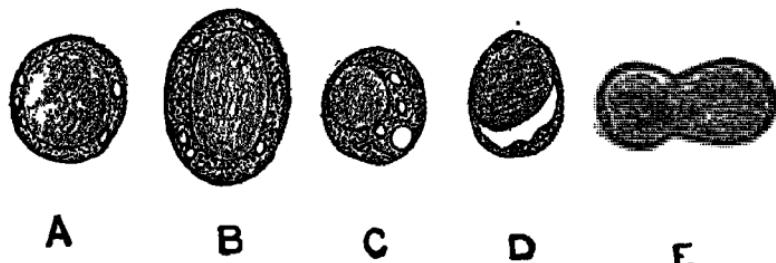


Fig. 190.—*Blastocystis hominis* in iodin solution.

A-D, different specimens showing a wide range in the thickness of the protoplasmic wall and in the size of the reserve mass. E, dividing specimen. $\times 2000$. (Original.)

according to Dobell and O'Connor (1921), varies in diameter from 5μ to over 30μ with the largest proportion of organisms falling between 8μ and 14μ . In structure, *B. hominis* is a hollow sphere of protoplasm of varying thickness containing nuclei which surround a large central vacuole (Fig. 190). The latter is filled with a reserve mass which sometimes stains and sometimes appears colorless. Dividing specimens have a peculiar hour-glass appearance (Fig. 190, E). *B. hominis* has been cultivated by Barret (1921) in a simple medium consisting of various dilutions of inactivated human blood serum in 0.5 per cent. NaCl solution, the most favorable strength being 10 per cent.

3. YEASTS

Practically all stools contain intestinal yeasts, most of which show the typical yeast structure and characteristic ellipsoidal shape (Fig. 191), although spherical ones are sometimes encountered. Reproducing forms exhibit terminal budding (Fig. 191, C). Beginners often mistake the larger yeasts for cysts of *Endolimax nana*. A careful study, how-

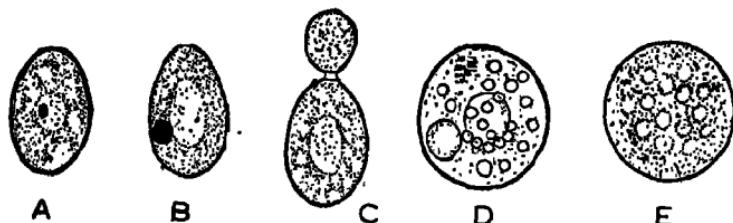


Fig. 191.—Yeasts and mold in iodin-eosin.

A, B and C, common intestinal yeast. D, large spheroidal yeast with central vacuole. E, spheroidal phase of a fecal mold $\times 2000$. (After Kofoid, Kornhauser and Swezy.)

ever, always reveals their relationships, and, furthermore, in fresh specimens treated with eosin or neutral red, the yeasts stain promptly whereas the protozoan cysts remain unstained.

4. FECAL MOLDS

In stools which have been kept for a day or more, a grey covering of mold often appears. The spores of some species of these molds are often superficially like amœbic cysts (Fig. 191, E). They are spheroidal or ellipsoidal in shape and vary in diameter from 7μ to 15μ (Kofoid, Kornhauser and Swezy, 1919 a). They may, however, be distinguished from the amœbic cysts by the absence of typical nuclei, and their source may, of course, be revealed by an examination of the mold on the surface of the stool.

C. Coprozoic Protozoa

I. INTRODUCTION

It has already been noted that stools which are kept may contain coprozoic protozoa. When the stool has been moistened, or when the weather is warm, these forms appear in the stools in a few hours and before very long simply swarm in numbers. They represent non-entozoic free-living species which find their way into the feces in two ways: (1) Their cysts may be swallowed with food and water, pass through the alimentary canal unchanged and hatch in the feces after passage through the body. (They do not hatch in the body, due probably to the high temperature and lack of oxygen.) (2) They may represent contaminations of the feces after passage from the body.

