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Protozoan Parasites of Domestic Animals and of Man

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by

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DEDICATED TO MY PARENTS:

To Max Levine, whose example guided me to a career in science

To Adele Levine, whose example helped me to an interest in the humanities

Preface

The importance of protozoan parasites as causes of disease in domestic animals is well recognized, yet the literature on them is still widely scattered and the books now available provide little more than an introduction to the subject. The present book was written to serve as a text and reference work for veterinarians, protozoologists, parasitologists, zoologists and also for physicians. As our knowledge of the relations between human and animal parasites has increased, the list has also increased of parasites which were once thought to be confined to domestic and wild animals but which are now known to occur also in man. The area of overlap between the fields of human and animal disease is becoming continually greater, and the zoonoses are receiving more and more attention. For this reason, the protozoan parasites of man are included in this book, and their relations to those of lower animals are indicated.

When this book was begun, it was intended to be a revision of the pioneering Veterinary Protozoology by the late Banner Bill Morgan and the late Philip A. Hawkins, the second edition of which was published in 1952. However, it soon became apparent that far more than this was necessary, and the result has been an entirely new book.

It is planned to follow this volume with others on veterinary helminthology and entomology. The first chapter, therefore, deals with the general principles of parasitology, while the second is an introduction to protozoology. The different groups of protozoa are discussed in the succeeding chapters, and the final chapter deals with laboratory diagnostic technics. This systematic organization based on parasite groups is used rather than one based on host animals because it is more efficient, avoids repetition, and makes the subject easier to present and to understand. However, it is also useful to know which parasites one can expect to find in each host. Lists of parasites by host have therefore been prepared and are incorporated in the index. E. A. Benbrook (1958. Outline of parasites reported for domesticated animals in North America. 5th ed. Iowa State Univ. Press) has listed the parasites both by host and by location in the host.

The world today is too small to permit a provincial approach to parasitism and disease. Katanga and Uttar Pradesh, Kazakhstan and Luzon are only a step from New York and San Francisco, and their problems and their diseases are becoming more and more our concern. The scope of this book, therefore, is world-wide, and parasites are discussed regardless of where they occur. However, major attention is given to the parasites of those domestic animals which occur in the temperate zones, and relatively little is included on parasites of animals like the elephant, camel, llama, reindeer and yak, even tho they are important domestic animals in some regions.

When C. M. Wenyon wrote his classic Protozoology in 1926, he remarked that one of his chief difficulties had been that hardly a week passed without the publication of some paper of importance; that difficulty is far greater today than it was then. The number of published papers has been increasing exponentially, and there is no sign that the logarithmic phase of the curve is near its end. Even if one tries to read the current journals faithfully and to use the abstract journals assiduously, important papers may escape his notice. I am sure that some have escaped mine, and I should appreciate having them called to my attention. In addition, to help me in preparing future editions, I should appreciate receiving reprints of pertinent papers.

A favorite saying of Jean Baer is that textbooks perpetuate errors by copying them from one to another. I have tried to avoid this by going to the original papers as much as possible, but in so doing I may well have introduced some errors of my own. I should appreciate having these called to my attention also.

Papers are appearing so fast that, unless one is forced to it, he cannot take the time to read and ponder those outside his own immediate field of interest and to try to integrate them into a coherent whole. Writing this book has made me do so, and the process has taught me a great deal. Not only have I learned many things which I did not know, but I have come to realize more clearly how much information we still lack, even about parasites which have been studied extensively. This book reflects that situation. The reader will find at least one question, one gap in our knowledge on each page. Each is a challenge for future research which I hope will be accepted by many who read this book.

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Contents

Chapter 1

INTRODUCTION TO PARASITOLOGY

Parasitology is the science which treats of parasites. The word "parasite" is derived from the Greek and means, literally, "situated beside." It was used by the ancient Greeks originally for people who ate beside or at the tables of others, and referred both to sycophants or hangers-on and to priests who collected grain for their temples. While the social meaning of the term has been partially retained, it has been given a new connotation by scientists. *Parasites* are defined as organisms which live on or within some other living organism, which is known as the *host*. *Parasitism* is the association of two such organisms.

Parasites may be either animals or plants--viruses, rickettsiae, spirochetes, bacteria, yeasts, fungi, algae, mistletoe, dodder, protozoa, helminths, arthropods, molluscs, and even certain vertebrates such as the cuckoo. The general principles of parasitology apply to all. However, in this book we shall deal primarily with animal parasites, leaving the plant parasites to textbooks of microbiology.

In our everyday thinking we consider that animals can live in three main habitats--land, fresh water and sea water. A fourth habitat is the parasitic one, which is quite different from the other three. As a matter of fact, there are quite a few different parasitic habitats, each with its own characteristics. Parasites are found in the lumen of the intestinal tract, on the outside of the body, in the skin, in various tissues, in the blood plasma, inside different types of cells, and even inside cell nuclei.

Parasites have arisen from free-living animals. Some parasites closely resemble their free-living relatives, but others have undergone structural changes which make them more suited to their changed environment. Since these changes have in many cases been the loss of some power which their free-living relatives possess, parasites have some-

times been considered degenerate creatures. The opposite is true. Parasites are highly specialized organisms. Those powers which were unnecessary, they have lost. For instance, the adults of most parasitic worms have relatively little ability to move around. But they don't need it. Too much activity might even lead to their reaching the point of no return and being discharged from their host's body.

As another example, tapeworms have no intestinal tract. But, since they obtain their nourishment directly thru the body wall, an intestine would be superfluous. Thus, in the case of parasites as with all other animals and plants, the useless has been eliminated in the course of evolution.

In contrast, the reproductive system of parasites is often tremendously developed. Since the chances of an egg or larva leaving one host and infecting another are very small, the numbers of eggs produced must be very large. Many parasitic worms produce thousands of eggs a day. The female of *Ascaris suum*, the large roundworm of swine, lays about 1,400,000 eggs per day (Kelley and Smith, 1956). Assuming that she lives 200 days, which is not an excessive life, she will have laid 280 million eggs in her lifetime. Since the number of *Ascaris* in the world is staying more or less the same, we can conclude that on the average only two of these eggs will produce adult worms--a male and a female. The chance of any particular egg ever becoming a mature worm is thus about 1 in 140 million, which is much less than a man's chance of being struck by lightning.

The broad fish tapeworm of the dog, man and other animals, *Dibothriocephalus latus*, will produce over 4 miles of segments containing 2 billion eggs during a 10-year life span, and again the number of these tapeworms is not increasing. Since these tapeworms are hermaphrodites, each egg can become an egg-laying worm, but its chances of doing so are a hundred times less than those of the *Ascaris* egg. Parasites are continually being confronted by odds of this sort and are continually surmounting them.

Life of one sort or another seems to have flowed into every possible niche. Parasites live in some of the most difficult niches, and it is remarkable how they have succeeded in surviving in them. Parasites have tremendous problems to solve--problems of nutrition, of respiration, of excretion, of getting from one host to another--and the different and often ingenious ways in which different parasites have solved these problems are amazing. Some of their adjustments are almost perfect; others are less satisfactory. In general, we may say that the more satisfactory the solution, the more abundant are the parasites. The rare ones are the less successful ones.

We can think of parasitism as related basically to the solution of the problem of nutrition, and we can think of the other problems as somewhat secondary. This is obviously an incomplete and defective view, but nevertheless it has some value.

Living organisms have four general types of nutrition. *Holophytic* nutrition is typical of plants; it involves synthesis of carbohydrates by means of chlorophyll. *Holozoic* nutrition is animal-like; it involves ingestion of particulate food thru a permanent or temporary mouth. *Saprozoic* or *saprophytic* nutrition (the choice of term depending upon whether the organism is an animal or plant) involves absorption of nutrients in solution thru the body wall. The fourth type of nutrition is that employed by viruses, which synthesize their proteins directly from the host's amino acids and do not have a true body wall during their parasitic phase.

The terms *saprophyte* and *saprophytic* are often used by bacteriologists in another sense also, to refer to non-pathogenic, non-parasitic organisms. The terms *saprozoite* and *saprozoic* are also similarly used with reference to free-living animals, but much less frequently.

Coprozoic or *coprophilic* organisms are animals which live in feces. They

may be either saprozoic or holozoic or both, and are sometimes mistaken for true parasites.

Parasites resemble predators in some respects; indeed, one grades into the other. In general, we think of predators as larger than or as large as their prey, while we think of parasites as considerably smaller. A lion seizing an antelope is a predator, as is a spider capturing a fly. But there is a distinction only in size of prey between a predatory assassin bug capturing another insect and sucking out its juices and the closely related, parasitic kissing bug sucking blood out of a man. And a mosquito is just as much a predator as the kissing bug. The distinction is one of degree. As Elton (1935) put it, "The difference between a carnivore and a parasite is simply the difference between living upon capital and income, between the burglar and the blackmailer. The general result is the same although the methods employed are different."

TYPES OF PARASITISM

There are several types of parasitism. *Parasitism* itself is defined as an association between two specifically distinct organisms in which one lives on or within the other in order to obtain sustenance.

Symbiosis is the permanent association of two specifically distinct organisms so dependent upon each other that life apart is impossible under natural conditions. The relation between many termites and their intestinal protozoa is symbiotic. The termites eat wood, but they cannot digest it; the protozoa can digest wood, turning it into glucose, but they have no way of obtaining it; working together, the termites ingest wood particles, the protozoa break the cellulose down to glucose, and the termites then digest the glucose. Lichens furnish another example of symbiosis. They are composed of certain species of algae and fungi living together.

Many insects, ticks and mites have symbiotic bacteria and rickettsiae. The symbiotic organisms are found either in special cells, the mycetocytes, in modified parts of the Malpighian tubules, or in special organs, the mycetomes. It is significant that, among blood-sucking arthropods, symbiosis occurs in those which live on blood thruout their life cycles (ticks, lice, bedbugs, kissing bugs, tsetse flies, hippoboscid flies) but not in those in which only the adults suck blood while the larvae are free-living (fleas, mosquitoes, phlebotomines, tabanids and stable flies). Blood lacks some metabolites which the arthropods are unable to synthesize themselves and for which they depend on their symbiotes. These metabolites appear to include vitamins of the B group and probably other substances as well (Buchner, 1953; Koch, 1956; Weyer, 1960).

Mutualism is an association of two organisms by which both are benefited. It differs from symbiosis in that it is not obligatory for both partners. One example often cited is that of a sea anemone living upon the back of a crab. The anemone is benefited by being moved to new hunting grounds and by obtaining morsels of food torn off by the crab, while the crab is protected by the bulk and stinging tentacles of the anemone. Another marine example is that of the scorpion fish of Indo-Malaya. It lives on the bottom of the sea, where it lies in wait for passing fish. It is covered with a crust of hydroids which camouflage it so that it can seize its unwary prey more easily. The hydroids presumably benefit by being moved to new sources of food and by being provided with a dwelling-place. However, since they can live other places beside the scorpion fish's back, their relation is mutualistic.

Another example of mutualism, and one closer to us, is the relationship between ruminants and the cellulose-digesting bacteria and other micro-organisms in their rumens. The latter are furnished a favorable home by their hosts and aid them by breaking down cellulose to usable compounds. The rumen-dwelling bacteria which produce B group vitamins and thus make an outside source of them unnecessary for ruminant nutrition probably

belong here too, altho they verge on the symbiotic. The bacteria which produce these vitamins in the large intestine of swine are more nearly mutualistic, since the pigs cannot absorb the vitamins thru the colon wall but must re-ingest their feces to obtain them. The same is true of rabbits, and is undoubtedly responsible for their coprophagy.

The bizarre protozoa which swarm in the rumen and reticulum are almost certainly mutualistic. Their host can get along without them, but they may benefit it by providing a better type of protein than it ingests. In addition, they are an important source of volatile fatty acids, and they smooth out the carbohydrate fermentation process.

Commensalism is an association between host and parasite in which one partner is benefited and the other is neither benefited nor harmed. Many intestinal bacteria such as *Escherichia coli* are normally commensals, as are many intestinal protozoa such as *Entamoeba coli* and *Trichomonas* spp.

The next two terms both refer to potentially pathogenic parasites. *Parasitosis* is the association between two organisms in which one injures the other, causing signs and lesions of disease. *Parasitiasis* is the association between two organisms in which one is potentially pathogenic but does not cause signs of disease.

The difference between parasitosis and parasitiasis is quantitative. In parasitiasis the host is able to repair the damage caused by the parasite without noticeable injury, while in parasitosis it cannot. As Whitlock (1955) put it, "Parasitiasis is a state of balance. Parasitosis is a state of imbalance." Applying the concept to ruminant helminths, Gordon (1957) said, "Helminthiasis is almost universal and continuous, helminthosis is more restricted and sporadic. However, one shades imperceptibly into the other in subclinical infestations." The same organism can cause either parasitosis or parasititiasis, depending upon the number present or upon the nutritional condition, age, sex, immune state, etc. of the host. Failure to recognize this distinction may cause many false diagnoses--the mere presence of a potentially pathogenic species of parasite does not necessarily mean that it is causing disease.

The *carrier state* furnishes a good example of parasitiasis. *Carriers* are animals which have a light infection with some parasite but are not harmed by it, usually due to immunity resulting from previous exposure, but which serve as a source of infection for susceptible animals. Thus, adult sheep and cattle may be lightly infected with gastrointestinal nematodes without noticeable effect, but their lambs and calves may become heavily parasitized from grazing with them. The condition in the adults is parasitiasis; that in the young is parasitosis. Adult chickens rarely suffer from coccidiosis because they have recovered from a clinical or subclinical attack when young. However, they are usually still lightly infected and continue to shed a few oocysts; they have coccidiasis. Cattle which have aborted as a result of *Brucella* infection may continue to shed the bacteria in their milk without ordinarily suffering further clinical attacks. The aborting cow has brucellosis, while the carrier has brucelliasis.

These endings can also be applied to the names of the disease agents, as has already been done above. Thus, *Haemonchus contortus* may cause haemonchosis or haemonchiasis, *Taenia* may cause taeniosis or taeniasis, *Histomonas meleagridis* may cause histomonosis or histomoniasis, depending on the circumstances.

It was mentioned earlier that the solutions different parasites have made of their problems of living have varied in satisfactoriness. We might consider this in regard to type of parasitism. Symbiosis is a highly specialized type of association which occurs only in certain groups. Mutualism is a much looser association, also fairly uncommon. It could well be a step on the road to symbiosis. The most

common types of parasitism are the last three. Of these, commensalism is clearly the most desirable, both from the standpoint of the host (which isn't harmed) and of the parasite. Parasitosis, which harms the host, is in the long run harmful to the parasite also. By injuring their hosts, parasites harm their environment, and if they are so indiscreet as to kill their hosts, they die too. Parasitiasis is intermediate between parasitosis and commensalism in some cases, but not in all.

HOST-PARASITE RELATIONS

Depending on their species, parasites may live in any organ or tissue of the host; they may live on its surface, or they may spend most of their time away from it. Special terms have been applied to these relationships. An *endoparasite* is a parasite that lives within the host's body. An *ectoparasite* is one that lives on the outside of the body. An *erratic* (or *aberrant*) *parasite* is one that has wandered into an organ in which it does not ordinarily live. An *incidental parasite* is a parasite in a host in which it does not usually live. A *facultative parasite* is an organism that is capable of living either free or as a parasite. An *obligatory parasite* is an organism which must live a parasitic existence. A *periodic parasite* is one which makes short visits to its host to obtain nourishment or other benefits. A *pseudoparasite* is an object that is mistaken for a parasite. Parasites may themselves be parasitized by *hyperparasites*.

An organism which harbors a parasite is its *host*. There are several types of host. A *definitive host* is the host which harbors the adult stage of a parasite. An *intermediate host* is the host which harbors the larval stages of the parasite. A *first intermediate host* is the first host parasitized by the larval stages of the parasite. A *second intermediate host* is the host parasitized by the larval stages at a later period in the life cycle. A *paratenic* or *transport host* is a second (or third) intermediate host in which the parasite does not undergo any development but usually remains encysted until the definitive host eats the paratenic host.

The *vector* of a parasite or disease agent is an arthropod, mollusc or other agent which transmits the parasite from one vertebrate host to another. If the parasite develops or multiplies in the vector, it is called a *biological vector*. If the parasite does not develop or multiply in it, it is called a *mechanical vector*.

Intermediate hosts of helminths are biological vectors, but biological vectors are not necessarily intermediate hosts. Indeed, the latter term has no application to protozoa, bacteria, rickettsia or viruses, none of which have larvae. Mosquitoes are biological vectors of malaria and of yellow fever, and the tsetse fly is a biological vector of *Trypanosoma brucei*, for the parasites must develop in them to become infective for the next vertebrate host. However, tabanid flies are merely mechanical vectors of *Trypanosoma evansi*, since the parasites undergo no development in them.

The terms *infection* and *infestation* are used by different people in different ways. The former term originally referred to internal agents of disease, while the latter was used with reference to external harassing agents, including not only ectoparasites but also rodents, pirates and thieves. This usage was current during the latter part of the nineteenth century. Later on, it was felt desirable to distinguish between parasites which multiplied in their hosts and those which did not. "Infection" was then used for the former type of parasitism, and "infestation" for the latter. This usage was popular for a time, but it was never universally accepted. More recently there has been a trend toward the older usage. Most American parasitologists have accepted it, but most British ones prefer to speak of helminth infestations. In this book *infection* will be used to refer to parasitism by internal parasites, and

infestation to parasitism by external parasites.

The term *life cycle* refers to the development of a parasite thru its various forms. It may be simple, as in an organism which multiplies only by binary fission, or it may be extremely complex, involving alternation of sexual and asexual generations or development thru a series of different larval forms. A *monogenetic* parasite is one in which there is no alternation of generations. Examples of this type are bacteria, flagellate protozoa such as *Trichomonas*, nematodes such as *Ascaris* and *Ancylostoma*, and the ectoparasitic fish trematodes of the order Monogenorida (= Monogenea). A *heterogenetic* parasite is one in which there is alternation of generations. Examples of this type are malarial parasites and coccidia, in which sexual and asexual generations alternate, the endoparasitic trematodes of higher vertebrates of the order Digenorida (= Digenea), in which there may be several larval multiplicative stages before the adult, and the nematode, *Strongyloides*, in which one generation is parasitic and parthenogenetic while another is free-living and sexual.

Depending on their type, parasites may live in only one or in a number of different types of hosts during the course of their normal life cycles. A *monoxenous* parasite has only one type of host--the definitive host. Examples are coccidia, amoebae, hookworms, fish trematodes, horse bots, streptococci and most pox viruses. A *heteroxenous* parasite has two or more types of host in its life cycle. Examples are the malarial parasites, most trypanosomes, trematodes of higher vertebrates, filariae, tapeworms, the rickettsiae, yellow fever virus and various encephalitis viruses.

These two pairs of terms are independent of each other. Parasitic amoebae and hookworms are monogenetic and monoxenous. Filariid and spirurid nematodes are monogenetic and heteroxenous. *Strongyloides* and most coccidia are heterogenetic and monoxenous. Malarial parasites and trematodes of birds and mammals are heterogenetic and heteroxenous.

Another group of terms deals with *host range*, i.e., the number of host species in which a particular parasite may occur. These parasites can be either monoxenous or heteroxenous, monogenetic or digenetic. Indeed, there may be a difference in host-restriction between the definitive and intermediate hosts of the same parasite. For example, the blood fluke, *Schistosoma japonicum*, can become adult in a rather wide range of mammals, but its larval stages will develop in only a few closely related species of snails.

The term, monoxenous parasite, is used by some authors for a parasite which is restricted to a single host species. Such parasites undoubtedly exist, but they are fewer than our present records indicate. The human malarial parasites were once thought to be monoxenous in this sense of the word, but they have more recently been found capable of infecting apes, and it is now known that chimpanzees in West Africa are naturally infected with *P. malariae*, the cause of quartan malaria in man (Garnham, 1958). Many species of coccidia are also known from but a single host, but for the most part closely related wild hosts have not been examined nor have cross transmission experiments been attempted with them. Because of this and because of the confusion arising between this usage of monoxenous and the one defined above, this usage should be avoided.

A *stenoxenous* parasite is one which has a narrow host range. Among the coccidia, members of the genus *Eimeria* are generally stenoxenous, as are the human malaria parasites and cyclophyllidorid tapeworms. Many nematodes such as the hookworms, nodular worms, filariids and spirurids tend to be stenoxenous. Both biting and sucking lice are stenoxenous, and many are even limited to specific areas on their host. Relatively few bacteria are stenoxenous, but *Streptococcus agalactiae*, *Mycobacterium leprae*, *Vibrio*, *Mycoplasma*, the spiro-

chete, *Treponema*, the rickettsiae, *Anaplasma*, *Eperythrozoon*, *Haemobartonella* and *Cowdria*, and the viruses of hog cholera, duck hepatitis and yellow fever are stenoxenous.

An *euryxenous* parasite is one which has a broad host range. Among the coccidia, members of the genus *Isospora* are often euryxenous. So are most trypanosomes, most *Plasmodium* species (but not those affecting man), and many species of *Trichomonas*. Most trematodes are euryxenous, as are *Trichinella*, *Dracunculus* and *Dioctophyma* among the nematodes. Fleas, chiggers and many ticks are euryxenous. Most parasitic bacteria are euryxenous; examples are most species of *Salmonella*, *Escherichia*, *Brucella*, *Erysipelothrix* and *Listeria*. Among euryxenous rickettsiae are *Rickettsia*, *Coxiella* and *Miyagawanella psittacii*. Among euryxenous viruses are those of rabies and many encephalitides. *Leptospira* and *Borrelia* are euryxenous spirochetes.

The use of these two terms, however, may be deceptive. There exist in nature all intergrades between them, and all we have done has been to pick out the two extremes of a continuum and give them names.

Actually, the host range of most parasites is broader than generally supposed. The fact is that most animal species have not been examined for parasites. For example, the genus *Eimeria* is one of the commonest and best known among parasitic protozoa. Becker (1956) listed 403 species, of which 394 were from chordates and 202 from mammals. This is quite impressive, especially to someone who wishes to study their taxonomy. However, according to Muller and Campbell (1954), there are 33,640 known living species of chordates and 3552 of mammals. Some hosts have more than one species of *Eimeria*, but some coccidian species occur in more than one host. Assuming that these more or less cancel out, we can calculate that *Eimeria* has been described from only 1.17% of the world's chordates and from 5.7% of its mammals. If all these possible hosts were to be examined, one might expect to find some 3500 species of *Eimeria* in mammals and 34,000 in chordates.

So far only the qualitative aspect of the host range has been discussed. However, altho a parasite may be capable of living in more than one host, it is much more common in some hosts than in others. The *principal hosts* of a parasite are those hosts in which it is most commonly found. The *supplementary hosts* are those of secondary importance, and the *incidental hosts* are those which are infected only occasionally under natural conditions. To these should be added *experimental hosts*, which do not normally become infected under natural conditions but which can be infected in the laboratory. This last category may include both incidental and supplementary hosts and also hosts never infected in nature.

In order to take into account this quantitative aspect of the host-parasite relationship, the terms *quantitative host spectrum* or *quantitative host range* are used. These give the amount of infection present in each infected species.

Several factors affect the quantitative host spectrum. One is geographic distribution. The natural quantitative spectrum may be quite different in one locality than in another. The species of animals present may be different, or the incidence of infection may be different. For example, a number of nematodes parasitize both domestic and wild ruminants. However, since the wild ruminants of North America and Africa are not the same, the quantitative host spectra of the same parasites on the two continents are different. The spectrum is still different in Australia, where there are no wild ruminants but where wild rabbits are susceptible to infection with a few ruminant nematodes.

A second factor is climate. Many of the same host species may be present in different areas but climatic conditions in one area may prevent or favor a para-

site's transmission. For instance, the common dog hookworm in most parts of the United States is *Ancylostoma caninum*, but in Canada it is *Uncinaria stenocephala*. This is due to a difference in temperature tolerance of the free-living larval stages.

Local conditions such as ground cover are also important. If the vegetation is open so that the sunlight can get down to the surface of the soil where a parasite's eggs, cysts or free-living stages are found, survival will be much less, transmission will be reduced and the numbers of affected hosts will be fewer than if the vegetation is thick and protective. Or the kinds and numbers of parasites in a herd of animals confined to a low, moist pasture may be quite different from those in a herd kept on a hill pasture or on drylot.

A fourth factor is that of the distribution of acceptable intermediate hosts. *Trypanosoma brucei* occurs only in Africa because its tsetse fly intermediate hosts occur only there. The fringed tapeworm of sheep, *Thysanosoma actinioides*, is found in the western United States but not in the east despite the fact that infected sheep have repeatedly been introduced onto eastern pastures. A suitable intermediate host does not occur on these pastures, so the parasite cannot be transmitted.

A fifth factor is that of chronologic time. The quantitative host spectrum may be quite different in the same locality at different periods, particularly if an eradication campaign has been carried out in the interim. Echinococcosis is a case in point. At one time it was extremely common in the dogs, sheep and people in Iceland, but it has now been eradicated. Gapeworms were once common in poultry in the United States, but as the result of modern poultry management practices they are now exceedingly rare in chickens and turkeys, altho they are not uncommon in pheasants.

A sixth factor is that of the ethology or habits of the host. A species may be highly susceptible to infection with a particular parasite, yet natural infections may seldom or never occur. The habits of the host may be such that it rarely comes in contact with a source of infection even tho both exist in the same locality. For example, wild mink in the midwestern United States are not infrequently infected with the lung fluke, *Paragonimus kellicotti*. It is easy to infect dogs with this fluke experimentally, yet it is extremely rare in midwestern dogs. The reason is that dogs rarely eat the crayfish which are the fluke's intermediate host.

Because of these factors, we must speak of *natural* and *potential* host spectra. The latter term refers to the absolute infectability of potential hosts and not to the natural situation. The natural host spectrum is an expression of the actual situation at a particular time and place. The two spectra may be quite different, and of course the natural one will vary considerably, depending on the circumstances. The complete host spectrum has not been worked out for any parasite, and to do so would be a very time-consuming process. However, it will have to be done, at least for the more important parasites, before we can fully understand their ecology and the epidemiology of the diseases they cause.

Certain parasites and diseases occur in man alone, others in domestic animals alone, and others in wild animals alone. Still others, including some important ones, occur in both man and domestic animals, man and wild animals, domestic and wild animals, or in all three. A knowledge of their host relations is important in understanding their ecology and epidemiology.

A disease which is common to man and lower animals is known as a *zoonosis*. Zoonoses were redefined in 1958 by the Joint WHO/FAO Expert Committee on Zoonoses as "those diseases and infections which are naturally transmitted between vertebrate animals and man" (World Health Organization, 1959). Less than 20 years ago it was said that there were 50 zoonoses, but in the above report the World Health Organization listed more

than 100, of which 23 were considered of major importance. Many more are certain to be revealed by future investigations.

Our thinking about parasites and diseases is ordinarily oriented toward either man or domestic animals. In this context, it is convenient to have a special term for hosts other than those with which we are primarily concerned. A *reservoir host* is a vertebrate host in which a parasite or disease occurs naturally and which is a source of infection for man or domestic animals, as the case may be. Wild animals are reservoirs of infection for man of relapsing fever, yellow fever and moist Oriental sore, while domestic animals are reservoirs for man of trichinosis and classical Oriental sore. Wild animals are reservoirs of infection for domestic animals of many trypanosomes, while man is a reservoir for domestic animals of *Entamoeba histolytica.*

Parasites and diseases may continue to exist indefinitely in their reservoir hosts, and man or domestic animals may become infected when they enter the locality where the parasites or diseases exist. Such a locality is known as a *nidus* (literally, "nest"). This term is used primarily in connection with vector-borne diseases, altho it need not be restricted to them.

Natural nidi may be elementary or diffuse (Palovsky, 1957). An *elementary nidus* is confined within narrow limits. A rodent burrow containing rodents, argasid ticks and relapsing fever spirochetes or a woodrat nest containing woodrats, kissing bugs and *Trypanosoma cruzi* is an elementary nidus. In a *diffuse nidus* the donors, vectors and recipients are distributed more widely over the landscape. A wooded region in which ticks circulate *Rickettsia rickettsii* among the rodents and lagomorphs is a diffuse nidus of Rocky Mountain spotted fever, as is an area where tsetse flies transmit trypanosomes among wild game. The *nidality* of a disease refers to the distribution and characteristics of its nidi.

The concept of the *deme* is useful in discussing host-parasite relationships, epidemiology, taxonomy, evolution, etc. (see Hoare, 1955). A *deme* is a natural population within a species. It lies more or less below the subspecies level, but it is not a formal taxon and is not given a Latin name. There are different types of deme. *Nosodemes* differ in their clinical manifestations. One example is *Leishmania donovani*, which has five nosodemes, Indian, Mediterranean, Sudanese, Chinese and South American, which produce different types of disease. *Serodemes* differ serologically. These are best known among the bacteria and viruses, but also occur among the animal parasites. *Tritrichomonas foetus*, for example, has several serological types or serodemes. *Xenodemes* differ in their hosts, and *topodemes* differ in geographic distribution.

There are also other types of demes. The population of a parasite species within a single host animal is a *monodeme*, and that in a single flock or herd is an *agelederne*. Thus, a population of the stomach worm, *Haemonchus contortus*, in a single sheep is a monodeme, the population in all the sheep of a single flock is an agelederne, that in all sheep is a xenodeme. The population in all cattle is another xenodeme and that in all goats is a third, the population of *H. contortus* in all hosts in North America is a topodeme, etc.

Each of these demes may differ morphologically and physiologically, and a large part of the taxonomist's work consists in determining the limits of their variation and deciding whether they are really demes or different species. Since the judgments of all taxonomists do not agree, there is some variation in the names which different parasitologists use. Demes are advance guards in the march of evolution, and no sharp line can be drawn beyond which they become subspecies or species. Taxonomists have been able to arrive at no better statement of how species are defined than to say that a species is what a specialist on its group says it is. And since some scientists are splitters and others are lumpers, their definitions vary with their temperaments. For most of us, the best rule is the pragmatic one of using those names which

make for the greatest understanding of the organisms we study and of their relations with each other and with their hosts.

Parasite evolution: Parasites have evolved along with their hosts, and as a consequence the relationships between the parasites of different hosts often give valuable clues to the relationships of the hosts themselves. Certain major groups of parasites are confined to certain groups of hosts. Sucking lice are found only on mammals. Biting lice occur primarily on birds, but a few species are found on mammals. The monogenetic trematodes are found almost without exception on fish; some of the more highly evolved digenetic trematodes are found in fish, but more occur in higher vertebrates. There is a tendency, too, for the more advanced digenetic trematodes to occur in the higher host groups.

One would expect that, as evolution progressed in different host groups, there would develop in each one its own group of parasites. This has often occurred. Thus, of the 48 families of digenetic trematodes listed by Dawes (1956), 17 occur only in fish, 8 only in birds, 3 only in mammals, 2 in fish and amphibia, 3 in reptiles and birds, 6 in birds and mammals, 1 in fish, amphibia and reptiles, 2 in reptiles, birds and mammals, 1 in amphibia, reptiles and birds, 3 in all but fish, and 2 in all five classes of vertebrates. Of the 11 classes of tapeworms recognized by Wardle and McLeod (1952), 4 are found only in elasmobranch fish, 3 only in teleosts, 1 only in birds, 1 in teleosts, amphibia and reptiles, 1 in teleosts, birds and mammals, and 1 in amphibia, reptiles, birds and mammals.

This same tendency is apparent even in parasitic groups which are quite widely distributed. For example, many reptiles and mammals (but not birds) have pinworms of the family Oxyuridae, but each group has its own genera. Iguanas have *Ozolaimus* and *Macracis*, other reptiles have *Thelandros*, *Pharyngodon* and several other genera, rodents have *Aspiculuris*, *Syphacia* and *Wellcomia*, rabbits have *Passalurus*, equids have *Oxyuris*, ruminants have *Skrjabinema*, and man and other primates have *Enterobius*.

On the other hand, there are many exceptions to this general rule, and it cannot be used without corroboration as the sole criterion of host relationship. Many fish-eating birds and mammals have the same species of trematodes for which fish act as intermediate hosts. And the fact that the pig and man share a surprising number of parasites is no proof of their close relationship despite their similarity of character and personality; it simply reflects their omnivorous habits and close association.

Adaptation to parasitism: Adaptation to a parasitic existence has required many modifications, both morphological and physiological. Locomotion, at least of the parasitic stages, has often become restricted. Certain organs and organ systems may be lost. Tapeworms lack an intestine altho their ancestors presumably had one, and adult trematodes have no eyespots altho their turbellarian ancestors and many of their larvae have them. Parasitic amoebae have no contractile vacuoles altho their free-living relatives do.

In contrast, many structures are modified or hypertrophied for the parasitic life. Many helminths have hooks and suckers to help them hold their position. The protozoon, *Giardia*, has turned most of its ventral surface into a sucking disc. The mouthparts of many insects and mites have become highly efficient instruments for tapping their hosts' blood supply. The chigger, which does not suck blood, has developed a method of liquefying its hosts' tissues. The food storage organs of many parasites have been enlarged. Many blood-sucking arthropods which are unable to obtain all the nutrients they need from blood, have established symbiotic relationships with various microorganisms and have formed special organs for them.

The reproductive system of many parasites has been hypertrophied to produce tremendous numbers of eggs. Other parasites, such as the trematodes, have

developed life cycles in which the larvae also multiply.

In the parasites with high reproductive rates, infection is left largely to chance. Many other parasites, however, have developed life cycles in which chance is more or less eliminated. In these, the reproductive rate is low. The larva of the sheep ked, *Melophagus ovinus*, develops to maturity in the body of its mother and pupates immediately after emerging. The pupa remains in its host's wool. The female tsetse fly, too, produces fully developed larvae. The tropical American botfly, *Dermatobia hominis*, captures a mosquito and lays her eggs on it. These hatch when the mosquito lights to suck blood, and the larvae enter the host.

Morphological and developmental modifications are the most obvious ones, but biochemical ones are even more important. How do parasites survive in their hosts without destruction? What keeps those which live in the intestine from being digested along with the host's food? Why is it that morphologically similar species are restricted to different hosts which themselves may be morphologically quite similar?

The second question has been answered by saying that the same mechanism operates which prevents the hosts from digesting themselves, that the parasites protect themselves by producing mucus or that mucoproteins in their integument protect them, that they secrete antienzymes, or that the surface membrane of living organisms is impermeable to proteolytic enzymes. However, much more research must be done before a satisfactory answer can be given. Answers given to the first and third questions are vague. Compatibility of host and parasite protoplasm is invoked, but all this does is put a name to the beast. The question of how this compatibility is brought about remains unanswered, and a great deal of biochemical and immunochemical research must be done before it can be answered (see Becker, 1953; Read, 1950; von Brand, 1952).

Injurious effects of parasites on their hosts. Parasites may injure their hosts in several ways:

1. They may suck blood (mosquitoes, hookworms), lymph (midges) or exudates (lungworms).
2. They may feed on solid tissues, either directly (giant kidney worms, liver flukes) or after first liquefying them (chiggers).
3. They may compete with the host for the food it has ingested, either by ingesting the intestinal contents (ascarids) or by absorbing them thru the body wall (tapeworms). In some cases they may take up large amounts of certain vitamins selectively, as the broad fish tapeworm does with Vitamin B_{12}.
4. They may cause mechanical obstruction of the intestine (ascarids), bile ducts (ascarids, fringed tapeworm), blood vessels (dog heartworm), lymphatics (filariids), bronchi (lungworms) or other body channels.
5. They may cause pressure atrophy (hydatid cysts).
6. They may destroy host cells by growing in them (coccidia, malaria parasites).
7. They may produce various toxic substances such as hemolysins, histolysins, anticoagulants, and toxic products of metabolism.
8. They may cause allergic reactions.
9. They may cause various host reactions such as inflammation, hypertrophy, hyperplasia, nodule formation, etc.
10. They may carry diseases and parasites, including malaria (mosquitoes), trypanosomosis (tsetse flies), swine influenza (lungworms), salmon poisoning of dogs (flukes), heartworms (mosquitoes) and onchocercosis (blackflies).
11. They may reduce their hosts' resistance to other diseases and parasites.

A great deal more could be said about this subject. Additional information is given in the symposium on mechanisms of microbial pathogenicity of the Society for General Microbiology (Howie and O'Hea, 1955).

Resistance and Immunity to Parasites. This is such a tremendous subject that its facets can only be hinted at. The general principles of immunology apply to animal parasites as much as they do to bacteria, viruses and other microorganisms. However, since the association of many of the larger parasites with their hosts is not as intimate as that of microorganisms, the hosts' immune responses may not be as great. This is especially true with regard to the formation of circulating antibodies.

Immunity or resistance may be either *natural* (*innate*) or *acquired*. Natural resistance is the basis of host-parasite specificity, but, as mentioned above, little is known of its mechanism. Acquired immunity may be either *active* or *passive*. Active immunity results from the body's own action. It follows exposure to living or dead disease agents, and can result from natural infection or artificial administration of virulent, attenuated or killed organisms.

One type of active immunity is *premunition*. This is immunity due to the continued presence of the disease agent. It occurs in such diseases as babesiosis and anaplasmosis.

Passive immunity results from the introduction of antibodies produced by some other animal. It may be acquired naturally, thru the colostrum or milk in mammals or thru the egg yolk in birds, or artificially by injection of antiserum. Passive immunity is seldom as long-lasting as active immunity.

Immunity against parasites and disease agents generally increases with age. There are exceptions, however. Young cattle, for instance, are more resistant to *Babesia* and *Anaplasma* than are adults. Age immunity may be either developed as the result of previous exposure or it may be natural. Not all the factors operating in the latter case are known. An important one is that very young animals cannot mobilize their body defenses against invasion as efficiently as adults. For instance, they do not produce antibodies at first, depending on those acquired from their mothers. Another factor, discovered by Ackert and his co-workers (cf. Ackert, Edgar and Frick, 1939) to explain the relative resistance of older chickens to *Ascaridia galli*, is that these birds have more intestinal goblet cells than do young birds. The goblet cells secrete mucus which inhibits the development of the worms. For further information on immunity in parasitic infections, see Taliaferro (1929), Culbertson (1941) and Soulsby (1960).

Genetic constitution is also important in determining resistance to parasites. For instance, Ackert *et al.* (1935) showed that Rhode Island Red and Plymouth Rock chickens are more resistant to *Ascaridia galli* than are Buff Orpingtons, Minorcas and White Leghorns. Cameron (1935) found that in a mixed flock of sheep, Cheviots were less heavily parasitized with gastrointestinal nematodes than Shetlands and Scottish Blackface, and that these in turn were less heavily parasitized than Border Leicesters. Stewart, Miller and Douglas (1937) found that Romney sheep were markedly resistant to infection with *Ostertagia circumcincta*, while Rambouillets were less so and Southdowns, Shropshires and Hampshires were least resistant. Certain individuals among the more susceptible breeds, however, were just as resistant as the Romneys. Whitlock (1958) has studied genetic resistance to trichostrongylidosis in sheep in some detail.

The nutritional status of the host may affect its resistance. Poorly nourished animals are usually more susceptible to infection and suffer more severely from its effects. Protein depletion or protein starvation is particularly important. Lack of specific vitamins and minerals generally decreases resistance, but there are cases in which lack of a certain vitamin which the parasite requires may affect the parasite adversely. Thus, Becker and Smith (1942) found that when calcium pantothenate was added to a ration containing restricted vitamins B_1, B_6 and pantothenate, the number of oocysts produced by *Eimeria nieschulzi* infections in the rat was increased.

Geographic Distribution. Some parasites, particularly those of man and his domestic animals, are worldwide in distribution, but others are much more restricted. But even a widely distributed species may be much more prevalent in one region than another. Many factors are responsible, some of which have already been discussed (pp. 7-8). A parasite which originated in a particular place in a particular host species may never have been introduced into some other locality or host where it could develop perfectly well. It may have been introduced but may have died out because a suitable vector was lacking or because the climate was not suitable. The ox warble has not been able to establish itself in the southern hemisphere because the reversal of seasons has prevented it from completing its life cycle.

Whenever domestic animals are introduced into a new region, there is a good possibility that they will pick up some of the parasites of their wild relatives there. The parasite spectrum of cattle in Africa differs from that in North America, both of these differ from the spectrum in Europe, and all three differ from the spectrum in Australia. Wild animals, too, may acquire parasites from domestic ones or from other wild species. Hence, the parasite spectrum of animals in zoos may be quite different from that in their normal habitat, and the success of an attempt to introduce a new game bird or mammal into a region may depend in part on the parasites and diseases that it encounters.

The importance of wildlife as a parasite reservoir for domestic animals is well illustrated by the report of Longhurst and Douglas (1953) on the interrelationships between the parasites of domestic sheep and Columbian black-tailed deer in the north coastal part of California, where the two live on the same range. They found in their survey of 63 sheep and 81 deer that 1 species of trematode, 5 of cestodes, and 13 out of 18 species of nematodes were common to both hosts.

Origin of Parasitism. Parasites originated from free-living ancestors. The process probably began soon after the first living forms appeared. The change from a free-living to a parasitic habitat has taken place many times in the course of evolution. It has occurred as new major groups appeared, it has taken place independently many times in each group, and it is undoubtedly still occurring. Once established, the parasites evolved along with their hosts.

In some cases, the parasites first invaded the host thru the integument, like *Pelodera* and related rhabditid nematodes. In other cases, the parasites were swallowed along with their host's food. Parasites with life cycles involving two or more hosts became established first in one host, and later on developed their more complicated life cycles. The trypanosomes, for instance, were originally gut parasites of insects and only later became blood parasites of vertebrates.

Preadaptation was necessary for parasites to become established. They must have had the ability to survive and reproduce in the host before they entered it. By far the great majority of free-living forms which entered the alimentary canal of some larger animal were killed and digested, but some of them were able to resist this process and a few were able to live there. Some of the factors involved have already been discussed (p. 10).

Economic Importance. Parasites are responsible for heavy economic losses to the livestock industry. These are due in part to death, but even more important are the losses due to illness, reduced growth rate, decreased meat, milk, egg and wool production and, in working animals, loss of working energy. It is impossible to quantitate these losses accurately, but rough estimates can be made. The U. S. Department of Agriculture (1954) made such an estimate for losses in agriculture during the ten-year period, 1942-1951. The figures on parasite losses in Table 1 are taken from this publication. Further details are given in the publication itself and by Schwartz *et al.* (1955).

TABLE I

ANNUAL LOSSES DUE TO PARASITES OF LIVESTOCK IN THE U.S., 1942-1951 (from USDA, 1954)

Class of Livestock	Average Annual Value of Production	Annual Losses	
		Dollars	% of Production
Cattle	$3,431,539,000	$420,658,000	12.3
Sheep	404,162,000	64,626,000	16.0
Goats	16,375,000	1,886,000	11.5
Swine	3,473,817,000	279,826,000	8.1
Horses and Mules	835,852,000*	26,320,000	3.1
Poultry	3,149,002,000	126,532,000	4.0
All Livestock (screw-worms only)		20,000,000	
TOTAL	$11,310,747,000	$939,848,000	8.3

*Average annual value of animals.

Parasites caused an estimated loss of $939,848,000 per year. All other diseases, both infectious and nutritional, were estimated to cause a total annual loss of $1,748,594,000, so parasites are considered to be responsible for about 35% of the losses in the American livestock industry. A billion dollars a year is a sizeable figure. We can hardly expect to eliminate this loss completely, but if every animal owner took advantage of our present knowledge, a half billion dollars a year, or even more, could be saved.

Scientific Names. There are several million species of animals in the world. Many of them are well enough known and easy enough to recognize to have received common names. However, these names vary from one language to another and from one locality to another among people who speak the same language. Furthermore, the same common name is often applied to different species in different regions.

In the United States, "cattle" refers to the ox, *Box taurus*, but in India it refers to the zebu, *Bos indicus*, and in England and some other countries (and in the Bible) to domestic livestock in general. "Fowl" has more than one meaning. It may refer to the chicken, *Gallus domesticus*, but it may refer to any bird raised for food, including the turkey, *Meleagris gallopavo*, and ducks. Most domestic ducks are *Anas platyrhynchos*, but the Muscovy duck is *Cairina moschata*. One of the worst offenders is "rabbit" which is applied indiscriminately to many quite different species. Rabbits are not rodents, but lagomorphs; they have four upper incisors, whereas rodents have only two. The domestic rabbit is the common wild rabbit of Europe, *Oryctolagus cuniculus*. The common wild rabbit of North America, however, is the cottontail, *Sylvilagus*, of which there are 13 species. In addition, there are several species of jack rabbits belonging to the genus *Lepus*. A list of scientific names of domestic and common wild animals is given in Appendix I.

In order to prevent the confusion which would be inevitable in dealing with these myriad species, a system of scientific names has been worked out. This system was first established by Linnaeus in the eighteenth century, and the starting point for the names of animals is the tenth edition of Linnaeus' *Systema Naturae*, which was published in 1758. An International Code of Zoological Nomenclature was adopted in 1904; it was reviewed at a colloquium held in Copenhagen in 1953, and a new, revised code was adopted by another colloquium held in London in 1958. This code establishes rules for naming animal species and for indicating their relationships.

In the system of *binomial nomenclature* used for scientific names, each species is given two names. The first name, which is capitalized, is used for a group of closely related species; this group is called a and its name is the *generic name*. The second name, which is not capitalized, is used for a single species within the genus and is called the *specific name*. A particular generic name can be used for only a single group of species in the animal kingdom, but the same specific name can be applied to species in different genera. The generic and specific names are often derived from Latin or Greek, but they may also be based

on the names of persons, geographic localities, etc. They must, however, have latinized endings. Both names are written in italics.

The name of the person who first named each species and the date when he did it are also part of its scientific name, altho these are often omitted in non-taxonomic writing. If the namer assigned the species to a different genus from the one which is accepted as correct, then his name and date are enclosed in parentheses and are followed outside the parentheses by the name of the person who assigned the species to its present genus with the date when he did it. If there has been no change in the genus designated by the original author, parentheses are not used. Thus, the common large roundworm of the dog, *Toxocara canis*, was first described by Werner in 1782, but he assigned it to the same genus as the earthworm, *Lumbricus*. In 1905, Stiles established a new genus, *Toxocara*, for this species. The original name, then, was *Lumbricus canis* Werner, 1782, and the presently accepted name is *Toxocara canis* (Werner, 1782) Stiles, 1905. Similarly, in the early days of parasitology almost all tapeworms were assigned to a single genus, *Taenia*. As knowledge increased, more and more genera were split off from it. The common sheep tapeworm was called *Taenia expansa* by Rudolphi in 1805, but in 1891 Blanchard established a new genus, *Moniezia*, for it, so that its correct name is now *Moniezia expansa* (Rudolphi, 1805) Blanchard, 1891.

Genera are grouped together into families, families are grouped into orders, orders into classes, and classes into phyla. Each of these categories, and also each of the lower ones, is known as a *taxon* (pl., *taxa*). Subfamilies and superfamilies, suborders and superorders, etc. are often used, and in some cases so many relationship levels are recognized that it is necessary to introduce cohorts, tribes, etc.

Each family is based on one of its genera, known as the *type genus*, and the name of the family is obtained by attaching the ending, -idae, to the root of the name of the genus. Thus, *Strongylus* belongs to the family Strongylidae, and *Trichomonas* to the family Trichomonadidae. The subfamily ending is -inae.

While the botanists long ago adopted a system of uniform endings for the names of their higher taxa, the zoologists have never been able to agree on one. As a consequence, it is impossible to determine the ranks of the higher taxa with certainty from their names. In the present book, however, the system of uniform endings proposed by Levine (1959) is used, so this problem does not arise. These are: Superclass, -asica; Class, -asida; Subclass, -asina; Superorder, -orica; Order, -orida; Suborder, -orina; Supercohort, -icohica; Cohort, -icohida; Subcohort, -icohina; Superfamily, -icae; Family, -idae; Subfamily, -inae; Supertribe, -ibica; Tribe, -ibida; Subtribe, -ibina.

Many scientific names appear quite formidable at first glance. They have definite meanings, however, and it helps in remembering them to know what these meanings are. Since most scientific names are based on Latin or Greek, a knowledge of some of the descriptive words from these languages is helpful. Much information can be obtained from a dictionary of derivations such as that of Jaeger (1955). The thorny-headed worm of swine is *Macracanthorhynchus hirudinaceus*. This name is derived from the Greek. The generic name means "large (*macr-*) thorny (*acantho-*) proboscis (*-rhynchus*)." The specific name is derived from the scientific name of the leech (*Hirudo*) and means "leechlike"; it was given because the worm is firmly attached to the intestinal wall and looks vaguely like a leech. The name of the whipworm, *Trichuris*, commemorates an error. This nematode looks a good deal like a buggy-whip, with a sturdy body and a long, whip-like anterior end about as thick as a hair. T[illegible]me, however, means hair-tail an[illegible]ir-head. This mistake was so offe[illegible] some scientists that they propos[illegible]o substitute *Trichocephalus* for *Trichuris*. This is not permissible according to the rule of priority of the International Code of Zoological Nomenclature, so the error remains.

It is often discouraging to students and scientists alike to see the many changes in scientific names which continue to be made. These, however, appear to be inevitable. As new knowledge is gained, some species must be split up, others recombined and still others shifted from one genus to another. It is sometimes found that a name which has been long used and accepted must be dropped in favor of an unfamiliar one, either because it had been used first for some other species or because the less familiar name had been given earlier but overlooked.

Another reason for these changes lies in human nature itself. No satisfactory criteria have ever been established for the definition of species, and some taxonomists go into finer differences than others in separating them.

The taxonomists' difficulties arise because what they are dealing with are individual organisms, and all taxonomic schemes are the result of man's attempts to arrange these individuals in a system which shows their relationships. All taxa, whether species, subspecies, genera, families or whatnot, are products of this abstraction process and have no real existence outside the human mind. Many taxonomists, however, refuse to accept this idea, believing that species are real and external, and that their task is simply to discover and differentiate them. It is easy to understand why they do not like to believe that they are devoting their lives to figments of the imagination.

Without the labors of the systematists we should be in a state of hopeless confusion. Their scientific names and their taxonomic schemes are absolutely necessary if we are to carry out reproducible experimental work or understand practically all biological phenomena.

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Chapter 2

INTRODUCTION TO THE PROTOZOA

Protozoa form the most primitive group in the animal kingdom. The bodies of all other animals are composed of many units, or cells, but those of the protozoa are a single cell. No matter how complex their bodies may be, and many of them are very much so, all the different structures are contained in a single cell. This complexity has made some investigators maintain that, instead of being considered single cells, protozoa should be thought of as non-cellular (see, for example, Boyden, 1957). This argument is essentially a verbal issue--a matter of how one wants to define "cell."

Protozoa are microscopic in size, only a few being visible to the naked eye. They differ from the Metazoa in being unicellular, but this difference is not as clearcut as might be supposed. Some protozoa have a syncytial stage in their life cycle in which there are no cell walls between the nuclei, and some species form colonies which swim as a unit and which contain somatic and reproductive organisms which look different. The difference between these and Metazoa is again partly a matter of definition, and gives a clue to how the Metazoa could have arisen.

The boundary between the Protozoa and certain one-celled plants, too, is not clearcut. For example, the whole group of slime molds are considered by protozoologists to be protozoa and assigned to the order Mycetozoorida in the class Sarcodasida, but botanists consider them fungi and assign them to the class Myxomycetes.

A still more confusing situation involves the plant-like protozoa which contain chlorophyll. Protozoologists assign them to the subclass Phytomastigasina, but botanists consider them green algae. The problem is that there are many species of colorless protozoa which differ from green ones only in that they lack chromatophores. Loss of chromatophores can be produced experimentally. It has

been done in *Euglena*, for instance, by treatment with streptomycin or simply by growing the organisms at 34 to 35° C (Pringsheim and Pringsheim, 1952). This change of a plant into an animal would be just as astounding as the metamorphosis of Cinderella's pumpkin into a golden coach if the differences between the lower forms were as great as those between higher plants and animals. However, the principal difference is one of nutrition, and many species are quite plastic, their form of nutrition depending on circumstances. Indeed, many of the metabolic pathways of the phytoflagellate, *Ochromonas malhamensis*, aside from those in which its chlorophyll takes part, are so similar to those of men that Hutner has facetiously called it a humanoid!

In recognition of this situation, Ernst Haeckel proposed that the name Protista be applied to all single-celled organisms and that the group be considered intermediate between the animal and plant kingdoms. Relatively few modern taxonomists subscribe to this idea, perhaps less because of any defect in the idea itself than because they have been trained either as botanists or zoologists and not as biologists.

Since their discovery by Leeuwenhoek, some 30,000 species of protozoa have been described. They occur in practically all habitats where life can exist and are among the first links of the food chain on which all higher life depends. Floating in the plankton of tropic seas, they cause the luminous glow of waves and ship-wakes. Blooming off our coasts, they cause the red tide which deposits windrows of dead fish on shore. They abound in ponds and streams and in the soil. Their role in sewage purification is just beginning to be understood. Their skeletons cover the ocean floor and form the chalk we use in classrooms.

As parasites, protozoa play a double role. Malaria is still the world's most important disease. Trypanosomes have interdicted vast African grazing lands for livestock. Amoebae cause dysentery in man, and coccidia cause it in his domestic animals. But other protozoa, packing the termite's hind-gut almost solidly, digest the cellulose that it eats and feed it with their wastes and dead bodies. Fabulous numbers of protozoa swarm in the rumen and reticulum of cattle and sheep and in the cecum and colon of the horse, but their role is still not clear.

In this book we are concerned with the protozoan parasites of domestic animals. Our understanding of these forms can be enhanced by knowledge of the parasites of other animals and of free-living forms. For further information on the protozoa in general, reference is made to Doge'l (1951), Grassé (1952-53), Grell (1956), Hall (1953), Hyman (1940), Kudo (1954), Reichenow (1949-53) and Wenyon (1926).

STRUCTURES

The structures of protozoa are not referred to as organs as in higher animals but as *organelles*, organs being composed of cells and organelles being differentiated portions of a cell.

NUCLEI

Protozoa contain one or more nuclei, which may be of several types. In the protozoa other than ciliates, the nucleus is *vesicular*, and all the nuclei in the same individual look alike. There are two types of vesicular nuclei. In one type, an *endosome* is present. The endosome is a more or less central body with a negative Feulgen reaction and therefore without deoxyribonucleic acid. The chromatin, which is Feulgen positive and which forms the chromosomes, lies between the nuclear membrane and the endosome. This type of nucleus is found in the trypanosomes, parasitic amoebae and phytoflagellates. In the other type of vesicular nucleus, there is no endosome, but there may be one or more Feulgen-positive *nucleoli*. In these, the chromatin is distributed thruout the nucleus. This type of nucleus is found in the Telosporasida, hypermastigorid flagellates, opalinids, dinoflagellates, and radiolaria.

In the ciliates there are two types of nucleus which look different, and each individual has at least one of each. The *micronucleus* is relatively small; it divides by mitosis at fission and apparently controls the reproductive functions of the organism. The *macronucleus* is relatively large; it divides amitotically at fission and apparently has to do with the vegetative functions of the organism. Both these nuclei appear quite homogeneous in composition in contrast to the vesicular nuclei of other protozoa.

LOCOMOTION

Protozoa move by means of flagella, cilia or pseudopods. A *flagellum* is a whip-like organelle composed of a central *axoneme* and an outer *sheath*. The axoneme arises from a *basal granule* or *blepharoplast* in the cytoplasm. The axoneme has been shown by electron microscopy to be composed of 9 peripheral and 2 central fibrils. In some species a flagellum may pass backward along the body, being attached to it along its whole length or at several points to form an *undulating membrane*. Flagella are found in the Mastigasida and in the flagellate stages of the Sarcodasida and Telosporasida.

A *cilium* is an eyelash-like organelle resembling a small flagellum. It has a sheath, basal granule and axoneme. In *Paramecium* and other forms, the axoneme is composed of 9 peripheral and 2 central fibrils. Cilia are found in the Ciliasida. The less specialized ciliates have large numbers of cilia which are arranged in rows and beat synchronously. In the more specialized ciliates, special locomotory organelles have been developed by fusion of cilia. A *cirrus* is a tuft of fused cilia embedded in a matrix. A *membranelle* is a more or less triangular flap formed by the fusion of two or more transverse rows of cilia; membranelles are found especially around the mouth. An *undulating membrane* (not to be confused with the undulating membrane of flagellates) is formed by the fusion of one or more longitudinal rows of cilia; they occur in the oral groove of some ciliates.

A *pseudopod* is a temporary locomotory organelle which can be formed and retracted as needed. There are four types of pseudopod. A *lobopod* is a relatively broad pseudopod with a dense outer layer and a more fluid inner zone; lobopods are found in the amoebae and some flagellates. A *filopod* is a slender, hyaline pseudopod which tapers from its base to its pointed tip; filopods tend to anastomose and may fuse locally to produce thin films of cytoplasm; they contain no cytoplasmic granules. A *myxopod* (*rhizopod, reticulopod*) is a filamentous pseudopod with a dense inner zone and a more fluid outer layer in which cytoplasmic granules circulate; myxopods branch and anastomose to form complex networks which are used for trapping food and also for locomotion; they are found in the Foraminiferorida. An *axopod* is a slender pseudopod which projects from the body without branching or anastomosing; it is composed of a thin outer layer of fluid cytoplasm and an axial filament composed of a fibrillar tube containing a homogeneous core; axopods are found in the Heliozoorida and Radiolariorida.

Locomotion can also be effected by bending, snapping or twisting of the whole body. A number of protozoa employ this method.

There is still another type of locomotion, *gliding*, exemplified by *Toxoplasma*, *Sarcocystis*, coccidian merozoites, gregarines and *Labyrinthula*, in which the body glides smoothly along without benefit of any apparent locomotor organelles, change in shape or other visible cause. Kummel (1958) found by means of electron micrographs that the outer surface of certain gregarines (*Gregarina cuneata, G. polymorpha, Beloides* sp.) is thrown into a series of deep, microscopic folds which he thought produce mucus. Beneath these folds in the pellicle are fibrils which he thought contract to move the organism along a mucous track. Jarosch (1959) thought that the gliding of *Gregarina*, *Euglena* and various single-celled plants is caused by superficial fibrils thrusting against extruded mucus. Beams *et al.* (1959) found numerous ultramicroscopic

folds in the surface membrane and an ultramicroscopic network of fibrils about 50 to 200 Å in diameter in the ectoplasm of the trophozoites of *Gregarina rigida* from the grasshopper. They believed that gliding is probably accomplished by movement of the body surface in contact with the substrate and that the mucus which is secreted may possibly provide a suitable surface for locomotion. Ludvik (1958) observed superficial, longitudinal fibrils in electron micrographs of *Sarcocystis tenella*. However, a definitive explanation of the mechanism of gliding still eludes us.

ORGANELLES ASSOCIATED WITH NUTRITION

Nutrition among the protozoa may be of several types. Rather elaborate classifications have been proposed by some authors, but three types are sufficient for our purposes. In *holophytic* nutrition, which is characteristic of the phytoflagellates, carbohydrates are synthesized by means of chlorophyll which is carried in *chromatophores*, which vary considerably in size, shape and number.

In *holozoic* nutrition, particulate food material is ingested thru a temporary or permanent mouth. A temporary mouth is formed by amoebae when they engulf their food. A permanent mouth is a *cytostome*. It may be simple or it may lead into a *cytopharynx*. In many ciliates the area around the cytostome forms a *peristome*, and there may be a number of other specialized structures associated with it. Particulate food passes into a *food vacuole* in the cytoplasm, where it is digested. The indigestible material may be extruded from the body either thru a temporary opening or thru a permanent *cytopyge*.

In *saprozoic* nutrition, no specialized organelles are necessary, nutrients being absorbed thru the body wall. This type is found in many protozoa, and may be present along with holophytic or holozoic nutrition.

EXCRETORY ORGANELLES

Excretion in the Protozoa is either thru the body wall or by means of a *contractile vacuole* which may be simple or may be associated with a system of feeder canals or vacuoles. Contractile vacuoles are probably more important as osmoregulatory organelles than for excretion. They maintain water balance by removing excess water from the cytoplasm and passing it out of the body. They are found in fresh-water protozoa but are absent in most marine and parasitic protozoa. However, some of the latter, including *Balantidium* and trypanosomes, contain them.

OTHER ORGANELLES

Protozoa have many other specialized organelles which are found in different groups. These will be described in the appropriate places below.

REPRODUCTION AND LIFE CYCLES

Reproduction in the Protozoa may be either asexual or sexual. The commonest type of asexual reproduction is *binary fission*, i.e., each individual divides into two. The plane of fission is longitudinal in the flagellates and transverse in the ciliates. Cytoplasmic division follows nuclear fission and separation of the daughter nuclei. Vesicular nuclei and micronuclei divide mitotically; macronuclei divide amitotically.

Multiple fission or *schizogony* is found mostly in the Telosporasida. In this type of fission, the nucleus divides several times before the cytoplasm divides. The dividing cell is known as a *schizont*, *agamont* or *segmenter*, and the daughter cells are *merozoites* or *schizozoites*. Nuclear division, again, is mitotic.

A third type of asexual division is *budding*. In this process, a small daughter individual is separated off from the

side of the mother and then grows to full size.

Internal budding or *endodyogeny* has been described in *Toxoplasma* and *Besnoitia*. Two daughter cells are formed within the mother cell and then break out, destroying it (Goldman, Carver and Sulzer, 1958).

Several types of sexual reproduction have been described, but only two occur in parasitic protozoa. In *conjugation*, which is found among the ciliates, two individuals come together temporarily and fuse along part of their length. Their macronuclei degenerate, their micronuclei divide a number of times, and one of the resultant haploid pronuclei passes from each conjugant into the other. The conjugants then separate, and nuclear reorganization takes place.

In *syngamy*, two gametes fuse to form a zygote. If the gametes are similar in appearance, the process is called *isogamy*; if they are different, it is *anisogamy*, the smaller gamete being the *microgamete* and the larger one the *macrogamete*. The gametes may be produced by special cells, the *microgametocytes* and *macrogametocytes*, respectively. These are also sometimes called *gamonts*. The zygote may or may not then divide by multiple fission to form a number of *sporozoites*. The process of gamete formation is known as gametogony. It may differ in different groups, and will be described in the appropriate places below.

Some protozoa form resistant cysts or spores. A *cyst* results from the formation of a heavy wall around the whole organism. *Spores* are produced within the organism by the formation of heavy walls around a number of individuals which have been produced by multiple fission or otherwise. This process, known as *sporogony*, ordinarily follows syngamy. Each spore may contain one or more individual organisms or *sporozoites*.

The vegetative, motile stage of a protozoon is known as a *trophozoite*.

HISTORY

The first person to see protozoa was the Dutch microscopist, Antony van Leeuwenhoek (1632-1723). He used simple lenses which he ground himself and which gave magnifications as high as 270 times. His letters to the Royal Society are a classic of biology. Between 1674 and 1716, Leeuwenhoek described many free-living protozoa, among them, according to Dobell (1932), being *Euglena*, *Volvox* and *Vorticella*. Huygens in 1678 was the first to describe *Paramecium*. Classic work on free-living protozoa was done by O. F. Müller (1786), Ehrenberg (1830, 1838) and Dujardin (1841).

The first parasitic protozoon to be discovered was *Eimeria stiedae;* Leeuwenhoek found its oocysts in the gall bladder of an old rabbit in 1674. Later, in 1681, Leeuwenhoek found *Giardia lamblia* in his own diarrheic stools, and in 1683 he found *Opalina* and *Nyctotherus* in the intestine of the frog.

The first species of *Trichomonas*, *T. tenax*, was found by O. F. Müller in 1773 in the human mouth; he named it *Cercaria tenax*. Donné found *T. vaginalis* in the human vagina in 1837, and Davaine found *Trichomonas* and *Chilomastix* in the stools of human cholera patients in 1854.

The first trypanosome was discovered in the blood of the salmon by Valentin in 1841, and the frog trypanosome by Gluge and Gruby in 1842. Lewis found the first mammalian trypanosome, *T. lewisi*, in the rat in 1878. Evans discovered the first pathogenic one, *T. evansi*, in 1881 in India, where it was causing the disease known as surra in elephants. Bruce discovered *T. brucei* in Africa in 1895 and described its life cycle and transmission by the tsetse fly in 1897. In 1902, Dutton discovered that African sleeping sickness of man was caused by *T. gambiense*. *Leishmania tropica* was first seen by Cunningham in India in 1885 and was first described and identified as a protozoon by Borovsky in Russia in 1898. Leishman and Donovan independently discovered *Leishmania donovani* in India in 1903.

Histomonas meleagridis, the cause of blackhead of turkeys, was discovered by Theobald Smith in 1895. Its transmission in the eggs of the cecal worm was discovered by Tyzzer and Fabyan in 1922 and described in detail by Tyzzer in 1934.

The first parasitic amoeba, *Entamoeba gingivalis*, was found in the human mouth by Gros in 1849. Lewis found *E. coli* in India in 1870, and Lösch found *E. histolytica* in Russia in 1875.

Balantidium coli was discovered by Malmsten in 1857.

It was not until 154 years after Leeuwenhoek saw *Eimeria stiedae* that any other telosporasids were found. Then, in 1828, Dufour described gregarines in the intestines of beetles, and in 1838 Hake rediscovered the oocysts of *E. stiedae*. The most extensive early study of the coccidia was that of Eimer (1870), who described a number of species in various animals. Schaudinn and Siedlecki (1897) described the gametocytes and gametes of coccidia and showed that they formed zygotes. Further studies on the life cycle of coccidia were published by Schaudinn in 1898 and 1899. Classic work on the coccidia of gallinaceous birds was done by Tyzzer (1929) and Tyzzer, Theiler and Jones (1932).

The human malaria parasite was discovered in 1880 by the French army doctor, Alphonse Laveran. Golgi (1886, 1889) reported on its schizogony and distinguished the types of fever caused by the different species. MacCallum (1897), working with the closely related *Haemoproteus* of birds, recognized that the exflagellation which had been seen by Laveran was microgamete formation, and later observed fertilization and zygote formation in *Plasmodium falciparum*.

Ross worked out the life cycle of the bird malaria parasite, *Plasmodium relictum* (*P. praecox*), in India in 1898, showing that it was transmitted by the mosquito, *Culex fatigans*. Working independently in Italy, Grassi and his collaborators (1898) almost immediately afterward found that human malaria is transmitted by *Anopheles* mosquitoes.

Babesia bovis was discovered by Babes in 1888. Theobald Smith and Kilborne described the cause of Texas fever of cattle, *B. bigemina* in 1893; they showed that it was transmitted by the tick, *Boophilus annulatus*, being passed thru its eggs to the next generation of ticks which then infected new cattle. This was the first demonstration of arthropod transmission of a protozoon.

The present century has seen many advances in protozoology, but there are many more ahead. Several times more species of parasitic protozoa have been described since 1900 than were known before, but these are only a fraction of the total number. Exciting new discoveries are being made every year on the physiology and nutritional requirements of protozoa (Lwoff, 1951; Hutner and Lwoff, 1955), and the life cycles, host-parasite relations, and pathogenesis of many species are only now being worked out. The electron microscope and the phase microscope have opened up a whole new field for morphologic study, chemotherapy is progressing rapidly, and new discoveries are being made even in taxonomy, which most people used to consider a dead field.

CLASSIFICATION

Various classifications have been proposed for the Protozoa. They have been discussed by Hall (1953) and also by Biocca (1957). The classification used in the present book is based on those used by Jahn and Jahn (1949) and Hall (1953), with certain modifications; the classification of the Ciliasida is based on Corliss (1956, 1959). The uniform endings for the names of higher taxa proposed by Levine (1959) are used. Most of the groups not of veterinary or medical interest are omitted. In addition, some genera

once thought to be protozoa but now known to be otherwise are not included. Among these are *Anaplasma*, *Eperythrozoon* and *Haemobartonella*, all of which are rickettsiae; *Bartonella*, which is a bacterium; and *Pneumocystis*, which is a yeast.

Class MASTIGASIDA

With 1 or more flagella. Nucleus vesicular.

Subclass PHYTOMASTIGASINA

Typically with chromatophores. Nutrition typically holophytic.

Order CHRYSOMONADORIDA

With 1 to 3 flagella. Chromatophores, if present, yellow, brown, orange, or occasionally blue. Stored reserves include leucosin (presumably a polysaccharide) and lipids, but no starch.

Suborder EUCHRYSOMONADORINA

Flagellate stage dominant. Without siliceous skeleton or peripheral zone of coccoliths.

Family CHROMULINIDAE

With 1 flagellum.

Caviomonas
Oikomonas
Sphaeromonas

Family OCHROMONADIDAE

With 1 long and 1 short flagellum.

Monas

Family PRYMNESIIDAE

With 3 flagella.

Prymnesium

Order EUGLENORIDA

With 1 to 4 flagella. Chromatophores, if present, green. Stored reserves composed of paramylum.

Suborder EUGLENORINA

Pellicle rigid. Flagellar sheath not swollen at base. Seldom holozoic.

Family ASTASIIDAE

Without chromatophores or stigma. With 1 flagellum. Body highly plastic, altho usually elongate spindle-shaped.

Copromonas

Order DINOFLAGELLORIDA

With 2 flagella, 1 of which is transverse. Marine forms.

Suborder GYMNODINIORINA

Unarmored dinoflagellates (without theca).

Family GYMNODINIIDAE

With well-developed girdle and sulcus. Transverse flagellum typically flattened. Tentacle and ocellus absent.

Gymnodinium

Suborder PERIDINIORINA

With a theca of a cellulose-like material, composed of separate plates.

Family GONYAULACIDAE

Thecal plates distinct. One antapical plate is characteristic.

Gonyaulax

Order PHYTOMONADORIDA

With 1 to 8 flagella. Typically with 1 green chromatophore. Body wall contains cellulose. Starch and lipids stored as food.

Family CHLAMYDOMONADIDAE

Solitary, with a well-developed membrane.

Polytoma

Subclass ZOOMASTIGASINA

Without chromatophores. Nutrition holozoic or saprozoic.

Order RHIZOMASTIGORIDA

With both flagella and pseudopods.

Family MASTIGAMOEBIDAE

With 1 to 3, rarely 4 flagella.

Histomonas

Order PROTOMASTIGORIDA

With 1 or 2 flagella.

Family TRYPANOSOMATIDAE

With 1 flagellum. Body characteristically leaf-like but may be rounded. With a single nucleus and a kinetoplast. With a basal granule from which a flagellum arises. Exclusively parasitic.

Blastocrithidia
Crithidia
Herpetomonas
Leishmania
Leptomonas
Phytomonas
Trypanosoma

Family BODONIDAE

With 2 flagella originating anteriorly, one directed anteriorly and the other posteriorly. Anterior end more or less drawn out. With 1 to several contractile vacuoles.

Bodo
Cercomonas
Pleuromonas
Proleromonas

Family AMPHIMONADIDAE

Body naked or loricate, with 2 equal flagella.

Spiromonas

Order POLYMASTIGORIDA

With 3 to about 12 flagella (2 in *Retortamonas*) and 1, 2 or several nuclei. Without costa, axostyle (except in some Hexamitidae and Polymastigidae) or parabasal body.

Family TETRAMITIDAE

With 4 flagella, 1 or 2 of which may be trailing.

Enteromonas
Tetramitus

Family RETORTAMONADIDAE

With 2 or 4 flagella, of which 1 is trailing. With 1 nucleus. Cytostome with supporting fibrils present.

Chilomastix
Retortamonas

Family CALLIMASTIGIDAE

With a compact antero-lateral group of flagella which beat as a unit. With 1 nucleus.

Callimastix
Selenomonas

Family POLYMASTIGIDAE

With 4 anterior flagella and axostyle. With 1 nucleus. Apparently without parabasal body.

Monocercomonoides

Family COCHLOSOMATIDAE

With 6 anterior flagella, 1 axostyle and a single nucleus. Apparently without parabasal body.

Cochlosoma

Family HEXAMITIDAE

With 6 or 8 flagella, 2 nuclei and sometimes axostyles and median or parabasal bodies. Bilaterally symmetrical.

Giardia
Hexamita
Octomitus
Trepomonas

Order TRICHOMONADORIDA

With 3 to 6 flagella, of which 1 is trailing and may form part of an undulating membrane. With 1 or many nuclei (the forms in vertebrates have only 1 nucleus), but not with 2. With axostyle and parabasal body.

Family MONOCERCOMONADIDAE

With either a free or an adherent trailing flagellum but no undulating membrane or costa.

Chilomitus
Hexamastix
Monocercomonas
Protrichomonas

Family TRICHOMONADIDAE

With an undulating membrane and a costa. Sometimes with a pelta.

Pentatrichomonas
Trichomonas
Tritrichomonas

Order HYPERMASTIGORIDA

With many flagella, 1 nucleus and often multiple axostyles and parabasal bodies. Intestinal parasites of termites and roaches.

Trichonympha

Class SARCODASIDA

With pseudopods but without flagella or cilia. Nucleus vesicular.

Subclass RHIZOPODASINA

With lobopods, filopods or myxopods but without axopods.

Order AMOEBORIDA

With lobopods. Without test.

Family NAEGLERIDAE

With amoeboid and flagellate stages.

Naegleria
Trimastigamoeba

Family AMOEBIDAE

Free-living or coprozoic amoebae without a flagellate phase.

Acanthamoeba
Harlmannella
Sappinia
Vahlkampfia

Family ENDAMOEBIDAE

Parasites in the digestive tract of vertebrates and invertebrates.

Dienlamoeba
Endamoeba
Endolimax
Entamoeba
Iodamoeba

Order TESTACEORIDA

With a single-chambered test.

Family ARCELLIDAE

Test simple and membranous. Pseudopods filose or simply branched.

Chlamydophrys

Class TELOSPORASIDA

With simple spores containing 1 to many sporozoites but without polar filaments. Without pseudopods, cilia or flagella (except for flagellated microgametes in some groups). Locomotion by body flexion or gliding. Reproduction both sexual and asexual. All parasitic.

Subclass GREGARINASINA

Mature trophozoite extracellular, large. Parasites of digestive tract and body cavity of invertebrates.

Subclass COCCIDIASINA

Mature trophozoite ordinarily intracellular, small.

Order EUCOCCIDIORIDA

Parasites of epithelial and blood cells of vertebrates and invertebrates. Life cycle involves both sexual and asexual phases. Schizogony present.

Suborder ADELEORINA

Macrogamete and microgametocyte associated in syzygy during differentiation. Microgametocyte usually produces few microgametes. Sporozoites enclosed in an envelope. Monoxenous or heteroxenous.

Superfamily ADELEICAE

Zygote inactive, may or may not develop a typical oocyst.

Family ADELEIDAE

Sporocysts formed in oocyst. In epithelium of gut and its appended organs. Chiefly in invertebrates.

Klossia

Family KLOSSIELLIDAE

Typical oocyst not formed; a number of sporocysts, each with many sporozoites, develops within a membrane which is perhaps laid down by the host cell. Two to 4 non-flagellate microgametes formed by microgametocyte. Monoxenous, gametogony and schizogony occurring in different locations in the same host. In kidney and other organs of host.

Klossiella

Superfamily HAEMOGREGARINICAE

Zygote active (ookinete), secreting a flexible membrane which is stretched during development. Heteroxenous. Life cycle involves 2 hosts, one vertebrate and the other invertebrate. In cells of circulatory system of vertebrates and digestive system of invertebrates.

Family HAEMOGREGARINIDAE

Oocysts small, without sporocysts.

Haemogregarina

Family HEPATOZOIDAE

Oocysts large, containing many sporocysts, each with 4 to 12 or more sporozoites. Microgametes non-flagellate.

Hepatozoon

Family KARYOLYSIDAE

Sporoblasts become sporokinetes which invade the egg of a mite before secreting sporocyst membrane. Sporocysts with numerous sporozoites. Gametocytes in erythrocytes of vertebrate host.

Karyolysus

Suborder EIMERIORINA

Macrogamete and microgametocyte develop independently. Syzygy absent. Microgametocyte typically produces many microgametes. Zygote not motile. Sporozoites typically enclosed in a sporocyst. Monoxenous or heteroxenous.

Family EIMERIIDAE

Development in host cell proper. Oocysts and schizonts without attachment organ. Oocysts with 0, 1, 2, 4 or many sporocysts, each with 1 or more sporozoites. Monoxenous. Schizogony in the host, sporogony typically outside. Microgametes with 2 flagella.

Dorisiella
Eimeria
Isospora
Tyzzeria
Wenyonella

Family CRYPTOSPORIDIIDAE

Development on the surface of the host cell or within its striated border and not in the cell proper. Oocysts and schizonts with a knob-like attachment organ at some point on their surface. Oocysts without sporocysts. Monoxenous. Microgametes without flagella.

Cryptosporidium

Family AGGREGATIDAE

Development in host cell proper. Oocysts typically with many sporocysts. Heteroxenous. Schizogony in one host, sporogony in another.

Merocystis

Family LANKESTERELLIDAE

Development in host cell proper. Oocysts without sporocysts, but with 8 or more sporozoites. Heteroxenous, with schizogony, gametogony and sporogony in a vertebrate host. Sporozoites in blood cells, transferred without developing by an invertebrate (mite or leech). Microgametes with 2 flagella, so far as is known.

Lankesterella
Schellackia

Suborder HAEMOSPORORINA

Macrogamete and microgametocyte develop independently. Syzygy absent. Microgametocyte produces moderate number of microgametes. Zygote motile (ookinete). Sporozoites naked. Heteroxenous. Schizogony in vertebrate host, sporogony in invertebrate. Pigment (hematin) formed from host cell hemoglobin.

Family PLASMODIIDAE

With the characters of the suborder.

Haemoproteus
Hepatocystis
Leucocytozoon
Plasmodium

Class PIROPLASMASIDA

Small, piriform, round, amoeboid or rod-shaped parasites of vertebrate erythrocytes and also in some cases of leucocytes or histiocytes. Pigment (hematin) not

formed from host cell hemoglobin. Without spores. Nucleus vesicular. Without flagella or cilia. Locomotion by body flexion or gliding. Reproduction asexual, by binary fission or schizogony. Existence of sexual reproduction dubious. All parasitic. Heteroxenous. Vectors (if known), ixodid or argasid ticks.

Order PIROPLASMORIDA

With the characters of the class.

Family BABESIIDAE

Relatively large, piriform, round or oval parasites occurring in erythrocytes of vertebrate host. Asexual reproduction in erythrocytes by binary fission or schizogony.

Aegyptianella
Babesia
Echinozoon

Family THEILERIIDAE

Relatively small, round, oval, irregular or rod-shaped parasites of erythrocytes and lymphocytes or histiocytes of vertebrate host. The forms in the erythrocytes may or may not reproduce; in the former case they divide into 2 or 4 daughter cells. Asexual reproduction by schizogony (or a series of binary fissions) in lymphocytes or histiocytes followed by invasion of erythrocytes.

Cytauxzoon
Gonderia
Theileria

Class TOXOPLASMASIDA

Without spores. With cysts or pseudocysts containing many naked trophozoites. Nucleus vesicular. Without flagella or cilia. Locomotion by body flexion or gliding. Reproduction asexual, by binary fission or endodyogeny (and possibly by schizogony in young cysts). All parasitic. Monoxenous.

Order TOXOPLASMORIDA

With the characters of the class.

Family SARCOCYSTIDAE

With cysts. Multiplication by binary fission, and possibly also by schizogony in young cysts.

Sarcocystis

Family TOXOPLASMATIDAE

With pseudocysts and probably true cysts as well. Multiplication by binary fission or endodyogeny and possibly by schizogony in young pseudocysts.

Besnoitia
Encephalitozoon
Toxoplasma

Class CNIDOSPORASIDA

With spores containing polar filaments. Nucleus vesicular. All parasitic.

Order MYXOSPORORIDA

Spores comparatively large, with bivalve shell and 1 to 4 polar capsules. Parasites of lower vertebrates, especially fish.

Order MICROSPORORIDA

Spores comparatively small, with 1-piece shell and 1 or 2 polar filaments. Typically parasites of invertebrates and fish.

Class CILIASIDA

With two types of nucleus--macronucleus and micronucleus. With cilia at some stage of the life cycle.

Subclass HOLOTRICHASINA

Without or with poorly developed adoral zone of membranelles (except in Peritrichorida).

Order GYMNOSTOMORIDA

Cytostome opens directly at surface or else into a slight depression which lacks well-developed peristomial ciliature.

Family BUETSCHLIIDAE

Cytostome usually at anterior end. Anterior concretion-vacuole (possibly a statocyst), one or more contractile vacuoles and posterior cytopyge present. Cilia uniformly distributed over body or restricted to certain areas.

Alloiozona
Ampullacula
Bundleia
Didesmis

Blepharoconus
Blepharoprosthium
Blepharosphaera
Blepharozoum
Buetschlia
Holophryoides
Paraisotrichopsis
Polymorphella
Prorodonopsis
Sulcoarcus

Family PYCNOTRICHIDAE

Body completely ciliated. A long groove usually leads to the cytostome, which may lie near the middle or at the posterior end of the body.

Buxtonella
Infundibulorium

Order SUCTORIORIDA

Young with cilia; adults with tentacles.

Family ACINETIDAE

With endogenous budding. Tentacles capitate, usually arranged in groups. Lorica often present. Stalk present or absent.

Allantosoma

Order TRICHOSTOMORIDA

Cytostome usually at base of well-defined oral groove or pit, the wall of which bears 1 or more dense fields of adoral cilia; in some primitive forms the cytostome is almost at the anterior end, but more often it is shifted posteriorly on the ventral surface. Spiral torsion of the body occurs in some genera.

Family BLEPHAROCORYTHIDAE

Somatic ciliation reduced to a few anterior and posterior fields, with 1 or 2 groups of anal cilia near the cytopyge and 2 or 3 distinct anterior groups. Cytostome anteroventral, opening into a long ciliated pharynx.

Blepharocorys
Charonina
Qchoterenaia

Family CYATHODINIIDAE

Cilia limited to anterior half of body. Peristome a nonciliated, rather long triangular groove. Slender trichites

extend from a row of papillae along left rim of peristome, and an adoral cilium arises from each papilla.

Cyathodinium

Family ISOTRICHIDAE

Mouth terminal or subterminal. Pharynx ciliated, with longitudinal striations in its wall. Somatic ciliation complete and practically uniform.

Dasytricha
Isotricha

Family PARAISOTRICHIDAE

Mouth subterminal, opening just posterior to concretion vacuole. Somatic ciliation complete, with an anterior tuft of longer cilia.

Paraisotricha

Family BALANTIDIIDAE

Somatic ciliation complete, with cilia arranged in approximately longitudinal rows. Peristome a pouch with a triangular opening, thru which the short adoral band of membranelles is not easily seen from the outside. Numerous long fibrils extend into the endoplasm from the basal granules of cilia and membranelles. Concretion vacuole absent.

Balantidium

Order HYMENOSTOMORIDA

Adoral cilia fused in membranes, the number, size and arrangement of which vary in different genera.

Family OPHRYOGLENIDAE

With a ciliated vestibule (peristome), an invagination of the body wall, and a pharynx which opens into the vestibule. A refractile body ("body of Lieberkühn", "watch-glass body") lies just to the left of the vestibule. Reproduction takes place within a cyst. The resultant young ciliates (tomites) leave the cyst, develop into trophic therontes and then into large trophonts which encyst.

Ichthyophthirius

Family PARAMECIIDAE

With oral groove extending from the anterior end toward the middle of the body. Somatic ciliation complete and essentially uniform. Adoral ciliature including a dorsal zone of long cilia (quadripartite membrane) and 2 peniculi (dense bands of cilia extending in a shallow spiral toward the cytostome).

Paramecium

Family TETRAHYMENIDAE

Adoral ciliature composed of 3 membranelles lying to the left in the oral pouch; a fourth, paroral membrane extends along its right margin. One or more stomatogenous rows of cilia end at the posterior margin of the oral pouch.

Tetrahymena

Subclass SPIROTRICHASINA

Bases of adoral zone membranelles usually at right or oblique angle to long axis of adoral zone; this series of membranelles extends anteriorly from the left margin of the cytostome; the basal plate of each membranelle contains 2, 3 or rarely 4 rows of basal granules.

Order HETEROTRICHORIDA

Somatic ciliation usually complete. Peristome usually elongated and fairly narrow, with adoral zone of membranelles along left wall. An undulating membrane often extends for some distance along right margin of peristome.

Family PLAGIOTOMIDAE

Body densely ciliated. Adoral zone of membranelles well defined. Undulating membrane at right margin of peristome.

Nyctotherus

Order ENTODINIORIDA

Ciliation may be limited to the adoral zone; there may be 1 or more additional bands or groups of membranelles. Skeletal plates usually present.

Family OPHRYOSCOLECIDAE

With not more than 1 band of membranelles in addition to adoral zone.

Amphacanthus	*Enoploplastron*	*Eudiplodinium*
Caloscolex	*Entodinium*	*Meladinium*
Cunhaia	*Eodinium*	*Ophryoscolex*
Diplodinium	*Epidinium*	*Opisthotrichum*
Diploplastron	*Epiplastron*	*Ostracodinium*
Elytroplastron	*Eremoplastron*	*Polyplastron*

Family CYCLOPOSTHIIDAE

With 2 or more bands of membranelles in addition to adoral zone.

Cochliatoxum	*Prololapirella*	*Trifascicularia*
Cycloposthium	*Spirodinium*	*Tripalmaria*
Ditoxum	*Tetratoxum*	*Triplumaria*
Elephantophilus	*Thoracodinium*	*Troglodytella*
Polydiniella	*Triadinium*	

Class PROTOCILIASIDA

With cilia. Nucleus vesicular.

Opalina

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Chapter 3

THE HEMOFLAGELLATES

The flagellates belong to the class Mastigasida. They have 1 or more flagella, and a few have pseudopods as well. Their nutrition is holophytic, holozoic or saprozoic. They multiply by longitudinal binary fission, and many produce cysts.

The class is divided into 2 subclasses, Phytomastigasina and Zoomastigasina. The former contains the phytoflagellates, the great majority of which are free-living and holophytic. Those of parasitic interest will be discussed in Chapter 6.

It is convenient for our purposes to divide the Zoomastigasina into 2 groups, the hemoflagellates which live in the blood, lymph and tissues, and the other flagellates which live in the intestine and other body cavities.

FAMILY TRYPANOSOMATIDAE

The hemoflagellates all belong to the family Trypanosomatidae. Members of this family have a leaf-like or sometimes a rounded body containing one nucleus. They have a single flagellum which arises from a basal granule or blepharoplast posterior to the end of an elongate blind pouch or reservoir and passes anteriorly, usually extending beyond the body. A contractile vacuole opens into the reservoir, but both of these structures can be seen only with the phase microscope and not with the ordinary light microscope (Ketterer, 1952; Cosgrove and Kessel, 1958; Clark, 1959). The flagellar axoneme is composed of 9 peripheral and 2 central fibrils (Anderson, Saxe and Beams, 1956). An undulating membrane is present in some genera; the flagellum lies in its outer border. Posterior to the basal granule is a rod-shaped or spherical kinetoplast containing deoxyribonucleic acid. The structure of the kinetoplast as seen in electron micrographs has been interpreted in different ways. According to Anderson, Saxe and Beams (1956), it consists of lamellae oriented at right angles to its long axis;

Meyer and Queiroga (1960) called it an apparently lamellar mass located in a vacuole-like space; Hans Ris (unpublished) said that the lamellae represent sections of a continuous spiral; Clark and Wallace (1960) considered the kinetoplast to be a mitochondrion containing antero-posteriorly oriented anastomosing fibers. Under the ordinary light microscope, the kinetoplast and blepharoplast may appear to be fused. Mitochondria and volutin granules have also been seen in electron micrographs.

Members of this family were originally parasites of the intestinal tract of insects, and many are still found only in insects. Others are heteroxenous, spending part of their life cycle in a vertebrate and part in an invertebrate host.

In the course of their life cycles, members of one genus may pass thru forms morphologically similar to those of other genera. These stages are named for the genera which they resemble. In the *trypanosome* form, which is perhaps the most advanced, the kinetoplast and basal granule are near the posterior end and the undulating membrane runs the length of the body. In the *crithidial* form, the kinetoplast and basal granule are just anterior to the nucleus and the undulating membrane runs forward from there. In the *leptomonad* form, the kinetoplast and basal granule are still further forward in the body and there is no undulating membrane. In the *leishmanial* form, the body is rounded and the flagellum has degenerated into a tiny fibril which remains inside the body (Fig. 1). Further information on life cycles and morphology is given by Noble (1955).

There are several genera in the family Trypanosomatidae. Members of the genus *Trypanosoma* are heteroxenous and pass thru leishmanial, leptomonad, crithidial and trypanosome stages in their life cycle. In some species, only trypanosome forms are found in the vertebrate host, while in other, more primitive ones, both leishmanial and trypanosome forms are present.

Members of the genus *Blastocrithidia* are monoxenous in arthropods and other

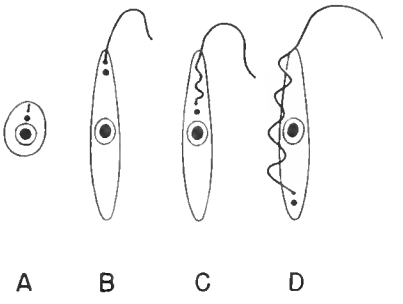

Fig. 1. Forms of the Trypanosomatidae. A. Leishmanial form. B. Leptomonad form. C. Crithidial form. D. Trypanosome form. (Original)

invertebrates. They pass thru crithidial, leptomonad and leishmanial stages in their life cycle. This generic name was introduced by Laird (1959) for the crithidial species commonly and erroneously assigned to the genus *Crithidia*. As both Laird (1959), Wallace (1943) and Clark (1959) have shown, the type species of *Crithidia*, from mosquitoes, *C. fasciculata*, is a short, truncate form with a stiff flagellum emerging from a funnelled anterior depression, and never has an undulating membrane. However, the term crithidial is so deeply embedded in our terminology as referring to forms with an undulating membrane that it is best to retain it.

Members of the genus *Crithidia* are monoxenous in arthropods. Despite their name, they have only a leptomonad stage.

Members of the genus *Leptomonas* are monoxenous in invertebrates. They pass thru leptomonad and leishmanial stages in their life cycle.

Members of the genus *Leishmania* are heteroxenous, passing thru the leishmanial stage in their vertebrate host and the leptomonad stage in their invertebrate host or in culture.

Members of the genus *Herpetomonas* are monoxenous in invertebrates. They pass thru trypanosome, crithidial, leptomonad and leishmanial stages in their life cycle. The trypanosome form in this genus differs from that of *Trypanosoma*

in that the undulating membrane lies in a long reservoir which runs the whole length of the body and opens at the anterior end, whereas in *Trypanosoma* the reservoir is very short and opens laterally near the posterior end so that the undulating membrane runs along the side of the body (Clark, 1959).

Members of the genus *Phytomonas* are heteroxenous in the latex of plants and hemipterous insects, passing thru leptomonad and leishmanial stages in their life cycle. They are found in milkweeds and related plants, and cause the normally milky sap to become colorless.

The only genera parasitic in domestic animals and man are *Trypanosoma* and *Leishmania*. Since, however, their stages in the invertebrate vector are morphologically similar to those of the genera confined to invertebrates, one cannot be positive, when he finds an infected invertebrate, whether it is infected with a parasite of vertebrates or with one of its own. It is possible, too, that some of the forms which we now think are confined to invertebrates may actually be normal parasites of some wild vertebrates.

Genus *TRYPANOSOMA* Gruby, 1843

Members of this genus occur in all classes of vertebrates. They are parasites of the circulatory system and tissue fluids, but some, such as *T. cruzi*, may actually invade cells. Almost all are transmitted by blood-sucking invertebrates. Most species are probably non-pathogenic, but the remainder more than make up for their fellows.

Trypanosomosis is one of the world's most important diseases of livestock and man. Trypanosomes cause African sleeping sickness and Chagas' disease in man and a whole series of similar diseases in domestic animals. They are relatively unimportant in North America, but they make it practically impossible to raise livestock in many parts of the tropics which would otherwise be ideal. According to Hornby (1949), "Trypanosomiasis is unique among diseases in that it is the only one which by itself has denied vast areas of land to all domestic animals other than poultry. The areas of complete denial are all in Africa and add up to perhaps one quarter of the total land surface of this continent." Some, but not all, of the African species are transmitted by tsetse flies. These flies occupy almost 4 million square miles, an area larger than the United States, and this whole region is under the threat of trypanosomosis.

In a recent Hollywood epic on South African history, there is a scene in which a line of Boer covered wagons on the Great Trek to the north is attacked by Zulus. The warriors pour over the hills, the wagons form a circle with the women and children in the center, and the Boer men prepare to fight. It is just like a scene from the American Wild West, with the Indians attacking a wagon train of pioneers. But there is one difference--the Zulus had no horses, and made their attack on foot. The reason? Trypanosomosis.

A large number of species of *Trypanosoma* has been named. At one time it was customary, and still is to some extent, to give different names to trypanosomes from different hosts. Many of these names are still valid, but as we learn more and more about the host-parasite relations and epidemiology of the trypanosomes, many other names have fallen into synonymy. No attempt will be made here to list all the synonyms of each species, but the more important ones will be mentioned.

Trypanosomes are classified in groups on the basis of their morphology, life cycles and other biological characteristics. The validity of this grouping is shown by the fact that their metabolic characteristics, which vary widely, fall into the same groups.

The following outline classification of trypanosomes of veterinary and medical importance is based on Hoare (1957, 1959). Sections on metabolism based on von Brand (1956), Ryley (1956) and von Brand and Tobie (1959), and a section on avian trypanosomes have been added.

I. PARASITES OF MAMMALS

A. Morphology in Mammal

Kinetoplast not terminal, large. Free flagellum always present. Posterior end of body pointed. Division in crithidial, leishmanial or trypanosome stages.

Biology

Multiplication in mammal typically discontinuous. Development of metacyclic trypanosomes in hind gut (posterior station) of vector (in *T. rangeli* also in salivary glands: anterior station). Transmission contaminative thru feces (in *T. rangeli* also inoculative). Except for *T. cruzi*, trypanosomes slightly or not pathogenic.

Metabolism

Blood forms have high respiratory quotient and low sugar consumption, producing acetic, succinic and lactic acids aerobically and succinic, lactic, acetic and pyruvic acids anaerobically. Cyanide markedly inhibits oxygen consumption. Sulfhydryl antagonists moderately inhibit oxygen consumption. Culture forms have high respiratory quotient and moderately high sugar consumption, producing acetic and succinic acids aerobically and succinic and acetic acids anaerobically; cyanide markedly inhibits oxygen consumption; sulfhydryl antagonists moderately to markedly inhibit oxygen consumption. Cytochrome pigments, cytochrome and succinic oxidase activity are present in *T. cruzi* and *T. lewisi*.

1. *LEWISI GROUP*

   a. Mode of reproduction unknown.

      T. melophagium of sheep.

   b. Reproduction by binary fission in crithidial stage.

      T. theileri of cattle.

   c. Reproduction by multiple fission in crithidial stage.

      T. lewisi of rats.

      T. duttoni of mice.

   d. Reproduction by binary fission in leishmanial stage.

      T. cruzi of man, dog, opossum, monkeys.

   e. Reproduction by multiple fission in leishmanial stage.

      T. nabiasi of rabbits.

   f. Reproduction by binary fission in trypanosome stage.

      T. rangeli of man, dog, opossum, monkeys.

B. Morphology in Mammal

Kinetoplast terminal or subterminal. Posterior end of body blunt. Division in trypanosome stage.

Biology

Multiplication in mammal continuous. Development of metacyclic trypanosomes in proboscis or salivary glands (anterior station) of vector (except *evansi* subgroup). Transmission inoculative, thru bite (except *T. equiperdum*). Trypanosomes pathogenic.

1. *VIVAX GROUP*

Morphology in Mammal

Monomorphic forms. Posterior end of body typically rounded. Free flagellum always present. Kinetoplast large, terminal. Undulating membrane inconspicuous.

Biology

Development in *Glossina*, in proboscis only. *T. vivax* is also transmitted mechanically by tabanids.

Metabolism

Blood forms have high respiratory quotient and high sugar consumption, producing pyruvic, acetic, lactic acids and glycerol aerobically and glycerol, pyruvic, lactic, and acetic acids anaerobically. Cyanide does not inhibit oxygen consumption. Sulfhydryl antagonists markedly inhibit oxygen consumption. Cytochrome and succinic oxidase activity are present.

a. Long forms

T. vivax of cattle, sheep, goats, antelope.

b. Short forms

T. uniforme of cattle, sheep, goats, antelope.

2. *CONGOLENSE GROUP*

Morphology in Mammals

Monomorphic or polymorphic forms. Free flagellum absent or present. Kinetoplast medium, typically marginal.

Biology

Development in *Glossina*, in midgut and proboscis.

Metabolism

Blood forms have high respiratory quotient and high sugar consumption, producing acetic acid, succinic acid, glycerol and pyruvic acid aerobically

and succinic acid, glycerol, acetic acid and pyruvic acid anaerobically. Cyanide and sulfhydryl antagonists moderately inhibit oxygen consumption. Cytochrome pigments are absent but cytochrome and succinic oxidase activity are present. Culture forms of *T. congolense* have R. Q. of 0.9, produce pyruvate, acetate and smaller amounts of lactate, succinate and glycerol aerobically, and pyruvate, acetate and succinate with small amounts of glycerol and no carbon dioxide anaerobically. Cyanide and sulfhydryl antagonists (iodoacetate and sodium arsenite) inhibit oxygen consumption, but Krebs cycle inhibitors (fluoroacetate and malonate) do so only slightly.

a. Monomorphic (free flagellum absent or short, undulating membrane inconspicuous).

(1) Short forms (means 12.2-14.4 μ).

T. congolense of cattle, equids, swine, sheep, goat, dog.

(2) Long forms (means 15.3-17.6 μ).

T. dimorphon of horse, cattle, sheep, goat, pig, dog.

b. Polymorphic (short forms without free flagellum, and long forms, of which some are stout, with conspicuous undulating membrane and some are slender with inconspicuous undulating membrane; free flagellum absent or present).

T. simiae of swine, camels, cattle, horse, warthog.

3. *BRUCEI GROUP*

Morphology in Mammal

Monomorphic or polymorphic forms. Free flagellum present or absent. Kinetoplast small, subterminal (absent in *T. equinum*). Undulating membrane conspicuous.

Biology

Development in *Glossina*, in midgut and salivary glands (except *evansi* subgroup).

Metabolism

Blood forms have very low respiratory quotient and very high sugar consumption, producing pyruvic acid and sometimes glycerol aerobically and pyruvic acid and glycerol anaerobically. Cyanide does not inhibit oxygen consumption. Sulfhydryl antagonists markedly inhibit oxygen consumption. Culture forms have high respiratory quotient and moderately high sugar consumption, producing acetic, succinic, pyruvic and lactic acids aerobically. Cyanide moderately inhibits oxygen consumption. Sulfhydryl antagonists markedly inhibit oxygen consumption. Cytochrome pigments have not been found in *T. rhodesiense* or *T. equiperdum*, but cytochrome and succinic oxidase activity are present in *T. rhodesiense*.

a. Monomorphic (stout forms with short free flagellum).

(1) *SUIS SUBGROUP*

T. suis of swine.

b. Polymorphic (slender, intermediate and stumpy forms).

(1) *BRUCEI SUBGROUP*

Polymorphism constant (stumpy forms always present).

T. brucei of all domestic animals, antelope.

T. rhodesiense of man, bushbuck and probably antelope.

T. gambiense of man.

(2) *EVANSI SUBGROUP*

Polymorphism inconstant (stumpy forms rare or sporadic); no cyclic development in vector host.

(a) Transmission mechanical by insects.

T. evansi of cattle, camels, equids, dogs, etc.

T. equinum of equids.

(b) Transmission by contact (coitus) from mammal to mammal.

T. equiperdum of equids.

II. PARASITES OF BIRDS

Very polymorphic, sometimes attaining great size. Relatively easy to cultivate. Cyclic development probably in biting arthropods.

AVIUM GROUP

T. avium of various birds.

T. calmettei of chickens.

T. gallinarum of chickens.

T. hannai of pigeons.

T. numidae of guinea fowl.

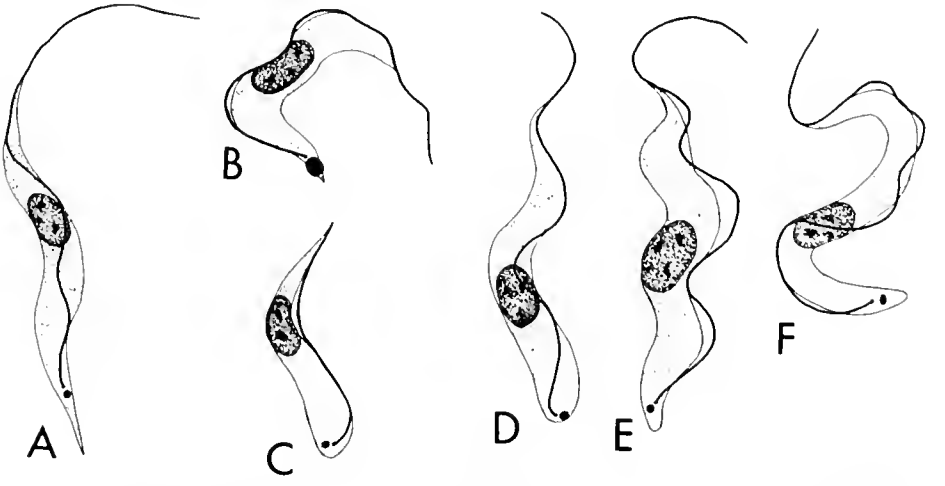

Fig. 2. Species of *Trypanosoma*. A. *T. theileri*. B. *T. cruzi*. C. *T. congolense*. D. *T. vivax*. E. *T. equiperdum*. F. *T. brucei*. X 2800. (Original)

In the discussion which follows, each trypanosome species is taken up separately, but special attention is paid to *T. brucei* and *T. cruzi* as representatives of different types.

TRYPANOSOMA BRUCEI PLIMMER AND BRADFORD, 1899

Synonym: *T. pecaudi.*

Disease: Trypanosomosis, nagana.

Hosts: Horse, mule, donkey, ox, zebu, sheep, goat, camel, pig, dog, and many wild game animals. Antelopes are the natural hosts of *T. brucei* and serve as reservoirs of infection for domestic animals. Experimental attempts to infect man have failed (Ashcroft, 1959a).

Location: Blood stream, lymph, cerebrospinal fluid.

Geographic Distribution: Widely distributed in tropical Africa between 15° N and 25° S latitude, coinciding with the distribution of its vector, the tsetse fly.

Prevalence: *T. brucei* is one of the commonest and most important parasites of domestic animals in Africa. It has prevented the raising of livestock in vast areas.

Morphology: Polymorphic, with slender, intermediate and stumpy forms. Undulating membrane conspicuous. Kinetoplast small, subterminal. Slender forms average 29 μ in length but range up to 42 μ; posterior end usually drawn out, tapering almost to a point, with kinetoplast up to 4 μ from posterior end, with a long, free flagellum. Stumpy forms stout, averaging 18 μ in length with a range of 12 to 26 μ; posterior end broad, obtusely rounded, with kinetoplast almost terminal; free flagellum typically absent. Intermediate forms average 23 μ in length; body of medium thickness, with blunt posterior end; moderately long free flagellum always present. A fourth form with a posterior nucleus often appears in laboratory animals.

Life Cycle: When it is first introduced into the body, *T. brucei* multiplies in the blood and lymph by longitudinal binary fission in the trypanosome stage, being particularly common in the lymph glands. Later the trypanosomes pass into the cerebrospinal fluid and multiply here and between the cells of the brain. Leishmanial forms have also been reported from the heart muscle of infected monkeys (Noble, 1955).

The vector is a tsetse fly of the genus *Glossina*. *T. brucei* is generally transmitted by members of the *morsitans* group of this genus, i.e., *G. morsitans*, *G. swynnertoni* and *G. pallidipes*. Both males and females feed on blood and act as vectors. Only a small percentage of the tsetse flies which feed on an infected animal become infected, most being apparently resistant. In experimental studies, 10% or less become infected, while less than 1% of wild flies caught in endemic areas are infected.

When ingested by a tsetse fly, *T. brucei* localizes in the posterior part of the midgut and multiplies in the trypanosome form for about 10 days. At first the trypanosomes are relatively broad, up to 35 μ long, with a kinetoplast about halfway between the posterior end of the body and the nucleus, with a less pronounced undulating membrane than the blood form, and with a free flagellum. On the 10th to 12th day, slender forms appear and migrate slowly toward the proventriculus, where they are found on the 12th to 20th days. They then migrate forward into the esophagus and pharynx, thence into the hypopharynx and finally into the salivary glands. Here they attach themselves to the walls by their flagella or lie free in the lumen, and turn into the crithidial form. These multiply further and then transform into the metacyclic trypanosome form, which is small, stumpy, and may or may not have a short free flagellum.

The metacyclic trypanosomes are the infective forms. They are injected into the blood with the saliva when the fly bites; up to several thousand may be introduced by the bite. The whole life cycle in the tsetse

fly takes 15 to 35 days, and the flies are not infective until the metacyclic trypanosomes have appeared in the salivary glands.

This type of development, in which the trypanosomes are found in the anterior part of the vector and are introduced by its bite, is known as development in the anterior station to contrast it with development in the posterior station or hindgut. In the latter, exemplified by the *lewisi* group, infection is by contamination with feces.

In addition to the cyclical transmission described above, *T. brucei* may occasionally be transmitted mechanically by tsetse flies or other biting flies. In this case, the trypanosomes remain alive in the proboscis for a short time and are transferred to a new host if the fly bites it soon enough after having bitten an infected one.

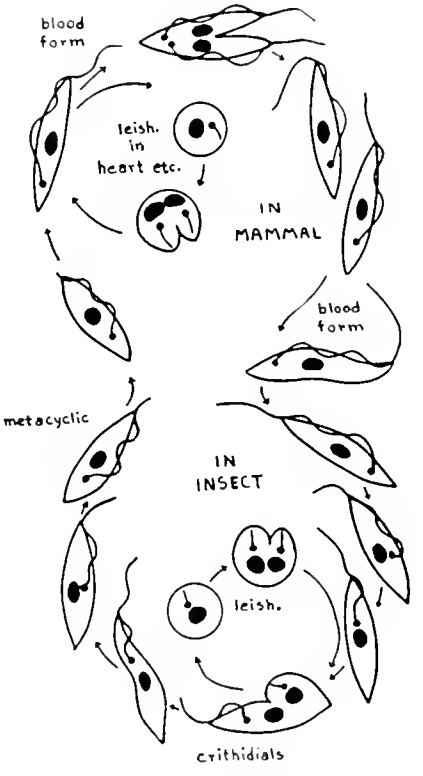

Fig. 3. Simplified life cycle of *Trypanosoma brucei*. (From Noble, 1955)

Pathogenesis: The signs and pathogenesis of the trypanosomoses of domestic animals are more or less similar. Different hosts are affected to different degrees. Horses, mules and donkeys are very susceptible to *T. brucei*. Affected animals have a remittent fever, edematous swellings of the lower abdomen, genitalia and legs, a watery discharge from the eyes and nose, and anemia. The animals become emaciated altho their appetite is good. Muscular atrophy sets in, and eventually incoordination and lumbar paralysis develop, followed by death. The course of the disease is 15 days to 4 months, and untreated animals rarely recover.

The disease in sheep, goats, camels and dogs is also severe. The signs are much the same as in horses. In the dog, fever may appear as shortly as five days after infection, and the parasites often cause conjunctivitis, keratitis and blindness.

The disease is usually more chronic in cattle. There is remittent fever with swelling of the brisket, anemia, gradual emaciation, and discharge from the eyes and nose. The animals may survive for several months. Swine are more resistant than cattle and usually recover.

Following infection, the trypanosomes appear first in the blood and lymph, causing fever, edema, anemia, etc. and only later on are they able to invade the central nervous system, causing incoordination, paralysis and meningo-encephalitis.

The exact way in which they act to kill their victims is unknown, altho several theories have been advanced. It is known that they have a high glucose metabolism, and one theory was that they rob the body of glucose so that death is due to hypoglycemia. In experimental animals, life can be prolonged by feeding glucose and shortened by injecting insulin. This theory, however, has been discredited.

It is known that the serum potassium level increases in trypanosomosis, and another theory was that the effects are due to the high potassium level. However, the latter is a result of the disease and not a

cause. It is due to the destruction of red cells with consequent release of potassium into the plasma, and the observed levels are not too harmful.

Epidemiology: The epidemiology of the diseases caused by *T. brucei* and other tsetse-borne trypanosomes depends upon the bionomics and distribution of their vectors. This is such a vast subject that no attempt will be made to cover it here. In general, tsetse flies occupy almost 4 million square miles of Africa. They occur in woodlands, bush or forested areas where there is ample rainfall and where the mean annual temperature is above about 70° F. Not all species are good vectors, and trypanosomosis does not occur every place that tsetse flies do. For further information on tsetse flies and the epidemiology of trypanosomosis, see Buxton (1948, 1955, 1955a), Hornby (1949, 1952), Davey (1958) and Ashcroft (1959).

Diagnosis: In the acute or early stage of the disease, trypanosomes can be found in the peripheral blood. Thick blood smears are preferable to thin ones. The protozoa are found even more often in the lymph glands. They can be detected in fresh or stained smears of fluid obtained by puncture of the glands. In the later stages of the disease, trypanosomes can be found in the cerebrospinal fluid. Laboratory animals such as the rat can also be inoculated. The complement fixation test can also be used; it is not specific for *T. brucei* infections, but also reacts in a number of other trypanosomoses.

Cultivation: Trypanosomes can be successfully cultivated in a number of media. A common one is NNN medium, which is essentially a 25% blood agar slant. Another medium is that of Weinman (1946), which contains beef extract, peptone, washed erythrocytes and plasma. Still another is that of Tobie, von Brand and Mehlman (1950). A discussion of problems of cultivation and diagnosis is given by Weinman (1953).

Trypanosomes can also be cultivated in developing chick embryos or in tissue culture. See Pipkin (1960) for a review of this subject.

Treatment: Many different drugs have been used in the treatment of trypanosomosis. Indeed, the first synthetic organic compound of known composition ever used to cure an experimental disease was trypan red, which was developed by Ehrlich and Shiga (1904). Since that time thousands of drugs have been found to show some activity, but the number of satisfactory ones is very small. The chemotherapy of trypanosomiasis has been reviewed by Findlay (1950), Ing (1953), Browning (1954), Goodwin and Rollo (1955), Davey (1957), and others.

Altho much of the earlier work on chemotherapy was done on members of the *brucei* subgroup, most of that since World War II on trypanosomosis of livestock has dealt with the *vivax* and *congolense* groups.

Antrycide methyl sulfate is perhaps the drug of choice for *T. brucei* in horses. It is injected subcutaneously at the rate of 5 mg/kg body weight; two treatments may be given 4 days apart. Antrycide is also effective against *T. brucei* in dogs, cattle and other animals.

Suramin (Germanin, Naganol, Antrypol, Moranyl, Bayer 205, Fourneau 309, etc.) has been used for many years. A single dose of 4 g per 1000 lb body weight is given intravenously to horses, but it may be toxic in some animals. In dogs, 5 mg/kg is given intravenously.

The diamidines, pentamidine and stilbamidine, have been used extensively against *T. gambiense* and *T. rhodesiense* in man, but have been used very little in veterinary medicine. Another diamidine, Berenil, appears promising against *T. brucei*, but needs more study.

Control: Preventive measures against trypanosomosis include measures directed against the parasite, measures directed against the intermediate hosts, livestock management, elimination of reservoir hosts, and avoidance of accidental, mechanical contamination.

Measures directed against the parasite include continuous survey and treat-

ment or slaughter of all affected animals and periodic mass prophylactic treatment of all animals. The latter is discussed in the section on treatment of *T. congolense*.

Fly traps and fly repellents have been used without much success in attempting to control tsetse flies. Elimination of breeding places has been practiced on a wide scale in many areas. Since the tsetse flies breed under brush along streams or in other localities, such measures consist essentially of brush removal. Two methods are used:

Eradicative clearing aims at eradication of tsetse flies thruout an area. All the species of trees and shrubs under which the flies survive thru the dry season are removed. When this is done thoroughly over a large area, the flies disappear completely.

Protective clearing is more limited. It is designed to break the contacts between tsetse flies and domestic animals and man at the places where transmission is taking place. Fly-free belts wide enough so that the flies cannot cross them are established. In addition, inspection stations known as deflying houses may be set up on traffic routes to remove flies which may be carried across on vehicles or animals.

Bush clearing can be quite successful. The incidence of trypanosomosis was reduced by 92% between 1938 and 1944 in the Kamba area of Africa by this means (Morris, 1946). However, it is expensive, requires a large amount of labor, and the initial clearing must be followed up faithfully as new growth occurs.

A potentially much more satisfactory control measure is the spraying of insecticides on fly breeding places by means of aircraft. DDT and benzene hexachloride are highly effective for this purpose. *Glossina pallidipes* was eradicated from Zululand by airplane spraying with these insecticides at a total cost of 2.5 million pounds, or slightly less than 2 shillings per acre (DuToit, 1959).

Since tsetse flies bite only in the daytime, night grazing has been practiced by African natives to avoid their bites. The animals are held in a protected corral during the day.

Cattle can be sprayed with DDT or another insecticide in order to kill any tsetse flies which light on them.

The elimination of reservoir hosts, e.g., wild game in Africa, has been advocated and practiced in some regions despite the protests of many people interested in game preservation. The Trypanosomiasis Committee of Southern Rhodesia (1946) has described and defended the practice. It claims that if a zone 10 miles wide with its ends in fly-free country is fenced off and all the game within it is killed, *Glossina morsitans* will disappear in less than 10 years. The fences can then be removed and the game allowed to return into the area.

Since trypanosomes can be transmitted mechanically by inoculation of infected blood or lymph, there is danger of its transmission by the use of contaminated instruments in bleeding, castrating, etc.

A great deal has been written on trypanosomosis control. For further information, see Hornby (1949, 1952), Morris (1946), Buxton (1948, 1955) and the proceedings of the meetings of the International Scientific Committee for Trypanosomiasis, which held its sixth meeting in 1956.

TRYPANOSOMA GAMBIENSE DUTTON, 1902

TRYPANOSOMA RHODESIENSE STEPHENS AND FANTHAM, 1910

These two species cause African sleeping sickness in man. *T. rhodesiense* is thought to occur also in antelopes (Hoare, 1955) and was isolated once from a bushbuck (Heisch, McMahon and Manson-Bahr, 1958). *T. gambiense* does not occur in wild game. Neither occurs in domestic animals. They are morphologically indistinguishable from each other and from *T. brucei*, and for this reason some people prefer to consider all three as subspecies of *T. brucei*. However, the biological and

epidemiologic differences between them make it more convenient to retain separate names. Whatever the names used, it is clear that these species arose from strains of *T. brucei* which became adapted to man.

Human trypanosomosis occurs in tropical Africa, roughly between 15° N and 15° S latitude. *T. rhodesiense*, which causes an acute form of the disease, occurs in Rhodesia, Tanganyika, Nyasaland, Bechuanaland and Portuguese East Africa, while *T. gambiense*, which causes a chronic form of the disease, occurs in a large part of the remainder of the area. Kunert (1953) prepared a map of the distribution of human sleeping sickness in Africa together with climatologic and other information. Ashcroft (1959a) and Morris (1960) reviewed its epidemiology.

In general, *T. gambiense* causes a "domesticated" type of disease, transmitted by tsetse flies from man to man in regions of human habitation, while *T. rhodesiense* causes more of a woodland disease and people become infected with it away from their village areas.

Altho it is certain that some wild animals must serve as reservoirs of *Trypanosoma rhodesiense*, it has been isolated from them only once. Heisch, McMahon and Manson-Bahr (1958) isolated it from a bushbuck (*Tragelaphus scriptus*) in Kenya by inoculation of a human volunteer.

Epidemiologic evidence for a wild animal reservoir is exemplified by the observation that every year fishermen and honey hunters become infected with *T. rhodesiense* near the Ugalla River in the Western Province of Tanganyika, yet this is an uninhabited region, and no people are there at all during the 6-month rainy season. The Ugalla River is part of the Malagarasi river system of the Western Province. It runs thru a sparsely populated, woodland region inhabited by many wild animals and infested with *Glossina morsitans*. Jackson (1955) described 25 cases of sleeping sickness in fly-boys stationed in remote outposts in this area between 1935 and 1939, and concluded that there was strong evidence that game was acting as a reservoir. Over half the cases of *T. rhodesiense* infection diagnosed in Africa in 1953, 1954 and 1955 were contracted in this region (Ashcroft, 1958); 2069 cases were reported in the Western Province in these years (Apted, 1955).

The only way to be positive that a *brucei*-like strain of trypanosome isolated from wild animals is actually *T. rhodesiense* is to inoculate human volunteers with it, and very few such attempts have been made. In one of the latest of these, Ashcroft (1958) inoculated a strain which he had isolated from a Coke's hartebeest (*Alcelaphus cokei*) in Tanganyika into 2 African volunteers, but no infection resulted and he concluded that the organism was *T. brucei*.

The life cycles of the human trypanosomes are the same as that of *T. brucei*. The vectors are species of tsetse flies of the genus *Glossina*. The chief vectors of *T. gambiense* are the riverine tsetse flies, *G. palpalis* and *G. tachinoides*, while those of *T. rhodesiense* are the game tsetse flies, *G. morsitans*, *G. swynnertoni* and *G. pallidipes*.

Human trypanosomosis is similar to nagana in its manifestations. For further information, any human parasitology text may be consulted.

TRYPANOSOMA EVANSI (STEEL, 1885) BALBIANI, 1888

Synonyms: *T. soudanense*, *T. elephantis*, *T. annamense*, *T. cameli*, *T. marocanum*, *T. ninae kohl-yakimov*, *T. aegyptum*, *T. hippicum*, *T. venezuelense*.

Disease: Trypanosomosis due to *T. evansi* has been given different names in different localities. The most widely used name, surra, is applied to the disease in all hosts. The disease in camels is called el debab in Algeria and mbori in Sudan. That in horses is called murrina in Panama and derrengadera in Venezuela.

Hosts: Camels, horse, donkey, ox, zebu, goat, pig, dog, water buffalo, elephants, capybara, tapir, and (in Mauritius) deer.

Location: Blood, lymph.

Geographic Distribution: Northern Africa (north of 15° N latitude in the west and central part of the continent, but extending almost to the equator in the east), Asia Minor, U.S.S.R. from the Volga River east into Middle Asia, India, Burma, Malaya, Indochina, parts of southern China, Indonesia, Philippines, Central America, South America. Hoare (1956) has shown how the original distribution of *T. evansi* coincided with that of the camel. In Africa, its southern boundary coincides roughly with the northern boundary of tsetse fly distribution. It now extends far to the east of the camel's range in the Old World. It is often associated with arid deserts and semi-arid steppes, but may occur in other types of climate as well. In India, it is most common in the Punjab, which is mostly in the northwestern dry region (Basu, 1945; Basu, Menon and Sen Gupta, 1952). It was probably introduced into the New World in infected horses by the Spanish conquerors during the 16th century.

Prevalence: *T. evansi* is an important cause of disease over a large part of its range.

Morphology: The morphology of the Old World strains of *T. evansi* has been studied intensively by Hoare (1956). The mean length of different host and geographic strains varies considerably. However, the typical forms are 15 to 34 μ long, with a mean of 24 μ. Most are slender or intermediate in shape, but stumpy forms occur sporadically. All forms are morphologically indistinguishable from the corresponding ones of *T. brucei*. Strains which lack a kinetoplast have occasionally arisen spontaneously or can be produced by treatment with certain dyes (Hoare, 1954).

Life Cycle: *T. evansi* is transmitted mechanically by biting flies. No cyclic development takes place in the vectors, the trypanosomes remaining in the proboscis. The usual vectors are horseflies of the genus *Tabanus*, but *Stomoxys*, *Haematopota* and *Lyperosia* can also transmit it. In Central and South America, the vampire bat is a vector, the disease in this case being known as murrina.

Pathogenesis: Surra is nearly always fatal in horses in the absence of treatment; death occurs in a week to six months. The disease is also severe in dogs and elephants. It is less severe in cattle and water buffalo. Cattle may carry the parasites without showing signs of disease for months. However, occasional outbreaks of acute disease occur in cattle and water buffalo. Surra in camels is similar to the disease in horses but more chronic. In dogs, *T. evansi* causes a chronic disease with a high mortality rate; untreated dogs usually die in 1 to 2 months (Gomez Rodriguez, 1956).

The signs of surra include intermittent fever, urticaria, anemia, edema of the legs and lower parts of the body, loss of hair, progressive weakness, loss of condition and inappetence. Conjunctivitis may occur, and abortion is common in camels.

The lesions include splenomegaly, enlargement of the lymph glands and kidneys, leucocytic infiltration of the liver parenchyma, and petechial hemorrhages and parenchymatous inflammation of the kidneys.

Diagnosis: Same as for *T. brucei*.

Cultivation: Same as for *T. brucei*.

Treatment: Treatment of *T. evansi* is similar to that of *T. brucei*. Antrycide methyl sulfate is less toxic than suramin for horses; a single subcutaneous dose of 5 mg/kg or even less is effective. A dose of 3 mg/kg has given good results in cattle. A single injection of 2 g is effective in camels.

The dose of suramin for horses is 4 g per 1000 lb body weight intravenously. Camels tolerate suramin well, and a single intravenous injection of 4 to 5 g is effective against surra in these animals. Tartar

emetic, which has been largely superseded in other animals, is still used in treating surra in the camel; a single intravenous injection of 200 ml of a 1% solution is given. This drug is also widely used in cattle in India because of its cheapness.

Control: Essentially the same measures used in the control of *T. brucei*, except of course those directed against the tsetse fly, can be used in the control of *T. evansi* infections. Control of horseflies and other biting flies is important.

Remarks: Hoare (1956, 1957) has discussed the phylogeny of *T. evansi*. This species undoubtedly arose from *T. brucei*, being introduced into camels when they entered the tsetse fly belt and then becoming adapted to mechanical transmission by tabanids.

TRYPANOSOMA EQUINUM VOGES, 1901

This species occurs in South America, where it causes a disease known as mal de Caderas in horses. The disease is similar to surra. *T. equinum* differs morphologically from *T. evansi* only in lacking a kinetoplast. However, strains of *T. evansi* without a kinetoplast have appeared in the laboratory, and *T. equinum* undoubtedly originated in this way.

T. equinum is transmitted mechanically by tabanids. Both antrycide methyl sulfate and suramin can be used in treating it. The former is less toxic. A single subcutaneous dose of 5 mg/kg or less of antrycide methyl sulfate or a single intravenous dose of 4 g per 1000 lb body weight of suramin can be used. Control measures are the same as for *T. evansi*.

TRYPANOSOMA EQUIPERDUM DOFLEIN, 1901

This species is morphologically indistinguishable from *T. evansi*. It causes a disease of horses and asses known as dourine. This is a venereal disease, transmitted by coitus. Dourine is similar to nagana, but runs a more chronic course of 6 months to 2 years. The incubation period is 2 to 12 weeks.

The first sign of the disease is edema of the genitalia and often of the dependent parts of the body. There is slight fever, inappetence, and a mucous discharge from the urethra and vagina. Circumscribed areas of the mucosa of the vulva or penis may become depigmented.

The second stage of the disease, characterized by urticaria, appears after 4 to 6 weeks. Circular, sharply circumscribed, urticarial plaques about 3 cm in diameter arise on the sides of the body, remain 3 or 4 days, and then disappear. They may reappear later. Muscular paralysis later ensues. The muscles of the nostrils and neck are affected first, but the paralysis spreads to the hind limbs and finally to the rest of the body. Incoordination is seen first, and is followed by complete paralysis. Dourine is usually fatal unless treated, altho mild strains of the parasite may occur in some regions.

T. equiperdum is found in Asia, North and South Africa, southern and eastern Europe and the U.S.S.R. It was once common in western Europe and North America, but has been eradicated from these regions. The last place where it was known to occur in North America, the Papago Indian Reservation in Arizona, was released from quarantine in 1949.

Dourine can be diagnosed by finding the parasites in smears of fluid expressed from the urticarial swellings, lymph, the mucous membranes of the genitalia or blood. The signs of the typical disease are characteristic enough to permit diagnosis in endemic areas. Inoculation of mice, rats, rabbits or dogs may also be practiced, but it is often difficult to demonstrate the parasites on the first passage. The complement fixation test is invaluable in detecting early or latent infections, and it was only by its use that dourine was eradicated from North America. All horses imported into the United States must be tested for dourine before they are admitted.

To treat dourine in horses, a single subcutaneous dose of 5 mg kg antrycide methyl sulfate or two intravenous injections of 2 g suramin each 15 days apart can be used.

TRYPANOSOMA SUIS OCHMANN, 1905

This species, which was once thought to be the same as *T. simiae*, was rediscovered in the Belgian Congo by Peel and Chardome (1954). It is a member of the *brucei* group, but differs from the others in being monomorphic, having only stout forms 14 to 19 μ long, with a short, free flagellum. The kinetoplast is very small and marginal.

T. suis occurs in pigs, causing a chronic infection in adults and a more acute disease with death in less than 2 months in young pigs. Peel and Chardome attempted without success to transmit *T. suis* to the goat, sheep, dog, white rat, guinea pig, *Cricetomys gambianus*, *Dendrohyrax*, chimpanzee, cat, rabbit, cattle, monkey and ass. It is transmitted by the tsetse fly, *Glossina brevipalpis*, in which it develops first in the intestine and proventriculus and then in the salivary glands. Metacyclic infectious trypanosomes appear in the hypopharynx on the 28th day.

TRYPANOSOMA CONGOLENSE BRODEN, 1904

Synonyms: *Trypanosoma nanum*, *T. confusum*, *T. pecorum*, *T. somaliense*, *T. cellii*, *T. frobeniusi*, *T. montgomeryi*, *T. ruandae*.

Disease: The South African disease of cattle known as nagana is ordinarily caused by *T. congolense*. Other names which have been given to the disease are paranagana, Gambia fever, ghindi and gobial.

Hosts: Cattle, equids, sheep, goats, camels, dogs and, to a lesser extent, swine. Antelopes, giraffes, zebras, elephants and wart hogs are also infected and act as reservoirs.

Location: This species develops almost exclusively in the blood. It does not invade the lymph or central nervous system.

Geographic Distribution: Widely distributed in tropical Africa between 15° N and 25° S latitude, coinciding with the distribution of the tsetse flies which act ad its vectors.

Prevalence: *T. congolense* is the commonest and most important trypanosome of cattle in tropical Africa.

Morphology: This species is small, being 8 to 20 μ long; the mean lengths of different populations range from 12.2 to 14.4 μ (Hoare, 1959). It lacks a free flagellum or has a short one, has an inconspicuous undulating membrane, and a medium-sized kinetoplast which lies some distance from the posterior end and is typically marginal.

Life Cycle: The vectors of *T. congolense* are various species of *Glossina*, including *G. morsitans*, *G. palpalis*, *G. longipalpis*, *G. pallidipes* and *G. austeni*. After the trypanosomes have been ingested by the tsetse flies, they develop in the midgut as long trypanosomes without a free flagellum. They then migrate to the proventriculus and thence to the proboscis, where they assume a crithidial form without a free flagellum. These are attached at first to the wall of the proboscis and multiply for a time. Later they pass into the hypopharynx, where they turn into metacyclic, infective trypanosomes similar in appearance to the blood forms. These are injected into the blood stream when the flies bite.

T. congolense can also be transmitted mechanically by other biting flies in tsetse-free areas.

Pathogenesis: Many strains which differ markedly in virulence and also in antigenic properties are united under *T. congolense* (Fiennes, 1950). In cattle,

the parasite may cause an acute, fatal disease resulting in death in about 10 weeks, or a chronic condition with recovery in about a year, or a mild, almost asymptomatic condition (Hornby, 1949). The disease is similar in sheep, goats, camels and horses. Swine are more resistant.

The signs of trypanosomosis due to this species are similar to those caused by other trypanosomes, except that the central nervous system is not affected.

Fiennes (1953) described the lesions observed in untreated *T. congolense* infections of cattle. The lymph nodes are edematous, the liver is congested, the marrow of the long bones is largely destroyed, and there are hemorrhages in the heart muscle and renal medulla. In cattle treated with antrycide or dimidium, the lesions are more chronic. The spleen is enlarged, the liver is swollen and sometimes fibrous, the lymph nodes are hypertrophied, edematous and somewhat fibrotic, the kidneys show chronic degeneration, the hemolymph tissue is hyperplastic, and the marrow of the long bones is largely destroyed.

Fiennes (1950), described a cryptic form of trypanosomiasis in cattle, usually following drug prophylaxis or unsuccessful drug therapy, in which severe lesions occur in the heart. These lesions were associated with degenerate or lysed trypanosomes, but some normal forms were also present. This is probably similar to the condition described by Curasson (1943) and Reichenow (1952), in which masses of degenerating trypanosomes plug the capillaries.

Diagnosis: This disease can be diagnosed by detection of the parasites in blood smears. Repeated examinations may be necessary in chronic cases. Inoculation of rats or guinea pigs may give positive results when blood examinations are negative.

Cultivation: Same as for *T. brucei*.

Treatment: No effective treatment was known for either *T. congolense* or *T. vivax* until after World War II. Several drugs have been introduced since then, and active research is still going on. The general pattern has been similar. Each new drug was introduced with glowing accounts of its effectiveness, later its limitations were discovered, and it was either dropped or assumed its place in the trypanocidal armamentarium while the search passed on to a new field. The review articles listed under treatment of *T. brucei* may be consulted for further information, but progress is being made so rapidly that both they and some of the recommendations below may soon be out of date.

Ethidium is the most effective and safest of several phenanthridinium derivatives which have been used. Cattle are treated by intramuscular injection of 1 mg/kg ethidium bromide or chloride. The trypanosomes disappear from the blood within 2 days. The earlier phenanthridinium compounds caused photosensitization and liver damage, but Ethidium apparently does not.

Antrycide methyl sulfate is also effective against *T. congolense*. Cattle are treated with a single subcutaneous injection of 4.5 to 5.0 mg/kg, while 3 to 5 mg/kg is used in horses and dogs. Antrycide causes a painful local reaction when given subcutaneously, and may sometimes also cause increased salivation, sweating and tremors. In addition, there are a number of reports of drug-fastness developing to antrycide.

The diamidine, Berenil, has been used with success in preliminary experiments, but has yet to be completely evaluated. The dosage for cattle is about 2 mg/kg subcutaneously or intramuscularly.

The above recommendations deal with curative treatment. A great deal of work has also been done on chemical prophylaxis of trypanosomosis. The idea here is to inject drugs in relatively insoluble form so that they will be released slowly over a long period of time and will protect animals for months.

Antrycide chloride, which is much less soluble than antrycide methyl sulfate, is

used for prophylaxis. In actual use, a mixture of 3 parts of the methyl sulfate and 4 of the chloride, known as Antrycide prosalt, is employed. The methyl sulfate eliminates any trypanosomes that might be present at the time of treatment, and the chloride provides the prophylaxis. The prosalt is injected subcutaneously in amount sufficient to give 5 mg/kg of the methyl sulfate. In areas where there are relatively few tsetse flies (defined as an apparent density (AD) of less than 10 flies caught per 10,000 yards of patrol, using a standardized catching technic), treatment every 2 months is effective, but under heavy challenge (defined as an AD of 40 or more) this protection may break down.

Prothidium (R.D. 2801), which contains the pyrimidine moiety of Antrycide linked to a phenanthridinium instead of a quinoline nucleus, was introduced in 1956 as a prophylactic agent. According to Robson and Cawdery (1958), it is better than Antrycide prosalt, a single subcutaneous dose of 4 mg/kg protecting zebus naturally exposed to *T. congolense*, *T. vivax* and *T. brucei* injections for 110 or more days.

Complexes or salts of suramin with Ethidium, Antrycide and other trypanocides were introduced by Williamson and Desowitz (1956) for prophylactic use. They obtained more than 7 months' protection against *T. congolense* and *T. vivax* by subcutaneous injection of Ethidium suraminate. However, Robson and Cawdery (1958) considered that the local reactions which it produced were so severe as to preclude its use even tho at 5 mg/kg it protected naturally exposed zebus for 113 days or more. Further work with such complexes may be rewarding.

Pentamidine has been used extensively in prophylaxis of human trypanosomosis, but is not used in domestic animals.

Whenever a drug is used continuously for prophylaxis, there is danger that drug-fast strains of parasites may appear because the blood level becomes so low that relatively resistant individuals can survive. This has happened particularly with Antrycide and also with the phenanthridinium derivatives. Unfortunately, too, strains which have become resistant to Antrycide are also resistant to phenanthridinium compounds. No drug resistance has appeared so far to Berenil.

Control: Same as for *T. brucei*.

TRYPANOSOMA DIMORPHON LAVERAN AND MESNIL, 1904

This species was once thought to be a synonym of *T. congolense*, but Hoare (1959) restudied Laveran and Mesnil's original slides, measuring 1200 individuals and analyzing the data statistically, and showed that it differs in length. *T. dimorphon* is 11 to 24 μ long with a mean of 16.2 μ; the means of different populations ranged from 15.3 to 17.6 μ. Despite its name, Hoare (1959) found that it is actually monomorphic. It is slender, without a free flagellum, and its undulating membrane is not pronounced. The posterior end is rounded (chiefly in the shorter forms) or pointed (chiefly in the longer forms). The nucleus is in the middle or posterior part of the body. The kinetoplast is fairly large and typically subterminal and marginal.

T. dimorphon occurs in Gambia, French Guinea, Ivory Coast, Belgian Congo, Sudan, Somalia, Southern Rhodesia, Portuguese East Africa, Zululand and possibly Nigeria, and has been found in the horse, sheep, goat, cattle, pig and dog. It is transmitted by tsetse flies in the same way as *T. congolense*.

TRYPANOSOMA SIMIAE BRUCE *ET AL.*, 1912

Synonyms: *T. ignotum*, *T. rodhaini*, *T. porci*.

T. simiae was first found in a monkey, but its natural reservoir host is the wart-hog (*Phacophoerus aethiopicus*). It is highly pathogenic for the pig and camel, causing a peracute disease with death usually in a few days. This is the most important trypanosome of domestic swine.

It is only slightly pathogenic for sheep and goats, and apparently non-pathogenic for cattle, horses or dogs, altho it may infect them. The rabbit appears to be the only susceptible laboratory animal. There is a great deal of variation in pathogenicity between strains, and indeed marked changes can occur in the pathogenicity of a single strain.

T. simiae occurs mostly in East Africa and the Belgian Congo, but it has also been found in other parts of Africa where *T. congolense* occurs.

T. simiae differs morphologically from *T. congolense* in being polymorphic instead of monomorphic. It varies in length from 12 to 24 μ. About 90% of its forms are long and stout, with a conspicuous undulating membrane, about 7% are long and slender with an inconspicuous undulating membrane, and about 3% are short, with an inconspicuous undulating membrane. A free flagellum is usually absent, but has been reported in from 1 to 4% of different strains.

This species is transmitted in the warthog reservoir host by tsetse flies, including *Glossina morsitans* and *G. brevipalpis*, in which it develops in the midgut and proboscis. Tsetse flies also transmit it to swine, but once it has been introduced into a herd, it can apparently be transmitted mechanically by horseflies and other blood-sucking flies (Unsworth, 1952).

T. simiae is more resistant to drugs than the other African trypanosomes. Antrycide methyl sulfate is probably the best drug, but it may not be completely effective. It is injected subcutaneously at the rate of 5 mg/kg; more than one injection is probably necessary.

Control measures are the same as for *T. brucei*. In addition, horseflies and other biting flies should be controlled.

TRYPANOSOMA VIVAX ZIEMANN, 1905

Synonyms: *T. cazalboui, T. viennei, T. bovis, T. angolense, T. caprae.*

Disease: Souma. *T. vivax* is also sometimes found in mixed infections of cattle with *T. congolense* and *T. brucei*.

Hosts: Cattle, sheep, goats, camels, horse. Antelopes and the giraffe are reservoir hosts in Africa, and the deer (*Odocoileus gymnotis*) in Venezuela (Faisson, Mayer and Pifano, 1948). The pig, dog and monkey are refractory to infection. The small laboratory rodents are difficult to infect.

Location: Blood stream, lymph. Central nervous system infections have been described in sheep.

Geographic Distribution: *T. vivax* is found thruout the tsetse fly belt in Africa. It has, however, spread beyond this region to other parts of Africa and to Central America, South America, the West Indies and Mauritius.

Morphology: *T. vivax* is 20 to 27 μ long and monomorphic. The posterior end is typically rounded, a free flagellum is always present, the kinetoplast is large and terminal, and the undulating membrane is inconspicuous (Fairbairn, 1953).

Life Cycle: The original vectors of *T. vivax* and still the most important in Africa are tsetse flies, including *Glossina morsitans, G. tachinoides* and other species. Development takes place only in the proboscis. The trypanosomes turn into the crithidial form, multiply in this form and then turn into metacyclic, infective trypanosomes which pass to the hypopharynx and infect new hosts when the tsetse flies bite. The flies become infectious as early as 6 days after they themselves were infected.

Horseflies and other tabanids may act as vectors; they are the only ones in the New World and in Africa outside the tsetse zone. In this case transmission is mechanical.

Pathogenesis: *T. vivax* is most important in cattle, in which the disease is similar to that caused by *T. congolense*. According to Fairbairn (1953), *T. vivax* infections of cattle in East Africa usually

cause a mild disease, but in West Africa they are usually fatal in some types of cattle. Virulent strains may also occur in East Africa. Unsworth (1953) found that *T. vivax* is highly pathogenic for zebu cattle in laboratory infections, and that when these cattle were exposed to infection under natural conditions in Nigeria, all of them died.

Camels are less seriously affected than cattle. *T. vivax* is apparently more pathogenic for sheep than other trypanosomes, and may be found in the central nervous system. It is apparently less pathogenic for goats. It causes a chronic disease, often with spontaneous recovery, in horses. It is not pathogenic for dogs, pigs and monkeys, and only slightly so for the common laboratory rodents.

The signs of disease are similar to those caused by *T. congolense*. There is a wide variation in virulence between different strains, but the virulence of any particular strain tends to remain constant.

Diagnosis: *T. vivax* is detected most readily in lymph node smears. Large numbers are found in the blood only in early infections. Inoculation of laboratory animals is not particularly satisfactory; inoculation of sheep or goats is better, the trypanosomes appearing in 7 to 10 days.

Cultivation: Same as for other trypanosomes.

Treatment: *T. vivax* can be successfully treated with the same drugs and in the same dosages as *T. congolense*. It is perhaps slightly more resistant, but not significantly so.

Control: Control measures are the same as those for *T. congolense* infections. In areas where tabanids are the vectors, measures directed against these flies should be practiced.

TRYPANOSOMA UNIFORME
BRUCE *et al.*, 1911

This species is similar to *T. vivax*, differing from it morphologically only in being smaller. It is 12 to 20 μ long, with an average of about 16 μ. It occurs in cattle, sheep, goats and antelopes, causing a disease similar to that caused by *T. vivax* (Wilson, 1949). Laboratory rodents are refractory to infection. *T. uniforme* occurs only in Uganda and the Belgian Congo. It is transmitted by tsetse flies in the same way as *T. vivax*.

TRYPANOSOMA CRUZI
CHAGAS, 1909

Synonyms: *Schizotrypanum cruzi*.

Disease: American human trypanosomosis, Chagas' disease.

Hosts: Many species of wild and domestic animals have been found naturally infected with *Trypanosoma cruzi*, and probably most mammals are susceptible. Man is also susceptible, infants and young children being most often affected. The most important wild reservoir hosts are probably armadillos (*Dasypus*) in South America, opossums (*Didelphis*) in South and Central America and the United States, and woodrats (*Neotoma*) and possibly raccoons (*Procyon*) in the United States. The dog, cat and possibly the pig are considered of some importance as reservoirs of infection for man in South America.

Location: The trypanosomes are found in the blood early in an infection. Later, they invade the cells of the reticuloendothelial system, heart and striated muscles and other tissues. In central nervous system infections, they are found in the neuroglial cells. Trypanosome forms occur in the blood, and leishmanial forms within the cells.

Geographic Distribution: *T. cruzi* occurs in South America from Argentina north, in Central America and in southern United States. Dias (1953) published maps of the distribution of Chagas' disease in South and Central America together with climatologic and other information.

In the United States, *T. cruzi* had been thought until recently to be confined to the southwestern states, including Texas,

Arizona, New Mexico and southern California, but Walton *et al.* (1956) discovered it in raccoons in Maryland, and it appears that it may be rather widely distributed in the southeastern states. McKeever, Gorman and Norman (1958) found it in 17% of 552 opossums, 2% of 118 grey foxes (*Urocyon cinereoargenteus*), 1.5% of 608 raccoons and 1% of 306 striped skunks (*Mephitis mephitis*) from Georgia and Florida. Walton *et al.* (1958) found it in 5 of 400 raccoons from Maryland. Norman *et al.* (1959) reported that their strains were typical *T. cruzi* of relatively low virulence for mice.

Morphology: The forms in the blood are monomorphic, 16 to 20 μ long, with a pointed posterior end and a curved, stumpy body. The kinetoplast is subterminal and larger than that of any other trypanosome of domestic animals or man, often causing the body to bulge around it. The undulating membrane is narrow, with only 2 or 3 undulations. There is a moderately long free flagellum. The leishmanial forms in the muscle and other tissue cells are 1.5 to 4.0 μ in diameter and occur in groups. Electron microscope studies of this species have been made by Meyer and Porter (1954), Meyer, Musacchio and Mendonca (1958) and Meyer and Queiroga (1960).

Life Cycle: Altho the trypanosome form of *T. cruzi* is common in the blood in the early stages of Chagas' disease, it does not multiply in this form. The trypanosome forms enter the cells of the reticulo-endothelial system, striated muscles and especially of the heart muscle, where they round up and turn into leishmanial forms. These multiply by binary fission, destroying the host cells and forming cyst-like nests of parasites. There does not appear to be conclusive proof that they turn into the crithidial form in mammals, as was once believed. The leishmanial forms turn into trypanosome forms which re-enter the blood. Among recent studies or reviews of the life cycle of *T. cruzi* in the vertebrate host are those of Elkeles (1951), Noble (1955), Romaña (1956) and Wood (1951, 1951a, 1953).

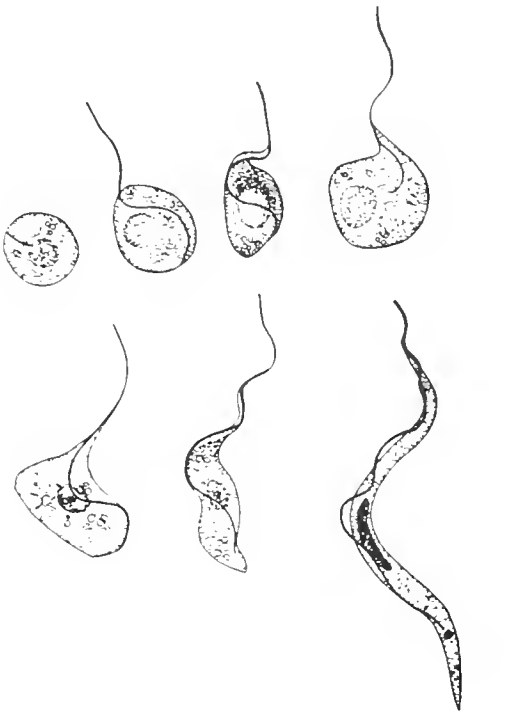

Fig. 4. Successive stages in the transformation of a leishmanial form of *Trypanosoma cruzi* into a metacyclic trypanosome form. The metacyclic trypanosome (lower right) is from a Giemsa stained smear; the other stages are from living preparations of culture material viewed with the phase microscope. (From Noble, 1955)

The vectors of *T. cruzi* are kissing bugs or conenose bugs, members of the hemipteran family Reduviidae. Natural infections have been found in at least 36 species of these bugs. They get their first name from the fact that in sucking blood they prefer to attack the thinner parts of the skin such as the lips or eyelids.

The most important vector in South America is probably *Panstrongylus* (syn., *Trialoma*) *megistus*. Other important vectors in South and Central America are *P. geniculatus*, *Eutriatoma sordida*, *Triatoma infestans*, *Rhodnius prolixus* and *Eratyrus cuspidatus*.

According to Faust (1949), 15 naturally infected species of reduviids have been found in the United States. Dias (1951,

1951a) listed *Triatoma protracta*, *T. sanguisuga* (= *T. gerstaeckeri*), *T. lectularius*, *T. longipes*, *T. neotomae* and *T. rubida* as having been found infected in the U. S. Mehringer and Wood (1958) found *T. cruzi* in 24% of 383 *Triatoma protracta* collected in the Boy's and Girl's Camp areas in Griffith Park, Los Angeles, Calif. Most of the conenose bugs were taken in human habitations.

Both the nymphs and adults of these reduviids can be infected and can transmit the disease. In addition, it is possible to infect sheep keds (Rodhain and Brutsaert, 1935), ticks (*Ornithodoros*)(Brumpt, 1939) and bedbugs (Wood, 1951a) experimentally.

