HOST-PARASITE RELATIONS BETWEEN MAN AND HIS INTESTINAL PROTOZOA

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Host-Parasite Relations Between Man and His Intestinal Protozoa

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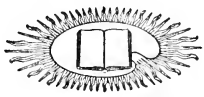

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PREFACE

Many books and monographs devoted to protozoölogy have appeared within the past few years; most of these are excellent and valuable additions to the subject. Various phases of protozoölogy are emphasized in the books; some of them stress morphology, classification, and life-cycles; others are devoted more particularly to the biology of the free-living species; and several treat especially the parasitic forms with emphasis on those that live in man. Besides these contributions to protozoölogy alone, should be mentioned books and treatises on Tropical Medicine, a large section of each being usually concerned with the disease-producing protozoa of man. The monographs are for the most part collections that bring together the data regarding one important species or group of organisms, whereas the books generally cover the entire phylum Protozoa.

Because of the numerous recent contributions to this subject the writer feels under obligation to provide a very good reason for adding another volume to the already large library of protozoölogical books. He believes he not only has a valid reason for the preparation of this volume but was practically forced by circumstances to undertake the task. The organization of work in protozoölogy in a school of hygiene and public health revealed at once a lack of coördination between the zoölogical and medical phases of the subject and be-

tween these and methods of prevention and control. Free-living protozoa comprise an enormous and exceedingly interesting group of animals, but their parasitic relatives are likewise numerous in species and fundamentally similar, as is shown in part 2 of the Introduction in Chapter I. The control of the parasitic species, to be effective, must be based on a knowledge of the relations between the parasite and its host. What these relations are is discussed in Section II of Chapter I, and how little we know about them is evident from the vast number of lacunæ disclosed in the accounts presented in Chapters II, III, IV and V of the groups of intestinal protozoa that have representatives living in man.

It is, therefore, the purpose of this book to gather together the more relevant data regarding the host-parasite relations of the intestinal protozoa of man and to present them in logical order in such a way as to bring out the state of our knowledge with special reference to the desirability of further studies. It has seemed unnecessary to include detailed accounts of the history, nomenclature, morphology, life-cycles, geographical distribution, pathology, symptomatology, treatment and cultivation of the various species discussed since this information has been presented in several recently published books. The writer has not attempted to include all of the data contained in the literature relating to the subjects discussed, but has selected examples, particularly from recent publications, as illustrative material.

One of the most striking features of this volume is the inadequacy of the data available on the particular phases of protozoölogy considered. Information is widely scat-

tered and usually in the form of isolated researches begun because chance happened to place favorable material in the hands of the investigators, and carried out with no larger program in view. More is known regarding *Endamœba histolytica* than of any other intestinal protozoön, largely because this species more frequently brings about distressing symptoms and even death. The severe pathogenic effects of this species also make it of particular interest since an excellent opportunity to study the interactions of host and parasite is thus afforded. The other species discussed are of less practical importance because of their apparent harmlessness or their comparative rarity. Scientifically, however, they are all intensely interesting and protozoölogists will not be satisfied until the complete story of their lives in relation to that of their hosts is known. Many of the species of intestinal protozoa have been described within the past decade and their systematic position, morphology and exact habitat are still in doubt. Many other forms have been reported from man, but must await further study before they can definitely be admitted to be "good" species; and an even larger number have been described as new species that were abnormal forms of species already known, were coprozoic and not real inhabitants of the human intestine, or were free-living species present in human material because of contamination.

Much of the work of preparing this volume was done in the laboratory of protozoölogy at the London School of Hygiene and Tropical Medicine during the spring of 1926, where the author was serving as exchange professor, and in the laboratory of Professor E. Brumpt in

the University of Paris, during the summer of 1926. He wishes to express his indebtedness to Dr. Andrew Balfour of London and Professor Brumpt of Paris and to various members of their respective institutions for the many courtesies extended to him.

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CHAPTER I

THE BIOLOGY OF HOST-PARASITE RELATIONS BETWEEN MAN AND HIS INTESTINAL PROTOZOA

I. *Introduction*

1. Protozoölogy

Protozoölogy is a subject of interest to students of various branches of biology. It is taught in the zoölogy departments of many colleges and universities and in medical schools, especially in schools of tropical medicine. Investigations in this field are carried on principally by zoölogists and medical men. Zoölogists who are interested in protozoölogy usually direct their attention primarily to the parasite, whereas most medical men tend to emphasize the reactions of the host. The zoölogical protozoölogist, for the most part, is concerned with morphology, systematics, and life-history studies, and the medical protozoölogist with symptomatology, pathology and therapeutics. Only when these two phases of the subject are brought together and studied experimentally and when the aspects of the subject peculiar to public health activities are added is a complete program realized; then protozoölogy becomes the Biology of Host-Parasite Relations.

Protozoölogy is one of the youngest of the sciences but nevertheless has had an interesting and important history. Protozoa were first discovered by Anton von Leeuwenhoek (1632-1723) in 1675. This famous Dutch microscopist not only saw free-living protozoa, but, as Dobell (1920) has pointed out, was the first to observe intestinal protozoa, having provided an account of a human intestinal flagellate, *Giardia lamblia,* that is easily recognizable. In 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 pissabed running up against a wall. But although they made a rapid movement with their feet, yet they made but slow progress" (from Dobell, 1920). It is also clear from Leeuwenhoek's words that he discovered the fact that the vegetative, motile giardias, thus described, 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.

For many years after Leeuwenhoek's discoveries large numbers of excellent biologists were engaged in describing and classifying protozoa and soon hundreds of species were known. Knowledge of the life-cycles, physiology and behavior of the protozoa also accumulated. Most of

this work was done with free-living species, but, fortunately, the fundamental structure, life-cycles and activities of the free-living and parasitic protozoa are the same; hence the information gained from the study of the former can be applied almost in its entirety to the parasitic forms.

Many of the intestinal protozoa of lower animals were described and studied in a more or less haphazard way before those living in man were considered of any importance, and even to-day it is necessary in certain cases to base our account of human infections on what we know of near relatives in animals. For example, the human coccidium, *Isospora hominis,* is known only in the oöcyst stage, and we are forced to guess at its activities within the host with the help of our knowledge of a closely related species, *Isospora felis,* in cats and dogs. The discovery by Lösch in 1875, of *Endamœba histolytica* in the feces of a patient suffering from dysentery, and the gradual accumulation of evidence that this amœba is the etiological agent of a certain type of dysentery, stimulated the study of this and other species of human intestinal protozoa, so that we believe we have to-day a fairly good idea of the species present in man although their host-parasite relations are very inadequately known.

The term protozoölogy is not difficult to define; it includes all we know about the protozoa, both free-living and parasitic. The term protozoölogist, however, is not so easily disposed of, and it seems worth while in this place to point out that a protozoölogist is one who devotes himself to the study of the protozoa as a special group of

animals, but that every one who studies protozoa is not necessarily a protozoölogist. Thus, many of the investigators who have added valuable data to our knowledge of the protozoa were or are biologists who have employed protozoa in their researches because these organisms seemed to be favorable for the study of problems of general biological significance. Both types of investigators are necessary in order to build up protozoölogy as a science.

2. HOMOLOGIES AND ANALOGIES BETWEEN FREE-LIVING AND PARASITIC PROTOZOA

Parasitic protozoa are often considered by biologists apart from free-living species as though there existed some more fundamental difference between them than that of habitat. A comparison of the structure, life-cycles, habitats and activities of the free-living and parasitic protozoa prove, however, that the same principles govern both types of organisms.

The activities of all animals may be separated into (1) those necessary for the maintenance of the individual, and (2) those necessary for the maintenance of the race. The individual must be able to protect itself in its environment, to escape enemies, to reach a favorable situation in which to live, to find, capture, ingest, digest and assimilate food, to egest undigested material, to secrete protective substances, digestive juices, etc., to carry on respiration and to excrete waste products. Races are maintained by the asexual reproduction of the individuals of which they are composed or by sexual reproduction or by both of these processes.

The phylum Protozoa is usually divided into four classes, Sarcodina, Mastigophora, Sporozoa and Infusoria. The Sporozoa are all parasitic; the other three classes include both free-living and parasitic species. It seems probable that the parasitic habit has evolved from the free-living habit independently in each of these three classes, and this type of evolution has no doubt taken place many times within each class. Changes from a marine to a fresh-water habitat and vice versa, involving the formation of new species, have doubtless similarly occurred among free-living species.

Amœba proteus vs. Endamœba coli. The most common amœba of man is *Endamœba coli,* which lives in the lumen of the large intestine and occurs in about 50 per cent of the general population. The best known free-living amœba is *Amœba proteus.* Morphologically these two species resemble each other very closely. Both consist of cytoplasm, which is differentiated into an external layer of clear ectoplasm and an internal mass of granular endoplasm. Both possess a single nucleus; the nuclei of the two species differ from each other in shape, size and the distribution of the chromatin, but the differences are no greater than those between nuclei of species belonging to different genera of free-living amœbæ. Both carry on locomotion and capture food by means of pseudopodia; and there is no reason to believe that the fundamental process of amœboid movement differs in the two species.

The food of both species consists, so far as we know, of solid particles in the medium in which they live, and these food substances appear to be selected in both species;

Amœba proteus feeds on minute aquatic plants, other protozoa, bacteria and other animal and vegetable matter that it encounters in fresh water, whereas *Endamœba coli* feeds on bacteria and animal and vegetable matter that occur in the contents of the intestine. Food vacuoles are formed in both species into which digestive juices are secreted from the surrounding cytoplasm and in which digestion takes place, the digested material being assimilated and the undigested material extruded through the surface of the body. Respiration takes place through the general body surface. The waste products of metabolism are excreted through the ectoplasm. The only striking difference between the two species morphologically and physiologically is the presence of a contractile vacuole in *Amœba proteus* and its absence in *Endamœba coli.* The functions of the contractile vacuole are supposed to be principally respiratory and excretory,—functions that in parasitic species are satisfactorily performed through the surface of the body.

The habitats in which *Amœba proteus* and *Endamœba coli* live differ in many respects. The factors of the environment of the former are well known to every student of biology but not so those of *Endamœba coli.* This parasitic species has for its habitat the lumen of the large intestine. Here it lives in total darkness in the liquid contents, which consist of digested food substances, bacteria of various sorts, the products of bacterial decomposition and more or less changed secretions from the digestive glands. This medium is more viscid than water and chemically much more complex. The temperature is rela-

tively high and constant (37°C.). Peristalsis, which transports the intestinal contents towards the rectum, tends to carry the amœbæ out of the body, just as currents of water may transport *Amœba proteus* from place to place. On the whole the environment of *Endamœba coli* is much more constant than that of *Amœba proteus,* but the important points are that each species maintains itself successfully in its own particular environment and that there are no fundamental differences between these environments. If either species is transferred to the environment of the other it is very quickly killed, but both species may be grown in artificial cultures. *Amœba proteus* may be grown in the laboratory in a flat dish containing pond weed immersed in water. The cultivation of *Endamœba coli* requires more care, but has recently been accomplished by several investigators. The culture medium consists of hens' eggs and Ringer's solution and is very easily prepared. Specimens of *Endamœba coli* are placed in the culture fluid and incubated at 37°C. Because of the rapid growth of bacteria new cultures must be made and inoculated at frequent intervals (approximately twenty-four to forty-eight hours).

The processes of reproduction are not fully known in either *Amœba proteus* or *Endamœba coli.* We know that both of them multiply asexually by binary division and that this division is by a sort of mitosis but without the dissolution of the nuclear membrane. Sexual phenomena may be exhibited, but none has yet been established with certainty. Cysts have been described in the life cycle of *Amœba proteus,* but appear to be of uncommon occur-

rence. The animal is reported to become spherical and then to secrete a resistant wall about itself. Within this cyst a large number of nuclei (500 to 600) are formed by repeated division of the original nucleus, and each of these nuclei becomes the center of a minute cell or amœbula. These amœbulæ break out of the cyst and develop into recognizable *Amœba proteus* in about three weeks. The cysts of *E. coli* are similarly formed. All food material is first extruded; then a cyst wall is secreted; and finally the nucleus undergoes three successive divisions resulting in eight daughter nuclei. At this point in the life cycle the cysts are carried out of the body in the feces of the host and no further development occurs unless they are ingested by a proper host and are in this way again brought into a favorable environment. The cysts of *Amœba proteus* must likewise encounter a favorable environment before they will develop normally. The process of excystation has not been satisfactorily worked out in *E. coli,* but presumably each cyst gives rise to eight small amœbæ which, as in the case of the amœbulæ of *A. proteus,* grow into adult amœbæ in their natural habitat—the contents of the large intestine. In *E. coli* the cyst wall undoubtedly protects the organisms from injury during their life outside of the body; one of the functions of the cyst wall of *A. proteus* is probably also to carry this species unharmed through periods of adverse circumstances.

The environment of *Endamœba coli* within the intestine changes from time to time according to the nature of the food taken in; for example, the intestinal flora may be changed from one consisting almost entirely of acidophil-

ous bacteria to one made up of almost 100 per cent of putrefactive bacteria by changing from a vegetarian diet to a meat diet for a few days. The intestinal environment may also be modified by the infection of the host with other parasitic organisms, such as other species of amœbæ, intestinal flagellates, intestinal worms or vegetable organisms such as yeast and *Blastocystis hominis.* Drugs of various sorts and other agents may likewise change the medium in which *E. coli* lives, for better or for worse. How similar are the conditions that exist in the free-living environment of *A. proteus!* The medium in which it lives may be diluted by rain or concentrated by drought. The nature and numbers of other organisms with which it must share its habitat differ from time to time; and pollution of the water may alter unfavorably its surroundings, just as the administration of drugs may modify the intestinal contents of man to the detriment of *E. coli.*

We know nothing about the behavior of *E. coli* within its habitat, but it is safe to assume that this species reacts to stimuli in its environment and that these reactions are such as to insure its continued existence, otherwise the race would cease to exist. These reactions are no doubt different from those of *A. proteus,* but they lead to the same result. The conclusion is inevitable that in morphology and in every process and activity that occurs during their life-cycles, no essential differences are evident between the free-living *Amœba proteus* and the parasitic *Endamœba coli.*

A consideration of the geographical distribution and methods of dissemination of the free-living and parasitic

amœbæ is also of interest. *Amœba proteus* seems to be very widespread, having been found in bodies of fresh water in many countries. *Endamœba coli* is likewise cosmopolitan in its distribution, occurring in man wherever it has been looked for. The factors that control the distribution of the two species are different in certain respects, but the end result is the same. Both species are spread by running water, *A. proteus* mostly in the active stage and *E. coli* in the cyst stage whenever water is polluted with cyst-containing feces. There is evidence that *E. coli* is also carried to the food or drink of man by flies and we know it to be transported to all parts of the world by its human host. *A. proteus* is no doubt carried from one pond to another by aquatic birds and by other organisms and may also be transported by man on aquatic animals or plants.

Similar comparisons could be made with similar results between other species of free-living and parasitic protozoa. It seems unnecessary, however, to describe in detail the similarities and differences between these types of protozoa, but certain characteristics in the lives of these organisms may be referred to with profit.

Tissue invasion. Balantidium coli is an occasional inhabitant of the large intestine of man, especially in tropical and subtropical countries. It is very similar to the common free-living ciliate, *Paramœcium caudatum,* and could be compared with this species just as *Endamœba coli* has been compared above with *Amœba proteus.* One very interesting activity in the life-cycle of *B. coli* is its invasion of the tissues of the intestinal wall and the production of ulcers and dysenteric symptoms. The evidence

available indicates that it actively bores its way into the tissues, which are apparently dissolved by ferments secreted by the ciliate. Other species of human protozoa also invade the tissues of the host and are thus pathogenic. There is no activity among free-living protozoa exactly like this; otherwise they would be classed with the parasitic species. The invasion of tissue is thus a characteristic peculiar to the latter.

Symptoms. The effects of parasitic protozoa on their environment, the host, is in many cases very striking, since not only are changes which constitute what we call disease produced but often the death of the host results. Symptoms are nothing but the results of the functional modification of the organs of the host. These modifications in the medium in which they live are well known and the changes observed in the medium correspond to the symptoms resulting from parasitic activities. In other words, the host is an environment just as a body of water is an environment.

Natural resistance. Each host offers certain obstacles which must be overcome by the parasite before invasion is accomplished; in many cases, in fact, hosts do not become infected at all, because of the natural resistance of the body, although parasites succeed in gaining entrance to the intestine or blood stream. We may compare the host with a pool of water which contains various substances in solution and also various species of plants and animals. Not all free-living protozoa succeed in populating such a pool of water due to the natural resistance offered by the composition of the water and the other organisms present—the physical, chemical and biological

factors of the environment. Those that are able to live and reproduce may be said to have successfully invaded this particular habitat. Each species of animal has an optimum habitat; this for a parasitic protozoön is a favorable host and for a free-living protozoön a body of water with certain physical, chemical and biological characteristics.

Acquired resistance. One of the effects of the infection of animals with parasitic organisms is the production by the host of an active (acquired) resistance which may result in the destruction of many and often all of the parasites and the immunity of the host to subsequent infection. There is no type of resistance similar to this that may be acquired by the environment of free-living protozoa; only living organisms are capable of this type of reaction. However, both parasitic and free-living protozoa may "foul their own nests" by their secretions and excretions to such an extent as to make their environments unfit for further life activities. In this way cultures of free-living species may die out and infections with parasitic species may come to an end.

Latency and relapse. An interesting phenomenon characteristic of many parasitic infections is the cessation of symptoms for a time, followed by the appearance of symptoms again, that is, a relapse. Every one who has collected protozoa from any particular pond at various times from year to year knows that a condition resembling relapse exists in such an environment. Sometimes a certain species is very abundant; at other times specimens can be found only by patient search.

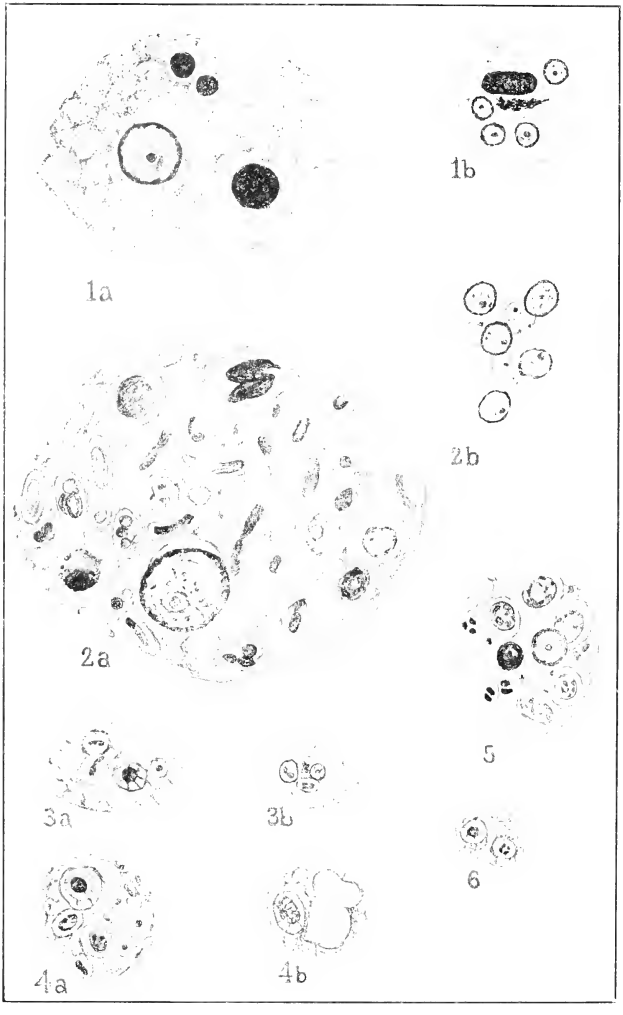

PLATE I
(Figures 1-6)

Host-parasite specificity. This phenomenon, which is one of the most interesting characteristics of animal parasites, is fully discussed later (see page 45).

Habitat restrictions. The situation as regards free-living protozoa is quite similar. A particular species has its optimum habitat; it may even exist although the environmental factors depart considerably from the optimum. There is, however, a point beyond which existence is impossible; life in a habitat where this point has been reached for one or more factors therefore ceases and the species concerned cannot grow and multiply in such a habitat, no matter how frequently and abundantly specimens may be introduced into it. Many of the terms familiar to parasitologists might with equal force be applied to free-living protozoa; for example, a tolerant host or habitat is one in which a protozoön can live and multiply successfully, whereas in a refractory host or habitat life and multiplication are difficult; a "natural" host or habitat is one in which a certain protozoön generally is to be found in nature, whereas a host or habitat in which a species usually does not live is considered "foreign." Conditions are similar as regards accidental

PLATE I

AMŒBÆ LIVING IN MAN

(All figures magnified about 2000 diameters)

1a and 1b. *Endamœba histolytica.* 1a, trophozoite. 1b, cyst. (After Dobell.)
2a and 2b. *Endamœba coli.* 2a, trophozoite. 2b, cyst. (After Dobell.)
3a and 3b. *Endolimax nana.* 3a, trophozoite. 3b, cyst. (After Taliaferro and Becker.)
4a and 4b. *Iodamœba williamsi.* 4a, trophozoite. 4b, cyst. (After Taliaferro and Becker.)
5. *Endamœba gingivalis.* (After Dobell.)
6. *Dientamœba fragilis.* (After Taliaferro and Becker.)

or transitory hosts or habitats and facultative or obligatory species of protozoa.

Control. And finally, if we wish to control the protozoa either in man or in a free-living habitat, we apply similar methods. For example, when a person has an attack of amœbic dysentery due to the presence in his intestine of large numbers of specimens of *Endamœba histolytica* he is treated with a therapeutic agent, such as emetin or yatren; and when a reservoir of water becomes overrun with flagellates of the genus Synura, thus causing obnoxious odors and tastes, it is treated with a dose of copper sulfate.

Cases could be multiplied almost indefinitely bringing out homologies and analogies between free-living and parasitic protozoa but the data presented are sufficient to prove that the same principles govern both these types of organisms as regards morphology, physiological processes, life-cycles and their relations to their physical and biological environments. The relations between parasitic protozoa and their hosts must, therefore, be studied as biological phenomena just as we are accustomed to study the relations between free-living protozoa and their environment.

3. THE INTESTINAL PROTOZOA LIVING IN MAN

Each of the four classes of the protozoa include "intestinal" species that live in man; these are represented in the accompanying figures and referred to by number below. To the Class Sarcodina belong six species of amœbæ, *Endamœba gingivalis* (Fig. 5) in the mouth, and *Endamœba coli* (Figs. 2a, 2b), *Endamœba histo-*

lytica (Figs. 1a, 1b), *Endolimax nana* (Figs. 3a, 3b), *Iodamœba williamsi* (Figs. 4a, 4b), and *Dientamœba fragilis* (Fig. 6) in the large intestine.

The Class Mastigophora contains seven species of "intestinal" flagellates, *Giardia lamblia* (Figs. 13a, 13b), an inhabitant of the duodenum; *Chilomastix mesnili* (Figs. 10a, 10b), *Embadomonas intestinalis* (Figs. 11a, 11b), *Tricercomonas intestinalis* (Figs. 12a, 12b), *Trichomonas hominis* (Fig. 9), which live in the large intestine; *T. buccalis* (Fig. 8), an inhabitant of the mouth; and *T. vaginalis* (Fig. 7) which occurs in the vagina.

The Class Sporozoa is represented by one species of coccidium, *Isospora hominis* (Fig. 15) which penetrates the intestinal epithelium. *Eimeria wenyoni* (Fig. 16) and *Eimeria oxyspora* (Fig. 17), which were named by Dobell as human species, have been shown by Thomson and Robertson (1926a) to be *E. clupearum and E. sardinæ* respectively,—species that occur in fish.

Only one species of the Class Infusoria is known with absolute certainty to be a parasite of man; this is the ciliate *Balantidium coli* (Fig. 18), which lives in the large intestine and gives rise sometimes to balantidial dysentery.

Besides these species, which are recognized by all protozoölogists, there is a long list of protozoa that have been described from the digestive tract of man about the authenticity of which there is still some doubt.

4. TERMS USED IN THE STUDY OF PARASITIC PROTOZOA

Those who have not interested themselves particularly in parasitology may not be familiar with some of the

terms in common use; to these the following definitions may be of value. It is perhaps desirable first to distinguish between parasitism and predatism. A parasite is an organism that lives on or in and at the expense of another organism without immediately destroying it. A predaceous animal also lives at the expense of other animals but kills them directly and devours them. There are in nature a continuous series of intermediate stages between parasitism on the one hand and predatism on the other.

The term symbiosis was proposed by deBary in 1879 for the constant, intimate and mutually beneficial association of two organisms. Etymologically, symbiosis means simply "living together" and hence should include parasitism, mutualism, commensalism and all other types of consociation, but the term now implies the permanent association of two specifically distinct organisms so dependent on each other that life apart is impossible. When the association is less intimate but each partner benefits the other the term mutualism is sometimes employed. The terms commensalism and inquilinism are often used for still looser associations. Commensalism is applied to the regular association of two definite species of organisms which "eat together at the same table" but not at each other's expense. Very similar in meaning is inquilinism, which is used to describe the condition where one animal lives with another as a co-tenant but usually not at its expense.

The origin of these various types of association is, of course, not definitely known, but can be inferred without much difficulty because of the existence of a large num-

ber of intermediate stages. That they have arisen many times is indicated by their wide distribution among the phyla in the animal kingdom. The evolution of parasitism is one of the most interesting of all biological problems and, as has been pointed out by several writers, parasites offer particularly favorable material for the study of the course of evolution, since parasites undoubtedly originated from free-living organisms from which they have become differentiated by a sort of superimposed evolution, and in many cases the free-living ancestors of these parasites still exist.

One very striking effect of the parasitic habit is that generally called degradation. This term implies that the parasite has degenerated, but although some of the parts of the parasite undergo degeneration, others become more highly developed. It seems better therefore to speak of the parasitic condition as a specialization rather than a degradation or regression, especially since most parasites are marvelously adapted to their mode of life.

II. *General Account of the Biology of Host-Parasite Relations between Man and His Intestinal Protozoa*

Before discussing the various species of intestinal protozoa of man in detail it seems desirable to present a general account which will serve more or less as an outline. The subject matter has been arranged as nearly as possible in the order of the series of events that occur during an infection, and illustrative material has been taken principally from data available regarding intestinal protozoa.

I. EPIDEMIOLOGY OF TRANSMISSION

(1) INFECTIVE STAGE. Perhaps the most satisfactory point at which to begin the study of the biology of host-parasite relations is the infective stage of the parasite. The organisms during the period of the infective stage are usually subjected to various environmental factors from the time they escape from the body until they gain entrance to a new host. The idea maintained by many of the older authorities that disease-producing organisms may multiply outside of the host and bring about foci of infection in soil or water has been abandoned since it has been abundantly proved that very little if any increase in numbers occurs under these conditions.

Most of the disease-producing protozoa of man escape from the body in a cyst-like condition and spend part of their life-cycle outside of the host. The problems encountered during this period are extremely serious and very few of the organisms survive the vicissitudes of a free-living existence. The two principal problems encountered by the parasites are (1) that of withstanding the factors in their new environment and (2) that of developing to the infective stage. The first problem is particularly difficult for protozoa such as the intestinal flagellate, *Trichomonas hominis* (Fig. 9), that do not form resistant cysts but pass from one host to another in the active, trophozoite stage. These trophozoites can exist only in a liquid medium and are presumably very susceptible to modifications of temperature, to chemical changes in the environment and to mechanical injury. Most protozoa, however, are protected by one or more

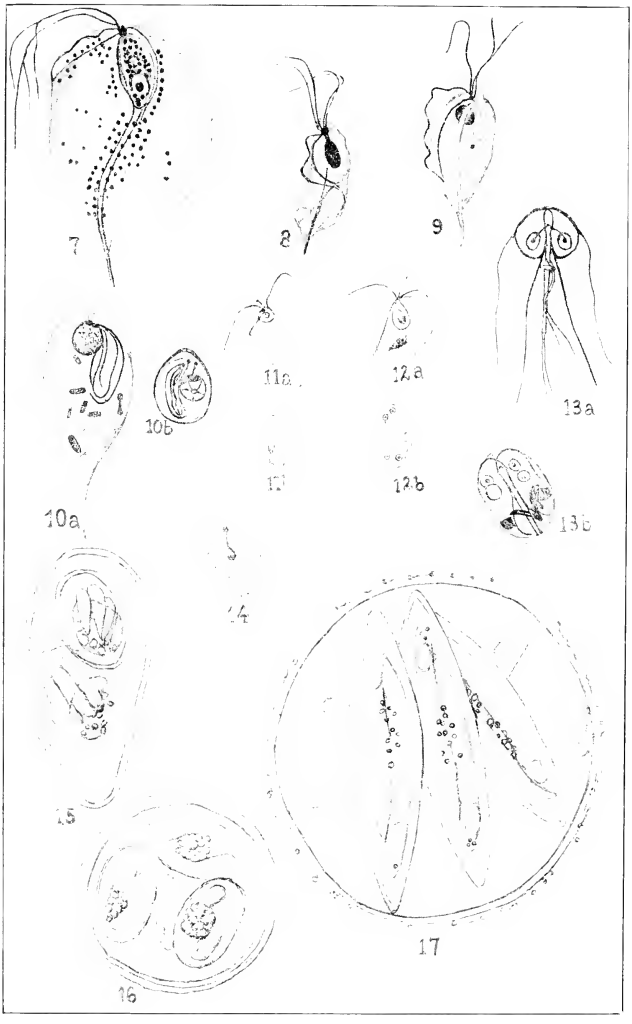

PLATE 2
(Figures 7-17)

resistant walls secreted by the organism, which help prevent loss of water; keep out injurious substances; and guard against molar agents. The terms applied to these resistant bodies are "cyst" (Fig. 1b) in the case of intestinal amœbæ, flagellates and ciliates, and "oöcyst" (Fig. 15) in the coccidia.

In most cases, probably no development occurs within the cysts after they escape from the body. The oöcysts of coccidia, however, continue their development if discharged when still immature. Presumably not until sporozoites are fully developed do the oöcysts become infective.

It seems clear from the evidence available (1) that no foci of infection of disease-producing protozoa exist outside of the host and (2) that in the majority of cases certain stages are already infective when they escape from the body and all other stages including trophozoites and immature cysts die outside of the host.

PLATE 2

Intestinal Flagellates (Figs. 7 to 14) and Coccidia (Figs. 15 and 17) Living in Man.

(All figures of flagellates magnified about 2000 diameters and of coccidia about 1600 diameters)

7. *Trichomonas vaginalis.* (After Hegner.)
8. *Trichomonas buccalis.* (After Goodey and Wellings.)
9. *Trichomonas hominis.* (After Faust.)
10a and 10b. *Chilomastix mesnili.* 10a, trophozoite. 10b, cyst. (10a, after Boeck; 10b, after Kofoid and Swezy.)
11a and 11b. *Embadomonas intestinalis.* 11a, trophozoite. 11b, cyst. (After Hegner.)
12a and 12b. *Tricercomonas intestinalis.* 12a, trophozoite. 12b, cyst. (After Wenyon and O'Connor.)
13a and 13b. *Giardia lamblia.* 13a, trophozoite. 13b, cyst. (After Simon.)
14. *Enteromonas hominis.* (After Fonseca.)
15. *Isospora hominis.* (After Dobell.)
16. *Eimeria clupearum* (= *E. wenyoni*). (After Wenyon.)
17. *Eimeria sardinæ* (= *E. oxyspora*). (After Dobell.)

(2) AVENUES OF INFECTION. Reaching and invading a new host is perhaps the most serious problem in the entire life-cycle of a parasitic protozoön so far as the maintenance of the species is concerned. Only the smallest fraction of the total number of infective organisms can possibly reach a susceptible animal in which to live, and only the almost inconceivable fecundity of the parasites prevents the various species from dying out. In the most simple cases the infective stage of the parasite is ingested with the food or drink of the proper host. The parasite is passively carried in the medium by which it is surrounded and it is the behavior of the host that leads to invasion. This is the contaminative method of parasite transmission. Laboratory experiments, mostly with lower animals, have established this as an effective method and there is no other obvious way in which infective cysts in nature can obtain entrance to the host.