A large number of free-living species of protozoa may occasionally be found living in the feces of man and animals. Many of them have not been sufficiently studied to identify and give accurate descriptions and a number of others occur only infrequently. Dobell and O'Connor (1921) have given an admirable introduction to the subject by describing twelve species which they consider representative. These are listed below.

Coprozoic Amœbæ

1. *Dimastigamoeba gruberi*.

One of the most easily recognized species which also occurs commonly in soil.

2. *Hartmannella hyalina*.

Probably a very widespread species which is often obtained from human feces.

3. *Sappinia diploidea*.

Found rarely in human feces but more commonly in those of other animals.

A Coprozoic Thecamœba

4. *Chlamydophrys stercorea.*

Dobell and O'Connor have never found this form in human feces, but Schaudinn (1903) maintains that it is very common. Dobell (1909) has studied it from the feces of frogs and toads.

Flagellates

5. *Bodo caudatus.*

The most common coprozoic flagellate in human feces.

6. *Bodo edax.*

Far less common than the preceding species.

7. *Cercomonas longicauda.*

Common in infusions and occasionally found in human feces.

8. *Cercomonas crassicauda.*

Less common in human feces than the preceding species.

9. *Copromonas subtilis.*

Found in the feces of frogs and toads and a similar, if not identical species, occurs occasionally in human feces.

10. *Helkesimastix faecicola..*

Found by Dobell and O'Connor in a single sample of human feces.

11. "Copromastix prowazekii."

Cultured from feces of frog and man by Aragão (1916, 1916 a).

12. "Toxobodo intestinalis."

A coprozoic form which Sangiorgi (1917) recently described as a new intestinal flagellate of man.

For the nomenclature and description of these species the reader is referred to the work of Dobell and O'Connor

(1921). In the scope of the present chapter a brief description can only be given of the more representative species most commonly encountered in human feces.

Before describing these forms it is well to note that Dobell and O'Connor consider it doubtful whether any true coprozoic ciliates occur in human feces, while in this laboratory we have never encountered any. A number will live, however, in diluted human excrement—for example, our standard medium for the culture of *Vorticella* is a mixture of human feces with water. On the other hand, free-living ciliates are often accidentally introduced into feces and cause subsequent confusion in diagnosis. Therefore, when a problematical ciliate is found in a fecal sample, it is always best immediately to examine the solution with which the sample is diluted. Ciliates are often found in saline solution that has stood for some time and even, occasionally, occur in bottles of "distilled water."

2. DIMASTIGAMŒBA GRUBERI

This amœba, commonly found in soil and water, and, according to Dobell and O'Connor (1921), readily cultivable in hay and soil infusion, in diluted egg albumin, or on agar plates, is the most easily recognized species of those which occur coprozoically in human feces. It has been studied by various investigators, but the most detailed account is that of Miss Wilson (1916) to which the reader is referred and from which this account is mainly taken. This amœba may readily assume a free-swimming flagellate form; it may also encyst. According to Miss Wilson, the body of the active amœba "has one constant character, namely, that it progresses by means of one blunt, broadly rounded or lobose, anterior pseudopodium" thrust out alternately from each side. Its general form (Fig. 192, A) varies, but is usually elongated, with the longitudinal diameter varying from $6\ \mu$ to $46\ \mu$, and the transverse one from $4\ \mu$ to about $11.5\ \mu$. The ectoplasm

is only differentiated from the endoplasm when the animal is moving. A contractile vacuole, formed by the fusion of three or four small ones, and generally one or a few food vacuoles containing bacteria are present. There is a single vesicular nucleus about 2μ to 4μ in diameter with a large, centrally located, spherical, compact karyosome which may have a delicate stainable network running from it out to the