After they have been ingested by the triatomids along with a blood meal, the trypanosomes pass to the midgut. Here they turn into leishmanial forms which multiply by binary fission and turn into either metacyclic trypanosomes or crithidial forms. The crithidial forms multiply further by binary fission, and extend into the rectum. Here they turn into metacyclic trypanosomes, which are unable to divide until they enter a vertebrate host. The life cycle in the invertebrate host takes 6 to 15 days or longer, depending on the insect species or stage and on the temperature.

The infective trypanosome forms pass out in the feces. They can penetrate the mucous membranes or skin actively. Triatomids commonly defecate after feeding, and most human infections occur when feces are rubbed into the eyes or mucous membranes following a bite. Animals can become infected by licking their bites or by eating infected bugs or rodents.

Epidemiology: Human infections with *T. cruzi* are common in many parts of tropical America, including Brazil, Bolivia, northern Chile, northern Argentina, French Guiana, Paraguay, Uruguay and Venezuela. In some localities 10 to 20% or even 50% of the inhabitants are positive to the complement fixation (Machado) test, but in other localities where exposure to the vectors is minimal, there are very few positive reactions. As mentioned below, acute Chagas' disease occurs primarily in infants and children, and the number of acute cases is far lower than the numbers of chronic and unrecognized infections.

Chagas' disease becomes increasingly uncommon to the north of the endemic area even tho infected reservoir hosts and vectors may be common. Less than 140 cases of Chagas' disease had been reported from Guatemala, Salvador, Nicaragua, Costa Rica and Panama according to Dias (1952a) while only 9 cases were known from Mexico (Mazzotti and Dias, 1950). Only a single indigenous case has been reported from the United States, by Woody and Woody (1955) in Texas.

Chagas' disease is a zoonosis, infections occurring widely in animals and man. The armadillo is thought by Hoare (1949) to be the original source of the human disease in South America, but the opossum and many other wild animals are also infected. The most important wild reservoirs in the United States are woodrats of the genus *Neotoma*. Natural infections have been found in the southwestern states and southern California in *N. fuscipes*, *N. albigula*, *N. micropus*, in the deermouse, *Peromyscus boylii*, and also in the opossum, house mouse and nine-banded armadillo (*Dasypus novemcinctus*). The recent discovery of *T. cruzi* infections in raccoons (*Procyon lotor*), opossums, gray foxes and skunks in Maryland, Georgia and Florida (Walton *et al.*, 1956, 1958; McKeever, Gorman and Norman, 1958) raises the question how widespread the infection is in these animals.

Cats and dogs are often naturally infected in South America, and, because of their closer association with man, are probably more important as sources of human infection than wild animals. Naturally infected pigs have been found in South America, and sheep and goats can be infected experimentally with these South American strains.

In a study of the possible role of farm animals as reservoirs of North American strains of *T. cruzi*, Diamond and Rubin (1958) established low-grade infections in

young pigs, lambs, kids and calves with a strain isolated from a raccoon in Maryland. The infections persisted at least 57 days in the pigs, 53 to 85 days in the lambs, 38 days in a kid and 21 days in a calf.

Infection is common in the triatomid vectors of Chagas' disease. In the endemic regions of South and Central America, 40 to 60% of them are infected, while 20 to 25% are infected in Mexico and southwestern United States. The triatomids infest armadillo burrows and woodrat nests, and thus maintain the infection in these animals. They also infest houses, where they live like bedbugs; it is these triatomids which are responsible for the vast majority of human infections.

Because triatomids are rare in southeastern U. S. where *T. cruzi* is common in the opossums, and because they isolated the organism from the urine of infected opossums, McKeever, Gorman and Norman (1958) believed that infections may be passed from mammal to mammal by contact with infected urine.

For further information on the epidemiology of *T. cruzi* infections see Dias (1951, 1951a, 1951b, 1952, 1952a, 1952b), Dias and Chandler (1951), Dias and Laranja (1948), Dias, Laranja and Nobrega (1946) and other papers by these authors. For information on the epidemiology of *T. cruzi* in southwestern United States, see Elkins (1951) and particularly Wood (1950, 1953a, 1958), and Mehringer and Wood (1958).

Pathogenesis: Chagas' disease may be either acute or chronic in man. The great majority of acute cases occur in infants and young children. The first sign of disease is often swelling of the eyes and conjunctiva. This swelling may affect either one or both sides of the face. The tear glands become inflamed, and the cervical lymph nodes swell. Later on, swellings may appear in other parts of the body. Each swelling, known as a *chagoma*, is due to an inflammatory exudate in the region where the parasites are invading the tissue cells. In addition to this edema, there may be anemia, more or less continuous fever, prostration and severe headache.

If the patient survives the acute phase, the disease becomes more or less chronic. Some authorities believe that it persists for life. The lymph nodes are edematous and inflamed, and the liver and spleen are enlarged. The heart is affected in many cases. Electrocardiographic abnormalities are common. Inflammatory infiltration by phagocytes, fibrosis, separation of the muscle cells and partial destruction of the fibers by the multiplying parasites are present. The death rate due to cardiac conditions is increased in endemic areas.

T. cruzi may cause an acute or chronic disease in laboratory animals, depending on the strain of the parasite and the age of the host. Puppies and kittens are most susceptible, followed in order by mice and guinea pigs. The reservoir hosts are apparently not seriously affected, nor are farm animals. No clinical signs were observed in the infected young pigs, lambs, kids and calves studied by Diamond and Rubin (1958).

Diagnosis: In the acute stage of the disease, *T. cruzi* can be found in thick blood smears. In chronic or light infections, other methods must be used. One of the most important is *xenodiagnosis*, the inoculation of susceptible vector hosts. Laboratory-reared, parasite-free triatomids are allowed to feed on suspected individuals, and their droppings or intestines are examined 7 to 10 days later for developing trypanosomes. *Rhodnius prolixus* is often used for this purpose (Pifano, 1954a).

Laboratory animals can also be inoculated. In descending order of susceptibility, these are puppies, kittens, mice and guinea pigs. The trypanosomes can be cultivated in NNN medium, Weinman's (1946) medium, Diamond and Herman's (1954) SNB-9 (serum-neopeptone-blood) medium, or in a number of other media. The trypanosomes can also be found in biopsy examinations of affected lymph glands or, on necropsy, in sections of heart muscle.

A complement fixation test, the Machado reaction, has been used, but it is also positive in *Leishmania* infections and weakly positive in a number of other conditions. Other serologic tests which have been used are the precipitin reaction, an intradermal skin test and a slide agglutination test. *T. cruzi* can be differentiated from the non-pathogenic *T. rangeli* by its smaller size and large kinetoplast.

Treatment: No satisfactory drug is known for the treatment of *T. cruzi* infections, altho Bayer 7602 Ac is used.

Control: Prevention of human *T. cruzi* infection depends upon eliminating triatomids from houses. This will also largely prevent infections among domestic dogs and cats. Dusting or spraying houses with residual lindane or dieldrin has given good results.

TRYPANOSOMA RANGELI TEJERA, 1920

Synonyms: *Trypanosoma guatemalense, T. ariarii.*

T. rangeli was first found in the triatomid, *Rhodnius prolixus*, in Venezuela. It was later found in children in Guatemala and still later in Colombia, Chile and El Salvador. It is quite common in dogs, cats and man in certain areas of Venezuela, Colombia and Guatemala, and is sometimes found in mixed infections with *T. cruzi*. Groot, Renjifo and Uribe (1951) found it in 67 of 183 persons in the Ariari Valley and Groot (1951) found it in 1 of 30 persons, 2 of 27 dogs and an opossum in the Miraflores region of Colombia. It has also been found in the monkey, *Cebus fatuellus*. Young mice, rats and rhesus monkeys can be infected experimentally.

The trypanosomes in the blood are considerably larger than *T. cruzi*, being 26 to 36 μ long. The nucleus is anterior to the middle of the body, the undulating membrane is rippled and the kinetoplast is small and subterminal.

The most common vector is *Rhodnius prolixus*, but *Triatoma dimidiata* and other triatomids have also been found infected. A piriform stage about 7 μ long has been found in the foregut, and crithidial and metacyclic trypanosome forms develop in the hindgut. The crithidial stages may be extremely long, ranging from 32 to 70 or even over 100 μ in length. The metacyclic trypanosome forms have a well-developed undulating membrane and a long free flagellum. They may pass into the hemolymph and thence to the salivary glands. They can be transmitted either by bite or by fecal contamination.

T. rangeli does not appear to be pathogenic for vertebrates, but Grewal (1957) found that it was pathogenic for *R. prolixus* and also for experimentally infected bedbugs.

The blood forms of *T. rangeli* can be readily differentiated from those of *T. cruzi* by their larger size and their much smaller kinetoplast. The forms in the insect hosts can be distinguished by their small kinetoplast and giant crithidial forms.

T. rangeli can be easily cultivated in a modified NNN medium containing glucose, peptone and macerated meat (Pifano, 1948). The culture forms are similar to those in the triatomid intestine. For further information regarding this species, see Groot, Renjifo and Uribe (1951), Groot (1954), Pifano (1948, 1954) and Zeledon (1954).

TRYPANOSOMA THEILERI LAVERAN, 1902

Synonyms: *Trypanosoma franki, T. wrublewskii, T. himalayanum, T. indicum, T. muktesari, T. falshawi, T. scheini, T. americanum, T. rutherfordi.*

T. theileri occurs in the blood of cattle. It is worldwide in distribution. It is probably quite common, but is rarely found in blood smears. Crawley (1912) found it in blood cultures from 74% of 27 cattle around Washington, D. C. and Glaser (1922) found it in blood cultures from 25% of 28 New Jersey cattle. Neither found it in direct blood smears. Atchley (1951) found it in the blood of 1% of 500 South Carolina cattle.

T. theileri is relatively large, being ordinarily 60 to 70 μ long, but forms up to 120 μ long and smaller ones 25 μ long often occur; those found by Levine *et al.* (1956) in an Illinois heifer were 34 to 40 μ long exclusive of the flagellum. The posterior end is long and pointed. There is a medium-sized kinetoplast some distance anterior to it. The undulating membrane is prominent, and a free flagellum is present. Both trypanosome and crithidial forms forms may occur in the blood. Multiplication occurs by binary fission in the crithidial form in the lymph nodes and various tissues.

T. theileri is transmitted by various tabanid flies, including *Tabanus* and *Haematopota*. It reproduces in the fly intestine by binary fission in the crithidial stage.

T. theileri is ordinarily non-pathogenic, but under conditions of stress it may cause serious signs and even death. It has caused losses in cattle being immunized against rinderpest and other diseases, and has occasionally been accused of causing an anthrax-like disease. Carmichael (1939) found masses of *T. theileri* in the brain of a cow which had died with signs of "turning sickness" in Uganda.

T. theileri may also be associated with abortion, altho it has not proved that it causes this condition. Levine *et al.* (1956) found it in an Illinois heifer which had aborted, and Dikmans, Manthei and Frank (1957) found it in the stomach of an aborted bovine fetus in Virginia. Lundholm, Storz and McKercher (1959) found it as a contaminant in a primary culture of kidney cells from a bovine fetus in California. This was further evidence that intrauterine transmission may occur.

Ristic and Trager (1958) found *T. theileri* in three Florida dairy cattle with depressed milk production; it was not found in cows in the same herd with normal milk production. The affected cows had a marked eosinophilia.

Since *T. theileri* is rarely seen in the blood, diagnosis ordinarily depends on cultivation. It can be cultivated in NNN and other media at room temperature. Ristic and Trager (1958) also cultivated it at 37° C in a blood-lysate medium. Both crithidial and trypanosome forms were present in their cultures. Lundholm, Storz and McKercher (1959) found that it grew well in tissue culture medium containing 10% lamb serum, but better if bovine kidney cells were present.

No treatment is known for *T. theileri*. Infections can be prevented by elimination of the tabanid vectors.

TRYPANOSOMA MELOPHAGIUM (FLU, 1908)

This parasite is very common in sheep thruout the world. It is non-pathogenic, and infections are so sparse that it can ordinarily be found only by cultivation. The trypanosomes in the blood resemble *T. theileri* and are 50 to 60 μ long.

T. melophagium is transmitted by the sheep ked, *Melophagus ovinus*, and can readily be found in its intestine. Its life cycle has been described by Hoare (1923). Crithidial forms are abundant in the midgut, and leishmanial forms occur here also. Both multiply by binary fission. The crithidial forms change into small, metacyclic trypanosome forms in the hindgut. Nelson (1956) found that *T. melophagium* may kill the ked by blocking the midgut. Sheep are infected when they bite into the keds and the trypanosomes pass thru the intact buccal mucosa. Because infections in sheep are so sparse, it has been suggested that no multiplication occurs in this host.

TRYPANOSOMA THEODORI HOARE, 1931

This non-pathogenic species was found in goats in Palestine. It resembles *T. melophagium* and has a similar life cycle, except that its intermediate host is another hippoboscid fly, *Lipoptena caprina*. *T. theodori* may be a synonym of *T. melophagium*.

TRYPANOSOMA NABIASI
RAILLIET, 1895

This species occurs in the wild European rabbit, *Oryctolagus cuniculus*. It has been found sporadically in England, France and other European countries. It is 24 to 28 μ long. Its intermediate host is the flea, *Spilopsyllus cuniculi*, in which it develops in the gut. The metacyclic infective forms occur in the rectum. Infection is presumably by ingestion. Grewal (1956) described its life cycle briefly.

TRYPANOSOMA LEWISI
(KENT, 1880)
LAVERAN AND MESNIL, 1901

This species occurs quite commonly in the black rat, Norway rat and other members of the genus *Rattus* thruout the world. It is not normally transmissible to mice. It is 26 to 34 μ long. Its vector is the rat flea, *Nosopsyllus fasciatus*, in which it develops in the gut, and in which the metacyclic, infective forms occur in the rectum. Rats become infected by eating infected fleas or flea feces. *T. lewisi* is non-pathogenic.

A great deal of research has been done on this species, since it is easy to handle and its host is a convenient one.

TRYPANOSOMA DUTTONI
THIROUX, 1900

This species occurs in the house mouse and other species of *Mus* thruout the world. It is not normally transmissible to rats. It is 28 to 34 μ long. Its vector is the flea, *Nosopsyllus fasciatus*, and its life cycle is the same as that of *T. lewisi*. It is non-pathogenic.

TRYPANOSOMES OF BIRDS

Trypanosomes have been reported under a large number of names from many species of birds. They all look very much alike and probably belong to relatively few species. However, extensive cross transmission studies are needed to establish their relationships, and, until these are carried out, it is probably best to refer to them by the names under which they were first described.

Trypanosoma avium Danilewsky, 1885 was first described from owls (scientific name not given) and roller-birds (*Coracias garrulus*) in Europe, and has since been reported from a wide variety of birds, including crows (Baker, 1956) and Canada geese (Diamond and Herman, 1954). Baker (1956 a, b) transmitted it from the rook (*Corvus frugilegus*) and jackdaw (*C. monedula*) to canaries, but failed to transmit it to a single 3-day-old chick.

T. calmettei Mathis and Leger, 1909 was described from the chicken in southeast Asia; it is about 36 μ long. *T. gallinarum* Bruce *et al.*, 1911 was described from the chicken in central Africa; it is about 60 μ long. *T. hannai* was described from the pigeon, and *T. numidae* from the guinea fowl.

Avian trypanosomes are very polymorphic, sometimes attaining great size. They may be 26 to 60 μ long or even longer. The kinetoplast is generally a long distance from the posterior end. There is a free flagellum, and the body is often striated.

Blood-sucking arthropods such as mosquitoes and hippoboscids are believed to be the vectors of avian trypanosomes, but the only complete life cycle was worked out by Baker (1956,a, b) for *T. avium* from rooks and jackdaws. He found that in England the hippoboscid fly, *Ornithomyia avicularia*, acts as the vector. Upon ingestion with a blood meal, the trypanosomes change into the crithidial form in the midgut, multiply by binary fission in this form, and pass to the hindgut. They multiply further and then turn into a piriform stage which develops in turn into a small, metacyclic trypanosome form. Birds become infected when they eat infected insects. The metacyclic trypanosomes penetrate the membranes of the mouth, esophagus and/or crop and probably invade the lymphatic system, developing into large forms

which first appear in the blood 18 to 24 hours after infection.

According to Baker, there is no multiplication in the avian host, the trypanosomes simply becoming larger. This would account for their sparse numbers in the blood. They persist in the rook and jackdaw over-winter, being more or less restricted to the bone marrow, and reappear in the peripheral blood in the spring. Diamond and Herman (1954), too, found that *T. avium* could be isolated from the bone marrow of Canada geese much more readily than from the blood.

Nothing is known of the pathogenicity of the avian trypanosomes. They are presumably non-pathogenic.

Avian trypanosomes can be readily cultivated on several media, including NNN medium and the SNB-9 (saline-neopeptone-blood) medium described by Diamond and Herman (1954).

Genus *LEISHMANIA* Ross, 1903

Members of this genus occur primarily in mammals. They cause disease in man, dogs and various rodents including gerbils and guinea pigs. *Leishmania* is heteroxenous, being transmitted by sandflies of the genus *Phlebotomus*. It is found in the leishmanial stage in the cells of its vertebrate hosts and in the leptomonad stage in the intestine of the sandfly and in culture.

Morphology: All species of *Leishmania* look alike, altho there are size differences between different strains. The leishmanial stage is ovoid or round, usually 2.5 to 5.0 by 1.5 to 2.0 μ, altho smaller forms occur. Only the nucleus and kinetoplast are ordinarily visible in stained preparations, but a trace of an internal fibril representing the flagellum can sometimes be seen. This flagellum and the basal granule from which it arises can also be seen in electron micrographs (Chang, 1956; Pyne and Chakraborty, 1958). The leptomonad forms in culture and in the invertebrate host are spindle-shaped, 14 to 20 μ long and 1.5 to 3.5 μ wide.

Life Cycle: In the vertebrate host, *Leishmania* is found in the macrophages and other cells of the reticulo-endothelial system in the skin, spleen, liver, bone marrow, lymph nodes, mucosa, etc. It may also be found in the leucocytes, especially the large mononuclears, in the blood stream. It multiplies by binary fission in the leishmanial form.

The invertebrate hosts of *Leishmania* are sandflies of the genus *Phlebotomus*. When the sandflies suck blood they ingest the leishmanial forms. These pass to the midgut, where they assume the leptomonad form and multiply by binary fission. They may be either free in the lumen or attached to the walls.

Their further development varies with the particular species of *Phlebotomus* and strain of *Leishmania*. In good vectors like *P. argentipes*, *P. papatasii* and *P. sergenli*, they begin to extend their range forward to the esophagus and pharynx by the fourth or fifth day. They continue to multiply to such an extent that they plug up the esophagus and interfere with blood-sucking. When an infected sandfly bites, it clears the passage by injecting some of the leishmanial forms into its victim and thus transmits the parasite. *Leishmania* may also be transmitted when sandflies are crushed on the skin.

In other cases, the parasites remain in the sandfly midgut and do not pass forward into the pharynx. These can then be transmitted only by crushing the sandflies. A third type of development was described by Shoshina (1953), who found leptomonads in the hindgut of *P. minutus* var. *arparklensis* in Russia and suggested that feces containing them might be rubbed into the bite while scratching it.

In addition to transmission by sandflies, it has been suggested that direct infection by means of excretions of infected individuals might occur in kala azar.

Species of Leishmania: The speciation of *Leishmania* has been discussed by

Hoare (1949), Kirk (1949, 1950) and Biaga (1953) among others. While some 22 different specific or subspecific names have been given to mammalian leishmanias, and while different strains are associated with different types of disease, neither morphologic, cultural nor immunologic characters can be used to differentiate the species of *Leishmania*. In practice, the species are separated on the basis of pathologic and epidemiologic differences and, since most studies have been made by parasitologists oriented toward human disease, the pathologic characters used for each strain have been those seen in man. In the earlier days of our knowledge, when relatively few types were known, it was quite easy to delineate their characteristics and set up separate species, but as more studies were made, intermediate types were found and the boundaries between species tended to disappear.

Some parasitologists consider that all the leishmanias of man and dogs should be assigned to a single species. Others prefer to assign them to two species, and still others to three. One can justify each of these schemes, but in all of them each species is still composed of a number of strains or demes.

In this book, two species of *Leishmania* are recognized: *L. donovani*, causing various visceral forms of disease, and *L. tropica*, causing various cutaneous and mucocutaneous forms. The third species recognized by some authorities is *L. brasiliensis*, which causes a mucocutaneous form of the disease.

Maps of the world distribution of leishmanioses together with climatologic and other information have been published by Piekarski (1952), Piekarski and Sibbing (1954), Piekarski, Hennig and Sibbing (1956, 1958a), the American Geographical Society (1954) and May (1954).

LEISHMANIA DONOVANI (LAVERAN AND MESNIL, 1903) ROSS, 1903

Synonyms: *Piroplasma donovani*, *Leishmania infantum*, *L. canis*, *L. chagasi*.

Disease: Kala-azar; dum-dum fever; visceral leishmaniosis.

Hosts: Man and the dog are the principal hosts of *L. donovani*. Infections have also been reported in the cat by Sergent *et al.* (1912) and Bosselut (1948), in the sheep by De Paolis (1935) and in the horse by Richardson (1926).

Location: *L. donovani* occurs in the cells of the reticulo-endothelial system, including both the endothelial cells and the circulating monocytes and polymorphonuclear leucocytes. The parasites are found thruout the body, but particularly in the endothelial cells of the blood and lymph vessels of the spleen, liver, bone marrow, lungs, kidneys, mesenteric lymph nodes and skin.

Types of Disease, Geographic Distribution and Epidemiology:

Five types of visceral leishmaniosis can be recognized:

1. Indian kala-azar or dum-dum fever is the classical type of the disease. It is found in India and affects young adults (60%) and children 5 to 15 years old. It does not occur naturally in dogs altho they can be infected experimentally. It is transmitted by *Phlebotomus argentipes*.

2. Sudanese kala-azar is found in the Sudan and Abyssinia. It affects people of the same ages as Indian kala-azar and does not occur naturally in dogs. It was found once in a horse (Kirk, 1956). Oral lesions are frequently present, and this type of the disease is relatively refractory to treatment with antimony compounds. It is transmitted by *P. orientalis*. A similar form occurs in small, isolated pockets scattered thru Africa south of the Sahara. It may cause skin lesions in addition to the visceral ones. It is a zoonosis, and has been found in a gerbil (*Tatera vicina*) and a ground squirrel (*Xerus rutilus*)(Manson-Bahr, 1959).

3. Chinese kala-azar is found in northern China. It is more common in children

than in adults, and also occurs commonly in dogs. It is transmitted by *P. chinensis* and *P. sergenti*.

4. Mediterranean or infantile kala-azar is found in countries of the Mediterranean basin including southern Europe and in parts of tropical Africa. Dogs are much more commonly infected than man, and 90% of the affected people are children less than 5 years old. The incidence in dogs may reach 20% in some countries, and infection rates as high as 40% have been reported in Greece and Samarkand. Even in such countries, the infection rate in children is only 1 to 2%. Mediterranean kala-azar is transmitted principally by *P. perniciosus* and *P. major*.

5. South American kala-azar is found from Mexico to northern Argentina. It attacks human beings of all ages and also occurs in dogs and cats. In a monographic review of visceral leishmaniosis in Brazil, Da Silva (1957) stated that it is endemic and at times epidemic in certain areas, that it is transmitted by *Phlebotomus longipalpis* from a natural reservoir host such as the dog, and that it occurs mostly among persons with a low economic status and particularly among the children of that group. According to Deane (1956, 1958), the dog is the principal urban reservoir and the most important source of human infection, while the "bush-dog" (*Lycalopex vetulus*) is probably the principal rural one. The disease is also transmitted by *P. intermedius*.

Two cases of visceral leishmaniosis have been reported in dogs in the United States, one in Alabama by Thorson *et al.* (1955) and the other in Washington, D. C. by Gleiser, Thiel and Cashell (1957). Both dogs had been imported into this country from Greece.

Of the five types of visceral leishmaniosis, the Mediterranean, Chinese and South American are zoonoses while the Indian and Sudanese are not. The reasons for this are not clear, since dogs can be infected experimentally with the Indian and Sudanese demes of *L. donovani*. Adler and Theodor suggested that it may be because the Mediterranean type is transmitted by sandfly bites whereas the Indian type is transmitted when the sandfly is crushed on the skin. Since dogs and infants are not good flyslappers, they are not so likely to get Indian kala-azar.

Pathogenesis: Kala-azar is an important and highly fatal disease of man, particularly in India. After an incubation period of several months, it starts with an irregular fever lasting weeks to months. The spleen and liver hypertrophy. In advanced cases, there is ulceration of the digestive tract (mouth, nose, large intestine) resulting in diarrhea, and ulceration of the skin. There is great emaciation, but the abdomen is swollen. In untreated cases, the mortality is 75 to 95%, being a little higher in adults than in infants. Death occurs in a few weeks to several years, often resulting from intercurrent disease. In treated cases, 85 to 95% recover. Following recovery, whitish spots which develop into lentil-sized nodules may appear in the skin, particularly of the face and neck. This condition is known as post-kala-azar dermal leishmanoid.

Mediterranean kala-azar in children is similar to the above, but the disease usually runs a shorter course.

Kala-azar is essentially a reticulo-endotheliosis. The reticulo-endothelial cells are increased in number and invaded by the parasites. The cut surface of the enormously enlarged spleen is congested, purple or brown, with prominent Malpighian corpuscles. The liver is enlarged and there is fatty infiltration of the Kupffer cells. The macrophages, myelocytes and neutrophiles of the bone marrow are filled with parasites. The lymph nodes are usually enlarged and the intestinal submucosa is infiltrated with macrophages filled with parasites; these are especially numerous around the Peyer's patches. Intestinal ulceration, if present, is usually a secondary condition. There is progressive leucopenia accompanied by monocytosis.

There may be anemia due to blockage of the reticulo-endothelial system.

In dogs and also in the Brazilian bush-dog, *L. donovani* may cause either visceral or cutaneous lesions, but the latter are much more common. The disease is usually chronic with low mortality, altho an acute, highly fatal type is known. There may be emaciation and anemia. There is an abundant scurfy desquamation of the skin, and in some dogs more or less numerous cutaneous ulcers. In Chinese kala-azar, cutaneous lesions occur especially around the nose and ears. The hair is shed on parts of the body, particularly the head. The parasites occur in the macrophages in the subcutaneous tissues or in nodular lesions in the skin. They have also been recovered from healthy appearing skin. The visceral type of the disease is similar to that in man.

Diagnosis: The only sure diagnostic method is the demonstration of the parasites themselves, altho serologic and other tests have also been used and are of suggestive value. Smears made from biopsy samples of spleen pulp, liver pulp, superficial lymph nodes, bone marrow or thick blood smears can be stained with Giemsa's stain and examined microscopically. In visceral leishmaniosis, the spleen is most often positive, but a certain amount of danger is associated with puncturing a soft, engorged, enlarged spleen. Thick blood smears are more often positive in man than in dogs.

Examination of bone marrow obtained by sternal puncture is becoming increasingly popular. In the cutaneous form of the disease, scrapings should be made for staining from the lesions or from the dermis with as little bleeding as possible. This is probably the method of choice for dogs, since the cutaneous disease is more common than the visceral form in them. *L. donovani* can often be found in apparently normal skin in dogs and also, in the Sudanese and Middle Asiatic forms of the disease, in man (Manson-Bahr, 1959). Examination of the superficial lymph nodes is also valuable.

Leishmania can be cultivated readily in NNN medium or a similar medium. The medium is inoculated with spleen, lymph node or liver juice, bone marrow, blood, or excised dermis and incubated for a week to a month at 22 to 24° C. Leptomonad forms are present in culture. *Leishmania* can also be grown in chicken embryos (Trincão, 1948) and in tissue culture (Hawking, 1948); see Pipkin (1960) for a review of this subject.

Animal inoculation can also be practiced, but is not usually done because it takes several months. The golden hamster is the most susceptible laboratory animal.

The complement fixation test has been used with some success, particularly in man. It is often positive before the parasites themselves can be found.

The formol gel test (Napier's aldehyde test) is positive in more advanced cases. It is carried out by adding a drop of commercial formalin to 1 ml of serum. In a positive reaction the serum turns into a milky white gel; a clear gel is not positive. Organic antimony compounds, resorcinol, and many other compounds will also produce this reaction. It is due to an increase in euglobulin and decrease in albumin in the serum. It also occurs in diseases other than kala-azar.

Treatment: Leishmanial infections can be treated successfully with various organic antimony compounds. The cheapest is tartar emetic, which is administered intravenously. In man, at least 25 or 30 doses totaling at least 2.5 g must be administered daily or on alternate days. Pentavalent antimony compounds are more expensive, but they are less toxic, act more quickly, and most of them can be injected intramuscularly as well as intravenously. Even so, 10 or 12 doses totaling 2.7 to 4.0 g are needed. Among these compounds are neostibosan, neostam, solustibosan and urea stibamine. The aromatic diamidines, pentamidine and stilbamidine, have been used in treating human leishmaniosis, but they are apparently not very effective in dogs. Goodwin

and Rollo (1955) reviewed the chemotherapy of leishmaniosis briefly.

Control: Prevention of leishmanial infections depends on breaking the life cycle by elimination of sandflies. This can be done by residual spraying of houses, barns and outside resting places with DDT or other chlorinated hydrocarbon insecticides (Hertig, 1949; Corradetti, 1954; Deane, 1958). In addition, insect repellents such as dimethylphthalate can be rubbed on the skin, houses can be screened with very fine mesh wire, and decaying vegetation and other breeding places can be cleaned up.

In regions where kala-azar is a zoonosis, treatment of infected dogs and destruction of strays will eliminate the reservoir of infection for man.

LEISHMANIA TROPICA
(WRIGHT, 1903)
LÜHE, 1906

Synonyms: *Helcosoma tropicum, Sporozoa furunculosa, Ovoplasma orientale, Plasmosoma jerichaense, Leishmania wrighti, L. cunninghami, L. nilotica, L. recidiva, L. brasiliensis, L. peruviana.*

Disease: Cutaneous leishmaniosis, mucocutaneous leishmaniosis, Oriental sore, Aleppo button, Jericho boil, Delhi boil, espundia, uta, chiclero ulcer, buba, pian bois, American forest leishmaniosis.

Hosts: The usual hosts are man, the dog and, in parts of the Old World, gerbils (*Rhombomys opimus*) and other wild rodents.

Location: *L. tropica* occurs in the monocytes and other cells of the reticuloendothelial system, in cutaneous lesions and in the skin. It may also occur in the lymph nodes and in the mucous membranes.

Types of Disease, Geographic Distribution and Epidemiology:

Two forms of cutaneous leishmaniosis have been described in man in the Old World and 4 in the New. Separate subspecific names have been given to some of them:

1. Classical Oriental sore is found in regions with a hot, dry climate from the Mediterranean basin to central and northern India. It is caused by *L. tropica minor*. The incubation period is several months. The lesions are circumscribed, "dry" sores in the skin. They heal spontaneously and do not extend to the mucous membranes. The lymph nodes are involved in about 10% of the cases. Dogs are commonly infected, and the disease is urban in distribution. In Teheran, Iran, for example, 40 to 50% of the dogs have skin ulcers. The disease is transmitted by *Phlebolomus papatasii, P. sergenti, P. perfiliewi* and *P. longicuspis*.

2. "Moist" or "wet" Oriental sore is found in Central Asia and southern USSR. It is caused by *L. tropica major:* there is no cross-immunity between this subspecies and *L. t. minor*. The incubation period is 1 to 6 weeks. The lesions are wet and ulcerative, but do not extend to the mucous membranes. They heal spontaneously. The lymph nodes are often involved. The disease is rural in distribution. The reservoir hosts are various desert rodents, the gerbil (*Rhombomys opimus*) being the most important. The vector is *P. caucasicus*, which lives in the gerbil burrows.

3. Mucocutaneous leishmaniosis or espundia is found in the Brazilian rain forests. It is caused by *L. tropica brasiliensis*, which many authors consider a separate species, *L. brasiliensis*. The skin lesions are chronic and spreading, often invading the mucous membranes either by metastasis or extension, and sometimes causing great disfigurement. Spontaneous recovery is rare. The lymph nodes are seldom involved. Dogs and occasionally cats have been found naturally infected, but the true reservoir hosts have not been discovered; they are probably wild jungle mammals. The retus monkey and various squirrels can be readily infected, but the golden hamster is refractory. The vectors are

Phlebotomus intermedius (syn., *P. lutzi*), and also probably *P. migonei*, *P. whitmani* and *P. pessoai*.

4. Uta occurs in the mountains of Peru. It is a benign form of the disease, with numerous small skin lesions. Its reservoir hosts and vectors are apparently unknown.

5. American forest leishmaniosis, pian bois or buba is found in Panama, Costa Rica, the Guianas and other parts of northern South America. It is caused by *L. tropica guianensis*. The skin lesions are moderately ulcerated, and ordinarily heal spontaneously unless they involve the nose. About 5% of the patients have lesions of the mucous membranes which have arisen by extension rather than by metastasis. The lymph nodes are involved in about 10%. Dogs may be naturally infected, but the wild reservoirs are unknown. The vectors in Venezuela are believed to be *Phlebotomus evansi*, *P. migonei*, *P. parasinensis* and *P. suis*.

6. Chiclero ulcer or bay sore is found in Guatemala, southeastern Mexico and British Honduras. It gets its name because it is common among chicle and rubber hunters in rain forests. It is caused by *L. tropica mexicana*. The skin lesions are small. They heal spontaneously in a few weeks to a few months unless they involve the ear. In this location they cause chronic, disfiguring nodular ulcers which may persist many years. There is no metastasis to the mucous membranes, and cutaneous metastases are rare. The lymph nodes are involved in about 2%. Nothing is known of the wild reservoirs or of the vectors, altho the disease is clearly a zoonosis (Garnham and Lewis, 1959).

Both the Old World types of cutaneous leishmaniosis are zoonoses, but their epidemiology is quite different. The dry type is an urban disease common to dogs and man, while the moist type is a rural disease of gerbils and other rodents which affects man more or less incidentally. The American forms, too, occur primarily in wild animals, mostly unknown, of the tropical rain forests; both man and dogs are secondary hosts.

Pathogenesis: The ulcers or sores of classical, dry Oriental sore are found on exposed parts of the body in man. At first they resemble mosquito bites, but they do not go away. The lesion grows slowly, becoming covered with thick brown scales. It itches a great deal, and scratching produces a small ulcer which is covered with a crust. This enlarges slowly, and may finally be several centimeters in diameter. After some months or a year, connective tissue is formed, but a permanent scar is left. The disease is very seldom fatal.

In the central Asian form of the disease, the lesions are moist. They develop more rapidly, becoming ulcerative in one or two weeks, and then heal spontaneously. Relatively few parasites can be found in them.

In espundia, the ulcers are often worse than those of Oriental sore and may last much longer. They usually heal in 7 to 8 months, but sometimes last more than 20 years. In addition, in some cases they may extend to the mucosa of the mouth or nose either directly or by metastasis. When they do this, they may cause a great deal of disfigurement; in extreme cases the nose may even be completely eaten away.

The lesions in the dog are similar to those in man. They are probably confined to the skin. Visceral leishmaniosis due to *L. tropica* has been reported in dogs, but many observers believe that these are due to concurrent infections with *L. donovani*. In infected gerbils, cutaneous sores occur on the ears.

Immunity: Persons who have recovered spontaneously from classical Oriental sore have a solid immunity. This fact is so well known among the natives that they vaccinate themselves on the arm in order to avoid natural, disfiguring ulcers on the face. There is no cross-immunity between the wet and dry Old World types of the

disease, between these and the New World forms, or between *L. tropica* and *L. donovani* infections.

Diagnosis: The same methods are used in diagnosing *L. tropica* as *L. donovani* infections, except for the tissues examined. The parasites are usually abundant in dry Oriental sore, but are scanty in wet Oriental sore and New World mucocutaneous leishmaniosis.

A skin test, the Montenegro intradermal reaction, is used with considerable success in diagnosing American mucocutaneous leishmaniosis. A suspension of killed organisms from NNN culture is injected intradermally. In positive cases, an erythematous wheal appears in 48 hours and lasts 4 or 5 days. A small sterile papule which becomes vesicular or pustular develops in the center of the wheal.

Treatment: Organic antimony compounds are effective against cutaneous leishmaniosis. The same ones are used as for kala-azar.

Control: The same measures used to prevent kala-azar are effective against cutaneous leishmaniosis.

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Chapter 4

HISTOMONAS

Histomonas belongs to the order Rhizomastigorida, members of which possess both flagella and pseudopods. Within this order it belongs to the family Mastigamoebidae, members of which have 1 to 4 flagella. *Histomonas* is the only genus in this order occurring in domestic animals.

Genus *HISTOMONAS*
Tyzzer, 1920

The body is actively amoeboid, usually rounded, sometimes elongate, with a single nucleus, and with 1 to 4 extremely fine flagella arising from a basal granule close to the nucleus. A single species, *H. meleagridis* is recognized.

HISTOMONAS MELEAGRIDIS
(SMITH, 1895) TYZZER, 1920

Disease: Histomonosis, infectious enterohepatitis, blackhead.

Hosts: Chicken, turkey, peafowl, guinea fowl, pheasant, ruffed grouse, quail, chukar partridge.

Location: Ceca, liver.

Geographic Distribution: Worldwide.

Prevalence: This parasite is practically ubiquitous in chickens, altho it seldom causes disease in them. It is one of the most important causes of disease in turkeys. Before control measures were developed, it drove many turkey raisers out of the business, and even now the United States Department of Agriculture (1954) has estimated that it causes an annual loss of $3,815,000 in turkeys and $149,000 in chickens due to mortality alone.

Morphology: This parasite was first recognized by Theobald Smith in 1895. He thought it was an amoeba and named it accordingly. It was later confused with a number of other microorganisms. Some workers thought that it was one of the forms

assumed by a pleomorphic *Trichomonas*, others that it was part of the life cycle of a coccidium, and still others confused it with the budding fungus, *Blastocystis*. Finally Tyzzer (1919, 1920, 1920a) showed that the organism was a flagellate and described it in detail. His observations have been confirmed by DeVolt and Davis (1936), Bishop (1938) and Wenrich (1943) among others.

H. meleagridis is pleomorphic, its appearance depending upon its location and the stage of the disease. The forms in the tissues have no discernible flagella, altho there is a basal granule near the nucleus. Tyzzer described three stages. The invasive stage is found in early cecal and liver lesions and at the periphery of older lesions. It is extracellular. It is 8 to 17 μ long and is actively amoeboid, with blunt, rounded pseudopods. Its cytoplasm is basophilic with an outer zone of clear ectoplasm and finely granular endoplasm. Food vacuoles containing particles of ingested material but no bacteria are present.

The vegetative stage is found near the center of the lesions and in slightly older lesions than the invasive stage. It is larger, measuring 12 to 15 by 12 to 21 μ. It is less active than the invasive stage and has few if any cytoplasmic inclusions. Its cytoplasm is basophilic, clear and transparent. The vegetative forms are often packed tightly together, and cause disruption of the tissues.

Tyzzer called the third form the resistant stage, but it is actually no more resistant than the other stages. There are no cysts. This form is 4 to 11 μ in diameter, compact, and seems to be enclosed in a dense membrane. The cytoplasm is acidophilic and filled with small granules or globules. These forms may be found singly or they may be packed together so that their outlines appear rather angular. They, too, are extracellular, but they may be taken up by phagocytes or giant cells.

A fourth form of the parasite is flagellated and occurs in the lumen of the ceca. The same form is found in cultures. Its body is amoeboid and may be 5 to 30 μ in diameter. Wenrich (1943) found that

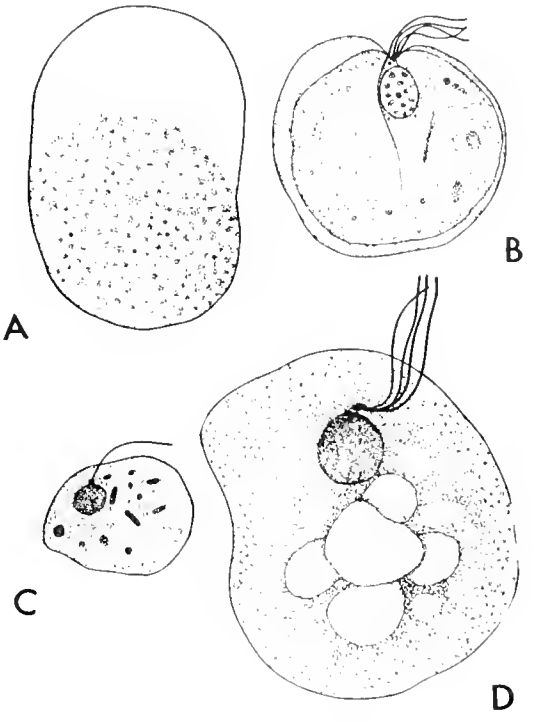

Fig. 5. *Histomonas meleagridis* trophozoites from cecum. A. Living trophozoite. B., C., D. Trophozoites fixed in Schaudinn's fluid and stained with iron-alum hematoxylin. X 2300 (From Wenrich, 1943, J. Morph. 72:279)

400 individuals from the ceca of 2 pheasants measured 9 to 28 μ in diameter with a mean of 13.9 μ, and that 400 individuals from the ceca of 2 chickens measured 5 to 18 μ in diameter with a mean of 7.9 μ. The cytoplasm is usually composed of a clear, outer ectosarc and a coarsely granular endosarc. It may contain bacteria, starch grains and other food particles, including an occasional red blood cell. The nucleus is often vesicular, with a single dense karyosome, or it may contain as many as 8 scattered chromatin granules. Near the nucleus is a basal granule or blepharoplast from which the flagella arise. There is typically a single, short flagellum, but as many as 4 may be present. Movement may be amoeboid, and there may be a pulsating, rhythmic,

intracytoplasmic movement. The flagella produce a characteristic, jerky, oscillating movement resembling that of trichomonads, but *Histomonas* can be differentiated from them because it lacks an undulating membrane and axostyle. Wenrich (1943) found peculiar, cylindrical feeding tubes in about 15% of the individuals from one of the 2 pheasants he examined and also in some individuals from a chicken. These sometimes extended out as much as the body diameter and often had internal extensions as long or longer.

This form is sometimes present in large numbers in the lumen of the ceca, but it is ordinarily absent or very difficult to find.

Life Cycle: Reproduction is by binary fission, and there is no evidence of a sexual cycle. Wenrich (1943) considered the larger, 4-flagellate forms in the ceca to be adult. There are no cysts.

The naked trophozoites are delicate and do not survive more than a few hours when passed in the feces. Turkeys can be infected by ingesting trophozoites, and this mode of infection plays a part in transmitting the parasites once disease has appeared in a flock (Tyzzer and Collier, 1925). However, large numbers of the parasites must be ingested. Tyzzer (1934) pointed out that oral infection with infected liver tissue or cecal discharges is somewhat unreliable because of the death of the protozoa during their passage thru the alimentary tract. Lund (1956) found that oral administration of 10,000 to 100,000 protozoa in saline caused infections in about 40% of 6- to 9-week-old poults, and illness in about 20%. However, when digestible materials were added to the inoculum, the infection and morbidity rates fell sharply. The protozoa remained in the gizzard and upper intestine longer in the presence of food, and were destroyed before they reached the cecum. Horton-Smith and Long (1956a) found that infections with trophozoite suspensions could be produced only in starved chickens or, in chickens that were feeding, by giving them an alkaline mixture just before dosing them. They believed that successful infection depends on the pH of the gizzard and possibly upper intestine. The pH of the starved gizzard is 6.3 to 7.0, that of chickens on feed is 2.9 to 3.3, and that of chickens on feed which have received alkali is 6.2 to 6.5.

By far the most important mode of transmission is in the eggs of the cecal worm, *Heterakis gallinarum*. Its discovery by Smith and Graybill (1920) was a milestone in the history of parasitology. This mode of transmission has been amply confirmed by many workers, and is the preferred method of producing experimental infections (Tyzzer and Fabyan, 1922; Tyzzer, 1926; Swales, 1948; McKay and Morehouse, 1948; Lund and Burtner, 1958). The parasites are carried inside the *Heterakis* eggs; eggs treated with disinfectants or other chemicals which do not kill them are still infective.

Infection of *Heterakis* eggs is so widespread that *Histomonas* infections can be produced with batches of eggs taken from a very high percentage of turkeys or chickens even if the hosts do not appear sick. Not every egg is infected, however. Lund and Burtner (1957) found that less than 0.5% of the embryonated eggs from experimentally infected chickens contained the protozoa, that less than half of the cecal worms they examined from these birds contained *Histomonas*-infected eggs, and that positive worms contained an average of only 2 infected eggs each.

The *Heterakis* eggs must hatch and liberate larvae in order to transmit the protozoa. *Histomonas* has never been seen in the infective eggs, its presence being inferred from the experimental results. However, Tyzzer (1926) found the protozoa in half-grown *Heterakis* from birds with histomonosis, and (1934) in the cells of the intestinal wall of 10-, 12-, and 21-day old worms from experimentally infected birds, and Kendall (1959) found them in a 4-day-old *H. gallinarum* larva.

The possibility that arthropods may transmit histomonosis has been considered by a number of authors. Mechanical transmission by flies and even grasshoppers is

possible (Frank, 1953), but it is of minor importance.

Epidemiology: *Histomonas* is extremely common in *Heterakis*-infected chickens, and these birds constitute the principal reservoir of infection for turkeys. This accounts for the fact that it is almost impossible to raise turkeys successfully on the same farm with chickens. In addition, wild gallinaceous birds such as the wild turkey, pheasant, quail and ruffed grouse may be infected, but their role as reservoirs of infection for domestic turkeys has not been properly assessed.

Birds become infected most commonly by ingesting infected *Heterakis* eggs. Infective eggs can survive for one to two years or even longer in the soil. Farr (1956) infected chickens and turkeys with *Histomonas* from eggs which had been in the soil in Maryland for 66 weeks.

Pathogenesis: Histomonosis can affect turkeys of all ages; the course and mortality of the disease vary with age. Poults less than 3 weeks old are refractory according to Swales and Frank (1948), but from this age to about 12 weeks, the disease is acute and may cause losses averaging 50% of the flock and ranging up to 100%. The birds often die 2 or 3 days after showing the first signs of disease. In older birds, the disease is more chronic, and recovery may take place. The mortality decreases with age, and losses in these birds rarely exceed 25%. However, even birds of breeding age may be affected.

Chickens are much less susceptible than turkeys. They ordinarily show no signs of disease, but serious outbreaks may occur in young birds. Histomonosis occasionally occurs in the peafowl (Graybill, 1925; Dickinson, 1930), guinea fowl (Graybill, 1925) and quail (Graybill, 1925). Serious outbreaks may occur in captive ruffed grouse (Tyzzer and Fabyan, 1920; Graybill, 1925) and chukar partridges (Honess, 1956). Altho the parasite occurs in pheasants, it is apparently not very pathogenic for them.

When the histomonads are released in the cecum, they enter the wall and multiply, causing characteristic lesions. Later they pass by way of the blood stream to the liver.

The incubation period is 15 to 21 days. The first sign of disease is droopiness. The birds appear weak and drowsy, and stand with lowered head, ruffled feathers and drooping wings and tail. There is a sulfur-colored diarrhea. The head may or may not become darkened. This sign, which is responsible for the name blackhead, may also occur in other diseases, so the term is a misnomer.

The principal lesions of histomonosis occur in the cecum and liver. One or both ceca may be affected. Small, raised pinpoint ulcers containing the parasites are formed first. These enlarge and may involve the whole cecal mucosa. Sometimes the ulcers perforate the cecal wall and cause peritonitis or adhesions. The mucosa becomes thickened and necrotic. It may be covered with a characteristic, foul-smelling, yellowish exudate which may consolidate to form a dry, hard, cheesy plug that fills the cecum and adheres tightly to its wall. The ceca are markedly inflamed and often enlarged.

The liver lesions are pathognomonic. They are circular, depressed, yellowish to yellowish green areas of necrosis and tissue degeneration. They are not encapsulated, but merge with the healthy tissue. They vary in diameter up to a centimeter or more and extend deeply into the liver. In older birds the lesions are often confluent.

Other organs such as the kidney and lung may occasionally be affected. P. P. Levine (1947), for example, described numerous white, round areas about 1 mm in diameter in the kidneys of an affected turkey.

The parasites can be readily found on histologic examination of the lesions. Hyperemia, hemorrhage, lymphocytic infiltration, and necrosis occur, and macrophages and giant cells are present. The pathology of histomonosis in turkeys has been described by Malewitz, Runnels and Calhoun (1958) among others.

McGuire and Cavett (1952) studied the effect of histomonosis on the blood picture of experimentally infected turkeys. The non-protein nitrogen, uric acid and hemoglobin levels declined progressively, but tended to recover just before death. The blood sugar rose during the incubation period but decreased during development of the liver lesions; severe hypoglycemia was present just before death. The total leucocyte count rose as the result of proliferation of heterophils, myelocytes and monocytes.

If the birds recover, the protozoa disappear from the tissues, and repair takes place. The exudate and necrotic tissue in the ceca are incorporated into the cecal plug, which becomes smaller and is finally passed. If the lesions were not too severe, the ceca may eventually appear entirely normal, but in other cases there may be so much scarring that the lumen is obliterated. In the repair process, the lesions are invaded by blood vessels, lymphoid cells and connective tissue. The liver lesions may be completely repaired or there may be extensive scar tissue.

Immunity: Birds which recover from histomonosis are immune to reinfection. In addition, as mentioned above, susceptibility decreases with age.

Lund (1959) found that infection of turkeys with a nonpathogenic strain of *Histomonas* did not protect the birds against subsequent infection with a pathogenic strain introduced by feeding *Heterakis* eggs, altho it did afford some protection against rectally introduced pathogenic histomonads.

Diagnosis: Histomonosis can be diagnosed from its lesions. Those in the liver are pathognomonic. In case of doubt and in order to differentiate the liver lesions from those caused by tumors, tuberculosis or mycotic infections, histologic examination is desirable. The cecal lesions can be distinguished from those caused by coccidia by microscopic examination of scrapings from the mucosa.

Cultivation: *Histomonas* was first cultivated by Drbohlav (1924) in a diphasic medium consisting of coagulated egg white slants overlaid with blood bouillon containing 1% peptone It has since been cultivated in a number of other media, both diphasic and monophasic (Tyzzer, 1934; DeVolt and Davis, 1936; Bishop, 1938). Delappe (1953, 1953a) found that addition of penicillin or streptomycin or both to Laidlaw's culture medium facilitated the initial isolation of the protozoa, but he was unable to obtain axenic cultures. When the bacteria disappeared, the protozoa did likewise.

Treatment: Since histomonosis can be prevented by proper management, drug therapy should be regarded as a secondary line of defense against the disease. The chemotherapy of this disease has been reviewed by Wehr, Farr and McLoughlin (1958).

While a number of phenylarsonic acid and quinoline derivatives have been used with some success in the past, the only one of them which is now used to any extent is 4-nitrophenylarsonic acid. When fed as 0.0125 to 0.075% of the mash or 0.006 to 0.04% of the drinking water for 3 days before and 21 days after experimental infection, this compound prevents death. However, there is a high relapse rate following cessation of treatment. Hence, to be effective this compound must be fed continuously until 5 days before slaughter. Mashes containing 0.01 to 0.03% of this compound stimulate growth, but 0.02% in the drinking water decreases egg production of adults and growth and livability of poults (Moreng and Bryant, 1956).

Thiazole derivatives are used most commonly against histomonosis. Three of these are enheptin, acetylenheptin, and nithiazide (Hepzide). The first is 2-amino-5-nitrothiazole, and the other two are derivatives of it. Enheptin was introduced by Waletzky, Clark and Marson (1950), and its activity was confirmed by a number of workers, including McGregor (1953), Jungherr and Winn (1950), DeVolt, Tromba and Holst (1954), and Joyner and Kendall (1955). Acetylenheptin (2-acetylamino-5-nitrothiazole) was found by Grumbles, Boney and Turk (1952, 1952a, 1952b) to be just as effective as enheptin; it was also studied by Brander and Wood (1955) and

Cooper and Skulski (1957) among others. Nithiazide (1-ethyl-3-[5-nitro-2-thiazolyl] urea) was introduced by Cuckler *et al.* (1956, 1957) and Cuckler and Malanga (1956).

These drugs have both prophylactic, suppressive and therapeutic value. Enheptin is usually fed continuously in the mash at the rate of 0.05% for prevention and suppression. If feeding is begun within 2 days after the infective dose of *Histomonas* is given in an experimental infection, it will almost completely prevent the disease. If it is begun later than this, it will suppress the disease as long as it is continued, but after it is withdrawn, histomonosis will reappear in the flock. If enheptin is to be used in treating turkeys which already show signs of disease, 0.1 to 0.2% of the drug is mixed in the feed. Not all the birds will recover, but quite a high percentage do. Acetylenheptin is used in much the same way. The preventive level of nithiazide in the feed recommended by the manufacturer in 1958 was 0.03%.

Potential hazards are often associated with feeding drugs continuously. Hudson and Pino (1952) and Pino, Rosenblatt and Hudson (1954) found that enheptin prevented or delayed sexual maturity in chickens and turkeys. When fed in the ration to chickens, it produced complete sexual involution or inhibition in both males and females. In young birds, sexual development did not take place, while in older ones the testes, ovary and oviduct atrophied. The effect was less marked in turkeys, altho 0.1% enheptin in the ration reduced the level of reproductive performance. This effect was found to be due to inhibition of gonadotropin secretion by the pituitary, and could be counteracted, at least in part, by simultaneous administration of gonadotropic hormone. Shellabarger and Schatzlein (1955) found that enheptin caused rats to have larger thyroid glands and to accumulate less iodine than normal rats. They suggested that these antithyroid properties might explain why enheptin inhibits the secretion of pituitary gonadotropin in the chicken.

Grumbles, Boney and Turk (1952) and Cooper and Skulski (1957) compared enheptin with acetylenheptin. The former found that 0.1% enheptin in the feed reduced production, fertility and hatchability in turkeys, but that acetylenheptin had no such effect. The latter found that enheptin decreased spermatogenesis and egg production and increased embryo mortality when fed to chickens at preventive levels. Acetylenheptin was less toxic. It had no effect on egg production, fertility or embryo mortality, and reduced sperm production only slightly.

According to Cuckler, Porter and Ott (1957), 0.1% nithiazide in the feed did not interfere with growth, maturation or reproduction of chickens or turkeys.

The nitrofuran, furazolidone (NF-180, Furoxone), was found by McGregor (1953a, 1954), Horton-Smith and Long (1955, 1956) and Costello and DeVolt (1956) to suppress histomonosis when fed at the rate of 0.01 to 0.04% in the feed. Even with the higher doses, however, some relapses occurred after medication was stopped, and slight lesions were found in treated birds killed during the experiments.

Cooper (1956) reported that feeding 0.02% furazolidone to pullets for 12 weeks had no effect on body weight, egg production, fertility or hatchability, but Cooper and Skulski (1955, 1956) found that feeding this drug to cockerels and roosters reduced the number of spermatozoa and decreased weight gains.

Control: Histomonosis can be prevented by good management. Turkeys should be kept separate from chickens, since chickens are carriers. Young turkeys should be kept separate from adults. The same attendants should not care for chickens and turkeys. Persons who go from one flock to another should take care not to carry the infection on contaminated shoes or equipment.

Young birds should be raised on hardware cloth, and the droppings should be removed regularly. When the poults are

old enough to move onto range, they should be placed on clean ground where neither turkeys nor chickens have been kept for 2 years. The length of time infective cecal worm eggs survive in the soil depends upon soil type, weather and amount of cover provided by vegetation. They will survive only a few weeks on barren soils in warm, dry regions, but may remain alive for several years in heavy soils in moist climates.

The range should be rotated at regular intervals. Different farmers use different intervals. Many of them move the birds along every week, not returning to the same place during the same season. Another rotation system which has been recommended is to move the birds thru a series of 4 lots, allowing them to remain on each for a month. The frequency of rotation depends on the climate. In cool, damp climates the birds should be moved at least every 10 days, but in hot, dry climates they need be moved less frequently, and it is even possible to raise turkeys successfully without changing the range if the area around the feeders, waterers, roosts and shelters is kept dry.

Low areas and streams that drain poultry yards should be fenced off.

The feeders and waterers should be placed on wire platforms. Most of the droppings are deposited around them, and this practice keeps the turkeys from getting at them. Wire should also be used beneath roosts and in shelters to keep the birds from their droppings.

Treating the birds with phenothiazine to prevent histomonosis by killing the cecal worms has been suggested. It is ineffective in controlling active outbreaks, but may help prevent future ones. Phenothiazine kills the cecal worms, but does not prevent their eggs from hatching and releasing the histomonads (Wehr and Olivier, 1946).

To eliminate *Heterakis*, 0.5% phenothiazine is mixed with the feed if the birds are not getting roughage, and 1.0% if they are on good range or getting supplementary roughage. The medicated ration is given for 5 to 7 days, the regular ration is fed for about 15 days, the medicated ration is then given again and alternated as before with regular feed until about 3 weeks before the birds are to be marketed. Phenothiazine should not be fed during these 3 weeks.

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Chapter 5

THE TRICHOMONADS

The trichomonads belong to the family Trichomonadidae within the order Trichomonadorida. The body is usually piriform, with a rounded anterior end and a pointed posterior end. There is a single nucleus in the anterior part of the body. Anterior to the nucleus is a *blepharoplast* composed of several *basal granules*. Two to five anterior flagella and a posterior flagellum arise from the blepharoplast. The posterior flagellum passes along the border of an *undulating membrane* which extends along the side of the body; a *secondary* or *accessory filament* may be associated with it. The posterior flagellum may or may not extend beyond the undulating membrane as a free flagellum. A filamentous *costa* arises from the blepharoplast and runs along the base of the undulating membrane. A *parabasal body* arises from the blepharoplast; there may or may not be a *parabasal filament* at its posterior end. A clear, rod-like *axostyle* also arises from the blepharoplast and passes thru the center of the body to emerge from the posterior end. The anterior end of the axostyle is enlarged to form a *capitulum*. There may or may not be a *chromatic ring* at the point of emergence of the axostyle. There may or may not be a *cytostome* near the anterior end. Just anterior to the blepharoplast and lying along the anterior margin of the body is a *pelta* which stains with silver. In addition to these structures, there may be various granules within or along the axostyle, along the costa, or in other locations.

An electron micrograph study of *Tritrichomonas muris* by Anderson (1955) revealed the fine structures of these organelles which may be taken to represent the group. He found that the blepharoplast appears to be limited by a membrane and to contain basal granules for each organelle. The anterior and posterior flagella are composed of 2 central and 9 peripheral fibrils. The accessory filament is composed of two differentiated meshwork areas. The undulating membrane is composed of a series of lamellae 300 to 400 Å thick; it is attached to the outer surface of the body by fine fibers 167 to 300 Å thick.

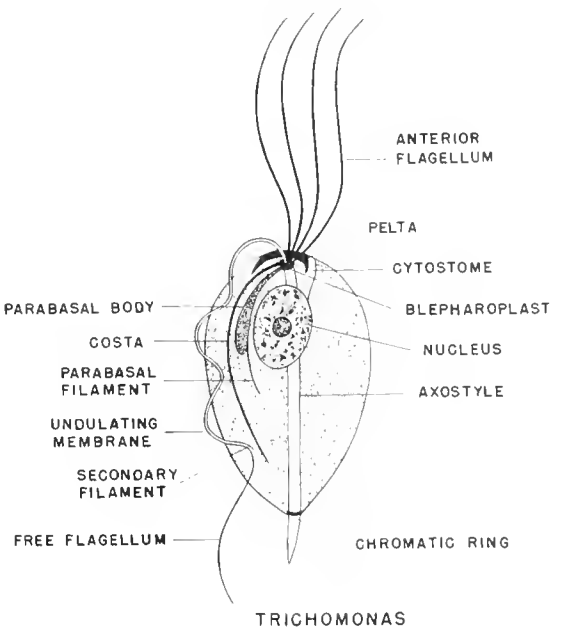

Fig. 6. Structures of *Trichomonas*. (Original)

The costa consists of a series of discs about 370 Å thick and 490 Å apart, embedded in a matrix; it is attached to the inner surface of the body wall by extensions of the discs. The axostyle is limited by a double, corrugated membrane. The chromatic ring is composed of a series of rods about 640 Å thick. The parabasal body consists of a series of filaments about 190 Å thick. The chromatic granules along the costa, inside the axostyle and scattered in the cytoplasm are irregular in shape and vacuolated. The mitochondria are spherical and contain a varying number of projections internally.

Trichomonads are divided into several genera on the basis of the number of their anterior flagella. *Ditrichomonas* has 2, *Tritrichomonas* has 3, *Trichomonas* has 4, and *Pentatrichomonas* has 5. These genera are closely related; Mehra, Levine and Reber (1960), for example, found in a column chromatographic study of the hydrolysates of *Tritrichomonas foetus*, *T. suis*, *Trichomonas gallinae*, *T. gallinarum* and *T. buttreyi*, that they are all composed of the same amino acids but that there are some differences in the amounts of each amino acid present in the different species.

Gabel (1954) established the genus *Paratrichomonas* for *P. marmotae* from the woodchuck and possibly *T. batrachorum* from the frog. *Paratrichomonas* differs from *Tritrichomonas* principally in having a ring-shaped parabasal body. *T. buttreyi* of the pig resembles it, but has 4 anterior flagella. There does not seem to be sufficient justification for accepting this genus, at least at present.

Morgan (1943, 1946) and Trussell (1947) have given host-parasite lists of the trichomonad species.

There are several species of trichomonads in domestic animals and man, but the nomenclatorial status and host-parasite relations of many of them are not yet clear. They have been found in the cecum and colon of practically every species of mammal or bird that has been examined for them, and they also occur in reptiles, amphibia, fish and many invertebrates. Those in the termite gut are particularly well known. Many of the cecal trichomonads look alike, and cross-transmission studies have shown that many of them can be easily transmitted from one host species to another. Some mammalian trichomonads have even been transmitted successfully to day-old chicks, altho they will not become established in older birds. Further and extensive studies are needed to establish the correct names and host spectra of all but a few trichomonads.

Most trichomonads are non-pathogenic commensals, but a few are important pathogens. None of the cecal trichomonads has ever been proven to be pathogenic, altho some people have thought that they were because they were found in animals which had enteritis or diarrhea. However, the mere presence of an organism in a diseased animal does not mean that the organism caused the disease. The latter may have set up conditions favorable to the organism's growth and multiplication. This is especially true of the cecal trichomonads, which flourish in a fluid or semi-fluid habitat.

The life cycle of trichomonads is simple. They reproduce by longitudinal binary fission. No sexual stages are known. There are no cysts, altho degenerating or phagocytized individuals (or entirely different organisms such as *Blastocystis*) have been mistaken for them.

Genus *TRITRICHOMONAS* Kofoid, 1920

Members of this genus have 3 anterior flagella.

TRITRICHOMONAS FOETUS (RIEDMÜLLER, 1928) WENRICH AND EMMERSON, 1933

Synonyms: *Trichomonas utero-vaginalis vitulae*, *T. bovis*, *T. genitalis*, *T. bovinus*, *T. mazzanti*.

Disease: Bovine trichomonad abortion, bovine genital trichomonosis.

Hosts: Ox, zebu, possibly pig, horse, roe deer.

Location: Genital tract.

Geographic Distribution: Worldwide. Morgan and Beach (1942) mapped the geographic distribution of bovine trichomonosis.

Prevalence: Altho *T. foetus* is known to be widely distributed, few studies have been made of its incidence. It is especially common in southern Germany and Switzerland, up to 30% of the cattle having been found infected in some areas. In the U. S., it is probably third to brucellosis and leptospirosis as a cause of abortion in cattle. In a survey of 383 beef bulls in Utah, Idaho and Colorado, Fitzgerald *et al.* (1958) found 6% to be infected.

The USDA Agricultural Research Service (1954) estimated that bovine trichomonosis causes an annual loss of $750,000 in the United States. This figure is probably low. Fitzgerald *et al.* (1958) estimated that losses in production because of reproductive disorders in cows bred by infected western range bulls may amount to about $800 annually per infected bull. On this basis, the 23 infected bulls they found in their survey would cost their owners about $18,000 a year. The annual loss due to an infected bull in an artificial insemination ring would be considerably greater than this.

Morphology: The morphology of *T. foetus* has been studied by Wenrich and Emmerson (1933), Kirby (1951) and Ludvik (1954), among others. The body is spindle- to pear-shaped, 10 to 25μ long and 3 to 15μ wide. It has 3 anterior flagella and a posterior flagellum which trails as a free flagellum about as long as the anterior flagella. The undulating membrane extends almost the full length of the body and has an accessory filament along its margin. The costa is prominent. The axostyle is thick and hyaline, with a capitulum containing endoaxostylar granules and a chromatic ring at its point of emergence from the posterior end of the body. The parabasal body is sausage- or ring-shaped. A cytostome is present. There is no pelta.

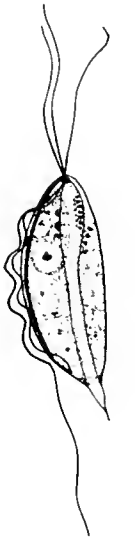

Fig. 7. *Tritrichomonas foetus*. X 3400 (From Wenrich and Emmerson, 1933, J. Morph. 55:193)

Pathogenesis: A great deal has been written about bovine trichomonosis.

Morgan's (1946) review listed 447 references, and many more papers have been published since then. Among more recent papers on its pathogenesis are those of Morgan (1947), Bartlett (1947), Bartlett and Dikmans (1949), Bartlett, Moist and Spurrell (1953), Laing (1956) and Gabel *et al.* (1956).

Bovine trichomonosis is a venereal disease, transmitted by coitus. It can also be transmitted by artificial insemination. Non-venereal transmission is very rare under natural conditions. After infection of the female, the trichomonads multiply at first in the vagina, causing a vaginitis. They are most numerous here 14 to 18 days after infection (Hammond and Bartlett, 1945). They invade the uterus thru the cervix. They may disappear from the vagina or they may remain there, producing low-grade inflammation and catarrh.

Early abortion is characteristic of bovine trichomonosis. Abortion usually occurs 1 to 16 weeks after breeding. The foetus is often so small that it is not observed by the owner, and he does not realize that abortion has occurred, believing that the animal failed to conceive and that its heat periods are irregular. Morgan and Hawkins (1952) knew of only 6 reports in the literature of abortion due to *T. foetus* after 6 months gestation.

If the placenta and fetal membranes are completely eliminated following abortion, the animal usually recovers spontaneously. This is the most common course. If, however, part of the placenta or membranes remain, a chronic catarrhal or purulent endometritis results which which may cause permanent sterility.

Sometimes the animal does not abort, but the fetus dies and becomes macerated in the uterus. Pyometra results, and the uterus may contain several quarts of a thin, greyish white fluid swarming with trichomonads. In the absence of bacteria, this fluid is almost odorless. The cervical seal may remain intact or it may allow a small amount of fluid to escape when the animal is lying down. Animals with pyometra seldom come in heat, and the owner may believe them to be pregnant. In long-standing cases, the trichomonads may disappear from the uterine fluid.

Occasionally normal gestation and calving may occur in an infected animal, but this is rare.

In the bull, the most common site of infection is the preputial cavity, altho the testes, epididymis and seminal vesicles may sometimes be involved. Spontaneous recovery is rare; bulls remain infected permanently unless treated. The numbers of trichomonads fluctuate, the intervals between peaks being 5 to 10 days according to Hammond *et al.* (1950).

Immunology: Cows or heifers which recover from infection are usually relatively immune, altho reinfections can occur.

A number of investigators have studied various immunological responses to trichomonad infection. Kerr and Robertson (1945) showed that there is more than one serological strain of *T. foetus*. McEntegart (1956) found that *T. foetus* var. *belfast* and *T. foetus* var. *manley* differed serologically from each other and from *T. vaginalis*. Menolasino and Hartman (1954) were unable to distinguish *T. foetus* from *T. vaginalis* serologically, but McDonald and Tatum (1948) and Schoenherr (1956) were able to do so. Both also found serological differences between *T.foetus* and *Pentatrichomonas hominis*, and the latter between *T. foetus* and *Trichomonas gallinae*. Sanborn (1955) found that *T. foetus* differed serologically from the large pig cecal trichomonad, *T. suis* and from the pig nasal trichomonad.

Kerr and Robertson (1941, 1943) and Pierce (1947) studied the agglutination test in cattle, and Feinberg (1952) described a capillary agglutination test. Kerr (1943) felt that his test was positive in about 60% of all infected cattle, but Morgan (1943a) considered it impractical. The wide distribution in the animal kingdom of non-specific antibodies against *T. foetus* was brought out by Morgan (1944), who showed that the sera of the carp, horned lizard

and leopard frog agglutinated *T. foetus* at 1:2, those of the gold fish, pigeon and domestic rabbit at 1:4, those of the guinea fowl and chicken at 1:8, those of the turkey and sheep at 1:16, those of deer and goat at 1:32, that of the cow at 1:128 and that of the horse at 1:1024.

Nakabayasi (1952) distinguished between agglutination and agglomeration. With immune rabbit and infected guinea pig sera, agglomeration reached its maximum within 30 minutes and then decreased gradually as the agglomerated individuals separated. On the other hand, the agglutination reaction reached its maximum within about an hour and did not reverse. Levine *et al.* (unpublished) have seen agglomeration of *T. foetus* following mixture with fresh culture media containing inactivated serum.

Kerr and Robertson (1954, 1956) found "normal agglutinin" in the blood of calves which they apparently acquired in the colostrum; this agglutinin disappeared after 17 to 55 days. Injection of calves less than 4 weeks old did not induce antibody formation, but instead caused impairment of antibody production (immunological paralysis) which persisted for about 2 years.

Complement fixation and precipitin reactions have been studied, but with unsatisfactory results (Svec, 1944; Morgan, 1948).

Kerr (1944) developed an intradermal test, using a trichloracetic acid-precipitated extract of *T. foetus* called "tricin." Positive reactions appear in 10 minutes, reach their peak within 30 minutes and disappear in about 6 hours. Fifty of 592 cows at an abattoir were positive to this test, and trichomonads were found in 11 of them on direct examination. Trichomonads were also found in 11 of 34 bulls which were positive to the skin test. Morgan (1948) obtained negative results with skin tests with a number of different antigens. Kerr, McGirr and Robertson (1949) found that cattle could be desensitized to the skin test by injecting antigen intramuscularly, instilling it into the uterus of non-pregnant cows, or by injecting adreno-cortical hormone or sphingomyelin at parturition. Absorption of antigen from acute uterine infections also desensitized the animals.

A local immune reaction takes place in the vaginal mucosa of infected animals. In addition, the uterine mucosa is sensitized (Kerr and Robertson, 1953). The presence of agglutinins in the vaginal mucus prompted the development of a mucus agglutination diagnostic test by Pierce (1947a, 1949) and Florent (1947, 1948, 1957). This test is considerably better than the blood agglutination test, but, according to Pierce (1949), must still be regarded as only a herd test because a number of infected animals fail to react. Unsatisfactory results are obtained with estral and post-estral vaginal mucus and with purulent uterine mucus containing trichomonads. Mucus from pregnant animals sometimes gives a false positive reaction. Schneider (1952), too, considered the mucus agglutination test simply an adjunct to other means of diagnosis.

Morgan (1947a) found that a series of 16 intramuscular or intravenous injections with living *T. foetus* over a period of 3 months apparently protected heifers temporarily against genital infection, but 6 intramuscular injections over a period of 3 weeks did not. This does not appear to be a practical method of prevention.

Epidemiology: Bovine trichomonosis is a venereal disease transmitted at coitus. *T. foetus* is known to occur in cattle, but whether it is also present in other animals and whether it may be transmitted from them to cattle by a non-venereal route remain to be determined.

With the introduction of the technic of preserving bovine semen by freezing in the presence of glycerol, the question arose whether *T. foetus* would survive in frozen semen. Several investigators have studied the problem, and have found that the protozoa may or may not survive freezing in the presence of glycerol, depending on the conditions (see Levine, Mizell and Houlahan, 1958 for a review of the literature).

They survive in some media but not in others. Rapid freezing and high salt concentration are deleterious (Levine and Marquardt, 1955; Levine, Mizell and Houlahan, 1958). The stage of the population growth curve is important, the protozoa being much more sensitive to injury when frozen during the initial and logarithmic phases than at the peak of the curve and for some time thereafter (Levine, McCaul and Mizell, 1959). Temperature fluctuation during storage is deleterious (Fitzgerald and Levine, 1961).

A particularly interesting fact is that glycerol appears to be toxic at refrigerator temperatures but not at either sub-freezing or incubator (37° C) temperatures (Joyner, 1954; Joyner and Bennett, 1956; Fitzgerald and Levine, 1961). It may be possible to develop a technic for freezing semen which would be sure to kill the protozoa, but at present the use of frozen semen from infected bulls cannot be recommended.

Many different laboratory animals can be infected experimentally in various ways with *T. foetus* (see Morgan, 1946 for review). Leaving aside other routes of infection, successful vaginal infections with *T. foetus* have been established in the rabbit by Witte (1933) and others, in the guinea pig by Riedmüller (1928) and several others, in the golden hamster by Kradolfer (1954) and Uhlenhuth and Schoenherr (1955), in the dog by Trussell and McNutt (1941), in the goat by Wittfogel (1935) and Hammond and Leidl (1957), in the sheep by Wittfogel (1935) and Andrews and Rees (1936), and in the pig by Hammond and Leidl (1957). The golden hamster is the laboratory animal of choice for experimental vaginal infections. Abortions were produced in some of the infected guinea pigs. Laboratory mice and rats are refractory to vaginal infection.

Küst (1936) found trichomonads similar to *T. foetus* in the genital tract and aborted fetuses of swine and horses in Germany. Petersen (1937) cultured trichomonads resembling *T. foetus* from the genital tracts of 13 mares with pyometra. He also found an infected stallion which had transmitted trichomonads to mares. Schoop and Oehlkers (1939) also found trichomonads in the genital tract of horses. Schoop and Stolz (1939) found trichomonads resembling *T. foetus* in the uteri of 4 out of 5 roe deer in Germany. The infections were associated with sterility, and the trichomonads produced vaginitis in guinea pigs. Schoop (1940) suggested that if the trichomonads from deer were *T. foetus*, deer might be a source of infection for cattle.

The relation of *T. foetus* to the trichomonads of swine still remains to be elucidated. The pig nasal trichomonad, *Tritrichomonas suis*, greatly resembles *T. foetus* morphologically (Buttrey, 1956) and in metabolic characteristics (Doran, 1957, 1959), and vaginal infections were readily established in cattle with it by Switzer (1951) and Fitzgerald *et al.* (1958). The infection reported by Switzer lasted 3 weeks and was accompanied by a mild catarrhal vaginitis. Those reported by Fitzgerald *et al.* lasted 46 to 133 days, and some infections appeared to interfere with breeding efficiency.

Vaginal infections of cattle with trichomonads from the cecum and stomach of swine have also been readily established (Switzer, 1951; Hammond and Leidl, 1957a; Fitzgerald *et al.*, 1958), and the latter two authors reported that bulls became infected by breeding infected heifers and then transmitted their infections to other heifers. The bulls later recovered spontaneously in both studies. Kerr (1958), too, infected heifers intravaginally with trichomonad from swine, using both strains obtained from Hammond and a strain of *T. suis* isolated in England. He found that the vaginal mucus agglutination test of heifers infected with porcine trichomonads was positive with *T. suis* and Belfast strain *T. foetus* antigens but not with Manley strain *T. foetus* antigen.

In the other direction, Fitzgerald *et al.* (1958) produced cecal infections with *T. foetus* in young pigs.

Robertson (1960) made a serologic comparison of the Belfast and Manley strains of *T. foetus* and Strains S2 and 414

of *T. suis*, isolated by Hammond and Leidl from the ceca of pigs in Germany and Utah, respectively. Using both the tube agglutination and precipitin tests and especially the gel diffusion precipitin test, she found considerable cross-reaction between the 4 strains. All had the same major protein antigens, but they shared their major polysaccharide antigens only partially. The 2 bovine strains were readily distinguished from each other, while the 2 porcine strains were very closely related but not identical. The porcine strains were more closely related to the Belfast than to the Manley strain of *T. foetus*. Robertson concluded that the serologic distinctions between the 4 strains did not justify separating them into 2 species, and she called them all *T. foetus*.

Diagnosis: Altho the mucus agglutination test and a number of other serological procedures have been suggested for diagnosing *T. foetus* infections, the only sure method is to demonstrate the protozoa microscopically either directly or in culture. Diagnostic procedures have been described by Hammond and Bartlett (1945), Morgan (1945), Bartlett (1949), Fitzgerald *et al.* (1952) and Thorne, Shupe and Miner (1955), among others.

In heavy infections, particularly of females, the trichomonads can be seen by direct examination of mucus or exudate from the vagina or uterus, amniotic or allantoic fluid, fetal membranes, placenta, fetus stomach contents, oral fluid or other fetal tissues, or, in bulls, of washings from the preputial cavity and rarely seminal fluid or semen. If trichomonads cannot be found on direct microscopic examination, cultures should be made in CPLM, BGPS or Diamond's media (see below).

Samples can be obtained from the vagina by washing with physiological salt solution in a bulbed douche syringe. They can be obtained from the preputial cavity with a cotton swab or, better, by washing with physiological salt solution in a bulbed pipette or syringe. The washings should be allowed to settle for 1 to 3 hours or centrifuged before examination.

The external genitalia should be cleaned thoroughly before the samples are taken in order to avoid contamination with intestinal or coprophilic protozoa which might be mistaken for *T. foetus*. Among these are *Tritrichomonas enteris*, *Monocercomonas ruminantium*, *Protrichomonas ruminantium*, *Bodo foetus*, *B. glissans*, *Spiromonas angusta*, *Cercomonas crassicauda*, *Polytoma uvella*, *Monas obliqua* and *Lembus pusillus*. In identifying *T. foetus*, it must be distinguished from these.

Trichomonads are most numerous in the vagina 2 to 3 weeks after infection. Their numbers fluctuate in bulls, the interval between peaks being 5 to 10 days (Hammond *et al.*, 1950).

A single examination is not sufficient to warrant a negative diagnosis. A cow can be considered uninfected if, after at least 3 negative examinations, she has 2 normal estrus periods and subsequently conceives and bears a normal calf; she should be bred by artificial insemination to avoid infecting the bull. A bull can be considered negative if, after at least 6 negative examinations at weekly intervals, he is bred to 2 or more virgin heifers and they remain negative.

Cultivation: *T. foetus* can be readily cultivated in a number of media. Among them are CPLM (cysteine-peptone-liver extract-maltose-serum) medium (Johnson and Trussell, 1943), BGPS (beef extract-glucose-peptone-serum) medium (Fitzgerald, Hammond and Shupe, 1954) and Diamond's (1957) trypticase-yeast extract-maltose-cysteine-serum medium.

T. foetus was first cultivated in tissue culture by Hogue (1938). It was cultivated in the chorio-allantoic sac of chicken embryos by Nelson (1938) and independently by Levine, Brandly and Graham (1939) and Hogue (1939).

Treatment: Since trichomonosis is ordinarily self-limiting in females, treatment is unnecessary. No satisfactory treatment is known for these infections.

Many investigators have reported on treatment of *T. foetus* infections in bulls,

including Bartlett (1948), Bartlett, Moist and Spurrell (1953), Mahoney, Christensen and Steere (1954), Thorne, Shupe and Miner (1955), Gabel *et al.* (1956) and Brodie (1960). Treatment is expensive, tedious and time-consuming; unless a bull is exceptionally valuable, it is best to sell it. Bartlett (1948) found that the German proprietary preparation, Bovoflavin-Salbe, cured 7 out of 8 infected bulls, and later workers have confirmed its effectiveness. This salve, which contains trypaflavine and surfen in an ointment base, is rubbed into the penis and prepuce following pudendal nerve block or relaxation of the retractor penis muscles with a tranquilizer. Brodie (1960) injected 200 to 1000 mg promazine hydrochloride intravenously for the latter purpose, and found that its ease of administration and quieting effect made it preferable to nerve block.

Massage is continued for 15 to 20 minutes, using 120 ml of the ointment. In addition, 30 ml of 1% acriflavine solution is injected into the urethra. Repeated treatment may be necessary. If the epididymis or testis are affected, this treatment will be ineffective.

For reasons which have not been determined, American bulls are much more refractory to treatment and much more difficult to infect experimentally than European bulls. Treatment with silver nitrate or by injecting 10 l of 3% hydrogen peroxide into the preputial cavity under pressure with the apparatus described by Hess (1949), which is successful in Germany and Switzerland (Jondet and Guilhon, 1957), has been found unsatisfactory in the United States.

Control: Control of bovine trichomonosis depends on proper herd management. Most infected bulls should be slaughtered. Infected cows should be given breeding rest, and should then be bred by artificial insemination to avoid infecting clean bulls.

Proper management of bulls used for artificial insemination is especially important, since they may spread the infection widely. They should be examined for *T. foetus* before purchase, and the herds from which they originated should be studied at the same time. In addition, they should be examined repeatedly while in use (Bartlett, Moist and Spurrell, 1953). Freezing the semen in the presence of glycerol cannot be expected to kill the trichomonads.

TRITRICHOMONAS SUIS (GRUBY AND DELAFOND, 1843)

Synonym: *Trichomonas suis* Gruby and Delafond, 1843.

Common Name: Large pig trichomonad, pig nasal trichomonad.

Disease: None.

Hosts: Pig.

Location: Nasal passages, stomach, cecum, colon, occasionally small intestine.

Geographic Distribution: Worldwide.

Prevalence: Switzer (1951) found this species in the nasal passages of 80% of swine affected with atrophic rhinitis and in only 3% of nonrhinitic pigs in Iowa. Shuman *et al.* (1953) found it in 27% of 36 pigs with atrophic rhinitis and in 17% of 32 unaffected pigs in a herd near Washington, D. C. Levine, Marquardt and Beamer (1954) found it in 91% of 11 pigs with atrophic rhinitis and in 39% of 23 pigs with normal nasal passages in Illinois. Hammond, Fitzgerald and Johnson (1957) found it in the nasal passages of 56% of 64 pigs from Utah, Nebraska and Idaho. Hibler *et al.* (1960) found it in the nasal passages of 55% of 100 pigs, the stomach of 8% of 512, the cecum of 43% of 496 and the small intestine of 3% of 100 pigs in Utah.

Morphology: This species was described in detail by Hibler *et al.* (1960), Marquardt (1954) and Buttrey (1956); the latter described it under the name *Tritrichomonas* sp. from the nasal passages. *T. suis* is characteristically elongate or spindle-shaped, occasionally piriform or rotund, 9 to 16 by 2 to 6 μ, with a mean of

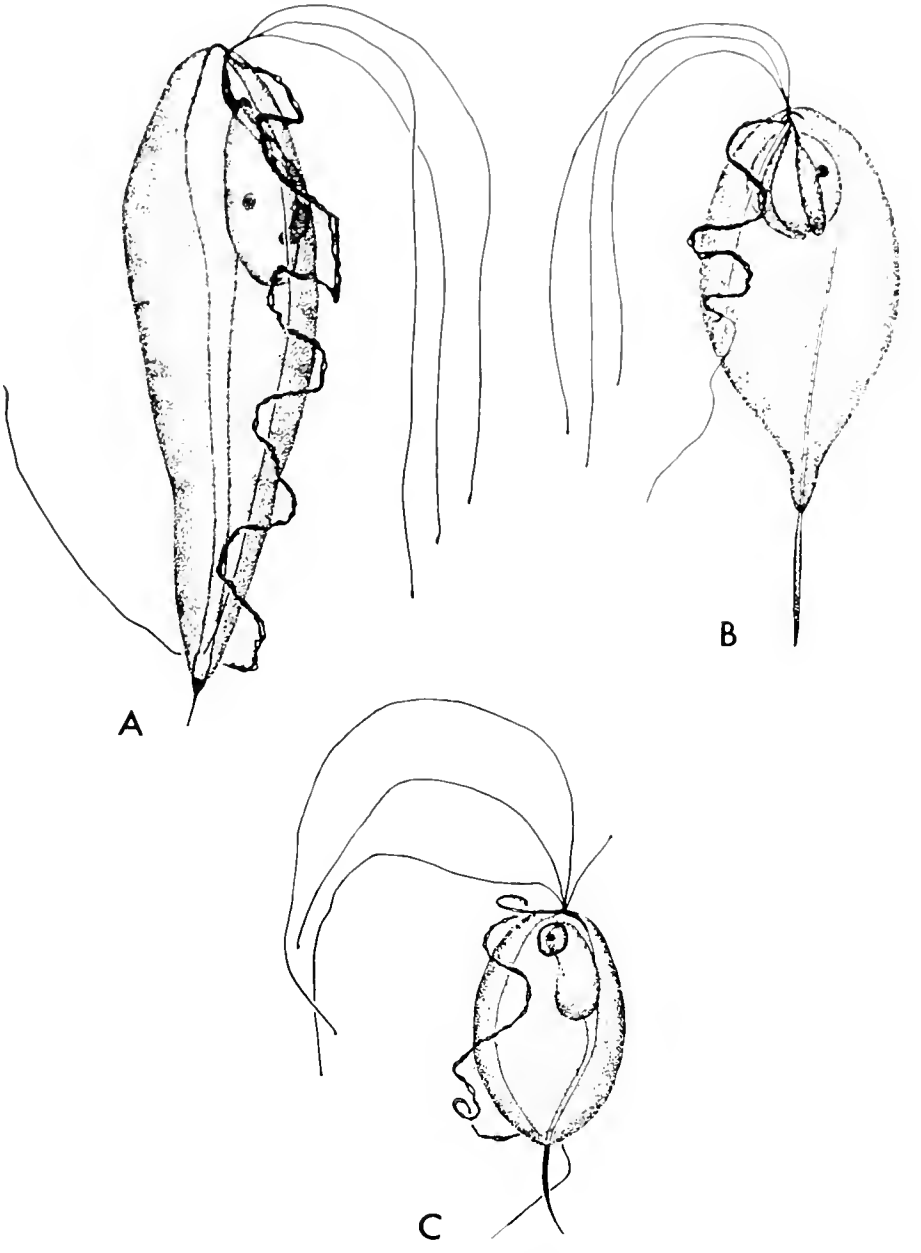

Fig. 8. Trichomonads of swine. A. *Tritrichomonas suis*. X 7700. B. *Tritrichomonas rotunda*. X 5100. C. *Trichomonas buttreyi*. X 5700. (From Hibler *et al.*, 1960)

11.3 by 3.4 μ. Buttrey described a cytostome, but Hibler *et al.* did not see one. The 3 anterior flagella are about equal in length, 7 to 17 μ long with a mean of about 12.6 μ, and end in a round to spatulate knob. The blepharoplast is composed of several granules. The undulating membrane runs the full length of the body and has 4 to 6 subequal folds. Its marginal filament continues as a posterior free flagellum 5 to 17 μ long. An accessory filament is present. The costa runs the full length of the body, and fine subcostal granules are present. The axostyle is a hyaline rod 0.6 μ in diameter with a bulbous capitulum 1.7 μ in diameter. It extends 0.6 to 1.7 μ beyond the body as a cone-shaped projection narrowing abruptly

to a short tip. There is a chromatic ring around its point of exit. The parabasal body is usually a single, slender, tube-like structure 2 to 5μ long. The nucleus is oval or elongated, 2 to 5 by 1 to 3μ, with a large, conspicuous endosome surrounded by a relatively clear halo.

Pathogenesis: The discovery of this trichomonad by Switzer (1951) in a high percentage of cases of atrophic rhinitis and in a relatively low percentage of normal pigs raised the question whether it was the cause of the condition. Spindler, Shorb and Hill (1953) produced the disease in young pigs with nasal washings containing trichomonads from pigs with atrophic rhinitis, but Switzer (1951), Levine, Marquardt and Beamer (1954) and Fitzgerald, Hammond and Shupe (1954a), among others, were unable to do so with axenic cultures of the protozoon. It is now generally agreed that this trichomonad is not pathogenic. Several other agents, including *Pasteurella multocida* and *Mycoplasma hyorhinis*, have been incriminated as causes of atrophic rhinitis, but their roles require further elucidation (see Switzer, 1955 for review).

While *T. suis* is not pathogenic for pigs in its natural locations, it may cause abortion in heifers with experimental infections of the reproductive tract (see below).

Cultivation: This trichomonad can be readily cultivated in any of the media used for *T. foetus*. In mixed cultures with other species of porcine trichomonads, it survives while the others die out, so that it sometimes seems as tho one species has taken on the appearance of another (Hibler *et al.*, 1960). Because of this fact, cultures of pig cecal trichomonad heretofore used in cross-transmission studies have most probably been this species.

Switzer (1959) cultivated *T. suis* from the nasal passages in pig kidney, nasal mucosa and lung tissue cultures.

Remarks: Uncertainty has existed for many years regarding which of the trichomonads known to occur in swine was *T. suis*. This specific name was originally given by Gruby and Delafond (1843) to a form found in the stomach. Since that time, trichomonads have been found in the cecum and nasal passages, but it was not certain what their relationship was to the form which Gruby and Delafond had named. However, Hibler *et al.* (1960) found that the species described above is the only one which occurs in the stomach and that it also occurs in the nasal passages, cecum and small intestine. They found the other 2 trichomonad species of swine only in the cecum.

The relationship between *T. suis* and *T. foetus* requires further study. Buttrey (1956) and Hibler *et al.* (1960) pointed out their great morphological similarity. Doran (1957, 1959) concluded on the basis of metabolic studies that *T. suis* is a highly adapted strain of *T. foetus*. The other way around would be more likely in terms of evolution, i.e., *T. foetus* may well have arisen from *T. suis* or may be an adapted strain of it.

Fitzgerald *et al.* (1958) produced vaginal infections in 3 heifers with *T. suis* from the pig nose; the infections lasted 46 to 133 days. They also produced vaginal infections in 2 heifers with *T. suis* from the pig cecum which lasted 33 and 84 days, respectively, and in another heifer with *T. suis* from the pig stomach which lasted 88 days. They produced abortion in a 4-month-pregnant heifer by intrauterine inoculation of *T. suis* from the pig cecum. In addition, a bull became infected by breeding an infected heifer. He remained positive for 4 months and transmitted the infection to a virgin heifer by coitus. Hammond and Leidl (1957a) infected the preputial cavity of bulls with *T. suis* from the pig cecum and found that the infections were transmissible by coitus. Kerr (1958) produced vaginal infections in heifers with Hammond's strains and also with a strain of *T. suis* which he isolated from pigs in England.

Hammond and Leidl (1957) produced vaginal infections with *T. suis* from the pig cecum in 4 of 5 sows; the infections

lasted 3 to 42 days. Fitzgerald *et al.* (1958) produced nasal and cecal infections in young pigs with cultures of *T. suis* from the pig nose, and they produced nasal, gastric and cecal infections with *T. suis* from the pig stomach and from the pig cecum.

Shaw and Buttrey (1958) were able to infect young chickens with *T. suis* from the pig nose by rectal inoculation but not by mouth.

Kerr (1958) found that the vaginal mucus agglutination test of heifers infected with *T. suis* was positive with *T. suis* and Belfast strain *T. foetus* antigens but not with Manley strain *T. foetus* antigen. Sanborn (1955) found by microagglutination tests that a strain of *T. suis* from the pig nose was antigenically different from a strain of *T. foetus* and that both differed from a pig cecal trichomonad.

As mentioned above under the discussion of *T. foetus*, Robertson (1960) found that the Belfast and Manley strains of *T. foetus* and Strains S2 and 414 of *T. suis* were serologically related and concluded that the differences between them did not justify separating them into 2 species.

Cattle and swine are often raised together, and the broad host ranges and morphologic, metabolic and serologic similarity between *T. suis* and *T. foetus* suggest that they may have had a common origin if they are not indeed the same. Robertson (1960) believed that they are the same and called them all *T. foetus*, but the correct name, as Hibler *et al.* (1960) pointed out, would be *T. suis*. Even so, however, it might still be worth-while to retain both names, simply as a matter of convenience.

TRITRICHOMONAS ROTUNDA HIBLER, HAMMOND, CASKEY, JOHNSON AND FITZGERALD, 1960

Synonym: *Tritrichomonas suis* pro parte.

Common Name: Medium-sized pig cecal trichomonad.

Disease: None.

Hosts: Pig.

Location: Cecum, colon.

Geographic Distribution: This species has been recognized so far only in North America, but presumably occurs thruout the world.

Prevalence: Hibler *et al.* (1960) found *T. rotunda* in the ceca of 10.5% of 496 pigs in Utah.

Morphology: This species was described in detail by Hibler *et al.* (1960) and by Buttrey (1956); the latter referred to it as "*T. suis*-like." *T. rotunda* is typically broadly piriform, and only occasionally ovoid or ellipsoidal. It measures 7 to 11 by 5 to 7 μ with a mean of 9.0 by 5.8 μ. Hibler *et al.* saw no cytostome. Cytoplasmic inclusions were frequently present. The 3 anterior flagella are about equal in length, being 10 to 17 μ long with a mean of 14.9 μ, and terminate in a knob or spatulate structure. The blepharoplast appears to consist of a single granule. The undulating membrane is relatively low. It and the costa extend about 1/2 to 2/3 of the length of the body according to Hibler *et al.* (the full length of the body, according to Buttrey), and its undulation pattern varies from smooth to tightly telescoped or coiled waves (with 3 to 5 indistinct folds, according to Buttrey). The accessory filament impregnates heavily with silver. The posterior free flagellum is generally shorter than the body. The axostyle is a narrow, straight, non-hyaline rod with a crescent- or sickle-shaped capitulum. It extends a relatively long distance beyond the body (0.6 to 6.3 μ with a mean of 4.3 μ). There is no chromatic ring at its point of exit from the body. The nucleus is practically spherical, 2 to 3 μ in diameter, with an endosome surrounded by a clear halo. The parabasal body measures 2.3 to 3.4 by 0.4 to 1.3 μ. It is composed of 2 rami which form a V; each ramus has a parabasal filament.

Pathogenesis: Non-pathogenic.

Cultivation: *T. rotunda* grows readily on primary culture in standard trichomonad media, but dies out on subculture and can no longer be found after the 4th or 5th subculture. However, it can be maintained indefinitely in a cecal extract-serum medium provided that *Pseudomonas aeruginosa* is present (Hibler *et al.*, 1960).

TRITRICHOMONAS ENTERIS
(CHRISTL, 1954) nov. comb.

Synonym: *Trichomonas enteris* Christl, 1954.

Hosts: Ox, zebu.

Location: Cecum, colon.

Geographic Distribution: Germany, India, probably worldwide.

Prevalence: Common in Bavaria, according to Christl (1954).

Morphology: The body is 6 to 12 μ long and 5 to 6 μ wide. Three anterior flagella of equal length arise from a single blepharoplast. The flagellum at the edge of the undulating membrane is single, without an accessory filament. The undulating membrane extends 3/4 of the body length, and a free flagellum extends beyond the undulating membrane. The axostyle is straight, slender, bent like a spoon around nucleus, and extends at the most 1/4 of the body length beyond the body. Subcostal granules are present.

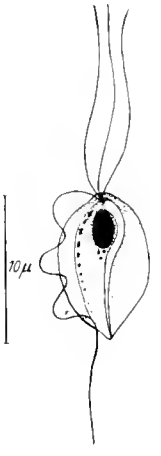

Fig. 9. *Tritrichomonas enteris*. X 1950. (From Christl, 1954 in *Zeitschrift für Parasitenkunde*, published by Springer-Verlag).

TRITRICHOMONAS SP.

Host: Ox.

Location: Feces.

Geographic Distribution: North America (Maryland).

Morphology: Diamond (1957) cultivated this form from calf feces. He did not describe it except to say that it resembled *T. batrachorum*.

TRITRICHOMONAS EQUI
(FANTHAM, 1921)

Synonyms: *Trichomonas equi*.

Host: Horse.

Location: Cecum, colon.

Geographic Distribution: Presumably worldwide, altho it has been reported specifically only from South Africa and the U.S. (Iowa).

Prevalence: Fantham (1921) found *T. equi* very rarely and in very small numbers in horses in South Africa. Hsiung (1930) found it on several occasions in Iowa.

Morphology: According to Hsiung (1930), *T. equi* measures about 11 by 6 μ and seems to possess 3 anterior flagella and an undulating membrane. The axostyle is slender.

TRITRICHOMONAS FECALIS
CLEVELAND, 1928

This species was isolated once from human feces by Cleveland (1928). It has 3 very long flagella, a heavy undulating

membrane, a long, coarse axostyle and a costa with 2 rows of granules. Like *T. buttreyi* and the form cultured from a calf by Diamond (1957), it resembles *T. batrachorum*. The relationship between the small trichomonads of mammals requires study.

TRITRICHOMONAS EBERTHI (MARTIN AND ROBERTSON, 1911) KOFOID, 1920

Synonyms: *Trichomonas eberthi*.

Hosts: Chicken, turkey. Kotlan (1923) reported *T. eberthi* from the duck.

Location: Ceca.

Geographic Distribution: Worldwide.

Prevalence: Common. McDowell (1953) found *T. eberthi* in 35% of a large number of chickens in Pennsylvania.

Morphology: The body is carrot-shaped, 8 to 14 by 4 to 7 μ, with vacuolated cytoplasm, and 3 anterior flagella. The undulating membrane is prominent, extending the full length of the body. The posterior flagellum extends about half of the body length beyond the undulating membrane. An accessory filament is present. The cytostome is difficult to demonstrate. The blepharoplast is composed of 4 equidistant granules, but tends to stain as a single body. Five to 12 or more subcostal granules are present. The axostyle is massive, hyaline, with its anterior end broadened to form a capitulum which contains siderophilic, argentophilic granules. Other endoaxostylar granules are also present. A ring of chromatic granules surrounds the axostyle at its point of emergence from the body. The parabasal body is shaped like a flattened rod, sometimes lumpy, of variable length. There are 5 chromosomes.

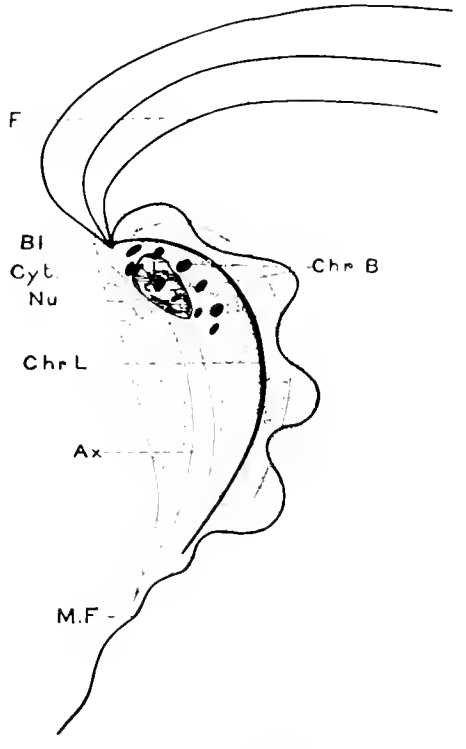

Fig. 10. *Tritrichomonas eberthi*. X 4700. (From Martin and Robertson, 1911)

Pathogenesis: Non-pathogenic.

Cultivation: Diamond (1957) cultivated *T. eberthi* axenically for the first time in Diamond's medium.

OTHER SPECIES OF *TRITRICHOMONAS*

Tritrichomonas muris (Grassi, 1879) occurs in the cecum, colon and sometimes small intestine of the Norway rat, black rat, house mouse, golden hamster and a large number of wild rodents. It measures 16 to 26 by 10 to 14 μ.

T. minuta (Wenrich, 1924) occurs in the cecum and colon of the Norway rat, house mouse and golden hamster. It measures 4 to 9 by 2 to 5 μ.

T. wenyoni (Wenrich, 1946) occurs in the cecum and colon of the Norway rat, house mouse, golden hamster, rhesus monkey and Chacma baboon. It measures 4 to 16 by 2.5 to 6 μ.

T. caviae (Davaine, 1875) occurs in the cecum and colon of the guinea pig. It measures 10 to 22 by 6 to 11 μ.

Tritrichomonas sp. Nie, 1950 occurs in the cecum of the guinea pig. It measures 6 to 13 by 4.5 to 6.5 μ.

Tritrichomonas criceti (Wantland, 1956 emend.) nov. comb. (syn., *Trichomonas cricetus* Wantland, 1956) occurs in the cecum and colon of the golden hamster. It measures 12 to 25 by 5 to 10 μ.

Genus *TRICHOMONAS* Donné, 1837

Members of this genus have 4 anterior flagella.

TRICHOMONAS TENAX (MÜLLER, 1773) DOBELL, 1939

Synonyms: *Cercaria tenax*, *Tetratrichomonas buccalis*, *Trichomonas buccalis*, *Trichomonas elongata*.

Disease: None.

Hosts: Man, monkeys (*Macaca mulatta*, *Papio sphinx*).

Location: Mouth, especially between gums and teeth.

Geographic Distribution: Worldwide.

Prevalence: Common. *T. tenax* has been found in 4% to 53% of persons examined in different surveys (Wenrich, 1947).

Morphology: The morphology of *T. tenax* has been studied by Wenrich (1947) and Honigberg and Lee (1959). The latter remarked on the close morphological resemblance of this species to *T. gallinae*. The body is ellipsoidal, ovoid or piriform, 4 to 16 μ long and 2 to 15 μ wide. Different strains differ in size; the smallest of 5 strains studied by Honigberg and Lee (1959) averaged 6.0 by 4.3 μ and the largest 8.4 x 6.0 μ. The 4 anterior flagella are 7 to 15 μ long. They originate in a basal granule complex anterior to the nucleus and terminate in little knobs or rods. The undulating membrane is shorter than the body; it ranged from 40 to 100% and averaged from 69 to 82% of the body length in the 5 strains studied by Honigberg and Lee (1959). An accessory filament is present. There is no free posterior flagellum. The costa is slender and accompanied by a group of large paracostal granules. The parabasal apparatus consists of a typically rod-shaped body and a long filament extending posteriorly from it. The axostyle is slender and extends a considerable distance beyond the body. There is no periaxostylar ring at its point of exit nor is it accompanied by paraxostylar granules. The capitulum of the axostyle is somewhat enlarged and spatulate. The pelta is of medium width. Wenrich (1947) said that a cytostome was present, but Honigberg and Lee (1959) found no evidence of one. Honigberg and Lee (1959) described the division process in detail.

Pathogenesis: None.

Cultivation: Honigberg and Lee (1959) cultivated *T. tenax* in Balamuth's yolk infusion medium. Diamond (1960) cultivated it axenically in a complex medium containing chick embryo extract.

Remarks: Hinshaw (1928) infected a dog which had gingivitis with *T. tenax*.

TRICHOMONAS EQUIBUCCALIS SIMITCH, 1939

Disease: None.

Hosts: Horse, donkey.

Location: Mouth, around gums and teeth.

Geographic Distribution: This species has apparently been reported only from Jugoslavia.

Prevalence: Simitch (1939) found *T. equibuccalis* by culture in 7 out of 22 horses and 2 out of 4 donkeys in Jugoslavia.

Morphology: The body is piriform or ovoid, 7 to 10 μ long. It has a single blepharoplast and 4 anterior flagella 10 to 15 μ long. The undulating membrane is relatively short, rarely reaching the posterior end. There is no free posterior flagellum. The costa is slender and not always visible. The axostyle is apparently slender and extends beyond the body.

Pathogenesis: Non-pathogenic.

Remarks: Simitch (1939) transmitted *T. equibuccalis* readily from the horse to the donkey and vice versa, but was unable to infect cattle, sheep and goats with it.

TRICHOMONAS FELISTOMAE
HEGNER AND RATCLIFFE, 1927

Hosts: Cat.

Location: Mouth.

Geographic Distribution: United States.

Prevalence: Hegner and Ratcliffe (1927) found this species in 2 out of 28 cats examined in Baltimore, Md.

Morphology: The body is piriform, 6 to 11 by 3 to 4 μ with a mean of 8 by 3 μ, and has 4 anterior flagella longer than body. The costa is illustrated as prominent. The undulating membrane extends most of the body length. There is a free posterior flagellum. The axostyle extends a considerable distance beyond the body.

Pathogenesis: Non-pathogenic.

TRICHOMONAS CANISTOMAE
HEGNER AND RATCLIFFE, 1927

Hosts: Dog.

Location: Mouth.

Geographic Distribution: United States, Europe.

Prevalence: Hegner and Ratcliffe (1927a), found this species in 22 out of 23 dogs examined in Baltimore, Md.

Morphology: The following description is based on Hegner and Ratcliffe (1927a). The body is piriform, 7 to 12 μ long and 3 to 4 μ wide. Four anterior flagella about as long as the body arise in pairs from a large blepharoplast. The undulating membrane extends almost the length of the body. The free posterior flagellum is about half as long as the body. The costa is apparently slender. The axostyle is thread-like, staining black with hematoxylin, and extends a considerable distance beyond the body. Subcostal granules are absent.

Pathogenesis: Non-pathogenic.

Remarks: An old dog with advanced gingivitis was infected with *T. tenax* by Hinshaw (1928); the infection was still present 14$\frac{1}{2}$ months later. Simitch and Kostitch (1938) were unable to infect humans with *T. canistomae* or to infect dogs with *T. tenax*. The morphological difference described between the two species indicates that they are different. *T. canistomae* and *T. felistomae*, however, may well be the same; further study is needed to determine this.

TRICHOMONAS VAGINALIS
DONNÉ, 1836

Hosts: Man.

The golden hamster can be infected intravaginally (Uhlenhuth and Schoenherr, 1955). Mice can be infected subcutaneously (Honigberg, 1959).

Location: Vagina, prostate gland, urethra.

Geographic Distribution: Worldwide.

Prevalence: *T. vaginalis* has been reported in 2% to as high as 80 to 90% of women and in 1 to 47% of men in various surveys (Wenrich, 1947; Kucera, 1957; Burch, Rees and Reardon, 1959).

Morphology: The body is piriform, 7 to 23 by 5 to 12 μ, and has 4 anterior flagella about as long as the body. The undulating membrane has 3 or 4 waves and extends a little more than half the body length. There is no free posterior flagellum. An accessory filament is present. The costa is very narrow. The parabasal body is long, cylindrical, and has a parabasal filament extending posteriorly from

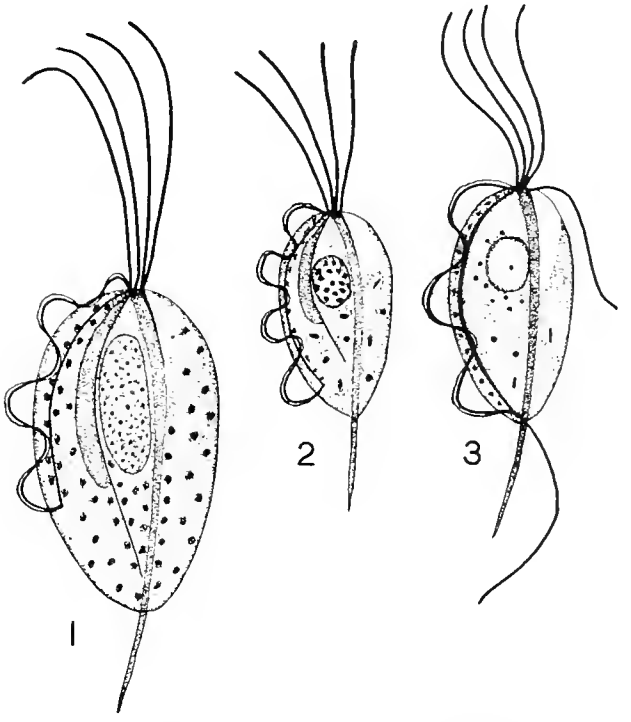

Fig. 11. Trichomonads of man. 1. *Trichomonas vaginalis*. 2. *Trichomonas tenax*. 3. *Pentatrichomonas hominis*. X 2500. (From Wenrich, 1947)

it. Paracostal and extra-axostylar granules are numerous; other siderophil granules are scattered in the cytoplasm. Four chromosomes are present. The axostyle is rather slender. The cytostome is inconspicuous.

Pathogenesis: *T. vaginalis* infections are often asymptomatic in womena and are usually so in the male. Trichomonad vaginitis is characterized by leukorrhea and vaginal and vulvar pruritis. *T. vaginalis* may occasionally cause purulent urethritis and prostato-vesiculitis in the male. Concomitant bacteria and yeasts may exacerbate the symptoms and lesions.

Epidemiology: *T. vaginalis* infections are essentially venereal in origin, the organism being transmitted during sexual intercourse. In exceptional cases, infants have been infected from their mothers. Transmission thru contaminated towels, underwear or toilet seats is extremely rare.

Diagnosis: *T. vaginalis* infections can be readily identified by microscopic examination of vaginal secretions or scrapings, sedimented urine or prostate secretions obtained by massaging the prostate gland.

Cultivation: *T. vaginalis* can be readily cultivated in any of the media used for trichomonads, such as CPLM medium.

Treatment: A number of preparations are used in treating trichomonad vaginitis. Among them are suppositories containing chiniofon, diodoquin, vioform, carbarsone or oxytetracycline. Lactic acid douches are often used to make the vaginal pH acid and provide conditions unsuitable for the protozoa. Infections in the male may be treated by introducing oxytetracycline ointment into the urethra or irrigating with a sulfonamide or antibiotic. To prevent reinfection, both husband and wife should be treated.

Remarks: Trussell (1947) has written a definitive monograph on this species.

TRICHOMONAS PAVLOVI NOM. NOV.

Synonym: *Trichomonas bovis* Pavlov and Dimitrov, 1957, non *Trichomonas bovis* Riedmüller, 1930.

Host: Ox.

Location: Large intestine.

Geographic Distribution: Bulgaria.

Morphology: This species was described by Pavlov and Dimitrov (1957). The trophozoites are piriform and usually measure 11 to 12 by 6 to 7 μ. The 4 anterior flagella are about the same length as the body. The undulating membrane is well developed, with 2 to 4 waves, and extends almost to the posterior end of the body. A posterior free flagellum, an accessory filament and a costa are present. The nucleus is round-oval or oval. The axostyle is relatively weak and slender, broadening to form a capitulum at the anterior end, and extending about 1/4 of its length from the posterior end of the body. There are many food vacuoles in the cytoplasm.

Pathogenesis: Pavlov and Dimitrov (1957) found this species in the feces of calves 5 days to 4 months old, all of which had diarrhea. They thought that the protozoa were the cause of the diarrhea, basing their opinion on their inability to find another cause and on the fact that the trichomonads disappeared from the feces when the diarrhea ceased. Needless to say, this is not sufficient justification for their view.

Remarks: Further study is necessary to be sure whether this species is valid. Pending such a study, it is considered best to retain it.

Pavlov and Dimitrov (1957) named this species *Trichomonas bovis*. This name is a homonym of *Trichomonas bovis* Riedmüller, 1930, which is in turn a synonym of *Tritrichomonas foetus* (Riedmüller, 1928). Hence I am renaming it *Trichomonas pavlovi* nom. nov.

TRICHOMONAS BUTTREYI HIBLER, HAMMOND, CASKEY, JOHNSON AND FITZGERALD, 1960

Common Name: Small pig cecal trichomonad.

Disease: None.

Host: Pig.

Location: Cecum, colon, rarely small intestine.

Geographic Distribution: This species has been recognized so far only in North America, but presumably occurs thruout the world.

Prevalence: Hibler *et al.* (1960) found *T. buttreyi* in the ceca of 25.4% of 496 pigs and in the small intestine of 1% of 100 pigs in Utah.

Morphology: This species was described in detail by Hibler *et al.* (1960) and by Buttrey (1956); the latter referred to it as a *Paratrichomonas*-like form resembling *P.* (or *Trichomonas*) *batrachorum*.

T. buttreyi is ovoid or ellipsoidal, 4 to 7 by 2 to 5 μ with a mean of about 5.9 by 3.4 μ. Cytoplasmic inclusions are frequently present, but Hibler *et al.* saw no cytostome. There are 4 or 3 anterior flagella which vary in length from a short stub to more than twice the length of the body and end in a knob or spatulate structure. The undulating membrane runs the full length of the body and has 3 to 5 undulations. The accessory filament is prominent and the costa relatively delicate. A posterior free flagellum is present. The axostyle is relatively narrow, with a spatulate capitulum, and protrudes 3 to 6 μ beyond the body. There is no chromatic ring at its point of exit. A pelta is present anteriorly. The nucleus is frequently ovoid but varies considerably in shape; it measures 2 to 3 by 1 to 2 μ and has a small endosome. The parabasal body is a disc 0.3 to 1.1 μ in diameter.

Pathogenesis: Non-pathogenic.

Cultivation: According to Hibler *et al.*, *T. buttreyi* grows readily on primary culture in standard trichomonad media, but dies out on subculture; they maintained it indefinitely in a cecal extract-serum medium provided *Pseudomonas aeruginosa* was present. Diamond (1957) however, established it in axenic culture.

Remarks: Doran (1958) studied the metabolism of this species, using Strain PC-287. It could not oxidize Krebs cycle intermediates, but produced carbon dioxide and other gas not absorbed by KOH anaerobically. It resembled *T. suis* more than other trichomonads, but differed in carbohydrate utilization and in having a generally lower respiratory rate.

TRICHOMONAS GALLINAE (RIVOLTA, 1878) STABLER, 1938

Synonyms: *Cercomonas gallinae*, *Cercomonas hepaticum*, *Trichomonas columbae*, *Trichomonas diversa*, *Trichomonas halli*.

Disease: Avian trichomonosis, upper digestive tract trichomonosis.

Hosts: The domestic pigeon is the primary host of *T. gallinae*, but it also occurs in a large number of other birds, including hawks and falcons which feed on pigeons. Its natural hosts besides the pigeon include the mourning dove (*Zenaidura macroura*), Indian dove (*Turtur suratensis*), wood pigeon (*Columba palumbus*), band-tailed pigeon (*C. fasciata*), ring dove (*Streptopelia risoria*), white-winged dove (*Zenaida asiatica*), turkey, chicken, Cooper's hawk (*Accipiter cooperi*), golden eagle (*Aquila chrysaetos*), duck hawk (*Falco peregrinus anatum*), Java sparrow (*Munia oryzivora*), zebra finch and orange-cheeked waxbill.

A number of other birds have been experimentally infected. They include the bobwhite quail, canary, English sparrow (Levine, Boley and Hester, 1941), barn swallow, goldfinch and song sparrow (Stabler, 1953), and Tovi parakeet and Verraux's dove (Callender and Simmons, 1937). Parenteral infections have also been produced experimentally in mammals --by Bos (1934) in mice and guinea pigs, by Wagner and Hees (1935), Wittfogel (1935), Miessner and Hansen (1936), Schnitzer, Kelly and Leiwant (1950) and Honigberg (1959) in mice, and by Rakoff (1934) in rats and kittens.

Prevalence: *T. gallinae* is extremely common in domestic pigeons, in which it often causes serious losses. It is fairly common in the turkey; the U.S. Dept. of Agriculture (1954) estimated that it causes an annual loss of $47,000 in these birds. It is rare in chickens. It is common in mourning doves, and may cause serious losses among them (Stabler and Herman, 1951). According to Stabler (1954), it was common in trained hawks during the heyday of falconry; they became infected because they were fed largely on pigeons. Stabler and Herman (1951) and Stabler (1954) give further information on incidence in domestic and wild birds.

Morphology: The following description is based on Stabler (1941, 1954). The body

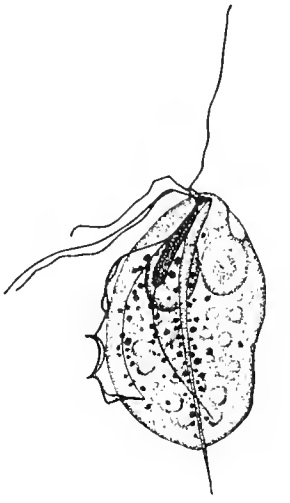

Fig. 12. *Trichomonas gallinae*. X 3400. (From Stabler, 1947)

is roughly piriform, 6 to 19 by 2 to 9 μ. Four anterior flagella 8 to 13 μ long arise from the blepharoplast. The axostyle is narrow and protrudes a short distance from the body. There is no chromatic ring around its point of emergence. The parabasal body is sausage-shaped, about 4 μ long, with a parabasal filament. The costa runs 2/3 to 3/4 of the body length. The undulating membrane does not reach the posterior end of the body. An accessory filament is present. A free trailing flagellum is absent. A cytostome is present. There are 6 chromosomes.

Pathogenesis: In the pigeon, trichomonosis is essentially a disease of young birds; 80 to 90% of the adults are infected but show no signs of disease. The severity of the disease varies from a mild condition to a rapidly fatal one with death 4 to 18 days after infection. This is due in part to differences in virulence of different strains of the trichomonad (Stabler, 1948). Severely affected birds lose weight, stand huddled with ruffled feathers, and may fall over when forced to move. A greenish fluid containing large numbers of trichomonads may be found in the mouth.

Lesions are found in the mouth, sinuses, orbital region, pharynx, esophagus, crop and even the proventriculus. They do not involve the digestive tract beyond the

proventriculus. They often occur in the liver and to a lesser extent in other organs, including the lungs, air sacs, heart, pancreas, and more rarely the spleen, kidneys, trachea, bone marrow, navel region, etc.

The early lesions in the mouth are small, yellowish, circumscribed areas in the mucosa. They increase in number and become progressively larger, finally developing into very large, caseous masses which may invade the roof of the mouth and sinuses and may even extend thru the base of the skull to the brain. The early lesions in the pharynx, esophagus and crop are small, whitish to yellowish caseous nodules which also grow. They may remain circumscribed and separate, or they may form thick, caseous, necrotic masses which may occlude the lumen. The circumscribed, disc-shaped lesions are often described as "yellow buttons". Those in the esophagus and crop may have central, spur-like projections. A large amount of fluid may accumulate in the crop. The lesions in the liver, lungs and other organs are solid, yellowish, caseous nodules ranging up to a centimeter or more in diameter.

In the turkey and chicken, the lesions occur mostly in the crop, esophagus and pharynx, and are relatively uncommon in the mouth and liver. The lesions in the mourning dove are similar to those in the pigeon.

Immunology: As mentioned above, different strains of *T. gallinae* differ greatly in virulence (Stabler, 1948; Florent, 1938; Gloor, 1943). Previous infection bestows more or less immunity; adult pigeons which have survived infection as squabs are symptomless carriers. Infection with a relatively harmless strain produces immunity against virulent strains (Stabler, 1948a, 1951). According to Florent (1938), pigeons are particularly susceptible at the time of weaning and of the first molt. Stabler (1953) found that immunity did not increase with age of uninfected birds. Certain breeds or strains of birds may be more sensitive than others. Miessner and Hansen (1936) felt that roller and tumbler pigeons were such, and Levine and Brandly (1940) were able to infect chicks from one source readily while chicks from other sources were very resistant.

Epidemiology: In pigeons and mourning doves, trichomonosis is transmitted from the adults to the squabs in the pigeon milk which is produced in the crop. The squabs are infected within minutes after hatching. Hawks and other wild raptors become infected by eating infected birds. Turkeys and chickens are infected thru contaminated drinking water. Feral pigeons and other columbid birds are the original source of infection. The trichomonads pass into the water from the mouths of infected birds, and not from the droppings (Stabler, 1954). *T. gallinae* has no cysts and is very sensitive to drying, so direct contamination is necessary.

Diagnosis: Upper digestive tract trichomonosis is readily diagnosed by observation of the lesions together with demonstration of the protozoa. It must be differentiated from other conditions which may cause more or less similar lesions, including fowl pox, vitamin A deficiency and moniliosis (thrush).

Cultivation: *T. gallinae* can be cultivated readily in any of the customary trichomonad media. Diamond (1954) compared 28 culture media for it and (1957) introduced a trypticase-yeast extract-maltose-cysteine-serum medium for it and other trichomonads.

Treatment: A number of workers have recommended the use of copper sulfate for 20 days or more in the drinking water to eliminate *T. gallinae* (see Stabler, 1954) but this is not particularly satisfactory. The optimal concentration for non-breeding pigeons is 1-1000 and that for breeding pigeons with squabs is 1-3000 according to Jaquette (1948), but it tends to make the birds sick, and Jaquette felt that all the treated birds may have suffered liver damage. Turkeys will not drink 1-2000 copper sulfate.

The best treatment for *T. gallinae* is 2-amino-5-nitrothiazole (enheptin).

Stabler and Mellentin (1953) recommended 7 daily doses of 28 mg/kg for homing pigeons and 45 mg/kg for commercial birds. This treatment cures both acute cases and carriers. Stabler, Schmittner and Harman (1958) used 6.3 g enheptin soluble per gallon of drinking water for 7 to 14 days in non-breeding pigeons. The birds consumed 9 to 27 mg of the drug per day--operation of the peck order may have cut down water consumption by some birds--and 53 of 61 infected birds were freed of their infections. Zwart (1959) obtained promising results with 0.125% enheptin in the drinking water of a Dutch aviary where the infection had been found in zebra finches and an orange-checked waxbill.

Control: Control of trichomonosis in pigeons depends upon elimination of the infection from the adults by drug treatment. Prevention in turkeys and chickens is based upon preventing wild pigeons and doves from drinking from their watering places.

TRICHOMONAS GALLINARUM MARTIN AND ROBERTSON, 1911

Synonym: *Trichomonas pullorum*.

Disease: None.

Hosts: Chicken, turkey, guinea fowl, and possibly other gallinaceous birds such as the quail, pheasant and chukar partridge. Diamond (1957) found a *T. gallinarum*-like form in the Canada goose (*Branta canadensis*).

Location: Ceca, sometimes liver.

Geographic Distribution: Worldwide.

Prevalence: Common. McDowell (1953) found *T. gallinarum* in over 60% of a large number of chickens in Pennsylvania.

Morphology: The body is piriform, 7 to 15 by 3 to 9 μ, with 4 anterior flagella and a posterior flagellum which runs along the undulating membrane and extends beyond it. An accessory filament is present. The axostyle is long, pointed and slender, without a chromatic ring at its point of emergence. The cytostome is prominent. Supracostal granules but no subcostal or endoaxostylar granules are present. The pelta is elaborate, ending abruptly with a short ventral extension more or less free from the ventral edge of the axostyle, according to McDowell (1953); Marquardt (1954), however, did not find a pelta in his cultures of a strain from a turkey. The shape of the parabasal body is highly variable, but it is usually a ring of variously spaced granules plus 1 or 2 fibrils or rami. The chromosome number is apparently 5. A rather uniform perinuclear cloud of argentophilic granules is usually present (McDowell, 1953).

The form originally described by Martin and Robertson (1911) had 4 anterior flagella. Allen (1940) described a trichomonad from the ceca and liver of chickens and turkeys which she considered to be this species but which had 5 anterior flagella. Walker (1948), too, illustrated the trichomonad he isolated from turkey livers with 5 anterior flagella. Further study is needed to determine the relationship of this form to *T. gallinarum*. McDowell (1953) insisted on the fact that the usual number of anterior flagella is 4, rarely 3 and in even rarer cases 5. He studied 1000 slides from a large number of chickens. Marquardt (1954), too, found only 4 anterior flagella in a culture strain from a turkey.

Pathogenesis: Allen (1936, 1941), Olsen and Allen (1942) and Walker (1948) isolated a trichomonad from turkey liver lesions resembling those of histomonosis and considered that the trichomonad had caused them. The disease they described resembled histomonosis, with cecal and liver lesions, pale yellow, cecal diarrhea, inappetance, loss of weight, and a mortality of 0 to 44%. The cecal lesions were said to be the same as those of histomonosis, but the liver lesions were said to be smaller, to have irregular outlines and to be raised or level with the liver surface instead of depressed below it. Wichmann and Bankowski (1956) described a similar condition in chukar partridges. However,

the mere presence of an organism in a lesion is no proof that it caused the lesion. There is no satisfactory proof that *T. gallinarum* by itself is capable of causing disease, and the weight of evidence is against it. Delappe (1957) infected chickens and turkeys experimentally with a strain of *T. gallinarum* isolated from liver lesions of a turkey with histomonosis, but was unable to produce either symptoms or lesions. The possibility has still not been completely eliminated, however, that a *Pentatrichomonas* may exist which is pathogenic (see below).

Epidemiology: Birds become infected by ingestion of trichomonads in contaminated water or feed. McLoughlin (1957) found that one-week-old turkey poults were more susceptible than 9-week-old ones. He also found that *T. gallinarum* survived for 24 hours but not for 48 hours in cecal droppings at 37° C, and for 120 hours at 6° C.

Cultivation: *T. gallinarum* is readily cultivated in the usual trichomonad media.

TRICHOMONAS ANATIS (KOTLAN, 1923)

Synonym: *Tetratrichomonas anatis*.

Host: Domestic duck.

Location: Posterior part of intestinal tract.

Geographic Distribution: Europe (Hungary).

Morphology: The body is broadly beet-shaped, 13 to 27 by 8 to 18 μ, with 4 anterior flagella, an undulating membrane extending most of the length of the body, a free trailing flagellum, a costa and a fibrillar axostyle.

TRICHOMONAS ANSERI HEGNER, 1929

Hosts: Domestic goose, baby chick (experimental).

Morphology: The body is oval, 6 to 9 by 3.5 to 6.5 μ with a mean of 8 by 5 μ. Four anterior flagella appear to arise in pairs from 2 blepharoplasts. The undulating membrane extends almost the full length of the body. A free trailing flagellum and a costa are present. The axostyle is broad and hyaline, extending a considerable distance beyond the body. There is no chromatic ring at its point of emergence from the body. The nucleus is characteristic, completely filled with minute chromatin granules and also with a single large karyosome usually at one side. The cytostome is prominent. Many specimens have large bacteria in the endoplasm.

Location: Ceca.

Geographic Distribution: United States (Maryland).

Prevalence: Unknown.

Remarks: Hegner (1929) found a very few of these trichomonads in cecal material from a goose. He inoculated 3-day-old chicks with the material *per os* and *per rectum* and the above description is based on material from the chicks.

OTHER SPECIES OF *TRICHOMONAS*

Trichomonas macacovaginae Hegner and Ratcliffe, 1927 occurs in the vagina of the rhesus monkey. It measures 8 to 16 by 3 to 6 μ and has a free posterior flagellum, a feature which differentiates it from *T. vaginalis*.

T. microti Wenrich and Saxe, 1950 occurs in the cecum of the Norway rat, house mouse, golden hamster, vole (*Microtus pennsylvanicus*) and other wild rodents. It is 4 to 9 μ long. Simitch, Petrovitch and Lepech (1954) transmitted it from the white mouse to the laboratory rat, guinea pig, ground squirrel (*Citellus citellus*), dog and cat, but were unable to infect the chicken and a human volunteer. Wenrich and Saxe (1950) transmitted if from the vole to the laboratory rat, hamster and guinea pig, but could not infect a human volunteer.

Genus *PENTATRICHOMONAS* Mesnil, 1914

Members of this genus have 5 anterior flagella.

PENTATRICHOMONAS HOMINIS (DAVAINE, 1860)

Synonyms: *Cercomonas hominis*, *Monocercomonas hominis*, *Trichomonas intestinalis*, *Trichomonas confusa*, *Pentatrichomonas ardin delteili*, *Trichomonas felis*, *Trichomonas parva*, *Pentatrichomonas canis auri*.

Disease: None.

Hosts: Man, gibbon, chimpanzee, orang-utan, rhesus monkey, pigtailed monkey (*Macaca nemestrina*), brown capuchin (*Cebus fatuellus*), weeping capuchin (*C. apella*), white-throated capuchin (*C. capucinus*), black spider monkey (*Ateles ater*), white-crested titi monkey (*Callicebus amictus*), Guinea baboon (*Papio papio*), Humboldt's woolly monkey (*Lagothrix lagotricha*), vervet monkey (*Cercopithecus pygerythrus*), dog, cat, rat, mouse, golden hamster. The primates were listed by Flick (1954).

Kessel (1928) infected kittens with trichomonads from man, the monkey and rat. Simitch (1932, 1932a, 1933) transmitted *P. hominis* from the rat to the cat, dog and man. Saxe (1954) transmitted it from the golden hamster to the laboratory rat and from the rat to the hamster. Simitch, Petrovitch and Lepech (1954) infected the white mouse, laboratory rat, guinea pig, ground squirrel (*Citellus citellus*), dog, cat and chicken with *P. hominis* from man.

Location: Cecum, colon.

Geographic Distribution: Worldwide.

Prevalence: Common.

Morphology: The following description is based primarily on Wenrich (1947) and Kirby (1945). The body is usually piriform, 8 to 20 by 3 to 14 μ. Five anterior flagella are ordinarily present, altho some organisms may have 4 and a few 3. Flick (1954) found in a study of more than 13,000 individuals from 13 *P. hominis* strains from 13 hosts that 77% had 5 flagella, 17% had 4, 5% had 3, and 1% had 6 or more anterior flagella. Four of the anterior flagella are grouped together and the fifth is separate and directed posteriorly. A sixth flagellum runs along the undulating membrane and extends beyond it as a free trailing flagellum. The undulating membrane extends the full length of the body. An accessory filament, a costa and paracostal granules are present. The axostyle is hyaline, thick, with a sharply pointed tip but without a chromatic ring at its point of exit. The parabasal body is small and ellipsoidal. The blepharoplast is composed of 2 granules. The pelta is crescent-shaped, prolonged dorsally in a filament which passes posteriorly in the cytoplasm dorsal to the nucleus. A cytostome is present. There are 5 or 6 chromosomes.

Pathogenesis: Non-pathogenic.

Cultivation: *P. hominis* is readily cultivable in the usual trichomonad media.

PENTATRICHOMONAS SP. ALLEN, 1936

Synonym: *Pentatrichomonas gallinarum* auct.

Hosts: Chicken, turkey, guinea fowl.

Location: Ceca, liver.

Geographic Distribution: Probably worldwide.

Prevalence: Unknown.

Morphology: *Pentatrichomonas* sp. resembles *T. gallinarum* morphologically except that it has 5 anterior flagella. Four of these are of equal length and the fifth is about half as long as the others. The body is usually spherical, sometimes more or less pear-shaped, fixed specimens

measuring 3 to 7 by 5 to 8 μ with a mean of 5 by 7 μ. The undulating membrane extends the full length of the body, with a free flagellum at its end. A costa is present (Allen, 1940 called it a parabasal body). A row of paracostal granules runs between the costa and the undulating membrane. The axostyle is slender, projecting from the posterior end, but not discernible in rounded-up specimens. A cytostome is present. The blepharoplast is composed of a group of small granules.

Pathogenesis: As mentioned in the discussion of *Trichomonas gallinarum*, Allen and others isolated this form from turkey liver lesions resembling those of histomonosis and attributed the disease to it. However, *post hoc* reasoning is not enough, and there is as yet no acceptable proof that this trichomonad is pathogenic.

Remarks: Allen (1936) first assigned this species to the genus *Pentatrichomonas* without naming it. She later (1940) described it as a five-flagellate *"Trichomonas gallinarum* Martin and Robertson, 1911". Later authors such as Morgan and Hawkins (1952) called it *Pentatrichomonas gallinarum*. The species described by Martin and Robertson has 4 anterior flagella, as does the form described by McDowell (1953). Further study is needed to determine the relationship between the two forms.

Genus *DITRICHOMONAS* Cutler, 1919

Similar to *Trichomonas*, but with 2 anterior flagella.

DITRICHOMONAS OVIS ROBERTSON, 1932

Host: Sheep.

Location: "Gut."

Geographic Distribution: England.

Prevalence: Robertson (1932) found this species in 1 out of 86 sheep in a London abattoir.

Morphology: The protozoa were described from cultures. The body is slightly ovoid or nearly spherical, 3 to 14 by 3 to 10 μ, with 2 anterior flagella, one 12 to 16.5 μ and the other 7.5 to 10.5 μ long. The undulating membrane is poorly developed but extends the whole length of the body. A free flagellum was described as present, but was absent in 7 out of 9 drawings. A costa is present. The axostyle extends beyond the body. Three blepharoplasts and another granule described as a parabasal body are present.

Pathogenesis: Non-pathogenic.

Cultivation: Robertson (1932) cultivated *D. ovis* in Tanabe's medium with or without added rice starch.

Remarks: Robertson's paper was apparently overlooked by Grasse, Reichenow and others who discussed the validity of the genus *Ditrichomonas*. Robertson insisted that there are only 2 anterior flagella except when the protozoa are dividing. No one else appears to have studied the intestinal trichomonads of sheep carefully, altho they are more common in the United States than Robertson found them to be in England. Whether they are the same species remains to be determined.

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Chapter 6

OTHER FLAGELLATES

In this chapter is discussed a miscellany of flagellates, most of which are found in the digestive tract. Only a few are pathogenic, the great majority being commensal. Some are not parasitic at all but are coprophilic or have been found as contaminants in washings from the sheath of bulls; these are mentioned because they must be differentiated from parasitic forms. A few other species are free-living toxin-producers.

TOXIC MARINE PHYTOFLAGELLATES

The great majority of phytoflagellates are free-living and holophytic. Some of them produce powerful toxins which may kill fish or even man.

Gonyaulax catanella is a marine dinoflagellate, found particularly off the coast of California, which causes a fatal disease of man known as mussel poisoning. Its toxin is one of the most powerful known. Under conditions still largely unknown, the protozoa multiply tremendously, forming a luminescent "bloom" in the ocean. Mussels and certain other shellfish feeding on plankton are not harmed by the toxin but accumulate it in their internal organs. People who eat these mussels may then be killed by the toxin.

The blooming of other dinoflagellates, including several species of *Gymnodinium*, cause the "red tide" or "red water" which sometimes kills huge numbers of fish, depositing them in rotting windrows on the shore. This condition is particularly common off the coast of Florida, where it is associated with the discharge of phosphates into the ocean, but it also occurs off the Texas coast and elsewhere (Hutner and McLaughlin, 1958).

A third marine phytoflagellate, the chrysomonad *Prymnesium parvum*, has killed fish *en masse* in brackish fish ponds in Israel, and has formed blooms accompanying fish kills in Holland and Denmark (McLaughlin, 1958).

A few phytoflagellates are coprophilic and may be mistaken for true parasites, and still fewer are parasitic. These will be discussed below.

PARASITIC FLAGELLATES

The parasitic mode of life has arisen independently a number of times in this group. Free-living species or genera in distantly related or unrelated families have found suitable living conditions in various hosts. Many of these were previously inhabitants of stagnant water. The fact that so few of them are pathogenic effectively refutes the notion that parasites tend to be pathogenic in their first association with a new host and that later on the host and parasite adjust to each other, the latter becoming less pathogenic and eventually commensal.

FAMILY MONOCERCOMONADIDAE

This family, like the Trichomonadidae, belongs to the order Trichomonadorida. Its members have either a free or adherent trailing flagellum, but lack the undulating membrane and costa found in the Trichomonadidae. Four genera, *Monocercomonas*, *Hexamastix*, *Protrichomonas* and *Chilomitus*, occur in the intestinal tracts of domestic animals.

Genus *MONOCERCOMONAS* Grassi, 1879

In this genus the body is piriform, with a rounded anterior end. There is a pelta. The cytostome and nucleus are anterior. There are 3 anterior flagella and a trailing one. The axostyle projects beyond the posterior end of the body. Travis (1932) showed that *Trichomastix* Blochmann, 1884 and *Eutrichomastix* Kofoid and Swezy, 1915 are synonyms of *Monocercomonas*. Morgan (1944) gave a checklist of species of the genus; it included 20 species, of which 4 were from mammals, 4 from birds, 2 from reptiles, 1 from amphibia, 2 from fish and 7 from insects and other arthropods. Others have been described since.

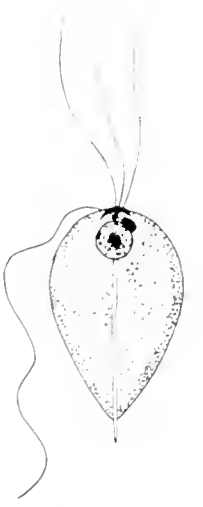

Fig. 13. *Monocercomonas*. X 2800. (Original)

Monocercomonas ruminantium (Braune, 1914) nov. comb. occurs in the rumen of cattle. In addition, Morgan and Noland (1943) found what was probably the same organism in material from the sheath of bulls.

The body is about 8 μ long, with 3 anterior flagella about 8 μ long and a trailing flagellum a little longer. The axostyle is curved and does not extend beyond the body, altho the posterior end is pointed. A line of granules runs beside the convex side of the axostyle. This species is nonpathogenic, but must be distinguished from *Tritrichomonas foetus*.

Synonyms of this species are *Trichomonas ruminantium* Braune, 1914; *Tricercomitus ruminantium* (Braune, 1914) Christl, 1954; and *Tritrichomonas ruminantium* (Braune, 1914); but not *Trichomastix ruminantium* Braune, 1914. Altho Braune (1914) assigned this species to the genus *Trichomonas* and succeeding workers have followed him in this or at the most have changed the generic name of *Tritrichomonas*, Christl (1954) pointed out that the absence of an undulating membrane made this assignment incorrect. Christl transferred it to the genus *Tricercomitus*, but it belongs more properly to the genus *Monocercomonas*.

Monocercomonas cuniculi (Tanabe, 1926) occurs in the cecum of the domestic rabbit. It is piriform, 5 to 14 μ long. Its axostyle is slender, hyaline, and projects from the body.

Monocercomonas gallinarum (Martin and Robertson, 1911) Morgan and Hawkins, 1948 is said to occur in the ceca of the chicken. Kotlan (1923) reported it from a single domestic duck. Its body is piriform, 5 to 8 by 3 to 4 μ. There is some question whether this is a valid species. It has been reported by Martin and Robertson (1911) in England, Kotlan (1923) in Hungary, and Morgan and Hawkins (1952) in Wisconsin, but McDowell (1953) failed to find it in 1000 slides from a large number of chickens in Pennsylvania. McDowell believed, along with Minchin (1917), Wenyon (1926), Doflein and Reichenow (1929) and others, that it is simply a degenerate *Trichomonas eberthi*.

Genus *HEXAMASTIX* Alexeieff, 1912

In this genus the body is piriform, with a rounded anterior end. The cytostome and nucleus are anterior. There are 6 flagella, of which 1 trails. (According to Nie, 1950, the number of anterior flagella varies in this genus from 2 to 6.) A pelta is present, the axostyle is conspicuous, and the parabasal body prominent. Members of this genus have been found in mammals, amphibia and insects. *Hexamastix caviae* Nie, 1950 and *H. robustus* Nie, 1950 occur in the guinea pig cecum, and *H. muris* (Wenrich, 1924) in the cecum of the Norway rat, golden hamster and other rodents.

Genus *CHILOMITUS* Da Fonseca, 1915

The body is elongate, with a convex aboral surface. The pellicle is well developed. The cuplike cytostome is near the anterior end. Four flagella emerge thru it from a bilobed blepharoplast. The nucleus and parabasal body are just below the cytostome. An axostyle is present but may be rudimentary. Cysts may occur. Only a few species have been described, all in mammals. *Chilomitus caviae* da Fonseca, 1915 and *C. conexus* Nie, 1950 occur in the guinea pig cecum.

Genus *PROTRICHOMONAS* Alexeieff, 1912

The body is piriform or beet-shaped, with 3 anterior flagella of equal length arising from an anterior blepharoplast, an anterior nucleus and an axostyle. Three species have been named, from birds, mammals and a fish.

Protrichomonas ruminantium (Braune, 1914) nov. comb. was originally assigned by Braune (1914) to the genus *Trichomastix* (now *Monocercomonas*), but the absence of a trailing flagellum makes this assignment incorrect. Its description agrees with that of *Protrichomonas*, altho it must be said that this genus is badly in need of redescription. *P. ruminantium* occurs in the rumen of cattle and sheep. It is about 8 μ long. Its nucleus is often surrounded by a clear zone. No cytostome was seen.

Protrichomonas anatis Kotlan, 1923 has been described from the large intestine of the domestic duck and other water birds. It is 10 to 13 μ long and 4 to 6 μ wide. Two distinct fibrillae arise from the anterior blepharoplast and pass back thru the body, separating to pass around the nucleus and finally passing out of the body as a pointed axostyle. The nucleus is often triangular.

ORDER POLYMASTIGORIDA

Members of this group have 2 to about 12 flagella and 1, 2 or several nuclei. They lack a costa, axostyle (except in some Hexamitidae and Polymastigidae) and parabasal body.

FAMILY TETRAMITIDAE

In this family there is a single nucleus and 4 flagella, 1 or 2 of which may be

trailing. *Enteromonas* is parasitic in domestic and laboratory animals, and *Tetramitus* is coprophilic.

Genus *TETRAMITUS* Perty, 1852

In this genus the life cycle involves flagellate and amoeboid forms; there are also uninucleate cysts. In the flagellate stage the body is ellipsoidal or piriform, with a large, trough-shaped cytostome at the anterior end, a vesicular nucleus with a large endosome, 4 anterior flagella, and a contractile vacuole. Nutrition is holozoic.

Tetramitus rostratus Perty, 1852 (syn., *Copromastix prowazeki* Aragao, 1916) is found in stagnant water and is also coprophilic. It has been found in human and rat feces. The flagellate stage is 14 to 18 μ long and 7 to 10 μ wide. The amoeboid stage is 14 to 48 μ long and usually has a single lobose pseudopod. The cysts are spherical, thin-walled, and 6 to 18 μ in diameter. The life cycle of this species has been studied by Bunting (1926), Bunting and Wenrich (1929) and Hollande (1942).

Genus *ENTEROMONAS* Da Fonseca, 1915

The body is spherical or piriform and is plastic. It has 3 short anterior flagella, 1 of which may be difficult to see, and a 4th, long flagellum which runs along the flattened body surface and extends free for a short distance at the posterior end of the body. A strand-like funis arises from the blepharoplast and extends posteriorly along the body surface; it stains faintly with iron hematoxylin and strongly with protargol. The nucleus is anterior, vesicular, with or without an endosome. There is no cytostome. The cysts are ovoid, and are tetranucleate when mature. A synonym of this genus is *Tricercomonas* Wenyon and O'Connor, 1917. This genus has been reported from a number of mammals.

Enteromonas hominis da Fonseca, 1915 (synonyms, *Octomitus hominis*, *Tricercomonas intestinalis*, *Diplocercomonas soudanensis*, *Enteromonas bengalensis*) occurs in the cecum of man, macaques (*Macaca mulatta*, *M. sinica*, *M. nemestrina*) the golden hamster and probably other animals thruout the world. Wantland (1955) reported it in 1% of 500 golden hamsters in the United States. Saxe (1954) transmitted it from the golden hamster to the laboratory rat. Dobell (1935) was unable to infect himself with a culture of *E. hominis* from the macaque, *M. sinica*, but believed that future work would show that the human and macaque forms are the same species. Simitch *et al.* (1959) reported failure to transmit *E. hominis* to 2 young pigs.

The trophozoite is oval, 4 to 10 by 3 to 6 μ, and has many food vacuoles containing bacteria. The cysts are ovoid or ellipsoidal; they are usually binucleate but have 4 nuclei when mature. *E. hominis* is readily cultivated on the usual media for enteric protozoa such as LES medium; cysts form in the cultures. It is non-pathogenic.

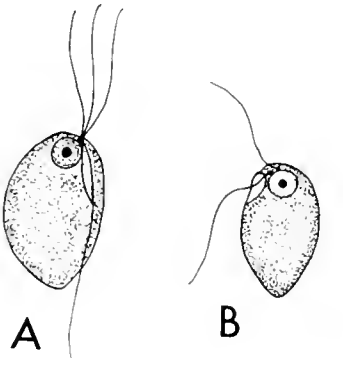

Fig. 14. A. *Enteromonas*. B. *Retortamonas*. X 2800. (Original)

Enteromonas suis (Knowles and Das Gupta, 1929) Dobell, 1935 (syn., *Tricercomonas suis*) was described from the cecum of a pig in India. It was cultivated easily in Dobell and Laidlaw's medium. It is shaped like a broad, ovate leaf with a more or less rounded anterior end and a pointed posterior end, and is 9 to 20 μ long and 6 to 14 μ wide. It moves sluggishly more or less directly forward and does not rotate like *Trichomonas*. The three anterior flagella are 8 to 18 μ long

with a mean of 14 μ, and the posterior flagellum is 9 to 26 μ long with a mean of 17 μ. Simitch *et al.* (1959) found it in 2% of 1800 pigs in Yugoslavia.

FAMILY RETORTAMONADIDAE

Members of this family have 2 or 4 flagella, of which 1 is trailing, a single nucleus and a cytostome with supporting fibrils. There are 2 genera of veterinary interest, *Retortamonas* and *Chilomastix*.

Genus *RETORTAMONAS* Grassi, 1879

The body is usually piriform or fusiform, drawn out posteriorly, and plastic. There is a large cytostome near the anterior end containing in its margin a cytostomal fibril which extends across the anterior end and posteriorly along each side. An anterior flagellum and a posteriorly directed, trailing flagellum emerge from the cytostomal groove. The cysts are piriform or ovoid, have 1 or 2 nuclei, and retain the cytostomal fibril. A synonym of this genus is *Embadomonas* Mackinnon, 1911. Species occur in various insects, amphibia, reptiles and mammals. (Ansari, 1955, 1956).