The problem of the parasite is to reach the mouth of the host before death results from drying, bacterial action, or starvation within the cyst. A moist, warm climate is therefore favorable for transmission. Insanitary conditions due to neglect on the part of the host are also favorable since this leads to the pollution of drinking water, milk and other food substances. Flies probably play an important rôle in the distribution of the cysts. In general it may be said that transmission by the contaminative method is more easily effected in rural than in urban communities, and in the tropics than in the temperate regions.

There are three other principal methods of reaching

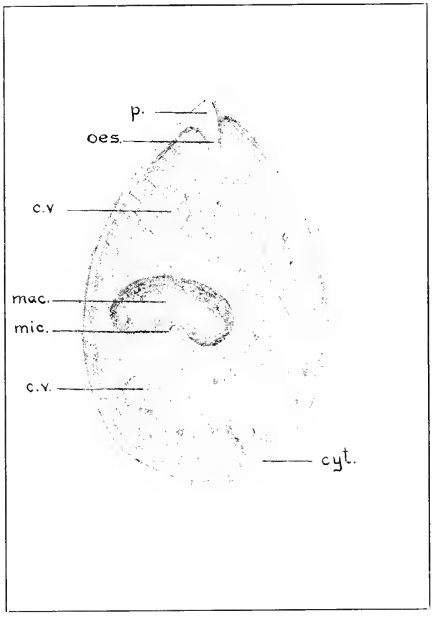

FIGURE 18

Balantidium coli from man. c.v., contractile vacuole; cyt., cytopyge; mac., macronucleus; mic., micronucleus; oes., oesophagus; p., peristome. (Original).

a new host. These are (1) by "contagion" or direct transference from definitive host to definitive host, (2) by inoculation through the agency of an intermediate host, and (3) by "inheritance." The "hereditary" method of transmission is not known to occur in any protozoön living in man, but takes place in a number of species of parasites of lower animals; inoculation by an intermediate host is a very common method of transmission among blood-inhabiting protozoa.

The direct or contagious method of transmission may be brought about in various ways. Among "intestinal" protozoa the amœba, *Endamœba gingivalis* (Fig. 5) and the flagellate, *Trichomonas buccalis* (Fig. 8), that live in the mouth, no doubt are transferred directly from one host to another by kissing. The flagellate, *Trichomonas vaginalis* (Fig. 7), which is apparently widespread among women and has been recorded from the urinary tract of man, may possibly be distributed during coitus.

2. CLINICAL AND PARASITOLOGICAL PERIODS DURING THE COURSE OF A NATURAL INFECTION

The term natural infection is used here to designate an infection in nature during which the parasite is able to pass through its life-cycle successfully and provide infective stages for the invasion of a new host or intermediate host. In contrast to natural infections, are conditions that result from the invasion of hosts that may be called foreign, refractory, accidental or casual,—terms that are fully explained below under the subheading Host-Parasite Specificity.

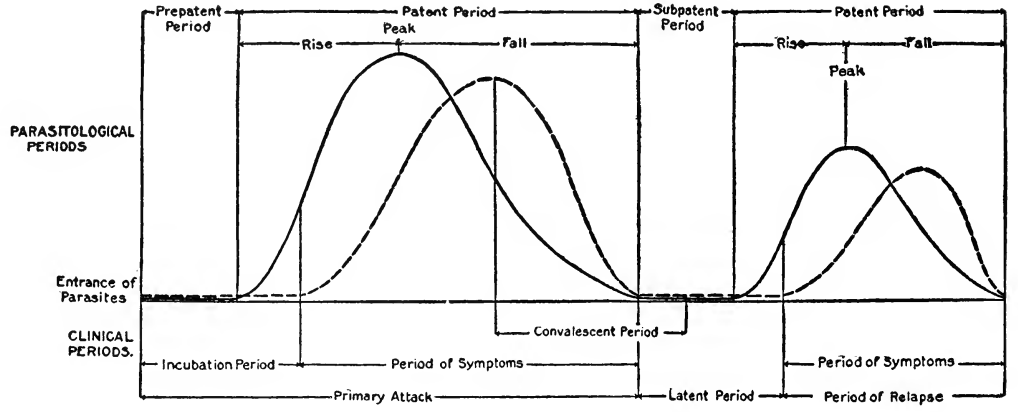

FIGURE 19

Parasitological and clinical periods in protozoan infections. This illustration is intended to represent by means of curves the parasitological and clinical periods in the course of infections with pathogenic protozoa. It represents in a general diagrammatic way the course of natural infections, consisting of a primary attack followed by apparent recovery of the host. This completes both the parasitological and clinical manifestations of disease in cases such as oriental sore in which one attack gives immunity. In other diseases, however, such as amœbic dysentery and malaria, the primary attack is often followed by a symptomless period when the parasites are latent. Eventually the parasite number again increases, symptoms reappear and the host is said to suffer a relapse. The terms used are fully described in the text. (From Hegner.)

(1) PARASITOLOGICAL PERIODS. (See Fig. 19.)

(a) The Prepatent Period * extends from the time the infective parasites enter the body of the host until their offspring can be recovered by specified laboratory methods. The length of this period obviously depends to some extent on the character of the laboratory technique employed.

(b) The Patent Period covers the interval during which the parasites can be demonstrated by the technique employed. The parasite number undergoes a Rise during this period, reaches a Peak and then suffers a Fall. The patent period ends when the parasites can no longer be found in the feces.

(c) In many infections the patent period is followed by a Subpatent Period of indefinite length. During this period parasites can not be recovered by the usual laboratory methods but their presence can be proved in various ways depending on the species of parasite. For example, protozoan cysts may disappear from the stools but reappear after a few days, weeks or months have elapsed.

The subpatent period may be followed by a second patent period during which the parasite number rises, reaches a peak, and falls, but often does not rise as high as in the primary attack.

(2) CLINICAL PERIODS. (See Fig. 19.)

(a) The Incubation Period extends from the time of the entrance of the parasites until symptoms appear. This period is usually longer than the prepatent period, but may be shorter. For example, in man infected with

* The terms prepatent, patent and subpatent were first suggested by Dr. Justin Andrews. The word *patent* is derived from the latin word *patens* meaning *evident, apparent, unconcealed.*

the coccidium, *Isospora hominis,* diarrheic symptoms appear before oöcysts are recoverable in the feces. As indicated in Fig. 19, the number of parasites usually increases considerably before symptoms become evident. The curves as given probably do not represent actual conditions in any specific protozoan infection but are meant to indicate that in general the increase in parasite number precedes the appearance of symptoms and that increases and decreases in the severity of the symptoms follow the rise and fall in parasite number.

(b) The Period of Symptoms begins when the incubation period ends and ends of course with the cessation of symptoms.

(c) The Convalescent Period is represented as beginning at the point of maximum symptoms. It ends, not when the symptoms disappear but later with the recovery of the host.

(d) In diseases characterized by relapses one or more Latent Periods may be present. During these periods the causative organisms are too few in number to bring about symptoms, but, after intervals of indefinite length, some change occurs in parasite or host or in both that results in an increase in parasite number and a reappearance of symptoms.

(e) The reappearance of symptoms following a latent period is known as the Period of Relapse.

3. DISTRIBUTION AND LOCALIZATION OF PARASITES WITHIN THE HOST

(1) DISTRIBUTION. As already noted intestinal protozoan parasites may gain entrance to their natural hosts

by way of the mouth in contaminated food or drink, or by direct contact. The distribution of the protozoa after they enter the body is at first due almost entirely to the activities of the host and is determined by the point and method of entrance. Intestinal-inhabiting species are transported with the food through the esophagus and stomach and into the intestine; species that are transmitted by contact remain usually in the region of entrance, e. g., in the mouth or genital cavities.

(2) PRIMARY SITE OF INFECTION. Different species of intestinal protozoan parasites become localized in different organs, tissues or cells of the body depending on various factors. The digestive tract is more frequently affected than others, a fact that is probably vitally associated with the necessity of the offspring to escape from the host. The parasites may be (1) coelozoic, *i. e.,* localized in cavities, such as the lumen of the digestive tract, and genito-urinary cavities, or (2) histozoic, *i. e.,* within the tissues, where they may live among the cells (intercellular), or within the cells (intracellular or cytozoic). The factors that are responsible for localization are not well known. The parasites are subjected to various conditions in their environment just as free-living protozoa are in theirs: they must protect themselves from injurious agents, such as secretions and excretions; must reach a location where the proper nutriment is available; must possess some means of fixing themselves in a favorable situation; and must be able to carry on reproductive processes. Certain intestinal flagellates of man, as will be pointed out later, are of particular interest for purposes of illustration.

Localization among protozoa that invade the tissues of the host offer many problems that are still unsolved. What factors influence the coccidia to attack the epithelial cells of the intestine? What causes *Endamœba histolytica* (Fig. 1a) and *Balantidium coli* (Fig. 15) to penetrate the intestinal wall, whereas *Endamœba coli* (Fig. 2a) and *Endolimax nana* (Fig. 3a), which are also inhabitants of the intestine do not? It may safely be said, that we really know almost nothing about the mechanism of localization of any human protozoön.

(3) SECONDARY SITES OF INFECTION. Many protozoa become localized in a particular organ or tissue and do not spread to other parts of the body. A few, however, bring about secondary foci of infection in other regions. This requires in the first place either movement on the part of the parasite from one place to another or activities within the host that passively transport the parasite to new situations. The circulatory system of the host is the most important agency. For example, the dysentery amœba, *Endamœba histolytica* (Fig. 1a), sets up a primary infection in the large intestine; specimens may enter the blood stream in the capillaries of the intestinal wall and are carried to all parts of the body; frequently the liver becomes a secondary site of infection and an amœbic liver abscess results. Less often amœbic abscesses occur in the lungs and brain, and amœbæ have been discovered in many other parts of the body. Recently Kofoid (1923) has described dysentery amœbæ from bone and believes that they are responsible for Ely's second type of arthritis; he believes also that these amœbæ may be the etiological factor in Hodgkin's disease and in some

cases of chronic neurasthenia and subnormal health; the evidence for this is as yet incomplete. It is obvious that amœbic abscesses may occur in any part of the body provided local conditions are favorable. Just what factors favor the localization and multiplication of the amœbæ in liver, lungs and brain and prevent infections in other parts of the body are not clear.

The details of the distribution and localization of parasites within the host are still to be determined, but it may be said in general that distribution is due primarily to the physiological activities of the host, and localization to host-parasite interactions, the parasites setting up an infection wherever favorable conditions exist.

4. PASSIVE (NATURAL) RESISTANCE OF THE HOST

The obstacles that must be met by the parasite at the beginning of an invasion constitute the passive, or natural, resistance of the host. This type of resistance is in part due to the nature of the host without respect to ancestral relations with the parasite or may in part have been built up during the course of evolution as a protection against infection. The changes in the environment of an intestinal flagellate, for example, which is carried from the outside into the digestive tract of a new host are very striking (see p. 148).

A parasitic protozoön may successfully withstand all the conditions encountered in a new host but still fail to bring about an infection because of the absence of factors necessary, for example, to weaken the wall of a protozoan cyst and allow the organism within to escape. The literature of protozoölogy contains many reports of

human "intestinal" protozoa on the basis of specimens found in the feces which were not active within the intestine but had passed through in the cyst stage and had emerged only after the feces were passed; these are "coprozoic" protoza, a considerable number of which have been described.

5. PASSIVE (NATURAL) RESISTANCE OF THE PARASITE

If some parasitic protozoa were not able to overcome the obstacles presented by the host, animals would be totally free from them. Thus far no such animal has been discovered. This passive resistance of the parasite, as in the case of that of the host, may have no relation to ancestral infections or may be due in part to evolutionary processes. Whether the resistant coverings of protozoan cysts serve to enable the parasite to reach a certain location within the host or are more important as a protection against injury during their life outside of the host it is not possible to decide with certainty. It is known that such cysts will survive when subjected to strong solutions of various chemicals (see p. 71) and might therefore easily withstand the body juices of the host. It is also known, as pointed out below in the case of *Trichomonas hominis* (see p. 142), that active trophozoites may pass unharmed through the digestive tract and set up an infection in the cecum. The value of the cyst wall as a means of resisting conditions within the host is therefore very doubtful.

Every animal is able to exist in an environment in which the various factors cover a considerable range,

i. e., the optimum conditions are not necessary, although they may be desirable, for the maintenance of either the individual or the race. Protozoan parasites that are natural to a particular host are accordingly able to withstand considerable changes in temperature; are not affected by the change from light to darkness or vice versa; and do not succumb to complex chemical changes nor to increases in the density of the surrounding medium when taken into that host; but why these same parasites are not able to live in nearly related hosts is a problem of great difficulty. A discussion of these questions is presented under the heading of Host-Parasite Specificity (p. 42).

6. THE PARASITE'S METHOD OF ATTACK

The character of the attack of the parasitic protozoön on the host is of vital importance not only to the host but also to the parasite. It is obvious that the degree of pathogenicity depends on which organs or tissues are invaded, and on the degree and rapidity of tissue destruction or the production of toxic substances, since slight injuries inflicted slowly are usually repaired by the host; whereas serious injuries that are quickly produced lead to symptoms. The association that exists in most cases of parasitism is such that the parasite is able to live and reproduce for many years within the host without apparent injury to it. If the host develops severe symptoms both it and the parasites are in danger and if the host dies the parasites die with it. This type of parasitic attack is unusual and is considered to represent a comparatively re-

cent association, since most parasites live in harmony with their hosts—a condition that is supposed to have developed during the course of evolution.

Types of parasitism are distinguished largely on the basis of the method of attack of the parasite. Among the amœbæ living in man, for example, is *Endamœba coli* (Fig. 2a) which inhabits the lumen of the large intestine where it probably lives on food taken in by the host; this type of parasite is known as a commensal and is sometimes termed a food-robber. It does not live in any other species of host nor outside of the body (except in the cyst stage) and is therefore a permanent, obligatory parasite and non-pathogenic. Another species of the same genus, *Endamœba histolytica* (Fig. 1a), also lives in the large intestine of man but feeds on tissue elements which it apparently dissolves with the aid of proteolytic enzymes that it secretes, or engulfs en masse. Usually the host is able to repair the tissue as rapidly as it is injured, but often the parasites gain the upper hand and amœbic dysentery results, sometimes ending fatally. This organism is a permanent, obligatory parasite that is apparently always pathogenic and sometimes lethal. Other parasites penetrate tissue cells and develop within them at the expense of the surrounding protoplasm. To this type belong the coccidia that live on cells of the intestinal epithelium.

Parasitic protozoa may produce toxic substances or zoötoxins. Almost nothing is known about these, but that they exist is certain, and that they act much like bacterial toxins is probable.

7. CHANGES IN THE HOST CAUSED BY THE PARASITE

The reactions of hosts to infection may be considered under the headings symptomatology, pathogenesis and immunology. If the parasite lives on the tissues of the body or injures the body in any way it is pathogenic. If the injuries are severe, changes occur in the functions of certain organs sufficient to bring about symptoms. In certain cases the body reacts to the infection by building up a resistance to the parasite which we call immunity.

(1) SYMPTOMATOLOGY. The symptoms that are characteristic of the various diseases due to parasitic protozoa are in most cases well known. Comparatively little is known, however, regarding the genesis of these symptoms. The Century Dictionary defines a symptom as "one of the departures from normal function or form which a disease presents, especially one of the more evident of such departures." In other words, symptoms arise when the function of an organ is modified. Usually one species of parasitic protozoön brings about a large number of symptoms since more than one organ may be disturbed. Some of these are localized at the point where injury is being done, whereas others appear at a distance.

The most recent and probably the most successful attempt to determine the mechanism of symptom production is that of Sir James Mackenzie (1923). His argument is as follows: Symptoms are produced by organs whose functions are stimulated to unusual activity, or depressed or suspended. Functional activity is dependent on the cells of the organ and on the nerves and other

agents that regulate the activity of these cells. There is formed a reflex arc consisting of a receptor which receives the stimulus, an afferent nerve which carries it to a nerve center, and an efferent nerve which conveys it to the effector. A modification of any part of this arc results in an abnormal response, *i. e.,* a symptom. The effector may be a muscle, gland, or the sensorium. The blood stream no doubt also plays an important rôle in the production of symptoms by transporting toxic substances from one part of the body to another. The genesis of symptoms in protozoan diseases is open to experimental study, but very little is known about the subject.

(2) PATHOGENESIS. Many of the protozoa of man are not pathogenic so far as is known; this is true of *Endamœba coli* and other commensals. A few, such as *Endamœba histolytica* and the coccidia are apparently unable to exist without direct attacks on the host tissues. These attacks bring forth more or less definite responses on the part of the host and usually a rather definite series of changes occur during the course of the infection. This series never proceeds beyond the earlier stages in contact carriers (see p. 107) and stops short of the end when the patient is treated or undergoes spontaneous recovery. Only when the death of the host ensues may the final stages be observed. Each parasite maintains its own method of attack and the host responds usually in a perfectly definite way to the inroads of each species of protozoön. The changes in the host have definite effects upon the progress of the parasitic attack and it is thus possible to obtain a dynamic view of host-parasite reactions during the course of the infection. The complete

pathogenesis has not been worked out for any human protozoön. Experimental studies are possible in lower animals, *e. g.,* amœbiasis in cats, and most of what we know of the pathogenesis of protozoan diseases has been gained in this way, but the complete story for any one of them is not yet possible because of lack of observations.

(3) IMMUNOLOGY. The subject of immunity to protozoan infections is in its infancy and the little we know about it at present is based principally on epidemiological observations and on animal experiments. That hosts differ considerably with respect to natural resistance to various protozoan diseases is evident since many individuals do not become infected although they are undoubtedly invaded by the parasites. Acquired resistance has been demonstrated in certain cases.

8. CHANGES IN THE PARASITE DUE TO RESIDENCE IN THE HOST

(1) IMMUNOLOGY. Residence in a host may bring about the building up of an active, acquired resistance on the part of the parasite in certain cases. The well-known hypothesis of Welch (1902), that bacteria are stimulated to protect themselves by the production of antibodies when they are subjected to the defensive forces of the host, no doubt holds true for protozoa. That this should occur is not at all strange since parasites are living organisms and doubtless react to various stimuli much as does the host, but they appear to be capable of modifications that enable them to resist harmful therapeutic agents. Thus races of parasites are supposed to develop

that are "fast" to substances ordinarily destructive. For example, it is customary to speak of emetin fastness in amœbiasis. Loss of resistance may also result from subjection to various environmental factors.

(2) AGGRESSIVITY. The term aggressivity is applied to the invasive powers of a parasite. Changes in parasite aggressivity due to residence within a host have been reported although the situation is not yet clear. Thus differences in invasive powers have been noted among the cysts of the dysentery amœba, *Endamœba histolytica,* which may have been due to changes during residence in the human host. For example, Baetjer and Sellards (1914) state that "chronic cases of long standing, with mild symptoms, often produced an attack in animals which was of comparatively short duration and eventually ended in recovery"; and Wagener and Thomson (1924) were able to infect kittens without difficulty with amœbæ from an acute case of amœbiasis but succeeded in only one of fourteen kittens when amœbæ were used from a chronic case of amœbiasis. The supposition is that the conditions of chronicity modified the aggressivity of the amœbæ until a strain with very little invasive powers was developed.

9. HOST-PARASITE ADJUSTMENTS DURING AN INFECTION

(1) CARRIERS. During the course of a natural infection as outlined above various adjustments occur between host and parasite. Continued reproduction by the parasite without check would obviously result in the death of the host; this would be a disadvantage to the parasite,

since it is thus prevented from further growth and multiplication and especially from dissemination. Spontaneous recovery results in most cases of protozoan infection, but by recovery is meant the cessation of symptoms and not the total disappearance of parasites from the body. Frequently the host, by means of its acquired resistance, is able to destroy most but not all of the parasites, and hence to bring about the carrier condition.

A carrier is a host in which parasites live and by which they are disseminated but which exhibits no visible symptoms of infection. Walker and Sellards (1913) distinguished two types of carriers in their work with human amœbæ, (1) contact carriers who are parasitized but never have exhibited symptoms, and (2) convalescent carriers who have recovered from the disease but are still infected. This is the ideal condition for the parasite since it is not in danger of losing its host and is ensured of the distribution of its offspring. As a matter of fact, most host-parasite relations are of this type; hosts become infected but never show symptoms and are apparently none the worse because of the presence of the parasite, a sort of equilibrium between host and parasite being established. Certain species of hosts are almost universally infected in nature by certain species of parasites and the parasitized condition might almost be considered the normal state for these species.

Infection without symptoms is supposed to be the result of long periods of association. According to this view the length of parasitism of a certain species of host by a certain species of parasite can be determined ap-

proximately by the host-parasite reactions. For example, if a parasite is pathogenic and lethal for a certain host the association is supposed to be recently acquired whereas the absence of symptoms indicates a long period of consociation. Frequently carriers are spoken of as reservoirs since they are storehouses for the organisms that are responsible for the spread of the parasite to new hosts. In certain cases the parasite is infective both to man and to lower animals and one or both kinds of hosts may serve as reservoirs. Very little is known at present regarding the conditions underlying the carrier state.

(2) LATENCY. Similar in certain respects to the carrier condition is the state known as latency. When parasites are present in a host but do not make themselves manifest, they are said to be latent. Latency, however, does not necessarily require the dissemination of the parasites by the host. In some cases a host may be parasitized and show no symptoms; in other cases a host may recover from symptoms but still harbor parasites; both types of conditions may be included under the term latency. Certain changes in host or parasite may bring on symptoms in a host that had never previously exhibited evidences of infection; such a case might be considered one with an extended incubation period.

(3) RELAPSE. Symptoms may appear in a host that had previously shown symptoms but had apparently recovered; such a reappearance of symptoms is known as a relapse, if the latent period is short, and a recurrence, if the latent period is long. Relapses may be induced in certain infections by definite stimuli but the physiological bases for this have not been determined.

10. THERAPEUTICS

Host-parasite relations may be profoundly modified by treating the host. This may take the form of building up the resistance of the host (biological therapy) or of destroying the parasite either directly or through the host (chemical therapy).

(1) BIOLOGICAL THERAPY. Constitutional, biological therapy involves the usual procedures for maintaining or increasing natural host resistance, such as rest, minimum movement, and the treatment of other infections present. Often the host may be aided by a certain type of nourishment. For example, a milk diet is prescribed in cases of intestinal amœbiasis since milk produces a small amount of putrefaction and is practically all absorbed before it reaches the large intestine where the amœbic ulcers are located.

Vaccines, antitoxins, etc., that increase the active resistance of the host are not available for protozoan diseases. There seem, however, to be no insurmountable obstacles to their preparation and use.

(2) CHEMOTHERAPY. Drugs may be used to aid the host either as parasiticides by destroying the parasites or as agents for building up host resistance. In many cases it is difficult to determine which of these processes is taking place. There seems to be no reason why, in the case of intestinal infections, enemas containing drugs toxic to protozoa should not be effective parasiticides. Thus rectal irrigations with a solution of tannic acid or of bihydrochloride of quinine have been recommended in cases of amœbic dysentery, and rectal injections of

iodine solution or of methylene blue for flagellate infections. Such treatment might be efficacious against organisms such as the flagellate, *Trichomonas hominis,* which lives in the lumen of the large intestine, but probably does not destroy organisms such as endamœbæ and coccidia that live in the wall of the intestine. In a similar fashion certain investigators advise vaginal douches with a saturated solution of sodium bicarbonate to destroy the flagellate, *Trichomonas vaginalis* (Fig. 7), which ordinarily lives in the acid secretions of the vagina.

It seems possible that drugs taken by mouth may act directly upon the parasites within the intestine. *Iodamœba williamsi* (Fig. 4a) is destroyed by the administration of emetin although it lives in the lumen of the intestine. In this case the emetin may kill the parasite by actual contact.

Progress in the chemotherapy of protozoan infections has been most gratifying within the past two decades. Quinine was already in use in the seventeenth century as a cure for malaria, but only recently have satisfactory therapeutic agents for other protozoan diseases been discovered. Emetin was introduced by Sir Leonard Rogers for amœbic dysentery in 1912 and soon came into general use; more recently yatren and stovarsol have both been proved to be specific agents for the cure of this disease. In 1905 Thomas inaugurated the treatment of trypanosomiasis with atoxyl and to this have since been added tartar emetic, tryparsamide, Bayer 205 and Pasteur 309. Tartar emetic which was discovered by Vianna in 1913 to be efficacious against American leishmaniosis, has been found to be equally valuable for the treatment

of kala-azar and oriental sore. No therapeutic agents are yet available for intestinal flagellates, ciliates, and coccidia.

Whether these drugs act directly on the parasite or through the host is still in doubt. In a recent illuminating address Dale (1924) states the situation in the following words. "The conception of a remedy not killing the parasites immediately, but modifying their virulence, or lowering their resistance to the body's natural defences; of a remedy not acting as such, but in virtue of the formation from it in the body of some directly toxic product, either by a modification of its structure or by its union with some tissue constituent; of an affinity of the remedy for certain cells of the host's body, leading to the formation of a depot from which, in long persistent, never dangerous concentration, the curative substance is slowly released; all these conceptions present themselves, again and again, as necessary for our present rationalisation of the effects observed. It can hardly be doubted that they will potently influence the methods by which, in the immediate future, new and still better specific remedies are sought. But though our practical aim, in relation to the affinities of a remedy for the parasite and for the host's tissues, may be radically changed the meaning of these specific affinities, so delicately adjusted to a precise molecular pattern, remains dark."

II. ROUTE TAKEN BY PARASITES IN ESCAPING FROM THE HOST

As already noted, parasites must not only reproduce within the host but their offspring must be able to escape

and set up new infections in order to maintain the race. In most cases escape is easy since the parasites attack parts of the host from which natural channels lead to the outside, *e.g.,* intestinal protozoa pass out with the feces. The escape of sufficient trichomonads from the mouth and vagina to keep these races from dying out is probably brought about by kissing and coitus respectively.

III. *Host-Parasite Specificity*

By host-parasite specificity is meant the character of the relations between species of parasites and species of hosts with respect to host susceptibility and parasite infectivity. Hosts and parasites may be divided into groups and labeled according to their interspecific relations, since observations and experiments have built up a considerable body of facts regarding this subject; but what environmental conditions and host and parasite characteristics are responsible for the facts observed are still very obscure.

I. HOST SUSCEPTIBILITY

Parasitologists have long recognized different types of hosts with respect to their susceptibility to various parasites. Thus if a host is easily parasitized by a certain species it is said to be tolerant, whereas if it is difficult to parasitize it is classed as refractory. A host that is frequently found parasitized by a certain species in nature is known as a natural or autochthonous host; whereas one that does not become so parasitized may be considered a foreign host. If a species of parasite that

habitually lives in a certain host species is found in a host that is very seldom infected, that host is spoken of as an accidental or casual host. A host may become infected but throw off the infection after a short time, in which case it is known as a provisional or transitory host; or it may serve as a host for a short stage in the life-cycle of a parasite, thus becoming a temporary host.

An infection may be acute, malignant, fulminating, chronic or benign, but the evidence does not indicate that the susceptibility of the host to an infection has any bearing on the character of the infection induced. That is to say, a host may be more susceptible to infection, and probably usually is, by a species of parasite that never calls forth symptoms than by a pathogenic or lethal species.

2. PARASITE INFECTIVITY

If a host is easily parasitized by a species, the parasite is said to be highly infective. How much its infectivity is due to the host and how much to the parasite it is impossible to say. Several of the terms noted above with respect to hosts are also commonly used to designate different types of parasites. Thus, we speak of natural parasites, accidental parasites, and provisional, transitory or temporary parasites. Parasites are also classified according to the necessity of existence within a certain host as facultative, when this is not required, and obligate, when the parasite is unable to live in any other host. The invasive powers of a parasite are indicated by such terms as virulent or aggressive and the degree of infectivity with respect to the effects on the host as pathogenic, sublethal, and lethal.

Two extremes of host-parasite specificity may be illustrated by the relations which have been found in the writer's laboratory to exist between (1) the giardias of mammals and (2) the herpetomonad flagellates of flies. Within the past few years we have been carrying on a series of investigations (Simon, 1921, 1922; Hegner, 1922a, 1922b, 1923b, 1924d, 1925c) which seem to indicate that the giardias found in each species of host differ specifically from those found in every other species of host and only in a few cases is more than one species of host infected by one species of giardia. Thus morphologically distinct species have been described from tadpoles, house mice and rats, field mice, rabbits, cats, dogs, guinea-pigs, and ground squirrels as well as from certain birds and reptiles. Here then is an example of very rigid host-parasite specificity.

In contrast to this are the results of Becker's (1923) studies on the herpetomonad flagellates that live in the intestine of flies. Investigators previous to Becker's work assumed that each species of fly was infected with its own peculiar species of herpetomonad and hence when a new species of fly was found to be infected the organism was given a new specific name. Becker carried on experiments with six species of muscoid flies belonging to six different genera and found that each species could be infected with herpetomonads from each of the other five species. Because of these results and of the fact that no morphological differences could be observed between the various so-called species, Becker concludes that the flagellates from these six species of flies are all of the same species,—that first described from the house fly,

Musca domestica, as *Herpetomonas muscæ-domesticæ.* These results have been confirmed and extended by Drbohlav (1925b).

3. SOME PROBLEMS IN HOST-PARASITE SPECIFICITY AMONG INTESTINAL PROTOZOA

Biological studies of the relations between protozoan parasites and their hosts, especially man, have within the past thirty years brought about a marked change in our ideas regarding host-parasite specificity. Until quite recently the belief was prevalent that cross-infection is the rule in nature; for example, that man is infected with protozoa of lower animals and that lower animals are regularly parasitized by human protozoa. Thus, where several decades ago one species was supposed to inhabit a number of species of hosts we know to-day that in many cases each species of host is parasitized by its own species of parasites, which appear to be rigidly adjusted to it and unable to live in any other species of host. Some of the problems involved in the study of host-parasite specificity are stated in the following paragraphs and suggestions are presented to account for the facts observed. The conclusion reached is that we know very little about this interesting and important subject, but that further experimental study is possible and desirable.

(1) To what extent does the behavior of the host and that of the parasite determine host-parasite specificity? This problem involves particularly the question of transmission (Hegner, 1926d). It is obvious that host and parasite must be brought together under favorable conditions when the host is sus-

ceptible and the parasite infective. This can be done in the laboratory with hosts and parasites that do not ordinarily encounter each other in nature. A study of protozoan transmission in nature, however, reveals the fact that the parasite is passive during its passage from one host to another and that it is the behavior of the host or intermediate host that is responsible for transmission. "Intestinal" protozoa are transmitted in the active (trophozoite) stage or in the form of cysts. Those inhabiting the mouth and vagina are probably transferred by contact, entirely by the host, during kissing or coitus. These appear to be present in from one-third to one-half of the general population. Those that live in the intestine are transmitted by the contamination of food or drink with feces containing cysts or trophozoites. The host, man, is responsible for the proper disposal of his own feces so that food or drink may not become contaminated. By certain methods of control, such as the elimination of infected food handlers, of the common towel, of soil pollution and house flies, he can to a considerable degree prevent the spread of infection. That insanitary conditions are prevalent is indicated by the high incidence of infection among the general population, which is estimated approximately as follows: 50 per cent with *Endamœba coli,* 25 per cent with *Endolimax nana,* 10 per cent with *Endamœba histolytica,* 10 per cent with *Iodamœba williamsi,* 15 per cent with *Giardia lamblia,* and 10 per cent with *Chilomastix mesnili.* Frequently one individual is infected with two or more of these species at the same time. Fortunately *Endamœba histolytica* is the only pathogenic species of great importance in this list.