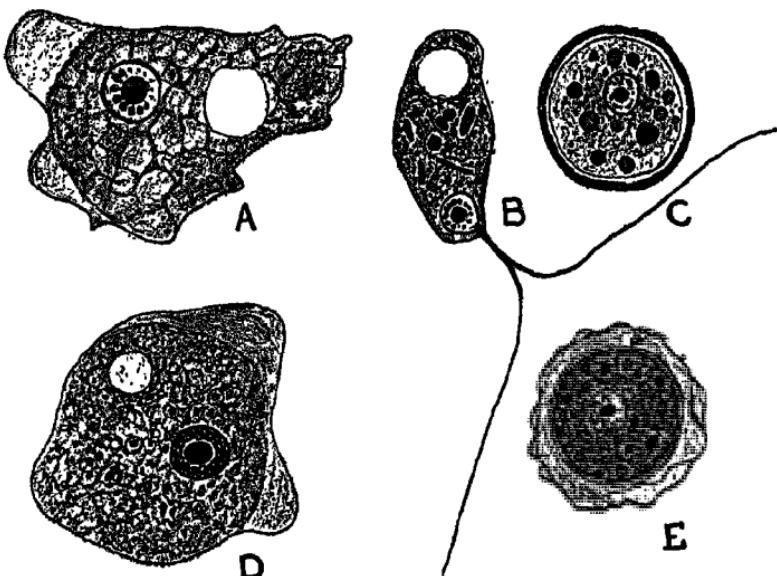


Fig. 192.—*Coprozoic amoebæ.*

A, *Dimastigamœba gruberi*, active amoeboid form. B, the same, flagellated stage. C, the same, cyst. D, *Hartmannella hyalina*, active amoeboid form. E, the same, cyst. $\times 2000$. (After Dobell and O'Connor.)

periphery. Around the karyosome there are a few granules comparable to the "peripheral chromatin" in *Iodamœba williamsi* (Fig. 192, A).

The flagellate stage (Fig. 192, B), which may be easily induced in about 3 hours by simply flooding a culture of the amoebæ with distilled water and exposing it to the air, is oval or pyriform in shape and smaller in size, with two equal

flagella slightly longer than the body situated at the narrower, anterior end and apparently arising from the blepharoplast which is situated just anterior to the nucleus. This stage is of short duration, rarely exceeding twenty-four hours. Slight changes in temperature or mechanical disturbance is sufficient for this stage to become actively amoeboid.

Encystment regularly occurs after a period of binary fission. The cyst (Fig. 192, C) is spheroidal (7μ to 14μ in

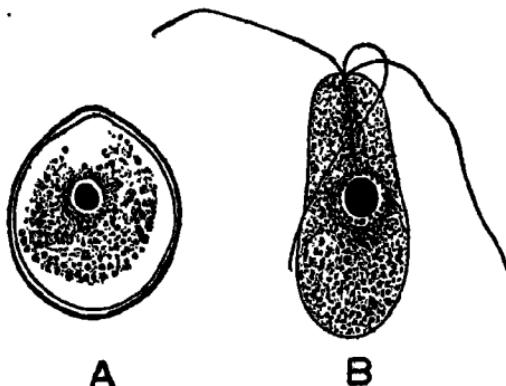


Fig. 193.—A coprozoic amoeba, *Trimastigamoeba philippinensis*.
A, cyst. B, flagellated stage. $\times 3000$. (From Lemmermann after Whitmore.)

diameter), translucent, uninucleate, and free from food and contractile vacuoles. A thin outer membrane is first formed and later within this a heavy hyalin wall containing three to eight minute openings, each in a local thickening. These openings are extremely characteristic and it is through one of these that the amoeba emerges during excystation. The newly formed cysts contain numerous deeply staining, spherical chromatoid bodies.

3. TRIMASTIGAMOEBA PHILIPPINENSIS

Whitmore (1911, a) has described a very similar flagellating amoeba (Fig. 193) from human feces which has three instead of two flagella in its flagellate stage.

