The Institute of Biology's
Studies in Biology no. 138

The Biology of Parasitic Protozoa

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Because it is no longer possible for one textbook to cover the whole field of biology while remaining sufficiently up to date, the Institute of Biology proposed this series so that teachers and students can learn about significant developments. The enthusiastic acceptance of 'Studies in Biology' shows that the books are providing authoritative views of biological topics.

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Readers' comments will be welcomed by the Education Officer of the Institute.

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Preface

Parasitology is a branch of ecology (from Greek *oikos*, a house and *logia*, branch of knowledge). Concentrating on either parasite or host results in a one-sided view of what is really a dynamic tension. Parasites and their hosts live in a more-or-less stable, though precarious, equilibrium. A small tilt in one direction can lead to the death of the host; a small tilt the other way can result in the death of the parasite. Either consequence damages the parasite – and perhaps the host also. A host supporting a limited number of uninvited guests with little or no untoward effect, in a balanced relationship, may benefit from a state of active immunity ("premunition") which prevents its inundation by re-infection from the environment. However, the biological situation is vastly more complex, and an understanding of it, in spite of about 100 years of classical parasitology, is only just beginning. This book is an attempted introduction to a study of the inter-relationships between parasitic protozoa and their hosts.

Cambridge, 1982

J.R.B.

Contents

1 Introduction

1.1 The concept of parasitism

Parasitism has been variously defined and subdivided. A simple scheme is to divide it into three categories. Firstly, *commensalism*, in which one organism (the parasite) uses another (the host) essentially for shelter only, though it often benefits by using some of the host's own food supply. The host does not suffer significantly from this type of association. Common examples include many of the protozoan inhabitants of the intestines of vertebrates, including humans. A few hundred amoebae of, say, the genus *Endolimax* living in the intestine discommode us only marginally by consuming an infinitesimal amount of our daily food intake. Secondly, there is the association called *symbiosis*. In this, both partners benefit: one, or both, may be unable to survive without the other. Common examples are the myriad ciliates (Fig. 1–1) inhabiting the rumen of herbivorous mammals like sheep and cattle; the ciliates depend absolutely on the vertebrate host for shelter and food supply, while the vertebrate benefits from protein and other nutrients synthesized by the protozoa from the otherwise

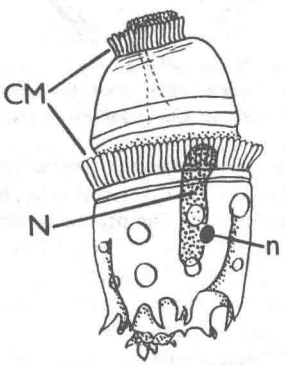

Fig. 1–1 Symbiotic ciliate *(Ophryoscolex caudatus)* from bovine rumen, × 250. CM. ciliary membranelles (fused cilia); N, macronucleus; n, micronucleus. (From BAKER, 1973, after Levine, 1961, *Protozoan Parasites of Domestic Animals and of Man*. Burgess Publishing Co., Minneapolis.)

indigestible portions of their food. The ruminants can, however, survive without the protozoa, though they do so less well. Another, more extreme example is provided by certain flagellates (Hypermastigida) living in the guts of wood-eating insects – cockroaches (Orthoptera) and termites (Isoptera). The

flagellates (Fig. 1–2) cannot survive without the shelter and food supplied by the insects, while the latter are equally dependent on their gut fauna since they do not secrete a cellulolytic enzyme and therefore cannot digest their sole food – wood. If the flagellates are killed by warming the host, the latter starves to death unless it can be re-infected with protozoa. The third kind of association is *tissue parasitism*. Some writers restrict the term 'parasitism' to this kind of association alone. Tissue parasitism is an association between two animals of different species in which one (the parasite) lives, temporarily or permanently, in the body of another (the host) and feeds on the latter's tissues. Whereas nearly all pathogenic (i.e. disease-producing) parasitic protozoa are tissue parasites, not

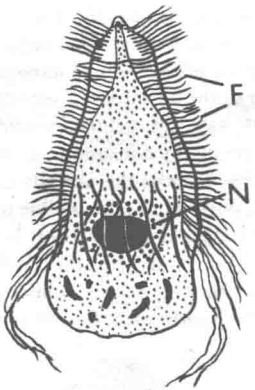

Fig. 1–2 Symbiotic flagellate (*Trichonympha campanula*) from termite's gut, × 200. F, flagella; N, nucleus. (From BAKER, 1973, after Mackinnon and Hawes, 1961, *An Introduction to the Study of Protozoa*, Clarendon Press, Oxford.)

all (probably not even most) tissue parasites are harmful. A high proportion of the British wild fauna, like that of other countries, harbours tissue parasites (often trypanosomes, or haemosporidan protozoa resembling those that cause malaria in humans), without obvious ill effect. A few tissue parasites may even be beneficial (e.g. *Trypanosoma lewisi* in *Rattus* spp., Fig. 1–3). Some parasites may be tissue parasites at one stage of their life cycle, and commensals at another. The balance between living harmlessly in the host and causing disease of the latter may be very delicate – easily tipped one way or the other by, for example, the host's general health or nutritional state. Parasites often have two

Fig. 1–3 *Trypanosoma lewisi*, × 2000 (see Fig. 1–7 for labelling of organelles). (From BAKER, 1973.)