The differences in the percentages noted above and the small numbers of infections that have been recorded for the other species of intestinal protozoa that occur in man are probably due principally to two factors: first, the success of the species in gaining entrance to the digestive tract, and second, the infectivity of the species in the human host. *Trichomonas hominis,* for example, does not possess a cyst stage in its life-cycle and hence must pass from man to man in the trophozoite stage (Hegner, 1924a). The trophozoite stage is not as resistant as the cyst stage; hence it is more often destroyed before ingested by man than are cysts. This may account for the fact that less than 10 per cent of the general population seems to be infected with this species. But great differences exist between species that are spread by cysts. *Endamœba coli,* with infections in about 50 per cent of its possible hosts, seems much more successful than *Endamœba histolytica* with an incidence of infection of only about 10 per cent. This is true in spite of the fact that *E. histolytica* cysts are apparently more abundant in fecal material from a host than those of *E. coli.* The chances of reaching new, susceptible hosts seems about the same for the two species. There may be a difference in the resistance of the cysts of the two species while outside of the body, especially since the degree of resistance depends somewhat on the thickness of the cyst wall. Perhaps *E. coli* is more successful because its ripe cysts normally contain eight nuclei and presumably give rise to eight offspring within the intestine, whereas the cysts of *E. histolytica* possess only four nuclei. This would give *E. coli* a better chance of starting an infection. The

activities of the two species are also different within the intestine. *E. coli* lives in the lumen on bacteria and food particles, whereas *E. histolytica* apparently depends on tissue elements from the intestinal wall. *E. histolytica* must therefore gain access to this tissue and successfully attack it against the resistance of the host, whereas *E. coli* is continually bathed in a favorable nutrient medium. Furthermore, *E. histolytica* often brings about a diarrheic or dysenteric condition during which no infective (cyst) stages are passed by the host, and sometimes this species actually brings about the death of the host, thus destroying its own chances of further distribution.

The conclusion is reached that the behavior of the host or intermediate host plays an important rôle in host-parasite specificity since the infective stage of a parasite can reach its specific host only by being passively transferred by the latter; and this must occur regularly in nature in order that the race of parasites may continue to exist.

(2) DO SPECIES OF PROTOZOAN PARASITES THAT ARE RESTRICTED TO ONE SPECIES OF HOST GAIN ACCESS TO OTHER SPECIES OF HOSTS? The answer to this question differs for the different species of parasites and depends, as above, on the behavior of the hosts and intermediate hosts. Man's food and drink, for example, are no doubt frequently contaminated by the feces of rats, mice, cats, dogs, and other domestic animals that contain living, infective cysts of various intestinal protozoa, such as *Endamœba muris* of the rat, *Giardia canis* of the dog, and *Isospora felis* of the cat. No human beings, however,

have ever been reported with infections due to these species.

We may conclude therefore that the infective stages of human protozoa frequently gain access to lower animals and that those of the latter gain access to man. The entrance of the infective stages of a species of parasite into a host is necessary for host-parasite specificity, but is only one factor in this relationship.

(3) WHAT FACTORS WITHIN A HOST ENABLE NATURAL PARASITES AND PREVENT FOREIGN PARASITES FROM BRINGING ABOUT AN INFECTION? To answer this question we should consider that part of the host in which the parasite lives as its particular habitat, just as we look upon certain fresh-water ponds as the habitat of free-living species. Both free-living and parasitic protozoa are at times subjected to certain factors in their habitats that are harmful, and successful life and reproduction depend on the severity of these harmful factors. The digestive juices of the host, for example, have been considered destructive to trophozoites even of natural protozoan parasites. The cyst wall of intestinal protozoa protects the organism from many conditions outside of the body and may play an important rôle in the initiation of an infection; for example, it may react to the digestive juices of the host, or to secretions of the parasite within the cyst stimulated by the intestinal environment, so as to liberate the enclosed parasite and give it a chance to maintain itself there; or it may fail to liberate the parasite and thus prevent infection. We know so little about excystation that nothing definite can be said on this subject.

Among the variable conditions within the intestine are those due to the character of the diet. Carnivorous animals are very seldom parasitized by intestinal protozoa (Hegner, 1923a, 1924b) and omnivorous species such as the rat and man can be relieved of some of these organisms if fed on a carnivorous diet. Such a change of diet brings about many profound changes in the intestinal contents, which apparently make them unfit as a medium for the growth and multiplication of certain protozoa. The character of the intestinal contents resulting from the normal dietary of the host may thus prevent a foreign species from initiating an infection even if it succeeded in reaching the normal location in the host unharmed.

The character of the digestive juices, failure of cysts to excyst, the character of the diet, and various other factors may, therefore, encourage or prevent parasites that gain access to the body of the host from setting up an infection.

(4) How may we account for laboratory infections in foreign hosts? It is possible in certain cases to bring about an infection in a certain host species in the laboratory that appears never to become parasitized in nature. Several explanations suggest themselves to account for this phenomenon. In the first place, the host or intermediate host may behave in such a way as never to encounter the infective stages of the parasite in nature. For example, we would hardly expect an animal that does not live in association with man to become infected with human parasites, although it might be susceptible as indicated by laboratory experiments. The

number of parasites that gain access to a host may be an important factor; that is, a few specimens may not succeed in bringing about an infection, whereas large numbers of specimens might. The necessity for the presence of large numbers of parasites may account for the great number of clinical cases of amœbiasis that occur in the tropics, where the ingestion of large numbers of cysts is favored by meteorological and insanitary conditions.

The method of entrance of the parasites may play a rôle in the initiation of an infection. For example, cats apparently do not often become parasitized by *Endamœba histolytica* in nature but may be infected in the laboratory in several ways. The method that results in the greatest success seems to be that of Sellards and Theiler (1924), who produce stasis by surgical ligature of the large intestine and then inoculate cysts anterior to the ligature. Their experiments show that excystation occurs at the point of stasis and that no excystation might take place if stasis was not induced. Many infections are no doubt prevented in nature by the rapid passage of the cysts through the digestive tract.

We can thus account for laboratory infections in foreign hosts by the bringing together of a host and parasite that do not ordinarily become associated in nature; or by the use of very large numbers of parasites; or by procedures not possible in nature.

(5) WHAT CONDITIONS ARE RESPONSIBLE FOR DIFFERENCES IN SUSCEPTIBILITY BETWEEN YOUNG AND ADULT ANIMALS? The greater susceptibility of young animals to infection has been abundantly demonstrated in the

case of many species of protozoa. Surveys of intestinal protozoa in various parts of the world have established the fact that children, as a rule, are more highly infected than adults. Perhaps hosts that are infected while young acquire immunity before the adult stage is reached; but a protozoön that is able to live in a host for only a brief period cannot be considered entirely successful as a specific parasite. Similar results have been obtained in laboratory experiments; for example, kittens become infected with *Endamœba histolytica* much more readily than adult cats. There are no essential differences among the cysts used in such experiments; hence the factors involved must reside in the hosts. Some type of resistance develops with age. Are the cysts of intestinal protozoa unable to excyst? Are the trophozoites prevented in some way from entering the tissues? Does the medium (intestinal content) become unfavorable as the host grows older?

Finally the point may be emphasized that the subject of host-parasite specificity is one that needs and is worthy of careful investigation, and that this section is intended merely to indicate some of the interesting problems involved.

IV. *Problems in Host-Parasite Relations among Intestinal Protozoa*

The succeeding chapters in this book are devoted to a discussion of the Intestinal Amœbæ, Intestinal Flagellates, Intestinal Coccidia, and Intestinal Infusoria of man. An attempt is made to follow the plan indicated

in this introductory chapter so far as the material is available. In doing this many problems that are awaiting solution are indicated. An examination of these groups abundantly demonstrates that the ratio between what we know and what we do not know about the host-parasite relations between man and these intestinal protozoa is decidedly in favor of the latter. Furthermore, a survey of the literature shows that contributions to our knowledge of this subject are due largely to chance and not to concerted activities and without any well thought-out program in mind. As a rule, the investigator uses the material that happens to be available at the moment and undertakes the study of problems that occur to him without respect to any of the larger questions involved. This situation is, of course, due partly to the fact that most investigators are able to spend only part of their time on research and that very seldom are more than one or two investigators at work at the same institution along similar lines. The adoption of a program, such as that discussed in this book, by a group of protozoölogists variously trained in zoölogy, medicine and public health would without doubt be most helpful and economical, since it would furnish a general objective and at the same time allow as much individual initiative as any investigator could desire. It is perhaps too much to expect a combination of circumstances to arise that will put into effect such a Utopian situation as that described, but at any rate it can do no harm to provide a program that presents the problems involved according to a logical plan. The subjects that especially need investigation are

in general the same as those discussed in Section II above. It may be worth while, however, to repeat them here.

(1) The viability of trophozoites and cysts outside of the body of the host
(2) Methods of transmission
(3) The factors involved in excystation
(4) Excystation in the host
(5) Localization within the host
(6) Factors of the intestinal habitat in relation to trophozoites and cysts
(7) Pathogenesis
(8) Resistance and susceptibility of the host
(9) Acquired immunity of the host
(10) Resistance and infectivity of the parasite
(11) Acquired resistance and aggressivity of the parasite
(12) Host-parasite adjustments
(13) Therapeutics
(14) Host-parasite specificity
(15) Prevention and control

No special section in this chapter is devoted to the practical aspects of host-parasite relations. These, however, are always kept in mind and any new data obtained are always scrutinized for possible applications to prevention and control. Investigations of host-parasite relations are of importance from the standpoint of personal hygiene since they furnish the knowledge necessary to protect the individual from protozoan infections. Of even greater significance are the data of use to workers in

the field of public health since whole communities may be protected as a result of these scientific investigations.

In addition there is always before us the possibility of throwing light on the greatest of all of our problems, that of the origin and evolution of parasitism. The materials available for observation and experiment are particularly favorable for attacks on this problem, and as an added incentive is the knowledge that the elucidation of the changes that occur during the development of the parasitic from the free-living habit may furnish the key to the solution of the problem of the method of evolution.

CHAPTER II

INTESTINAL AMŒBÆ

1. *Generic Characteristics*

Practically all protozoölogists agree that there are at least six "good" species of amœbæ that are natural parasites of man. Besides these there are a number of doubtful species that have been described from man but about which there is as yet no general agreement. The six good species are illustrated in Figs. 1-6. They have been placed in four genera although there is still some doubt about the validity of several of these genera. Any one who wishes to become informed regarding the classification of the parasitic amœbæ should consult the following books: Dobell (1919a), Hegner and Taliaferro (1924), and Wenyon (1926). Detailed accounts of the morphology and life histories of these amœbæ are also to be found in these books, hence only a brief statement of their distinguishing characteristics will be included here. The structure of the nucleus is the most important criterion used in distinguishing the different genera.

1. ENDAMŒBA

This genus possesses a spherical nucleus with a small karyosome of chromatin and a superficial layer of chromatin granules lying on the inside of the nuclear membrane. In fixed material a clear area is present around

the karyosome and between this and the nuclear membrane is a network of linin fibers.

2. ENDOLIMAX

In this genus the nucleus is vesicular but not always spherical; there is no layer of chromatin granules on the nuclear membrane; the karyosome is large and irregular in shape and may consist of several portions attached to each other by strands; and often linin fibers extend from the karyosome to the nuclear membrane.

3. IODAMŒBA

The nucleus of this genus likewise has no chromatin granules on the nuclear membrane; the single karyosome is very large and is surrounded by a layer of globules that do not stain as deeply as the karyosome; often delicate linin fibers extend from the karyosome to the nuclear membrane.

4. DIENTAMŒBA

This genus is characterized by the presence in the majority of specimens of two nuclei; according to Jepps and Dobell (1918) 80 per cent are binucleate, whereas Kudo (1926) found only 12.2 per cent of 2000 specimens with two nuclei, and Craig (1926b) reports 67 per cent of cultural forms of this type. The nuclei are spherical and the chromatin consists of several granules embedded in a matrix of plastin. Linin fibers may connect this mass with the nuclear membrane.

Among the genera of amœbæ that have been described from man but are not yet well established are *Councilmania* (Kofoid and Swezy, 1921), *Caudamœba* (Faust,

1923), and *Karyamœbina* (Kofoid and Swezy, 1924a, 1925a).

II. *Specific Characteristics*

1. ENDAMŒBA HISTOLYTICA

Trophozoite. The active or trophozoite stage of *E. histolytica* (Fig. 1a) varies greatly in size but is usually from 20μ to 30μ in diameter. The size variations are due principally to two factors (1) growth following binary division and (2) heritably diverse size races. The clear ectoplasm around the periphery of the body may be distinguished from the more granular endoplasm; and the pseudopodia, which are entirely of ectoplasm, are thin and blade-like and formed in an explosive manner. Within the cytoplasm are usually food vacuoles containing red blood cells, leucocytes or other tissue elements and a single nucleus, which, however, is rarely distinctly visible in the living specimens. The nuclear structure is revealed in fixed and stained preparations. The nucleus is of the endamœba type (see above); it is from 4μ to 7μ in diameter; is "poor" in chromatin; and has a small centrally located karyosome and a layer of fine chromatin granules on the nuclear membrane.

Precystic stage. Before encysting, *E. histolytica* loses its food inclusions; decreases in size; becomes sluggish; and rounds up. Elmassian in 1909 believed this stage to be a distinct species and gave to it the name *Entamœba minuta.* Frequently vacuoles containing glycogen and rod-like refractile (chromatoid) bodies appear before encystation occurs.

Cyst. A thin peripheral wall is secreted by the precystic organism thus forming a spherical body, the cyst (Fig. 1b), which ranges from 5μ to 20μ in diameter. Different size races are indicated by differences in the size of the cysts. The mature cyst contains 4 nuclei, each of which appears like that of the trophozoite, but cysts with 1, 2, and 3 nuclei are frequently passed. Often glycogen vacuoles and chromatoid bodies are present in young cysts, but these are usually absorbed later.

Life-cycle. The life-cycle of *E. histolytica* appears to be very simple. Trophozoites occur in the large intestine in about 10 per cent of the general population; often in the liver, where they may bring about the formation of liver abscesses; and rarely in the small intestine, brain, lungs, spleen and other parts of the body. A culture method of diagnosis of intestinal amœbæ similar to that originated for intestinal flagellates by Hegner and Becker (1922) is advocated by Craig and St. John (1927). These investigators have found the Locke-Serum medium the best for this purpose. One microscopic preparation from each culture inoculated with fecal material from 71 individuals resulted in 39 positives or an incidence of 54.92 per cent. The various amœbæ were found in the following percentages: *Endamœba histolytica,* 15.49 per cent; *E. coli,* 29.57 per cent; *Endolimax nana,* 12.67 per cent; and *Iodamœba williamsi,* 5.63 per cent. It remains to be proved that this method can be employed successfully under field conditions as Hill (1926) has done for the diagnosis of intestinal flagellates.

Reproduction of the trophozoite is by binary fission.

The character of the nuclear division of *E. histolytica* is of particular importance since the identification of this amœba in the lesions of arthritis deformans and Hodgkin's disease (Kofoid, 1923) has been based largely on the mitotic figures found in certain of the cells. Dobell (1919a) has described division in specimens obtained by sectioning tissues from freshly killed cats that had been experimentally infected. He was unable to satisfy himself that chromosomes were present. Kofoid and Swezy (1922a) described mitosis in specimens of *E. histolytica* found in the bone marrow in arthritis deformans and later (1925b) published a detailed account of this process in both trophozoites and cysts from human cases of amœbiasis. They recognize an "interphase with normal resting nucleus, the prophase in which the daughter centrosomes form and the chromosomes emerge and divide, the modified amphiaster in which the divided chromosomes assemble in the equatorial region of the spindle, the anaphase in which they migrate toward the poles, and the telophase in which the nucleus constricts into two which then return to the interphase" (p. 333). The nuclear membrane remains intact throughout. The number of chromosomes is six; these divide in the metaphase and migrate to the poles in the anaphase. Then the nucleus constricts into two. They claim that mitosis in *E. histolytica* is in all essential particulars of the type normal to the parasitic Amœbida.

Multiplication of the nucleus occurs within the cyst; that this leads to an increase in the number of organisms seems probable from the work of Yorke and Adams (1926a; see p. 66). The quadrinucleated cysts no doubt

excyst in the intestine and give rise to four young amœbæ (see p. 88).

Hyperparasitism. Both free-living and parasitic amœbæ are sometimes parasitized by other organisms. One of these, a vegetable organism of the genus *Sphærita,* is known to invade the intestinal amœbæ of man. How destructive it is to its host, and whether it plays a significant rôle in control, are points on which we have no evidence.

2. ENDAMŒBA COLI

Trophozoite. The trophozoite of this species (Fig. 2a) also varies greatly in size but averages larger than that of *E. histolytica;* it ranges from about 18μ to 40μ being usually 20μ to 30 in diameter. The ectoplasm isμ meager in amount and not sharply separated from the endoplasm; the latter is very granular giving the organism a grayish appearance. Locomotion is sluggish and no rapidly forming ectoplasmic pseudopodia occur. Food vacuoles are usually abundant, containing bacteria and various materials from the intestinal contents, but ordinarily no red cells or other tissue elements. Cleft-like vacuoles sometimes appear. The nucleus is usually visible in the living organism; it is larger and coarser than that of *E. histolytica* with a thicker membrane, more chromatin on the membrane and a larger karyosome eccentrically placed.

Precystic stage. This resembles the similar stage of *E. histolytica* but averages larger.

Cyst. The cysts (Fig. 2b) range from 10μ to 30μ or more in diameter, the usual size being between 15μ and

20μ. The nuclei are 8 in number in the mature cyst, but cysts with 1, 2 and 4 are commonly found and more rarely with 16 or more. Glycogen occurs in early stages in greater amount than in cysts of *E. histolytica;* it is rare in 8-nucleated cysts. Chromatoid bodies are often present especially in early stages; they may resemble splintered glass or be filamentous.

Life-cycle. This species has been found only in the large intestine of man and about 50 per cent of the general population is infected. The stages described above are the only ones known with certainty to occur. The trophozoite undergoes binary division during which the nucleus probably divides by mitosis. The cysts are supposed to give rise to 8 amœbulæ on hatching. Nuclear division within the cyst has been described by Swezy (1922) as mitotic involving the formation of probably six chromosomes.

3. ENDAMŒBA GINGIVALIS

Trophozoite. The trophozoites of *E. gingivalis* (Fig. 5) range from 6μ to 60μ in diameter but are rarely over 20μ. The clear ectoplasm is distinct from the granular endoplasm and locomotion is fairly active. Kofoid and Swezy (1924c) describe a distinct pellicle. The nucleus is of the endamœba type, smaller than that of *E. coli,* and with a karyosome either centrally located or eccentric. The food vacuoles contain bacteria, leucocytes, etc.; red cells have rarely been reported in them.

Life-cycle. So far as we know the life-cycle of this species contains only the trophozoite stage. The mouth is the normal habitat and although exact figures are not

available no doubt a large proportion of mankind is infected. The only method of reproduction is probably binary division of the trophozoites.

4. ENDOLIMAX NANA

Trophozoite. This is a comparatively small species, the trophozoite (Fig. 3a) measuring only 6μ to 12μ in diameter. It has clear, blunt pseudopodia but is usually sluggish. The food vacuoles contain bacteria and other food bodies. The nucleus is like that characteristic of the genus.

Precystic stage. As in *E. histolytica,* the precystic stage loses its food bodies, but does not become much smaller than the adult trophozoite.

Cyst. The cysts (Fig. 3b) are typically ovoidal but sometimes spherical or irregular in shape. They are from 8μ to 10μ in length and about 6μ in breadth. The fully developed cysts contain 4 nuclei but younger cysts with 1, 2, or 3 nuclei occur. No chromatoid bodies are present but diffuse glycogen masses may occur from the precystic to the 4-nucleate stage.

Life-cycle. E. nana probably lives only in the large intestine of man and is present in about 25 per cent of the general population. Increases in number no doubt result from binary fission of trophozoites and from division of the 4-nucleated cyst into 4 uninucleate amœbulæ when excystation occurs.

5. IODAMŒBA WILLIAMSI

Trophozoite. This is generally from 9μ to 14μ in diameter, although specimens have been reported that were smaller or larger (Fig. 4a). There is no clear distinction

between ectoplasm and endoplasm. Locomotion is sluggish. The endoplasm is usually crowded with food vacuoles containing bacteria and intestinal debris but no red cells. The nucleus is like that characteristic of the genus.

Precystic stage. As in other species the trophozoites before encysting lose their food vacuoles, but decrease very little in size.

Cyst. The cysts (Fig. 4b) are spherical or irregular in shape and measure from 6μ to 16μ in diameter, averaging about 9μ. They contain a single nucleus except on rare occasions when two are present. The karyosome of the nucleus lies on one side and the rest of the space is filled with globules. Large glycogen vacuoles are seldom absent from the cysts. No chromatoid bodies are present.

Life-cycle. Iodamœba williamsi inhabits the intestine of man and is present in about 10 per cent of the general population. Binary fission of the trophozoite has been reported but no other method of reproduction is known.

6. DIENTAMŒBA FRAGILIS

Trophozoite. This rare species (Fig. 6) is only 3.5μ to 12μ in diameter. It is active, has clearly defined ectoplasm and endoplasm and sends out leaflike, hyaline pseudopodia. Two nuclei with the characteristics already described are usually present although specimens with one nucleus are not uncommon. The food consists of bacteria and yeasts.

Cyst. Only one observer has noted cysts (Kofoid, 1923). These "are spherical with glycogen vacuoles and small chromatoidal bodies."

Life-cycle. D. fragilis lives in the intestine of man, but less than 100 cases have been recorded. Its process of reproduction has not been described.

III. *Host-Parasite Relations between Man and Endamœba histolytica*

1. EPIDEMIOLOGY OF TRANSMISSION

(1) INFECTIVE STAGE. The general idea regarding the infective stage of intestinal protozoa is expressed admirably by Dobell (Dobell and O'Connor, 1921) in the following words, "Infection with any intestinal protozoön is, in nature, always acquired through the mouth, by swallowing a living cyst containing the resting form of the particular organism. In ordinary circumstances the free forms cannot live outside the body for more than a very short time, and they die if swallowed—in other words, they are non-infective" (p. 6).

Are trophozoites capable of bringing about an infection? Just how long trophozoites of *E. histolytica* ordinarily live in fecal material outside of the body is not known. Rivas (1926) reports that active specimens remained alive for over 24 hours in fecal material and for from 32 hours to 3 days when sealed in capillary tubes and kept at a temperature of 5° C. At 22° C. they lived for at least several hours, at 37° C. for a shorter period and at 45° C. for only 5 minutes. It seems probable that trophozoites are seldom ingested by man in a living condition although this might happen under unusual circumstances. That they are not quickly killed by the digestive juices is indicated by an experiment of the writer

(Hegner, 1926c). Trophozoites from an artificial culture were injected into the stomach of a guinea-pig. One hour later the pig was killed and the stomach and small intestine carefully examined. None were found in the stomach, but specimens alive and moving were recovered in the small intestine 6, 12, 20, 26, 28, 34, 38, 44, and 51 inches posterior to the stomach. Dobell and Laidlaw (1926b) report that trophozoites of *E. histolytica* will withstand 0.2 per cent HCl for 30 minutes. Whether trophozoites could successfully pass through the stomach and small intestine of man is doubtful, since no one has been able to infect human beings with them per os, nor has any one been able to infect lower animals by adding trophozoites to their food. Hence, although the trophozoites are more resistant than usually supposed, it seems probable that infections are never brought about in nature except by the ingestion of cysts.

Are immature cysts infective? Not all of the cysts passed are supposed to be infective. As noted above cysts may possess 1, 2, 3 or 4 nuclei when they escape from the body. One patient may pass mostly uninucleate or binucleate cysts at one time and quadrinucleate cysts at another time, and cysts passed by different hosts may differ with respect to their nuclear number. It is generally believed that only the quadrinucleate cysts are capable of infection, and that those with 1, 2 or 3 nuclei do not continue development outside of the body, but soon die. Recently, Yorke and Adams (1926a) have shown that nuclear division may occur when immature cysts are placed in artificial culture media. This suggests that uninucleate and binucleate cysts that have been swallowed

by a host may also continue their development; if so, then all cysts may be infective regardless of the number of nuclei they contain.

Viability of cysts outside of the body. Desiccation. From the public health viewpoint it is important to know how long cysts remain viable outside of the body under various conditions. In the first place moisture is necessary for long-continued existence, since cysts, although provided with a resistant wall, are really very delicate and soon perish if dried. Thus Kuenen and Swellengrebel (1913) found that desiccation killed the cysts instantly, a result confirmed by Wenyon and O'Connor (1917) both for cysts allowed to dry in fecal material in the laboratory and for cysts contained in the dried droppings of flies that had fed on infected stools. The distribution of viable cysts in a dried condition in dust is therefore impossible.

The dispersion of cysts in nature. Most cysts in nature remain in the raw feces in latrines until they die or are carried away by flies and other animals, or on the surface of the soil where they are destroyed by desiccation, are carried away by animals, or are washed into the soil or into ponds and streams by the rain. Boeck (1924a) recovered *E. histolytica* cysts from 10 of 201 privies examined in one of our Southern cities. The viability of cysts in raw and diluted feces thus becomes an important public health subject. Another very important point is the possibility and probability of their dissemination by flies and other animals.

Tests of the viability of cysts. Some method of determining whether or not cysts are alive is the first requisite

for a study of their viability. The eosin test has been used for this purpose more than any other. Kuenen and Swellengrebel (1913) were the first to use this method to determine whether cysts are alive or dead. Eosin in a concentration of 1:1000 appeared to penetrate and stain dead cysts but not those that were alive. Later work by Wenyon and O'Connor (1917), Cutler (1920), Yoshida (1920), Root (1921), Boeck (1921a, 1921b), Bercovitz (1924), Kessel (1925b) and others indicates that this test is not infallible; dead cysts are usually stained but some of them are not. Bercovitz (1924), for example, found that cysts of *Endamœba coli* that were killed by chemicals did not, as a rule, take the eosin stain, and on this account, employs hæmatoxylin as a stain and cytological changes as a criterion of death. Boeck (1921) and Kessel (1925b) showed that cysts that have undergone plasmolysis do not become stained and Kessel was able to prove that neither stained cysts nor the plasmolyzed cysts of *Hartmannella hyalina* excyst in culture medium, whereas most of the "green" (viable) cysts do. Root (1921) found neutral red more satisfactory than eosin since it stained a larger proportion of the cysts. Congo red has also been experimented with by Bercovitz (1924). The best method of determining whether cysts are alive or dead is to test them in susceptible animals or in artificial culture. Now that culture methods have been perfected by Boeck and Drbohlav (1925a, 1925b) and others, and excystation may be brought about in the test tube, it will be possible to obtain more definite results than heretofore regarding the longevity of cysts outside of

the body under various conditions, and the effects of temperature, disinfectants, etc., on them.

Viability of cysts when tested on animals and in artificial culture. Data on the longevity of amœba cysts have been furnished by various investigators. Walker and Sellards (1913) infected men with cysts of *E. histolytica* after they had been outside the body in fecal material for two days at tropical temperature, and with cysts of *E. coli* after 10 days under similar conditions. Experiments of Sellards and Theiler (1924) with kittens indicate that cysts are still infective after six days when left in the original stool at 2° C. but lose their infectiveness after two weeks. Cysts of *E. histolytica* that were 10 days old and had been kept in an ice box in the original stool for 8 days of that period were found by St. John (1926) to excyst when incubated in culture medium. And Dobell and Laidlaw (1926b) discovered that cysts will not excyst in culture until they have been held outside of the host's body at a lower temperature for two days.

Viability of cysts when tested with eosin, etc. Some of the results obtained with the eosin test are as follows. Kuenen and Swellengrebel (1913) found that some histolytica cysts lived for over 7 days in water containing many bacteria and for at least 29 days in water containing few bacteria; Penfold, Woodcock and Drew (1916) kept cysts alive in slowly running water for 15 days; Thomson and Thomson (1916a) observed living cysts in formed, moist feces 16 days after they were passed, and state that they can live considerably over a month

in feces that are kept moist and for weeks in feces diluted with drinking water. Wenyon and O'Connor (1917) record cysts still alive in diluted fecal material at the end of a month; and Dobell (1919a) states that they will survive for several weeks in feces that are kept moist and cool and for five weeks if placed in water.

A comprehensive experimental study of this subject was carried out by Boeck (1921b). Washed cysts of *E. histolytica* when kept in bottles in distilled water at 12° to 22° C. were still viable at the end of 153 days, and those of *E. coli* at the end of 244 days; cysts of *E. histolytica* in eosin-stained wet preparations under a cover glass sealed with vaseline were still unstained after 211 days and those of *E. coli* after 124 days. Yorke and Adams (1926b) were able to obtain cultures from cysts that had remained in raw feces at room temperature for 9 days, in washed suspensions at room temperature for 10 days, and in saline or water at 0° C. for 17 days.

Conclusions from viability tests. These results indicate that when kept moist the cysts of these amœbæ remain alive for long periods outside of the body. They live longer in water than in fecal material. It may be assumed, therefore, that dilution of the infected feces is favorable for the continued existence of the cysts. Cysts that reach drinking water or water used for washing vegetables, etc., or milk or moist food are thus in a comparatively advantageous position both as regards length of life and the chances of being ingested by a susceptible human being.

Resistance of cysts to temperature. Very few of the factors encountered by cysts outside of the body have

been studied. We know something, however, about the effects of temperature on the cysts of *E. histolytica* and *E. coli.* As noted above cysts remain alive for a considerable period at room temperature in both the tropics and temperate zones and when stored at 2° C. Boeck (1921a) attempted to find the upper temperature limit with the aid of the eosin test. He found that all cysts of *E. histolytica* were killed at 68° C., of *E. coli* at 76° C., of *Endolimax nana* at 64° C., and of *Iodamœba williamsi* at 64° C.; many of the cysts were killed at temperatures 10° below these. The temperatures recorded are not ordinarily encountered in nature, but Boeck held cysts at these temperatures for only 5 minutes, whereas in nature high temperatures often continue for long periods and it is well known that organisms can withstand a certain temperature for a short time that would destroy them if maintained for a longer period. Yorke and Adams (1926b) found that cysts would withstand a temperature of 45° C. for 30 minutes but were all killed in 5 minutes at 50° C. The possibility of destroying parasites in night soil by means of the heat from the sun has recently been discussed by Barnes (1925) who obtained temperatures of over 60° C. in glass-covered de Saussure boxes over a continuous period of at least 4 hours (11:00 A. M. to 3:00 P. M.). Such a temperature would doubtless be fatal to all protozoan cysts and the method suggested by Barnes might therefore be employed to prevent the spread of intestinal protozoa where night soil is used as fertilizer.

Resistance of cysts to chemicals. Various investigators have tested the effects of chemicals on protozoan cysts,

but no comprehensive study of this subject has been made. Kuenen and Swellengrebel (1913) found that mercuric chloride, 1 in 1000, killed histolytica cysts in 4 hours; that cresol, 1 in 250, killed them in from 5 to 10 minutes; but that formalin, 10 per cent, does not destroy them if allowed to act only a few minutes. Emetin, 1 in 100, was not fatal to some cysts when allowed to act for an hour. Cresol, 1 in 20, was found by Wenyon and O'Connor (1917) to kill histolytica cysts immediately; in one minute, in a strength of 1 in 30; in half an hour, in 1 in 100; in one hour, 1 in 200; but not at all in a strength of 1 in 2,000. They consider cresol the best disinfecting agent for dysenteric stools and for the hands of those who are exposed to contamination. These authors record the death of cysts in 15 minutes in carbolic acid, 1 in 40; in 7 hours, 1 in 100; but state that some cysts were still alive after 8.5 hours, 1 in 200. Eosin failed to stain cysts subjected to formalin, 1 in 100, for 4 hours, although the cysts appeared shrunken and distorted. Acid sodium sulphate in tablet form and chlorinated lime tabloids as used for the purification of water had no effect on the cysts, hence drinking water thus treated is not freed from any infective cysts that may be present.

Bercovitz (1924) treated cysts of *Endamœba coli* and *Councilmania lafleuri* with many of the most common disinfectants and then stained them with hematoxylin to determine whether changes of a lethal nature had occurred. Cysts were placed in vials containing the disinfectant and allowed to remain there for periods of 15 seconds, 15 minutes and one hour. All agents used seemed to kill the cysts within an hour. HCl, 1 per cent, killed

them in 15 minutes or less; formalin 1 per cent, destroyed them; in sodium hydroxide they became swollen at once; death seemed to follow treatment with chlorinated lime, 1 per cent, bichloride of mercury, 1 per cent, and carbolic acid; and lysol, 1 per cent, killed quickly not only cysts of *E. coli* and *C. lafleuri* but also those of *E. histolytica.* The conclusion seems justified that these various disinfectants are powerful destructive agents for amœba cysts; and it seems probable that those of *E. histolytica* are at least as susceptible as are the cysts of the two species principally studied. As Bercovitz suggests, however, culture experiments in addition to the eosin and hematoxylin tests, are necessary to prove that the cysts were really destroyed.