Retortamonas intestinalis (Wenyon and O'Connor, 1917) Wenrich, 1932 (syns., *Embadomonas intestinalis, Waskia intestinalis*) occurs in the cecum of man and probably also in the chimpanzee, macaques and other monkeys. Dobell (1935) was unable to infect a *Macaca mulatta* and a *M. sinica* with cultures of *R. intestinalis* from man, but nevertheless believed it likely that the *Retortamonas* of man and macaques belong to the same species. It is not common in man, and is non-pathogenic.

The trophozoites of *R. intestinalis* are elongate piriform, 4 to 9 μ long and 3 to 4 μ wide. The cysts are uninucleate, piriform, 4.5 to 7 μ long and 3 to 4.5 μ wide and have a rather thick wall. This species can be cultivated in the usual culture media for intestinal protozoa.

Retortamonas ovis (Hegner and Schumaker, 1928) (syn., *Embadomonas ovis*) was described from trophozoites and cysts in cultures from sheep feces in Maryland. The trophozoites are piriform and average 5.2 by 3.4 μ.

Retortamonas cuniculi (Collier and Boeck, 1926) (syn., *Embadomonas cuniculi*) occurs in the cecum of the rabbit. The trophozoites are generally ovoid but occasionally have a tail-like process; they measure 7 to 13 by 5 to 10 μ. The cysts are oval and measure 5 to 7 by 3 to 4 μ. Collier and Boeck (1926) found this species in 1 of 50 rabbits. It is apparently non-pathogenic.

Genus *CHILOMASTIX* Alexeieff, 1912

The body is piriform and plastic, with a large cytostomal groove near the anterior end containing in its margin a cytoplasmic fibril which extends across the anterior end and posteriorly along each side. The nucleus is anterior. There are 3 anteriorly directed flagella and a short fourth flagellum which undulates within the cytostomal cleft. Cysts are formed. Synonyms of this genus are *Macrostoma* Alexeieff, 1909 and *Fanapepea* Prowazek. *Chilomastix* is found in mammals, birds, reptiles, amphibia, fish, insects and leeches. All species are apparently non-pathogenic.

Chilomastix mesnili (Wenyon, 1910) Alexeieff, 1912 (syns., *Macrostoma mesnili, Chilomastix suis, Chilomastix hominis*) is found in the cecum and colon of man, the orang-utan, chimpanzee, a number of monkeys (*Macaca, Cercopithecus, Cebus, Pithecus*) and the pig. It is quite common in man, having been found in 1 to 28% in various surveys; according to Belding (1952), it was found in 3.4% of 35,577 persons in recent surveys in the United States, and in 6.1% of 19,006 persons elsewhere in the world. Frye and Meleney (1932) found it in 3 of 127 pigs in Tennessee. Kessel (1928) found it in pigs in California, and Reichenow (1952) in Hamburg, Germany. Simitch *et al.* (1959)

found it in 1.7% of 1800 pigs in Yugoslavia.

Kessel (1924) transmitted *C. mesnili* from man to monkeys, and Deschiens (1926) from the chimpanzee to *Macaca sinica*. However, Simitch *et al.* failed to transmit *C. mesnili* from man to 2 young pigs and consequently named the pig form *C. suis*.

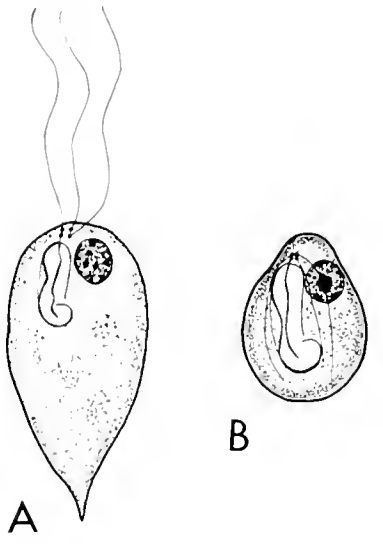

Fig. 15. *Chilomastix*. A. Trophozoite. B. Cyst. X 2800 (Original)

The trophozoites of *C. mesnili* are asymmetrically piriform, with a spiral groove running thru the middle half of the body. The posterior end is drawn out when the protozoa are moving. The trophozoites are 6 to 24 μ long and 3 to 10 μ wide. The cytostomal cleft is about 6 to 8 μ long and 2 μ wide. A complex of 6 minute blepharoplasts lies anterior to the nucleus; from them come the 3 free anterior flagella (of which 2 are short and the third is relatively long), the cytostomal flagellum, and the 2 cytostomal fibrils. The cysts are lemon-shaped, 6.5 to 10 μ long, and contain a single nucleus and the organelles of the trophozoite.

C. mesnili is ordinarily considered non-pathogenic. However, Mueller (1959) suggested that it might possibly be a mild pathogen occasionally. He referred to an outbreak of watery diarrhea in very young children in Czechoslovakia and to his own experience with watery diarrhea accompanied by swarms of *Chilomastix* following a visit to Mexico. This species can be cultivated in the usual media used for intestinal protozoa.

C. cuniculi da Fonseca, 1915 occurs in the cecum of the domestic rabbit. It is morphologically similar to *C. mesnili*. The trophozoite is ordinarily 10 to 15 μ long, but may range from 3 to 20 μ.

C. caprae da Fonseca, 1915 was reported from the rumen of the goat in Brazil. Das Gupta (1935) found it in India. It is morphologically very similar to *C. mesnili* and is 8 to 10 μ long and 4 to 6 μ wide.

C. gallinarum Martin and Robertson, 1911 occurs in the ceca of the chicken and turkey. McDowell (1953) found it in 40% of a large number of chickens in Pennsylvania. The body is pear- or carrot-shaped, 11 to 20 by 5 to 12 μ. The nucleus is pressed against the anterior end of the body. The cytostomal pouch is 8-shaped, spirals toward the left on the ventral side, and extends 1/2 to 2/3 of the body length. Cysts are rare in cecal material but common in culture. They are lemon-shaped, measure 7 to 9 by 4 to 6 μ, and have a single nucleus. McDowell (1953) cultivated *C. gallinarum* easily in Ringer's solution with 0.2% gastric mucin at 39 to 40 C.

C. intestinalis Kuezynski, 1914 and *C. wenrichi* Nie, 1948 occur in the cecum of the gunea pig, and *C. bettencourti* da Fonseca, 1915 in that of the laboratory rat, domestic mouse and golden hamster.

FAMILY CALLIMASTIGIDAE

Members of this family have a single nucleus and a compact antero-lateral group of flagella which beat as a unit. There are 2 genera, *Callimastix* and *Selenomonas*.

Genus *CALLIMASTIX* Weissenberg, 1912

The body is ovoid, with a compact central or anterior nucleus. There are

12 to 15 long flagella near the anterior end which beat in unison. One species occurs in the body cavity of copepods and the others in ruminants and equids. They are non-pathogenic.

Callimastix frontalis Braune, 1913 occurs in the rumen of cattle, sheep and goats thruout the world. Becker and Talbot (1927) reported it in Iowa. The body is spherical or ovoid, about 12 to 14 μ in diameter. The nucleus has a large central endosome. The 12 flagella are about 30 μ long; they arise from a row of basal granules on the anterior margin of the body and join to form a single unit distally. This species has been found in material submitted for diagnosis of *Tritrichomonas foetus* infections (Morgan and Hawkins, 1952).

Callimastix equi Hsiung, 1929 occurs in the cecum and colon of the horse. The body is kidney-shaped with the hilus at its anterior third; it is 12 to 18 μ long and 7 to 10 μ wide with a mean of 14 by 8 μ. Just behind the hilus is a clear, granule-free area on the margin of which are 12 to 15 basal granules which give rise to flagella 25 to 30 μ long; these unite distally and function as a unit. The rest of the cytoplasm is filled with deeply staining granules. The nucleus is 3 μ in diameter has a large endosome and lies near the center of the body.

Genus *SELENOMONAS* Von Prowazek, 1913

The body is kidney- to crescent-shaped, with blunt ends. One or more flagella are attached to the middle of the concave side. The flagella are thicker at the base than at the free end and are usually 1 to 1.5 times as long as the body. The nucleus is highly refractile and lies on the concave side near the base of the flagella. Reproduction is by transverse binary fission thru the flagellar region. This genus has been placed by many authors in the Spirillaceae among the bacteria, but Jeynes (1955, 1956) showed that it is actually a protozoon. It is not pathogenic.

Selenomonas ruminantium (Certes, 1889) Wenyon, 1926 (syns., *Ancyromonas ruminantium*, *Selenomastix ruminantium*) occurs in the rumen of cattle, sheep, goats and various wild ruminants including the gazelle, giraffe, antelope (*Cephalophus maxwelli*) in Africa and the pronghorn antelope (*Antilocapra americana*), deer (*Odocoileus hemionus*) and elk (*Cervus nannodes*) in the United States (California). It was also found in the blood of the African antelope by Kerandel (1909), of the pronghorn antelope by Chattin, Herman and Kirby (1944) and of the deer (*O. hemionus*) by Herman and Sayama (1951) in California. According to Lessel (1957), *S. ruminantium* is the predominant organism found on microscopic examination of the rumen juices.

The body of *S. ruminantium* is crescent-shaped, 9.5 to 11 by 2 to 3 μ, with a tuft of flagella arising from the center of the concave side. The nucleus is in the center of the concave side. There are no cysts. This species has not been cultivated.

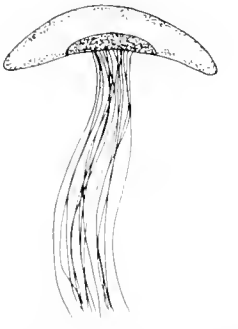

Fig. 16. *Selenomonas ruminantium*. X 2800. (Original)

Selenomonas palpitans Simons, 1922 occurs in the cecum and upper part of the colon of the guinea pig.

S. sputigena (Flugge, 1886) Dobell, 1932 occurs in the mouth of man. It grows well in thioglycollate broth.

FAMILY POLYMASTIGIDAE

Members of this family have 4 anterior flagella, an axostyle and a single

nucleus. They apparently lack a parabasal body. The only genus found in domestic animals is *Monocercomonoides*.

Genus *MONOCERCOMONOIDES* Travis, 1932

Members of this genus have 4 anterior flagella in 2 pairs, a pelta and an axostyle which is generally filamentous. Nie (1950) described 1 to 4 strand-like funises which stain with protargol in 4 species of this genus from the guinea pig. The funis is a costa-like structure extending backwards just beneath the body surface. Members of this genus occur in insects, amphibia, reptiles and a number of mammals. They are non-pathogenic.

Monocercomonoides caprae (Das Gupta, 1935) (syn., *Monocercomonas caprae*) was described from the rumen of the goat in India. The body is ovoid, 6 to 12 μ long and 4 to 8 μ wide.

Monocercomonoides caviae (Cunha and Muniz, 1921) Nie, 1950, *M. quadrifunilis* Nie, 1950, *M. wenrichi* Nie, 1950 and *M. exilis* Nie, 1950 occur in the cecum of the guinea pig.

Monocercomonoides sp. was found by Saxe (1954) in the laboratory rat and golden hamster. He transmitted it from the hamster to the rat. This species awaits morphologic study and specific characterization.

FAMILY COCHLOSOMATIDAE

In this family there are 6 anterior flagella, an axostyle, an anteroventral sucker, and a single nucleus. There may or may not be a parabasal body. The only genus so far reported from domestic animals is *Cochlosoma*, but *Cyathostoma* Tyzzer, 1930 and *Ptychostoma* Tyzzer, 1930 have been described from the ruffed grouse (*Bonasa umbellus*) in North America.

Genus *COCHLOSOMA* Kotlan, 1923

The body is ovoid, broadly rounded anteriorly and narrowly rounded posteriorly. Six flagella of unequal length arise from a blepharoplastic complex at the anterior end; 2 of them are trailing and lie in a longitudinal groove. The nucleus is near the middle of the body. A slender, fibrillar axostyle and a more lateral costa arise from the blepharoplastic complex. On the anteroventral surface is a large sucker which opens on the left side and has a marginal filament. A parabasal body is present.

Cochlosoma anatis Kotlan, 1923 (syn., *Cochlosoma rostratum*) occurs in the cloaca, large intestine and sometimes the ceca of the domestic duck, Muscovy duck and also in the wild mallard and various other wild ducks. It has been reported in Hungary by Kotlan (1923), in California by Kimura (1934), in Iowa by Travis (1938), and is probably worldwide in distribution. Kimura (1934) found it in 23 of 30 White Pekin and Muscovy ducks in central California.

The body of *C. anatis* is beet-shaped, 6 to 12 μ long and 4 to 7 μ wide. The sucker covers 1/3 to 1/2 the body length. The organism swims forward with an erratic, jerky motion, rotating on its long axis but with very little of the dipping motion of *Giardia*. The parabasal body is sausage-shaped. Reproduction is by longitudinal fission. *C. anatis* has not been cultivated.

The pathogenicity of *C. anatis* in waterfowl is unknown. Kimura (1934) found it in both healthy and sick birds, but the condition of the latter was due to bacterial or nutritional disturbances, and even in heavy *Cochlosoma* infections there was no intestinal inflammation. Travis (1938) found no lesions in the infected domestic and wild ducks which he examined.

McNeil and Hinshaw (1942) reported finding a *Cochlosoma* morphologically indistinguishable from *C. anatis* in turkeys

in California. In young poults it was present thruout the intestinal tract, and in adults in the region of the cecal tonsil. Campbell (1945) found *Cochlosoma* in large numbers in the intestinal tracts of a flock of young turkeys in Scotland affected with a disease clinically and pathologically indistinguishable from infectious catarrhal enteritis due to *Hexamita meleagridis*. Both McNeil and Hinshaw and Campbell considered the turkey form to be the same species found in ducks, but experimental and further morphological studies are needed to be sure of this.

It has not been established whether this form is pathogenic for turkeys. Campbell believed that it was the cause of the enteritis which he saw, but *Hexamita* was also usually detectable in his affected birds. In the turkey poults studied by McNeil and Hinshaw, *Cochlosoma* was always found in association with *Hexamita* or with *Hexamita* and *Salmonella*.

FAMILY HEXAMITIDAE

Members of this family are bilaterally symmetrical, with 2 nuclei, 6 or 8 flagella and sometimes with axostyles and parabasal bodies. Three genera are of veterinary interest: *Hexamita*, *Giardia* and *Trepomonas*.

Genus *HEXAMITA* Dujardin, 1838

The body is piriform, with 2 nuclei near the anterior end, 6 anterior and 2 posterior flagella and 2 independent axostyles (which may possibly be hollow tubes rather than rods). The body is quite symmetrical, three anterior flagella and 1 posterior one arising on each side. Free-living forms have 1 or 2 contractile vacuoles. The cytostome is obscure if present. Some species form cysts. Some members of this genus are free-living, while others are parasitic in insects, other invertebrates and all classes of vertebrates. The taxonomic relations of the various species are greatly confused, and much work is needed before they will be understood. Reasons why some workers use the spelling *Hexamitus* are given by Kirby and Honigberg (1949).

HEXAMITA MELEAGRIDIS McNEIL, HINSHAW AND KOFOID, 1941

Disease: Hexamitosis, infectious catarrhal enteritis.

Hosts: Turkey, peafowl, California valley quail, Gambel's quail, chukar partridge, ring-neck pheasant, golden pheasant. See Levine, Beamer and McNeil (1952) for references. *H. meleagridis* has been transmitted from the turkey to the chicken, quail and domestic duck, and from the ring-neck pheasant, quail and chukar partridge to the turkey.

Location: Duodenum and small intestine of younger birds; some occur in the cecum and bursa of Fabricius, especially in adults.

Geographic Distribution: United States, Canada, Great Britain, South America (Uruguay). The distribution of hexamitosis in California has been discussed by Hinshaw, McNeil and Kofoid (1938). It has been reported from Connecticut by Jungherr and Gifford (1944), from Indiana by Doyle, Cable and Moses (1947), from Virginia by Farr, Wehr and Jaquette (1948), from Alberta by Vance and Bigland (1956), from Scotland by Campbell (1945) and from England by Slavin and Wilson (1953). It also occurs in Illinois.

Prevalence: The published reports of outbreaks of hexamitosis are too few to give a true picture of its importance. It occurs in all major turkey producing areas in the United States and in other countries as well. It appears to be particularly important in California. The U. S. Dept. of Agriculture (1954) estimated that it causes an annual loss of $667,000 in turkeys in the United States.

Morphology: The body is 6 to 12μ long and 2 to 5μ wide, with a mean of 9

by 3 μ. The two nuclei contain round endosomes 2/3 the diameter of the nucleus. Anterior to each nucleus is a large blepharoplast or group of blepharoplasts from which 2 anterior and 1 anterolateral flagella arise. Just behind this blepharoplast is another from which the caudal flagellum arises. The caudal flagella pass posteriorly in a granular line of cytoplasm to their points of emergence near the posterior end of the body. *Hexamita* moves rapidly without the spiralling characteristic of trichomonads.

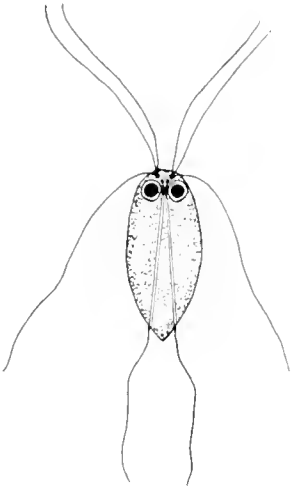

Fig. 17. *Hexamita meleagridis*. X 2800. (Original)

Life Cycle: Multiplication is by longitudinal binary fission. Slavin and Wilson (1953) and Wilson and Slavin (1955) described what they believed to be schizogony and cyst formation, but Hoare (1955) considered their idea to be purely speculative and inacceptable.

Pathogenesis: Hexamitosis is a disease of young birds; adults are symptomless carriers. The mortality in a flock may be as high as 70 to 80%, but heavy losses seldom occur in poults over ten weeks old. Affected poults appear nervous at first, have a stilted gait, ruffled, unkempt feathers, and a foamy, watery diarrhea. They usually continue to eat, but chirp continually. They lose weight rapidly, become listless, weak and finally die. Birds often do not appear to be ill until shortly before death, but examination will reveal that they are thin and have lowered temperatures. Birds which recover grow poorly, and an outbreak may leave many stunted birds in its wake.

The incubation period is 4 to 7 days. Poults may die within a day after signs appear. In acute outbreaks, the mortality reaches a peak in the flock in 7 to 10 days after the first birds die; in other flocks, deaths may continue for 3 weeks.

The principal pathological changes are found in the small intestine. Catarrhal inflammation with marked lack of tone is present in the duodenum, jejunum and ileum. The intestinal contents are usually thin, watery and foamy, with localized bulbous swellings filled with watery fluid. The small intestine, especially the anterior part, is inflamed and edematous. The cecal contents are usually fluid, and the cecal tonsils are congested.

Epidemiology: *Hexamita* is transmitted thru contaminated feed and water. Carrier adult birds which have survived earlier attacks are the most important source of infection for turkey poults. Sometimes the disease does not appear in the earlier hatches but strikes the later ones after the adults have been sold. This may come about because the infections in the earlier hatches were very light or perhaps because the virulence of the strains was too low to cause noticeable disease. According to Hinshaw (1959) it may take several passages in poults of a strain from carrier turkeys before an acute outbreak occurs.

Wild quail, pheasants and chukar partridges sharing the range with turkeys may also be a source of infection.

Hot weather and overcrowding may also contribute to the severity of an outbreak. In addition, the role of flies deserves study. Turkey poults are excellent fly-catchers, and these insects might carry the protozoa from one pen to another.

Diagnosis: Hexamitosis can be diagnosed by finding the protozoa in scrapings from the small intestine, and particularly

from the jejunum and duodenum. The smears should be mixed with physiological salt solution and examined while fresh. *Hexamita* can be readily differentiated from *Trichomonas*, *Giardia* or *Cochlosoma* by its small size, absence of a sucker or undulating membrane, and characteristic motion. Impression smears can also be made of cross sections of fresh small intestine, dried rapidly and stained with Giemsa's stain; the protozoa are often found in groups in the crypts. *Hexamita* can also be found in the bursa of Fabricius and cecal tonsils in carrier birds.

Cultivation: *Hexamita meleagridis* has not been cultivated in artificial culture media, but Hughes and Zander (1954) cultured it axenically in the chorioallantoic fluid of chick embryos.

Treatment: No treatment has apparently been uniformly successful for hexamitosis. McNeil (1948) recommended replacing the drinking water for several days with a mixture of 3% dried whey in 1-2000 aqueous copper sulfate solution. It must be begun early in an outbreak to be effective, and Wilson and Slavin (1955) did not find it to be of value in their studies.

Almquist and Johnson (1951) found in preliminary tests that streptomycin was ineffective, but that penicillin, chlortetracycline and oxytetracycline were of some value. Enheptin also gave good results when fed as 0.1% of the mash for 14 days. Wilson and Slavin (1955) said that they tested every antiprotozoal drug available commercially in England (including antimalarials, trypanocides, amoebicides and antiluetics) without success. Enheptin was only about 50% effective in experimentally infected poults. The most promising drug was di-n-butyl tin dilaurate, which appeared to control mild field outbreaks and to lower the death rate in more severe ones.

Mangrum *et al.* (1955) reported that furazolidone reduced mortality in experimentally infected turkey poults. McNeil (1958) mentioned that nithiazide had been used successfully by Merck, Sharp & Dohme Research Laboratories in a combined outbreak of histomonosis and hexamitosis.

Prevention and Control: Hexamitosis can be prevented by proper management and sanitation. Poults should be separated from adults, and separate caretakers should be used for the two groups. If feasible, breeding birds should be sold 2 weeks before any poults are hatched. Separate equipment should be used for different groups of birds. Attendants should keep out of the pens, and the feeders and waterers should be placed where they can be reached from the outside. Feeders and waterers should be placed on wire platforms. Young birds should be kept on wire. Ranges frequented by pheasants, quail and chukar partridges should be avoided. General sanitation and fly control should be practiced.

OTHER SPECIES OF *HEXAMITA*

Hexamita columbae (Nöller and Buttgereit, 1923) (syn., *Octomitus columbae*) occurs in the duodenum, jejunum, ileum and large intestine of the pigeon. It is 5 to 9 μ long and 2.5 to 7 μ wide. It is pathogenic, causing a catarrhal enteritis. Nöller and Buttgereit (1923) found it in great masses from the gizzard to the anus in a pigeon with catarrhal enteritis in Germany, and McNeil and Hinshaw (1941) found it in affected pigeons in California. They were unable to infect turkeys with this species.

Hexamita was found by Kotlan (1923) in the intestinal mucus of the domestic duck in Hungary. He called it "*Hexamitus intestinalis* (?)". It was usually piriform, 10 to 13 μ long and 4 to 5 μ wide. Kimura (1934) found *Hexamita* in the ceca and large intestine of domestic ducks in California. McNeil and Hinshaw (1941) infected domestic ducks experimentally with *H. meleagridis* from the turkey. It is uncertain whether the duck form is a separate species. It has not been adequately described, and its pathogenesis is unknown.

Hexamita muris (Grassi, 1881) (syns., *Octomitus muris*, *Syndyomita muris*) occurs in the posterior small intestine and cecum

of the Norway rat, house mouse, golden hamster and various wild rodents. It measures 7 to 9 by 2 to 3 μ.

Wenrich (1933) described *Hexamita* sp. from the feces of a rhesus monkey. It measured 4 to 6 by 2 to 4 μ.

Genus *OCTOMITUS* Von Prowazek, 1904

The body is piriform, with 2 nuclei near the anterior end, and 6 anterior and 2 posterior flagella. The body is quite symmetrical, 3 anterior flagella and 1 posterior one arising on each side. There are 2 axostyles which originate at the anterior end and fuse as they pass posteriorly, emerging as a single central rod from the middle of the posterior end. This genus differs from *Hexamita*, of which it was formerly considered a synonym, in the structure of its axostyles (Gabel, 1954).

Octomitus pulcher (Becker, 1926) Gabel, 1954 (syn., *O. intestinalis*) occurs in the cecum of the Norway rat, house mouse, golden hamster, ground squirrels and other wild rodents. It measures 6 to 10 by 3 to 7 μ.

Genus *GIARDIA* Kunstler, 1882

The body is piriform to ellipsoidal, and bilaterally symmetrical. The anterior end is broadly rounded, and the posterior end is drawn out. There is a large sucking disc on the ventral side; the dorsal side is convex. There are 2 anterior nuclei, 2 slender axostyles, 8 flagella in 4 pairs, and a pair of darkly staining median bodies. The cysts have 2 or 4 nuclei and a number of fibrillar remnants of the trophozoite organelles. A synonym of this generic name is *Lamblia* Blanchard, 1888.

The names given the species of *Giardia* depend on the authorities concerned. Traditionally, it has been believed that *Giardia* is highly host-specific, and different names have been given to almost all the forms in different hosts. Thus, if we accept the names in Ansari's (1951, 1952) review, the species in cattle is *G. bovis*, that in goats and sheep *G. caprae*, that in the dog *G. canis*, that in the cat *G. cati*, that in the rabbit *G. duodenalis*, that in the guinea pig *G. caviae*, those in the Norway rat *G. muris* and *G. simoni*, and those in the house mouse *G. muris* and *G. microti*. However, Filice (1952) was unable to find any morphological difference between the giardias of the laboratory rat and a number of wild rodents, and on reviewing the literature discovered that almost no acceptable cross-transmission studies exist between some species. Altho he did not discuss them all, he appears to have concluded that there are only two species of *Giardia* in mammals, each with a number of races. *G. muris* occurs in the mouse, rat and hamster, and *G. duodenalis* in the rabbit, rat, chinchilla, ground squirrel, deermouse, pocket mouse, man and presumably ox, dog, cat and guinea pig, among others. The essential difference between these two forms is that the median bodies of *G. muris* are small and rounded while those of *G. duodenalis* are long, resemble somewhat the claw of a claw-hammer, and lie approximately transversely across the body.

In this connection, Hegner's (1930), Armaghan's (1937) and Haiba's (1956) success in infecting laboratory rats with *Giardia* from man suggests that Filice's view may eventually prevail. However, careful cross-transmission studies must be carried out before a decision can be made. In the meantime, it is more convenient to use different specific names for most of the forms from different hosts.

Associated with this nomenclatorial problem is an important epidemiological one. If it turns out that *Giardia* can be freely transmitted from one host to another, we shall have to revise our ideas about the danger to man of infections in laboratory and domestic animals, and of infections in one domestic animal to others. Here is an area of ignorance which deserves exploration.

Giardia has not yet been cultivated in artificial media, a fact which has hampered

studies both of its epidemiology and pathogenicity. However, Karapetyan (1960) cultivated *G. lamblia* in chicken fibroblast tissue cultures.

GIARDIA LAMBLIA STILES, 1915

Synonyms: *Cercomonas intestinalis*, *Lamblia intestinalis*, *Giardia intestinalis*, *Megastoma entericum*, *Giardia enterica*. European writers still call this species *Giardia intestinalis*, but there was so much confusion about the availability of the specific names *intestinalis* and *enterica* that Stiles (1915) established the present name.

Disease: Giardiosis.

Hosts: Man, Old and New World monkeys, pig. Hegner (1930) and Armaghan (1937) infected laboratory rats and Bonestell (1935) infected woodrats (*Neotoma fuscipes*) with *G. lamblia* from man. Haiba (1956) infected wild and laboratory *Rattus norvegicus*, but failed to infect wild *R. rattus* and laboratory mice with *G. lamblia* from man.

Location: Duodenum, jejunum, upper small intestine.

Geographic Distribution: Worldwide.

Prevalence: *G. lamblia* is common in man. In 86 surveys of 134,966 people thruout the world summarized by Belding (1952), its prevalence ranged from 2.4 to 67.5% with a mean of 10.4%. It was found in 7.4% of 35,299 persons in 24 surveys in the United States, and in 6.9% of 65,295 persons in 20 surveys in the rest of the world. It is about 3 times as common in children as in adults.

G. lamblia was reported from a pig in Tennessee by Frye and Meleney (1932). Its prevalence in swine is unknown.

Morphology: The trophozoites are 9 to 21 μ long, 5 to 15 μ wide and 2 to 4 μ thick; they are usually 12 to 15 μ long. The median bodies are curved bars of the *duodenalis* type. The cysts are ovoid, 8 to 12 μ long and 7 to 10 μ wide, and contain 4 nuclei.

Pathogenesis: There was considerable controversy for many years whether *Giardia* is pathogenic in man, but it is now generally agreed that it may be in some individuals. Most infections are symptomless, but in a fairly small number there is a chronic diarrhea. The feces contain a large amount of mucus and fat but no blood. The diarrhea is accompanied by dull epigastric pain and flatulence. Affected persons have a poor appetite and lose weight. In some cases the gall bladder may be invaded and cholecystitis may be present, but there is no proof that the protozoa caused this condition. Pizzi (1957) reviewed some of the literature on the pathogenicity of *G. lamblia* and concluded that in heavy infections it may also interfere with fat absorption and produce a deficiency in fat-soluble vitamins. It is more often pathogenic in children than adults.

The pathogenicity of *G. lamblia* for swine is unknown.

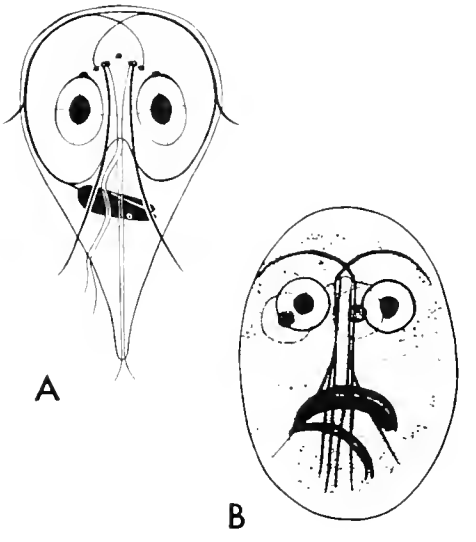

Fig. 18. A. *Giardia* trophozoite. X 2800. (After Filice, 1952). B. *Giardia bovis* cyst. X 2900. (From Becker and Frye, 1929)

Diagnosis: *Giardia* infections can be diagnosed by recognition of trophozoites or cysts in stained fecal smears. Fixation with Schaudinn's fluid and staining with iron hematoxylin are recommended. Trophozoites alone are generally found in diarrheic stools. The cysts can be concentrated by the flotation technic. Zinc sulfate solution should be used for flotation; sugar and other salt solutions distort the cysts and make them unrecognizable.

Cultivation: Neither *G. lamblia* nor any other species of *Giardia* has been cultivated in artificial media. Karapetyan (1960) cultured it in chicken fibroblast tissue cultures along with the yeast, *Candida guilliermondi*. The protozoon did not develop without the yeast, which led him to believe that there may be a synergistic relation between the 2 organisms.

Treatment: *Giardia* infections in man can be successfully treated with either quinacrine or chloroquine. Three oral doses of 0.1 g each are given daily for 5 days. Amodiaquin is considered even better than these (Lamadrid-Montemayor, 1954); a single dose of 0.6 g is given to adults.

Prevention and Control: These depend on sanitation. Cerva (1955) found that 2 to 5% phenol or lysol would kill *G. lamblia* cysts, but that chloramine, mercuric chloride, formalin and a number of other disinfectants were ineffective in the concentrations commonly used. The cysts were killed by temperatures above 50° C and, after 10 hours, by freezing below -20° C. They remained viable in water for over 3 months.

GIARDIA CANIS HEGNER, 1922

Host: Dog.

Location: Duodenum, jejunum, upper small intestine. Tsuchiya (1932) found the optimum location to be 10 to 30 inches posterior to the stomach in puppies on a carbohydrate diet and 25 to 40 inches from the stomach in puppies on a high protein diet.

Geographic Distribution: North America (United States, Canada), South America (Uruguay).

Prevalence: Catcott (1946) found *G. canis* in 17.7% of 113 dogs in Ohio. Choquette and Gelinas (1950) found it in 9.0% of 155 dogs in Montreal, Canada. Craige (1948) found it in 8.8% of 160 dogs in California. We have seen it a number of times in dogs in Illinois, but have not attempted a survey.

Morphology: The trophozoite is 12 to 17 μ long and 7 to 10 μ wide. The median bodies are curved bars of the *duodenalis* type. The cysts measure 9 to 13 by 7 to 9 μ.

Pathogenesis: The pathogenicity of *G. canis* for the dog has still to be incontrovertibly determined. Catcott (1946) noted diarrhea in one-third of his positive dogs. Craige (1948) found *Giardia* in 17 of 71 dogs with dysentery, but in 13 of them other organisms which he considered pathogenic were also present. Choquette (1950) found *Giardia* in several cases of dysentery, but some of these were complicated by other conditions. Tsuchiya (1931) reported that diarrheic stools alternated with formed stools in a number of experimentally infected puppies, but was uncertain whether it was due to an existing pathological condition or to the flagellates. According to Tsuchiya (1932), a carbohydrate diet is more favorable for *G. canis* than a high protein diet.

Diagnosis: Same as for *G. lamblia*.

Treatment: Quinacrine has been found effective against *G. canis*. Craige (1949) gave dogs 50 to 100 mg twice daily for 2 or 3 days, repeating if necessary after 3 to 4 days. Choquette (1950) gave large dogs 0.2 g three times the first day and twice a day for 6 more days; he gave small dogs 0.1 g twice the first day and once a day for 6 more days. Chloroquine has also been found effective in man; 0.1 g is given 3 times a day for 5 days.

Prevention and Control: The standard sanitary measures should be used in preventing the transmission of *Giardia*.

GIARDIA CATI
DESCHIENS, 1925

Synonyms: *Giardia felis*.

Host: Cat.

Location: Small intestine, large intestine. Hitchcock and Malewitz (1956) noted *G. cati* trophozoites thruout the small intestine, cecum and colon (except at the pyloric valve) of a naturally infected 64-day-old kitten in Michigan. They were most numerous in the lower part of the small intestine.

Geographic Distribution: North America (United States), Europe (France).

Prevalence: Hitchcock (1953) found *Giardia* in 8 of 14 kittens in Michigan.

Morphology: It is quite likely that this species is a synonym of *G. canis*. The trophozoites are 10 to 18 μ long and 5 to 9 μ wide with a mean of 13 by 7 μ. The median bodies are bars of the *duodenalis* type. The cysts are 10.5 μ long and 7 μ wide.

Pathogenesis: Unknown. The infected cats studied by Hitchcock (1953) and Hitchcock and Malewitz (1956) apparently had no signs of enteritis.

GIARDIA BOVIS
FANTHAM, 1921

Host: Ox.

Location: Duodenum, jejunum, ileum.

Geographic Distribution: North America (United States), Europe (England, Holland, Austria, Italy), South Africa.

Prevalence: Unknown. Becker and Frye (1927) found this species in the feces of cattle in Iowa, Nieschulz (1923) saw it in a calf in Holland, Graham (1935) found it alive and active in the digestive tract of 6 of 21 female *Cooperia oncophora* from a calf from New Jersey, and we have found it from time to time in casual examinations in Illinois.

Morphology: The trophozoites are 11 to 19 μ long and 7 to 10 μ wide. The median bodies are curved bars of the *duodenalis* type. The cysts are 7 to 16 μ long and 4 to 10 μ wide.

Pathogenesis: The pathogenicity of *G. bovis* is unknown. Supperer (1952) found it in a calf in Austria with a mucous diarrhea. The calf was killed for necropsy diagnosis and was found to have catarrhal duodenitis and jejunitis; the mucosa was dark red, thickened and lay in folds. Botti (1956, 1956a) found it in calves with hemorrhagic diarrhea in Italy. On the other hand, the cattle in which we saw the organism in Illinois did not appear to be affected by it.

OTHER SPECIES OF *GIARDIA*

Giardia caprae Nieschulz, 1923 (syn., *G. ovis*) was reported from the anterior part of the small intestine of 2 goats in Holland. Nieschulz (1924) described it further. Its trophozoites are 9 to 17 μ long and 6 to 9 μ wide with a mean of 13.5 by 7.5 μ. The median bodies are curved bars of the *duodenalis* type. The cysts have 4 nuclei and measure 12 to 15 by 7 to 9 μ with a mean of 14 by 8 μ.

Giardia caprae was found by Grassi (1881) in sheep in Italy and by Turner and Murnane (1932) in the small intestine of sheep in Australia. The Australian sheep had been losing weight gradually for several months. Deas (1959) found it in a lamb with enteritis in England. D. A. Willigan (unpubl.) found *Giardia* in 3 of 24 lambs brought to the University of Illinois Veterinary Diagnostic Service. All came from a single flock in which many of the lambs were suffering from diarrhea and loss of weight, but coccidiosis and salmonellosis were also found. Dissanaike (1954) found live and active *G. caprae* in the intestines of 50 female and no male *Nematodirus filicollis* from 5 sheep in England.

Giardia equi Fantham, 1921 was originally found in the large intestine of a horse in South Africa. Varela and Salsamendi (1958) found it in the feces of a

horse with colic in Venezuela. Its trophozoites measure 17 to 21 by 9 to 12 μ, and its cysts measure 12 to 16 by 8 to 9.5 μ.

Giardia duodenalis (Davaine, 1875) (syns., *Hexamita duodenalis*, *Lamblia cuniculi*) occurs in the anterior small intestine of Old and New World rabbits and also in *Cocnda villosus* in Brazil. It occurs sporadically and is apparently not pathogenic. Its trophozoites measure 13 to 19 by 8 to 11 μ with a mean of 16 by 9 μ. The median bodies are curved bars resembling the claws of a claw-hammer; th they lie transversely across the body. The cysts contain 2 to 4 nuclei.

Giardia simoni Lavier, 1924 occurs in the anterior small intestine of the Norway rat, golden hamster and probably various wild rodents. Its trophozoites measure 11 to 19 by 5 to 11 μ. Its median bodies are curved bars of the *duodenalis* type.

G. muris (Grassi, 1879) occurs in the anterior small intestine of the house mouse, Norway rat, black rat, golden hamster and various wild rodents. It is common in laboratory rats and mice. Its trophozoites measure 7 to 13 by 5 to 10 μ. Its median bodies are small and rounded.

G. caviae Hegner, 1923 occurs in the anterior small intestine of the guinea pig. Its trophozoites measure 8 to 15 by 6.5 to 10 μ. Its median bodies are curved bars of the *duodenalis* type.

Giardia chinchillae Filice, 1952 emend. (syn., *Giardia duodenalis* race *chinchillae* Morgan, 1949 of Filice, 1952; altho he gave the first description of this form, Morgan did not give it a specific name; the name *chinchillae* was introduced by Filice) occurs frequently in the chinchilla. It is found thruout the small intestine, but more commonly in the duodenum and anterior jejunum. Its trophozoites measure 11 to 20 by 6 to 12 μ. Its median bodies are curved bars of the *duodenalis* type. This species has been accused by various workers of causing diarrhea and even death (Shelton, 1954; Gorham and Farrell, 1955). Treatment with 6 to 9 mg quinacrine for 5 to 7 days was found by Hagan (1950) to eliminate the infection. Attempts to transmit *G. chinchillae* to the golden hamster, white mouse, domestic rabbit or guinea pig have been unsuccessful (Morgan, 1949; Shelton, 1954).

Genus *TREPOMONAS* Dujardin, 1841

These are free-swimming protozoa with a more or less rounded, bilaterally symmetrical body and with a cytostomal groove on each side of the posterior half. There are 8 flagella, of which 1 long and 3 short ones are present on each side. A horseshoe-shaped structure near the anterior margin contains the 2 nuclei. Members of this genus are free-living in fresh water, coprophilic or parasitic in amphibia, fish and turtles.

Trepomonas agilis Dujardin, 1841 occurs in stagnant water and the intestine of amphibia and is also coprophilic. It is 7 to 25 μ long and 2 to 15 μ wide, with a flattened body and with the posterior end wider than the rounded anterior end. The flagella come off near the middle of the body at the anterior end of the cytostome.

ORDER PROTOMASTIGORIDA

Members of this order have 1 or 2 flagella.

FAMILY BODONIDAE

Members of this family have 2 flagella which originate anteriorly; one is directed anteriorly and the other posteriorly. The anterior end is more or less drawn out. There are 1 to several contractile vacuoles. There are several genera of free-living and parasitic forms in this family.

Genus *BODO* Stein, 1875

These are small, more or less ovoid, plastic forms with an anterior cytostome and a central or anterior nucleus. Cysts are formed.

Bodo caudatus (Dujardin, 1841) Stein, 1878 is a common coprophilic form and also occurs in stale urine and stagnant water. It is 8 to 18 μ long and 2.5 to 6 μ wide, with a polymorphic body ranging in shape from spherical to elongate ovoid. It has a tiny contractile vacuole, a single vesicular nucleus with a large endosome and a rounded parabasal body. This species and also *B. foetus* and *B. glissans* have been found in material from bulls submitted for *Tritrichomonas foetus* diagnosis.

Genus *CERCOMONAS* Dujardin, 1841

These are small forms with a plastic body, one flagellum directed anteriorly and the other running backward over the body to become a trailing flagellum. The nucleus is piriform and is connected with the basal granule of the flagella. The cysts are spherical and uninucleate. A number of freshwater and coprophilic species have been described, but it is not clear whether all the species are valid.

Cercomonas longicauda Dujardin, 1841 is a common coprophilic flagellate. Its trophozoites are amoeboid, 2 to 15 μ long, have 2 contractile vacuoles, and ingest food by means of pseudopods. Its cysts are 4 to 7 μ in diameter.

Cercomonas heimi (Hollande) is similar to *C. longicauda* but is piriform and has longer flagella.

Cercomonas equi (Sabrazes and Muratet, 1908) (syn., *C. asini*) was described from the large intestine of the horse and donkey and also occurs in their feces.

Cercomonas faecicola (Woodcock and Lapage, 1915) (syn., *Helkesimastix faecicola*) was found in the feces of the goat. It is ovoid, with a rigid, pointed anterior end. The anterior flagellum is very short and easily overlooked. The trophozoites are 4 to 6 μ long and the cysts 3 to 3.5 μ in diameter.

Cercomonas crassicauda Dujardin, 1841 occurs in fresh water and is also coprophilic. It has been found in material from bulls submitted for examination for *Tritrichomonas foetus* (Morgan and Hawkins, 1952). Its trophozoites measure 10 to 16 by 7 to 10 μ.

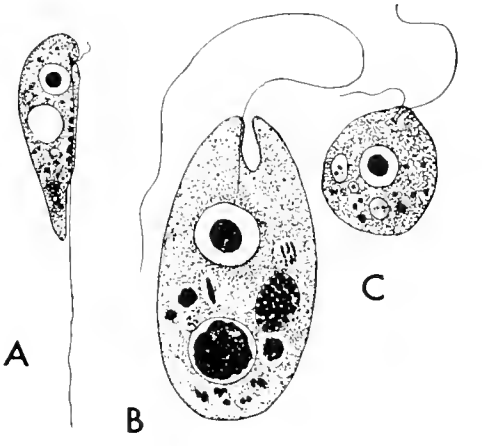

Fig. 19. Coprophilic flagellates. A. *Cercomonas* sp. X 4200. B. *Copromonas subtilis*. X 5100. C. *Monas* sp. X 4200. (From Noble, 1956)

Noble (1956) found *Cercomonas* sp. in fresh bovine and porcine feces, and cultivated them in feces in the refrigerator at 4° C for 5 months. Noble (1958) found that *Cercomonas* sp. appeared in fecal specimens from Wyoming elk, bison, cattle, horses and sheep after storage at 4°C for 6 to 7 days. They persisted for several weeks and then died out. They failed to survive in soil alone or in soil mixed with boiled feces, nor could they be found in soil samples taken from areas where elk, sheep or horses were present. Noble concluded that this and other coprophilic protozoa may require certain essential metabolites produced by bacteria.

The *Cercomonas* sp. trophozoites observed by Noble (1958) were 5.4 by 2.5 μ, somewhat tadpole-shaped, with a broad anterior end tapering to a highly flexible tail-like posterior end. An extremely short anterior flagellum, visible only with phase contrast, extended from a minute subterminal cytostome. Another flagellum arose from the anterior region, passed thru the cytoplasm ventral to the nucleus, emerged about 2/3 of the body length from

the anterior end, and continued as a long trailing whip. Eight to 10 large, dark cytoplasmic granules were arranged along this flagellum. The cytoplasm contained a large contractile vacuole and many food vacuoles. The nucleus was vesicular.

Genus *PLEUROMONAS* Perty, 1852

The body is somewhat amoeboid. The 2 flagella often appear to emerge separately from the body. The anterior flagellum is very short and often rolled up into a ring. The posterior flagellum is very thick and more than 2 to 3 times the length of the body. There is a single vesicular nucleus. The cyst is spherical, and 4 to 8 young individuals apparently emerge from it.

There is a single species in this genus, *Pleuromonas jaculans* Perty, 1852, which occurs in stagnant water. It is 6 to 10μ long and about 5μ wide. Uribe (1921) found large numbers of this protozoon in the ceca of young chickens which he had fed *Heterakis* material, and believed that it could become adapted to parasitism.

Genus *PROTEROMONAS* Kunstler, 1883

The body is spindle-shaped. An anterior and a free trailing flagellum arise from 2 blepharoplasts at the anterior end. The nucleus is anterior to the middle of the body and contains scattered chromatin granules but no endosome. A rhizoplast runs from the blepharoplast to a centrosome on the nuclear membrane. A perirhizoplastic ring surrounds the rhizoplast a short distance behind the blepharoplast; this is considered a parabasal body. A paranuclear body the same size as the nucleus lies beside the nucleus; it divides when the nucleus divides, and stains with hematoxylin but not with protargol. All the species are parasitic. They are common in the intestines of reptiles and amphibia. Synonyms of this genus are *Prowazekella* Alexeieff, 1912 and *Schizobodo* Chatton, 1917.

Proteromonas brevifilia Alexeieff, 1946 occurs in the cecum of the guinea pig.

FAMILY AMPHIMONADIDAE

Members of this family have a naked or loricate body with 2 equal flagella. There are several genera, mainly in fresh water.

Genus *SPIROMONAS* Perty, 1914

Members of this genus have an elongate, spirally twisted body with 2 anterior flagella. They form spherical cysts in which division into 4 daughter individuals takes place. They live in fresh water. A synonym is *Alphamonas* Alexeieff.

Spiromonas angusta (Dujardin) Alexeieff lives in stagnant water or is coprophilic. It has also been found in bull sheath washings. It is spindle-shaped and about 10μ long.

SUBCLASS PHYTOMASTIGASINA

Members of this subclass typically have chromatophores and holophytic nutrition. Some are colorless but closely resemble other holophytic forms and are derived from them or from a common ancestor. A few are coprophilic and still fewer are parasitic. In each group the parasitic mode of life has undoubtedly arisen anew.

ORDER CHRYSOMONADORIDA

In this order the chromatophores, if present, are yellow, brown, orange or occasionally blue. The stored reserves include leucosin (presumably a polysaccharide) and lipids, but no starch. There are five suborders in the Chrysomonadorida, but only one of them, Euchrysomonadorina, contains forms of veterinary or medical interest. In this suborder the flagellate stage is dominant, and neither a siliceous skeleton nor a peripheral zone

of coccoliths is present. This suborder contains 4 families, 2 of which contain parasitic or coprophilic species.

FAMILY CHROMULINIDAE

Members of this family have a single flagellum.

Genus *OIKOMONAS* Kent, 1880

Members of this genus lack chromatophores, lorica or test. They are solitary. The nucleus is near the center of the body. The single flagellum arises from a basal granule near the body surface. Cysts are formed, at least in the free-living species. This genus is the colorless homolog of *Chromulina*. Its parasitic species are poorly known.

Oikomonas communis Liebetanz, 1910 and *Oikomonas minima* Liebetanz, 1910 were both described from the rumen of cattle. They are said to differ in size, the former being up to 11 μ long and the latter more than 4 μ long; this is probably not a valid difference. Das Gupta (1935) found *O. communis* in the rumen of goats in India.

Oikomonas equi Hsiung, 1930 was found in the cecum of 8 horses in Iowa. It is usually spherical or ovoid and swims in a jerky manner. The nucleus has a large, central endosome and the cytoplasm is filled with small, dark-staining granules. The body is 3.5 to 7 μ long and 3 to 5.5 μ wide. The flagellum is about 20 μ long.

Genus *SPHAEROMONAS* Liebetanz, 1910

The body is spherical or ellipsoidal, with a more or less central nucleus. A single, long flagellum arises from a basal granule on the nuclear membrane. This genus is poorly known and has apparently not been studied by modern methods. It is closely related to *Oikomonas* and may even be a synonym of that genus. Several species have been named, all parasitic, but most of them are probably the same.

Sphaeromonas communis Liebetanz, 1910 (syns., *S. minima*, *S. maxima*, *S. liebetanzi*, *S. rossica*) occurs in the rumen of the ox and goat and in the cecum and feces of the guinea pig. It may also be coprophilic. Liebetanz (1910) and Braune (1914) found it in the rumen of cattle in Europe, Becker and Talbott (1927) found it in the rumen of a few cattle in Iowa (calling it, however, *Monas communis*), and Fonseca (1916) found it in cattle and goats and also in the guinea pig in Brazil. Yakimoff *et al.* (1921) found it in the guinea pig in Russia. The body is spherical or ellipsoidal, 3 to 14 μ in diameter. The cytoplasm contains many dark-staining granules.

Genus *CAVIOMONAS* Nie, 1950

The body is naked, without chromatophores and with a vesicular nucleus at the anterior end. One flagellum arises from the nuclear membrane. A band-like peristyle arises from the nuclear membrane opposite to the origin of the flagellum and extends posteriorly along the periphery of the body surface; it stains with hematoxylin and protargol. Cytostome and contractile vacuoles are absent.

Caviomonas mobilis Nie, 1950 occurs in the cecum of the guinea pig. The body is ovoid to elongate carrot-shaped and the posterior end is often pointed. It measures 2 to 7 by 2 to 3 μ with a mean of 4 by 3 μ.

FAMILY OCHROMONADIDAE

Members of this family have 1 long and 1 short flagellum.

Genus *MONAS* Müller, 1773

The body is active and plastic. Chromatophores are absent. This genus is the colorless homolog of *Ochromonas*. Reynolds (1934) recognized 13 free-living species, and in addition there is at least 1 coprophilic one.

Noble (1956) cultivated *Monas* sp. in bovine feces at 4° C in the refrigerator for 5 months. He also (1958) found that *Monas* sp. appeared in fecal samples from Wyoming elk, bison and bear after storage at 4° C for 7 to 27 days. The protozoa persisted for several weeks and then died out. They failed to survive in soil or in soil mixed with boiled feces, nor could they be found in soil samples taken from areas where elk, sheep or horses were present. Noble concluded that this and other coprophilic protozoa may require certain essential metabolites produced by bacteria. The form which Noble studied was spherical and 4 μ in diameter. He assigned it to the "*Monas communis*" reported by Becker and Talbott (1927) from the rumen of cattle, but the latter had only a single flagellum and was *Sphaeromonas communis*.

Monas obliqua Schewiakoff has been found in material from bulls submitted for *Tritrichomonas foetus* diagnosis (Morgan and Hawkins, 1952).

ORDER EUGLENORIDA

In this order the chromatophores, if present, are green. The stored reserves include lipids and paramylum. There is a reservoir or "gullet" from the posterior or postero-dorsal wall of which the flagella arise. There are 3 suborders in the Euglenorida, of which the Euglenorina includes one genus containing coprophilic forms.

FAMILY ASTASIIDAE

Members of this family have a single flagellum. They lack chromatophores or a stigma.

Genus COPROMONAS Dobell, 1908

The body is elongate ovoid, with an elongate reservoir at the anterior end into which a contractile vacuole discharges. The single flagellum arises from a blepharoplast at the base of the reservoir. The nucleus is vesicular, with a large endosome. Permanent fusion followed by encystment takes place. Nutrition is holozoic on bacteria.

Copromonas subtilis Dobell, 1908 (syn., *Copromonas ruminantium*) was first described from the feces of frogs and toads, but has since been found in the feces of man and various domestic and wild mammals. Wenyon (1926) and Noble (1956) found it in pig feces. Woodcock (1916) found it in goat feces. Noble (1958) found that it appeared in fecal samples from Wyoming elk, bison, cattle, horses, sheep and moose after storage at 4° C for 7 to 11 days. It persisted from 2.5 months in the bison samples to more than 18 to 24 months in the elk and cattle samples. It failed to survive in soil or in soil mixed with boiled feces, nor could it be found in soil samples taken from areas where elk, sheep or horses were present.

The trophozoites of *C. subtilis* are 7 to 20 μ long. They are usually ovoid, but can change from spindle-shaped to almost round. The flagellum is 1 to 2 times the length of the body. When the animal swims straight, only the tip of the flagellum moves; the flagellum is sometimes used like a highly flexible probe. The cysts are ovoid or spherical and 7 to 8 μ long. They have a thin wall and clear contents with a single vesicular nucleus.

Reichenow (1952) and Grassé (1952) considered that *Copromonas subtilis* is a synonym of *Scytomonas pusilla* Stein, 1878, which was incompletely described by Stein.

ORDER PHYTOMONADORIDA

In this order a single large green chromatophore is typical. The stored reserves are starch and sometimes lipids. No members of this order are parasites of domestic animals or man, but one species deserves mention.

Polytoma uvella Ehrenberg, 1838 occurs in infusions and stagnant water, and has been found in bull sheath washings submitted for *Tritrichomonas foetus*

diagnosis (Morgan and Hawkins, 1952). Its body is ovoid to piriform, 15 to 30 by 9 to 20 μ, without chromatophores and with numerous starch granules in the posterior part of the body. A red or pink stigma may or may not be present. There are 2 anterior flagella of equal length.

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Chapter 7

THE AMOEBAE

The amoebae belong to the class Sarcodasida. Members of this class move by means of pseudopods. They have no cilia and, except in rare instances, no flagella. The group is named for *sarcode*, a term introduced by Dujardin for what was later called protoplasm. Most sarcodasids are holozoic, ingesting their prey by means of their pseudopods. Their cytoplasm is usually divided into endoplasm, containing the food vacuoles, nucleus, etc., and relatively clear ectoplasm. The fresh water forms contain one or more contractile vacuoles; these are absent in the salt water and parasitic species. With a few exceptions, reproduction is asexual, by binary fission or rarely by multiple fission, by budding or by plasmotomy. Most species form cysts.

The Sarcodasida originated from the Mastigasida. The group did not arise from a single progenitor, but is polyphyletic. One line, for example, passes from *Tetramitus* thru *Naegleria* to *Vahlkampfia*. In *Tetramitus*, which is usually classified among the flagellates, the life cycle includes flagellate and amoeboid stages, and the flagellate stage has a permanent cytostome. In *Naegleria*, which is usually classified among the amoebae, the life cycle also includes flagellate and amoeboid stages, but there is no permanent cytostome. In *Vahlkampfia*, there is no flagellate stage, but the amoebae are very similar to those of *Naegleria*. Another line passes from the amoeboid flagellate, *Histomonas*, to the very similar but non-flagellate amoeba, *Dientamoeba*.

Only a few of the Sarcodasida are parasitic. The free-living forms include the most beautiful protozoa of all, the pelagic Radiolaria with their delicate, latticework siliceous skeletons. One group of Radiolaria has skeletons of strontium sulfate--perhaps some day protozoologists will be asked to develop ways of using them to eliminate strontium 90 pollution. Another marine group, the Foraminifera, has calcareous shells.

Their skeletons form our chalk, and they and the Radiolaria are of great geological interest. They are used, too, as indicators in oil well drilling. More species of Foraminifera have probably been named than of all the other protozoa put together; 493 new species and 53 new genera of Foraminifera were listed in the Zoological Record for 1956, and of these, 470 species and 48 genera were fossil. In contrast, 57 new species and 4 new genera of parasitic protozoa were listed. And this was not an exceptional year.

ORDER TESTACEORIDA

Members of this order have a single-chambered shell or test.

FAMILY ARCELLIDAE

The test is simple and membranous, without foreign bodies, platelets or scales. The pseudopods are filose or simply branched and do not anastomose. There are many genera and species of free-living protozoa in this family. They are found commonly in fresh water, swamps, etc. One species is coprophilic.

Genus *CHLAMYDOPHRYS* Cienkowski, 1876

The test is rigid and circular in cross section. The nucleus is vesicular, with a prominent endosome. The cytoplasm is differentiated into distinct zones, and refractile waste granules are present in it. The pseudopods are branching.

Chlamydophrys stercorea Cienkowski, 1876 has an oval, white porcelaneous, thin, smooth shell open at the pointed end. It measures about 20 by 14 μ. The pseudopods are filose. Somewhat smaller naked amoebae may also be seen. The cysts are uninucleate, 12 to 15 μ in diameter, with thick, irregular, brownish walls. Multiplication is by budding. *C. stercorea* is coprophilic and may also be found in fresh water.

ORDER AMOEBORIDA

Members of this order have lobopodia and are naked, without a test. All of the parasitic and all but one of the coprophilic Sarcodasida are found in this order.

FAMILY NAEGLERIIDAE

This family is transitional between the Mastigasida and the Sarcodasida. Both amoeboid and flagellate stages occur in its life cycle.

Genus *NAEGLERIA* Alexeieff, 1912 emend. Calkins, 1913

The flagellate stage has 2 flagella. The amoeboid stage has lobopodia and resembles *Vahlkampfia*. The nucleus is vesicular, with a large endosome. The contractile vacuole is conspicuous. The cysts are uninucleate. *Naegleria* lives on bacteria and is free-living in stagnant water or coprophilic.

Naegleria gruberi (Schardinger) (syn., *Dimastigamoeba gruberi*) is found in stagnant water and is also coprophilic. The active amoebae are 10 to 36 by 8 to 18 μ and have a single vesicular nucleus 3 to 4 μ in diameter. The nucleus has a central endosome and sparse granules of peripheral chromatin. The flagellate stage is 18 by 9 μ, ovoid, and has 2 equal anterior flagella. It can be produced from the amoeboid stage by flooding the culture with distilled water and exposing it to air. The cysts are spherical, 8 to 12 μ in diameter, translucent, with a single nucleus and several large spherical chromatoid bodies when first formed. The cyst wall is double, and the outer wall is perforated by 3 to 8 pores.

Genus *TRIMASTIGAMOEBA* Whitmore, 1911

The nucleus is vesicular, with a large endosome. The flagellate stage has 3 flagella (4 according to Bovee, 1959) and the

amoeboid stage is relatively small. The cysts are uninucleate, with a smooth wall.

Trimastigamoeba philippinensis Whitmore, 1911 was first found in human feces. Bovee (1959) rediscovered it in sewage-seepage into a spring in Florida and redescribed it. According to Whitmore (1911), the flagellate stage has 3 (occasionally 2 or 4) anterior flagella and measures 16 to 22 by 6 to 8 μ. Bovee, however, found that there are actually 4 anterior flagella which arise in pairs from 2 basal granules adjacent to the nucleus. The flagella lie in an anterior, gullet-like, cylindrical invagination and extend 20 to 25 μ beyond it. According to Bovee, the fully formed flagellate stage is 17 to 20 μ long, its larger rear end is 6.5 to 7.5 μ in diameter, the narrower anterior end is 4.5 to 5.5 μ in diameter and the anterior pocket is 7 μ deep.

The amoeboid stage was said by Whitmore (1911) to be 16 to 18 μ in diameter. Bovee (1959) said that it is 12 to 18 μ in diameter when at rest and 30 to 40 μ long and 14 to 20 μ wide during rapid locomotion. It moves quickly by means of rapidly-extruded eruptive waves at its frontal margin. It feeds principally on bacteria and has a contractile vacuole which is formed by the union of several small vacuoles in about 2 minutes. The cysts are oval to subspherical. Whitmore (1911) gave their dimensions as 13 to 14 by 8 to 12 μ.

FAMILY AMOEBIDAE

Members of this family are free-living or coprophilic. They have no flagellate phase. This family contains, besides *Amoeba* and a number of other free-living genera, several coprophilic species and one which can produce disease with a little human help.

Genus *ACANTHAMOEBA* Volkonsky, 1931

These are relatively small amoebae without well developed ectoplasm. The nucleus is vesicular, with a large endosome. During mitosis, the nuclear membrane disappears at prophase. The mitotic figure at the end of metaphase is a straight or concave spindle with sharply pointed poles. The cysts are angular and polyhedric, with 2 membranes, the outer one being highly wrinkled and mammillated.

Acanthamoeba hyalina (Dobell and O'Connor, 1921) Volkonsky, 1931 (syn., *Hartmannella hyalina*) is a common coprophilic form. It occurs in soil and fresh water and is easily cultivated from old human and animal feces. Its trophozoites are 9 to 17 μ in diameter when rounded. It has a single contractile vacuole and a single vesicular nucleus with a central endosome and peripheral chromatin. The cysts are spherical, 10 to 15 μ in diameter, with a thin, smooth inner wall and a thick, wrinkled, brownish outer wall.

Walker (1908) described an amoeba from the intestinal tract of the turkey in Massachusetts under the name *Ameba gallopavonis* which Chatton (1953) listed as *Acanthamoeba gallopavonis* Walker. It had angular cysts and may be synonymous with *A. hyalina*.

Acanthamoeba has occasionally been encountered as a contaminant of tissue cultures and, because of its pathogenicity on injection and the resistance of its cysts to virucidal agents, it is a potential hazard in vaccines prepared from viruses grown in tissue culture.

Jahnes, Fullmer, and Li (1957) and Culbertson, Smith and Minner (1958) isolated an *Acanthamoeba* sp. from tissue cultures of trypsinized monkey kidney cells. The latter first recognized the amoebae in the lesions of monkeys which had died following inoculation of tissue culture fluid thought to contain an unknown virus but later shown to contain only *Acanthamoeba*. Following intracerebral and intraspinal inoculation into cortisonized monkeys, the amoebae caused extensive choriomeningitis, destructive encephalomyelitis and death in 4 to 7 days. Following intracerebral inoculation into mice, they caused destructive encephalitis and death in 3 to 4 days. Following intranasal instillation into mice, they

produced ulcers in the nasal mucous membrane and invaded the adjacent base of the skull, involving the frontal lobes of the brain and causing death in about 4 days. Following intravenous inoculation into mice, they caused perivascular granulomatous lesions in the lungs. These were associated with severe pneumonia, extensive fibrinopurulent exudate containing polymorphonuclear leucocytes and monocytes, hemorrhage, and invasion of the pulmonary veins followed by the formation of thrombi containing the amoebae.

McCowen and Galloway (1959) also isolated *Acanthamoeba* sp. from tissue cultures of trypsinized monkey kidney cells. They studied the pathogenicity for mice of this strain and of others isolated from the same source. The average survival time of intracerebrally inoculated mice was approximately 5 days. Cysts remained virulent for mice after storage at -67°C for 15 months.

Genus *SAPPINIA* Dangeard, 1896

In this genus the trophozoites have 2 closely associated nuclei with large endosomes. The cysts are binucleate also.

Sappinia diploidea (Hartmann and Nägler, 1908) Alexeieff, 1912 is a common coprophilic amoeba in the feces of man and other animals. Its trophozoites are 10 to 60 μ long, with a thick, smooth, hyaline pellicle; according to Noble (1958), the ectoplasm has fine lines sometimes resembling wrinkles in cellophane. Two nuclei are present, usually pressed tightly together. Each nucleus has a large endosome and frequently a crescentic mass of granules between the endosome and the nuclear membrane. The cytoplasm is usually filled with many food vacuoles. A contractile vacuole is present, formed by the enlargement and coalescence of smaller vacuoles. A single, clear, broad pseudopod is characteristic, altho occasionally many pseudopods may be present. Cytoplasmic granules and food vacuoles are concentrated between the pseudopod and the rest of the body. Movement is quite sluggish. The cytoplasmic granules usually move rapidly. The cysts are typically binucleate, 12 to 18 μ or more in diameter, with thick, uniform walls. The cysts are formed from 2 individuals which come together and secrete a common cyst wall; their nuclei fuse so that each one has a single nucleus, their cytoplasm fuses, each nucleus gives off reduction bodies, and the 2 remaining nuclei come into contact to make the cyst binucleate.

Noble (1958) found that *S. diploidea* appeared in fecal samples from Wyoming elk and bison (but not from cattle, horses and sheep) after storage at 4° C for a few days to a few weeks. It failed to survive in soil or in soil mixed with boiled feces, nor could it be found in soil samples taken from areas where elk, sheep or horses were present.

S. diploidea is readily cultivated. Noble (1958), for example, cultivated it both at 4° C and at room temperature on the surface of agar plates containing 1.5% agar, 0.05% yeast extract and 0.05% peptone. The cultures held at room temperature became moldy after 6 weeks. *Sappinia* was present for 2 to 3 weeks in the cultures at 4° C.

Genus *VAHLKAMPFIA* Chatton and Lalung-Bonnaire, 1912 emend. Calkins, 1913

These are small amoebae with a nucleus containing a large endosome and peripheral chromatin, with polar caps during nuclear division. The trophozoites have a single broad pseudopod and move like a slug. The cysts have a perforated wall. The nucleus of this genus closely resembles that of *Naegleria*, but the latter has both flagellate and amoeboid stages. A number of species have been described from fresh water, old feces, lower vertebrates and invertebrates, but the taxonomy, nomenclature and validity of some of them are not certain.

Vahlkampfia punctata (Dangeard, 1910) Chatton and Lalung-Bonnaire, 1912 has been found in human feces. Its cysts have punctate markings.

Vahlkampfia lobospinosa (Craig, 1912) Craig, 1913 is another coprophilic species. Becker and Talbott (1927) found it in the rumen of a cow in Iowa. Its trophozoites are 10 to 24 μ long. Its cysts have 1 or 2 nuclei and are 7 to 11 μ in diameter.

Noble (1958) found that *Vahlkampfia* sp. appeared in fecal samples from Wyoming elk, bison, cattle, horses, sheep, moose and marmots after storage at 4° C for a few days to a few weeks. The protozoa persisted for several months. They failed to survive in soil, nor were they present in soil samples taken from areas where elk, sheep or horses were present. The trophozoites were 20 to 40 μ in diameter, with finely granular cytoplasm filled with food vacuoles and other particles. A contractile vacuole was present. The pseudopods were broad, usually sluggish but sometimes formed almost explosively; often there was only a single, large pseudopod. The nucleus was vesicular, with a large, central endosome occasionally appearing to be composed of several closely packed granules. Peripheral chromatin was rarely present, altho a ring of minute granules was often present just within the nuclear membrane. The cysts were 8 to 15 μ in diameter and almost exclusively mononucleate. The nucleus was different from that of the trophozoite. Its central endosome was usually smaller than in the trophozoite and often composed of several granules, and the peripheral chromatin was distinct, arranged in irregular clumps and often forming a crescent. A large vacuole and irregular chromatoid bodies, many of which resembled those of *Entamoeba histolytica*, were present. Noble believed that many of the cysts found in animal and human feces and described as those of *Entamoeba* are actually of the *Vahlkampfia* type.

Noble (1958) cultured this species at both 4° C and room temperature on the surface of agar plates containing 1.5% agar, 0.05% yeast extract and 0.05% peptone. The cultures held at room temperature became moldy after 6 weeks and were discarded, but *Vahlkampfia* was present for 3 months without attention in those held at 4° C.

FAMILY ENDAMOEBIDAE

Members of this family are parasitic in the digestive tract of vertebrates and invertebrates. The genera are differentiated on the basis of nuclear morphology. Four genera contain parasites of domestic animals and man, but only two of these contain pathogenic species. However, it is important to be able to identify the various species in order to know whether an infection with a pathogenic one is present or not.

Genus *ENTAMOEBA* Casagrandi and Barbagallo, 1895

The nucleus is vesicular, with a comparatively small endosome located at or near its center, with or without periendosomal granules around the endosome, and with a varying number of granules around the periphery of the nucleus. Cysts are formed; they contain 1 to 8 nuclei and may or may not contain chromatoid bodies (rod-like bodies which stain with hematoxylin and which are absorbed and disappear as the cysts mature). This genus occurs in both vertebrates and invertebrates.

The name of this genus was the subject of one of the most famous taxonomic controversies in protozoology (Dobell, 1938; Kirby, 1945). The genus *Endamoeba* was established by Leidy in 1879 for an amoeba of the cockroach, *Endamoebae blattae*. In 1895 and in ignorance of this name, Casagrandi and Barbagallo introduced the name *Entamoeba* for the human amoeba, *E. coli*. The nuclei of these two forms are very different, that of the cockroach species lacking a central endosome. Since the appearance of the nucleus is the most important differentiating character between genera in this family, it is obvious to a protozoologist that these forms belong in different genera. However, the first syllable of their names is derived from the same Greek root. Hence the International Commission of Zoological Nomenclature was asked to decide whether the name that had been given second should be changed to something else (i.e., whether the two names were homonyms).

The Commission, which had no protozoologists among its members, went beyond this, and decided that *Entamoeba* was a synonym of *Endamoeba* and that both protozoa belonged to the same genus. Altho the latter is obviously not true, this dictum was accepted by many protozoologists. Finally, after much agitation and several long, involved papers by various authorities, the International Commission finally reversed itself, and *Entamoeba* is now universally recognized as the correct name for the species occurring in vertebrates.

The nomenclature and taxonomy of the species of *Entamoeba* are about as confused as it is possible to make them. Some of the problems are explained below, but puzzle addicts are referred to the cited papers for the details.

Members of the genus found in domestic animals and man can be divided into 4 groups on the basis of trophozoite and cyst morphology. A fifth group includes species about which insufficient morphological information is available to determine which of the other groups they belong in. Most of the species within each group are morphologically indistinguishable; they are differentiated on the basis of size, hosts, pathogenicity, etc. Criteria of this type are given different weights by different taxonomists, and this fact combined with a lack of cross-transmission studies for many species accounts for some of the confusion. More is due to the fact that the original descriptions of some of the species were so poor as to make it difficult or impossible to be sure what forms the authors were dealing with.

The groups of *Entamoeba* will be described first, and then the individual species.

1. HISTOLYTICA GROUP. The nucleus has a small, central endosome, a ring of small peripheral granules and a few scattered chromatin granules between them. The cysts have 4 nuclei when mature, and their chromatoid bodies are rods with rounded ends. Glycogen vacuoles, when present in the cyst, are usually diffuse and ill-defined.

   Entamoeba histolytica of man, other primates, the dog, cat and rarely the pig.
   Entamoeba hartmanni of man and presumably also of the other hosts of *E. histolytica*.
   Entamoeba equi of the horse.
   Entamoeba anatis of the duck.
   Entamoeba moshkovskii of sewage.

2. COLI GROUP. The nucleus has a somewhat larger, eccentric endosome than that of the histolytica group and has a ring of coarse peripheral granules and some scattered chromatin granules between them. The cysts have 8 nuclei when mature, and their chromatoid bodies are splinter-like. Glycogen vacuoles, when present in the cyst, may be fairly well defined.

   Entamoeba coli of man, other primates, the dog and possibly the pig.
   Entamoeba wenyoni of the goat.
   Entamoeba muris of mice, rats, hamsters, and other rodents.
   Entamoeba caviae of the guinea pig.
   Entamoeba cuniculi of rabbits.
   Entamoeba gallinarum of the chicken, turkey, guinea fowl, duck, and goose.

3. BOVIS GROUP. The endosome of the nucleus varies in size; it may be as small as that of the histolytica group, but is generally larger than that of the coli group. The ring of peripheral granules in the nucleus may be fine or coarse, evenly or irregularly distributed. Periendosomal granules may be present. The cysts have 1 nucleus when mature, and their chromatoid bodies are either rods with rounded ends or less often splinter-like. Glycogen granules, when present in the cyst, are usually fairly well defined.

   Entamoeba bovis of cattle.
   Entamoeba ovis of sheep and goats.
   Entamoeba dilimani of goats.
   Entamoeba suis of the pig and perhaps man.
   Entamoeba bubalis of the carabao.
   Entamoeba chattoni of monkeys and probably man.

4. GINGIVALIS GROUP. The nucleus has a small, central endosome and a ring of small peripheral granules. There are no cysts. Members of this group are found in the mouth.

   Entamoeba gingivalis of man, other primates, the dog and cat.
   Entamoeba equibuccalis of the horse.
   Entamoeba suigingivalis of the pig.

5. INSUFFICIENTLY KNOWN SPECIES. This group includes *Entamoeba gedoelsti* of the horse and *E. caudata* of the dog. The nucleus of *E. caudata* resembles that of *E. coli*, while the nucleus of *E. caudata* resembles that of *E. histolytica*. The cysts of these species are unknown.

The only species of *Entamoeba* pathogenic for mammals is *E. histolytica*. The fact that it has been recorded in an average of about 18% of the people examined in various surveys thruout the world and yet only about 1/5 of them have signs or symptoms of disease has puzzled epidemiologists for many years. Two other facts contribute to the problem. One is that there are two different sizes of these amoebae, the smaller of which is not associated with disease; it has been encountered in about 1/3 of the people in these surveys. The other is that amoebic dysentery occurs mostly in the tropics. Autochthonous cases occur so seldom in western Europe that many European parasitologists believe that cases of amoebic dysentery which occur in their countries have been imported from the tropics either directly or thru contact with infected persons. These parasitologists have not had the benefit of the American experience with the disease. There is no question that indigenous cases occur in the temperate zone of this country.

Several hypotheses have been advanced in explanation (see Hoare, 1958). The first theory, suggested about 1913 and still held by perhaps the majority of parasitologists, is based on an unwillingness to assign separate specific names to protozoa which differ only in size and pathogenicity. According to this view, the species *Entamoeba histolytica* is composed of a small race and a large race. The small race is not pathogenic, while the large race may or may not be. In its virulent phase the latter invades the tissues; the trophozoites of this phase are large--the "magna" form. In its commensal phase it remains in the lumen of the intestine, feeding on bacteria and saprozoically. The trophozoites of this phase are small--the "minuta" form. Under proper conditions, this non-pathogenic "minuta" form can invade the intestinal mucosa and turn into the pathogenic "magna" form. The trophozoites of the small race of *E. histolytica* are usually 12 to 15μ in diameter and the cysts are 5 to 9μ in diameter with a mean of 7 to 8μ. The trophozoites of the "magna" form of the large race are 20 to 30μ and those of the "minuta" form 12 to 15μ in diameter. However, the cysts of both the "magna" and "minuta" forms are the same size, 10 to 20μ in diameter, with a mean of about 12μ.

The second theory was proposed by Brumpt in 1925. He recognized 3 species. He called the small, non-pathogenic race *Entamoeba hartmanni*, and divided the large race into 2 species. Of these, *E. dispar* is non-pathogenic and occurs thruout the world, while *E. dysenteriae* is pathogenic, altho it may cause no apparent symptoms in carriers, and occurs only in warm and hot countries.

The third theory was formulated formally by Hoare in 1957. It calls the small, non-pathogenic race *E. hartmanni*, but retains the name *E. histolytica* for the large race of the first theory. It then divides *E. histolytica* into an avirulent race (corresponding to Brumpt's *E. dispar*) and a virulent race (corresponding to Brumpt's *E. dysenteriae*) which may invade the gut wall or live in the lumen without causing symptoms.

This view has a great deal to recommend it. By giving the non-pathogenic small form a separate name, it makes it easier for the physician to interpret laboratory reports and prevents faulty diagnoses and needless treatment. However, the question whether there actually are completely non-pathogenic strains of *E.*

histolytica (sensu stricto) which cannot be induced to become pathogenic has not yet been answered satisfactorily. Concomitant bacteria, nutritional deficiencies and other factors affect the pathogenicity of the amoebae. Indeed, Phillips *et al.* (1955) found it impossible to infect bacteria-free guinea pigs with *E. histolytica* at all, altho normal guinea pigs or those infected with *Escherichia coli* or *Aerobacter aerogenes* could be readily infected and subsequently developed intestinal lesions.

Some of the amoebae reported as *E. histolytica* from domestic animals may well have been actually *E. hartmanni*, but unless they were specifically described as having small cysts, it is impossible to know which they were.

The above discussion has to do primarily with a matter of nomenclature. In addition, another species morphologically identical with *E. histolytica* has been found in sewage. This is *E. moshkovskii* It has not been found in fresh feces, but nevertheless its existence must be taken into consideration in diagnosis. It is not infective for rats, kittens, guinea pigs or frog or salamander larvae and its optimum temperature is about 24° C, altho it will grow poorly at 37° C.

Before beginning a systematic account of the species of *Entamoeba*, a word is in order regarding the bovis group. All of these look alike, with minor differences which may not be of taxonomic significance. Different names have been given to the forms in different hosts, but no cross-transmission studies have been attempted, and it is quite likely that when they are, some of these forms will be found to be synonyms. In this case, *Entamoeba bovis* will have precedence over the other names.

The name *Entamoeba polecki* has been used for members of the bovis group from the pig and goat, but it is a *nomen nudum*. Prowazek's (1912) original description and figures of it are so poor that it is impossible to know whether he was dealing with a member of the genus *Entamoeba* at all.

Noble and Noble (1952) and Hoare (1959) have reviewed the amoebae of domestic animals.

ENTAMOEBA HISTOLYTICA SCHAUDINN, 1903

Synonyms: *Amoeba coli, Amoeba dysenteriae, Entamoeba tetragena, Entamoeba dispar, Entamoeba venaticum.*

Disease: Amoebic dysentery.

Hosts: Man, orang-utan, gorilla, chimpanzee, gibbon, many species of macaques, baboons, spider monkeys and other monkeys, dog, cat, pig, rat, possibly cattle. The rat, mouse, guinea pig and rabbit are often infected experimentally.

Location: Large intestine, sometimes liver, occasionally lungs, and rarely other organs including the brain, spleen, etc.

Geographic Distribution: Worldwide. Maps of the world distribution of amoebic dysentery and *E. histolytica*, together with climatological and other information, were published by Piekarski and Westphal (1952) and Westphal (1955).

Prevalence: *E. histolytica* is most important as a parasite of man. It also occurs in monkeys and higher primates. According to Belding (1952), it was found in an average of 17.6% of 42,713 persons (range, 0.8 to 50%) in 37 surveys thruout the world from 1941 to 1948. In 10 surveys of 10,867 persons in the United States from 1941 to 1948, it was found in an average of 13.6% (range, 0.8 to 38%).

According to Benson, Fremming and Young (1955), it has given considerable trouble in their chimpanzee colony at the Univ. of Texas.

Sporadic cases of amoebic dysentery have been reported in dogs; these animals are generally considered to have acquired their infections from human contacts. Kartulis (1891, 1913) found *E. histolytica* causing dysentery in 3 dogs in Egypt; in

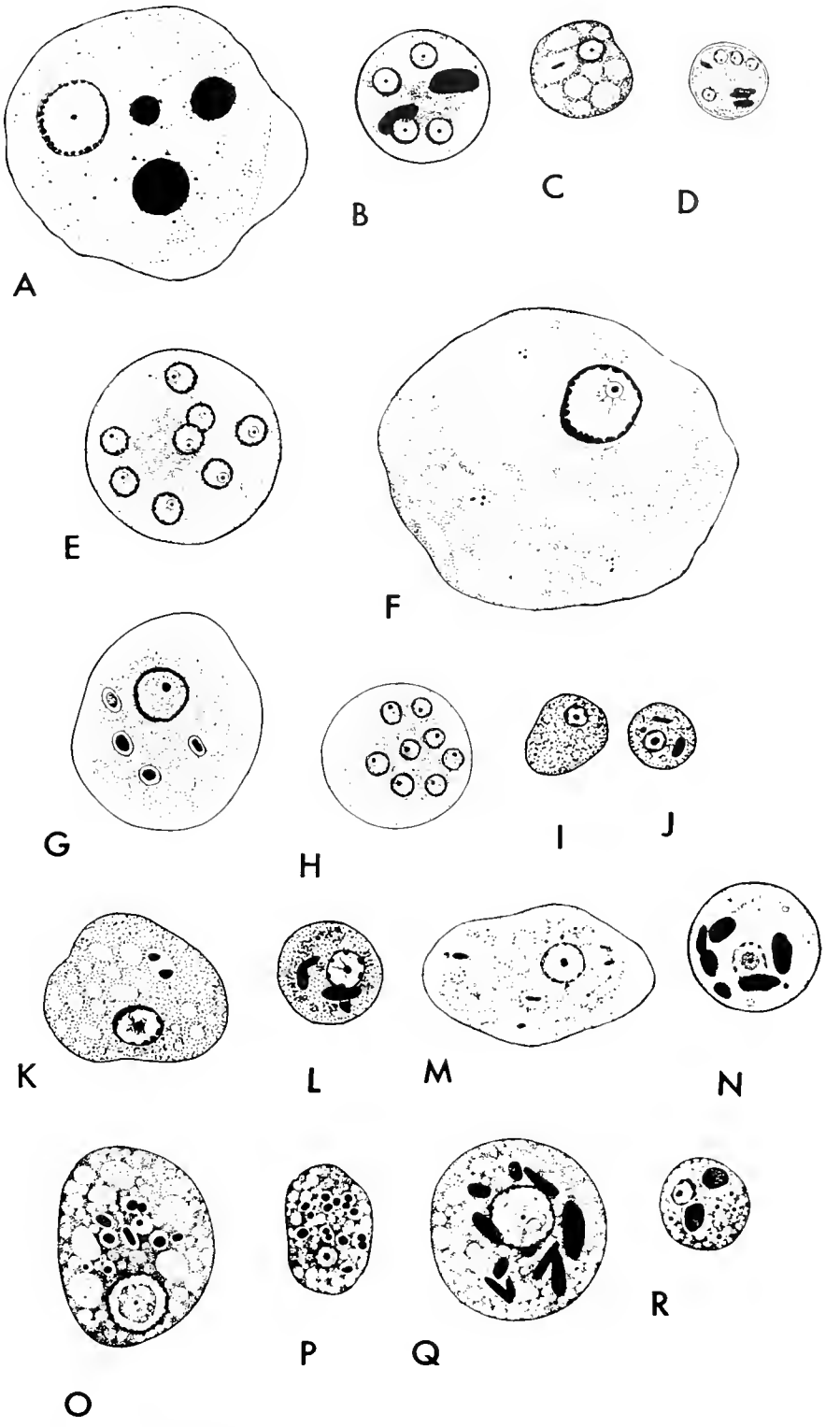

Fig. 20. Species of *Entamoeba*. A. *E. histolytica* trophozoite. B. *E. histolytica* cyst. C. *E. hartmanni* trophozoite. D. *E. hartmanni* cyst. E. *E. coli* cyst. F. *E. coli* trophozoite. G. *E. gallinarum* trophozoite. H. *E. gallinarum* cyst. I. *E. bovis* trophozoite. J. *E. bovis* cyst. K. *E. ovis* trophozoite. L. *E. ovis* cyst. M. *E. dilimani* trophozoite. N. *E. dilimani* cyst. O. *E. suis*, large trophozoite. P. *E. suis*, small trophozoite. Q. *E. suis*, large cyst. R. *E. suis*, small cyst. X 1700. (From Hoare, 1959, in *Veterinary Reviews and Annotations*)

one of these, a liver abscess was also present. Darling (1915) reported a fatal infection in a dog in Panama. Ware (1916) reported an outbreak in a pack of foxhounds in the Nilgiri Hills of India, Boyd (1931) reported an outbreak in another pack of hounds in India, and more recently Ganapathy and Alwar (1957) reported 2 cases of amoebic dysentery in dogs in India. Fischer (1918) reported a case of amoebic dysentery in a dog in China, Bauche and Motais (1920) reported one in Indochina, and Morcos (1936) found 5 cases in Egypt. In the United States, Faust (1930) found 2 dogs in New Orleans with amoebic dysentery, Andrews (1932) found *E. histolytica* in the feces of a diarrheic dog in Baltimore, and Thorson, Seibold and Bailey (1956) reported a case of systemic amoebosis in a puppy which also had distemper. *E. histolytica* was found in large numbers in the lungs and amoebae were also seen in the liver, kidneys and spleen.

In surveys of presumably normal dogs, Kubo (1936) found *E. histolytica* in 8% of 85 street dogs in Mukden, China, while Yamane (1938) found it in 3% of 60 street dogs from the same city. Chary *et al.* (1954) stated that amoebic dysentery occurs frequently in dogs in Indochina. Eyles *et al.* (1954) found *E. histolytica* in 8.4% of 143 dogs in the Memphis, Tennessee dog pound. The protozoa were so scarce that cultural methods were required to reveal them. This finding suggests that amoebic dysentery may be more common in dogs than is generally believed.

Natural *E. histolytica* infections are apparently rare in cats, but Kessel (1928) found the protozoon in 3 of 150 kittens in China.

E. histolytica is rare in swine. Frye and Meleney (1932) found it in 1 of 127 pigs which they examined in Tennessee; this animal came from a farm where an infected woman lived.

There are 2 reports of what may have been *E. histolytica* in cattle. Walkiers (1930) saw it in the feces of dysenteric cattle in the Belgian Congo. Thiery and Morel (1956) found it in the lungs of a young zebu in Dakar which was slaughtered on account of generalized streptothricosis.

Natural infections in rats have been reported by a number of workers. Chiang (1925) found *E. histolytica* in 7 laboratory rats. Brug (1919) found it in 2 of 50 wild rats in Batavia, Nagahana (1934) found it in 3 of 274 wild rats in Mukden, China, and Epshtein and Avakian (1937) found it in 7 of 515 wild *Rattus norvegicus* in Moscow. In the United States, Lynch (1915) saw it in a wild rat, Tsuchiya and Rector (1936) found it in 2 of 100 wild rats in St. Louis, and Andrews and White (1936) found it in 28 (1.1%) of 2515 wild rats in Baltimore.

Morphology: The trophozoites of the large, pathogenic race of *E. histolytica* are 20 to 30 μ and those of the small race are 12 to 15 μ in diameter. They have a thick, clear layer of ectoplasm and granular endoplasm. They move rapidly when warm, usually moving forward in a straight line with a single clear pseudopod at the anterior end. When the feces have cooled, the amoebae stay in one place and throw out large, clear pseudopods from various parts of their body. The trophozoites often ingest erythrocytes, a feature which differentiates them from those of other amoebae. The nucleus is indistinct in living amoebae. When stained with hematoxylin, it has a small, central endosome, a ring of small peripheral granules and a few scattered chromatin granules in between. The cysts of both the large and small races are 10 to 20 μ (average, 12 μ) in diameter. They have 4 nuclei when mature and often contain rod-like chromatoid bodies with rounded ends. Diffuse glycogen is present in the young cysts.

Life cycle: *E. histolytica* multiplies in the trophozoite stage by binary fission. It has 6 chromosomes. Before encysting, the amoebae round up, became smaller and eliminate their food vacuoles. They lay down a cyst wall, and the nucleus divides into 2 and then into 4 small nuclei. After the 4-nucleate amoebae emerge from the cyst, both the nuclei and cytoplasm divide so that 8 small amoebulae result. Each then grows into a normal trophozoite.

Pathogenesis: As mentioned above, only the large forms of *E. histolytica* are generally considered pathogenic, altho there are reports of mild disease and slight lesions associated with the small form (Shaffer *et al.*, 1958). They may cause diarrhea or dysentery, and may invade the wall of the cecum and colon, forming ragged, undermining or flask-shaped ulcers which may be pinpoint in size or may become large and confluent. The amoebae invade the mucosa at first and multiply to form small colonies. These colonies then extend into the submucosa and even into the muscularis. In the absence of bacterial invasion, there is little tissue reaction, but in complicated infections there is hyperemia, inflammation and infiltration with neutrophiles.

Some of the amoebae may pass into the lymphatics or even the mesenteric venules. Those entering the hepatic portal system pass to the liver, where they may cause abscesses. Those which enter the lymph ducts are generally filtered out by the lymph nodes. Abscesses may be formed in various other organs, including the lungs, brain, etc., depending on the host's resistance.

The relation of parasite strain to pathogenicity has already been mentioned. The species of concomitant bacteria present may also affect the amoeba's pathogenicity, as may the nutritional status of the host and other environmental factors. Dysentery is much more common in the tropics than in the temperate zone.

In most cases, *E. histolytica* causes minor symptoms or none at all. Infections may last 40 years or even more. There may be recurring mild to severe gastrointestinal symptoms, including intermittent diarrhea, bowel irregularity, abdominal pain, nausea and flatulence. Sometimes affected persons tire easily, have headaches or feel nervous. Appendicitis or symptoms resembling it may occur. These symptoms generally clear up after treatment.

In acute amoebic dysentery, the feces consist almost entirely of blood and mucus filled with amoebae and blood cells. The patient is wracked by waves of severe abdominal pain and spends a large part of his time on the stool, straining and passing blood and mucus every few minutes. In contrast to bacillary dysentery, there is no fever in uncomplicated cases.

Epidemiology: As mentioned above, *E. histolytica* is primarily a parasite of primates, and man is the reservoir of infection for his domestic animals. This is one of the few zoonoses which man gives to his associated animals in return for the many which he receives from them.

Infection is due to ingestion of cysts. Since trophozoites alone are passed by dysenteric individuals, these are not important sources of infection, while cyst-producing chronic cases and carriers are.

The cysts are relatively resistant. They are not affected by water chlorination, but can be removed by sand filtration. They survive for at least 8 days in soil at 28 to 34° C (Beaver and Deschamps, 1949), but live only an hour at 46 to 47° C and less than a minute at 52° C (Jones and Newton, 1950). They survive longest at refrigerator temperatures (40 days at 2 to 6° C according to Simitch, Petrovitch and Chibalitch, 1954; 62.5 days at 0° C according to Chang, 1955). They will not excyst after 24 hours at temperatures of -15° C or lower (Halpern and Dolkart, 1954), and die in 7.5 hours or less in the deep freezer at -28° C (Chang, 1955).

The cysts are usually transmitted with food or water. Raw vegetables may be a source of infection. Flies may transmit the cysts also. Pipkin (1949) was able to cultivate cysts from the vomitus of filth flies (*Musca domestica, Lucilia pallescens, Cochliomyia macellaria, Phormia regina* and *Sarcophaga misera*) 39 to 64 minutes after ingestion and from their feces 172 to 254 minutes after ingestion.

Faulty plumbing and water systems may cause water-borne transmission. The most striking case of this kind occurred during the Chicago World Fair in 1933. An outbreak of amoebic dysentery occurred

among guests at two neighboring hotels from which over 1000 cases with 58 deaths were tracked down in 44 states and 3 Canadian provinces (Bundesen *et al.*, 1936). Cross connections between the water and sewage pipes, back siphonage from toilet bowls into the water supply and leakage from an overhead sewage pipe in the kitchen were involved.

Food handlers may play an important role in transmission of amoebae, even tho the cysts rarely survive more than 10 minutes on the hands, except under the fingernails (Spector and Buky, 1934). Thus, Schoenleber (1940, 1941) reported that in a group of Americans living in a Standard Oil Co. camp in Venezuela, the prevalence of amoebic infection was reduced in 3 years from 25.6% to 1.9% and the amoebic dysentery rate from 36.84 to 0.61 per 1000 per year by inspection and treatment of food handlers. Winfield and Chin (1939), in a comparison of the prevalence of amoebic infection with food habits in different parts of China, concluded that transmission by food handlers is probably more important than by other means in that country. *E. histolytica* is much commoner in North China than in South and Central China. This is correlated with the serving and eating of cold bread with the hands in North China as contrasted to the handling of hot rice with a serving spoon and chopsticks in South and Central China. On the other hand, Sapero and Johnson (1939, 1939a) found no evidence that carriers were important in the transmission of amoebae in a study of 919 persons in the U.S. Navy. The sanitary habits of American sailors probably had something to do with this.

Diagnosis: The laboratory diagnosis of amoebiasis has been discussed in detail by Brooke (1958). Live amoebae can be found in wet smears made with physiological salt solution. These smears may be stained with Lugol's iodine solution diluted 1:5 to bring out the nuclei of the cysts and stain glycogen. However, for accurate identification and differentiation from other species of amoebae, staining with hematoxylin is essential. The smears are generally fixed in Schaudinn's fluid and stained with Heidenhain's iron hematoxylin. Sapero and Lawless's (1953) MIF (merthiolate-iodine-formaldehyde) stain-preservation technic can also be used.

For concentration of cysts, flotation in zinc sulfate solution (Faust *et al.*, 1938) can be used. The cysts are distorted beyond recognition, however, by the other salt and sugar solutions in common use for flotation of helminth eggs. For concentration by sedimentation, the FTE (formalin-triton-ether) sedimentation technic (Ritchie, Pan and Hunter, 1952, 1953) or MIFC (merthiolate-iodine-formaldehyde-concentration) technic (Blagg *et al.*, 1955) can be used.

Cultivation can be helpful in diagnosis of amoebiasis, but only if fresh specimens are used and if the laboratory personnel are expert. Cultivation is not recommended for general use.

E. histolytica cannot be differentiated morphologically from *E. hartmanni*, and its differentiation from other intestinal amoebae, and especially from *E. coli*, is not an easy task. There is a surprising amount of discrepancy even among those who should be qualified. Thus, in an evaluation by the USPHS Communicable Disease Center of the diagnostic ability of 42 state health department laboratories (Brooke and Hogan, 1952), an average of 4.1 out of 18 *E. histolytica* infections was missed among 98 stool samples sent to the laboratories for examination, and an average of 4.4 false positive reports was made among the 80 negative samples. Furthermore, in an analysis of responses by members of the American Society of Tropical Medicine to a questionnaire on the clinical and laboratory diagnosis of amoebiasis, Brooke *et al.* (1953) found a surprising lack of agreement in statements concerned with the identification of *E. histolytica* cysts and trophozoites.

Goldman (1959, 1960) was able to differentiate between *Entamoeba histolytica*, *E. hartmanni*, *E. moshkovskii* and *E. coli* by a fluorescence antibody technic. Three originally invasive strains of *E. histolytica* which he studied differed significantly from a non-invasive strain.

Cultivation: *E. histolytica* was first cultivated by Boeck and Drbohlav (1925). Their medium was composed essentially of a coagulated egg slant overlaid with Locke's solution containing serum. Various modifications of this medium are still in use. Cleveland and Collier (1930) used a liver infusion agar slant overlaid with serum and physiological salt solution. Balamuth (1946) introduced an all-liquid egg infusion-liver extract medium.

Treatment: Amoebiasis can be treated with a number of drugs (cf. Balamuth and Thompson, 1955). The old standard drug, emetine, is not used as much as formerly because of its toxicity. Other drugs from which one can choose include (1) the arsanilic acid derivatives, carbarsone, glycobiarsol (bismuth glycoarsanilate, Milibis) and thiocarbarsone; (2) the iodoquinoline derivatives, diodohydroxyquin (Diodoquin), chiniofon (Yatren) and iodochlorhydroxyquin (Vioform); (3) the antimalarial drug, chloroquine; and (4) the antibiotics, erythromycin, fumagillin, tetracycline, chlortetracycline and oxytetracycline.

The particular drug selected depends in part on the type of amoebic infection involved. For acute or subacute dysentery, erythromycin, oxytetracycline or chlortetracycline may be used. Erythromycin is administered to man by mouth at the rate of 15 mg/kg daily in divided doses for 14 days. The usual human course of treatment with oxytetracycline or chlortetracycline is 0.5 g 4 times a day by mouth for 10 days.

For chronic cases or to eradicate intestinal infections, one of the arsanilic acid or iodoquinoline derivatives may be used. The usual adult human course of treatment with carbarsone is 0.25 g 2 or 3 times a day by mouth for 10 days. That with glycobiarsol is 0.5 g 3 times a day by mouth for 8 days. That with chiniofon is 1.0 g 3 times a day by mouth for 7 days. That with diodohydroxyquin is 0.65 g 3 times a day by mouth for 20 days.

For amoebic hepatitis or liver abscesses, chloroquine is used. A loading dose of 1 g chloroquine phosphate (0.6 g base) by mouth on each of 2 successive days followed by 0.5 g daily for 2 to 3 weeks is the recommended adult human course of treatment.

Diodohydroxyquin has also been recommended as a prophylactic drug for use by travellers in areas of high endemicity.

While relatively little work has been done on the treatment of amoebiasis in domestic animals, the same drugs are in general effective in them. Benson, Fremming and Young (1955) found that for chimpanzees the most successful drugs were carbarsone (0.25 g twice daily for 10 days) and fumagillin (20 to 30 mg twice daily for 10 days) administered in fruit or fruit juice. They also frequently gave a course of emetine hydrochloride (1 mg/kg body weight up to a maximum of 60 mg, injected intramuscularly daily for a maximum of 6 days) prior to carbarsone or fumagillin therapy (see also Fremming *et al.*, 1955). Herman and Schroeder (1939) successfully treated amoebic diarrhea in a 21-lb. orang-utan with carbarsone. They gave 2 courses of treatment 11 days apart, each course consisting of 0.05 g carbarsone in milk or a slice of banana 3 times a day for a week.

Prevention and Control: Infection with amoebae can be prevented by sanitation. Water supply systems should be built without cross connections to sewage systems. Water which may be polluted should be boiled or filtered thru sand, since ordinary chlorination does not kill the cysts. Food handlers should wash their hands thoroughly after using the toilet. Vegetables grown on polluted ground should be cooked, or, if they are to be eaten raw, should be scalded or soaked in vinegar containing 5% acetic acid for 15 minutes at 30° C or in vinegar containing 2.5% acetic acid for 5 minutes at 45° C (Beaver and Deschamps, 1949). Diodohydroxyquin may also be used prophylactically.

ENTAMOEBA HARTMANNI
VON PROWAZEK, 1912

As mentioned above, *E. hartmanni* closely resembles the small race of *E. histolytica*. It can be differentiated by careful examination of hematoxylin-stained preparations. Burrows (1959) compared the two species. Most trophozoites of *E. hartmanni* are smaller than those of *E. histolytica*. Rounded trophozoites of *E. hartmanni* range from 3 to 10.5 μ in diameter, while those of *E. histolytica* are 6.5 μ or more in diameter. The trophozoite nucleus of *E. hartmanni* is usually 2.0 to 2.5 μ in diameter but may range from 1.5 to 3.2 μ, while that of *E. histolytica* is usually 3.0 to 3.5 μ in diameter but may range from 2.8 to 3.8 μ. The peripheral chromatin of *E. hartmanni* is more variable in its arrangement than that of *E. histolytica* and may consist of discrete granules with wide spaces between them, a crescent of granules on one side of the nucleus, or a single large bar of chromatin with several small granules around the membrane; the peripheral chromatin of *E. histolytica* is generally distributed uniformly along the nuclear membrane.

Most cysts of *E. hartmanni* are smaller than those of *E. histolytica*. They range from 3.8 to 8.0 μ in diameter while those of small race *E. histolytica* are 5.5 μ or more in diameter. The cyst nuclei of *E. hartmanni* are 1.8 to 3.0 μ in diameter in uninucleate cysts, 1.3 to 2.0 μ in binucleate cysts and 0.7 to 1.7 μ in tetranucleate cysts; those of small race *E. histolytica* are 2.4 to 2.8 μ in diameter in uninucleate cysts, 2.0 to 2.8 μ in binucleate cysts and 1.4 to 2.2 μ in tetranucleate cysts. The cysts of *E. hartmanni* seldom contain large glycogen bodies, but nearly all of them have a few to many small vacuoles; the cysts of *E. histolytica* generally have one large glycogen vacuole or no vacuoles. The chromatoid bodies of the two species are similar.

Freedman and Elsdon-Dew (1959) suggested that, until an accurate, practical method of separation is devised, mean sizes of 12 μ for trophozoites and 10 μ for cysts be used as the dividing line between *E. histolytica* and *E. hartmanni*. The latter criterion has been used for some time to distinguish between the cysts of large and small race *E. histolytica* by those who do not accept the name *E. hartmanni* (Shaffer *et al.*, 1958).

The incidence of *E. hartmanni* in animals and man is unknown because in the past it has ordinarily been lumped with *E. histolytica*. According to Burrows (1957, 1959) about half of the reported cases of *E. histolytica* in the United States were actually *E. hartmanni*. Further studies in which the two species are separated will throw light on this point, which is important because *E. hartmanni* is nonpathogenic.

ENTAMOEBA MOSHKOVSKII
CHALAYA, 1941

This species occurs in sewage. It is not a parasite of animals, but of the municipal digestive tract. It was found in the sewage disposal plant and sewer system of Moscow by Chalaya (1941, 1947), in sewage in Leningrad by Gnezdilov (1947), in sewage in Brazil by Amaral and Azzi Leal (1949), in sewage in London by Neal (1950, 1953), and in sewage in Quebec by Lachance (1959). Probably the same organism was found in sewage in California by Wright, Cram and Nolan (1942), altho they did not name it. Chalaya (1947) cultivated it from the water of 2 ponds and a river in Russia. Altho *E. moshkovskii* is not parasitic, the possibility of its accidental presence in fecal samples is of concern in diagnosis.

E. moshkovskii resembles *E. histolytica* morphologically. The trophozoites are active, 9 to 29 μ (usually 11 to 13 μ) in diameter. The nucleus has a small, central endosome and a peripheral layer of fine granules. The cysts are generally spherical, 7 to 17 μ in diameter. They contain a very large glycogen vacuole at first which is eventually absorbed as the cysts age. The chromatoid bodies are large, rather elongate, and have rounded ends. The mature cysts have 4 nuclei.

The cysts remain viable at 4° C up to 10 months if they are not allowed to dry out.

E. moshkovskii can be cultivated in the usual *Entamoeba* media. Its optimum temperature is about 24° C and it grows poorly at 37° C. The ability to grow at room temperature differentiates this species from *E. histolytica.*

Chalaya (1941) was unable to infect kittens with *E. moshkovskii*, and Neal (1953) could not infect rats, frog (*Rana temporaria*) tadpoles or salamander (*Salamandra maculosa*) larvae by feeding.

ENTAMOEBA EQUI FANTHAM, 1921

Fantham (1921) found this amoeba in the feces of 2 horses with signs of intestinal disturbance in South Africa. It is unusually large, fully extended trophozoites measuring 40 to 50 by 23 to 29 μ and rounded ones 28 to 35 μ in diameter. The nucleus is of the *histolytica* type, but is oval rather than round. Erythrocytes are ingested. The cysts are 15 to 24 μ in diameter and contain 4 nuclei and chromatoid bars.

ENTAMOEBA ANATIS FANTHAM, 1924

Fantham (1924) found this amoeba in the feces of a duck which had died of acute enteritis in South Africa. It resembles *E. histolytica* morphologically, and its trophozoites ingest erythrocytes. The cysts are spherical or subspherical, thin-walled, 13 to 14 μ in diameter, and contain 1 to 4 nuclei and thin, needle-like chromatoid bodies.

ENTAMOEBA COLI (GRASSI, 1879) CASAGRANDI AND BARBAGALLO, 1895

Synonyms: *Amoeba coli, Endamoeba hominis, Councilmania lafleuri.*

This is the commonest species of amoeba in man. According to Belding (1952), it was found in 28% in 19 surveys of 17,733 persons thruout the world and occurs in about 30% of the population of the United States. It also occurs in the gorilla, orangutan, chimpanzee, gibbon and in various species of macaques and other monkeys (Mackinnon and Dibb, 1938). Smith (1910) saw an amoeba similar to *E. coli* in pigs, and Kessel (1928a) found it in a Chinese pig. Kessel (1928a) also infected pigs experimentally with *E. coli* cysts from man, but the infections lasted less than 6 weeks.

Entamoeba coli occurs in the cecum and colon. It can be cultivated on the usual media. It is non-pathogenic, and therefore must be differentiated from *E. histolytica.*

Its trophozoites are 15 to 50 μ (usually 20 to 30 μ) in diameter. The cytoplasm is filled with bacteria and debris, and the ectoplasm is thin. The organism moves sluggishly. The nucleus has an eccentric endosome larger than that of *E. histolytica*, and a row of relatively coarse chromatin granules around its periphery. There may also be a few scattered chromatin granules between the endosome and the nuclear membrane. The cysts are 10 to 33 μ in diameter and have 8 nuclei when mature. The cysts contain slender, splinter-like chromatoid bodies with sharp, fractured or square ends; these disappear as the cysts age. The young cysts also may contain a large, well-defined glycogen globule; it usually disappears before the cyst is mature.

ENTAMOEBA WENYONI GALLI-VALERIO, 1935

Wenyon (1926) reported that he had seen 8-nucleate amoeba cysts of the *E. coli* type in the feces of goats. Galli-Valerio (1935) described this form, naming it *Entamoeba wenyoni.* The few trophozoites which he saw measured 12 by 9 μ, their protoplasm was fairly granular with no distinction between ectoplasm and endoplasm, and they contained numerous bacteria. They moved very slowly with short, rounded pseudopods. The cysts were spherical, 6 to 9 μ in diameter, and contained 8 nuclei.

ENTAMOEBA MURIS
(GRASSI, 1879)

Synonyms: *Amoeba muris*, *Councilmania muris*, *Councilmania decumani*.

E. muris occurs commonly in the cecum and colon of rats, mice and the golden hamster thruout the world. Andrews and White (1936) found it in 10.4% of 2515 wild rats in Baltimore. Fry and Meleney (1932) found it in 48% of 48 wild *Rattus norvegicus* and 24.1% of 54 grey mice captured in a rural area of Tennessee. Tsuchiya and Rector (1936) found it in 8% of 100 rats in St. Louis. Elton, Ford and Baker (1931) reported it in 50% of 440 long-tailed field mice (*Apodemus sylvaticus*), 41% of 116 bank voles (*Clethrionomys glareolus*) and 41% of 51 short-tailed field mice (*Microtus hirtus*) in England. Wantland (1955) found it in 33% of 412 golden hamsters from several American suppliers and laboratories. Mudrow-Reichenow (1956) found *E. muris* in 7% of 14 golden hamsters, 35% of 21 laboratory rats and 39% of 92 laboratory mice in Germany.

Kessel (1924) transmitted *E. muris* from the rat to the mouse and vice versa. Neal (1947, 1950a) and Saxe (1954) infected rats with *E. muris* from the golden hamster and mouse. Saxe (1954) infected the golden hamster with *E. muris* from the rat.

E. muris is morphologically similar to *E. coli*. Its trophozoites are 8 to 30 μ long. Its cysts are 9 to 20 μ in diameter and have 8 nuclei when mature. Its nuclear structure and division were studied by Wenrich (1940). He found that the nucleus is intermediate in structure between those of *E. histolytica* and *E. coli* but more nearly resembles the latter. It varies in diameter from 3 to 9 μ with a mean of 4 to 5 μ. In division, approximately 8 chromosomes are formed. Binucleate cysts almost always contain a large glycogen vacuole, and mononucleate cysts very frequently do.

E. muris is non-pathogenic. It is important to the research worker because it must be differentiated from other amoebae introduced in experimental infections.

ENTAMOEBA CAVIAE
CHATTON, 1918

This species is often referred to as *Entamoeba cobayae* (Walker, 1908) Chatton, 1917. However, the form which Walker (1908) called *Amoeba cobayae* was seen in cultures from a guinea pig intestine and was not an *Entamoeba* at all. Hoare (1959) considered this species a synonym of *E. muris*.

E. caviae is common in the ceca of laboratory guinea pigs. Nie (1950) found it in 14% of 84 guinea pigs in Pennsylvania and Mudrow-Reichenow (1956) found it in 46% of 13 guinea pigs in Germany.

E. caviae resembles *E. coli*. Its morphology has been studied by Nie (1950). The trophozoites are 10 to 20 μ in diameter with a mean of 14.4 μ. The nucleus is 3 to 5 μ in diameter. Its endosome varies in size and shape and may be central or eccentric. In some cases it is composed of several granules. There is a ring of coarse chromatin granules inside the nuclear membrane. The cysts are 11 to 17 μ in diameter with a mean of 14 μ and have 8 nuclei (Holmes, 1923). They are rare.

E. caviae is non-pathogenic. Because it is so common, it must be differentiated from other amoebae in experimentally infected animals.

ENTAMOEBA CUNICULI
BRUG, 1918

This species occurs in the cecum and colon of the domestic rabbit. It is not pathogenic. It resembles *E. coli*, and Kheisin (1938) has even suggested the name *Entamoeba coli* forma *cuniculi* for it. Hoare (1959) considered it a synonym of *E. muris*. It is apparently quite common in rabbits, altho there seem to be relatively few reports on it. Kheisin (1938) found it in 25% of the rabbits he examined in Russia. The trophozoites range from 12 to 30 μ in length with means of 13 to 17 μ in different rabbits. The cysts have 8 nuclei. They

range in diameter from 7 to 21 μ with means of 10 to 15 μ in different rabbits.

ENTAMOEBA GALLINARUM
TYZZER, 1920

This non-pathogenic species was described from the ceca of the chicken and turkey by Tyzzer (1920). By cecal inoculation of parasite-free baby chicks, Richardson (1934) found what appeared to be the same species in the ceca of the domestic duck, turkey and goose. *E. gallinarum* is common. McDowell (1953) found it in about 30% of a large number of chickens he examined in Pennsylvania.

E. gallinarum closely resembles *E. coli*. The trophozoites are 9 to 25 μ in diameter, most measuring 16 to 18 μ. The endoplasm is highly vacuolated and contains many food vacuoles. Altho Tyzzer (1920) said that *E. gallinarum* did not ingest bacteria, McDowell (1953) found that bacteria were its main food, altho it also ingested *Trichomonas* among other foods. The ectoplasm is clear or granular. The nucleus is 3 to 5 μ in diameter, with an eccentric endosome and a row of granules around the outside. The mature cysts are 12 to 15 μ in diameter and contain 8 nuclei.

Richardson (1934) transferred infection from chick to chick by association in the same cage. She found that the minimum oral infective dose of *E. gallinarum* for the chick was 240 cysts, and observed that the cysts remained viable in raw feces for 10 days and in feces diluted with water for at least 28 days.

ENTAMOEBA BOVIS
(LIEBETANZ, 1905)

Synonym: *Amoeba bovis*.

This non-pathogenic species occurs commonly in the rumen and feces of cattle thruout the world. Noble and Noble (1952) found it in the feces of all of 34 cattle from California, Pennsylvania, Korea and Japan. Mackinnon and Dibb (1938) found it in the feces of 4 gnus (*Connochaetes taurinus*) in the London zoo. It has been described most recently by Noble (1950) and Noble and Noble (1952). The trophozoites are 5 to 20 μ in diameter. The cytoplasm is smoothly granular and filled with vacuoles of various sizes. The nucleus is large, with a large, central endosome made up of compact granules and a conspicuous row of chromatin granules of different sizes around its periphery. The cysts are 4 to 14 μ in diameter and contain a single nucleus when mature. Their chromatoid bodies are irregular clumps of varying size and rods, splinters or granules. A large glycogen vacuole may or may not be present.

Noble and Noble (1952) found that the uninucleate entamoebae from the feces of cattle, goats, sheep and swine were morphologically indistinguishable. However, since their physiological characteristics have not been studied and cross infections have not been attempted, they considered it best not to assign them all to the same species. If future work should show that they are all the same, their correct name would be *E. bovis*.

ENTAMOEBA OVIS
SWELLENGREBEL, 1914

Synonym: *Entamoeba debliecki, pro parte*.

This non-pathogenic species was first described from the intestines of sheep in Sumatra, but it is common thruout the world. Noble and Noble (1952) found it in the feces of all of 25 sheep from California and Washington. Triffitt (1926) reported it from the feces of the sable antelope (*Hippotragus niger*) and common waterbuck (*Cobus ellipsiprymus*) in Africa.

By a historical accident, the name of the pig entamoeba rather than that of the sheep entamoeba has been used for the entamoeba of the goat. Nieschulz (1923) gave the first description of *E. debliecki* (a synonym of *E. suis*) from the pig and soon after (1923a) found what appeared to be the same species in the large intestine

of the goat in Holland. Hoare (1940) found it in the feces of 10 out of 14 goats in England and redescribed it under the name *E. debliecki*. Noble and Noble (1952) found it in the feces of 27 out of 28 goats in the United States and called it *E. polecki* (the name they used for the pig entamoeba). However, they considered the uninucleate entamoebae of cattle, goats, pigs and sheep to be morphologically indistinguishable. Since goats share a great many parasites with sheep but relatively few with swine, and in the absence of cross-infection experiments to the contrary, the best name for the goat entamoeba is *E. ovis*.

The trophozoites of *E. ovis* measure 11 to 12 by 13 to 14 μ. The nucleus typically contains a large, pale endosome generally composed of several granules, a ring of peripheral chromatin, and numerous small granules between the endosome and the nuclear membrane. In some cases there is very little peripheral chromatin and in others the endosome may be very small. The cysts are 4 to 13 μ in diameter with a mean of 7 μ and contain a single nucleus when mature. They usually contain numerous chromatoid bodies of varying size, shape and abundance and a glycogen vacuole.

The cysts of the form from the goat are 4 to 13 μ in diameter. Hoare (1940) found 2 races which differed in size. The cysts of one ranged from 5 to 9 μ in diameter with a mode of 6.7 μ, while the cysts of the other ranged from 9 to 13 μ with a mode of 10.4 μ Noble and Noble (1952), however, found only a single race with cysts ranging in diameter from 4 to 12 μ with a mean of 6.4 μ.

It is quite likely that *E. ovis* is a synonym of *E. bovis*, but until cross infection experiments have been carried out, it is thought best to retain it as a separate species.

ENTAMOEBA DILIMANI NOBLE, 1954

Noble (1954) found this species in the feces of all of 12 goats he examined on Luzon in the Philippines. He saw only 2 trophozoites. They were 12 μ across, had broad, rounded pseudopods whose ends had fairly clear ectoplasm, and food vacuoles containing bacteria. The cysts are 5 to 16 μ in diameter with a mean of 9.7 μ, and contain a single nucleus. The endosome is usually a small, central dot but may be eccentric. Peripheral chromatin is often absent or may appear as a few large, irregular granules. The entire nucleus is filled with fine granules which may form a ring around the endosome. The cyst contains 1 or more large glycogen vacuoles and from one to a large number of chromatoid bodies varying in shape from small, irregular masses to a single, large, sausage-shaped body. Noble considered this species to differ from the *Entamoeba* in American goats in that the peripheral chromatin rarely forms a heavy ring, the endosome is usually a single, small dot, and a periendosomal ring of chromatin is usually present.

ENTAMOEBA SUIS HARTMANN, 1913

Synonym: *Entamoeba debliecki, pro parte.*

A number of authors have used the name, *Entamoeba polecki* Prowazek, 1912, for this species, but this name must be considered a *nomen nudum* because Prowazek's description was so poor as to be unrecognizable (see Hoare, 1940, 1959).

E. suis occurs in the cecum and colon of swine. Chang (1938) found it in 71% of 209 pigs in China. Pavloff (1935) found it in 26 of 1840 pigs in France and Bulgaria. Simitch *et al.* (1959) found it in 8% of 1800 pigs in Yugoslavia. Frye and Meleney (1932) found it in 63% of 80 pigs, Alicata (1932) found it in 43% of 35 pigs, and Noble and Noble (1952) found it in all of 30 pigs in the United States. Mackinnon and Dibb (1938) found it in the European wild boar (*Sus scrofa*), giant forest hog (*Hylochoerus meinertzhageni*) and Indian boar (*Sus cristatus*) in a London zoo. Kessel and Johnstone (1949) and Kessel and Kaplan (1949) reported "*E. polecki*" from the rhesus monkey but remarked that it

appeared identical with *E. chattoni* of monkeys; this is the species to which their form should be assigned. Ten human infections have been reported (Kessel and Johnstone, 1949; Lawless, 1954; Burrows and Klink, 1955). However, altho no human cross-infection experiments have been attempted, *E. suis* does not seem to be readily transmissible to man. Chang (1939) observed that it was not present in 27 Chinese butchers, altho their methods of slaughtering provided ample opportunity for infection. Pavloff (1935) was unable to infect kittens with it by intrarectal inoculation.

E. suis has been described by a number of authors, including Noble and Noble (1952) in domestic animals, and by Burrows (1959) in man. The following description is based on Noble and Noble. The trophozoites are 5 to 25 μ long. Some authors (e.g., Hoare, 1959; Simitch *et al.* 1959) have considered the small forms to be a separate species, *E. debliecki*, but such a separation does not appear to be justified.

The nucleus varies in appearance. The endosome is central and is usually quite large. It may sometimes almost fill the nucleus, but it may also sometimes be small and similar to that of *E. histolytica*. There is a rather homogeneous ring of peripheral chromatin within the nuclear membrane. There are ordinarily no chromatin granules between the endosome and the peripheral ring. The cytoplasm is granular and vacuolated, and contains bacteria in its food vacuoles. The cysts are 4 to 17 μ in diameter and have a single nucleus when mature. The chromatoid bodies in the cysts vary markedly in shape from stout rods with rounded ends similar to those of *E. histolytica* to irregular granules of varying size. There may or may not be a glycogen vacuole. Cysts without chromatoid bodies or glycogen vacuoles are also common.

E. suis is probably non-pathogenic. Smith (1910) found amoebae in sections of intestinal ulcers in swine. Hartmann (1913), who studied Smith's preparations, named the amoeba *E. suis*. Ratcliffe (1934) observed amoebae associated with necrosis in sections of the colon of pigs which had died of experimental hog cholera. However, altho *E. suis* is very common in swine, it has never been found in sections of intestinal lesions of hundreds of swine examined by University of Illinois pathologists.

E. suis can be cultivated in the usual media. It is apparently less sensitive than *E. histolytica* to amoebicidal drugs, but Frye and Meleney (1932) eliminated it from pigs by feeding 50 mg/kg carbarsone in the milk daily for 10 days.

ENTAMOEBA BUBALUS NOBLE, 1955

Noble (1955) found this species in the feces of 12 of 15 carabao (*Bubalus bubalis*) from several islands in the Philippines. Only 2 trophozoites were seen. They averaged 12 μ in diameter. The cysts are 5 to 9 μ in diameter with a mean of 8 μ. They contain 1 or more vacuoles, but usually a single large one which crowds the cyst contents to its periphery. The chromatoid bodies are usually small and irregular in shape but may occasionally be large, with rounded ends, similar to those of *E. histolytica*. The cysts contain a single nucleus 2.6 μ in diameter with a large endosome 1.4 μ in diameter which often appears to be a cluster of 4 granules. There is usually a distinct peripheral ring of chromatin, but the amount of peripheral chromatin may vary from practically none to a ring of dots to a few isolated clumps. There is no periendosomal chromatin. Noble (1955) considered *E. bubalus* to differ from other entamoebae with uninucleate cysts in the character of its nucleus--the heavy, usually uniform outer ring of chromatin and the large, prominent endosome.

ENTAMOEBA CHATTONI SWELLENGREBEL, 1914

Synonym: *Entamoeba polecki, pro parte.*

This species occurs in the large intestine of macaques and a number of other monkeys. It was first seen by Chatton (1912), who called it *Loeschia* sp., and was given its present name by Swellengrebel (1914), who found it in the rhesus monkey. This name was thought to be one of the many synonyms of *E. histolytica* until Salis (1941) showed that it was not. Kessel and Johnstone (1949) found *E. chattoni* and *E. polecki* to be morphologically similar, and used the older name, *E. polecki*, for the species. However, in the absence of cross-infection experiments between pigs and monkeys, it is best to retain the name, *E. chattoni*, for the monkey form. In any case, *E. polecki* is a *nomen nudum* and should be replaced by *E. suis*. The proper name for the forms in the 10 human cases which have been reported (see Burrows and Klink, 1955) is uncertain. Perhaps it should depend in each case on the source of infection, whether pig or monkey, or perhaps both these names will eventually be dropped in favor of *E. bovis*. However, for the present *E. chattoni* is preferable.

E. chattoni is probably much more common in monkeys than *E. histolytica*, from which it must be distinguished. Mudrow-Reichenow (1956) found it in 6 of 7 rhesus monkeys in Germany. The trophozoites of *E. chattoni* are 9 to 25 μ long. The cysts are 6 to 18 μ in diameter. Salis described two size races with cysts averaging 10.9 and 13.1 μ, respectively but other workers have not made this differentiation. The nucleus varies a great deal in morphology. It may be indistinguishable from that of *E. histolytica*, with a small, central endosome and a row of fine, peripheral chromatin granules. On the other hand, the endosome may be large or small, central or eccentric, compact or diffuse, and composed of one to many granules, while the peripheral chromatin may be fine or coarse, uniform, irregular or diffuse, and there may or may not be chromatin granules between the endosome and the peripheral chromatin. The cysts are almost always uninucleate when mature. Less than 1% are binucleate, and they are never tetranucleate. The chromatoid bodies are usually irregular and small, but may also be rod-shaped with round or pointed ends, oval or round. A glycogen vacuole may or may not be present.

E. chattoni is generally considered non-pathogenic, altho 2 of the human patients studied by Burrows and Klink (1955) had diarrhea which may or may not have been caused by the amoebae.

ENTAMOEBA GINGIVALIS
(GROS, 1849)
BRUMPT, 1914

Synonyms: *Amoeba gingivalis, Amoeba buccalis, Entamoeba buccalis, Amoeba dentalis, Amoeba kartulisi, Entamoeba maxillaris, Entamoeba canibuccalis.*

This species occurs commonly in the human mouth, where it lives between the teeth, in the gingival margins of the gums and in the tartar. It has occasionally been found in infected tonsils. *E. gingivalis* is present in perhaps 50% of all humans, but in up to 95% of those with pyorrhea. It was once thought to be the cause of pyorrhea, but is now known to be a harmless commensal which finds an ideal home in diseased gums.

Hinshaw (1920) transmitted *E. gingivalis* to 5 dogs with gingivitis. In one of them the infection was still present after 14 1/2 months, but in the others it died out within 4 months. Kofoid, Hinshaw and Johnstone (1929) established persistent infections in 5 of 11 dogs with *E. gingivalis* from cultures. They could not infect dogs with healthy mouths, but only those with gingivitis, pus pockets or loose gums.

Goodrich and Moseley (1916) found amoebae indistinguishable from *E. gingivalis* in pyorrheic ulcers in the mouths of 2 dogs and a cat in England. Nöller (1922) found it in dogs in Germany. Simitch (1938) found a small amoeba in the saliva of 3 out of 165 dogs in Serbia and named it *E. canibuccalis*. The trophozoites were 8 to 16 μ long but became as long as 25 μ in culture. Simitch infected 2 old

dogs with cultured protozoa but failed to infect 3 young dogs, a young wolf and 2 humans. In view of the affection with which some dog and cat owners treat their pets, there is no reason to believe that the entamoebae in the mouths of these animals are a different species from that of man.

Kirby (1928) found *E. gingivalis* in the mouths of 2 chimpanzees with pyorrhea. Kofoid, Hinshaw and Johnstone (1929) found it in the mouths of *Macaca mulatta* and *M. irus*. Deschiens and Gourvil (1930) found it in the *M. mulatta* and *Papio sphynx*. Hegner and Chu (1930) found it in the mouths of 37 out of 44 wild *M. philippinensis*.

E. gingivalis has no cysts. The trophozoites are usually 10 to 20 μ long, but may range from 5 to 35 μ. The cytoplasm consists of a zone of clear ectoplasm and granular endoplasm containing food vacuoles. The amoebae usually feed on leucocytes, epithelial cells, sometimes on bacteria and rarely on red blood cells. There are usually a number of pseudopods. The nucleus is 2 to 4 μ in diameter, with a moderately small endosome, a peripheral layer of chromatin granules and some delicate achromatic strands extending from the endosome to the nuclear membrane.

Reproduction is by binary fission. It was described in detail by Child (1926), Stabler (1940) and Noble (1947). Child said that 6 chromosomes are present, but Stabler and Noble found only 5.

ENTAMOEBA EQUIBUCCALIS SIMITCH, 1938

Synonym: *Entamoeba gingivalis* var. *equi*.

Nieschulz (1924) found this amoeba in the mouths of several horses in Holland, and Simitch (1938a) cultured it from the mouths of 16 out of 22 mares and 3 out of 4 donkeys in Serbia. It is morphologically identical with *E. gingivalis*, except that its trophozoites are somewhat smaller, measuring 7 to 14 μ in diameter. It has no cysts. Simitch (1938a) was unable to infect horses with *E. gingivalis* (syn., *E. canibuccalis*) from the dog or to infect dogs with the horse form. Hence he considered the latter to be a new species. Further study is needed to learn whether this view is correct.

ENTAMOEBA SUIGINGIVALIS TUMKA, 1959

Tumka (1959) found this amoeba on the coating of the teeth of 6 out of 32 domestic pigs from the vicinity of Leningrad. It resembles *E. gingivalis* but is in its lower size range, measuring 7 to 12 μ with a mean length of 9 μ when fixed and stained. It is questionable whether this is a separate species.

ENTAMOEBA CAUDATA CARINI AND REICHENOW, 1949

This species was found in the feces of a dog in Brazil. No cysts were seen. The trophozoites were 10 to 36 μ long. Their pseudopods and nuclei resembled those of *E. histolytica*, but they differed from it in containing many ingested bacteria and in having a sac-like appendage at the posterior end containing dense, darkly staining cytoplasm and undigested bacteria.

ENTAMOEBA GEDOELSTI (HSIUNG, 1930)

Synonym: *Endamoeba gedoelsti*.

Hsiung (1930) found this amoeba in the cecum or colon of 7 out of 46 horses in Iowa. What was probably the same species had been seen in the horse by Gedoelst (1911) in Belgium and Fantham (1920) in South Africa. No cysts have been seen. The trophozoites are 7 to 13 μ long and contain bacteria in their food vacuoles. The nucleus is similar to that of *E. coli*, with an eccentric endosome surrounded by a halo and a row of peripheral chromatin granules.

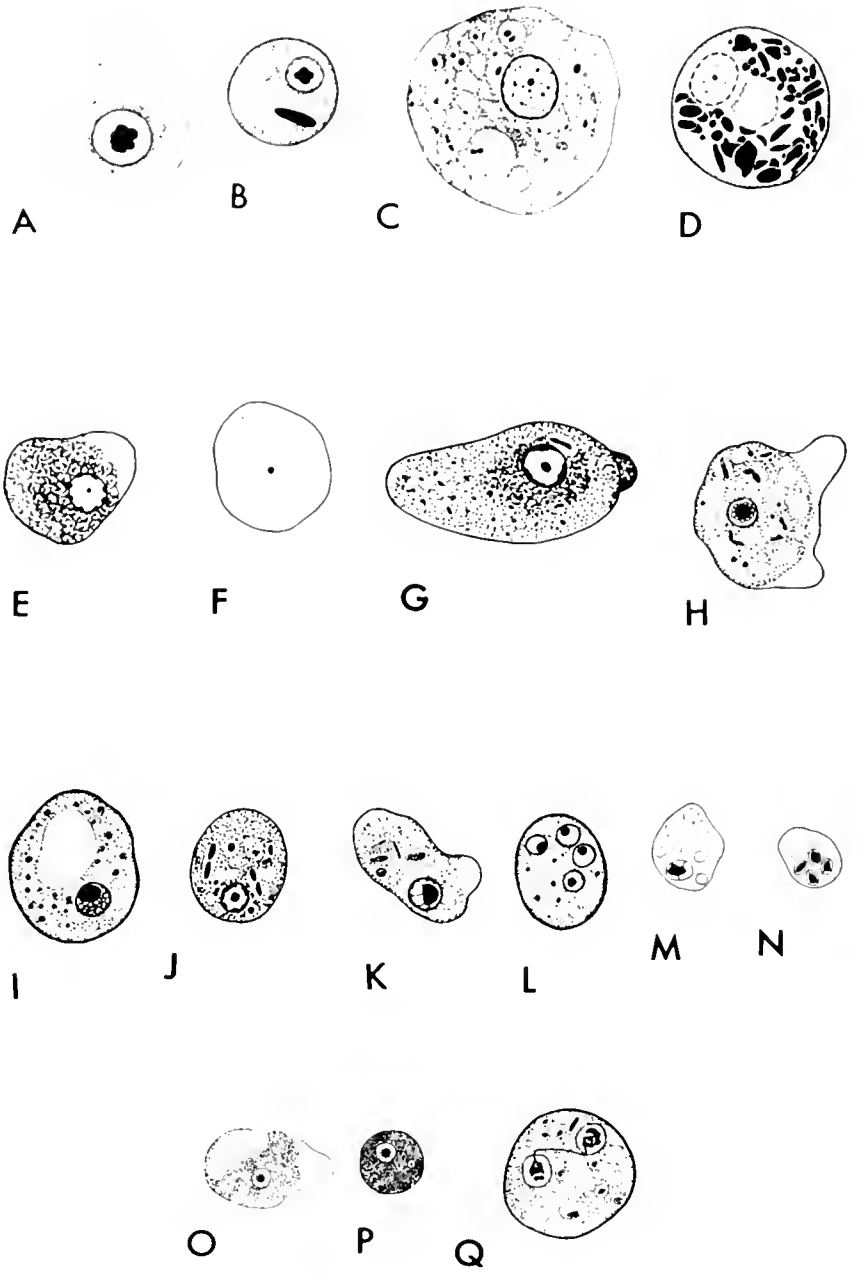

Fig. 21. A. *Entamoeba bubalus* trophozoite. B. *E. bubalus* cyst. C. *Entamoeba chattoni* trophozoite. D. *E. chattoni* cyst. E. *Entamoeba gingivalis* trophozoite from dog. F. *Entamoeba gedoelsti* trophozoite. G. *Entamoeba caudata* trophozoite. H. *Iodamoeba buetschlii* trophozoite. I. *I. buetschlii* cyst. J. *Entamoeba equibuccalis* trophozoite. K. *Endolimax nana* trophozoite. L. *E. nana* cyst. M. *Endolimax gregariniformis* trophozoite. N. *E. gregariniformis* cyst. X 1700. (From Hoare, 1959, in *Veterinary Reviews and Annotations*). O. *Vahlkampfia lobospinosa* trophozoite. P. *V. lobospinosa* cyst. X 1050. (From Becker and Talbott, 1927). Q. *Dientamoeba fragilis* trophozoite. X 1700. (From Wenrich, 1944, J. Morph. 74:467)

ENTAMOEBA CAPRAE
FANTHAM, 1923

Fantham (1923) described this species from the intestine and reticulum of a lightly infected goat in South Africa. It is very large, one streaming individual measuring 34 by 24 μ. The pseudopods are short and lobose, and red cells may be ingested. The nucleus is oval, 9 to 10 μ in diameter, with an eccentric endosome. No cysts were seen. The relationship of this form to other goat amoebae remains to be determined.

ENTAMOEBA SP.

Brenon (1953) tabulated 3 deaths from amoebic dysentery among the causes of death he observed in 1005 chinchillas in California. Since the amoebae of chinchillas have apparently not been described, they cannot be assigned to any species.

Genus *IODAMOEBA* Dobell, 1919

In this genus the nucleus is vesicular, with a large endosome rich in chromatin, a layer of lightly staining globules surrounding the endosome, and some achromatic strands between the endosome and nuclear membrane. The cysts contain a large glycogen body which stains darkly with iodine. They are ordinarily uninucleate. This genus occurs in vertebrates. A single species is recognized.

IODAMOEBA BUETSCHLII
(VON PROWAZEK, 1912)
DOBELL, 1919

Synonyms: *Entamoeba williamsi pro parte, Endolimax williamsi, Endolimax pileonucleatus, Iodamoeba wenyoni, Iodamoeba suis, Endolimax kueneni.*

Hosts: Pig, man, chimpanzee, gorilla, macaques and other monkeys and baboons, including *Macaca mulatta, M. irus, M. sancti-johannis, M. lasiotis, M. philippinensis, Cercocebus aethiops, Cercopithecus mona, C. ascanius, Papio papio,* kra monkey, green monkey, Anubis baboon, Gelada baboon and mandrill, (Mackinnon and Dibb, 1938; Wenrich, 1937). In addition, Mackinnon and Dibb (1938) found this species in the giant forest hog, *Hylochoerus meintritz-hageni.* Smith (1928) infected rats with *I. buetschlii* from man, and Pavloff (1935) did so with a strain from the pig. However, Simitch *et al.* (1959) were unable to infect man with cysts from fresh pig feces or to infect the pig with cysts from fresh human feces; they gave no details of their experiments.

Location: Cecum and colon.

Geographic Distribution: Worldwide.

Prevalence: *I. buetschlii* is the commonest amoeba of swine, and the pig was probably its original host. Frye and Meleney (1932) found it in 24% of 127 pigs in Tennessee. Alicata (1932) found it in 25% of 35 pigs in the U.S. Cauchemez (1921) estimated that it was present in 50 to 60% of the pigs he examined in France. Nöller (1922) found it in about 20% of those he examined in Germany. Pavloff (1935) found it in 29% of 530 pigs in France and 30% of 1310 pigs in Bulgaria. Simitch *et al.* (1959) found it in 8% of 1800 pigs in Yugoslavia. Kessel (1928a) found it in 42% of the pigs he examined in China, and Chang (1938) found it in 51% of 209 pigs in China.

According to Belding (1952), *I. buetschlii* was found in 8.4% of 17,568 persons in 20 surveys thruout the world, and in 4% of the people in American surveys. Wenrich (1937) found it in 44% of 55 apes and monkeys which he examined.

Morphology: Wenrich (1937), among others, has studied the morphology of *I. buetschlii.* The trophozoite is usually 9 to 14 μ long but may range from 4 to 20 μ. It has clear, blunt pseudopods which form slowly, and it moves rather slowly. The ectoplasm is clear, but not well separated from the granular endoplasm. Food vacuoles containing bacteria and yeasts are present in the cytoplasm. The nucleus is relatively large, and ordinarily contains a large, smoothly rounded, central

endosome surrounded by a vesicular space containing a single layer of periendosomal granules about midway between the endosome and the nuclear membrane. Fibrils extend to the nuclear membrane, but there are no peripheral granules inside the membrane. Stabler (1945) described tube-like processes which may be used for feeding in 12% and 27%, respectively, of the trophozoites of 2 human strains.

The cysts are often irregular in form. They are usually 8 to 10 μ long, but may range from 5 to 14 μ. They contain a single nucleus in which the periendosomal granules have usually aggregated into a crescent-shaped group at one side of the endosome, pushing it to one side. They contain a large, compact mass of glycogen which stains deeply with iodine. The glycogen disappears after 8 to 10 days in feces held at room temperature, and at the same time the cysts die and disintegrate (von Brand, 1932). There are no chromatoid bodies in the cysts, but they may contain small, deeply staining granules something like volutin granules.

Life Cycle: *I. buetschlii* reproduces by binary fission. Pan (1959) studied nuclear division in the trophozoites. He considered the process unique; his paper should be read for the details. The haploid number of chromosomes is usually more than 10--possibly 12.

Pathogenesis: *I. buetschlii* is nonpathogenic except under unusual circumstances. These have never been noted in the pig, but Andrew (1947) reported symptoms similar to those of chronic *E. histolytica* in a few persons, and Derrick (1948) described a fatal generalized infection in a Japanese soldier captured in New Guinea in which there were ulcers in the stomach, small intestine, large intestine, lymph nodes, lungs and brain.

Bionomics and Epidemiology: *I. buetschlii*, like other intestinal amoebae, is transmitted by cysts.

Cultivation: This species can be cultivated in the usual media.

Treatment: Little is known about treatment for *I. buetschlii*, but it can be eliminated by emetine.

Prevention and Control: The same preventive measures recommended for *E. histolytica* will also prevent *I. buetschlii* infections.

Genus *ENDOLIMAX* Kuenen and Swellengrebel, 1917

These are small amoebae. The nucleus is vesicular, with a comparatively large, irregularly shaped endosome composed of chromatin granules embedded in an achromatic ground substance, and with several achromatic threads connecting the endosome with the nuclear membrane. Cysts are present. This genus occurs in both vertebrates and invertebrates.

ENDOLIMAX NANA (WENYON AND O'CONNOR, 1917) BRUG, 1918

Synonyms: *Amoeba limax, Entamoeba nana, Endolimax intestinalis, Endolimax cynomolgi, Endolimax suis, Councilmania tenuis.*

Hosts: Man, pig, gorilla, chimpanzee, gibbon, macaques and other monkeys and baboons, including *Macaca mulatta, M. irus, M. sinica, M. sancti-johannis, M. lasiotis, M. philippinensis, Papio papio, Cercocebus aethiops, Cercopithecus ascanius, Gamadrillus* sp., and *Erythrocebus patas* (see Mackinnon and Dibb, 1938). These authors also reported a morphologically indistinguishable form from the capybara (*Hydrochoerus capybara*) and tree porcupine (*Coendou prehensilis*).

It is quite likely that *E. ratti* (see below) may be a synonym of *E. nana*, so that the latter's host range may be even broader than that given above. Chiang (1925) considered *E. ratti* a separate species because he was unable to infect 14 rats with *E. nana* from man. However, Kessel (1928) succeeded in doing so, and Smith (1928) found

an *E. nana*-like amoeba in 4 of 63 rats which had been fed human feces but did not know whether it had already been present in the rats.

Dobell (1933) transmitted *E. nana* from *Macaca sinica* to a man (himself) and from man to *M. mulatta*. Kessel (1928) infected *M. irus* with *E. nana* from man. However, Simitch *et al.* (1959) were unable to infect 4 young pigs with *E. nana* from man and consequently named the pig form *E. suis*; they gave no details of their experiments.

Location: Cecum, colon. Hegner (1929) and Dobell (1933) found *E. nana* in the vagina of macaques, where it was most likely of fecal origin.

Geographic Distribution: Worldwide.

Prevalence: *E. nana* is common in man. According to Belding (1952), it was found in 20.5% of 18,333 persons in 20 surveys thruout the world and in about 14% of those examined in the United States. Frye and Meleney (1932) found it in 5.5% of 127 pigs in Tennessee, Alicata (1932) found it in 1 of 35 pigs in the U.S., Kessel (1928) found it in 14% of the pigs examined by him in China, and Chang (1938) found it in 15% of 209 pigs in China. Simitch *et al.* (1959) found it in 8% of 1800 pigs in Yugoslavia.

Morphology: The trophozoites are 6 to 15 μ in diameter with an average of 10 μ. They move sluggishly by means of a few blunt, thick pseudopods. The endoplasm is granular, vacuolated and contains bacteria and crystals. The nucleus contains a large, irregular endosome composed of a number of chromatin granules. Several achromatic fibrils run from the endosome to the nuclear membrane. There are ordinarily no peripheral chromatin granules, but Stabler (1932) noted that they are formed after fixation in Schaudinn's fluid containing 20% acetic acid. The cysts are oval, often irregular, and thin-walled; they are usually 8 to 10 μ long but may range from 5 to 14 μ. The mature cysts contain 4 nuclei, and they may contain ill-defined glycogen bodies. They have no chromatoid bodies but may have small granules resembling volutin and occasionally a few filaments of uncertain nature.

Life Cycle: Reproduction is by binary fission in the trophozoite stage. The amoeba which leaves the cyst is multinucleate, but it divides into uninucleate amoebulae which grow into ordinary trophozoites.

Pathogenesis: *E. nana* is non-pathogenic.

Bionomics and Epidemiology: *E. nana* is transmitted in the same way as other enteric amoebae. Dobell (1933) found that its cysts could live at least 2 weeks at room temperature (15-24° C) and at least 3 weeks at 10° C, while all the trophozoites died in 24 hours at both temperatures. Frye and Meleney (1932) found *E. nana* cysts in 1 out of 46 lots of flies which they examined in Tennessee.

Cultivation: This species can be cultivated in the usual media.

Treatment: Little is known about the treatment of *E. nana*. Dobell (1933) and others found that emetine has no effect on it.

Prevention and Control: The same preventive measures recommended for *E. histolytica* will also prevent *E. nana* infections.

ENDOLIMAX RATTI
CHIANG, 1925

This species, which may be a synonym of *E. nana*, occurs in the cecum and colon of laboratory and wild rats. Andrews and White (1936) found it in 1 out of 2515 wild rats in Baltimore, and Baldassari (1935) found it in 1 of 225 wild rats in Toulon, France. Chiang (1925) did not describe it, but merely stated that it was morphologically identical with *E. nana*.

ENDOLIMAX CAVIAE
HEGNER, 1926

This species occurs commonly in the cecum of the guinea pig. Hegner (1926) found it in Baltimore and Hegner and Chu (1930) found it in the Philippines. Nie (1950) found it in 18% of 84 guinea pigs in Pennsylvania. It is somewhat smaller than *E. nana*, the trophozoites measuring 5 to 11 by 5 to 8 μ, but otherwise resembles it. Nie saw one specimen with an ingestion tube. The cysts are apparently unknown.

ENDOLIMAX GREGARINIFORMIS
(TYZZER, 1920)
HEGNER, 1929

Synonyms: *Pygolimax gregariniformis*, *Endolimax janisae*, *Endolimax numidae*.

This species is found in the ceca of the chicken, turkey, guinea fowl, pheasant, domestic goose, domestic duck and various wild birds, including the black duck (*Anas rubripes tristis*), black-crowned night heron (*Nycticorax nycticorax*) and screech owl.

E. gregariniformis was first described by Tyzzer (1920) from the turkey; he transmitted it easily to the chicken. Hegner (1926) described it from the chicken, naming it *E. janisae*. Hegner (1929a) found the same species and another form which he named *E. numidae* in the guinea fowl. The latter was smaller than *E. gregariniformis*, averaging 4 by 3 μ, but nevertheless fell within its size range and did not differ from it morphologically. Hegner (1929a) infected chicks with both sizes of *Endolimax* from the guinea fowl and also with *Endolimax* from the domestic goose, domestic duck and screech owl. Richardson (1934) infected chicks with *Endolimax* from the duck, goose, pheasant, black duck and black-crowned night heron.

E. gregariniformis occurs thruout the world and is non-pathogenic. McDowell (1953) found it in over 50% of a large number of chickens he examined in Pennsylvania.

The trophozoites of *E. gregariniformis* are usually 4 to 13 μ long with a mean of 9 by 5 μ, altho Hegner (1929a) found a small race in the guinea fowl. The trophozoites are oval, often with a posterior protuberance, and move sluggishly. The ectoplasm is not clearly separated from the endoplasm. The food vacuoles contain bacteria. The nucleus is very similar to that of *E. nana* but tends to have a larger endosome and a more apparent nuclear membrane, often with chromatin granules at the juncture of the achromatic threads with the membrane. The cysts have 4 nuclei when mature; they measure 7 to 8 by 8 to 11 μ with a mean of 10 by 7 μ (McDowell, 1953). They tend to be somewhat lima bean-shaped instead of truly ovoid, and are often highly vacuolated.

Genus *DIENTAMOEBA* Jepps and Dobell, 1918

These are small amoebae, usually with 2 nuclei. The nuclei are vesicular, with a delicate membrane and an endosome consisting of several chromatin granules connected to the nuclear membrane by delicate strands. No cysts are known. Dobell (1940) considered that this genus might be an aberrant flagellate closely related to *Histomonas*.

DIENTAMOEBA FRAGILIS
JEPPS AND DOBELL, 1918

This species occurs in the cecum and colon of man and also of some monkeys. According to Belding (1952), it was found in 4.2% of 7120 persons in 14 surveys thruout the world. Hegner and Chu (1930) found *D. fragilis* in 2 out of 44 *Macaca philippinensis* in the Philippines, and Knowles and Das Gupta (1936) found it in 1 out of 4 *M. irus* in India. In addition, Noble and Noble (1952) mentioned finding a *Dientamoeba* in sheep feces in California.

Only trophozoites are known for this species. They are very sensitive to

environmental conditions, bursting in water and becoming degenerate in older fecal samples. In order to identify them, smears of fresh feces should be fixed in Schaudinn's fluid containing 10 to 20% acetic acid or in Bouin's fluid and stained with iron hematoxylin. Their morphology has been studied by Wenrich (1936, 1939, 1944) and Dobell (1940), whose accounts do not always agree.

The trophozoites range from 3 to 22 μ but are usually 6 to 12 μ in diameter. The ectoplasm is distinct from the endoplasm, which contains food vacuoles filled with bacteria, yeasts, starch granules, and parts of cells. In fresh feces there may be a single clear, broad pseudopod. About 3/5 of the protozoa contain 2 nuclei which are connected by a filament or desmose. This appears to be one of the first structures to disappear during degeneration. Each nucleus is vesicular and has an endosome composed of 4 to 8 granules from which a few delicate fibers radiate to the nuclear membrane. There is no peripheral chromatin.

Reproduction is by binary fission. There are 4 chromosomes.

At one time *D. fragilis* was thought to be non-pathogenic, and this is true in most cases. However, in some persons it causes a mucous diarrhea and gastrointestinal symptoms. It does not invade the tissues, but may cause low-grade irritation of the intestinal mucosa, excess mucus secretion and hypermotility of the bowel. There may be mild to moderate abdominal pain and tenderness or discomfort. There may also be an increase in eosinophiles.

The mode of transmission of *D. fragilis* is not clear, since there are no cysts and the trophozoites are so delicate. Dobell (1940) was unable to infect himself by mouth or 2 monkeys by mouth or rectally and suggested, by analogy with *Histomonas*, that *D. fragilis* might possibly be transmitted by an intestinal nematode such as *Trichuris*. This idea has been partially confirmed by Burrows and Swerdlow (1956), who found small, amoeboid organisms resembling *D. fragilis* in the eggs of *Enterobius vermicularis* and suggested that the pinworm might be the vector.

D. fragilis can be readily cultivated in the usual culture media. It is sensitive to most amoebicidal drugs, including carbarsone, diodoquin and erythromycin.

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Chapter 8

THE TELOSPORASIDA AND THE COCCIDIA PROPER

All members of the class Telosporasida are parasitic. They have simple spores, without polar filaments. (The spore has been lost secondarily in a few genera.) Each spore contains 1 to many sporozoites. Pseudopods, cilia and flagella are absent, except for flagellated microgametes in some groups. Locomotion is by body flexion or gliding. Reproduction is both sexual and asexual, and there may or may not be alternation of generations. Most Telosporasida are saprozoic, but a few, including the trophozoites of the malaria parasite, are holozoic.