4. HARTMANNELLA HYALINA

This organism has probably been described—although incompletely—by Dangeard (1900), Brodsky (1910), Hartmann and Chagas (1910) and others, but the following brief description is taken from Dobell and O'Connor (1921). It is probably one of the species of amoeba cultivated from human feces by Whitmore (1911 a). It is readily cultivable on agar and in other media. The organism differs considerably from *D. gruberi*; although the active amoeba is closely similar to it, especially with regard to its nucleus (Fig. 192, D). *H. hyalina* possesses but a single contractile vacuole and measures from about $9\ \mu$ to $17\ \mu$ in diameter when rounded. Unlike *D. gruberi*, it does not possess a flagellated stage, and, furthermore, the cyst (Fig. 192, E) is larger (usually from $10\ \mu$ to $14\ \mu$ in diameter) with a thick crinkled outer wall and a thin smooth inner one. Neither wall possesses any pores. The newly formed cysts contain small spherical chromatoid bodies which are sometimes very numerous but which later disappear.

5. TETRAMITUS ROSTRATUS

This organism was first described by Perty (1852) and has been known to a number of investigators as a flagellate. Recently, however, Miss Bunting (1922) has adduced considerable evidence that it is the flagellated stage of a coprozoic amoeba which she first cultivated directly from the cæcal contents of a rat. In her work pure line cultures were started from a single flagellate, a single amoeba and a single cyst, all of which gave the same results and leave little doubt¹ that the life-history given in figure 194 is correct and that a genetic relationship exists between this amoeba and flagellate.

¹ Miss Bunting's work has appeared only in preliminary form but there is enough data to indicate that it is a very careful investigation.

The following account of the various stages in the life-cycle is taken from Miss Bunting and can be better understood if

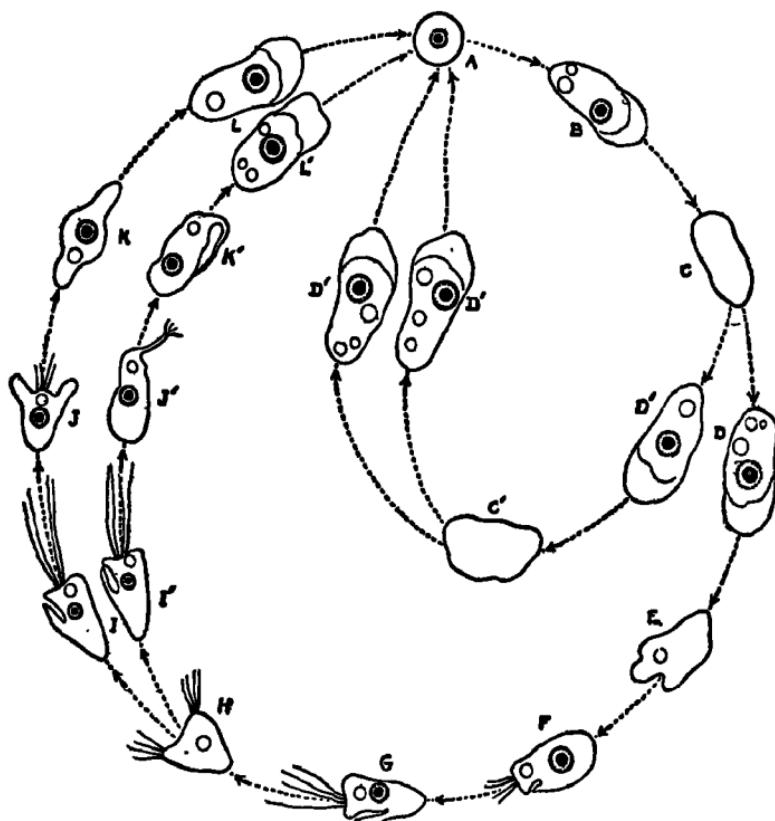


Fig. 194.—Diagram of life-cycle of a coprozoic amoeba with *Tetramitus* as its flagellate phase.