The culture method was employed by Kessel (1925b) in his study of the effects of chlorin water on cysts of *Hartmannella hyalina.* No excystation occurred in cultures within 72 hours after the cysts had been subjected for 10 minutes to chlorin water containing free chlorin of from 2.2 to 4.0 per cent, and very few cases of excystation were noted until a dilution of 0.4 per cent was reached. No excystation took place in cysts kept in 0.2 per cent chlorin water for 24 hours, and none in 0.15, 0.1, and 0.08 per cent chlorin water for 48 hours. Cysts did not hatch after 96 hours in 0.06 per cent chlorin water, but a few excysted in 0.009 per cent and many in 0.006 per cent chlorin water.

The culture method was also used by Yorke and Adams (1926b) in their studies of *E. histolytica.* The cysts were subjected to the chemical and then washed and placed in culture medium. They found them very

resistant to yatren and emetin; they withstood 5 per cent HCl for 30 minutes, but were not very resistant to various disinfectants.

Conclusions regarding the disinfection of feces, milk, and drinking water. As a result of these various experiments we may conclude that a simple method of disinfecting feces containing histolytica cysts is available, namely, the use of cresol in a strength of 1 in 20, but that no practical method has yet been discovered of sterilizing drinking water. The pasteurization of milk probably raises the temperature to a high enough point (60° C.) for a sufficient time to destroy any protozoan cysts present and of course the boiling of water would quickly kill all cysts.

2. AVENUE OF INFECTION. *How do living cysts of E. histolytica reach the digestive tract of man?* There is only one conceivable method of entrance, in nature, and that is by way of the mouth. It seems probable that they usually reach the mouth in contaminated food and drink but they might also find their way there on soiled hands. Among the most important factors that bring about the dissemination of cysts, and especially their presence in food and drink, are probably the handling of food in homes, restaurants, hotels and markets by infected persons who are passing cysts; the use of night soil as fertilizer in vegetable gardens; the common use of toilet, washbowl, and towel; and the presence of insects, such as flies, ants and cockroaches, and of domestic animals, such as rats, mice, dogs and cats.

The dissemination of cysts by flies. That flies may play an important rôle in the dissemination of histolytica and

other protozoan cysts has been recognized for many years. As long ago as 1913 Stiles and Keister recovered giardia cysts from flies that had been fed on fecal matter containing cysts of this flagellate. Kuenen and Swellengrebel (1913) did not find cysts in or upon flies that were allowed access for 48 hours to fecal matter containing histolytica cysts; and cysts that were present on the outside of flies that they soiled with infected material soon died because of dessication; they therefore concluded that flies are unimportant carriers. Several years later, however, Thomson and Thomson (1916b) noted that flies may ingest cysts and deposit them in their feces. A number of interesting and important experiments were conducted by Wenyon and O'Connor (1917) with flies belonging to several genera. They found that a fly that had not fed for 2 or 3 hours could ingest one milligram of feces in half an hour; Root (1921) records a larger capacity, a single house fly (*Musca domestica*) ingesting 0.0068 cc. of fluid and a single blow fly (*Calliphora erythrocephala*) 0.022 cc. after being without food for from 17 to 21 hours. Flies are thus able to ingest a large number of cysts at a single meal. With the use of the eosin test, Wenyon and O'Connor found that cysts are not killed by conditions in the fly's digestive tract, but may remain alive there for as long as 24 hours, and that the fly may deposit living cysts in its feces from 5 minutes to at least 16 hours after feeding. Of particular interest is their discovery that 18 of 229 wild flies captured in a hospital compound passed cysts or eggs of parasites in their feces; of these, 6 deposited coli cysts and 5 histolytica cysts. Cysts were not found in material

regurgitated by flies that had fed on infected fecal matter.

Roubaud's (1918) data confirm certain of these results. He found viable histolytica and coli cysts in flies' feces for over 24 hours but only rarely after 40 hours. Cysts in drowned flies lived for about a week but were all dead by the time the flies decomposed sufficiently to liberate them, which requires a month or more. Similar experiments were carried out by Root (1921) who found that half of the histolytica cysts ingested by house flies and blow flies were dead after 15 hours and that some of the cysts lived at least 49 hours. In Mesopotamia wild house flies were found by Buxton (1920) to be carriers of parasites that occur in human feces. Flies were collected from Arab compounds, Indian latrines and incinerators, and British mess and cook houses. The dissection of 1,027 flies revealed that 63 per cent had apparently ingested human feces; that 4.09 per cent contained human intestinal parasites; and that 0.3 per cent contained cysts of *E. histolytica.* Cysts of *E. coli* and *Giardia lamblia* were also found in the flies.

Not all experiments with flies have been as successful as those described above. Jausion and Dekester (1923b), working at Fez, fed 40 flies on stools containing cysts and trophozoites of *E. histolytica* but recovered only a few amœbæ from them in a very bad state of preservation. No amœbæ were found in seventy-three flies caught in the laboratory and in the latrines at a time when 23 per cent of the inhabitants were infected. The injection into the rectum of a young cat of 50 flies that were caught in the latrines did not result in an infection. The con-

clusion reached by these investigators is that flies play a very small rôle in the transmission of intestinal amœbæ. A similar attempt to infect kittens with flies that had fed on fecal matter containing histolytica cysts had previously been made by Wenyon and O'Connor (1917). Flies were fed by them to two kittens but no infections resulted.

The data available seem to prove conclusively that flies both in the laboratory and in nature ingest fecal material containing protozoan cysts; that the cysts are not quickly killed in the flies' intestine; that living cysts may be deposited in the feces of the fly from 5 minutes to 49 hours after feeding; and that these cysts may be deposited in the food or drink of man where they may remain viable until ingested by a human host. The conclusion seems inevitable that flies play an important rôle in the dissemination of histolytica cysts and that food and drink should be protected from their visits, especially in localities where amœbic dysentery occurs and sanitary conditions are such as to allow flies access to infected fecal matter. It may be pointed out here that the fly does not become infected but is a "passive" carrier, in contrast to the rat described below which is an "active" carrier.

The dissemination of cysts by other animals. Under favorable circumstances other insects, such as ants and cockroaches, and even other animals, may serve as distributors of protozoan cysts. Several investigators have incriminated rats and mice. The data furnished by Lynch (1915b) on spontaneous and experimental infections with *E. histolytica* in rats in South Carolina cannot be

accepted at their face value since he did not take into account the fact that rats are naturally infected with other species of amœbæ. Brug (1919a) noted what were apparently infections with *E. histolytica* in two wild rats in Java and seems to have been successful in infecting a specimen of *Mus rattus*. Kartulis (1891) also reported spontaneous infection in rats. The most successful experimental infections in rats and mice are those described by Kessel (1923). Rats were fed human feces containing histolytica cysts; 8 of 29 specimens became infected. The infection was of the chronic type; cysts were passed in the feces; and other rats were infected by feeding them these cysts. One of 10 mice was also infected by ingesting histolytica cysts; this mouse passed cysts in its feces. Rats and mice, according to Kessel's results, not only may become infected and pass cysts, but histolytica cysts may pass through their digestive tract and appear in their feces in a viable condition. The fecal pellets of such animals might bring about the contamination of food or drink and hence infection in any susceptible human host. However, it seems probable that infection is very seldom spread in this way, even if Kessel's results should be confirmed by other investigators. It has been found possible to infect other domestic animals, such as cats and dogs, with *E. histolytica* (see p. 113). Cysts may pass through the digestive tract of the cat unharmed (Dobell, 1919a) but since infected dogs and cats do not ordinarily pass cysts these animals play a minor rôle, if any, in the transmission of intestinal amœbæ.

The activity of the host and passivity of the parasite in transmission. An important fact that has been brought

out by studies on the transmission of protozoan cysts is that the host is entirely responsible for his own infection. The parasite remains passive and can only reach the intestine of a new host by the activities of the host. The discussion of the methods of protecting individuals and communities from infection is reserved until later (see p. 116).

Transmission as a result of association with carriers. The principal reservoirs of *E. histolytica* are human carriers who are passing cysts. Observations indicate that the association of such carriers with susceptible hosts result in infections. For example, Kofoid (1923) emphasizes the spread of the infection in families, and Garin and Lépine (1924) noted that 50 per cent of indigenous cases of amœbiasis near Lyons, France, had associated with colonial troops during the war. The migration of troops to France and their return to the United States furnished an excellent opportunity to study this subject. Stiles (1922) summarized the results of a very extensive study by Boeck and himself (Boeck and Stiles, 1923) in part as follows.

Examinations were made of

13,043 specimens from 8,029 persons in 22 states and D. C.

44 per cent (3,533) were infected with both protozoa and worms.

39.9 per cent (3,208) were infected with protozoa.

9.6 per cent (775) were infected with worms.

4.1 per cent (333) were infected with *E. histolytica.*

Positive cases were found in persons from every state that sent in a fair number of specimens. These data give

a very adequate idea of the incidence of infection on the basis of one or two examinations of each person. The results of the examinations of the soldiers are of particular interest. They are as follows.

Soldiers that did not go to Europe (1.3 examinations each).

2584 individuals, 93 positive = 3.5 per cent positive.

Soldiers after their return from Europe (1.1 examinations each).

3536 individuals, 100 positive = 2.8 per cent positive.

Soldiers of unknown military history (1.5 examinations each).

362 individuals, 11 positive = 3.0 per cent positive.

These results indicate that soldiers who may have come into contact with other soldiers, some of whom had come from the tropics, were no more frequently infected than those who remained in the United States.

An attempt was also made to determine by means of questionnaires whether the return of about 3,000,000 soldiers from overseas had had any effect upon the number of cases of clinical amœbiasis. The data are as follows (Stiles, 1922):

Letters of enquiry were sent to 607 hospitals and 115 medical schools.

Replies were received from 468 hospitals and 71 medical schools, representing all but 4 states.

190 negative replies were received from 28 states.

174 negative replies, 8 indefinite, and 24 positive replies, were received from 13 other states. Three states gave negative or indefinite replies.

Total—532 replies from 44 state; 24 or 4.5 per cent reported increase but not serious.

The conclusion reached was that the increase in clinical amœbiasis, if any really occurred, was not serious enough to warrant the attempt to eliminate the carriers among the returned soldiers by treatment.

Epidemics of amœbiasis. That clinical amœbiasis may exist in epidemic form is claimed by several writers. Thus Craig (1926a) states that epidemics of amœbic dysentery occurred among the U. S. troops in the Philippine Islands during the Philippine Insurrection, and a series of cases reported by Voss (1925) indicate that the presence of carriers may bring about an epidemic of amœbiasis in a northern climate (Norway) even at an unfavorable time of the year (the middle of winter). A young man contracted dysentery in Calcutta; three or four months later he arrived at his home in Norway; three days after his arrival his father was taken ill, developed amœbic dysentery and died; the day after the father's death his nurse came down with dysentery.

The relation between climate and infections with E. histolytica. Amœbiasis has for many years been included among the tropical diseases because of the great number of acute cases that occur in the tropics as compared with the temperate regions; but surveys have within the past decade been made in almost all parts of the world and we now know that infection of man with *E. histolytica* is much more common than generally supposed and exists throughout the entire globe. There may be local areas that are free from it but these have not yet been circumscribed. The incidence of infection varies in differ-

ent regions; it appears to be greater in the tropics than in the temperate zone but not enough greater to account for the much larger number of clinical cases encountered in the tropics. A fair estimate from all the data available indicates an average rate of infection throughout the world of about 10 per cent.

Three principal solutions have been offered to the question why there is more clinical amœbiasis in the tropics. One is climate, another, lack of sanitation, and the third, more virulent strains.

Brug (1925) has attempted to establish climate as the responsible factor. Jaeger (1902) reported epidemics of amœbic dysentery in Königsberg during August to October 1900, and August to September 1901; Brug found the greatest number of cases in 1900 to 1901 in the E. Asiatic Expeditionary Corps to be in the late summer; Viereck (1907) records amœbiasis as most abundant at Hamburg in 1900 to 1905 in July and August; Woodcock (1918), in the region of the Suez Canal, found August and September to be the two months when amœbic dysentery was most prevalent; Jouveau-Dubreuil (1919) in Setchouan, China, reports the highest incidence of the disease from July to September; Ledingham (1920) found amœbic dysentery most abundant in Mesopotamia during the hotter months; Vallardi (1920) recorded 840 of 1,671 cases among the Italian Expeditionary Corps in Macedonia in July; Garin and Lépine (1924) state that near Lyons, France, relapses are most frequent in June and July, and September and October; and Scott (1924), in Tientsin, noted that fresh cases of amœbic dysentery are rare in winter. Brug adds to these

reports data obtained by him in tropical Batavia in 1917 to 1924; these indicate August, October and November as the months of greatest incidence. The conclusion reached is that climate is responsible for the change from the carrier condition to that of acute dysentery and that the greater incidence of clinical amœbiasis in the tropics is explained by the fact that hot weather is favorable and colder weather unfavorable for the production of symptoms. Just how differences in temperature operate to bring about these results is not indicated.

2. PARASITOLOGICAL AND CLINICAL PERIODS

The prepatent period. Usually the exact time when infective cysts were ingested by persons infected with *E. histolytica* is not known. On this account we must depend on the very few human experiments that have been reported and on animal experiments for our data. The best work on human beings is that of Walker (Walker and Sellards, 1913). Specimens, mostly cysts, in gelatine capsules, were fed to 20 different persons whose stools had previously been found to be free from this species. These men were then kept under observation for periods of time ranging from 2 months to one year—14 for the latter period. Eighteen of the 20 became infected; no specimens were recovered from the other two during the year following the initiation of the experiment. The prepatent periods of those who became infected were as follows: in 2 cases, 1 day; in 2 cases, 2 days; in 1 case, 3 days; in 4 cases, 4 days; in 3 cases, 5 days; in 1 case, 8 days; in 2 cases, 11 days; in 1 case, 21 days; in 1 case, 33 days; and in 1 case, 44 days. The shortness of the

prepatent period is surprising; it suggests that some of the specimens noted on the first and second days after ingestion might have been among those fed to the patients and not the offspring of the original specimens that had succeeded in establishing themselves in the intestine.

The patent period. In all cases specimens were passed from the time they first appeared to the termination of the experiments. The duration of the patent period was therefore at least as long as the patients were kept under observation.

The incubation period. Only 4 of the 18 positives suffered from dysenteric symptoms, the incubation periods being 20, 57, 87, and 95 days in length. The other 14 positives may, however, have exhibited symptoms after observations ceased.

The carrier period. Apparently all persons infected with *E. histolytica* become carriers; those who never exhibit symptoms are "contact" carriers according to Walker's terminology, and those who pass cysts after suffering from dysentery are "convalescent" carriers. Unless rid of their amœbæ by the aid of drugs, carriers remain infected for many years. During this time they are always in danger of a disturbance in the equilibrium between host and parasite which will bring about the appearance of symptoms which, in the case of convalescent carriers, would constitute a relapse.

The incubation period in cats. Infections with *E. histolytica* from man have been obtained by many investigators in kittens. The course of the infection in this host is very different from that in man. The infected cat exhibits symptoms, but does not pass cysts and hence does

not become a carrier. Dale and Dobell (1917) state that the incubation period lasts about two weeks if cysts are fed to kittens but averages only about two days if infections are brought about by rectal injections of motile specimens. Wagener (1924) records the incubation period in kittens, following rectal injections, as from 2 to 5 days and in adult cats as at least one week.

3. DISTRIBUTION AND LOCALIZATION WITHIN THE HOST

Cysts are passively carried to the primary site of infection. Natural infections with *E. histolytica* are brought about by the ingestion of infective cysts, although under extraordinary circumstances (see p. 65) it is possible that trophozoites may be responsible for an infection. Cysts have no powers of locomotion and no hooks or other structures that might anchor them to the wall of the digestive tract, hence they are carried along passively with the food that is swallowed. Since the primary site of infection is the large intestine, they must pass through the stomach and small intestine in a living condition. Food is known to pass through the small intestine of man in about 4 hours; hence the cysts may reach the large intestine in this length of time. If the contents of the large intestine are not well formed, that is, if the bowels are loose, many of the cysts and trophozoites (if excystation has taken place) may be carried directly out of the body. As Dobell and Low (1922) have pointed out, infection is most frequent in those parts of the intestine where stasis occurs; and Sellards and Theiler (1924) have emphasized the fact that stasis is an important condition in experimental infections in kittens. Trophozoites

that succeed in escaping from cysts and that find suitable conditions in the large intestine may start an infection.

Where does excystation occur and what factors are responsible? The conditions that bring about excystation are not well known and no one has yet determined exactly where it occurs in the human host. It is generally believed that cysts do not hatch outside of the body and Dobell (Dobell and O'Connor, 1921) goes so far as to state that "cysts never hatch in the colon, where they are formed, or outside the body." Darling (1913), however, as pointed out by Yorke and Adams (1926a), noted the disappearance of cysts and the appearance of trophozoites in feces containing histolytica cysts that were kept in a moist chamber. It is not at all certain that the trophozoites observed came from the cysts since amœbæ of other species often appear in fecal material kept under similar conditions.

Excystation in laboratory animals. Several investigators have attempted to study excystation by feeding cysts to laboratory animals and then killing the animals after intervals of various lengths and examining the cysts present in various parts of the intestine. Dobell (1919a) had no success with this method, but Chatton (1917b) observed what he considered to be excystation of cysts fed to cats. In the stomach the chromatoid bodies disappeared from the cysts but no hatching occurred. Excystation took place, however, in the small intestine. A single quadrinucleate amœba emerged from each cyst. These amœbæ ingested bacteria; their cytoplasm became vacuolated; and their nuclei clumped together.

The effects of digestive juices on cysts outside of the body. Other investigators have treated cysts outside of the body with various substances. For example, Ujihara (1914) found that pancreatic juice acted upon the cyst wall but not gastric juice at 37° C. for 24 hours; Penfold, Woodcock and Drew (1916) also found pancreatic extract effective but had no success with either pepsin in an acid medium, or bile. Cutler (1919b) records excystation after the action of liquor pepticus followed by liquor pancreaticus. The trophozoites that emerged possessed four nuclei but were supposed to divide later into 4 uninucleate amœbæ.

Excystation in artificial culture. The perfection of methods of cultivating *E. histolytica* in artificial media by Boeck and Drbohlav (1925a, 1925b) and others has made it possible to study excystation under more favorable conditions. Boeck and Drbohlav report what seemed to be a case of excystation in one of their cultures and St. John (1926) states that he obtained trophozoites in cultures from a stool that was 10 days old and had been in the icebox for 8 days; this material must have been free from trophozoites. No observations were recorded by these investigators on the hatching of the cysts. Yorke and Adams (1926a) and Dobell and Laidlaw (1926b) have published more extensive studies on excystation in cultures.

Yorke and Adams found that uninucleate and binucleate cysts continued to develop in Locke-egg-serum medium when incubated at 37° C. For example, material containing 42 per cent uninucleate, 18 per cent binucleate

and 27 per cent quadrinucleate cysts at the time cultures were made had changed after 2.5 hours to 8 per cent uninucleate, 9 per cent binucleate and 75 per cent quadrinucleate cysts. This indicates that development of immature cysts may take place outside of the body contrary to the general belief at present. The glycogen, which occurred in most of the uninucleate cysts, disappeared in the cultures, and chromatoid bodies, which were rare in the uninculeate cysts, increased during the first few hours of cultivation but decreased again later as trophozoites began to appear. It was noted by Dobell (Dobell and O'Connor, 1921) that cysts when kept outside of the body in moist feces or water gradually lose their glycogen and chromatoid bodies.

Yorke and Adams describe excystation in the following words. "An individual which is about to excyst presents a characteristic appearance. The cytoplasm is more or less homogeneous and appears to be of a faintly-greenish tint, and is frequently very finely-alveolar; the nuclei in the living individual can be distinguished only with the greatest difficulty. Careful examination shows that the amœba is retracted in places from the cyst envelope and is evidently loose inside it; from time to time vigorous pseudopodial movements can be seen to take place. Finally a rent apparently occurs in the cyst envelope, and a clear bead of ectoplasm is protruded; this progressively enlarges in a spasmodic manner, more and more of the amœba protruding from the envelope, until finally the creature has escaped completely. It then proceeds to move about in an active, usually slug-like manner, frequently drawing behind it the empty cyst envelope

or fæcal debris. . . . Although at the moment of emergence the cytoplasm is either practically homogeneous or, at most, very finely alveolar, with minute granules, it quickly becomes definitely alveolar, and as it ingests bacteria digestive vacuoles appear in large numbers."

Yorke and Adams also found that cysts would develop at 37° C. and excyst in Locke-serum medium, broth, and physiological saline and would develop even in water at 37° C. up to the rupture of the cyst wall, but that in water the amœba is killed either before, during, or immediately after its escape. They conclude that "moisture and a suitable temperature (preferably about 37° C.) are essential for the occurrence of excystation; the passage of cysts through such solutions as *liquor pepticus* or *liquor pancreaticus* is unnecessary for excystation." A similar conclusion was reached by Sellards and Theiler (1924) from experiments with histolytica cysts in kittens; they state that "For the excystation of amœbæ, a suitable temperature and a reasonable supply of water are obvious necessities."

Dobell and Laidlaw (1926b) bring out the interesting fact that histolytica cysts which have formed in culture or have been freshly passed by human or simian hosts are incapable of excysting in cultures until they have been cooled below body temperature for one or two days. When kept cool they retain their ability to hatch for approximately two weeks.

Autogamy, gamete formation and the production of amœbulæ. The evidence at present available indicates that the amœba escapes from the cyst in a quadrinucleate condition, the nuclei being closely agglomerated. Autog-

amy within the cyst as described in *E. coli* by Schaudinn (1903), and in *E. muris* by Wenyon (1907) probably does not occur. In fact Wenyon (1926) states that "it is abundantly evident that no such autogamy process occurs in the development of the cysts of any entamœba." Recent work also seems to disprove the observation of Darling (1913) that four uninucleate amœbulæ are formed within the cyst before hatching; of Yoshida (1920) that the fusion of nuclei occurs after excystation (in *E. tetragena* and *E. coli*); and of Mathis and Mercier (1917) that eight-nucleated cysts produce uninucleate gametes that conjugate in pairs. Yorke and Adams have shown by careful experiments that in culture the young quadrinucleate amœba usually either divides into two binucleate animals and these subsequently into unincleate forms, or uninucleate amœbulæ separate one by one from the quadrinucleate animal. Their cultures inoculated with cysts gave for example at the end of 6.5 hours 19 per cent of amœbæ with 4 nuclei, 1 per cent with 3, and 5 per cent with 2, the rest of the original cysts being still unhatched; whereas at the end of 24 hours there were 3 per cent with 4 nuclei, 1 per cent with 3, 14 per cent with 2, 74 per cent with 1, 6 per cent with many, and 2 per cent still in the cyst stage. The detailed statistics show a gradual change from quadrinucleate to uninucleate forms. Multinucleate specimens were of frequent occurrence (up to 24 per cent) indicating that nuclear division without cell division sometimes takes place in quadrinucleate amœbæ after hatching.

In what part of the digestive tract does excystation take place? The data presented above dispose effectively

of the general belief that cysts must be subjected to the digestive juices in the stomach and small intestine before they will hatch. The idea that cysts hatch in the small intestine and not in the large intestine is also coming under suspicion. Kessel (1923), for example, found trophozoites of *E. histolytica* most commonly in the cecum and none in the small intestine of rats that were fed cysts of this species. Only once were they encountered in the colon. He concludes from this that excystation occurs in the cecum of this animal. As regards hatching in the colon, Sellards and Theiler (1924), contrary to the experience of Izar (1914b), have shown that histolytica cysts injected into the large intestine of kittens will excyst there provided stasis is produced and Hoare (1925) found amœbæ in the intestinal mucosa of a kitten that had been injected rectally with material containing cysts only of *E. histolytica.* Drbohlav (Wenyon, 1926) has also noted the hatching of cysts in the large intestine of kittens. Presumably excystation may also take place in the large intestine of man.

Sellards and Theiler (1924) have gone even further and suggest that cysts may hatch in the colon of the same individual in which they are formed. Relapse in amœbiasis may, according to this view, result from the hatching of resistant cysts held for long periods in the intestine. However, the observation of Dobell and Laidlaw (1926b) that cysts must be cooled below body temperature for several days before they will hatch indicates that cysts could not hatch until they had passed out of the body of the host in which they encysted.

4. THE PRIMARY SITE OF INFECTION

The large intestine is the primary site of infection with *E. histolytica.* This is an excellent location for the parasite since escape from the body of the host, which is of great importance for the maintenance of the race, is easy from this habitat. Access to the tissues of the wall was for many years considered necessary since the amœbæ were supposed to live only on tissue elements, but the discovery that they feed on bacteria in cultures suggests that they are able to exist in the lumen of the intestine or on the outside of its wall. Those trophozoites or cysts that become embedded in the fecal matter are carried out of the body, hence it seems probable that only those excysted amœbæ that are able to reach the intestinal wall to which they can attach themselves are able to withstand the peristaltic movements of the bowel. These, therefore, are the specimens responsible for bringing about an infection.

Experiments with *E. histolytica* on cats indicate that the primary site of infection is located in that part of the large intestine where stasis first occurs. Stasis evidently prevents the organisms from being carried down the intestine and gives them sufficient time to reach and anchor themselves to the wall of the intestine. Data regarding the location of ulcers in human cases of amœbiasis favor this hypothesis. Thus a résumé of records of 6800 post-mortem examinations made in the Panama Canal Zone from 1905 to 1923 principally by Darling and Clark has been published by the latter (Clark, 1924). Of these, 186 died of amœbiasis or had amœbic ulcers

in their intestine (in 27 cases). The ulcers were scattered throughout the colon in 113 cases (60.7%) but in 63 cases (33.8%) were limited to certain regions. These

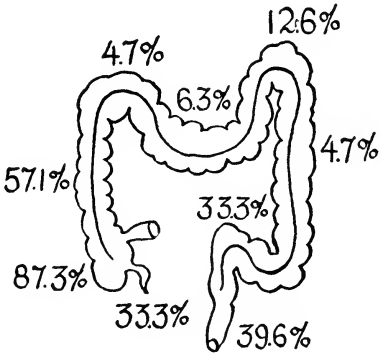

FIG. 20. Appendix, colon and rectum of man showing the regional distribution of lesions in 63 cases of amœbic dysentery. (After Clark).

regions are indicated in Fig. 20; they are dependent portions where the greatest stasis exists, *i.e.*, the cecum, ascending colon, rectum, sigmoid and appendix.

5. SECONDARY SITES OF INFECTION

Infections in the ileum. E. histolytica has been noted on several occasions anterior to the ileo-cecal valve. Cases have been reported in man by Harris (1898), Kuenen (1909), Allen (1924) and Craig (1926a). Craig states

that he has "observed several cases in which typical amœbic ulcerations containing the amœbæ were present in the lower portion of the ileum in the region just above the ileocaecal valve." Sellards and Theiler (1924) working with cats found that in several of the older animals with histolytica infections of several weeks the amœbæ spread into the small intestine above the ileo-colic sphincter. They found that by ligating the large intestine and then forcing water into the digestive tract with a stomach tube they were able to cause the distension of the ileo-colic sphincter. In 3 kittens thus treated the amœbæ infected the lower part of the small intestine, this result suggesting that the sphincter ordinarily mechanically prevents the passage of the amœbæ into the ileum. In 3 other kittens no infection was obtained when the ileum was ligated and trophozoites injected above the ligature. Why the wall of the ileum is not as readily attacked as that of the large intestine is not known.

The distribution of amœbæ to other parts of the body. A number of other secondary sites of infection with *E. histolytica* have been noted besides the ileum; many of these have been in parts of the body at a considerable distance from the large intestine and can be explained only on the assumption that amœbæ are carried there in the blood or lymph. When an amœbic ulcer is formed in the wall of the large intestine blood vessels are opened, thus giving the amœbæ access to the blood stream; specimens have actually been observed in the capillaries of the intestinal wall. Such amœbæ may obviously be carried to any part of the body and may initiate infections wherever suitable conditions are encountered.

Infections in the liver. The liver is the most frequent secondary site of infection, the amœbæ gaining access to this organ by way of the portal vein and giving rise there to one or more abscesses. These abscesses are most often in the right lobe but may be situated in any part of the liver. Sometimes they are very large, containing over a gallon of pus. They may rupture into the lung or other neighboring regions and thus be responsible for spreading the infection. Various investigators have reported the percentage of liver abscesses in cases of intestinal amœbiasis. For example, Kartulis (1887) noted 55 per cent of 500 cases at autopsy; Councilman and Lafleur (1891) record 21 cases among 1429 patients suffering from amœbic dysentery; Craig (1911) noted 33 per cent of 78 cases at autopsy; Clark (1924) records 51 per cent of 186 cases of intestinal amœbiasis that were autopsied in Panama between the years 1905 and 1923; and Ludlow (1926) reports the extremely high incidence of 11.2 per cent among Korean females.

Other secondary sites of infection. Abscesses of amœbic origin may also occur in the lung, brain, spleen, etc. Pulmonary abscesses may be due to rupture of a liver abscess or to amœbæ carried to the lungs in the circulation. The lower lobe of the right lung is the most common location. Abscesses in the lung may rupture into the air passages and the pus, containing amœbæ, may be coughed up by the patient. Amœbæ carried into the brain may give rise to cerebral abscesses of which about 50 cases have been recorded, all ending fatally. Several cases of splenic abscess have been reported. Amœbæ have also been reported from the urinary tract, the testis, the

skin, the eye, the bone marrow, and the lymph glands. Whether the "organisms" described in all of these cases were amœbæ of the species *E. histolytica* and whether they were responsible for the various diseased conditions ascribed to them are questions that will be discussed later (see p. 109).

6. CHANGES IN THE HOST DUE TO THE PRESENCE OF THE PARASITE

(1) THE GENESIS OF SYMPTOMS. Human hosts of *E. histolytica* do not usually exhibit symptoms; presumably the parasites in most cases do not interfere sufficiently with the normal functions of the body to bring about obvious changes. Occasionally, however, symptoms appear, their character depending on the location and severity of the lesions produced by the amœbæ. Thus primary or intestinal amœbiasis may give rise to amœbic diarrhea or amœbic dysentery, and secondary amœbiasis to the various symptoms characteristic of amœbic liver abscess, of amœbic abscesses in the lungs, brain and spleen, and of lesions in other parts of the body. The symptoms that result from the invasion of these organs are rather well known and are described in various books on protozoölogy and tropical medicine (see p. 198).

The origin of the symptoms observed, however, is practically unknown. For example, the most common symptom of intestinal amœbiasis is diarrhea; this is "abnormal frequency and liquidity of fecal discharges." Two factors are involved here, (1) the exudation into the intestinal lumen of serous fluid with a tendency to putrefy and (2) an increase in peristaltic activity. Auer-

bach's plexus is recognized as the center for intestinal peristalsis and its abnormal stimulation may be brought about in various ways. In the case of amœbic diarrhea the most probable stimulus is the irritation of the intestinal wall. If mild, such irritation would result in an increased production of mucus; if more severe, a more copious transudation might ensue. The exact modus operandi in this and other types of symptoms due to amœbic invasion is, however, unknown, and hence it would be fruitless to elaborate the subject further in this place. The fact that the origin of symptoms in this and other protozoan diseases is open to experimental study and offers a fascinating field for investigation cannot be too strongly emphasized.

(2) PATHOGENESIS. As in the case of symptomatology, the pathology of amœbiasis has been described many times. Descriptions of the pathogenesis of amœbiasis are also available but these are based largely on assumptions; it is easy to imagine what might take place but quite another thing to determine exactly the relations between the parasite and the host that result in a diseased condition. Probably some of the newly hatched amœbæ succeed in reaching the wall of the large intestine to which they attach themselves by means of their pseudopodia, and thus escape being carried out of the body. The presence of only a few specimens would interfere extremely little with the functions of the intestinal wall, but large numbers of amœbæ might exert an effect by simply decreasing the amount of epithelial surface in contact with the intestinal contents. The combined effects of large numbers of amœbæ may thus become of suffi-

cient magnitude to be noticed by the host or his physician. It is well known that whether or not symptoms appear in many parasitic infections depends on the number of parasites present.