Most of the Telosporasida probably arose from the Mastigasida, but some may have arisen from the Sarcodasida. However, it is difficult to be sure of their origin because of their lack of the usual organelles of locomotion.

The classification of this and related groups is still the subject of considerable difference of opinion among taxonomists, and that used in this book is not considered definitive. It may require several generations of parasitologists to work out a universally acceptable one. This class is divided into 2 subclasses, of which the Gregarinasina parasitize invertebrates and the Coccidiasina occur in both vertebrates and invertebrates. In the latter group, the mature trophozoite is ordinarily intracellular and comparatively small.

The coccidia and their relatives belong to the order Eucoccidiorida. In this order, schizogony is present and the life cycle involves both sexual and asexual phases. Members of the order are found in the epithelial and blood cells of vertebrates and invertebrates.

The coccidia proper belong to the suborder Eimeriorina, which is differentiated from the other 2 suborders by several features of its life cycle. The macrogamete and microgametocyte develop independently, the microgametocyte produces many microgametes, the zygote is not motile, and the

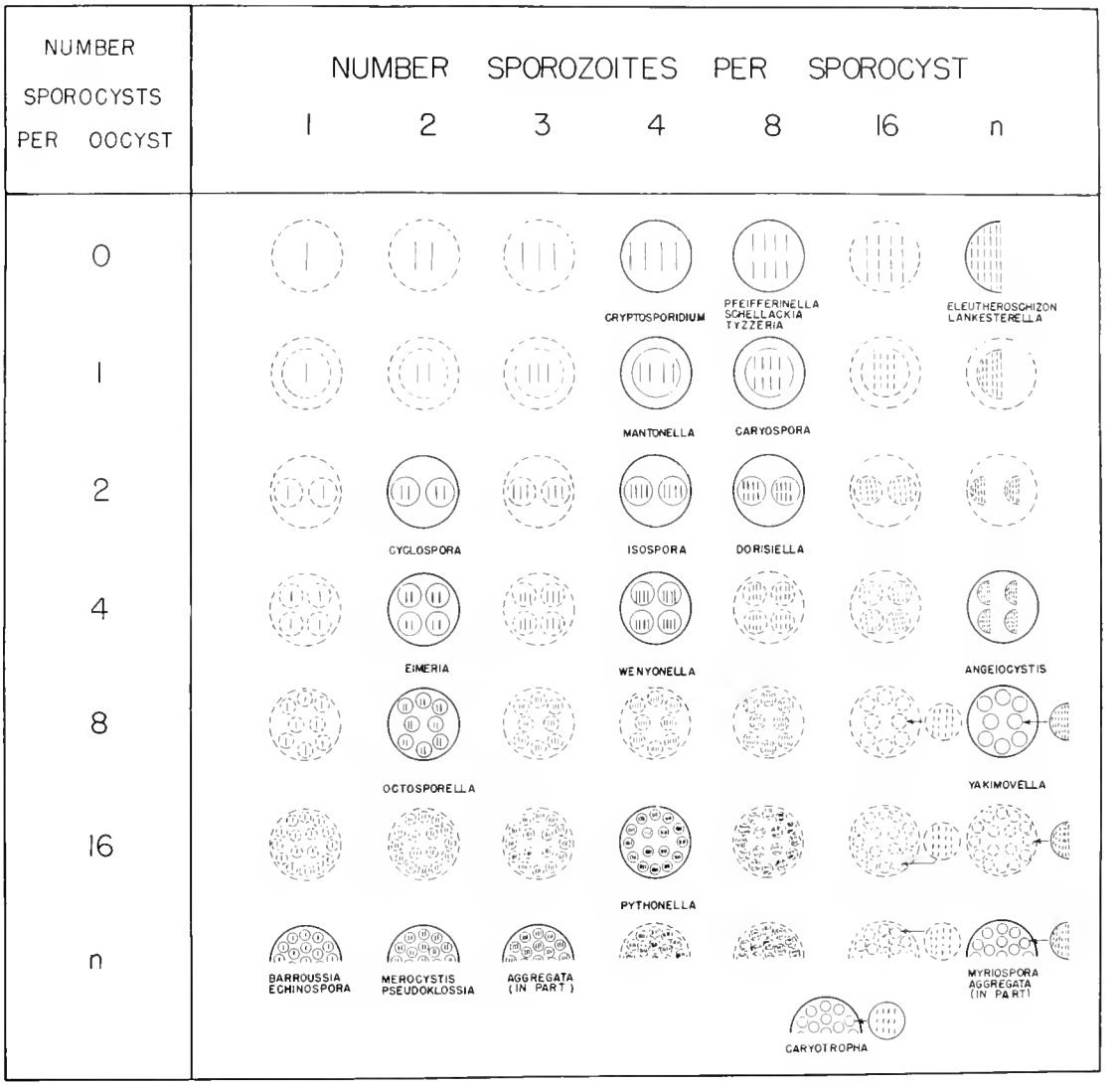

Fig. 22. Numbers of sporocysts per oocyst and of sporozoites per sporocyst in the genera of the suborder Eimeriorina. (In the genera without sporocysts, the numbers of sporozoites per oocyst are given.) (Original)

sporozoites are typically enclosed in a sporocyst. All the coccidia of domestic animals and man, with one possible exception, belong to two families, the Eimeriidae and Cryptosporidiidae. Another family, the Lankesterellidae, is of considerable interest. Becker (1934) wrote a classic review of the coccidia. Orlov (1956) discussed those of domestic animals, but was seriously handicapped by lack of information about non-Russian work. Becker (1956) and Pellérdy (1956, 1957) have given checklists of the species of coccidia. The coccidia of the avian orders Galliformes, Anseriformes and Charadriiformes were reviewed by Levine (1953).

FAMILY EIMERIIDAE

Members of this family have a single host. Schizogony and gametogony take place within the host cells, and sporogony ordinarily occurs outside the host's body. The oocysts and schizonts lack an attachment organ. The oocysts contain 0, 1, 2, 4 or many sporocysts, each containing 1

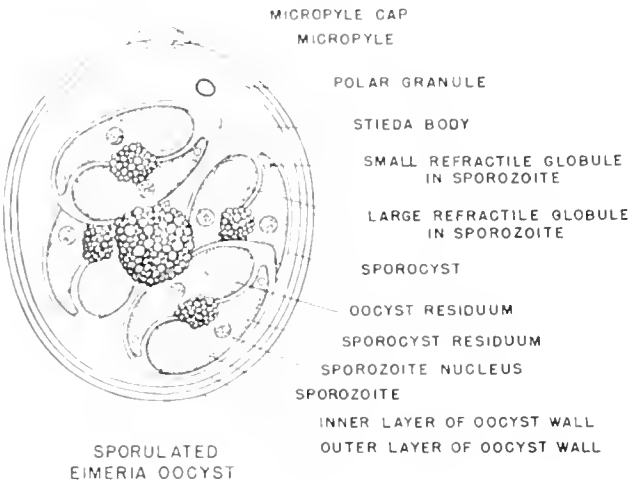

Fig. 23. Structures of sporulated *Eimeria* oocyst. (Original)

or more sporozoites. The microgametes have 2 flagella. The genera are differentiated by the number of sporocysts in their oocysts and the number of sporozoites in each oocyst.

Morphology. The morphology of a typical oocyst, that of *Eimeria*, is shown in Fig. 23. The oocyst wall is composed of 1 or 2 layers and may be lined by a membrane. It may have a micropyle, which may be covered by a micropylar cap. Within the oocyst in this genus are 4 sporocysts, each containing 2 sporozoites. There may be a refractile polar granule in the oocyst. There may be an oocyst residuum or a sporocyst residuum in the oocyst and sporocyst, respectively; these are composed of material left over after the formation of the sporocysts and sporozoites. The sporocyst may have a knob, the Stieda body, at one end. The sporozoites are usually sausage- or comma-shaped, and may contain 1 or 2 clear globules.

Location. Most coccidia are intracellular parasites of the intestinal tract, but a few occur in other organs such as the liver and kidney. Each species is usually found in a specific location within the intestinal tract; some are found in the cecum, others in the duodenum, still others in the ileum, etc. They may invade different cells in these locations. Some species are found in the mucosal cells at the tips of the villi, others in the crypts and still others in the interior of the villi. Their location within the host cell also varies. Some species are found above the host cell nucleus, while others are found beneath it and a few occur inside it. Some species enlarge the host cell only slightly, while others cause it to become enormous. The host cell nucleus is also often greatly enlarged even tho it may not be invaded.

Life Cycle: The life cycles of the Eimeriidae are similar, and can be illustrated by that of *Eimeria tenella*, which is found in the ceca of the chicken (Fig. 24). It was first worked out in a classic paper by Tyzzer (1929). The oocysts are passed in the feces; at this time they contain a single cell, the sporont. They must have oxygen in order to develop to the infective stage, a process known as sporulation or sporogony. The sporont, which is diploid, undergoes reduction division and throws off a refractile polar body. The haploid number of chromosomes is 2 (Walton, 1959). The sporont divides to form 4 sporoblasts, each of which then develops into a sporocyst. Two sporozoites develop within each sporocyst. Sporulation takes 2 days at ordinary temperatures. The oocysts are then infective and ready to continue the life cycle.

When eaten by a chicken, the oocyst wall breaks, releasing the sporozoites. The factors which cause excystation have not been determined. Itagaki and Tsubokura (1958) found that pancreatic juice did not cause excystation of *E. tenella*, and Landers (1960) was unable to induce excystation by treating the oocysts of *E. nieschulzi* from the rat with pepsin, trypsin, pancreatin, pancreatic lipase or bile. Ikeda (1960), however, reported that pancreatic juice did cause excystation of *E. tenella*, and that trypsin was the responsible enzyme.

According to Challey and Burns (1959) and Pattillo (1959), the sporozoites first enter the cells of the surface epithelium. Pattillo (1959) observed passageways, which he called penetration tubes, in the striated border and epithelium thru which the sporozoites passed. They deploy along

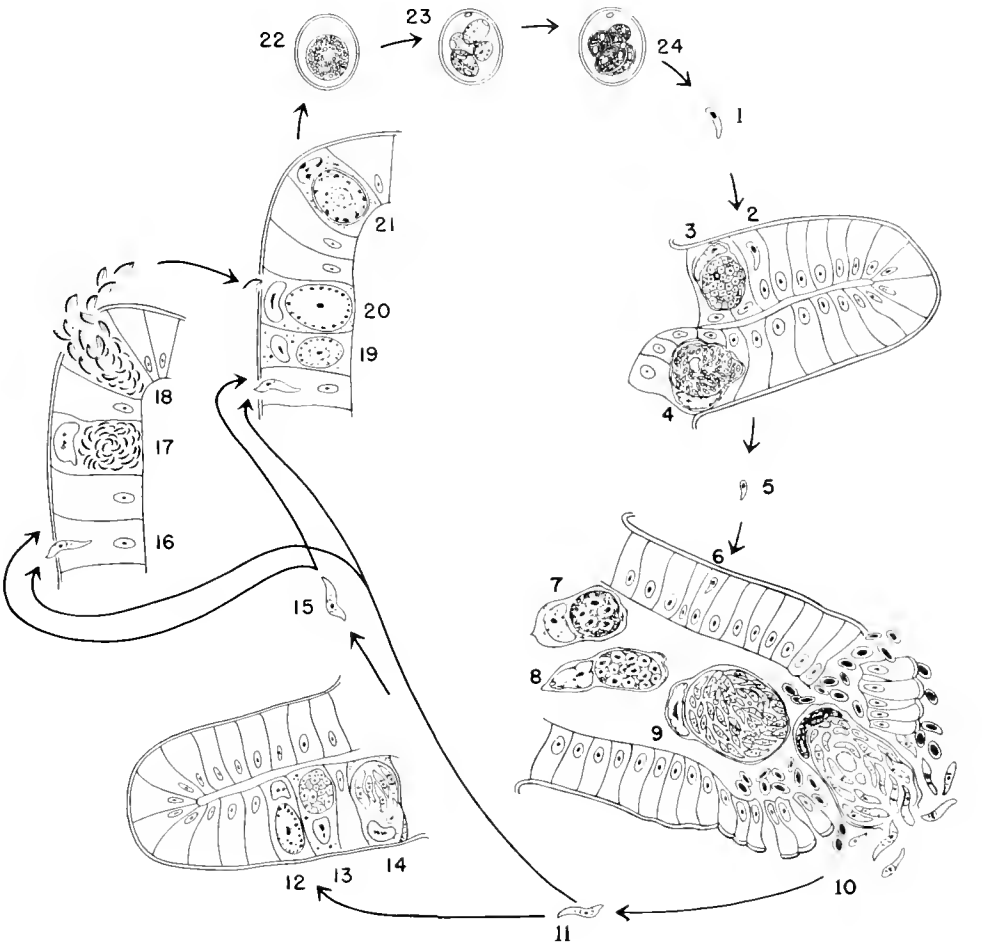

Fig. 24. Life cycle of the chicken coccidium, *Eimeria tenella*. A sporozoite (1) enters an intestinal endothelial cell (2), rounds up, grows, and becomes a first generation schizont (3). This produces a large number of first generation merozoites (4), which break out of the host cell (5), enter new intestinal endothelial cells (6), round up, grow, and become second generation schizonts (7, 8). These produce a large number of second generation merozoites (9, 10), which break out of the host cell (11). Some enter new host intestinal endotheliat cells and round up to become third generation schizonts (12, 13), which produce third generation merozoites (14). The third generation merozoites (15) and the great majority of second generation merozoites (11) enter new host intestinal endothelial cells. Some become microgametocytes (16, 17), which produce a large number of microgametes (18). Others turn into macrogametes (19, 20). The macrogametes are fertilized by the microgametes and become zygotes (21), which lay down a heavy wall around themselves and turn into young oocysts. These break out of the host cell and pass out in the feces (22). The oocysts then sporulate. The sporont throws off a polar body and forms 4 sporoblasts (23), each of which forms a sporocyst containing 2 sporozoites (24). When the sporulated oocyst (24) is ingested by a chicken, the sporozoites are released (1). (Original)

the basement membrane and then pass thru it into the lamina propria. Here they are engulfed by macrophages and carried by them to the glands of Lieberkühn. They then leave the macrophages and enter the epithelial cells of the glands, where they are found below the host cell nucleus, i.e., on the side away from the lumen. We do not know how common this method of penetration is among the coccidia; Van Doorninck and Becker (1957) first found it in *E. necatrix* of the chicken.

Once in a glandular epithelial cell, each sporozoite rounds up and becomes a first generation schizont. By a process of asexual multiple fission (schizogony), each schizont forms about 900 first generation merozoites, each about 2 to 4 μ long. These get their name from the Greek word for mulberry, which they resemble before they separate. They break out into the lumen of the cecum about 2.5 to 3 days after infection. Each first generation merozoite enters a new host cell, and rounds up to form a second generation schizont, which lies above the host cell nucleus. By multiple fission it forms about 200 to 350 second generation merozoites about 16 μ long. These are found 5 days after infection. Some of them enter new intestinal cells, round up to form third generation schizonts, which lie beneath the host cell nuclei and produce 4 to 30 third generation merozoites about 7 μ long.

Most of the second generation merozoites, however, enter new host cells and begin the sexual phase of the life cycle, known as gametogony. Most of these merozoites turn into female gametes (macrogametes), which simply grow until they reach full size. Some of the merozoites turn into male gametocytes (microgametocytes). Both the macrogametes and microgametocytes lie below the host cell nuclei. Within each microgametocyte a large number of tiny biflagellate microgametes are formed. These break out and fertilize the macrogametes.

The resultant zygote lays down a wall around itself in the following way: The macrogametes contain one or two layers of eosinophilic plastic granules in their cytoplasm; these are composed of muco-protein (Kheisin, 1958). They pass to the periphery, flatten out and coalesce to form the oocyst wall after fertilization. The formation of this wall marks the transition of a fertilized macrogamete into an oocyst. According to Monne and Hönig (1954), the outer layer of the oocyst wall is a quinone-tanned protein and the inner layer is a lipid coat firmly associated with a protein lamella.

The oocysts then break out of their host cells, enter the intestinal lumen, and pass out in the feces. The prepatent period, from the time of infection to the appearance of the first oocysts in the feces, is 7 days. Oocysts continue to be discharged for a number of days thereafter, due to the fact that the sporozoites do not all enter the host cells immediately but may remain in the lumen for some time, and also because many of them are retained in a plug of material in the ceca for some days before they are eliminated.

In the absence of reinfection, coccidial infections are self-limiting. Asexual reproduction does not continue indefinitely as it does, for example, in *Plasmodium*. In *E. tenella*, 3 generations of merozoites are produced; in other species there may be 1, 2 or 4. After this, the life cycle enters its sexual phase; the oocysts are formed, eliminated from the body, and the infection is over. Reinfection may take place, but the host develops more or less immunity following primary infection.

The number of oocysts produced in an animal per oocyst fed depends in part on the number of merozoite generations and the number of merozoites per generation. A single oocyst of *E. tenella* containing 8 sporozoites is theoretically capable of producing 2,520,000 second generation merozoites (8 x 900 x 350), each of which can develop into a macrogamete or microgametocyte.

In *E. bovis* of cattle, there is only a single asexual generation, but a giant schizont containing about 120,000 merozoites is formed (Hammond *et al.* 1946). In the rat, *E. nieschulzi* is theoretically capable of producing 1,500,000 oocysts per oocyst fed, *E. miyairii* 38,016, and *E. separata* only 1536 (Roudabush, 1937). In *E. nieschulzi* there are 4 generations of merozoites, while in the latter two species there are only 3, and fewer merozoites are usually produced in each than in *E. nieschulzi*. In the rabbit, *E. magna* produces 800,000 oocysts per oocyst fed, *E. media* produces 150,000 and *E. coecicola* 100,000 (Kheisin, 1947, 1947a).

The actual numbers of oocysts produced per oocyst fed are usually considerably lower than the theoretical ones. If the host is resistant or immune, it destroys many merozoites, and many others pass out in the feces before they have time to enter host cells. The infecting dose is also an important factor in determining the number of oocysts produced. The greater the infecting dose, the smaller the number of oocysts usually produced per oocyst fed. For example, Hall (1934) obtained a yield of 1,455,000 oocysts of *E. nieschulzi* per oocyst fed when the infecting dose was 6 oocysts, 1,029,666 when it was 150 oocysts, and 144,150 when it was 2000 oocysts. If the infecting dose is too small, however, smaller numbers of oocysts are produced. Hall (1934) found that when only a single oocyst was fed, the yield was 62,000.

Similarly, Brackett and Bliznick (1950, 1952) found that with *E. acervulina* of the chicken, 9000 oocysts were produced per oocyst fed when the infecting dose was 200 oocysts, 35,000 to 72,000 when it was 2000 oocysts, 35,000 when it was 10,000 oocysts, and 7,600 when it was 20,000 oocysts. With *E. maxima* of the chicken, they found that 11,500 oocysts were produced per oocyst fed when the infecting dose was 200 oocysts, 2,250 when it was 2000 oocysts, and 940 to 2900 when it was 10,000 oocysts. With *E. necatrix* of the chicken, they found that 50,000 oocysts were produced per oocyst fed when the infecting dose was 200 oocysts, and 2400 when it was 2000 oocysts. With *E. tenella* of the chicken, they found that the maximum number of oocysts produced per oocyst fed in numerous experiments was 400,000. However, in one series of 2-week-old chicks this figure ranged from 1200 for chicks fed 40,000 oocysts to 80,000 when the infecting dose was 50 oocysts.

All the factors responsible for these results are not known. More effective mobilization of the host's defenses is probably important, but lack of enough epithelial cells to parasitize, sloughing of patches of epithelium, increased intestinal motility with resultant diarrhea and elimination of merozoites before they can reach a cell, and entrapment of merozoites in tissue debris and cecal cores may also play a part.

Pathogenesis. While many species of coccidia are pathogenic, many others are not. Pathogenicity depends on a number of factors, some of which are probably still unknown. Among those which might be mentioned are the number of host cells destroyed per infecting oocyst (which depends upon the number of merozoite generations and the number of merozoites per generation) and the location of the parasite in the host tissues and within the host cells. The size of the infecting dose or doses, the degree of reinfection, and the degree of acquired or natural immunity of the host are also important.

Even with a pathogenic species, the final effect on the host depends on the interplay between many factors; it may range from rapid death in susceptible animals to an imperceptible reaction in immune ones.

If disease is present, the signs are those of a diarrheal enteritis. There may or may not be blood in the feces, depending on the parasite species and severity of infection. Affected animals gain weight poorly, become weak and emaciated, or may even die, depending again on the parasite species and the size of the infecting dose. Young animals are much more commonly affected than older ones. Those animals which recover develop an immunity to the particular species which infected them. However, this is not an absolute immunity, and recovered adult animals are often continuously reinfected so that they carry light infections which do not harm them but which make them a source of infection for the young. In addition, under conditions of stress their immunity may be broken down and they may suffer from the disease again.

Differentiation of Species. Both morphological and biological characters are used to separate the species of coccidia. Both the endogenous and exogenous stages of the life cycle may differ morphologically. However, since the endogenous

stages of many species are unknown, the structure of the oocyst is most commonly used. The feeling is sometimes expressed that the oocysts have so few structures that not many species can be distinguished morphologically, but conservative calculation shows that at least 2,654,208 morphologically different oocysts are possible in the genus *Eimeria* alone (Levine, 1961).

A second group of criteria is the location of the endogenous stages in the host. This has been discussed above. Host specificity is a third criterion. This varies with the protozoan genus and to some extent with the species. In general, the host range of *Isospora* and *Tyzzeria* species is relatively broad. Several members of the same host order may be infected by the same species of these genera. For example, *Isospora bigemina* occurs in the dog, cat, ferret and mink, while *Tyzzeria anseris* has been found not only in the domestic goose and several other members of the genus *Anser*, but also in the Canada goose and Atlantic brant (both *Branta*) and whistling swan (*Olor*). On the other hand, the host range of *Eimeria* species is relatively narrow. A single species rarely infects more than one host genus unless the latter are closely related.

Cross-immunity studies are also used in differentiating the coccidia of a particular host species from each other. Infection of an animal with one species of coccidium produces immunity against that species but not against other species which occur in the same host.

Diagnosis: Coccidiosis can be diagnosed by finding the coccidia on microscopic examination. There are several pitfalls in diagnosis. Each species of domestic and laboratory animal has several species of coccidia, some of which are pathogenic and some of which are not. Since an expert is often needed to differentiate between some of the species, the mere presence of oocysts in the feces, even in the presence of disease signs, is not necessarily proof that the signs are due to coccidia and not to some other agent.

Following recovery from a coccidial infection, an animal is relatively immune to reinfection with the same species. This immunity is not so solid that the animal cannot be reinfected at all, but it does mean that the resultant infection will be low-grade (except possibly under conditions of stress) and will not harm the host. Such low-grade infections are extremely common, i.e., the animals have coccidiasis rather than coccidiosis. Hence, the presence in the feces of oocysts of even highly pathogenic species of coccidia does not necessarily mean that the animal has clinical coccidiosis.

On the other hand, coccidia may cause severe symptoms and even death early in their life cycle before any oocysts have been produced. This occurs commonly, for example, with *E. tenella* of the chicken and *E. zurnii* of the ox. Consequently, failure to find oocysts in the feces in a diarrheal disease does not necessarily mean that the disease is not coccidiosis.

The only sure way to diagnose coccidiosis, then, is by finding lesions containing coccidia at necropsy. Scrapings of the lesions should be mixed on a slide with a little physiological salt solution and examined microscopically. It is not enough to look for oocysts, but schizonts, merozoites, gametes and gametocytes inside the host cells must be sought for and recognized.

Some species of coccidia can be identified from their unsporulated oocysts, but study of the sporulated oocysts is often desirable. Oocysts can be sporulated by mixing the feces with several volumes of 2.5% potassium bichromate solution, placing the mixture in a thin layer in a Petri dish and allowing it to stand for 1 day to 2 weeks or more, depending on the species. The potassium bichromate prevents bacterial growth which might kill the protozoa, and the thin layer is necessary so that oxygen can reach the oocysts.

Treatment. The first compound found effective against coccidia was sulfur, which was introduced by Herrick and

Holmes (1936). Later, Hardcastle and Foster (1944) introduced borax. Neither of these compounds was a satisfactory anticoccidial drug. Sulfur interferes with calcium metabolism, causing a condition known as sulfur rickets in chickens, while borax is only partially effective and in addition is toxic in therapeutic doses.

The first practical anticoccidial drugs were the sulfonamides, of which the first to be used was sulfanilamide, introduced by P. P. Levine (1939). Since that time many different drugs have been used, particularly against *Eimeria tenella* of the chicken. These include not only sulfonamides but also derivatives of phenylarsonic acid, diphenylmethane, diphenyldisulfide, diphenylsulfide, nitrofuran, triazine, carbanilide, imidazole and benzamide. Several thousand papers have probably been published on coccidiostatic drugs, and their use in poultry production is so common in the United States that it is difficult to obtain a commercial feed which does not contain one or another of them. They are used to a considerably lesser extent for other classes of livestock.

None of these drugs will cure a case of coccidiosis once signs of the disease have appeared. They are all prophylactic. They must be administered at the time of exposure or soon thereafter in order to be effective. They act against the schizonts and merozoites and occasionally against the sporozoites, preventing the life cycle from being completed. They are not effective against the gametes. Hence, since exposure in nature is continuous, these drugs must be fed continuously. This is usually done by mixing them with the feed or water.

Nowhere is a knowledge of the normal course of the disease more important than in interpreting the results of treatment of coccidiosis, and nowhere is the controlled experiment more important than in research in this field. This disease is self-limiting not only in the individual patient but also in a flock or herd. In a typical outbreak of coccidiosis, signs of disease appear in only a few animals at first, the number of affected animals builds up rapidly to a peak in about a week, and then the disease subsides spontaneously. In the early stages, most farmers do little, thinking that the condition is unimportant and will soon be over. Once more animals become affected and losses increase, it takes a little time to establish a diagnosis, so treatment is often not started until the outbreak has reached its peak. Under these circumstances, it matters little what treatment is used--the disease will subside. This is the reason why so many quack remidies used to get glowing testimonials from satisfied users.

A similar course of events is encountered by the small animal practitioner. The patient with coccidiosis is not brought to him until it is already sick. By this time it is too late for any anticoccidial drug to be of value, altho supportive treatment and control of secondary infections may be helpful. If the patient recovers, however, whatever drug happened to be used is often given undeserved credit. Such drugs are like Samian clay, which was Galen's favorite remedy. He said that it cured all diseases except those which were incurable, in which case the patient died.

Collins (1949) described the "four-pen test" which should be used in evaluating coccidiostats and other drugs. The birds in one pen are infected with coccidia and treated with the compound under test. Those in the second pen are infected and untreated, those in the third pen are uninfected and treated, and those in the fourth pen are uninfected and untreated. Comparison of the first 2 pens determines whether the compound has any effect on the coccidia; the third and fourth pens are used to determine whether the drug has any effect on the chickens themselves and to make sure that no extraneous infection has taken place.

After an animal has been receiving a coccidiostatic drug for some time during exposure to infection, it develops an immunity to the coccidia. This occurs because the sporozoites are not affected by the drug but invade the tissue cells and stimulate the host's defenses.

After coccidiostats had been mixed in poultry feeds for a number of years, it was inevitable that drug resistant strains of coccidia would appear. The first report of this was by Waletzky, Neal and Hable (1954), who found a field strain of *Eimeria tenella* resistant to sulfonamides. Cuckler and Malanga (1955) reported on 40 field strains of chicken coccidia which were resistant to one coccidiostat or another, and drug resistance is now a well-known complicating factor in the use of these agents. A race has developed between the coccidia and the pharmaceutical houses, and some day, *horribile dictu*, we may be reduced to sanitation to control coccidia.

Mixed Infections. All domestic animals have more than one species of coccidia. Some are highly pathogenic, others less so, and still others practically nonpathogenic. Pure infections with a single species are rare in nature, so the observed effect is the resultant of the combined actions of the particular mixture of coccidia and other parasites present, together with the modifying effects of the nutritional condition of the host and environmental factors such as weather and management practices.

In the remainder of this chapter, each species of coccidium in a particular host animal will be taken up first, and then a general discussion of coccidiosis in the host will follow.

Genus *EIMERIA* Schneider, 1875

In this genus the oocyst contains 4 sporocysts, each of which contains 2 sporozoites.

EIMERIA ALABAMENSIS CHRISTENSEN, 1941

Hosts: Ox.

Location: Posterior half of ileum, especially within a few feet forward from the ileo-cecal valve. In heavy infections, the cecum and upper colon may be involved.

Geographic Distribution: North America.

Prevalence: Davis, Boughton and Bowman (1955) found this species in 93% of 102 dairy calves in 6 herds in southeastern United States in a weekly fecal survey; they found it in 24% of 135 animals from which only a single fecal sample was taken; it was present in all of 26 herds from which at least 5 animals were examined. Hasche and Todd (1959) found it in 42% of 355 cattle in Wisconsin.

Morphology: The oocysts have been described by Christensen (1941). They measure 13 to 24 by 11 to 16 μ with a mean of 18.9 by 13.4 μ. They are typically piriform but may also be subellipsoidal or subcylindrical. The oocyst wall is thin, delicate, homogeneous, transparent, colorless to greyish-lavender to pale brownish yellow, slightly thinner at the narrow end but without a perceptible micropyle. During sporulation there is a parachute-shaped cap at each end of the sporoblasts. Sporulation takes 4 to 5 days. The sporocysts are elongate and gently tapered. Neither oocyst nor sporocyst residua are present. A polar granule is presumably absent.

Life Cycle: Davis, Bowman and Boughton (1957) described the life cycle of *E. alabamensis*. It is unusual in being an intranuclear parasite, occurring within the nuclei of the epithelial cells at the tips of the villi. Excysted sporozoites were seen in the cytoplasm of the intestinal epithelial cells 2 days after infection. They enter the nuclei and round up, forming schizonts. These are present as early as 2 days after infection and are uncommon by the 8th day. They form 16 to 32 merozoites, which are slender and spindle-shaped while still within the parent schizont but appear short, with bluntly rounded ends in the intracellular spaces and crypts. Davis, Bowman and Boughton (1957) thought that there is probably more than one generation of schizonts.

Macrogametes and microgametocytes first appear 4 days after infection. Most are found in the lower third of the small intestine, but they may invade the cecum

and upper colon in heavy infections. Young oocysts still in the host cell nuclei first appear 6 days after infection. Multiple infections are common, as many as 3 schizonts or microgametocytes and 4 or 5 macrogametes or oocysts having been found in a single host cell nucleus. This crowding may affect the shape of the oocysts in heavy infections, making some of them wedge-shaped or asymmetrical.

The prepatent period in experimentally infected calves was found by Davis, Boughton and Bowman (1955) to range from 6 to 13 days with a mean of 8 to 9 days. The patent period ranged from 1 to 10 days with a mean of 4.6 days in 21 low-grade infections, and from 1 to 13 days with a mean of 7.2 days in 72 heavy infections.

Pathogenesis: Under field conditions, *E. alabamensis* is considered essentially non-pathogenic. However, Boughton (1943) produced clinical coccidiosis in 5 young calves by feeding them 200 million oocysts. Within 5 days they developed a severe diarrhea, with yellowish feces having a characteristic acrid odor. They become thin, and 1 calf died on the 8th day and another on the 14th. In the first calf the lower half of the small intestine was hyperemic and there was massive tissue involvement with merozoites and macrogametes. In the second calf there was enteritis in only the last 3 feet of the ileum, and only a few parasites remained in the tissues, most of these being within 1 foot of the ileocecal valve.

Davis, Boughton and Bowman (1955) fed two 14-month-old calves 140 million oocysts. One became diarrheic on the fifth day. Its feces were watery, yellowish green, with some bloody mucus and a sharp, acrid odor. The diarrhea gradually subsided. In the second calf the feces were soft toward the end of the prepatent period. A 7-month-old heifer which had previously been exposed to coccidial infection had a slight diarrhea on the 9th and 11th days following similar exposure, and a 2-year-old cow remained normal.

Immunity: Reinfection is considered common in the field. Davis, Boughton and Bowman (1955) reported that in 58 attempts to reinfect calves 2 or more times, there were 39 high-grade infections, 11 low-grade infections and 8 failures. Nine of the low-grade infections and 7 of the failures followed the third or subsequent inoculations. Some animals were reinfected as many as 4 times before reinfection attempts failed.

EIMERIA AUBURNENSIS CHRISTENSEN AND PORTER, 1939

Synonym: *Eimeria ildefonsoi* Torres and Ramos, 1939.

Hosts: Ox. In addition, Böhm and Supperer (1956) reported finding this species in a wild roe deer in Austria, but gave no morphological information on it.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: North America, South America (Brazil), Europe (Austria, Spain, England).

Prevalence: *E. auburnensis* is one of the commonest coccidia of cattle in North America. Davis and Bowman (1952) found it in all of 20 calves in Alabama. Hasche and Todd (1959) found it in 45% of 355 cattle in Wisconsin. Torres and Ramos (1939) found it in 32% of 146 cattle in Brazil. Supperer (1952) found it in 3% of 130 cattle in Austria. According to Lapage (1956), Watkins found it in 91% of the calves he examined in Devonshire.

Morphology: The oocysts have been described by Christensen and Porter (1939) and Christensen (1941). They measure 32 to 46 by 20 to 26 μ with a mean of 38.4 by 23.1 μ. Their length-width ratios range from 1.32 to 2.08 with a mean of 1.67. They are typically elongate ovoid, but vary between sub-ellipsoidal and markedly tapered. The micropyle appears as a thin, pale area at the small end in unstained specimens, but when stained with iodine-eosin in physiological salt solution, a definite gap covered by a narrow black line which may be a flat operculum is seen.

A membrane lines the oocyst wall, which is illustrated as composed of a single layer. The oocyst wall is 1 to 1.5 μ thick, typically smooth, homogeneous, transparent and yellowish brown; relatively rarely it may be semi-transparent and heavily mammillated, and all gradations between these two conditions occur. The smooth-walled forms are more common than the rough.

The sporulation time at room temperature in Alabama is 2 to 3 days. There is no oocyst residuum or polar granule. The sporocysts were illustrated by Christensen and Porter (1939) as elongate with one end pointed. The sporozoites lie lengthwise, head to tail, in them and contain 3 clear globules, 1 of which may be the nucleus. The sporocyst residuum consists of rounded masses or individual granules between the sporozoites.

Christensen and Porter (1939) showed that the rough and smooth forms were the same species by infecting a calf with rough oocysts and recovering all types, but predominantly smooth ones, from it.

Life Cycle: The endogenous stages of this species are unknown. Christensen and Porter (1939) found that the prepatent period in one calf was 24 days. Large numbers of oocysts were discharged for 3 days, and small numbers for the next few weeks.

Pathogenesis: Christensen and Porter (1939) produced a profuse, watery, green diarrhea accompanied by slight apathy in a 2-week-old calf following administration of 8000 sporulated oocysts. The signs appeared 9 days after infection (i.e., 15 days before the first oocysts appeared in the feces) and continued for 5 days. According to Davis and Bowman (1952), infections with *E. auburnensis* are usually accompanied by straining and the passing of visible blood and mucus, especially following experimental inoculation with large numbers of oocysts or in natural outbreaks where contamination is heavy.

EIMERIA BOVIS (ZÜBLIN, 1908) FIEBIGER, 1912

Synonyms: *Coccidium bovis*, *Eimeria canadensis* (pro parte), *Eimeria smithi*, *Eimeria thianethi*, *Globidium fusiformis* (?).

Hosts: Ox, zebu, water buffalo. Wilson (1931) was unable to infect pigs or goats with this species.

Location: The schizonts are mostly in the small intestine and the sexual stages in the cecum, colon and terminal ileum.

Geographic Distribution: Worldwide.

Prevalence: This is one of the commonest coccidia of cattle. Boughton (1945) found it in 41% of 2492 bovine fecal samples in south-eastern U.S. Hasche and Todd (1959) found it in 41% of 355 cattle in Wisconsin. Supperer (1952) found it in 66% of 130 cattle in Austria. Cordero del Campillo (1960) reported it and other bovine species in Spain. Torres and Ramos (1939) found it in 49% of 136 cattle in Brazil. Yakimoff, Gousseff and Rastegaieff (1932) found it in 40% of 126 cattle in Uzbekistan. Yakimoff (1933) found it in 47% of 17 zebus, 23% of 30 water buffaloes and 39% of 44 cattle in Azerbaidzhan. Marchenko (1937) found it in 54% of 137 cattle in the North Caucasus. Rao and Hiregaudar (1954) stated that it is common in Bombay State, India. Ruiz (1959) found it in 7% of 100 adult cattle in the San Jose, Costa Rica abattoir.

Morphology: The oocysts of *E. bovis* were described by Christensen (1941). Five hundred oocysts measured 23 to 34 by 17 to 23 μ with a mean of 27.7 by 20.3 μ. Their length-width ratios ranged from 1.1 to 1.8 with a mean of 1.37. They are typically stoutly ovoid and somewhat blunted across the narrow end, but vary considerably in shape, especially in heavy infections, subellipsoidal, asymmetrical and elongated, tapered oocysts also occurring.

The micropyle is a lightened area at the small end. The oocyst wall is smooth, homogeneous, transparent, pale cloudy, greenish brown to yellowish brown, and slightly thinner toward the micropylar end. The wall is not so delicate as that of *E. alabamensis*. It is darker than that of *E. alabamensis* and lighter than that of *E. auburnensis*. Christensen (1941) illustrated the wall as composed of a heavy inner layer and a very thin, transparent outer layer, but he did not mention layers in his description. An oocyst residuum and polar granule are absent. A sporocyst residuum is present.

The sporulation time is 2 to 3 days.

Life Cycle: Hammond *et al.* (1946) described the endogenous stages of the life cycle of *E. bovis* in detail. There is a single asexual generation. The sporozoites invade the endothelial cells of the lacteals of the villi in the posterior half of the small intestine. These cells become detached from the lacteal lining and lie free and greatly swollen in the lumens of the lacteals. The schizonts are first found 5 days after infection. They grow to giant size, becoming mature 14 to 18 days after infection. A few may still be found as long as 30 days after inoculation, but most of these are degenerate. The mature schizonts measure 207 to 435 by 134 to 267 μ with a mean of 281 by 303 μ and contain 55,000 to 170,000 (mean, 120,000) merozoites. They are easily visible to the naked eye as whitish balls, and their presence was first pointed out by Boughton (1942) as a macroscopic lesion which could be used in diagnosing coccidiosis.

The merozoites are 9 to 15 μ (mean, 11.6 μ) long and about 2 μ wide. They are rounded at one end and taper abruptly to a point at the other. The nucleus is near the pointed end.

The sexual stages usually occur only in the cecum and colon, but in heavy infections they may be found in the terminal 3 or 4 feet of the small intestine. They occur in the epithelial cells of the intestinal glands. The cells at the base of the glands are invaded first, and later the rest of the gland becomes involved. The first sexual stages appear 17 days after inoculation. The macrogametes contain plastic granules in their cytoplasm, there being 1 layer of small granules near the surface and a less distinct layer of larger granules beneath it. Fertilization was not seen, but 2 stages in the union of nuclei were seen before formation of the oocyst wall.

According to Walton (1959), the haploid number of chromosomes in *E. bovis* is 2.

Oocysts appear 16 to 21 days after experimental infection. Large numbers are discharged for 5 to 7 days, and smaller numbers are present in the feces for 2 to 3 weeks. In 28 calves studied by Senger *et al.* (1959), oocysts were discharged for 7 to 15 days with a mean of 11.5 days.

Pathogenesis: *E. bovis* is one of the 2 most pathogenic of the bovine coccidia. Hammond, Davis and Bowman (1944) studied its effects in experimentally infected calves. An infective dose of 125,000 oocysts or more was generally needed to cause marked signs. These appeared about 18 days after infection, and consisted of diarrhea and/or bloody diarrhea, tenesmus, and temperatures as high as 106.6° F. One of 4 calves given 125,000 oocysts become moribund due to coccidiosis, while single calves given 250,000 to 1,000,000 oocysts all died or became moribund 24 to 27 days after infection.

The most severe pathologic changes occur in the cecum, colon and terminal foot of the ileum. They are due to the sexual stages of the coccidia. At first the mucosa is congested, edematous and thickened, with petechiae or diffuse hemorrhages. Its lumen may contain a large amount of blood. Later, the mucosa is destroyed and sloughed, and a patchy or continuous membrane forms over its surface. The submucosa may also be destroyed. If the animal survives, both mucosa and submucosa are later replaced.

Immunity: Senger *et al.* (1959) found that inocula of 10,000, 50,000 or 100,000 oocysts of *E. bovis* produced a good deal of immunity to reinfection. The immunity developed rapidly, calves being resistant to challenge 14 days after immunization. Immunity persisted to a moderate degree for 2 to 3 months in young calves, and in one group of yearlings there was apparently a high degree of immunity 7 months after the last inoculation. An inoculum of 10,000 oocysts did not produce as great an immunity as 50,000 or 100,000 oocysts; there was no significant difference in the degree of immunity produced by the higher doses. All these immunizing doses caused diarrhea and bloody feces; the greater the number of oocysts administered, the more severe and longer-lasting the resultant disease.

Hammond *et al.* (1959) found that this immunity was not directed against the schizonts but against the sexual stages or merozoites. They found no significant differences in numbers or size of schizonts between immunized and non-immunized calves, but the latter had many more sexual stages than the former.

Remarks: Hassan (1935) described the sporozoites and schizonts of an organism which he named *Globidium fusiformis* from 5 zebus with dysentery and rinderpest in India. The schizonts were found in the abomasum, duodenum and ileum; they often occurred anterior to the ileocecal valve, but were not found in the large intestine. They were whitish and measured 0.4 to 1.0 by 0.8 mm. The merozoites were elongate, spindle-shaped, slightly curved; with one end bluntly rounded and the other finely pointed, 13 by 2 to 2.5 μ. This form may well be *Eimeria bovis*. However, the fact that schizonts were found in the abomasum as well as in the small intestine made Hammond *et al.* (1946) hesitate to assign it to this species, since they never found schizonts of *E. bovis* in the abomasum.

EIMERIA BRASILIENSIS TORRES AND RAMOS, 1939

Synonyms: *Eimeria boehmi* Supperer, 1952; *Eimeria orlovi* Basanov, 1952.

Hosts: Ox, zebu. In addition, Böhm and Supperer (1956) reported finding this species in several chamois in Austria, but gave no morphological information on which a comparison could be based.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: North America, South America (Brazil), Europe (Austria), Africa (Nigeria), USSR (Kazakhstan).

Prevalence: Davis and Bowman (1952) stated that this species is uncommon in Alabama. Hasche and Todd (1959) found it in 6% of 927 cattle in Wisconsin. Torres and Ramos (1939) found it in 3% of 146 cattle in Brazil. Supperer (1952) found it in 7% of 130 cattle in Austria. Lee and Armour (1958) saw it frequently in cattle in Nigeria. Basanov (1952) found it in Kazakhstan, USSR.

Morphology: The oocysts are ellipsoidal, colorless to yellowish or pinkish, smooth, 31 to 49 by 22 to 33 μ, with a mean of 36 to 38 by 26 to 27 μ. The oocyst wall is composed of a single layer. The micropyle is 5 to 6 μ in diameter, and is covered by a micropylar cap 8 to 12 μ wide and 1.5 to 3 μ high; this cap tends to collapse on storage in unprepared feces in the refrigerator. An oocyst polar granule is present. An oocyst residuum is absent. The sporocysts are elongate ovoid (with a fine "operculum", according to Torres and Ramos), 16 to 22 by 7 to 9 μ (Supperer). A sporocyst residuum is present.

The sporulation time is 6 to 7 days at 27° C (Lee and Armour, 1958) or 12 to 14 days at 20° C (Supperer, 1952).

Life Cycle: Unknown.

Pathogenesis: Unknown.

EIMERIA BUKIDNONENSIS
TUBANGUI, 1931

Synonyms: *Eimeria wyomingensis* Huizinga and Winger, 1942; *Eimeria khurodensis* Rao and Hiregaudar, 1954.

Hosts: Ox, zebu.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: North America, Philippines, USSR, Africa (Nigeria), South America (Brazil).

Prevalence: This species is relatively uncommon. Baker (1938, 1939) and Christensen (1938) reported it in a heifer in New York, Christensen (1941) found it infrequently in Alabama, Huizinga and Winger (1942) found it in 10 cattle in Wyoming, and Hasche and Todd (1959) found it in 5% of 355 cattle in Wisconsin. Tubangui (1931) found it in 1 of 28 zebus in the Philippines. Yakimoff, Gousseff and Rastegaieff (1932) found it in 2 of 126 oxen in Uzbekistan. Yakimoff (1933) found it in 2 of 17 zebus and 1 of 41 oxen in Azerbaidzhan. Marchenko (1937) found it in 0.7% of 137 cattle from the North Caucasus. Yakimoff (1936) found it in 1 of 49 cattle in Brazil, Torres and Ramos (1939) reported it from 8% of 146 cattle in Brazil. Lee (1954) found it in a Fulani calf (zebu) in Nigeria.

Morphology: The oocysts are piriform, yellowish brown to dark brown, 33 to 54 by 24 to 35 μ. Their length-width ratio is 1.3 to 1.8 with a mean of about 1.4. The oocyst wall is about 2 to 4 μ thick except at the micropylar end, where it is thin. It is composed of 2 layers (3 according to Yakimoff, 1933), the outer one thick and the inner one a tough membrane. Tubangui (1931), Yakimoff (1933), and Lee (1954) described the wall as radially striated, but the only American author to note this feature was Baker (1939). The oocyst wall is speckled, and rather rough. The micropyle is conspicuous, 3.5 to 7 μ in diameter. An oocyst residuum and polar granule are absent. The sporocysts are elongate lemon-shaped, 14 to 22 by 9 to 12 μ. A Stieda body is possibly present. Definite sporocyst residual material is absent. The sporozoites were described by Tubangui as more or less roundish or reniform and illustrated without refractile globules. According to Huizinga and Winger, refractile globules are prominent, and it is possible that Tubangui mistook these for the sporozoites proper.

Rao and Hiregauder (1954) described a new species, *E. khurodensis*, from zebus in India. It failed to sporulate, and there is nothing in their description which differs from that of *E. bukidnonensis*.

The sporulation time is 4 to 7 days according to Christensen (1941), 5 to 7 days according to Huizinga and Winger (1942), 24 to 27 days according to Baker (1939).

Life Cycle: Unknown. Baker (1939) found that oocysts first appeared in an experimentally infected calf on the 10th day.

Pathogenesis: Baker (1939) observed a tendency toward a diarrheic condition from the 7th to 15th days after experimental infection of a 70-day old calf with 55 oocysts.

EIMERIA CANADENSIS
BRUCE, 1921

Synonyms: *Eimeria zurnabadensis*.

Hosts: Ox, zebu.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: North America, USSR (Azerbaidzhan).

Prevalence: This species is quite common in the United States. Hasche and Todd (1959) found it in 35% of 355 cattle in Wisconsin.

Morphology: This species has been described by Christensen (1941). The oocysts are 28 to 37 by 20 to 27 μ with a

mean of 32.5 by 23.4 μ. Their length-width ratio is 1.2 to 1.6 with a mean of 1.39. They are typically ellipsoidal, but vary from nearly cylindrical to stoutly ellipsoidal and occasionally slightly tapered. The oocyst wall is transparent, about 1 μ thick in the middle, slightly thinner at each end, delicately yellowish brown (paler toward the ends), normally smooth, and apparently composed of a single layer lined by a membrane. The micropyle is an inconspicuous gap in the wall at one end, appearing covered with a thin, dark refraction line. An oocyst residuum and polar granule are absent. The sporocysts were not described by Christensen (1941). The sporulation time is 3 to 4 days.

Life Cycle: Unknown.

Pathogenesis: Apparently slight.

EIMERIA CYLINDRICA
WILSON, 1931

Hosts: Ox, zebu. Wilson (1931) was unable to infect pigs or goats with this species.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: North America, Europe (Austria), India.

Prevalence: This species is quite common. Hasche and Todd (1959) found it in 20% of 355 cattle in Wisconsin. Supperer (1952) found it in 4% of 130 cattle in Austria. Rao and Hiregaudar (1954) considered it quite prevalent in zebu calves in Bombay. Ruiz (1959) found it in 1% of 100 adult cattle in the San Jose, Costa Rica abattoir.

Morphology: This species has been described by Wilson (1931) and Christensen (1941). The oocysts are 16 to 28 by 12 to 16 μ with a mean of about 23 by 14 μ. They are typically cylindrical, their sides being nearly parallel thruout their middle third, but they may vary from ellipsoidal to narrow cylinders twice as long as wide. The oocyst length-width ratio is 1.3 to 2.0 with a mean of 1.67. The oocyst wall is thin, smooth, homogeneous, transparent, colorless to slightly tinted, and presumably composed of a single layer. A micropyle is absent, altho the wall is slightly paler at one end. An oocyst residuum and polar granule are absent. A sporocyst residuum is present, but there is no sporocyst Stieda body. The sporozoites are elongate, lying lengthwise in the sporocysts and filling them. (According to Rao and Hiregaudar, 1954, the sporocysts measure 6 to 8 by 2 to 4 μ and the sporozoites are very small, rounded bodies.) The sporulation time is 2 days.

The oocysts of *E. cylindrica* intergrade to some extent with those of *E. ellipsoidalis* in size and shape, but other characters indicate that they are separate species.

Life Cycle: Unknown. Wilson (1931) found oocysts in a calf from the eleventh to twentieth days after experimental infection.

Pathogenesis: This species appears to be somewhat pathogenic. Wilson (1931) observed blood in the feces of an experimentally infected calf 6 days after infection. Rao and Hiregaudar (1954) considered this species pathogenic in zebu calves.

EIMERIA ELLIPSOIDALIS
BECKER AND FRYE, 1929

Hosts: Ox, zebu, water buffalo.

Location: Small intestine.

Geographic Distribution: North America, Europe (Austria, Spain), USSR.

Prevalence: This species is common in cattle. Boughton (1945) found it in 45% of 2492 bovine fecal samples from southeastern United States and remarked that its oocysts comprised 40 to 50% of the total oocyst population in 959 samples from over 100 calves 3 to 12 weeks old. Christensen (1941) found its oocysts more frequently than those of any other species in the feces of healthy calves in Alabama during early natural infection. Hasche and Todd (1959)

found it in 43% of 355 cattle in Wisconsin. Supperer (1952) found it in 15% of 130 cattle in Austria. Yakimoff, Gousseff and Rastegaieff (1932) found it in 23% of 126 oxen in Uzbekistan. Yakimoff (1933) found it in 27% of 41 oxen, 6% of 17 zebus and 52% of 21 water buffaloes in Azerbaidzhan. Marchenko (1937) found it in 16% of 137 cattle in the North Caucasus. Ruiz (1959) found it in 3% of 100 adult cattle in the San Jose, Costa Rica abattoir.

Morphology: The oocysts have been described by Becker and Frye (1929) and Christensen (1941), among others. They are 12 to 27 by 10 to 18 μ with a mean of 17 by 13 μ. Their length-width ratio is 1.0 to 1.6 with a mean of 1.30. They are predominantly ellipsoidal, but vary in shape from spherical to almost cylindrical, the spherical and subspherical oocysts occurring in the smaller size range. The oocyst wall is thin, smooth, presumably composed of a single layer, homogeneous, transparent, colorless to pale lavender or pale yellowish, and slightly thinner and paler at one end, suggesting a possible micropyle. A true micropyle is apparently absent, however. An oocyst residuum and polar granule are absent. A sporocyst residuum is present. The sporocysts were illustrated by Becker and Frye (1929) without a Stieda body. The sporocysts (in the zebu) measure 13 to 14 by 4.5 μ according to Yakimoff (1933). The sporozoites were illustrated by Becker and Frye (1929) without clear globules.

The sporulation time is 2 to 3 days.

Life Cycle: Unknown. The endogenous stages occur in the epithelial cells of the small intestine mucosa, according to Boughton (1945).

Pathogenesis: According to Boughton (1945), this species often causes nonbloody diarrhea in calves 1 to 3 months old.

EIMERIA PELLITA
SUPPERER, 1952

Hosts: Ox.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: Europe (Austria).

Prevalence: Supperer (1952) found this species in 5% of 130 cattle in Austria.

Morphology: This species has been described by Supperer (1952). The oocysts are 36 to 41 by 26 to 30 μ, ovoid, with a flattened small end. There is a micropyle at the small end. The oocyst wall is relatively thick and dark brown. The surface of the oocyst bears numerous small, uniformly distributed protuberances in the form of small, blunt points which give the wall a velvety appearance. An oocyst polar granule and residuum are absent. The sporocysts are elongate ovoid, 14 to 18 by 6 to 8 μ, without a Stieda body. A sporocyst residuum is present, usually compact. The sporozoites lie lengthwise in sporocysts, with 2 refractile globules. The sporulation time is 10 to 12 days.

It is possible that *E. pellita* is a synonym of *E. bukidnonensis*. However, it differs from it in the velvety appearance described for its oocyst wall; the oocyst wall of *E. bukidnonensis* has been described as speckled, and as a matter of fact Supperer's drawing of *E. pellita* looks speckled, too. Other differences are that a sporocyst residuum has not been described in *E. bukidnonensis*, while *E. pellita* has a prominent one, and the sporocysts of *E. bukidnonensis* are somewhat pointed at one end (with a Stieda body?), while those of *E. pellita* are not.

Life Cycle: Unknown.

Pathogenesis: Unknown.

EIMERIA SUBSPHERICA
CHRISTENSEN, 1941

Hosts: Ox.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: North America.

Prevalence: This species is relatively uncommon. Christensen (1941) found it in 6 calves in Alabama, never in large numbers. Hasche and Todd (1959) found it in 11% of 355 cattle in Wisconsin.

Morphology: This species has been described by Christensen (1941). The oocysts are 9 to 13 by 8 to 12 μ with a mean of 11.0 by 10.4 μ. Their length-width ratio is 1.00 to 1.3 with a mean of 1.06. They are typically subspherical, but vary from spherical to bluntly ellipsoidal. A micropyle is absent. The oocyst wall is thin, smooth, homogeneous, transparent, of uniform thickness thruout, and colorless to faintly yellowish. An oocyst residuum and polar granule are absent. The sporocysts are pale, spindle-shaped, without a sporocyst residuum. The sporulation time is 4 to 5 days.

The oocysts of this species might be confused with the smaller, subspherical oocysts of *E. ellipsoidalis* or *E. zurnii*, but Christensen (1941) considered that they can be differentiated by their more fragile appearance, their more delicate wall, and by their requiring 2 days longer to sporulate.

Life Cycle: Unknown.

Pathogenesis: Unknown.

EIMERIA ZURNII (RIVOLTA, 1878) MARTIN, 1909

Synonyms: *Cytospermium zurnii*, *Eimeria bovis* (pro parte), *Eimeria canadensis* (pro parte).

Hosts: Ox, zebu, water buffalo. Dahlberg and Guettinger (1956) reported *E. zurnii* in 2 white-tailed deer in Wisconsin, and Salhoff (1939) reported it in a roe deer in Germany. Wetzel and Enigk (1936) found it in a wisent in Germany. Honess and Winter (1956) recorded it from the elk in Wyoming.

Location: Cecum, colon, rectum, thruout small intestine.

Geographic Distribution: Worldwide.

Prevalence: This is one of the commoner coccidia of cattle. Boughton (1945) found it in 42% of 2492 bovine fecal samples in southeastern U.S., and Hasche and Todd (1959) found it in 26% of 355 cattle in Wisconsin. Supperer (1952) found it in 11% of 130 cattle in Austria. Yakimoff, Gousseff and Rastegaieff (1932) found it in 13% of 126 oxen in Uzbekistan. Marchenko (1937) found it in 20% of 137 cattle in the North Caucasus. Yakimoff (1933) found it in 18% of 41 oxen, 6% of 17 zebus and 37% of 30 water buffaloes in Azerbaidzhan. Tubangui (1931) found it in 3 of 28 zebus and 1 of 11 carabaos in the Philippines. Torres and Ramos (1939) found it in 38% of 156 cattle in Brazil. Ruiz (1959) found it in 1% of 100 adult cattle in the San Jose, Costa Rica abattoir.

Morphology: The oocysts have been described by Christensen (1941) among others. They are 15 to 22 by 13 to 18 μ with a mean of 17.8 by 15.6 μ. Their length-width ratio is 1.0 to 1.4 with a mean of 1.14. They are spherical to bluntly ellipsoidal, without a micropyle. The oocyst wall is thin, homogeneous, transparent, and colorless to faint greyish-lavender or pale yellow. An oocyst polar granule and residuum are absent. The sporocysts are 9 to 12 by 6 to 7 μ according to Yakimoff, Gousseff and Rastegaieff (1932). A sporocyst residuum is absent.

Complete sporulation occurs in 9 to 10 days at 12° C, 6 days at 15°, 3 days at 20°, 40 hours at 25° and 23 to 24 hours at 30 to 32.5° C; a few oocysts may sporulate at temperatures as low as 8° C in several months, but sporulation is not normal above 32° C (Marquardt, Senger and Seghetti, 1960).

Life Cycle: The endogenous stages of *E. zurnii* were described by Davis and Bowman (1957). Schizonts are found 2 to 19 days after experimental infection in the epithelial cells of the upper, middle and lower small intestine, cecum and colon.

When mature they measure about 10 by 13 μ and contain 24 to 36 merozoites. They lie distal to the host cell nucleus. Merozoites are first seen 7 days after infection. They are about 5 by 12 μ, have their nucleus near the tapering end and contain 2 refractile globules. Davis and Bowman did not determine the number of asexual generations, but believed that there is more than one. The mature schizonts late in the cycle are slightly larger than the early ones.

Macrogametes are first seen 12 days after infection. They occur in the epithelial cells of the glands and to a lesser extent of the surface of the lower small intestine, cecum, colon and rectum, and rarely in the upper small intestine. They are about 11 by 14 μ and contain 1 or 2 rows of plastic granules. Microgametocytes are first seen 15 days after infection in the same location as the macrogametes. They measure about 10 by 14 μ when mature. Immature oocysts are first seen 12 days after infection.

Pathogenesis: *E. zurnii* is the most pathogenic coccidium of cattle. In acute infections it causes a bloody diarrhea of calves. At first the feces are streaked with blood. The diarrhea becomes more severe, bloody fluid, clots of blood and liquid feces are passed, and straining and coughing may cause this mixture to spurt out as much as 6 to 8 feet. The animal's rear quarters may look as tho they had been smeared with red paint. Anemia, weakness and emaciation accompany the dysentery, and secondary infections, especially pneumonia, are common. This acute phase may continue for 3 or 4 days. If the calf does not die in 7 to 10 days, it will probably recover.

E. zurnii may also be associated with a more chronic type of disease. Diarrhea is present, but there may be little or no blood in the feces. The animals are emaciated, dehydrated, weak and listless, with rough hair coats, drooping ears and sunken eyes.

The lesions of coccidiosis have been described by Boughton (1945) and Davis and Bowman (1952) among others. A generalized catarrhal enteritis involving both the small and large intestines is present. The lower small intestine, cecum and colon may be filled with semi-fluid, bloody material. Large or smaller areas of the intestinal mucosa may be eroded and destroyed, and the mucous membrane may be thickened, with irregular whitish ridges in the large intestine or smooth, dull grey areas in the small intestine or cecum. Diffuse hemorrhages are present in the intestines in acute cases, and petechial hemorrhages in mild ones.

EIMERIA BOMBAYANSIS RAO AND HIREGAUDAR, 1954

Host: Zebu.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: India.

Prevalence: Unknown. Rao and Hiregaudar (1954) stated that its prevalence was great in calves in a dairy herd near Bombay.

Morphology: The oocysts measure 32 to 40 by 20 to 25 μ with a mean of 37 by 22.4 μ. They are ellipsoidal, tending toward the cylindrical, some with parallel sides and others with 1 side straight and the other slightly convex. The micropyle is 2 to 4 μ in diameter, with a thickened wall around it. The oocyst wall is smooth, transparent, homogeneous, pale yellowish brown, 1 to 1.5 μ thick. The sporocysts are 12 to 15 μ long, oval, with 1 end a little more pointed than the other. An oocyst residuum is absent, but a sporocyst residuum is present. The sporozoites are 4 to 6 μ long. The sporulation time is 2 to 3 days.

Pathogenesis: Unknown.

EIMERIA MUNDARAGI HIREGAUDAR, 1956

Host: Zebu.

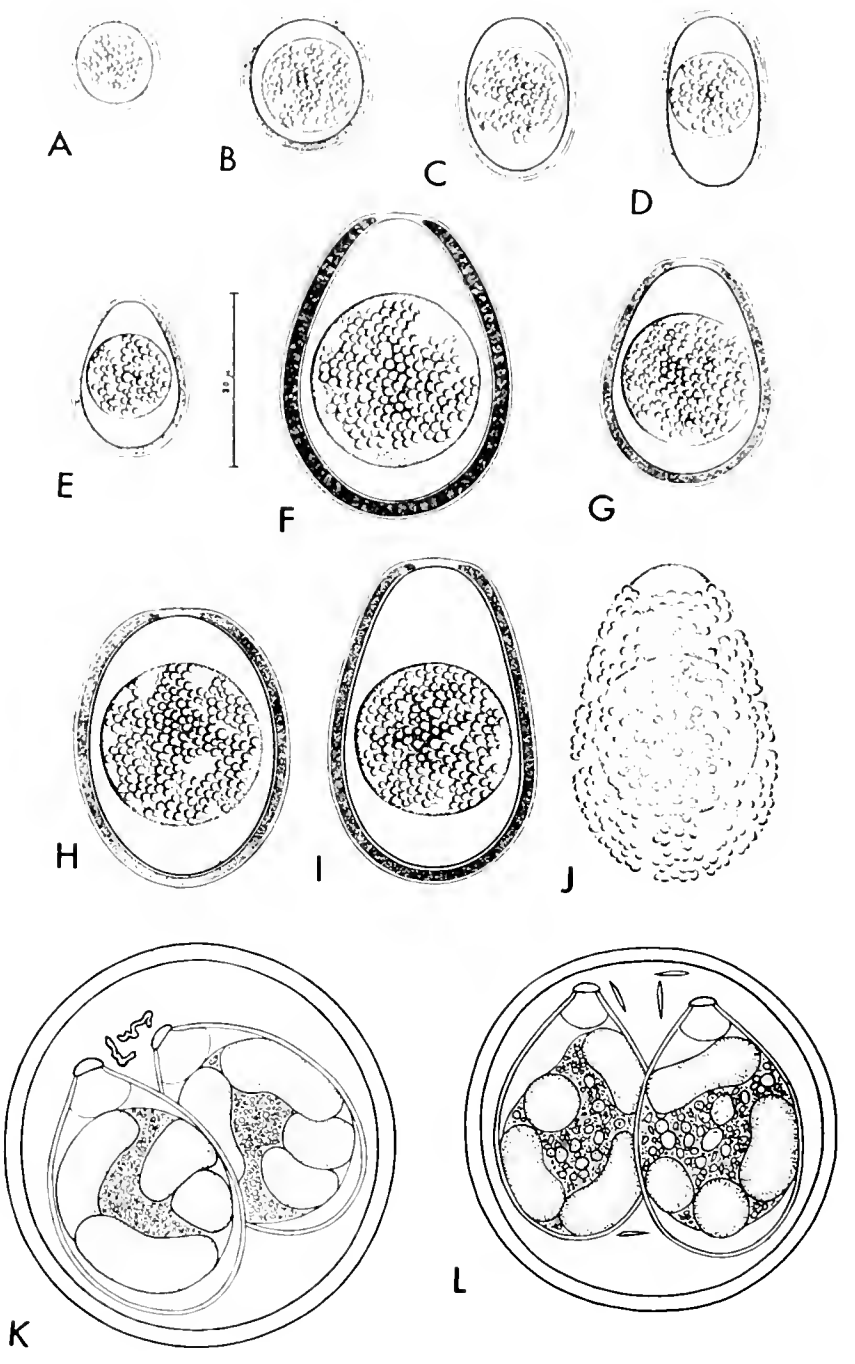

Fig. 25. Coccidia of cattle. A. *Eimeria subspherica* unsporulated oocyst. B. *E. zurnii* unsporulated oocyst. C. *E. ellipsoidalis* unsporulated oocyst. D. *E. cylindrica* unsporulated oocyst. E. *E. alabamensis* unsporulated oocyst. F. *E. bukidnonensis* unsporulated oocyst. G. *E. bovis* unsporulated oocyst. H. *E. canadensis* unsporulated oocyst. I. *E. auburnensis* unsporulated oocyst. J. *E. auburnensis* unsporulated oocyst with mammillated wall. K. *Isospora* sp. sporulated oocyst from cattle. L. *Isospora lacazei* sporulated oocyst from English sparrow; note the close resemblance to Fig. K. (A.-J., X 1150, from Christensen, 1941; K.-L., X 2300, from Levine and Mohan, 1960)

Location: Unknown. Oocysts found in feces.

Geographic Distribution: India (Bombay).

Prevalence: Hiregaudar (1956) described this species from a single calf.

Morphology: The oocysts are ovoid, 36 to 38 by 25 to 28 μ. The oocyst wall is 0.3 μ thick, slightly thicker toward micropylar end, smooth, transparent, and pale yellow or yellow. The micropyle is distinct, 0.5 μ in diameter. The sporocysts are oval, 15 to 9 μ, thinning at the pointed end. The sporozoites are 4 to 6 by 1 to 3 μ and finely granular. An oocyst residuum and polar granule are absent. A sporocyst residuum is present. The sporulation time is 1 to 2 days during the summer. The extremely thin wall and the tiny, distinct micropyle may differentiate this species from other bovine coccidia. However, the possibility must not be overlooked that these oocysts may be those of a species such as *E. bukidnonensis* from which the thick, brittle outer wall has cracked off.

Life Cycle: Unknown.

Pathogenesis: Unknown.

COCCIDIOSIS IN CATTLE

Epidemiology: Infections with a single species of coccidium are rare in nature; mixed infections are the rule. *Eimeria zurnii* and *E. bovis* are the most pathogenic species, but *E. auburnensis* and the other species may contribute to the total disease picture, and some of them may cause marked signs by themselves if they are present in large enough numbers.

Bovine coccidiosis is primarily a disease of young animals. It ordinarily occurs in calves 3 weeks to 6 months old. Older calves and even adult animals may be affected under conditions of gross contamination, but they are usually symptomless carriers.

Calves become infected by ingesting oocysts along with their feed or water. The severity of the disease depends upon the number of oocysts they receive. If they get only a few, there are no symptoms, and repeated infections produce immunity without disease. If they get more, the disease may be mild and immunity may also develop. It they get a large number, severe disease and even death may result.

Crowding and lack of sanitation greatly increase the disease hazard. Successive passage of coccidia from one animal to another often builds up infection to a pathogenic level, since in each passage the recipient receives more oocysts than in the previous one. This is the reason for the common observation that calves placed in a lot where others are already present may suffer more from coccidiosis than those which were there first. This successive passage from a carrier to a symptomless "multiplier" to a sub-clinical case to a fatal case was described by Boughton (1945) as typical of the transmission of bovine coccidiosis. In addition, it is likely that recycling by repeated infections of a single individual may also play an important part.

A little-understood type of bovine coccidiosis is winter coccidiosis. This occurs when it is so cold that oocyst sporulation should be minimal if it occurs at all. Presumably there is enough heat in the bedding to permit sporulation. Another explanation which has been advanced is that the stress of winter conditions exacerbates a latent infection. This explanation is not easy to validate, however, in view of the self-limiting nature of coccidial infections.

Davis, Herlich and Bowman (1959, 1959a, 1960, 1960a) found that concurrent infections of cattle with the nematodes, *Trichostrongylus colubriformis* or *Cooperia punctata*, exacerbated the effects of coccidia in calves, but that *Ostertagia ostertagi* and *Strongyloides papillosus* had no such effect.

Diagnosis: Bovine coccidiosis can be diagnosed from a combination of history,

signs, gross lesions at necropsy and microscopic examination of scrapings of the intestinal mucosa and of feces. Diarrhea or dysentery accompanied by anemia, weakness, emaciation and inappetance are suggestive of coccidiosis in calves. Secondary pneumonia is often present. The lesions found at necropsy have already been described.

Microscopic examination is necessary to determine whether the lesions are due to coccidia or to some other agent. However, diagnoses will often be missed if one relies only on finding oocysts in the feces. There may be none there at all in the acute stage of zurnii coccidiosis. Similarly, the mere presence of oocysts in the feces is not proof that coccidiosis is present; it may be coccidiasis. To be sure of a diagnosis, scrapings should be made from the affected intestinal mucosa and examined under the microscope. It is not enough to look for oocysts, however, but schizonts, merozoites and young gametes should be recognized.

Treatment: A number of investigators (Boughton, 1943; Boughton and Davis, 1943; Davis and Bowman, 1952, 1954; Hammond *et al.*, 1956; Senger *et al.*, 1959) have found that the sulfonamides have some value against bovine coccidiosis.

Other types of compounds which are used in avian coccidiosis are unsatisfactory. For example, Hammond *et al.* (1957) found that nicarbazin was effective in preventing experimental coccidiosis due to *E. bovis* in calves only at doses which were toxic to the animals. Gardner and Wittorff (1955) found that 0.1 to 0.3% furacin in the ration was toxic to dairy calves, causing nervous signs and reducing or preventing weight gains. Even 0.01% of the drug had some toxic effect. It injures the myelin sheaths and causes cerebral damage.

Gasparini, Roncalli and Ruffini (1958) claimed that drenching with 4 g per 100 kg ammonium sulfate plus 2 ml lactic acid in a liter of milk twice a day for 4 consecutive days cured coccidiosis due to *E. zurnii* in 2 herds of cattle in Italy. They believed that the ammonium sulfate worked by releasing ammonia, and added the lactic acid to prevent release from taking place in the stomach. However, their work was improperly controlled, and the efficacy of this compound remains to be determined.

Sulfamethazine and sulfamerazine appear to be better than sulfaquinoxaline or other sulfonamides. They are only partially effective, however. They do not prevent the diarrhea, but they do reduce the severity of the disease. Thus, Davis and Bowman (1954) found that sulfamethazine reduced the severity of experimental infections with *E. zurnii* or mixed species in calves and that treated calves gained slightly more weight than the controls. Drug treatment was started before infection, and no immunity to subsequent exposures was produced. Hammond *et al.* (1956) found that sulfamerazine or sulfaquinoxaline, given to calves at the rate of 0.143 g per kg body weight for 2 days and 0.072 g per kg for 2 more days, decreased the severity of coccidiosis due to *E. bovis* if they were administered between 13 and 17 days after experimental infection. They were not effective earlier or later than this. The drugs presumably act on the merozoites after their release from the schizonts. Senger *et al.* (1959) found that a mixture of equal parts sulfamerazine and sulfamethazine given by mouth at the rate of 213, 143 and 70 mg per kg body weight 13, 14 and 15 days, respectively, after inoculation reduced the severity of the disease and did not interfere with the development of immunity.

Hammond *et al.* (1959) found that a single treatment with 0.215 g per kg sulfamethazine or sulfabromomethazine 13 days after experimental inoculation with *E. bovis* effectively controlled coccidiosis. Administration of either compound on alternate days at the rate of 0.0215 g per kg for as short a period as 10 to 18 days after inoculation also effectively controlled coccidiosis, while in 1 experiment treatment at this rate 12 and 14 days after inoculation suppressed the disease. This treatment did not interfere with the development of immunity.

Since the sulfonamides are generally only partially effective, preventive measures are more important than curative ones.

Prevention: Sanitation and isolation are effective in preventing coccidiosis. Beef calves should be dropped and kept on clean, well drained pastures. Overstocking and crowding should be avoided. Feed and water containers should be high enough to prevent fecal contamination. Feed lots should be kept dry and should be cleaned as often as possible. Concrete or small gravel are preferable to dirt.

Dairy calves should be isolated within 24 hours after birth and kept separately. Individual box stalls which are cleaned daily may be used. Slat-bottom pens are also effective and require less cleaning. Allen and Duffee (1958) described a simple, raised, wooden home-made stall with a 4 by 2-1/2 foot slatted floor in which dairy calves can be raised separately for the first 3 months. Davis (1949) and Davis and Bowman (1952) described a 5 x 10 x 3 foot outdoor portable pen which can be moved to a fresh site once a week and thus eliminates the need for cleaning. It is made primarily of net wire and 1 x 4 lumber, with a removable roof and siding at one end. The pens should not be returned to the same ground for a year.

These methods will not eliminate all coccidia, but they will prevent the calves from picking up enough oocysts to harm them. In addition, they will greatly reduce lice, helminth parasites, pneumonia, white scours and other diseases.

The unsporulated oocysts of *E. zurnii* are killed by sunlight in 4 hours or by drying at 25% relative humidity or below in several days. They are not harmed by freezing at -7 to -8° C for as long as 2 months, and half of them survive as long as 5 months; at -30°C, however, only 5% survive 1 day. The oocysts are killed by 10^{-6} M mercuric chloride, 0.05 M phenol, 0.25 M formaldehyde, 1.25% sodium hypochlorite, or 0.5% cresol (Marquardt, Senger and Seghetti, 1960).

EIMERIA AHSATA
HONESS, 1942 emend.

Synonym: *Eimeria ah-sa-ta* Honess, 1942.

Hosts: Sheep, Rocky Mountain bighorn sheep.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: North America (Wyoming, Alabama).

Prevalence: Unknown.

Morphology: The oocysts are ellipsoidal and faint pink. The oocyst wall is faint straw-colored and lined by a membrane. A micropyle and micropylar cap are present. Oocysts from the bighorn sheep are 30 to 40 by 20 to 30 μ with a mean of 32.7 by 23.7 μ; their length-width ratio is 1.1 to 1.8 with a mean of 1.40; the micropylar cap is 0.4 to 4.2 μ high and 2.1 to 12.5 μ wide with a mean of 2.1 by 7.5 μ. Oocysts from domestic sheep are 29 to 37 by 17 to 28 μ with a mean of 33.4 by 22.6 μ; their length-width ratio is 1.2 to 1.8 with a mean of 1.48; the micropylar cap is 1.7 to 4.2 μ high and 5.9 to 13.4 μ wide with a mean of 3.0 by 8.4 μ. An oocyst residuum is present in some oocysts. Oocyst polar (?) granules are almost always present. The sporocysts are 15.4 by 7.8 μ and have a sporocyst residuum.

This species is difficult to distinguish from *E. arloingi*, and Morgan and Hawkins (1952) and Lotze (1953) considered it of doubtful validity. However, Smith, Davis and Bowman (1960) rediscovered it in Alabama and confirmed its distinctiveness.

Life Cycle: Unknown. The prepatent period is 18 to 20 days according to Smith, Davis and Bowman (1960).

Pathogenesis: Smith, Davis and Bowman (1960) considered this the most pathogenic of all sheep coccidia. They produced fatal infections in 4 out of 9 lambs 1 to 3 months old by feeding 100,000 oocysts.

The intestines of infected lambs had thickened, somewhat edematous areas in the upper part. The Peyer's patches and the last 8 to 10 inches of the small intestine above the ileocecal valve were inflamed.

EIMERIA ARLOINGI
(MAROTEL, 1905)
MARTIN, 1909

Hosts: Sheep, goat, Rocky Mountain bighorn sheep, *Ovis musimon*, *O. polii*, *Capra ibex*, *Hemmitragus jemlaicus*, roe deer.

Location: Small intestine.

Geographic Distribution: Worldwide.

Prevalence: This is probably the most common coccidium in sheep. Christensen (1938a) found it in 90% of 100 sheep from Idaho, Maryland, New York and Wyoming. Balozet (1932) found it in 52% of 63 sheep and 56% of 41 goats in Tunisia. Jacob (1943) found it in 58% of 100 sheep and 18% of 11 goats in Germany. Svanbaev (1957) found it in 52% of 302 sheep and 52% of 48 goats in Kazakhstan.

Morphology: The oocysts are usually elongate ellipsoidal, but are sometimes asymmetrical, with one side curved more than the other, or slightly ovoid. They are 17 to 42 by 13 to 27 μ with a mean of 27 by 18 μ; their length-width ratio is 1.1 to 1.9 with a mean of 1.49 (Christensen, 1938a). The oocyst wall is 1.0 to 1.5 μ thick, transparent, almost colorless to yellowish-brown, and composed of 2 layers, the outer one being half as thick as the inner, according to Christensen (1938a). The oocyst wall is apparently lined by a membrane. A micropyle 2 to 3 μ in diameter is present. A micropylar cap is present; it varies from an inconspicuous, flat structure to a prominent, transparent, pale yellow to yellowish-green rounded cone or crescent, 0.2 to 2 μ high by 3 to 8 μ wide with a mean of 1.2 by 5 μ. This cap is a tough, lid-like structure which is easily dislodged and may be lost in some specimens. An oocyst residuum is absent. An oocyst polar granule is present according to Balozet (1932). The sporocysts are ovoid, 13 by 6 μ. A sporocyst residuum is present. The sporulation time is 1 to 2 days (Christensen, 1938a) to 3 to 4 days (Balozet, 1932).

Life Cycle: Lotze (1953a) studied the life cycle of *E. arloingi* in experimentally infected lambs. The sporozoites emerge from the oocysts in the small intestine, enter the crypts of Lieberkuehn, and penetrate thru the tunica propria into the interior of the villi. Here they enter the endothelial cells lining the central lacteals and grow. The host cell also grows, and its nucleus becomes very large. There is apparently only 1 generation of schizonts and merozoites. The schizonts become mature 13 to 21 days after infection. At this time they are about 122 to 146 μ in diameter and contain a large number (probably millions) of merozoites about 9 μ long and 2 μ wide.

The merozoites break out of the schizonts and enter the epithelial cells of the small intestine. Sometimes only a small group of cells at the bottom of the crypts is parasitized, but in heavy infections practically all the epithelial cells of the villi are invaded. The infected villi are enlarged and greyish. Some of these merozoites become microgametocytes; these form many microgametes, leaving a large mass of residual material. Most of the merozoites become macrogametes, which contain large plastic granules when mature.

Following fertilization, the macrogametes turn into oocysts, which break out of the host cells and are first seen in the feces on the 20th day after infection. Their numbers increase for about 5 days and then decrease at about the same rate for the next 5 days. Thus the prepatent period is 19 days and the patent period about 10 days following a single exposure.

Pathogenesis: Lotze (1952) studied the pathogenicity of *E. arloingi* in 3-month-old lambs experimentally infected with 200,000 to 60 million oocysts. No visible signs were produced by infections

with 1 million oocysts or less. In lambs infected with 3 or 5 million oocysts, the feces became soft on the 13th day and then returned to normal during the next 6 days. The health, general condition and weight gains of these animals were not affected.

Severe diarrhea was produced with higher doses, but none of the animals died altho one was killed in extremis. In general, the experimentally infected lambs appeared normal up to the 13th day after inoculation, when their feces became soft. In the more heavily infected lambs the feces then became watery, and diarrhea was severe beginning on the 15th day. Blood-tinged mucus was passed by affected lambs only occasionally. The feces began to return to normal on the 17th day and were usually nearly normal by the 20th day. Lambs with marked diarrhea became weak and refused feed.

At necropsy, only a few small, slightly hemorrhagic areas scattered thruout the lining of the small intestine were seen up to the 13th day. From the 13th to 19th days the small intestine was more or less thickened and edematous, and thick, white opaque patches made up of groups of heavily parasitized villi were present.

The villi containing the schizonts become thin-walled sacs and are presumably destroyed. The sexual stages are clustered in the epithelial cells of the villi and destroy these cells when they emerge. However, they do not do as much damage as the asexual stages, since the condition of affected animals appears to improve before oocysts are shed.

E. arloingi is less pathogenic than *E. ninakohlyakimovae.*

Epidemiology: This species has been reported not only from domestic sheep and goats but also from the Rocky Mountain bighorn sheep (*Ovis canadensis*), moufflon (*O. musimon*), argali (*O. ammon polii*), ibex (*Capra ibex*), and *Hemmitragus jemlaicus* (see especially Yakimoff, 1933a). Ullrich (1930) found it in the roe deer. Whether the forms from all these animals are really *E. arloingi* remains to and cross-transmission experiments. According to Lotze (1953), no cross-transmission studies, even between domestic sheep and goats, have been reported up until the time of his paper, and he attempted none.

EIMERIA CRANDALLIS HONESS, 1942

Hosts: Sheep, Rocky Mountain bighorn sheep. This species was originally described from the bighorn sheep, but Hawkins (in Morgan and Hawkins, 1952) found it in domestic sheep.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: North America (Wyoming, Michigan).

Prevalence: Unknown.

Morphology: The oocysts are spherical to broadly ellipsoidal to ovoid, colorless to faint pink or greenish, and are 17 to 23 by 17 to 22 μ with a mean of 21.9 by 19.4 μ. Their length-width ratio is 1.00 to 1.35 with a mean of 1.11. A micropyle and micropylar cap are present. The micropylar cap measures from a trace to 1.7 μ high and 3.3 to 6.6 μ wide with a mean of 0.8 μ high and 4.9 μ wide. The oocyst wall has a distinctly demarcated outer edge, a feature which Honess (1942) considered to distinguish this species from *E. arloingi.* The sporocysts are 8 to 11 by 5 to 8 μ with a mean of 9.5 by 6.4 μ. No information is available on oocyst polar granule, oocyst residuum or sporocyst residuum.

Lotze (1953) considered that this species was of doubtful validity, since its oocysts fall into the size range of *E. arloingi,* but Morgan and Hawkins (1952) accepted it, stating that Hawkins had found that its peak of infection in Michigan occurred at a different time from that of *E. arloingi.*

Life Cycle: Unknown.

Pathogenesis: Unknown.

EIMERIA FAUREI
(MOUSSU AND MAROTEL, 1902)
MARTIN, 1909

Synonym: *Eimeria aemula.*

Hosts: Sheep, goat, Rocky Mountain bighorn sheep, *Ovis ammon polii*, *O. musimon*, *O. orientalis*, *Capra ibex*, *Rupicapra rupicapra (chamois)*, *Ammotragus lervia (Barbary sheep)*.

Location: Small intestine.

Geographic Distribution: Worldwide.

Prevalence: This species is fairly common. Christensen (1938a) found it in 11% of 100 sheep from Idaho, Maryland, New York and Wyoming. Balozet (1932) found it in 21% of 63 sheep and 2% of 41 goats in Tunisia. Jacob (1943) found it in 40% of 100 sheep and 18% of 11 goats in Germany. Svanbaev (1957) found it in 43% of 302 sheep and 40% of 48 goats in Kozakhstan.

Morphology: The following description is based primarily on those of Christensen (1938a) and Balozet (1932). The oocysts are ovoid, 25 to 35 by 18 to 24 μ, with a mean of 28.9 by 21.0 μ according to Christensen or 31.5 by 22.1 μ according to Balozet. The oocyst wall is transparent, delicate salmon pink to pale yellowish brown, 1 μ thick at the most according to Balozet; with a faint, yellowish-green "external coat ... about half as thick as wall" according to Christensen. The micropyle is conspicuous, 2 to 3 μ in diameter, at the small end. A micropylar cap is absent. An oocyst polar granule was illustrated by Balozet (1932). Oocyst and sporocyst residua are absent. The sporulation time is 1 to 2 days according to Christensen, 3 to 4 days according to Balozet.

Life Cycle: The life cycle of *E. faurei* does not seem to have been worked out in detail. According to Lotze (1953), its schizonts are about 100 μ in diameter and contain thousands of merozoites.

Pathogenesis: This species is only mildly pathogenic. Lotze (1954) found that single infections of 3-month-old lambs with 5 million oocysts produced only a temporary softening of the feces without significantly affecting the general health or physical condition of the animals, and infections with 50 million oocysts failed to cause death.

Epidemiology: This species has been reported not only from the domestic sheep and goat but also from the Rocky Mountain bighorn sheep (*Ovis canadensis*), moufflon (*O. ammon*), urial or shapo (*O. orientalis*), Barbary sheep (*Ammotragus lervia*), ibex (*Capra ibex*) and chamois (*Rupicapra rupicapra*) (see especially Yakimoff, 1933). Whether the forms from these species are all *E. faurei* remains to be proven by careful study of their oocysts and cross-transmission experiments. According to Lotze (1953), no cross-transmission studies, even between domestic sheep and goats, had been reported up until the time of his paper, and he attempted none.

EIMERIA GILRUTHI
(CHATTON, 1910)
REICHENOW AND CARINI, 1937

Synonyms: *Gastrocystis gilruthi*, *Globidium gilruthi*.

Hosts: Sheep, goat.

Location: Abomasum, seldom small intestine.

Geographic Distribution: Worldwide.

Prevalence: This form is very common in Europe. Chatton (1910) and Triffitt (1928) found it in the abomasa of almost all the sheep they examined in France and England, respectively. Alicata (1930) found it in 9% of 78 sheep in Indiana, 11% of 101 sheep from West Virginia and 8% of 72 sheep from Idaho. It has also been seen in Montana, Wyoming, Michigan (Morgan and Hawkins, 1952) and Illinois. Sarwar (1951) found it in 34% of the sheep and goats slaughtered at the Lahore, Pakistan abattoir, and found it in as many as 94% in other parts of East Punjab. Soliman (1958) found it in 18% of 250 sheep and 28% of 150 goats slaughtered in Egypt. Soliman (1960) found it in 32% of 425 sheep and 40% of 240 goats

in the Sudan. Rac and Willson (1959) reported it in Australia.

Morphology: Only the schizonts and merozoites of this form have been described. The schizonts occur in the connective tissue of the abomasal wall. They are 300 to 700 μ long and 300 to 465 μ wide, and are easily visible to the naked eye as whitish nodules. The host cell nucleus is flattened and greatly enlarged. The mature schizonts are filled with many thousands of crescent-shaped merozoites about 4.5 to 7.5 μ long and 1.2 to 2.0 μ wide. One end of the merozoites is rounded and the other pointed. The nucleus is near the broad end, and a heavily staining granule is in the center.

These schizonts are undoubtedly those of a species of *Eimeria* presently known from its oocysts alone, but we do not know which species it is. Reichenow (1940) said that it was very probably *E. intricata*. Becker (1956) agreed and, since the specific name *gilruthi* has priority, synonymized *E. intricata* with it. However, Kotlán, Pellérdy and Verséenyi (1951, 1951a) found two types of giant schizonts in sheep. One type, which measured 64 to 256 by 48 to 179 μ and contained straight, slender merozoites 10 to 12 μ long, they found to be those of *E. parva*. The other type of schizont was larger and contained merozoites about 16 μ long which were bent like a hoe at one end ("hackenformigen"). These they said were those of *E. intricata*. However, they saw both schizonts in the small intestine and not in the abomasum, and they used only 2 lambs in their work. Hence, it is felt best for the present not to attempt to assign the *gilruthi* schizonts to any other species of *Eimeria*.

EIMERIA GRANULOSA CHRISTENSEN, 1938

Hosts: Sheep, Rocky Mountain bighorn sheep.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: North America, Europe (Germany).

Prevalence: This species is relatively uncommon in sheep. Christensen (1938a) found in in 10% of 100 sheep from Maryland and New York. Jacob (1943) found it in 1% of 100 sheep in Germany. Honess (1942) remarked that it was more frequent and numerous in bighorn sheep than in domestic sheep in Wyoming.

Morphology: This species has been described by Christensen (1938a). The oocysts are piriform or shaped like a stout, broad-shouldered urn, with the micropyle and micropylar cap at the broad end; small oocysts are often bluntly ellipsoidal. The oocysts are 22 to 35 by 17 to 25 μ with a mean of 29.4 by 20.9 μ. Their length-width ratio is 1.2 to 1.7 with a mean of 1.41. The micropyle is distinct, 3 to 5 μ in diameter. The micropylar cap is prominent, 5 to 10 μ wide and 1 to 2.5 μ high with a mean of 7.5 by 1.7 μ; it is shaped like a broad truncated cone with a flat or slightly convex top, and is easily dislodged. The oocyst wall is transparent, pale brownish-yellow to yellowish-brown, and composed of 2 layers, the outer one being transparent, pale yellow to yellowish-green, and half as thick as the inner, heavier layer. The oocyst wall is lined by a membrane. An oocyst polar granule and oocyst residuum are absent. The sporocysts are ovoid, with a sporocyst residuum. The sporozoites have a refractile globule at each end. The sporulation time is 8 to 4 days.

Life Cycle: Unknown.

Pathogenesis: Unknown.

EIMERIA INTRICATA SPIEGL, 1925

Hosts: Sheep, Rocky Mountain bighorn sheep, moufflon, roe deer.

Location: Uncertain, presumably abomasum and small intestine.

Geographic Distribution: Worldwide.

Prevalence: This species is fairly common. Christensen (1938a) found it in 14% of 100 sheep from Maryland, New York and Wyoming. Jacob (1943) found it in 13% of 100 sheep in Germany. Balozet (1932) found it in 3% of 63 sheep in Tunisia. Svanbaev (1957) found it in 4% of 302 sheep in Kazakhstan.

Morphology: This species has been described by Spiegl (1925), Balozet (1932) and Christensen (1938a). The oocysts are ellipsoidal, 39 to 54 by 27 to 36 μ with a mean of 47.0 by 32.0 μ (Christensen) or 45.6 by 33.0 μ (Spiegl). Their length-width ratio is 1.3 to 1.8 with a mean of 1.47. The oocyst wall, as described by Henry (1932), is composed of 3 layers; the outer layer is a transparent, colorless membrane which is very difficult to see; the middle layer is thick, rough, brown, transversely striated, 2.0 to 2.5 μ thick, and somewhat thinner at the micropylar end; the inner layer is colorless, 0.8 to 1.0 μ thick. The micropyle is prominent, 6 to 10 μ in diameter; it does not extend to the inner layer. The micropylar cap is prominent, transparent, colorless to yellowish-green, crescent-shaped, detachable, and 6 to 11 μ wide and 1 to 3 μ high with a mean of 9 by 2 μ. An oocyst polar granule and residuum are absent. The sporocysts are elongate ovoid, 17 to 18 by 9 to 13 μ, with a small Stieda body and a sporocyst residuum. The sporozoites are wedge-shaped, with several refractile globules. The sporulation time is 3 to 5 days.

Life Cycle: The life cycle of *E. intricata* has not been worked out. As mentioned above, Reichenow (1940) and Becker (1956) considered that the giant schizonts described from sheep under the name *E. gilruthi* are those of *E. intricata*. According to Kotlán, Pellérdy and Versényi (1951), the merozoites of *E. intricata* are about 16 μ long and bent like a hoe at one end.

Pathogenesis: Unknown. These oocysts are rarely found in large numbers.

Epidemiology: Honess (1952) found this species in the Rocky Mountain bighorn sheep, and Wetzel and Enigk (1936) reported it from the roe deer in Germany.

EIMERIA NINAKOHLYAKIMOVAE YAKIMOFF AND RASTEGAIEFF, 1930 emend.

Synonyms: *Eimeria galouzoi* (pro parte), *E. nina-kohl-yakimovi* Yakimoff and Rastegaieff, 1930.

Hosts: Sheep, goat, Rocky Mountain bighorn sheep, mouflon (*Ovis musimon*), *O. orientalis*, Siberian ibex (*Capra ibex sibirica*), Barbary sheep (*Ammotragus lervia*), Persian gazelle (*Gazella subgutturosa*).

Location: Small intestine, especially the posterior part, and also cecum and colon.

Geographic Distribution: Worldwide.

Prevalence: This species is fairly common. Christensen (1938a) found it in 3% of 100 sheep from Maryland and Idaho. Jacob (1943) found it in 5% of 100 sheep in Germany. Balozet (1932) found it in 35% of 63 sheep and 34% of 41 goats in Tunisia. Svanbaev (1957) found it in 52% of 302 sheep and 31% of 48 goats in Kazakhstan.

Morphology: This species has been described by Yakimoff and Rastegaieff (1930), Balozet (1932) and Christensen (1938a). The oocysts are usually ellipsoidal, sometimes spherical, occasionally slightly ovoid. They are 16 to 27 by 13 to 22 μ with means of 23.1 by 18.3 μ (Christensen) or 19.8 by 16.5 μ (Balozet). Their length-width ratio is 1.1 to 1.5 with a mean of 1.27 according to Christensen (1938). The oocyst wall is 1 to 1.5 μ thick, transparent, almost colorless to pale brownish yellow, and composed of two layers of which the outer is half as thick as the inner. A micropyle is absent or imperceptible (occasionally visible under bright light if the oocyst is tilted, according to Christensen). There is no micropylar cap. An oocyst polar granule and oocyst residuum

are absent. The sporocysts are ovoid; 4 to 11 by 4 to 6 μ. A sporocyst residuum is present. The sporozoites have one end slender and pointed and the other thick and rounded; they measure 4 to 5 by 2 μ and lie lengthwise, head to tail, in the sporocysts. The sporulation time is 1 to 2 days according to Christensen (1938a) or 3 to 4 days according to Balozet (1932).

Life Cycle: The life cycle of this species has been described by Balozet (1932) in the goat and briefly by Lotze (1954) in the sheep. Their accounts differ, and are given separately below.

Lotze (1954) found in sheep that the sporozoites enter the epithelial cells at the base of the glands of Lieberkuehn in the small intestine, where they form schizonts about 300 μ in diameter containing thousands of merozoites. The sexual stages occur in the epithelial cells of the ileum, cecum and upper part of the large intestine, appearing 15 days or more after infection.

Balozet (1932a) found in a kid killed 39 days after infection that the schizonts were only 15 to 35 μ in diameter and contained only 40 to 200 merozoites. However, these schizonts were found very late in the infection. They were associated with macrogametes and microgametocytes, and it is possible either that they may have belonged to a second generation not mentioned by Lotze or that they may have belonged to some other species.

The prepatent period was found by Shumard (1957) to be 15 days in lambs and by Balozet (1932) to be 10 to 13 days.

Pathogenesis: This is one of the most pathogenic species of coccidium in sheep. Lotze (1954) found that as few as 50,000 oocysts caused diarrhea in a 3-month-old lamb, and as few as 500,000 oocysts caused death. Dysentery was produced in a 2-year-old sheep by inoculation with as few as 1 million oocysts.

Lotze (1954) found that in lambs the feces became soft in 12 to 17 days after experimental infection. They became watery a day or 2 later and remained so for a week or more. In the more heavily infected lambs, the feces contained blood-stained mucus beginning the 15th day after infection or soon thereafter. In those animals which recovered, the feces remained soft for some weeks.

The lambs with severe diarrhea lost their appetites at first, altho they appeared to drink more. After about a week they drank very little. There was rapid loss of weight at the onset of diarrhea. If the lambs recovered, they gained weight about as rapidly as the controls, but of course had taken a setback in growth. About 2 months after severe coccidiosis, the wool began to break off over extensive areas, starting on the flanks; this may have been due to nutritional disturbance caused by the infection.

The diarrheic feces attracted flies, and affected animals quickly became fly-struck. Some animals which would otherwise have recovered died of flystrike if they were not treated for this condition.

At necropsy, petechial hemorrhages were found in the small intestine 3 to 7 days after infection. The small intestine then became thickened and inflamed. Extensive hemorrhage was present in the posterior part of the small intestine of severely affected lambs by the 15th day. The cecum and upper part of the large intestine became thickened and edematous, and were hemorrhagic by the 19th day. In heavily infected lambs, vast areas of the posterior part of the small intestine were denuded of epithelium. Thus, one can say that the lesions consisted at first of petechial hemorrhages, followed by thickening, edema and inflammation, and finally by epithelial denudation. The small intestine, especially its posterior part, cecum and upper colon were affected.

Shumard (1957a) studied a somewhat less pathogenic strain. He reported lowered feed consumption, lassitude, generalized incoordination and slight diarrhea with some bleeding in lambs experimentally infected at 50 days of age with 7 million

oocysts of *E. ninakohlyakimovae* and 100,000 oocysts of *E. faurei*. There was no decrease in water consumption. Clinical signs appeared on the 9th day after infection and ended about the 22nd day. One out of the 4 lambs died on the 15th day. There were decreases in percentage of feed protein digested and inorganic serum phosphorus, increases in serum globulins and blood glucose, and no significant changes in total serum protein, blood hemoglobin and hematocrit values. Oocysts of both species appeared in the feces on the 15th day, increased until the 21st day and then decreased gradually.

Balozet (1932) observed a case of muco-sanguineous diarrhea followed by death in a naturally affected adult goat, and produced the disease experimentally in 2 newborn kids. A mucous diarrhea appeared on the 22nd day after infection, became bloody, and persisted until about the 39th day.

Remarks: In one of the very few cross-transmission experiments attempted with sheep and goat coccidia, Balozet (1932) was unable to infect a recently weaned lamb with *E. ninakohlyakimovae* from a goat, altho he did infect 2 newborn kids. He thought the lamb was too old.

EIMERIA PALLIDA
CHRISTENSEN, 1938

Host: Sheep.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: North America.

Prevalence: Christensen (1938a) found this species in 10% of 100 sheep from Maryland and Wyoming.

Morphology: This species has been described by Christensen (1938a). The oocysts are ellipsoidal, 12 to 20 by 8 to 15μ with a mean of 14.2 by 10.0μ. Their length-width ratio is 1.2 to 1.7 with a mean of 1.43. A micropyle is imperceptible and there is no micropylar cap. The oocyst wall is thin, homogeneous, colorless to pale yellow to yellowish green, appears fragile and pallid, and is composed of 2 layers of which the outer is half as thick as the inner, with a single dark refraction line marking its inner edge. An oocyst polar granule and oocyst residuum are absent. The sporocysts are ovoid, without a sporocyst residuum. The sporozoites lie lengthwise, head to tail, in the sporocysts, and contain a spherical globule at each end. The sporulation time is 1 day.

Life Cycle: Unknown.

Pathogenesis: Unknown.

Remarks: In describing *E. pallida*, Christensen (1938a) said that it differed from *E. parva* is being narrower, pale, inconspicuous and colorless, and in having only a single black refraction line on the inner surface of the oocyst wall instead of 2 black refraction lines, one on each side of the inner wall. However, Kotlán, Pellérdy and Versényi (1951) considered *E. pallida* a synonym of *E. parva*.

EIMERIA PARVA
KOTLÁN, MÓCSY AND VAJDA, 1929

Synonyms: *Eimeria galouzoi* (pro parte).

Hosts: Sheep, goat, Rocky Mountain bighorn sheep, Barbary sheep (*Ammotragus lervia*), Siberian ibex (*Capra ibex sibirica*), roe deer.

Location: Schizonts are found thruout the small intestine, and gametes and gametocytes in the cecum, colon and small intestine.

Geographic Distribution: Worldwide.

Prevalence: This species is common in sheep, less common in goats. Christensen (1938a) found it in 50% of 100 sheep from Idaho, Maryland and Wyoming. Jacob (1943) found it in 52% of 100 sheep and 9% of 11 goats in Germany; he also found it in

a roe deer. Balozet (1932) found it in 21% of 63 sheep and 22% of 41 goats in Tunisia. Svanbaev (1957) found it in 9% of 302 sheep in Kazakhstan.

Morphology: This species has been described by Kotlán, Mócsy and Vajda (1929), Balozet (1932) and Christensen (1938a). The oocysts are subspherical, ellipsoidal or spherical, 12 to 22 by 10 to 18 μ with a mean of 16.5 by 14.1 μ (Christensen) or 17.2 by 13.5 μ (Balozet). Their length width ratio is 1.0 to 1.5 with a mean of 1.18 (Christensen). The oocyst wall is smooth, homogeneous, pale yellow to yellowish green, and composed of 2 layers of which the outer is half as thick as the inner; there is a heavy, black refraction line on each side of the inner layer, according to Christensen. According to Balozet, the wall appears to be lined by a membrane. The sporont is clear. A micropyle is absent; according to Christensen, the oocyst wall occasionally appears slightly paler at one end than the other. An oocyst polar granule and oocyst residuum are absent. The sporocysts are oval. The sporocyst residuum is indistinct if present at all. The sporulation time is 1 to 2 days (Christensen) or 7 to 8 days (Balozet).

Life Cycle: The life cycle of *E. parva* in sheep has been described by Kotlán, Pellérdy and Versényi (1951). The schizonts are found thruout the small intestine. They measure up to 185 to 256 by 128 to 179 μ and are easily visible to the naked eye as whitish bodies. They lie in the mucosa, usually near the surface but sometimes as far down as the muscularis mucosae. They invade endothelial cells and enlarge both the host cell and its nucleus enormously. They are surrounded by a rather thick layer of connective tissue which becomes thinner as they increase in size. Each schizont produced thousands of straight merozoites 10 to 12 μ long.

Kotlán, Pellérdy and Versényi (1951) also found a second, much smaller type of schizont in the small intestine. It occurred in the superficial epithelial cells, was 10 to 12 μ in diameter and contained about 10 to 20 merozoites 2.5 to 3 μ long. They were not sure, however, whether it was part of the life cycle of *E. parva*.

The sexual stages occur mostly in the cecum and colon and to a lesser extent in the small intestine. They are found in the epithelial cells and measure 15 to 19 by 10 to 16 μ.

Pathogenesis: This species is apparently not very pathogenic. Most of the damage is caused by the sexual stages in the large and small intestines. In a lamb killed by Kotlán, Pellérdy and Versényi (1951) 16 days after experimental infection, the contents of the cecum and colon were semifluid, dark and mixed with blood in places. The wall was thickened and its surface uneven and denuded of epithelium in places. By histologic examination of the cecum, it was found that the mucosa had been stripped from the glandular layer in places and the tissue had become necrotic and infiltrated with lymphocytes and neutrophiles but no eosinophiles. Sharply separated from these necrotic areas were other areas in which most of the epithelial cells contained microgametocytes, macrogametes or young oocysts.

EIMERIA PUNCTATA
LANDERS, 1955

Synonym: *Eimeria honessi* Landers, 1952.

Host: Sheep.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: North America (Wyoming).

Prevalence: Landers (1952) found this species in 2 of 9 sheep in Wyoming.

Morphology: This species has been described by Landers (1952). The oocysts are subspherical, 18 to 25 by 16 to 21 μ with a mean of 21.2 by 17.7 μ. Their length-width ratio is 1.1 to 1.3 with a mean of 1.20. The oocyst wall has

conspicuous, uniform, cone-shaped pits about 0.5 μ in diameter. It is 1.2 to 1.8 μ thick (mean, 1.5 μ); its outer layer is colorless to yellowish, and its inner layer greenish, with two dark refraction lines, one at either surface. There is a conspicuous micropyle. A micropylar cap is present, sometimes imperceptible, up to 6.5 μ wide and 1.6 μ high with a mean of 4.1 by 0.8 μ. An oocyst residuum and oocyst polar granule are absent. The sporocysts are spherical to ellipsoidal, and average 8.1 μ in diameter or 10.4 by 6.9 μ. The presence or absence of a sporocyst residuum was not mentioned.

Life Cycle: Unknown.

Pathogenesis: Unknown.

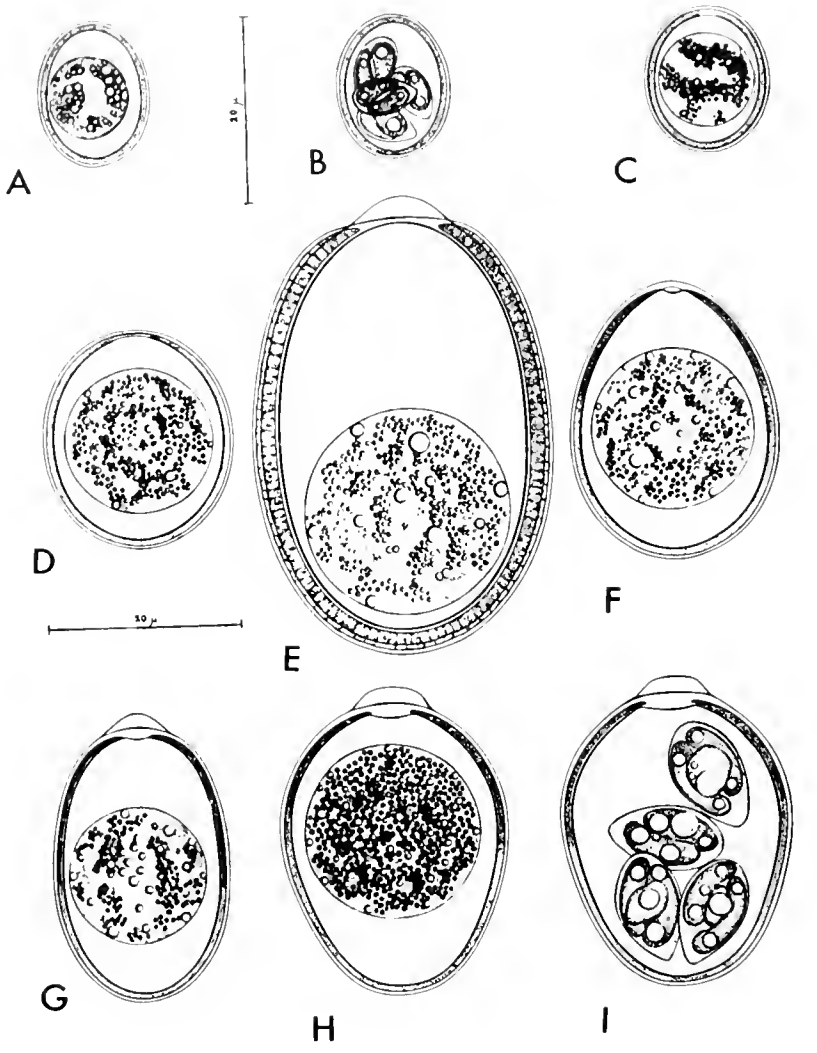

Fig. 26. Coccidia of sheep. A. *Eimeria pallida* unsporulated oocyst. B. *E. pallida* sporulated oocyst. C. *E. parva* unsporulated oocyst. D. *E. ninakohlyakimovae* unsporulated oocyst. E. *E. intricata* unsporulated oocyst. F. *E. faurei* unsporulated oocyst. G. *E. arloingi* unsporulated oocyst. H. *E. granulosa* unsporulated oocyst. I. *E. granulosa* sporulated oocyst. X 1270. (From Christensen, 1938)

COCCIDIOSIS IN SHEEP AND GOATS

Coccidiosis in sheep is primarily a disease of feedlot lambs. It has been studied by Newsom and Cross (1931), Deem and Thorp (1939, 1940) and Christensen (1940) among others. It appears 12 days to 3 weeks after the lambs arrive in the feedlot. Diarrhea, depression and inappetance appear, followed by weakness and loss of weight. The diarrhea continues for several days up to about 2 weeks, and some lambs may die during this period. Most, however, recover. The mortality varies, but is seldom more than 10%. In a group of 16,000 New Mexico feeder lambs studied by Christensen (1940) on a Nebraska feedlot, the mortality was 3.4%, but another 9.8% of scouring, emaciated lambs were removed to a hospital lot for special diet and care.

Even if there are no deaths, there may be loss of weight or reduced weight gains. Thus, Shumard (1957) found that 80 lambs experimentally infected with a sublethal mixture of coccidian oocysts (mostly *E. ninakohlyakimovae* and *E. arloingi*) lost an average of 0.205 pounds per pound of feed consumed during the 24 days following infection, as compared with an average gain of 0.062 pounds per pound of feed consumed for 40 control, uninfected lambs.

When lambs are brought into the feedlot, they are usually shedding small numbers of coccidian oocysts. As the result of crowding, and under conditions which promote fecal contamination of the feed, the coccidial infections build up. The number of oocysts in the feces rises for about a month, remains stationary for 1 to 3 weeks and then decreases rather rapidly, only a few oocysts being present at the end of the feeding period. Whether or not disease will appear depends upon the number and species of oocysts which the lambs ingest during the crucial first week or two. By the end of the first month, there is little danger of coccidiosis. The lambs have been infected, but the exposing dose of oocysts has been small enough to permit immunity to develop. In other words, there has been coccidiasis but no coccidiosis.

Feeding of chopped feed in open troughs low enough to be contaminated with feces promotes coccidiosis. Christensen (1941a) found that corn silage provided an amount of moisture which favored oocyst sporulation, while chopped alfalfa, grain and molasses also permitted sporulation.

Dunlap, Hawkins and Nelson (1949) followed oocyst production from the time of birth in lambs running with their mothers. The ewes were the source of infection, and lambs became infected by ingesting sporulated oocysts from the bedding. The first oocysts appeared when the lambs were 5 to 8 weeks old; they built up to a peak which lasted 1 to 4 weeks, and then declined.

Temperature affects oocyst sporulation. Dunlap, Hawkins and Nelson (1949) found the first sporulated oocysts in the bedding when the mean temperature was 49° F. Christensen (1939) found that the optimum sporulation temperature for the oocysts of *E. arloingi* was 20-25° C, the sporulation time being 2 to 3 days at that temperature in a thin layer of water or in fecal pellets. The oocysts survived less than 4 months in fecal sediment at this temperature. Sporulation was slow at 0 to 5° C, altho oocysts remained alive for at least 10 months in fecal sediment or moist pellets. No sporulation took place at 40° C and the oocysts were killed within 4 days. If the fecal sediment was allowed to putrefy, however, no sporulation took place at any temperature.

Landers (1953) found that the oocysts of *E. arloingi*, *E. ninakohlyakimovae* and *E. parva* did not survive 24 hours in sheep pellets when frozen directly to -30° C, and survived less than 2 days when conditioned at -19° C prior to freezing to -30°. They survived without essential mortality when frozen directly to -25° C for 7 days, but only about half of the first two species and one quarter of *E. parva* survived 14 days. Repeated freezing and thawing at -19 or -25° C up to 6 or 7 times had no significant effect on survival. Landers said that in an average winter at Laramie, Wyoming the minimum soil surface temperature would probably be between -15 and -20° C and that unsporulated oocysts would not normally be killed by such temperatures.

Diagnosis: Coccidiosis in sheep and goats can be diagnosed from a combination of history, signs, gross lesions at necropsy and microscopic examination of the intestinal mucosa and feces. However, recognition of coccidia in the lesions at necropsy is necessary for positive diagnosis. The mere presence of oocysts in the feces does not necessarily mean that the disease is due to coccidia. On the other hand, acute coccidiosis may be present before any oocysts appear.

Treatment: Relatively few studies have been carried out on the treatment of coccidiosis in sheep. A distinction must be made between preventive and curative treatments. Several sulfonamides and sulfur are of value in preventing coccidiosis in lambs, but no drugs are known to cure the disease once signs appear. However, oxytetracycline and related antibiotics may be of value in controlling secondary infections.

Foster, Christensen and Habermann (1941) found that 2 g sulfaguanidine a day prevented the acquisition of natural coccidiosis in 5 lambs and reduced the level of oocyst output in 4 subclinical infections with unnamed species. Christensen and Foster (1943) reported that 0.2% sulfaguanidine in the feed for 20 days beginning 1 day after an infective feeding with 500,000 sporulated oocysts from lambs with clinical coccidiosis prevented severe coccidiosis in lambs, but that 0.45% sulfaguanidine failed to affect the course of the disease when it was begun the day after clinical signs had appeared. Steward (1952) found that sulfamethazine and sulfadiazine had some value in an outbreak of coccidiosis in sheep, reducing the numbers of oocysts passed, but that quinacrine was valueless. Whitten (1953) found in a controlled experiment that neither 0.01 g per kg quinacrine hydrochloride nor 0.01 g per kg sulfamethazine daily for 3 days had any significant effect on oocyst production or weight gains in naturally affected lambs. However, oocyst production decreased markedly markedly in both treated lambs and controls following treatment, so that if no controls had been used, it would have been assumed that the treatment had been of value.

Christensen (1944) found that 0.5 to 1.5% sulfur fed in a ration of chopped alfalfa and ground corn held together by molasses and water prevented coccidiosis in feeder lambs. He fed this amount of sulfur for 72 days without ill effects, but higher concentrations caused diarrhea and decreased weight gains.

Tarlatzis, Panetsis and Dragonas (1955) claimed that furacin was effective against coccidiosis in sheep and goats, but their work was uncontrolled.

Prevention: Good sanitation will largely prevent coccidiosis in lambs. Coccidiosis is not a problem in suckling lambs on the western range, but appears when the animals are brought together in the feedlot. Feedlots should be kept dry and clean. Clean water and feed should be supplied, and feed troughs should be so constructed that they cannot be contaminated with feces.

Coccidiosis is a potential hazard if lambing takes place in a barn or restricted area, and the bedding is the most common source of infection. Shumard and Eveleth (1956) recommended as a practical method for raising lambs with their ewes that the animals be kept in concrete pens with straw bedding, that the pens be cleaned twice a week, and that 1 pint of a 3.45% sulfaquinoxaline solution be added to each 50 gallons of drinking water. In their studies, coccidian oocysts did not appear in the lambs until 18 days after treatment had been discontinued.

EIMERIA DEBLIECKI DOUWES, 1921

Synonyms: *Eimeria brumpti, Eimeria jalina, Eimeria suis.*

Host: Pig.

Location: Small intestine, and, to a lesser extent, large intestine.

Geographic Distribution: Worldwide.

Prevalence: *E. debliecki* is the commonest coccidium of swine. De Graff (1925) found it in 51% of 500 pigs in the

Netherlands. Yakimoff *et al.* (1936) found it in 92% of 141 pigs in Russia. Yakimoff (1936) found it in 27% of 53 pigs from Brazil. Novicky (1945) found it in 27% of 62 pigs in Venezuela. Nieschulz and Ponto (1927) found it in all of about 50 pigs in Java.

Morphology: The oocysts are ovoid to ellipsoidal or subspherical, 13 to 29 by 13 to 19 μ. The oocyst wall is 1.0 to 1.5 μ thick, smooth, colorless to brownish, and composed of 2 layers. A micropyle is absent. An oocyst polar granule is absent (present according to Paichuk, 1953). An oocyst residuum is absent. The sporocysts are ellipsoidal or ovoid, 14 to 18 by 6 to 8 μ with a Stieda body. A sporocyst residuum is present.

The sporulation time is 6 to 9 days. The sporulation process has been described in detail by de Graaf (1925).

Life Cycle: De Graaf (1925) and others have described the endogenous stages of this species. The schizonts produce 14 to 16 banana-shaped merozoites. These are 8 to 10 μ long and 3 to 4.5 μ wide; one end is rounded and the other pointed. The nucleus is usually in the middle of the merozoites. The microgametocytes are 7 to 22 μ in diameter when mature. The microgametes are 3.5 μ long and 0.6 μ wide and have 2 flagella. The macrogametes are similar to those of other *Eimeria* species.

Biester and Schwarte (1932) found that the prepatent period in experimentally infected pigs was about 7 days and that oocysts were present in the feces for 10 to 15 days in the absence of reinfection.

Pathogenesis: *E. debliecki* is only slightly pathogenic if at all in adult animals, but it may cause diarrhea and even death in young pigs. Biester and Murray (1929) found that young pigs fed large numbers of sporulated oocysts developed severe diarrhea. They became emaciated and some even died. Some had constipation, but dysentery was never observed. The pigs which recovered usually failed to do well. Alicata and Willett (1946) found that pigs experimentally infected with 20 to 30 million mixed sporulated oocysts of *E. debliecki* and *E. scabra* developed a profuse diarrhea lasting 2 to 15 days, inappetance and did not gain weight. Swanson and Kates (1940) described an outbreak of coccidiosis in a litter of 4.5 month old pigs in Georgia. The pigs had a profuse diarrhea and gained weight poorly despite ravenous appetites, excellent rations and good care. Novicky (1945) described several outbreaks of swine coccidiosis in Venezuela. The mortality was relatively low, but the young animals which survived were retarded.

Immunity: Biester and Schwarte (1932) produced complete immunity in pigs by feeding them oocysts daily for 100 days or more. Light infections produced partial immunity. As with other coccidia, adult pigs are often carriers, shedding a few oocysts in their feces.

Remarks: Brug (1946) found *E. debliecki* as a pseudoparasite of man in Holland. Four out of 13 persons in a psychiatric ward passed oocysts in their feces on one day. They had probably been ingested with liver sausage, the casing of which was made from pig intestines.

EIMERIA PERMINUTA HENRY, 1931

Host: Pig.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: Worldwide.

Prevalence: Yakimoff *et al.* (1936) found this species in 18% of 141 pigs in Russia. Yakimoff (1936) found it in 45% of 53 pigs in Brazil.

Morphology: This species was first described by Henry (1931) from pigs in California. The oocysts are ellipsoidal to spherical, 11 to 16 by 10 to 13 μ. The oocyst wall is rough, yellowish, and apparently composed of a single layer. A micropyle is absent. An oocyst polar

granule is present. No other morphological information is known. The sporulation time is 11 days.

Life Cycle: Unknown.

Pathogenesis: Unknown.

EIMERIA POLITA
PELLÉRDY, 1949

Host: Pig.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: Europe (Hungary), North America (Alabama).

Prevalence: Unknown.

Morphology: This species has been described by Pellérdy (1949) in Hungary and by Lesser and Davis (1958) in Alabama. The oocysts are ellipsoidal, rarely broadly ovoid, 17 to 36 by 13 to 24 μ with a mean of 23.8 by 17.9 μ. The oocyst wall is smooth or occasionally roughened, yellowish brown or pinkish brown, 1.0 to 1.5 μ thick. The micropyle is imperceptible or is seen only when the oocyst lies in a favorable position. A polar granule is present in about half the oocysts. There is no oocyst residuum. The sporocysts are ellipsoidal, 15 to 19 by 6 μ. A sporocyst residuum is present. The sporulation time is 8 to 9 days.

Life Cycle: Unknown. Pellérdy (1949) found that the prepatent period in 3 experimentally infected pigs was 8 to 9 days.

Pathogenesis: Unknown.

EIMERIA SCABRA
HENRY, 1931

Host: Domestic and wild pigs.

Location: Intestine. The sexual stages are found in the epithelial cells of the villi.

Geographic Distribution: Worldwide.

Prevalence: Yakimoff *et al.* (1936) found this species in 33% of 141 pigs in Russia.

Morphology: The oocysts are ovoid to ellipsoidal, 22 to 36 by 16 to 28 μ. The oocyst wall is brown, rough, and 1.5 to 2.0 μ thick, becoming thinner at the narrow end. A micropyle is present according to Pellérdy (1949), but was not mentioned by Henry (1931). An oocyst polar granule is present. There is no oocyst residuum. The sporocysts are ellipsoidal, 15 to 19 by 6 μ, and have a sporocyst residuum. The sporulation time is 9 to 12 days.

Life Cycle: The endogenous stages of this species have not been described. Pellérdy (1949) found that the prepatent period in 2 experimentally infected pigs was 9 days.

Pathogenesis: Uncertain. Alicata and Willett (1946) found that pigs experimentally infected with 20 to 30 million mixed sporulated oocysts of *E. debliecki* and *E. scabra* developed a profuse diarrhea lasting 2 to 15 days, lost their appetites and did not gain weight. How much of this effect was due to *E. scabra* is not known.

EIMERIA SCROFAE
GALLI-VALERIO, 1935

Host: Domestic pig.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: Europe (Switzerland).

Prevalence: Unknown.

Morphology: This species has been described only by Galli-Valerio (1935). The oocysts are cylindroid, with one end slightly flattened, and measure 24 by 15 μ. There is a distinct micropyle. Oocyst and sporocyst residua are absent. The sporont is finely granular. Pellérdy (1949) considered this a rather doubtful species

resembling *E. debliecki*. However, the absence of a sporocyst residuum and presence of a micropyle differentiate it from this species.

Life Cycle: Unknown.

Pathogenesis: Unknown.

EIMERIA SPINOSA
HENRY, 1931

Host: Pig.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: North America (California, Minnesota, Maryland, Georgia), Hawaii, USSR (North Caucasus).

Prevalence: Unknown. This species appears to be relatively uncommon.

Morphology: This species was described by Henry (1931). The oocysts are ovoid or ellipsoidal, 16 to 22 by 10 to 13 μ. The oocyst wall is brown, opaque, and studded with spines about 1 μ long and 1 μ apart. A micropyle is absent. An oocyst polar granule is present. An oocyst residuum is apparently absent. The sporocysts have a Stieda body. A sporocyst residuum is apparently present (Henry's description is ambiguous). The sporulation time is 11 to 12 days.

Pathogenesis: This species is only slightly pathogenic if at all. Andrews and Spindler (1952) observed no diarrhea or other signs in an infected pig which passed as many as 7 million oocysts per gram of feces.

COCCIDIOSIS IN SWINE

Epidemiology: Coccidia are common in swine, but we know little about the prevalence and importance of the disease, coccidiosis. Enteritis is so common in young pigs and is caused by so many different agents that they have not all been sorted out and their importance assessed. Coccidia are among the least known of these agents.

Coccidiosis is primarily a disease of young pigs. Adults are carriers. *Eimeria debliecki* is probably the most pathogenic species, but *E. scabra* and *Isospora suis* may also cause disease.

Pigs become infected by ingesting sporulated oocysts along with their feed or water. The presence or severity of the disease depends upon the number of oocysts they receive. Crowding and lack of sanitation greatly increase the disease hazard.

Avery (1942) found that the oocysts of *E. debliecki* and *E. scabra* could survive and remain infective in the soil for 15 months. The soil surface temperature varied between -4.5° and 40° C during this period. Unsporulated oocysts withstood continuous freezing at -2° to -7° C or alternate freezing and thawing at 0.5° and -3° C for at least 26 days, altho subsequent sporulation was somewhat decreased.

Immunity: Repeated infections over a period of time confer immunity to coccidiosis. Biester and Schwarte (1932) produced complete immunity in pigs by feeding oocysts daily for 100 days or more. Light infections produced partial immunity.

The coccidia of swine are not transmissible to other farm animals, and pigs cannot be infected with their coccidia.

Diagnosis: Coccidiosis in swine can be diagnosed by finding the endogenous stages in lesions in the intestine. The presence of oocysts in the feces does not necessarily mean that coccidiosis is present, nor does their absence necessarily mean that it is absent, since oocysts may not be produced until 2 or 3 days after the first signs of disease appear.

Treatment: Little is known about treatment of coccidiosis in pigs. Alicata and Willett (1946) found that when 1 g

sulfaguanidine per 10 lb body weight was administered to pigs daily with their feed for 7 or 10 days beginning 2 days before experimental infection with 20 to 30 million sporulated oocysts of *E. debliecki* and *E. scabra*, very few if any oocysts were produced and the pigs did not become ill. Similar treatment with sulfaguanidine for 3 days beginning on the 2nd day of oocyst discharge reduced the numbers of oocysts produced and the period of discharge. Presumably other sulfonamides would also be of value.

Prevention and Control: Sanitation will prevent coccidiosis in swine. Pens should be cleaned frequently, overcrowding should be avoided, and pigs should be raised under conditions which prevent them from eating many infective oocysts.

EIMERIA LEUCKARTI
(FLESCH, 1883)
REICHENOW, 1940

Synonyms: *Globidium leuckarti.*

Hosts: Horse, ass.

Location: Small intestine.

Geographic Distribution: Europe, India.

Prevalence: Apparently uncommon.

Morphology: The sporulated oocysts have been described by Reichenow (1940a) and Hiregaudar (1956a). They are ovoid, somewhat flattened at the smaller end, and 75 to 88 by 50 to 59 μ. The oocyst wall is composed of 2 layers, of which the outer is dark brown, 5 to 7 μ thick, opaque and granular, and the inner layer is about 1 μ thick and colorless. The micropyle is distinct. An oocyst residuum is absent. An oocyst polar granule is apparently absent. The sporocysts are elongate, 30 to 42 by 12 or 14 μ with a Stieda body. A sporocyst residuum is present. The sporozoites are elongate, up to 35 μ long, with a clear globule at the large end. The sporulation time is 21 days at 20 to 22° C in Germany (Reichenow) or 15 days during the hot days of October in India (Hiregaudar).

Life Cycle: The schizonts and merozoites of this species have not been described. The sexual stages were described most recently by Hemmert-Halswick (1943). They are found beneath the epithelium in the villi of the small intestine. The microgametocytes measure up to 300 by 170 μ when mature. The macrogametes contain both eosinophilic and basophilic plastic granules which later form the wall of the oocyst.

Pathogenesis: Diarrhea, loss of weight and even death have been reported in heavily infected animals. Hemmert-Halswick (1943) described marked inflammatory changes in the small intestine mucosa.

Diagnosis: Diagnosis can be made by finding the endogenous stages of this coccidium in association with lesions in the intestine. The oocysts are seldom seen in feces because they are so heavy that they do not rise to the surface in the salt solutions used for flotations.

EIMERIA SOLIPEDUM
GOUSSEFF, 1935

Hosts: Horse, ass.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: USSR (Azerbaidzhan, Volga basin, Leningrad).

Prevalence: Gousseff (1935) found this species in 1.4% of 3355 horses, 3% of 251 donkeys and 1% of 161 mules in Russia.

Morphology: The oocysts are spherical, bright orange to yellowish brown, and 15 to 28 μ in diameter. The oocyst wall is double contoured, without a micropyle. An oocyst residuum and polar granule are absent. The sporocysts are ellipsoidal or oval, 5 by 3 μ. The sporozoites are piriform. The presence or

absence of a sporocyst residuum could not be determined.

Life Cycle; Unknown.

Pathogenesis: Unknown.

EIMERIA UNIUNGULATI
GOUSSEFF, 1935

Hosts: Horse, ass.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: USSR (Azerbaidzhan, western RSFSR, Volga region, Leningrad, Siberia, Tadzhikistan, Uzbekistan).

Prevalence: Gousseff (1935) found this species in 0.8% of 3355 horses, 3% of 251 donkeys and 1% of 161 mules in Russia.

Morphology: The oocysts are oval (ellipsoidal?), bright orange, and 15 to 24 by 12 to 17 μ. The oocyst wall is double contoured. A micropyle, oocyst residuum and polar granule are absent. The sporocysts are 6 to 11 by 4 to 6 μ. A sporocyst residuum is present.

Life Cycle: Unknown.

Pathogenesis: Unknown.

COCCIDIOSIS IN HORSES, ASSES AND MULES

Coccidiosis is such a rarity in horses, asses and mules that little can be said about it. The same measures which are effective in cattle should control coccidiosis in equids.

EIMERIA CANIS
WENYON, 1923

Hosts: Dog, cat, dingo.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: Europe (England, Holland), North America (Nebraska, Quebec), Australia.

Prevalence: This form is uncommon. It was found by Brown and Stammers (1922) and Wenyon (1923) in England, Nieschulz (1924) in Holland, Skidmore and McGrath (1933) in Nebraska and Bearup (1954) in a dingo in Australia. Choquette and Gelinas (1950) reported it in 10% of 155 dogs in Quebec.

Morphology: The oocysts are ovoid or ellipsoidal, 17 to 45 by 11 to 28 μ, pink, red or colorless. The oocyst wall is fairly thick, rough, and composed of 2 layers. A micropyle is present. The sporocysts measure 9.5 by 2.5 μ. The sporulation time is 1 to 4 days.

Remarks: It is far from certain that this is a valid species. Wenyon (1926) remarked that in many respects *E. canis* resembles a mixture of *E. stiedae* and *E. perforans* of the rabbit, and Goodrich (1944) considered it to be a rabbit form which the dogs had eaten.

EIMERIA CATI
YAKIMOFF, 1933

Hosts: Cat, dog.

Location: Intestine.

Geographic Distribution: USSR.

Prevalence: Rare.

Morphology: The oocysts are ovoid or spherical; the ovoid oocysts are 18 to 24 by 14 to 20 μ with a mean of 21 by 17 μ; the spherical oocysts are 16 to 22 μ in diameter with a mean of 18 μ. A micropyle is absent. An oocyst polar granule is present. An oocyst residuum is absent. A sporocyst residuum is present.

Life Cycle: Unknown.

Pathogenesis: Unknown.

EIMERIA FELINA
NIESCHULZ, 1924

Hosts: Cat, lion.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: Europe (Holland).

Prevalence: Rare.

Morphology: The oocysts are ellipsoidal, 21 to 26 by 13 to 17 μ with a mean of 24 by 14.5 μ. The oocyst wall is about 1 μ thick, smooth, colorless, and double contoured. A definite micropyle is absent. An oocyst polar granule is absent. An oocyst residuum is present. The sporocysts are elongate ovoid, with a Stieda body. A sporocyst residuum is present. The sporozoites are comma-shaped, with a large vacuole at the large end and usually a small one at the small end.

Life Cycle: Unknown.

Pathogenesis: Unknown.

EIMERIA STIEDAE
(LINDEMANN, 1865)
KISSKALT AND HARTMANN, 1907

Synonyms: *Monocystis stiedae*, *Coccidium oviforme*, *Coccidium cuniculi*.

Hosts: Domestic rabbit, European hare (*Lepus europaeus*), varying hare (*L. americanus*), black-tailed jack rabbit (*L. californicus*), alpine hare (*L. timidus*), *L. variabilis*, cottontails (*Sylvilagus floridanus*, *S. nuttalli*).

Location: Liver. The coccidia are found in the bile duct epithelial cells.

Geographic Distribution: Worldwide.

Prevalence: This is the most common and most important coccidium of domestic rabbits. It also occurs in hares (*Lepus*), but is less common in cottontails than other species.

Morphology: The oocysts are ovoid, sometimes ellipsoidal, with a flat micropylar end, and measure 28 to 40 by 16 to 25 μ with a mean of 37 by 21 μ. The oocyst wall is smooth and salmon-colored. A micropyle is present. An oocyst polar granule and oocyst residuum are absent. The sporocysts are elongate ovoid, 18 to 10 μ, with a Stieda body. A sporocyst residuum is present. The sporulation time is 3 days.

Life Cycle: The life cycle of this species has been studied by a number of workers (see Becker, 1934, for a review). The sporulated oocysts excyst in the small intestine. The sporozoites penetrate the intestinal mucosa, enter the hepatic portal system, and pass to the liver. Here they enter the epithelial cells of the bile ducts. The liver parenchyma cells are only rarely invaded. Development takes place above the host cell nucleus. Each sporozoite rounds up and becomes a schizont which produces 6 to 30 or more (usually 8 to 16) merozoites which measure about 8 to 10 by 1.5 to 2.0 μ. The number of asexual generations is not known. Later, some merozoites become microgametocytes which produce large numbers of comma-shaped, biflagellate microgametes, while others become macrogametes. These are fertilized, lay down an oocyst wall, break out of their host cell, pass into the intestine with the bile and thence out of the body. The prepatent period is 18 days.

Pathogenesis: In mild cases of liver coccidiosis there may be no signs, but in more severe ones the animals lose their appetites and grow thin. There may be diarrhea, and the mucous membranes may be icteric. The disease is more severe in young animals than in old. It may be chronic, or death may occur in 21 to 30 days.

Some of the symptoms are due to interference with liver function. The liver may become markedly enlarged, and white circular nodules or elongated cords appear in it. At first they are sharply circumscribed, but later they tend to coalesce. They are enormously enlarged bile ducts filled with the developing parasites. There is tremendous hyperplasia of the

bile duct epithelial cells. Instead of forming a simple, narrow tube, the epithelium is thrown into great, arborescent folds, and each cell contains a parasite.

Dunlap, Dickson and Johnson (1959) found that infection with *E. stiedae* increased the serum β- and γ-globulin and β-lipoprotein and decreased the α-lipoprotein.

EIMERIA MAGNA PÉRARD, 1925

Synonyms: *Eimeria perforans* var. *magna*.

Hosts: Domestic rabbit, California jack rabbit (*Lepus californicus*), varying hare (*L. timidus*), European hare (*L. europaeus*), cottontail (*Sylvilagus floridanus*) (experimental).

Location: Jejunum, ileum.

Geographic Distribution: Worldwide.

Prevalence: This species is quite common. Kessel and Jankiewicz (1931) found it in 19% of over 2000 rabbits in California.

Morphology: The oocysts are ovoid or ellipsoidal, becoming subspherical toward the end of the patent period, smooth, orange-yellow or brownish, and 27 to 41 by 17 to 29 μ with a mean of 35 by 24 μ. The micropyle is large and surrounded by prominent shoulders. An oocyst polar granule is absent. An oocyst residuum is present. The sporocysts are elongate ovoid, with a Stieda body. A sporocyst residuum is present. The sporulation time is 2 to 3 days. Oocyst variation was studied carefully by Kheisin (1947).

Life Cycle: Rutherford (1943) described the life cycle of this species. The endogenous stages are found below the epithelial cell nuclei of the villi and also in the submucosa. There are 2 asexual generations of merozoites followed by microgamete and macrogamete production. It takes 7 days for completion of the endogenous cycle, and the prepatent period is 7 to 8 days. According to Kheisin (1947), *E. magna* produces 800,000 oocysts per oocyst fed.

Pathogenesis: This is one of the most pathogenic of the intestinal coccidia of the rabbit. Only a few hundred oocysts of some strains may produce symptoms, and 300,000 may cause death (Lund, 1949). Other strains are less pathogenic, 1 million oocysts not causing death. The principal signs are loss of weight, inappetance and diarrhea. A good deal of mucus may be passed. The animals lose their appetites and grow thin. The intestinal mucosa is hyperemic and inflamed, and epithelial sloughing may occur.

EIMERIA PERFORANS (LEUCKART, 1879) SLUITER AND SWELLENGREBEL, 1912

Synonyms: *Coccidium perforans*, *Eimeria exigua*, *E. lugdunumensis*.

Hosts: Domestic rabbit, varying hare (*Lepus americanus*), east Greenland hare (*L. arcticus groenlandicus*), California jack rabbit (*L. californicus*), European hare (*L. europaeus*), Brazilian cottontail (*Sylvilagus brasiliensis*), cottontail (*Sylvilagus floridanus*) (experimentally).

Location: Thruout small intestine and also in cecum.

Geographic Distribution: Worldwide.

Prevalence: Common. Kessel and Jankiewicz (1931) found it in 30% of over 2000 rabbits in California.

Morphology: The oocysts are ellipsoidal, sometimes ovoid, smooth, colorless to pinkish, 24 to 30 by 14 to 20 μ with a mean of 26 by 16 μ. A micropyle is absent. An oocyst polar granule is absent. An oocyst residuum is present. The sporocysts are ovoid, with a Stieda body. A sporocyst residuum is present. The sporulation time is 2 days.

Life Cycle: Rutherford (1943) described the life cycle of this species. The endogenous stages are found above the nuclei of the epithelial cells of the intestine. There are 2 asexual generations of merozoites, followed by microgamete and macrogamete production. Completion of the endogenous cycle takes 5 days, and the prepatent period is 5 to 6 days.

Pathogenesis: *E. perforans* is one of the less pathogenic intestinal coccidia of rabbits, but it may nevertheless cause mild to moderate signs if the infection is heavy enough. The duodenum may be enlarged and edematous, sometimes chalky white; the jejunum and ileum may contain white spots and streaks, and there may be petechiae in the cecum.

EIMERIA MEDIA
KESSEL, 1929

Synonym: *Eimeria flavescens.*

Hosts: Domestic rabbit, California jack rabbit (*Lepus californicus*), cottontail (*Sylvilagus floridanus*), Wyoming cottontail (*S. nuttallii grangeri*).

Location: Thruout small and large intestines.

Geographic Distribution: Worldwide.

Prevalence: Quite common. Kessel and Jankiewicz (1931) found it in 12% of over 2000 rabbits in California.

Morphology: The oocysts are ovoid, smooth, 19 to 33 by 13 to 21 μ. A micropyle is present. An oocyst polar granule is absent. An oocyst residuum is present. The sporocysts are elongate ovoid, with a Stieda body. A sporocyst residuum is present. The sporulation time is 2 days.

Life Cycle: Rutherford (1943) and Pellérdy and Babos (1953) described the life cycle of this species. The endogenous stages are found above or below the epithelial cell nuclei of the intestinal villi and also occur in the submucosa. There are 2 asexual generations of merozoites followed by microgamete and macrogamete production. Completion of the endogenous cycle takes 6 days, and the prepatent period is 5 to 6 days. According to Kheisin (1947), *E. media* produces 150,000 oocysts per oocyst fed.

Pathogenesis: This species is moderately pathogenic (Pellérdy and Babos, 1953). It may cause the usual signs of intestinal coccidiosis. The affected parts of the intestine may be edematous, with greyish foci.

EIMERIA IRRESIDUA
KESSEL AND JANKIEWICZ, 1931

Hosts: Domestic rabbit, California jack rabbit (*Lepus californicus*), white-tailed jack rabbit (*L. townsendii*).

Location: Thruout small intestine.

Geographic Distribution: Worldwide.

Prevalence: Quite common. Kessel and Jankiewicz (1931) found this species in 10% of over 2000 rabbits in California.

Morphology: The oocysts are ovoid, smooth, and 38 by 26 μ. The micropyle is prominent. An oocyst polar granule and oocyst residuum are absent. The sporocysts are elongate ovoid, with a Stieda body. A sporocyst residuum is present. The sporulation time is 2 to 2.5 days.

Life Cycle: Rutherford (1943) described the life cycle of this species. The endogenous stages are found above or below the epithelial cell nuclei of the intestinal villi and also occur in the submucosa. There are 2 asexual generations of merozoites followed by microgamete and macrogamete production. Completion of the endogenous cycle takes 9 to 10 days, and the prepatent period is 9 to 10 days.

Pathogenesis: This is one of the more pathogenic of the intestinal coccidia of rabbits. It causes the usual signs of intestinal coccidiosis. The affected areas are hyperemic, there may be extravasation of blood, and the epithelium may slough and become denuded.

EIMERIA PIRIFORMIS
KOTLAN AND POSPESCH, 1934

Host: Domestic rabbit.

Location: Small and large intestines.

Geographic Distribution: Europe (France, Hungary).

Prevalence: Relatively uncommon. This species has been found in both wild and captive domestic rabbits.

Morphology: The oocysts are piriform to ovoid, smooth, yellowish brown, 26 to 32 by 17 to 21 μ with a mean of 29 by 18 μ. A micropyle is present. An oocyst polar granule and oocyst residuum are absent. A sporocyst residuum is present. The sporulation time is 2 days.

Life Cycle: The life cycle of this species does not appear to have been worked out in detail. The life cycle described by Pellérdy (1953) for "*E. piriformis*" is actually that of *E. intestinalis*. The prepatent period is 9 days.

Pathogenesis: Unknown.

EIMERIA NEOLEPORIS
CARVALHO, 1942

Hosts: Cottontail (*Sylvilagus floridanus*), domestic rabbit (experimentally).

Location: Posterior part of small intestine, large intestine.

Geographic Distribution: North America.

Prevalence: Common in cottontails. Ecke and Yeatter (1956) found this species in 31% of 32 cottontails in Illinois.

Morphology: The oocysts are subcylindrical or elongate ellipsoidal to ovoid, smooth, pinkish yellow, 33 to 44 by 16 to 23 μ with a mean of 39 by 20 μ. A micropyle is present. An oocyst polar granule is absent. An oocyst residuum is usually absent. A sporocyst residuum is present. The sporozoites are elongate ovoid, with a Stieda body. The sporulation time is 2 to 3 days.

Life Cycle: The endogenous cycle of this species has not been described.

Pathogenesis: This species is slightly to markedly pathogenic, depending upon the extent of the infection. The affected intestinal mucosa is inflamed and hyperemic, and caseous necrosis may be present.

Remarks: Pellérdy (1954a) found a coccidium which he believed to be *E. neoleporis* in domestic rabbits in Hungary and described its pathogenic effects. He believed that *E. coecicola* was a synonym of this species and he may be right. Carvalho (1942) transmitted *E. neoleporis* from the cottontail to the domestic rabbit. However, for the present I am using the name *E. neoleporis* for the cottontail form alone.

EIMERIA COECICOLA
CHEISSIN, 1946

Host: Domestic rabbit.

Location: Posterior ileum, cecum.

Geographic Distribution: Europe (Hungary), USSR.

Prevalence: This species is apparently rare in captive domestic rabbits, but is common in wild ones.

Morphology: The oocysts are ovoid, sometimes ellipsoidal, smooth, light yellow, 25 to 40 by 15 to 21 μ. A micropyle is present. An oocyst polar granule is absent. Oocyst and sporocyst residua are present. The sporulation time is 3 days.

Life Cycle: Kheisin (1947) described the life cycle of this species. The schizonts are found in the epithelial cells of the villi of the posterior ileum, and the gametes and gametocytes are below the host cell nuclei of the crypt cells of the cecum. The prepatent period is 9 days,

and 100,000 oocysts are produced per oocyst fed.

Pathogenesis: According to Kheisin (1947), this species is slightly if at all pathogenic. He saw small white spots in the cecum which were groups of developing oocysts.

EIMERIA ELONGATA
MAROTEL AND GUILHON, 1941

Synonym: *Eimeria neoleporis* Carvalho, 1942 (?).

Host: Domestic rabbit.

Location: Unknown, presumably intestine.

Geographic Distribution: Europe (France).

Prevalence: Unknown.

Morphology: The oocysts are slightly greyish, elongate ellipsoidal with almost straight sides, 35 to 40 by 17 to 20 μ. The oocyst wall is thin. The micropyle is broad and easily visible. An oocyst polar granule and oocyst residuum are absent. The sporocysts are elongate. A sporocyst residuum is present, and almost as long as the sporocysts. The sporulation time is 4 days.

Life Cycle: Unknown.

Pathogenesis: Unknown.

Remarks: Becker (1956) believed that this species and *E. neoleporis* from the cottontail might be the same, and I agree. However, it is probably best to retain both names pending further research.

EIMERIA INTESTINALIS
KHEISIN, 1948

Synonyms: *Eimeria agnosta, Eimeria piriformis* Gwéléssiany and Nadiradze, 1945; non *E. piriformis* Kotlan and Pospesch, 1934.

Host: Domestic rabbit.

Location: Small intestine except anterior duodenum.

Geographic Distribution: Europe (Hungary), USSR.

Prevalence: Relatively uncommon.

Morphology: The oocysts are piriform, smooth, yellowish, 21 to 36 by 15 to 21 μ. A micropyle is present. An oocyst polar granule is absent. An oocyst residuum is present. The sporulation time is 1 to 2 days. Kheisin (1958) made a cytochemical study of the oocysts and endogenous stages of this species.

Life Cycle: Pellérdy (1953) described the life cycle of this species under the name *E. piriformis*. The endogenous stages occur above or sometimes beside the nuclei of the epithelial cells of the small intestine. There are at least 2 generations of merozoites. The prepatent period is 9 days.

Pathogenesis: According to Pellérdy (1953, 1954), experimental infections with this species cause more or less severe intestinal catarrh and diarrhea, and may kill young rabbits. At necropsy, edema and greyish-white foci which may coalesce to form a homogeneous, sticky, purulent layer may be found in the intestine.

EIMERIA MATSUBAYASHII
TSUNODA, 1952

Host: Domestic rabbit.

Location: Primarily ileum.

Geographic Distribution: Japan.

Prevalence: Unknown.

Morphology: The oocysts are broadly ovoid, 22 to 29 by 16 to 22 μ with a mean of 25 by 18 μ. A micropyle is present. An oocyst residuum is present. The presence or absence of an oocyst polar granule and a sporocyst residuum

are unknown. The sporulation time is 1.5 to 2 days.

Life Cycle: Unknown.

Pathogenesis: According to Tsunoda (1952), this species may be slightly to moderately pathogenic, causing a diphtheritic enteritis.

COCCIDIOSIS IN DOMESTIC RABBITS

Epidemiology: The most important species of rabbit coccidium is *Eimeria stiedae*, which occurs in the liver. All the other species are found in the intestine. Of these, the most important are *E. residua*, *E. magna*, *E. media* and *E. perforans*. Kheisin (1957) has assembled information on the localization of the intestinal species.

Coccidiosis is primarily a disease of young rabbits; adults are carriers. Rabbits become infected by ingesting oocysts along with their feed or water. The severity of the disease depends upon the number of oocysts they ingest and also upon the species involved. Mixed infections are the rule, infections with a single species usually being seen only under laboratory conditions. Crowding and lack of sanitation greatly increase the disease hazard.

Some of the coccidia of the domestic rabbit (*Oryctolagus cuniculus*) also occur in cottontails (*Sylvilagus* spp.). Some have also been reported from jack rabbits and hares (*Lepus* spp.). However, after reviewing the cross-transmission studies carried out to date, Pellérdy (1956a) concluded that, except for *E. stiedae*, none of the coccidia of jack rabbits and hares occur in domestic rabbits and cottontails, and none of the coccidia of the latter two genera occur in *Lepus*. If this conclusion is confirmed, the listing above of *Lepus* as a host of *E. magna*, *E. perforans*, *E. media* and *E. irresidua* would be incorrect. Further cross-transmission experiments are needed to settle this matter.

Diagnosis: Liver coccidiosis can be diagnosed by finding the characteristic lesions containing coccidia. Intestinal coccidiosis can be diagnosed by finding the coccidia on microscopic examination. However, the mere presence of these parasites in a case of enteritis does not mean that they caused it. Many rabbits carry a few coccidia without suffering any noticeable effects. In a 3-year study of mortality among hutch-raised domestic rabbits in California, Lund (1951) considered coccidiosis to be the cause of enteritis in only 80 out of 1541 affected animals.