A, the cyst; B, vegetative amoeba; C, C', division ("gel" stage); D, D', amoeba after division; E, F, phases in transformation to *Tetramitus*; G, fully formed *Tetramitus*; H, *Tetramitus* previous to division ("gel" stage); I, I', *Tetramitus* after division; J, K, L and J', K', L', series of transformation stages, *Tetramitus* to amoeba. (After Bunting.)

read in conjunction with figure 194. The cyst is uninucleate, rarely binucleate, and measures 6 μ to 18 μ in diameter (Fig. 194, A). From it emerges an amoeba, and never a flagellate,

which is of the general form shown in figure 194 B. It measures 14μ to 48μ in length, contains a single contractile vacuole and a vesicular nucleus with a central karyosome and a layer of chromatin around the periphery between which there may be a few granules (probably the so-called "peripheral chromatin"). It reproduces by fission, at which time a change in the physical nature of the cytoplasm, termed by Miss Bunting the "gel" state (Fig. 194, C and C¹), obscures the nucleus. After several divisions, this amoeba may either encyst or become converted into the flagellated stage (Fig. 194, C, D, C¹, D¹ and A). The flagellate (Fig. 194, G) measures 14μ to 18μ in length and 7μ to 10μ in greatest width, and possesses a single nucleus, a contractile vacuole and four flagella, corresponding in every way to *Tetramitus rostratus*. It multiplies by binary fission (Fig. 194, H and I), eventually becoming converted into the amoeba (Fig. 194, K and L), which in turn may later encyst.

The cysts may exist in old media indefinitely, but when planted on favorable media, the amoebæ emerge in two or three hours. These may either encyst after having remained active for several days or become transformed into flagellates after eight or nine hours, which, in turn, after two days, become transformed into amoebæ or die.

Dobell and O'Connor (1921) think that it is almost certain that "*Copromastix prowazekii*," which is noted above, and which Aragão (1916 and 1916 a) cultivated from the feces of man and the frog, belongs to the genus *Tetramitus*. They also suggest that "*Tetratricomastix intestinalis*," which Sangiorgi (1917) cultivated from human feces, belongs to this genus.

6. CERCOMONAS LONGICAUDA

Besides occurring coprozoically in human feces, this species is common in various infusions. The body is amoeboid and food is ingested by means of pseudopodia (Fig. 195, C).

There are two flagella, one of which is free and is directed anteriorly during locomotion, and the other of which adheres to the body and is directed posteriorly. The two blepharoplasts from which the flagella arise are located on the anterior pole of the single nucleus where the latter is drawn out as shown in figure 195, A. No contractile vacuole has been

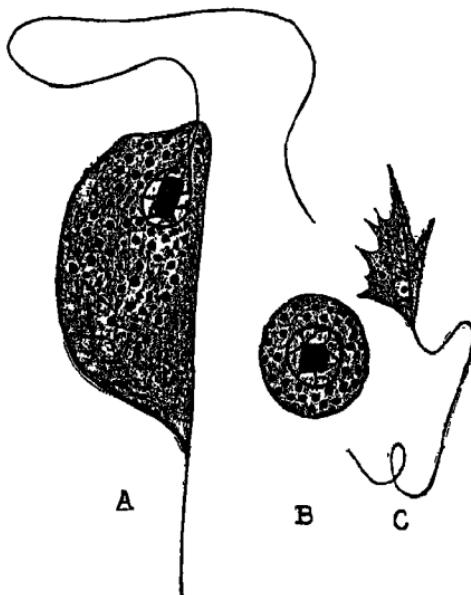


Fig. 195.—*Cercomonas longicauda*.

A, motile flagellate stained with iron haematoxylin. B, cyst stained in same manner. C, living motile flagellate. Note that the anterior end of A is directed upwards while that of C is directed downwards. A, magnification not stated; B \times about 2000; C \times 2000. (A and B after Wenyon; C after Dobell and O'Connor.)

seen, but food vacuoles and other vacuoles may be present in the cytoplasm. Sometimes the cytoplasm, especially in the region of the nucleus, contains a number of refractive granules, which stain deeply with haematoxylin (Fig. 195, A). According to Wenyon (1910) the flagellates are very variable in size, ranging in length, exclusive of the flagella, from $2\ \mu$ to $15\ \mu$ or more.