Sections of the intestinal wall of both man and experimental animals infected with *E. histolytica* have revealed large numbers of amœbæ in the glands of Lieberkühn. Here again, functions may be disturbed merely by the presence of the parasites. Amœbæ also invade the tissues of the intestinal wall. They are supposed to do this by dissolving away the cells with the aid of a cytolytic ferment which they secrete or by pressure due to their rapid multiplication and the consequent blocking of the opening of the glands into which they have migrated. They do not appear to ingest red blood cells or other tissue elements while within the tissues. Continued multiplication of the amœbæ and cellular destruction leads to the formation of a nodule which eventually bursts into the intestinal lumen, thus producing an ulcer. Some of the amœbæ that escape invade neighboring glands and repeat the process, thus spreading the infected area. At the same time, those that remain in the ulcer continue the destruction of the tissues at the sides and bottom. Further pathological effects result from a continuation of this process. These tissue-invading amœbæ are large and never of the precystic type; the latter, as well as cysts, occur only in the lumen of the intestine. What modifications occur in other parts of the body due to the formation of ulcers in the intestine are very little understood and their genesis unknown.

Much of this story, as mentioned above, is based on

assumptions; but the pathogenesis of amœbiasis is open to experimental study and not until properly conducted experiments have been carried out can we state definitely what actually occurs. We know even less about secondary amœbiasis than we do about amœbic infection of the intestine, and any attempt to describe its pathogenesis at present would be futile.

7. RESISTANCE AND SUSCEPTIBILITY OF THE HOST

The problems involved. Our knowledge of the resistance and the susceptibility of the host to infection with *E. histolytica* is fragmentary and scattered and mostly based on experiments on animals. Some of the questions involved are as follows: Do the members of the various human races differ in resistance to infection and in severity of the symptoms? Does the age of the host have an influence on susceptibility and the character of the disease? Does an infection with *E. histolytica* or with some other parasite increase the resistance of the host to subsequent infection? What relation exists between host resistance and the invasion of the tissues of the intestinal wall? Does the character of the climate influence the resistance of the host or the aggressivity of the parasite or both? What immunological reactions have been obtained by experiments with *E. histolytica?*

Racial differences. The influence of race upon susceptibility to amœbic infection is not very clear at present. Fletcher and Jepps (1924) found that Tamils are much more susceptible than Chinese to both *E. histolytica* and *E. coli.* The Tamils gave 19.1 per cent and the Chinese 5.6 per cent of infection with *E. histolytica,* and 11.9 per

cent and 1.7 per cent respectively with *E. coli*. Interesting comparisons between Chinese and foreigners as regards susceptibility to infection with *E. histolytica* and with respect to the severity of the infections have been provided by Kessel and Svensson (1924) and Kessel and Willner (1925). A survey in Peking by the former showed that infection in Chinese (29.5 per cent) was considerably greater with this species than in foreigners (16.5 per cent). Kessel and Willner (1925) report a careful study of 1800 patients admitted to the Peking Union Medical College Hospital between October 1, 1923, and May 31, 1924. Of these 129 were positive for *E. histolytica* on stool examination. Five groups were recognized by these investigators, according to the character of the symptoms as follows.

Symptoms	Chinese		Foreigners	
	No.	Per cent	No.	Per cent
1. Acute or chronic colitis	16	16.7	10	30
2. Abscess in liver, lung or brain	3	3.1		
3. Undefined gastrointestinal symptoms suggestive of gastric neurosis, ulcer or neoplasm	11	11.5	17	52
4. Multiform symptoms of what may be termed diminished vitality: loss of weight and strength, diminished resistance to commonplace infections, chronic infections and cachexia from other causes	32	33.3	3	9
5. No symptoms, healthy carriers	34	35.4	3	9

These results show that foreigners exhibit both more severe and more mild symptoms than Chinese and are correspondingly low in the number of healthy carriers when compared with the Chinese. These data indicate that although the Chinese are more highly infected their

infections are not as severe as are those of foreigners and that, therefore, a real difference exists in susceptibility and resistance of the different races to *E. histolytica.*

Age. The relation between age in man and host-susceptibility to *E. histolytica* is not well known. Young animals seem to be more frequently infected with parasites than adults. Kessel and Svensson (1924), however, report conditions among the Chinese that do not coincide with this view. Of 100 individuals between the ages of 1 and 15 years, 25.3 per cent were infected, between 16 and 50, 29.2 per cent, and over 51, 34.4 per cent were infected. Kessel (1923) in an earlier report noted that older rats are more resistant to infection with human amœbæ than younger rats. It is well known that kittens are more readily infected with *E. histolytica* than are older cats.

The character of the infection may also differ with age. Kittens usually come down with an acute infection whereas Wagener and Thomson (1924) obtained chronic infections in 3 adult and 3 half-grown cats with amœbæ from an acute human infection; evidently the amœbæ encounter greater resistance in the older animals. This resistance affects the size and number of amœbæ; those from acute cases being large and numerous, whereas those from chronic cases are smaller and few in number. Experiments to determine the relative susceptibility of old and young cats were also carried out by Eguchi (1925). Of 15 kittens that were fed cysts of *E. histolytica,* 6 or 40 per cent developed dysentery and one liver abscess; but of 23 cats over 500 gm. in weight only 2 or 8.6 per cent became infected. Eguchi believes this result

is due to a deficiency of protective bodies in the mucous membrane of the young hosts and the development of immunity in the older animals.

Wagener (1924) secured evidence that the severity of the infection depends on both the age of the host and the amount of material injected. Thus young kittens exhibited symptoms in from 2 to 5 days and frequently died on the fourth day; whereas adult cats did not become dysenteric for a week or more and lived for over two weeks after definite symptoms developed. Two adult cats remained alive and passed amœbæ for six weeks and a half-grown cat recovered and its stools became amœba-free. The infection in kittens although more severe was limited to the rectum; in adult cats the entire area from the ileo-cecal valve to the anus was eroded.

It seems certain that resistance to infection and to the pathological effects of tissue invasion is acquired as the host grows older, but the nature of this resistance is unknown.

Does infection with amœbæ of the same or a different species add to the resistance of the host? Kessel (1923) answers this in the affirmative. He found that young rats that were infected with rat amœbæ were more difficult to infect with human amœbæ than those that were amœba-free, indicating that resistance to human amœbæ is built up by a foreign host that is infected with its "normal" species.

Climate. Climate (see p. 81) is often called upon to explain host susceptibility to histolytica infection. The fact to be explained is the greater number of clinical cases of amœbiasis in the tropics than in temperate re-

gions although the incidence of infection is apparently not much greater in the former. The consensus of opinion seems to be that the resistance of the host is so greatly lowered in the tropics that the amœbæ are able to invade the tissues sufficiently to bring about symptoms. Other possible explanations are increases in the aggressivity of the parasites due to rapid passage through the hosts (see p. 105), the presence of more aggressive strains in the tropics, mass infections due to favorable weather and insanitary conditions, and the character of the diet.

8. IMMUNOLOGICAL REACTIONS

Complement fixation. Very little attention has been directed toward the study of immunity reactions in amœbic infections and the data on this subject are therefore few and indefinite. Izar (1914a) claims to have obtained positive complement fixation, using aqueous antigens from liver abscess pus and from the feces of infected cats. Successful results were secured with the serum from three cats and five persons infected with *E. histolytica.* Hage (1920) was unable to confirm Izar's work. He used antigens from liver abscess pus and from the feces of infected human beings. He accounts for his failure on the ground that any antigen present is too small in amount to be extracted easily. Now that methods of cultivating *E. histolytica* have been devised, a new source of antigens is available.

Precipitin tests. Wagener (1924) prepared antigen from scrapings of ulcerated areas in infected cats and obtained a positive precipitin test in cats infected for

a week or longer after amœbæ appeared in their stools, but a negative result when serum from normal cats or from cats infected less than a week were used.

Intradermal reactions. Scalas (1923) obtained remarkably successful results in his experiments with intradermal reactions. He used an antigen prepared from the fresh feces of a case of acute dysentery which he injected intracutaneously into patients suffering from acute, subacute and chronic stages of amœbic dysentery and into non-dysenteric subjects. The 9 infected patients gave a positive result, *i. e.,* a swelling which itched and was hot; the 23 non-infected persons gave only negative results, —erythema without itching or heat.

9. CHANGES IN THE PARASITE DUE TO RESIDENCE IN THE HOST

(1) AGGRESSIVITY. *The problems involved.* As already mentioned, many persons are "contact carriers" who never have shown symptoms but "carry" the amœbæ in their intestine and pass cysts in their feces. The discovery that *E. histolytica* can live, grow and reproduce in culture without access to tissue elements indicates that this organism can live in the lumen of the intestine and suggests that the intestinal wall of contact carriers may never be invaded by the amœbæ. This brings up the important problem of parasite aggressivity. Are strains that inhabit contact carriers lacking in aggressivity? Are they capable of bringing about acute infections? Can the aggressivity of a strain be increased by rapid passage through a number of hosts? Are there adolescent and senile strains or periods in the life-cycle

of one strain? Can a strain retain its aggressivity against the acquired resistance of the host or against drugs?

Human experiments. The experiments of Walker and Sellards (1913) seem to prove that the amœbæ in contact carriers are sufficiently aggressive to give rise to acute amœbiasis and that the condition of the host is the important factor, not the aggressivity of the parasite. They found that 2 of the 20 men employed in their experiments never became infected; that several of the remaining 18 had to be fed cysts more than once before positive results were obtained; and that cysts from a convalescent carrier produced a contact carrier when fed to a second man, and that cysts from him produced another contact carrier when fed to a third man, but when cysts from this individual were fed to a fourth man an acute case developed 20 days later. It is possible that rapid passage through several hosts increased the aggressivity of the strain to such an extent as to bring about an acute attack in the fourth man but this does not seem probable.

Animal experiments. Several investigators, on the other hand, have found that when kittens are used as experimental animals the percentage of infections obtained and the severity of the attack depend upon the character of the amœbæ used. The evidence indicates that the amœbæ differ in virulence and that material from chronic cases is not as infective as that from acute cases. Thus Baetjer and Sellards (1914) state that "chronic cases of long standing, with mild symptoms, often produced an attack in animals which was of comparatively short duration and eventually ended in recovery." Wagener and Thomson (1924) had no difficulty in producing

amœbiasis in kittens when they used amœbæ from an acute case but only one of 14 kittens became infected when motile amœbæ from a chronic human case were injected into the rectum; this single case developed into a chronic infection which was difficult to transfer to other kittens and exhibited no lesions on autopsy.

(2) RESISTANCE TO DRUGS. Many cases have been described in the literature of drug resistant strains of *E. histolytica.* An attempt was made by Dobell and Laidlaw (1926a) to determine whether this species when grown in culture is able to build up a resistance to emetin. A strain that survived a medium containing emetin in dilution of 1 to 50,000 was subcultured in a similar medium for over a month, and also in media containing larger amounts of emetin. The cultures were kept going in the 1 to 50,000 dilution only with difficulty; no continued growth was obtained in media of greater emetin concentration; and no evidence was obtained of an increase in resistance to the drug. These investigators believe that "emetin-resistant" cases of amœbiasis are not due to a resistant strain of amœbæ but are the result of some physiological idiosyncrasy of the host that prevents the emetin from reaching the large intestine, possibly being excreted in the urine, as appears to be true when the drug is administered to cats. That differences may exist, however, as regards sensitiveness to emetin in strains both from man and monkey was noted by these investigators; one human strain was more sensitive to the drug than the other, and this was true also of the two monkey strains used.

Kofoid and Wagener (1925) also report studies with

drugs on *E. histolytica* in culture. The lethal dilution of these drugs ranged from 1 to 250 for yatren casein, to 1 to 100,000 for arsphenamine. Of particular interest are the results obtained in experiments with emetin hydrochloride. Amœbæ were unable to live for 24 hours in culture tubes containing dilutions of 1 to 5000 or less and were successful only at dilutions of 1 to 16,600 or greater. At the end of 48 hours no amœbæ were still living in dilutions of 1 to 16,600 or less and only a few in dilutions of from 1 to 20,000 to 1 to 100,000. The size of the amœbæ in cultures containing the lowest dilution in which they were able to exist was two or three times that of normal specimens, which suggests that the presence of emetin in such quantities prevents division. This suggestion, that emetin in certain dilutions inhibits reproduction, is also made by Dobell and Laidlaw but their results show that this inhibition, if it exists, is only temporary since amœbæ transferred from such cultures to normal cultures grow and multiply and show no ill effects from their previous subjection to emetin.

10. HOST-PARASITE ADJUSTMENTS DURING AN INFECTION

(1) THE CARRIER CONDITION. *Contact and convalescent carriers.* Infections with *E. histolytica* seldom end fatally and in most cases never exhibit an acute phase. The reactions of host and parasite may result in various conditions such as acute amœbiasis, the carrier condition, latency and relapse. In the majority of cases the infected host never experiences what we are accustomed to consider symptoms of amœbiasis; such a host is known

as a "contact" carrier. Spontaneous recovery or recovery following the administration of drugs does not necessarily include the elimination of the amœbæ from the body but only the cessation of symptoms.

As Walker (Walker and Sellards, 1913) pointed out, the acute stage is followed by the "convalescent" carrier condition during which the amœbæ still multiply within the host and escape with the feces usually in the form of cysts. Such hosts may suffer relapses during which acute symptoms reappear.

The period of infection. How long a host remains infected, unless cured by the administration of drugs, it is difficult to state. It seems probable that an infection once established persists throughout the life of the host. Various investigators have attempted to determine the length of infections in particular hosts (Low, 1916; Wenyon and O'Connor, 1917; Dobell and Stevenson, 1918), and although they seem to prove that infections persist for many years, the chances of reinfection are so favorable that no definite conclusion can be reached.

Host-parasite relations during the carrier period. There are three principal points of view with regard to host-parasite relations during the carrier period. The first is that the amœbæ live as harmless commensals in the lumen of the intestine. This was considered impossible until recent cultivation experiments proved that tissue elements are not necessary for the growth and reproduction of the trophozoites.

The second view is that the amœbæ require access to the tissue of the intestinal wall; that they must have tissue elements for their growth and reproduction; and

that they therefore always injure the body of the host. These injuries, however, because of host resistance, are so slight that they are repaired by the regeneration of tissue as rapidly as they are incurred, the result being a sort of equilibrium between host and parasite.

The third view is that a large proportion of carriers suffer from "chronic" amœbiasis. Kofoid and his colleagues have been studying this phase of the subject for several years. They state that "It has been possible for the last two years to say that there is a definite disease entity that can be recognized by clinical means as chronic amœbiasis. According to our own records, this is so definite that fully 95 per cent of cases can be diagnosed clinically, before laboratory confirmation is obtained." "Over a period of years, constant search has been made for a true carrier, but as yet only one individual has been found by the medical author in more than 500 cases who showed no visible tissue damage attributable to *E. dysenteriæ*. . . . In symptomatology, one of the salient facts is that persons with amebiasis commonly complain of fatigability. They go to bed tired, and arise in the morning tired; they are tired whether they work or rest; many of them are so tired that they are in actual anguish; others merely say they have lost their 'pep.' Very commonly associated with fatigability is constipation, or constipation interspersed with evanescent diarrheas. Occasionally one will elicit the passing of considerable quantities of mucus or blood. A normal man knows hunger and the desire for evacuation; otherwise his bowel does not obtrude on his consciousness. In amebiasis the patient often has no actual pain. On the

other hand, he is not comfortable in the abdominal region. He is, as we say, 'bowel conscious.' He may have considerable flatulence. He may or may not complain of definite soreness in the right lower quadrant, in the right upper quadrant, or in the region of the splenic flexure. He may complain of neuritic symptoms, pain along the course of some particular nerve or nerves. He may complain of basal headache, or of pain and aching in the region of joints. He may have considerable digestive disturbance, referable to the upper abdomen, that leads to the clinical suspicion of hyperacidity, hypoacidity, gastric and duodenal ulcers, chronic pancreatitis, chronic cholecystitis, chronic duodenitis or chronic hepatitis. He may complain of cough and expectoration, with bloody sputum. He may complain of rapid pulse, and one may see mild tachycardias. He may complain of much nervousness, and one may find in him symptoms of a subacute or chronic thyroiditis. He may complain of defective vision, and one may find greatly impaired vision with or without definite iritis. Some may complain of disabling loss of memory, while others show various neuroses. One may find also the grosser lesions of liver abscess, lung abscess, brain abscess, skin ulcers, etc. The matter can be summed up by saying that in chronic amebiasis we are dealing with a disease entity as protean as syphilis." Boyers, Kofoid & Swezy (1925).

Acton and Knowles (1924) are also among those who believe the "healthy" carrier is not always free from clinical symptoms. They "recognize two well marked types of *E. histolytica* carriers; the first the thin, lean, cadaverous individual whose food assimilation is inade-

quate, who tends to be faddy and irritable, who is vaguely ill, without knowing what is wrong with him; the second the fat jovial type, with good food assimilation, a bon viveur, who soon discovers, however, that indulgence in 'short drinks' at the club bar is apt to be followed by trouble. Together with minor ulceration of the colon mucosa by the entamœbæ goes a train of ill-defined symptoms," which they describe in detail under the subheads, irregularity in the state of the bowels, pain, fever, bacterial embolism, and absorption of poisonous pressor bases from the ulcerated gut.

Craig (1927) states that his experience indicates that more than 50 per cent of the so-called carriers of *E. histolytica* present some symptoms apparently caused by the presence of these amœbæ in the intestine. These symptoms are largely confined to the digestive and nervous systems. Constipation and diarrhea are perhaps the most common; lack of appetite is very frequent and is associated with loss of weight; and evanescent neuralgic pains in the lower portion of the abdomen and other symptoms are characteristic. The symptoms referable to the nervous system are of the neurasthenic type. A mild degree of anemia and occasional subnormal temperature may occur. Craig expresses belief in the routine examinations of stools and in efforts to cure carriers.

(2) LATENCY AND RELAPSE. Latency in amœbiasis exists only in the sense that parasites may be present without the appearance of symptoms. If fecal specimens from hosts in which *E. histolytica* is living in a "latent" condition are examined daily, eventually cysts or trophozoites will be found. As noted above (p. 108) a host

when once infected may remain infected throughout the rest of his life. He may never show symptoms, but is always liable to come down with an acute infection. If an acute infection appears he may recover with or without treatment. Such a recovery, however, is often followed by one or more relapses as in many other protozoan diseases. Just what modifications in the host or parasite are responsible for relapses are not known with certainty.

II. HOST-PARASITE SPECIFICITY

No comprehensive study has yet been made of the host-parasite specificity of *E. histolytica.* Spontaneous infections with this species have been reported in certain species of lower animals and experimental infections have been obtained in primates, carnivores and rodents.

Primates. Both spontaneous amœbic dysentery and amœbic liver abscess have been reported in monkeys, and Suldey (1924) has recently noted acute dysentery in a 3-year-old chimpanzee. Many investigators have described amœbæ from primates other than man among which are types resembling *E. histolytica* and *E. coli* so closely that they cannot be separated by means of morphological or cultural characteristics. This fact throws doubt on all data obtained as a result of attempts to infect these animals with *E. histolytica.* Of particular interest is the recent observation of Dobell (1926b) that histolytica-like amœbæ from the monkey when cultivated in artificial media and injected into cats produces dysentery that differs from that similarly obtained by injections of *E. histolytica,* thus indicating a physiological

difference between the types found in man and monkey. (Discussions of the amœbæ of monkeys may be found in the books of Dobell (1919a) and Wenyon (1926), and in the recent papers by Brug (1923), Mello (1923), Suldey (1924), Kessel (1924), Dobell (1926b) and Dobell and Laidlaw (1926b).

Carnivores. Spontaneous amœbic dysentery has also been reported in cats and dogs, and kittens have proved to be more easily infected with *E. histolytica* than any other lower animal (see pp. 101, 105). Infections may be brought about by feeding cysts to the experimental animal or injecting trophozoites per anum. When an infection is obtained in cats it is usually acute and recovery is rare. Kruse and Pasquale (1894) claim to have infected a cat with amœbæ from a liver abscess. Liver abscesses have been reported in both cats and dogs.

Rodents. Rats and Mice. Recent experiments on the infection of rats with *E. histolytica* have been carried on by Brug (1919a), Kessel (1923), Wagener and Thomson (1924) and Chiang (1925a, 1925b). Brug found two wild rats in Java which were infected with amœbæ that apparently belonged to the species *E. histolytica,* and reported the experimental infection of a specimen of *Mus rattus* with *E. histolytica* from man. Kessel had no difficulty in infecting young rats and mice by feeding them human feces containing cysts of *E. histolytica* and of other human amœbæ. Transfer of these human amœbæ from one rat to another was also successfully accomplished. No morphological or racial differences could be found between the amœbæ before and after they had been established in the rat hosts. The infections in

both rats and mice were chronic instead of acute as in kittens. The experiments of Wagener and Thomson were performed with motile amœbæ injected into the rectum of amœba-free rats. Negative results were obtained with 16 rats when thus treated and with 4 rats into the cecum of which motile amœbæ were injected by laparotomy. Chiang extended Kessel's work and not only succeeded in infecting rats but in obtaining dysenteric symptoms in cats fed on cysts passed by the rats. He also found that clean rats became parasitized when placed in the same cage with infected rats. Several species of amœbæ have been described as normal inhabitants of the rat's intestine; hence there is always danger of confusion between these and introduced species. Rat experiments must therefore be carried on with extreme care and great familiarity with both the normal and foreign amœbæ is necessary to insure reliable data. The evidence indicates that rats and mice may become infected with *E. histolytica,* but that they are often parasitized by this species in nature, or play anything but a very minor rôle in transmission is doubtful.

Guinea-pigs. Of the many investigators who have attempted to infect guinea-pigs with *E. histolytica* only Baetjer and Sellards (1914) and Chatton (1917a, 1918) have reported positive results. Chatton first obtained infections by feeding cysts to guinea-pigs and then infected other pigs by the rectal injection of trophozoites from these. No dysenteric symptoms were noted although several of the experimental animals died, one in 20 days and another in 9 days. The site of infection was the cecum where a hyperplasia of the epithelium of the glands of

Lieberkühn developed. The differences between the reactions of two species of hosts (man and guinea-pig) to the same parasite are strikingly brought out by these experiments. Wagener and Thomson (1924) attempted to repeat these experiments without success. Since the colon of the guinea-pig is 30 inches long they doubt if Chatton obtained cecal infection by rectal injections.

Rabbits. Huber (1909), until recently, was the only investigator who claimed to have infected rabbits with *E. histolytica.* Four of the 8 rabbits were positive. The amœbæ produced ulcers in the cecum but did not bring on diarrhea or dysentery and were not passed in the feces. Thomson (1926) has likewise succeeded in infecting rabbits. Rectal injections of motile amœbæ from cats failed but one of 3 rabbits fed cysts of *E. histolytica* from a chronic human case became infected and died 30 days after the initial feeding. No cysts were observed in the feces nor found at autopsy. The cecum alone was parasitized, and that invasion of the tissues occurred was proved by the discovery of amœbæ in the mucosa, submucosa and circular muscular layer.

No doubt many other species of animals could be infected with *E. histolytica* in the laboratory but probably very few of these ever become parasitized in nature except under extraordinary conditions. However, by carefully performed experiments it may be possible to determine why cysts hatch in one species of host and not in another; why newly hatched trophozoites are able to live and multiply in one species of host and not in another; why one host becomes infected and another of

the same species does not; and the many other problems that are involved in the study of host-parasite specificity (see p. 42).

12. PREVENTION AND CONTROL

Carriers and transmitting agents. The methods of transmission of histolytica cysts have already been described (pp. 74-83). Amœbiasis is a preventable disease just as are typhoid fever, bacillary dysentery, cholera, etc. Patients suffering from an acute attack are not ordinarily dangerous, since only trophozoites appear in their stools and these are probably seldom if ever responsible for new infections (p. 65); it is the carrier who is passing cysts that must be guarded against. Such a carrier may discharge as many as 300,000,000 cysts in a single day (Kofoid, 1923). These cysts, as already pointed out (p. 67), cannot be conveyed through the air since they are killed by dessication; they must enter a new host by way of the mouth in a moist condition. The problems of prevention and control, therefore, concern methods of transmission and the destruction or control of transmitting agents.

The protection of individuals. Individuals are probably usually infected by cysts ingested in food or drink. These cysts may reach drinking water because of soil pollution or contamination in some other way; they may be transferred to food by infected food-handlers who are passing cysts, by flies or other animals that have fed upon infected human feces, or by the use of night soil in the fertilization of vegetable gardens or the use of contaminated water to wash uncooked vegetables. Trans-

mission by association has already been mentioned (p. 79); this apparently often occurs in families, a member who is a carrier by uncleanly habits contaminating the food, drinking water, towels, wash bowl, etc. The danger from uncooked vegetables is especially great in countries such as China where night soil is used as fertilizer. Recently Mills, Bartlett and Kessel (1925) have concluded from their experiments that "Dipping fruits and vegetables for 10 seconds in boiling water, or water which remains above 80° C. during the immersion, is the only method thus far discovered, which will uniformly kill all pathogenic bacteria, protozoan cysts, and helminth eggs which might be found contaminating such food products, and render them safe for human consumption in an uncooked condition." Protection might also be secured by a favorable diet. For example, Kessel and K'e-Kang (1926) find that an exclusive diet of raw milk always brings about a reduction in the number of specimens present in the intestine of the host and in certain cases entire freedom from the amœbæ resulted.

The protection of communities. Community efforts for the prevention and control of amœbic infection should be directed primarily toward improvements in water supplies, and general sanitation. An excellent example of community protection by the provision of a pure water supply is afforded by statistics from Panama given by Clark (1924). Adequate water systems were installed in Panama in 1914-1915. During the period from 1905-1914, 170 cases of amœbiasis (4.25 per cent) were noted among 4,000 autopsies, whereas from 1914 to 1923 only 16 cases (0.57 per cent) were recorded among 2,800

autopsies. Educational campaigns and the automobile have already lessened the danger from dissemination by flies. The use of cresol to kill cysts in fecal material before they could be ingested by flies and other animals might be effective. Any efforts to bring about a decrease in soil pollution would also be helpful. The control and treatment of carriers are difficult problems that have been discussed freely of late. Stiles (1922), for example, has attempted to determine the feasibility of diagnosing United States veterans of the World War and of treating those who are found to be infected. He estimated that the microscopic work alone would cost $5,000,000 and that the hospitalization, treatment, etc., would add $25,000,000 more. The sterilization of food-handlers in markets, hotels and restaurants has also been advocated but the difficulties and expense involved make this proposal likewise impracticable.

IV. *Host-Parasite Relations between Man and Other Species of Amœbæ*

The species of amœbæ, other than *E. histolytica,* that live in man are considered by practically every protozoölogist to be harmless commensals. For this reason they have not been the subject of such intensive study as their near relative, *E. histolytica.* There is consequently nothing or very little to be said regarding them with respect to such subjects as pathogenesis, symptomatology, immunology, resistance to infection, susceptibility of the host, aggressivity of the parasite, relapse, etc. Furthermore, many of the subjects presented under

E. histolytica regarding transmission, infection, prevention, etc., are omitted here in order to avoid repetition. Instead, therefore, of following the outline used in discussing *E. histolytica,* only those phases of the host-parasite relations will be referred to in the case of the other amœbæ of man that call for special attention.

1. ENDAMŒBA COLI

Epidemiology of transmission. As in the case of *E. histolytica, E. coli* is no doubt usually transmitted in the cyst stage, although occasionally trophozoites may bring about the colonization of a new host (see p. 65). Experiments designed particularly to test the viability of histolytica cysts outside of the body under various conditions have contributed also to our knowledge of this subject with respect to cysts of *E. coli.* Certain of the results of these experiments are included in the account of *E. histolytica* (see p. 71); these indicate that the same principles obtain in both species and hence a detailed statement regarding the cysts of *E. coli* seems unnecessary. No doubt the cysts of *E. coli* reach the digestive tract of man in contaminated food or drink and are carried about by flies and possibly by lower animals such as mice, rats, cats and dogs. The very high incidence of infection with *E. coli* among the general population, which appears to be at least 50 per cent, indicates that fecal contamination of our food and drink is very prevalent. All persons in whom *E. coli* lives are carriers who are more or less constantly passing cysts capable of bringing about the colonization of new hosts.

Distribution and localization in the host. The cysts of *E. coli* are passively carried with the food into the intestine. Where they hatch is not known; none have ever been found in the small intestine; but excystation may occur there and the young amœbæ may then pass on into their normal habitat, the large intestine. The process of excystation in the host has not been described, but the writer (Hegner, 1927b) has observed the hatching of coli cysts in vitro. Washed cysts either in water or in weak saline solution were sealed under a cover glass and placed on the stage of a microscope confined in a warm chamber. The protoplasm within the cyst is at first finely granular and the 8 nuclei are usually clearly visible, but later the nuclei become invisible and a number of larger granules of various sizes appear. The first evidence of activity preceding excystation is the movement of the cytoplasm in the center of the cyst. No large, free area exists between the cyst contents and the cyst wall, such as described by Smith (1927) in *Iodamœba williamsi.* Pseudopodia first appear through an opening in the cyst wall. This opening is small and the protoplasm streams through it rapidly in a thin strand. The amœba does not leave the cyst wall at once but usually, after from one-half to three-fourths of the protoplasm has escaped, movement begins in the opposite direction and most or all of the animal streams back again into the cyst. This egress and return of the protoplasm may occur as often as ten times before complete escape is effected and the liberated amœba moves away from the deserted cyst wall.

After excystation the amœba moves at first slowly, but soon flows across the field by means of rapidly form-

ing pseudopodia. These pseudopodia are somewhat similar to those of *E. histolytica* being formed rapidly and more or less explosively, and being at first free from granules although not so clear and hyaline as those of *E. histolytica.* In every case the entire contents of the cyst emerged as a single amœba. Excysted amœbæ were watched for more than 6 hours but no division stages were observed. The newly hatched amœbæ probably succeed in maintaining their position in the intestine against the movement of peristalsis by clinging to the intestinal mucosa wth their pseudopodia. The trophozoites occur in the upper part of the colon where the contents are liquid and the precystic stages and cysts further down where the feces become firmer.

Food. The food of *E. coli* consists of bacteria, yeast, starch grains and other protozoa, and all sorts of debris contained in the large intestine. It differs markedly from *E. histolytica* in its failure to ingest red blood cells. Lynch (1924a) has reported a case in which an amœba that formed an 8-nucleated cyst indistinguishable from cysts of *E. coli* ingested red cells. Fecal material was added to a medium of salt solution to which a small amount of human blood had been added and placed in a "warm incubator." Two hours later the majority of the amœbæ from the bottom of the tube were found to have ingested red cells. Amœbæ from the same patient at a later date failed to ingest red cells.

Tissue invasion. There is some evidence that *E. coli* under certain conditions may invade the tissues of the intestinal wall. Brumpt (1926a) has gathered together the scattered data available from reports on human be-

ings and has added facts obtained by himself from experiments on kittens. Brumpt found specimens of *E. coli* in small ulcerations in the intestinal mucosa of a kitten that had been given a rectal injection of material containing both *E. coli* and *E. dispar,* the latter being the name given by him for what he believes to be a species resembling *E. histolytica* morphologically but differing from it in being non-pathogenic. If this work and the observations of Lynch are confirmed, it must be admitted that *E. coli* may under certain conditions eat red blood cells and invade the tissues of the intestine.

Host-parasite specificity. That *E. coli* finds the human intestine a favorable habitat is evident from the high incidence of infection; about 50 per cent of the general population are carriers of this species. The ease with which infection takes place is indicated by the results of the experiments reported by Walker and Sellards (1913). Twenty men were fed cysts obtained from 5 different hosts; 17 of them became infected, cysts being passed in from 1 to 11 days. No symptoms were observed in any of the infected men. Why *E. coli* should be more successful than *E. histolytica* is difficult to understand; the latter is parasitic in only about one-fifth as many persons (10 per cent) as is *E. coli* (50 per cent).