Treatment: Some of the sulfonamides have been found helpful in preventing coccidiosis if given continuously in the feed or drinking water. Succinylsulfathiazole, sulfamerazine or sulfamethazine mixed with the feed at the rate of 0.5% have been recommended (Horton-Smith, 1947; Gerundo, 1948), as have been 0.02 to 0.05% sodium sulfaquinoxaline or sodium sulfamerazine in the drinking water. Lund (1954) found that the administration of 0.03% sulfaquinoxaline in the feed controlled *E. stiedae* infections effectively if begun not more than 4 days after experimental infection. The drug was not completely effective at this level, but it did give practical control. Lund stated further that this drug had not been found to harm rabbits when fed continuously.

However, long-term, continuous feeding of such drugs is not particularly desirable, nor is it usually necessary. It has been the usual experience with poultry, and there is evidence that the same thing is true with rabbits (see Horton-Smith, 1947), that if the hosts are exposed to coccidiosis during the drug-feeding period (as they usually are), an aborted infection occurs which is sufficient to induce immunity. The drug can then be safely stopped.

Prevention: Coccidiosis can be prevented by proper management (see Lund, 1949). Feeders and waterers should be designed so that they do not become contaminated with droppings, and should be kept clean. Hutch floors should be self-cleaning or should be cleaned frequently

and kept dry. Manure should be removed frequently. The animals should be handled as little as possible, and care should be taken not to contaminate either the animals themselves or their food, utensils or equipment. In addition, the rabbitry should be kept as free as possible of insects, rodents and other pests.

EIMERIA TENELLA
(RAILLIET AND LUCET, 1891)
FANTHAM, 1909

Synonyms: *Eimeria avium*, *Coccidium tenellum*, *Coccidium globosum*, *Eimeria bracheti*.

Host: Chicken.

Location: Ceca.

Geographic Distribution: Worldwide.

Prevalence: Very common.

Morphology: The oocysts are broadly ovoid, smooth, 14 to 31 by 9 to 25 μ with a mean of 22.9 by 19.1 μ. A micropyle is absent. An oocyst polar granule is present. An oocyst residuum is absent. The sporocysts are ovoid, without a sporocyst residuum.

The sporulation time is 1 to 2 days. Edgar (1954) found that the minimum sporulation time is 18 hours at 29° C, 21 hours at 26.5 to 28° and 24 hours at 20, 24 and 32°. Maximum sporulation was reached in 22 to 24 hours at 29°, the optimum temperature. Some sporulation took place at 41°. When the oocysts were kept at 8° they failed to sporulate in 8 weeks and most were killed, so that only a few sporulated when they were subsequently held at 28°.

Life Cycle: The life cycle of *E. tenella* has already been described as an example of coccidian life cycles (p. 160).

Pathogenesis: This is the most pathogenic of the chicken coccidia and is responsible for heavy losses. Together with the other species, it was estimated by the USDA (1954) to cause an annual loss of $38,229,000 in the United States due to death and disease alone. To this should be added the cost of the medicated feeds which are generally fed to poultry, and various labor and other costs entailed by disease outbreaks.

Cecal coccidiosis is found most frequently in young birds. Chicks are most susceptible at 4 weeks of age, while chicks 1 to 2 weeks old are more resistant (Gardiner, 1955). However, day-old chicks can be infected (Gordeuk, Bressler & Glantz, 1951). Older birds develop immunity as the result of exposure.

Coccidiosis due to *E. tenella* may vary in severity from an inapparent infection to an acute, highly fatal disease, depending upon the infective dose of oocysts. The pathogenicity of different strains of *E. tenella* varies, and it is affected also by the breed and age of the chickens and their state of nutrition. Thus, Jankiewicz and Scofield (1934) found that less than 150 sporulated oocysts produced no signs, 150 to 500 oocysts produced slight hemorrhagic diarrhea, 1000 to 3000 oocysts produced moderate hemorrhage and a few deaths, 3000 to 5000 oocysts produced marked hemorrhage and moderate mortality, and more than 5000 oocysts produced severe hemorrhage and high mortality. However, Horton-Smith (1949) found that infections with 15,000 oocysts caused no mortality in week-old birds, 30,000 oocysts caused 32% mortality and 60,000 oocysts caused 45% mortality. Swales (1944) found that in 6-week-old chickens 15,000 oocysts caused 40% mortality, 30,000 oocysts caused 44% mortality and 200,000 oocysts caused 80% mortality, while in 4.5-week-old chicks 120,000 oocysts caused 90% mortality and in 12-week-old chicks 100,000 oocysts caused 50% mortality. Waletzky and Hughes (1949) found that in one experiment 20,000 oocysts produced 18% mortality and 100,000 oocysts 36% mortality in 4-week-old chicks, while in other experiments 50,000 oocysts produced 45% mortality in 7-week-old chicks, 100,000 oocysts produced 67% mortality in 4- to 5-week-old chicks and 500,000 oocysts produced 48% mortality in 3- to

6-week-old chicks. Gardiner (1955) found that 200,000 oocysts were required to produce mortality in 1- to 2-week-old chicks, while 50,000 to 100,000 oocysts produced mortality in older birds.

Cecal coccidiosis is an acute disease characterized by diarrhea and massive cecal hemorrhage. The first signs appear when the second generation schizonts begin to enlarge and produce leakage of blood into the ceca. Blood first appears in the droppings 4 days after infection. At this time the birds appear listless. They may become droopy and inactive, and eat little, altho they still drink. The greatest amount of hemorrhage occurs on the 5th and 6th days after infection. It then declines, and oocysts appear in the feces 7 days after infection if the birds live that long. The oocysts increase to a peak on the 8th or 9th day and then drop off very rapidly. Very few are still being shed by the 11th day. A few oocysts may be found for several months.

Coccidiosis is self-limiting, and if the birds survive to the 8th or 9th day after infection, they generally recover.

The lesions of cecal coccidiosis depend upon the stage of the disease. They have been described by Tyzzer (1929), Tyzzer, Theiler and Jones (1932) and Mayhew (1937). On the fourth day after infection, hemorrhage is present thruout the cecal mucosa. On the fifth day, the cecum is filled with large amounts of unclotted or only partly clotted blood. This increases on the sixth day. Cecal cores of fibrinous and necrotic material begin to form on the 7th day. They adhere tightly to the mucosa at first, but soon come loose and lie free in the lumen.

About 7 days after infection, the wall of the cecum changes color from red to mottled reddish or milky white due to the formation of oocysts. It is greatly thickened. The cecal core, which was at first reddish, becomes yellowish or whitish. If it is small enough, it may be passed intact in the feces, but usually it is broken up into small pieces. In a few days the cecum becomes normal in appearance or at most slightly enlarged and thickened. Occasionally the cecum may rupture or adhesions may form.

About the 4th day, when the second generation schizonts are developing, the lamina propria becomes infiltrated with eosinophiles, there is marked congestion, and the cecal wall is thickened. The epithelium may be torn and coccidia, blood and tissue cells may be released into the lumen in areas where there are large numbers of parasites. On the 5th day, when the second generation merozoites are released, their host cells are ruptured and there is extensive epithelial sloughing. The sloughed material and cecal contents consolidate to form the cecal core, which loosens from the wall as the epithelium is regenerated.

Epithelial regeneration is complete in light infections, but in severe ones it may not be. There is a marked inflammatory reaction, with extensive lymphoid and plasma cell infiltration, and there may be some giant cells. Connective tissue is increased. The epithelium may not be replaced between the glands, and cysts formed by constriction of the glands during the inflammatory stage may persist.

The loss of blood into the ceca causes anemia. Using the microhematocrit technic, Joyner and Davies (1960) found that the packed red cell volume decreased markedly beginning 5 days after experimental infection. From an original level of 26 to 29% it decreased to 18% and 14%, respectively, 7 days after infection with 2000 and 10,000 oocysts. It had returned to normal 5 days later.

Natt (1959) found that *E. tenella* causes marked changes in the leucocyte picture. He observed lymphopenia and heterophilia on the 5th day, and eosinophilia on the 10th day after infection. A marked leucocytosis began on the 7th day and persisted thru the recovery phase.

Birds which recover from coccidiosis may suffer ill effects for some time or even permanently. Gardiner (1954) found an inverse correlation between growth

rate and severity of cecal coccidiosis. Cnicks which recovered following severe infection made much poorer weight gains than mildly affected ones. Mayhew (1932, 1932a, 1934) found that it took 10 weeks to 6 months after recovery before infected birds regained the weight they had lost in comparison with uninfected controls. He found, too, that pullets which had been infected when 6 to 8 weeks old laid 19.25% fewer eggs than the controls. In addition, severely affected birds began to lay 6 to 7 weeks later than the controls. Davidson, Thompson and Morre (1936) compared a group of chickens which was passing oocysts with another group which was not. Over a period of 11 months, the positive group had a 12.1% higher mortality, while the negative group averaged 0.44 pounds heavier than the positive one and had a 15.2% higher egg production. Bressler and Gordeuk (1951) found, in a flock of Single Comb White Leghorn chickens which had survived a mortality of 8.3% due to cecal coccidiosis, that weight gains were slightly less than in a "control" group fed 0.0125% sulfaquinoxaline continuously which had not suffered an outbreak of the disease, but that neither egg production nor hatchability were impaired.

EIMERIA NECATRIX
JOHNSON, 1930

Host: Chicken.

Location: The first and second generation schizonts are found in the small intestine and the third generation schizonts, gametes and gametocytes in the ceca.

Geographic Distribution: Worldwide.

Prevalence: Common.

Morphology: The oocysts are oblong ovoid, 12 to 29 by 11 to 24 μ with a mean of 20 by 17 μ (Becker *et al.*, 1956). The oocyst wall is smooth and colorless, without a micropyle. An oocyst polar granule is present. An oocyst residuum is absent. The sporocysts are elongate ovoid, with a Stieda body. A sporocyst residuum is absent. The sporulation time is 2 days (18 hours at 29° C according to Edgar, 1955).

Life Cycle: Chickens become infected by ingesting sporulated oocysts. When the sporozoites emerge, Van Doorninck and Becker (1957) found that they first enter the epithelial cells of the villi in the small intestine, pass thru the epithelium into the lamina propria or core of the villus and migrate toward the muscularis mucosae. Most of them are engulfed by macrophages en route and are transported by them to the epithelial cells of the fundus. The macrophages invade these cells and appear to disintegrate during or after the invasion process, leaving the sporozoites unharmed. These then round up to form first generation schizonts.

The remainder of the life cycle has been studied by Johnson (1930) and Tyzzer, Theiler and Jones (1932). Both the first and second generation schizonts are found above the host cell nuclei in the epithelial cells of the gland fundi. The first generation merozoites are liberated 2.5 to 3 days after infection and enter adjacent epithelial cells. The second generation schizonts are relatively large, measuring 39 to 66 by 33 to 54 μ with a mean of 52 by 38 μ. Most of the second generation merozoites are liberated 5 to 8 days after infection, but a few may still be liberated as long as 23 days after infection. They measure 8 to 11 by 1.5 to 2.0 μ with a mean of 9 by 2 μ. They pass to the cecum, where they penetrate the epithelial cells, coming to lie below the host cell nuclei, and turn into third generation schizonts. Most of them are found in the surface epithelium, but some enter the glandular epithelium. Multiple infections of a cell with 3 or 4 schizonts may occur. These third generation schizonts are relatively small and contain only 6 to 8 or a maximum of 16 third generation merozoites. It is not certain whether there is more than one asexual generation in the cecum.

The third generation and some of the second generation merozoites enter other cecal epithelial cells and become macrogametes or microgametocytes. These

also lie below the host cell nuclei. Microgametes develop from the microgametocytes, fertilization takes place, and oocysts form and are released. The prepatent period is 7 days, and the patent period is about 12 days.

Brackett and Bliznick (1952a) reported that the number of oocysts produced by *E. necatrix* per oocyst fed ranged from 15 in a group of chicks infected with 35,000 oocysts each to 58,000 in another group in which the infective dose was 50 oocysts.

Pathogenesis: Next to *E. tenella*, this is the most pathogenic and important species of chicken coccidium. Indeed, with the decrease in importance of *E. tenella* due to the use of coccidiostatic drugs, *E. necatrix* has come to the fore in many areas as the cause of more losses than *E. tenella*.

E. necatrix is often said to cause a more chronic type of coccidiosis than *E. tenella*. This is not because it runs a longer course, but because it produces so much scar tissue in the small intestine that its effects are more lasting.

The pathogenesis of *E. necatrix* has been studied especially by Tyzzer, Theiler and Jones (1932). The principal lesions are in the small intestine, the middle third of which is most seriously affected. Small, white, opaque foci are found here by the fourth day after infection. They are composed of second generation schizonts developing deep in the mucosa. They are so deep that they can be seen thru the serosa but not from the mucosal surface of the intestine. They are seldom more than a millimeter in diameter, but may coalesce and thus appear larger. Severe hemorrhage may appear on the 5th or 6th days. The small intestine may be markedly swollen and filled with clotted or unclotted blood. Its wall is greatly thickened, dull red, and many petechial hemorrhages appear in the white, opaque foci which by now contain second generation merozoites. The gut wall may lose contractility, become friable and even appear gangrenous. The epithelium may slough, and by the end of the 6th day a network of fibrin containing mononuclear cells appears in the destroyed areas. This is later replaced by connective tissue, and permanent scarring results which interferes with intestinal absorption.

There is less anemia than in *E. tenella* infections. Using the microhematocrit technic, Joyner and Davies (1960) found that the packed red cell volume decreased from 28% to 23% seven days after experimental infection with 20,000 oocysts, and to 25% after infection with 10,000 oocysts, but that there was no significant decrease after infection with 1000 oocysts. The hematocrit levels had not returned to the original level 12 days after infection.

The ceca are not seriously affected. They may be contracted and their contents may be dehydrated.

Death usually occurs 5 to 7 days after infection. Many of the birds which recover remain unthrifty and emaciated. The after-effects of this type of coccidiosis are often so long-lasting that it is not worthwhile to keep birds which have recovered from severe attacks.

Brackett and Bliznick (1950, 1952) found that inoculation with 25,000 to 50,000 oocysts (a relatively small number) caused a high degree of mortality in young chickens. Following inoculation with equal numbers of oocysts, young birds are more severely affected than older ones, but if the inocula are calculated on a weight basis, older birds may be more severely affected than younger ones. In 3-week-old chicks, 25,000 oocysts caused a mortality of 87%, while in 4-week-old chicks, 18,000, 37,000, 75,000 and 150,000 oocysts caused mortalities of 8, 75, 85 and 61%, respectively.

EIMERIA BRUNETTI LEVINE, 1942

Host: Chicken.

Location: First generation schizonts occur thruout the small intestine. Second generation schizonts, gametes and gametocytes occur in the posterior small intestine, rectum, ceca and cloaca.

Geographic Distribution: North America.

Prevalence: Uncommon.

Morphology: P. P. Levine (1942) described this species, and Becker, Zimmermann and Pattillo (1955) made a biometric study of its oocysts. The oocysts are ovoid, smooth, 14 to 34 by 12 to 26 μ with a mean of 23 by 20 μ. A micropyle is absent. An oocyst polar granule is present. An oocyst residuum is absent. The sporocysts are elongate ovoid, about 13 by 7.5 μ, with a Stieda body. A sporocyst residuum is present. The sporulation time is 1 to 2 days. Edgar (1955) found infective oocysts as early as 18 hours at 24° C.

Life Cycle: The life cycle of this species was described by Boles and Becker (1954). The sporozoites are liberated in the intestine and invade the epithelial cells of the villi. They round up to become first generation schizonts, which lie below the host cell nuclei on the sides of the villi of the upper, middle and lower small intestines. They are present 51 to 76 hours after infection, measure 30 by 20 μ, and contain approximately 200 first generation merozoites when mature. These invade other cells in the posterior small intestine, rectum, tubular part of the ceca and cloaca. They are found primarily at the tips of the villi, and usually lie below the host cell nuclei. They turn into second generation schizonts, which are present 4 days after infection. These average 30 by 16 μ and contain 50 to 60 merozoites. Small schizonts about 10 by 9 μ were also seen on the 4th day, but their significance was not determined.

The second generation merozoites invade fresh cells in the lower small intestine, ceca, rectum and cloaca and turn into sexual stages. These first appear on the 5th day and lie at the tips and sides of the villi, either above the host cell nuclei or on the basement membrane. The microgametocytes have a multicentric appearance, and are larger than the macrogametes, which measure about 25 by 22 μ. The macrogametes contain eosinophilic plastic granules which later coalesce and form the oocyst wall.

The prepatent period is 5 days.

Brackett and Bliznick (1952) found that *E. brunetti* could produce a maximum of 400,000 oocysts per oocyst fed. This figure was obtained in 2- to 3-week-old chickens fed 50 oocysts each. With larger inocula, relatively fewer oocysts were obtained. With inocula of 250, 1250, 6250, 20,000 and 40,000 oocysts, respectively, 150,000, 26,000, 7000, 800 and 400 oocysts were produced per oocyst fed.

Pathogenesis: *E. brunetti* is markedly pathogenic, but its effects depend upon the degree of infection. In light infections, no gross lesions may be seen. In heavier infections, Levine (1942) found that the gut wall becomes thickened and a pink or blood-tinged catarrhal exudate appears 4 or 5 days after experimental infection; the droppings are quite fluid and contain blood-tinged mucus and many mucus casts. The birds become somewhat depressed. These signs continue for 5 days and then subside if the birds recover.

In early or light infections, hemorrhagic, ladder-like streaks are present on the mucosa of the lower intestine and rectum. In heavy infections, a characteristic necrotic enteritis appears. It may involve the entire intestinal tract, but is more often found in the lower small intestine, large intestine and tubular part of the ceca. A patchy or continuous, dry, caseous necrotic membrane may line the intestine, and the intestine may be filled with sloughed, necrotic material. Circumscribed white patches may be visible thru the serosa, and there may even be intestinal perforation with resultant peritonitis.

Boles and Becker (1954) did not observe the extensive coagulation necrosis described by Levine (1942) in their experimentally infected chicks, but the other lesions were similar to those of his moderately infected birds. The birds became listless 82 hours after infection, and petechial hemorrhages were found, mostly in the lower small intestine but also in the

middle and upper small intestine. These became more severe the next day but had disappeared from the upper and middle intestine. The lower small intestine and large intestine were hyperemic and hemorrhagic, there was epithelial sloughing, and the intestinal contents were watery and blood-tinged. The tubular part of the ceca was involved, and the dilated portion was plugged with dehydrated material. The epithelial denudation was most probably caused by the asexual stages, and was most prominent on the 4th day. Signs of illness continued until the 6th day.

Field outbreaks of the disease were studied by Levine (1943). The disease occurs most commonly in chicks 4 to 9 weeks old. The mortality is high, and typical necrotic lesions are present. We have seen the same condition in field outbreaks in Illinois.

EIMERIA ACERVULINA
TYZZER, 1929

Host: Chicken.

Location: Anterior small intestine.

Geographic Distribution: Worldwide.

Prevalence: Common.

Morphology: The oocysts are ovoid, smooth, 12 to 23 by 9 to 17 μ with a mean of 16 by 13 μ. A micropyle is absent. An oocyst polar granule is present. An oocyst residuum is absent. The sporocysts are ovoid, with a Stieda body but without a sporocyst residuum. The sporulation time is 1 day. Edgar (1955) found that the minimum sporulation time for this species at 28° C was 17 hours.

Life Cycle: The life cycle of this species was described by Tyzzer (1929). The schizonts are found in the epithelial cells of the villi of the anterior small intestine, where they lie above the host cell nuclei. The gland cells may also be invaded. Sometimes more than one parasite is found in a cell. The schizonts produce 16 to 32 merozoites which measure about 6 by 0.8 μ. There are at least 2 and possibly more asexual generations. Asexual reproduction lasts longer than in *E. tenella*.

The sexual stages occur above the host cell nuclei in the epithelial cells of the villi and to a lesser extent in the gland cells in the anterior small intestine. They first appear 4 days after infection. The microgametocytes are relatively small, measuring 11 by 9 μ.

The prepatent period is 4 days, and oocysts continue to be produced for relatively longer than with some other chicken coccidia.

Brackett and Bliznick (1950) found that the maximum number of oocysts produced per oocyst fed in their studies was 72,000. This occurred in a group of 3-week-old birds fed 2000 oocysts each. In another experiment in which similar birds were fed the same number of oocysts, only 35,000 oocysts were produced per oocyst fed. Oocyst production was lower with both larger and smaller inocula. Following inoculation with 200, 10,000 and 20,000 oocysts, respectively, 9000, 35,000 and 7,600 oocysts were produced per oocyst fed.

Pathogenesis: *E. acervulina* is generally considered only slightly pathogenic, but very large inocula may cause severe signs and even death. Generally, however, this species causes only a temporary setback. Dickinson (1941) found that administration of as many as 25 million oocysts to pullets produced only a temporary drop in weight and temporary cessation of egg production. Between 4 and 9 days after infection, the birds were droopy, ate relatively little and passed slimy, mucoid feces. Peterson (1949) reported losses from *E. acervulina* infection in the Pacific Northwest in older birds 3 to 4 weeks after they had been brought in off the range and placed in houses. The birds lost weight, egg production ceased, the combs shriveled and keratin pigment disappeared. There were few if any deaths. After about 6 weeks the birds recovered and egg production returned to normal.

Brackett and Bliznick (1950) found that inoculation with 500,000 oocysts reduced weight gains of 2-week-old chicks. Moynihan (1950) obtained similar results. Becker (1959) found that 300,000 oocysts produced only loss of appetite for 2 or 3 days and watery feces on the third day after infection in White Leghorn chicks. Morehouse and McGuire (1958) found that infection of chicks with 100,000 oocysts retarded weight gains somewhat but did not affect the final weight. Larger inocula produced increasingly severe effects. Single and multiple doses of 5 million or more oocysts caused 6 to 75% mortality.

The lesions produced by *E. acervulina* are not as marked as with *E. necatrix*. The intestine may be thickened and a catarrhal exudate may be present, but hemorrhage is rare. The maturing oocysts lie massed in limited areas, and form whitish or grey spots or streaks running transversely in the intestinal mucosa. In heavy infections the entire mucosa may be involved and may appear greyish, mottled and somewhat thickened. Morehouse and McGuire (1958) described a severe inflammatory reaction in chicks infected with 1 to 20 million oocysts. The intestine was edematous and thickened, with extensive vasodilation and marked reddening of the mucosa, and there was also degeneration or necrosis and sloughing of the intestinal epithelium.

EIMERIA MAXIMA TYZZER, 1929

Host: Chicken.

Location: Middle and posterior small intestine.

Geographic Distribution: Worldwide.

Prevalence: Common.

Morphology: The oocysts are ovoid, smooth or somewhat roughened, yellowish, 21 to 42 by 16 to 30 μ with a mean of 29 by 23 μ. A micropyle is absent. An oocyst polar granule is present. An oocyst residuum is absent. The sporocysts are elongate ovoid, 15 to 19 by 8 to 9 μ, with a Stieda body. A sporocyst residuum is absent. The sporozoites are 19 by 4 μ, with a conspicuous refractile globule (Long, 1959). The sporulation time is 2 days. Edgar (1955) and Long (1959) found some infective oocysts as early as 30 hours at 28 C.

Life Cycle: The life cycle of this species has been studied by Tyzzer (1929), Scholtyseck (1959) and Long (1959), among others. The schizonts are found above the host cell nuclei or occasionally beside them in the epithelial cells of the tips of the villi of the duodenum and upper ileum. There are 2 generations of schizonts, both of which are relatively small, measuring about 10 by 8 μ; they produce only about 8 to 16 merozoites each. Schizonts may be present thru the 5th day. The second generation merozoites enter new epithelial cells, where they round up and enter the sexual phase of the life cycle.

The sexual stages are found beneath the host cell nuclei. As they become larger, the host cells are displaced toward the center of the villus and come to lie in its interior. The mature microgametocytes measure 30 to 39 by 22 to 33 μ and form a large number of biflagellate microgametes. The macrogametes are somewhat smaller, averaging 19 by 15 μ (Long, 1959). After fertilization, they lay down an oocyst wall, break out of the villus and are passed in the feces. The prepatent period is 5 to 6 days, and the patent period is only a few days.

Brackett and Bliznick (1950, 1952) reported that the maximum number of oocysts produced per oocyst fed in their experiments was 12,000. In a series of 3-week-old chicks, they found that 11,500, 2250 and 940 to 2900 oocysts were produced per oocyst fed when the inoculating doses were 200, 2000 and 10,000 oocysts, respectively.

Long (1959) found that the number of oocysts produced per oocyst fed varied with the age of the birds and the inoculum. With an inoculum of 10,000 oocysts it averaged 128, 33, 176, 448, 1049 and

3294, respectively, in chicks 3, 7, 14, 21, 28 and 42 days old, while with an inoculum of 80,000 oocysts it was 9, 31 and 169, respectively in chicks 7, 14 and 21 days old.

Pathogenesis: *E. maxima* is slightly to moderately pathogenic. Tyzzer (1929), Brackett and Bliznick (1950), Scholtyseck (1959) and Long (1959) studied its effects on chickens. The asexual stages cause relatively little damage, the most serious effects being due to the sexual stages. Brackett and Bliznick (1950) observed a mortality of 35% in one group of young chicks infected with 500,000 oocysts each, but there were no deaths in another group. The survivors lost some weight and then gained less than the controls for a time, but infection with 100,000 oocysts had no significant effect on weight gains. Long (1959) observed no deaths in a group of 6-week-old chicks infected with 500,000 oocysts each or in three 17-day-old chicks infected with 1 million oocysts each, altho diarrhea was present and the infected birds gained less than the controls. Immunity is quickly produced.

Berg, Hamilton and Bearse (1951) found that inoculation of White Leghorn laying pullets with 8000 oocysts each produced a mild infection and temporary cessation of egg-laying.

The principal lesions are hemorrhages in the small intestine. The intestinal muscles lose their tone, and the intestine becomes flaccid and dilated, with a somewhat thickened wall. Short, fine, hair-like hemorrhages in the intestinal wall are sometimes present. There is a catarrhal enteritis and the intestinal contents are viscid and mucoid, greyish, brownish, orange or pinkish, occasionally but not usually with flecks of blood.

Birds which recover soon return to normal.

EIMERIA MITIS
TYZZER, 1929

Host: Chicken.

Location: Anterior small intestine, occasionally middle and lower small intestine or even tubular part of ceca.

Geographic Distribution: Worldwide.

Prevalence: Common.

Morphology: The oocysts are subspherical, smooth, colorless, 10 to 21 by 9 to 18 μ with a mean of 16 by 13 μ. A micropyle is absent. An oocyst polar granule is present. An oocyst residuum is absent. The sporocysts are ovoid, 10 by 6 μ, with a Stieda body, but without a sporocyst residuum. The sporulation time is 2 days. Edgar (1955) found some infective oocysts as early as 18 hours at 29° C.

Life Cycle: The life cycle of this species has been studied by Tyzzer (1929) and Joyner (1958), the latter using a strain derived from a single oocyst. The endogenous stages occur in the epithelial cells of the villi and occasionally in the glands. They lie against the host cell nuclei, and below them more often than above according to Tyzzer. However, Joyner stated that the schizonts are nearly always superficial; he illustrated the sexual stages as below the host cell nuclei. The schizonts produce 6 to 24 or rarely 30 merozoites, but the number of schizont generations is not known. The merozoites are crescent-shaped, with blunt ends, and measure about 5 by 1.5 μ.

The microgametocytes are about 9 to 14 μ long and the macrogametes are somewhat larger. In contrast to most other coccidian species, in which development following a single inoculum is quite synchronous, both asexual and sexual stages occur together. The prepatent period is 4 to 5 days, and the patent period 10 days (Joyner, 1958).

Joyner (1958) found that chicks infected with 1000 oocysts produced 61,709 oocysts per oocyst fed, while chicks fed 100,000 oocysts produced 2253 oocysts per oocyst fed.

Pathogenesis: This species is slightly pathogenic, but is unlikely to be

of pathological significance under normal field conditions. Tyzzer (1929) observed neither signs nor gross lesions in young chickens given tremendous and repeated doses of sporulated oocysts. Becker (1959) found neither lesions nor diarrhea in infected chickens. Joyner (1958) found that weight gains of 6- to 26-day-old birds fed 500,000 oocysts were reduced, while 38% of 29 6-day-old chicks died after being fed 2.5 million oocysts.

EIMERIA PRAECOX
JOHNSON, 1930

Host: Chicken.

Location: Upper third of small intestine.

Geographic Distribution: Probably worldwide.

Prevalence: Common.

Morphology: The oocysts are ovoid, smooth, colorless, 20 to 25 by 16 to 20 μ with a mean of 21 by 17 μ. A micropyle is absent. An oocyst polar granule is present. Oocyst and sporocyst residua are absent. The sporulation time is 2 days.

Life Cycle: Tyzzer, Theiler and Jones (1932) studied the life cycle of this species. The endogenous stages occur in the epithelial cells of the villi, usually along the sides of the villi and below the host cell nuclei. There are 2 generations of schizonts, the second of which appears as early as 32 hours after infection. Later development is irregular, both sexual and asexual stages being seen together. The prepatent period is 4 days, and the patent period is short, 4 days or a little more in the absence of reinfection.

Pathogenesis: This species is essentially non-pathogenic. Tyzzer, Theiler and Jones (1932) were unable to cause death with heavy doses of oocysts, altho they did observe a mucous cast containing large numbers of oocysts at the end of the infection. Becker (1959) found that *E. praecox* is probably no more pathogenic than *E. mitis*.

EIMERIA HAGANI
LEVINE, 1938

Host: Chicken.

Location: Anterior half of small intestine.

Geographic Distribution: North America, India.

Prevalence: Rare. This species has apparently been reported in the United States only by P. P. Levine (1938) in New York and Edgar (1955) in Alabama. Gill (1954a) found it in 2.5% of 120 chickens in India.

Morphology: The oocysts are broadly ovoid, smooth, 16 to 21 by 14 to 19 μ with a mean of 19 by 18 μ (18 by 16.5 μ according to Edgar, 1955). A micropyle is apparently absent. An oocyst polar granule is present. No other morphological data are known; this species was differentiated from other chicken coccidia by cross-immunity tests. The sporulation time is 1 to 2 days. Edgar (1955) found sporulated oocysts as early as 18 hours at 29° C.

Life Cycle: Unknown. The prepatent period is 7 days according to Levine (1938) or almost 6 days according to Edgar (1955).

Pathogenesis: This species is only slightly pathogenic. Levine (1938) observed pin-head size hemorrhages and catarrhal inflammation in the anterior half of the small intestine. There were also a few hemorrhages in the lower small intestine. Later, however, Levine (1942a) referred to this species as non-pathogenic, stating that 300,000 oocysts had no effect on experimentally infected birds.

COCCIDIOSIS IN CHICKENS

Epidemiology: Infections with a single species of coccidium are rare, and mixed infections are the rule. *Eimeria*

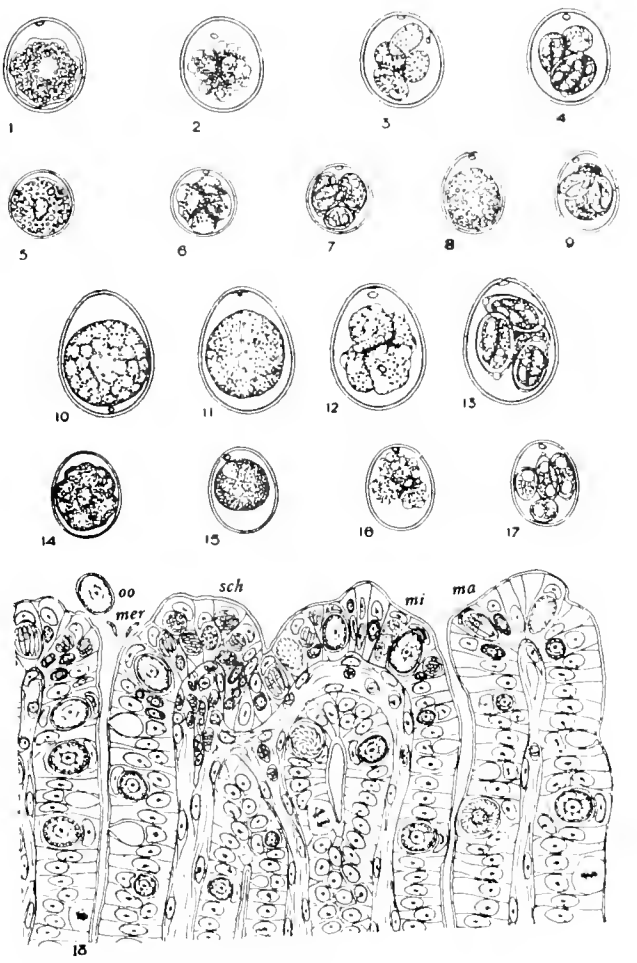

Fig. 27. Morphology and developmental stages of species of *Eimeria* from the chicken. 1-4. Stages in development of oocysts of *Eimeria tenella*. 5-7. Stages in development of oocysts of *Eimeria mitis*. 8-9. Stages in development of oocysts of *Eimeria acervulina*. 10-13. Stages in development of oocysts of *Eimeria maxima*. 14-17. Stages in development of oocysts of *Eimeria necatrix*. 18. Developmental stages in cecal epithelium 7 to 9 days after infection. oo = oocyst. sch = third generation schizont. mer = third generation merozoite. mi = microgametocyte. ma = macrogamete. (From Tyzzer, 1929 in the *American Journal of Hygiene*, published by the Johns Hopkins Press).

tenella is the most pathogenic and important species. In recent years, however, control of this species with coccidiostats has revealed more and more coccidiosis due to *E. necatrix*. The other species may contribute to the total picture. *E. brunetti* is markedly pathogenic but uncommon. *E. maxima* and *E. acervulina* are slightly to moderately pathogenic. Both are common. *E. mitis* and *E. praecox* are common but non-pathogenic. *E. hagani* is rare and only slightly if at all pathogenic. *Wenyonella gallinae* is rare but moderately pathogenic; it has been found so far only in India. *Cryptosporidium tyzzeri* is rare and non-pathogenic. *Isospora gallinae* is rare if it is a chicken parasite at all, and is presumably non-pathogenic.

Coccidiosis is primarily a disease of young birds. Older birds are carriers. Birds become infected by ingesting oocysts along with their food or water. Under farm conditions, and even in the laboratory unless extreme precautions are taken, it is practically impossible to avoid exposure to at least a few oocysts.

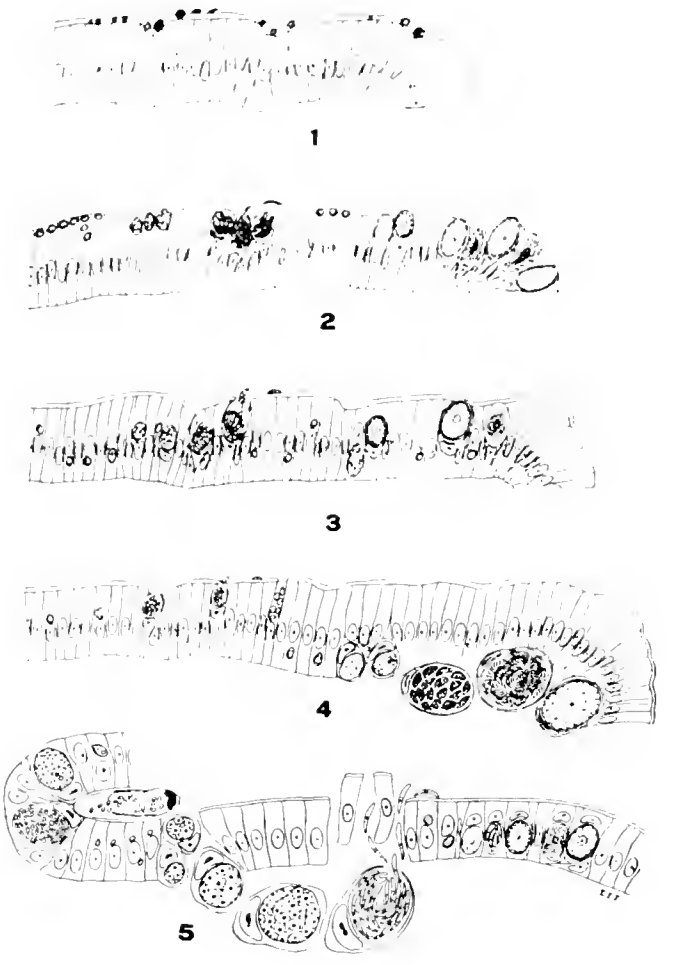

Fig. 28. Location of avian coccidia in intestinal epithelium of chicken. 1. *Cryptosporidium tyzzeri*. 2. *Eimeria acervulina*. 3. *Eimeria mitis*. 4. *Eimeria maxima*. 5. *Eimeria tenella*. (From Tyzzer, 1929 in the *American Journal of Hygiene*, published by the Johns Hopkins Press)

The disease picture depends upon the number of oocysts of each species which the birds ingest. If they get only a few, there are no signs, and repeated infections produce immunity without disease. If they get more, the disease may be mild and the birds will become immune. Only if they get a large number of oocysts do severe disease and death result.

Crowding and lack of sanitation greatly increase the disease hazard. As the oocysts accumulate, the birds receive heavier and heavier exposures, and the disease becomes increasingly severe in each successive batch of birds placed in contaminated surroundings.

Immunity: Coccidiosis is a self-limiting disease, and birds which have recovered become immune. The speed with which immunity develops depends upon the species of *Eimeria* and on the intensity and frequency of infection. Immunity develops rapidly following infections with *E. maxima*, *E. praecox* and probably *E. hagani*, somewhat more slowly following infections with *E. tenella* and *E. brunetti*, and is delayed following infections with *E. mitis*, *E. acervulina* and *E. necatrix*.

Immunity is species-specific. Chickens which have become immune to one species are susceptible to all the others. This fact makes it possible to differentiate

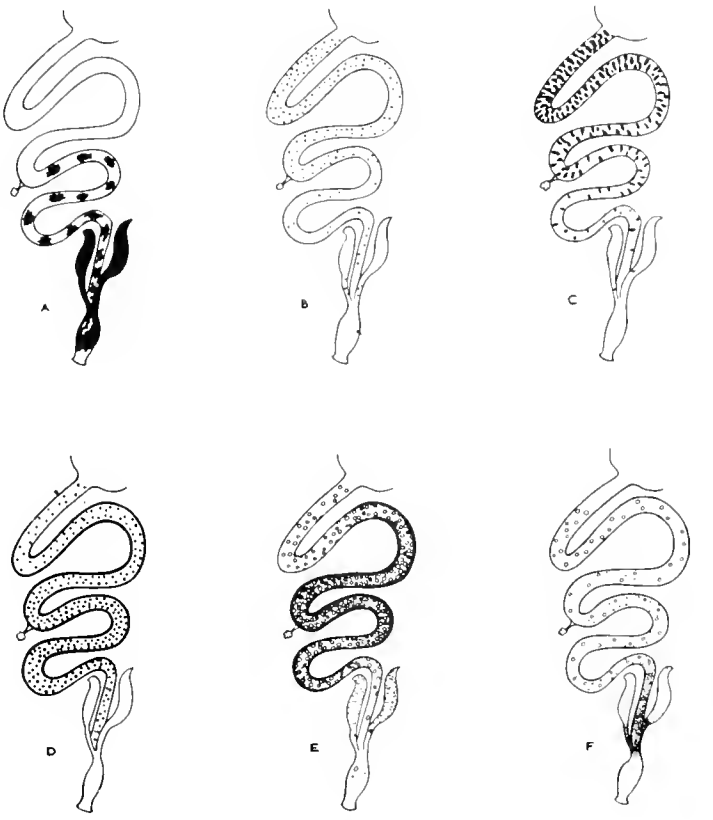

Fig. 29. Location of chicken coccidia in regions of the intestinal tract. A. *Eimeria tenella*. B. *E. mitis*. C. *E. acervulina*. D. *E. maxima*. E. *E. necatrix*. F. *E. brunetti*. (A-D after Tyzzer, 1929; E after Tyzzer, Theiler and Jones, 1932; F after Boles and Becker, 1954)

between species by cross-immunity studies, and indeed it was by means of such studies that Levine (1938), for instance, was able to show that *E. hagani* was a valid species.

Immunity against coccidia is seldom solid. Birds which have recovered may be reinfected, but such infections are light and do not cause disease. Carriers are extremely common and are a source of infection for other birds. Thus, Levine (1940) found *E. mitis*, *E. acervulina* or both in 53%, *E. praecox* in 33%, *E. maxima* in 28%, *E. necatrix* in 38% and *E. tenella* in 23% of 39 pullets 8 months or more old, but only 8% of them had gross lesions.

Heredity is a factor in resistance to coccidiosis. Herrick (1934) found that chicks from resistant parents were about 100% more resistant to *E. tenella* than unselected chicks. Champion (1954) and Rosenberg, Alicata and Palafox (1954) established *E. tenella*-resistant and susceptible lines of chickens by selective breeding. They found that sex linkage, passive transfer of immunity thru the egg and cytoplasmic inheritance did not play a significant part in resistance and susceptibility. Champion considered that they were controlled in large part by non-dominant, multiple genetic factors which presumably act additively. Rosenberg *et al.* also thought that the factor or factors for resistance or susceptibility did not show marked dominance.

Immunity in older birds is due mostly to previous infection. The birds are exposed repeatedly and almost continuously, and their immunity is continually being reinforced. Coccidiasis is thus extremely common--and indeed normal under natural conditions--while coccidiosis is the result

of imbalance between infection rate and resistance. Actually, the best type of environment to control coccidiosis is one in which the chickens become infected lightly enough to develop an immunity without suffering any disease.

Many workers have studied the development of immunity to coccidiosis (Waletzky and Hughes, 1949; Brackett and Bliznick, 1950). Most of this research has been done with *E. tenella*. Farr (1943) immunized chickens with 1000 oocysts daily for 15 days or with 3 doses of 1000, 5000 and 9000 oocysts given 5 days apart. She also carried out 5 similar experiments with differing numbers of oocysts, all of which showed that repeated small doses of *E. tenella* oocysts would produce immunity. Horton-Smith (1949), Waletzky and Hughes (1949) and Gordeuk, Bressler and Glantz (1951) found that single doses of oocysts would also produce immunity, and that the degree of protection was proportional to the intensity of the initial infection. Babcock and Dickinson (1954) found that a total of 1600 sporulated oocysts, given either in one or several doses, would produce practical immunity that withstood severe challenge. The number of individual doses required to make the total did not materially affect the immunity produced. It took 4 days longer for immunity to result following exposure to 1050 sporulated oocysts than to 2125. Gordeuk, Bressler and Glantz (1951) found that day-old chicks could develop a certain degree of immunity. They found, too, that feeding the oocysts in the mash resulted in higher mortality than when a similar dose was given by mouth.

Many workers have shown that immunity will develop against coccidiosis in birds on suppressive therapy (Waletzky and Hughes, 1949; Johnson, Mussell and Dietzler, 1949, 1949a; Grumbles *et al.*, 1949; Bankowski, 1950; Kendall and McCullough, 1952). The drugs are ineffective against the sporozoites or first generation schizonts, at least in the concentrations used, but they do kill the merozoites or later stages. The coccidia are thus able to invade the host tissues and stimulate the development of immunity, but are killed before they can multiply enough to harm the host.

A number of workers have attempted to produce immunity by infecting birds with oocysts attenuated in different ways. Jankiewicz and Scofield (1934) heated the oocysts to 46° C for 15 minutes before sporulation, and found that when they were then sporulated and fed to chickens, they stimulated the production of immunity with a minimum of injury. Waxler (1941) produced mild infections with oocysts irradiated with 9000 r of x-rays. Following recovery, the chicks were almost as resistant as those which had had a severe attack after infection with normal oocysts. Uricchio (1953) produced marked immunity by feeding chicks 100,000 oocysts which had been held at -5° C for 5 days, and a lesser degree of immunity with oocysts which had been heated at 45° C for 12 hours.

It is well known that cultures slowly lose their infectivity upon storage. Babcock and Dickinson (1954), for example, observed reduced pathogenicity in a culture of *E. tenella* after storage for 236 days, and reduced immunogenicity at 344 days. Using a standard immunizing procedure in which 600 oocysts were fed the first day and 1000 the second, they found that it took 3 days to produce immunity with a culture less than 150 days old and 6 days with a culture more than 300 days old.

There is an unanswered question whether such treatments produce true attenuation or whether the observed results are due simply to the death of some of the oocysts. Invasion must take place for immunity to result, and attempts to immunize birds with killed antigens have not succeeded.

Most attempts to find circulating antibodies have failed. However, McDermott and Stauber (1954) found agglutinins against merozoites in the serum of experimentally infected chickens and also produced them in rabbits and roosters by injecting formalinized merozoite suspensions. Becker and Zimmermann (1953) found that infected chicks injected intravenously with

an alcoholic horse kidney extract produced fewer oocysts than untreated, infected controls. Burns and Challey (1959) found that when chicks which had been previously infected thru a fistula into a cecal pouch which had been isolated from the intestine were challenged with *E. tenella* orally, they were somewhat more resistant than the controls, indicating that there is some generalized host response.

Less research has been done on the development of immunity in other species of coccidia. Tyzzer, Theiler and Jones (1932) found that chickens which had recovered from *E. necatrix* infections were immune, as did Grumbles and Delaplane (1947). Dickinson (1941) and Brackett and Bliznick (1950) showed that immunity developed following infection with *E. acervulina*. The latter found the same thing with *E. maxima*. Similar results have been obtained for the other species (Brackett and Bliznick, 1950).

Diagnosis: Avian coccidiosis can be diagnosed by finding lesions containing coccidia at necropsy. Diarrhea with or without blood in the droppings, inappetence and emaciation are suggestive, but scrapings of the affected intestinal mucosa must be examined microscopically to determine whether coccidia are present. It is not enough to look for oocysts, but schizonts, merozoites and young gametes should be recognized also.

Coccidiasis is much more common than coccidiosis; hence the mere presence of oocysts in the feces cannot be relied upon for diagnosis. Conversely, the absence of oocysts does not necessarily mean that coccidiosis is not present, since the disease may be in too early a stage to produce oocysts.

Since some species of coccidia are highly pathogenic for the chicken while others are practically non-pathogenic, the species present must be identified to establish a diagnosis. This can often be done in a rough way from the type and location of the lesions.

Treatment: Many hundreds of papers have been written on the treatment of coccidiosis in chickens, and there is no space here for more than a relatively brief discussion. By far the greatest part of the research has been done on *E. tenella*.

The first compound found effective against coccidia was sulfur, which Herrick and Holmes (1936) introduced. When 2 to 5% sulfur is mixed with the feed, coccidiosis is largely prevented in young chicks. The use of sulfur had a certain vogue, but it was soon found unsatisfactory because it causes a condition known as sulfur rickets. Even tho the chicks are on an ordinarily adequate diet, the sulfur interferes with calcium utilization and causes rickets.

The use of borax in *E. tenella* coccidiosis was introduced by Hardcastle and Foster (1944). Several others have done research on it (Wehr, Farr and Gardiner, 1949), and the consensus is that 0.3 to 0.5% borax in the feed prevents death from coccidiosis if administered beginning 1 or 2 days after experimental infection and continued for 3 days or longer. However, it does not prevent cecal hemorrhage or weight losses. It is also toxic, causing loss of weight even when fed alone.

P. P. Levine (1939) was the first to use sulfonamides against coccidiosis. His discovery that sulfanilamide was active opened up the field. Many different--probably several hundred--sulfonamides were tested, and a number of them were found of practical value. Sulfaguanidine was introduced after sulfanilamide. It was followed by sulfamerazine and sulfamethazine (called sulfamezathine in England), and still later by sulfaquinoxaline and N^4-acetyl-N^1-(4-nitrophenyl) sulfanilamide. All of these compounds are effective against *E. tenella*, the last 2 are quite effective against *E. necatrix*, and sulfaquinoxaline and sulfaguanidine are quite effective against *E. acervulina*. Sodium sulfadimidine is active against *E. mitis*, but does not completely eliminate it (Joyner, 1958).

Sulfaguanidine is fed at the rate of 0.5% in the mash, sulfamethazine and sulfamerazine at 0.1 to 0.25%, and sulfaquinoxaline at 0.025%. Sodium sulfamethazine and sodium sulfadimidine are given in the

drinking water at 0.2%, and sodium sulfaquinoxaline at about 0.04% (see Grumbles, *et al.*, 1949; Farr, 1949; Dickinson, 1949; Kendall and McCullough, 1952; Peterson and Munro, 1949; Peterson and Hymas, 1950; Davies and Kendall, 1954; Bankowski, 1950; Horton-Smith and Long, 1959; and McLoughlin and Chester, 1959 for reviews and further information).

The sulfonamides are in general more effective against the schizonts and merozoites than against the gametes, gametocytes and sporozoites. Bankowski (1950) found that 0.5% sulfaguanidine was coccidiostatic against the first generation schizonts of *E. tenella* but that 2% sulfaguanidine was required to kill the second generation schizonts in the lamina propria and even this concentration had no effect on the sporozoites. He concluded that this drug must act against the merozoites in the lumen of the ceca, since 0.5% is the usual concentration in the feed. Kendall and McCullough (1952) found that 0.25 to 0.375% sulfamethazine in the feed affected the later stages in the life cycle, but that 0.5 to 1.0% was required to affect the early stages. Farr and Wehr (1947) found that 1% sulfamethazine almost completely destroyed the second generation schizonts and their merozoites, somewhat affected the first generation schizonts but did not completely destroy them, and either damaged or destroyed the young gametes. It did not injure the larger gametes, oocysts or sporozoites. The action of sulfaquinoxaline is similar.

All of the sulfonamides are coccidiostatic rather than truly curative. None will cure coccidiosis once signs of disease have appeared. When fed continuously in the feed, they abort the disease. Sulfaquinoxaline will protect birds when given as late as 4 days after experimental infection. Since the sporozoites are not affected, they invade the intestinal cells and stimulate the development of immunity. However, if too much of a sulfonamide is given, immunity will not develop. Thus, Kendall and McCullough (1952) found that when 0.25 to 0.375% sulfamethazine was given in the feed, immunity developed, but when the concentration was raised to 0.5 to 1.0% it did not.

When given in the recommended amounts, the sulfonamides are not generally harmful. Sulfaquinoxaline does not depress the growth rate of chicks when fed for a long period at rates of 0.01 to 0.02%, but 0.03% gives variable results and higher concentrations are usually toxic. Delaplane and Milliff (1948) found that when 0.05% sulfaquinoxaline was fed continuously to pullets in egg production, signs of poisoning appeared and some birds died. They found greyish-white nodules in the spleens of most birds and in the livers, kidneys, hearts and lungs of some. There were also hemorrhages beneath the skin of the legs and in the combs. Davies and Kendall (1953) found that 0.0645% sodium sulfaquinoxaline in the drinking water was toxic to chickens when fed for as short a period as 5 days. The principal lesions were hemorrhages, especially in the spleen, and accumulation of fluid in the peritoneal cavity. On the other hand, Cuckler and Ott (1955) reported that the continuous administration of 0.05% sulfaquinoxaline in the feed or of 0.025% in the water for as long as 12 weeks had no adverse effects on chickens. The blood clotting time was prolonged and the prothrombin time increased slightly by feeding 0.4% sulfaquinoxaline for 3 to 12 weeks.

Several organic arsenic compounds have been found effective against *E. tenella*, but not against the other species (Morehouse and Mayfield, 1946; Goble, 1949). All are derivatives of phenylarsonic acid. All are coccidiostatic, and none will cure coccidiosis once signs of disease have appeared. The most widely used of these is perhaps 3-nitro-4-hydroxyphenylarsonic acid, which is generally administered in the feed at a concentration of 0.01%. It apparently acts against the earlier endogenous stages, but not against the sporozoites, and birds which are exposed while under prophylactic treatment become immune. At the recommended dosage it has no harmful effect on the host but is actually a growth stimulant. A mixture of this compound and N^4-acetyl-N^1-(4-nitrophenyl) sulfanilamide is sold under the name Nitrosal to suppress both cecal and intestinal coccidiosis. Another active organic arsenic compound is arsanilic acid.

A number of alkylidenediphenols, which are diphenylmethane derivatives, are effective against *E. tenella*, (Johnson, Mussell and Dietzler, 1949, 1949a; Groschke *et al.*, 1949). One of these, Parabis-90, is 2,2'-methylene-bis-4-chlorophenol. It is used in the starter feed at a concentration of 0.15%, and later on, when the chicks are 6 to 8 weeks old, in the grower feed at a concentration of 0.12%. These compounds are also coccidiostatic and will not cure coccidiosis once signs of the disease have appeared. They appear to act primarily against the earlier endogenous stages but not against the sporozoites, and birds which are exposed while getting the drug become immune. They do not appear to harm chickens when fed at the recommended levels.

A diphenyl disulfide derivative which has been widely used as a coccidiostat against both *E. tenella* and *E. necatrix* is nitrophenide (Megasul). It is 3,3'-dinitrodiphenyldisulfide (Waletzky, Hughes and Brandt, 1949; Peterson and Hymas, 1950; Dickinson, Babcock and Osebold, 1951; Gardiner, Farr and Wehr, 1952; Horton-Smith and Long, 1959). It is mixed with the feed at the rate of 0.025 to 0.05%. It is coccidiostatic and will not cure coccidiosis once signs of the disease have appeared. It acts against both the sporozoites and later stages, but is more effective against the latter and especially against the second generation schizonts. Immunity does not appear to develop if chickens are treated before infection, but it does if treatment begins at the time of infection or later. Nitrophenide is not harmful if fed in therapeutic concentrations. At higher doses Newberne and McDougle (1956) found that it may cause postural and locomotor disturbances, lowered weight gains, liver degeneration and bone marrow changes.

Another coccidiostat is the diphenylsulfide derivative, bithionol, or 2,2'-dihydroxy-3,3',5,5'-tetrachlorodiphenyl sulfide. The commercial coccidiostat, Trithiadol, is a mixture of 5 parts bithionol and 1 part methiotriazamine. The latter is 4,6-diamino-1-(4-methylmercaptophenyl)-1,2-dihydro-2,2-dimethyl-1,3,5-triazine. Bithionol is not only coccidiostatic but also antibacterial and antifungal. Methiotriazamine is coccidiostatic at high concentrations and is also an active antimalarial agent. In combination, these drugs are effective against coccidia at lower concentrations than when used alone. A mixture containing 60% active ingredients is fed in the feed at the rate of 2 pounds per ton. The recommended use level is 0.05% bithionol plus 0.01% methiotriazamine. It is effective against *E. tenella*, *E. necatrix*, *E. maxima* and *E. acervulina*. Chickens fed it develop immunity to these coccidia. McLoughlin and Chester (1959) found that 0.06% Trithiadol gave relatively good protection from mortality due to *E. tenella*. It was not as good as glycarbylamide and nicarbazin but was about as effective as nitrofurazone and Bifuran and somewhat better than sulfaquinoxaline. Trithiadol is not harmful to growing chickens when fed at the recommended levels (Arnold and Coulston, 1959). It does not appear to affect egg production or egg shell color or quality, but it does affect hatchability to some extent and is not recommended for use in laying mashes.

Two nitrofurans are currently used as coccidiostats. Nitrofurazone (5-nitro-2-furaldehyde semicarbazone) was introduced by Harwood and Stunz (1949, 1949a, 1950) and has been studied further by Peterson and Hymas (1950), Gardiner and Farr (1954), Horton-Smith and Long (1952, 1959) and McLoughlin and Chester (1959), among others. It is mixed with the feed at the rate of 0.011%. It is effective against *E. tenella* and to a lesser extent against *E. necatrix* and *E. maxima*. Higher concentrations give better results against the intestinal species. Nitrofurazone is coccidiostatic and will not cure coccidiosis once signs of the disease have appeared. It acts against the schizonts, and birds infected while receiving the drug develop immunity to reinfection.

Nitrofurazone is not harmful if fed in therapeutic amounts, but 0.04 to 0.05% in the feed is definitely toxic, and an adverse effect on the growth rate has been noted even at 0.022% (Gardiner and Farr, 1954;

Peterson and Hymas, 1950). Newberne and McEuen (1957) found that 0.05 to 0.1% of nitrofurazone in the feed produced stunted growth, curled-toe paralysis, clinical polyneuritis, atrophy of the follicles of the bursa of Fabricius, renal tubular degeneration and pulmonary ossification in young chicks. The blood picture remained essentially normal. McLoughlin and Chester (1959) found that 0.0055% nitrofurazone was less effective than glycarbylamide or nicarbazin but more effective than 0.0125% sulfaquinoxaline against *E. tenella*.

Another nitrofuran coccidiostat, Bifuran, was introduced quite recently. It is a mixture of nitrofurazone and furazolidone (NF 180, or N-(5-nitro-2-furfurylidene)-3-amino-2-oxazolidone). The final concentrations in the feed are 0.0055% nitrofurazone and 0.0008% furazolidone. McLoughlin and Chester (1959) found that it was less effective than glycarbylamide and nicarbazin, about as effective as nitrofurazone and more effective than sulfaquinoxaline against *E. tenella* infections in chicks. Horton-Smith and Long (1959) found that it was effective against *E. necatrix* when fed at double the above level. Kantor and Levine (unpublished) found that furazolidone by itself was valueless against *E. necatrix*.

Both nitrofurazone and furazolidone are also antibacterial agents. Furazolidone is used against *Salmonella* infections in poultry, and also has some effect against *Histomonas meleagridis* (Harwood and Stunz, 1954) and *Trichomonas gallinae* (Stabler, 1957).

The anticoccidial properties of substituted carbanilide complexes were discovered by Cuckler *et al.* (1955). They introduced nicarbazin, which is an equimolar complex between 4,4'-dinitrocarbanilide and 2-hydroxy-4,6-dimethylpyrimidine. A simple mixture is no better than the carbanilide alone. Nicarbazin is fed at a concentration of 0.01 to 0.0125% in the feed, or 0.008% in replacement flocks. It is effective against *E. tenella*, *E. acervulina* and *E. necatrix* (Cuckler, Malanga and Ott, 1956; Rubin *et al.*, 1956; Cuckler, Ott and Fogg, 1957; Horton-Smith and Long, 1959). McLoughlin and Chester (1959) found that nicarbazin was about as effective as glycarbylamide against *E. tenella*, and more effective than nitrofurazone, Bifuran, sulfaquinoxaline or Trithiadol.

Nicarbazin is coccidiostatic, and will not cure coccidiosis once signs of the disease have appeared. It acts against the second generation schizonts and their merozoites (Cuckler and Malanga, 1956), and birds which are infected while receiving the drug develop immunity to reinfection (Cuckler and Malanga, 1956; Marthedal and Velling, 1957; McLoughlin, Rubin and Cordray, 1957, 1958).

Nicarbazin is not recommended for laying hens. When fed at the recommended level, it makes the egg shells pale (McLoughlin, Wehr and Rubin, 1957). At higher levels the yolks become mottled, blotchy, enlarged and sometimes even brown, the whites may become cloudy, hatchability is affected, and production may be reduced (Snyder, 1956; Sherwood, Milby and Higgins, 1956; Baker *et al.*, 1956; Lucas, 1958).

Other pyrimidine derivatives besides the one in nicarbazine may have a synergistic effect on sulfonamide coccidiostats. Lux (1954) found that pyrimethamine (Daraprim; 2,4-diamino-5-p-chlorophenyl-6-ethyl pyrimidine), which is a powerful antimalarial drug, acted synergistically with sulfanilamide and other sulfonamides against *E. tenella*. Joyner and Kendall (1955) found that as little as 0.0025% pyrimethamine allowed the effective concentration of sulfamethazine against *E. tenella* to be reduced to 1/8 to 1/16 of that normally required for protection. Marthedal and Velling (1957) found that pyrimethamine acted synergistically with two other sulfonamides, sulfabenzpyrazine and sulfadimidine, against *E. tenella*.

Most recently, a quaternized derivative of pyrimidine, amprolium, has been introduced. This compound is 1-(2-*n*-propyl-4-amino-5-pyrimidinylmethyl)-2-methylpyridinium chloride hydrochloride.

According to Rogers *et al.* (1960), 0.0125% amprolium in the feed is effective against *E. tenella*, *E. necatrix* and *E. acervulina*. It is a thiamine antagonist, and 0.003% thiamine in the feed markedly decreased its activity against coccidia. Another name for amprolium is mepyrium, the discovery of which was announced by Aries (1960).

According to Rogers *et al.* (1960), many other 1-(2-alkyl-4-amino-5-pyrimidinylmethyl)-alkyl pyridinium salts have marked prophylactic activity in coccidiosis of poultry. Analogous 3-thiazolium compounds are also effective.

The imidazole derivative, glycarbylamide (4, 5-imidazoledicarboxamide) was introduced as a coccidiostat by Cuckler *et al.* (1958). It is fed in a concentration of 0.003% in the feed. It is effective against *E. tenella*, *E. necatrix* and *E. acervulina*, but Horton-Smith and Long (1959a) found that it is inferior to sulfaquinoxaline against the last. McLoughlin and Chester (1959) found that it is about as effective as nicarbazin against *E. tenella*, and more effective than nitrofurazone, Bifuran, sulfaquinoxaline or Trithiadol.

Glycarbylamide is coccidiostatic, and will not cure coccidiosis once signs of the disease have appeared. It acts against the stages prior to the second generation schizonts, and birds which are infected while receiving the drug develop immunity to reinfection. It is apparently non-toxic when fed at the recommended level.

Several benzamide derivatives are effective coccidiostats. Morehouse and McGuire (1957, 1959) found that 3, 5-dinitrobenzamide and several aliphatic N-substituted derivatives are effective against *E. tenella* and somewhat less effective against *E. necatrix*. They found that Unistat, a "coccidiostatic growth stimulant" mixture containing 30% N^4-acetyl-N^1-(4-nitrophenyl) sulfanilamide, 25% 3, 5-dinitrobenzamide and 5% 3-nitro-4-hydroxyphenylarsonic acid in an inert carrier, when fed at a concentration of 0.1% in the feed, prevented death and permitted normal or near normal weight gains in chicks infected with potentially lethal doses of *E. tenella*, *E. necatrix* and *E. acervulina*.

Another benzamide derivative is zoalene (3, 5-dinitro-o-toluamide). Hymas, Stevenson and Shaver (1960) reported that it prevents mortality and weight losses from infections with *E. tenella*, *E. necatrix*, *E. acervulina*, *E. maxima* and *E. brunetti* when fed continuously in the ration of chicks at levels ranging from 0.0025 to 0.015%. They recommended a level of 0.0125% for broilers and lower levels for replacement pullets. This compound is most effective against *E. necatrix*.

The benzamide derivatives are coccidiostatic and will not cure the disease once signs have appeared. Birds which are infected while receiving them develop immunity to reinfection.

Hemorrhage is an important cause of death from cecal coccidiosis, and its control will ameliorate the disease. Harms and Tugwell (1956) and Tugwell, Stephens and Harms (1957) found that the vitamin K activity of alfalfa meal or menadione sodium bisulfite complex (Klotogen F) prevented deaths from cecal coccidiosis in birds on a basic vitamin K-deficient diet. Otto *et al.* (1958) confirmed their work, finding that 1.0 g of the water soluble menadione sodium bisulfite complex per ton of feed was just as effective as 3 g per ton of menadione.

Sulfonamides and other coccidiostats have been mixed in poultry feed for so many years that it was inevitable that drug resistant strains of coccidia would develop. The first report of this was by Waletzky, Neal and Hable (1954), who found that a field strain of *E. tenella* from a Delaware broiler flock was more than 40 times as resistant to sulfaquinoxaline and 5 times as resistant to sulfamethazine as ordinary strains. It was unaffected by 1.0% sulfaquinoxaline in the feed. Cuckler and Malanga (1955) studied 40 field strains of allegedly drug-resistant cecal or mixed intestinal and cecal coccidia from chickens. They found that 43% were resistant

to nitrophenide, 45% to sulfaquinoxaline and 57% to nitrofurazone. Twenty-two percent were resistant to all 3 drugs, 18% to 2 and 18% to 1. None were resistant to nicarbazin, which had only recently been placed on the market. They produced resistance against sulfaquinoxaline in 1 strain of *E. acervulina* and 2 strains of *E. tenella* by exposure to suboptimal dosages of the drug during 15 serial passages, but 1 strain of *E. tenella* was not rendered resistant to nitrophenide, nitrofurazone or nicarbazin by the same method for 15 serial passages.

Drug resistance is becoming increasingly common. It seems to develop with especial ease against glycarbylamide. As a consequence, we are in a race between the discovery of new coccidiostats and the development by the parasites of resistance against the older ones. In the long run, prevention of coccidiosis without reliance on drugs appears to hold more promise.

Prevention and Control: Coccidian oocysts are extremely resistant to environmental conditions. They may remain alive in the soil for a year or more (Warner, 1933; Farr and Wehr, 1949; Koutz, 1950). They will not sporulate in the absence of oxygen, and they are killed in time by subfreezing temperatures. Thus, Edgar (1954) found that the oocysts of *E. tenella* were dead after 7 days at -12° C.

Ordinary antiseptics and disinfectants are ineffective against them. Pérard (1924), for instance, found that the oocysts of rabbit coccidia would sporulate unharmed in 5% formalin, 5% phenol, 5% copper sulfate, or 10% sulfuric acid. Horton-Smith, Taylor and Turtle (1940) confirmed this with *E. tenella* and added 5% potassium hydroxide and 5% potassium iodide to the list. Indeed, the standard storage solutions for coccidian oocysts are 2.5% potassium bichromate or 1% chromic acid solution.

The oocysts may be destroyed by ultra-violet light, heat, desiccation or bacterial action in the absence of oxygen. Long (1959) found that exposure to a temperature of 52° C for 15 minutes killed the oocysts of *E. tenella* and *E. maxima*. However, Horton-Smith and Taylor (1939) found that even a blowtorch did not kill all the oocysts on the floors of poultry houses unless it was applied long enough to make the wood start to char. The problem is to reach and maintain a lethal temperature at the spot where the oocysts are.

While formaldehyde fumigation is ineffective against coccidia, Horton-Smith, Taylor and Turtle (1940) showed that ammonia fumigation is of practical value. *E. tenella* oocysts were killed by an 0.0088% solution of ammonia in 24 hours, by an 0.044% solution in 2 hours and by an 0.088% solution in 45 minutes. They fumigated poultry houses successfully with 3 oz. ammonia gas per 10 cu. ft. For satisfactory results, the houses should be sealed so that the gas does not leak out.

Boney (1948) found that methyl bromide is also an effective fumigant. It inactivated sporulated oocysts of *E. tenella* in the litter or soil when applied at the rate of approximately 1 lb. per 1000 square feet (0.3 ml per sq. ft.). It prevented infection in brooder houses using artificially contaminated cane pulp litter on wooden floors when used as a space fumigant at the rate of 2 lb. per 1000 cu. ft.

Since it is practically impossible under farm conditions to prevent chickens from picking up at least a few oocysts, prevention of coccidiosis depends upon preventing a heavy enough infection to produce disease while at the same time permitting a symptomless infection (coccidiasis) to develop and to produce immunity. This can be accomplished by proper sanitation and management. Strict sanitation is effective alone, but it is usually supplemented by the use of a coccidiostatic drug.

Young chickens should be raised apart from older birds, since the latter are a source of infection. If birds are raised on the floor, each new brood of chicks should be placed in a clean house containing clean, new litter. The litter should be kept dry, stirred frequently and removed when wet. The feeders and waterers should be washed in boiling water before use, and should be

cleaned at least weekly with hot water and detergent. The waterers should be placed on wire platforms over floor drains, and the feeders should be raised high enough to prevent their being fouled. Enough feeders should be provided so that all the birds can feed at once without crowding.

Chicks raised on wire have much less chance of contamination than those raised on the floor. However, the wire should be cleaned regularly.

Flies, rats and mice around the poultry houses and yards should be eliminated, since they may carry coccidia mechanically. Damp areas around the poultry house should be filled in or drained.

Feeding a coccidiostat during times when the birds are especially susceptible may also be helpful. The drug may be fed until the birds are 8 or 9 weeks old, after which they have ordinarily become immune. In addition, it is often recommended that a coccidiostat be fed to pullets for the first 2 or 3 weeks after they have been moved into laying houses.

If an outbreak of coccidiosis occurs, all sick birds should be removed from the flock and placed in a separate pen. They should be given ample food and water, but it is useless to attempt to treat them. The remaining, apparently healthy birds should be treated with a coccidiostat in the dosage recommended by the manufacturer. Birds which become ill should be removed. The litter should be kept dry and stirred frequently.

All dead birds should be burned. The litter should also be burned or put someplace where chickens will never have access to it.

Care should be taken not to track coccidia from sick birds to healthy ones. Special rubbers or overshoes should be put on before entering pens containing sick birds, and should be cleaned thoroughly after each use. Veterinarians going from one farm to another should disinfect their boots before leaving each premises.

The use of old, built-up, deep floor litter has been recommended by Kennard and Chamberlin (1949) and others to reduce losses from coccidiosis. By this method, the litter is not changed when new batches of birds are placed in a house, but some fresh litter may be added from time to time as needed to keep it in good condition. The litter is stirred every 2 or 3 days for the first 8 weeks and every day thereafter. Every 2 to 4 weeks, hydrated lime may be mixed in with the litter at the rate of 10 to 15 lb. per 100 square feet of litter, but this is not necessary. The litter will keep dry for 8 to 16 weeks. Using this method, Kennard and Chamberlin (1949) observed a mortality of 7% as compared to a mortality of 19% in chickens kept on fresh litter removed and changed every 2 weeks.

On the other hand, Koutz (1952, 1952a) found that many coccidian oocysts and nematode eggs remain alive in deep litter. Horton-Smith (1954), too, pointed out the dangers inherent in its use. He noted, however, that the ammonia produced would kill many oocysts. Long and Bingstead (1959) found that chicks on old, built-up litter did not gain as well as chicks on wire or new wood shavings, and that coccidia appeared in them earlier. Because of the dust, ammonia fumes, and danger of other diseases, the use of built-up litter in raising chickens is not recommended.

Edgar (1955a) developed a coccidiosis "vaccine" which is said to be highly successful in immunizing chicks. It is a mixture of sporulated oocysts of *E. tenella, E. necatrix, E. maxima, E. acervulina* and *E. hagani* (Libby, Bickford and Glista, 1959). It is recommended for use when the chicks are 3 to 5 days old. They are starved for about 3 hours and then given feed freshly mixed with the commercially prepared oocyst culture. The chicks are supposed to develop light infections and seed the litter with the oocysts which they produce. These oocysts produce reinfections in turn. It is recommended that a coccidiostat be fed at a low level until 5 weeks after vaccination, i.e., until the birds are $5\frac{1}{2}$ to 6 weeks old. Under these conditions, the birds are said to become

immune without suffering disease. While this system often works well, failures have been encountered too often to justify recommending its general use at present.

EIMERIA MELEAGRIDIS TYZZER, 1927

Hosts: Domestic and wild turkey.

Altho Steward (1947) and Gill (1954) claimed to have transmitted this species experimentally to the chicken, Tyzzer (1929) was unable to transmit it to the chicken, ring-necked pheasant or bobwhite quail, Hawkins (1952) was unable to transmit it to the bobwhite quail or Hungarian partridge, and Moore, Brown and Carter (cited by Moore, 1954) and Clarkson (1959a) were unable to transmit it to the chicken.

Location: The first generation schizonts, which are relatively few in number, are found only in the small intestine a short distance on either side of the yolk stalk. They lie below the host cell nuclei in the epithelial cells, mostly in those near the base of the villi but not in the deep glands.

The second generation schizonts occur in the cecum, where they lie above the host cell nuclei in the epithelial cells of the tips of the villi.

The sexual stages are found in the cecum, rectum and, to a slight extent, ileum. They lie above the host cell nuclei deep in the glands of the cecum as well as in the surface epithelium (Clarkson, 1959a).

Geographic Distribution: Worldwide.

Prevalence: Common. Kozicky (1948) found "*E. meleagridis*" in the droppings of 40% of 95 wild turkeys in Pennsylvania.

Morphology: This species was first described by Tyzzer (1927). The oocysts are ellipsoidal, smooth, 19 to 31 by 14 to 23 μ with a mean of 24 by 17 μ. The oocysts measured by Clarkson (1959a) were 22.5 $\pm$ 2.3 by 16.25 $\pm$ 1.23 μ. A micropyle is absent. One or 2 oocyst polar granules are present. An oocyst residuum is absent. The sporocysts are ovoid, with a Stieda body. A sporocyst residuum is present. The sporulation time is 1 day. Edgar (1955) found some sporulated oocysts as early as 15 hours at 28 C.

Life Cycle: Hawkins (1952) and Clarkson (1959a) described the life cycle, the latter using a strain which he had derived from a single oocyst. The first generation schizonts are present 2 to 5 days after infection, being found in greatest numbers at 60 hours. They measure 20 by 15 μ and contain 50 to 100 merozoites measuring 7 by 1.5 μ. The second generation schizonts first appear 60 hours after infection, and mature ones are present after 70 hours; they are seen in greatest numbers at 84 hours. They are about 9 μ in diameter and contain 8 to 16 merozoites which measure 10 by 2 μ. Hawkins stated that there may be a third asexual generation, but that most of the second generation merozoites develop into sexual stages; Clarkson did not describe third generation schizonts.

Macrogametes and microgametocytes appear at 91 hours and become mature 9 days after infection. They measure about 18 by 13 μ. The microgametes are biflagellate.

According to Hawkins, oocysts appear in the feces 5 days after infection; Clarkson found that the prepatent period was 108 to 112 hours.

Pathogenesis: This species is practically non-pathogenic. Hawkins (1952) observed only a slight drop in weight in poults experimentally infected with 400,000 to 1 million sporulated oocysts. Moore and Brown (1951) infected poults with "enormous numbers" of fresh, sporulated oocysts without producing clinical evidence of coccidiosis. Clarkson (1959a) found that doses of up to 1 million oocysts produced no signs of disease in 2-week-old poults.

The serosal surface of the ceca of heavily infected birds is cream colored. The ceca contain a non-adherent, mucoid or caseous, yellow plug on the 5th and 6th days. Caseous material composed of oocysts and epithelial cells is sometimes found in the feces on the 6th day, but the ceca appear quite normal in another day or two. Hawkins noted petechial hemorrhages in the cecal mucosa.

Immunity: Turkeys which have recovered from an infection with *E. meleagridis* have a high degree of immunity according to Hawkins (1952). Clarkson (1959a) found no cross immunity between this species and *E. adenoeides*.

EIMERIA MELEAGRIMITIS TYZZER, 1929

Host: Turkey.

Hawkins (1952) was unable to transmit this species to the bobwhite quail or Hungarian partridge. Gill (1954) claimed to have transmitted it to the chicken.

Location: The asexual stages occur mainly in the upper jejunum, but a few are present in the duodenum and ileum as far as the yolk stalk. The first generation schizonts lie below the host cell nuclei of the epithelial cells of the glands. The second generation schizonts develop in colonies in the epithelial cells of the deep glands but also spread up the sides of the villi. They usually lie just beneath the brush border of the cell but are sometimes found below the host cell nucleus. The third generation schizonts are found in the epithelial cells of the villi but never in the glands. Most of them lie above the host cell nucleus, but some are below it.

The sexual stages are found mainly in the epithelial cells at the tips of the villi but also spread down the sides. The great majority lie above the host cell nucleus (Clarkson, 1959).

Geographic Distribution: Presumably worldwide.

Prevalence: Quite common. Four out of 22 outbreaks studied by Clarkson and Gentles (1958) in Great Britain were due to this species, and 3 to a mixture of it and *E. adenoeides*.

Morphology: The morphology of this species has been studied especially by Tyzzer (1929), Hawkins (1952) and Clarkson (1959). The oocysts are subspherical, smooth, 16 to 27 by 13 to 22 μ with a mean of 19 by 16 μ; 150 oocysts measured by Clarkson (1959) were 20.1 ± 1.95 by $17.3 \pm 1.7 \mu$. A micropyle is absent. One to 3 oocyst polar granules are present. An oocyst residuum is absent. The sporocysts are ovoid, with a Stieda body. A sporocyst residuum is present. The sporozoites have a colorless globule at the large end. The sporulation time is 2 days according to Hawkins (1952), 1 day at 26° C according to Clarkson (1959).

Life Cycle: Tyzzer (1929), Hawkins (1952) and Clarkson (1959) studied the life cycle of this species, the last using a strain derived from a single oocyst. The account below is that of Clarkson, which is the most complete. The sporozoites invade the tips of the villi and migrate down the villi in the lamina propria until they reach the glands. Young first generation schizonts can be found in the gland epithelial cells as early as 12 hours after infection, and many are mature by 48 hours. They usually measure 17 by 13 μ and enlarge the host cell, pushing its nucleus into the gland lumen. They contain 80 to 100 merozoites which measure about 4.5 by 1.5 μ and have the nucleus at the larger end.

The first generation schizonts rupture and release the merozoites, which invade the adjacent epithelial cells, forming colonies of second generation schizonts. Most of these are mature by 66 hours after infection. They measure 8 by 7 μ and contain 8 to 16 merozoites which measure about 7 by 1.5 μ and have the nucleus near the center.

Third generation schizonts may be recognized as early as 72 hours after

infection and reach maturity at about 96 hours. They measure about 8 by 7 μ and differ from the second generation schizonts in having a residuum. They produce 8 to 16 merozoites which measure about 7 by 1.5 μ and have the nucleus much nearer the large end than do the second generation schizonts.

Macrogametes and microgametocytes first appear 114 hours after infection. They measure about 15 by 11 μ, and the microgametocytes contain a rounded residuum. The microgametes have 2 long flagella.

According to Hawkins (1952), the prepatent period is 6 days. Clarkson (1959) found that it ranged from 114 to 118 hours with an average of 116 hours.

Pathogenesis: This species is moderately to markedly pathogenic, causing catarrhal enteritis. The death rate is high in young poults up to 6 weeks of age, but older birds are more resistant. Hawkins (1952) found that infection with 50,000 sporulated oocysts produced a high mortality in young poults, in some instances killing 100% of 2- to 3-week-old poults. Clarkson and Gentles (1958) and Clarkson (1959) observed mortalities of 62%, 36% and 0%, respectively, in poults 1.5, 3 and 4 weeks old fed 100,000 oocysts; of 40% and 100%, respectively, in 4-week-old poults fed 300,000 and 400,000 oocysts; and of 0% in 5- and 10-week-old poults fed 200,000 and 2 million oocysts, respectively. Food utilization is reduced in infected birds, and those which recover do not gain weight well for some time.

Lesions first appear at the end of the 4th day after infection (Hawkins, 1952; Clarkson and Gentles, 1958; Clarkson, 1959). The jejunum is slightly thickened, dilated, and contains an excessive amount of clear, colorless fluid or mucus containing merozoites and small amounts of blood and other cells. Five to 6 days after infection the duodenum is enlarged, its blood vessels are engorged, and it contains a reddish brown, necrotic core which adheres firmly to the mucosa and extends a little way into the upper small intestine. The duodenal mucosa occasionally seems to have undergone coagulation necrosis, and pieces of caseous material may be scattered in the lumen of the entire intestine along with a large amount of fluid which may have a pinkish tinge. The remainder of the intestine is congested, and petechial hemorrhages may be present in the mucosa of most of the small intestine.

Regeneration of the mucosa begins on the 6th or 7th day. A few petechiae are present in the duodenum and jejunum, and there are a few minute streaks of hemorrhage and spotty congestion in the ileum. The posterior part of the jejunum and ileum may contain greenish, mucoid casts 5 to 10 cm long and 3 to 6 mm in diameter, and necrotic material may be found in the ileum or feces.

Feed consumption begins to drop 2 to 3 days after infection, and 4 days after infection the birds huddle together with closed eyes, drooping wings and ruffled feathers. Their droppings at this time are scanty and slightly fluid. At the peak of the disease, 5 to 6 days after infection, some of the feces form cylinders 1 to 2 cm long and 3 to 6 mm in diameter. The droppings are not bloody, altho a few flecks of blood may occasionally be seen. Death usually occurs 5 to 7 days after infection.

The first reaction of the host is local infiltration of the whole intestine with eosinophiles (Clarkson, 1959). This begins within 2 hours after infection, reaches a maximum in 1 to 2 days, and persists at least 10 days. There are no striking abnormalities at 4 days, but at 5 days many of the infected villi appear to have lost their tips, all the duodenal blood vessels are congested, and many of the epithelial cells around the villi stain poorly and appear necrotic. These changes are present also in birds which die on the 6th or 7th days, but resolution is rapid in recovered birds, and Clarkson (1959) saw very little abnormality by the 8th day except for increased cellularity of the lamina propria.

Immunity: According to Hawkins (1952), the immunity produced by infections with this species is not as solid as that

produced by *E. meleagridis*, *E. dispersa* and *E. gallopavonis*, but it is still considerable.

EIMERIA DISPERSA
TYZZER, 1929

Hosts: Turkey, bobwhite quail, ring-necked pheasant, ruffed grouse (?), sharp-tailed grouse (?).

This species was first described by Tyzzer (1929) from the bobwhite quail. He also found it in the ring-necked pheasant. Hawkins (1952) first found it in the turkey. Boughton (1937) reported it from the ruffed grouse (*Bonasa umbellus*) and sharp-tailed grouse (*Pedioecetes phasianellus campestris*). Tyzzer (1929) transmitted it from the bobwhite to the turkey, chicken (producing a light infection) and possibly to the pheasant. Venard (1933) and Patterson (1933) were unable to infect chickens with strains from the bobwhite. Tyzzer (1929) transmitted it from the pheasant to the bobwhite. Hawkins (1952) infected the bobwhite and Hungarian partridge (*Perdix perdix*) with *E. dispersa* from the turkey, but was unable to infect the pheasant or chicken. Moore and Brown (1952) infected the bobwhite with a turkey strain, but, according to Moore (1954), were unable to infect the pheasant.

Location: Primarily duodenum, but also small intestine.

Geographic Distribution: North America.

Prevalence: Presumably relatively uncommon.

Morphology: The morphology of this species was studied especially by Tyzzer (1929) and Hawkins (1952). The oocysts are broadly ovoid, smooth, 22 to 31 by 18 to 24 μ with a mean of 26 by 21 μ. The oocyst wall is composed of a single layer and lacks a micropyle. An oocyst polar granule and oocyst residuum are absent. The sporocysts are ovoid, with a Stieda body. The sporulation time is 2 days.

Life Cycle: Tyzzer (1929) and Hawkins (1952) studied the endogenous stages of this species. They are found above the nuclei of the epithelial cells near the tips of the villi. There are apparently two types of first generation schizonts. Much the commoner is a small type about 6 μ in diameter which produces 15 or fewer merozoites each 4 to 6 μ long and 1 μ wide. The other type measures up to 24 by 18 μ and produces at least 50 merozoites. The first generation merozoites are formed by the end of the second day of infection.

The second generation schizonts are about 11 to 13 μ in diameter and produce 18 to 23 merozoites each 5 to 6 μ long and 1.5 to 2 μ wide about 4 days after infection.

There are a few third generation schizonts and merozoites, but most of the second generation merozoites develop into sexual stages. The macrogametes are 18 to 20 μ in diameter when mature, and the microgametocytes are slightly smaller. The microgametes have 2 flagella. Oocysts first appear in the feces late on the fifth or on the sixth day after infection.

Pathogenesis: This species is only slightly pathogenic in the turkey. Hawkins (1952) found the most severe lesions on the fifth and sixth days after experimental infection. The entire small intestine was markedly dilated and the duodenum and anterior jejunum were creamy white when seen thru the serosal surface. The anterior half of the small intestine was filled with creamy, yellowish, sticky, mucoid material. The wall of the anterior intestine was edematous, but there was little epithelial sloughing. The intestinal tract was virtually normal by the eighth day after infection.

The only signs Hawkins saw in infected turkeys were a slight tendency to produce somewhat liquid feces and a slight depression in weight gains.

Immunity: According to Hawkins (1952), turkeys which have recovered from infection are strongly immune to reinfection.

EIMERIA GALLOPAVONIS
HAWKINS, 1952

Host: Turkey.

Hawkins (1952) transmitted this species experimentally to the Hungarian partridge but not to the pheasant or bobwhite quail. Gill (1954) claimed to have transmitted it from the turkey to the chicken.

Location: Ileum, rectum and, to a lesser extent, ceca.

Geographic Distribution: North America, India.

Prevalence: Uncommon.

Morphology: This species was described by Hawkins (1952, 1952a), who remarked that its oocysts cannot be differentiated with any certainty from those of *E. meleagridis*. The oocysts are ellipsoidal, smooth, 22 to 33 by 15 to 19 μ with a mean of 27 by 17 μ, without a micropyle. An oocyst polar granule is present. There is no oocyst residuum. The sporocysts are ovoid, with a Stieda body. A sporocyst residuum is present. The sporulation time is 1 day.

Life Cycle: Hawkins (1952) described the life cycle of this species. The endogenous stages are found in the epithelial cells at the tips of the villi, where they lie mostly above the host cell nuclei. The first generation schizonts occur in the ileum and rectum. They produce approximately 8 merozoites and a residual mass 3 days after infection. There are apparently two sizes of second generation schizonts. The smaller ones occur in the rectum, ileum and rarely in the ceca. They produce 10 to 12 merozoites and a residual mass 4 to 5 days after infection. The larger second generation schizonts occur only in the rectum. They are 20 μ in diameter and produce a large, undetermined number of merozoites 4 days after infection.

There are a few third generation schizonts and merozoites in the rectum. They produce about 10 to 12 merozoites. These and most of the second generation merozoites develop into sexual stages. These are found primarily in the rectum and only occasionally in the ileum and ceca. The macrogametes and microgametocytes are similar to those of other turkey protozoa. Some oocysts are passed in the feces on the sixth day after infection, but most appear on the seventh day.

Pathogenesis: Little is known of the pathogenicity of this species. Hawkins (1952) noted marked edema, sloughing and lymphocytic infiltration in the intestines, but did not have sufficient material to make a thoro study.

Immunity: According to Hawkins (1952), infection with *E. gallopavonis* produces a more solid immunity than that elicited by *E. meleagridis*, *E. meleagrimitis* or *E. dispersa*.

EIMERIA ADENOEIDES
MOORE AND BROWN, 1951

Host: Turkey.

Moore and Brown (1951) were unable to transmit this species to the chicken, guinea fowl, ringnecked pheasant or bobwhite quail. Clarkson (1959a) was unable to transmit it to the chicken.

Location: The first generation schizonts (Clarkson, 1958) occur in the neck of the ceca and in the terminal inch or so of the ileum, where 80% of them lie below the host cell nuclei of the epithelial cells. The second generation schizonts occur thruout the ceca, and some are found in the rectum and posterior ileum. They lie above the host cell nuclei of the epithelial cells, just beneath the brush border. The sexual stages occur thruout the ceca, rectum and posterior third of the small intestine. A few are found even more anteriorly, but none more than halfway to the yolk sac stalk. They invade the epithelial cells of the crypts and deep glands, a location which distinguishes them from *E. meleagridis* and *E. gallopavonis*, and also apparently the epithelial cells of the villi. Clarkson

(1958) illustrated them as lying above the host cell nuclei.

Geographic Distribution: North America, Great Britain.

Prevalence: Quite common. Fifteen out of 22 outbreaks studied by Clarkson and Gentles (1958) in Great Britain were caused by this species and 3 by a mixture of it and *E. meleagrimitis*.

Morphology: The oocysts have been described by Moore and Brown (1951) and Clarkson (1958). They are similar to those of *E. meleagridis* and *E. gallopavonis*. They are ellipsoidal, sometimes ovoid, smooth, 19 to 31 by 13 to 21 μ with a mean of 26 by 17 μ. A micropyle is sometimes present. One to 3 oocyst polar granules are present. An oocyst residuum is absent. The sporocysts are elongate ovoid, apparently with a Stieda body. A sporocyst residuum is present. The sporozoites contain a clear globule at the large end. The sporulation time is 1 day. Edgar (1955) found sporulated oocysts as early as 18 hours at 29° C.

Life Cycle: Clarkson (1958) studied the life cycle of this species, using a strain derived from a single oocyst. First generation schizonts can be found in the epithelial cells as early as 6 hours after infection. They become mature 60 hours after infection; by 66 hours most of them have released their merozoites, altho a few remain up to 84 hours. The mature first generation schizonts measure 30 by 18 μ and contain about 700 merozoites measuring 4.5 to 7 by 1.5 μ, with a central nucleus.

The second generation schizonts become mature 96 to 108 hours after infection. They measure 10 by 10 μ and contain 12 to 24 merozoites measuring about 10 by 3 μ, with the nucleus a little nearer the rounded than the pointed end.

Sexual stages can be found as early as 114 hours and recognized as early as 120 hours after infection. The mature macrogametes measure about 20 by 18 μ and contain many large, plastic granules which stain black with Heidenhain's hematoxylin. The mature microgametocytes are about the same size as the macrogametes.

The prepatent period was given by Moore and Brown (1951) as 112 hours. Edgar (1955) found oocysts in the feces as early as 104 hours, and Clarkson (1958) found that the prepatent period varied from 114 to 132 hours in 30 birds.

The patent period is 7 to 8 days according to Moore and Brown (1951). Clarkson (1958) found that very few oocysts were passed more than 14 days after infection, and none after the 20th day.

Pathogenesis: This species is highly pathogenic. Moore and Brown (1951) were able to kill 100% of experimental poults up to 5 weeks of age with large doses of sporulated oocysts. Older poults developed a severe enteritis with few or no deaths. Clarkson (1958) and Clarkson and Gentles (1958) observed mortalities of 0%, 0%, 45% and 100%, respectively, in 3-week-old poults fed 10,000, 25,000, 100,000 and 200,000 oocysts; of 33% in 6-week-old poults fed 1 million oocysts; and of 0% in 11-week-old poults fed 3 million oocysts. Birds which did not die had decreased food consumption and weight gains.

Poults develop signs of anorexia, droopiness and ruffled feathers during the 4th day after experimental infection. If death occurs, it is usually on the 5th or 6th days but may be a little later (Moore and Brown, 1951).

The gross lesions have been studied by Moore and Brown (1951), Clarkson (1958) and Clarkson and Gentles (1958). The intestines appear quite normal until the 4th day. The walls of the lower third of the small intestine, ceca and rectum become swollen and edematous, petechial hemorrhages which are visible from the mucosal but not from the serosal surface appear, and the lower intestine becomes filled with mucus.

During the 5th day, most of the terminal intestine is congested and contains large numbers of merozoites and long

streaks of blood. By the end of the day, the intestine contains caseous material composed of cellular debris, gametes, and a few immature oocysts. A little later the caseous exudate is composed largely of oocysts. The feces in severe cases are relatively fluid and may be blood-tinged and contain mucous casts 1 to 2 inches long. Caseous plugs are sometimes present in the ceca.

On the 6th to 8th days in birds infected with 10,000 oocysts, the terminal intestine contains white, creamy mucus, and petechiae are present in the mucosa. By the 9th day the intestinal contents appear normal, altho they still contain large numbers of oocysts (Clarkson, 1958).

Infiltration with eosinophiles commences as early as 2 hours after infection, and enormous numbers of eosinophiles may be found in the terminal small intestine, ceca and rectum from the 3rd to the 10th days.

Beginning 4 days after infection, edematous changes are seen in the intestine, and infected epithelial cells begin to break off, leaving the villi denuded. The blood vessels become engorged, and cellular infiltration of the submucosa and epithelial denudation increase progressively until the 6th day. In birds which recover from the disease or which have received relatively few oocysts, resolution is very rapid. Vascularity is greatly reduced, the deep glands are almost free of parasites by the 7th day, and the intestine is almost normal by the 9th or 10th day (Clarkson, 1958).

Clarkson (1958) found no changes in the blood picture of infected poults.

Immunity: Moore and Brown (1951) produced solid immunity to *E. adenoeides* by infecting turkey poults with 25 doses of sporulated oocysts over a period of 2 months. These birds were not immune to *E. meleagridis*. Conversely, poults which had been immunized against *E. meleagridis* were not immune to *E. adenoeides*. Clarkson (1959a), too, found no cross immunity between *E. meleagridis* and *E. adenoeides*. Since *E. adenoeides* is found in the same locations as *E. meleagridis* and *E. gallopavonis*, and since its oocysts are apparently similar to theirs, this lack of reciprocal immunity is an important differentiating criterion. The only other differences are its greater pathogenicity and its location in the crypts and deep glands rather than only in the tips of the villi.

EIMERIA INNOCUA MOORE AND BROWN, 1952

Host: Turkey.

Moore and Brown (1952) were unable to infect the chicken, guinea fowl, ring-necked pheasant and bobwhite quail with *E. innocua*.

Location: Thruout the small intestine.

Geographic Distribution: North America (New York).

Prevalence: Apparently uncommon.

Morphology: The oocysts of this species were described by Moore and Brown (1952). They are subspherical, smooth, 19 to 26 by 17 to 25 μ with a mean of 22 by 21 μ, and without a micropyle or oocyst polar granule. No other morphological information was given. The sporulation time is 2 days.

Life Cycle: Unknown. The endogenous stages occur in the epithelial cells of the villi. The tips of the villi are most heavily parasitized, while the crypts and deep glands are never affected. According to Moore and Brown (1952), oocysts first appear in the feces 5 days after infection, and the patent period is up to 9 days.

Pathogenesis: This species is nonpathogenic according to Moore and Brown (1952). They observed no macroscopic lesions, even in heavy infections; poults less than 5 weeks old showed no signs of illness and had no diarrhea.

Immunity: Moore and Brown (1952) immunized turkey poults by infecting them

with 4 to 7 doses of oocysts over a period of 22 to 29 days. The immunized birds were not immune to *E. dispersa*, the species which *E. innocua* most closely resembles, and turkeys immunized against *E. dispersa* were susceptible to infection with *E. innocua*.

EIMERIA SUBROTUNDA MOORE, BROWN AND CARTER, 1954

Host: Turkey.

Moore, Brown and Carter (1954) were unable to infect the chicken, guinea fowl, ringnecked pheasant or bobwhite quail with this species.

Location: Duodenum, jejunum and upper ileum as far as 2 inches anterior to the yolk stalk rudiment.

Geographic Distribution: North America.

Prevalence: Apparently uncommon.

Morphology: This species closely resembles *E. innocua*, according to Moore, Brown and Carter (1954). The oocysts are subspherical, smooth, 16 to 26 by 14 to 24 μ with a mean of 22 by 20 μ, without a micropyle or polar granule. No other morphological information was given. The sporulation time is 48 hours.

Life Cycle: Unknown. According to Moore, Brown and Carter (1954), the endogenous stages occur in the epithelial cells of the tips of the villi, extend along the sides of the villi to some extent, but never invade the crypts and deep glands. Oocysts first appear in the feces 96 hours after infection, and the patent period is 12 to 13 days.

Pathogenesis: This species is apparently non-pathogenic. Moore, Brown and Carter (1954) observed no signs of infection, diarrhea or gross lesions in poults less than 5 weeks old which had been infected with massive doses of sporulated oocysts.

Immunity: Moore, Brown and Carter (1954) immunized turkey poults by feeding them 10,000 to 15,000 sporulated oocysts every 4 days until they ceased to shed oocysts; this occurred in less than a month. Poults which had been immunized against *E. subrotunda* were not immune to *E. innocua* and *E. dispersa*, and poults which had been immunized against the latter two species were not immune to *E. subrotunda*. This was the primary basis for separating *E. subrotunda* from *E. innocua*.

COCCIDIOSIS IN TURKEYS

Epidemiology: Coccidiosis in turkeys has been discussed by Morehouse (1949), Hawkins (1952), Moore (1954) and Becker (1959) among others. The U. S. Dept. of Agriculture (1954) estimated that it caused an annual loss of $466,000 from 1942 to 1951, and it is becoming of increasing importance to the turkey grower.

Of the 7 species of *Eimeria*, 1 of *Isospora* and 1 of *Cryptosporidium* reported from turkeys, by far the most important are *E. meleagrimitis* and *E. adenoeides*. The former affects the jejunum and the latter the lower ileum, ceca and rectum.

Coccidiosis is primarily a disease of young birds. Older birds are carriers. Poults become infected by ingesting oocysts along with their feed or water. The severity of the disease depends on the number of oocysts they receive. If they ingest relatively few, they may develop immunity without ever showing signs of illness, while if they ingest large numbers, they may become seriously ill or die. Crowding and lack of sanitation greatly increase the disease hazard.

Diagnosis: Coccidiosis of turkeys can be diagnosed in the same way as coccidiosis of chickens by finding endogenous stages of the coccidia in scrapings of the affected parts of the intestinal tract of birds which show signs of the disease. The mere presence of coccidia in the absence of disease cannot be relied on. Since several species of turkey coccidia

(*E. innocua*, *E. subrotunda* and *E. meleagridis* in particular) are non-pathogenic or nearly so, they must be differentiated from the pathogenic *E. meleagrimitis* and *E. adenoeides*. The sporulated oocysts of both the latter have polar bodies, which differentiates them from all but *E. meleagridis* and *E. gallopavonis*. The oocysts of *E. meleagrimitis* are ellipsoidal, but apparently only pathogenesis and absence of cross-immunity differentiates *E. adenoeides* from the other two. This last is hardly a practical diagnostic test, since it requires a colony of turkeys immunized against the various species.

Treatment: The sulfonamides are effective against a number of the turkey coccidia. Morehouse (1949a) found that only one of 6 sulfonamides was ineffective against *E. meleagridis*. Peterson (1949a) found that several sulfonamides were effective against *E. meleagrimitis*. Moore (1949) found that 0.031% sulfaquinoxaline, 1% sulfaguanidine or 0.5% sulfamerazine in the feed was effective against turkey coccidiosis. Wilson (1951) reported that 0.06% sodium sulfaquinoxaline in the drinking water stopped losses from *E. meleagridis* and *E. meleagrimitis* in a natural outbreak. (Their cultures of *E. meleagridis* may have contained *E. adenoeides*, a species which had not yet been named at the time.) Boyer and Brown (1953) found that 0.0175% acetylsulfaquinoxaline in the feed or 1-1000 to 1-2000 sulfamethazine in the water was effective against *E. adenoeides*, *E. gallopavonis*, *E. meleagridis*, *E. innocua*, *E. subrotunda*, *E. dispersa* and *E. meleagrimitis*. Horton-Smith and Long (1959) found that 0.0125% sulfaquinoxaline in the feed was effective against *E. meleagrimitis*.

Other coccidiostats used in treating chickens have not been found so useful in turkeys. Morehouse (1949) found that sodium 4-chlorophenyl arsonate was the most effective of 10 organic arsenic compounds to be tested against *E. meleagridis*, but that its effective dose was too close to the toxic one. Another organic arsenic compound, 3-nitro-4-hydroxyphenyl arsonic acid was of less value.

Boyer and Brown (1953) found that nitrophenide, 2-amino-5-nitrothiazole, sulfisoxazole, nitrofurazone and furoxone were not effective coccidiostatic agents in the turkey. Cuckler *et al.* (1955) reported that nicarbazin was effective against *E. gallopavonis* and *E. meleagrimitis*, but Horton-Smith and Long (1959) found that it was ineffective against *E. meleagrimitis* and in addition found that nitrofurazone and glycarbylamide were also ineffective against this species.

Prevention and Control: The same measures should be used for the prevention and control of coccidiosis in turkeys as in chickens.

EIMERIA TRUNCATA (RAILLIET AND LUCET, 1891) WASIELEWSKI, 1904

Synonym: *Coccidium truncatum*.

Hosts: Domestic goose, greylag goose (*Anser anser*), Ross's goose (*A. rossi*), Canada goose (*Branta canadensis*) (see Levine, 1953; Hanson, Levine and Ivens, 1957). In addition to these, Pavlov (1942) reported finding *E. truncata* in domestic ducks in Bulgaria, and Christiansen (1948, 1952) found oocysts resembling *E. truncata* but smaller in the kidneys of young swans (*Cygnus olor*) and common eiders (*Somateria mollissima*) in Denmark.

Location: Kidneys.

Geographic Distribution: Worldwide.

Prevalence: Relatively uncommon in domestic geese, at least in North America.

Morphology: This species has been described by Kotlan (1933) and Lerche (1923) among others. The oocysts are ovoid, with a narrow, truncate, small end, and measure 14 to 27 by 12 to 22 μ. The oocyst wall is smooth and delicate, shrinking quickly during concentration in hypertonic solutions. A micropyle with a polar cap is present. An oocyst residuum is sometimes present. A sporocyst residuum is present. The sporulation time is 1 to 5 days.

Life Cycle: The endogenous stages occur in the epithelial cells of the kidney tubules. The life cycle has not been studied in detail. The prepatent period is 5 to 6 days according to Kotlán (1933).

Pathogenesis: *E. truncata* is highly pathogenic for goslings, sometimes wiping out whole flocks within a few days. The disease is usually acute, lasting only 2 or 3 days. Affected birds are extremely weak and emaciated. Their kidneys are greatly enlarged, light-colored, with small, yellowish white nodules, streaks and lines on the surface and thruout the parenchyma. The infected epithelial cells are destroyed, and adjacent, uninfected cells are also destroyed by pressure. The infected tubules are so filled with urates and oocysts that they are enlarged to 5 to 10 times the diameter of normal tubules.

Epidemiology: *E. truncata* occurs only sporadically in domestic geese in North America. It was first described in the United States by McNutt (1929) in Iowa, and has since been reported by Allen (1933) in Washington, D. C., Adler and Moore (1948) in Washington state, Levine, Morrill and Schmittle (1950) in Illinois, Lindquist, Belding and Hitchcock (1951) in Michigan, Farr and Wehr (1952) in Maryland, and McGregor (1952) in Ontario. It has also been found in New York and Quebec.

The epidemiology of *E. truncata* in wild geese is especially interesting (Hanson, Levine and Ivens, 1957). It has been found in the greylag goose (*Anser anser*) in Europe by Christiansen and Madsen (1948), and in Ross's goose (*A. rossi*) and the Canada goose (*Branta canadensis*) in North America. However, of the 6 wild goose flyways which form vertical bands across North America, *E. truncata* has been found only in the South Atlantic and Pacific flyways, and not from the flyways in between. It is common among Canada geese of the South Atlantic flyway, and has been associated with losses at their winter quarters at Pea Island, North Carolina (Critcher, 1950). Its apparent absence from wild geese in the interior flyways does not seem due to the examination of too few birds, since Hanson, Levine and Ivens (1957) failed to find it in 258 wild geese from these flyways altho they recognized it in birds from both coasts. Perhaps *E. truncata* was originally a parasite of greylag and domestic geese in Eurasia and has reached North American wild geese relatively recently, entering from both the east and west.

EIMERIA ANSERIS
KOTLÁN, 1932

Hosts: Domestic goose, blue goose (*Anser caerulescens*), Richardson's Canada goose (*Branta canadensis hutchinsi*).

Location: Small intestine, mainly posterior part.

Geographic Distribution: Europe, North America.

Prevalence: *E. anseris* has been reported from domestic geese only in Europe (Kotlán, 1933; Cerna, 1956) and is apparently not particularly common there. Hanson, Levine and Ivens (1957) found it in 4% of 73 blue geese from Ft. Severn and Weenusk, Ontario and in 33% of 6 Richardson's Canada geese from York Factory, Manitoba.

Morphology: This species was described in detail by Hanson, Levine and Ivens (1957). The oocysts have the form of a sphere surmounted by a truncate cone, with a micropyle at the truncate end, and measure 20 to 24 by 16 to 19 μ with a mean of 22 by 17 μ (16 to 23 by 13 to 18 μ according to Kotlán, 1933). The oocyst wall is smooth, colorless, composed of a single layer about 1 μ thick, and slightly thickened around the micropyle but incised sharply to form a plate or shelf across the micropyle itself. The oocyst residuum is a mass of amorphous material just beneath the micropyle and forming a seal beneath it. An oocyst polar granule is absent. The sporocysts are ovoid and almost completely fill the oocyst. The sporocyst wall is slightly thickened at the small end.

The sporocysts are 10 to 12 by 7 to 9 μ. A sporocyst residuum is present. The sporozoites often lie more or less transversely at the anterior and posterior ends of the sporocyst. The sporulation time is 1 to 2 days according to Kotlán (1933).

Life Cycle: The endogenous stages have been described by Kotlán (1933). They occur in compact clumps under the intestinal epithelium near the muscularis mucosae and also in the epithelial cells of the villi. The schizonts are spherical, 12 to 20 μ in diameter, and contain 15 to 25 slightly curved, crescent-shaped merozoites. There is probably only a single asexual generation. The sexual stages are found mostly in the subepithelial tissues of the villi, but invade the epithelium in heavy infections. The macrogametes measure 12 to 16 by 10 to 15 μ. The microgametocytes are spherical and about the same size. Oocysts first appear in the feces 7 days after infection, and the patent period is 2 to 8 days.

Pathogenesis: Kotlán (1933) reported that experimental infections in 2.5- to 3-month-old geese were harmless, but described two outbreaks of intestinal coccidiosis in goslings which he considered due to a combination of *E. anseris* and *E. nocens*.

EIMERIA NOCENS KOTLÁN, 1933

Hosts: Domestic goose, blue goose (*Anser caerulescens*).

Location: Posterior part of small intestine.

Geographic Distribution: Europe, North America.

Prevalence: *E. nocens* has been reported from the domestic goose only in Europe (Kotlán, 1933; Cerná, 1956), and is apparently not particularly common there. Hanson, Levine and Ivens (1957) found it in blue geese from Ft. Severn and Weenusk, Ontario.

Morphology: The sporulated oocysts were described by Hanson, Levine and Ivens (1957). They are ovoid but flattened at the micropylar end, 29 to 33 by 19 to 24 μ with a mean of 31 by 22 μ (25 to 33 by 17 to 24 μ according to Kotlán, 1933). The oocyst wall is smooth and composed of 2 layers, the outer one 1.3 μ thick and pale yellow, the inner one 0.9 μ thick and almost colorless. A prominent micropyle is present. A true micropylar cap is absent, but the micropyle appears to be present only in the inner wall and is covered by the outer wall. An oocyst polar granule and oocyst residuum are absent, but part of the oocyst wall often forms one or more roundish protuberances just below the micropyle. The sporocysts are broadly ellipsoidal, with a thin wall and sometimes with a very small Stieda body. The sporocysts are 10 to 14 by 8 to 10 μ with a mean of 12 by 9 μ. The sporozoites usually lie head to tail in the sporocysts and contain 2 or more large, clear globules which almost obscure their outline. The sporocyst residuum fills the space between sporozoites.

Life Cycle: According to Kotlán (1933), the endogenous stages are found primarily in the epithelial cells at the tips of the villi, but they may also occur beneath the epithelium. The younger developmental stages lie near the host cell nuclei, but as they grow they not only displace the nuclei but also destroy the host cell and come to lie free and partly beneath the epithelium. The schizonts are spherical, 15 to 30 μ in diameter, and contain 15 to 35 merozoites. The macrogametes are usually ellipsoidal or irregularly spherical, uniformly coarsely granular, and measure 20 to 25 by 16 to 21 μ. The microgametocytes are spherical or ellipsoidal and measure 28 to 36 by 23 to 31 μ.

Pathogenesis: Kotlán (1933) described 2 outbreaks of intestinal coccidiosis in goslings in Hungary in which he found both *E. nocens* and *E. anseris*. Since the latter is apparently non-pathogenic, the disease was presumably due to *E. nocens*.

EIMERIA PARVULA
KOTLÁN, 1933

Host: Domestic goose.

Location: Small intestine, primarily posterior part.

Geographic Distribution: Europe.

Prevalence: This species is common in geese in Hungary, according to Kotlán (1933).

Morphology: This species was described by Kotlán (1933). The oocysts are spherical or subspherical, smooth, colorless, delicate, 10 to 15 by 10 to 14 μ, without a micropyle. No other morphological details were given.

Life Cycle: Unknown. According to Kotlán (1933), the endogenous stages are found almost exclusively in the epithelial cells of the villi. Oocysts first appear in the feces 5 days after infection.

Pathogenesis: According to Kotlán (1933), this species is non-pathogenic.

EIMERIA ANATIS
SCHOLTYSECK, 1955

Host: Wild mallard (*Anas platyrhynchos*). Scholtyseck did not find this species in 6 domestic ducks, which he called *A. domestica*.

Location: Small intestine.

Geographic Distribution: Europe (Germany).

Prevalence: Scholtyseck (1955) found this species in 5 of 32 wild mallards.

Morphology: The oocysts are ovoid, 14 to 19 by 11 to 16 μ with a mean of 17 by 14 μ. The oocyst wall is smooth, about 0.7 to 1.0 μ thick, with a thickened ring forming shoulders around the micropyle. An oocyst residuum and polar granule are absent. The sporocysts are elongate ovoid or ellipsoidal, with a slight thickening at the small end but not a true Stieda body. A few sporocyst residual granules are present between the sporozoites.

Life Cycle: Unknown.

Pathogenesis: Unknown.

Remarks: Tiboldy (1933) reported *Eimeria* sp. oocysts in domestic ducks in Hungary. They were ovoid, elongate ovoid or occasionally spherical are measured 11 to 25 by 8 to 13 μ. Their relationship to *E. anatis* is unknown.

EIMERIA LABBEANA
PINTO, 1928

Synonyms: *Coccidium pfeifferi*, *Eimeria pfeifferi*, *Eimeria columbarum*.

Hosts: Domestic pigeon, ring dove (*Columba palumbus*), turtle dove (*Streptopelia turtur*), *Streptopelia orientalis meena* (see Scholtyseck, 1956).

Location: Small and large intestine.

Geographic Distribution: Worldwide.

Prevalence: Common.

Morphology: The oocysts are subspherical to spherical, colorless or slightly yellowish brown, 13 to 24 by 12 to 23 μ. The oocyst wall is composed of 2 layers, the inner one darker than the outer. There is no micropyle. An oocyst polar granule is present. An oocyst residuum is absent. The sporocysts are elongate ovoid, with a Stieda body. A sporocyst residuum is present. The sporozoites lie lengthwise, head to tail, in the sporocysts. They are slightly crescent-shaped, with one end wider than the other, a vacuole at each end and the nucleus near the middle.

Nieschulz (1935) separated this species into two on the basis of size. He retained the name *E. labbeana* for the smaller form, which measured 13 to 24 by 12 to 22 μ (usually 15 to 18 by 14 to 16 μ) with a mean of 18 by 15 μ. He named the larger form *E. columbarum*; it measured 17 to 24 by

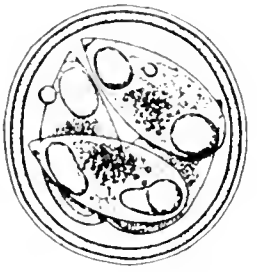

Fig. 30. Sporulated oocyst of *Eimeria labbeana* of the pigeon. X 1700. (From Nieschulz, 1935)

16 to 22 μ (usually 19 to 21 by 17.5 to 20 μ) with a mean of 20 by 19 μ. On the other hand, Duncan (1959), in a study of infections in more than 300 pigeons, measured a large but unspecified number of oocysts at various times during the patent period and found that the overall range was 14.5 to 24 by 13 to 22.5 μ with an overall mean of 19 by 17 μ. However, smaller oocysts appeared early in the infection in 13 birds, and in 10 of them they increased in size to approximately the overall average by the end of the patent period. These small oocyst strains averaged 15 to 18 by 14 to 17 μ. It would appear, therefore, that *E. columbarum* is a synonym of *E. labbeana*.

Life Cycle: Nieschulz (1925a) described the endogenous stages and also gave one of the few descriptions extant of early sporogony in the coccidia. Soon after the macrogametes are fertilized and the oocysts are formed, the zygote contracts into a ball within the oocyst wall. A fertilization spindle then forms; it is a clear band which passes thru the center of the sporont and forms extensions which reach to the oocyst wall. This band then disappears and the sporont rounds up again, but a refractile granule is left in the oocyst. Altho Nieschulz did not recognize it as such, this was undoubtedly reduction division with the throwing off of a polar granule. Four prominences form on the sporont, which then divides to form 4 spherical sporoblasts. These become rather triangular or elongate ovoid, and a clear area appears at the pointed end (pyramid stage). The sporoblasts round up again, and finally elongate to form elongate ovoid, pointed sporocysts in which the sporozoites develop. The sporulation time is 4 days or less (Duncan, 1959a).

After the sporulated oocysts are ingested, the sporozoites are released and invade the epithelial cells of the intestine. They round up and grow into mature schizonts in 3 days. Each schizont produces about 15 to 20 merozoites, often leaving a residual body. The merozoites are somewhat crescent-shaped, pointed at the ends, and 5.5 to 9 μ long. There is a second generation of schizonts which Nieschulz thought might be extracellular. These are elongate, up to 18 by 5 μ, and form up to 16 merozoites.

The microgametocytes form a large number of biflagellate microgametes about 3 μ long with flagella 10 μ long. The macrogametes have a row of large plastic granules around their periphery. Nieschulz figured what was probably a fertilized macrogamete in which a microgamete nucleus was approaching the macrogamete nucleus in a clear pathway thru the cytoplasm. After fertilization, the plastic granules coalesce to form the oocyst wall. Oocysts first appear in the feces 6 days after infection.

Pathogenesis: *E. labbeana* is slightly to markedly pathogenic, depending in part upon the age of the birds (Levi, 1957). Adults are fairly resistant, altho fatal infections have been seen. The birds become weak and emaciated, eat little but drink a great deal, and have a greenish diarrhea. The heaviest losses occur among squabs in the nest. A high percentage of the squabs may die, and those which recover are often somewhat stunted.

The principal gross lesion is inflammation thruout the intestinal tract.

Diagnosis: Diagnosis depends on recognizing the oocysts and other stages in the intestine in association with the signs and lesions of the disease.

Treatment: According to Lindsay (cited by Levi, 1957), sulfaquinoxaline is effective against *E. labbeana*.

Prevention and Control: The same measures used to control coccidiosis in chickens are effective against the disease in pigeons. General sanitation and dry quarters are especially important.

EIMERIA COLUMBAE
MITRA AND DAS GUPTA, 1937

Host: Indian pigeon (*Columba livia intermedia*).

Location: Intestine.

Geographic Distribution: India.

Prevalence: Unknown.

Morphology: The oocysts of this species have not been completely described. They are subspherical, have a maximum size of 16 by 14 μ, and differ from *E. labbeana* in having an oocyst residuum, according to Mitra and Das Gupta (1937).

Life Cycle: Unknown.

Pathogenesis: Unknown.

Genus *ISOSPORA* Schneider, 1881

In this genus the oocyst contains 2 sporocysts, each of which contains 4 sporozoites.

ISOSPORA AKSAICA
BASANOV, 1952

Host: Ox.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: USSR (Kazakhstan).

Prevalence: Unknown. This species was found only in calves 12 to 30 days old.

Morphology: The oocysts are 26 μ in diameter, spherical, dark silver under low magnification and light, pinkish grey under high. The oocyst wall is 1.6 μ thick, smooth and double-contoured, with a light blue outer layer and a greenish, dingy rose inner layer. The sporocysts are ellipsoidal or spherical, 22 by 15 μ. Micropyle, oocyst residuum and sporocyst residuum are presumably absent. Polar granules are possibly present. The sporozoites are spherical, bean-shaped or ellipsoidal, 15 by 11 μ.

Life Cycle: Unknown.

Pathogenesis: Unknown.

Remarks: There is a question whether this is actually a valid species of bovine coccidium or whether it is a pseudoparasite, i.e., an avian or other foreign coccidium which the cattle had ingested along with its host's feces. Further work will be necessary to decide this point. The subjacent discussion of the *Isospora* species found by Levine and Mohan (1960) in cattle has a bearing on *I. aksaica* also.

ISOSPORA sp.
LEVINE AND MOHAN, 1960

Hosts: Ox and ox-zebu hybrids.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: North America (Illinois).

Prevalence: Levine and Mohan (1960) found this form in 6 out of 54 beef cattle on 3 farms in central Illinois.

Morphology: The oocysts are usually subspherical, occasionally spherical, 21 to 33 by 20 to 32 μ with a mean of 27 by 25 μ. The oocyst wall is smooth, colorless, pale lavender or pale yellowish, composed of a single layer about 1 μ thick. In some oocysts, the wall appeared to be lined by a thin membrane. A micropyle and oocyst residuum are absent. Several oocyst polar granules are present. The sporocysts are lemon-shaped, quite thick-walled, 14 to 20 by 10 to 12 μ with a mean

of 17 by 11 μ. The sporocyst Stieda body is a button-shaped cap, with a dependent, globular hyaline mass protruding into the interior of the sporocyst. The sporocyst residuum is finely granular. The sporozoites are sausage-shaped, not arranged in any particular order in the sporocyst. The sporocyst residuum and sporozoites are enclosed in a membrane, forming more or less of a ball within the sporocyst.

Life Cycle: Unknown.

Pathogenesis: Unknown.

Remarks: Levine and Mohan (1960) compared this form with *I. lacazei* of the English sparrow, which they redescribed. They found that the 2 forms were practically indistinguishable and concluded that the oocysts found in bovine feces were most likely those of *I. lacazei* and were pseudoparasites of cattle. They calculated that, in a steer which produced about 20 pounds of feces per day, the presence of 1 oocyst per gram of feces would represent contamination of the feed with about 9000 oocysts, assuming that the oocysts were mixed uniformly with the ingesta and passed thru the animal unchanged. Assuming again that a flotation was carried out with about 2 g of feces and that about 10% of the oocysts present were recovered, they calculated that every oocyst found might represent an initial contamination of a day's feed with about 45,000 oocysts. Since Boughton (1933) quite frequently obtained counts of 200,000 to 2 million oocysts per gram of dried sparrow feces, they considered it quite likely that sparrow coccidia could be detected in a calf's feces if it ingested only a single fecal deposit from a single sparrow in the course of a day.

ISOSPORA SUIS BIESTER, 1934

Host: Pig.

Location: Small intestine, from the lower third of duodenum to 2 or 3 feet from the ileocecal valve.

Geographic Distribution: North America (Iowa), USSR (Kazakhstan).

Prevalence: Unknown.

Morphology: This species has been described by Biester (1934) and Biester and Murray (1934). The oocysts are subspherical to ellipsoidal, becoming more ellipsoidal on sporulation. The oocyst wall is often stretched by the oocysts and pinched in between them. It is smooth, composed of 2 layers, brownish yellow, and 1.5 μ thick. A micropyle is absent. The unsporulated oocysts measure 20 to 24 by 18 to 21 μ with a mean of 22.5 by 19.4 μ. An oocyst polar granule is present. An oocyst residuum is absent. The sporocysts are ellipsoidal, 16 to 18 by 10 to 12 μ with a mean of 16.4 by 11.2 μ. The sporocyst wall is double, 0.7 μ thick. The sporozoites are elongate. A sporocyst residuum is present. A Stieda body is absent. The sporulation time is 4 days.

Life Cycle: According to Biester and Murray (1934), *I. suis* invades the epithelial cells of the intestine. Many of these invaded cells migrate to a subepithelial position, but often both the host cells and the parasite appeared to undergo retrogressive changes and to be desquamated.

The prepatent period after experimental infection is 6 to 8 days, and oocysts continue to be eliminated for about 8 days after a single infective feeding.

Pathogenesis: According to Biester and Murray (1934), *I. suis* causes a catarrhal enteritis. The epithelium of the crypts is destroyed except near the intestinal lumen. The substantia propria of the tips of the villi is destroyed, leaving a reticular honeycomb without cells or nuclei. Interstitial inflammation with marked eosinophilic infiltration is present, but there is no gross hemorrhage.

Diarrhea began about the 6th day af after experimental infection, continued for 3 or 4 days, and was followed by constipation. *I. suis* infections are apparently not fatal, but they may retard growth and produce unthriftiness.

Cross-Transmission Studies: Biester and Murray (1934) and Biester (1934) reported that attempts to transmit *I. suis* to guinea pigs, rats, and dogs were unsuccessful.

ISOSPORA ALMATAENSIS
PAICHUK, 1953

Host: Pig.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: USSR (Kazakhstan).

Prevalence: Unknown.

Morphology: This species was described by Paichuk (1953). The oocysts are short-oval, subspherical or spherical, and grey. The short-oval forms are 25 to 32 by 23 to 29 μ with a mean of 27.9 by 26.0 μ; the spherical forms are 26 to 32 μ in diameter with a mean of 27.7 μ. The oocyst wall is smooth, bright yellow, 3 μ thick, and composed of 3 layers. A micropyle is apparently absent. The oocysts sometimes have 2 sporoblasts when passed. Oocyst polar granules are present. An oocyst residuum is absent. The sporocysts are oval or ovoid with a pointed end, 12 to 19 by 9 to 12 μ with a mean of 15.5 by 10.8 μ. A sporocyst residuum is present. The sporozoites are short-oval, 6 by 4 μ. The sporulation time is 5 days.

Life Cycle: Unknown.

Pathogenesis: Unknown.

ISOSPORA BIGEMINA
(STILES, 1891)
LÜHE, 1906

Synonyms: *Coccidium bigeminum*, *Lucetina bigemina*.

Hosts: Dog, cat, fox, polecat (*Putorius foetidus*), mink (*Mustela vison*), man (?).

Location: Thruout small intestine.

Geographic Distribution: Worldwide.

Prevalence: This species is quite common in dogs and cats. Gassner (1940) found it in 74% of 320 dogs in Colorado. Catcott (1946) found it in 3% of 113 dogs in Ohio. Choquette and Gelinas (1950) found it in 2% of 155 dogs in Montreal. Ehrenford (1953) found it in 0.7% of 377 dogs in Indiana and other midwestern states. Hitchcock (1953) found it in 1% of 147 kittens in Michigan. Levine (1948) reviewed reports of this species in Mustelidae.

Morphology: The oocysts are very thin-walled, spherical to ellipsoidal when unsporulated, but with the wall stretched around the sporocysts and usually constricted somewhat between them when sporulated. The oocyst wall is smooth, colorless, and composed of a single layer. Two sizes of oocyst have been reported. The larger ones measure 18 to 20 by 14 to 16 μ, and the smaller, more common ones 10 to 14 by 7 to 9 μ. Micropyle, oocyst polar granule and oocyst residuum are absent. The sporocysts are ellipsoidal, 7.5 to 9 by 5 to 7 μ, without a Stieda body. A sporocyst residuum is present. The oocysts are sporulated when passed. The oocyst wall is often ruptured so that the sporocysts are found free in the feces. In acute infections, the oocysts may be unsporulated when passed; their sporulation time is about 4 days.

Life Cycle: The life cycle of this species has been studied by Wenyon (1926a) and Wenyon and Sheather (1925). The endogenous stages occur thruout the small intestine. Altho the course of infection has not been followed consecutively in a series of experimentally infected animals, it appears that the epithelial cells are invaded first, followed later on by the subepithelial cells. At any rate, Wenyon and Sheather (1925) found coccidia only in the epithelial cells of a dog killed during the acute phase of the infection. The schizonts of this stage contain 8 merozoites. Later on, the coccidia are found in the subepithelial cells and cores of the villi. The schizonts

here contain about 12 merozoites. Sexual stages appear to be produced in both locations. The oocysts produced in the epithelial cells during the acute phase are unsporulated when passed in the feces. They appear 6 to 7 days after infection. The oocysts produced in the subepithelial cells are sporulated when passed. A number of unanswered questions are raised by this account, and the whole life cycle deserves re-investigation.

Pathogenesis: This species is markedly pathogenic for both cats and dogs. Its effects on the dog, cat and fox were studied by Lee (1934). Puppies and kittens are most seriously affected, while adults are usually carriers, having developed an immunity following earlier infection.

The first signs usually begin 4 to 6 days after infection. Their severity depends on the degree of infection. In severe cases, catarrhal or bloody diarrhea, rapid emaciation and anemia occur. Affected animals are weak, depressed and lose their appetite. There may be a rise in temperature or muscular tremors of the hind legs. If the animal survives the acute phase, the dysentery is replaced by mucous stools for 2 to 4 days and the other signs subside, disappearing 7 to 10 days after their onset. Recovered animals may continue to shed oocysts for a time.

In severe cases, hemorrhagic enteritis is present thruout the small intestine; it is most severe in the lower ileum and becomes progressively less so anteriorly. Petechiae are present in light infections, and diffuse hemorrhages in more severe ones. There may be ulcers in addition. The mucosa is thickened, and there may be extensive desquamation. A circulating eosinophilia may be present, and the parasitized region is infiltrated with eosinophiles.

Cross-Transmission: Lee (1934) transmitted *I. bigemina* from the dog to the cat and fox, but failed to infect rabbits or guinea pigs with it.

Remarks: The oocysts of *I. hominis* of man are apparently indistinguishable from those of *I. bigemina*, and a number of investigators believe that they are the same species (Elsdon-Dew and Freedman, 1953; Routh, McCroan and Hames, 1955; Becker, 1956). Cross-transmission experiments are needed to determine whether they are.

ISOSPORA FELIS
WENYON, 1923

Synonyms: *Isospora cati*, *Coccidium bigeminum* var. *cati*, *Lucetina cati*, *Lucetina felis*.

Hosts: Dog, cat, lion and possibly other carnivores.

Location: Small intestine, sometimes cecum, occasionally colon.

Geographic Distribution: Worldwide.

Prevalence: This species in common in dogs and cats. Gassner (1940) found it in 6% of 320 dogs in Colorado. Catcott (1946) found it in 3.5% of 113 dogs in Ohio. Choquette and Gelinas (1950) found it in 9% of 155 dogs in Montreal. Hitchcock (1953) found it in 75% of 147 kittens in Michigan. Alves da Cruz, de Sousa and Cabral (1952) found it in 10% of 40 stray cats in Lisbon, Portugal.

Morphology: The oocysts are ovoid, 32 to 53 by 26 to 43 μ with a mean of 43 by 33 μ. The oocyst wall is smooth and colorless, without a micropyle. An oocyst polar granule and residuum are absent. The sporocysts are ellipsoidal, 20 to 27 by 18 to 21 μ. A sporocyst residuum is present. The sporozoites are 10 to 15 μ long. The sporulation time is 3 days or less.

Life Cycle: The life cycle of *I. felis* in experimentally infected kittens was described in detail by Hitchcock (1955) and Lickfeld (1959). It is similar in dogs. The parasites are found above or beside the host cell nuclei of the epithelial cells of the villi and sometimes in the subepithelial tissues. There are 2 asexual generations. The first generation schizonts

are found in the small intestine and cecum from the second to fourth day after experimental infection. They are ellipsoidal and about 20 μ long. They produce 40 to 60 merozoites according to Hitchcock, or 12 or less according to Lickfeld. These merozoites are relatively large, measuring 16 to 18.5 by 5 to 8 μ.

The second generation schizonts are found on the 5th and 6th days after infection in the small intestine and less commonly in the large intestine. According to Hitchcock, they produce up to 24 merozoites, but most contain 12 to 16; according to Lickfeld there are 30 to more than 100 of these merozoites, and they measure 7.5 by 2.5 μ.

The sexual stages are found on the 7th and 8th days after infection. They occur in the small intestine and less commonly in the cecum. According to Hitchcock (1955), the macrogametes average 25 to 22 μ, but other workers have recorded dimensions up to 56 by 48 μ. The microgametocytes average 28 by 19 μ according to Hitchcock, but other workers have recorded dimensions up to 50 by 30 μ, and Lickfeld said that they are 73 μ in diameter in life. Well over 2000 spindle-shaped, curved, biflagellate microgametes are formed in each microgametocyte. The oocyst wall is laid down following fertilization while the zygotes are still within the host cells. The young oocysts then break out and are passed in the feces. The prepatent period was found by Hitchcock (1955) to be 7 to 8 days.

According to Walton (1959), the haploid number of chromosomes in *I. felis* is 2. Lickfeld (1959) described a cryptomitotic type of schizogony, but saw no chromosomes.

Pathogenesis: This species is slightly to moderately pathogenic, depending on the host species, age, degree of infection, etc. It is less serious in cats than in dogs. None of 18 four- to nine-week-old kittens infected by Hitchcock (1955) with 100,000 sporulated oocysts showed signs of disease. Andrews (1926), however, observed enteritis, emaciation, weakness, depression, dysentery and even death in kittens and dogs experimentally infected with *I. felis*. Hitchcock thought that these signs and deaths in the kittens might well have been due to feline distemper.

The gross pathologic lesions are similar to those caused by *I. bigemina*. There is hemorrhagic enteritis, frequently with ulceration, thickened mucosa and epithelial desquamation.

Immunity: Animals which have recovered from *I. felis* infections are resistant to reinfection.

Cross Transmission: Lee (1934) infected dogs with *I. felis* from the cat, and a fox with *I. felis* from the dog.

ISOSPORA RIVOLTA (GRASSI, 1879)

Synonyms: *Coccidium rivolta, Lucetina rivoltai.*

Hosts: Dog, cat, dingo, and probably other wild carnivores.

Location: Small intestine.

Geographic Distribution: Worldwide.

Prevalence: This species is common in dogs and cats. Gassner (1940) found it in 20% of 320 dogs in Colorado. Catcott (1946) found it in 4% of 113 dogs in Ohio. Ehrenford (1953) found it in 72% of 377 dogs from Indiana and nearby states. Choquette and Gelinas (1950) found it in 13.5% of 155 dogs in Montreal. Hitchcock (1953) found it in 13% of 147 kittens in Michigan.

Morphology: The oocysts are ovoid, 20 to 25 by 15 to 20 μ. The oocyst wall is smooth, with a micropyle at the small end. An oocyst polar granule and residuum are absent. The sporocysts are 16 by 10 μ. A sporocyst residuum is present. The sporulation time is 4 days.

Life Cycle: The endogenous stages of *I. rivolta* are poorly known. They are said to resemble those of *I. felis* but to be smaller. They are found in the epithelial cells and sometimes in the subepithelial tissues of the small intestine. Oocyst development ordinarily takes place outside the body, but occasionally occurs in the subepithelial tissues.

Pathogenesis: Altho experimental studied on *I. rivolta* alone have apparently not been carried out, it is presumably as pathogenic as *I. bigemina* and *I. felis*.

Cross Transmission: Lee (1934) infected a fox with *I. rivolta* from the dog.

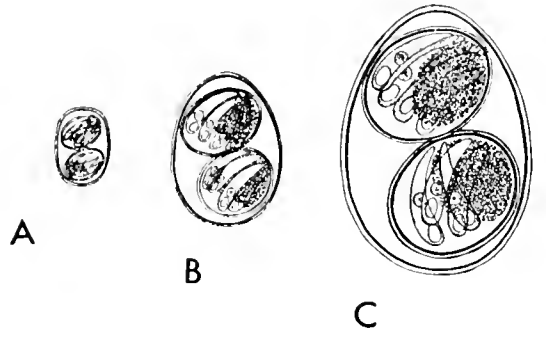

Fig. 31. Sporulated oocysts of coccidia of dog and cat. A. *Isospora bigemina*. B. *Isospora rivolta*. C. *Isospora felis*. X 850. (From Becker, 1934, after Wenyon, 1926, *Protozoology*)

COCCIDIOSIS IN DOGS AND CATS

Epidemiology: Coccidiosis is common in dogs and cats, and is a not infrequent cause of diarrhea and even death in puppies and kittens. Crowding and lack of sanitation promote its spread. Coccidia sometimes seed a breeding kennel, boarding kennel or veterinarian's wards so heavily that most of the puppies born or brought there become infected.

Diagnosis: Coccidiosis can be diagnosed at necropsy by finding coccidia in the intestinal lesions. It can be diagnosed in affected animals by finding oocysts in association with diarrhea or dysentery. However, care must be taken to differentiate coccidiosis from coccidiasis, since many animals may be shedding oocysts without suffering from disease. Other disease agents should be searched for and found absent. The presence of a wave of oocysts during and shortly after an attack of enteritis and their marked diminution or disappearance soon thereafter would suggest that coccidia caused the attack.

The oocysts of *Isospora bigemina* are usually sporulated when they are passed in the feces. They are often ruptured, releasing the sporocysts. These are very small, and will often be overlooked unless the high dry power of the microscope is used in making a fecal examination. In addition, they resemble *Cryptosporidium* oocysts and might be mistaken for them.

Treatment: There is no good treatment for coccidiosis in dogs and cats once the signs of disease have appeared. All the coccidiostatic agents on the market are preventive rather than curative in action. The fact that coccidiosis is a self-limiting disease has often led to the belief that some ineffective drug, administered at the time natural recovery was due to begin, was responsible for the cure. Uncontrolled studies on coccidiosis therapy, such as that of Duberman (1960) with nitrofurazone, are worse than useless, since they may lead to false conclusions regarding a drug's value.

Craige (1949), a clinician with considerable experience in handling canine coccidiosis, considered treatment in an unsatisfactory state. Sometimes the animals would respond to sulfonamides, but he had better success by combining a sulfonamide with quinacrine, sulfocarbolates, tannin-yeast, iodine preparations, etc. McGee (1950) used sulfamethazine. Altman (1951) used chlortetracycline. Supportive treatments such as these, and particularly the use of antibiotics such as chlortetracycline and oxytetracycline to control secondary infections, may be helpful even tho they do not act on the coccidia themselves.

Prevention: Sanitation and isolation are effective in preventing coccidiosis. Animal quarters should be cleaned daily. Runways should be concrete. Ordinary disinfectants are ineffective against coccidian oocysts, but boiling water, if it is still boiling when it reaches the oocysts, will kill them.

ISOSPORA BELLI WENYON, 1923

Host: Man.

Location: Presumably small intestine. Elsdon-Dew, Roach and Freedman (1953) found oocysts in material from a duodenal intubation.

Geographic Distribution: Presumably worldwide, but more common in the tropics than in the temperate zone.

Prevalence: This species is quite rare in man. However, Elsdon-Dew and Freedman (1953) found it in 32 persons in Natal, and considered that it was often missed because it was not looked for.

Morphology: This species has often been confused with *I. hominis* (see Elsdon-Dew and Freedman, 1953), but is clearly different. The oocysts are elongate ellipsoidal, 20 to 33 by 10 to 19 μ (mean, 30 by 12 μ according to Elsdon-Dew and Freedman, 1953). One or both ends of the oocyst may be somewhat narrow. The oocyst wall is smooth, thin, and colorless. A very small micropyle is sometimes visible. An oocyst polar granule may be present in young, incompletely sporulated oocysts, but quickly disappears. An oocyst residuum is absent. The sporocysts are subspherical to ellipsoidal, without a Stieda body, 12 to 14 by 7 to 9 μ (mean 11 by 9 μ according to Elsdon-Dew and Freedman, 1953). A sporocyst residuum is present. The sporozoites are slender, somewhat crescent-shaped, with the nucleus at one end. Both immature and mature oocysts may be passed in the feces. The sporulation time is up to 5 days.

Life Cycle: Unknown.

Pathogenesis: Most infections with *I. belli* appear to be subclinical and self-limiting. However, it may cause a mucous diarrhea. In 31 of the 33 cases of *Isospora* infection studied by Barksdale and Routh (1948), anorexia, nausea, abdominal pain and diarrhea were present. Matsubayashi and Nozawa (1948) reported that symptoms appeared 1 week after experimental infection of 2 human volunteers, presumably with *I. belli*, and that oocysts appeared in the feces 10 days after infection and persisted for a month.

Cross-Transmission Studies: Jeffery (1956) failed to transmit *I. belli* from man to 2 monkeys, 2 dogs, 2 pigs, 12 mice, 4 rats, a guinea pig and a rabbit. Robin and Fondimare (1960) were unable to transmit it from man to the guinea pig, rabbit, mouse or rat.

ISOSPORA HOMINIS (RAILLIET AND LUCET, 1891) WENYON, 1923

Synonyms: *Coccidium bigeminum* var. *hominis*, *Lucetina hominis*.

Host: Man.

Location: Small intestine.

Geographic Distribution: Worldwide, but more common in the tropics than in the temperate zone.

Prevalence: This species is quite rare in man. However, Elsdon-Dew and Freedman (1953) found it in 23 persons in Natal, and thought that it was often missed because people did not look for it.

Morphology: The oocysts are sporulated when passed. The oocyst wall is very thin, stretched around the sporocysts and usually constricted between them, and sometimes not visible. It is often ruptured, releasing the sporocysts. The oocysts are about 20 by 15 μ. Micropyle, oocyst polar granule and residuum are absent. The sporocysts are ellipsoidal or with one side flattened, about 15 by 10 μ, without a Stieda body. A sporocyst residuum is present.

Life Cycle: Unknown.

Pathogenesis: Most infections appear to be subclinical and self-limiting. However, *I. hominis* may cause a mucous diarrhea. In 31 of 33 cases of *Isospora* infection studied by Barksdale and Routh (1948), anorexia, nausea, abdominal pain and diarrhea were present.

Remarks: This species resembles *I. bigemina* very closely, and it may well be the same species (see Becker, 1956, Elsdon-Dew and Freedman, 1953; Routh, McCroan and Hames, 1955). Elsdon-Dew (1954) failed to infect a dog with *I. hominis* from man, but the animal was an adult and could have been immune.

ISOSPORA NATALENSIS
ELSDON-DEW, 1953

Host: Man.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: Africa (Natal).

Prevalence: Elsdon-Dew (1953) found this species in 2 persons in Natal.

Morphology: The oocysts are subspherical, 25 to 30 by 21 to 24 μ. The oocyst wall is smooth and thin, without a micropyle. An oocyst polar granule and oocyst residuum are absent. The sporocysts are ellipsoidal, 17 by 12 μ, without a Stieda body. A sporocyst residuum is present. The sporulation time is 1 day.

Life Cycle: Unknown.

Pathogenesis: Unknown.

Remarks: When Elsdon-Dew and Freedman (1953) first saw this form, they thought that it was *I. rivolta*. However, it differs morphologically from that species.

COCCIDIOSIS IN MAN

Coccidiosis is quite rare in man, and the relation of the species described from man to those in lower animals is still not clear. *Isospora belli* appears to be confined to man, and *I. natalensis* may be also. However, further research may show that *I. hominis* is a synonym of *I. bigemina* and that man acquires his infections with this parasite from dogs and cats.

In addition to the above species which produce infections in man, a number of other coccidia have been found in human feces and mistaken for parasites of man. Perhaps the most famous of these were *Eimeria wenyoni*, *E. oxyspora* and *E. snijdersi*, which Dobell (1919) described as human parasites. The first turned out to be *E. clupearum*, a coccidium of herring, sprats and mackerel, and the second two were both *E. sardinae*, a parasite of sardines, herring and sprats. In addition, oocysts of *E. stiedae* of the rabbit have been found in a mental hospital patient who liked to eat raw rabbit livers, and oocysts of *E. debliecki* of the pig were found by Brug (1946) in several others who probably acquired them in sausage casings.

ISOSPORA GALLINAE
SCHOLTYSECK, 1954

Host: Chicken.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: Europe.

Prevalence: Unknown, presumably rare.

Morphology: The oocysts are ellipsoidal, 19 to 27 by 15 to 23 μ with a mode of 24 by 19 μ. A micropyle is absent. Oocyst polar granules are present. An oocyst residuum is absent. The sporocysts are piriform.

Life Cycle: Unknown.

Pathogenesis: Unknown.

Remarks: The validity of this species is dubious. It is more likely a parasite of some wild bird, such as *I. lacazei* of the English sparrow.

ISOSPORA HEISSINI
SVANBAEV, 1955

Host: Domestic turkey.

Location: Unknown. Oocysts found in feces.

Geographic Distribution: USSR (Kazakhstan).

Morphology: The oocysts are spherical or rarely broadly ovoid, 25 to 33 μ in diameter, with a mean of 31 by 30 μ. The oocyst wall is greenish, smooth, double contoured (illustrated with a single layer), and 1.5 to 1.7 μ thick. A micropyle is apparently absent. An oocyst polar granule is present. An oocyst residuum is absent. The sporocysts are spherical or ovoid and pointed at one end, 15 by 10 μ. A sporocyst residuum is absent. The sporozoites are oval, 7 to 9 by 4 to 5 μ. The sporulation time is 16 to 20 hours at 20 to 25° C.

Life Cycle: Unknown.

Pathogenesis: Unknown.

Remarks: Svanbaev (1955) found this species only in turkeys up to 4 months of age.

Genus *WENYONELLA* Hoare, 1933

In this genus the oocyst contains 4 sporocysts, each of which contains 4 sporozoites.

WENYONELLA GALLINAE
RAY, 1945

Host: Chicken.

Location: Terminal part of intestine.

Geographic Distribution: India.

Prevalence: Uncommon. Gill (1954) found this species in 1.7% of 120 chickens near Mukteswar.

Morphology: The oocysts are ovoid, rough, punctate, 29 to 34 by 20 to 23 μ. The sporocysts are flask-shaped, 19 by 8 μ. No other morphological information was given. The sporulation time is 28° C is 4 to 6 days.

Life Cycle: Unknown.

Pathogenesis: According to Ray (1945), this species causes a diarrhea with blackish-green, semisolid excreta. The terminal part of the intestine is thickened and congested, and there are pinpoint hemorrhages in the mucosa.

Genus *TYZZERIA* Allen, 1936

In this genus the oocyst contains 8 naked sporozoites and no sporocysts.

TYZZERIA PERNICIOSA
ALLEN, 1936

Host: Domestic Pekin duck.

Location: Thruout the small intestine, but especially in the upper half.

Geographic Distribution: North America.

Prevalence: Uncommon. This species has been reported from domestic ducks only by Allen (1936) on Long Island. However, its relationship to *T. alleni*, which Chakravarty and Basu (1946) described from the cotton teal (*Cheniscus coromandelianus*) in India, to *Tyzzeria* sp. which Farr (1952) reported from the black duck (*Anas rubripes*), to *T. anseris* reported by Farr (1959) from the lesser scaup duck (*Nyroca affinis*) in Michigan, and to *T. anseris* from domestic and wild geese and the whistling swan (see Hanson, Levine and Ivens, 1957) remains to be determined.

Morphology: The oocysts are ellipsoidal, 10 to 13 by 9 to 11 μ. The oocyst wall is thick, colorless, composed of an outer thin, transparent layer and an inner thicker layer. A micropyle is absent. The sporozoites are curved, with one end rounder and broader than the other, about 10 μ long and 3.5 μ wide at the larger end. The oocyst residuum is large, usually spherical. The sporulation time is 1 day.

Life Cycle: According to Allen (1936), the endogenous stages are found in the mucosal and submucosal cells. There are at least 3 asexual generations. The first generation schizonts are relatively small, about 12 by 8 μ, and contain relatively few, small merozoites. The later schizonts measure about 15 to 16 by 14 to 15 μ and contain more and larger merozoites than the first generation ones. Schizogony continues long after the formation of gametes.

The first microgametocytes appear 2 days after infection. They measure about 7.5 by 6 μ and produce a large number of tiny microgametes. The macrogametes are somewhat irregular in shape. Oocysts first appear in the feces 6 days after infection.

Pathogenesis: According to Allen (1936), *T. perniciosa* is highly pathogenic for ducklings. All of 7 experimentally infected, week-old ducklings died.

Affected birds stop eating, lose weight, become weak and cry continuously as if in distress. At necropsy, inflammation and hemorrhagic areas were found thruout the small intestine and especially in its upper half. The intestinal wall was thickened, and round, white spots were visible thru its serosal surface. In severe cases the lumen was filled with blood and often contained a cheesy exudate. The intestinal epithelium sloughed off in long pieces, sometimes forming a tube which could easily be lifted out.

TYZZERIA ANSERIS NIESCHULZ, 1947

Hosts: Domestic goose, white-fronted goose (*Anser albifrons*), blue or snow goose (*A. caerulescens*), Ross's goose (*A. rossi*), Canada goose (*Branta canadensis*), Atlantic brant (*B. bernicla hrota*), whistling swan (*Olor columbianus*), lesser scaup duck (*Nyroca affinis*).

Location: Small intestine.

Geographic Distribution: North America, Europe.

Prevalence: This species is apparently rare in domestic geese, having been reported in them only by Nieschulz (1947) in Holland and by Farr and Wehr (1952) in Maryland. It is common, however, in wild geese, and has been found in all the species from which coccidia have been reported and from all 6 North American flyways (Hanson, Levine and Ivens, 1957). It is most likely a parasite of wild geese which occasionally occurs in domestic ones as the result of accidental contamination.

Morphology: The oocysts were described by Levine (1952). They are ellipsoidal, 10 to 16 by 9 to 12 μ with a mean of about 13 by 11 μ. The oocyst wall is smooth, colorless, about 0.6 μ thick, and usually appears to be composed of a single layer altho in some oocysts a second inner line is visible; this may perhaps be a membrane which has pulled away from the wall. A micropyle is absent. The sporozoites are banana-shaped. The oocyst residuum is large, irregular, granular, and often surrounded by the sporozoites.

Life Cycle: Unknown.

Pathogenesis: Unknown in very young birds; negligible in adults.

COCCIDIOSIS IN DUCKS AND GEESE

Our knowledge of the coccidia of ducks and geese is extremely deficient. Except for renal coccidiosis of the goose caused by *E. truncata*, coccidiosis appears to be of little importance in these birds, and coccidia have seldom been reported from them. A few outbreaks of intestinal coccidiosis have been reported,

however, Jansen (1931), for example, described one in Holland in which more than 10% of a flock of 700 ducks died in 2 days.

Treatment: Little is known of the treatment of coccidiosis of ducks and geese. McGregor (1952) reported that *E. truncata* infections of geese seemed to respond to sodium sulfamethazine, and the urinary excretion of sulfonamides in general would suggest that they should be particularly effective against this species.

Prevention and Control: The same measures should be used for the prevention and control of coccidiosis in ducks and geese as in chickens.