The cysts are spherical and possess a single nucleus and a number of the same type of refractive granules as noted in the motile forms (Fig. 195, B). Wenyon (1910) gives the diameter of the cysts as $6\ \mu$ or $7\ \mu$, and Dobell and O'Connor as from $4\ \mu$ to $6\ \mu$.

7. *BODO CAUDATUS*

Dobell and O'Connor (1921) assign variously named organisms to this species, such as *Bodo urinarius*, Hassall,

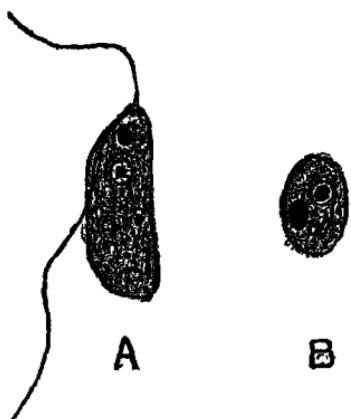


Fig. 196.—*Bodo caudatus*.

A, free-swimming stage. B, cyst. $\times 2000$. (Original, drawn from Dr. E. R. Whitmore's slides of "*Prowazekia asiatica*."

1859, *Bodo asiaticus*, Castellani and Chalmers, 1910, *Prowazekia cruzi*, Hartmann and Chagas, 1910, *Prowazekia javanensis*, Flu, 1912, etc., although they add that many of the published accounts are such that absolute identification cannot be made with certainty. The following account is taken from Dobell and O'Connor (1921) and Castellani and Chalmers (1919).

This form is the commonest coprozoic flagellate found in human feces, but it seems probable that it is unable to live within the human body, since it is easily cultivable in many liquid media or on agar plates, seems to be aerobic, and cannot live long in cultures at 37° C .

The active flagellate form (Fig. 196, A) is polymorphic and variously described as slender, sausage-, carrot-, or pear-shaped, oval or rounded, ranging in size from $2.5\ \mu$ to $6\ \mu$ in breadth by $8\ \mu$ to $25\ \mu$ in length. The maximum length for most specimens is $18\ \mu$. It is generally broadest at the anterior end where a slight projection extends over the small

mouth aperture. Within the cytoplasm lies a minute contractile vacuole just posterior to the cytostome, behind this an oval parabasal body connected by short rhizoplasts to two small blepharoplasts, a more or less central vesicular nucleus with a large central karyosome, and food vacuoles containing bacteria generally located in the posterior portion. From the blepharoplasts two flagella arise, the anteriorly directed one being short—about the same length as the body—and the posteriorly directed one being longer—about twice as long and sometimes attached to the body along the anterior portion.

The cyst (Fig. 196, B) is thin-walled and oval, measuring about $5\ \mu$ to $7\ \mu$ in length, and contains a single nucleus, a single parabasal body, and the remains of the two flagella, although these may appear paired, as they occasionally divide. Sometimes it may also contain some deeply stainable granules.

8. COPROMONAS SUBTILIS

This flagellate was probably first described by Dobell (1908) (see note, page 106, for a discussion of the possibility that it is identical with some previously described species) from the feces of frogs and toads and has been found once by Dobell and O'Connor in human feces. *Copromonas* belongs to the order *Euglenoidina* and has already been noted in the discussion of this order. The following description is taken from Dobell (1908). The body is ovoid or pyriform in shape and ranges in length from $7.5\ \mu$ to $20\ \mu$ with an average length of $16\ \mu$. Dobell and O'Connor note that specimens as small as $4\ \mu$ to $5\ \mu$ occur in cultures and that in cultures from human feces there occur very minute forms which were never seen by Dobell in his original cultures from frogs. At the anterior end there is a true mouth or cytostome (Fig. 197, c. s.) through which bacteria and other solid particles are ingested (*cp.* with *Peranema* and *Jenningsia*, page 116). Leading from the cytostome to the posterior part of the body there is a cytopharynx (Fig. 197, c. p.). The

single flagellum originates from a blepharoplast (Fig. 197, *bl.*) and runs along the wall of the cytopharynx and passes to the exterior through the cytostome. Closely associated