Many attempts have been made to infect lower animals with *E. coli* but only recently have any of them been successful. Kessel (1923) reported positive results with rats and later (Kessel, 1924) with monkeys. There is some doubt about the latter since monkeys are apparently naturally infected with an amœba indistinguishable from

E. coli. No careful experiments have been carried out to determine just what happens to coli cysts within the digestive tract of foreign hosts.

2. ENDOLIMAX NANA

This species appears to be a harmless commensal that lives in the large intestine of man, although its exact location in the human host is not known. About 25 per cent of the general population have been found to be infected. The cysts are apparently responsible for the infection of new hosts and since they probably differ from those of *E. histolytica* in no essential feature with respect to transmission, etc., and, since our knowledge of them is even more meager than that of histolytica cysts, it seems useless to discuss them here. No evidences of pathogenicity have been discovered; Wenyon (1926) has reported them from the lumen of the intestinal glands but there were no signs of tissue invasion. Hegner (1927b) has described the excystation of *Endolimax nana* in vitro.

Species belonging to the genus *Endolimax* occur in certain lower animals. Amœba-like organisms that have been described from the malpighian tubules of rat and dog fleas and in the vagina of the leech may be species of *Endolimax*. The frog, domestic fowl and monkey also seem to be infected with members of this genus. Tyzzer (1920) described as *Pygolimax gregariniformis* a species that he found in the cecum of fowls. Hegner (1926a) redescribed this species as *Endolimax janisœ*. Tyzzer's specific name is valid but the organism undoubtedly belongs to the genus *Endolimax*. Very few attempts to

infect lower animals with *Endolimax nana* have been made. Kessel (1923) claims to have infected rats by feeding them cysts. This author (Kessel, 1924) also reports positive results with monkeys, but the fact that Brug (1923) found specimens in the monkey indistinguishable from those of man throw some doubt on these results. Chiang (1925) was unable to infect rats with cysts from man, but found amœbæ in the rat similar to *E. nana* of man which he thinks represents a new species, *E. ratti,* on physiological grounds.

3. IODAMŒBA WILLIAMSI

The so-called "iodine cysts" described by Wenyon in 1916 were later found to belong to another harmless commensal, *Iodamœba williamsi.* This species has been found in man in various parts of the world and occurs in about 10 per cent of the general population. Its exact habitat is not known but it probably lives only in the large intestine where it feeds on bacteria. Species of *Iodamœba* similar to the one in man have been recorded from pigs by O'Connor (1920) and others, and it is possible that the specimens found in man and pig may belong to one species. *Iodamœba suis* was the scientific name suggested by O'Connor for the pig form. Brug (1921) found an *Iodamœba* in the feces of a monkey, *Macacus cynomolgus,* Hegner and Taliaferro (1924) in another species of monkey, *Cebus variegatus,* and Wenyon (1926) in a gorilla. Kessel (1923) claims to have infected rats with *Iodamœba* from man.

Excystation of *Iodamœba williamsi* from man in vitro and in guinea-pigs has recently been described by Smith

(1927). Apparently moisture and a suitable temperature are the required stimuli; cysts in a weak saline solution on a slide under a cover glass when maintained at a temperature of 37° C. for about 5 hours were seen to excyst on a number of occasions. When injected into the stomach of guinea-pigs cysts are carried into the small intestine where they may excyst in the jejunum within a period of three hours.

4. DIENTAMŒBA FRAGILIS

Only a few cases of infection with this species are on record, and cysts have been reported by only one observer (Kofoid, 1923). The rarity of cysts and the apparent delicacy of the trophozoites probably account for the small number of infections that have been noted; in fact, its seems strange that this species can continue to exist. It has been suggested that man is not the "normal" host of *D. fragilis,* but no one has yet discovered this or similar species in any lower animal. Obviously no discussion of its host-parasite relations is possible until further data are obtained.

5. ENDAMŒBA GINGIVALIS

Transmission. Although *E. gingivalis* is probably not pathogenic, as once supposed, it is of considerable interest because its habitat differs from that of all other amœbæ of man. It has been suggested that this form and *E. histolytica* belong to the same species, but recent studies, especially those of Kofoid and Swezy (1924c), render this highly improbable. Cysts have been described but there is no evidence that they really exist although Wen-

yon (1926) states that they "probably occur." If they do, they must play a minor rôle in the life-cycle of the organism. Transmission from host to host no doubt takes place in the trophozoite stage and plenty of opportunity is afforded for direct passage during kissing. It is thus easy to account for the high incidence of infection in the general population which probably averages at least 50 per cent. This species of amœba, although in the active stage when disseminated, is probably passively carried from mouth to mouth. The absence of a cyst stage in the only amœba of man that is transmitted by direct contact is worthy of note.

Pathogenicity. E. gingivalis lives in various places in the mouth, but especially in the tartar of the teeth and in the materia alba around them; it has been reported from many suppurative and inflamed conditions of the mouth and throat. That this species does not require access to the tissues is indicated by the fact that Lynch (1915c) found them in the crevices between the false teeth of persons with healthy gums. Various new species have been described from abscesses in the jaw, tonsils, etc., but these were probably all somewhat modified *E. gingivalis*. The presence of large numbers of these amœbæ in the lesions of pyorrhea alveolaris led Smith and Barrett (1915) and Bass and Johns (1915) to conclude that they are responsible for this disease, and on their recommendation emetin was widely used in its treatment. More recent studies indicate that the species is harmless. Its food consists principally of leucocytes and a few bacteria (Kofoid and Swezy, 1924c). Smith and Barrett (1915) record the ingestion of red cells and

Howitt (1926b) finds that washed red cells of the guinea-pig are eaten by specimens in artificial cultures, but erythrocytes are probably very seldom devoured in nature. The colonization of the intestine by this species as a result of swallowing trophozoites seems impossible according to the work of Howitt (1926b), who found that they were unable to withstand human gastric juice containing the normal amount of acid, and quickly exploded when subjected to human bile.

Host-parasite specificity. Amœbæ have been found in the mouths of certain lower animals. Goodrich and Moseley (1916) reported them from the dog and cat suffering from pyorrhea, and Nieschulz (1924) described what he considers a variety of the human species *E. gingivalis* var. *equi* from around the teeth of the horse. It remains to be determined whether these are specifically distinct from the species occurring in man. Hecker (1916) found it impossible to infect guinea-pigs with amœbæ from the human mouth and Drbohlav (1925a) was equally unsuccessful with a kitten into the intestine of which he injected specimens grown in culture and in a young dog into the gingivæ of which similar material was inoculated.

CHAPTER III

INTESTINAL FLAGELLATES

I. *Generic Characteristics*

There is still some doubt as to the number of distinct species of intestinal flagellates that live in man. No question exists, however, regarding the generic and specific rank of *Giardia lamblia, Chilomastix mesnili, Embadomonas intestinalis* and *Tricercomonas intestinalis.* There is some doubt, however, about *Enteromonas hominis,* and opinions differ with respect to the genera and species of the trichomonads. The classification of the flagellates is not in a satisfactory state, due largely to their small size, the difficulty of making adequate preparations, and the apparent inconstancy of various characteristics.

1. TRICHOMONAS

Members of this genus (Figs. 7, 8, 9) possess three to five anterior flagella; an undulating membrane to which an axoneme is attached, a chromatic basal rod, a cytostome, an axostyle, a nucleus situated near the anterior end, a group of blepharoplasts, and, at least in some species, a parabasal body. The vaginal trichomonad, *T. vaginalis,* Donné (1837), is the type species; it has four flagella. If the number of flagella is considered of generic importance, then trichomonads with 4 flagella must be included in the genus *Trichomonas.* The genus *Tritri-*

chomonas was suggested by Kofoid (1920) for trichomonads with 3 anterior flagella and *Pentatrichomonas* by Mesnil (1914) for those with five. Certain protozoölogists (*e.g.,* Wenyon, 1926) prefer to consider trichomonads that differ with respect to the number of flagella as varieties of the genus *Trichomonas.* In the following pages the terms *Tritrichomonas, Trichomonas* and *Pentatrichomonas* will be used to indicate the 3, 4 and 5 flagellated types.

2. CHILOMASTIX

This genus (Fig. 10a) is characterized by the presence of three anterior flagella, a large cytostome in which is located a short flagellum, two cytostomal fibers, a large anteriorly placed nucleus and a group of blepharoplasts.

3. EMBADOMONAS

Embadomonads (Fig. 11a) possess two flagella; one is long and slender and directed anteriorly, the other short and thick and directed posteriorly. There is a large cytostome, and an anteriorly located nucleus, on the membrane of which are two blepharoplasts.

4. TRICERCOMONAS

This type (Fig. 12a) has three anterior flagella and a trailing flagellum which is attached to the surface of the body. The nucleus is large and contains a massive karyosome. There is no cytostome. Considerable confusion exists with respect to this genus and the genus *Enteromonas* of Fonseca (1915, 1920). The latter was described as a minute spherical organism with three

anterior flagella. Several investigators have reported *Enteromonas* from man and Lynch (1922b) described a species belonging to this genus in the guinea-pig. Further evidence is necessary, however, before *Enteromonas* can be recognized with certainty as a separate genus.

5. GIARDIA

The members of this genus (Fig. 13a) are bilaterally symmetrical, have two nuclei, a ventral sucking disc and four pairs of flagella.

II. *Specific Characteristics*

1. TRICHOMONAS VAGINALIS (Fig. 7)

This is the type species of the genus *Trichomonas* established by Donné in 1837. No cyst stage is known. The trophozoite is comparatively large with an average length of 16μ and an average breadth of 11μ. The anterior flagella are four in number and usually emerge from the body in pairs, the members of each pair becoming separate some distance from the body. They arise from blepharoplastic granules situated near the anterior end of the body. A fifth flagellum arises also from one of these blepharoplastic granules; it is fastened to the edge of the short undulating membrane but does not extend beyond the side of the body. A thin, chromatic basal rod lies along the base of the undulating membrane. Spherical chromatic granules lie on either side of this rod, often arranged in a single row. The nucleus is large and spindle-shaped, and contains many chromatic granules em-

bedded in an achromatic matrix. A thick, thread-like rod, the axostyle, extends from the nucleus through the body and emerges near the posterior end. Spherical chromatic bodies are arranged about it, often in rows, or embedded in it. On the side of the nucleus opposite the undulating membrane a clear slit-like area appears in specimens prepared by the Schaudinn-iron-hematoxylin method and probably represents the cytostome. This area is bordered by what is apparently a cytostomal fibril. The division of *T. vaginalis* has never been described fully. No other stages in the life-cycle of this species are known.

2. TRICHOMONAS BUCCALIS (Fig. 8)

The trichomonas from the human mouth may or may not be a distinct species. No cyst form is known. The trophozoite varies greatly in size, measuring from 3.8μ to 7.6μ in breadth and 5μ to 21μ in length. An average specimen measures about 10μ long and 5μ broad. The anterior flagella are usually four in number and emerge from the two blepharoplasts in pairs. The undulating membrane extends posteriorly from the anterior end about two-thirds the length of the body. A flagellum is attached along its outer edge but does not extend beyond its posterior end. The chromatic basal rod is not conspicuous. The axostyle is thread-like and stains deeply in iron-hematoxylin. Hogue (1926) has described a clear area at the side of the nucleus similar to that noted by Hegner (1925d) in *T. vaginalis* and considered by him to be the cytostome. Hogue, however, finds in some specimens of *T. buccalis* a clear funnel-shaped area near the

anterior end which she suggests may be a cytostome. A similar clear area is noted by Hinshaw (1926b). Ohira and Noguchi (1917) observed binary longitudinal fission and multiple fission of this species in cultures, and Hinshaw (1926b) has described both nuclear division and cell division. No other stages in the life-cycle have been found.

3. TRICHOMONAS HOMINIS (Fig. 9)

The trichomonads of the human intestine are very difficult to prepare for microscopic study and their morphology, therefore, has not been worked out in as great detail as that of the other types. The size varies greatly, from 8μ to 15μ in length by 3μ to 5μ in breadth. The flagella appear to arise from two or more blepharoplasts near the anterior end. The undulating membrane extends almost the entire length of the body and its flagellum projects out freely at the posterior end for a considerable distance. The axostyle is a clear rod of considerable thickness, part of which protrudes from the posterior end of the body. On the other side opposite the nucleus at the anterior end is a well defined cytostome. At the base of the undulating membrane is a heavy, deeply-staining chromatic basal rod. This rod is considered by Kofoid and his students to be a parabasal body, but Cutler (1919a) in *Ditrichomonas termitis* from termites, Wenrich (1921) in *Tritrichomonas muris* from rats and Andrews (1925) in *Trichomonas termopsidis* find another body, which appears when specimens are fixed with osmic or chromic acid, which is apparently the parabasal body. The fact that one of Kofoid's stu-

dents (Hinshaw, 1926b) has recently described this organelle as a chromatic basal rod indicates that Kofoid has changed his mind regarding its homology with the parabasal body of other flagellates. Both binary longitudinal fission and multiple fission have been observed; but no other stages are known in the life-cycle.

4. CHILOMASTIX MESNILI (Fig. 10a)

The large intestine is the habitat of this species. The trophozoite (Fig. 10a) is usually from 8μ to 14μ long and from one-half to one-fourth the total length in breadth. Three flagella extend out from the anterior end and a fourth lies within the large cytostome. On either side of the cytostome is a supporting fibril. There is a large nucleus near the anterior end and three blepharoplasts from which the flagella arise. The cyst (Fig. 10b) is lemon-shaped and measures from 7μ to 9μ in length and from 4μ to 6μ in breadth; nucleus, cytostome, cytostomal fibrils and flagellum, and blepharoplasts are visible within it. Longitudinal and multiple fission of the trophozoite have been described and also nuclear division within the cyst (Hegner, 1923c; Grassé, 1926).

5. EMBADOMONAS INTESTINALIS (Fig. 11a)

This species lives in the large intestine of man where it occurs both in the trophozoite and cyst stages. The trophozoite usually measures from 5μ to 6μ in length and from 3μ to 4μ in breadth. On one side near the anterior end is a very large cytostome resembling somewhat a sucking disc. There are two anterior flagella that arise from separate blepharoplasts situated on the

nuclear membrane; one flagellum is much thicker than the other. Longitudinal fission has been observed and also stages that suggest multiple fission (Broughton-Alcock and Thomson, 1922). The cysts of *E. intestinalis* (Fig. 11b) are pear-shaped and range from 4μ to 9μ in length and from 2.5μ to 4.8μ in breadth. These stages constitute all we know about the life-cycle of this species.

6. TRICERCOMONAS INTESTINALIS (Fig. 12a)

This species also lives in the large intestine of man but has been recorded from less than 100 persons. It measures from 4μ to 10μ in length and from 3μ to 6μ in breadth. The ovoid nucleus contains a large central karyosome. Two blepharoplasts are located on the nuclear membrane. From the anterior blepharoplast three free flagella arise, two of which are often fastened together; and from the posterior blepharoplast a single flagellum arises, passes posteriorly through the cytoplasm and emerges near the posterior end of the body. No cytostome has been discovered. The cyst (Fig. 12b) is ovoid and averages about 7μ in length and 4.5μ in breadth. It possesses two nuclei at one end, or four nuclei, two at either end. Binary division occurs, but there is no evidence of multiple fission.

7. GIARDIA LAMBLIA (Fig. 13a)

The optimum habitat of this species is in the duodenum. The trophozoite is bilaterally symmetrical. It measures on the average, 13.7μ in length and 6.6μ in breadth. The shape of the body in front view is indicated in figure 13a. The ventral anterior portion is a

large sucking disc bordered by anterior and posterior peristomal fibers. Beneath the sucking disc are two oval nuclei. A pair of slender axostyles extend through the center of the body and four pairs of flagella arise as shown in the figure. The posterior peristomal fibers and the posterior lateral flagella delimit on either side a thickened area, the lateral shields, between which is a diamond-shaped region that thins out into a "tail." The cysts (Fig. 13b) are oval in shape and average 10.7μ in length and 7.47μ in breadth. Cysts with 2, 4, 8 and 16 nuclei occur; they are spherical and are usually distributed two near the anterior end, two near either end, four near the anterior end or four near either end. Axostyles and many of the fibrils present in the trophozoite persist in the cyst. Frontal longitudinal division of the trophozoite has been described (Kofoid and Swezy, 1922b) and multiple fission has been reported (Noc, 1909). Excystation has recently been described (Hegner, 1927a).

III. *Host-Parasite Relations between Man and His Intestinal Flagellates*

1. TRICHOMONAS VAGINALIS

As in the case of intestinal amœbæ there is no other conceivable method of infection of the human host with intestinal flagellates but by the ingestion of living trophozoites and cysts. However, *Trichomonas vaginalis* and *T. buccalis,* which are usually included with the intestinal flagellates proper, must reach their primary sites of infection in some other way. These two species will be considered first, and then the other species described above.

Trichomonas vaginalis in women. This species (Fig. 7) is widespread and a high incidence of infection has been reported by several investigators. It not only lives in the vagina but has been reported in the urinary tract of males. Recent surveys furnish the following data. Brumpt (1913) found it in over 10 per cent of the women examined at a gynecological clinic in Paris; Barlow (1916) in 5 per cent of 100 women at a similar clinic in St. Louis; Reuling (1921) in 18.4 per cent of 250 women in a clinic in Heidelberg; Ponoschina (1923) in 30 per cent of 55 women, but not in 22 girls from 2 to 14 years of age; and Hegner (1925d) in 50 per cent of 32 women in Honduras and Costa Rica.

Trichomonas vaginalis in men. The trichomonads that have been reported on several occasions from men presumably belong to this species. The first authentic case seems to be that of Marchand (1894) who discovered them in the urine of a man sixty years of age suffering from a fistula in the perineum; the flagellates were noted in the urine daily for some time. In the same year Miura (1894) found them in the urine of a Japanese man; he concluded they were located in the urethra and that the infection came from the man's wife who was found to harbor flagellates in her vagina. Dock (1896), who described the above cases, reports a third case in a student at Ann Arbor, Michigan, 27 years of age, who passed trichomonads in large numbers in his urine that presumably came from the infected bladder. This young man denied coitus, and how he became infected was not determined. Hegner (Hegner and Taliaferro, 1924) saw trichomonads in the urine of a man but had no

record of the case. Katsunuma (1924) describes *T. vaginalis* in the urine of a Japanese boy only 3 years old. No trichomonads were present in the feces and the urinary tract was apparently normal. The flagellates were thought to be located in the terminal portion of the ureter, and the boy to have been infected by his mother or some other woman attendant. Finally, Dastider (1925) during the routine examination of fresh urine from about 1000 persons found trichomonads in that of three men and one woman. In all four the urine was acid and that of the three men contained pus cells; in two of the latter the flagellates and pus cells disappeared at the same time which indicates some relation between the trichomonads and the pathological condition present.

Trichomonads of vagina, mouth and intestine. Certain investigators believe that the trichomonads that occur in the vagina, mouth and intestine of man all belong to one species. Thus both Lynch (1922a) and Wenyon (1926) failed to find distinctive differences when specimens from these habitats were grown in culture and compared. It has been suggested that vaginal infections are due to contamination with specimens from the intestine. The absence of intestinal trichomonads in certain reported cases of vaginal infections is opposed to this theory, but it is not always an easy matter to detect an intestinal infection with *Trichomonas.*

Methods of infection. How *T. vaginalis* reaches the vagina is uncertain. Specimens from the vagina may easily gain access to the urinogenital tract of men during coitus. The vagina may become infected during coitus but this has still to be proved. Contamination with speci-

mens from the intestine, as suggested above, or during homosexual practices are also possibilities (Dickinson and Pierson, 1926). It seems probable that the incidence of infection among men is higher than reports now available indicate. The exact distribution of these flagellates in the various parts of the urinogenital tract is not known.

Host-parasite relations. The relations of *T. vaginalis* to its host cannot be stated with certainty. It is reported to be commonly present when the vaginal mucous membrane is in an abnormal condition and when the reaction of the vaginal mucus is acid. Treatment with sodium bicarbonate is therefore recommended by some physicians so as to change the vaginal contents to an alkaline condition. Whether the trichomonads are pathogenic and bring about a diseased condition in the vagina or this condition is favorable for the growth and multiplication of the flagellates is a question not yet solved.

Host-parasite specificity. So far as the writer is aware, no trichomonads have been reported from the vagina of lower animals. Blockmann (1884) and Dock (1894) were unable to infect dogs, rabbits, and guinea-pigs with specimens from man. Under the writer's direction, vaginal mucus from the following freshly slaughtered animals has been examined with negative results: 35 sows, 100 cows, 103 calves, and 108 sheep. Recently the writer has obtained trichomonads from the vagina of several monkeys, *Macacus rhesus*, maintained in the Department of Embryology of the Carnegie Institution of Washington. These have been described as a new species,

Trichomonas macacovaginæ, by Hegner and Ratcliffe (1927b).

2. TRICHOMONAS BUCCALIS

Incidence of infection. This species (Fig. 8) inhabits the human mouth, but what is no doubt the same species has been recorded from diseased tonsils, lungs, and stomach. A large proportion of the general population are probably infected. Jepps (1923) examined scrapings from the gingival space at the base of the teeth of 50 coolies in Kuala Lumpur, Federated Malay States, and records 16 positive cases, an incidence of 32 per cent. Hogue (1926) considers the culture method more satisfactory for purposes of diagnosis. She inoculated scrapings into culture tubes which were then incubated for 48 hours at 30° C. She obtained 7 positive cases from 32 dental patients with pyorrhea or acute gingivitis and 2 positive cases from 18 persons taken at random. Hinshaw (1926a) likewise seldom found these flagellates in smears but secured 37 positive cases of 120 examined by the culture method.

T. buccalis and T. hominis. The possibility has been suggested that the trichomonads of the human mouth and intestine may belong to the same species, the latter being specimens from the mouth swallowed by the host. Lynch (1915c), however, could find no specimens in the intestine of a person who harbored them in the mouth; Wenyon and O'Connor (1917) report a case of oral infection which they followed for months but no trichomonads were ever found in the stools; and in four of Hogue's

(1926) cases stool examinations by both smear and culture methods proved negative.

Infection and resistance. Transfer from one host to another is no doubt the result of kissing and the flagellates at some time probably reach the oral cavity of practically everyone. Failure to infect is due to the resistance of the host, in other words, to the unfavorable conditions that exist in certain individuals, or to non-aggressive strains of parasites. That strains differ with respect to their ability to withstand various conditions is indicated by the work of Hogue (1926). Material was subjected to heat for a certain period and then inoculated into culture tubes. Two strains withstood a temperature of 40° C. for 5 minutes, 2 strains 40° C. for 10 minutes, and 2 strains 45° C. for 5 minutes. Furthermore, certain strains lived for over 7 months whereas others died out in 3 months in the same medium; some persisted for 96 hours in a medium of pH 7.2-7.8 but others were killed; and one strain was able to live in normal saline solution to which horse serum was added.

Host-parasite relations. Recent investigations indicate a definite relation between the presence of *T. buccalis* and a diseased condition of the oral region. Thus Hogue (1926) found that all of her positive cases gave a history of pyorrhea, acute gingivitis or abscessed teeth, and Hinshaw (1926a) found no specimens in normal mouths but records 37 positives from 49 patients with advanced pyorrhea and no positives from 71 persons with normal mouths or incipient pyorrhea. This flagellate, therefore, seems to be associated with pathological conditions but it is yet to be convicted of being responsible for pyorrhea.

Host-parasite specificity. Trichomonads have been described from the mouths of lower animals by Hegner and Ratcliffe (1927a, 1927b). A species, named by these investigators *Trichomonas canistomæ,* was reported from 22 of 23 dogs examined. Since this report was prepared the twenty-third dog and 26 other dogs examined have all been found to be positive; thus 100 per cent infection existed in these 49 dogs from Baltimore. Trichomonads were obtained also from the mouths of 2 cats; these have been named *Trichomonas felistomæ* (Hegner and Ratcliffe, 1927b).

3. TRICHOMONAS HOMINIS

(1) EPIDEMIOLOGY OF TRANSMISSION. *Transmission by trophozoites.* As noted above, intestinal trichomonads have been recorded with three, four, and five anterior flagella. These may be considered varieties of the species *Trichomonas hominis* (Fig. 9), or separate species of the genus *Trichomonas* or representatives of different genera, *Tritrichomonas, Trichomonas* and *Pentatrichomonas* respectively; but at present it seems best to discuss them all as members of the species *Trichomonas hominis.*

The problem of the transmission of the intestinal trichomonads from one human host to another is particularly interesting because there is no cyst stage known in the life-cycle of the species that live in man and, therefore, transmission must take place by the ingestion of living trophozoites, usually, no doubt, in food or drink. However, the prevailing idea, as already pointed out (p. 65), is that trophozoites of intestinal protozoa are destroyed in the digestive tract if swallowed. Wenyon

(1915b) states that some specimens of human trichomonads become spherical and motionless when removed from the body and in this condition "will withstand the action of gastric juice for a considerable time," and expresses the opinion "that it appears probable that it is such contracted spherical forms which are responsible for the spread of the infection," but apparently did not carry out any infection experiments. Woodcock (1917) also suggested that "infection with trichomonas can take place by means of the active, unencysted forms." Experimental evidence that trophozoites of trichomonads are capable of passing unharmed through the stomach and small intestine and of setting up an infection in the large intestine of a mammalian host is now available.

Experiments with rats, guinea-pigs and cats. Infection experiments carried out by Hegner (1924a) with *Trichomonas muris* of the rat prove that trophozoites of this species are capable of remaining actively motile for at least one hour after being injected into the stomach of the rat; that they may pass from the stomach into the duodenum apparently unharmed within half an hour; that they may reach the cecum through the stomach and 780 mm. of small intestine within half an hour and still be actively motile; and that a rat free from trichomonads may acquire a cecal infection within four days after trophozoites are injected into the stomach. Later experiments (Hegner, 1926c) prove that the trophozoites of *Trichomonas caviæ* and *T. flagelliphora* of the guinea-pig are able to pass through the stomach and small intestine of a guinea-pig and reach the cecum apparently

unharmed within an hour after being injected into the stomach.

Other investigators have recently confirmed these results. Thus Brumpt (1925) has reported the infection of cats by the ingestion of trophozoites of *Trichomonas felis* and Wenrich and Yanoff (1927) have shown that four species of rat trichomonads and one species from man may likewise be infective in the trophozoite stage. The species studied were *T. muris, T. parva, T. minuta,* and *Pentatrichomonas* sp. of the rat and *Pentatrichomonas* from man. Cysts are known to occur in the life-cycle of *T. muris* and were found also by Wenrich and Yanoff in *T. minuta* but the trophozoites of these species are evidently infective as well as the cysts.

Incidence of infection. The chances of the trophozoites being ingested before they are killed by conditions outside of the body are no doubt less than those of cysts and probably account for the low incidence of infection with this species reported in various surveys. Wenyon's (1926) statement that "*Trichomonas hominis* is probably the commonest intestinal flagellate of man" is contrary to the results reported by most investigators. The incidence of infection is certainly much greater, however, than the data available show. This is probably due to the fact that most of the studies have been made with stools that were many hours old. For example, Boeck and Stiles (1923) report an incidence of only .07 per cent of *T. hominis* from 8,029 individuals, but much of their material was sent to them by post from considerable distances (from 22 States). There are no cysts to reveal infection

such as exist in the case of *Giardia, Chilomastix,* etc., and the trophozoites round up and become quiescent soon after leaving the bodies and in this condition it is almost impossible to find them.

Fresh material and culture methods. When fresh fecal material is examined by the smear method or when comparatively fresh feces are cultured the percentage of positive cases increases at once. Hegner and Becker (1922) found that the smear method revealed only one infection with *T. hominis* in 110 specimens from different persons; but four were discovered by the culture method. Similar results were obtained by Reichenow (1923). The examination of fresh fecal specimens by the smear method in tropical America (Hegner, 1925a) gave an incidence of infection with trichomonads of 20.6 per cent. This result was probably due in part to a high rate of infection among the persons examined but the fact that the stools were fresh was an important factor. Recently Hill (1926) reported a more extensive comparison of the smear and culture methods in Porto Rico under field conditions. Of 912 persons examined, the smear method revealed 16 infections with trichomonads and the culture method 84 and all of the 16 specimens found positive when smears were examined were also found positive when cultures were made. Hill also found that smears from fresh stools gave a higher incidence than those from older stools; thus, smears from feces that were 6 to 20 hours old gave 3 positive cases, or 0.5 per cent, from 612 persons; whereas smears from feces that were 2 to 6 hours old gave 13 positive cases, or 4.3 per cent, from 300 persons. The latter were all children

between the ages of 6 months and 6 years which may have had some influence on the results obtained. Paulson and Andrews (1927) also used fresh stools in their work and obtained an incidence of 4.3 per cent in 210 persons in Baltimore which is high when compared with the results reported by Boeck and Stiles. The ease of manipulation and uniformly excellent results obtained by various investigators indicate that the fear expressed by Lynch (1924b) that the culture method in inexpert hands "could only increase the existing confusion" is without foundation.

Viability of trophozoites. Trichomonas hominis is very resistant in the trophozoite stage in fecal material. Hegner and Becker (1922) kept an infected stool in a covered glass container and inoculated culture tubes at intervals for four days; positive cultures were obtained 79 hours but not 95 or 103 hours after the stool was passed. Smears were positive 37½ hours but not 47 hours or more after defecation. *Pentatrichomonas ardindelteili,* according to Kofoid and Swezy (1924b), will remain alive in liquid stools for 24 days. These flagellates, therefore, have considerable opportunity to reach the food or drink of man so long as the fecal material remains moist. That high temperatures do not destroy trichomonads in nature is evident from the experiments of Pringault (1920) and Andrews (1926a). Pringault found that *T. (intestinalis) hominis* dies in 2 hours and 30 minutes at 0° C., in 45 minutes at 50° C., and in 7 minutes at 65° C.; and Andrews showed the thermal death point of *Pentatrichomonas ardindelteili* and *Trichomonas*

hominis to be 49° C. These temperatures are higher than any the flagellates would ordinarily encounter in nature.

Favorable conditions for transmission. Although *Trichomonas hominis* reaches new hosts in the trophozoite stage the locomotor powers of the flagellates play no part in transmission; that is, the flagellates are passively conveyed to new hosts in various ways but not by their own activities. A moist climate is particularly favorable since fecal material does not dry as rapidly and rains may dilute the feces or wash the flagellates into ponds or streams that are sources of drinking water. It is probable that *T. hominis* will live longer in diluted than in raw feces as is true of the cysts of certain other intestinal protozoa (Boeck, 1921b). Insanitary conditions are also conducive to transmission. Flies and possibly other insects that visit both fecal material and human food and drink play a rôle of undetermined importance in the transfer of the flagellates. Wenyon and O'Connor (1917) found that living, motile trichomonads were present in the droppings of flies 5 minutes after being fed on infected feces, which is an interval sufficiently long to allow the flies to carry the organisms a considerable distance. The host, therefore, brings about his own infection without any effort on the part of the trichomonads and entirely because of insanitary habits. Cleanliness, the prevention of soil pollution, the screening of fecal material from flies and other insects, and the abolition of infected food handlers would do much to lower the incidence of infection.

(2) DISTRIBUTION AND LOCALIZATION WITHIN THE HOST. *The digestive tract.* It is evident that the tropho-

zoites of certain trichomonads, probably including the human intestinal species, are infective. They are carried through the anterior portion of the alimentary canal by the action of peristalsis into the large intestine. Here peristalsis is comparatively weak and the current down the intestine is so slow that colonization is possible. Very likely the organisms react to the current (rheotropism) and are able to escape being carried out of the body by swimming against it. Any condition, such as diarrhea or dysentery or even looseness of the bowels, that increases the speed of the current in the intestine tends to overcome the locomotor powers of the flagellates and to prevent the colonization of newly ingested specimens. The greater abundance of flagellates in fecal material when the bowels are loose, for example, after the administration of a purgative, is thus accounted for. On the other hand, anything that tends to bring about the production of formed stools and constipation are of advantage to the parasites since under these conditions they are allowed sufficient time to establish themselves in the intestine. The passage of the trichomonads, therefore, from the mouth to their definitive focus of infection in the large intestine is due entirely to the activities of the digestive tract of the host. Whether or not an infection is established in the large intestine is also largely due no doubt to the consistency of the intestinal contents of the host. The share the flagellates play in their own distribution and localization within the host is thus very slight indeed. That the trichomonads of the cat may migrate from the rectum into the small intestine has been suggested by Brumpt (1925). Migration also may occur from the

cecum into the ileum of rats and other animals. When trichomonads are fed to experimental animals movement of the trophozoites appears to be more or less inhibited in the anterior part of the small intestine but gradually regained as the organisms approach the cecum (Hegner, 1924a). The factors that cause cessation of movement in the duodenum and jejunum are, of course, sufficient to render these habitats untenable for the flagellates.