FAMILY CRYPTOSPORIDIIDAE

Members of this family are monoxenous. Development takes place on the surface of the host cells or within their striated border, and not in the cells proper. The oocysts and schizonts have a knob-like attachment organ at some point on their surface. The oocysts contain no sporocysts. The microgametes have no flagella. There is a single genus, *Cryptosporidium*.

Genus *CRYPTOSPORIDIUM* Tyzzer, 1907

In this genus the oocyst contains 4 naked sporozoites.

CRYPTOSPORIDIUM TYZZERI NOM. NOV.

Synonyms: *Cryptosporidium parvum* Tyzzer, 1912 pro parte.

Host: Chicken.

Location: All stages occur in the striated border (cuticular layer) of the surface epithelial cells of the tubular part of the ceca.

Geographic Distribution: North America (Massachusetts).

Prevalence: Rare.

Morphology: Tyzzer (1929) did not describe this form in detail, but illustrated it and said that it appeared morphologically identical with *C. parvum* of the mouse. The following description is based primarily on that given by Tyzzer (1912) for *C. parvum*. The oocyst is ovoid or spherical, 4 to 5 by 3μ. The oocyst wall is smooth, composed of a single layer, with a small, knob-like attachment organ. A micropyle is absent. An oocyst residuum is present. The sporozoites are slender, bow- or boomerang-shaped, 5.5 to 6μ long, with a rod-shaped, slender nucleus near the anterior end.

Life Cycle: The following description is based primarily on that given by Tyzzer (1912) for *C. parvum*, which is morphologically identical with *C. tyzzeri*. The schizonts are 3 to 5μ in diameter when mature and have an attachment organ. They are attached to the cell surface or embedded in its striated border. They form 8 falciform merozoites 2.5 to 5 by 0.5 to 0.7μ, with a nucleus near the thicker end, and a small residual mass. The microgametocytes are smaller than the schizonts and also have an attachment organ. They give rise to 16 tiny microgametes and a spherical mass of residual material. The microgametes are chromatin rods about 1μ long and not more than 0.4μ wide, without visible flagella. The macrogametes are larger than the schizonts and microgametocytes, and contain tiny, refractile granules. They have a thin, dense limiting membrane and an attachment organ.

Pathogenesis: Apparently non-pathogenic.

Remarks: Tyzzer (1929) thought that this was the same species he had previously found in mice, but he attempted no cross-infection experiments. He said that even if such experiments failed, the morphological agreement was such that the chicken and mouse forms could only be regarded as biological varieties of the same species. However, such a narrow species concept is no longer held, and it seems

best to draw attention to the chicken form by giving it a name of its own. Consequently it is named *Cryptosporidium tyzzeri*.

In this connection, too, it might be mentioned that Tyzzer (1910) was unable to infect the laboratory rat with the closely related *C. muris* from the laboratory mouse.

CRYPTOSPORIDIUM MELEAGRIDIS SLAVIN, 1955

Host: Domestic turkey.

Location: All stages occur on the villus epithelium of the terminal third of the small intestine.

Geographic Distribution: Scotland.

Prevalence: Unknown; found in 1 flock.

Morphology: This species was described by Slavin (1955). The oocysts are oval, 4.5 by 4.0 μ, with very foamy cytoplasm and an eccentric, faint, poorly defined wisp of nucleus. No sporulated oocysts were seen.

Life Cycle: The young schizonts (trophozoites) are attached to the epithelium of the villi, often in enormous numbers. They have an attachment organ which penetrates the striated border of the epithelial cells. Slavin also saw these forms in the goblet cells, between cells as far down as the basement membrane, and in surface depressions between the epithelial cells. The mature schizonts measure 5 by 4 μ and contain 8 merozoites. These are falciform, 5 by 1 μ, and taper toward the ends, with one end blunter than the other. The nucleus is subterminal.

The microgametocytes are rounded or oval, 4 μ in their greatest diameter, and contain 16 intensely staining rod-like microgametes. These measure 1 by 0.3 μ and have no flagella. The macrogametes are roughly oval, 4.5 to 5.0 by 3.5 to 4.0 μ.

Pathogenesis: According to Slavin (1955), *C. meleagridis* may cause illness with diarrhea and a low death rate in 10- to 14-day-old turkey poults.

CRYPTOSPORIDIUM SP.

Tyzzer (1929) remarked in passing that he had found a *Cryptosporidium* morphologically similar to *C. parvum* in the rabbit's intestine, but did not discuss it further. No one else appears to have recognized this form.

FAMILY AGGREGATIDAE

Members of this family are heteroxenous, with two hosts. Schizogony takes place in one and sporogony in the other. Development takes place in the host cell proper. The oocysts typically contain many sporocysts. With one dubious exception, the Aggregatidae are parasites of marine annelids, molluscs and crustacea.

Genus *MEROCYSTIS* Dakin, 1911

In this genus the oocysts contain numerous sporocysts, each with 2 sporozoites. A single species, *M. kathae*, has been named. It occurs in the kidney of the whelk, *Buccinum undatum*.

In addition, Paichuk (1953) described oocysts in the feces of several pigs in Kazakhstan which he called *Merocystis* sp. The oocysts are short-oval, almost spherical, 34 to 43 by 30 to 37 μ with a mean of 38.7 by 33.0 μ. The oocyst wall is smooth, 2 μ thick, composed of 3 layers of which the outer is dark brown, the middle bright green and the inner yellow-green or bright brown. The oocyst wall is very fragile. The number of sporocysts is unknown, but more than 13. The sporocysts are spherical, 9 to 13 μ in diameter with a mean of 9.1 μ. The presence of an oocyst polar granule is unknown. The oocyst and sporocyst residua are composed of dispersed granules. The sporozoites are spherical, 4.3 μ in diameter. Altho Paichuk assigned this form to the

genus *Merocystis*, it is much more probably *Adelea* or *Adelina*, both of which occur in arthropods, and it may well be a parasite of some arthropod which the pigs had eaten.

FAMILY LANKESTERELLIDAE

Members of this family are heteroxenous, with 2 hosts. Schizogony, gametogony and sporogony all take place in a vertebrate host. The sporozoites enter the blood cells and are taken up by a blood-sucking invertebrate (a mite or leech). They do not develop in this host, but are transferred to the vertebrate host when the latter eats the invertebrate, or possibly by injection. In the vertebrate host, development takes place in the host cells proper. The oocysts contain no sporocysts, but have 8 or more sporozoites, the number depending on the genus. The microgametes have 2 flagella so far as is known. There are 2 genera in this family: *Lankesterella*, which occurs in birds and amphibia, and *Schellackia*, which occurs in reptiles.

Genus *LANKESTERELLA* Labbé, 1899

In this genus the oocysts contain 32 or more naked sporozoites. The vectors are leeches or mites.

The type species, and the only one known for a long time, is *L. minima*, a parasite of the frog. However, Lainson (1959) recently showed that the genus *Atoxoplasma* Garnham, 1950 is a synonym of *Lankesterella*, enlarging the genus considerably and clearing up a question which has puzzled parasitologists for years.

The parasites now known to be sporozoites of *Lankesterella* are found frequently in the lymphocytes and other blood cells of wild birds. They had been thought to be *Haemogregarina* or *Toxoplasma*, but Garnham (1950) showed that they were definitely not the latter and therefore called them *Atoxoplasma*.

The names and accepted species of the genus are still in a highly confused state (Laird, 1959; Lainson, 1959).

Lankesterella adiei (Aragão, 1933) Lainson, 1959 (syns., *L. passeris* Raffaele, 1938; *L. garnhami* Lainson, 1959) is a common parasite of the English sparrow thruout the world. Lainson (1959) found it in all of 99 adult and 150 fledgling English sparrows in England, Manwell (1941) and Manwell *et al.* (1945) reported that it was common in passerine birds, and D. D. Myers (unpublished) found it commonly in English sparrows in Illinois. The sporozoites occur in the lymphocytes and monocytes, and often cause a pronounced indentation of the host cell nucleus. They are typically sausage-shaped with rounded ends, stain weakly and lack a well defined periplast, so that it is often difficult to differentiate their cytoplasm from that of the host cell. Their nucleus is diffuse and granular, with a tiny karyosome. They measure 4 to 5 by 2 to 4 μ according to Lainson (1959).

The life cycle of *L. adiei* was described (under the name *L. garnhami*) by Lainson (1959). Schizogony takes place in the lymphoid-macrophage cells of the spleen, bone marrow and liver. There are 2 types of schizont, one producing 10 to 30 (average 16) oval merozoites measuring 4 by 2 μ, and the other producing a smaller number of larger merozoites measuring 6 by 3.5 μ. Gametogony and sporogony take place in the lymphoid-macrophage cells of the liver, lungs and kidney. The microgametocytes resemble those of *Eimeria* and produce 60 to 100 microgametes. The macrogametes are about 14.5 μ in diameter when mature and produce a large but unspecified number of sporozoites measuring about 3.6 by 1.8 μ. The vector is presumably the common red mite, *Dermanyssus gallinae*, but Lainson was unable to prove this because he had no uninfected receptor birds.

According to Lainson (1958), *Lankesterella* may cause congestion and hemorrhage of the blood vessels and inflamma-

tory foci in the liver and lungs of infected English sparrows. Manwell (1941) stated that infections seemed to spread rapidly among adult English sparrows from New York after they had been captured and kept in relatively close quarters in the laboratory. He found that the disease was not infrequently fatal, but that chronic cases also occurred. The liver and spleen were greatly enlarged and very dark in one bird which he necropsied, but there were no hematin granules in either organ. D. D. Myers (unpublished) also observed deaths from this infection in captured English sparrows in Illinois.

Lankesterella serini Lainson, 1959 was discovered in canaries when Lainson wanted to infect them with *L. adiei* from the English sparrow and found that they already had an infection of their own. It apparently resembles *L. adiei*. Nothing is known about its pathogenicity. Perhaps the "x-bodies" or "Einschlüsse" which occur in the macrophages of the lungs, liver and spleen of canaries (Manwell *et al.*, 1945) belong to this species.

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Chapter 9

KLOSSIELLA AND HEPATOZOON

These genera belong to the suborder Adeleorina, which is differentiated from the Eimeriorina and Haemospororina by the fact that the macrogamete and microgametocyte are associated in syzygy (i.e., they lie up against each other) during development. Correlated with this is the fact that the microgametocytes produce very few microgametes. The zygote may or may not be motile, and the sporozoites are enclosed in an envelope.

The great majority of the Adeleorina are parasites of lower vertebrates and invertebrates, but a few occur in domestic and laboratory animals.

FAMILY KLOSSIELLIDAE

In this family, the zygote is not motile. A typical oocyst is not formed, but a number of sporocysts each containing many sporozoites develop within a membrane which is perhaps laid down by the host cell. Each microgametocyte forms 2 to 4 non-flagellated microgametes. The life cycle involves a single host, gametogony and schizogony occurring in different locations. There is a single genus, *Klossiella*.

Genus *KLOSSIELLA* Smith and Johnson, 1902

This genus has the characters of the family.

Infection takes place by ingestion of sporulated sporocysts, and the sporozoites pass into the blood stream and enter the endothelial cells of the capillaries and arterioles of the kidneys, lungs, spleen and other organs. Here they turn into schizonts, and these then produce merozoites. There are probably several asexual generations.

Eventually some merozoites enter the epithelial cells of the convoluted tubules

of the kidney, where they become gamonts and where gametogony and sporogony take place. A macrogamete and microgametocyte are found together in syzygy within a vacuole in the host cell. The microgametocyte divides to form 2 to 4 microgametes, one of which fertilizes the macrogamete. The resultant zygote (sporont or mother sporoblast) divides by multiple fission to form a number of sporoblasts. Each of these develops into a sporocyst containing 8 to 25 or more sporozoites. The sporocysts are enclosed within a membrane, but all authorities do not agree whether it is a true oocyst or simply the remnant of the host cell.

The sporocysts are released into the lumen of the kidney tubules by rupture of the host cell, and pass out in the urine.

KLOSSIELLA EQUI BAUMANN, 1946

Synonym: *Eimeria utinensis* (?).

Hosts: Horse, ass.

Location: Kidneys.

Geographic Distribution: Europe, Turkey, North America.

Prevalence: Unknown. This species has been encountered only in the course of histopathologic examinations of the kidney for some other reason. Baumann (1946) found it in the kidney of a horse from Hungary which had died of pneumonia, Seibold and Thorson (1955) found it in the kidney of a jackass in Alabama which had died of spinal injuries incurred while he was being roped. Akcay and Urman (1954) found it on histopathologic examination of the kidneys of 72 out of 117 donkeys in the course of an experiment on infectious anemia.

Morphology: The stages in the kidney are the only ones known. These are found in the epithelial cells lining the thick limbs of Henle's loops in the medullary rays. Schizonts and merozoites have not been recognized. The macrogametes and microgametocytes develop in syzygy. The latter form 4 microgametes (Baumann, 1946). After fertilization, the zygote grows to 38 to 46 by 32 to 39 μ and produces a large number of sporoblasts by multiple nuclear fission followed by budding from a large, central residual mass. Each sporoblast develops into a sporocyst. The fully developed "oocysts" are thin-walled sacs 50 to 90 by 35 μ containing as many as 40 ovoid sporocysts measuring 8 to 10 by 4 to 5 μ. Each sporocyst contains 8 to 12 sporozoites. Seibold and Thorson (1955) found 40 sporocysts in a cross section of one of the largest sacs they saw, so there must have been many more actually present.

Pathogenesis: Apparently non-pathogenic.

Remarks: Pachinger (1886) described parasites resembling *Eimeria falciformis* in the kidneys of 3 horses. These were almost certainly *K. equi*. Selan and Vittorio (1924) described a parasite from the lungs and gall bladder of a horse in Italy which they called *Eimeria utinensis*. Their description was too poor to be sure what they actually saw, but it may perhaps have been a stage of *K. equi*.

OTHER SPECIES OF KLOSSIELLA

Klossiella muris Smith and Johnson, 1902 is apparently fairly common in laboratory mice thruout the world, but has been reported only once in wild house mice. In the laboratory colonies in which it is found, 20 to 100% of the mice are infected. Each microgametocyte forms 2 microgametes. Each sporont forms 12 to 16 sporocysts, each of which contains about 25 to 34 banana-shaped sporozoites. *K. muris* is ordinarily non-pathogenic, altho in heavy infections the kidneys may have minute, greyish, necrotic foci over their entire surface, and the epithelium of the infected kidney tubules is destroyed (Smith and Johnson, 1902). Otto (1957) described a perivascular, follicular, lymphocytic infiltration in the region of the medullary cortex which he considered of diagnostic significance. There is no

inflammatory reaction. No fatal infections have been reported.

Klossiella cobayae Seidelin, 1914 occurs sporadically in the guinea pig thruout the world. Each microgametocyte forms 2 microgametes. Each sporont forms 30 or more sporocysts, each of which contains about 30 sporozoites. *K. cobayae* is apparently non-pathogenic and produces slight if any pathologic changes in the kidney. However, it may be encountered in sections of the kidney or other organs which are being examined for something else, as C. C. Morrill and I (unpublished) did in some guinea pigs at the University of Illinois.

FAMILY HEPATOZOIDAE

In this family the zygote is active (an ookinete), secreting a flexible membrane which is stretched during development. The life cycle involves 2 hosts, 1 of which is vertebrate and the other invertebrate. The parasites are found in the cells of the circulatory system of vertebrates and of the digestive system of invertebrates. The oocysts are large and contain many sporocysts, each with 4 to 12 or more sporozoites. There is a single genus, *Hepatozoon*.

Genus *HEPATOZOON* Miller, 1908

In this genus schizogony takes place in the viscera of a vertebrate, and the gametocytes are either in the leucocytes or erythrocytes, depending on the species. Fertilization and sporogony occur in a tick, mite, louse, tsetse fly, mosquito or other blood-sucking invertebrate, depending again on the species. The microgametocyte forms 2 microgametes. A synonym of this generic name is *Leucocytogregarina*.

Species of *Hepatozoon* have been described from mammals, reptiles and birds. They are especially common in rodents.

The vertebrate hosts become infected by eating the invertebrate hosts. The sporozoites are released in the intestine, penetrate its wall and pass via the blood stream to the liver, lungs, spleen or bone marrow; different species prefer different organs. The sporozoites enter the tissue cells and become schizonts, which divide by multiple fission to produce a number of merozoites. There are several asexual generations in the visceral cells, but their number is known in only a few cases. The last generation merozoites enter the blood cells and become gamonts. These look alike; presumably the female is a macrogamete and the male a microgametocyte, but no evidence is available on this point.

No further development takes place until the parasites reach the alimentary tract of the intermediate host. The gamonts then leave their host cells, associate in syzygy, and the microgametocyte forms 2 non-flagellate microgametes. These are relatively large, but smaller than macrogametes. One of them fertilizes the macrogamete, and the resultant ookinete penetrates the intestinal wall and comes to lie in the haemocoel. Here it grows considerably and becomes an oocyst. Several nuclear divisions take place in the sporont within the oocyst wall. The daughter nuclei migrate to its periphery, and each one buds off to form a sporoblast, leaving a large residual mass. The sporoblasts then form a wall around themselves, becoming sporocysts. Sporozoites develop in the sporocysts, their number depending on the species. When the vertebrate host ingests the invertebrate one, the oocysts and sporocysts rupture in its intestine, releasing the sporozoites.

It is possible that trans-placental infection may also occur, at least in some species. At any rate, Clark (1958) found a full-blown infection with *H. griseisciuri* in a 36-hour-old grey squirrel which had been born in a mite-free environment.

HEPATOZOON CANIS (JAMES, 1905)

Synonyms: *Leucocytozoon canis, Haemogregarina canis, Haemogregarina rotundata, Haemogregarina chattoni, Hepatozoon felis.*

Disease: Hepatozoonosis.

Hosts: Dog, cat, jackal, hyena and palm civet or musang (*Paradoxurus hermaphroditus*). The forms described from the cat, jackal and hyena under the names *H. felis*, *H. rotundata* and *H. chattoni*, respectively, are practically indistinguishable morphologically, and are probably all the same species. Laird (1959) believed that the form he found in the palm civet in Malaya was *H. canis*.

Location: The schizonts are in the spleen, bone marrow and to a lesser extent in the liver. The gamonts are in the polymorphonuclear leucocytes.

Geographic Distribution: India, Malaya, Singapore, Indochina, Central Africa, North Africa, Middle East, Italy. This species is well known in dogs, but has been reported from cats only by Patton (1908) in Madras (Laird, 1959).

Morphology: The gamonts in the leucocytes are elongate rectangular bodies with rounded ends measuring about 8 to 12 by 3 to 6 μ, and with a central, compact nucleus. Their cytoplasm stains pale blue and their nucleus dark reddish with Giemsa stain. They are surrounded by a delicate capsule. They may emerge from the leucocytes and capsule and lie free in citrated blood. Leitão (1945) saw schizonts in the circulating blood which he said were difficult to distinguish from platelets.

Life Cycle: The life cycle of *H. canis* was worked out by Christophers (1906, 1907, 1912) and Wenyon (1911). Schizogony takes place in the spleen and bone marrow, and Rau (1925) saw it in the liver also. There are several types of schizonts. One type produces a small number (usually 3) of large merozoites, another type produces a large number of small merozoites, and intermediate types produce merozoites of intermediate numbers and size. The small merozoites are the ones which enter the leucocytes to form gamonts.

The vector is the brown dog tick, *Rhipicephalus sanguineus*. Both the nymph and adult can transmit the infection, but there is no transovarian transmission. The oocysts are found in the haemocoel. They are about 100 μ in longest diameter and contain 30 to 50 sporocysts 15 to 16 μ long, each containing about 16 banana-shaped sporozoites and a residual body. Dogs become infected by eating infected ticks.

Pathogenesis: *H. canis* has often been found in apparently healthy dogs, but it may also cause serious disease and death (Rau, 1925; Rahimuddin, 1942). The principal signs are irregular fever, progressive emaciation, anemia and splenomegaly. Lumbar paralysis has also been reported. Affected dogs may die in 4 to 8 weeks.

Diagnosis: Hepatozoonosis can be diagnosed by identifying the gamonts in stained blood smears or in stained smears of spleen pulp, bone marrow or liver.

Treatment: Unknown.

Prevention and Control: Since *H. canis* is transmitted by the brown dog tick, elimination of ticks will eliminate the disease.

OTHER SPECIES OF HEPATOZOON

Hepatozoon muris (Balfour, 1905) occurs in the wild and laboratory Norway rat and black rat thruout the world. Schizogony takes place in the parenchymal cells of the liver, and the gamonts are found in the monocytes and rarely in the polymorphonuclear leucocytes. The vector is the spiny rat mite, *Echinolaelaps echidninus*. Massive infections may cause marked degenerative changes in the liver and death, but little or no effect has been observed in lightly infected wild rats.

Hepatozoon musculi (Porter, 1908) was reported from the white mouse in England. It differs from *H. muris* in that schizogony takes place only in the bone marrow.

Hepatozoon cuniculi (Sangiorgi, 1914) was reported from the domestic rabbit in

Italy. Its gamonts are found in the leucocytes and its schizonts in the spleen.

Hepatozoon grisceisciuri Clark, 1958 is common in the grey squirrel (*Sciurus carolinensis*) in the United States. Clark (1958) described its life cycle. Schizogony takes place in the spleen, liver and bone marrow, and the gamonts are found in the monocytes. The natural vector is the mite, *Euhaemogamasus ambulans*, but *Echinolaelaps echidninus* can act as a vector experimentally.

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Chapter 10

PLASMODIUM HAEMOPROTEUS AND LEUCOCYTOZOON

These genera belong to the suborder Haemospororina, which is differentiated from the Eimeriorina and Adeleorina by the facts that the microgametocyte produces a moderate number of microgametes and the sporozoites are naked. The gamonts are similar and develop independently. The zygote is motile (i.e., it is an ookinete). All species are heteroxenous; schizogony takes place in a vertebrate host, and sporogony in an invertebrate. If the erythrocytes are invaded, pigment (hemozoin) is formed from the host cell hemoglobin.

This suborder was customarily divided into 2 families, the Plasmodiidae containing the genus *Plasmodium*, and the Haemoproteidae containing the genera *Haemoproteus* and *Leucocytozoon*. The principal difference was that in the Plasmodiidae schizogony was thought to take place only in the erythrocytes, while in the Haemoproteidae it takes place in the lungs, liver, spleen, kidneys and other internal organs. However, when the complete life cycles of several species of avian and human *Plasmodium* were worked out (Huff and Coulston, 1944, 1946; Shortt and Garnham, 1948; Short *et al.*, 1951; Garnham, 1954; Bray, 1957), it was realized that schizogony may occur both within the erythrocytes and exoerythrocytically. The distinction between the two families is thus an artificial one, and there is no point in retaining more than a single family in the suborder.

It is likely, as Manwell (1955) has suggested, that the Haemospororina may well have arisen from the coccidia of vertebrates rather than from those of insects, as had been more commonly supposed. Genera like *Lankesterella* and *Schellackia*, in which schizogony, gametogony and sporogony all take place in the vertebrate host and in which the sporozoites invade the blood cells and are transmitted by mites or other blood-suckers, could well be the starting-point for the transition from the Eimeriorina to the Haemospororina.

FAMILY PLASMODIIDAE

This family has the characters of the suborder. Its taxonomy has been reviewed by Garnham (1953) and Bray (1957). These authors preferred to split the classical genus *Plasmodium* into several genera, based on the life cycles of their species, but it is simpler not to do so.

Genus *PLASMODIUM* Marchiafava and Celli, 1885

The gametocytes occur in the erythrocytes. Schizogony takes place in the erythrocytes and also in various other tissues, depending on the species. The exoerythrocytic ("e.e.") schizonts are solid or, at the most, vacuolated bodies. Members of this genus are parasites of mammals, birds and lizards. They are transmitted by mosquitoes, *Anopheles* transmitting the mammalian species, and culicines or sometimes *Anopheles* the avian and reptilian ones.

Members of this genus cause malaria, which is still the most important disease of man. They also cause a similar disease in birds. Coatney and Roudabush (1949) have cataloged the species of *Plasmodium*, and other species are discussed by Bray (1957). Man has 4 species, higher apes 4, lower apes and lemurs 7, rodents 2, and bats 1. Birds have 14 or 15 valid species (Hewitt, 1940; Bray, 1957; Laird and Lari, 1958).

Life Cycle: The life cycle of *Plasmodium vivax* of man is representative. The sporozoites enter the blood thru a mosquito bite. They stay in the blood less than an hour, quickly entering liver parenchymal cells. Here they become schizonts (known as cryptozoites from their location), which enlarge and divide by multiple fission to form metacryptozoites (a type of merozoite). These enter new liver parenchymal cells, undergo multiple fission, and form new metacryptozoites. This process may go on indefinitely in *P. vivax*, but in another human species, *P. falciparum*, there is only a single generation of metacryptozoites.

The metacryptozoites break out of the liver cells, pass into the blood stream and enter the erythrocytes about a week to 10 days after infection. Here they round up and develop a large vacuole in their center. They are called ring stages because in Romanowsky stained smears they resemble a signet ring, with a red nucleus at one edge and a thin ring of blue cytoplasm around the vacuole. These grow and are now called schizonts or trophozoites.

The trophozoites were formerly thought to obtain their nutriment saprozoically, but Rudzinska and Trager (1957) showed in an electron microscope study of *P. lophurae* of the duck that they are holozoic as well. They form food vacuoles containing host cell cytoplasm by invagination. The hematin pigment granules are formed within these food vacuoles by digestion of the hemoglobin. This study, incidentally, settled once and for all the question which is raised perennially as to whether *Plasmodium* occurs within or on the surface of the host cell; it is within it.

The trophozoites undergo schizogony to produce merozoites, the number depending on the species. These break out of the erythrocytes, enter new ones, and repeat the cycle indefinitely.

The length of each cycle depends on the parasite species. It is 2 days in *P. vivax* and *P. falciparum*, and 3 days in another human species, *P. malariae*. Practically all the parasites are generally in the same stage of the cycle at the same time, so all the merozoites break out of the red cells and pass into the blood at the same time. Along with them go the hematin granules and other waste products produced by the parasites' metabolism. These are toxic, and cause a violent reaction or paroxysm in the host--the chills and fever characteristic of malaria.

After the infection has been present for some time and after an indefinite number of asexual generations, some merozoites entering the erythrocytes develop into macrogametes and others develop into microgametocytes. The former are

customarily called macrogametocytes, but this name is incorrect since they are haploid from the start (see below). They remain in this stage until the blood is ingested by a mosquito.

In the stomach of the mosquito, microgametes are produced. The changes in the microgametocytes are striking. Within 10 to 15 minutes the nucleus divides, and 6 to 8 long, heavy flagellum-like microgametes are extruded. This process is known as exflagellation. The microgametes break off and swim freely until they find a macrogamete. Fertilization takes place, and a motile zygote (ookinete) is formed.

The ookinete penetrates into the stomach wall and grows into an oocyst, which forms a ball 50 to 60 μ in diameter on the outer surface of the stomach. The oocyst nucleus divides repeatedly and a number of sporoblasts are formed. The nucleus of each sporoblast then divides repeatedly, and eventually each oocyst comes to contain 10,000 or more slender, spindle-shaped sporozoites about 15 μ long with a nucleus in the center. These break out of the oocyst into the body cavity and migrate to the salivary glands. They are then injected into a new host when the mosquito bites again. The process of sporozoite development takes 10 days to 3 weeks or longer, depending on the species of *Plasmodium*, the species of mosquito and the temperature.

Once infected, a mosquito remains infected for life, and can transmit the parasites every time it bites. There is a case on record (James, 1927) of a mosquito which lived from August 5 to November 16 and infected more than 40 general paresis patients as part of their therapy.

In vivax and malariae malaria, relapses are common and may occur for a number of years after the individual has had his first attack. Between attacks the parasites are ordinarily not found in the blood. What apparently happens is that all the parasites do not leave the liver when the metacryptozoites emerge into the blood stream, but a few remain there and continue to multiply in secret until such time as the body's defenses have decreased sufficiently so that the parasites can again invade the blood.

There are several variations of the above general pattern. In *P. falciparum* of man, there is only a single generation of metacryptozoites in the liver, and relapses rarely occur. In addition, the schizonts and merozoites of this species are rarely seen in the peripheral blood. Instead, the infected red cells become viscid and clump together in the internal organs.

In the avian species, exoerythrocytic schizogony does not take place in the liver parenchyma, but either in the endothelial cells (*P. gallinaceum*, *P. relictum*, *P. cathemerium*, *P. lophurae*, *P. fallax*, *P. circumflexum*, *P. durae*, *P. juxtanucleare*, *P. hexamerium*) or largely in the haemopoietic cells (*P. elongatum*, *P. vaughani* and probably *P. huffi* and *P. rouxi*).

In bird malaria also, but not in mammalian malaria, some of the merozoites which have been formed in the erythrocytes are able to enter the tissue cells and develop exoerythrocytically. They are known as phanerozoites, but they do not differ morphologically from the forms derived from sporozoites.

Plasmodium is haploid thruout its life cycle except for a brief period following fertilization and zygote formation. In a cytologic study of the early oocysts of 7 species of *Plasmodium* in mosquitoes, Bano (1959) found that the oocysts undergo meiosis 2 to 3 days after the infective blood meal, the time depending on the species. For *P. vivax* it was 48 hours, for *P. gallinaceum* 53 to 55 hours, and for *P. inui* 72 to 79 hours. After that, division is by mitosis.

The haploid number of chromosomes is 2 for *P. falciparum*, *P. malariae*, *P. ovale*, *P. lophurae*, *P. relictum*, *P. floridense* (Wolcott, 1955, 1957), *P. vivax*, *P. knowlesi*, *P. berghei* (Wolcott, 1955, 1957; Bano, 1959), and *P. gallinaceum* (Bano, 1959); it is 3 for *P. gonderi* and

4 for *P. cynomolgi* and *P. inui* (Bano, 1959).

Cultivation: Various species of *Plasmodium* have been cultivated in fluid media (Trager, 1947; Anfinsen *et al.*, 1946; Geiman *et al.*, 1946) and in avian embryos and tissue culture (see Pipkin and Jensen, 1958 for a review).

HUMAN MALARIA

The following discussion of human malaria is necessarily brief. Further details and references can be found in any textbook of human parasitology and, in more detail, in Boyd (1949) and Macdonald (1957).

Man has 4 recognized species of *Plasmodium*. *P. falciparum* (Welch, 1897) Schaudinn, 1902 is the cause of malignant tertian, aestivo-autumnal or falciparum malaria. Paroxysms of chills and fever occur every other day (i.e., on days 1 and 3, which accounts for the name "tertian"). The ring forms are about 1/6 to 1/5 the diameter of a red blood cell. The schizonts and merozoites ("segmenters") rarely occur in the peripheral circulation, but are found in clumped erythrocytes in the viscera. The schizonts are usually compact and rounded, with coarse, blackish pigment. The segmenters occupy 2/3 to 3/4 of the host cell and form 8 to 32 merozoites. The host erythrocyte is not enlarged but contains reddish clefts known as Maurer's dots and may also have bluish stippling. The macrogametes and microgametocytes are crescent- or bean-shaped, with pigment granules clustered around a central nucleus or scattered except at the poles. The microgametocytes have pale blue cytoplasm and a relatively large, pink nucleus when stained with Giemsa's stain. The macrogametes have darker blue cytoplasm and a more compact, red nucleus.

Plasmodium vivax (Grassi and Feletti, 1890) Labbé, 1899 is the cause of benign tertian or vivax malaria. Paroxysms occur every other day as in falciparum malaria. The ring forms are about 1 3 to 1 2 the diameter of the host cell. The schizonts are highly active and sprawled out irregularly over the host cell, with small, brown pigment granules usually collected in a single mass. The host cell is enlarged, pale, and contains red dots known as Schüffner's dots. The segmenters nearly fill the host cell and produce 15 to 20 or occasionally up to 32 irregularly arranged merozoites. The macrogametes and microgametocytes are rounded, 10 to 14μ in diameter (i.e., larger than normal erythrocytes), and have fine, brown, evenly distributed pigment granules. The microgametocytes have pale blue cytoplasm and a relatively large, pink nucleus when stained with Giemsa's stain. The macrogametes are slightly larger, with darker blue cytoplasm and a small, red nucleus.

Plasmodium malariae (Laveran, 1881) Grassi and Feletti, 1890 is the cause of quartan or malariae malaria. This species also occurs naturally in chimpanzees in West Africa (Garnham, 1958). Paroxysms occur every 3 days (i.e., on days 1 and 4). The ring forms are similar to those of *P. vivax*. The schizonts are more compact and rounded or are drawn out in a band across the host cell; their pigment granules are blacker and coarser than those of *P. vivax*. The host cell is not enlarged and does not contain Schüffner's dots. The segmenters nearly fill the host cell and produce 6 to 12 (usually 8 or 9) merozoites arranged in a rosette. The macrogametes and microgametocytes are rounded and smaller than those of *P. vivax*. They do not quite fill the host cell and contain blacker and coarser pigment granules.

Plasmodium ovale Stephens, 1922 is a rare species which causes a tertian type of malaria. Its ring forms are similar to those of *P. vivax*. The schizonts are usually round, with brownish, coarse, somewhat scattered pigment granules. The host cell is oval, often fimbriated, not much enlarged, and contains Schüffner's dots. The segmenters occupy 3/4 of the host cell and produce 8 to 10 merozoites

in a grape-like cluster. The macrogametes and microgametocytes are rounded, occupy 3/4 of the host cell and have coarse, black evenly distributed pigment granules.

A *vivax*-type *Plasmodium*, *P. cynomolgi*, occurs in macaques. Eyles, Coatney and Getz (1960) recently described accidental laboratory infections of 2 humans with *P. c. bastianellii* originally isolated from *Macaca irus* from Malaya. They were able to infect 2 other humans experimentally by allowing them to be bitten by infected *Anopheles freeborni* mosquitoes. This finding and the presence of *P. malariae* in chimpanzees suggest that more than one of the human malarias may be zoonoses.

Pathogenesis: The malarial paroxysm is highly characteristic. It begins with a severe chill. The patient shivers uncontrollably, his teeth chatter, and he has gooseflesh, altho his temperature is actually above normal. The chill is followed by a burning fever, headache and sweating. This gradually subsides, the temperature falls, and after 6 to 10 hours the patient feels much better--until his next paroxysm. The destruction of erythrocytes causes anemia.

After a certain number of paroxysms, the attack of malaria subsides. Relapses may occur over a period of years in vivax and malariae malaria, but this is rarely the case in falciparum malaria.

In general, mortality from malaria is higher in children than in adults in endemic areas, because by the time the people become adult they have had repeated attacks, and those who have survived have developed a good deal of immunity. For this reason, if one wants to determine the incidence of malaria in an area, it is better to examine children than adults.

A highly fatal, cerebral form of malaria may occur in falciparum infections. It is due to clogging of the capillaries of the brain by agglutinated, infected erythrocytes. If enough clogging takes place in the viscera, a severe gastro-intestinal disease resembling typhoid, cholera or dysentery may occur. Another complication of falciparum malaria is blackwater fever, which gets its name from the color of the urine. There is tremendous destruction of the erythrocytes--60 to 80% may be destroyed in 24 hours--accompanied by fever, intense jaundice and hemoglobinuria. Severe attacks are usually fatal. The cause of blackwater fever is not known, but it may involve some sort of immunological reaction which hemolyzes the erythrocytes.

Epidemiology: Malaria is transmitted by *Anopheles* mosquitoes. There are about 200 species of this genus, but not all are equally good vectors, and the epidemiology of the disease in any particular locality depends not only upon the terrain and climatic conditions, but also upon the particular vectors present, their breeding habits, food preferences, susceptibility to infection, etc. The subject is an extremely complex one and cannot be discussed in detail here. Three examples will suffice.

The principal malaria vector in southeastern United States is *Anopheles quadrimaculatus*. This species breeds best in clean, open water with dense aquatic vegetation and abundant flotage. It prefers bovine to human blood, however, so that the ratio of livestock to men in an area is an important factor in the transmission rate.

The principal malaria vector in the Solomon Islands is *Anopheles farauti*. It breeds in small ponds and puddles. During the Guadalcanal campaign of World War II, the profusion of shell holes, fox holes, road ruts, etc. provided ideal conditions for its propagation, and the result was an explosive outbreak of malaria. It was controlled by eliminating or draining the breeding places or spraying them with fuel oil.

These measures would not work in the Philippines, where the principal vector is *Anopheles minimus flavirostris*. This species breeds at the edges of slow-moving streams in the plains, hence quite different measures, such as stream clearing, straightening and flushing, must be used to prevent its breeding.

Malaria is primarily a disease of warmer climates nowadays, but at one time it was common in the temperate zone. Nevertheless, malaria is still the most important human disease from a global standpoint. Of the 1955 world population of 2,653 million, 1070 million lived in malarious areas, and 696 million of these were protected poorly or not at all from malaria. In 1955 there were still 200 to 225 million cases of malaria in the world, with more than 2 million deaths (Diehl, 1955).

Malaria control has eliminated or almost eliminated malaria from many parts of the world (Pampana and Russell, 1955; Russell, 1956, 1958; Anonymous, 1956), largely by use of residual spraying with DDT and other insecticides. At the end of World War II there were about 4 million cases of malaria a year in southern Europe from Spain to Bulgaria. In 1956 there were less than 10,000 cases in the same area.

Malaria was one of the causes of the decline of the Roman empire. The swampy land of the Roman Campagna made it almost uninhabitable because of the disease. There were 411,602 cases of malaria in Italy in 1945. Systematic spraying with DDT was begun in 1946, and as a result only 12 cases of indigenous malaria (both primary cases and relapses) were reported in 1953. In 1955 there were only 3.

During World War I, the British and French landed armies at Salonika, Greece, with the objective of driving into Germany thru the back door. Malaria wrecked their plans and immobilized their armies. There were 2 million cases of malaria in Greece in 1942. In 1950 there were 50,000, and in 1952 only 408.

In the Eastern Mediterranean countries, with a population of about 170 million, there were about 40 million cases of malaria a year in 1949. There are now about 14 million, and it has been shown that it is technically possible to eliminate malaria from the area.

Malaria has been completely eliminated from Sardinia and Sicily, and it is practically gone from Venezuela, Brazil, British Guiana, Argentina, Cyprus, Ceylon and parts of India, to mention a few of the places.

In the United States there were a million cases of malaria a year among a population of 25 million in 12 southern states during 1912 to 1915. Before that, malaria was an important disease thruout the midwest. Ackerknecht (1945) has given its history in that region from 1760 to 1900. The decrease of malaria in this country was due only in small part to measures aimed directly at the disease, but more to agricultural development and to other, still unknown, factors. It was almost entirely eliminated from the midwest, for instance, by farm land drainage.

After World War II an intensive campaign was started to wipe out malaria from this country. Residual spraying of dwellings, outhouses, barns, etc. was practiced in malarious areas. Mosquito larval control measures were intensified. An attempt was made to follow up every case diagnosed as malaria, to get a blood smear in order to be sure that it actually was malaria, and to treat it immediately in order to prevent it from being a source of further cases.

During 1949 less than 5000 cases were reported in the U. S. During 1955, 477 cases were reported. Of these, 64 were appraised by the U. S. Public Health Service, and only 29 were confirmed by blood smear as malaria. Only 4 were primary indigenous cases. Two were in California, 1 in Arizona, and the fourth--acquired by blood transfusion--in Illinois. In 1957, 157 cases were reported, of which 138 were appraised by the Public Health Service; 102 were confirmed, and 11 of these were primary indigenous cases (Dunn and Brody, 1959). In 1958, 94 cases were reported, of which 61 were confirmed. Seven of these were primary indigenous cases, 4 of them resulting from blood transfusions, and 1 natural case each originating in California, Arizona and possibly Pennsylvania (Brody and Dunn, 1959).

One outbreak of malaria illustrates what can happen if conditions are right (Brunetti, Fritz and Hollister, 1954). It

occurred at a Campfire Girl camp at Lake Vera, California. A marine visited the camp on July 4, 1952, and spent the night. He had a malarial relapse while he was there, and was sleeping without a mosquito bar. Within a few weeks the first case appeared among the girls, and cases continued to appear until January or February. A total of 35 cases of vivax malaria occurred among that group of girls as the result of one night's exposure from one infected marine.

There are no obvious technical or economic reasons why malaria could not be eradicated from the Americas, Europe, Australia and much of Asia during the next quarter century, altho the situation is not so promising in tropical Africa (Williams, 1958). This can be done almost entirely by residual spraying of dwellings. The cost of protection has been found to vary from 11 to 45 cents per capita per year. Properly conducted, residual house spraying for 2 to 3 years will eradicate *Plasmodium*, altho the mosquito vector may persist. Some mosquitoes have developed a resistance to DDT, but this has always taken at least 6 years, so malaria can be eliminated before the mosquitoes become resistant.

New problems result from disease control, however. These are well illustrated by the effect of malaria control on the population of Ceylon. The birth rate on that island was 40 per 1000 in 1920, and it was still about the same in 1950. But the death rate in 1920 was 32 per 1000, while in 1950 it was 12 per 1000, and this decrease was due primarily to the elimination of malaria. This means that if both the present birth and death rates are maintained, the population of Ceylon will double in about 26 years. And how can all these additional millions be fed? (Stone, 1954).

Diagnosis: Malaria can be diagnosed with certainty only by finding and identifying the causative organisms in the blood. This is done by microscopic examination of smears stained with one of the Romanowsky stains; Giemsa's stain is best. At one time thin smears were used almost entirely, but thick, laked smears are much better, since they permit a much larger amount of blood to be examined in a given time. Identification of the species and stages requires skill and practice. An excellent guide with outstanding colored illustrations is that of Wilcox (1960).

Treatment: A number of drugs have been used in treating malaria. The first one was quinine, the most active ingredient of cinchona bark, which was identified in 1820 by Pelletier and Caventou. It is both suppressive and curative, but does not prevent relapses. Chemically it is 6-methoxy-alpha-(5-vinyl-2-quinuclidyl-4-quinoline-methanol).

Quinacrine (Atebrin, Atabrine, mepacrine) was discovered by Mauss and Mietsch (1933) in Germany. It is 2-chloro-5-diethylamino-isopentylamino-7-methoxy-acridine dihydrochloride. It was used extensively during World War II when the Indonesian cinchona plantations were taken over by the Japanese. It is actually better than quinine. It is prophylactic against falciparum malaria and suppressive against vivax and malariae malarias. It cures attacks of the disease, but does not prevent relapses. One disadvantage is that it is a dye and stains the skin yellow.

Chloroquine (Aralen) is 7-chloro-4, 4-dimethylamino-1-methylbutylaminoquinoline. It was developed thru a crash drug-testing program during World War II in which the Americans tested over 14,000 compounds and the British about half as many. The results of the American effort are summarized by Wiselogle (1946). Chloroquine appeared too late to be used in that war except experimentally. It is the most effective drug known for the treatment and suppression of all types of malaria. The recommended therapeutic dose is 1.5 g in 3 days. Following its use, fever subsides in a day, and the parasites disappear from the blood in 2 or 3 days. The suppressive dose is 0.3 g weekly. Chloroquine does not prevent vivax malaria relapses, however.

Primaquine appeared even later than chloroquine, having been introduced in

1949. It is 8-(4-amino-1-methylbutyl-amino)-6-methoxyquinoline. It is most useful as a truly curative agent against vivax malaria, since it not only cures attacks but prevents relapses. It is best used in combination with chloroquine if the patient is having an attack, but can be used alone in between relapses to prevent further relapses. The dosage is 15 mg daily for 14 days. The effectiveness of this drug in preventing relapses was proven in returning Korean veterans.

Chlorguanide (Paludrine, Proguanil) was developed by the British during World War II. It is N-p-chlorophenyl-N-5-isopropylbiguanide. It showed a great deal of promise, but after it had been used for a while, resistant strains of *Plasmodium* appeared, and it is no longer being used.

Pyrimethamine (Daraprim, Malocide) was introduced by the British in 1951. Its discovery grew out of the World War II study. It is 2,4-diamino-5-p-chlorophenyl-6-ethylpyrimidine. It is perhaps the best suppressive drug known, altho it is not recommended for the treatment of malarial attacks. In single weekly doses of 25 mg it completely suppresses all *Plasmodium* species and is prophylactic against *P. falciparum* and some strains of *P. vivax*. In addition, it destroys *P. falciparum* gametocytes, so that it has value in the epidemiological control of this type of malaria. It is being mixed with the salt for prophylaxis in some parts of the Americas. Unfortunately, resistant strains have appeared in some areas where it has been used, and its eventual value is uncertain.

Many other drugs have been used for treating malaria, but these are the most important. At present, the ones generally recommended are chloroquine, primaquine and pyrimethamine.

BIRD MALARIA

A tremendous amount of work has been done on the bird malarias. The avian species of *Plasmodium* lend themselves well to experimentation, and, until the discovery of *P. berghei* in rodents in 1948, birds were the only experimental animals in which malaria could be conveniently studied. All the drug screening for antimalarials in World War II was carried out in birds (Wiselogle, 1946).

About 40 species of *Plasmodium* have been described from birds, but only 14 or 15 are accepted as valid (Hewitt, 1940; Bray, 1957; Laird and Lari, 1958). Many wild birds are commonly infected. The most complete general review of bird malaria is that of Hewitt (1940), altho it is now somewhat out of date. Herman (1944) and Coatney and Roudabush (1949) have given catalogs and host-indices of the species of *Plasmodium* in birds. Levine and Hanson (1953) tabulated reports of *Plasmodium* from waterfowl, and Levine and Kantor (1959) did the same for birds of the order Columbiformes. Other more recent general papers are those of Becker (1959), Bray (1957), Herman *et al.* (1954), Huff (1954) and Wolfson (1941).

Bird malaria is not of great veterinary importance, but it may occasionally cause losses, especially in pigeons. Most of the species are not strongly host specific and can infect several species of birds. Most laboratory studies have been carried out with *Plasmodium cathemerium* and *P. relictum* in the canary, *P. gallinaceum* in the chicken and *P. lophurae* in the duck.

The avian species of *Plasmodium* fall into 2 groups, depending upon whether their gametocytes are round or elongate. Among those with round gametocytes are *P. cathemerium*, *P. relictum* and *P. gallinaceum*. Among those with elongate gametocytes are *P. circumflexum*, *P. nucleophilum*, *P. rouxi*, *P. elongatum*, *P. hexamerium*, *P. vaughani* and *P. polare*. *P. lophurae* is somewhat different; its gametocytes are elongate at first but continue to grow and come to fill up the whole host cell except for the nucleus.

Cutting across these morphological groups are the two groups based on the type of cell invaded by the exoerythrocytic forms mentioned on page 261.

PLASMODIUM GALLINACEUM
BRUMPT, 1945

Disease: Chicken malaria.

Hosts: Chicken.

Crawford (1945) thought that jungle fowls are the natural hosts of *P. gallinaceum*. These are *Gallus lafayetti* in Ceylon, *G. sonnerati* in Sumatra and *G. bankiva* in India. Brumpt (1936), however, thought that the natural, wild host is still unknown. Jungle fowls are relatively resistant, but outbreaks of disease occur in domestic chickens introduced into areas where the parasite is endemic in wild birds.

Pheasants, partridges, peacocks and geese have been infected experimentally, but the duck, guinea fowl, pigeon, turtle dove, quail, buzzard, canary, English sparrow, Java sparrow (*Padda oryzivora*) and finch are resistant (Brumpt, 1936).

Location: Erythrocytes. The exo-erythrocytic stages are in endothelial cells.

Geographic Distribution: Southern Asia, Indonesia. *P. gallinaceum* was first seen by Crawford in Ceylon and named by Brumpt (1935) from material sent to him from Indochina. It has also been found in India (Rao, Das and Ramnani, 1951; Das, Rao and Ramnani, 1952) and Java, Sumatra and Celebes (Kraneveld and Mansjoer, 1953). It was reported from Egypt by Haiba (1948), but this record requires confirmation.

Morphology: The gametocytes and schizonts are round or irregular. The host cell nucleus is displaced but seldom expelled. The pigment granules in the gemetocytes are rather large and not very numerous. The schizonts produce 8 to 30 merozoites.

Life Cycle: The life cycle is similar to that of other *Plasmodium* species. The exo-erythrocytic stages in the endothelial or reticulo-endothelial cells of the spleen, brain, liver, etc. have been described by James and Tate (1937, 1938), James (1939) and Huff and Coulston (1944). The natural vectors are unknown, but various mosquitoes, including *Aedes aegypti*, *A. albopictus*, *A. geniculatus* and *Culex quinquefasciatus*, are potential vectors (Brumpt, 1936, 1936a; Vargas and Beltran, 1941). Huff (1954) listed 29 susceptible and 1 questionable species of which 19 are *Aedes*, 5 *Armigeres*, 2 (possibly 3) *Culex*, 1 *Anopheles*, 1 *Culiseta* and 1 *Mansonia*.

Pathogenesis: *P. gallinaceum* causes a serious disease with a high mortality rate in domestic chickens. The body temperature fluctuates, and anemia and splenomegaly are present. The birds may become paralyzed and die due to blocking of the brain capillaries by the exoerythrocytic stages.

Remarks: Beltran (1941, 1943a) and Crawford (1945) reviewed the history of research on this species. Because it lends itself well to experimental study and because the chicken is such an excellent laboratory animal, hundreds of papers have been written on it--according to Brumpt (1949), more than 600 between 1935 and 1948.

PLASMODIUM JUXTANUCLEARE
VERSIANI AND GOMES, 1941

Disease: Chicken malaria.

Hosts: Chicken. Versiani and Gomes (1941) infected 1 of 3 turkeys experimentally, but were unable to infect the duck, guinea fowl, pigeon, canary, domestic sparrow, tico tico, or 3 other species of wild birds.

Location: Erythrocytes. The exo-erythrocytic stages are in endothelial cells.

Geographic Distribution: South America (Brazil), Mexico.

Morphology: This species has been described from Minas Gerais, Brazil by Versiani and Gomes (1941, 1943) and from Chiapas, Mexico by Beltran (1941a, 1943). The gametocytes and schizonts are round

or irregular, relatively small, and usually in contact with the red cell nucleus. The schizonts produce 3 to 7 (usually 4) merozoites. The host cell is often distorted.

Life Cycle: The life cycle has not been completely studied. According to Beltran (1943), schizogony takes about 24 hours and its synchronicity is low. The prepatent period may vary from 2 to 38 days. Paraense (1947) saw exoerythrocytic stages in the endothelial cells. *Culex quinquefasciatus* was found to be a suitable experimental vector by Paraense (1944), but *Aedes aegypti* and *A. lepidus* are not.

Pathogenesis: This species is highly pathogenic. The Brazilian strain killed 75% of the infected young birds and 68% of the adults in 15 days to 9 months, and the Mexican strain killed 12 of 13 birds in 1 to 8 months. Affected birds do not show any marked signs. Shortly before death they appear listless and weak, with pale combs. Their temperature is not elevated. There are deposits of pigment in the liver and spleen. Versiani and Gomes (1943) observed a large amount of pericardial fluid, but Beltran (1943) did not.

PLASMODIUM DURAE HERMAN, 1941

Disease: Turkey malaria.

Hosts: Turkey. Purchase (1942) produced a transient infection in baby chicks. Simpson (1944) infected ducks of various ages. This is probably a natural parasite of some as yet unknown wild African bird.

Location: Erythrocytes. The exoerythrocytic stages are in endothelial cells.

Geographic Distribution: Africa (Kenya).

Prevalence: Herman (1941) found *P. durae* in 1 of 75 domestic turkeys in Kenya.

Morphology: This species was described by Herman (1941). The gametocytes are elongate, at the end or side of the host cell, and often displace the host cell nucleus when oriented obliquely to it. This tendency to take an oblique position differentiates *P. durae* from other avian species of *Plasmodium*. The pigment granules are usually large, round and black. The host cell is not enlarged. The trophozoites are more or less amoeboid. Presegmenters are often at the end of the host cell. The mature schizonts rarely displace the host cell nucleus. The pigment granules are round, black, up to 8 in number, and tend to become clumped together in the mature schizonts. Six to 14 (usually 8) merozoites are formed. The host cell is not distorted.

Life Cycle: Purchase (1942) and Simpson (1944) found exoerythrocytic stages of *P. durae* in the endothelial cells of the spleen, liver, lungs and brain of turkeys. The prepatent period is 3 days to 2.5 weeks after intravenous injection and 12 days to 40 days after intramuscular injection (Herman, 1941). Schizogony in the erythrocytes apparently takes 24 hours. The vectors are unknown (Huff, 1954).

Pathogenesis: *P. durae* causes an acute, often fatal disease in turkeys less than a year old. Twelve out of 14 young poults infected by Purchase (1942) died. They showed some malaise and ruffled feathers, but usually died without any acute signs. Two naturally affected adult birds showed signs of cerebral involvement, emaciation, edema of the legs and high blood pressure.

At necropsy of acute cases (Purchase, 1942) the liver, spleen and kidneys are dark and congested, the lungs slightly edematous, and the pericardial cavity contains an excess of clear fluid. The lumen of the duodenal loop is light chocolate in color and its villi are heavily laden with pigment. There is injection of the superficial vessels of the brain and meninges.

In chronic, naturally infected birds, the spleen is reduced in size, hard and fibrous, the liver is firm, with chronic congestion and much fibrosis. The villi of the duodenal loop are packed with large pigment granules.

PLASMODIUM RELICTUM (GRASSI AND FELETTI, 1891)

Synonyms: *Plasmodium praecox* Grassi and Feletti, 1890. (Bray, 1957 considered the correct specific name to be *praecox*, but he continued to use *relictum*.)

Disease: Pigeon malaria.

Hosts: Pigeon, mourning dove, pintail, cinnamon teal, falcated duck, black swan, and various passerine and other wild birds. This species was first described from the English sparrow. Experimental infections have been studied in the canary, duck, chicken and other birds.

Location: Erythrocytes. The exoerythrocytic stages are in endothelial cells.

Geographic Distribution: Worldwide.

Prevalence: This species is common in wild passerine birds. It occurs occasionally in domestic pigeons, having been found in them by Sergent and Sergent (1904) in Algeria, Coatney (1938) in Nebraska, Herman *et al.* (1954), Mathey (1955) and Graue (cited by Levi, 1957) in California, Becker, Hollander and Pattillo (1956) in Iowa, Pelaez *et al.* (1951) in Mexico, Cassamagnaghi (1950) in Uruguay, Haiba (1946, 1948) in Egypt, and Rousselot (1943) in the French Sudan.

Morphology: The gametocytes and schizonts are round or irregular. The host cell nucleus is displaced and often expelled by the larger forms. The pigment granules of the gametocytes are relatively fine and dot-like. The schizonts produce 8 to 32 merozoites, the number depending on the particular strain.

Life Cycle: The life cycle of this species has been studied extensively (Bray, 1957). The exoerythrocytic stages occur in the endothelial cells. The asexual cycle has been reported to take from 12 to 36 hours in different strains; some have a very high and others a low degree of synchronicity (Hewitt, 1940). Many species of mosquitoes can act as vectors. Huff (1954) listed 12 of *Culex*, 4 of *Anopheles*, 3 of *Aedes* and 2 of *Culiseta*, and remarked that 93% of the species which had been tested had been found susceptible.

Pathogenesis: *P. relictum* is highly pathogenic for the pigeon but less so for the mourning dove and canary. Affected squabs become weak and anemic, with enlarged and heavily pigmented spleens and livers. Pigment may also be deposited in the fat. Hill (1942) showed that anemia is the principal cause of death.

PLASMODIUM CIRCUMFLEXUM KIKUTH, 1931

Hosts: This species is quite common in a wide variety of hosts. The type host is a German thrush, *Turdus pilaris*. It was found in the ruffed grouse in Canada by Fallis (1945, 1946), and a similar form was found in the Canada goose in Illinois by Levine and Hanson (1953).

Location: Erythrocytes. The exoerythrocytic stages are in endothelial cells.

Morphology: The gametocytes and trophozoites are elongate; they tend to encircle the host cell nucleus but are generally not in contact with it and do not displace it. The schizonts produce 13 to 30 merozoites (average 19).

Life Cycle: The life cycle is similar to that of other avian species of *Plasmodium*. Exoerythrocytic stages occur in the endothelial cells. The known vector mosquitoes are *Culex tarsalis*, *Culiseta annulata* and *C. melaneura*. Two other species of *Culex* and 5 of *Aedes* have been found insusceptible (Huff, 1954).

PLASMODIUM CATHEMERIUM HARTMAN, 1927

Disease: Canary malaria.

Hosts: This species was first found in the English sparrow. It is common in passerine birds and has also been found in canaries.

Location: Erythrocytes. The exoerythrocytic stages are in endothelial cells.

Morphology: The gametocytes and schizonts are more or less round, displacing and often expelling the host cell nucleus. The pigment granules in the gametocytes are coarse, often elongate and rod-like. The schizonts produce 6 to 24 merozoites.

Life Cycle: The life cycle of this species has been studied extensively (Bray, 1957). It is similar to that of other avian species of *Plasmodium*. Exoerythrocytic stages occur in the endothelial cells. The asexual cycle takes 24 hours, and synchronicity is high. Huff (1954) listed 8 species of *Culex*, 3 each of *Aedes* and *Anopheles* and 1 of *Psorophora* which can act as vectors. However, he remarked that only 46% of the mosquito species which had been tested were susceptible.

Pathogenesis: *P. cathemerium* causes a highly fatal disease in canaries. Herman and Vail (1942) reported it in a canary in California, and Mathey (1955a) described an outbreak in a canary breeding establishment in that state in which possibly 165 out of 700 birds died.

Affected canaries have subcutaneous hemorrhages, anemia, splenomegaly and hepatomegaly. Mathey (1955a) described swelling in the region of the eyes. Hewitt (1939) found splenic infarcts in 47% of his experimentally infected canaries.

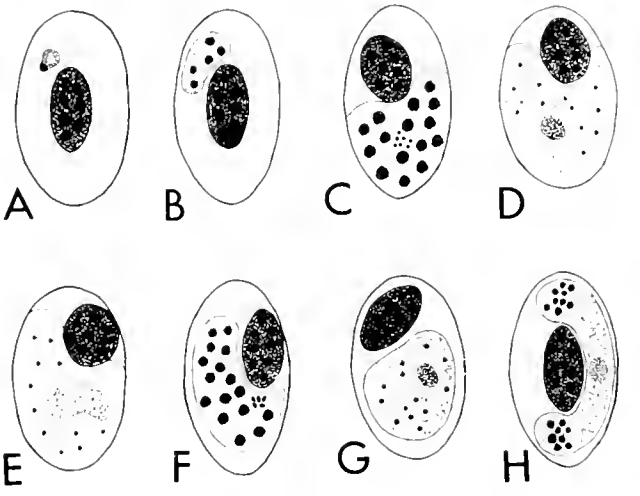

Fig. 32. Avian *Plasmodium* and *Haemoproteus* in erythrocytes. A. *Plasmodium gallinaceum* young trophozoite (ring stage). B. *P. gallinaceum* older trophozoite. C. *P. gallinaceum* mature trophozoite (segmenter). D. *P. gallinaceum* macrogamete. E. *P. gallinaceum* microgametocyte. F. *P. relictum* mature trophozoite (segmenter). G. *P. relictum* macrogamete. H. *Haemoproteus columbae* macrogamete. X 2800. (Original)

BIRD MALARIA

Diagnosis: Bird malaria can be diagnosed by finding and identifying the protozoa in stained blood smears. If schizonts or merozoites are present, it is easy to differentiate *Plasmodium* from *Haemoproteus*, since these stages are not found in the peripheral blood in the latter. However, if elongate gametocytes alone are found, differentiation is usually not possible.

Treatment: The bird malarias respond to treatment with quinacrine, chloroquine and other antimalarial drugs. Indeed, these were discovered by screening against bird malarias. Chloroquine at the rate of 5 mg per kg, chlorguanide at 7.5 mg per kg and pyrimethamine at 0.3 mg per kg protect chickens against *P. gallinaceum* infections. However, as a practical matter,

treatment is usually hardly worthwhile, and preventive measures are recommended instead.

Prevention and Control: Since bird malaria is carried by mosquitoes, prevention depends upon mosquito control. Residual spraying of poultry houses with insecticides such as DDT or lindane should be effective. Birds can also be raised in screened quarters where mosquitoes cannot get to them.

Genus *HAEMOPROTEUS* Kruse, 1890

The gametocytes occur in the erythrocytes and are usually halter-shaped. Schizogony takes place in the endothelial cells of the blood vessels, especially in the lungs, and not in the erythrocytes. The known vectors are louse-flies (Hippoboscidae) and midges (*Culicoides*). Members of this genus are parasites of birds and reptiles. A synonym of this generic name is *Halteridium* Labbé, 1894.

Members of this genus are extremely common in wild birds and also occur in domestic pigeons, ducks and turkeys. They are not an important cause of disease. Coatney (1936) gave a checklist and host index of the species of *Haemoproteus*, and Herman (1944) listed the species reported from North American birds.

Life Cycle: The life cycle of *Haemoproteus* is similar to that of *Plasmodium* except that schizogony does not take place in the erythrocytes but in the endothelial cells of the blood vessels, and the vectors are not mosquitoes but hippoboscid flies or midges.

HAEMOPROTEUS COLUMBAE KRUSE, 1890

Synonyms: *Haemoproteus maccallumi, Haemoproteus melopeliae, Haemoproteus turtur, Haemoproteus vilhenai* (?).

Hosts: Domestic and wild pigeons, mourning dove, turtle dove and a large number of other wild columbiform birds. Levine and Kantor (1959) tabulated reports of *Haemoproteus*, all of which were probably *H. columbae*, from 45 species belonging to 19 genera of columbiform birds.

H. maccallumi was first described from the mourning dove (*Zenaidura macroura*). It is morphologically indistinguishable from *H. columbae*. Huff (1932) transmitted it from the mourning dove to the pigeon, but Coatney (1933) was unable to transmit it from the pigeon to the mourning dove; both used the hippoboscid fly, *Pseudolynchia canariensis*, as the vector. There may be strain differences between the different hosts, but until greater differences than these are brought out, it is probably better to use the name *H. columbae* for the species from columbiform birds.

Location: The gametocytes are in the erythrocytes. Schizogony occurs in the endothelial cells of the blood vessels.

Geographic Distribution: Worldwide.

Prevalence: Common. Thirty-eight reports of *H. columbae* from the domestic pigeon were tabulated by Levine and Kantor (1959). Among those in which relatively large numbers of birds were examined, Coatney (1935) found it in all of about 28 pigeons in midwestern United States, Kartman (1949) found it in 82% of 101 pigeons in the Honolulu zoo, Giovannoni (1946) found it in 58% of 159 pigeons in southern Curitiba, Brazil, Acton and Knowles (1914) found it in all of 75 pigeons from the plains of India, and Singh, Nair and David (1951) found it in 22% of 214 pigeons in Delhi, India.

Herman (1938) found it in 8% of 86 mourning doves (*Zenaidura macroura carolinensis*) on Cape Cod, Massachusetts, Huff (1939) found it in 47% of 188 mourning doves, mostly from Illinois, Couch (1952) found it in 56% of 213 mourning doves in Texas, and Hanson *et al.* (1957) found it in 30% of 392 immature and 43% of 72 adult mourning doves in Illinois; its incidence in this last survey increased steadily from 7 to 8% in very young doves to 70% in the oldest immatures, and varied markedly in

different parts of the state and in different years. Wood and Herman (1943) found it in 93% of 27 western mourning doves in Arizona and California.

Morphology: The only stages found in the peripheral blood are macrogametes and microgametocytes. When mature, these are elongate and sausage-shaped. They partially encircle the host cell nucleus; they may displace it to some extent, but they do not push it to the edge of the host cell. They contain a variable number of dark brown pigment granules. The host cell is not enlarged.

When stained with a Romanowsky stain, the cytoplasm of the microgametocytes is pale blue or almost colorless and their nuclei are pale pink and diffuse, while the cytoplasm of the macrogametes is darker blue and their nuclei are compact and dark pink or red.

Life Cycle: The life cycle of *H. columbae* has been studied by Aragão (1908), Adie (1915, 1924) and Huff (1942) among others. Birds become infected when bitten by the dipteran vector. The sporozoites enter the blood stream and invade the endothelial cells of the blood vessels of the lungs, liver and spleen. Here they round up to form schizonts. Each schizont undergoes multiple fission to form 15 or more small, unpigmented bodies, the cytomeres, each with a single nucleus. Each cytomere grows still further, and its nucleus undergoes multiple fission. Finally, the host cell becomes considerably hypertrophied and is filled with a number of multinucleate cytomeres.

The endothelial cells break down, releasing the cytomeres. These vary in size, but may reach 60 μ in diameter. They accumulate in the capillaries, which they sometimes block completely. They are irregularly shaped and tortuous, and may send out branches along the capillaries, becoming bifurcate, trifurcate or even multiradiate. Each cytomere produces an enormous number of merozoites, which break out and pass into the blood stream.

According to Wenyon (1926), the schizonts do not necessarily form cytomeres but may produce merozoites directly. Presumably, too, schizogony is repeated a number of times.

Following schizogony, the merozoites enter red blood cells and become macrogametes and microgametocytes. These first appear 28 to 30 days after infection. At first they resemble ring stages of *Plasmodium*, but grow to the mature, elongate form in a few days. Multiple infections of erythrocytes with immature parasites are not uncommon, sometimes as many as 12 being found in a single host cell, but infections with more than 1 mature gamete or gametocyte are rare.

The only proven vector is the hippoboscid fly, *Pseudolynchia canariensis* (syns., *Lynchia maura, L. lividicolor, L. capensis*). In addition, Aragão (1916) stated that *Microlynchia pusilla* is a vector in South America, but gave no experimental evidence. Baker (1957) found that *H. columbae* from the English wood pigeon (*Columba palumbus*) would undergo sporogony in *Ornithomyia avicularia*, but 6 attempts to infect domestic pigeons by bite or injection of infected louse-flies failed.

It is highly unlikely that hippoboscids are the only vectors of this species, however. As Hanson *et al.* (1957) pointed out, hippoboscids are extremely rare on mourning doves, yet *H. columbae* is common in them. The discovery by Fallis and Wood (1957) that biting midges (*Culicoides*) are the vectors of *H. nettionis* of ducks suggests that they may also transmit *H. columbae*.

In the stomach of the hippoboscid vector, the microgametocytes produce 4 or more snake-like microgametes by exflagellation. They fertilize the macrogametes, and the resultant zygotes are ookinetes which crawl to the midgut wall and form oocysts on its outer surface. These grow, reaching a diameter of about 36 μ. They become mature in 10 to 12 days, producing very large numbers of slender, falciform sporozoites up to 10 μ long and

similar to those of *Plasmodium*. These break out of the oocysts into the body cavity and pass to the salivary glands, where they accumulate and are injected into a new host when the fly bites it.

Pathogenesis: *H. columbae* is only slightly pathogenic. Infected birds usually show no signs of disease. In relatively heavy infections the birds may appear restless and go off feed, and anemia may result from destruction of erythrocytes, but this is unusual. The liver and spleen of affected birds may be enlarged and dark with pigment.

HAEMOPROTEUS SACHAROVI NOVY AND MacNEAL, 1904

Hosts: Domestic pigeon, mourning dove (*Zenaidura macroura*), turtle dove (*Streptopelia turtur*). This species is primarily a parasite of wild doves which may also infect pigeons.

Location: Gametocytes in erythrocytes. Schizogony in endothelial cells of blood vessels.

Geographic Distribution: North America, Europe (Italy).

Prevalence: *H. sacharovi* is common in mourning doves. Levine and Kantor (1959) tabulated 12 reports from this host from coast to coast in the United States. Among those studies in which relatively large numbers of birds were examined, Herman (1938) found it in 7% of 86 mourning doves on Cape Cod, Massachusetts, Huff (1939) found it in 56% of 188 mourning doves, mostly from Illinois, Coatney and West (1940) found it in 67% of 18 mourning doves in Nebraska, Couch (1952) found it in 27% of 213 mourning doves in Texas, Wood and Herman (1943) found it in 41% of 27 western mourning doves in Arizona and California, and Hanson *et al.* (1957) found it in 58% of 392 immature and 43% of 72 adult mourning doves in Illinois. In this last study, its incidence was 31% in very young doves and 52% to 69% in older birds. Its incidence varied markedly in different parts of the state and in different years.

H. sacharovi was found in 22% of 50 domestic pigeons in Nebraska by Coatney and West (1940) and in 15% of 20 domestic pigeons in Iowa by Becker, Hollander and Pattillo (1956).

Morphology: The macrogametes and microgametocytes are found in the erythrocytes. They differ from those of most species of *Haemoproteus* in that when mature they completely fill the host cell, enlarging and distorting it, and often pushing the host cell nucleus to the edge of the cell. In addition, they contain very little pigment. When stained with a Romanowsky stain, the microgametocytes have pale blue to almost colorless cytoplasm and a light pink, diffuse nucleus, while the macrogametes have dark blue cytoplasm and a dark pink to red, compact nucleus.

The young gametocytes are ring-forms, and all stages between these and mature gametocytes can be found in the blood.

Life Cycle: Huff (1932) transmitted *H. sacharovi* from the mourning dove to the pigeon by means of the hippoboscid fly, *Pseudolynchia canariensis*. However, the natural vectors of this protozoon are still unknown. In view of its high incidence in mourning doves and the extreme rarity of hippoboscid flies on these birds, the natural vector must be some other ectoparasite, possibly *Culicoides*.

Pathogenesis: *H. sacharovi* is only slightly if at all pathogenic in the mourning dove. Becker, Hollander and Pattillo (1956) considered that it caused the enlarged, purplish livers which they encountered in dressing domestic pigeon squabs from an infected flock; there was apparently no other evidence of disease.

HAEMOPROTEUS NETTIONIS (JOHNSTON AND CLELAND, 1909) COATNEY, 1936

Synonyms: *Haemoproteus anatis*, *Haemoproteus hermani*.

Hosts: Domestic duck, domestic white Chinese goose, and over 23 species

of wild ducks, geese and swans, including the Canada goose, whistling swan, wood duck, pintail, green-winged teal, Australian teal, blue-winged teal, mallard, black duck, white-winged duck, cotton teal, Australian sheldrake, wattle duck, shoveller, Baer's pochard, ring-necked duck, white-eyed duck, rufous-crested duck, baldpate, common goldeneye, surf scoter, old squaw and common merganser (Levine and Hanson, 1953; Herman, 1954; Fallis and Wood, 1957).

Location: The gametocytes are in the erythrocytes. Schizogony occurs in the endothelial cells of the blood vessels.

Geographic Distribution: Worldwide.

Prevalence: Common. This species is a parasite of wild waterfowl which may infect domestic ducks in heavily endemic regions.

Morphology: Only the sexual stages are found in the red blood cells. Except early in an infection, young stages are absent or rare. The mature macrogametes and microgametocytes are elongate and sausage-shaped, partially (or sometimes completely) encircling the host cell nucleus, often displacing it. There is frequently a narrow band of cytoplasm between the parasite and the host cell nucleus, Free macrogametes and microgametocytes may occasionally be found; these are usually round. The macrogametes and microgametocytes contain a few to 30 or more (usually 12 to 24) pigment granules which are usually coarse and round and tend to be grouped at the ends of the cell. The host cell is not enlarged.

When stained with a Romanowsky stain, the cytoplasm of the microgametocytes is pale blue or almost colorless and their nuclei are pale pink and diffuse, while the cytoplasm of the macrogametes is darker blue and their nuclei are compact and dark pink or red.

Life Cycle: The vector of *H. nettionis* was first discovered by Fallis and Wood (1957). It is the biting midge, *Culicoides*. The prepatent period in experimentally infected birds is 14 to 21 days. Schizogony has not been described, and the details of sporogony in the midge are still to be worked out. Fallis and Wood found ookinetes in the midge stomach 36 hours after ingestion; they found structures which they regarded as oocysts on the stomach wall, and other structures which they regarded as sporozoites in the salivary glands.

Pathogenesis: *H. nettionis* is only slightly if at all pathogenic.

HAEMOPROTEUS MELEAGRIDIS N. SP.

Hosts: Domestic and wild turkey.

Location: The gametocytes are in the erythrocytes.

Geographic Distribution: North America.

Prevalence: Uncommon. *Haemoproteus* sp. was reported from 1 out of 4 domestic turkeys in the District of Columbia and vicinity by Wetmore (1941), from a turkey poult from Texas by Morehouse (1945), from 5 of 97 eastern wild turkeys (of which 4 had been reared in captivity) in Pennsylvania by Kozicky (1948), from a flock of turkeys in North Dakota by Goldsby (1951), from 3 out of 10 turkeys in South Carolina by Atchley (1951), from 1 out of 2 wild turkeys in Georgia by Love, Wilkin and Goodwin (1953) and from 42% of 52 birds in a flock of domestic turkeys in South Carolina by Bierer, Vickers and Thomas (1959).

Morphology: Only Morehouse (1945) described the macrogametes and microgametocytes. They are elongate, sausage-shaped, curve around the host cell nucleus and occupy about 1/2 to 3/4 of the host cell. Their surface is usually in close contact both with the host cell nucleus and host cell wall. The macrogametes measure 14 to 19 by 2 to 4 μ with a mean of 17 by 3 μ. They contain 18 to 48 (mean, 27) round or irregular pigment granules. Their nuclei measure 2 to 6 by 2 to 3 μ with a mean of 4 by 2 μ and are ovoid or irregular in shape.

The microgametocytes stain less intensely than the macrogametes. They measure 13 to 18 by 3 to 4 μ with a mean of 16 by 3 μ. They contain 11 to 24 (mean, 18) pigment granules. Their nuclei measure 5 to 10 by 2 to 4 μ with a mean of 8 by 3 μ. The host cells are not enlarged. Morehouse also observed occasional extracellular macrogametes.

Life Cycle: Unknown.

Pathogenesis: Unknown.

Remarks: Altho *Haemoproteus* is relatively rare in turkeys, it has been seen enough times and has been described well enough to warrant having a name of its own. I am therefore naming it *H. meleagridis* n. sp.

HAEMOPROTEUS INFECTIONS IN BIRDS

Diagnosis: *Haemoproteus* infections can be diagnosed by finding and identifying the protozoa in stained blood smears. However, not all infections in which gametocytes alone are found are necessarily *Haemoproteus* infections. Some of them may be *Plasmodium*.

Treatment: Little is known about treatment of *Haemoproteus* infections. According to Coatney (1935), quinacrine inhibits the development of young gametocytes of *H. columbae*, while pamaquine destroys the mature ones. Neither is effective against the schizonts. However, in view of the slight pathogenicity of *Haemoproteus*, treatment does not seem warranted.

Prevention and Control: Prevention of *Haemoproteus* infections depends on control of their hippoboscid and midge vectors, or, at least in the latter case, in preventing the birds from being bitten.

Genus *LEUCOCYTOZOON* Danilewsky, 1890

The macrogametes and microgametocytes occur in the leucocytes or, in some species, also in the erythrocytes. Schizogony takes place in the parenchyma of the liver, heart, kidney or other organs, the schizonts forming large bodies divided into cytomeres. There is no schizogony in the erythrocytes or leucocytes. The vectors are blackflies (*Simulium*). Members of this genus are parasites of birds.

Leucocytozoon is common in many wild birds and also causes disease in ducks, geese, turkeys and chickens. Coatney (1937) gave a catalog and host-index of the genus, and Herman (1944) listed the species occurring in North American birds.

LEUCOCYTOZOON SIMONDI MATHIS AND LEGER, 1910

Synonyms: *Leucocytozoon anatis*, *Leucocytozoon anseris*.

Disease: Leucocytozoonosis.

Hosts: Domestic ducks, domestic goose and many wild anseriform birds. Levine and Hanson (1953) tabulated reports of *L. simondi* from 23 species of wild waterfowl, including the grey-lag goose, white-fronted goose, Canada goose, wood duck, American pintail, green-winged teal, teal duck, blue-winged teal, falcated teal, mallard, black duck, baldpate, shoveller, scaup, lesser scaup, ring-necked duck, redhead, canvasback, American goldeneye, old squaw duck, hooded merganser, American merganser and red-breasted merganser.

Fallis, Pearson and Bennett (1954) transmitted *L. simondi* from domestic ducks to domestic geese, but failed to infect ruffed grouse, chickens, turkeys and pheasants with it.

Location: The gametocytes are in the lymphocytes, monocytes and also erythrocytes. Schizogony takes place in the liver, heart, brain, spleen, lungs, lymph nodes and pancreas.

Geographic Distribution: North America, Europe, Indochina.

Prevalence: This species is common in northern United States, Canada and other mountainous or hilly areas where cold, rapid streams permit suitable blackfly vectors to breed.

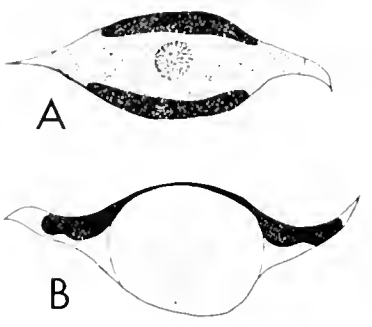

Fig. 33. Species of *Leucocytozoon* in avian leucocytes. A. *L. smithi* macrogamete from turkey. B. *L. simondi* microgametocyte from duck. X 1400. (Original)

Morphology: The mature macrogametes and microgametocytes are more or less elongate, and 14 to 22 μ long. Their host cells are ordinarily elongate, up to 45 to 55 μ long, with their nucleus forming a very long, thin, dark band along one side and with pale cytoplasmic "horns" extending out beyond the parasite and the nucleus. In some cases, round macrogametes and microgametocytes in rounded host cells have been reported (Fallis, Davies and Vickers, 1951; Rawley, 1953; Cook, 1954); both types are mature and able to exflagellate. Briggs (1960) noted that there were approximately equal numbers of round and elongate forms in white Pekin ducks but that elongate forms were rare in Muscovy ducks, never constituting more than 5% of the total number. He suggested that this might be due to the influence of the host species.

The cytoplasm of the macrogametes is rather dark blue and the nucleus compact and red when stained with a Romanowsky stain. The cytoplasm of the microgametocytes is very pale blue and the nucleus diffuse and pale pink. The microgametocytes are more delicate and more subject to distortion than the macrogametes.

A good deal of controversy has existed as to the type of cell parasitized by *L. simondi*. The host cells of the mature gametocytes are so distorted that they cannot be recognized. Huff (1942) considered them to be lymphocytes or stages in transformation between them and monocytes. Levine and Hanson (1953) found young and developing forms only in lymphocytes or monocytes. On the other hand, Fallis, Davies and Vickers (1951) and Cook (1954) found very young forms in both lymphocytes and erythrocytes. Using the benzidine-peroxide stain for hemoglobin, Cook found no hemoglobin in the host cells containing mature gametocytes, but she found at least some hemoglobin in all of the 191 host cells she saw which contained developing gametocytes. She concluded that, while the ring stages may invade both erythrocytes and lymphocytes, they develop to maturity only in cells of the red blood series. Whatever the host cell may be, the gametes and gametocytes never contain hematin pigment granules.

Life Cycle: The life cycle has been studied by O'Roke (1934), Huff (1942), Fallis, Davies and Vickers (1951), Fallis, Anderson and Bennett (1956) and Cowan (1955) among others. Birds become infected when bitten by a blackfly vector. The sporozoites enter the blood stream, invade various tissue cells, round up and become schizonts.

Two types of schizont occur in the duck. Hepatic schizonts 11 to 18 μ in diameter occur in the liver cells; they form a number of cytomeres which in turn form small merozoites by multiple fission.

Megaloschizonts 60 to 164 μ in diameter when mature are found in the brain, lung, liver, heart, kidney, gizzard, intestine and lymphoid tissues 4 to 6 days after exposure. They are more common than the hepatic schizonts. The megaloschizonts develop in cells, possibly lymphoid cells or macrophages, within or outside the blood vessels. They contain numerous cytomeres and a large, conspicuous central body which may be either a primordium off of which the cytomeres

have budded (Cowan) or perhaps a hypertrophied host cell nucleus (Huff).

According to Cowan, spherical primary cytomeres are first formed. Their chromatin first diffuses and then proliferates to form peripheral clusters, which separate to form secondary cytomeres, which in turn multiply in the same manner. The multiplying cytomeres become smaller and more granular, their chromatin becomes more concentrated, and finally merozoite-like bodies are formed. These reproduce until the central body is greatly compressed and the megaloschizont membrane is ruptured, releasing the merozoites into the blood. Many thousands of bipolar merozoites are produced by each megaloschizont.

In addition to the hepatic schizonts and megaloschizonts, small structures thought to be schizonts were found by R. C. Ritchie (cited by Fallis, Anderson and Bennett, 1956) in the Kupffer cells of the liver of a duck killed 3 days after exposure.

On the basis of these observations, Fallis, Anderson and Bennett postulated the following life cycle: The first asexual generation occurs in the Kupffer cells of the liver. Some of the merozoites from these schizonts may develop into gametocytes; this explains the presence of a few large parasites in the blood 5 to 6 days after infection. Other merozoites from the first generation schizonts develop into hepatic schizonts, megaloschizonts and perhaps other Kupffer cell schizonts. Merozoites arising from megaloschizonts and hepatic schizonts develop into gametocytes which flood into the peripheral circulation beginning 6 to 7 days after infection. Some of these merozoites presumably develop into another asexual generation.

The development of the gametocytes in the blood cells has already been mentioned in the section on morphology. According to Chernin (1952), the gametocytes may disappear from the blood about 30 days after they first appear. Following this primary parasitemia, which begins in mid-summer in northern Michigan, only an occasional parasite is seen in the blood during the fall and winter (O'Roke, 1934; Huff, 1942; Chernin, 1952a). With the development of sexual activity in the spring, gametocytes reappear in the blood and in some cases continue to be present thruout the summer.

It is clear from this account that schizogony continues in the internal organs for an indefinite, long time, altho at a much reduced rate. There are about 1000 times fewer gametocytes in the relapse phase than in the primary infection, and these adult birds are not seriously affected. However, they serve as the source of infection for the new crop of ducklings.

According to Chernin (1952a), the early season infections in ducklings are comparatively light, but the heavier pool of gametocytes provided by these primary infections in the first crop ducklings ensures the heavier and highly fatal infections which occur during midsummer.

The vectors of *L. simondi* are various species of blackflies (*Simulium*). O'Roke (1934) showed that *S. venustum* is the vector in Michigan. Fallis, Anderson and Bennett (1956) found that *S. croxtoni* and *S. euryadminiculum* are the important vectors during the early part of the blackfly season (May to June) in Ontario, while *S. rugglesi* is the important vector in late June and July.

In the blackfly's stomach (O'Roke, 1934; Fallis, Davies and Vickers, 1951; Rawley, 1953), 4 to 8 microgametes are formed within a few minutes by exflagellation from the microgametocytes. These fertilize the macrogametes to form a motile zygote or ookinete about 33 μ long and 5 μ wide. Ookinetes are present in the blackfly stomach 2 to 6 hours after ingestion of infected blood. They develop into oocysts both in the stomach wall and in the stomach itself.

The oocysts are 10 to 13 μ in diameter. They can be found 2 to 3 days after infection, and complete their development 2.5 to 4 days after infection. They produce relatively few sporozoites compared with

Plasmodium. The sporozoites are 5 to 10 μ long, slender, with one end rounded and the other pointed. They break out of the oocysts and pass to the salivary glands, where they accumulate. Viable sporozoites can be found for at least 18 days after infective feeding.

Pathogenesis: *L. simondi* is markedly pathogenic for ducks and geese. The heaviest losses occur among young birds. O'Roke (1934) reported mortalities of 35%, 57% and 85% among young ducks in 3 different years in Michigan, but noted that the death rate among adults was very low. Knuth and Magdeburg (1922) and Stephan (1922) described serious outbreaks in young geese in Germany. According to Chernin (1952b), about 68% of the deaths in ducklings occur 11 to 19 days after exposure.

Briggs (1960) found that Muscovy ducklings were more resistant to *L. simondi* infections than white Pekin ducklings under conditions of natural exposure in Michigan. Altho both became readily infected, the mortality and number of sexual forms in the blood were much lower among the Muscovies than the white Pekins. In addition, deaths were delayed in the Muscovies.

The outstanding feature of an outbreak of leucocytozoonosis is the suddenness of its onset. A flock of ducklings may appear normal in the morning, may become ill in the afternoon, and may be dead by the next morning. Acutely affected ducklings are listless and do not eat. Their breathing is rapid and labored due to obstruction of the lung capillaries with schizonts. They may go thru a short period of nervous excitement just before death. Adult birds are more chronically affected. They are thin and listless, and the disease develops more slowly in them. If they die at all, it is seldom in less than 4 days after the appearance of signs. Ducklings which have recovered often fail to grow normally. Recovered birds, as mentioned above, remain carriers.

The principal lesions of leucocytozoonosis are splenomegaly and liver hypertrophy and degeneration. Anemia and leucocytosis are present, and the blood clots poorly. Cowan (1957) described the tissue reactions of infected ducks against the megaloschizonts. These include destruction by phagocytes and inflammatory cells, necrosis and possibly encapsulation.

Diagnosis: Leucocytozoonosis can be diagnosed by finding and identifying the gametocytes in stained blood smears or the schizonts in tissue sections.

Treatment: No effective treatment is known. Fallis (1948) found that quinacrine, sulfamerazine and chlorguanide were ineffective.

Prevention and Control: Prevention depends upon blackfly control--ordinarily a difficult task--or on raising ducks and geese under conditions which prevent them from being bitten by blackflies. In blackfly areas this means raising them in screened quarters. Blackflies pass readily thru ordinary, 16 mesh per inch window screening, and 32 to 36 mesh screen is needed to keep them out. Since this type of screening is expensive, a good grade of cheesecloth has been recommended for a single season's use.

This disease can be avoided entirely by raising ducks and geese in regions where blackflies do not occur in significant numbers. Since wild ducks and geese are reservoirs of infection for domestic birds, the latter should not be raised close to places where wild waterfowl congregate.

LEUCOCYTOZOON SMITHI LAVERAN AND LUCET, 1905

Disease: Leucocytozoonosis.

Hosts: Domestic and wild turkeys.

Location: The gametocytes are in the leucocytes. Schizogony occurs in the liver.

Geographic Distribution: North America, Europe (France, Germany, Crimea).

Prevalence: This species is common in northern and southeastern United States, along the Gulf Coast and Pacific Coast and in Canada in mountainous or hilly areas wherever cold, rapid streams permit suitable blackfly vectors to breed. It was first seen by Smith (1895) in eastern United States, and has been encountered in North Dakota, Minnesota, Nebraska, Wisconsin, Illinois, Virginia, South Carolina, Georgia, Alabama, Florida, Pennsylvania, Missouri, Texas, California, Ontario and Manitoba (Volkmar, 1929; Skidmore, 1932; Johnson, 1942, 1945; Johnson *et al.*, 1938; Hinshaw and McNeil, 1943; Banks, 1943; Stoddard, Humlin and Cooperrider, 1952; Travis, Goodwin and Gambrell, 1939; Mosby and Handley, 1943; Wehr and Coburn, 1943; Kozicky, 1948; West and Starr, 1940; Atchley, 1951; Bierer, 1950; Simpson Anthony and Young, 1956; Savage and Isa, 1945; Fallis, Pearson and Bennett, 1954).

Travis, Goodwin and Gambrell (1939) found it in 81% of 357 adult domesticated turkeys in Georgia, Florida, Alabama and South Carolina. Mosby and Handley (1943) found it in 40% of 268 captivity-reared wild turkeys, wild turkeys and domestic turkeys in Virginia. Kozicky (1948) found it in 21% of 92 captivity-reared and all of 5 native wild turkeys in Pennsylvania. Atchley (1951) found it in all of 10 domestic turkeys in South Carolina.

Morphology: The mature macrogametes and microgametocytes are rounded at first but later become elongate, averaging 20 to 22 μ in length. Their host cells are elongate, averaging 45 by 14 μ, with pale cytoplasmic "horns" extending out beyond the enclosed parasite. The host cell is elongate, forming a long, thin, dark band along one side of the parasite; often it is split to form a band on each side of the parasite. The cytoplasm of the macrogametes is rather dark blue and the nucleus compact and red when stained with a Romanowsky stain. The cytoplasm of the microgametocytes is very pale blue and the nucleus diffuse and pale pink.

Life Cycle: The life cycle of *L. smithi* is similar to that of *L. simondi*, but is not known in nearly so much detail. The prepatent period is about 9 days. Newberne (1955) and Richey and Ware (1955) described hepatic schizonts in the liver parenchyma of infected turkeys. According to Newberne, they measure 10 to 20 by 7 to 14 μ, with a mean of 13.5 by 10.5 μ. The earliest stage he saw contained round and crescent-shaped, basophilic cytomeres. These developed into masses of deeply staining merozoites which completely filled the host cell cytoplasm. Megaloschizonts have not been seen.

The vectors of *L. smithi* are various species of blackflies (*Simulium*). Skidmore (1932) found that *S. occidentale* transmitted it in Nebraska, Johnson *et al.* (1938) found *S. nigroparvum* to be the vector in Virginia, and Richey and Ware (1955) showed that *S. slossonae* could transmit it in South Carolina. The stages in the blackflies are similar to those of *L. simondi*.

Pathogenesis: *L. smithi* is markedly pathogenic for turkeys, and extremely heavy losses have been reported. Savage and Isa (1945) described an outbreak in Manitoba in which more than 3000 out of 8000 birds died in 2 months. Not more than 10% of the birds which became ill recovered. Stoddard, Humlin and Cooperrider (1952) described an outbreak in Georgia in which 75% of 1600 5-month-old turkeys died within a week. Adult birds are less seriously affected than poults, and the disease runs a slower course in them, but even they may die.

Affected poults fail to eat, appear droopy and tend to sit. They move with difficulty when disturbed; in the later stages there may be incoordination, and the birds may suddenly fall over, gasp, become comatose and die. If the birds do not die within 2 or 3 days after signs of disease appear, they recover.

Recovered birds continue to carry parasites in their blood. Some may show no serious after-effects, but others develop a chronic type of the disease. They never regain their vigor, and the males pay little attention to the females and rarely strut. They often have moist

tracheal rales, and cough and repeatedly try to clear their throats when disturbed. They may die suddenly under stress caused by undue excitement or handling.

The spleen and liver of affected birds are enlarged, and the duodenum is more or less inflamed. This enteritis may sometimes extend thruout the small intestine. The birds are anemic and emaciated, their flesh is flabby, and their muscles may be brownish. There are no gross lesions in adult carriers, but the liver may occasionally be icteric, enlarged and cirrhotic. Newberne (1955) saw no local tissue reaction around the hepatic schizonts, but noted hepatic hemosiderosis and lymphocytic infiltration.

According to Johnson *et al.* (1938), death is due to obstruction of the circulatory system by large numbers of parasites.

Diagnosis, Treatment, Prevention and Control: Same as for *L. simondi*.

LEUCOCYTOZOON CAULLERYI MATHIS AND LEGER, 1909

Synonyms: *Leucocytozoon andrewsi* Atchley, 1951; *Leucocytozoon schueffneri* Prowazek, 1912 pro parte.

Hosts: Chicken.

Location: The gametocytes are in the leucocytes and erythrocytes.

Geographic Distribution: Indochina, Malaya, India, Sumatra, North America (South Caroiina).

Prevalence: This species is relatively uncommon except perhaps in Malaya. Atchley (1951) found it in 15% of 400 adult domestic chickens in South Carolina, but his is the only report of it in North America. It has been found in Indochina by Mathis and Leger (1909), in Sumatra by Prowazek (1912), in Malaya by Kuppusamy (1936), and in India by Ramanujachari and Alwar (1953), Ramaswami (1955), and Biswal and Naik (1958). In addition, Hamerton (1929) reported a *Leucocytozoon* without describing it from a domestic chicken and a jungle fowl (*Gallus lafayettei*) in the London zoo.

Morphology: The mature gametocytes are round, measuring 15.5 by 15.0 μ according to Mathis and Leger (1909). According to Atchley (1951) the macrogametes are 12 to 14 μ in diameter with a nucleus generally 3 to 4 μ in diameter, and the microgametocytes are 10 to 12 μ in diameter with a nucleus 10 to 12 μ in diameter occupying most of the cell. The host cell is round, about 20 μ in diameter according to Mathis and Leger and 13 to 17 μ in diameter according to Atchley. The host cell nucleus forms a narrow, dark band extending about a third of the way around the parasite. The macrogametes stain more darkly with Romanowsky stains than the microgametocytes.

Life Cycle: Unknown. Atchley (1951) described exflagellation of the microgametocytes, and figured one with what appeared to be 6 microgametes.

Pathogenesis: This species is presumably pathogenic, but accounts of it have been so mixed up with those of *L. sabrazesi* (see below) that its pathogenicity is uncertain.

Remarks: Another species of *Leucocytozoon*, *L. sabrazesi*, with elongate gametocytes, has been described from the chicken. There has been a good deal of uncertainty as to whether *L. caulleryi* may not be merely an immature stage of *L. sabrazesi*. Many of the infections which have been seen have been mixed ones. However, Mathis and Leger, who first described both species, found pure infections of each, and Atchley found only round forms in the 61 infected chickens which he studied, some of which he kept under observation for a year. In addition, Atchley's observation of exflagellation leaves no doubt that the round forms are mature.

LEUCOCYTOZOON SABRAZESI MATHIS AND LEGER, 1910

Synonyms: *Leucocytozoon schueffneri* Prowazek, 1912 pro parte.

Hosts: Chicken.

Location: The gametocytes are in the leucocytes and erythrocytes.

Geographic Distribution: Indochina, Malaya, India, Sumatra, Java.

Prevalence: Relatively uncommon except perhaps in Malaya. *L. sabrazesi* has been found in Indochina by Mathis and Leger (1910), in Malaya by Kuppusamy (1936), and in India by Ramanujachari and Alwar (1953), Ramaswami (1955) and Biswal and Naik (1958). In addition, de Haan (1911) reported a *Leucocytozoon* in the chicken in Java which he assigned to *L. nearei* (a species with elongate gametocytes occurring in the guinea fowl) but which was undoubtedly *L. sabrazesi*.

Morphology: The mature gametocytes are elongate and measure about 24 by 4 μ according to Mathis and Leger (1910). According to Ramanujachari and Alwar (1953), the macrogametes average 22 by 6.5 μ and the microgametocytes 20 by 6 μ. The host cells are spindle-shaped, with long, cytoplasmic "horns" extending beyond the parasites, and measure about 67 by 6 μ according to Mathis and Leger (1910). The host cell nucleus forms a narrow, darkly staining band along one side of the parasite. The macrogametes stain more darkly with Romanowsky stains than the microgametocytes, and have a more compact nucleus.

Life Cycle: Unknown.

Pathogenesis: According to Kuppusamy (1936), this species causes a disease in chickens characterized by anemia, pyrexia, diarrhea, paralysis of the legs and a ropy discharge from the mouth. Ramanujachari and Alwar (1953) observed similar signs in the bird they studied.

Remarks: Prowazek (1912) gave the name *L. schueffneri* to the forms he found in the chicken in Sumatra. He saw and illustrated both spindle-shaped and round host cells, but gave dimensions only for the spindle-shaped ones. These ranged in length from 45 by 66 μ. He also observed granules in the host cell cytoplasm which stained red with Giemsa's stain. He stated that these granules were partially missing in *L. caulleryi* and *L. sabrazesi* and that he was establishing his new species because of this and also because of the difference in size between them and his form. However, the dimensions he quoted for *L. sabrazesi* were those of the parasite itself and not those of the host cell, and the dimensions he gave for *L. schueffneri* were those of the host cell and not those of the parasite itself. There is actually no significant difference in size between the two forms, and Prowazek's name becomes a synonym of *L. sabrazesi* and also, in part, of *L. caulleryi*. Prowazek also saw *Trypanosoma* in the same chicken, and thought it was a stage of *Leucocytozoon*.

The type of cell parasitized by *Leucocytozoon* has been the subject of some discussion (see under *L. simondi*, p. 276). The host cells containing mature gametocytes are so distorted as to be unrecognizable. Both Ramanujachari and Alwar (1953) and Ramaswami (1955) considered them to be erythrocytes. In the slide sent to me by Biswal, I saw one very young parasite in a cell which appeared to be an erythrocyte, but the host cells of other, somewhat older parasites did not appear to be. Further study is needed on this point. At any rate, the parasites do not form hematin granules from hemoglobin.

LEUCOCYTOZOON MARCHOUXI MATHIS AND LEGER, 1910

Synonyms: *Leucocytozoon turtur*.

Hosts: Various doves and pigeons. Levine (1954) and Levine and Kantor (1959) assembled reports of *Leucocytozoon* from 17 species of 7 genera of columbiform birds. All but one were probably *L. marchouxi*. There is only a single report of this species in the domestic pigeon, by Jansen (1952) in South Africa.

Location: The gametocytes are in the white blood cells.

Geographic Distribution: Worldwide.

Prevalence: This species is fairly common in wild doves. Hanson *et al.* (1957), for example, found it in 1.2% of 392 immature and 6.5% of 72 adult mourning doves (*Zenaidura macroura*) in Illinois.

Morphology: Levine (1954) redescribed this species. The macrogametes are rounded or elliptical, 8 to 15 by 7 to 11 μ with a mean of 12 by 9 μ; they stain dark blue with Giemsa's stain and have a compact, reddish nucleus. The microgametocytes are often distorted or ruptured by the smearing process, but if not badly damaged measure 8 to 15 by 5 to 11 μ with a mean of 11 by 8 μ. They stain pale blue with Giemsa's stain and have a very diffuse, pale pink nucleus. Host cell cytoplasm is rarely seen surrounding the microgametocytes and was found in only 26% of the cells parasitized by macrogametes. When present, it forms a narrow border around part of all of the parasite's periphery. The host cell nucleus forms a dark-staining band along about 1/3 of the periphery of the parasite.

Young gametocytes were seen only in lymphocytes or, in one case, in a monocyte.

Life Cycle: Unknown.

Pathogenesis: Unknown. There were no signs of illness in the infected mourning doves seen by Levine (1954), even though 4 of them were nestlings and 1 was only 14 days old.

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Chapter 11

THE PIROPLASMASIDA

Members of this class constitute a fairly cohesive group of blood cell parasites of vertebrates. They are small in comparison with the Plasmodiidae. They are piriform, round, amoeboid or rod-shaped, depending in part on the genus. They occur in the erythrocytes, and some genera occur in the leucocytes or histiocytes as well. Pigment (hemozoin) is not formed from the host cell hemoglobin. No spores are formed, and no flagella or cilia are present. Locomotion is by body flexion or gliding. Reproduction is asexual, by binary fission or schizogony. Budding has also been said to occur, but the processes described under this name are actually binary fission with the formation of 2 daughter cells or schizogony with the formation of 4. The existence of sexual reproduction is dubious, altho it has been described by earlier authors. The Piroplasmasida are heteroxenous; the known vectors are ixodid or argasid ticks.

The systematic position of this group is still uncertain, and varies with the authority. The position given it here seems reasonable.

There is a single order, Piroplasmorida. It contains 2 families, both of which contain parasites of domestic animals.

FAMILY BABESIIDAE

Members of this family are relatively large, piriform, round or oval parasites. They occur in the erythrocytes, where they reproduce asexually by binary fission or schizogony. The vectors are ixodid or argasid ticks. Binary fission, schizogony and sexual reproduction have been described in the tick, but the existence of sexual reproduction is dubious, and Reichenow (1953) believed that schizogony is simulated by repeated binary fissions.

By far the most important genus in the family is *Babesia*, species of which

cause piroplasmosis or babesiosis, a group of highly fatal and economically important diseases of livestock. Other genera are *Echinozoon* and *Aegyptianella*.

Genus *BABESIA* Starcovici, 1893

In this genus the trophozoites multiply by binary fission in the erythrocytes, forming pairs, or by schizogony, forming tetrads. A "blepharoplast" from which a rhizoplast arises has been described in the trophozoites. However, Rudzinska and Trager (1960) did not report seeing either of these structures in electron micrographs of *Babesia rodhaini* from the mouse.

If present, the blepharoplast and rhizoplast may betray a flagellate origin for the group (Dennis, 1932). However, Reichenow (1953) thought that it originated from the amoebae. Another possibility is that it is related to *Plasmodium*. This is suggested by Rudzinska and Trager's (1960) finding in *B. rodhaini* of structures composed of concentric membranes (possibly representing primitive mitochondria) similar to those they had previously seen in *Plasmodium berghei*, and by their observation that *B. rodhaini* apparently engulfs bits of host cell cytoplasm by phagotrophy like *Plasmodium*.

There are two opposing schools of thought as to the speciation of this genus. One breaks it up into several genera or subgenera, each with a number of species (e.g., Sergent *et al.*, 1945; Antipin *et al.*, 1959), while the other prefers a single genus with a relatively small number of species, each of which may include several strains (e.g., Wenyon, 1926; Neitz, 1956). The second system seems preferable. The taxonomy of the group has been discussed by Reichenow (1953), Poisson (1953) and Laird and Lari (1957) in addition to the above authors.

Synonyms of *Babesia* include *Piroplasma*, *Achromaticus*, *Nicollia*, *Nuttallia*, *Smithia*, *Rossiella*, *Microbabesia*, *Babesiella*, *Francaiella*, *Luhsia*, *Pattonella*, *Rangelia*, and *Gonderia* in part.

For practical purposes, one can divide the genus into 2 groups of species, large forms more than 3μ long and small forms less than 3μ long. In general, infections with the large forms can be successfully treated with trypan blue, while infections with the small ones cannot.

Babesia and babesiosis occur in most parts of the world where there are ticks, except in countries such as the United States where they have been wiped out by a concerted effort. They are most important in the tropics, where, along with the trypanosomoses, they often dominate the livestock disease picture. However, they also occur in the temperate zone. Bovine babesiosis nearly reaches the Artic circle in Norway, and Thambs-Lyche (1943) reported that it was increasing in that country.

Babesiosis was once an extremely important disease of cattle in the United States, but it has now been eliminated, and the only domestic animal species left in this country is *B. canis*, which occurs in dogs in Florida, Virginia and Texas. However, *Babesia* is still important in livestock in Central and South America. It occurs in most of Europe, being especially important in the countries bordering the Mediterranean Ocean. It is one of the most important diseases of livestock in the Middle East, thruout Africa, and also in parts of India and the Far East. Its importance in the USSR, and especially in its southern part, is attested by the fact that 61% of the protozoan section of Antipin *et al.*'s (1959) textbook on veterinary parasitology is devoted to it and a related disease, theileriosis. It also occurs in Australia.

Veterinarians and livestock owners in the United States today do not know what it is to have to contend with babesiosis, but other parts of the world are not so fortunate. The disease is of great economic importance in the tropics and subtropics; indeed, Curasson (1943) believed that it was no exaggeration to say that the babesioses are the most formidable diseases of livestock in these regions and that they are taking a more and more

important place in the animal disease picture as we discover new manifestations of their activity

Among recent discussions of babesiosis and its manifestations are those by Curasson (1943), Sergent *et al.* (1945), Muromtseva and Dobrokhotova (1955), Henning (1956), Malherbe (1956) and Antipin *et al.* (1959).

Life Cycle: The trophozoites of *Babesia* occur in the erythrocytes, where they multiply by binary fission or by schizogony. In some species, two trophozoites are formed, which break out of the erythrocytes and enter new red cells, while in others tetrads composed of 4 trophozoites are formed. Some authors place the latter in a separate genus, *Nuttallia*. The formation of more than 4 trophozoites by schizogony has also been described in the erythrocytes (Dschunkowsky, 1937; Ivanic, 1942; Delpy, 1946), but most workers (e.g., Reichenow, 1953) consider that it is merely simulated by repeated binary fissions or by multiple invasion of a host cell.

The above asexual cycle continues indefinitely, the animals sometimes remaining infected for life.

Babesia is transmitted by ticks. The discovery of this fact--by Smith and Kilborne (1893) for *B. bigemina* of cattle--was a milestone in the history of parasitology, since it was the first demonstration that an arthropod was the vector of any disease.

Dennis (1932) described sexual reproduction of *B. bigemina* from cattle in the tick, *Boophilus annulatus*, and Petrov (1941) did the same for *B. bovis* in *Ixodes ricinus*. However, Regendanz and Reichenow (1933) denied its existence in the life cycle of *B. canis* from the dog in *Dermacentor reticulatus*, and Regendanz (1936) and Muratov and Kheisin (1959) found no evidence of sexual reproduction in *B. bigemina* in *Boophilus microplus* and *B. calcaratus*, respectively, nor could Polyanskii and Kheisin (1959) for *B. bovis* in *Ixodes ricinus*. It is likely that Dennis and Petrov may have been misled by trying to draw an analogy with the life cycle of *Plasmodium*. Pending final settlement of the question, however, both accounts are given below.

According to Regendanz and Reichenow (1933), most of the *B. canis* ingested by the female tick die in its intestine. Some of them become vermiform and enter the intestinal epithelial cells, coming to lie against the basal membrane, and grow into large amoeboid forms. These then multiply by a series of binary fissions, producing more than 1000 individuals in 2 to 3 days. These lie together loosely at first, but finally fill the whole host cell. They then become vermiform and pass into the body cavity.

The vermiform stages are broadly rounded at the anterior end and pointed posteriorly, about 16 μ long, and have a gliding motion. They enter the ovary, where they penetrate the eggs. Here they round up and divide a few times, forming very small round individuals. They do not develop further in the larval tick which hatches from the egg, but when it molts they enter the salivary glands and continue their development. This first occurs in the nymphal stage, but is much more active in the adults, both male and female. The parasites undergo a series of binary fissions and enter the cells of the glandular acini. Here they multiply further, becoming smaller and filling the whole host cell, so that it finally contains thousands of minute parasites. These become vermiform, break out of the host cell, come to lie in the lumen of the gland, and are injected into the host when the tick sucks blood. The developmental process in the salivary glands takes 2 to 3 days.

The tick larvae are not able to infect new hosts. The nymphs may do so, but generally it takes so long for the parasites to reach the salivary glands that most transmission, in this species at least, is by the adults.

Regendanz (1936) found that the development of *Babesia bigemina* from cattle in the intestinal wall of *Boophilus microplus*

corresponded completely with that of *B. canis* in *Dermacentor reticulatus*. After numerous binary fissions, the protozoa turn into the motile, vermiform stage and enter the developing eggs of the female tick, where development continues. He found no evidence of sexual stages.

Muratov and Kheisin (1959) described a similar process for *B. bigemina* in *Boophilus calcaratus*, except that they said that schizogony occurs. They studied only the stages in the females and their eggs. On the day after the tick drops from its host, the protozoa begin to reproduce in its intestine by binary fission or by schizogony, producing club-shaped forms which penetrate into the epithelial cells of the intestine. Here they develop and undergo atypical multiple fission, characterized by asynchronous segmentation, into amoeboid or round agamonts. These become club- or cigar-shaped, penetrate other intestinal cells and repeat the asexual cycle. The dividing stages in the intestinal cells are up to 30 to 45μ in diameter and produce about 250 daughter parasites.

Some of the club-shaped stages enter the body cavity and divide further. They penetrate all the organs of the female, including the ovary, and continue to divide. In the ovary they enter the eggs and divide by binary or multiple fission just as in the intestine, producing round or amoeboid agamonts which turn into club-shaped stages. Their number increases as the eggs develop, and they are distributed thruout the organs of the developing larvae. Muratov and Kheisin found no evidence of copulation or sexual reproduction.

Polyanskii and Kheisin (1959) found essentially the same pattern for *B. bovis* in *Ixodes ricinus*. They said that it reproduces by binary fission or schizogony in the tissues of the tick and in the eggs of infected females, and found no stages of sexual reproduction or sporogony.

Quite a different process was described by Dennis (1932) for *B. bigemina* in *Boophilus annulatus*. According to him, when a female tick ingests blood, most of the parasites in the blood are destroyed, but some of them turn into vermiform bodies about 6μ long which he considered to be gametes and which he called isogametes because they all look alike. They move actively by bending or gliding. Two of them unite to form a motile, club-shaped zygote or ookinete 7 to 12μ long. The ookinetes pass thru the intestinal wall, enter the ovaries and then the eggs. Here they round up to form sporonts 7.5 to 12μ in diameter. The sporonts grow, and then divide by multiple fission into 4 to 32 amoeboid sporoblasts. The nuclei of the sporoblasts divide repeatedly, forming small, multinucleate, amoeboid sporokinetes which are distributed thruout the tissues of the developing tick embryo. The sporokinetes vary in shape, being round, elongate, club- or ribbon-shaped, and may be as much as 15μ long. They contain a varying number of granular nuclei 0.4μ in diameter. In the course of the embryonal development of the tick, the sporokinetes multiply, probably by plasmotomy. All the tissues of the tick may be invaded, and sometimes the cytoplasm of a host cell is almost entirely supplanted by the parasites, particularly in the salivary glands. Toward the end of the tick's development, some sporokinetes produce sporozoites, which are the infectious stage. Others produce them only after the larva has hatched. The sporozoites resemble minature trophozoites; they are piriform and have a blepharoplast. They are particularly numerous in the salivary glands, in the coelenchymatous tissue at the base of the legs and around the viscera. They are inoculated into the blood with the saliva when the tick feeds.

Petrov (1941) described a similar process for *B. bovis* in *Ixodes ricinus*. According to him, the isogametes fuse in the tick's intestine to form an ookinete which passes thru the intestinal wall and enters a developing ovum. Here it rounds up, forms sporoblasts, and these in turn form sporozoites which pass to the salivary glands. The larvae, nymphs and adults of the succeeding generation can all transmit the parasite.

It should be said that Reichenow (1953) considered some of the stages described by Dennis to be normal, intracellular symbionts (cf. Buchner, 1953; Koch, 1956) rather than *Babesia*.

In the life cycles described above, the adult tick picks up the infection, but does not transmit it. This is done by the next generation. *Babesia* can also be transmitted by different stages in the same generation; it can be picked up by a larval tick and transmitted by the nymph, or it can be picked up by the nymph and transmitted by the adult. The occurrence of this stage-to-stage transmission depends upon both the species of *Babesia* and the species of tick. Neitz (1956) has assembled information on this subject, and it is given below in the discussion of the individual species.

The life cycle in the tick in stage-to-stage transmission was studied carefully by Shortt (1936) for *B. vogeli* (*B. canis*) of the dog in *Rhipicephalus sanguineus* in India. He saw no evidence of sexual stages. After the nymph has taken a blood meal, the parasites do not multiply in the gut epithelium, but in the phagocytes next to the hypodermis in the body cavity. Here they reproduce by multiple fission to form what Shortt called pseudocysts--clumps of up to 200 organisms contained within the envelope of the parasitized host cell. These are fully developed about 7 days after the nymph has left its host. They are 14 to 35 μ in diameter. The stages within these pseudocysts are at first more or less spherical and 1.7 to 3.3 μ in diameter. They become club-shaped in 4 to 8 more days, at which time they measure about 9 by 2 μ. The club-shaped stages then break out of the host cell and migrate to the muscles and muscle-sheaths. They penetrate the cells, round up, and divide by repeated binary fissions to form a large number of relatively small, ovoid or slightly elongate parasites about 1.2 μ long. This stage is reached about 20 days or more after the nymph has fed. This phase of the life cycle corresponds to that which takes place in the eggs of the adult.

The muscles remain unchanged during metamorphosis. When the adults begin to feed on a dog, the parasites migrate to the salivary glands and enter their cells. Development then continues as described by Reichenow and Regendanz (1933) for *B. canis*. The parasites multiply by repeated binary fissions to form large numbers of spherical or ovoid infective stages about 1.9 μ or less in diameter.

Pathogenesis: Babesiosis is a highly pathogenic disease in most hosts. It is unusual in that the death rate is much higher in adults than in young animals.

The various species of *Babesia* cause a similar disease in different hosts. In most cases there are fever, malaise and listlessness. Affected animals do not eat, or eat little. There is severe anemia, and destruction of the erythrocytes is accompanied by hemoglobinuria. The mucous membranes become pale, and icterus develops. The spleen is greatly enlarged, with soft, dark red pulp and prominent splenic corpuscles. The liver is enlarged and yellowish brown. The lungs may be slightly edematous. There may be diarrhea or constipation, and the feces are yellow except in very early or peracute cases. Affected animals lose condition, become emaciated, and often die.

The signs of babesiosis may vary markedly from this typical picture, however. As Malherbe (1956) said, "Anybody with extensive experience of these diseases...is forcibly struck by the deviate and protean manifestations of the disease picture as it is encountered from time to time. There is almost no guise under which the disease does not masquerade at some time or another, and it is therefore no accident that the majority of South African veterinarians have a pronounced attachment to their microscopes." Malherbe remarked on the similarity of the clinical and pathological manifestations of babesiosis to those of malaria, stating that "in spite of the differences in the life cycle of the parasites, their effect on the body is capable of exactly similar potentialities."

Death, if it occurs, is due to organic failure which, in turn, is due not only to the destruction of erythrocytes with resultant anemia, edema and icterus, but also to the clogging of the capillaries of various organs by parasitized cells and free parasites (Malherbe and Parkin, 1951;

Malherbe, 1956). The stasis resulting from this sludging (Knisely *et al.*, 1947) causes degeneration of the endothelial cells of the small blood vessels, anoxia, accumulation of toxic metabolic products, capillary fragility, and eventually perivascular escape of erythrocytes and macroscopic hemorrhage. Purpura may result, the great majority of such cases in dogs being due to babesiosis. The signs of the disease depend in part on the location where the most serious stasis takes place. Cerebral babesiosis similar to cerebral falciparum malaria may occur. Gilles, Maegraith and Andrews (1953) described liver damage in *B. canis* infections, beginning with early damming of the blood in the sinusoids around the central vein, thru centrilobular atrophy and degeneration of the hepatic cells, to necrosis of the cells. Kidney damage is also present.

Immunity: Cattle which have recovered from an attack of babesiosis due to *B. bigemina* remain infected for life, and are immune to reinfection. This type of immunity, due to continuing low-grade infection, is known as premunition. Premunition in cattle due to species other than *B. bigemina*, and in sheep, swine and dogs, lasts up to 2 years. Premunized animals do not show signs of disease except under stress of one sort or another. For instance, an attack of foot and mouth disease may reactivate babesiosis in cattle, and distemper may do the same in dogs.

The spleen plays an important role in maintaining immunity, and it is a common observation that splenectomy is often followed by a severe or fatal relapse in premunized animals. In addition, splenectomized animals are much more susceptible to infection with *Babesia* and much more seriously affected than normal ones.

Calves, foals, young pigs and kids are much less seriously affected by babesiosis than are adult animals. This is the reason that cattle can often be raised in highly endemic areas without being seriously affected, whereas imported animals usually die. The native cattle were infected as calves and are premunized. Lambs and puppies, however, are highly susceptible.

There is no cross-immunity between the different species of *Babesia*.

Treatment: The treatment of babesiosis has been reviewed by Goodwin and Rollo (1955), Carmichael (1956) and Richardson and Kendall (1957), among others. There is an interesting relationship between the chemotherapy of babesiosis and that of trypanosomosis. Many of the compounds effective against *Trypanosoma* are also effective against *Babesia*. This may perhaps indicate a phylogenetic relationship, but I hasten to warn that a similar line of reasoning was once used to suggest a relationship between the trypanosomes and the spirochetes.

Nuttall and Hadwen (1909) introduced the first effective drugs, the azo-naphthalene dyes, trypan red and trypan blue. The latter is still used in some areas. It is the sodium salt of ditolyl diazo-bis-8-amino-1-naphthol-3,6-disulfonic acid. It must be given intravenously, since abscessation and sloughing follow subcutaneous injection. It stains the tissues blue-green for several months after injection. It does not eliminate all parasites, so that recovered animals are premunized.

The acridine derivative, acriflavine (trypaflavine, gonacrine, flavin, euflavin) was introduced by Stephan and Esquibel (1929). It is a mixture of 2,8-diamino-10-methylacridinium chloride with a small amount of 2,8-diaminoacridinium chloride. It, too, is still being used, especially against *B. equi* in South Africa and in cattle in North Africa. It does not eliminate all parasites, and recovered, treated animals are premunized.

The quinoline derivative, acaprin (Acapron, Pirevan, Babesan, Piroparv, Zothelone, Piroplasmin) was introduced by Kikuth (1935) and also by Carmichael (1935). It is 6,6'-di-(N-methylquinolyl) urea dimethosulfate. It is administered subcutaneously. In large doses it elim-

inates all parasites, but in small ones it leaves some so that recovered animals are premunized (Kikuth, 1938). It affects the parasympathetic nervous system, and may cause alarming reactions, including salivation, vasodilation, sweating, copious urination, diarrhea, panting, a drop in blood pressure and even collapse and death. Adrenaline and calcium gluconate can be given as antidotes. To avoid such reactions, the drug is often given in 2 or 3 divided doses a few hours apart. Dogs are much more sensitive than cattle. Animals showing reactions usually recover rather quickly. Despite these reactions, acaprin is still one of the most widely used drugs for treating babesiosis in all animals thruout the world.

Lourie and York (1939) found that a number of aromatic diamidines were effective against *Babesia*. Adler and Tchernomoretz (1940) found that stilbamidine (4, 4'-diamidinostilbene) was effective against *B. bigemina*, and *B. ovis*, and it is also used for *B. canis* and *B. caballi* (Daubney and Hudson, 1941). Propamidine (4, 4'-diamidino-1, 3-diphenoxypropane) has been used against *B. canis* in dogs (Carmichael and Fiennes, 1941). Pentamidine (lomidine; 4, 4'-diamidino-1, 5-diphenoxypentane) is used quite widely, especially in North Africa, for babesiosis in all animals. Phenamidine (4, 4'-diamidinodiphenyl ether) was introduced by Carmichael (1942) for canine babesiosis and is now used in cattle and other animals as well. Berenil (4, 4'-diamidino diazoaminobenzene diaceturate) was introduced by Bauer (1955), and is effective against babesiosis in cattle, dogs and other animals. Amicarbalide (M & B 5062A; 3, 3'-diamidinocarbanilide diisethionate) was introduced by Ashley, Berg and Lucas (1960). Preliminary studies indicate that it is effective against babesiosis in cattle (Beveridge, Thwaite and Shepherd, 1960; Lucas, 1960).

The diamidines are injected subcutaneously or intramuscularly, depending upon the compound. Many of them tend to cause a fall in blood pressure, but it soon returns to normal. Subcutaneous injection of concentrated solutions may cause irritation. Transitory swelling of the face and lips which is anaphylactic in nature sometimes occurs with phenamidine.

Prevention and Control: Since babesiosis is transmitted by ticks, prevention and control depend primarily on tick elimination. This can be done by regular dipping, which should be carried out on an area basis for livestock, at least. Dogs and riding horses can be treated individually.

Artificial premunization of young animals has been practiced with a good deal of success, especially in North Africa (Sergent *et al.*, 1945). A mild strain of the organism is ordinarily used. This practice is not necessary if the animals are raised in an endemic area where they will all become naturally infected at an early age, but it is worthwhile in areas where only a certain proportion of the animals become infected or for animals which are destined to be shipped to endemic areas later on.

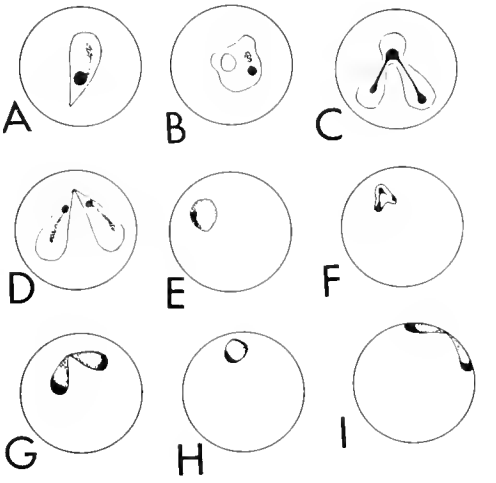

Fig. 34. Bovine species of *Babesia* in erythrocytes. A., B., C., D. *Babesia bigemina*. E., F., G. *Babesia bovis*. H., I. *Babesia divergens*. X 2800. (A., B., C., D. after Nuttall and Graham-Smith, 1908 in *Parasitology*, published by Cambridge Univ. Press; E., F., G., H., I. after Davies, Joyner and Kendall, 1958 in *Annals of Tropical Medicine and Hygiene*, published by Liverpool School of Tropical Medicine).

BABESIA BIGEMINA
(SMITH AND KILBORNE, 1893)

Synonyms: *Pyrosoma bigeminum*, *Apiosoma bigeminum*, *Piroplasma bigeminum*, *Piroplasma australe*, *Babesia ludsonius bovis*.

Disease: Bovine babesiosis, piroplasmosis, redwater, Texas fever.

Hosts: Ox, zebu, water buffalo, deer (*Mazama americana reperticia*) (syn., *M. sartorii reperticia*), white-tailed deer (*Odocoelus virginianus chiriquensis* (syn., *O. chiriquensis*).

Location: Erythrocytes.

Geographic Distribution: Central America, South America, Europe, North, Central and South Africa, Australia, formerly North America (U.S.).

Prevalence: This species causes one of the most important diseases of cattle in the tropics and subtropics.

Morphology: The trophozoites in the erythrocytes are piriform, round, oval or irregularly shaped. The piriform trophozoites occur characteristically in pairs, a feature which gives the species its name. *B. bigemina* is relatively large. The round forms are 2 to 3 μ in diameter and the elongate ones 4 to 5 μ long.

Life Cycle: This has been described above (p. 287). The tick vectors are *Boophilus annulatus* in North America, *B. microplus* in South and Central America, *B. australis* in Australia, *B. calcaratus* in North Africa and the USSR, *B. decoloratus* in South Africa, *Haemaphysalis punctata* in Europe, *Rhipicephalus appendiculatus* and *R. evertsi* in South Africa, and *R. bursa* in North Africa. Transmission takes place thru the egg in all species; stage-to-stage transmission also takes place in *Haemaphysalis* and *Rhipicephalus*.

Intrauterine transmission may also take place (Enigk, 1942).

Pathogenesis: *B. bigemina* is highly pathogenic for adult animals but much less so for calves. The incubation period is 8 to 15 days or less. The first sign of disease is a rise in temperature to 106 to 108 F. The temperature persists for a week or more. Affected animals are dull, listless, fail to eat and stop ruminating. The feces are yellowish brown. Severe anemia is caused by the invasion and destruction of the erythrocytes; up to 75% of them may be destroyed. Hemoglobinuria is ordinarily present, but may be absent. Affected animals become thin, emaciated and icteric. In chronic cases the temperature is not very high and there is usually no hemoglobinuria, but diarrhea or constipation with hard, yellowish feces is present.

The initial febrile response is associated with the appearance of parasites in the peripheral blood.

Death may occur in 4 to 8 days in acute cases. The mortality is as high as 50 to 90% in untreated cases, but treatment reduces it markedly. Calves less than a year old are seldom seriously affected.

Chronically affected animals lose condition quite rapidly and remain thin, weak and emaciated for weeks before finally recovering.

The principal lesions are splenomegaly with soft, dark red splenic pulp and prominent splenic corpuscles. The liver is enlarged and yellowish brown. The gall bladder is distended with thick, dark bile. The mucosa of the abomasum and intestine is edematous and icteric, with patches of hemorrhage. The subcutaneous, subserous and intramuscular connective tissues are edematous and icteric, and the fat is yellow and gelatinous. The blood is thin and watery, the plasma may be tinged with red, and the urine in the bladder is usually red.

Immunity: As mentioned in the general discussion of immunity, recovered cattle are premunized, and premunition due to latent infection persists for life.

Diagnosis: Fever associated with hemoglobinuria, anemia and icterus is suggestive of babesiosis. The diagnosis can be confirmed by finding *B. bigemina* by microscopic examination of stained blood smears.

Treatment: Trypan blue was the first effective drug used against babesiosis, and is still used in some areas. It is administered intravenously in 1 to 2% aqueous solution; up to 200 ml may be given at a time. Two treatments on successive days may be needed, but 1 is often enough. The tissues turn blue, and recovery is relatively slow.

Acriflavine (trypaflavine) is also used to some extent, 50 to 100 ml of a 1% aqueous solution being given intravenously. Neither acriflavine nor trypan blue eliminates all parasites, and recovered animals remain premunized.

A number of aromatic diamidines are effective against *B. bigemina*. Stilbamidine was found by Adler and Tchernomoretz (1940) to be effective in calves when injected subcutaneously at a dosage of 2 to 4 mg per kg. Phenamidine is used quite widely. Randall and Laws (1947) gave 15 mg per kg phenamidine isethionate subcutaneously; the drug was well tolerated in doses up to 22.5 mg per kg. Berenil is the most recent of these drugs to be introduced (Bauer, 1955). It is injected intramuscularly at a dosage rate of 1 to 3 mg per kg body weight.

The quinoline derivative, acaprin, is also effective. The dosage for cattle is 0.02 ml per kg of a 5% aqueous solution subcutaneously.

The diamidines and acaprin eliminate all the parasites, so that treated animals are no longer premunized.

Prevention and Control: Since *B. bigemina* is transmitted only by ticks, infection can be prevented by tick control. This can be done by dipping the cattle regularly. This is the way in which Texas fever was eliminated from the United States.

Another measure which has been used is artificial premunization of young animals with a mild strain, especially before shipping them to endemic areas.

Remarks: Spindler *et al.* (1958) found a *Babesia* which resembled *B. bigemina* in a white-tailed deer (*Odocoileus virginianus couesi*) in New Mexico. The animal was weak and had lesions characteristic of babesiosis. Blood smears made from several other white-tailed deer, mule deer, cattle and a few antelope from the same region were negative, but this finding raises a question as to the existence of a possible reservoir of *Babesia* in wild deer in the southwestern states.

BABESIA BOVIS
(BABES, 1888)
STARCOVICI, 1893

Synonyms: *Haematococcus bovis*, *Piroplasma bovis*, *Babesiella bovis*, *? Babesiella berbera*.

Disease: Bovine babesiosis, piroplasmosis, redwater.

Hosts: Cattle, roe deer, stag.

Location: Erythrocytes.

Geographic Distribution: Europe, USSR, Africa.

Prevalence: This species is the most important cause of European babesiosis. It is common in many regions, but information on its true prevalence must await a decision as to whether *B. berbera* is a synonym and must also await new surveys in the light of the recent recognition that *B. divergens* is a separate species (see below).

Morphology: The trophozoites in the erythrocytes are piriform, round or irregular. Vacuolated "signet-ring" forms are especially common. *B. bovis* is a small form, with trophozoites measuring about 2.4 by 1.5 μ (Davies, Joyner and Kendall, 1958).

Life Cycle: The life cycle was described above (p. 287). The tick vectors are *Ixodes persulcatus* in the USSR and, according to Simitch, Petrovitch and Rakovec (1955), *Boophilus calcaratus* and *Rhipicephalus bursa* in Europe. The tick ordinarily considered the vector in Europe is *Ixodes ricinus*, but the species it actually transmits may be *B. divergens* (see below). Transmission takes place thru the egg in all ticks, and from stage to stage in *I. ricinus*.

Intra-uterine transmission has also been reported (Neitz, 1956).

Pathogenesis: The disease caused by *B. bovis* is similar to that caused by *B. bigemina*, but is not generally as severe. The incubation period is 4 to 10 days, and the first sign is a temperature of 104 to 106° F which usually lasts 2 to 3 days. Hemoglobinuria, anemia, icterus, diarrhea and rapid heart beat are present, and affected animals may die.

Immunity: Same as for *B. bigemina*, except that premunition does not last more than about 2 years. There is no cross-immunity between *B. bigemina* and *B. bovis*.

Diagnosis: Babesiosis due to *B. bovis* can be diagnosed on the basis of the history, clinical signs and presence of ticks together with identification of the parasites in stained blood smears. However, they are easily found only during the febrile period.

Treatment: In contrast to *B. bigemina*, *B. bovis* does not respond to trypan blue. Acaprin, acriflavine, phenamidine and berenil are effective, however; the same dosages are used as against *B. bigemina*.

Prevention and Control: Same as for *B. bigemina*.

Remarks: Recent work has reopened the question of synonymy in this and related species. *B. berbera* has generally been considered a separate species from *B. bovis*. It was thought to be the common small *Babesia* of North Africa, whereas *B. bovis* was thought to be primarily European. However, Simitch and Nevenitch (1953) and Simitch, Petrovitch and Rakovec (1955) found a *Babesia* in Yugoslavia just across the Danube River from the area where Babes (1888) has described *B. bovis* which corresponded completely with *B. berbera*. The latter authors also found another, morphologically different species in Yugoslavia which corresponded completely with *B. divergens*. This latter species had originally been described in England by M'Fadyean and Stockman (1911), and had generally been considered a synonym of *B. bovis*. Simitch, Petrovitch and Rakovec (1955) concluded that *B. berbera* is a synonym of *B. bovis* and that it occurs in North Africa and southern Europe in association with *Boophilus calcaratus* and *Rhipicephalus bursa*. They also concluded that the species which occurs in western and central Europe in association with *Ixodes ricinus* is not *B. bovis*, but *B. divergens*.

Davies, Joyner and Kendall (1958) compared a British strain of *Babesia* with a strain of *B. bovis* sent to them from Yugoslavia, and concluded that they were indeed morphologically different and that the correct name for the British strain was *B. divergens*.

Sergent, Donatien and Parrot (1954) felt that final proof as to the identity of *B. bovis* and *B. berbera* must await cross-immunity experiments. I am retaining both names for the present, with the strong suspicion that they are synonymous.

BABESIA BERBERA (SERGENT, DONATIEN, PARROT, LESTOQUARD, PLANTUREUX AND ROUGEBIEF, 1924)

Synonyms: *Babesiella berbera*, *Francaiella caucasica*, ? *Francaiella occidentalis*.

Hosts: Cattle.

Location: Erythrocytes.

Geographic Distribution: North Africa, USSR, probably southern Europe.

Morphology: Same as *B. bovis*.

Life Cycle: Same as *B. bovis*. The vectors in North Africa are *Boophilus calcaratus* and *Rhipicephalus bursa*. Transmission occurs thru the egg in the former and stage-to-stage in the latter. The vector in the USSR is *Ixodes ricinus*.

Pathogenesis: Same as *B. bovis*.

Treatment: Same as *B. bovis*.

Remarks: As mentioned above, *B. berbera* is probably a synonym of *B. bovis*.

BABESIA DIVERGENS
(M'FADYEAN AND STOCKMAN, 1911)

Synonym: *Piroplasma divergens*.

Hosts: Cattle, rarely man.

Location: Erythrocytes.

Geographic Distribution: Western and central Europe.

Prevalence: This is probably the commonest if not the only species of *Babesia* in western and central Europe, but further investigation is needed to confirm this statement. *B. divergens* is now definitely known to occur in Yugoslavia, Austria and England (Simitch, Petrovitch and Rakovec, 1955; Davies, Joyner and Kendall, 1958).

Morphology: This species is smaller than *B. bovis*. The trophozoites usually occur as paired, club-shaped organisms about 1.5 by 0.4 μ; the angle between the members of the pair is relatively large, so that they diverge more from each other than the trophozoites of *B. bovis;* in addition, they tend to lie along the circumference of the host erythrocyte (the so-called accolé position). Other trophozoites are stouter and piriform (about 2 by 1 μ), circular (about 1.5 μ in diameter), or vacuolated and circular (up to 2 μ in diameter) (Davies, Joyner and Kendall, 1958).

Life Cycle: Same as that of *B. bovis*. The vector tick is *Ixodes ricinus*.

Pathogenesis: Same as for *B. bovis*.

Immunity: Same as for *B. bovis*.

Diagnosis: Same as for *B. bovis*.

Treatment: Same as for *B. bovis*. Amicarbalide was found by Beveridge, Thwaite and Shepherd (1960) and Lucas (1960) to be effective against *B. divergens*. The dosage is about 5 to 20 mg per kg subcutaneously or intramuscularly.

Remarks: Skrabalo and Deanovic (1957) described a fatal human case of babesiosis accompanied by blackwater due to *B. divergens* in Yugoslavia. The patient had had a splenectomy 11 years before and lived on a tick-infested farm where the cattle had babesiosis.

Garnham and Bray (1959) infected 2 splenectomized chimpanzees and a splenectomized rhesus monkey with the British strain of *B. divergens* described by Davies, Joyner and Kendall (1958), but were unable to infect 2 splenectomized rabbits. The parasites in the rhesus monkey had the typical accolé form, but those in the chimpanzees did not. Garnham and Bray suggested that latent babesiosis might exist in man on a large scale in rural populations in infected regions.

BABESIA ARGENTINA
(LIGNIERES, 1903)

Synonyms: *Piroplasma argentinum, Francaiella argentina*.

Hosts: Cattle.

Location: Erythrocytes.

Geographic Distribution: South America, Central America, Australia.

Morphology: The trophozoites resemble those of *B. bovis*. They are piriform, about 2.0 by 1.5 μ, and usually lie in the center of the host erythrocyte.

Life Cycle: Similar to that of *B. bovis*. The vector in South America is *Boophilus microplus* and that in Australia

is *B. australis*. Transmission takes place thru the egg.

Pathogenesis: In Australia, *B. argentina* is more pathogenic than *B. bigemina* (Pierce, 1956). Daly and Hall (1955) found that the mortality in Australian cattle inoculated with *B. bigemina* was 30% and that of cattle inoculated with *B. argentina* was 70 to 80%. The clinical signs, lesions, etc. are similar in both diseases.

Immunity: Premunition following recovery from *B. argentina* infections lasts less than 2 years, and the minimum time at which cattle regain susceptibility is 5 to 6 months (Pierce, 1956). Cattle infected with *B. bigemina* are resistant to infection with *B. argentina* (Legg, 1935; Seddon, 1952), but those infected with *B. argentina* are susceptible to infection with *B. bigemina* (Seddon, 1952).

Diagnosis: Same as for *B. bovis*. The trophozoites can be found more easily in smears from the heart or kidney than in the peripheral blood.

Treatment: Same as for *B. bovis*.

BABESIA MAJOR
(SERGENT, DONATIEN, PARROT, LESTOQUARD AND PLANTUREUX, 1926)

Synonyms: *Babesiella major*, *Francaiella colchica*.

Hosts: Cattle.

Location: Erythrocytes.

Geographic Distribution: Europe, USSR.

Morphology: The trophozoites resemble those of *B. bovis*, but are larger. The piriform, paired forms measure 2.6 μ by 1.5 μ, and the round ones are 1.8 μ in diameter. The parasites lie in the center of the host erythrocyte.

Life Cycle: Similar to that of *B. bovis*. The vector in the USSR is *Boophilus calcaratus*.

Pathogenesis: This species is considerably less pathogenic than *B. bovis*. There is little fever, relatively slight anemia, and experimentally infected animals show no clear clinical signs of illness.

Immunity: This species can be differentiated from *B. bigemina* and *B. berbera* by cross-immunity studies.

Treatment: Same as for *B. bovis*. Trypan blue is ineffective against *B. major*.

BABESIA MOTASI
WENYON, 1926

Synonyms: *Haematococcus ovis* pro parte, *Piroplasma ovis*.

Hosts: Sheep, goats.

Location: Erythrocytes.

Geographic Distribution: Southern Europe, Middle East, USSR, Indochina, Africa, and other parts of the tropics.

Morphology: This is a large form, measuring 2.5 to 4 by about 2 μ. The trophozoites resemble those of *B. bigemina* and are usually piriform. They occur singly or in pairs; the angle between members of a pair is acute.

Life Cycle: Similar to that of *B. bigemina*. The vector in Roumania is *Rhipicephalus bursa*, that in Sardinia is *Haemaphysalis punctata*, and those in the USSR are *Dermacentor silvarum* and *Haemaphysalis otophila*. Transmission occurs both thru the egg and stage-to-stage in *R. bursa*.

Pathogenesis: This species may cause either an acute or chronic disease. Fever, prostration, marked anemia and hemoglobinuria are present in the acute disease, and affected animals often die. There may be no characteristic signs in the chronic disease.

Immunity: Sheep which are immune to *B. motasi* are not immune to *B. ovis* and vice versa.

Diagnosis: Same as for *B. bigemina*. The parasites are abundant in the peripheral blood during an attack.

Treatment: Trypan blue is effective against this species, as is acaprin. The latter is administered subcutaneously, 0.2 ml per kg of a 0.5% aqueous solution being given.

Prevention and Control: Same as for other species of *Babesia*.

BABESIA OVIS
(BABES, 1892) STARCOVICI, 1893

Synonyms: *Haematococcus ovis* pro parte, *Piroplasma ovis*, *Piroplasma hirci*, *Babesiella ovis*.

Hosts: Sheep, goats.

Location: Erythrocytes.

Geographic Distribution: Southern Europe, USSR, thruout the tropics and in some subtropical regions.

Morphology: This is a small species, about 1 to 2.5 μ long. Most of the parasites are round, and they usually lie in the margin of the host erythrocytes. The angle between the paired, piriform trophozoites is usually obtuse.

Life Cycle: Similar to that of *B. bovis*. The vectors in the USSR are *Rhipicephalus bursa* and *Ixodes persulcatus* (Rastegaeva, 1940).

B. ovis was found in 2 sheep fetuses by Donatien, Lestoquard and Kilcher-Maucourt (1934).

Pathogenesis: This species is less pathogenic than *B. motasi*, but it may cause fever, anemia and icterus. Usually not more than 0.6% of the erythrocytes are infected.

Immunity: There is no cross-immunity between *B. ovis* and *B. motasi*.

Diagnosis: Same as for *B. bovis*.

Treatment: Trypan blue is ineffective against *B. ovis*. Acaprin can be used in the same way as for *B. motasi*, but it is not as effective. Acriflavine is recommended, a single intravenous injection of 0.15 g being given.

BABESIA FOLIATA
RAY AND RHAGHAVACHARI, 1941

Host: Sheep.

Location: Erythrocytes.

Geographic Distribution: India.

Morphology: This species resembles *B. ovis*, but differs in being leaf-shaped and in lying more centrally in the host erythrocytes.

Life Cycle: The vectors are unknown.

Remarks: Richardson and Kendall (1957) considered this to be probably a synonym of *B. ovis*, but Neitz (1956) accepted it as a valid species.

BABESIA TAYLORI
(SARWAR, 1935)

Synonym: *Piroplasma taylori*.

Host: Goat.

Location: Erythrocytes.

Geographic Distribution: India.

Morphology: This is a small species, the trophozoites measuring about 2 by 1.5 μ when there is a single one per host cell, down to 1 μ or less in diameter when there are several. The trophozoites are mostly ovoid or round, rarely piriform. The host cell is enlarged. Division is by binary or quadruple fission. Many erythrocytes contain 8 or even 16 parasites, which Sarwar thought were produced by multiple fission. The host erythrocytes are enlarged. Extracellular dividing forms are common.

Life Cycle: Unknown.

Pathogenesis: According to Sarwar (1935), this species is probably pathogenic. Hemoglobinuria is not produced, however.

BABESIA CABALLI
(NUTTALL, 1910)

Synonym: *Piroplasma caballi.*

Hosts: Horse; transmissible to mule and donkey.

Location: Erythrocytes.

Geographic Distribution: Southern Europe thru Asia, USSR, North and South Africa, Central America.

Morphology: This is a large species, resembling *B. bigemina.* The trophozoites are piriform and 2.5 to 4 μ long, or round or oval and 1.5 to 3 μ in diameter. The piriform trophozoites are often found in pairs at an acute angle to each other.

Life Cycle: Similar to that of *B. bigemina.* The vectors in Europe and the USSR are *Dermacentor marginatus* (syn., *D. reticulatus*), *D. pictus, D. silvarum, Hyalomma anatolicum* (syn., *H. excavatum*), *H. marginatum* (syn., *H. detritum*), *H. volgense, Rhipicephalus bursa* and *R. sanguineus.* The vector in North Africa is *Hyalomma dromedarii.* Transmission thru the egg occurs in *D. marginatus, D. silvarum, H. marginatum, H. volgense, R. sanguineus* and *H. dromedarii.* Stage-to-stage transmission occurs in *D. marginatus, D. pictus, H. anatolicum, H. marginatum, R. bursa* and *R. sanguineus.*

B. caballi has also been found in fetuses (Neitz, 1956).

Pathogenesis: The symptomatology of this disease varies markedly. The disease may be either acute or chronic; in either case it may be relatively mild or severe, ending in death. Hemoglobinuria is rare, but fever, anemia and icterus are present. Gastro-enteritis is common. Locomotor signs are usually present, and posterior paralysis may occur. The incubation period is 7 to 19 days. In fatal cases death occurs a week to about a month after the appearance of symptoms.

Immunity: Young animals are less susceptible than old ones. There is no cross-immunity between *B. caballi* and *B. equi.*

Diagnosis: Because of the varied symptomatology, diagnosis depends upon identification of the parasites in stained blood smears. They are most numerous in the blood during the first febrile attack.

Treatment: Trypan blue is quite effective against *B. caballi,* but acaprin and acriflavine are better. Trypan blue is given intravenously, 50 to 75 ml of a 1% aqueous solution being injected. Acaprin is given subcutaneously, 1.2 ml of a 5% solution being injected per 100 kg. Acriflavine is injected intravenously, 20 ml of a 5% solution being given.

Prevention and Control: Same as for *B. bigemina.*

BABESIA EQUI
(LAVERAN, 1901)

Synonyms: *Piroplasma equi, Nuttallia equi, Nuttallia asini, ? Nuttallia minor.*

Hosts: Horse, mule, donkey, Burchell's zebra (*Equus burchelli*).

Location: Erythrocytes.

Geographic Distribution: Europe, USSR, Central Asia, North and South Africa, India, South America. This species is more widely distributed than *B. caballi.*

Morphology: This species is relatively small, being 2 μ long. The trophozoites in the erythrocytes are rounded, amoeboid or most often pear-shaped.

The last are usually found in a group of 4 joined together in the form of a cross. Because of this, some authorities prefer to use a separate generic name, *Nuttallia*, for this and similar species.

Life Cycle: Division in the erythrocytes is unlike that of most other species of *Babesia* in that 4 daughter trophozoites are formed at one time.

The vectors are *Dermacentor marginatus* (syn., *D. reticulatus*), *D. pictus*, *Hyalomma marginatum* (syn., *H. detritum*), *H. uralense* and *Rhipicephalus bursa* in the USSR, *H. anatolicum* (syn., *H. excavatum*) and *H. marginatum* in Greece, *H. dromedarii* and *R. sanguineus* in North Africa, *R. evertsi* in South Africa, and *H. marginatum* and *R. sanguineus* in central Asia. Transmission is thru the egg in *H. anatolicum*, and stage-to-stage in all the others.

Intra-uterine transmission may also occur (Neitz, 1956).

Pathogenesis: This species is more pathogenic than *B. caballi*. Mixed infections are not rare, however, so that it is sometimes difficult to be sure which species is causing the symptoms. The incubation period following an infective tick bite is 10 to 21 days. The first sign of disease is a rise in temperature. This is followed by listlessness, depression, marked thirst, inappetence, watering of the eyes and swelling of the eyelids. The most characteristic sign is icterus. There is marked anemia, more than half the erythrocytes often being destroyed. Hemoglobinuria is present, but in contrast to *B. caballi* infections, posterior paralysis is absent. Edema of the head, legs, and ventral part of the body is sometimes present. Affected animals are constipated, passing small, hard balls of feces covered with yellow mucus; they lose condition fairly rapidly, and may become extremely emaciated. Hemorrhages are present on the mucous membranes of the nasal passages, vagina and third eyelid.

The disease usually lasts 7 to 12 days, but it may be peracute, with death occurring in 1 to 2 days, or it may be chronic and last for weeks. The mortality is generally not more than 10%, but may sometimes reach 50%. Recovery is slow, and it may be several weeks or even months before the animal returns to normal.

At necropsy, emaciation, icterus, anemia and edema are present. There are accumulations of fluid in the pericardial sac and body cavities, and the fat is gelatinous and yellow. The spleen is enlarged, with soft, dark brown pulp. The lymph nodes are swollen and sometimes inflamed. The liver is swollen, engorged, and brownish yellow; the hepatic lobules are yellow in the center and greenish yellow around the edges. The kidneys are pale yellow and may contain petechial hemorrhages. There are hemorrhages or red streaks on the mucosa of the intestine and stomach.

Immunity: There is no cross-immunity between *B. equi* and *B. caballi*. Young animals are less seriously affected than adults.

Diagnosis: Babesiosis can be diagnosed by identifying the parasites in stained blood smears. Examinations should be made as early as possible, since the parasites begin to disappear from the peripheral blood after the fifth day.

Treatment: Trypan blue is ineffective against *B. equi*. Acriflavine has been recommended; it is injected intravenously, 10 ml of a 2% aqueous solution being given per 100 kg body weight.

BABESIA TRAUTMANNI (KNUTH AND DU TOIT, 1918)

Synonyms: *Piroplasma trautmanni*, *Piroplasma suis*.

Host: Pig.

Location: Erythrocytes.

Geographic Distribution: Southern Europe, Central and South Africa, USSR.

Morphology: This is a large form, the trophozoites being 2.5 to 4 μ long and 1.5 to 2 μ wide. They are oval, piriform, or less commonly round. They often occur in pairs. The host cells usually contain 1 to 4 or occasionally 5 to 6 parasites. From a very few to 65% of the erythrocytes may be invaded.

Life Cycle: The vector is *Rhipicephalus sanguineus* (syn., *R. turanicus*). Transmission occurs thru the egg. Other ticks have also been incriminated.