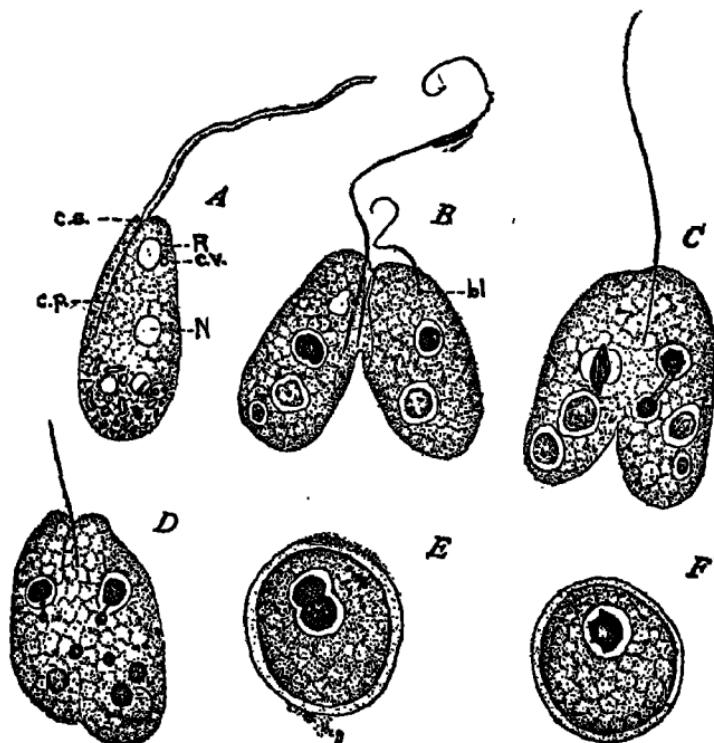


Fig. 197.—*Copromonas subtilis*.

A, ordinary adult before division (living). B, fusion of two individuals prior to conjugation. C, First reduction division of nuclei. D, second reduction of nuclei. E, fusion of nuclei and encystment. F, resting cyst. *bl.*, blepharoplast; *c. p.*, cytopharynx; *c. s.*, cytostome; *c. v.*, contractile vacuole; *N*, nucleus; *R*, reservoir. This figure should be compared with figure 48. A of figure 197 corresponds with 1 of figure 48, B with 6, C with 7, D with 8, E with 10 and F with 11. \times about 1500. (From Calkins after Dobell.)

with the base of the flagellum and the cytopharynx there is a comparatively large reservoir and a small contractile vacuole which periodically empties its contents into it (Fig. 197, *R*

and *c. v.*). Asexual reproduction by longitudinal fission, conjugation and encystment have already been noted (page 106 and figure 48). Figure 197 shows some of the cytological details of conjugation and encystment. The cysts are spherical or ovoid and generally measure from 7μ to 8μ in diameter. Besides the single nucleus very few cytological details can be made out in them (Fig. 197, F).

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CHAPTER I INTRODUCTION TO THE ORGANIZATION OF THE PROTOZOA

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CHAPTER II

A GENERAL CONSIDERATION OF THE SARCODINA

Extensive lists of literature on the Sarcodina may be found in the general works of Bütschli (1880-1889), Calkins

(1901), Doflein (1916), Lankester (1903, 1909), and Minchin (1912). The present list contains only (1) the references, exclusive of the general textbooks, cited in the text, and (2) a few of the recent contributions to our knowledge of the group.