The blood stream. Many reports have been published of the presence of intestinal flagellates in the blood of various animals. Among these are listed trichomonads. For example, Lanfranchi (1908) found trichomonas in the blood of a pigeon, Plimmer (1912) in the blood of snakes, Sangiorgi (1922) in the blood of a mouse, and Pentimalli (1923) in the blood of man. Kessel (1925a) found *T. hominis* in the pus of an amœbic liver abscess and believes that it reached this location by way of the blood stream from the intestine, but had no evidence on this point except the presence of the organism in the liver at a distance from its normal habitat. In most of these cases the trichomonads were found at autopsy and there was some danger of contamination, as well as opportunity for the flagellates to enter the blood stream in some way after death. It does not seem safe at present, therefore, to state definitely that *Trichomonas* ever lives in the blood stream of man or any other animal while alive.

Factors within the intestine. What conditions within the large intestine of man are favorable for the growth and multiplication of *Trichomonas hominis?* Tempera-

ture is a factor that can be disposed of at once since it does not change much even in patients suffering from diseases accompanied by fever, and culture experiments have shown that the organisms are able to live and reproduce within a wide temperature range. The normal temperature of man is evidently favorable. The digestive juices also probably play a minor rôle. The degree of moisture is no doubt a more important factor since the density of the intestinal contents must affect the activities of the flagellates.

Diet. Perhaps the character of the food of the host has the greatest influence on the flagellates since diet determines to a considerable extent the character of the bacteria and the products of bacterial decomposition within the large intestine. There seems to be a definite relation between the presence or absence of intestinal protozoa and the character of the diet, whether herbivorous or carnivorous. A survey of the literature available (Hegner, 1924b) indicates that intestinal protozoa are rare in strictly carnivorous mammals, less rare in omnivorous mammals, but common in herbivorous species. Feeding experiments with rats (Hegner, 1923a) show a carnivorous diet to be unfavorable for intestinal flagellates. When laboratory rats infected with trichomonads were fed for one week on a carnivorous diet that was favorable for the growth and reproduction of the rats, the number of trichomonads decreased to less than one-fiftieth of the number present in control rats. Changes in the hydrogen-ion concentration of the intestinal contents do not account for this loss (Hegner and Andrews, 1925), but the conclusion was reached that the

almost complete reversal from acidophilus to putrefactive bacteria within the intestine due to the carnivorous diet effected changes in the contents unfavorable for the flagellates. Tsuchiya (1925), however, has found large numbers of trichomonads in certain human cases irrespective of whether the food was largely of a carbohydrate or protein nature and his conclusion is that "The type of intestinal flora does not alter the number of flagellates." Herbivorous and omnivorous animals no doubt more frequently ingest food contaminated by the feces of others of their kind than do carnivorous species; but the latter would probably become infected if flagellates were capable of living in their intestines. Conditions within the large intestine of carnivores must be particularly unfavorable to bring about a resistance so extreme as to prevent flagellates from obtaining a foot hold in this habitat during the course of evolution.

(3) PATHOGENICITY. Many protozoölogists believe that trichomonads are merely commensals or "food robbers" that have found the large intestine of man a favorable place in which to live. In this habitat they feed on food particles taken in by the host, or on bacteria, and absorb the products of digestion through their body wall. Growth and reproduction occur until enormous numbers of organisms are present, some of which are carried out of the body in the feces and keep the race from dying out by infecting new hosts. On the other hand, a considerable body of evidence has been accumulated, principally by physicians, that these flagellates actually attack the host and bring about what is known as trichomoniasis or flagellate diarrhea. Many cases of

intestinal disturbances have been reported in which no causative organisms could be discovered except trichomonads. Most of the individuals, however, who are infected with intestinal trichomonads never exhibit symptoms of any kind. A study was made by Tsuchiya (1925) of 20 persons in whom *T. hominis* was abundant and 10 persons who had a mild infection. Examinations of the feces, urine and blood and studies of the gastro-intestinal, nervous, nutritional and circulatory symptoms led him to the conclusion that this species is not pathogenic. We cannot conclude from this, however, that the flagellates are not pathogenic in some instances since their hosts may be carriers such as we are familiar with in the case of certain pathogenic bacteria and other protozoa.

Pentatrichomonas. Another argument in favor of the pathogenicity of trichomonads is the frequent association of pentatrichomonads with diarrheic conditions and the habit of these five-flagellated organisms of ingesting red blood cells. Derrieu and Raynaud (1914) first reported this type in dysentery cases in Algiers; Chatterjee (1915, 1917) found it in 30 cases of chronic dysentery in Bengal; Wenyon and O'Connor (1917) cite one case in the Near East; Haughwout and de Leon (1919) found it in a case of acute dysentery in Manila; and Kofoid and Swezy (1923) describe it from three patients with histories of chronic diarrhea, all of whom had lived in the tropics or had come into close contact with persons who had. Up to this time this five-flagellated type had been found only in hosts who had diarrhea or dysentery. The presence of an organism under conditions of pathogenicity and the ingestion of red blood cells by it, is not

enough to convict it of being pathogenic. The diarrheic or pathogenic condition may have rendered the intestinal contents particularly favorable for the growth and reproduction of the flagellate and the presence of the red-blood cells in the intestinal contents may have offered the flagellates an opportunity to take them in along with other kinds of food particles and does not prove that the flagellates actually attack the host tissues. Furthermore, cases of infection with *Penatrichomonas* that have never exhibited evidence of diarrhea or dysentery have been reported (Hegner, 1925e). Kessel (1925a) has reported the ingestion of red blood cells by the four-flagellated trichomonad, *Trichomonas hominis,* found in the stool of a patient suffering from bacillary dysentery and in *Trichomonas hominis* (*vaginalis?*) from the urine of another patient passed at the time of menstruation; Reichenow (1925a) finds that in culture both the four-flagellated and five-flagellated types will ingest red blood cells; and Wenyon (1926) states that he has observed them in specimens passed by a patient suffering from bacillary dysentery and in cultures containing rabbit blood. It thus seems probable that all types of trichomonads may under certain conditions take in erythrocytes.

The relationship between the pentatrichomonads of rat and man is a problem of considerable interest. Wenrich and Yanoff (1927) go so far as to state that the organisms from these two species of hosts belong to the same species, and that the rat serves as a reservoir for the parasite of man. These authors also find that infection in the rat with pentatrichomonads "is usually accompanied by a great many leucocytes in the

cæcal contents, and this leucocytosis suggests pathological conditions which may be caused by this flagellate."

There has been considerable discussion regarding the tissue-invading powers of trichomonads (Haughwout, 1918; Hadley, 1917), but very little actual data are available. Wenyon (1920) made a histological study of the intestinal wall of 5 patients who had died of pneumonia and were infected with trichomonas. "There were no noticeable lesions of the intestine which one could attribute to the flagellates." Not only were the flagellates distributed over the surface of the mucosa but were also found in the lumen of the glands of Lieberkühn, in some cases in large numbers. Definite ruptures of the glandular epithelium were noted in one case and the trichomonads "were evidently passing through these." "The flagellates were scattered about in the interglandular loose connective tissue, so that there was a definite invasion of the tissues of the gut." "There never appeared to be an extension of the invasion beyond the mucous layer. Furthermore, there did not seem to be any reaction on the part of the tissue as regards cell proliferation or invasion." If we consider an organism pathogenic that injures the tissues of its host, then *Trichomonas hominis* may in certain cases be pathogenic; but we do not know how often it invades the tissues nor whether this invasion is sufficient to bring about the condition known as flagellate diarrhea.

(4) HOST-PARASITE SPECIFICITY. Many of the lower animals, such as rats, mice, dogs, cats, and guinea-pigs, are infected with trichomonads apparently belonging to species that differ from those in man. Thus far attempts

to infect lower animals with trichomonads from man have not succeeded beyond a reasonable doubt. Escomel (1913) claims to have infected the dog, cat, rabbit and guinea-pig, Lynch (1915a) the rabbit, Boyd (1919) the rat, Kessel (1924) the monkey, and Escomel (1925) the frog. In every one of these cases there is the possibility that the infection obtained was already present before the experimental animal was inoculated. Kessel (1923) found trichomonads in a rat to which he had fed cysts of *Endamœba histolytica* and recovered later motile flagellates "smaller than *Tritrichomonas* of rat, apparently *Trichomonas hominis*," but this case is also very doubtful. Pringault (1920) was unable to infect the cat, rabbit, guinea-pig and rat; Hogue (1922) likewise failed to infect cats and rabbits that were free from trichomonads. Kessel (1926b) on the other hand was successful in infecting 10 of 14 kittens with *T. hominis* from man either per os or per rectum; and the diarrheic symptoms that developed in the positive animals were accepted by him as due to the presence of the trichomonads. The host-parasite specificity of the human intestinal *Trichomonas* seems, however, quite rigid.

4. GIARDIA LAMBLIA

(1) Epidemiology of transmission. *Infection by trophozoites. Giardia lamblia* (Figs. 13a, 13b) enters the human digestive tract by way of the mouth either in the trophozoite or cyst stage. Although infections are probably usually brought about by the ingestion of cysts, recent experiments (Hegner, 1926c) indicate that trophozoites may also be infective. Active motile speci-

mens of *Giardia canis* from dogs were injected into the stomach of guinea-pigs; they became distributed throughout the small intestine within one or two hours, remaining active during this period. Of particular interest is the fact that they appeared to congregate in the duodenum which is their optimum habitat. These experiments suggest that trophozoites may be infective. They may be ingested by the host with contaminated food or drink and carried through the digestive tract in the intestinal contents. It seems probable that conditions in the duodenum stimulate them to attach themselves to the epithelial cells of the intestinal wall by means of their sucking discs. Those that do not succeed in doing this are carried down and out of the intestine. Entrance to the body and distribution within the body are thus primarily due to the host, but the establishment of the flagellates in the primary site of infection probably depends on the reactions of the parasite.

Infection by cysts. Very little is known regarding the length of life of the trophozoites of giardias while outside the body, but it is apparently very short and hence active specimens are probably very seldom ingested in a living condition. The cysts, therefore, are more important in bringing about infections. Cysts of *Giardia lamblia* are of common occurrence. The incidence of infection among human beings, and consequently the number of hosts passing cysts, differs in various parts of the world, according to the results of various surveys. Data from 35 surveys published during the years 1916-1919 gave an average incidence of about 12 per cent in 20,000 persons (Hegner and Payne, 1921). Boeck and Stiles

(1923) record 6.5 per cent of 8,029 persons infected. It is rather strange to find less infection in the tropics, where conditions for the spread of the parasites seem particularly favorable, than in the temperate zone. Thus Jepps (1923) records giardia in only 4.2 per cent of 1024 persons in the Federated Malay States and Hegner (1925a) in only 2.1 per cent of 286 persons in tropical America.

Viability of cysts outside the body. The cysts of giardia are resistant to various factors encountered outside of the body of the host. Experiments have been based on the assumption that cysts that become stained in a weak solution of eosin are dead and that those that do not become stained are alive. No one has determined whether these unstained "living" cysts are capable of infecting a new host. Boeck (1921a) found that giardia cysts are killed at a temperature of 64° C., which is a temperature higher than any they normally are subjected to in nature. In raw feces, the cysts seldom remain alive more than 10 days, but washed cysts kept at room temperature were still alive over two months after they were passed. Thus plenty of time is allowed for distribution before death occurs. Presumably, however, cysts are incapable of withstanding drying; hence the length of life noted above is dependent upon the presence of moisture.

Flies as transmitting agents. Giardia cysts resist conditions in the digestive tract of flies for considerable periods. Stiles and Keister (1913) first proved that flies may ingest fecal material containing cysts. Wenyon and O'Connor (1917) recovered living cysts from flies 24 hours after such a meal; also from the droppings of flies

40 minutes after feeding; and from the droppings of one out of 229 wild flies. Root (1921) confirmed certain of these results. He found giardia cysts still viable after 16 hours in the intestine of *Musca domestica* and after 4 days in drowned flies.

(2) LOCALIZATION WITHIN THE HOST. *Excystation.* What happens to the cysts after they enter the human digestive tract is unknown. No one has determined where or in what manner excystation occurs in man. Hegner (1925b) described some peculiar specimens that seemed to be excysting, passed by a patient who had been under observation for several years and, who, both before and after this occasion, passed cysts normal in every way. The trophozoites that seemed to be emerging from these cysts were much smaller than normal trophozoites and their nuclei were larger than those in normal cysts and contained chromatin scattered throughout the nuclear substance. Cysts swallowed by man probably excyst in the duodenum, and this process must be extremely rapid or the cysts would be carried through the duodenum and thus out of their normal habitat. It is of course possible that the newly escaped trophozoites may be able to progress against the action of peristalsis and colonize the duodenum from the ileum or jejunum but this hardly seems possible. The writer (Hegner 1927a) has recently obtained excystation of the cysts of *Giardia lamblia* in the intestine of rats and guinea-pigs. Washed cysts in water were injected through the esophagus into the stomach by means of a syringe and small rubber tube. No excystation was ever found in the stomach but in a number of animals it occurred in the small intestine, usually

from 30 to 60 c.m. posterior to the stomach, and within about 40 minutes after the cysts were injected into the stomach. The process was studied in the living organism and also on prepared slides. Activity within the cyst is stimulated by unknown factors and the organism can be seen moving about apparently by means of its axostyles. The cyst wall at the posterior end seems to become weakened and the posterior end of the animal, which has assumed the approximate shape of the trophozoite, breaks through. Cysts were observed with only the posterior flagella extruded; these were active and moved the entire cyst about in the medium. Then the organism gradually squeezes through the opening at the posterior end. Division of the organelles occurs before the trophozoite emerges but actual division takes place after it becomes free. In all those observed the four nuclei remained at the anterior end and did not separate into two pairs, one pair at either end, as stated by Wenyon (1926). Cell division proceeded from the anterior end posteriorly, the organism, during the process, moving about by means of the posterior flagella and the other flagella that appeared to be forming. The excysted trophozoites after division measured from 8.5 μ to 10.5 μ in length (average about 9. 5 μ) and from 4.5 μ to 7 μ in breadth (average about 5.5 μ).

Wenyon (1926) suggests that cysts may excyst in the same host in which they are formed; this would account for the increase in the number of specimens present in the host, since division during the trophozoite stage seems to be rare, but does not appear probable to the writer.

Localization in the duodenum. What factors are responsible for the localization of giardias in the duodenum is a question still unsolved. All of the other intestinal flagellates proper of man live in the large intestine, except when a few specimens under extraordinary conditions succeed in migrating forward into the ileum. It is interesting here to refer again to the apparent aggregation of the trophozoites of *G. canis* in the duodenum of the guinea-pig into the stomach of which they were inoculated (see p. 155). The sucking disc no doubt is an important organelle of attachment and some such means of maintaining the organisms against the downward force of peristalsis is certainly necessary before the duodenum can be used as a habitat. The lumen of the glands are also of assistance, and, that this haven of refuge is taken advantage of by giardias, is shown by their presence there in sections of infected duodenum. Perhaps these flagellates react strongly to currents and are capable of moving up the intestine until stopped by the pyloric sphincter. Giardias have no visible method of ingesting solid food particles and no such material has been observed in them; they must therefore absorb nutriment through the general surface of the body. What substances are necessary for this purpose is unknown, and up to this time all efforts to duplicate conditions in the duodenum so as to cultivate the organisms outside of the body have resulted in failure.

(3) PATHOGENICITY. *Giardiasis.* The terms lambliasis, giardiasis and flagellate diarrhea all refer to a pathological condition supposed to be due to the presence of giardias. Most of the infected hosts do not exhibit any symp-

toms; they are carriers. A few individuals, however, are afflicted with a diarrheic condition that is very persistent; the presence of large numbers of giardias in their stools and the absence of any other organisms that are known to be the etiological agents of diarrhea indicate but do not prove that the giardias are responsible. Diarrhea is known to result from the irritation of the epithelial cells of the intestinal wall (see p. 96) and no doubt such irritation is brought about when vast numbers of giardias are moving about in the duodenum and attaching themselves by means of their sucking discs to the surface of the cells. When few in number the symptoms produced in this mechanical way would be so slight as to pass unnoticed but millions of specimens present at one time might aggravate conditions and bring about diarrhea. Giardias in large numbers might also be injurious to the host as a result of the excretion of waste products or possibly some specific toxic substance, and might even hinder digestion and absorption because of interference with the glands and epithelial cells as Haughwout (1918) has suggested.

Cholecystitis. What appear to be secondary sites of infection for *Giardia lamblia* are the bile ducts and gall bladder. Many reports have appeared in the literature during recent years of infections in these organs, but these reports are confusing and it is difficult at present to arrive at definite conclusions. Boyd (1921) and Silverman (1923) reported cases in which the microscopic examination of the liquid obtained by duodenal tubage revealed numerous trophozoites of giardia; this was soon followed by a description of three similar cases by Libert

and Lavier (1923). In the same year Westphal and Georgi (1923) also found giardias in the duodenal fluid of a patient suffering from cholecystitic symptoms. On removing the gall bladder, large numbers of giardias were found in this organ. Less convincing evidence is that of Felsenreich and Satke (1923) who maintain that various disturbances of the liver and gall bladder are due to giardias that occurred in large numbers in duodenal juice. Similar evidence is offered by Labbé, Nepveux and Gavrila (1925) and Pappalardo (1925) to explain symptoms of what they designate "vesicular lambliasis." On the other hand, Chiray and Lebon (1925) report a case of cholecystitis in which giardias were abundant in the duodenal juice but absent from the excised gall bladder, and Gaivoronsky (1925) described two similar cases, one of cholecystitis and the other of gall-stone. The data available indicate that giardias may migrate through the bile ducts and into the gall bladder but do not prove that they are responsible for the disturbances associated with their presence.

(4) HOST-PARASITE SPECIFICITY. *Historical.* The giardia of man, as Dobell (1920) has shown, was first described by Leeuwenhoek in 1681. It was not again noted in the literature until Lambl (1859) found it in the feces of children. From that time on cases of human infection were frequently reported. That these flagellates also occurred in mice, rats and cats was reported by Grassi (1879, 1881). Twenty years later, Metzner (1901) gave a good description of giardias from the rabbit. During all this time only one species was recognized in man and lower animals and cross-infection between

these different host species was taken for granted. Bensen, in 1908, however, distinguished three species which we know now as *G. lamblia,* from man, *G. muris,* from rats and mice, and *G. duodenalis* from the rabbit. Practically all of those who accepted Bensen's work still believed that cross-infection occurred. Many investigators have carried on cross-infection experiments, but circumstances in many cases rendered the results obtained of little value principally because of the difficulty of obtaining clean experimental animals. Even as recently as 1923 (Galli-Valerio) the possibility of human infection with giardias from rats and mice has been urged.

Species of giardias in lower animals. Careful morphological studies carried out especially during the past decade indicate that the giardias of lower animals differ from those in man specifically and that each species of animal is infected with its own peculiar species of giardia. Cross-infection experiments when properly controlled indicate that this specificity is very rigid; hence the members of this genus furnish very favorable material for studies of host-parasite specificity. Up to the present time the following giardias have been described, chiefly on the basis of morphology and host association.

Mammals

Giardia lamblia (Lambl, 1859) Stiles, 1914	Man
Giardia duodenalis Devaine, 1875	Rabbit
Giardia muris Grassi, 1879	Rats & Mice
Giardia microti Kofoid and Christiansen, 1915	Field mice

Giardia canis Hegner, 1922	Dog
Giardia caviæ Hegner, 1923	Guinea-pig
Giardia viscaciæ Lavier, 1923	Viscacha
Giardia simoni Lavier, 1924	Wild rats
Giardia capræ Nieschulz, 1923	Goat
Giardia equi Fantham, 1921	Horse
Giardia bovis Fantham, 1921	Cattle
Giardia sp. Hegner	Wild cat
Giardia sp. Deschiens, 1925	Lion
Giardia cati Deschiens, 1925	Cat
Giardia sp. Hegner, 1924	*Ateleus geoffroyi* Kuhl
Giardia sp.	Sheep
Giardia sp. Nieschulz, 1923	Calf
Giardia suricatæ Fantham, 1923	Meercat
Giardia beckeri Hegner, 1926	Ground Squirrel

Birds

Giardia sanguinis Gonder, 1911	*Elanus cœruleus* (blood)
Giardia ardeæ Nöller, 1920	Herons
Giardia sp. Kotlán, 1922	*Lanius cœllurio*
Giardia sp. Kotlán, 1922	*Recurvirostra avocetta*
Giardia sp. Hegner, 1925.	Black-crowned night heron
Giardia sp. Hegner, 1925	Great blue heron

Reptiles	
Giardia varani Lavier, 1923	*Varanus niloticus*
Amphibia	
Giardia agilis Kunstler, 1882	Tadpole
Giardia xenopodis Fantham, 1923	Clawed frog
Fish	
Giardia denticis Fantham, 1919	Fish (blood)
Nematode	
Giardia sp. Thomson, 1925	*Viannella sp.*

Criteria of species. Some of the giardias from the different host species differ so greatly in morphology that there can be little question regarding their specific rank. The differences between others are slight but constant. Several species listed, such as *G. equi* and *G. bovis,* have not been described in detail and careful study is necessary in order to establish them as distinct. Further study is also required to determine the position of the specimens reported from the wild cat, lion, sheep, birds and nematode. One species is known to live about equally well in two hosts, namely, *G. muris,* which occurs in both rats and mice. Two of the species, *G. muris* and *G. simoni,* live in a single host, *Epimys norvegicus. G. simoni* is morphologically identical with *G. lamblia* but is considered a distinct species by Lavier because it is capable of infecting rats, whereas *G. lamblia* is not. The characteristics that have been used by certain investigators in determining the specific rank of the trophozoites of the different species of giardias are length, breadth, ratio

of length to breadth, distance from the anterior end of the body to the center of the nucleus, from the center of the nucleus to the lateral shields, from the end of the lateral shields to the posterior end of the body; distance across the body at the center of the nuclei and at the ends of the lateral shields; the contour of the body, whether narrow or broad at the anterior end and across the lateral shields; distance of nuclei from the median line and from the posterior edge of the sucking disc; size and shape of the nuclei; number, size, shape and location of the parabasal bodies; staining characteristics; and infectivity when cysts are fed to experimental animals.

Cross infection. The results of cross-infection experiments are briefly as follows: Grassi (1882) was unable to infect himself by swallowing cysts from lower animals. Calandroncio, however, according to Piccardi (1895) was more successful, since he found motile specimens in his stools 25 days after ingesting cysts. Moritz and Hölzl (1892), on the contrary, did not succeed in infecting a human being with cysts from mice. Results just as contradictory have resulted from attempts to infect lower animals with giardias from man. Perroncito (1888) claims to have infected 2 white mice and Stiles (1902) a guinea-pig with human cysts. Only negative results were obtained by Bohne and Prowazek (1908) when trophozoites and cysts from man were injected both per os and per rectum into rabbits, rats and kittens.

The experiments of Fantham and Porter (1916) seem remarkably successful. Eight kittens and nine mice previously found to be free from giardias were fed washed

cysts from man. Six of the eight kittens suffered from diarrhea and died in from one to eight weeks, and 6 of the 9 mice became infected, but their symptoms were not so severe. Surprising results were later obtained by Porter (1919), who induced diarrhea in rats by feeding them on a supply of drinking water containing cysts; and infected rats on cysts that had passed through the digestive tract of flies and cockroaches. Deschiens (1921) was also surprisingly successful in his cross-infection experiments. Four cats, 2 of which were fed cysts of *G. muris* and the other 2 cysts of *G. lamblia,* became infected, developed dysentery and three of them died. Infection followed by symptoms was induced in 2 cats by the intrarectal injection of trophozoites of *G. muris.* Four of 5 mice fed on cysts of *G. lamblia* became infected and died; whereas 5 mice that were already infected with *G. muris* suffered no ill effects from similar feedings. Cysts of *G. muris* from a cat infected with this species brought about a fatal infection in 4 of 5 clean mice, whereas 5 mice already infected with *G. muris* suffered no ill results. These results have not yet been confirmed. Simon (1922) on the contrary was unable to infect rats with human giardia cysts. Five laboratory rats and 5 wild rats were fed washed cysts but no diarrhea resulted and no giardias were found at autopsy. He succeeded, however, in infecting rats by feeding them the entrails of mice containing *G. muris.* Negative results were also reported by Deschiens (1925b) when he attempted to infect 2 kittens per os with cysts from a lion. Wenyon (1926) likewise failed to infect 4 kittens with giardia cysts from man.

According to Thomson (1926) Howitt has succeeded in infecting dogs with giardias from human feces.

Most of the experimental work on cross infection has been done with the idea of determining merely whether or not infection is possible. A more interesting and biologically more important question, however, is what factors favor or hinder infection? In this direction lies the solution of the real problem of cross infection. After the writer (Hegner, 1927a) found that cysts of *G. lamblia* excyst in the rat (see p. 157), attempts were made to bring about infection. Ten rats were fed washed cysts of *G. lamblia* from man on 11 of 12 successive days, and killed at intervals during the succeeding 19 days. Temporary infections were apparently established in 4 rats; these infections were highest on the 6th and 7th days, decreased by the 12th and 14th days, and disappeared by the 16th and 19th days. The trophozoites recovered from these animals were smaller than those of *G. lamblia:* this may have been due to their unusual habitat. The distribution of the giardias differed from that of *G. muris;* the latter are most abundant in the duodenum, whereas the trophozoites of *G. lamblia* were absent from the duodenum and most numerous in the small intestine from 40 cm. to 90 cm. posterior to the stomach. No cysts were passed by the experimentally infected rats, which indicates that the rat is not an important transmitting agent of this species.

The difficulties involved in cross-infection experiments are obvious and the results of such investigations will be unsatisfactory until some method is found of securing experimental animals that are absolutely free from in-

fection. It is unnecessary here to point out the possibilities of error in the work described. The conclusion seems warranted that *Giardia lamblia* has not been proved capable of living in any other animal; and that it is rigidly limited to one host, man. How many of the species of *Giardia* listed above are "good" species remains to be determined by more careful study and experimental infections.

Relation of age and susceptibility. It is generally recognized that the young of both man and the lower animals are more susceptible to infection and more frequently infected with protozoa than are adults. Thus it has become the custom to select young animals for laboratory experiments. In the case of *Giardia lamblia* considerable information exists on this subject. Dobell (1921b) has collected the following data:

Investigator	Place	Per cent *G. lamblia*	
		Adults	Children
Matthews and Smith	Liverpool	7.0	14.1
Campbell	Bristol	3.9	16.3
Miss Nutt	Leeds	3.8	39.8
Miss Nutt	Sheffield	7.2	15.8
McLean	Reading	9.3	17.4

Similar results have been obtained by Maxcy (1921) in the United States and by Simon (1924) in Germany. Maxcy found 17 per cent of infection in children one to 5 years of age, but almost 40 per cent in children from 6 to 12 years old. Simon (1924) found 23.4 per cent of infection in a group of 137 persons; 77.4 per cent of those who were under 15 years of age gave an incidence of 27.4 per cent. His youngest case was that of a child 9 months

old; Miss Nutt records infections in children 3 weeks, 3 months, 9 months, 11 months and 12 months old. Dobell suggests that the higher incidence among children may be due to the greater ease of finding the organisms in their stools. It seems more probable that some type of host resistance develops with age. The cysts may be unable to excyst or the escaping trophozoites may find the intestinal contents of adults less favorable than those of children for their growth and reproduction.

5. OTHER INTESTINAL FLAGELLATES

(1). CHILOMASTIX MESNILI

Not so much is known regarding the host-parasite relations of *Chilomastix mesnili, Embadomonas intestinalis* and *Tricercomonas intestinalis* as of the species already described. *Chilomastix mesnili* (Fig. 10a, 10b) is a common inhabitant of man and has been reported from many parts of the world. The average incidence of infection as indicated by the results of various surveys is about 10 per cent. Transmission takes place no doubt usually in the cyst stage and infections result from the ingestion of contaminated food or drink. That flies may play a rôle in transmission was shown by Root (1921) who found that trophozoites might pass through the digestive tract of *Musca domestica* within 7 minutes apparently unharmed, and that cysts could live in the intestine of this species for 80 hours. Washed cysts, according to Boeck (1921b) may live for six months or more in water at room temperature and can withstand temperatures up to 72° C.

The primary site of infection is the large intestine; the small intestine may also be inhabited, and Pons (1925b) has reported specimens from the vagina, but this was probably due to contamination from the intestine which was infected in this case. Much of what has been stated above regarding the cysts of other protozoan species and of flagellate diarrhea applies likewise to *C. mesnili;* on this account and because of the meager condition of our knowledge regarding this species it would be fruitless to discuss these subjects further.

Chilomastix has been reported from various species of lower animals but so far as we know these are all specifically distinct from that living in man. Kessel (1924) seems to have inoculated monkeys with *C. mesnili* from man, but there is always a possibility when monkeys are used for experimental purposes that they may already have had an infection since *Chilomastix* has been recorded in monkeys by both Bach (1923) and Hegner (1924d).

(2). EMBADOMONAS INTESTINALIS

This is apparently a rare species in man (Fig. 11a, 11b), but has been found in widely separated regions and hence probably occurs in all parts of the world. Nothing is known regarding its relations to man except that both trophozoites and cysts pass out of infected hosts in the feces, and that infections may last for at least six weeks (Wenyon and O'Connor, 1917). *Embadomonas* has been reported from a number of lower animals but the specific rank of these forms is still in doubt. A second species from man was described by Faust and

Wassell (1921) as *E. sinensis,* but it seems probable that the specimens observed belonged to the species already known.

(3). TRICERCOMONAS INTESTINALIS

This flagellate (Fig. 12a, 12b) has been reported in less than 100 cases. It appears in the feces in both the trophozoite and cyst stages. Nothing is known regarding its relations to the human host. Much confusion exists regarding this species and *Enteromonas hominis* (Fig. 14) described by Fonseca (1915); the reader is referred to Wenyon (1926) for a discussion of this subject.

CHAPTER IV

INTESTINAL INFUSORIA

I. *Balantidium coli*

1. MORPHOLOGY

Balantidium coli (Fig. 18), which lives in the large intestine, is the only ciliate known with certainty to be an inhabitant of man. It is very large compared with other human protozoa, ranging from 30μ to 200μ or more in length and from 20μ to 70μ in breadth. The usual range in size is 50μ to 70μ long by 40μ to 60μ wide. This extraordinary range in size may be due in part to heritably diverse races as regards size but is more probably the result of the presence of specimens in various stages of growth. The organism seems very little modified by its parasitic habit being very similar in structure to *Paramœcium caudatum*. It is in general oval in shape, but broader at the posterior end and more pointed at the anterior end. Cilia emerge from minute basal granules beneath the cuticle; cover the entire surface; and are arranged in parallel rows. Near the anterior end is a funnel-shaped cytostome, which can be expanded and contracted and into which food particles are driven by the surrounding cilia which are longer than on the rest of the body. An excretory pore, the cytopyge, is located near the posterior end. The cytoplasm is separated into

a thin, clear peripheral layer of ectoplasm and a central granular mass of endoplasm. Within the cytoplasm are a kidney-shaped macronucleus, in the concave side of which lies a minute spherical micronucleus, and two contractile vacuoles situated as indicated in the figure.

2. LIFE-CYCLE

The life-cycle of *B. coli* is not fully known. Asexual reproduction is by transverse fission involving the mass division of the macronucleus into two apparently equal parts and a sort of mitotic division of the micronucleus. Rapid division sometimes results in the formation of "nests" of small specimens in the tissues of the intestinal wall. Sporulation (Walker, 1909) and budding (Ohi, 1924) have been described but have not been satisfactorily confirmed.