Pathogenesis: This species may cause either a mild disease or a fatal one with fever, listlessness, inappetence, anemia, hemoglobinuria, icterus, edema and incoordination. Infected sows may abort. The spleen is enlarged and engorged, the liver is enlarged, there are pulmonary, renal and gastrointestinal hyperemia and edema, petechiae are present on the serous membranes, and there are subepicardial and subendocardial hemorrhages.

Treatment: Trypan blue, acaprin and phenamidine are all effective. Acaprin is injected subcutaneously, 2 ml of a 5% solution being administered per 100 kg to large pigs and 1 ml of a 0.5% solution per 10 kg to small pigs. Lawrence and Shone (1955) injected phenamidine subcutaneously at the rate of 1.5 ml of a 40% aqueous solution per 100 pounds body weight.

Prevention and Control: Same as for other babesioses.

BABESIA PERRONCITOI (CERRUTI, 1939)

Synonym: *Babesiella perroncitoi.*

Host: Pig.

Location: Erythrocytes.

Geographic Distribution: Europe (Sardinia), French Sudan.

Morphology: This is a small form. It is usually annular, 0.7 to 2 μ in diameter, with a thin ring of cytoplasm surrounding a vacuole, but it may also be oval, quadrangular, lanceolate or piriform, measuring 1.2 to 2.6 by 0.7 to 1.9 μ. The trophozoites usually occur singly in the host cells, but sometimes 2 or more may be present.

Life Cycle: The vector is unknown.

Pathogenesis: The disease caused by this species is similar to that caused by *B. trautmanni.*

Treatment: Acaprin is effective against this species, but trypan blue is presumably not.

BABESIA CANIS (PIANA AND GALLI-VALERIO, 1895)

Synonyms: *Pyrosoma bigeminum* var. *canis, Piroplasma canis, Babesia rossi, Rossiella rossi, Babesia vitalii, Rangelia vitalii.*

Disease: Canine babesiosis, canine piroplasmosis, biliary fever, malignant jaundice, nambiuvu.

Hosts: Dog, wolf, side-striped jackal (*Thos adustus*), black-backed jackal (*T. mesomelas*). In addition, the red fox, and jackal (*Canis lupaster*) have been infected experimentally. However, Thomas and Brown (1934) were unable to infect the cat even after splenectomy.

Location: Erythrocytes.

Geographic Distribution: North America (Florida, Virginia, Texas, Puerto Rico), Central America, South America, southern Europe, USSR, Africa, Asia.

Prevalence: This parasite is common in many tropical regions. It is uncommon in the U.S., but has been reported from Florida by Eaton (1934) and Sanders (1937), from Texas by Merenda (1939) and from Virginia by Grogan (1953).

Morphology: This is a large form. The trophozoites are piriform and 4 to 5 μ long, or amoeboid and 2 to 4 μ in

diameter. They generally contain a vacuole. Multiple infections of the erythrocytes are common. In addition, masses of 30 to 100 "merozoites" have been described for *Rangelia vitalii* (a synonym of *B. canis*) in the endothelial cells of the lungs and kidneys. However, these were much more likely agglomerations of organisms in the small blood vessels.

Life Cycle: The life cycle has been described above (p. 287). The vectors are *Rhipicephalus sanguineus* thruout the world, *Dermacentor marginatus* (syn., *D. reticulatus*), *D. pictus* and *D. venustus* in Europe, *D. pictus* and *Hyalomma marginatum* in the USSR, and *Haemaphysalis leachi* in South Africa. Transmission takes place thru the egg in all but *D. pictus*, and stage-to-stage in this species, *R. sanguineus* and *H. leachi*.

Pathogenesis: The severity of infections with *B. canis* varies considerably with the strain. In some localities it is a comparatively mild disease, while in others it may be highly pathogenic. Both young and old dogs are susceptible. In countries where the disease is endemic, the indigenous dogs usually become infected while young and do not suffer such a severe disease, while the mortality is high among imported dogs.

The incubation period is 10 to 21 days in naturally infected dogs. The first sign of disease in acute cases is fever. This is quickly followed by marked anemia, with icterus, inappetence, marked thirst, weakness, prostration and often death. Hemoglobinuria is sometimes but not usually present.

In chronic cases the fever is not high and seldom lasts more than a few days and there is little icterus. Anemia is severe, and the dogs are listless and become very weak and emaciated.

Canine babesiosis is protean in its manifestations, and may take on many different clinical forms. Involvement of the circulatory system may produce edema, purpura and ascites; there may be stomatitis and gastritis; and involvement of the respiratory system causes catarrh and dyspnea. Keratitis and iritis are seen if the eyes are affected, and myositis and rheumatic signs if the muscles are involved.

Central nervous system involvement causes locomotor disturbances, paresis, epileptiform fits, etc. (Malherbe and Parkin, 1951; Malherbe, 1956). A cerebral form of the disease was described by Purchase (1947) in which parasites were rare in the blood but abundant in the brain capillaries. This tendency to clog the capillaries is common to many species of *Babesia*. In cerebral babesiosis the signs may be confused with those of rabies.

In South America, the disease is called nambiuvu, meaning "bloody ears" in the Guarani language. As the name suggests, it is a hemorrhagic disease. There is bleeding from the edges of the ears and from the muzzle, particularly in young dogs in summer. There are also internal hemorrhages.

The spleen is enlarged, with dark red, soft pulp and prominent splenic corpuscles. The liver is enlarged and yellow, with pathological changes ranging from congestion to centrilobular necrosis (Gilles, Maegraith and Andrews, 1953). The heart is pale and yellowish. The kidneys are yellowish, and show considerable nephrosis or nephritis histologically. The muscles are pale and yellow, and the fat and mucous membranes may be yellowish. There may be a variable amount of fluid in the pleural, pericardial and peritoneal cavities. Small hemorrhages are sometimes present on the heart, pleura, bronchi and intestines. There is less icterus in chronic than in acute cases.

Immunity: Recovered animals remain infected in a state of premunition. This persists for life if they are kept in an endemic area, but the parasites die out in a year or more in the absence of reinfection.

Diagnosis: In endemic areas, symptoms of fever, anemia, and icterus, with or without hemoglobinuria, are suggestive of canine babesiosis. The diagnosis can ordinarily be confirmed by finding the

parasites in stained blood smears. They are often present in capillary blood when they cannot be found in venous blood.

Treatment: Trypan blue is effective against *B. canis*. It is injected intravenously, the dosage for a 35-pound dog being 4 to 5 ml of a 1% solution. Acriflavine has also been recommended. It is injected intravenously in 0.1 to 2.0% solution, the dosage being 1 to 3 ml of the drug per kg body weight. Acaprin is safer than acriflavine. It is injected subcutaneously in 0.5% solution at the rate of 0.05 ml per kg body weight. Phenamidine has given excellent results. It is injected subcutaneously in 5% solution at a dosage rate of 10 mg per kg (i.e., 0.2 ml per kg); a single dose is usually effective, but it may be repeated the next day.

Prevention and Control: As for other *Babesia* infections, these depend upon tick control.

BABESIA VOGELI REICHENOW, 1937

Synonym: *Babesia major* Reichenow, 1935.

Host: Dog.

Location: Erythrocytes.

Geographic Distribution: Southern Asia, North Africa.

Morphology: This species is somewhat larger than *B. canis*.

Life Cycle: Similar to that of *B. canis*. The vector is *Rhipicephalus sanguineus* (Shortt, 1936). Transmission occurs thru the egg and stage-to-stage.

Pathogenesis: This species is less pathogenic than *B. canis*, but the disease it causes is otherwise similar.

Immunity: Dogs infected with this species are not resistant to infection with *B. canis* transmitted by *Dermacentor*, which is the reason that Reichenow (1935) separated the two species. Some authors, however, (e.g., Poisson, 1953) consider them synonymous.

Treatment: Same as for *B. canis*.

BABESIA GIBSONI (PATTON, 1910)

Synonyms: *Piroplasma gibsoni*, *Achromaticus gibsoni*, *Babesiella gibsoni*, *Pattonella gibsoni*, *Nuttallia bauryi*.

Disease: Canine babesiosis, Lahore canine fever, tick fever.

Hosts: Dog, jackal (*Canis aureus*), wolf, Indian wild dog (*Cuon dukhensis*), fox. The jackal is the natural host in India.

Location: Erythrocytes.

Geographic Distribution: India, Ceylon, parts of China, occasionally North Africa.

Morphology: This species is smaller than *B. canis* and does not have its characteristic paired, piriform trophozoites. The trophozoites of *B. gibsoni* are usually annular or oval and not more than 1/8 of the diameter of the host erythrocyte. Occasionally, large ovoid forms half the diameter of the host cell or thin, elongate forms reaching almost across the cell may be found.

Life Cycle: Similar to that of *B. canis*. The vectors in India are *Haemaphysalis bispinosa* and *Rhipicephalus sanguineus*. Transmission is thru the egg and stage-to-stage in the former, and stage-to-stage in the latter.

Pathogenesis: This species is only slightly pathogenic for its natural host, the jackal, but is highly pathogenic for the dog, causing marked anemia, remittent fever, hemoglobinuria, constipation, marked splenomegaly and hepatomegaly. The disease usually runs a chronic course, with remissions and relapses of fever, and death may not occur for many months. In imported dogs, however, death is said to occur in 3 to 4 weeks.

Immunity: Dogs which are immune to *B. canis* are still susceptible to *B. gibsoni*.

Treatment: Neither trypan blue nor acaprin is effective against *B. gibsoni*. Treatment with arsenicals such as novarsenobillon or tryparsamide has been suggested, but they are apparently not too satisfactory.

BABESIA FELIS
DAVIS, 1929

Synonyms: *Babesiella felis*, *Nuttallia felis* var. *domestica*.

Hosts: Domestic cat, wild cat (*Felis lybica*), puma (*F. concolor*), lion (*F. leo*), American lynx (*Lynx rufus*), Indian leopard (*Panthera pardus*).

Location: Erythrocytes.

Geographic Distribution: Africa, India, ? North America (California). This species was first found in a wild cat in the Sudan and has since been found in domestic cats in India and South Africa, in the lion in the French Sudan and in the Indian leopard. In addition, it was found in 2 pumas imported into Egypt from California and in an American lynx in the London zoo. It has not been found in animals still in North America, so its existence on this continent is still problematical.

Morphology: This is a small form. Most of the trophozoites are round or irregularly round and 1.5 to 2 μ in diameter. Some are elongate and 2 to 3, or rarely 4 μ long. Piriform trophozoites are rare. Division is quadruple, forming a cruciform schizont, or binary.

Life Cycle: The vectors are unknown, altho *Haemaphysalis leachi* has been incriminated in South Africa.

Pathogenesis: Feline babesiosis is less severe than the canine disease, and affected animals usually recover without treatment. It is characterized by anemia, slow respiration, somnolence, listlessness, emaciation, constipation with yellow or orange feces, splenomegaly, and sometimes icterus and hemoglobinuria.

Treatment: Both trypan blue and acaprin are effective against *B. felis*.

Genus *AEGYPTIANELLA* Carpano, 1928

This genus contains small, round, oval or piriform parasites of the erythrocytes of birds. The host cell is not deformed. Schizogony occurs in the erythrocytes, with the formation of 4 to 16 or 20 merozoites. Laird and Lari (1957) have questioned the justification for separating this genus from *Babesia*, but for the present it is probably best to do so.

AEGYPTIANELLA PULLORUM
CARPANO, 1928

Synonyms: *Balfouria gallinarum*, *Balfouria anserina*.

Disease: Aegyptianellosis, avian piroplasmosis.

Hosts: Chicken, goose, duck, turkey. This species has been transmitted experimentally to the turtle dove, ringdove, crowned crane, quail, pigeon, canary and other birds (Curasson, 1943). The chicken is probably the most important host.

Location: Erythrocytes.

Geographic Distribution: North Africa, South Africa, Indochina, India, USSR (Transcaucasia), southeast Europe.

Morphology: The trophozoites are usually small, ranging in size from 0.5 to 3 or even 4 μ, depending upon the stage of development. They are round, oval or piriform, sometimes with a vacuole. They multiply by schizogony, producing a variable number--up to 20--of very small merozoites.

Life Cycle: The natural vector is the fowl tick, *Argas persicus*. Transmission does not take place either thru the egg or stage-to-stage. After the

adult tick becomes infected by feeding on an infected bird, it takes 26 days or more before it is able to transmit it to another bird (Bedford and Coles, 1933). Ticks can remain infective for as long as 162 days. The stages of development in the tick have not been described. *A. pullorum* can be transmitted experimentally by intravenous, intraperitoneal, subcutaneous or intramuscular injection or by scarification.

The incubation period in chickens is 12 to 15 or more days.

Pathogenesis: *A. pullorum* may cause either a latent, chronic, subacute or acute disease in chickens. The acute form occurs primarily in young or imported birds in endemic regions, while the chronic and latent forms occur primarily in adult birds in endemic regions. Severe outbreaks have been reported in chickens in Algeria, Egypt, South Africa and Greece. Ducks and geese are apparently less seriously affected.

The principal signs are anemia, fever, icterus, diarrhea and anorexia. Necropsy findings include splenomegaly, liver degeneration, characteristic greyish yellow kidneys, intestinal congestion, petechial hemorrhages on the serosa, and sometimes pericarditis. Adult birds usually recover.

Immunity: Birds which have recovered from infection are premunized, but their latent infections can be reactivated by splenectomy or by intercurrent disease.

Diagnosis: *A. pullorum* infections can be diagnosed by identifying the parasites in stained blood smears. They are difficult to stain, however, so the staining time must be prolonged. Affected birds are often simultaneously infected with *Borrelia anserina*, the cause of fowl spirochetosis, which is also transmitted by *Argas persicus*.

Treatment: Trypan blue and acriflavine are ineffective against *A. pullorum*, and variable results have been obtained with stovarsol and quinacrine. Ichthargan is said to be highly effective, but must be given intravenously.

Prevention and Control: These depend upon elimination of the tick vectors.

AEGYPTIANELLA MOSHKOVSKII (SCHURENKOVA, 1938) POISSON, 1953

Synonyms: *Sogdianella moshkovskii*, *Babesia ardeae*, *Nuttallia shortti*, *Babesia moshkovskii*.

Hosts: Chicken, turkey (?), pheasant (?), eagle (*Gypaetus barbatus*), Indian house crow (*Corvus splendens*), heron (*Ardea cinerea*), Egyptian kestrel (*Falco tinnunculus*).

Location: Erythrocytes.

Geographic Distribution: Indochina, USSR (Tadzhikistan), Egypt, Pakistan, India, United States (?), South Africa (?), Iran (?).

This species was first described by Schurenkova (1938) from *Gypaetus barbatus* in Tadzhikistan. Laird and Lari (1957) found what they considered the same species in an Indian house crow in Pakistan, reviewed the literature on avian babesioid hematozoa, and concluded that the following should be assigned to this species: The form described from the chicken in Indochina by Henry (1939), the form described under the name *Babesia ardeae* by Toumanoff (1940) from *Ardea cinerea* in Indochina, and the form described under the name *Nuttallia shortti* by Mohamed (1952) from *Falco tinnunculus* in Egypt. They were not sure of its relationship to the forms reported from chickens in Philadelphia, New York and South Africa by Coles (1937), from chickens in the Punjab by Abdussalam (1945), from turkey poults in California by McNeil and Hinshaw (1944), and from the pheasant in Iran by Rousselot (1947), all of which they considered insufficiently described. I am including these latter forms here as a matter of convenience, without prejudice as to their final disposition.

Laird and Lari (1957) assigned this species to the genus *Babesia*, considering that the differences between the various members of the Babesiidae, including *Aegyptianella*, might best be dealt with at the subgeneric level. They may well be correct. However, until more is known about the avian babesiids, I prefer to leave them in the genus *Aegyptianella*.

Morphology: The form from the chicken described from Henry (1939) is 0.2 to 2.5 μ in diameter, occurring as *Anaplasma*-like granules, as small rings and as elongate bodies with a terminal dot of chromatin and a thin tail of cytoplasm. Both binary fission and schizogony were seen. The nuclei of the schizonts are either strung on a thin cytoplasmic ring or are at the angles of triangular or lozenge-shaped figures. The schizonts usually produce 4 merozoites, altho some have as many as 6.

The form described by Schurenkova from the eagle produces 4 merozoites and also has large, homogeneous bodies which she took to be gametocytes.

The form described by Laird and Lari from the crow has anaplasmoid bodies 0.2 to 0.6 μ in diameter, elongate forms 0.7 to 0.9 by 0.1 μ composed of a terminal dot of chromatin and a slender cytoplasmic tail, ring forms measuring up to 2.1 by 1.4 μ, and large, solid, oval or irregular forms 0.9 to 5.3 μ in diameter. All stages could divide by binary fission. Cruciform and fan-shaped schizonts were also present. Four merozoites are formed.

The form described by McNeil and Hinshaw (1944) from turkey poults was roundish, oval or piriform, 0.5 to 2 μ in diameter, and occurred singly or in pairs. They thought it resembled *Sauroplasma thomasi*, a blood parasite described from a lizard in South Africa by DuToit (1937).

Life Cycle: Unknown.

Pathogenesis: Unknown.

FAMILY THEILERIIDAE

Members of this family are relatively small, round, ovoid, irregular or bacilliform parasites. They occur in the erythrocytes and lymphocytes or histiocytes. Schizogony takes place in the lymphocytes or histiocytes, and is followed by invasion of the erythrocytes. The forms in the erythrocytes may or may not reproduce; in the latter case they divide into 2 or 4 daughter cells. Reichenow (1940, 1953) maintained that schizogony does not occur in the vertebrate host but is simulated by repeated binary fissions. However, observations on the protozoa in tissue culture (Tsur-Tchernomoretz, 1945; Brocklesby and Hawking, 1958) indicate that schizogony does occur.

The vectors are ixodid ticks. Binary fission, schizogony and sexual reproduction have been said to occur in the tick, but the existence of sexual reproduction is dubious, and Reichenow (1940, 1953) believed that schizogony is simulated by repeated binary fissions.

Members of this family cause an important group of diseases, known collectively as theilerioses, in cattle, sheep and goats. These have caused heavy losses in Africa, southern Europe and Asia.

This group has been reviewed by Reichenow (1953), Poisson (1953), and most comprehensively by Neitz (1956, 1957, 1959). Most authors place all members of the family in the genus *Theileria* (e.g., Poisson, 1953), while some accept the genus *Cytauxzoon* as well (e.g., Reichenow, 1953). However, Neitz and Jansen (1956) divided the group into 3 genera on the basis of biological characteristics. They even placed them in 2 families in a new suborder Leucosporidea, but this latter treatment does not seem justified.

In the genus *Theileria* as redefined by Neitz and Jansen, the forms in the erythrocytes do not divide, the parasites cannot be transmitted by blood inoculation, and recovered animals do not remain car-

riers (i.e., there is no premunition). In the redefined genus *Gonderia*, the forms in the erythrocytes do divide, the parasites can be transmitted by blood inoculation, and recovered animals remain carriers for life (i.e., premunition is present). In the genus *Cylauxzoon*, schizogony takes place in the histiocytes rather than in the lymphocytes as in the other 2 genera, and the forms in the erythrocytes reproduce by binary fission.

Neitz (1959) recognized 1 species of *Theileria* and 5 of *Gonderia* in domestic animals, and 10 named and 36 unnamed species of *Theileria*, 1 of *Gonderia* and 2 of *Cylauxzoon* in wild animals. The great majority occur in African ruminants. They are all tabulated by Neitz (1957). Since the forms in the erythrocytes of all 3 genera look alike, and since practically all the species in wild animals are known only from these forms, their assignment to the genus *Theileria* is clearly provisional.

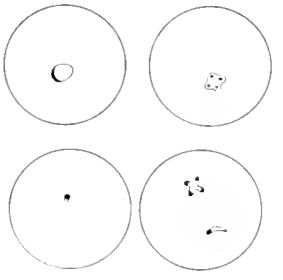

Fig. 35. *Theileria parva* in bovine erythrocytes. X 2800. (After Nuttall, 1913 in *Parasitology*, published by Cambridge Univ. Press).

Genus *THEILERIA* Bettencourt, França and Borges, 1907

In this genus the parasites multiply by schizogony (or possibly by a series of binary fissions) in the lymphocytes and finally invade the erythrocytes. The forms in the erythrocytes do not reproduce. Infection cannot be transmitted by blood inoculation, and there is no premunition. There is one valid species in domestic animals.

THEILERIA PARVA (THEILER, 1904) BETTENCOURT, FRANÇA AND BORGES, 1907

Synonyms: *Piroplasma kochi*, *Piroplasma parvum*, *Theileria kochi*.

Disease: East Coast fever, bovine theileriosis, African Coast fever, Rhodesian tick fever, Rhodesian redwater.

Hosts: Ox, zebu, water buffalo, African buffalo (*Syncerus caffer*).

Location: Lymphocytes, erythrocytes.

Geographic Distribution: East, Central and South Africa.

Prevalence: East Coast fever is one of the most important cattle diseases in the regions where it is found. According to Neitz (1959), it occurs enzootically in the Belgian Congo, Uganda, Kenya, Tanganyika, Nyasaland, Zanzibar and Swaziland. It has been eliminated from most parts of South Africa.

Morphology: The forms in the erythrocytes are predominantly (over 80%) rod-shaped, and measure about 1.5 to 2.0 by 0.5 to 1.0 μ. Round, oval and comma-shaped forms also occur. When stained with a Romanowsky stain, they have a red nucleus at one end and blue cytoplasm. Several parasites are often found in a single host erythrocyte.

The multiplying forms occur in the lymphocytes and occasionally in the endothelial cells. They are found especially in the lymph nodes and spleen, where they are usually very numerous. They are known as Koch's blue bodies or Koch's bodies, and are circular or irregularly shaped bodies averaging 8 μ in diameter and ranging up to 12 μ or more. They may be intracellular or free in the gland or spleen juice. When stained with a Romanowsky stain, their cytoplasm is blue and they contain a varying number of red chromatin granules.

Two types of these schizonts are recognized. Macroschizonts (sometimes

called agamonts) contain chromatin granules 0.4 to 2.0 μ in diameter with a mean of 1.2 μ and produce macromerozoites 2.0 to 2.5 μ in diameter. Microschizonts (sometimes called gamonts because they are thought to produce sexual stages) contain chromatin granules 0.3 to 0.8 μ in diameter with a mean of 0.5 μ and produce micromerozoites 0.7 to 1.0 μ in diameter.

Life Cycle: The life cycle of this species has been studied more than that of other members of the family, but its details in the tick are still uncertain. The most important vector is *Rhipicephalus appendiculatus*. Other vectors are *R. ayrei*, *R. capensis*, *R. evertsi*, *R. jeanelli*, *R. neavei*, *R. simus*, *Hyalomma excavatum*, *H. dromedarii* and *H. truncatum*. Transmission is stage-to-stage in all cases, and not thru the egg. *R. appendiculatus*, for instance, acquires the infection as a larva and transmits it as a nymph, or acquires the infection as a nymph and transmits it as an adult. The parasite will not survive in the ticks thru more than 1 molt.

Reichenow (1940), who made a careful study of the life cycle in cattle and in *R. appendiculatus*, said that the great majority of parasites die in the tick intestine. A few succeed in passing thru the intestinal wall into the body cavity and thence to the salivary glands, where they invade the secretory cells. Here they lie dormant until after the tick has dropped off its host, molted, attached itself to a new host and started to suck blood. The parasites then begin to multiply by repeated binary fissions, filling the interstices between the secretory droplets. They continue to multiply, and finally the host cell is greatly enlarged and filled with something over 30,000 tiny parasites. This requires 15 successive binary divisions. Very few secretory droplets remain. The host cell ruptures, and the parasites are released into the lumen of the salivary ducts and are injected into the host when the tick sucks blood. It takes 3 days for the developmental process to be completed in nymphs and 4.5 days in adult ticks.

The above process is completely asexual. Gonder (1910, 1911), however, thought that there was a sexual stage in the tick, and described a process of syngamy. Cowdry and Ham (1932) also thought that sex was involved, altho they admitted they found no proof of it. According to their account of the life cycle, two types of parasite, large and small, emerge from the erythrocytes in the tick's gut and become applied to the surface of the gut epithelial cells. Cowdry and Ham thought that fertilization probably takes place here. They said, "Very careful search was made for fertilisation stages without conspicuous success. Large and small parasites were, however, occasionally observed in contact, but it was difficult to tell whether this was merely optical superposition or whether actual union was taking place. Such appearances were detected in 0.1 per cent or less of the parasites."

The parasites then enter the intestinal cells, the small forms disappear, and the large forms grow and give rise to a stage without distinct nuclei which they called a zygote. The zygote grows, a nucleus reappears in it, and also a central concentration of material. This central concentration becomes more marked and turns into a large, elongated, nucleated organism which they called an ookinete. The ookinete breaks out of the zygote into the gut cell, enters the body cavity, makes its way to the salivary glands, and enters a salivary gland cell. Here it rounds up and grows, surrounded by a colorless halo of host cell cytoplasm, becoming so large that it distends the host cell. Buds appear about its periphery which Cowdry and Ham called sporoblasts; the parent cell they called a sporont. The sporoblasts develop rapidly and produce sporozoites about their periphery. These are discharged into the lumen of the salivary gland acinus and are introduced into the animal when the tick feeds on it.

Reichenow (1940) criticized the work of Cowdry and Ham (1932) severely. He said that the bodies in the intestinal cells (the "zygotes"), could be found in both infected and clean ticks and were therefore not a stage in the parasite's life cycle. He found no structures which resembled ookinetes. He considered the "sporonts" to be degenerated tissue cells

phagocytized by the salivary gland cells, and the "sporoblasts" to be masses of coalesced droplets secreted by the salivary gland cells. Gonder's work has been discredited not only by Reichenow but also by Cowdry and Ham, Wenyon (1926) and others. According to Cowdry and Ham, Gonder did not distinguish between *Theileria* and the symbionts which are present in all ticks, and substantiating details in his account were conspicuous by their absence. Wenyon said that his "account was so obscured by such theoretical bias that it is difficult to separate fact from theory."

A definitive study is badly needed to clear up the life cycle of *T. parva*, and one may hope that 20 more years do not pass before someone carries it out. In the meantime, Reichenow's account is the most convincing.

Pathogenesis: *T. parva* is highly pathogenic. From 90 to 100% of affected cattle die, altho the mortality is lower in endemic areas. In East Africa, for instance, immature cattle are more resistant than adults, and the mortality among calves varies from 5 to 50%. In Kenya, the mortality varies considerably among calves, but adults usually die.

The incubation period following tick transmission is 8 to 25 days, with a mean of 13 days. The disease itself lasts 10 to 23 days, with a mean of 15 days. Acute, subacute, mild and inapparent forms of the disease have been described, of which the acute type is the usual one.

In the acute form, the first sign is fever. The body temperature varies from 104 to 107° F; it may continue high or it may decrease after 7 to 11 days and then increase again. Other clinical signs usually appear a few days after the initial rise in temperature. The animals cease to ruminate and to eat. Other signs are a serous nasal discharge, lachrymation, swelling of the superficial lymph nodes, sometimes swelling of the eyelids, ears and jowl region, rapid heart beat, general weakness, decreased milk production, diarrhea, frequently with blood and mucus in the feces, emaciation, coughing, and sometimes icterus. Breathing becomes rapid and dyspnea is pronounced just before death. An oligocythemic anemia is present, but there is no hematuria in uncomplicated cases.

In the subacute form, which is often encountered in calves and sometimes in adults in the endemic areas of East Africa, the signs resemble those in the acute form but are not so pronounced. Affected animals may recover, but it takes them several weeks to return to normal.

In the mild form, little is seen but a relatively mild fever lasting 3 to 7 days, listlessness and swelling of the superficial lymph nodes. An inapparent form of the disease has been produced by injection of blood, coarsely ground spleen and lymph node emulsions or suspensions from partially engorged, infected ticks.

The lymph nodes are usually marked swollen, with a variable degree of hyperemia. The spleen is usually enlarged, with soft pulp and prominent Malpighian corpuscles. The liver is enlarged, friable, brownish yellow to lemon yellow, with parenchymatous degeneration. The kidneys are either congested or pale brown, with a variable number of hemorrhagic "infarcts" or greyish white lymphomatomata. The meninges may be slightly congested. The heart is flabby, with petechiae on the epicardium and endocardium. The lungs are often congested and edematous. There may be hydrothorax and hydropericardium, and the kidney capsule may contain a large amount of serous fluid. There may be petechiae in the visceral and parietal pleura, adrenal cortex, urinary bladder, and mediastinum. There are characteristic ulcers 2 to 5 mm or more in diameter in the abomasum, and similar ulcers together with red streaks or patches may be present thruout the small and large intestines. These ulcers consist of a central, red or brown necrotic area surrounded by a hemorrhagic zone. The Peyer's patches are swollen, and the intestinal contents are yellowish.

Immunity: Animals which recover from *T. parva* infections are solidly immune. The parasites disappear completely, and there is no premunition. There is no cross-immunity between *T. parva* and *Gonderia mutans*, but there is partial cross-immunity between *T. parva* and *G. lawrencei*.

Diagnosis: Diagnosis is based upon finding the parasites in the erythrocytes in stained blood smears or in stained smears made from the lymph nodes or spleen. Differential diagnosis between East Coast fever and the gonderioses is not always easy, however, and depends upon knowledge of the geographic distribution of the parasites, symptomatology, pathology, pathogenicity, degree of parasitemia, epidemiology and results of cross-immunity tests. The last is the best test in case of doubt.

Cultivation: Tsur-Tchernomoretz, Neitz, and Pols (1957) cultivated *T. parva* up to 15 days in ox spleen, liver or lymph node tissue cultures. The Koch bodies developed during the first 10 days but then died out. Brocklesby and Hawking (1958) also grew *T. parva* in tissue cultures, but could not maintain them more than 14 days. The parasites occurred mostly in lymphoid cells.

Treatment: No drug is effective against *T. parva* once signs of disease have appeared. However, chlortetracycline and oxytetracycline seem to prevent clinical disease if given repeatedly during the incubation and reaction periods, and treated animals become solidly immune (Neitz, 1957; Barnett, 1956).

Prevention and Control: These depend upon tick control and quarantine measures. Immunization by intravenous injection of a suspension of spleen and lymph node material from affected animals was practiced in South Africa around 1912 to 1914, but was then discontinued.

Repeated, regular dipping of cattle in arsenical dips has been found effective, even tho some arsenic-resistant strains of ticks have appeared. Other dips, such as lindane and toxaphene, have also been used.

Quarantine measures are also effective in preventing the spread of East Coast fever. In isolated outbreaks, the whole herd may be slaughtered and the farm kept free of cattle for 18 months before restocking.

Genus *GONDERIA* Du Toit, 1918

In this genus the parasites multiply by schizogony (or possibly by a series of binary fissions) in the lymphocytes and finally invade the erythrocytes. The forms in the erythrocytes reproduce by binary fission into 2 or 4 daughter individuals. Infection can be transmitted by blood inoculation, and recovered animals are premunized.

GONDERIA ANNULATA (DSCHUNKOWSKY AND LUHS, 1904)

Synonyms: *Piroplasma annulatum, Theileria annulata, Theileria dispar, Theileria turkestanica, Theileria sergenti.*

Disease: Tropical gonderiosis, tropical theileriosis, tropical piroplasmosis, Egyptian fever, Mediterranean Coast fever.

Hosts: Ox, zebu, water buffalo. In addition, an American bison in the Cairo zoo died of a natural infection (Carpano, 1937).

Location: Lymphocytes, erythrocytes.

Geographic Distribution: North Africa, southern Europe, southern USSR, India, western China.

Prevalence: Tropical gonderiosis is one of the most important diseases of cattle in North Africa, southeastern Europe, southern USSR and Asia.

Morphology: The forms in the erythrocytes are predominantly (70 to 80%) round or oval, but may also be rod-shaped, comma-shaped or even anaplasma-like. The round forms are 0.5 to 1.5μ in diameter, the oval ones about 2.0 by 0.6μ, the comma-shaped ones about 1.6 by 0.5μ, and the anaplasma-like forms 0.5μ in diameter. Binary fission with the formation of 2 daughter individuals or quadruple fission with the formation of 4 individuals in the form of a cross takes place.

The Koch bodies in the lymphocytes of the spleen and lymph nodes, or free in these organs, are similar to those of *T. parva*; they average 8μ in diameter but

range up to 15 μ or even 27 μ. Two types are recognized: Macroschizonts, which contain chromatin granules 0.4 to 1.9 μ in diameter and produce macromerozoites 2.0 to 2.5 μ in diameter; and microschizonts, which contain chromatin granules 0.3 to 0.8 μ in diameter and produce micromerozoites 0.7 to 1.0 μ in diameter.

Life Cycle: The vectors of *G. annulata* are *Hyalomma detritum* (syn., *H. mauretanicum*) in North Africa and the USSR, *H. truncatum* in parts of Africa, *H. dromedarii* in Central Asia, *H. excavatum* (syn., *H. anatolicum*), *H. turanicum* (syn., *H. rufipes glabrum*) and *H. marginatum* (syns., *H. savignyi*, *H. aegyptium*) in Asia Minor, and *H. marginatum* in India. Transmission is stage-to-stage in all cases, and not thru the egg. Ray's (1950) and Kornienko and Shmyreva's (1944) claim of passage thru the egg has been disproved by Delpy (1949) and Daubney and Sami Said (1951).

The life cycle of *G. annulata* has been studied in *H. detritum* by Sergent *et al.* (1936). They admitted that they found no stages which could be identified as macrogametes or microgametes and that they saw nothing which could be recognized as fertilization, but they nevertheless believed that these must be present and called the subsequent stage a zygote. According to their account, the forms ingested by the tick are gametocytes. These form gametes in the tick's intestine, and the gametes in turn give rise to zygotes. The zygotes enter the intestinal epithelial cells, encyst, and remain in the lumen of the intestine for 6 to 8 months until after the nymphal tick has hibernated and molted to the adult stage. (*H. detritum* is a 2-host tick, with the larva and nymph on one host and the adult on the other.) At this time they leave the cyst and enter the salivary gland acini, where they penetrate the gland cells and turn into sporonts. These give rise to sporoblasts in 3 or 4 days, and the sporoblasts in turn produce a multitude of sporozoites which break out of the cells, enter the salivary ducts and are injected into a new host when the tick feeds. This life cycle is similar to that described by Cowdry and Ham (1932) for *T. parva*, and is subject to the same criticisms.

Pathogenesis: Tropical gonderiosis is similar to East Coast fever in most respects. The mortality varies considerably, from 10% in some areas to 90% in others. It is about 20 to 40% in Algeria, up to 90% in enzootic regions of the USSR and 13 to 23% in indigenous calves in India.

The incubation period following tick transmission is 9 to 25 days, with a mean of 15 days. The disease itself lasts 4 to 20 days, with a mean of 10 days. Peracute, acute, subacute, mild and chronic forms have been described. The acute form is the usual one. The first sign is fever, the body temperature rising to 104 to 107° F. The fever is continuous or intermittent, and persists for 5 to 20 days. A few days after it begins, other signs appear. These include inappetence, cessation of rumination, drooling, serous nasal discharge, lachrymation, rapid heart beat, weakness, decreased milk production and swelling of the superficial lymph nodes and of the eyelids. Marked anemia develops in a few days, and there may be hemoglobinuria. Bilirubinemia and bilirubinuria are always present. Diarrhea appears, and the feces contain blood and mucus. The conjunctiva is icteric and may bear petechial hemorrhages. Affected animals become greatly emaciated, and their erythrocyte count may drop below 1 million per cu mm. Death, if it comes, usually occurs 8 to 15 days after the onset.

In the peracute form of the disease, the animals may die in 3 or 4 days. In the subacute form, the fever is usually irregularly intermittent and lasts up to 10 or 15 days, after which the animals usually recover; pregnant animals sometimes abort. In the chronic form, intermittent fever, inappetence, marked emaciation and more or less anemia and icterus may persist for 4 weeks or longer, but it may take 2 months before the animals return to normal; in some cases, the acute form may suddenly supervene and the animals may die in a day or two. In the mild form, little is seen but mild fever, inappetence, listlessness, slight digestive disturbances and lachrymation lasting a few days. There may be moderate anemia.

The lymph nodes are often but not always swollen; the spleen is often much enlarged. The liver is usually enlarged. Infarcts are usually present in the kidneys. The lungs are usually edematous, and characteristic ulcers are present in the abomasum and often in the small and large intestines.

Mixed infections with *Babesia* and/or *Anaplasma* are not uncommon; the resultant signs and lesions are then due to a combination of diseases and may differ from those described above.

Immunity: Animals which recover from *G. annulata* infections are premunized. There is no cross-immunity between *G. annulata*, *G. mutans* and *T. parva*.

Diagnosis: This is based upon finding and identifying the parasites in the erythrocytes in stained blood smears or in stained smears made from the lymph nodes or spleen. As mentioned under *T. parva*, differential diagnosis between theileriosis and the gonderioses is not always easy.

Cultivation: Tsur-Tchernomoretz (1945) cultivated the Koch bodies of *G. annulata* in ox tissue cultures thru 10 subcultures over a period of 2 months. Brocklesby and Hawking (1958) grew *G. annulata* in tissue culture for over 59 days, and the cultures were infective for cattle when tested after 42 days.

Treatment: No reliable drug is known for the treatment of tropical gonderiosis (Neitz, 1959).

Prevention and Control: Tick control by regular, repeated dipping is the most important control measure. Quarantine measures, particularly with respect to importation of livestock from endemic areas into regions where suitable tick vectors exist, are also of great importance.

Immunization with a strain of low virulence has been used with success in North Africa and Israel (Sergent *et al.*, 1945). The vaccine strain is maintained by serial passage in tick-free cattle. Animals are vaccinated by subcutaneous injection of 5 to 10 ml of citrated blood collected at the height of the febrile reaction. The blood should be used within 3 days after collection. The mortality following vaccination is usually less than 5%.

GONDERIA MUTANS (THEILER, 1906)

Synonyms: *Piroplasma mutans*, *Theileria mutans*, *Theileria buffeli*, *Theileria orientalis*.

Disease: Benign bovine gonderiosis, benign bovine theileriosis, Tzaneen disease, Marico calf disease, mild gallsickness.

Hosts: Ox, zebu. The water buffalo and African buffalo (*Syncerus caffer*) can be infected experimentally but without causing death.

Location: Lymphocytes, erythrocytes.

Geographic Distribution: Africa, Asia, southern Europe, England, USSR, Australia, North America.

Prevalence: *G. mutans* is endemic thruout Africa, in the great part of Asia, and in many areas of the USSR and southern Europe. It has been reported by Splitter (1950) in Kansas.

Morphology: The forms in the erythrocytes are round, oval, piriform, comma-shaped or anaplasma-like. About 55% are round or oval. The round forms are 1 to 2 μ in diameter and the oval ones about 1.5 by 0.6 μ. Binary and quadruple fission occur in the erythrocytes.

There are relatively few Koch bodies in the lymphocytes of the spleen and lymph nodes or free in these organs. They average 8 μ in diameter but may range up to 20 μ. They contain 1 to 80 chromatin granules from 1 to 2 μ in diameter, and are practically all of the macroschizont

type. Merozoites have apparently not been seen, but they must occur.

Life Cycle: The vectors of *G. mutans* in Africa are *Rhipicephalus appendiculatus* and *R. evertsi*. In addition, *Boophilus annulatus* has been found to be able to transmit this species experimentally. Transmission is stage-to-stage.

The stages in the tick vectors are unknown.

Pathogenesis: *G. mutans* is seldom more than slightly if at all pathogenic, altho an acute form of the disease may develop in cattle imported into an endemic area and exposed to massive tick infestation. The mortality is less than 1%.

The signs, course of the disease and lesions resemble those of mild *G. annulata* infections. Anemia, if present, is slight. Icterus is sometimes present, and the lymph nodes are moderately swollen. In acute cases the spleen and liver are swollen, the lungs may be edematous, there are characteristic ulcers in the abomasum, and infarcts may be present in the kidneys. Hematuria is absent.

The incubation period following tick transmission is 10 to 20 days with a mean of 15 days. The disease lasts 3 to 10 days with a mean of 5 days.

Splenectomy may cause the appearance of parasites in the blood, and indeed Splitter (1950) first observed them in a splenectomized calf.

Immunity: Animals which have once been infected with *G. mutans* are premunized. There is no cross-immunity between *G. mutans* and *G. annulata*, *G. lawrencei* and *T. parva*.

Diagnosis: Same as for other species of *Gonderia* and *Theileria*.

Treatment: None known.

Prevention and Control: These depend upon tick control.

GONDERIA LAWRENCEI
(NEITZ, 1955)
NEITZ AND JANSEN, 1956

Synonyms: *Theileria lawrencei*, *Gonderia bovis*.

Disease: Corridor disease, buffalo disease, malignant syncerine gonderiosis, Rhodesian malignant bovine gonderiosis.

Hosts: Cattle, African buffalo (*Syncerus caffer*). The buffalo is the natural host.

Location: Lymphocytes, erythrocytes.

Geographic Distribution: Union of South Africa, Southern Rhodesia.

Prevalence: This disease is widely distributed in Southern Rhodesia, both in cattle and African buffaloes. In the Union of South Africa its distribution is much more restricted, and it occurs only in cattle which have come in contact with ticks from premune African buffaloes. It takes its name from the fact that it was first found here in the Corridor, a stretch of 100 square miles of land between the Hluhluwe and Umfolozi Game Reserves where buffalo abound. It has also been found around Krüger National Park.

Morphology: The erythrocytic stages are oval, round, piriform or comma-shaped, and indistinguishable from those of *G. mutans*. About 55% are round or oval.

There are relatively few Koch bodies in the lymphocytes of the spleen and lymph nodes or free in these organs. They average 5 μ in diameter but may range up to 10 μ. They contain 1 to 16 or 32 reddish purple granules 0.5 to 2 μ in diameter and are practically all of the macroschizont type. The mature macromerozoites are 2.0 to 2.5 μ in diameter, and the mature micromerozoites are 0.7 to 1.0 μ in diameter.

Life Cycle: The vector is *Rhipicephalus appendiculatus*, and transmission is stage-to-stage. The parasite stages in the tick are unknown.

Pathogenesis: Corridor disease is similar to East Coast fever and tropical gonderiosis in its manifestations. *G. lawrencei* is highly pathogenic for cattle, the mortality being about 80%. African buffaloes, however, are highly resistant and serve as the reservoir of infection for cattle.

The incubation period following tick transmission is 12 to 20 days, with a mean of 15 days. The disease itself lasts 5 to 15 days, with a mean of 10 days. Peracute, acute, subacute and mild forms have been described. The acute form is the usual one.

There is usually no anemia, altho oligocythemia may occur. Icterus may be present, but hematuria is not. The lymph nodes, spleen and liver are often swollen, edema of the lungs is pronounced, characteristic ulcers are usually present in the abomasum, and infarcts are sometimes present in the kidneys.

Immunity: Animals which recover from infection with *G. lawrencei* are premune. There is no cross-immunity between this species and *G. mutans*, but there is partial or complete cross-immunity between it and *T. parva*.

Diagnosis: Same as for other species of *Gonderia* and *Theileria*.

Treatment: No effective drugs are known for the treatment of Corridor disease, but there is some evidence that chlortetracycline may suppress the disease if given repeatedly during the incubation and patent periods.

Prevention and Control: These depend upon tick control and upon prevention of association between cattle and African buffaloes.

GONDERIA HIRCI (DSCHUNKOWSKY AND URODSCHEVICH, 1924)

Synonyms: *Theileria hirci, Theileria ovis* du Toit, 1918; non *T. ovis* Rodhain, 1916.

Disease: Malignant ovine and caprine gonderiosis, malignant ovine and caprine theileriosis.

Hosts: Sheep, goat.

Location: Lymphocytes, erythrocytes.

Geographic Distribution: North Africa, southeastern Europe, southern USSR, Asia Minor.

Morphology: The erythrocytic stages are about 80% round or oval, 18% rod-shaped and 2% anaplasma-like. The round forms are 0.6 to 2.0 μ in diameter and the more elongate ones about 1.6 μ long. Binary or quadruple fission takes place in the erythrocytes.

Koch bodies are common in the lymphocytes of the spleen and lymph node smears or free in these organs. They average 8 μ in diameter but may range up to 10 or even 20 μ. They contain 1 to 80 reddish purple granules from 1 to 2 μ in diameter. Both macroschizonts and microschizonts can be found. These produce merozoites 1 to 2 μ in diameter.

Life Cycle: The vector is unknown, but is possibly *Rhipicephalus bursa*.

Pathogenesis: This species is highly pathogenic for sheep and goats, mortalities of 46 to 100% having been reported in these animals. The disease is relatively mild in young lambs and kids in endemic areas.

The incubation period is unknown. The disease itself lasts 5 to 42 days. Acute, subacute and chronic forms have been described, the acute form being the usual one.

The disease resembles tropical bovine gonderiosis in its manifestations. There is fever following by listlessness, nasal discharge, atony of the rumen and weakness. Affected animals are anemic, and icterus is frequently present. There is often a transitory hemoglobinuria. The lymph nodes are always and the liver

usually swollen, the spleen is markedly enlarged, the lungs are edematous, infarcts are often present in the kidneys, and there are petechiae on the mucosa of the abomasum and irregularly disseminated red patches on the intestinal mucosa, particularly in the cecum and large intestine.

Immunity: Animals which recover from the disease are premune. There is no cross-immunity between this species and *G. ovis*.

Diagnosis: This depends upon identification of the parasites in stained blood, lymph node or spleen smears. In contrast with *G. ovis*, the erythrocytic stages are usually present in relatively large numbers, and Koch bodies are common in the lymph nodes and spleen. Inoculation of susceptible sheep or goats may also be resorted to.

Treatment: None known.

Prevention and Control: These depend upon tick control.

GONDERIA OVIS (RODHAIN, 1916) LESTOQUARD, 1929

Synonyms: *Theileria ovis* Rodhain, 1916; *Babesia sergenti, Theileria recondita, Theileria sergenti.*

Disease: Benign ovine and caprine gonderiosis, benign ovine and caprine theileriosis.

Hosts: Sheep, goat.

Location: Lymphocytes, erythrocytes.

Geographic Distribution: Africa, Europe, USSR, India, western Asia. This species is much more widely distributed than *G. hirci*.

Morphology: The erythrocytic stages resemble those of *G. hirci* in shape and size, but are much sparser in infected animals, less than 2% of the erythrocytes being infected in non-splenectomized animals. The Koch bodies resemble those of *G. hirci*, but have been found only in the lymph nodes and then only after prolonged examination.

Life Cycle: The vectors are *Rhipicephalus bursa* in the USSR, North Africa and Asia, and *R. evertsi* in South Africa. Transmission with *Ornithodoros lahorensis, Dermacentor silvarum* and *Haemaphysalis sulcata* has been claimed in the USSR (Bitukov, 1953), but this claim is dubious (Neitz, 1959).

The stages in the tick are unknown.

Pathogenesis: This species is nonpathogenic or practically so. The incubation period following tick transmission is 9 to 13 days, and the disease lasts 5 to 16 days. The only signs are fever, swelling of the lymph nodes in the region of tick attachment, and slight anemia. These would normally be overlooked in the field.

Immunity: Animals which have been infected are premune. There is no cross-immunity between *G. ovis* and *G. hirci*.

Diagnosis: This depends upon identification of the parasites in stained blood or lymph node smears. *G. ovis* is morphologically indistinguishable from *G. hirci*, but the small number of parasites present and their lack of pathogenicity may help to differentiate them. Cross-immunity tests may be carried out if desired.

Treatment: None known.

Prevention and Control: These depend upon tick control.

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Chapter 12

SARCOCYSTIS TOXOPLASMA AND RELATED PROTOZOA

The members of the class Toxoplasmasida have been and still are a headache to taxonomists. Their affinities to other protozoa are uncertain, and some people have even questioned whether some of them are protozoa at all, preferring to assign them to the fungi. Until recently, no relationship was recognized between *Sarcocystis* and *Toxoplasma*, the 2 main genera, but it has become increasingly clear that they have many resemblances (Manwell and Drobeck, 1953). Even the names given to the trophozoites and to the order have been wrong; they were influenced by the belief (shown to be mistaken by Perrier as long ago as 1907) that the trophozoites are spores. Much of our difficulty is due to lack of information. As we learn more and more, and as new facts fall into place, our understanding of the group will improve and we can expect that some of our present ideas may change. The classification adopted here is considered reasonable and useful, but it is not necessarily definitive.

All members of this class are parasitic. They have no spores. They produce cysts or pseudocysts containing many naked trophozoites (sometimes called schizozoites, altho the existence of schizogony is dubious, and often erroneously called spores or sporozoites). They are monoxenous and reproduce asexually. They lack pseudopods, flagella and cilia, and move by body flexion or gliding.

There is a single order, Toxoplasmorida, with the characters of the class. It contains 2 families, Sarcocystidae and Toxoplasmatidae, both of which contain parasites of domestic animals and man. (It is possible that the distinction between these families is artificial, but, pending further research, it is probably best to retain them.)

FAMILY SARCOCYSTIDAE

Members of this family form cysts. They multiply by binary fission and

perhaps also in the young cysts by schizogony. There is a single genus, *Sarcocystis*. The group has been reviewed by Babudieri (1932), Scott (1930, 1943), Barreto (1940), Erickson (1940), Grassé (1953) and Eisenstein and Innes (1956).

Genus *SARCOCYSTIS* Lankester, 1882

In this genus the cysts are found in the striated and heart muscles, and are usually divided into compartments by internal septa. Synonyms of this name are *Miescheria* Blanchard, 1885 and *Balbiania* Blanchard, 1885.

Sarcocystis is common in many species of animals. It is found in the great majority of sheep, cattle, horses and swine, and is often seen in wild ducks. It is extremely rare, however, in carnivores such as the dog and cat, and the reports of its presence in these animals require verification. Dogs cannot be infected experimentally (Pfeiffer, 1891).

More than 50 species of *Sarcocystis* have been named, but it is not at all certain that they are all valid. They are differentiated on the basis of the host in which they occur, the structure of the cyst wall and the size of the trophozoites. However, *Sarcocystis* is not very host-specific. The rat and guinea pig can be infected with the form from the mouse, the mouse, guinea pig, chicken and duck with that from the sheep, and the rat and mouse with that from the pig. In addition, the same species does not look the same in all hosts. For example, in the guinea pig the trophozoites of the form from the mouse are only half their former size and the cysts do not have alveoli. Finally, the structure of the cyst wall may vary with its age even in the same host. The specific names below are therefore used more as a matter of convenience and custom than from any conviction that they are all necessarily valid.

Morphology: The cysts are known as Miescher's tubules and are easily visible to the naked eye. They are usually cylindroid or spindle-shaped, running lengthwise in the muscles, but they may also be ellipsoidal or rather irregular. They vary in size depending in part on the host. The ellipsoidal cysts in the sheep may reach 1 cm in diameter, but considerably smaller ones are the rule. Those in the duck are 1 or 2 mm in diameter and 1 cm or more long.

The cyst wall varies in appearance with the species. There are 3 types. In one, e.g., *Sarcocystis muris* of the mouse, it is smooth. In another, e.g., *S. platydactyli* of the gecko, it has an outer layer of radial spines, villi or fibrils called cytophaneres. In a third, e.g., *S. tenella* of the sheep, the wall is smooth in the young cysts, acquires a layer of cytophaneres as the cysts develop, and then loses it when they become old.

The cyst wall of *S. tenella* is composed of 2 thick layers (Ludvik, 1958). The inner one is homogeneous and contains nuclei. Extensions from it form septa between the compartments in the cyst. The outer layer contains no nuclei and appears spongy in electron micrographs. It forms the cytophaneres. The inner layer contains RNA and the outer a polysaccharide. The cyst wall is essentially negative to the periodic acid-Schiff test, altho the cytophaneres stain slightly according to Frenkel (1956a).

The cyst wall of *S. miescheriana* differs from that of *S. tenella* in being composed of only a single layer with a complicated surface structure (Ludvik, 1960). The cytoplasm of the wall is granulated, and fine septa project from its inner surface to divide the interior of the cyst into small compartments. The outer surface of the cyst wall is spongy, with a fine honeycomb structure. It sends numerous parallel, hollow, finger-like projections or villi into the surrounding muscle tissue. These villi may be as much as 8 to 10 μ long, and are circular or ellipsoidal in cross section and about 0.7 to 0.8 μ in diameter. They contain slender, long double fibrils 100 Å thick.

There is a difference of opinion as to whether the cyst wall is formed by the

parasite or the host. A few authors, such as Wang (1950), think that the host forms both layers, but this view is probably not correct. Some, such as Chatton and Avel (1923) and Barretto (1940), think that the parasite forms both layers. Others, such as Scott (1943), think that the parasite forms the inner layer and the host the outer; still others, such as Babudieri (1932), think that the whole cyst wall is formed by the parasite in *S. muris* and similar species, and that one layer is formed by the parasite and the other by the host in *S. tenella* and similar species. According to Ludvik (1960), the single-layered cyst wall of *S. miescheriana* is quite certainly formed by the parasite, and the villi which project into the muscle tissue take up nutritive material from the host.

The trophozoites are banana-shaped when mature, with the anterior end slightly pointed and the posterior end rounded. They are 6 to 15 μ long and 2 to 4 μ wide, varying in size with the species. They move by gliding or body flexion, twisting, turning, or following a spiral path.

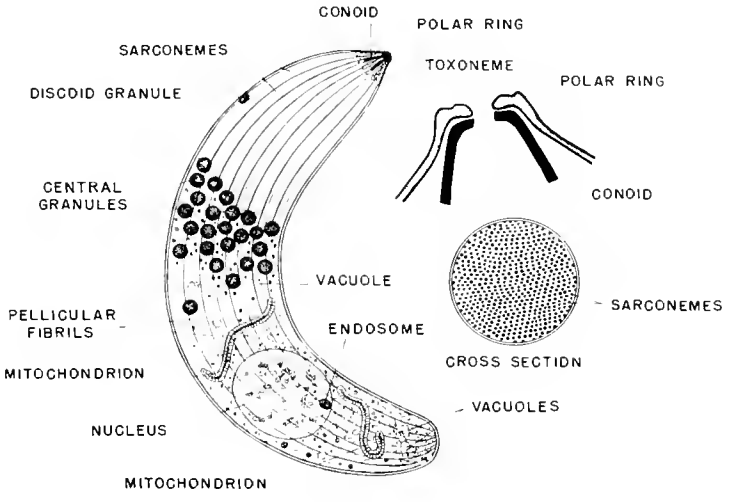

Fig. 36. Trophozoite of *Sarcocystis tenella*. (After Ludvik, 1958)

Ludvik (1958, 1960) described their structure in *S. tenella* and *S. miescheriana* on the basis of electron microscope, cytochemical and light microscope studies. At the anterior end within the pellicle is a polar ring 0.4 to 0.5 μ in diameter, and within it is a hollow, truncate cone 0.3 to 0.4 μ long known as a conoid. From the polar ring 22 to 26 fine fibrils run backwards in the pellicle the full length of the body. In some individuals short, club-shaped structures similar to the toxonemes of *Toxoplasma* can be seen in the cytoplasm beneath the pellicle.

The cell body is divisible into 3 zones. The anterior third of the body, the so-called fibrillar zone, is filled with a large number (about 300 to 350) of parallel, equidistant fibrils or perhaps channels about 50 mμ in diameter, the sarconemes. They probably arise from the conoid, and they end abruptly. Just under the pellicle on the dorsal (convex) side about the middle of the fibrillar zone is a disc-shaped granule which stains with Bodian silver.

The middle third of the body contains a large number of spherical granules 0.4 to 0.5 μ in diameter, the so-called central granules. They impregnate with osmium and stain intensely with Heidenhain's hematoxylin but not with Giemsa. In the same region are many minute granules, some of which contain volutin and others RNA. There are also 1 or 2 large vacuoles which stain with neutral red.

The posterior third of the body contains the nucleus. It is an ellipsoidal vesicle almost as wide as the body, and contains a relatively small number of chromatin granules and an endosome which stains with Bodian silver. The nucleus is surrounded by a large number of small vacuoles and granules, many of which contain glycogen, and these extend to the posterior end of the body. Among them lie 1 to 3 serpentine mitochondria 0.15 to 0.2 μ in diameter and 2 μ or more long.

In addition to the above structures, a network of fibrils forming a characteristic rectangular pattern can be seen on the surface following silver impregnation by the Klein or Chatton technics.

Life Cycle: Several differing accounts have been given of the life cycle of *Sarcocystis*. Pitfalls in its study have been discussed by Scott (1943). There is now general agreement that the life cycle is simple, without sexual stages, and that no intermediate host is involved.

Animals become infected by ingesting trophozoites, either in unbroken cysts in the muscles or free in the feces of other animals. Smith (1901, 1905) was the first to show that infection took place by the oral route, and was able to maintain the infection with *S. muris* in mice for 7 years by feeding infected mouse muscle.

The trophozoites presumably pass thru the intestinal wall, enter the blood stream and are carried to the striated muscles, where they enter the muscle cells. They are found in the striated and heart muscles. They are especially common in the wall of the esophagus, but are also found in the tongue, masseter muscle, diaphragm, throat, neck, body and limb muscles, and even in the eye muscles and Purkinje fibers of the heart among other places. In ducks they are most commonly found in the breast muscles.

There is a latent period of a month to 6 weeks or more during which almost nothing is known of what happens. The first stage in the muscle cell is a one-celled, irregularly rounded ("amoeboid") naked parasite. This divides by repeated binary fissions (Scott, 1943) into a number of rounded cells 4 to 8 μ in diameter which are enclosed in a cyst wall. Betegh and Dorcich (1912), Erdmann (1914) and Arai (1925) thought that schizogony takes place at this stage, but Scott (1943) did not agree, and Frenkel (1956a) considered its existence doubtful.

The rounded cells have been called sporoblasts, pansporoblasts or prosporoblasts, but these names all carry the connotation that the trophozoites are spores, and the cells are better called cytomeres (Grassé, 1953) or trophoblasts. They continue to reproduce by binary fission, and become pressed together and polygonal. Later they change into ellipsoidal and then into banana-shaped trophozoites.

As multiplication proceeds, the cyst grows and is divided into chambers or compartments by septa arising from the inner layer of the cyst wall. The process continues, new trophoblasts are formed at the periphery of the cysts, produce new trophozoites, and new septa are laid down and new compartments formed.

The trophozoites themselves also reproduce by binary fission. This process was described by Ludvik (1958). The nucleus first begins to enlarge and the dispersed chromatin forms large granules and variously curved structures. The nucleus is indented in the middle of its anterior edge and becomes horseshoe-shaped. The cell loses its banana shape and becomes broadly spindle-shaped, with a rounded posterior end. The central granules become dispersed thru the whole cell and diminish in size. A medial sac-like structure begins to be separated off from the posterior part of the horseshoe-shaped nucleus, and the central granules disappear. The sac-like structure becomes detached from the nucleus and gradually divides into 2 halves which later, after the true nuclear division has been completed, disappear. The horseshoe-shaped nucleus divides into 2 longitudinal segments. The conoid and cytoplasm in the anterior third of the cell also divide into 2 longitudinal halves with a clear streak between them. The newly formed nuclei become shorter and their chromatin

gradually disperses. Cell division now begins, starting from the conoid at the anterior end and proceeding posteriorly. The nuclei round up, their nucleoplasm becomes thicker, and they move toward the posterior part of the newly forming cells. New central granules appear in the cytoplasm in front of the nuclei. The daughter cells remain attached at their posterior ends for a time and then separate entirely.

Finally, as the cyst itself becomes older, the trophozoites in the central compartments degenerate and disappear. After the cyst becomes mature, its wall breaks down and the trophozoites are released. They enter the blood stream, reach the digestive tract, and pass out in the feces. They have also been found in the nasal secretions of sheep (Scott, 1943).

Quite a different account has been given by Spindler and his associates, who believe *Sarcocystis* to be a fungus rather than a protozoon. Spindler and Zimmerman (1945) reported that they had isolated an *Aspergillus*-like fungus from sarcocysts from pig muscles, and that 25 out of 50 pigs injected with or fed material from the cultures had sarcocysts in their muscles 4 to 6 months later, while the control pigs were negative. They also said that pigs, rats and mice fed the cysts passed yeast-like bodies in their urine or feces which produced a similar fungus upon culture, and they found these bodies in the kidneys of infected mice and in clumps attached to the walls of the ileum and cecum of infected rats and mice.

Spindler, Zimmerman and Jaquette (1946) were unable to infect pigs directly with sarcocysts in pig muscles, but they observed that the pigs became infected if they ate their own feces. They fed pork containing sarcocysts to pigs, dogs, cats, rats, mice and chickens. These subsequently passed a stage in their feces and/or urine which was infective for swine. Their observation, incidentally, may perhaps explain the remark of Scott (1943) that feeding experiments in sheep indicate that the trophozoites of *S. tenella* must undergo some change before they can infect other sheep.

Spindler (1947) described a network of jointed, hypha-like structures in cysts from a sheep and a duck, and said that the trophozoites appeared to be exogenous growths on these structures. However, Grassé (1953) commented that his illustrations were not convincing, and that the structures he described appeared to be the result of marked alterations in the true ones. Frenkel (1956a), too, disagreed with Spindler. He found no fungal characteristics in morphological studies of organisms from man, the sheep, mouse, rabbit, squirrels and the duck. Unlike fungi, the trophozoites and cyst walls did not give a positive reaction with the periodic acid-Schiff stain. *Sarcocystis* from cottontail rabbits and house mice failed to grow on the media customarily used for fungi. Frenkel concluded that these organisms neither look nor behave like fungi.

Scott (1943), too, and others cited by him were unable to cultivate organisms from the cysts. Only Ciesla (1950) has reported positive results. He observed "sporozoites" in cultures from cysts from cattle, and said that these eventually turned into round corpuscles with a quick, convulsive type of movement which budded into branched chains of mycelia.

The weight of the evidence thus indicates that *Sarcocystis* is a protozoon and not a fungus.

Pathogenesis: *Sarcocystis* is not generally considered very pathogenic. However, Scott (1943a) believed that it is of greater economic importance than is usually supposed.

Light or moderate infections produce no noticeable signs, but in very heavy infections there may be lameness, weakness, emaciation, paralysis and even death.

The sarcocyst destroys that part of the muscle fiber which it occupies, and as it grows it may cause pressure atrophy of adjacent cells. Calcification may also occur. There is ordinarily little if any cellular reaction around the cysts. Focal myocarditis and myositis develop when the cysts break down. Destombes (1957) described a marked inflammatory reaction

around the cysts followed by necrosis and calcification in swine, but saw no such reaction in cattle. Spindler, Zimmerman and Jaquette (1946) found that pigs with 40 or more cysts per gram of diaphragm were unthrifty and showed signs of muscular stiffness.

Gastrointestinal signs and lesions may occur after ingestion of the cysts. Scott (1943) reported extensive destruction of the epithelium together with a bloody serous exudate in the ileum of young rats fed sarcocysts from sheep, and the animals appeared ill and disinclined to move about. Spindler, Zimmerman and Jaquette (1946) observed vomiting, diarrhea, inappetence and temporary posterior paralysis in pigs fed infected muscles, urine or feces.

The cysts contain a powerful endotoxin known as sarcocystin, which is highly toxic for rabbits, mice, and sparrows, but probably less toxic for rats, sheep and some other animals. Sarcocystin acts on the central nervous system and also affects the heart, adrenal glands, liver and intestinal wall. It is filtrable, and is destroyed by heat. Small amounts cause a febrile reaction in the rabbit, while large amounts produce collapse, severe diarrhea and death. According to Sato (1926), the intravenous minimum lethal dose for the rabbit of the extract from *S. fusiformis* from the ox is 0.05 mg per kilogram body weight.

Immunity: Animals can be immunized against sarcocystin by repeated injections of untreated or formalin-treated toxin. The serum of immunized animals will protect other animals against the toxin.

The close relationship between *Sarcocystis* and *Toxoplasma* is attested by the fact that both react with cytoplasm-modifying antibody in the Sabin-Feldman dye test (described below under *Toxoplasma*). As a matter of fact, cross reactions between the two are not uncommon. Muhlpfordt (1951) and Awad and Lainson (1954) found that the sera of laboratory animals fed *S. tenella* from sheep reacted positively to the dye test with *Toxoplasma* trophozoites. The sera of sheep naturally infected with *S. tenella* also gave positive reactions. Awad (1954) went a step further, and developed a modified dye test for *Toxoplasma*, using *S. tenella* trophozoites. These trophozoites gave positive results with the sera of animals infected with either *Toxoplasma* or *Sarcocystis*.

Epidemiology: Seasonal infection during the late spring, summer and early fall has been reported in sheep, swine and horses in the temperate zone (Scott, 1943). Repeated infections of sheep in successive seasons were reported by Scott (1943). He had the impression that the older the animals, the more heavily they were parasitized.

Diagnosis: Because of the absence of recognizable signs, *Sarcocystis* infections are almost always diagnosed after death. The larger cysts are easily seen with the naked eye, and the small ones can be found by histologic examination.

Cultivation: Sarcocystis has not been cultivated, unless the claims of Spindler and Zimmerman (1945) and Ciesla (1950) are accepted.

Treatment: None known.

Prevention and Control: Since *Sarcocystis* infections are acquired thru fecal contamination of food or drink, infections can be prevented by measures designed to prevent such contamination. Sanitation and good management should be effective.

SARCOCYSTIS MIESCHERIANA (KÜHN, 1865) LANKESTER, 1882

Synonyms: *Synchytrium miescherianum*.

Host: Pig.

Location: Striated and heart muscles.

Geographic Distribution: Worldwide.

Prevalence: This species is extremely common thruout the world, having been reported in as high as 98.5% of pigs examined. Alicata (1932) found it in 75%

of 180 garbage-fed hogs in California. Jacobs, Remington and Melton (1960a) found it in 44% of 50 pigs from a Baltimore slaughter house. Musfeldt (1950) found it in 6% of 264 swine diaphragms in British Columbia. Sysoev (1955) reported it in 9.2% of 319,492 swine diaphragms in the USSR.

Morphology: The cysts are 0.5 to 4 mm long and up to 3 mm wide. They are compartmented, and their wall is striated with cytophaneres.

Remarks: This is the type species of the genus. If it eventually turns out that the various species reported from different hosts are actually the same, then their correct name would be *S. miescheriana*.

SARCOCYSTIS FUSIFORMIS RAILLIET, 1897

Synonyms: *Sarcocystis blanchardi*, *Miescheria cruzi*.

Hosts: Ox, water buffalo.

Location: Striated and heart muscles.

Geographic Distribution: Worldwide.

Prevalence: This species is extremely common thruout the world. Wilson and McDonald (1938) found it in the hearts of 86% of 35 cattle in Virginia. Wang (1950) found it in 75% of 48 cattle in Illinois. Jacobs, Remington and Melton (1960a) found it in 98% of 60 cattle from a Baltimore slaughter house. Skibsted (1945) found it in 94% of 100 cows and 18.5% of 97 calves in Denmark.

Morphology: The cysts are up to 1 cm or more long. They are compartmented when mature. The cyst wall may be thin and smooth or may contain cytophaneres. The trophozoites are about 10 μ long.

SARCOCYSTIS TENELLA RAILLIET, 1886

Synonym: *Balbiania gigantea*.

Hosts: Sheep, goat, bighorn sheep (Honess, 1956).

Location: Striated and heart muscles. This species is especially common in the wall of the esophagus.

Geographic Distribution: Worldwide.

Prevalence: This species is extremely common in sheep thruout the world, having been reported from 50 to 100% of the sheep examined (Scott, 1943; Destombes, 1957; Grassé, 1953). Jacobs, Remington and Melton (1960a) found it in 98% of 86 sheep from a Baltimore slaughter house. It is uncommon in goats (Reichenow, 1953).

Morphology: The cysts are relatively ellipsoidal and up to 1 cm long. The cyst wall is smooth at first, acquires a layer of cytophaneres as it grows, and loses them again as it ages. The cysts are compartmented. The trophozoites measure 8 to 11 by 2 to 4 μ.

SARCOCYSTIS CERVI DESTOMBES, 1957

This species was described by Destombes (1957) from an unidentified species of deer in Vietnam. Honess (1956) found *Sarcocystis* in the mule deer (*Odocoileus hemionus*) and elk (*Cervus canadensis*) in Wyoming. However, it is likely that *S. cervi* is a synonym of *S. tenella*.

SARCOCYSTIS BERTRAMI DOFLEIN, 1901

Hosts: Horse, ass.

Location: Striated and heart muscles.

Geographic Distribution: Worldwide.

Prevalence: This species is extremely common thruout the world.

Morphology: This species closely resembles *S. miescheriana* of the pig. The cysts are up to 10 mm long and are compartmented. The cyst wall has a layer of cytophaneres.

SARCOCYSTIS LINDEMANNI
(RIVOLTA, 1878)

Synonyms: *Gregarina lindemanni, Sarcocystis hominis.*

Host: Man.

Location: Striated and heart muscles.

Geographic Distribution: Worldwide.

Prevalence: This name has been given to various sarcocysts of different sizes which have been found on rare occasions in man. It is extremely doubtful that man has a species of his own, and these cases were most probably infections with forms from domestic animals. The differences in size of trophozoites given in different reports bear this out (cf. Kean and Grocott, 1945).

Morphology: The cysts are up to 5.3 cm long, but are usually much smaller. Compartmentation has been described. The cyst wall is apparently smooth. The trophozoites vary in size; most are in the range of 7 to 10 by 2 to 3 μ, but small ones 5 by 2 μ have also been reported.

SARCOCYSTIS MURIS
BLANCHARD, 1885

Hosts: House mouse, Norway rat, black rat.

Location: Striated and heart muscles.

Geographic Distribution: Worldwide.

Prevalence: At one time this species was common in laboratory mice, but it is less so today. According to Deschiens, Levaditi and Lamy (1957), it is quite rare in laboratory mice, and they did not encounter it in laboratory rats in France during the previous 4 years.

Morphology: The cysts are elongate, range in length up to several centimeters, and are apparently not compartmented. The cyst wall is smooth. The trophozoites are 9 to 15 μ long and 2.5 to 3 μ wide.

SARCOCYSTIS CUNICULI
BRUMPT, 1913

Synonym: *Sarcocystis leporum.*

Hosts: Domestic rabbit, cottontail.

Location: Striated and heart muscles, especially in the hind legs, flanks and loins.

Geographic Distribution: Worldwide.

Prevalence: This species is common in cottontails. Erickson (1946) found it in 38% of 78 cottontails in Minnesota. It is apparently not common in domestic rabbits. Deschiens, Levaditi and Lamy (1957) said that it is very rare in France.

Morphology: The cysts are up to 5 mm long. They are compartmented, and their walls have a layer of cytophaneres. The trophozoites usually measure 12 to 13 by 4 to 5 μ, but range in length from 6 to 16 μ.

SARCOCYSTIS RILEYI
(STILES, 1893)

Synonyms: *Balbiania rileyi, Sarcocystis anatina, Sarcocystis horwathi, Sarcocystis gallinarum.*

Hosts: Domestic duck and various wild ducks, including the mallard, black duck, gadwall, American pintail, blue-winged teal and shoveller. In addition, this species has been found in the chicken (Hawkins, 1943), sage grouse (Salt, 1958) and a number of other wild birds. Erickson (1940) listed 20 species of birds belonging to 8 orders in which *Sarcocystis* had been found.

Location: Striated and heart muscles, especially of the breast, neck and legs.

Geographic Distribution: Worldwide.

Prevalence: This species is especially common in surface-feeding wild ducks, but not in diving ducks. Erickson (1940) reported it from 3% of 312 wild ducks in Minnesota. It is not uncommon for hunters

to bring infected birds which they have shot to a diagnostic laboratory to learn whether they are safe to eat.

Morphology: The cysts are several millimeters long and are compartmented. The cyst wall is smooth. The trophozoites measure about 8 by 2 μ.

FAMILY TOXOPLASMATIDAE

Members of this family form pseudocysts, i.e., the "cyst" wall is formed by the host and not by the parasite. A true cyst may be formed as well (cf. Lainson, 1958). Multiplication is by binary fission or endodyogeny, and possibly by schizogony in the young pseudocysts.

This family includes the genera *Toxoplasma*, *Besnoitia* and *Encephalitozoon*. Its taxonomy has been reviewed by Westphal (1954), Van Thiel (1956), Biocca (1949, 1957), and Goldman, Carver and Sulzer (1958), among others.

Genus TOXOPLASMA
Nicolle and Manceaux, 1908

In this genus the pseudocyst wall is thin. A single, euryxenous species, *T. gondii* is recognized.

Because of its importance as a cause of human disease, *T. gondii* has been studied intensively and the literature on it is vast. Eyles and Frenkel (1952) published a bibliography which listed 920 papers and then supplemented it (1954) with 400 more. A great many more papers have been published since that time. It is obviously impractical to attempt to refer to them all here. Various aspects of *Toxoplasma* and toxoplasmosis have been reviewed by Weinman (1952), Habegger (1953), Jacobs (1956, 1957), Feldman and Miller (1956), Siim (1956), Eichenwald (1956), Frenkel (1956a), Hoare (1956), Eyles (1956), de Roever-Bonnet (1957) and Siim (1960).

TOXOPLASMA GONDII
NICOLLE AND MANCEAUX, 1908

Synonyms: *Toxoplasma cuniculi, T. caviae, T. canis, T. musculi, T. ratti, T. laidlawi, T. sciuri, T. pyrogenes, T. hominis.*

Disease: Toxoplasmosis.

Hosts: *T. gondii* was first found in the gondi (*Ctenodactylus gondi*), a North African rodent, but it has since been found in many species of mammals and birds. Its host list includes the gondi, house mouse, Norway, black, climbing and water rats, squirrel, ground squirrel, vole, guinea pig, chinchilla, marmot, the Chilean rodent, *Octodon degus*, the Uruguayan rodent, *Ctenomys torquatus*, rabbit, hare, mole, shrew, hedgehog, dog, cat, fox, weasel, ferret, mink, wombat, bandicoot, brush-tail possum, marsupial rat, pig, sheep, ox, baboon, chimpanzee, macaque (*Macaca tantala*), whiteface monkey (*Cebus capucinus*), cotton-topped marmoset (*Oedipomidas oedipus*), squirrel monkey (*Saimiri sciurea*), man, pigeon, chicken, crow, canary, penguin and partridge (*Perdrix perdrix*) (Ratcliffe and Worth, 1951; Christen and Thiermann, 1953; Talice, Perez-Moreira and Mossera, 1954; Jacobs, 1956; Finlay and Manwell, 1956; Van den Akker, Bool and Spitseshuis, 1959; Cook and Pope, 1959; Benirschke and Richart, 1960). In addition, organisms which resemble *Toxoplasma* morphologically have been seen in reptiles; and turtles, lizards, geckos and chameleons can be infected experimentally (Jacobs, 1956). On the other hand, most of the organisms reported as *Toxoplasma* from the blood of various wild birds are probably *Lankesterella*.

Location: *Toxoplasma* is an intracellular parasite of many types of cells, including neurons, microglia, endothelium, reticulum, liver parenchyma cells, lung and glandular epithelial cells, cardiac and skeletal muscle cells, fetal membranes and leucocytes. In acute infections, the parasites may be found free in the blood and peritoneal exudate.

Geographic Distribution: Worldwide.

Prevalence: Toxoplasmiasis is apparently extremely common in man and also in many domestic animals. As Jacobs (1957) said, there is a sea of *Toxoplasma* infection around us. However, toxoplasmosis is far less common. Most infections are inapparent, and the disease itself appears only under special circumstances, many of which are still unknown.

Most of the surveys which have been made for *Toxoplasma* have been serologic and indicate either previous or present infections. In some cases, particularly in sheep and other domestic animals in which *Sarcocystis* infection is common and in which the Sabin-Feldman dye test was used, they may indicate merely the presence of cross-reacting antibodies (Muhlpfordt, 1951; Awad, 1954; Awad and Lainson, 1954). Hence surveys in which the organism itself was isolated are more reliable, altho much more time-consuming and expensive.

The prevalence of antibodies varies widely in man in different geographic locations. For instance, according to Jacobs (1957), there is relatively less infection in California than in the eastern United States. Feldman and Miller (1956a) observed positive dye tests in 68% of 121 persons on Tahiti, 64% of 266 in Honduras, 36% of 104 in Haiti, 35% of 144 in Pittsburgh, Penn., 31% of 270 in New Orleans, 26% of 184 in St. Louis, 17% of 293 in Portland, Ore., 11% of 108 on Iceland, 4% of 236 Navajo Indians in Arizona, and none of 21 Eskimos in Alaska. In a study of 1072 urban and rural Negroes 11 to 19 years old in the region of Memphis, Tennessee, Gibson (1956) found that the Sabin-Feldman dye test was positive in 20.4% of the urban and 18.9% of the rural group. Balozet (1955) found that 12% of 125 humans in Algiers were positive to the complement fixation test. Thiermann and Naquira (1958) found that the dye test was positive in 43% and the complement fixation test in 11% of 284 normal medical students in Santiago, Chile; the dye test was positive in 48% and the complement fixation test in 2% of 131 blood donors, mostly over 30 years old. Orio *et al.* (1958) found that the sera of 10.2% of 1139 Africans in Middle Congo were positive to the complement fixation test. The above results give some idea of the range of positive reactions which may be expected in different surveys.

Among domestic animals, the first spontaneous case of toxoplasmosis in the dog was reported by Mello (1910) in Turin, Italy. In reviewing the animal reservoir of toxoplasmosis, Habegger (1953) stated that only something more than 50 cases had been reported in dogs thruout the world. However, more recent reports have raised this figure considerably.

Miller and Feldman (1953) found dye test antibodies in 59% of 51 dogs in Pennsylvania. Feldman and Miller (1956a) found them in 28% of 51 dogs from New York, 30% of 23 dogs from Arizona and 86% of 7 dogs from Honduras. Siim (1950) found that 18.5% of 54 dogs in Copenhagen had dye test titers of 1:250 or more. Otten, Westphal and Kajahn (1950) found that 36% of 84 dogs in Hamburg, Germany were positive to the dye test. Borgen and Berg (1957) found that 44.5% of 20 dogs in Norway were positive to the dye test. De Roever-Bonnet (1957) found that 1 of 75 dogs in Amsterdam was positive to the dye test at a titer above 1:100. Makstenieks and Verlinde (1957) found that 14% of 29 dogs from households in the Netherlands where human toxoplasmosis existed were positive to the dye test at 1:64 or above. Eyles *et al.* (1959) found that 8.3% of 809 dogs from the Memphis pound or slums were positive to the dye test at a titer of 1:64 or above, and they isolated *Toxoplasma* by mouse inoculation from 3 of 200 of the dogs. Gibson and Jumper (1960) found that the sera of 16% of 800 dogs from the Memphis pound were positive to the dye test at a titer of 1:16 or above; they found *Toxoplasma* by mouse inoculation in only 2 out of 75 of these animals.

Morris, Aulisio and McCown (1956) found that 25% of 180 dogs in the Middle Atlantic stages were positive to the complement fixation test. Lainson (1956) found that 42.5% of 113 dogs in London were positive to the complement fixation test. Balozet (1955) found that 30% of

105 pound dogs in Algiers were positive to the complement fixation test.

In the United States, Cole *et al.* (1953) described an outbreak of toxoplasmosis in a kennel of 104 dachshunds, in which 69 pups and 17 adults (of which 14 were bitches) died. In another outbreak in a kennel of 47 chihuahuas, 14 pups and 15 adults died. They also found toxoplasmosis in 11 pet dogs, each owned by a different family. Langham and Sholl (1949) reported a case in a young fox terrier in Michigan. Moulton and Linton (1953) reported a fatal canine case in California. Krause (1954) found *Toxoplasma* in 1 out of 30 dogs by inoculating mice with brain tissue. Seibold and Hoerlein (1955) reported a case of renal toxoplasmosis associated with distemper in a puppy.

Hulland (1956) described 8 fatal cases of canine toxoplasmosis in Canada. Wickham and Carne (1950) reported 3 cases in Australia. Grocott (1950) reported a case from the Canal Zone. Sjolte (1948) reported the first case of canine toxoplasmosis in Denmark. Fankhauser (1950, 1951) found it in 6 dogs in Switzerland. Kardevan and Kapp (1957) found *Toxoplasma* in 2 of 20 dogs in Hungary. Bonser (1950) described a case of toxoplasmatal intussusception in a 3-year-old bitch in England. Campbell, Martin and Gordon (1955) found it by histological examination in 6% of 268 dogs in Glasgow, Scotland. Flir (1954) described 3 cases in dogs in Germany. Van den Akker, Bool and Spitseshuis (1959) found it in a dog in Holland. Blanc and Hintermann (1948) reported it in a dog in Morocco. Orio *et al.* (1959) found *Toxoplasma* in a dog in Brazzaville, Middle Congo.

Toxoplasmosis has been reported in single cats by Wickham and Carne (1950) in Australia, Holzworth (1954) in Massachusetts, Jones (1955) in the U.S. and Hulland (1956) in Canada. Jones, Eyles and Gibson (1957) found *T. gondii* by mouse inoculation in 24% of 140 cats in Memphis, Tennessee, and in 11% of 35 cats in Columbia, South Carolina. They reviewed the literature on isolation of *Toxoplasma* from the cat; theirs was the tenth report. Gibson and Eyles (1957) found *T. gondii* by mouse inoculation of brain tissue in 20% of 35 cats from the neighborhood of a house in Memphis where a newborn child had died of congenital toxoplasmosis.

Feldman and Miller (1956a) found that 33% of 79 cats from Massachusetts and New York were positive to the dye test for *Toxoplasma*. Makstenieks and Verlinde (1957) found that 15% of 33 cats from households in the Netherlands where human toxoplasmosis existed were positive to the dye test at a titer of 1:64 or above. Havlik and Hubner (1959) found that 34% of 200 cats in central Bohemia were positive to the dye test at a titer of 1:16 or above; they isolated *Toxoplasma* by mouse inoculation from 2 out of 23 of the positive cats.

The first cases of toxoplasmosis in swine were reported by Farrell *et al.* (1952) in Ohio. They found the disease in 8 pigs from a farm where an undiagnosed disease had recurred for many years. Sanger and Cole (1955) isolated *T. gondii* from 2 newborn pigs collected aseptically from the vagina as well as from the milk and heart of a naturally infected sow which showed no signs of disease. They also isolated *Toxoplasma* from the milk and from 3 of 4 pigs from another apparently healthy, naturally infected sow. Momberg-Jørgensen (1956) isolated *Toxoplasma* from a litter of 8-day-old pigs in Norway, 6 of which had died of pneumonia, enteritis, hepatitis, nephritis and splenitis; he also found *Toxoplasma* in tissue sections of some 18-day-old pigs that had died of a similar pneumonia.

In a serologic survey of hog sera from a slaughterhouse in New Haven, Conn., Weinman and Chandler (1956) found that 42% of 88 sera were positive to the dye test. Most of the positive pigs were from one farm where the pigs were fed uncooked garbage. Feldman and Miller (1956a) found that 30% of 73 pigs from the midwest and New York were positive to the dye test. De Roever-Bonnet (1957) found that 12% of 25 hogs from an Amsterdam slaughterhouse were positive to the dye test at a titer above 1:1000. Eyles *et al.* (1959) found that 2% of 178 pigs from Memphis, Tenn. slaughterhouses were positive to the dye test at

a titer of 1:64 or above, and isolated *Toxoplasma* by mouse inoculation from 1 out of 129 of them. By inoculation of mice with peptic digests of diaphragm samples, Jacobs, Remington and Melton (1960a) found *Toxoplasma* infection in 24% of 50 pigs from a Baltimore slaughterhouse.

Toxoplasmosis was first reported from sheep by Olafson and Monlux (1942) in New York. It was later found in sheep in Australia by Wickham and Carne (1950) and Osborne (1959), in Ohio by Cole *et al.* (1954) in New Zealand by Hartley and Marshall (1957), and in England by Beverley and Watson (1959). It was associated with abortions and perinatal mortality in the last 4 reports; indeed, Hartley and Marshall considered toxoplasmosis to be the most wide-spread and probably the most important cause of ovine perinatal mortality in New Zealand. It may be important in England, too; Beverley and Watson (1959) found it in 6 of 39 aborted lambs from a number of flocks in that country, and found dye test titers of 1:128 or above in 43 of 549 ewes from 93 flocks, including 22 of 158 ewes which had aborted from causes other than viral or bacterial.

Feldman and Miller (1956a) found that 5% of 66 sheep from Arizona, 56% of 9 sheep from Kentucky and 43% of 65 goats from New York were positive to the dye test for *Toxoplasma*. De Roever-Bonnet (1957) found that 35% of 23 sheep from an Amsterdam slaughterhouse were positive to the dye test at a titer above 1:100. He also (1957a) isolated *Toxoplasma* by mouse inoculation from the brains of 4 out of 30 slaughtered sheep picked at random. Rawal (1959) found that 3 of 100 sheep sera from a Sheffield, England slaughterhouse were positive to the dye test at a titer of 1:64 or above. He found *Toxoplasma* by mouse inoculation in the brains of 6 out of 21 sheep whose sera had reacted to the dye test at a titer of 1:4 or above. Jacobs, Remington and Melton (1960a) found *Toxoplasma* infection in 9% of 86 sheep from a Baltimore slaughterhouse.

Sanger *et al.* (1953) found *Toxoplasma* in 4 herds of cattle in Ohio. Miller and Feldman (1953) and Feldman and Miller (1956) found that 19% of 132 cattle from New York were positive to the dye test. De Roever-Bonnet (1957) found that 6% of 31 cattle from an Amsterdam slaughter house were positive to the dye test at a titer above 1:100. Jacobs, Remington and Melton (1960a) found *Toxoplasma* infection in 2% of 60 beef cattle from a Baltimore slaughterhouse.

Toxoplasma has been found in lagomorphs not infrequently. Perrin (1943) found it in a laboratory rabbit in Bethesda Md., Christiansen (1948) found it in 8.75% of 2411 hares in Denmark, Lainson (1955) found it in the brains of 5% of 113 domestic rabbits in England, and Orio *et al.* (1959) isolated it from 57% of 14 rabbits from Brazzaville, Middle Congo, either from the Pasteur Institute animal colony there or from the environs of the city itself. Miller and Feldman (1953) found that 5% of 22 laboratory rabbits were positive to the dye test, and Morris, Aulisio and McCown (1956) found that 19% of 107 cottontails from the Middle Atlantic states were positive to the same test.

Toxoplasma has been found several times in guinea pigs. Among others, Mariani (1941) found it in guinea pigs sent from Italy to Ethiopia. De Rodaniche (1949) found it in guinea pigs purchased in the suburbs of the city of Panama. Varela, Martinez and Trevino (1953) found it in a guinea pig in Mexico. Orio *et al.* (1959) found it in 23% of 31 adult guinea pigs in the Pasteur Institute animal colony at Brazzaville, Middle Congo. Miller and Feldman (1953) found that 27% of 51 laboratory guinea pigs in the U.S. were positive to the dye test. Makstenieks and Verlinde (1957) found that 33% of 174 guinea pigs from animal dealers in the Netherlands were positive to the dye test at a titer of 1:64 or above.

Toxoplasma was found by mouse or guinea pig inoculation of brain tissue in over 3% of the wild Norway rats in Memphis by Eyles (1952). He also found that the dye test was positive in 20% of 100 rats, but observed no correlation between the dye test and the results of tissue inoculation. Lainson (1957) found *Toxoplasma*

in 1 of 99 wild Norway rats in England. Miller and Feldman (1953) found no positive dye test reactors among 54 albino rats which they studied.

Toxoplasma has been found in laboratory mice by Nicolau and Balmus (1934) and Mooser (1950). Gibson and Eyles (1957) found it by mouse inoculation of brain tissue in 6% of 121 wild house mice captured in the neighborhood of a house in Memphis where a new-born infant had died of congenital toxoplasmosis. Lainson (1957) failed to find it in 399 wild house mice in England. Makstenieks and Verlinde (1957) found that none of 4097 laboratory mice from animal dealers in the Netherlands was positive to the dye test even at a titer of 1:4.

Toxoplasmosis is so common in voles (*Microtus agrestis*) in England that it is said to be a population-limiting factor (Findlay and Middleton, 1934; Elton, Davis and Findlay, 1935).

Among other mammals, toxoplasmosis has been reported in mink by Hulland (1956) and Pridham and Belcher (1958) in Canada, and by Momberg-Jørgensen (1956a) in Norway. In the last case, a severe outbreak of distemper was also present. Lainson (1957) found it in a weasel (*Mustela nivalis*), a ferret, and 2 ferret-polecat hybrids in England. *Toxoplasma* was reported in 3 chinchillas in Washington by Gorham and Farrell (1956), and in 3 chinchilla ranches in Canada by Hulland (1956).

Among domestic birds, *Toxoplasma* was found in a hen in Switzerland by Fankhauser (1951a), in a flock of chickens in Norway by Erichsen and Harboe (1954), and in 35 hens from 21 flocks in Denmark by Biering-Sørensen (1956).

Manwell and Drobeck (1951) isolated *T. gondii* from a pigeon caught in Syracuse, N. Y., while Jacobs, Melton and Jones (1952) isolated it from 4 of 80 wild pigeons caught in Washington, D. C.; the dye test was positive in 7 of these birds, including 1 of those from which the organism was isolated.

Rosenbusch (1931) found *T. gondii* in a canary in Argentina, and Sergent and Poncet (1954) found it in one in Algeria. Finlay and Manwell (1956) have reviewed the literature on *Toxoplasma* in birds.

Fig. 37. *Toxoplasma gondii* trophozoites from mouse peritoneal exudate. Giemsa stain. X 2800. (Original)

Morphology: The trophozoites of *T. gondii* are crescentic or banana-shaped, with one end pointed and the other rounded, and measure 4 to 8 by 2 to 4 μ. The nucleus is vesicular and more or less central. There are no flagella, cilia or pseudopods. Locomotion is by body flexion whereby the protozoa follow a corkscrew path, rotate on their longitudinal axis or somersault (Manwell and Drobeck, 1953), or by gliding.

The morphology of the trophozoites has been studied following silver protein staining by Goldman, Carver and Sulzer (1957, 1958) and with the electron microscope by Gustafson, Agar and Cramer (1954), Bringmann and Holz (1954), Ludvik (1956) and Meyer and Mendonca (1957). They resemble the trophozoites of *Sarcocystis* in a number of ways. At the anterior end within the pellicle is a short, truncate, hollow cone 0.15 to 0.25 μ in diameter and 0.2 to 0.3 μ long, called a conoid. There is sometimes a distinct, spike-like extension at the anterior end. A number of fine, longitudinal fibrils run posteriorly in the pellicle from the region of the conoid; they extend for about 1/5 of the body length according to Ludvik (1956) or 2/3 of it according to Bringmann and Holz (1954). Running longitudinally in the body from the conoid are 5 to 18 cylindrical or club-shaped structures known as toxonemes. They are of variable length, some extending nearly to the posterior end and others not reaching the level of the nucleus; they become very slender and tortuous as they approach the conoid, and seem to enter its base. They

are 0.02 μ in diameter when they leave the conoid and then thicken to form a club or sausage-shaped structure 0.08 to 0.2 μ in diameter. In addition to these, there are 1 or 2 central fibrils which frequently form a large loop or run posteriorly in a zigzag.

The cytoplasm is somewhat vacuolated and contains a number of osmiophilic granules about 0.5 μ in diameter, mitochondria and often a cluster of fine granules around the nucleus. Goldman, Carver and Sulzer (1958) found a mass of argyrophilic granules at the very posterior end. The nucleus is usually round or oval, but lobed and horseshoe shapes have also been seen in electron micrographs. In the latter, the open end faces anteriorly as in *Sarcocystis*. The nucleus is about 1.0 to 1.5 μ in diameter when circular and up to 2 μ in diameter when elongated. Inside the nucleus is a large endosome which can be seen both in electron micrographs and after silver protein staining.

In addition to the above structures, Goldman, Carver and Sulzer (1958) described long, thread-like appendages in trophozoites treated with dilute (0.1 to 1.0%) formalin in saline before fixation. These may have been detached pellicular fibrils.

The parasites occur within vacuoles in their host cells. According to Gustafson, Agar and Cramer (1954), there is a definite space between the parasite and the vacuole wall. The space often contains a filamentous or granular precipitate, and concentrations of mitochondria are often present in the host cell at the edge of the vacuole.

As the parasites multiply, they form a cyst-like structure. Frenkel (1956a) emphasized that there is a difference between the terminal colonies which represent the final stage of parasitization in the leucocytes and the cysts which are found in the central nervous system, eye and myocardium. The wall of the latter is argyrophilic and weakly positive to the periodic acid-Schiff stain (PAS), while that of the former is not. Some authorities believe that the wall is formed by the host, so that the "cyst" is actually a pseudocyst, but Frenkel and Friedlander (1951) considered it likely that the wall is derived from the parasite. Lainson (1958), too, distinguished between the cyst-like structures formed in the acute and chronic stages of the infection. The former he considered to be pseudocysts and the latter true cysts.

The trophozoites in the cysts differ slightly from the proliferative ones in the pseudocysts. They contain large glycogen granules, are more resistant to external agents, and multiply slowly. Dasgupta and Kulasiri (1959) found that PAS-positive granules were abundant in the stages in the "pseudocysts" from the brains of mice, but that they were not universally present in the intracellular and extracellular trophozoites at all days of infection.

Life Cycle: Reproduction in *Toxoplasma* has generally been considered to take place by binary fission. However, Goldman, Carver and Sulzer (1958) reported on the basis of silver protein staining that *T. gondii* reproduces by a process of internal budding which they named endodyogeny. In this process, 2 daughter cells are formed within the parent cell. They are small at first, but grow until they destroy the parent cell and are released.

The natural mode of infection is unknown except in congenital toxoplasmosis, but experimental infections can be established by intravenous, intraperitoneal or any other type of parenteral inoculation or even by feeding. Weinman and Chandler (1954) transmitted toxoplasmosis to swine and rodents, and Makstenieks and Verlinde (1957) transmitted it to mice and a cynomolgus monkey by feeding infected tissue or peritoneal fluid. However, Schmidtke (1956) and van Thiel and van der Waaij (1956) considered that infection by feeding can occur only when there are epithelial lesions in the mouth or esophagus.

Jacobs, Remington and Melton (1960) found that the cysts of *T. gondii* are not able to survive freezing and drying, but they survive as long as 68 days at 4° C. Proliferative forms are destroyed within a few minutes by artificial gastric juice,

but the cysts remain infective in tissue up to 3 hours, while trophozoites liberated by peptic juice from isolated cysts survive 2 hours. Trypsin destroys the cyst wall immediately, but the liberated trophozoites survive at least 6 hours; proliferative forms survive at least 3 but less than 6 hours. Thus, parasites encysted in tissues could survive the normal digestive period in the stomach and should survive even longer in the duodenum.

Following experimental inoculation, the protozoa proliferate for a time at the site of injection and then invade the blood stream and cause a generalized infection. Susceptible tissues all over the body are invaded, and the parasites multiply in them, causing local necrosis. The parasitemia continues for some time, until antibodies appear in the serum, after which the parasites disappear from the blood and more slowly from the tissues. They finally remain only in pseudocysts or cysts, and only in the most receptive tissues. In general, the spleen, lungs and liver are cleared of parasites relatively rapidly, the heart somewhat more slowly and the brain much more slowly. These residual infections may persist for a number of years.

Following experimental infection of rats, Ruchman and Fowler (1951) reported that *Toxoplasma* could be found in the blood regularly for the first week and then occasionally during the next 9 days. It could be found in the spleen for 2 weeks, in the liver and lungs for 10 weeks and in the brain for 2 years after infection. Other workers found it as long as 3 years after infection in the brain of rats, mice and pigeons, and 10 months after infection in that of the dog (Jacobs, 1956).

Toxoplasma trophozoites have been found in the urine and feces of mice and dogs with acute toxoplasmosis, in the milk of mice, dogs, cows and sows, in a serous exudate from the conjunctiva of a pigeon, and in the saliva of mice, rabbits and man. However, these are the proliferative forms and are very delicate. They are rarely able to infect other animals. Mice can be raised in the same jar with infected mice without becoming infected. Olafson and Monlux (1942) reported transmission to uninfected puppies caged with a littermate dying of toxoplasmosis, but Jacobs (1957) was unable to repeat this observation under similar circumstances. He was also unable to infect rabbits by spraying large numbers of proliferative forms into a confined space with them.

Transmission via the placenta occurs in congenital toxoplasmosis. It is generally considered to be an accidental complication of an inapparent primary infection of a pregnant female (Feldman and Miller, 1956). Foci of infection are set up in the placenta, and the fetus is infected from them. Koestner and Cole (1960) reported the occurrence of congenital toxoplasmosis in 2 consecutive litters whelped by the same bitch.

Other than placental transmission, as mentioned above, the natural mode of transmission is unknown. Weinman and Chandler (1954) suggested that toxoplasmosis might be acquired in the same way as trichinosis, by eating infected pork. However, the epidemiological evidence does not appear to support this, altho there is suggestive evidence that dogs might perhaps become infected by eating chronically infected rodents (Jacobs, 1957). Arthropod transmission has been postulated without any substantiation.

One possibility which deserves investigation is that a concurrent disease of some sort may be required for infection to succeed. Campbell, Martin and Gordon (1955) found *T. gondii* in 6% of 268 dogs in Glasgow, Scotland with clinical evidence of distemper or its neurological sequellae. They found distemper virus inclusion bodies in all these dogs, mentioned that the association of distemper with toxoplasmosis had been noted by several earlier workers, and remarked that they themselves had never seen a case of "pure" canine toxoplasmosis or of canine toxoplasmosis associated with any infection other than distemper.

Jacobs, Melton and Cook (1955) studied experimental *T. gondii* infections in dogs and found that only young puppies given relatively large inocula succumbed. Since

it is hardly likely that dogs are exposed to such enormous numbers of parasites, they considered that canine toxoplasmosis is most frequently subclinical or asymptomatic. They believed that the chance of dogs spreading the disease to man under ordinary circumstances is small. On the other hand, Cole *et al.* (1953), in a study of 37 people in a household containing *Toxoplasma*-infected dogs, found that the sera of 9 of them were serologically positive and 5 of them ranged in titer from 1:80 to 1:1024. Of these 5 persons, 2 had toxoplasmic encephalitis and neuroretinitis, while 1 had *Toxoplasma* parasitemia. Makstenieks and Verlinde (1957) found evidence of concurrent infection in man and cats or dogs in a number of households in the Netherlands. These results suggest that there is a relationship between toxoplasmosis in man, dogs and cats, altho there is no proof of communicability.

Kimball *et al.* (1960) found that 44% of their obstetrical patients who had lived on farms were positive to the dye test as compared with only 21% of those who had never lived on farms. They observed a significant association between a positive dye test and contact with farm animals (cattle, chickens, ducks and geese), and suggested that domesticated fowls may be an important source of human *Toxoplasma* infections.

Pathogenesis: Toxoplasmosis may vary from an inapparent infection to an acutely fatal one. Asymptomatic toxoplasmiasis is the most common type.

In man, the most common form of the disease is the congenital type found in newborn infants. It is characterized by encephalitis, rash, jaundice and hepatomegaly, usually associated with chorioretinitis, hydrocephalus and microcephaly, and the mortality rate is high (Feldman, 1953; Feldman and Miller, 1956).

Acquired (i.e., non-congenital) human toxoplasmosis has many different manifestations. Siim (1956) divided them into 4 main types. The most common is characterized by lymphadenopathy. It may be febrile, non-febrile or subclinical. In the first, the onset may be acute, with chills and fever, or gradual. The temperature may last for 2 to 4 weeks or even longer. The lymph nodes are enlarged, the throat is often sore, and the patients suffer from malaise. Fatigue may persist for some time following recovery, and the lymph nodes remain enlarged for months.

The main characteristic of the non-febrile form is lymphadenitis. Its course is benign, but the lymph nodes remain enlarged for months. In the subclinical form, the only characteristic is the presence of swollen but not tender lymph nodes.

The second type of acquired human toxoplasmosis is a typhus-like, exanthematous disease. In addition to the exanthema, there may be atypical pneumonia, myocarditis and meningoencephalitis, and the termination is often fatal. Lymphadenopathy may or may not be present.

The third type is a cerebrospinal form, characterized by fever, encephalitis, convulsions, delirium, lymphadenopathy and a mononuclear pleocytosis, followed by death. This form is quite rare.

The fourth type is an ophthalmic form, characterized by chronic chorioretinitis. Hogan (1950) described ocular toxoplasmosis in detail.

Remington, Jacobs and Kaufman (1960) reviewed toxoplasmosis in the human adult.

The disease in domestic animals is similar to that in man. In dogs (cf. Cole *et al.*, 1953), the disease is most serious in puppies altho adults may also die. Signs include fever, cough, anorexia, weakness, depression, ocular and nasal discharges, pale mucous membranes, dyspnea, premature birth and abortion. The resistance of dogs to experimental infection (Jacobs, Melton and Cook, 1955) and the possible association of the disease with distemper (Campbell, Martin and Gordon, 1955) have already been mentioned.

At necropsy, lesions of pneumonitis are common. The liver may be swollen

and contain grey, necrotic foci. There may be ulcers in the oral, gastric and intestinal mucosa; this ulceration is perhaps more common in dogs than in other animals. Lymphadenitis, hydrothorax, ascites, nephritis, pancreatitis and vaginitis may also be present.

None of the 16 cases described by Campbell, Martin and Gordon (1955) had clinical signs which could be regarded as specific for *Toxoplasma* infection, altho the effects of this parasite may have been masked by intercurrent distemper. They found *Toxoplasma* in the lungs of 7 dogs, the mediastinal lymph nodes of 6, the mesenteric lymph nodes of 2, the heart muscle of 8, the liver of 4, the pancreas of 3, the spleen of 4, the kidneys of 3, the urinary bladder of 3 and the brain of 10.

Makstenieks and Verlinde (1957) reported encephalitis in one infected cat and abortions in another. However, Simitch *et al.* (1960) reported that the cat is relatively refractory to infection. They could not infect adult cats with 3 strains of *T. gondii* by either intravenous, intraperitoneal, subcutaneous or oral inoculation, and only part of the kittens less than 2 to 3 months old which were exposed by these routes became infected.

The disease in swine is similar to that in dogs. Pneumonitis, ulcerative enteritis, focal hepatitis, nephritis and splenitis have been described. Young pigs are much more susceptible than adults.

The disease in cattle is similar to that in dogs, and may vary considerably in its manifestations. In 1 herd described by Sanger *et al.* (1953), 3 cows developed nervous signs and died, and a fourth, asymptomatic cow which reacted positively to the toxoplasmin skin test was found to have the organisms in her colostrum, uterine wall, spleen and lung. In addition, 3 of 31 calves in this herd were born dead, and 4 developed an obscure disease of which 2 died. In a second herd, 45 of 78 calves died between the ages of 1 day to 6 months with signs of dyspnea, coughing, sneezing, nasal discharge, frothing at the mouth, trembling, headshaking, dehydration and occasionally diarrhea with blood and mucus. *Toxoplasma* was recovered from the lungs of 1 calf. In a third herd, a bull died a week after the onset of illness characterized by anorexia, weakness, ataxia, prostration, chewing movements and bicycling; *Toxoplasma* was found in his brain. In a fourth herd, *Toxoplasma* was found in various tissues of a 7-year-old cow which had died 2 weeks after parturition with signs of anorexia, diarrhea, depression, fever and mastitis. Some calves in this herd later died of an undiagnosed disease.

In sheep, Olafson and Monlux (1942) and Wickham and Carne (1950) described cases of non-suppurative encephalomyelitis with nervous signs. Cole *et al.* (1954) isolated *Toxoplasma* from a flock of sheep in which several ewes and lambs died of a disease with respiratory and nervous signs. Hartley and Marshall (1957) found that toxoplasmosis is an important cause of perinatal mortality in sheep in New Zealand. The overall perinatal mortality rate in sheep in this country is 10 to 15% and at least 1/5 of the deaths are due to potentially pathogenic organisms. Of these *Toxoplasma* is considered the most widespread and important. In a study of 30 lambs which died of toxoplasmosis on 15 farms, Hartley and Marshall considered that 2/3 died before birth and the other third died either at the end of an apparently normal parturition or a few hours afterwards. The cotyledons of the fetal membranes bore small, necrotic foci which contained clumps of proliferative trophozoites.

Ratcliffe and Worth (1951) described an epidemic of toxoplasmosis in squirrel monkeys (*Saimiri sciurea*) in the Philadelphia Zoo, and Benirschke and Richart (1960) described a fulminating acute case in a young cotton-topped marmoset (*Oedipomidas oedipus*).

In naturally affected chickens, Biering-Sorensen (1956) reported that emaciation and central nervous system signs were the principal signs. Necrosis of the optic chiasma and of the retina with cellular infiltration were characteristic. Erichsen and Harboe (1954) described

lesions of necrotizing pneumonitis, peri- and myocarditis, necrotizing hepatitis, focal necrotizing encephalitis and ulcerative gastroenteritis.

Altho natural infections occur in the chicken, Jones *et al.* (1959) found that this bird is remarkably tolerant to the parasite. Disease can be produced experimentally only by large inocula in mature birds, and even very young chicks can survive inoculation of enough parasites to kill rabbits, guinea pigs and hamsters. Parasitemia appears in 2 to 3 days after inoculation and disappears spontaneously, seldom persisting longer than 2 weeks. Even when enormous numbers of parasites were injected into large birds, *Toxoplasma* was rarely found in the tissues more than 40 days later.

The histopathology of toxoplasmosis has been reviewed by Frenkel (1956a) and Smith and Jones (1957). In the brain, *Toxoplasma* multiplies in the neurons and other cells and may cause cellular and interstitial necrosis. Sometimes infarction necrosis causes extensive lesions. Whenever aqueductal obstruction and internal hydrocephalus are present, periventricular vasculitis and necrosis are generally observed; these constitute a lesion unique for toxoplasmosis.

Koestner and Cole (1960) studied the neuropathology of canine toxoplasmosis in detail. They found lesions attributed to *Toxoplasma* in the central nervous systems of 47 out of 63 experimentally or naturally infected dogs with confirmed toxoplasmosis, and they found *Toxoplasma* itself microscopically in the central nervous systems of 25 of the animals. The parasites themselves were found in the cerebral cortex and basal ganglia of 17 dogs, in the midbrain of 12, the cerebellum of 9, the pons of 8, the medulla of 13 and the spinal cord of 4. Lesions were found in the cerebral cortex and basal ganglia of 47, the midbrain of 28, the cerebellum of 21, the pons of 20, the medulla of 29 and the spinal cord of 9. In acute cases, the lesions consisted of vascular damage and focal necrosis; extracellular trophozoites were found associated with the necrotic foci. In chronic cases, glial nodules and repair were seen, and intracellular parasites and cysts were present. In reactivated latent toxoplasmosis, ruptured cysts and a hyperergic response were present.

The lesions in the liver consist of small, sharply delimited areas of coagulation necrosis in any part of the hepatic lobules. The hepatic cells surrounding them are apparently normal, and there is little or no cellular reaction. The lungs contain small, grey, tumor-like nodules scattered thru 1 or all the lobes. These consist of alveoli filled with large mononuclear cells and leucocytes; the cells of the alveolar walls are cuboid or columnar and contain aggregations of *Toxoplasma*. The lymph nodes are usually involved. They are enlarged to several times their normal size and contain extensive areas of coagulation necrosis. These areas are irregular in outline, with sharply demarcated boundaries and slight leucocytic infiltration around their margins. *Toxoplasma* is present around these areas, in the endothelial cells of the veins, in monocytes or free in the tissues. There may be ulcers in the intestine. These may invade the muscularis, producing chronic, necrotizing lesions followed by granulation. Granulomatous chorioretinitis is sometimes seen in man, but ocular infections are apparently rare in animals.

Weinman and Klatchko (1950) found that a toxin which they called toxotoxin is produced in the peritoneal fluid of animals infected with *Toxoplasma*. It is heat stable and usually kills mice in 1 or 2 minutes following intravenous injection. Cook and Jacobs (1958), however, found no evidence of toxin production in tissue cultures of the organism.

Immunity: There is a definite age immunity against toxoplasmosis. Congenital infections are the most common, and the mothers usually do not show signs of disease themselves. Young animals are more susceptible than adults.

In infections acquired after birth, humoral antibodies appear at the time that the parasitemia disappears and are probably responsible in part for clearing the

blood of parasites. Humoral antibodies are not effective against intracellular parasites, however.

At least 2 types of humoral antibodies, complement fixing and cytoplasm modifying, are produced against *Toxoplasma*. The latter are revealed by the Sabin-Feldman dye test. They appear earlier in the course of the disease than complement fixing antibodies and persist much longer.

The dye test was introduced by Sabin and Feldman (1948) and has been described in detail by Sabin *et al.* (1952). It is based on the fact that both the cytoplasm and nucleus of *Toxoplasma* trophozoites stain deeply with alkaline methylene blue after incubation with normal serum, but that after incubation with antibody-containing serum only the nuclear endosome will stain. According to Lelong and Desmonts (1952), the dye test antibodies act by producing partial lysis of the organisms thru a modified Pfeiffer phenomenon in which the parasites lose those cytoplasmic constituents which are ordinarily stained by methylene blue. Kulasiri and Dasgupta (1959) found that ribonucleic acid disappears during incubation in a positive reaction, and suggested that this is the reason the organisms no longer stain.

The antibody itself is heat stable, but a fairly large amount of a heat-labile, complement-like "accessory" factor is also necessary. This is apparently a mixture of the C_2, C_3 and C_4 factors of complement plus properdin (Grönroos, 1956).

In carrying out the dye test, a series of serum dilutions is used, and a titer of 1:16 is considered diagnostic. The dye test titer usually reaches a high level by the end of the second week after infection; in active disease, titers above 1:1000 are found in a month or more. These antibodies usually persist for a number of years, probably for more than a decade, altho their titer declines slowly.

The trophozoites used in the dye test can be obtained from peritoneal exudate or tissue culture. These fluids sometimes contain a soluble antigen in sufficiently high titer to block the test partly or completely (Jacobs and Cook, 1954). Antibody in mouse peritoneal fluid may give rise to false positive tests (Frenkel, 1956). In addition, a prozone phenomenon may often occur, so that a full range of dilutions up to 1:1024 at least must be tested.

The dye test is not necessarily specific for *Toxoplasma*. Muhlpfordt (1951) and Awad and Lainson (1954) reported cross reactions with *Sarcocystis tenella*, and Awad (1954) even developed a modified dye test for *Toxoplasma*, using *S. tenella* trophozoites.

On the other hand, Cathie and Cecil (1957) were unable to confirm this latter test. Moscovici (1954) found no dye test cross reaction between *T. gondii* and *S. tenella*. Jacobs (1956) found no dye test cross reaction between *Toxoplasma* and *Trypanosoma cruzi*, *Plasmodium berghei*, *P. gallinaceum*, *Eimeria tenella*, *Hepatozoon* sp. in squirrels, or *Sarcocystis* in rhesus monkeys, but did observe cross reactions at titers up to 1:4 between *Toxoplasma* and *Encephalitozoon* in rats and up to 1:16 between *Toxoplasma* and *Besnoitia jellisoni* in rabbits. Cathie (1957) found the dye test to be specific for *Toxoplasma*, for human sera at least; the test sera should be inactivated.

The complement fixation test was developed by Warren and Sabin (1943) and Sabin (1949). Complement fixing antibodies rarely appear earlier than 1 month after infection, and decrease relatively rapidly with time. In 60 children with congenital toxoplasmosis studied by Eichenwald (1956), complement fixing antibodies had disappeared from 44 at 5 years of age and from 8 more at 7 years, altho all but 3 still had dye test antibodies. In 15 cases of active toxoplasmosis studied by Makstenieks and Verlinde (1957), the complement fixation reaction became negative in 6 to 9 months while the dye test was still positive at the end of 4 years.

A positive complement fixation titer of 1:32 or above is considered to indicate relatively recent infection.

The antigen for this test may be prepared from protozoa in chicken embryos, mouse brain, peritoneal exudate or tissue cultures. Eichenwald (1956) preferred chorioallantoic membrane or tissue culture because peritoneal exudate has a strong anticomplementary activity.

Jacobs and Lunde (1957) and Lunde and Jacobs (1958) reported on a hemagglutination test for toxoplasmosis. It agreed very closely with the dye test in a survey of 12 human serum specimens from Trinidad; 54.5% were positive by both tests. They considered that the hemagglutination test was adequate for survey purposes but that more work must be done to determine its usefulness in the diagnosis of acute infections.

A skin test using "toxoplasmin" was developed by Frenkel (1948, 1949). Positive reactions appear in man, rhesus monkeys and guinea pigs 3 to 4 weeks after infection. However, they do not appear in about 10% of the individuals, and the test remains negative in most infected rodents and in humans with highly active disease.

Hook and Faber (1957) found that antigenic activity in both the dye and complement fixation tests is associated with a protein component of sonically fragmented *T. gondii* which was precipitated by 30% saturated ammonium sulfate at pH 7.

Diagnosis: The most certain method of diagnosis of toxoplasmosis is by isolation of the parasites themselves by inoculation of experimental animals. Eichenwald (1956) considered mice, hamsters and guinea pigs the most sensitive animals in his experience, and recommended the administration of cortisone to the test animals for 3 to 5 days before inoculation in order to increase the chance of isolating the organisms. Jones *et al.* (1958), however, found no advantage in using cortisone. They recommended intraperitoneal inoculation of mice. Simitch, Petrovitch and Brodjochki (1956) considered the ground squirrel, *Citellus citellus*, to be the animal of choice, while Lainson (1957) found that the multimammate rat (*Mastomys coucha*) is more susceptible than the house mouse and suggested that it might prove more suitable. After isolation, the organism should be identified serologically.

Despite the disadvantages discussed above, the dye test still appears to be the most satisfactory serological test available at present. Eichenwald (1956) considered the complement fixation test useful only as an adjunct to it, and the hemagglutination test requires further study. A neutralization test was introduced by Sabin and Ruchman (1942). It is now carried out chiefly in tissue cultures. However, according to Eichenwald (1956), it is of use primarily as a research tool to study cell-parasite relationships.

Serologic studies with fluorescein-labelled *Toxoplasma* antibody have also been carried out (Goldman, Carver and Sulzer, 1957). This technic shows promise. The antibody does not agglutinate *Besnoitia*.

Toxoplasma can also be found in stained smears and sections of tissues and exudates. It must be differentiated from similar organisms, including *Sarcocystis*, *Besnoitia* and *Encephalitozoon*, and this is not always possible on morphological grounds alone.

Cultivation: *Toxoplasma* grows reaily in chicken embryos and tissue culture. It was first cultivated in both by Levaditi *et al.* (1929). Cook and Jacobs (1958) cultivated it in a wide variety of mammalian and avian tissue cultures, including various human, monkey, mouse, rabbit, guinea pig, rat, ox and chick normal tissues, and in human and mouse cancer cells. They also reviewed the literature on the subject.

Eyles, Coleman and Cavanaugh (1956) preserved *T. gondii* for as long as 209 days by freezing it in the presence of 5% glycerol and storing it at -70° C. They used the technic routinely for preservation of their strains.

Treatment: No satisfactory treatment for toxoplasmosis is known. Promising results have been obtained by the use of pyrimethamine and sulfonamides simul-

taneously; the two drugs act synergistically (Eyles, 1956).

For treatment of human ocular toxoplasmosis, Remington, Jacobs and Kaufman (1960) recommended that the patients receive 2 oral loading doses of 200 mg pyrimethamine and 2 g triple sulfonamides each on the first day of therapy, and that thereafter they be given 25 mg pyrimethamine and 2 g triple sulfonamides twice a day for 5 weeks.

Prevention and Control: In the absence of solid information regarding the mode of spread of toxoplasmosis, specific preventive measures cannot be recommended. The measures customarily employed to control infectious diseases should be used. In addition, since many wild mammals are apparently reservoir hosts, contact with them should be avoided and rodents should be controlled. Man and his domestic animals apparently receive their infections from the same source, but it is not clear whether they can give it to each other.

Genus *BESNOITIA* Henry, 1913

In this genus the pseudocysts are found in the subcutaneous and connective tissues, serosal membranes and elsewhere. They have a heavy wall containing nuclei, and are not divided into compartments. A synonym of this name is *Fibrocystis* Hadwen, 1922. The name *Globidium* has often been used instead of *Besnoitia* for members of this genus, but this is incorrect, since *Globidium* is a synonym of *Eimeria*.

The "cyst" wall is said by Pols (1954a) to be formed entirely by the host, so that it is actually a pseudocyst. The wall is composed of a thin inner layer containing a number of flattened, giant nuclei and a thick, homogeneous or concentrically laminated, eosinophilic outer wall. It is positive to the periodic acid-Schiff test, and the reaction is not affected by salivary digestion (Frenkel, 1956).

The trophozoites are banana-shaped, crescentic or elongate oval, and slightly pointed at one end. They move by body flexion. They reproduce by binary fission or endodyogeny; multiple fission has also been described.

This genus is poorly known and has often been confused with *Eimeria* and *Sarcocystis*. Species have been found in cattle, horses, reindeer, caribou, rodents and opossums. A somewhat similar organism described by Campbell (1954) as the cause of Bangkok hemorrhagic disease of chickens in Thailand is more probably a fungus.

BESNOITIA BESNOITI (MAROTEL, 1912) HENRY, 1913

Synonyms: *Sarcocystis besnoiti*, *Gastrocystis robini*, *Gastrocystis besnoiti*, *Globidium besnoiti*. The nomenclature of this species has been discussed by Jellison (1956).

Disease: Besnoitiosis, olifantvel.

Hosts: Cattle. Pols (1954) infected the domestic rabbit experimentally.

Location: The cysts are in the cutis, subcutis, connective tissue, fascia, serosae, mucosae of the nose, larynx and trachea, and other places. Trophozoites are in the blood, either extracellularly or in monocytes, and in smears of lymph nodes, lungs, testes, etc.

Geographic Distribution: Europe (southern France, Pyrenees, Portugal), Africa (South Africa, Belgian Congo, Angola, Sudan).

Prevalence: According to Hofmeyr (1945), *B. besnoiti* is endemic in South Africa thruout the whole of the Bushveld area from the Western Transvaal to Potgietersrust district and probably further north. Hérin (1952) found it in about 2% of the cattle he examined in Ruanda-Urundi, Belgian Congo. Leitao (1949) discussed its occurrence in Portugal.

Morphology: The pseudocysts are more or less spherical, without septa, and about 100 to 500 μ in diameter. The

pseudocyst wall is composed of a thin inner layer containing several flattened, giant nuclei and a thick, homogeneous or concentrically laminated outer wall. The trophozoites in the pseudocysts are crescentic or banana-shaped, with 1 end pointed and the other rounded. According to Pols (1954) the trophozoites in blood, lung and testis smears of experimentally infected rabbits measure 5 to 9 by 2 to 5 μ and are usually elongate oval and slightly pointed at one end. Banana-shaped and crescentic forms are found more rarely. The nucleus is more or less central.

Life Cycle: The natural mode of transmission is unknown, but it is probably thru ingestion. Hofmeyr (1945) gave circumstantial evidence that the infection is spread thru contaminated watering troughs in South Africa. Jellison, Fullerton and Parker (1956) transmitted the related *B. jellisoni* to house mice by feeding trophozoites from cysts of infected deermice or from peritoneal fluid of infected house mice.

Cuillé and Chele (1937), Barrairon (1938) and Pols (1954) transmitted *B. besnoiti* to cattle by intravenous injection of blood from cattle in the primary stage of the disease. Pols also infected an ox by intraperitoneal injection and rabbits by intravenous, intraperitoneal and subcutaneous injection of blood. He passed the protozoon from a rabbit thru 2 generations of cattle and back to a rabbit. Later (1954a) he reported having passed it thru 19 serial passages in the rabbit. He was unable to infect mice, rats and guinea pigs.

The incubation period in the cattle infected by Pols varied from 6 to 10 days, and that in the rabbits from 6 to 16 days. It was followed by a thermal reaction which lasted 2 to 5 days. Cysts were found in the skin of naturally and artificially infected cattle 6 to 28 days after the beginning of the temperature reaction.

Pols (1954a) described cyst formation in experimentally infected rabbits. The initial stages were seen as early as 16 to 18 days after inoculation. When a trophozoite invades a histiocyte, a vacuole is formed around it. The trophozoites in tissue sections measure about 3 by 1.5 μ, and the vacuoles are about 8 μ in diameter. The trophozoites multiply by binary fission; Pols saw a few cases of multiple fission but they were so rare that he considered them aberrant. It is possible that the trophozoites may actually divide by endodyogeny, since Goldman, Carver and Sulzer (1958) stated that this takes place in *B. jellisoni*.

The nucleus of the host cell begins to divide at the same time that the trophozoites do, forming a multinucleate cell. As the parasites multiply within the vacuole, the latter becomes larger and the host cell cytoplasm is compressed to form a narrow rim. This is the middle layer of the pseudocyst wall. Within it is an inner membrane which can be seen only if 2 trophozoites have invaded the same host cell, in which case it forms a thin line between the resultant cysts; it is uncertain whether it is formed by the parasite, the host or both. Concentric layers of collagenous fibers are laid down around the host cell to form a hyaline capsule around the whole; this is the outer layer of the pseudocyst.

Pathogenesis: The most complete description of bovine besnoitiosis has been given by Hofmeyr (1945). He found it in cattle of all ages from 6 months up. Aged animals were also affected. He recognized 3 stages in the course of the disease:

The febrile stage. The first sign of besnoitiosis is fever, up to 107° F but usually lower. The animal develops a photophobia and stays in the shade. The hair loses its luster, especially along the buttocks, limbs, flanks, lower abdomen and neck. Marked anasarca develops, especially along the lower line but sometimes over the whole body. The swellings are warm and tender. The animals have a stiff gait and are reluctant to move. The pulse is fast, respiration is rapid, and rumination decreases or ceases. Diarrhea is sometimes present, and abortions are not uncommon. The lymph nodes, especially the prescapular and precrural ones, are enlarged. Lachrymation and hyperemia of the sclera are present. The cornea is studded with whitish, elevated specks

which are *Besnoitia* cysts. The nasal mucosa becomes bright red and is also studded with cysts. The mucosa may be swollen and there may be a rapidly progressive rhinitis; it starts with a mucous discharge which later becomes thick, hemorrhagic and mucopurulent, forming dark brown crusts in the nostrils. If the pharynx and larynx are involved, there is a short cough. This stage may last 5 to 10 days. The acute stage then subsides and the second stage begins.

The depilatory stage. In this stage, the pathologic and clinical pictures are dominated by skin lesions. The skin becomes greatly thickened and loses its elasticity. The hair falls out over the swollen parts, and the skin on the flexor surfaces cracks and a sero-sanguinous fluid oozes out. Necrosis of the skin develops on the parts in contact with the ground when the animal lies down. Toward the end of this stage, hard sitfasts develop on the sides of the stifles, brisket and elbows. The anasarca subsides, leaving the skin with typical, broad wrinkles along the lower line. The photophobia decreases, and grazing is resumed in many cases. Death may occur at this stage. If not, the stage lasts 2 weeks to about a month and gradually passes into the third stage.

The seborrhea sicca stage. In this stage, most of the hair on the previously anasarcous skin has been lost, and the denuded parts are covered by a thick, scurfy layer. The sitfasts crack away from the underlying tissues, fissures remain in the flexor surfaces, the skin hardens, and deep scars show plainly. The hide resembles that of an elephant, and the animal looks as tho it has mange. The lymph nodes are permanently enlarged, the protozoan cysts remain, and the animal is listless and debilitated.

In light infections in which there has been little hair loss, the animals become practically normal in appearance, but in more severe cases recovery requires months or even years, and the changes in the cutis and subcutis and the loss of most of the hair are permanent. In convalescent animals the remaining hair forms patterns resembling the markings on a giraffe.

The morbidity in a herd varies from 1 to 20%, and the mortality is about 10%.

Diagnosis: Besnoitiosis can be diagnosed by biopsy examination of affected skin or other areas. The spherical, encapsulated cysts are pathognomonic. There may be a severe granulomatous reaction in young cysts or those which have broken and released their trophozoites, but there is usually little reaction except for the formation of the hyaline wall around the mature cysts.

Trophozoites are often found in blood smears, sometimes in large numbers, but most of them are introduced when a cyst is cut in obtaining blood.

Treatment: None known.

Prevention and Control: Until the mode of transmission is learned, the appropriate preventive measures must remain unknown. However, sanitary measures would prevent the spread of besnoitiosis if transmission is by ingestion, and insect control would prevent it if transmission is by biting insects, as some believe.

BESNOITIA BENNETTI BABUDIERI, 1932

Hosts: Horse, ass.

Location: Same as *B. besnoiti*.

Geographic Distribution: Africa (Sudan, South Africa), Europe (southern France, Pyrenees), North America (Mexico, United States).

Prevalence: Relatively uncommon. Bennett (1927, 1933) recorded this species from 3 horses in the Sudan, all of which originated in the Nuba Mountains of Southern Kordofan. Schulz and Thorburn (1941) found it in South Africa. Jones (1957) found it in the skin and other tissues of small burros which had been imported into the

United States from Mexico. Gorlin *et al.* (1959) found it in the lip of a burro of unspecified origin in the United States.

Morphology: Same as *B. besnoiti*. According to Bennett (1933), the trophozoites measure 10 by 4 μ.

Life Cycle: Presumably same as *B. besnoiti*.

Pathogenesis: According to Bennett (1933), the horse-owning tribes in Southern Kordofan know this disease quite well, can differentiate it from mange and ringworm and have given it a separate Arabic name. It was said that one tribe which not many years before had owned 600 to 800 horses, now had less than 50 due to besnoitiosis. On the other hand, the organism produces no grossly recognizable disease in burros, according to Jones (1957).

The disease as described by Bennett (1933) in horses is a chronic one, running a course of many months. Affected animals are weak and dejected, altho their appetite is good. The skin is scurfy and thickened, and contains many scabs and whitish scars. The hair may be destroyed by the lesions. The conjunctiva is a peculiar brick red color, with a few petechiae. The temperature is slightly elevated.

The muscles in advanced cases are pale brown and friable, but contain no parasites. The *Besnoitia* cysts are abundant in the skin and may also be found in the mucous membrane covering the larynx, nostrils, soft palate, etc.

Diagnosis: Same as for *B. besnoiti*.

Treatment: None known.

Remarks: It is possible that the same species of *Besnoitia* affects both cattle and horses, and that *B. bennetti* is a synonym of *B. besnoiti*. Until this is shown to be the case by cross-transmission studies, however, it is considered best to retain separate names for the forms in cattle and equids.

BESNOITIA TARANDI (HADWEN, 1922) NOV. COMB.

Synonyms: *Fibrocystis tarandi* Hadwen, 1922.

Disease: Besnoitiosis, corn-meal disease.

Hosts: Reindeer, caribou.

Location: The cysts occur in the fibrous connective tissues, especially in the periosteum and on the surface of the tendons.

Geographic Distribution: Alaska.

Prevalence: Hadwen (1922) found this parasite in a number of herds of reindeer and in caribou in Alaska.

Morphology: The cysts are spherical and 100 to 450 μ in diameter with a mean of 275 μ. They are composed of 3 layers, of which the outermost is thick and fibrous, with concentrically arranged fibers, the middle layer is clear and hyaline, and the inner layer forms a thin lining. The cysts are not compartmented. The cyst contents are dark brown. The trophozoites are spindle shaped, with a central nucleus, and measure 7 by 1.8 μ in alcohol-fixed material.

Life Cycle: Unknown.

Pathogenesis: Reindeer owners call besnoitiosis "corn-meal disease" because of the granular nature of the lesions. The cysts may be found in the periosteum of all of the bones. When the periosteum is stripped off, small pits corresponding to their position are found in the bone itself. They are also found on the surface of the tendons, where they cause similar pits.

BESNOITIA JELLISONI FRENKEL, 1955

This species was described from the deermouse, *Peromyscus maniculatus*, in Idaho by Frenkel (1955). He transmitted it by intraperitoneal or intravenous

inoculation of peritoneal fluid from acutely ill animals or by trophozoites from cysts of chronically affected animals to white mice, rats, hamsters, voles (*Microtus*), ground squirrels (*Citellus*) and chicken embryos, but not to guinea pigs, rabbits, the ox, rhesus monkey, baby chick, canary or pigeon (Frenkel, 1956a). Jellison, Fullerton and Parker (1956) transmitted it to mice by feeding trophozoites from cysts or peritoneal fluid of affected animals.

The cysts occur in the connective tissue and on the serosae of many of the viscera organs, including the intestine, liver, spleen, heart, testes, etc. They are spherical, up to 1 mm in diameter, with thick walls containing giant nuclei. The wall is positive to the periodic acid-Schiff reaction and is unaffected by salivary digestion.

The trophozoites are crescent-shaped, with a central nucleus. According to Goldman, Carver and Sulzer (1957, 1958), who studied them after staining with Bodian silver stain, they have a truncated, cap-like cone at the anterior end with 1 or more rod- or fibril-like structures extending posteriorly from it, a dark-staining posterior granule and a nucleus consisting of a larger, less dense portion and a smaller, more compact structure. They reproduce by endodyogeny.

B. jellisoni may cause an acute, fatal disease or a chronic one.

B. jellisoni is serologically and immunologically distinct from *Toxoplasma* and also from *B. besnoiti*. Goldman, Carver and Sulzer (1957) found that fluorescein-labelled *Toxoplasma* antibody did not stain *B. jellisoni*, and Frenkel (1955) found that sera from cows naturally infected with *B. besnoiti* did not react with *B. jellisoni* in the dye test.

Genus *ENCEPHALITOZOON* Levaditi, Nicolau and Schoen, 1923

This genus closely resembles *Toxoplasma* and may indeed eventually turn out to be a synonym of it, as Biocca (1949) believed. It is too poorly known, however, for a final decision to be made. It differs from *Toxoplasma* in that its trophozoites are smaller and rod-shaped; the clusters of trophozoites in the brain are not surrounded by an argyrophilic cyst wall, altho they are said to form a pseudocyst; *Encephalitozoon* stains very poorly with hematoxylin and eosin but stains dark red with Wright and Craighead's carbol fuchsin-methylene blue stain (decolorized with 37% formaldehyde solution), whereas *Toxoplasma* stains well with hematoxylin and eosin and stains blue with carbol fuchsin-methylene blue; *Encephalitozoon* stains black with Weigert's iron hematoxylin, whereas *Toxoplasma* does not; and *Encephalitozoon* survives rapid freezing and storage at -70° C or storage in 50% buffered glycerol at 4° C, while *Toxoplasma* does not (Perrin, 1943a; Frenkel, 1956).

Several species of *Encephalitozoon* have been named, and the name has also been mistakenly given to the Negri bodies of rabies. However, in view of the transmissibility of the organism from one host to another, only a single species deserves recognition. Even this has been questioned. Robinson (1954) claimed that the structures described under this name are actually ceroid pigment or hemofuscin. Unfortunately, none of the 11 photomicrographs of tissue sections in his paper shows these structures, so that there is no clear evidence of what he was actually dealing with.

ENCEPHALITOZOON CUNICULI LEVADITI, NICOLAU AND SCHOEN, 1923

Synonyms: *Encephalitozoon negrii.*

Disease: Encephalitozoonosis.

Hosts: Domestic rabbit, house mouse, Norway rat, cottontail, dog. The golden hamster has been infected experimentally. A few human infections with *Encephalitozoon* have been reported, but they were all actually *Toxoplasma.*

Location: *Encephalitozoon* occurs intracellularly in the brain, kidneys, peritoneal exudate, liver, spleen and other organs.

Geographic Distribution: Worldwide.

Prevalence: Perrin (1943) found *Encephalitozoon* in the brains of 5 of 502 Swiss mice, 2 of 283 Wistar strain albino rats, and 1 of 291 guinea pigs at the National Institutes of Health, Bethesda, Md. It has been encountered sporadically by a number of workers in laboratory rabbits, mice and rats (Frenkel, 1956; Perrin, 1943). In most cases it has been found during routine histologic examination of animals being studied for some other purpose.

Plowright (1952) described 3 cases in a litter of foxhounds in England, and Plowright and Yeoman (1952) found it in a litter of puppies in Tanganyika. They also reviewed the literature on previous reports of what may have been the same organism in dogs. Jungherr (1955) found it in a cottontail rabbit.

Morphology: The trophozoites measure 2.0 to 2.5 by 0.8 to 1.2 μ in tissue sections and up to 4.0 by 2.5 μ (mean, 2.0 by 1.2 μ) in smears (Perrin, 1943a). They are straight to slightly curved rods with both ends bluntly rounded but one end a little larger than the other. The body is sometimes slightly constricted at or near its midpoint. Round or oval forms occasionally occur. The nucleus is compact, round, oval or bandlike, about 1/4 to 1/3 the size of the parasite, and is not central. Pseudocysts containing up to 100 or more trophozoites are found within the nerve cells, macrophages or other tissue cells. Both they and the trophozoites are rarely extracellular.

Life Cycle: The mode of multiplication is unknown. The organism can be transmitted from the mouse, rabbit, rat or guinea pig to other laboratory animals by intracerebral, intravenous, intraperitoneal or other parenteral inoculation of infected brain, liver, spleen or peritoneal exudate (Perrin, 1943a). It has been found in the urine. Congenital infection undoubtedly occurs in mice (Perrin, 1943) and probably in rabbits (Smith and Florence, 1925) and dogs (Plowright, 1952).

Pathogenesis: *E. cuniculi* causes encephalitis and systemic disease associated with nephritis in rabbits and puppies, and an inapparent infection in laboratory rodents. The great majority of cases in rabbits are also inapparent, being discovered on histologic examination carried out for some other reason. Its true importance in dogs is unknown, since it has been seen very rarely in them.

Encephalitozoonosis is usually a mild, chronic, infection in rabbits, altho paralysis and death may occur. The principal lesions in the brain are tiny, focal granulomata made up of epithelioid cells surrounding a tiny area of necrosis. In fatal cases there may be large necrotic areas and perivascular lymphocytic cuffing. The parasites may occur in or around the necrotic areas. Similar granulomatous lesions may be present in the kidneys and other organs. In the kidneys they occur principally in the epithelial cells of the collecting tubules, which they distend and finally rupture, passing out in the urine (Smith and Florence, 1925).

In mice and rats the principal lesions are meningoencephalitis and, in experimentally infected animals, abdominal enlargement with ascites. Nodular, granulomatous lesions, sometimes with central necrosis, occur thruout the brain. There is slight to moderate focal perivascular infiltration by lymphocytes and a few large mononuclear and plasma cells in the meninges and also in the brain. The parasites may be either within or at the margins of the lesions or even in normal brain tissue at a distance from them. There may be moderate to marked interstitial lymphocytic infiltration in the kidneys, primarily in the cortex. The tubular epithelium may be degenerate or proliferative in the areas of infiltration, and parasites may occur either in the epithelial cells or within the collecting tubules. Similar areas of infiltration may be seen in other organs (Perrin, 1943).

According to Frenkel (1955), treatment with cortisone exacerbates the disease in mice. The parasites proliferate extensively in most organs, and the mice may die.

In the litter of six puppies described by Plowright (1952), the principal signs were posterior weakness and incoordination, apathy, rapid tiring, some loss of condition and signs of ocular involvement. All died between 6 weeks and 15 months of age. Two puppies were affected at 8 to 10 weeks of age in the litter described by Plowright and Yeoman (1952). Both had symptoms resembling those of rabies. They became vicious and bit or attempted to bite people. One had fits of rushing wildly around, and died on the 5th day with uncontrolled spasms of the limbs and jaws. The other had an epileptiform fit, became dull and off feed, but remained quiet under mild sedation. Its retina was dull and greyish, with darker "smoke-wisp" foci, and the optic disc was dull and ill-defined. It died 11 days after the onset.

The principal lesions in both litters were those of encephalitis or meningo-encephalomyelitis and interstitial or tubular nephritis similar to those described above in rabbits and rodents. *Encephalitozoon* was readily seen in the lesions.

Immunity: According to Frenkel (1955), there is no cross immunity between *Encephalitozoon* and *Toxoplasma*.

Diagnosis: Encephalitozoonosis is generally diagnosed by finding the causative organisms in tissue sections. They can be differentiated from *Toxoplasma* on the basis of size, shape and differential staining reactions, as described above.

Cultivation: *Encephalitozoon* has not been cultivated.

Treatment: None known.

Prevention and Control: In the absence of information on its mode of transmission, little can be said about prevention and control. Sanitation combined with elimination of affected litters, and possibly also of their mothers, may be helpful.

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Chapter 13

THE CILIATES

The ciliates of domestic animals all belong to the class Ciliasida. The nuclei of this group are unique in the animal kingdom. Every individual (except in a few amicronucleate strains) has a micronucleus which contains a normal set of chromosomes, and a macronucleus which contains an indeterminately large number of sets and is actually *n*-ploid rather than polyploid. The micronucleus is active in reproduction, while the macronucleus has to do with the vegetative functions of the organism.

The ciliates have either simple cilia or compound ciliary cirri or membranelles in at least one stage of their life cycle. They also have an infraciliature in the cortex beneath the pellicle, composed of the ciliary basal granules (kinetosomes) and associated fibrils (kinetodesmata). The infraciliature can be stained with silver, forming the so-called silver-line system. Reproduction is by transverse binary fission, in contrast to the longitudinal fission seen in the flagellates. True sexual reproduction, in which gametes fuse to form a zygote, is absent, but conjugation, in which there is an exchange of micronuclear material between two individuals, may be present.

The great majority of ciliates are free-living, but a number are parasitic. Their classification has recently undergone considerable overhauling, and they are now arranged in 26 orders and suborders belonging to 2 subclasses (Corliss, 1956, 1957, 1959). This classification is based on recent work by the French school, and particularly by Fauré-Fremiet, on the silver-line system, and is more natural than the earlier one. Terms used in describing the ciliates are defined by Corliss (1959). Corliss (1961) has reviewed the whole group.

The characteristics of the taxa found in domestic animals have been given on pp. 34-38. In the subclass Holotrichasina, the body ciliature is typically uniform and simple. Buccal ciliature (an adoral zone

of membranelles) is usually absent or inconspicuous. This subclass contains 4 orders of veterinary interest. In the order Gymnostomorida ("naked mouth"), the cytostome opens directly at the surface or else into a slight depression which has no oral ciliature. This order includes 2 families of which members occur in the large intestine of equids or ruminants or in the rumen and reticulum of ruminants.

In the order Suctoriorida only the young have cilia, while the adults have tentacles. All members of this order are free-living except for one genus which occurs in the large intestine of equids.

In the order Trichostomorida ("hair mouth"), the cytostome is usually at the base of a well-defined oral pit or vestibulum, which in turn may sometimes be preceded by an oral groove. The vestibular wall bears 1 or more dense fields of adoral (vestibular) cilia. The great majority of trichostomes are free-living, but there are 5 families which contain parasites of domestic animals.

In the order Hymenostomorida ("membrane mouth"), the adoral cilia are fused in membranes, the number, size and arrangement of which vary in different genera. The free-living genera *Paramecium* and *Tetrahymena* belong to this order; the latter is occasionally parasitic. The most important parasite in the order is *Ichthyophthirius*, which is often a serious pathogen of aquarium fish, causing a disease known as "ick."

In the subclass Spirotrichasina, the buccal ciliature, and especially the multipartite adoral zone of membranelles, is conspicuously developed. The body ciliature is typically sparse, and all the simple cilia may even be replaced by cirri; in one order, the Heterotrichorida, however, the body ciliation is usually complete.

This subclass contains 2 orders of veterinary interest. In the order Heterotrichorida the somatic ciliation is usually complete. One genus in this order, *Nyctotherus*, occurs in amphibia and various invertebrates, but has also been found in the feces of ruminants. The order Entodiniorida contains a group of remarkably bizarre genera which occur in ruminants and equids. In this order, ciliation may be limited to the adoral zone or there may be 1 or more additional bands or groups of membranelles. The internal anatomy is complex, and unique "skeletal plates" may be present. There are 2 families. The Ophryoscolecidae have not more than 1 "dorsal" or "metoral" band of membranelles in addition to the adoral zone and occur in the rumen and reticulum of ruminants. The Cycloposthiidae have 2 or more bands of membranelles in addition to the adoral zone, and occur in the large intestine of equids and also of anthropoid apes.

A. CILIATES OF RUMINANTS

A tremendous number and bewildering variety of ciliates swarm in the rumen and reticulum of ruminants, and a few species occur in the large intestine. Many are holotrichs, but the most bizarre ones are ophryoscolecids. No attempt will be made here to differentiate all the species, but the genera will be described and the principal species mentioned, and the relations of the different groups to their hosts will be discussed. Further taxonomic and morphologic information is given by Becker and Talbot (1927), Dogiel (1927), Kofoid and MacLennan (1930, 1932), Chavarria (1933), Polyansky and Strelkov (1938) and Lubinsky (1957).

FAMILY BUETSCHLIIDAE

In this holotrichasin gymnostomorid family, the cytostome is usually at the anterior end, there are ordinarily a posterior cytopyge, one or more contractile vacuoles and an anterior concretion vacuole which some authors think is a statocyst. Cilia are uniformly distributed over the body or are restricted to certain areas. This family includes a number of genera and species, the great majority of which occur in the cecum and colon of equids. One genus, however, is found in the rumen of cattle and camels.

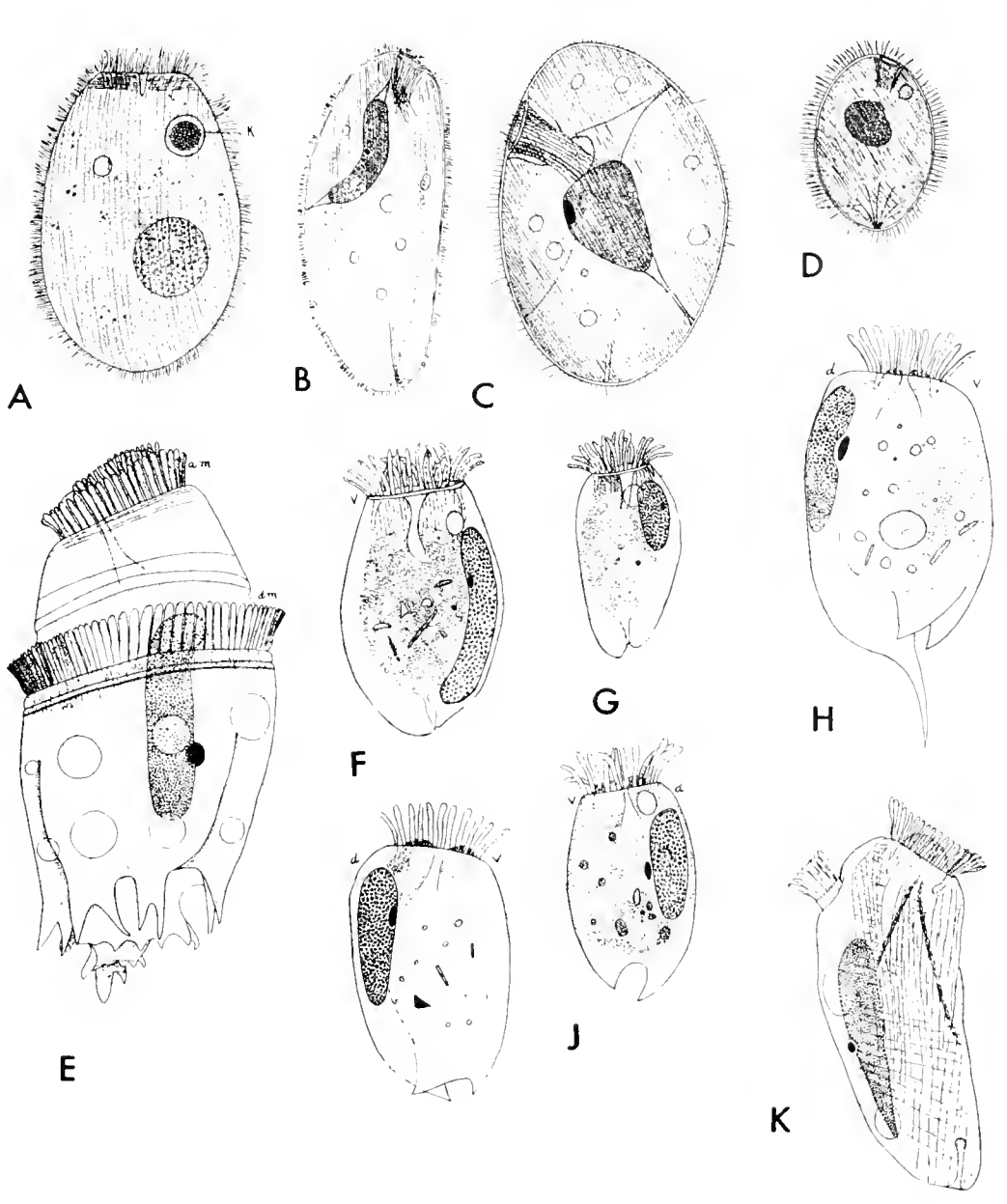

Fig. 38. Ciliates of ruminants. A. *Buetschlia parva*. X 1090. B. *Isotricha prostoma*. X 320. C. *Isotricha intestinalis*. X 640. D. *Dasytricha ruminantium*. X 420. E. *Ophryoscolex caudatus*. X 425. F. *Entodinium bursa*. X 640. G. *Entodinium minimum*. X 640. H. *Entodinium caudatum*. X 640. I. *Entodinium bicarinatum*. X 640. J. *Entodinium furca*. X 640. K. *Epidinium ecaudatum*. X 425. (From Becker and Talbott, 1927, in Iowa State College Journal of Science, published by Iowa State Univ. Press).