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CHAPTER III

THE ECTOZOIC AND ENTOZOIC SARCODINA

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CHAPTER IV

A GENERAL CONSIDERATION OF THE MASTIGOPHORA

Extensive lists of literature on the Mastigophora may be found in the general works of Bütschli (1880-1889), Calkins (1901), Doflein (1916), Lankester (1903) and Minchin (1912). The present list contains only (1) the references, exclusive of the general textbooks, cited in the text, and (2) a few of the recent contributions to our knowledge of the group.

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CHAPTER V

THE HÆMOFLAGELLATES AND ALLIED FORMS

Because of the great number of titles it is obviously impossible to present a comprehensive bibliography on the hæmoflagellates or even to list all of the references cited in the text. Most of the references, exclusive of general textbooks, cited under the genera *Trypanosoma*, *Criithidia* and *Herpetomonas*, are given in the following list. A monographic treatment of the literature on the trypanosomes prior to 1912 may be found in Laveran and Mesnil (1912). A large amount of the work of Sir David Bruce and other British workers may be found in the Reports of the Sleeping Sickness Commission of the Royal Society (Vol. 1, 1903, onwards, London). Furthermore, a bibliography of the literature on trypanosomiasis prior to April, 1909, is given in Thimm (1909) and abstracts and references of practically all subsequent works on trypanosomiasis are given in the Sleeping Sickness Bulletin (Vols. I-IV, 1908-1912. Sleeping Sickness Bureau, London) and the Tropical Diseases Bulletin (Vol. 1, 1912, onwards, Tropical Diseases Bureau, London). References in the text on leishmaniosis prior to 1917 are not listed but may be found in Laveran (1917). A review of the more recent literature is given in Wenyon (1922). A bibliography of work on leishmaniosis done prior to July, 1911, is given in Sheppard (1911) and abstracts and references of almost all subsequent work in the Kala Azar Bulletin (Nos. 1-3, 1911-1912, Sleeping Sickness Bureau,

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CHAPTER VI

THE INTESTINAL FLAGELLATES

Literature lists containing extensive references to contributions that involve studies of intestinal flagellates will be found especially in Dobell and O'Connor's "The Intestinal Protozoa of Man" and in Rodenwaldt's monograph (1921).

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CHAPTER VII

A GENERAL CONSIDERATION OF THE SPOROZOA

Readers are referred to general works on Protozoa for a general consideration of the Sporozoa, especially such books as Minchin's Introduction to the Study of the Protozoa, Calkin's Protozoa, Lankester's Treatise on Zoology, and the Cambridge Natural History.

CHAPTER VIII

THE GREGARINES AND COCCIDIA

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CHAPTER IX

THE ORDER HÆMOSPORIDIA EXCLUSIVE OF
THE MALARIAL PARASITES

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CHAPTER X

THE MALARIAL PARASITES

It is obviously impossible to present an extended bibliography on malaria because of the great number of titles. Only a few of the more recent papers are therefore listed. In these will be found references to the older literature. The books or monographs by Ascoli, Craig, Mühlens, Prowazek, Ross and Ziemann contain large literature lists.

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CHAPTER XI

THE NEOSPORIDIA

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CHAPTER XII

A GENERAL CONSIDERATION OF THE INFUSORIA

Extensive lists of the literature on the Infusoria may be found in the general works of Bütschli (1880-1889), Calkins (1901), Doflein (1916), Lankester (1903) and Minchin (1912). The present list contains only (1) the references, exclusive of the general textbooks, cited in the text, and (2) a few of the recent contributions to our knowledge of the group.

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CHAPTER XIII -

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CHAPTER XIV

GENETICS AND PHYSIOLOGY OF REPRODUCTION IN THE PROTOZOA

The reader is referred to the following works for extensive bibliographies and critical reviews of investigations on the genetics of the Protozoa: (1) Genetical work on trypanosomes prior to 1912 in Dobell (1912), (2) genetical work on the ciliates prior to 1914 in Dobell (1914), and genetics of the protozoa, in general, in Jennings (1920). The present list contains only those references cited in the text which are *not* given in Jennings (1920).

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CHAPTER XV

THE DIAGNOSIS OF THE INTESTINAL
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