Encystment may occur in the intestine. The cyst is provided with a double wall secreted by the organism. Conjugation cysts have been described by Brumpt (1913). Small specimens only 30μ in length, that result from rapid division, unite in pairs, secrete a cyst wall about themselves, throw out part of their substance, and then fuse into one. The further history of these cysts is unknown.

3. HOST-PARASITE RELATIONS

Viability. The trophozoites of *B. coli,* according to McDonald (1922), live for only a few hours outside of the host. He found that cooling the intestinal contents of the pig to room temperature causes the organisms to become spherical, in which condition they will live for 6 or 8 hours; when kept in an incubator at 37.5° C. they

will live for three days. Rees (1927), however, while working in the writer's laboratory, discovered that these ciliates do not round up at room temperature if kept under anærobic conditions; that they will continue to live at room temperature for as long as 10 days; that washings from trucks in which pigs were hauled contained trophozoites that lived at room temperature for 14 hours; and that active specimens could be recovered from solid feces of the pig that had been passed at least 4 hours. There is thus abundant opportunity for the living trophozoites to reach the digestive tract of man after they are passed by the pig. This is particularly true when a person works with pigs as in the case of a butcher (Cordes, 1921). Cysts are more resistant. Ohi (1924) states that when kept moist at room temperature they will live for 2 months; when dried in the shade, 1 to 2 weeks; and when exposed to direct sunlight, 3 hours. They withstand bile 15 days; urine, 10 days; gastric juice, 12 hours; 5 per cent carbolic acid, 3 hours; 1 per cent carbolic acid, 4 hours; and 10 per cent formalin, 4 hours (Ohi, 1924).

Distribution and localization within the host. B. Coli must reach its habitat in the large intestine by way of the mouth, stomach and small intestine. It may be ingested either in the trophozoite or cyst stages and is no doubt passively carried through the digestive tract by peristalsis and other movements of the host. Primary distribution and localization within the host is thus brought about by the host without any effort on the part of the ciliate. Where excystation takes place is not known. It is easy to understand why *B. coli* becomes localized at first in the large intestine since it has no special means of

maintaining itself in the small intestine against the action of peristalsis. In the large intestine, movement of the contents is so slow that the organism, which probably reacts positively to currents, is able to progress sufficiently against these to escape being carried out of the body in the feces. Many specimens do not succeed, hence trophozoites are frequent in the feces, especially when the stools are loose. Evidently movement of the intestinal contents is so rapid when the stools are loose that many of the ciliates are unable to swim against it and are thus extruded. The location of *B. coli* within the large intestine may have an influence on successful colonization. If the organisms reside in the mucus of the walls they are not in as much danger of being carried down as they are if they live within the mass of fecal material.

Under certain conditions *B. coli* may live in the ileum of man; for example, Reis (1923) records 4 cases in which the ciliates occurred both in the large intestine and in the ileum.

Passive resistance of the host. The conditions encountered by the balantidia within the digestive tract, which constitute the passive resistance of the host, are the same as those met by other protozoa that are ingested by man (see p. 30). The walls of the cysts no doubt protect the organisms during their passage to the large intestine. It is generally supposed that trophozoites are not infective. Thus Fantham, Stephens and Theobald (1916) state that "As they are killed by acids even when much diluted, they cannot pass through the normal stomach alive except under the most unusual circumstances." Experiments with *B. coli* from the pig, however, indicate that tro-

phozoites may also withstand the conditions in mouth, stomach, and small intestine (Hegner, 1926c). Active trophozoites from the pig were injected into the stomach of guinea-pigs. In one guinea-pig killed one hour after such an injection balantidia normal in appearance and actively swimming were found in all parts of the stomach and small intestine and in the cecum. In another guinea-pig killed four days after an injection, no balantidia were found in the stomach and small intestine but a few were present in the cecum. These resembled the specimens injected but may have been inhabitants of the cecum before the experiment was begun. Apparently the trophozoites may pass through the stomach and small intestine unharmed and set up an infection in the cecum but this has not been definitely proved. Similar results have been obtained by Rees (1927).

Excystation. Passive resistance includes all conditions that are unfavorable to excystation. In order to set up an infection encysted specimens must excyst or they are carried directly out of the body. When and how excystation occurs is not known. This process might be prevented if the cysts are carried through the intestine too quickly, for example under more or less diarrheic conditions; hence a certain degree of stasis is probably necessary. Other factors that may play a rôle in excystation are temperature, the degree of moisture and the character of the digestive fluids.

The intestinal environment. Another type of passive host resistance is the character of the digestive contents, especially as regards their effect upon the physiological processes of the parasite and upon its food supply. Tro-

phozoites that succeed in reaching the large intestine or in escaping from the cyst walls may find the intestinal environment lacking in certain elements essential for their metabolic processes, or find these elements in unsuitable quantities, or encounter harmful substances. These factors might not produce death immediately but might hinder or prevent growth and reproduction. Apparently *B. coli* does not exercise any selection as regards food (de Leon, 1919), but no doubt certain types of food particles are more satisfactory than others and thus the diet of the host becomes of importance in the successful colonization of the parasite. Even after a successful infection is established changes in the diet may affect the intestinal environment so adversely as to destroy the parasites (Greene and Scully, 1923). Our knowledge of free-living ciliates leads to the conclusion that optimum conditions are not necessary for the growth and reproduction of *B. coli,* but too wide a departure from these conditions would obviously result in the prevention of both these processes and constitute passive host resistance too great for the existence of the parasite.

Attack on the host. Most of the earlier cases of human infection with balantidia involved intestinal symptoms that were evidently due to the presence of the protozoa (Strong, 1904). The examination of the feces of healthy persons later revealed the fact that infection with *B. coli* does not necessarily result in symptoms. Apparently this organism is able to live and reproduce within the intestinal lumen without access to the tissues of its host, and may therefore be non-pathogenic. In this environment it probably feeds on digested or undigested food taken

in by the host and may be termed a commensal or food robber. The host does not suffer because of the small amount of food taken by the parasite. In some cases, however, the ciliates attack the intestinal wall and thus become pathogenic. This attack may be slight, in which case the host makes repairs as rapidly as the tissues are injured, or may be severe, in which case symptoms of balantidiosis ensue. Evidence that all individuals infected with *B. coli* exhibit symptoms is furnished by Aguilar (1926) who observed 40 cases at the Quirigua hospital in Guatemala during the period from August 30 to October 2, 1925; these 40 cases represented 10 per cent of the admissions for these months. All 40 gave a history of diarrhea and in 14 the stools contained pus or pus and blood. Stovarsol was used in treating these patients with apparently complete success.

Although the attack on the intestinal wall by *B. coli* may be very slight, every infected person is liable at some time to exhibit clinical symptoms. These symptoms vary in severity from diarrhea to dysentery which may be continuous or characterized by apparent recovery and subsequent relapse. The disease produced is known as balantidiosis, balantidial dysentery or ciliate dysentery and the symptoms have been described many times.

Pathogenesis. The early pathological changes appear to be due to irritation caused by penetration of the parasites into the tissues of the intestinal wall. This results in hyperæmia of the mucosa. The ulcers are at first visible as minute reddened areas of the mucosa; as these grow larger a necrotic area appears in the center. Later the ulcers become undermined at the edges. When ex-

amined microscopically the balantidia are found to occur in groups in the mucosa and submucosa where their food probably consists of tissues dissolved by ferments they secrete. They are located near the edges of the older ulcers associated with living cells and within the blood vessels. The more extensive ulcers involve the muscle. The abscesses produced by the balantidia appear to be sterile at first but become secondarily infected with bacteria when they rupture. Balantidia probably enter the blood stream in cases of intestinal ulceration and may be carried to various parts of the body but no secondary sites of infection have been determined with certainty.

Nothing is known regarding immunity (active resistance of the host) in cases of balantidial infection.

4. HOST-PARASITE SPECIFICITY

Host-parasite specificity appears to be less rigid in *B. coli* than in any other protozoön that lives in man. Soon after Malmsten (1857) decribed *B. coli* from man, Leuckart (1861) reported it from pigs and the following year Stein placed the ciliate in the genus *Balantidium* which had been established by Claperede and Lachmann in 1858 with *B. entozoön* of the frog as the type species. Since then what seems to be the same species has been found in several species of primates, and infection experiments have been carried on with various species of lower animals which indicate the possibilities in this direction.

Pig. B. coli probably exists in pigs wherever these animals are to be found. They have been reported from pigs in Germany, Sweden, Russia, Italy, France, the

Philippine Islands, China, Formosa, Cuba, South America, the United States, and from other countries. McDonald (1922) records them in 68 per cent of 200 pigs from the western United States and Ohi (1925) found from 36 to 96 per cent of the pigs at Taiwan in Southern Formosa infected, the incidence depending on the season, there being a higher percentage parasitized in summer than in winter. It is generally admitted that the species that occurs in the pig is the same as that in man. That two species are present in the pig is claimed by McDonald (1922) who describes one type that corresponds in size and other characteristics to *Balantidium coli* of man and another type which he has named *Balantidium suis;* this type averages the same as *B. coli* in length but is one-third less in breadth and possesses a rod or sausage-shaped macronucleus at least one-half the length of the body instead of a bean-shaped macronucleus about one-third the length of the body as in *B. coli.* How extensively *B. suis* is distributed among pigs geographically cannot be stated. *B. suis* has not been recorded from man, all specimens thus far described being of the *B. coli* type.

B. coli has been considered for many years a natural parasite of the pig and an accidental parasite of man. This is indicated by the widespread infection among pigs and the usual absence of pathogenic effects in these animals; whereas in man the infection is rare and is often accompanied by severe pathological conditions. The intestinal wall of the pig is apparently very seldom attacked by *B. coli.* Brumpt (1909), however, records one case in which a pig was given an injection of material rich in

balantidia from two monkeys on May 27. On May 30 the pig passed cysts; from June 2 to 23 large numbers of the ciliates were passed; diarrhea occurred on June 11 and red blood cells appeared in the stools on June 12. When killed and examined on June 23 lesions were found in the large intestine, presumably due to the presence of the ciliates, that were similar in every way to those previously described by various investigators in man. Ohi (1923) claims that the indigenous pigs of Southern Formosa that are infected with *B. coli* appear sickly as compared with uninfected animals. Six pigs injected with balantidial material either by mouth or rectum did not become infected but one pig that received injections by both mouth and rectum became infected, and, although it showed no symptoms, pathological changes supposedly due to the ciliates were found in the large intestine when the animal was killed 172 days later.

Besides the morphological evidence that *B. coli* in man and the pig belong to the same species, there is strong epidemiological evidence that human beings become infected by ingesting specimens from pigs rather than from man. In the first place, as Brug (1919b) has pointed out, cysts which no doubt are usually responsible for the infection of new hosts, appear to be more rare in man than in pigs. Secondly, a large percentage of human cases can be traced more or less definitely to pigs. Thus of 117 cases of balantidiosis listed by Strong (1904) twenty-five per cent had either been associated with pigs or had eaten or prepared fresh sausage. Recently reported cases offer similar evidence. Young and Walker (1918) report a patient who worked in a packing house as a gut-

stripper and frequently got fecal material in his mouth; DeBuys (1918) reports a case in a colored boy of 5 who "had been accustomed to help 'round up' the pigs in the pen every day and frequently would eat some food which he would hold in his hand while helping with the pigs. It was his habit also to go into the pen at times when he ate his food"; Cordes (1921) describes a case in a pork butcher who had been slaughtering pigs for thirty years; Graziader and Mario (1922) treated a case in a peasant who was accustomed to eat raw salad grown on soil fertilized with pig manure; and Jausion and Dekester (1923a) report two cases from Morocco in villagers who were closely associated with pigs. Direct infection of man with cysts of *B. coli* from the pig has been attempted (Grassi, 1888) but with negative results.

There is good evidence obtained from cross-infection experiments that the ciliate of the pig and that of certain apes belong to one species. Brumpt (1909) established infections in two young pigs by injecting material containing balantidia from monkeys of the species *Macacus cynomolgus* (see below) and succeeded in infecting a monkey of this species with rectal injections of balantidial material from pigs. Twelve days after the injections a few balantidia were found and 4 days later an enormous number were present, leaving no doubt that colonization had taken place in the monkey.

Primates. Balantidia were first reported from primates, other than man, by Brooks (1903) who found them in orang-utans in the New York Zoölogical Garden; they were the cause of fatal dysentery in these animals. In 1908, Noc recorded *B. coli* in a monkey,

Macacus cynomolgus, from the Pasteur Institute in Saigon, Cochin-China and Brumpt (1909) found them in six monkeys of the same species that probably came from Indo-China. As noted above two pigs were successfully infected with material from these monkeys and a clean monkey with material from pigs. *B. coli* was next noted by Joyeux (1913) in a baboon in French Guinea and successfully transferred to an uninfected baboon.

Extensive experiments with monkeys (name undetermined) were carried out by Walker (1913) in the Philippine Islands. Cysts or trophozoites or both from man or pig were fed to or injected per rectum into the monkeys. Of 13 monkeys that were fed cysts from pigs, 12 became infected; of 4 injected per rectum with trophozoites from pigs, one became infected; of 3 injected per rectum with trophozoites from man, one became infected; one injected per rectum with both cysts and trophozoites from man became infected but one fed cysts from man remained uninfected. Balantidia were found in the tissues of the monkey that received rectal injections of cysts and trophozoites from man and in one monkey that was fed cysts from pigs.

Balantidia have also been reported from the chimpanzee. Christeller (1922) describes fatal balantidial dysentery in two specimens in the Berlin Zoölogical Garden. In one the ciliates were found in the tissues at autopsy; in the other they occurred in the stools but were not found at autopsy because of decomposition. Six cases of balantidial infection in chimpanzees in the Berlin Zoölogical Garden were later reported by Ziemann (1925). In two of these the balantidia were found

to have penetrated the healthy tissue. Two attempts to infect himself per os with fecal material containing balantidia were unsuccessful.

Ziemann (1925) also found balantidia in a monkey, *Cercocebus fuliginosus,* which he considers to be *B. coli.* Several years before this, Hegner and Holmes (1923) discovered a balantidium in a South American monkey, *Cebus variegatus,* recently imported from Brazil. The morphology of the balantidia in this monkey differed from both that of *B. coli* and of *B. suis* but no decision was reached regarding its zoölogical position. Recently Rees (1927) has reported balantidia from *Macacus rhesus.*

Other lower animals. Balantidia were first found in frogs and species have been described from frogs, salamanders, fish, cœlenterates, flatworms, sand fleas, cockroaches, and snails as well as in several species of mammals: in guinea-pigs by da Cunha (1914), in horses by da Cunha (1917), in the agouti by Buisson (1923) in sheep by Hegner (1924d) and in cattle by Cooper and Gulati (1926). A thorough study of the genus must first be made before it will be possible to state how many of these are "good" species and whether any of them belong to the species *B. coli.* A study of the balantidium in the guinea-pig by Scott (1925) indicates that this form is indistinguishable from *B. coli.* Attempts to infect laboratory animals with cysts from other hosts have all been unsuccessful. Thus Graziader and Mario (1922) failed to infect the guinea-pig and cat per rectum with infected feces from man; Ohi (1923) likewise failed to infect the guinea-pig, rabbit and dog with pig

material; and Ziemann (1925) obtained only negative results in an attempt to transmit the infection from the chimpanzee to the cat.

General discussion. From the evidence available it seems certain that the balantidia occurring in pigs, man and certain other primates all belong to one species, *Balantidium coli.* This is in marked contrast to the situation that exists in the case of most of the other human protozoa, which normally live only in one species of vertebrate host, man. Does *B. coli* possess any morphological, physiological or life-history peculiarities that account for this weak host-parasite specificity?

It has been pointed out above that *B. coli* resembles free-living ciliates morphologically; that is, it has not become appreciably modified by its parasitic habit. For example, it takes in solid food particles by means of a well-developed ingesting apparatus; is supplied with two active contractile vacuoles; and swims about freely, there being no organs of attachment, undulating membranes nor obvious modifications of the locomotor organelles so characteristic of certain other parasitic protozoa. The fact that *B. coli* does not exhibit morphological and physiological peculiarities associated with its parasitic habit indicates that it is very resistant to changes in the environment; that is, it is able to withstand successfully a great range in the factors of its environment, such as temperature, density and chemical nature of the medium and food supply. The observations of Rees (1927) indicate that *B. coli* is really more resistant than most other human protozoa in spite of the view that ciliates, as Sütterlin (1921) has shown in his

chemical experiments on free-living protozoa, are particularly sensitive, since harmful substances may easily reach the interior of the body by way of the cytostome. The fact, however, that *B. coli* is able to live in the large intestine of man, monkey, and pig proves that it can withstand the different conditions encountered in these different species of hosts, and indicates an adaptability or resistance greater than that of other human species.

We have a fairly good idea regarding the incidence of infection with *B. coli* in pigs and man but not in monkeys. The pig seems to be most susceptible to infection and the usual absence of pathological conditions indicates that the association is an old one. Perhaps the high incidence of infection in pigs may be accounted for by the production in this animal of numerous infective cysts and by its uncleanliness which probably results in the ingestion of large numbers of cysts daily. In man, on the contrary, cysts are rare, hence the transfer of infective cysts from man to man is no doubt exceptional and man probably receives his infection in almost every case as a result of the ingestion of cysts from pigs. This type of infection, however, is also exceptional since only a very small percentage of those who work with pigs and who frequently swallow cysts from pigs become infected, thus indicating high powers of resistance in the human host.

Both man and monkeys may be infected with balantidia without exhibiting symptoms but both may also suffer from more or less severe diarrhea or dysentery which sometimes terminates fatally. The adjustments between the balantidia and man and monkey are therefore less perfect than between these ciliates and the pig.

This may be due not to the short length of association between the two but to the infrequency of the association; thus a period that has been long enough to bring about an apparently harmless association in the pig may not have been sufficient to accomplish this result in man and monkeys. The life-cycle of *B. coli* is not fully known, but there seems to be nothing peculiar about it that might account for the weak host-parasite specificity of this ciliate.

CHAPTER V

COCCIDIA

I. *Species Living in Man*

Historical. The coccidia are tissue parasites, but are generally included among the intestinal protozoa because they apparently are responsible for digestive disturbances and the infective stages, oöcysts, in their life-cycle escape from the body in the feces of the host. Between 1858 and 1890, 10 more or less authentic cases of human infection with coccidia were recorded in the literature (Dobell, 1919b). In five of these cases the coccidia were found in the liver, in 3 cases in the intestine and in 2 cases in the feces. No more infections with coccidiosis were discovered until 1915; then a number of cases were reported by British protozoölogists in soldiers from the Eastern Mediterranean war area, and within the succeeding 5 years (1915-1920) almost 150 cases were recorded. Since then infections from various parts of the world have been added until the number has now reached about 200. In 1919, Dobell concluded that "there are four distinct species of coccidia which may parasitize man. These are (1) *Isospora hominis* Rivolta, 1878 (emend.), discovered by Kjellberg in 1860, and recently investigated by Wenyon; (2) *Eimeria wenyoni* n. sp., a form discovered in 1915 by Wenyon; (3) *Eimeria oxyspora* n. sp., another new form, here described for

the first time; (4) an undetermined species of *Eimeria* (?) which was discovered by Gubler in 1858" (p. 193). In 1921, Snijders (1921) described oöcysts of an *Eimeria* in human feces which Dobell (1921a) considered a new species and named *E. snijdersi.* Snijders (1921) suggested that "The cysts might have been ingested with food or water and passed unaltered (or only slightly altered) through the alimentary canal," and Brug (1922) made a similar suggestion regarding this case because of the native custom of eating intestines and livers. The study of oöcysts of *E. oxyspora* (Fig. 17) led Thomson and Robertson (1922) to conclude that no specific difference exists between this form and *E. snijdersi,* and more recently these investigators (1926a) have shown that the oöcysts of *E. oxyspora* are morphologically like those of *E. sardinæ* that occur in the testes of sprats and herrings, and the oöcysts of *E. wenyoni* (Fig. 16) like those of *E. clupearum* from the livers of herrings, sprats and mackerel. Furthermore, Thomson and Robertson (1926b) have demonstrated that oöcysts of *E. sardinæ* may be cooked and eaten without being destroyed and will pass through the digestive tract of man and appear in good condition in the feces. Fish containing oöcysts of *E. sardinæ* and *E. clupearum* are eaten frequently by many people and there seems to be no doubt but that the three species of *Eimeria* named by Dobell must be considered synonyms of these two species previously described from fish.

Isospora hominis and I. belli. This leaves one of Dobell's four species, *Isospora hominis,* to be considered. Wenyon (1923) concluded that there are two species

of *Isospora* parasitic in man; one is a small species described by Virchow, which should be known as *Isospora hominis,* and the second, the larger species discovered in 1915 and called by Dobell (1919b) *I. hominis.* For this species Wenyon proposes the name *Isospora belli.* Dobell (1926a), however, still maintains that the correct scientific name of this species is *Isospora hominis.* Until this controversy is settled it seems best to continue to use the familiar name *Isospora hominis* for the most common species reported from man.

Causey (1926) has recently described as *Eimeria butkai* what he considers to be a new species from man. More convincing evidence is necessary before this species can be accepted.

Morphology ana ufe-cycle of Isospora hominis. Isospora hominis is known only in the oöcyst stage (Fig. 15). The oöcysts pass out of the host in the feces. Their shape is shown in Fig. 15. They measure from 25μ to 33μ long and from 12.5μ to 16μ broad. Usually their protoplasmic contents form a ball; this is protected by the oöcyst wall which consists of a thin inner layer and a thicker resistant outer layer. After leaving the body

FIG. 21. Diagrams illustrating the asexual and sexual cycles of *Isospora felis* of cats and dogs. Stages 26, 28, and 29 are oöcysts which pass out of the body with the feces; in each oöcyst two sporoblasts are formed (28) and in each sporoblast, 4 sporozoites (29). When ingested by a susceptible animal the sporozoites escape from the oöcyst (30), enter epithelial cells (1) where they undergo schizogony (2-8). The merozoites produced may repeat the asexual cycle (8 to 1 to 8) or initiate the sexual cycle (9-25). In the latter, female cells (♀) or macrogametes (19-22) and male cells (♂) or microgametes (11-18) develop. Fertilization (23) is followed by the formation of the oöcyst (24-26). (Drawn by Dr. Justin Andrews.)

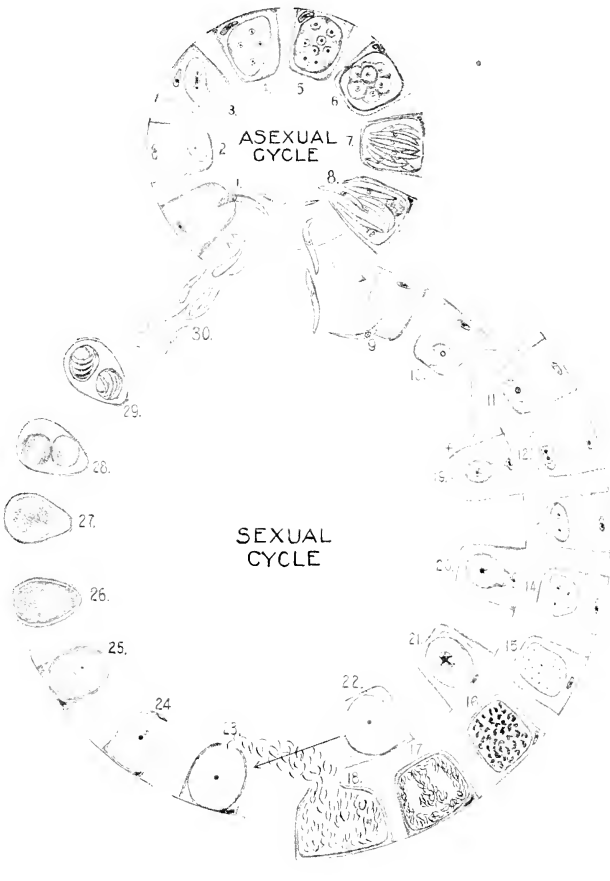

FIGURE 21

of the host, the nucleus divides; then the protoplasm separates into two sporoblasts, each with one of the daughter nuclei. Each sporoblast then secretes two walls (sporocysts) about itself and becomes a spore. Within the sporocysts two nuclear divisions occur and four sausage-shaped sporozoites are formed, each with a single nucleus. Part of the protoplasm is not included in the sporozoites but remains behind as a residue. One or two days are required for the development within the oöcyst.

The asexual and sexual cycles are probably similar to those of *Isospora felis* of the cat. Fig. 21 presents the stages in these cycles in diagramatic form.

II. *Host-Parasite relations of Isospora hominis*

Transmission. As is true of other intestinal protozoa, infections with coccidia no doubt are brought about by the ingestion of food or drink contaminated with the infective stages (oöcysts). These oöcysts are no doubt more resistant to conditions outside of the body than are the cysts of the intestinal amœbæ and flagellates. Haughwout (1921), for example, exposed oöcysts in the sporoblast stage to the sun for three hours every day for a week and found that at the end of this period some of them developed sporozoites when water was added; and Wenyon (1926) reports the completion of development within the oöcysts of *Eimeria stiedæ* from the rabbit after being subjected to a fixing solution of sublimate, stained with hæmotoxylin, dehydrated, cleared, and mounted in balsam.

Incidence. Only about 200 cases of human coccidiosis have been reported. This apparent low incidence of infection may be due to several factors: (1) the small number of oöcysts usually passed by the human host (Wenyon, 1926); (2) the fact pointed out by Andrews (1927) that cysts do not appear until the symptoms have disappeared and hence are probably frequently overlooked, and (3) the possibility that *I. hominis* may be a natural parasite of some lower animal and only occasionally brings about infection in man. To what extent these and probably other factors influence the incidence of infection in man can hardly at present be estimated.

Development and escape of sporozoites. When swallowed, the oöcysts are carried with the food or drink into the stomach and then into the intestine. No one knows where they hatch nor what is the primary site of infection. Presumably, however, the sporozoites escape in the small intestine and immediately penetrate the epithelial cells; this we know to be true of *Isospora felis* in the cat. They are thus always pathogenic although probably large numbers must be ingested before symptoms are produced. Several investigators have recently attempted to determine what factors are responsible for the development of the sporozoites within the oöcyst and for their subsequent liberation in the intestine of a new host. The oöcysts of *Isospora hominis* are passed either before or after the division of the protoplasmic contents into two sporoblasts. In Egypt, Wenyon and O'Connor (1917) found that complete development took place at room temperature in one day; in England (Wenyon, 1926) it requires 3 to 4 days. The oöcysts of the rabbit

coccidia, according to Kolpakoff (1925, 1926), develop in water and in physiological salt solution, and in some cases in gastric juice, but no sporogony was observed in pancreatic juice, bile or intestinal juice. The exit of sporozoites from the oöcysts of the rabbit coccidia may occur in diluted intestinal juice and also when subjected to gastric juice followed by intestinal enzymes (Krijgsman, 1926). That digestive juices have an influence on the escape of sporozoites from the oöcysts is also indicated by the experiments of Andrews (1927) on the coccidia of cats and dogs (see p. 196).

Course of human infections. There is still some question as to whether the various digestive disturbances associated with human coccidial infections are due to the coccidia or to some other cause. Connal (1922) has given us a connected account of what appears to be an infection with *Isospora hominis* accompanied by symptoms. His description agrees very closely with infections of cats with *Isospora felis* as described by Andrews (1926b).

The Connal infection. According to Connal, fecal material containing oöcysts of *Isospora hominis* was accidentally thrown over the face of a laboratory worker 40 years of age. Some of the oöcysts were probably swallowed. After an incubation period of 6 days the patient suffered from diarrhea, during which the feces appeared similar to those passed after a saline purge; this continued for 22 days and then the feces became more copious and of a thick oily consistency. Seven days later the stools became less fluid and during the next 2 days became formed. Oöcysts appeared in the feces 22 days

after diarrhea commenced, giving a prepatent period of 28 days. The patent period continued for 13 days, *i.e.*, during the second diarrheic period and 4 days after the feces became formed. No oöcysts were observed in the feces during the succeeding 6 months and no further diarrhea appeared. Evidently no relapse has been suffered by the patient since an account of it has not been reported.

Other human infections. Pons (1925a) has described two cases of coccidiosis in man that are interesting although not as simple as that of Connal. The first patient suffered with a choleric syndrome for 6 days; he subsequently appeared normal for 10 days; diarrhea, with stools containing blood and mucus, then commenced and continued for 9 days; a normal period of 20 days ensued; diarrhea again appeared, lasting 6 days. Giardias were noted but no dysentery bacilli or other pathogenic organisms. Oöcysts appeared 3 days before the end of the last diarrheic period and did not definitely disappear until 30 days later. The second case reported by Pons was that of a woman who had suffered from attacks of diarrhea for 10 months before coccidia were discovered in her feces. Oöcysts were passed for 16 days and then no more could be found.

These two cases resemble that of Connal and infections in cats and dogs in several respects: (1) diarrhea preceded the appearance of the oöcysts; that is, the incubation period was shorter than the prepatent period; (2) the oöcysts appeared for several weeks and then were apparently entirely eliminated, the patent period being 13 days in Connal's case, 30 and 16 days respectively in

Pons' cases, and 30 days in cats and dogs; (3) no relapses were reported. The literature contains descriptions of a number of other cases of human coccidiosis accompanied by symptoms, for example, Haughwout (1921) in an American in the Philippines who was suffering from occasional watery diarrhea, Boon van Ostade (1923) in a Malay who acquired it in Java or Sumatra and had chronic diarrhea, Petzetakis (1925) in a case of acute dysentery, and Leger (1926), also a dysenteric case, in Annam.

III. *Host-Parasite Specificity*

Cross-infection experiments. The results of recent investigations, especially those of Andrews (1927), indicate that the coccidia of mammals are very rigidly host-specific. According to Andrews, attempts had previously been made to infect kittens, mice and rats with oöcysts from man; horses, pigs, sheep, rabbits, guinea-pigs, and rats with oöcysts from cattle; man, kittens and rats with oöcysts from dogs; dogs and rats with oöcysts from cats; and cattle with oöcysts from rabbits. These experiments were all unsuccessful except those of Fantham (1917), who claims to have infected kittens with oöcysts from man. Fantham's work is open to question since no details are given and his experimental animals may already have been infected with their natural coccidial parasites. Andrews (1927) attempted to infect dogs, cats, skunks and opossums with oöcysts from rabbits; dogs with oöcysts from skunks; and cats with oöcysts from pigs, prairie-dogs and skunks but

failed in every case. He appears to have been successful, however, in infecting dogs with cat coccidia and cats with oöcysts from dogs. One kitten was infected with oöcysts of *Isospora felis* and *I. rivolta* from dogs, and exhibited intermittent diarrhea, and 4 of 8 dogs were infected with oöcysts of *I. felis* and *I. rivolta* from cats. Apparently, therefore, these two species are infective to both cats and dogs and represent, so far as we know at present, the only examples of coccidia that are able to live in more than one species of mammal.

Digestion experiments. Andrews (1927) tested the effects of the environment within the stomach and duodenum of cats and dogs on oöcysts of *Eimeria* from rabbits and on those of *Isospora* from cats and dogs. He found that very few of the *Eimeria* oöcysts were affected within 72 hours in the cat and within 24 hours in the dog, but that sporozoites were liberated from *Isospora* oöcysts in the cat within 48 hours and in the dog within 24 hours. Selective digestive action on the oöcysts enables the sporozoites of the coccidia natural to the host to escape more quickly than those foreign to the host; the foreign oöcysts thus have less chance to bring about an infection since they are passive bodies and are continually being carried further down the intestine. Andrews also attempted to determine whether the merozoites of foreign coccidia are able to bring about an infection by obtaining specimens of *Eimeria perforans* from the intestine of the rabbit and injecting them intraduodenally into dogs and cats. Experiments on 3 dogs and 3 cats were unsuccessful but a control cat into which merozoites of *Isospora felis* from a cat were injected

exhibited an infection 5 days later. This indicates that the intestinal epithelium probably allows the penetration of the merozoites of natural coccidia but prevents that of foreign coccidia. This method of attacking the problem of host-parasite specificity can be carried further and will doubtless lead to a better understanding of the subject.

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