Genus *BUETSCHLIA* Schuberg, 1888

The body is ovoid, with a truncate anterior end and a rounded posterior end. There is a circular cytostome at the anterior end, but no cytopyge. The body is uniformly ciliated except for long cilia surrounding the cytostome. The ectoplasm at the anterior end is thick. The macronucleus is spherical.

Buetschlia parva Schuberg, 1888 is 30 to 50 μ long and 20 to 30 μ wide. *B. neglecta* Schuberg, 1888 resembles

B. parva, but its posterior end is somewhat pointed and has 4 indentations, so that it looks like a cross in cross section; it measures 40 to 60 by 20 to 30 μ. *B. lanceolata* Fiorentini, 1890 is lanceolate, with a collar-like stricture in the anterior fifth of the body; it measures 48 by 20 μ. These species all occur in cattle but are apparently not common, at least in the United States. Becker and Talbott (1927) did not find them in 26 cows they examined in Iowa.

B. nana Dogiel and *B. omnivora* Dogiel are found in the rumen of the dromedary.

FAMILY PYCNOTRICHIDAE

In this holotrichasin gymnostomorid family, a long groove leads to the cytostome, which may lie near the middle or at the posterior end of the body. The body is completely ciliated. This family contains 2 genera in ruminants whose names may actually be synonymous.

Genus *BUXTONELLA* Jameson, 1926

The body is ovoid and uniformly ciliated, with a prominent curved groove bordered by 2 ridges running from end to end. The cytostome is near the anterior end.

Buxtonella sulcata Jameson, 1926 is common in the cecum of the ox, zebu and water buffalo. The trophozoites measure 60 to 138 by 46 to 100 μ with a mean of 100 by 72 μ, and have an oval or bean-shaped macronucleus measuring 18 to 36 by 10 to 18 μ with a mean of 28.5 by 14 μ (Lubinsky, 1957).

According to Lubinsky (1957), the reports of "*Balantidium*" in cattle were actually of *B. sulcata*.

Infundibulorium cameli Bozhenko, 1925, which was described from the diarrheic stools of a camel, may be the same species as *B. sulcata*. If so, the name will have to be changed, since *Infundibulorium* has priority (Lubinsky, 1957a).

FAMILY ISOTRICHIDAE

In this holotrichasin trichostomorid family, the mouth is terminal or subterminal, and the pharynx is ciliated, with longitudinal striations in its wall. Somatic ciliation is complete and practically uniform. This family contains the 2 most important holotrich genera of ruminants.

Genus *ISOTRICHA* Stein, 1859

The body is oval and flattened, with dense, longitudinal rows of cilia. The cytostome is at or near the anterior end. Several contractile vacuoles are present. The macronucleus is kidney-shaped; it and the micronucleus are connected to each other and suspended by fibrils which constitute the karyophore. Locomotion is toward the rear.

Isotricha prostoma Stein, 1859 is the most widely distributed of all the ruminant ciliates. It occurs in the rumen and reticulum of cattle, sheep and goats. Becker and Talbott (1927) found it in 58% of 26 cattle in Iowa. It measures 80 to 195 by 53 to 85 μ, and its cytostome is subterminal.

I. intestinalis Stein, 1859 also occurs in the rumen and reticulum of cattle, sheep and goats. Becker and Talbott (1927) found it in 19% of 26 cattle in Iowa. It measures 97 to 130 by 68 to 88 μ, and differs from *I. prostoma* in that its cytostome and nucleus are more posterior.

Genus *DASYTRICHA* Schuberg, 1888

The body is oval and flattened. The cilia are in spiral, longitudinal rows. There is no karyophore.

Dasytricha ruminantium Schuberg, 1888 occurs in the rumen and reticulum of cattle, sheep and goats. Becker and Talbott (1927) found it in 38% of 26 cattle in Iowa. It measures 50 to 75 by 30 to 40 μ.

FAMILY OPHRYOSCOLECIDAE

In this spirotrichasin entodiniorid family, there is not more than 1 "dorsal" ("metoral") band of membranelles in addition to the adoral zone. This family contains 18 or more genera which occur in the rumen of ruminants; 13 of these occur in cattle and sheep. The most important genera are *Entodinium*, *Diplodinium*, *Epidinium* and *Ophryoscolex*. The taxonomy of this group is complicated. Variations in structure, even within a clone, are common in *Entodinium* and *Diplodinium*. They make species identification difficult and have served to multiply unduly the number of different species which have been named (Polyansky and Strelkov, 1938; Hungate, 1943; Lubinsky, 1957, 1958).

The body in this family is often flattened, and another source of confusion results from the fact that different authors have used the same name for different sides of the body. Depending on whose terminology was used, every one of the four sides has been called the left, right, dorsal or ventral side. Lubinsky (1958) introduced a new system which has the advantages of eliminating the concept of dorsality and ventrality, which actually has no application in this group, and of making it possible to use the same terms both for *Entodinium* and for the higher genera in the family. In this system, which is used below, the observer orients the protozoon with its anterior (oral) end pointing away from him (toward 12 o'clock) and with the micronucleus to the left of the macronucleus (toward 9 o'clock). The sides are then designated left (the observer's left, i.e., the micronuclear side), right, upper and lower.

If this terminology is accepted, then the term, dorsal zone of membranelles (DZM), which is used in describing ophryoscolecids, is no longer appropriate. Lubinsky used "metoral membranelle zone" instead.

Genus *OPHRYOSCOLEX* Stein, 1858

The body is ovoid, with adoral and metoral zones of membranelles. The metoral zone is some distance from the anterior end and encircles 3/4 of the body circumference at its middle, being broken on the upper right side. There are 3 skeletal plates extending the length of the body on the upper right side, and 9 to 15 contractile vacuoles arranged in an anterior and a posterior circle. The macronucleus is simple and elongate. This genus occurs in the rumen and reticulum of cattle, sheep, goats and wild sheep. It is not common, and is seldom present in large numbers when it does occur, but is interesting because of its size and appearance.

Ophryoscolex inermis Stein, 1858 occurs principally in the goat. It measures 170 to 190 by 65 to 100 μ. Becker and Talbott (1927) found it in 1 of 26 cows in Iowa. It differs from the other species of *Ophryoscolex* in having a rounded posterior end, without spines.

O. purkinjei Stein, 1858 occurs in cattle. It measures 200 by 80 μ and has 2 or 3 terraces of thorn-like appendages or spines encircling the posterior end of the body except for a short gap on the right side; in addition, there is a bifid spine at the posterior end. Becker and Talbott (1927) did not find this species in Iowa cattle.

O. caudatus Eberlein, 1895 occurs in sheep and cattle. It resembles *O. purkinjei* but its terminal spine is long and not bifid. Becker and Talbott (1927) found it in 1 of 26 cattle in Iowa.

Genus *ENTODINIUM* Stein, 1858

The body is truncate anteriorly, with the adoral zone of membranelles at that end. There is no metoral zone of membranelles, and skeletal plates are likewise absent. The contractile vacuole is anterior. The macronucleus is cylindrical or sausage-shaped and dorsal. The micronucleus is anterior to the middle on the upper left side of the macronucleus. This is one of the commonest and most important genera in the rumen and reticulum of cattle, sheep, goats and other ruminants.

Many species of *Entodinium* have been named, but knowledge of the true number and of their correct names awaits some future exhaustive taxonomic study. In the earlier papers, great reliance was placed on caudal spination to differentiate species, but later it was found that this character varies within a species. Thus, *E. caudatum* was given its name because it has a long posterior spine on the right side in addition to 2 short, pointed lobes on the left. A second species, *E. lobosospinosum*, received its name because it has only a single lobe on the left (the upper one) in addition to the spine on the right. A third species, *E. dubardi*, has no posterior spines or lobes at all. However, it was later found that the caudal spination varies all the way from the *caudatum* type to the *dubardi* type in several species, and that other characters are more constant and more valuable in differentiating species.

Six types or classes of caudal spination have been set off along this series for *E. caudatum*. Three of them have received special names, and workers now speak of *E. caudatum* forma *caudatum*, *E. caudatum* forma *lobospinosum*, and *E. caudatum* forma *dubardi*, the last being a form without a tail at all! These same forma names are also used for *E. simulans*, and some of them for *E. rectangulatum* and *E. lobospinosum* (cf. Poljansky and Strelkow, 1938; Lubinsky, 1957).

Entodinium bursa Stein, 1858 has a flattened body which measures about 80 by 60 μ. (Because of the variation in caudal spination, measurements of *Entodinium* are made nowadays from the anterior end to the cytopyge, but early workers usually gave measurements to the end of the caudal spine.) The sausage-shaped macronucleus is 4/5 of the body length, and the micronucleus is pressed closely to it. The body surface has conspicuous longitudinal striations. The contractile vacuole is anterior.

E. minimum Schuberg, 1888 has a flattened body which measures about 40 by 22 μ. The right margin of the body is strongly convex and the left margin almost straight. The body surface has faint longitudinal striations. The macronucleus is about 1/3 of the body length. The contractile vacuole is anterior.

E. caudatum Stein, 1858 has a flattened body about 30 to over 80 μ long. The macronucleus is about half of the body length; it is broader anteriorly than posteriorly. The contractile vacuole is near the anterior end of the macronucleus. The upper side of the body is hollowed out to form a groove which broadens posteriorly. As mentioned above, there is great variation in the caudal spination.

E. bicarinatum Cunha, 1914 may be a synonym of *E. caudatum*. It measures about 61 by 35 μ, and the upper groove is not as deep as in *E. caudatum*.

E. furca Cunha, 1914, too, may be a synonym of *E. caudatum*. It has 2 unequal caudal projections, 1 on the left and the other on the right, and measures about 52 by 27 μ.

E. dentatum Stein, 1859 measures 60 to 90 by 30 to 50 μ and has 6 incurved, tooth-like posterior projections.

E. rectangulatum Kofoid and MacLennan, 1930 measures 23 to 47 by 23 to 39 μ. Its body is nearly rectangular as seen from above, except for the caudal spines. The macronucleus is about half the body length and is broader anteriorly than posteriorly. The contractile vacuole is about the middle of the body at the level of the esophagus, i.e., more to the left than that of *E. caudatum*. The upper groove is more marked than that of *E. caudatum*, and its anterior end separates the contractile vacuole from the macronucleus.

E. lobosospinosum Dogiel, 1927 measures 18 to 33 by 13 to 25 μ. Its body is rectangular as seen from above. The macronucleus is about half the body length. The contractile vacuole is on the mid-line of the upper side of the body on the level of the micronucleus and to the left of the broad upper groove.

E. simulans Lubinsky, 1957 measures 27 to 44 by 21 to 34 μ. Its body is ovoid as seen from above. The macronucleus is about half the body length. The contractile vacuole is on the mid-line of the upper side of the body at the level of the micronucleus and to the left of the upper groove. This groove is narrow and long, with a slit-shaped anterior half.

E. longinucleatum Dogiel, 1925 measures 39 to 64 by 27 to 46 μ and has an ellipsoidal, flattened body. The macronucleus usually extends the whole length of the body from the anterior end to the cytopyge. The contractile vacuole is close to the upper side of the macronucleus, slightly anterior to the micronucleus.

E. rostratum Fiorentini, 1889 measures 27 to 51 by 13 to 23 μ and has a rather long, slim, flattened body with a strongly convex right side and a concave left side. The macronucleus is narrow, bandlike, and about half the length of the body. The contractile vacuole is directly anterior to the macronucleus.

E. laterale Kofoid and MacLennan, 1930 measures 19 to 28 by 18 to 21 μ and has a short, fairly broad, truncated ellipsoidal, flattened body. The macronucleus is broad and wedge-shaped, less than half the length of the body, and lies in the anterior half of the body. The contractile vacuole is in the middle of the upper side.

E. nanellum Dogiel, 1922 measures 20 to 32 by 10 to 18 μ and has an ovoid, flattened body. The macronucleus is thin, wedge-shaped, and longer than half the body length. The contractile vacuole is above the anterior end of the macronucleus.

E. bimastus Dogiel, 1927 measures 30 to 46 by 28 to 40 μ and has a subspherical, flattened body. The macronucleus is flattened, wedge-shaped, and about 2/3 of the length of the body. The contractile vacuole is above the anterior end of the macronucleus.

E. exiguum Dogiel, 1925 measures 21 to 29 by 14 to 18 μ and has an elongate, oval body. The macronucleus is relatively short and thick, being shorter than half the body length, and lies in the middle of the body.

E. dubardi Buisson, 1923 (syn., *E. simplex* Dogiel, 1925 pro parte; the true *E. simplex* occurs in the reindeer) measures 30 to 50 by 20 to 29 μ and has an oval or elongate oval, flattened body. The macronucleus is more or less band-shaped, with a somewhat broader anterior end. It is about half as long as the body and lies anteriorly in it. The contractile vacuole is below the anterior end of the macronucleus.

E. vorax Dogiel, 1925 measures 60 to 121 by 40 to 83 μ and has an oval, plump, thick body. The anterior end is often smaller than the posterior. The macronucleus is sausage-shaped, about half as long as the body, and lies in the anterior part of the body. The contractile vacuole is to the right of the anterior end of the macronucleus.

Quite a few other species of *Entodinium* have been described from various ruminants. Among them are the following, which Kofoid and MacLennan (1930) described from the zebu: *E. ellipsoideum, E. acutonucleatum, E. pisciculum, E. biconcavum, E. bifidum* (Dogiel) *E. acutum, E. aculeatum, E. brevispinum, E. laterospinum, E. ovoideum, E. rhomboideum, E. gibberosum, E. tricostatum,* and *E. indicum.*

Genus *EPIDINIUM* Crawley, 1924

The body is elongate and twisted around its main axis. The adoral zone of membranelles is at the anterior end, and the metoral zone elsewhere. There are 3 skeletal plates with secondary plates. The macronucleus is simple and club-shaped. There are 2 contractile vacuoles. This genus occurs in the rumen and reticulum of cattle, sheep, goats, camels, reindeer, elk, and other ruminants.

Epidinium ecaudatum (Fiorentini, 1889) (syn., *Diplodinium ecaudatum*) measures 82 to 173 by 36 to 70 μ and has an elongate body with a convex left side and

a flat or slightly concave right side. This species has 9 formae which differ principally in their caudal spination: *E. ecaudatum* forma *ecaudatum* has no caudal spines, forma *caudatum* has a single long spine and the posterior end of its body is narrow, *hamatum* has a single long spine and the posterior end of its body is broad, *bulbiferum* has a bulb-shaped appendage instead of a spine, *bicaudatum* has 2 spines, *tricaudatum* has 3, *quadricaudatum* has 4, *cattencoi* has 5 short ones, and *fasciculus* has 5 very long ones with greatly swollen bases. All 9 formae occur in the rumen of cattle, the most common being *ecaudatum* and *caudatum*. *Ecaudatum*, *hamatum* and *cattaneoi* are found in sheep, and the last in goats as well. *Ecaudatum* and *caudatum* also occur in the reindeer, *caudatum* and *hamatum* in camels and *caudatum* in the elk.

Genus *EODINIUM* Kofoid and Maclennan, 1932

The metoral zone of membranelles is at the same level as the adoral zone. Skeletal plates are absent. The macronucleus is a straight, rod-like body near the left edge. Two contractile vacuoles are present. This genus occurs in the rumen and reticulum of cattle and sheep.

E. posterovesiculatum (Dogiel, 1927) Kofoid and MacLennan, 1932 measures 47 to 60 by 23 to 30 μ and has a relatively long, flattened body with rounded ends. The macronucleus is very long, straight, and has 2 deep depressions on its left side. The micronucleus lies in the posterior one, and a contractile vacuole in the anterior one. The second contractile vacuole is posterior to the macronucleus. This species occurs in cattle.

E. bilobosum (Dogiel, 1927) Kofoid and MacLennan, 1932 measures 46 to 60 by 30 to 44 μ and has a relatively short, flattened body with 2 caudal spines, 1 dorsal and the other ventral. The nuclei and vacuoles are similar to those of *E. posterovesiculatum*. This species occurs in cattle and sheep.

Eodinium lobatum Kofoid and MacLennan, 1932 measures 44 to 60 by 29 to 37 μ and has a narrow body. The macronucleus is narrow and rod-like, and is almost as long as the body. It has 3 large depressions in its left side; the micronucleus lies in the middle one, and the contractile vacuoles in the end ones. This species occurs in the zebu.

Genus *DIPLODINIUM* Schuberg, 1888

The metoral zone of membranelles is on the same level as the adoral zone. Skeletal plates are absent. The macronucleus is beneath the upper surface of the body; its anterior third is bent to the right at an angle of 30 to 90°. Two contractile vacuoles are present. This genus occurs in the rumen and reticulum of cattle, goats, sheep, camels, reindeer and various wild ruminants.

In this genus, as in *Entodinium*, there is also considerable variation in the caudal spination.

Diplodinium dentatum (Stein, 1858) Schuberg, 1888 measures 55 to 82 by 44 to 62 μ. Its body has a broad, truncate posterior end with 6 large, relatively heavy, incurved caudal spines. The left side is convex and the right one concave. The macronucleus is 25 to 50 μ long; it is heavy and rod-like, with the anterior end bent at a 45° angle. The 2 contractile vacuoles lie in the left rib slightly below the midline. This species occurs in the ox and zebu.

D. quinquecaudatum Dogiel, 1925 measures 57 to 73 by 47 to 65 μ. It resembles *D. dentatum*, but has 5 caudal spines. It occurs in cattle and sheep.

D. anacanthum Dogiel, 1927 measures 60 to 124 by 38 to 72 μ. The posterior end of its body tapers, giving it a somewhat conical appearance. The macronucleus varies a good deal in length. Its anterior third is bent at an angle of about 45°. The 2 contractile vacuoles are on the lower side. This species has 7 formae (which Kofoid and MacLennan,

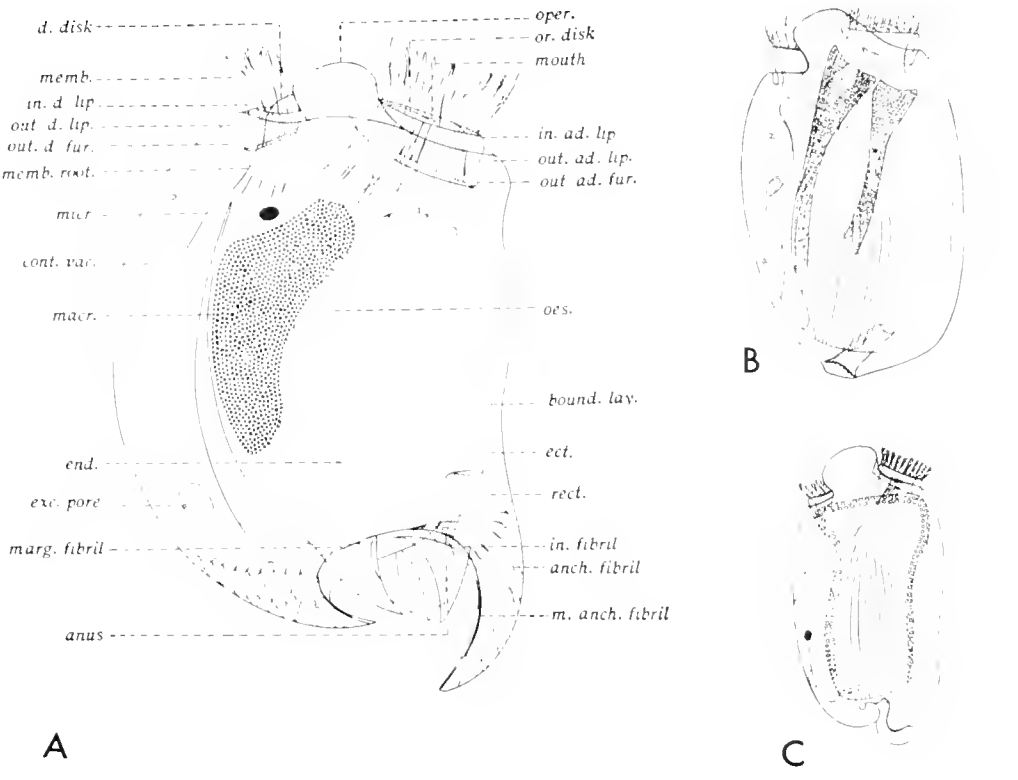

Fig. 39. Ciliates of ruminants. A. *Diplodinium dentatum*. X 850. *anch. fibril*, anchoring fibril; *anus*, cytopyge; *bound. lay.*, boundary layer; *cont. vac.*, contractile vacuole; *d. disk*, metoral disk; *ect.*, ectoplasm; *end.*, endoplasm; *exc. pore*, excretory pore; *in. ad. lip*, inner adoral lip; *in. d. lip*, inner metoral lip; *in. fibril*, inner fibril; *macr.*, macronucleus; *m. anch. fibril*, main anchoring fibril; *marg. fibril*, marginal fibril; *memb.*, membranelle; *memb. root*, membranelle root; *micr.*, micronucleus; *mouth*, mouth; *oes.*, esophagus; *oper.*, operculum; *or. disk*, oral disk; *out. ad. fur.*, outer adoral furrow; *out. ad. lip*, outer adoral lip; *out. d. fur.*, outer metoral furrow; *out. d. lip*, outer metoral lip; *rect.*, rectum. B. *Metadinium medium*. X 425. C. *Ostracodinium mammosum*. X 425. (From Kofoid and MacLennan, 1932)

1930, considered separate species): *anacanthum*, *monacanthum*, *diacanthum*, *triacanthum*, *tetracanthum*, *pentacanthum*, and *anisacanthum*, with 0, 1, 2, 3, 4, 5 and 6 caudal spines, respectively. It occurs in the ox and zebu.

D. psittaceum Dogiel, 1927 measures 95 to 155 by 59 to 105 μ and has a heavy, rounded, posteriorly tapering body with a thin ventral spine on the right and a narrow flange on the left of the posterior third of the body. The macronucleus is a stout rod-like body with its anterior end bent at a 40° angle. The contractile vacuoles lie near the left side. This species occurs in the ox and zebu.

D. bubalidis Dogiel, 1925 measures 104 to 195 by 58 to 98 μ and has an oval body with its largest diameter anterior, a strongly convex left side and a slightly convex right one. There is a small, longitudinal groove on the posterior part of the upper surface of the body, and a single, thin spine on the right. This species occurs in cattle and African antelope.

D. elongatum Dogiel, 1927 measures 177 to 205 by 73 to 100 μ and has an elongate body with weakly convex left and right sides and a narrow groove in the posterior end of the upper surface of the body. It occurs in the ox.

D. laeve Dogiel, 1927 measures 77 to 100 by 52 to 70 μ and has a roughly triangular body with no caudal projections except a small lobe on the right. It occurs in goats.

D. cristagalli Dogiel, 1927 measures 77 to 100 by 52 to 70 μ and has a triangular body with the lower side extended posteriorly to form a prominent fan with 2 to 7 spines. It occurs in goats.

D. flabellum Kofoid and MacLennan, 1932 measures 82 to 118 by 57 to 82 μ and has a roughly triangular body with the upper side extended posteriorly to form a prominent fan with 5 to 7 spines, and with 2 small spines on the posterior left side. It occurs in the zebu.

Genus *EREMOPLASTRON* Kofoid and MacLennan, 1932

The metoral and adoral zones of membranelles are at the same level. There is a single, narrow skeletal plate beneath the upper surface. The macronucleus is triangular or rod-like, often with its anterior end bent to the right. Two contractile vacuoles are present. This genus occurs in the rumen and reticulum of cattle, sheep, antelope and reindeer.

Eremoplastron rostratum (Fiorentini, 1889) Kofoid and MacLennan, 1932 (syn., *Diplodinium helseri* Becker and Talbott, 1927) measures 40 to 63 by 22 to 47 μ and has a proportionately long, compressed body with a thick flange on the left and a large caudal spine on the right. The macronucleus is rod-like. This species occurs in the ox and zebu.

E. neglectum (Dogiel, 1925) is 81 to 124 μ long and has an elongate oval body with the left side strongly convex, the right side slightly convex, a large lobe on the right, and a long, rod-like macronucleus. It occurs in cattle and African antelope.

E. bovis (Dogiel, 1927) (syn., *Diplodinium clevelandi* Becker and Talbott, 1927) measures 52 to 100 by 36 to 57 μ and has an ellipsoidal, compressed body with a somewhat flattened right side, a more strongly convex left side, and a small caudal lobe. The macronucleus is rod-shaped. This species occurs in the ox, zebu and sheep.

E. monolobum (Dogiel, 1927) is 58 to 83 μ long and has a nearly spherical body with a prominent right lobe and a low, blunt left lobe. The macronucleus is thick and rod-shaped. This species occurs in cattle.

E. dilobum (Dogiel, 1927) is 73 to 101 μ long and has an ellipsoidal, flattened body with 1 left and 1 right caudal lobe. The macronucleus is rod-shaped. This species occurs in cattle and sheep.

E. rugosum (Dogiel, 1927) is 69 to 90 μ long and has a short body with a flat or slightly concave right side, a convex left side, and a deep cuticular fold from the cytopyge along the left side of the macronucleus to the region of the metoral zone of membranelles. The right lobe is laterally compressed, with 8 to 10 shallow indentations in its left border. The macronucleus is long and rod-like. This species occurs in cattle.

E. brevispinum Kofoid and MacLennan, 1932 measures 72 to 92 by 42 to 53 μ and has an ellipsoidal, flattened body with 2 short caudal spines. The macronucleus is rod-shaped. This species occurs in the zebu.

E. magnodentatum Kofoid and MacLennan, 1932 measures 58 to 82 by 30 to 50 μ and has a rectangular, flattened body with a large, compressed caudal spine on the right and a similar caudal spine on the left. The macronucleus is rod-shaped. This species occurs in the zebu.

Genus *EUDIPLODINIUM* Dogiel, 1927 emend. Kofoid and MacLennan, 1932

The metoral and adoral zones of membranelles are at the anterior end. There is a single, narrow skeletal plate beneath the upper surface. The macronucleus is rod-like, with its anterior end enlarged to form a hook which opens to the left.

The pellicle and ectoplasm are thick. There are 2 contractile vacuoles with heavy membranes and prominent pores.

Eudiplodinium maggii (Fiorentini, 1889) measures 104 to 240 by 63 to 77 μ and has a roughly triangular body with a smoothly rounded posterior end. It occurs in the rumen and reticulum of the ox and zebu.

Genus *DIPLOPLASTRON* Kofoid and MacLennan, 1932

The metoral and adoral zones of membranelles are at the anterior end. There are 2 skeletal plates beneath the upper surface. The macronucleus is narrow and rod-like. There are 2 contractile vacuoles below the left surface, separated from the macronucleus.

Diploplastron affine (Dogiel and Fedorowa, 1925) measures 88 to 120 by 47 to 65 μ and is more or less ellipsoidal. It occurs in the rumen of cattle, sheep and goats.

Genus *METADINIUM* Awerinzew and Mutafowa, 1914

The metoral and adoral zones of membranelles are at the anterior end. There are 2 skeletal plates beneath the upper surface which are sometimes fused posteriorly. The macronucleus has 2 to 3 left lobes. There are 2 contractile vacuoles. The pellicle and ectoplasm are thick. There are conspicuous esophageal fibrils beneath the left and upper sides.

Metadinium medium Awerinzew and Mutofowa, 1914 measures 180 to 272 by 92 to 170 μ and has a heavy body with large membranelle zones. The skeletal plates are narrow. This species occurs in the rumen of the ox and zebu.

M. tauricum (Dogiel and Fedorowa, 1925) measures 185 to 288 by 70 to 160 μ and has a heavy body. The skeletal plates are fused posteriorly. The anterior and median lobes of the macronucleus are large, and the posterior lobe is small. This species occurs in the rumen of sheep, goats and cattle.

M. ypsilon (Dogiel, 1925) measures 110 to 152 by 60 to 72 μ and has an oval, flattened body with a rounded posterior end. The anterior lobe of the macronucleus is small, and there is no posterior lobe. The skeletal plates are fused posteriorly. This species occurs in the rumen of cattle.

Genus *POLYPLASTRON* Dogiel, 1927

The metoral and adoral zones of membranelles are at the anterior end. There are 2 separate or fused skeletal plates beneath the upper surface, and 3 longitudinal plates with anterior ends connected by cross bars beneath the lower surface. There is a longitudinal row of contractile vacuoles beneath the left surface, and others in other locations.

Polyplastron multivesiculatum (Dogiel and Fedorowa, 1925) measures 120 to 190 by 78 to 140 μ and has an oval body with a smoothly rounded posterior end. There is a row of 4 contractile vacuoles near the macronucleus, plus 2 beneath the left surface, 1 beneath the right surface and 2 beneath the upper surface. The 2 upper skeletal plates are separate. This species occurs in the rumen of cattle and sheep.

P. fenestratum Dogiel, 1927 resembles *P. multivesiculatum* except that the upper skeletal plates are partly fused. This species occurs in the rumen of cattle.

P. monoscutum Kofoid and MacLennan, 1932 resembles *P. multivesiculatum* except that the upper skeletal plates are completely fused to form a single broad plate. This species occurs in the rumen of cattle.

Genus *ELYTROPLASTRON* Kofoid and MacLennan, 1932

The metoral and adoral zones of membranelles are at the anterior end. There are 2 skeletal plates beneath the upper

surface, a small plate beneath the right surface and a long plate below the lower surface. The pellicle and ectoplasm are thick. There are conspicuous fibrils beneath the left and upper surfaces.

Elytroplastron bubali (Dogiel, 1928) measures 110 to 160 by 67 to 97 μ and has an ellipsoidal body with a smoothly rounded posterior end. There are 4 contractile vacuoles along the left midline. This species occurs in the rumen of the water buffalo and zebu.

Genus *OSTRACODINIUM* Dogiel, 1927

The metoral and adoral zones of membranelles are at the anterior end. There is a broad skeletal plate beneath the upper side of the body, and a row of 2 to 6 contractile vacuoles beneath the left surface. Heavy pharyngeal fibrils are present which extend to the posterior end.

Ostracodinium mammosum (Railliet, 1890) measures 41 to 110 by 25 to 68 μ and has a left caudal lobe and a right lobe which is hollow on the left side. The posterior part of the skeleton extends only 2/3 of the way across the upper side. The macronucleus has a large, shallow depression in the middle of its lower side. There are 3 contractile vacuoles. This species occurs in the rumen of the ox and zebu.

O. gracile (Dogiel, 1925) measures 90 to 133 by 42 to 60 μ and has a roughly triangular body with flat right and lower surfaces and convex left and upper surfaces, and with a smoothly rounded posterior end. The skeletal plate extends across the upper surface. The macronucleus has 2 lobes. There are 2 contractile vacuoles. This species occurs in the rumen of the ox, zebu, sheep and African antelopes.

O. tenue (Dogiel, 1925) measures 59 to 76 by 28 to 38 μ and has a slender body with a smoothly rounded posterior end. The skeletal plate extends across the upper surface. The macronucleus has an anterior and a median left lobe. There are 2 contractile vacuoles. This species occurs in the rumen of cattle and an African antelope.

O. trivesiculatum Kofoid and MacLennan, 1932 measures 78 to 100 by 42 to 60 μ and has a triangular body with a smoothly rounded posterior end. The skeletal plate extends across the upper side. The macronucleus has a small, shallow depression in the middle of the lower side. There are 3 contractile vacuoles. This species occurs in the rumen of the zebu.

O. quadrivesiculatum Kofoid and MacLennan, 1932 measures 92 to 112 by 43 to 56 μ and has a triangular body with a bluntly rounded posterior end. The skeletal plate extends across the upper side. The macronucleus is elongate and rod-like. There are 4 contractile vacuoles. This species occurs in the rumen of the zebu.

O. nanum (Dogiel, 1925) measures 47 to 70 by 30 to 41 μ and has an ellipsoidal body with a slender, right caudal spine. The skeletal plate extends between the macronucleus and the ventral surface. The macronucleus is short and stout. There are 2 small contractile vacuoles. This species occurs in the rumen of cattle and African antelopes.

O. gladiator (Dogiel, 1925) measures 78 to 112 by 40 to 55 μ and has a slender body with a long, very narrow, right caudal spine. The skeletal plate extends between the macronucleus and the right side. The macronucleus has a lobe on the left anterior end. There are 2 contractile vacuoles. This species occurs in the rumen of cattle and African antelopes.

O. crassum (Dogiel, 1925) measures 120 to 142 by 80 to 100 μ and has a heavy body with a smoothly rounded posterior end. The skeletal plate extends under only 1/2 of the upper side. The macronucleus is short and stout, with a wide, shallow depression in the anterior half of its left side. There are 2 contractile vacuoles. This species occurs in the rumen of cattle and the steenbock.

O. obtusum (Dogiel and Fedorowa, 1925) (syn., *Diplodinium hegneri* Becker and Talbott, 1927) measures 118 to 148 by 55 to 80 μ and has an ellipsoidal, only slightly flattened body, with a smoothly rounded posterior end. The posterior part of the skeleton extends across only 2/3 of the upper side. The macronucleus is elongate and rod-like. There are 6 contractile vacuoles. This species occurs in the rumen of cattle and reindeer.

O. venustum Kofoid and MacLennan, 1932 measures 76 to 115 by 41 to 60 μ and has a triangular body with a small posterior right lobe. The skeletal plate extends beneath the upper surface between the macronucleus and the right side. The macronucleus has 2 left lobes. There are 2 contractile vacuoles. This species occurs in the rumen of the zebu.

O. dogieli Kofoid and MacLennan, 1932 measures 92 to 130 by 48 to 63 μ and has an ellipsoidal body with a strongly convex left side, a slightly convex right side and a flattened right lobe lying below the cytopyge. The skeletal plate extends between the macronucleus and the right side. The macronucleus has 2 left lobes (1 anterior and 1 median). There are 2 contractile vacuoles. This species occurs in the rumen of the ox.

O. clipeolum Kofoid and MacLennan, 1932 measures 92 to 128 by 50 to 65 μ and has an ellipsoidal body with a flattened lobe projecting from the right posterior surface below the midline. The skeletal plate extends beneath the upper surface between the macronucleus and the right side. The macronucleus has 2 left lobes. There are 3 contractile vacuoles. This species occurs in the rumen of the zebu.

O. monolobum Dogiel, 1927 measures 105 to 150 by 55 to 77 μ and has a rectangular body with a large right lobe. The skeletal plate extends under only 2/3 of the left side. The macronucleus is elongate and rod-like. There are 5 contractile vacuoles. This species occurs in the rumen of the ox.

O. dilobum Dogiel, 1927 measures 88 to 140 by 54 to 78 μ and has an ellipsoidal body with a laterally flattened right lobe and a flattened left lobe. The skeletal plate extends under only 2/3 of the left side. The macronucleus is elongate and rod-like. There are 5 contractile vacuoles. This species occurs in the rumen of cattle.

O. rugoloricatum Kofoid and MacLennan, 1932 measures 84 to 125 by 37 to 58 μ and has a rectangular body with a flattened right lobe. The left side of the exceptionally large skeletal plate turns in and extends toward the middle of the body. The macronucleus is straight and rod-like. There are 3 contractile vacuoles. This species occurs in the rumen of the zebu.

Genus *ENOPLOPLASTRON* Kofoid and MacLennan, 1932

The metoral and adoral zones of membranelles are near the anterior end. There are 3 separate or partially fused skeletal plates beneath the upper and right surfaces of the body. There are 2 contractile vacuoles. The pharyngeal fibrils are heavy.

Enoploplastron triloricatum (Dogiel, 1925) measures 60 to 112 by 37 to 70 μ and has an ellipsoidal body with a smoothly rounded posterior end. The skeletal plates are separate. The macronucleus has a shallow depression in the anterior half of its left side. This species occurs in the rumen of the ox, reindeer and an African antelope.

RELATIONS OF RUMEN CILIATES TO THEIR HOSTS

Ciliates swarm in such tremendous numbers in the rumen and reticulum that everyone who has seen them has speculated on their role in their host's nutrition. This problem has been reviewed by Hungate (1950, 1955) and Oxford (1955, 1955a), to whose papers reference is made for further details. It should be said that in these reviews the name "*Diplodinium*" is used for practically all the ophryoscolecids except *Entodinium* and *Ophryoscolex*, but the other genera involved can often be determined from their specific names.

The rumen ciliates are obligate anaerobes. The holotrichs (*Isotricha* and *Dasytricha*) have been cultivated by Sugden and Oxford (1952), Gutierrez (1955) and others. *Diplodinium*, *Entodinium*, *Eudiplodinium*, *Polyplastron* and *Metadinium* have been cultivated by Hungate (1942, 1943), Sugden (1953), and others, but *Ophryoscolex* has not yet been cultivated.

The holotrichs absorb soluble carbohydrates from the medium and convert them into amylopectin, which is stored in ovoid granules measuring 3 by 2 μ and resembling small yeast cells. They are able to utilize glucose, fructose, sucrose, cellobiose, inulin and levans. In addition, both *Isotricha intestinalis* and *I. prostoma* rapidly ingest small starch granules and are able to metabolize them. *Dasytricha ruminantium* does not ingest starch. Gutierrez and Hungate (1957) found that *D. ruminantium* ingested small cocci and occasionally small rod-shaped bacteria; they were able to cultivate this species in a medium containing these types of bacteria, but not without them. Gutierrez (1958) showed that *Isotricha prostoma* feeds selectively only on certain rods among the many types of rumen bacteria, but that pure strains did not fulfill all the protozoon's growth requirements, since it divided once and then died out in a monobacterial culture.

The holotrichs produce hydrogen, carbon dioxide, lactic, acetic and butyric acids, and traces of propionic acid (Heald and Oxford, 1953; Gutierrez, 1955).

Many but not all species of *Entodinium* ingest and digest starch. According to Kofoid and MacLennan (1930), *E. longinucleatum* and *E. acutonucleatum* feed selectively on pollen grains. Certain species of *Entodinium* are the predominant starch-ingesters among the rumen protozoa and are the dominant protozoa in animals on full feed. Among those known to ingest starch are *E. caudatum*, *E. longinucleatum*, *E. minimum* and *E. dubardi*. Almost nothing is known about the products of starch fermentation by this genus. Granules of polysaccharide are stored in the outer zone of the endoplasm, but they have never been isolated and identified; it would be difficult to separate them from ingested starch granules.

It has been suggested that carbohydrate metabolism is dependent upon intracellular bacteria. Sugden (1953) was unable to cultivate *E. longinucleatum* in the presence of streptomycin except when streptomycin-resistant strains of bacteria were present. However, Appleby, Eadie and Oxford (1956), who found various bacteria in disintegrated *Entodinium*, concluded that there was so far no good reason for denying the existence of protozoan enzyme systems concerned with carbohydrate fermentation. Gutierrez and Davis (1959) found about 100 to 150 gram-positive diplococci (*Streptococcus bovis*) per ciliate in *E. caudatum*, *E. minimum*, *E. dubardi*, *E. longinucleatum*, *E. bursa*, *E. nanellum*, *E. exiguum* and *E. vorax* in cattle being fed a high starch ration. The ciliates sometimes ingested starch granules with adherent starch-attacking bacteria. *Entodinium* species could be cultivated in the presence of *S. bovis* but not without it. Thus, bacteria are ingested by *Entodinium* and appear to be necessary for its nutrition, but most likely as a source of nitrogen rather than of prefabricated enzymes.

Epidinium, like *Entodinium*, ingests starch and also bacteria; its metabolic products are also unknown. Gutierrez and Davis (1959) found that *E. ecaudatum* (syn., *Diplodinium ecaudatum*) ingested not only *Streptococcus bovis* but also other bacteria.

Diplodinium and related genera (*Eudiplodinium*, *Polyplastron*, *Eremoplastron*, *Metadinium*) ingest and digest cellulose in addition to starch and bacteria. Hungate (1942, 1943) cultured *Diplodinium dentatum* (syn., *D. denticulatum*), *Polyplastron multivesiculatum* and *Eudiplodinium maggii* in media containing dried grass and pure cellulose, but the protozoa failed to grow if the cellulose was omitted. These species and *Eremoplastron neglectum* all contained a cellulase. Sugden (1953) found that *Metadinium medium* also utilized cellulose. Gutierrez and Davis (1959) found that *E. neglectum* and a large unidentified species of "*Diplodinium*" contained gram-

positive diplococci and other bacteria on different occasions. Sugden and Oxford (1955) found that a "pure", washed, living suspension of *Metadinium medium* had no action on glucose in the Warburg apparatus.

Diplodinium and related species were found by Hungate (1946) to produce hydrogen, carbon dioxide and volatile acids.

The skeletal plates of all ophryoscolecids which have them stain brown with iodine and are polysaccharide in nature. According to Oxford (1955), Hirst *et al.* extracted enough of the storage polysaccharide from *Metadinium medium* to identify it as of the "glycogen-amylopectin" type, but they were not sure whether it was pure amylopectin.

The mode of nutrition of *Ophryoscolex* has not been determined, altho it is known to ingest starch granules and sometimes cellulose fibers.

Lubinsky (1957b) reported that accidental predation on smaller protozoa is a common trait of many of the larger species of Ophryoscolecidae, particularly of *Diplodinium* and related cellulose-feeding genera. Predation is rare in *Ophryoscolex*, however. The prey of these occasional predators consists primarily of spineless smaller species. The spines are thus of value in protecting the smaller ophryoscolecids against ingestion. Lubinsky gave a table listing cases of predation among ophryoscolecids from the Canadian reindeer, goat, sheep and Indian water buffalo, which included 8 genera and 9 species of predators and 7 genera and 9 species of prey.

The role of the rumen protozoa in their host's nutrition is still not clear. Young animals on a milk diet do not have them. As they grow older and begin to feed on hay and grass, they become infected from protozoa in the saliva of faunated animals. This is the only way in which transmission occurs. There are no resistant forms or cysts, and the protozoa are killed when they enter the abomasum.

The relation between the protozoa and their hosts is not symbiotic, since the host does not need the protozoa for survival, and indeed gets along perfectly well without them. Becker, Schulz and Emmerson (1929, 1930) and Winogradow *et al.* (1930) killed the protozoa in the rumens of goats without harming the goats. The defaunated animals continued to break down cellulose just as actively as the normal controls, due to the action of cellulolytic bacteria. Pounden and Hibbs (1950) raised calves successfully without protozoa.

The fact that defaunation is not harmful does not mean, however, that the protozoa are of no value to their hosts. It means simply that they are not essential.

It has been suggested that the protozoa might harm their hosts by excreting ammonia which may then not be utilized by the rumen bacteria for protein synthesis and which would therefore be lost to their hosts; by robbing the host of B vitamins; by feeding on and destroying valuable bacteria; or by producing lactic acid and other undesirable intermediate products of carbohydrate metabolism which the rumen bacteria cannot cope with (see Oxford, 1955). However, there is no proof that they are actually harmful, and this is simply speculation.

Rumen protozoa form about 20% of the protein which reaches the abomasum (Hungate, 1955). McNaught *et al.* (1954) found that the rumen protozoan and bacterial proteins both had a biological value for rats of 80 to 81, which is higher than that of brewer's yeast (72). Furthermore, the true digestibility of the protozoan protein was 91%, much higher than that of the bacterial (74%) or yeast (84%) proteins. Hence the protozoan protein is nutritionally superior. No amino acid analyses have been carried out on it.

While many of the protozoa store reserve starch (amylopectin), this stored starch is not of much importance for the host's nutrition. About 1% of the carbohydrate required by a mature sheep is supplied from this source (Hungate, 1955).

The protozoa are an important source of volatile fatty acids. Carroll and Hungate (1954) estimated that about 2.2 kg of volatile fatty acids are produced per 100 kg rumen contents in cattle. Gutierrez (1955) calculated that the fermentation acids produced by the rumen holotrichs would constitute a little more than 10% of this amount. If the ophryoscolecids produced an equal amount, then protozoa would provide about 20% of the fermentation products available to their host (Hungate, 1955). As Hungate (1955) remarked, Gruby and Delafond, who first discovered the rumen protozoa in 1843, guessed that they supplied 1/5 of the food used by their hosts, and the results of investigations during the next 110 years have not significantly modified that estimate.

Another advantage to the host lies in the fact that the holotrichs take up soluble carbohydrates from the medium and convert them into stored starch, withholding them for a while and then fermenting them for a long time. This smooths out the fermentation process, which would proceed much more irregularly if it depended upon bacteria alone (Hungate, 1955; Oxford, 1955). *Entodinium* and *Epidinium*, too, help smooth out the fermentation process by converting starch into reserve foods. In addition, as Hungate (1959) pointed out, when animals are shifted from hay to grain, there is a period of adaptation during which lactic acid is produced explosively by *Streptococcus bovis* and may be extremely harmful. The adaptation period may be due to the time needed for *Entodinium*, *Epidinium* and other bacteria-feeding protozoa to multiply enough to keep the streptococci in check.

B. CILIATES OF EQUIDS

Just as great a variety and number of ciliates swarm in the cecum and colon of equids as in the rumen and reticulum of ruminants. Hsiung (1930) gave descriptions of 51 species of 25 genera in his monograph, while Strelkov (1939) listed 87 species and forms. The fauna of the proximal large intestine (the cecum and ventral colon) differs from that of the distal large intestine (the dorsal and small colons). Strelkov (1939) listed 25 species and forms in the proximal fauna, 43 in the distal fauna, and 7 common to both. Mixing occurs at the pelvic flexure of the colon. All horses do not contain all species. Strelkov (1939) found an average of 7.7 species per horse in the proximal fauna and 16.6 species per horse in the distal fauna.

The highest populations of ciliates occur in the left dorsal colon and the lowest in the cecum (Adam, 1951). The ciliate population shows large daily variations. Adam (1953) obtained counts ranging from 1000 to 47,000 per ml in the cecum and from 14,000 to 3,072,000 per ml in the ventral colon of a single horse at different times and on different rations.

Almost nothing is known of the relationship of these protozoa to their host, but it is most likely that they are simply commensals. No cysts have been reported, and transmission is probably by mouth. Adam (1953) infected a horse with *Cycloposthium edentatum* and *C. dentiferum* by feeding fresh colon contents by stomach tube. Forsyth, Hirst and Oxford (1953) found that *Cycloposthium* stores a polysaccharide with a highly branched molecular structure closely similar to that of amylopectin.

FAMILY BUETSCHLIIDAE

The characters of this holotrichasin gymnostomorid family have been given above (p. 349).

Genus *ALLOIOZONA* Hsiung, 1930

The cilia are present in 3 zones--anterior, equatorial and posterior.

Alloiozona trizona Hsiung, 1930 is ovoid, with both ends rounded, and measures 50 to 90 by 30 to 60 μ. The cytostome is at the anterior end and is surrounded by a shallow groove provided with short cilia. The cytopharynx is funnel-shaped. The

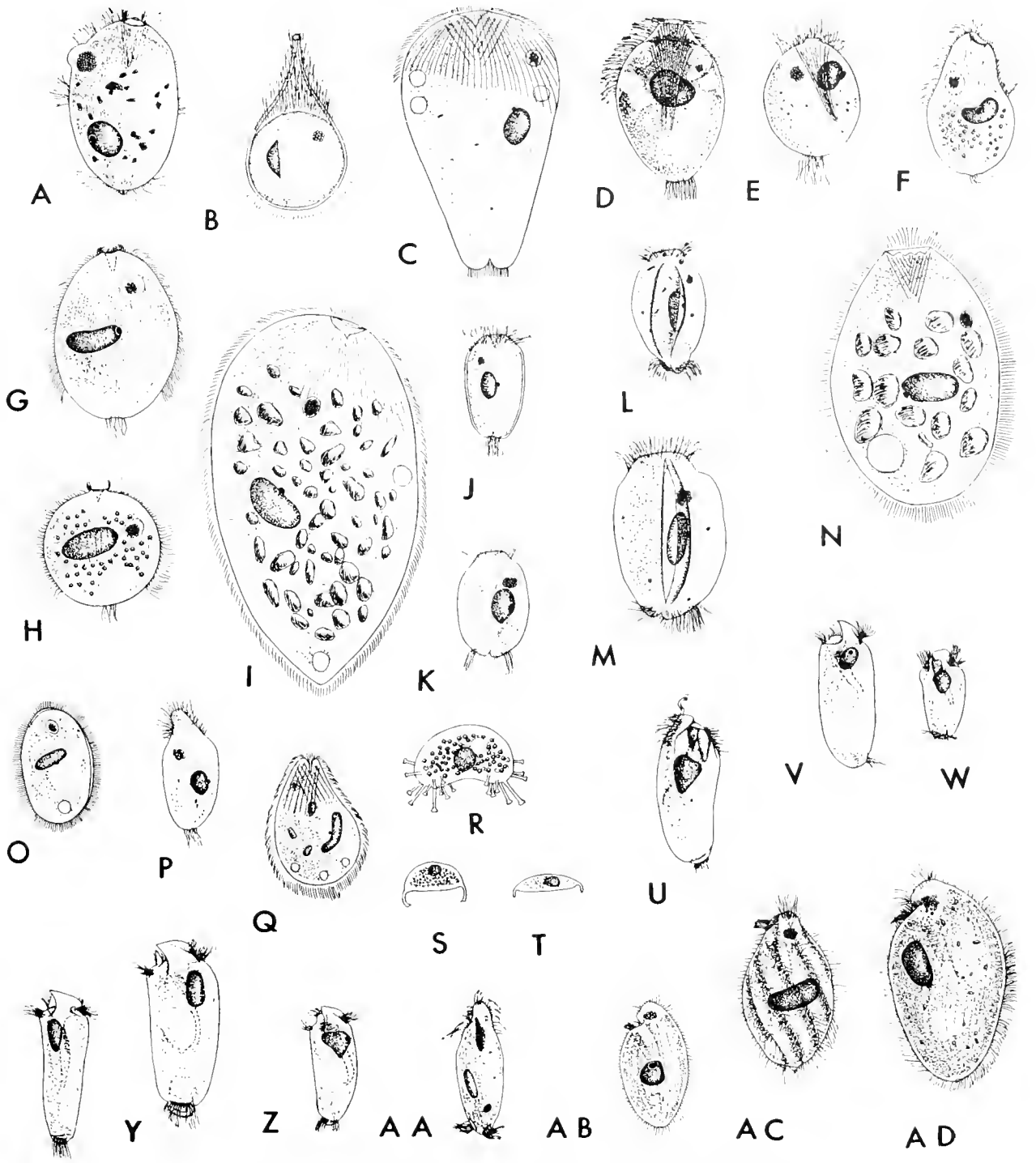

Fig. 40. Ciliates of equids. A. *Alloiozona trizona*. B. *Ampullacula ampulla*. C. *Blepharoconus hemiciliatus*. D. *Blepharoconus cervicalis*. E. *Blepharoconus benbrooki*. F. *Blepharoprosthium pireum*. G. *Blepharosphaera ellipsoidalis*. H. *Blepharosphaera intestinalis*. I. *Blepharozoum zonatum*. J. *Bundleia postciliata*. K. *Didesmis ovalis*. L. *Didesmis spiralis*. M. *Didesmis quadrata*. N. *Holophryoides ovalis*. O. *Paraisotrichopsis composita*. P. *Polymorphella ampulla*. Q. *Prorodonopsis coli*. R. *Allantosoma intestinalis*. S. *Allantosoma dicorniger*. T. *Allantosoma brevicorniger*. U. *Blepharocorys uncinata*. V. *Blepharocorys valvata*. W. *Blepharocorys jubata*. X. *Blepharocorys angusta*. Y. *Blepharocorys curvigula*. Z. *Blepharocorys cardionucleata*. AA. *Charonina equi*. AB. *Paraisotricha minuta*. AC. *Paraisotricha beckeri*. AD. *Paraisotricha colpoidea*. E., P. and AA., X 710. All others, X 340. (From Hsiung, 1930 in Iowa State College Journal of Science, published by Iowa State Univ. Press)

cytopyge is on a knob at the posterior end. The macronucleus is a more or less thick, distinctly granular disc, and is not constant in position. The concretion vacuole is large and is near the surface in the anterior third of the body. There is usually a small, posterior contractile vacuole. Hsiung (1930) found this species in the cecum or colon of 7 out of 46 horses in Iowa.

Genus *AMPULLACULA* Hsiung, 1930

The body is flask-shaped. Its posterior half is covered with fine, short cilia, and its neck with longer cilia.

Ampullacula ampulla (Fiorentini, 1890) Hsiung, 1930 measures about 110 by 40 μ. The cytostome is at the anterior end. This species occurs in the cecum of the horse.

Genus *BLEPHAROCONUS* Gassovsky, 1919

The body is ovoid. The cytostome is small, and the cytopharynx has rods in its wall. There are cilia on the anterior third to half of the body and at the caudal end. The macronucleus is ovoid. There are 3 contractile vacuoles.

Blepharoconus hemiciliatus Gassovsky, 1919 has a conical body and measures 83 to 135 by 45 to 65 μ. The macronucleus is nearly spherical. This species occurs in the colon of the horse.

B. cervicalis Hsiung, 1930 is ovoid, with a blunt anterior and a rounded posterior end, and measures 56 to 83 by 48 to 70 μ. There is usually a short neck which is formed by a slight groove. The macronucleus is more or less disc-shaped. The concretion vacuole is small and ellipsoidal. Hsiung (1930) found this species in the colon of 2 out of 46 horses in Iowa.

B. benbrooki Hsiung, 1930 is ovoid to ellipsoidal, with a knob-like anterior end and a rounded posterior one, and measures 21 to 37 by 17 to 26 μ. The macronucleus is a thick disc. The concretion vacuole is large and ellipsoidal. Hsiung (1930) found this species in the colon or feces of 2 out of 46 horses in Iowa.

Genus *BLEPHAROPROSTHIUM* Bundle, 1895

The body is piriform, with a contractile anterior half. There are cilia on the anterior half and at the posterior end. The macronucleus is kidney-shaped.

Blepharoprosthium pireum Bundle, 1895 measures 54 to 86 by 34 to 52 μ. The cytostome is anterior. The cytopharynx is funnel-shaped. The concretion vacuole contains numerous granules and is found in the anterior half of the body close to the surface. There is a contractile vacuole at the posterior end. Hsiung (1930) found this species in the cecum of 13 and the colon of 4 out of 46 horses in Iowa.

Genus *BLEPHAROSPHAERA* Bundle, 1895

The body is spherical or ellipsoidal. Cilia cover the anterior 3/4 of the body, and there is also a caudal tuft of cilia.

Blepharosphaera intestinalis Bundle, 1895 is spherical and 38 to 74 μ in diameter. Its macronucleus is a thick, ellipsoidal disc. Hsiung (1930) found this species in the cecum of 9 and the colon of 2 out of 46 horses in Iowa.

B. ellipsoidalis Hsiung, 1930 is ellipsoidal and measures 34 to 65 by 27 to 49 μ. Its macronucleus is sausage-shaped. Hsiung (1930) found this species in the cecum of 4 and the colon of 2 out of 46 horses in Iowa.

Genus *BLEPHAROZOUM* Gassovsky, 1919

The body is ellipsoidal, with an attenuated anterior end, and is uniformly ciliated. The cytostome is near the anterior tip. There are 2 to 4 contractile vacuoles.

The macronucleus is small and kidney-shaped.

Blepharozoum zonatum Gassovsky, 1919 measures 230 to 245 by 115 to 122 μ and has an anterior concretion vacuole. It occurs in the cecum of the horse.

Genus *BUNDLEIA* Da Cunha and Muniz, 1928

The body is ellipsoidal, with a small cytostome. There are cilia at the anterior and posterior ends, the latter being much less numerous than the former.

Bundleia postciliata (Bundle, 1895) da Cunha and Muniz, 1928 has a slightly flattened body with a sharply tapering, truncate anterior end and a truncate posterior end, and measures 30 to 56 by 17 to 32 μ. The cytopharynx is short and funnel-shaped. The macronucleus is ellipsoidal. The concretion vacuole is small and anterior. There is a small contractile vacuole. Hsiung (1930) found this species in the cecum, colon or feces of 7 out of 46 horses in Iowa.

Genus *DIDESMIS* Fiorentini, 1890

The anterior end of the body forms a neck behind the large cytostome. There are cilia at the anterior and posterior ends. The macronucleus is ellipsoidal.

Didesmis ovalis Fiorentini, 1890 is oval or rectangular and slightly flattened, with a blunt anterior end and a tapering posterior end. It measures 34 to 55 by 27 to 40 μ. The cytostome is at the middle of the anterior end, and the cytopharynx is short and funnel-shaped. There is a short neck behind the cytostome. The concretion vacuole is near the anterior end of the irregularly oval macronucleus. There are 1 or 2 contractile vacuoles. Hsiung (1930) found this species in the cecum of 16 and the colon of 6 out of 46 horses in Iowa.

D. quadrata Fiorentini, 1890 resembles *D. ovalis*, but has a deep, wide, highly refractive, longitudinal groove on the dorsal surface. It measures 50 to 90 by 33 to 68 μ and has a spindle-shaped macronucleus. Hsiung (1930) found this species in the cecum of 8 and the colon of 3 out of 46 horses in Iowa.

D. spiralis Hsiung, 1929 resembles *D. quadrata* except that it is spirally shaped. It measures 60 to 94 by 38 to 54 μ. The dorsal groove runs slightly diagonally to the longitudinal axis. The concretion vacuole contains less than 10 granules. Hsiung (1930) found this species in the cecum of 2 out of 46 horses in Iowa.

Genus *HOLOPHRYOIDES* Gassovsky, 1919

The body is ovoid and uniformly ciliated, with a comparatively large cytostome at the anterior end. The macronucleus is small and ellipsoidal. The contractile vacuole is subterminal.

Holophryoides ovalis (Fiorentini, 1890) Gassovsky, 1919 measures 95 to 140 by 65 to 90 μ. There is an accumulation of ectoplasm at the anterior part of the body. Hsiung (1930) did not find this species in Iowa horses.

Genus *PARAISOTRICHOPSIS* Gassovsky, 1919

The body is uniformly ciliated and has a spiral groove from the anterior to the posterior end.

Paraisotrichopsis composita Gassovsky, 1919 measures 43 to 56 by 31 to 40 μ, and has an elongate macronucleus. Hsiung (1930) did not find it in Iowa horses.

Genus *POLYMORPHELLA* Corliss, 1960

The body is flask-shaped, with cilia in the anterior region and a few at the caudal end. The macronucleus is disc-shaped, and the contractile vacuole terminal. The name *Polymorphella* replaces the original name, *Polymorpha*, given by

Dogiel (1929) because the latter is a homonym of the names previously given to a foraminiferan and a lepidopteran (Corliss, 1960).

Polymorphella ampulla (Dogiel, 1929) Corliss, 1960 measures 22 to 36 by 13 to 21 μ. Hsiung (1930) found it in the cecum of 3 and the colon of 1 out of 46 horses in Iowa.

Genus *PRORODONOPSIS* Gassovsky, 1919

The body is piriform and uniformly ciliated. The macronucleus is sausage-shaped. There are 3 contractile vacuoles.

Prorodonopsis coli Gassovsky, 1919 measures 55 to 67 by 38 to 45 μ. Hsiung (1930) did not find it in Iowa horses.

Genus *SULCOARCUS* Hsiung, 1935

The body is ovoid, compressed, with a short spiral groove at the anterior end. The cytostome is at the end of the groove. The cytopyge is terminal. The concretion vacuole is mid-ventral, with the contractile vacuole posterior to it. Cilia are present on the groove, mid-ventral region and posterior end.

Sulcoarcus pellucidulus Hsiung, 1935 measures 33 to 56 by 30 to 40 μ. Hsiung (1935) found it in the feces of the mule in China.

FAMILY ACINETIDAE

In this holotrichasin, suctoriorid family the tentacles are capitate and are usually arranged in groups. Endogenous budding occurs. A lorica is often present, and a stalk is present or absent.

Genus *ALLANTOSOMA* Gassovsky, 1919

The body is elongate, with 1 or more tentacles at each end, but without lorica or stalk. The macronucleus is ovoid or spherical, and the micronucleus is compact. There is 1 contractile vacuole. The cytoplasm is often filled with small spheroidal bodies.

Allantosoma intestinalis Gassovsky, 1919 has a sausage-shaped body with 3 to 12 tentacles at each end bearing distinct suckers. It measures 33 to 60 by 18 to 37 μ. The cytoplasm is filled with small, round bodies. The macronucleus is more or less spherical. Hsiung (1930) found this species in the cecum of 6 and the colon of 8 out of 46 horses in Iowa.

A. dicorniger Hsiung, 1928 has a more or less cycloid body with 1 incurved tentacle at each end, and measures 20 to 33 by 10 to 20 μ. The end of the tentacle is somewhat boot-shaped. The cytoplasm is filled with granules. The macronucleus is subspherical. Hsiung (1930) found this species in the colon of 2 out of 46 horses in Iowa.

A. brevicorniger Hsiung, 1928 has an elongate, cycloid body with 1 short, slender slightly incurved tentacle at each end. It measures 23 to 36 by 7 to 11 μ. The distal end of the tentacle is rounded. The cytoplasm is slightly granular. Hsiung (1930) found this species in the cecum of 9 out of 46 horses in Iowa.

FAMILY BLEPHAROCORYTHIDAE

In this holotrichasin, trichostomorid family, somatic ciliation is reduced to a few anterior and posterior fields, with 1 or 2 groups of anal cilia near the cytopyge and 2 or 3 distinct anterior groups. The cytostome is anteroventral, and opens into a long, ciliated cytopharynx.

Genus *BLEPHAROCORYS* Bundle, 1895

There are 3 (oral, dorsal and ventral) ciliary zones at the anterior end and 1 caudal ciliary zone. There is a deep oral groove near the anterior end.

Blepharocorys uncinata (Fiorentini, 1890) Bundle, 1895 is elongated and irregular in shape, with a slightly convex dorsal side, a slightly concave ventral side and more or less rounded ends; it measures 55 to 74 by 22 to 30 μ. A corkscrew-like anterior process which makes 2 turns projects from the anterior end and also passes thru the body dorsal to the cytopharynx, ending just behind it. There is a large, ciliated vestibule at the anterior end which leads to a cytostome opening into a ciliated cytopharynx which extends dorso-posteriad and then bends sharply ventrad and disappears at the posterior half of the body. The macronucleus is heart-shaped. There is a single posterior contractile vacuole. Hsiung (1930) found this species in the cecum of 21 and the colon of 4 out of 46 horses in Iowa.

B. valvata (Fiorentini, 1890) Bundle, 1895 is more or less elliptical and flattened bilaterally. It measures 52 to 68 by 20 to 27 μ. The vestibule is small and has a beak-like dorsal plate. The macronucleus is more or less kidney-shaped. Hsiung (1930) found this species in the cecum of 1 and the colon of 4 out of 46 horses in Iowa.

B. jubata Bundle, 1895 resembles *B. valvata,* but the dorsal plate guarding the vestibule has 2 teeth. It measures 33 to 60 by 17 to 23 μ. The cytopharynx extends backward and upward and then again turns backward. The macronucleus is more or less ovoid. Hsiung (1930) found this species in the cecum of 22 and the colon of 4 out of 46 horses in Iowa.

B. curvigula Gassovsky, 1919 also resembles *B. valvata,* but its dorsal plate is more or less rhomboid. The long cytopharynx extends backward and upward, and finally bends in a smooth, 180° curve. The macronucleus is more or less ovoid. Hsiung (1930) found this species in the colon of 12 out of 46 horses in Iowa.

B. angusta Gassovsky, 1919 resembles *B. valvata,* but is more elongate, measuring 58 to 78 by 20 to 25 μ. The dorsal plate is more or less rhomboid. The macronucleus is irregular. Hsiung (1930) found this species in the colon of 8 out of 46 horses in Iowa.

B. cardionucleata Hsiung, 1930 resembles *B. curvigula,* but its macronucleus is heart-shaped, with an anterior base and a posterior apex. It measures 48 to 62 by 17 to 23 μ. Hsiung (1930) found it in the colon of 1 out of 46 horses in Iowa.

Genus *CHARONINA* Strand, 1928

There are 2 caudal and 3 anterior ciliary zones, and an anterior knob is present on the body. This genus was originally named *Charon* by Jameson (1925), but this name is a homonym (Corliss, 1960).

Charonina equi (Hsiung, 1930) Strand, 1928 is lanceolate and measures 30 to 48 by 10 to 14 μ. The cytostome occupies nearly the whole ventral side of the anterior knob and leads to a prominent cytopharynx which extends straight down to the middle third of the body. The macronucleus is large and elongate. Hsiung (1930) found this species in the colon of 3 out of 46 horses in Iowa.

Genus *OCHOTERENAIA* Chavarria, 1933

There are 3 ciliary zones at the anterior end and 2 at the posterior end. One of the latter is borne on a caudal appendage which arises ventral to the cytopyge. There is a beak-like dorsal plate like that of *Blepharocorys.*

Ochoterenaia appendiculata Chavarria, 1933 is more or less elliptical and is flattened bilaterally. It measures 58 to 72 by 24 to 33 μ with a mean of 66 by 28 μ. The vestibule is prominent. The macronucleus is more or less kidney-shaped. Chavarria (1933a) found this species in the rectum of horses in Mexico.

FAMILY PARAISOTRICHIDAE

In this holotrichasin, trichostomorid family, somatic ciliation is complete, and there is an anterior tuft of longer cilia. The mouth is subterminal, opening just posterior to the concretion vacuole.

Genus *PARAISOTRICHA* Fiorentini, 1890

The cilia form more or less spiral longitudinal rows. The contractile vacuole is posterior.

Paraisotricha colpoidea Fiorentini, 1890 is ovoid, measures 70 to 100 by 42 to 60 μ and has 34 to 40 rows of cilia. The macronucleus is a thick, ellipsoidal disc. There is a large concretion vacuole at the anterior end. Hsiung (1930) found this species in the cecum of 21 and the colon of 6 out of 46 horses in Iowa.

P. beckeri Hsiung, 1930 resembles *P. colpoidea* but has only 11 rows of cilia. It measures 52 to 98 by 30 to 52 μ. Hsiung (1930) found it in the cecum of 8 and the colon of 1 out of 46 horses in Iowa.

P. minuta Hsiung, 1930 resembles *P. colpoidea* but has only 20 rows of cilia and measures 38 to 68 by 27 to 36 μ. Hsiung (1930) found it in the cecum of 31 and the colon of 3 out of 46 horses in Iowa.

FAMILY CYCLOPOSTHIIDAE

This spirotrichasin, entodiniorid family differs from the related Ophryoscolecidae in that its members have 2 or more bands of membranelles in addition to the adoral zone, instead of 1. Most members of this family occur in equids, but others occur in tapirs, rhinoceroses and elephants, which are related to them. One genus occurs in anthropoid apes.

Genus *CYCLOPOSTHIUM* Bundle, 1895

The body is large and elongate barrel-shaped. The cytostome is in the center of a retractile, conical elevation at the anterior end. The adoral zone of membranelles is conspicuous. There are open ring zones of membranelles near the posterior end on the dorsal and ventral sides. The pellicle is ridged. There is a club-shaped skeletal plate. A row of several contractile vacuoles runs along the band-formed macronucleus.

Cycloposthium bipalmatum (Fiorentini, 1890) Bundle, 1895 is more or less rectangular, slightly compressed laterally, with a truncate anterior end and a tapering posterior end with a tail-like structure. It measures 80 to 127 by 35 to 57 μ. A longitudinal groove and a light, linear skeletal plate are present on the left side. The macronucleus is hooked anteriorly, and the micronucleus is located near its middle. There are 4 contractile vacuoles. Hsiung (1930) found this species in the cecum of 38 and the colon of 8 out of 46 horses in Iowa.

C. dentiferum Gassovsky, 1919 measures 140 to 220 by 80 to 110 μ. It resembles *C. bipalmatum* but has a ventral dentiform projection, and the anterior end of its macronucleus is not hooked. The cuticle is not corrugated. A longitudinal groove is present on the left side, but the linear skeletal plate is quite indistinct. There are 4 to 6 contractile vacuoles. Hsiung (1930) found this species in the cecum of 16 and the colon of 2 out of 46 horses in Iowa.

C. ishikawai Gassovsky, 1919 differs from all other species of the genus in that the posterior arches of membranelles are nonretractile. It measures 230 to 280 by 110 to 130 μ. Hsiung (1930) did not find it in Iowa horses.

C. edentatum Strelkov, 1928 resembles *C. bipalmatum* but has 6 to 7 contractile vacuoles. It measures 146 to 230 by 68 to 93 μ. Hsiung (1930) found this species in the cecum of 11 and the colon of 2 out of 46 horses in Iowa.

C. piscicauda Strelkov, 1928 resembles *C. bipalmatum* but lacks both the longitudinal groove and skeletal plate on the left side. It measures 125 to 190 by

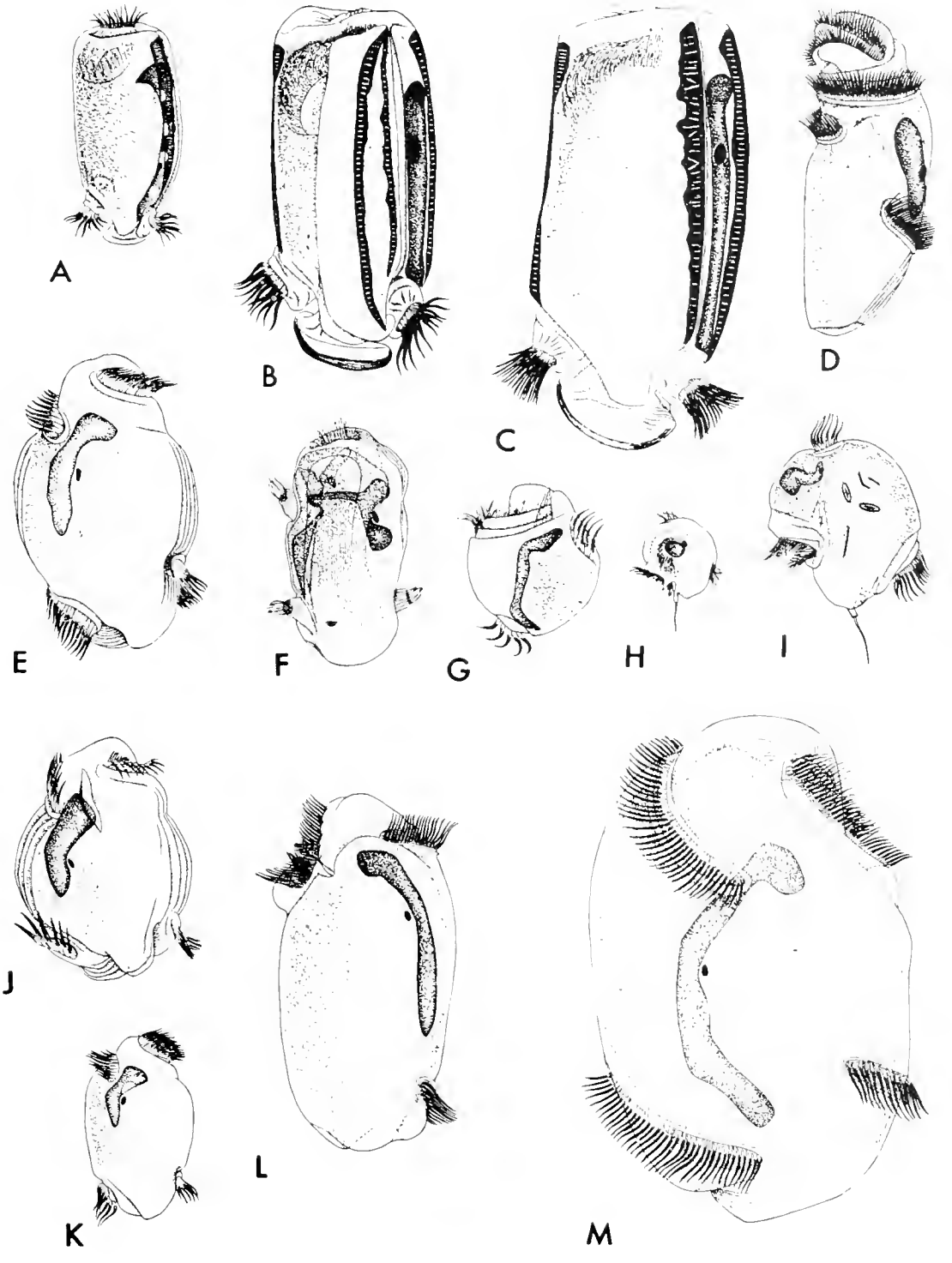

Fig. 41. Ciliates of equids. A. *Cycloposthium bipalmatum*. B. *Cycloposthium scutigerum*. C. *Cycloposthium edentatum*. D. *Spirodinium equi*. E. *Tetratoxum unifasciculatum*. F. *Tripalmaria dogieli*. G. *Triadinium galea*. H. *Triadinium minimum*. I. *Triadinium caudatum*. J. *Tetratoxum excavatum*. K. *Tetratoxum parvum*. L. *Ditoxum funinucleum*. M. *Cochliatoxum periachtum*. X 340. (From Hsiung, 1930, in Iowa State College Journal of Science, published by Iowa State Univ. Press)

44 to 80 μ. It has 4 or 5 contractile vacuoles. Its posterior end forms a tail resembling that of a fish. Hsiung (1930) did not find this species in Iowa horses.

C. scutigerum Strelkov, 1928 differs from *C. bipalmatum* in having a shield-like skeletal plate interrupted by 2 longitudinal grooves on the left side instead of a simple, narrow plate. It measures 132 to 210 by 63 to 90 μ and has 5 or 6 contractile vacuoles. Hsiung (1930) found this species in the cecum of 24 and the colon of 4 out of 46 horses in Iowa.

C. affinae Strelkov, 1928 differs from *C. bipalmatum* in having a heavy skeletal plate and in that the micronucleus is near the anterior end of the macronucleus. It measures 92 to 141 by 45 to 58 μ. Hsiung (1930) found this species in the cecum of 3 and the colon of 1 out of 46 horses in Iowa.

C. corrugatum Hsiung, 1930 measures 135 to 195 by 70 to 112 μ. It has a ventral dentiform projection, and its cuticle is corrugated. The anterior end of its macronucleus is not hooked. The linear skeletal plate is quite indistinct. There are 4 or 5 contractile vacuoles. Hsiung (1930) found this species in the cecum of 7 and the colon of 1 out of 46 horses in Iowa.

Genus *SPIRODINIUM* Fiorentini, 1890

The body is elongate and more or less fusiform, with an adoral zone of membranelles at the anterior end. An anterior ciliary zone encircles the body at least once, and a posterior ciliary arch spirals half-way around the body. There is a dorsal cavity of unknown function lined with stiff rods.

Spirodinium equi Fiorentini, 1890 measures 77 to 180 by 30 to 74 μ. Its macronucleus is elongated, with rounded ends. There is a large contractile vacuole just back of the anterior membranelles. Hsiung (1930) found this species in the colon of 3 out of 46 horses in Iowa.

Genus *TRIADINIUM* Fiorentini, 1890

The body is more or less helmet-shaped and compressed, with an adoral zone of membranelles at the anterior end. There are ventral and dorsal posterior zones of membranelles. There may or may not be a caudal projection.

Triadinium caudatum Fiorentini, 1890 measures 50 to 105 by 36 to 85 μ and has a long, slender tail. The macronucleus is bent like a question-mark. There is a single contractile vacuole. Hsiung (1930) found this species in the colon of 3 out of 46 horses in Iowa.

T. galea Gassovsky, 1919 measures 58 to 88 by 50 to 70 μ and lacks a tail. It has a long macronucleus running longitudinally along the left surface, and 2 contractile vacuoles. Hsiung (1930) found this species in the colon of 3 out of 46 horses in Iowa.

T. minimum Gassovsky, 1919 measures 32 to 50 by 31 to 42 μ and has a slender tail. The macronucleus is ellipsoidal. There is a single contractile vacuole. Hsiung (1930) found this species in the colon of 2 out of 46 horses in Iowa.

Genus *TETRATOXUM* Gassovsky, 1919

The body is slightly compressed and has 2 anterior and 2 posterior zones of membranelles.

Tetratoxum unifasciculatum (Fiorentini, 1890) Gassovsky, 1919 measures 104 to 168 by 62 to 100 μ. It is irregularly elliptical, with both ends rounded, and has 7 to 9 longitudinal, cuticular ridges on both the dorsal and ventral surfaces of the body. Lateral cuticular extensions at the posterior end form 2 caudal sheaths. The macronucleus is elongate, with a short hook at the anterior end. There is a large contractile vacuole under its curvature. Hsiung (1930) found this species in the colon of 2 out of 46 horses in Iowa.

T. excavatum Hsiung, 1930 measures 95 to 135 by 55 to 90 μ. It differs from *T. unifasciculatum* in having a deep elliptical excavation covered by a flap of cuticle at its anterior end, and its cuticular ridges are more prominent and the adjacent ones further apart. Hsiung (1930) found this species in the colon of 1 out of 46 horses in Iowa.

T. parvum Hsiung, 1930 measures 67 to 98 by 39 to 52 μ. It differs from the other 2 species in lacking longitudinal cuticular ridges. Hsiung (1930) found this species in the colon of 1 out of 46 horses in Iowa.

Genus *TRIPALMARIA* Gassovsky, 1919

There is an adoral zone of membranelles at the anterior end and also 2 dorsal and 1 ventroposterior tuft-formed zones of membranelles. The macronucleus is shaped like an inverted U. A synonym of this genus is *Tricaudalia* Buisson, 1923.

Tripalmaria dogieli Gassovsky, 1919 measures 77 to 123 by 47 to 62 μ. Beneath the right side it has skeletal plates forming a horseshoe with its open end directed posteriad. Hsiung (1930) found this species in the colon of 3 out of 46 horses in Iowa.

Genus *COCHLIATOXUM* Gassovsky, 1919

There is an adoral zone of membranelles at the anterior end and also 1 anterodorsal, 1 posterodorsal and 1 posteroventral zone of membranelles. The anterior end of the macronucleus is curved.

Cochliatoxum periachtum Gassovsky, 1919 is more or less cylindrical, with both ends rounded, and measures 210 to 370 by 130 to 210 μ. There is a contractile vacuole. Hsiung (1930) found this species in the colon of 1 out of 46 horses in Iowa.

Genus *DITOXUM* Gassovsky, 1919

There is a large adoral zone of membranelles near the anterior end and also anterodorsal and posterodorsal zones of membranelles. The macronucleus is curved and club-shaped.

Ditoxum funinucleum Gassovsky, 1919 is elliptical with both ends rounded, slightly flattened bilaterally, and measures 135 to 203 by 70 to 101 μ. It has a single contractile vacuole. Hsiung (1930) found this species in the colon of 2 out of 46 horses in Iowa.

C. OTHER CILIATES

FAMILY BALANTIDIIDAE

This holotrichasin, trichostomorid family was once considered to belong in the Heterotrichorida; Faure-Fremiet (1955) showed its proper position. Cilia are arranged in longitudinal rows over the whole body. The peristome forms a pouch with a triangular opening containing a short adoral zone of membranelles. There is no concretion vacuole.

Genus *BALANTIDIUM* Claparède and Lachmann, 1858

The body is ovoid, ellipsoid to subcylindrical. The macronucleus is elongated. There is a single micronucleus. The contractile vacuole and cytopyge are terminal.

Many species of *Balantidium* have been named, based on the host in which they occur and on the size and shape of their body and macronucleus (cf. Hegner, 1934; Kudo and Meglitsch, 1938). However, many of these are probably not valid. For instance, McDonald (1922) separated *B. suis* from *B. coli*, both from swine, on the basis of its slenderer body and straighter macronucleus, but Levine (1940, 1940a) showed that *Balantidium* from swine changed dimensions upon

cultivation, and that a single strain could resemble *B. coli* if it was full-fed and *B. suis* if it was starved. Lamy and Roux (1950) found both *suis* and *coli* forms in clone cultures started from single organisms and considered the *suis* forms to be conjugants and the *coli* forms trophozoites. Auerbach (1953) concluded from his cytological and cultural studies that the 2 forms were not different species.

BALANTIDIUM COLI
(MALMSTEN, 1857)
STEIN, 1862

Synonym: *Balantidium suis.*

Disease: Balantidiosis, balantidial dysentery.

Hosts: Pig, man, chimpanzee, orang-utan, rhesus monkey, cynomolgus monkey, other macaques, rarely dog and rat.

Location: Cecum, colon.

Geographic Distribution: Worldwide.

Prevalence: *B. coli* is extremely common in swine, having been reported in 21 to 100% of them in various surveys (Kennedy and Stewart, 1957), but the lower figures may reflect the examination technic rather than the true incidence (de Carneri, 1958). It is much less common in man, its incidence in 12 surveys comprising 24,837 fecal specimens thruout the world being 0.77% according to Belding (1952). Shookhoff (1951) found it in 0.6% of approximately 3000 Puerto Rican patients. Swartzwelder (1950) described 16 human cases in New Orleans; these represented more than 1/4 of all the available reports in the United States.

B. coli occurs in primates other than man, but is not common. Habermann and Williams (1957) found it at postmortem examination of 5 of 615 rhesus monkeys obtained by the National Institutes of Health from various importers; the animals had died of various diseases. They did not find it in 93 cynomolgus monkeys (*Macaca philippinensis*). Cockburn (1948) described an epidemic of enteritis among the larger primates at the London Zoo which appeared to be due to *Balantidium*. Benson, Fremming and Young (1955) reported it in captive chimpanzees.

Balantidium has been seen on rare occasions in the dog. Dikmans (1948) reported a case in a dog in North Carolina. Bailey and Williams (1949) reported one from Tennessee, and Hayes and Jordan (1956) reported one from Georgia.

Bogdanovich (1955) found *B. coli* in 6 out of 150 Norway rats in a Russian slaughter house.

"*Balantidium coli*" has been reported from the zebu (Cooper and Gulati, 1926) and water buffalo (Priestley, 1944), but Lubinsky (1957) considered it to be a late exconjugant of *Buxtonella sulcata*, which he had found commonly in the zebu. The longitudinal furrow is inconspicuous in this stage and is easily overlooked.

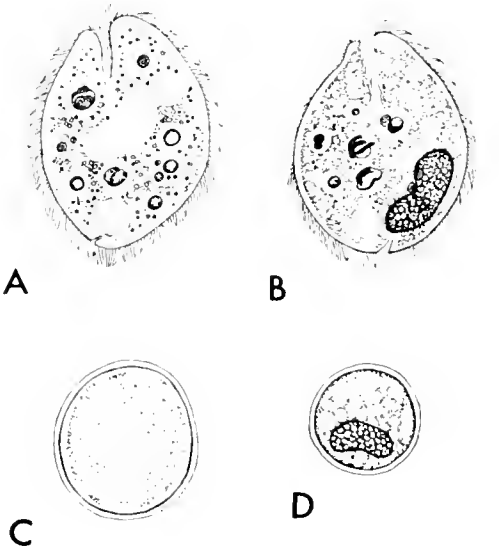

Fig. 42. *Balantidium coli*. A. Living trophozoite. B. Stained trophozoite. C. Fresh cyst. D. Stained cyst. X 450. (From Kudo, R. R., PROTOZOOLOGY 4th Ed., 1954. Courtesy of Charles C Thomas, Publisher, Springfield, Illinois)

Morphology: The trophozoites are ovoid, with a subterminal cytostome at the smaller end, and measure 30 to 150 by 25

to 120 μ. The cytopyge is near the posterior end. The macronucleus is sausage- or kidney-shaped, and the micronucleus lies near the center of 1 side. There are 2 contractile vacuoles, 1 terminal and the other near the center of the body. There are many food vacuoles containing starch grains, cell fragments, bacteria, erythrocytes, etc.; starch is the most important food. The surface is covered by slightly oblique longitudinal rows of cilia.

Krascheninnikow and Wenrich (1958) studied the morphology and division of *B. coli* in detail. Auerbach (1953), Sen Gupta and Ray (1955) and Lom (1955) reported on cytologic and cytochemical studies.

The cysts are spherical to ovoid and measure 40 to 60 μ in diameter. They are slightly yellowish or greenish, with hyaline cytoplasm. The cyst wall is composed of 2 membranes.

Life Cycle: *B. coli* reproduces by transverse binary fission (Krascheninnikow and Wenrich, 1958). Conjugation also takes place (Nelson, 1934; Svensson, 1955), and resistant cysts are formed.

Pathogenesis: In the pig, *Balantidium coli* is ordinarily a commensal in the lumen of the large intestine, where it lives on starch, other ingesta and bacteria. It does not seem able to penetrate the intact intestinal mucosa by itself. Enormous numbers of *Balantidium* may be found in the lumen of the cecum of pigs with normal cecal mucosae. However, once some other organism or condition has initiated a lesion, *Balantidium* may be a secondary invader and may be found deep in the ulcer. It produces hyaluronidase (Tempelis and Lysenko, 1957), which might help it to enlarge the lesions by attacking the ground substance between the cells, altho it would not help it to initiate the lesions.

Balantidium is pathogenic in man and other primates. It causes diarrhea or dysentery, and produces undermining lesions similar to those caused by *Entamoeba histolytica*. The protozoa may be found down to the muscularis mucosae, the ulcers are infiltrated with round cells, and coagulation necrosis and hemorrhagic areas may be present. The protozoa often occur in nests within the tissues or even in the capillaries, lymph ducts and neighboring lymph nodes. The lesions in the pig and other animals are similar. The disease in man has been reviewed by Swartzwelder (1950), Shookhoff (1951) and Arean and Koppisch (1956).

The infected dog described by Dikmans (1948) died of a severe diarrheal disease, and ulcers were found in the intestine at necropsy. In the case reported by Bailey and Williams (1949), the animal had dysentery for several days beginning several days after it ate the intestines of a hog, but it recovered.

Lesions in the ceca of some naturally infected rats were described by Bogdanovich (1955).

Bionomics and Epidemiology: *Balantidium* may be transmitted by ingestion of either cysts or trophozoites. The cysts are more resistant to environmental conditions. Svensson (1955) found that the trophozoites of different strains of *B. coli* from the pig differ in their resistance to heat and cooling. Most strains survive heating to 47° C for more than 15 minutes but survive at room temperature for less than 3 days. A cold-resistant strain survived heating for only 5 to 10 minutes, but remained alive at room temperature for 5 days or more. *B. coli* from man is similar to the latter. The cysts may remain alive for weeks in pig feces if they do not dry out.

The pig is the usual source of infection for man. Contact with swine has been noted in more than half the human cases reported (Aréan and Koppisch, 1956), and Shookhoof (1951) obtained a history of close contact with pigs in practically all the cases he observed in Puerto Rico.

Chimpanzees and other primates appear to have their own infection pool.

Diagnosis: *Balantidium* can be easily recognized by microscopic examination of

intestinal contents or by histologic examination of intestinal lesions.

Cultivation: *B. coli* was first cultivated by Barret and Yarbrough (1922) in a medium consisting of 1 part inactivated serum and 16 parts of 0.5% sodium chloride solution. It has since been cultivated by many workers. Schumaker (1931) and Levine (1940) used a medium consisting of 9 parts of Ringer's solution and 1 part of horse serum plus about 0.007 g rice starch per tube containing 10 ml of the medium. Tempelis and Lysenko (1957) used an agar slant of Difco *Entamoeba histolytica* medium overlaid with Balamuth's egg yolk infusion plus rice starch, 500 units per ml of streptomycin and 250 units per ml of penicillin; this medium was used successfully to establish clone cultures from single microorganisms.

Treatment: No treatment is necessary in swine. Carbarsone has been used in man. Young and Burrows (1943) administered 0.25 to 0.5 g twice a day for 10 days. However, DeLanney (1943) found that carbarsone did not eliminate all the parasites and recommended 2.1 g diiodohydroxyquin (diodoquin) daily for 20 days. Swartzwelder (1950) recommended diodoquin if carbarsone failed. More recently, chlortetracycline and oxytetracycline have been found effective (Areán and Koppisch, 1956; Neghme *et al.*, 1951).

Benson, Fremming and Young (1955) treated chimpanzees with 250 mg carbarsone daily for 10 days, concealing the drug in fruit or fruit juices.

Prevention and Control: Sanitary measures designed to prevent ingestion of cysts or feces should prevent balantidial infections.

FAMILY TETRAHYMENIDAE

In this holotrichasin, hymenostomorid family, the buccal ciliature is composed of 3 membranelles which lie to the left in the buccal cavity and a fourth, paroral membrane extending along its right margin. One or more stomatogenous rows of cilia end at the posterior margin of the buccal pouch.

Genus *TETRAHYMENA* Furgason, 1940

The body is piriform and uniformly ciliated with 17 to 42 rows of cilia. The piriform cytostome is near the anterior end. There is a single contractile vacuole.

Tetrahymena pyriformis (Ehrenberg, 1830) Lwoff, 1947 (syn., *T. geleii*) measures 40 to 60 by 15 to 30μ. It is extremely popular in protozoological research. According to Corliss (1954, 1957a), over 500 papers had been written on it and other members of the genus up to 1954, and another 186 papers were published in 1954 thru 1956. Altho *T. pyriformis* is normally free-living, it may on rare occasions be a facultative parasite. Knight and McDougle (1944) found it in the digestive tract, infraorbital sinuses and serous material under the eyelids of chickens in Missouri. It was found only in birds with a vitamin A deficiency.

Thompson (1958) infected chicken embryos with *T. pyriformis*, *T. corlissi* and *T. vorax*. He also infected guppies (*Lebistes reticulatus*) and tadpoles (*Rana palustris*) thru artificially produced wounds with *T. corlissi* but not with the other species. Various adult and larval insects proved excellent hosts, the protozoa teeming in the hemolymph of some of them.

D. COPROPHILIC CILIATES

A number of ciliates which live in water or soil may contaminate feces and develop coprophilically. They are common in old feces, especially if it has been in contact with the ground, but may also appear in feces taken directly from an animal. Cysts ingested by livestock in feeding or drinking may pass thru the intestinal tract unharmed, and trophozoites may emerge and develop as the feces stands. Horse and ruminant feces which have been cultured for nematode larvae often contain large numbers of small ciliates. Some of these are probably *Colpidium*, *Chilodonella* and *Cyclidium*.

Nyctotherus faba Schaudinn, 1899 has been found in human feces on occasion (Wichterman, 1938). It belongs to the heterotrichorid family Plagiotomidae. Its body is reniform, covered with cilia, and 26 to 28 μ long. The peristome begins at the anterior end, turns slightly to the right and ends in the cytostome at the middle of the body. The cytopharynx is a long tube and contains an undulating membrane. The macronucleus is about the middle of the body. It is spherical, and its chromatin is arranged in 4 or 5 large, solid bodies on the nuclear membrane, while the remainder of the nucleus is chromatin-free.

Noble (1958) found that a *Nyctotherus*-like ciliate about 15 to 30 μ long appeared in fecal samples from Wyoming sheep and elk after storage at 4° C for about 30 days. A smaller ciliate about 10 to 12 μ long also appeared in the elk feces at about the same time. The smaller ciliates persisted for a few weeks and the *Nyctotherus*-like ones for about twice as long.

Balantiophorus minutus Schewiakoff, 1893 (syn., *Balantidium minutum* Schaudinn) occurs occasionally in contaminated human feces (Watson, 1940, 1945, 1945a). It belongs to the holotrichorid family Pleuronemidae. It is ovoid, with the narrow end anterior and with the anterior end bent ventrad, giving the ventral surface a hollowed appearance. It measures 12 to 54 by 7 to 33 μ, but is usually 25 to 45 μ long. The peristome is in the middle of the anterior half of the body. The adoral zone of membranelles on its left, posterior and right borders forms a sac-like structure which is conspicuous when expanded but which can be retracted into the peristome and become invisible. The cytopharynx is funnel-shaped. The body is uniformly covered by 12 rows of setiform cilia, of which only 6 extend anterior to the peristome. The macronucleus is central and ellipsoidal. There is a posterior contractile vacuole.

The taxonomy and bionomics of these and other coprophilic protozoa have been reviewed by Alexeieff (1929) and Watson (1946). The latter listed 51 species of flagellates, 18 of amoebae and 18 of ciliates which have been found in feces, but many of these need further study.

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Chapter 14

LABORATORY DIAGNOSIS OF PROTOZOAN INFECTIONS

Many different technics have been used for the laboratory diagnosis of protozoan infections and for the study of parasitic protozoa. Only the commonest and those which have been found most useful in the author's laboratory are given here. Other routine and specialized technics are given by Craig (1948), Hoare (1949), Kirby (1950), and various textbooks of human parasitology.

Some of these technics are useful not only for protozoa but also for helminth eggs or larvae. If so, their value for these purposes is mentioned.

DIRECT MICROSCOPIC EXAMINATION OF WET FECAL SMEARS

Place a drop of physiological salt solution on a microscope slide. Take up a small amount of feces on the end of a toothpick and mix thoroughly with the salt solution. Do not make too heavy a suspension, or it will be impossible to see objects clearly under the microscope. An emulsion thru which newsprint can be read is about right. Place a coverslip on the drop. Examine under the low and high dry powers of the microscope.

Flagellates and ciliates can be seen moving about actively. Amoebae may move sluggishly or may remain still. Oocysts of coccidia and helminth eggs can be recognized from their shape and size. Many other objects will be seen, some of which may be mistaken for protozoan parasites. These include bacteria, yeasts, fungus spores, the fungus *Blastocystis*, pollen grains, undigested food particles such as starch grains and plant fibers, and ingested pseudoparasites such as grain mites or coccidian oocysts of animals which have been eaten by or have defecated in the feed of the animals under examination. In cases of enteritis, red or white blood cells or epithelial cells may be present.

In examining preparations under the microscope, move the slide systematically back and forth or up and down in order to bring every part of the preparation into view.

Iodine Staining. In order to bring out certain details which are not visible in the living protozoon, wet smears may be stained with iodine. Prepare a fecal suspension slightly heavier than that described above, and mix it with an equal amount of D'Antoni's aqueous iodine solution or of 1 part of Lugol's solution diluted with 4 parts of distilled water.

DIRECT MICROSCOPIC EXAMINATION OF INTESTINAL MUCOSA

This technic can be used only in animals which have been killed and have had their intestinal tracts opened. It permits a greater amount of material to be examined on a single slide than does the direct examination of diluted feces. It can be used to find the intracellular and extracellular stages of coccidia, other protozoa, small nematodes such as *Strongyloides* and *Capillaria*, small trematodes, cestodes or cestode scolices, and schistosome eggs.

Make a rather deep scraping of the suspected intestinal mucosa with a scalpel, toothpick or similar instrument, or even with the end of a slide. Place the material thus obtained on a microscope slide and cover with a coverslip. Press the coverslip down if necessary to flatten out the preparation and make it thin enough to see thru.

To search for *Trichomonas*, *Giardia*, *Hexamita* and other motile flagellates, mix a little physiological salt solution with the scraping before placing the coverslip on it.

MICROSCOPIC DIAGNOSIS OF *TRITRICHOMONAS FOETUS* INFECTIONS

In heavy infections of female cattle, *T. foetus* can be found by direct microscopic examination of mucus or exudate from the vagina or uterus. In aborted fetuses it can be found in the amniotic or allantoic fluid, fetal membranes, placenta, fetus stomach contents, oral fluid or other fetal tissues; it occurs most commonly in the stomach contents and the material around the base of the tongue. In bulls, it can be found in the sheath cavity.

Clean the external genitalia thoroughly before taking samples in order to avoid contamination with intestinal or coprophilic protozoa. Take samples from the vagina by introducing about 10 ml of physiological salt with a bulbed dose syringe and washing it back and forth several times by squeezing the bulb repeatedly. Take samples from the preputial cavity of bulls in the same way, using a long, bulbed pipette or syringe, or introduce a cotton swab into the cavity and rub it around to obtain a sample of exudate; in the latter case, wash off the swab in physiological salt solution.

Allow the washings to stand 1 to 3 hours or centrifuge them before examination. Place a drop of the sediment on a slide, cover with a coverslip, and examine under the microscope.

If trichomonads cannot be found on direct microscopic examination, inoculate some of the washings into CPLM, PGPS or Diamond's medium, and examine after 1, 2 and 4 days' incubation at 37° C.

SPORULATION OF COCCIDIAN OOCYSTS

In order to identify coccidia, it is often necessary to allow the oocysts to sporulate (i.e., to develop to the infective stage). To permit this, mix feces containing the coccidia with several volumes of 2.5% potassium bichromate solution and place the mixture in a thin layer in a petri dish. The potassium bichromate prevents bacteria from destroying the oocysts. Oxygen is necessary for the oocysts to develop, so the layer of fluid should never be more than a few millimeters thick. In most species, sporocysts and sporozoites form in a few days, but it is well to allow development to proceed for a week (or,

for a few species, even longer). If it is not desired to study the sporulated oocysts immediately, the fecal suspension can be transferred to a bottle and stored in the refrigerator. The oocysts will remain alive for several months or, in some species, as long as a year.

It is best to sporulate coccidian oocysts before they have been subjected to refrigeration, since in some species (apparently a minority), refrigeration of the unsporulated oocysts prevents subsequent sporulation altho it does not harm sporulated oocysts.

MIF (MERTHIOLATE-IODINE-FORMALDEHYDE) STAIN-PRESERVATION TECHNIC

This technic was first introduced by Sapero, Lawless and Strome (1951) and improved by Sapero and Lawless (1953). It was designed especially to permit identification of human protozoan trophozoites and cysts, but can also be used for helminth eggs and for parasites of domestic animals. It is simple and relatively cheap, permits rapid (almost immediate) wet-fixed staining of the smears, and preserves the parasites so that feces can be collected in the field or by untrained persons and shipped to the laboratory for later diagnosis. There is no appreciable loss or deterioration of parasites or cellular exudates for 6 months or more.

A. Direct Examination Technic for Fresh Fecal Specimens.

1. The MIF stain is composed of tincture of 1:1000 merthiolate No. 99 (Lilly), Lugol's solution (5%) and 40% formaldehyde solution (USP). Since Lugol's solution is unstable, it should be freshly prepared every 3 weeks, and the amount used should be varied with its age. The following amounts (in ml) are recommended:

	First Week	Second Week	Third Week
Lugol's solution	10.0	12.5	15.0
Formaldehyde solution	12.5	12.5	12.5
Tincture of merthiolate	77.5	75.0	72.5

2. Place 1 ml of the stain (sufficient for 25 to 30 fecal smears) in a Kahn tube. Place some distilled water in a second tube. Put a small caliber medicine dropper in each tube.
3. Place 1 drop of distilled water at 1 end of a slide. Add a drop of MIF stain. Mix.
4. Add a small amount of feces and mix. Do not use too much feces, or fixation and staining will be poor. The finished wet smear should be thin enough so that the slide can be tipped on edge without the coverslip sliding.
5. Add a coverslip and examine at once. If it is desired to examine the slide later, ring it with petrolatum to keep the preparation from drying out.

B. Collection and Preservation of Fecal Specimens in the Field for Subsequent Examination in the Laboratory.

1. Prepare the following stock MF solution:

Tincture of merthiolate	200 ml
Formaldehyde solution	25 ml
Glycerol	5 ml
Distilled water	250 ml

Store in a brown bottle

2. Measure 2.35 ml MF solution into a standard Kahn tube and stopper with a cork.
3. Measure 0.15 ml of 5% Lugol's solution into another Kahn tube and close with a rubber stopper. (Or keep the Lugol's solution in a bottle, and add the proper amount to the MF solution just before adding the feces in step 4 below.)
4. At the time the fecal sample is collected, pour the MF solution into the Lugol's solution. Within a few seconds, add an amount of feces equal to 2 medium-sized peas (about 0.25 g), and mix thoroughly with an applicator stick. Do not use too much feces. Stopper the tube and set aside for future examination.
5. To examine, draw off a drop of mixed supernatant fluid and feces from the top of the sedimented layer with a medicine dropper and place on a slide. Mix thoroughly, crushing any large particles. Add a coverslip and examine.

PERMANENT FIXING AND STAINING TECHNICS

It is often desirable to make permanent preparations of fecal smears or to make hematoxylin-stained slides for detailed study. For this purpose, smears must first be fixed, i.e., the protozoa must be killed by the action of a chemical or mixture of chemicals which will preserve their structures as nearly as possible in the same form as in life.

Many different technics are used for fixing and staining tissues, cells and small organisms. Those given below are especially suitable for protozoa. The standard hematoxylin and eosin stain used routinely for tissue sections is also valuable for protozoa in tissues, but it is so well known that it is not described here. For further information on fixing, sectioning, staining and mounting technics, any text or reference book on microscopic technic may be consulted.

FIXATION

Schaudinn's fluid is probably the best all-round fixative for intestinal protozoa, and it also serves well for other forms. Smears may be made on slides and stained in Coplin jars, or they may be made on coverslips and stained in Columbia jars. The latter method has the advantages that smaller amounts of reagents are necessary, a neater preparation is obtained (since there is no possibility of a portion of the smear extending beyond the coverslip), and in the completed slide the mounting medium is beneath the smear rather than above it, so that the microscope objective can come closer to the smear. This factor may be of importance when the oil immersion objective is used. Coverslips are fragile, however, and greater care must be exercised in handling them than in handling slides.

Clean the coverslips by dipping them in 95% alcohol, and dry them with a clean cloth before use. Be careful to handle them only by the edges in order not to leave fingerprints.

Place a tiny drop of albumen fixative in the center of the coverslip (or slide) and smear it over the surface with the little finger. (The finger should previously have been cleaned and rid of its oil by dipping it in 95% alcohol and wiping it with a clean cloth.) Albumen fixative is used to make the feces adhere to the glass.

Take up a small amount of feces on a toothpick (preferably a round, smooth one) and spread as evenly as possible in a very thin layer over the surface of the coverslip. Do not allow it to dry. Drop immediately into a Columbia jar containing Schaudinn's fluid at room temperature or 37° C. Allow to remain about 10 minutes and then transfer to 70% alcohol.

In some cases it may be necessary to mix the feces with a little physiological salt solution in order to make it thin enough to spread well. In other cases the feces are so fluid that if the coverslips are dropped edgewise into the fixative, all the material will come off. To prevent this, place the fixing solution in a small, flat vessel such as a petri dish, and place the coverslip face down on its surface. After a few seconds it can be transferred to a Columbia jar.

After fixation, wash the smear in 2 changes of 70% alcohol for at least 5 minutes each. Then transfer to 70% alcohol containing enough iodine to give it a port wine color. Allow to remain at least 10 minutes (preferably longer). This treatment takes out the excess mercuric chloride which may otherwise form crystals in the preparation. Then transfer to fresh 70% alcohol. Fixed material may be kept in 70% alcohol indefinitely without injury.

STAINING WITH HEIDENHAIN'S HEMATOXYLIN

In order to bring out many structures of organisms it is necessary to color them with a dye or dyes. The best and most commonly used dye employed in parasitologic and histologic work is hematoxylin, which is extracted from logwood. Hematoxylin alone has very poor staining

properties, and a mordant must be employed to make it effective. Many different formulas have been used for hematoxylin staining solutions. In some, the mordant is mixed with the hematoxylin, while in others it is used separately. Many different compounds are used as mordants, the great majority being salts of heavy metals such as iron, lead, copper, cobalt, tungsten and molybdenum. One of the best hematoxylins is Heidenhain's iron-hematoxylin. A modification of this technic is given below. Starting with the smears in 70% alcohol after passing thru iodine, the staining schedule is:

50% alcohol	5 minutes
30% alcohol	5 minutes
Distilled water	5 minutes
2% aqueous iron alum	1 hour
Distilled water	1 minute
0.5% aqueous hematoxylin	2 hours
Distilled water	Rinse
Saturated aqueous picric acid	Destain until the structures assume the proper intensity of color. This process should be controlled by microscopic examination at intervals. Ten minutes is usually good for intestinal amoebae, but a longer time is necessary for large protozoa such as *Balantidium*.
Distilled water	Rinse--2 changes
Tap water	Until all picric acid has come out of the smear. Change the water at intervals.
30% alcohol	5 minutes
50% alcohol	5 minutes
70% alcohol	5 minutes

Gradual changes in alcohol concentration are used in all staining and dehydration procedures to avoid distortion of tissues. Hematoxylin-stained smears and sections can be kept in 70% alcohol indefinitely.

In the classical Heidenhain's hematoxylin staining procedure, the stained smears are destained with iron alum. In the above procedure, saturated aqueous picric acid is used instead; this requires a minimum of observation (usually none) during the destaining process, and the resultant stain is dark blue instead of brownish black as with iron alum.

If desired, longer mordanting and staining times can be used. The smears can be mordanted for 2 hours and stained for 4 hours, or they can be mordanted for 4 hours and stained overnight. These give a little more precise staining, but not enough to make them worthwhile for routine purposes.

COUNTERSTAINING

If desired, the smears can be counterstained with eosin Y. However, this has a tendency to obscure fine nuclear detail somewhat. To counterstain the smears, transfer them from 70% alcohol to 0.5% solution of eosin Y in 90% alcohol. The pH of this solution should be brought to 5.4 to 5.6 by adding 4.0 ml of 0.1 N HCl per 100 ml. The acidified solution will not keep more than 10 days to 2 weeks. After that its pH will become too high for satisfactory use. Stain for 45 seconds to 3 minutes. Transfer to 95% alcohol to wash out excess dye and then proceed as directed below.

MOUNTING

Permanent slides are mounted in a medium which, quite fluid at first, later becomes hard. Most mounting media are immiscible with water, and many with alcohol. Hence, before mounting, all water and alcohol must be removed from the smears. This cannot be done simply by allowing the smears to dry, for such dehydration in air would ruin the preparations by distorting the protozoa. Mounting media which have been employed include natural resins such as Canada balsam and damar, and synthetic resins such as euparal, naphrax, permount and clarite.

Starting with stained coverslips in 70% alcohol, pass them thru the following solutions:

95% alcohol	5 minutes
100% alcohol	5 minutes
100% alcohol	5 minutes
Toluene	5 minutes
Toluene	5 minutes

Mount in permount: Place a drop of permount on a clean slide, place the coverslip slantingly, smear side down, alongside the drop, and gently lay it down on the drop, taking care to prevent air bubbles from forming.

Neutral xylene may be used in place of toluene, altho it hardens the tissues more. Neutral balsam or other resinous mounting media may be used in place of permount. Neutralize the xylene and balsam by placing marble chips in their containers. If this is not done, the stains will fade more or less after months to years.

FEULGEN STAIN

The Feulgen nucleal stain, which is used for the detection of deoxyribonucleic acid (DNA), is essentially a modification of the Schiff reaction for aldehydes. When DNA is hydrolyzed by hydrochloric acid, aldehyde-like substances are formed which, when treated with colorless fuchsin sulfite, stain a purplish red. Whether the reaction is limited to DNA is doubtful, but at any rate, when properly carried out, the Feulgen technic produces a preparation in which only chromatin is stained.

Not all samples of basic fuchsin are satisfactory for the Feulgen stain. Hence, care must be taken to use dye from a batch which has been found satisfactory and which has been certified as such by the Biological Stain Commission.

1. Fix material to be stained by this method for 24 hours in a saturated solution of mercuric chloride containing 2% acetic acid.
2. Wash in running water, and pass thru 30%, 50%, and 70% alcohol. Do not treat with iodine.
3. Cut sections in the usual manner.
4. Before staining, leave smears and sections in 95% alcohol 48 hours to remove "plasmalogen" substances which may take the stain.
5. To stain, run down thru the alcohols to distilled water, and place the smears or sections in 1 N HCl at 60° C for 4 minutes.
6. Wash in cold 1 N HCl, then rinse with distilled water.
7. Transfer to the decolorized fuchsin solution, and stain 1 to 3 hours.
8. Wash thoroughly in water containing a little sodium bisulfite plus a few drops of HCl.
9. Wash in distilled water.
10. Dehydrate by passing up thru the alcohols as described above, clear, and mount in permount.

BODIAN SILVER IMPREGNATION TECHNIC

This method is superior to ordinary stains for demonstration of flagella and other diagnostic structures of flagellates. The technic given below is essentially that described by Honigberg (1947). Not all batches of protargol are equally good for this stain, and care must be taken to use a sample which has been tested and found satisfactory.

1. Fix in Hollande's or Bouin's solutions for 10 minutes.
2. Wash in 50% alcohol.
3. Transfer to 30% alcohol and then to distilled water.
4. Bleach in 0.5% aqueous potassium permanganate for 5 minutes.
5. Wash in distilled water.
6. Bleach in 5% aqueous oxalic acid for 5 minutes.
7. Wash several times in distilled water.
8. Place in freshly prepared 1% aqueous protargol solution. (To prepare this solution, place the proper amount of distilled water in a beaker and scatter the protargol powder on its surface; do not stir, heat or disturb the vessel until the protargol has dissolved.)
9. Keep copper wire or thin copper sheeting in the vessel thruout the staining process. Use 5 g copper per 100 ml protargol solution. Columbia jars contain 10 ml of solution. If they are used, it is convenient to place a coil of copper wire weighing 0.5 g in the bottom of each jar before adding the protargol.
10. Stain for 1 to 2 days at room temperature or 37° C in the protargol-copper solution. The staining time and temperature will depend on the material being stained and the final intensity desired. If staining is continued for more than a day, transfer to fresh protargol solution containing fresh copper for the second day.

11. Wash in distilled water.
12. Place in a solution of 1% hydroquinone in 5% aqueous sodium sulfite for 5 to 10 minutes to reduce the silver.
13. Wash several times in distilled water.
14. Place in 1% (or more dilute) aqueous gold chloride for 4 to 5 minutes.
15. Wash in distilled water.
16. Place in 2% aqueous oxalic acid for 2 to 5 minutes until a purplish color appears.
17. Wash several times in distilled water.
18. Place in 5% sodium thiosulfate for 5 to 10 minutes.
19. Wash several times in distilled water.
20. Pass up thru a graded series of alcohols to dehydrate, clear in toluene or xylene, and mount in permount or balsam.

GIEMSA STAIN FOR TISSUE SECTIONS

The following technic is based on that described by Hewitt (1940) for staining tissue sections with Giemsa stain.

1. Fix small pieces of tissue in formol-Zenker's fluid for 18 to 24 hours.
2. Wash in running tap water overnight.
3. Place in 30% alcohol, 50% alcohol and 70% alcohol for 2 hours each.
4. Treat overnight with 70% alcohol containing enough iodine to give it a port-wine color. This removes the excess mercuric chloride.
5. Place in fresh 70% alcohol for 2 to 4 hours or longer to remove the iodine.
6. Finish dehydration, and infiltrate, embed, section and mount in the usual manner.
7. Run the sections down thru xylene and the alcohols into distilled water in the usual manner.
8. Mordant in 2.5% aqueous potassium bichromate solution 1/2 to 1 hour.
9. Wash quickly in distilled water.
10. Stain for 24 hours in the following solution:

0.5% aqueous sodium carbonate	2 to 4 drops
Methyl alcohol (CP)	3 ml
Giemsa stain	2.5 ml
Distilled water	100 ml

11. Wash in distilled water colored lemon yellow with 2.5% potassium bichromate to remove the excess stain.
12. Differentiate in 70% alcohol. This is the most critical step in the whole procedure. It usually takes 30 seconds to 2 minutes, but the time varies with the type of tissue and the thickness of the sections. Liver usually takes less time than enlarged, engorged spleen, which takes less time than normal spleen. Thick sections take longer than thin. Stop differentiating as soon as the stain is being removed in noticeable quantities. Tissues which contain a large amount of blood will show sharply differentiated red and blue areas macroscopically when they are properly differentiated.
13. Stop the differentiation by washing quickly in distilled water.
14. Dehydrate and mount. Alcohol cannot be used for the dehydrating process, since it will remove too much dye. The simplest and best method of dehydration is to pass the sections thru 3 changes of *anhydrous* tertiary butyl alcohol for 5 to 10 minutes each (Levine, 1939). (Ordinary samples of tertiary butyl alcohol contain water and cannot be used. A simple way of determining whether a sample is anhydrous is to place it in the refrigerator; its melting point is 25° C, and it will crystallize.)

Transfer the sections from the third tertiary butyl alcohol to 2 changes of xylene and then mount in permount or another resinous mounting medium. It is important that the mounting medium be neutral; if it is acid it will soon decolorize the preparations.

(The following dehydration procedure, recommended by Hewitt, can be used if tertiary butyl alcohol is not available:

Distilled water	Wash
5% xylene, 95% acetone	1 minute
30% xylene, 70% acetone	2 minutes
70% xylene, 30% acetone	2 minutes
Xylene	5 minutes)

MICROSCOPIC EXAMINATION OF BLOOD

In searching for blood protozoa, thick or thin smears of the blood are prepared and stained with one or another of the Romanowsky (methylene blue-eosin combination) stains. Thick smears are preferable to thin ones for mammalian blood because their use permits one to examine a relatively large amount of blood in a relatively short time. However, they cannot be used for avian blood because of its nucleated erythrocytes. The protozoa may be distorted in thick smears enough so that some practice is needed to differentiate species, especially of the malaria parasites.

Romanowsky stains may be either rapid (such as Wright's and Field's stains) or slow (such as Giemsa's stain). The rapid stains are satisfactory if speed is necessary, but they stain unevenly, particularly in thick smears, and they are not as precise as the slow stains. Giemsa's stain is best for most purposes. Mammalian blood should be stained at pH 7.0 to 7.2, and avian blood at pH 6.75. These pH's can be obtained by using Clark and Lubs phosphate buffers.

Trypanosomes, microfilariae and most protozoa can be found in fresh, wet, unstained smears, but for critical study they must be stained.

Preparation of Thin Blood Smears. Clean 2 slides by rinsing in 95% alcohol and wiping with a clean cloth. Handle the slides only by their edges to avoid leaving finger marks. Place a *small* drop of fresh blood at the end of one slide, place the other slide at a 30° angle to the first slide, touch the drop of blood with the end of the slanted slide so that the blood runs into the space beneath it, and then draw the slanted slide rather quickly over the length of the other slide. The blood should be pulled behind the slide and not pushed ahead of it as the smear is being made. A thin, even film of blood should result. Wave the slide in the air until it dries (a matter of a few seconds if the smear is thin enough). If the smear is to be stained in Giemsa's stain, fix it by dipping in absolute methyl alcohol (CP). If the smear is to be stained in Wright's stain, fixation is not necessary, since it will take place during the staining process. If the smear is to be stored for more than a day or so before staining, it should be fixed.

Preparation of Thick Blood Smears. Prepare slides as for thin smears. Place a medium-sized drop of blood or several tiny ones on the slide, and mix with a toothpick or the corner of another slide. Allow to dry in air or in an incubator at 37° C. A hair dryer can be used to speed up the drying process. Thick smears must be laked (i.e., the hemoglobin must be extracted) before being stained. This can be done by placing them in water until the color has disappeared. If Giemsa's stain is used and the smears are fresh, laking will take place during the staining process. If the smears are to be stored for more than a day or so before staining, they should be laked and then fixed with absolute methyl alcohol (CP) before storage, since it is often extremely difficult to remove the hemoglobin from smears which have been stored for some time.

While *Leucocytozoon,* microfilariae and sometimes *Trypanosoma* can be found with the low power of the microscope, the stained blood smears should be examined with the oil immersion objective for other protozoa. The faster thin smears have dried, the less distortion is produced. Hence, the most natural appearing protozoa will be found at the thin end and around the edges of the smear.

Cleaning Immersion Oil Off of Slides. Stained blood smears are customarily not covered with a coverslip, and immersion

oil is placed on them for examination. The immersion oil should be removed after the examination has been completed if the slides are not to be thrown away. Many people do this by rubbing the slide with lens paper as tho they were polishing silver, a procedure which removes not only the oil but also many of the blood cells. The following technic, which I first saw demonstrated by Dr. Joseph A. Long, permits one to remove the oil quickly and neatly without disturbing the blood cells. It can also be used for slides which have been covered by a coverslip; by its use, one can remove the oil from a newly mounted slide without also removing either the coverslip or the wet mounting medium beneath it.

Fold a small piece (about 5 cm square) of lens paper twice so that it is 4 layers thick. Place the lens paper on top of the immersion oil and allow it to take up the oil. Pull if off the slide sideways in a single motion; do not rub.

Fold a second piece of lens paper like the first. Place a drop of xylene on it. Place the wet lens paper on what remains of the oil. Leave it for a second or two, and then pull it off the slide sideways in a single motion; do not rub. When the xylene has evaporated, the slide will be clean and dry. (Sometimes it is necessary to repeat this second step with a fresh piece of lens paper.)

CONCENTRATION OF PROTOZOAN CYSTS FROM FECES

A number of technics have been developed for the concentration of protozoan cysts and helminth eggs from feces. They are of 2 general types, flotation and sedimentation. Each has certain advantages over the other.

FLOTATION TECHNICS

These technics make use of solutions of higher specific gravity than protozoan cysts or helminth eggs, but of lower specific gravity than most of the fecal debris. When feces are mixed with them, the cysts and eggs will float to the top while most of the fecal material remains at the bottom. Flotation technics are most useful for coccidian oocysts, other protozoan cysts, nematode eggs and some tapeworm eggs. They are not satisfactory for trematode, acanthocephalan and other tapeworm eggs.

Many different solutions have been used, and many variations in technic have been proposed. The methods described here all work satisfactorily.

Sugar Flotation

This technic is preferable for general use, but is not satisfactory for protozoan cysts other than those of coccidia. Sugar solution is preferable to sodium chloride, sodium nitrate or other salt solutions except zinc sulfate. It does not crystallize as readily, and causes less distortion than salt solutions, and it is just as efficient (Levine *et al.*, 1960). The following technic is a modification of the DCF (direct centrifugal flotation) technic introduced by Lane (1923).

1. Make a rather heavy suspension of feces in physiological salt solution in a shell vial or other container.
2. Strain thru 2 layers of cheesecloth into a test tube or centrifuge tube, filling the tube almost half full. The lip of the tube must be smooth, or an air bubble will form under the coverslip following centrifugation (#6 below).
3. Add an equal volume of Sheather's sugar solution, leaving a small air space at the top. Cover with a plastic coverslip or small piece of card, and invert repeatedly to mix.
4. Add enough additional Sheather's sugar solution to bring the surface of the liquid barely above the top of the tube.
5. Cover with a round coverslip.
6. Centrifuge for 5 minutes. (If a centrifuge is not available, let stand for 45 minutes to 1 hour.)
7. Remove the coverslip, place it on a slide, and examine under the microscope.

(If desired, Steps 2 to 4 can be modified by straining the fecal suspension into a second shell vial, mixing with an equal volume of Sheather's sugar solution, and then filling the centrifuge tube with the mixture.)

Zinc Sulfate Flotation

Zinc sulfate solution has the advantage of concentrating the cysts of protozoa such as *Entamoeba* and *Giardia* without distortion. The following technic is a modification of that introduced by Faust *et al.* (1938).

1. Make a suspension of feces in physiological salt solution in a shell vial or other container.
2. Strain 4 ml of the suspension thru 2 layers of cheesecloth into a test tube or centrifuge tube. The lip of the tube must be smooth.
3. Add tap water to within 1 cm of the top of the tube.
4. Mix thoroughly and centrifuge for 5 minutes.
5. Pour off the supernatant fluid.
6. Add a small amount of zinc sulfate solution and mix with an applicator stick. Add more zinc sulfate solution until the tube is almost full, cover with a plastic coverslip or a small piece of card, and invert repeatedly to mix.
7. Add enough additional zinc sulfate solution to bring the surface of the liquid barely above the top of the tube.
8. Cover with a round coverslip.
9. Centrifuge for 5 minutes.
10. Remove the coverslip, place it on a slide, and examine under the microscope.

SEDIMENTATION TECHNICS

Sedimentation technics can be used for concentration of protozoan cysts, and are necessary for the concentration of trematode, acanthocephalan and some tapeworm eggs, which sink to the bottom of the solutions used in the flotation technics. A few protozoan cysts such as those of *Eimeria leuckarti* also sink to the bottom.

Since they are essentially washing processes, sedimentation technics may not concentrate cysts and eggs as much as flotation technics. Many different sedimentation technics have been developed. The two described below appear to be among the best.

Formalin-Triton-Ether (FTE) Sedimentation Technic

This technic was introduced by Ritchie (1948) and modified by Maldonado, Acosta-Matienzo and Velez-Herrera (1954). The latter considered it the nearest to an all-round diagnostic procedure, since it is highly effective for the detection not only of schistosome, hookworm, whipworm and ascarid eggs but also of protozoan cysts.

1. Mark off a test tube at the 5 ml and 6 ml levels.
2. Place 5 ml of 10% formalin containing a drop of Triton NE in the tube.
3. Add 1 ml of feces.
4. Break up the feces thoroughly with a wooden applicator.
5. Strain the suspension thru 4 layers of cheesecloth into a 15 ml conical centrifuge tube. Squeeze the cloth to get out as much liquid as possible.
6. Add 5 ml of commercial ether to the suspension in the centrifuge tube. Cover the tube with a plastic coverslip and shake vigorously.
7. Centrifuge (at 2000 r.p.m. in a horizontal centrifuge with a radius from the center to the tip of the tube of 8 inches; if another type of centrifuge is used, change the speed of centrifugation accordingly) for 1 minute after the centrifuge has reached its terminal speed.
8. Loosen the plug of detritus at the formalin solution-ether interface with an applicator stick, pour off all the supernatant fluid rapidly, and, holding the tube slightly inverted, clean its walls carefully with a piece of clean, dry gauze. This is done to prevent the liquid and debris on the walls of the tube from sliding down to the bottom and diluting the sediment.

9. Add a drop of physiological salt solution to the sediment to facilitate its removal.
10. Take up the sediment with a pipette (a Stoll pipette works well), place on a slide, add a coverslip, and examine under the microscope.

MIFC (Merthiolate-Iodine-Formaldehyde Concentration) Technic

This technic was introduced by Blagg *et al.* (1955) as a modification of the MIF preservative stain. They found that the MIFC technic was positive for protozoan trophozoites in 74% of 110 positive human fecal specimens as compared with 55% for the MIF direct smear; it was positive for 92% of 226 specimens containing protozoan cysts, as compared with 58% positive with the MIF direct smear.

1. Prepare an MIF preserved fecal specimen as described above (p. 379).
2. When ready to examine, shake the specimen vigorously for 5 seconds.
3. Strain thru 2 layers of wet surgical gauze into a 15 ml centrifuge tube.
4. Add 4 ml cold (refrigerated) ether to the centrifuge tube, insert a rubber stopper, and shake vigorously. If ether remains on top after shaking, add 1 ml tap water and shake again.
5. Remove the stopper and let stand for 2 minutes.
6. Centrifuge 1 minute at 1600 r.p.m. Four layers will appear in the tube: (a) an ether layer on top, (b) a plug of fecal detritus, (c) an MIF layer, (d) the sediment containing protozoa and helminth eggs on the bottom.
7. Loosen the fecal plug by ringing with an applicator stick.
8. Quickly but carefully pour off all but the bottom layer of sediment.
9. Mix the sediment thoroughly, pour a drop on a slide, cover with coverslip, and examine.

PROTOZOAN CULTURE MEDIA

NNN (Novy, MacNeal and Nicolle) Medium

This medium was developed for the cultivation of *Leishmania*, but it can also be used for trypanosomes of the lewisi group.

1. Measure or weigh out:

Sodium chloride	6 g
Agar	14 g
Distilled water	900 ml

2. Mix, bring to the boiling point, and place in bacteriologic culture tubes in 5 ml amounts. Sterilize in the autoclave. This is the medium base, and can be stored in the refrigerator.
3. To use, melt the agar in the tubes and cool to 48° C. Add to each tube 1/3 of its volume of sterile, defibrinated rabbit blood. Mix thoroughly by rolling the tube between the palms of the hands.
4. Place the tube on a slant without leaving a butt of medium at the bottom, and allow to solidify. This is best done in the refrigerator or in ice, since more water of condensation is obtained in this way. (The protozoa develop best in the water of condensation at the bottom of the slant.)
5. Seal the tubes to prevent the water of condensation from evaporating, and incubate at 37° C for 24 hours to test for sterility before inoculating.
6. Inoculate suspected material into the condensation water and incubate at 22 to 24° C. Transfer cultures every week or two.

Weinman's Trypanosome Medium

This medium was developed by Weinman (1946) for the cultivation of *Trypanosoma gambiense* and *T. rhodesiense*. It can also be used for other trypanosomes.

1. The base medium is Difco nutrient agar (1.5%), which consists of:

Beef extract	3 g
Bacto peptone	5 g
Sodium chloride	5 g
Agar	15 g
	31 g

Dissolve in 1 liter distilled water, bring to pH 7.3, sterilize by autoclaving.

2. To prepare the culture medium, heat the base medium to melt the agar. Before it has resolidified, add the following aseptically to each 75 ml of the base:

Citrated human plasma previously inactivated at 56°C for 30 minutes	12.5 ml
Human red cells	12.5 ml

3. Dispense in Kolle flasks or slanted in test tubes. Stopper with rubber corks or seal with Parafilm to retard drying. Store in the refrigerator until used.
4. Inoculate with suspected material and incubate at room temperature. The trypanosomes grow on the surface as small, rounded, colorless, transparent, slightly raised, glistening, moist-appearing colonies 1 to 2 mm in diameter; they are detectable in 5 to 10 days or, exceptionally, in 3 to 4 weeks.

Tobie, von Brand and Mehlman's Trypanosome Medium

This medium was developed by Tobie, von Brand and Mehlman (1950) for African trypanosomes. It consists of a solid slant with a liquid overlay.

1. Solid slant. Measure or weigh out:

Bacto-beef (Difco)	1.5 g
Bacto-peptone (Difco)	2.5 g
Bacto-agar (Difco)	7.5 g
NaCl	4.0 g
Distilled water	500 ml

Mix the ingredients, dissolve by bringing to the boiling point, adjust to pH 7.2 to 7.4 with NaOH, and autoclave at 15 lbs. pressure for 20 minutes.

Cool to 45° C, and add 1 part of inactivated, citrated rabbit blood to each 3 parts of the above base. Place 5 ml amounts in test tubes, slant, and allow to cool. If desired, 25 ml amounts may be placed in flasks.

2. Fluid overlay (Locke's solution). Measure or weigh out:

NaCl	5.0 g
KCl	0.2 g
$CaCl_2$	0.2 g
KH_2PO_4	0.3 g
Glucose	2.5 g
Distilled water	1000 ml

Autoclave at 15 pounds pressure for 20 minutes.

3. Place 2 ml of the liquid overlay in each tube containing 5 ml of the base (or 10 to 15 ml in each flask), using aseptic technic.
4. Inoculate with suspected material and incubate at 24 to 25° C for 10 to 14 days.

RES (Ringer's-Egg-Serum) Medium for Enteric Protozoa

This medium was first introduced by Boeck and Drbohlav (1925). Many different modifications have been proposed which are as useful as the one described below. The serum may be replaced by egg albumen, for instance, or the Ringer's solution by Locke's solution.

The medium is essentially a coagulated egg slant overlaid with a fluid nutrient solution.

A. Egg slant.

1. Mix 12.5 ml Ringer's solution with each egg used. For best results, mix in a Waring blendor for 30 seconds. If a blendor is not used, filter the mixture thru cheesecloth.
2. Place 2 ml amounts of the mixture in cotton-stoppered test tubes.

(Other standard closures for bacteriologic work can also be used.)

3. Place the tubes upright in a vacuum desiccator. Evacuate the desiccator slowly. As evacuation proceeds, the egg mixture begins to bubble, and within 4 minutes a dense foam of egg begins to climb in the tubes. Stop the evacuation before the cotton plugs become wet, and allow the tubes to remain in the evacuated desiccator for an hour. The purpose of this treatment is to remove the dissolved air from the medium. If it is allowed to remain, it will bubble out during subsequent sterilization and coagulation, roughening and pitting the slant surface (Levine and Marquardt, 1954).
4. Release the vacuum, pack the tubes in baskets, slant them in the autoclave, and inspissate and sterilize them simultaneously at 15 pounds pressure for 20 minutes. Best results are obtained when no butt of medium is left in the tubes. When this is done, 2 ml of fluid makes a slant about 1.5 inches long in an 18 x 150 mm tube.

B. Fluid overlay.

1. Mix the following aseptically:

Sterile Ringer's solution	500 ml
Sterile 10% glucose solution	10 ml
Sterile serum (horse, rabbit, cow, etc.).	10 ml

2. Add sufficient fluid overlay to each egg slant to cover the whole slant. Aseptic technic must be used thruout. Incubate at 37° C for 2 days prior to inoculation to test for sterility.

Balamuth's Amoeba Medium

This medium was developed by Balamuth (1946) for enteric amoebae, but it can be used for other enteric protozoa as well.

1. Mix 288 g dehydrated egg yolk with 288 ml distilled water and 1000 ml physiological salt solution. Mix with a Waring blendor or similar instrument until the suspension is smooth.
2. Heat over an open flame in the upper part of a double boiler, stirring constantly, for 5 to 10 minutes until coagulation begins.
3. Continue heating over boiling water in the double boiler for 20 minutes until coagulation is complete. Add 160 ml distilled water to replace water lost by evaporation.
4. Filter thru a muslin bag. When the bag cools, squeeze it gently to obtain the maximum amount of filtrate.
5. Add enough physiological salt solution to the filtrate to bring its volume to 1000 ml.
6. Place 500 ml of filtrate in each of 2 Erlenmeyer flasks. Autoclave at 15 pounds pressure for 20 minutes.
7. Chill the flasks by refrigeration overnight or in some other way.
8. Filter while cold thru 2 layers of Whatman qualitative filter paper in a Buchner funnel, using negative pressure. Pour the mixture thru the funnel in small amounts, replacing the filter paper frequently.
9. Add an equal volume of Balamuth's buffer solution to the filtrate.
10. Add 5 ml of crude liver extract (Lilly, No. 408) to each liter of medium.
11. Dispense in 5 to 7 ml amounts in tubes.
12. Autoclave at 15 pounds pressure for 20 minutes.
13. Add a small amount of sterile rice powder to each tube. Incubate for 24 hours at 37° C to test for sterility. (If desired, the medium can be stored in large flasks in the refrigerator after autoclaving; it can be kept for a month or more without deteriorating, but any sediment which forms should be removed by filtration before use.)

CPLM (Cysteine-Peptone-Liver Infusion-Maltose) Medium

This medium was developed by Johnson and Trussell (1943) for *Trichomonas*, but it can also be used for other enteric protozoa.

A. Liver infusion.

1. Mix the following thoroughly, using a Waring blendor if available:

Bacto liver powder	20 g
Distilled water	330 ml

2. Infuse for 1 hour at about 50° C.
3. Heat with stirring at 80° C for 5 minutes to coagulate the protein.
4. Filter thru a Buchner funnel. About 320 ml of liver infusion are obtained.

B. Preparation of final medium.

1. Mix the following, using a Waring blendor if available:

Cysteine monohydrochloride	2.4 g
Peptone	32.0 g
Maltose	1.6 g
Agar	1.6 g
Ringer's solution	960 ml

2. Add the liver infusion from A above.
3. Adjust the pH to 7.0 (approximately 20 ml of 1.0 N NaOH are needed).
4. Heat to dissolve the agar.
5. Filter thru cotton into a 2000 ml flask.
6. Add 0.7 ml of 0.5% methylene blue solution.
7. Place 300 ml amounts in 500 ml Erlenmeyer flasks.
8. Autoclave for 15 minutes at 15 pounds pressure.
9. Add 75 ml sterile inactivated serum to each 300 ml flask.
10. Place 7 to 10 ml amounts aseptically in sterile, plugged test tubes.
11. Incubate for 2 days at 37° C to test for sterility before use.

BGPS (Beef Extract-Glucose-Peptone-Serum) Medium

This medium was introduced by Fitzgerald, Hammond and Shupe (1954) for use in the diagnosis of *Tritrichomonas foetus* infections, but it can also be used for other trichomonads.

1. Mix the following in a 3 liter flask:

Difco beef extract	3 g
Glucose	10 g
Bacto peptone	10 g
NaCl	1 g
Agar	0.7 g
Distilled water	1000 ml

2. Dissolve by boiling. After cooling, adjust the pH to 7.4 with 1.0 N NaOH solution.
3. Cover the mouth of the flask with heavy paper and autoclave for 30 minutes at 15 pounds pressure.
4. After cooling, add 20 ml inactivated (at 56° C for 30 minutes) beef serum aseptically, and mix thoroughly.
5. Dispense in 10 ml amounts into 15 ml culture tubes. Test for sterility by incubating at 37° C for 2 days.
6. Just before inoculation, add 500 to 1000 units of penicillin and 0.5 to 1.0 mg of streptomycin to each ml of medium, and mix thoroughly.
7. Pipette the inoculum on the top of the medium in such a way as to minimize mixing. The trichomonads migrate to the bottom of the tube, while yeasts and molds tend to remain near the top. Incubate at 39° C for 3 to 5 days. To examine, remove a sample from the bottom of the tube with a pipette.

Diamond's Trichomonad Medium

This medium was introduced by Diamond (1957) for the axenic cultivation of trichomonads. It can be used successfully for more species than other media.

1. Mix the following:

Trypticase (BBL)	2.0 g
Yeast extract	1.0 g
Maltose	0.5 g
L-cysteine hydrochloride	0.1 g
Ascorbic acid	0.02 g
K_2HPO_4	0.08 g
KH_2PO_4	0.08 g
Distilled water	90 ml

2. Adjust the pH to 6.8-7.0 with 1 N NaOH for all trichomonads except *T. vaginalis*; for this species, adjust the pH to 6.0 with 1 N HCl.
3. Add 0.05 g agar.
4. Autoclave for 10 minutes at 15 pounds pressure.
5. Cool to 48° C, and add the following:

Sheep serum (inactivated at 56°C for 30 min.)	10 ml
Potassium penicillin G	100,000 units
Streptomycin sulfate	0.1 g

(The penicillin and streptomycin can be made up in 1 ml distilled water beforehand.)

6. Place 5 ml amounts of the medium aseptically in sterile, stoppered test tubes. Store in the refrigerator up to 14 days or longer.
7. Prior to inoculation, incubate the tubes at 35.5° C for 1 hour.

All the trichomonads which Diamond (1957) cultivated except *T. gallinarum* and *T. gallinarum*-like species grew well at 35.5° C; the latter grew better at 38.5° C.

It has been found in the author's laboratory that the phosphates are not necessary for the growth of *T. foetus*, *T. suis*, *T. gallinae*, *T. gallinarum* and several other species.

RSS (Ringer's-Serum-Starch) Medium for Balantidium

The following medium is slightly modified from that introduced by Rees (1927) for the cultivation of *Balantidium coli*.

1. Add 25 ml of horse, rabbit or bovine serum aseptically to 500 ml of sterile Ringer's solution.
2. Tube in 8 ml amounts, using aseptic technic.
3. To each tube add a 5 mm loop of rice starch which has been sterilized in a large test tube for 30 minutes at 15 lb pressure.
4. Incubate at 37° C for 48 hours to test for sterility. Store in the refrigerator.
5. Before inoculation, warm the tubes to 37° C by placing them in the incubator. Incubate at 37°. The protozoa grow in the bottom of the tube.

FORMULAE

Physiological Salt Solution

NaCl	8.5 g
Distilled water	1000 ml

D'Antoni's Iodine Solution

Powdered iodine	1.5 g
1% aqueous KI solution	100 ml

Allow to stand 4 days before use. This is the stock solution and contains an excess of iodine. Filter small amounts before use. If tightly stoppered, the filtered solution will keep 4 weeks before too much iodine has volatilized for use.

Lugol's Iodine Solution

Potassium iodide	10 g
Powdered iodine	5 g
Distilled water	100 ml

Dissolve the potassium iodide in the water before adding the iodine.

Mayer's Albumen Fixative

Put the whites of several new-laid eggs in a shallow dish. Whip them a little with a fork or wire egg beater, 2 or 3 dozen strokes being sufficient. Do not beat them until they are white and stiff.

Allow them to stand for about an hour, and then skim the foam from the top and pour the remaining liquid into a graduated cylinder. Pour in an equal amount of glycerol, and add 1 g of sodium salicylate for each 100 ml of the mixture. Shake thoroughly and filter thru paper into a clean bottle. Filtration will require 1 to several weeks. It may be accelerated somewhat by pouring a rather small amount at a time into the filter and replacing the paper every few days. Keep a small quantity in a vial or bottle provided with a glass rod stuck into the cork and projecting into the albumen. A drop can easily be placed on a slide with this rod.

Hollande's Fixative

Picric acid	4 g
Copper acetate	2.5 g
Formalin	10 ml
Acetic acid	1.5 ml
Distilled water	100 ml

Schaudinn's Fixative

Saturated aqueous mercuric chloride	66 ml
95% alcohol	33 ml

Add 5% acetic acid immediately before use.

Iron Alum Solution

Ferric ammonium sulfate (violet crystals only)	2 g
Distilled water	100 ml

Filter immediately before use.

Heidenhain's Hematoxylin (Stock Solution)

Hematoxylin	10 g
100% alcohol	100 ml

Allow to remain 1 month in a loosely stoppered bottle before use. To make the staining solution, add 0.5 ml of the stock solution to 9.5 ml of distilled water.

Feulgen Stain

1. Dissolve 1 g basic fuchsin (certified as suitable for the Feulgen stain) in 200 ml boiling distilled water.
2. Cool to 50° C.
3. Filter.
4. Add 20 ml 1 N HCl to the filtrate.
5. Cool to 25° C.
6. Add 1 g dried sodium bisulfite ($NaHSO_3$); this liberates sulfurous acid.
7. Allow to stand at room temperature 24 hours until decolorized.
8. Store in the refrigerator in small glass-stoppered bottles filled to the top to exclude air. The solution will keep several weeks. It should be straw-colored; if it is red, it should be discarded.

Sørensen's Phosphate Buffers

To make M/15 Na_2HPO_4 solution, dissolve 9.5 g anhydrous Na_2HPO_4 or 11.9 g $Na_2HPO_4 \cdot 2\ H_2O$ in 1 liter distilled water. To make M/15 KH_2PO_4, dissolve 9.08 g KH_2PO_4 in 1 liter distilled water. Store separately in pyrex, glass-stoppered bottles.

To prepare buffered water for the Giemsa stain, mix the following amounts of the solutions (in ml):

	pH 6.8	pH 7.0	pH 7.2
M/15 Na_2HPO_4	50.0	61.1	72.0
M/15 KH_2PO_4	50.0	38.9	28.0
Distilled water	900.0	900.0	900.0

Balamuth's Buffer Solution

1.0 M K_2HPO_4 (174.180 g K_2HPO_4 in 1000 ml distilled water)	4.3 parts
1.0 M KH_2PO_4 (136.092 g KH_2PO_4 in 1000 ml distilled water)	0.7 parts

This is the stock solution. To prepare the final solution used in Balamuth's medium, add 14 parts of distilled water to 1 part of the stock solution.

Ringer's Solution

NaCl	6.5 g
$NaHCO_3$	0.2 g
$CaCl_2 \cdot 2H_2O$	0.16 g
KCl	0.14 g
$NaH_2PO_4 \cdot H_2O$	0.011 g
Distilled water	1000 ml

Sheather's Sugar Solution

Sucrose (ordinary cane or beet sugar)	500 g
Distilled Water	320 g
Phenol (melted in water bath)	6.5 g

Zinc Sulfate Flotation Solution

$ZnSO_4 \cdot 7H_2O$	331 g
Distilled water	1000 ml

The specific gravity of this solution is 1.180.

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APPENDIX

Scientific and Common Names of Some Domestic and Wild Animals

Class MAMMALASIDA

Order MARSUPIALORIDA

Didelphis marsupialis	Opossum

Order PRIMATORIDA

Alouatta villosa	Howler monkey
Ateles geoffroyi	Goeffroy's spider monkey
Cebus capucinus	Capuchin monkey
Ceropithecus aethiops	Green guenon, vervet monkey
Cercopithecus mona	Mona monkey
Gorilla gorilla	Gorilla
Homo sapiens	Man
Macaca irus	Cynomolgus macaque, kra monkey
Macaca mulatta	Rhesus monkey
Macaca philippinensis	Philippine macaque
Mandrillus sphinx	Mandrill
Pan troglodytes	Chimpanzee
Papio papio	Baboon
Pongo pygmaeus (syn., *Simia satyrus*)	Orang-utan

Order EDENTATORIDA

Dasypus novemcinctus	Nine-banded armadillo

Order LAGOMORPHORIDA

Lepus americanus	Snowshoe rabbit
Lepus californicus	Black-tailed jack rabbit
Lepus europaeus	European hare
Lepus townsendii	White-tailed jack rabbit
Oryctolagus cuniculus	Domestic rabbit, European wild rabbit
Sylvilagus floridanus	Eastern cottontail

Order RODENTORIDA

Apodemus sylvaticus	Long-tailed field mouse (European)
Cavia porcellus	Guinea pig
Chinchilla laniger	Chinchilla
Clethrionomys spp.	Red-backed mice
Cricetulus barabensis griseus	Chinese (striped) hamster
Dipodomys spp.	Kangaroo rats
Gerbillus gerbillus	Lesser Egyptian gerbil
Meriones unguiculatus	Mongolian gerbil, clawed jird
Mesocricetus auratus	Golden hamster
Microtus spp.	Voles

Mus musculus	Domestic mouse, house mouse
Neotoma spp.	Wood rats
Oryzomys palustris	Swamp rice rat
Peromyscus spp.	Deer mice
Rattus mastomys	Multimammate mouse
Rattus norvegicus	Norway rat
Rattus rattus	Black rat
Rhombomys opimus	Gerbil
Sciurus spp.	Tree squirrels
Sigmodon hispidus	Cotton rat
*Spermophilus** spp. (syn., *Citellus* spp.)	Ground squirrels, susliks, ziesels

Order CARNIVORIDA

Alopex lagopus	Arctic fox
Canis dingo	Dingo
Canis familiaris	Dog
Canis latrans	Coyote
Canis lupus	Grey wolf
Felis catus	Domestic cat
Felis concolor	Mountain lion, puma
Lynx canadensis	Lynx
Lynx rufus	Bobcat
Martes americana	Marten
Mephitis mephitis	Striped skunk
Mustela erminea	Ermine
Mustela frenata	Long-tailed weasel
Mustela putorius furo	Ferret
Mustela vison	Mink
Panthera leo	Lion
Panthera tigris	Tiger
Procyon lotor	Raccoon
Spilogale spp.	Spotted skunks
Urocyon cinereoargenteus	Grey fox
Ursus americanus	Black bear
Ursus horribilis	Grizzly bear
Vulpes fulva	Red fox (North American)
Vulpes vulpes	European common fox

Order PERISSODACTYLORIDA

Asinus asinus	Domestic ass
Equus caballus	Horse
Rhinoceros unicornis	Rhinoceros

Order ARTIODACTYLORIDA

Suborder SUIORINA

Sus scrofa	Pig

Suborder RUMINANTORINA

Alces alces	Moose
Antilocapra americana	Pronghorn

Bison bison	Bison
Bos indicus	Zebu
Bos taurus	Ox
Bubalus bubalis	Water buffalo, carabao
Bubalus (Syncerus) caffer	African buffalo
Camelus bactrianus	Bactrian camel, two-humped camel
Camelus dromedarius	Dromedary, one-humped camel
Capra hircus	Domestic goat
Capreolus capreolus	Roe deer
Cervus canadensis	Wapiti, elk
Cervus elaphus	Red deer (European)
Dama dama	Fallow deer
*Dama** (syn., *Odocoileus*) *hemionus*	Black-tailed deer, mule deer
*Dama** (syn., *Odocoileus*) *virginiana*	White-tailed deer
Lama glama	Llama
Mazama americana	Red brocket
Oreamnos americanus	Mountain goat
Ovibos moschatus	Musk ox
Ovis ammon	Argali
Ovis aries	Domestic sheep
Ovis canadensis	Mountain sheep, Rocky Mountain bighorn sheep
Ovis musimon	Mouflon
Ovis vignei	Urial
Rangifer tarandus	Caribou, reindeer
Rupicapra rupicapra	Chamois

Order PROBOSCIDORIDA

Elephas indicus	Indian elephant
Loxodonta africana	African elephant

Class AVEASIDA

Order ANSERORIDA

Anas platyrhynchos	Domestic duck, wild mallard
Anser anser (Anser cinereus)	Domestic goose
Anser albifrons	White-fronted goose
Branta canadensis	Canada goose
Cairina moschata	Muscovy duck
Cygnus olor	Swan

Order GALLORIDA

Alectoris graeca	Chukar partridge
Bonasa umbellus	Ruffed grouse
Colinus virginianus	Bobwhite
Gallus gallus	Chicken
Meleagris gallopavo	Turkey
Numida meleagris	Guinea fowl
Pavo cristatus	Peafowl
Perdix perdix	Grey partridge
Phasianus colchicus	Ring-necked pheasant

Order COLUMBORIDA

Columba fasciata	Band-tailed pigeon
Columba livia	Domestic pigeon
Streptopelia chinensis	Spotted dove
Streptopelia risoria	Ringed turtle dove
Streptopelia turtur	Turtle dove (European)
Zenaidura macroura	Mourning dove

Order PASSERORIDA

Passer domesticus	English sparrow
Serinus canarius	Canary

Order STRUTHIONORIDA

Struthio camelus	Ostrich

* The generic names *Spermophilus* and *Dama* were accepted rather than the more usual *Citellus* and *Odocoileus*, respectively, by E. R. Hall and K. R. Kelson (1959. The mammals of North America. 2 vols. Ronald Press, New York). Their book was seen too late for their usage to be incorporated in the text of the present volume.

Index and Host-Parasite Lists

(Numbers in italics refer to illustrations)