(or more, though not amongst the protozoa) hosts of different species during their life cycle; often one is a vertebrate and the other, called the vector, an invertebrate.

A recent book in this series (LYONS, 1978) has well summarized the advantages and disadvantages of parasitism for helminths; they are fundamentally the same for protozoan parasites. In this book I hope to show how the protozoa have used the advantages and overcome the disadvantages.

1.2 The scope of this book

This short book cannot be comprehensive: it is intentionally eclectic, illustrating certain aspects of the life of parasitic protozoa by referring to examples. The examples will be mainly among parasites of economic importance, if only because such parasites have been more fully studied. Other, less 'important' parasites will be included where they are the best available examples. More complete treatment of many of the aspects mentioned in this book can be found in the books and papers suggested for further reading or the relevant scientific journals, p. 56. Further information about the more economically important parasitic protozoa has been tabulated in the Appendix.

1.3 An outline of systematics

Lengthy discussion of systematics (classification) would be out of place here, and can be found in some of the general reference books listed. But it is convenient to have a brief classification of the parasites referred to in the book. The Protozoa are nowadays usually treated as a subkingdom, with the major subdivisions constituting phyla. If one feels that the absence of photosynthetic pigments from the protozoa is of major taxonomic significance, then the subkingdom can be regarded as part of the kingdom Animalia. If, however, one feels that it is the unicellularity of the protozoa which is significant, remembering that presence or absence of chlorophyll can be a very capricious feature even within a genus (e.g. *Euglena*, normally a green alga but with some colourless species or strains, Fig. 1–4), then it seems more satisfactory to unite the protozoa and unicellular algae in the kingdom Protista. This concept, however, if strictly adhered to, would result in excluding the multicellular myxosporea (Fig. 1–5) and the colonial algae, yet another rather arbitrary distinction. Perhaps the best available solution is to regard the organizational simplicity of non-differentiation into discrete tissues as the dividing line between 'lower' and 'higher' eukaryotes. (Eukaryotic organisms possess mitochondria, nuclear membranes, mitotic spindles, endoplastic reticulum, Golgi apparatus, and '9 + 2' flagella or cilia, and are differentiated from the prokaryotic Monera – bacteria and blue-green algae – which lack all these structures, and also plasmids, and differ biochemically also; Fig. 1–6.) The non-tissue level, including protozoa and algae (and, in some schemes, fungi), can then be accommodated as subkingdoms of the kingdom Protista (or, perhaps more correctly, though less euphoniously, Protoctista).

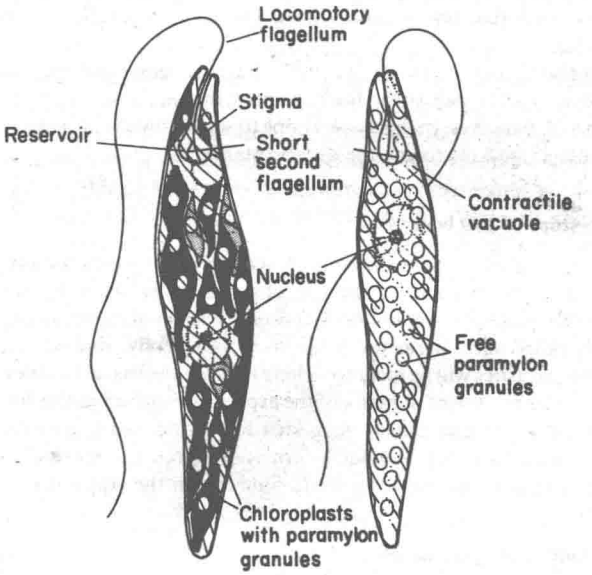

Fig. 1–4 Euglenoid flagellates with (left) and without (right) chloroplasts; note similarity in all other respects. (From Vickerman, K. and Cox, F. E. G., 1967, *The Protozoa*, Murray, London.)

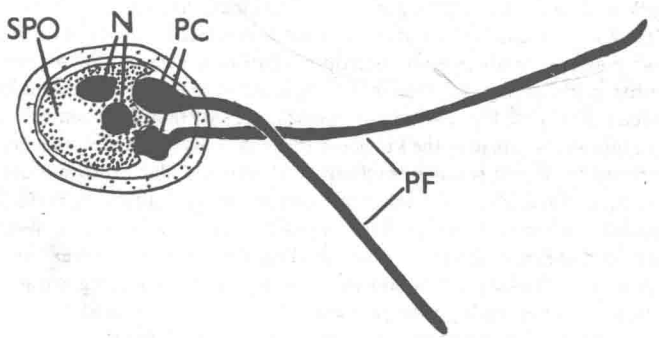

Fig. 1–5 Multicellular spore of *Myxobolus* sp., ×2000. N, nuclei of binucleate cell forming sporoplasm (SPO); PC, unicellular polar capsules, each producing a polar filament (PF); the spore wall is formed from 2 other cells. (From BAKER 1973.)

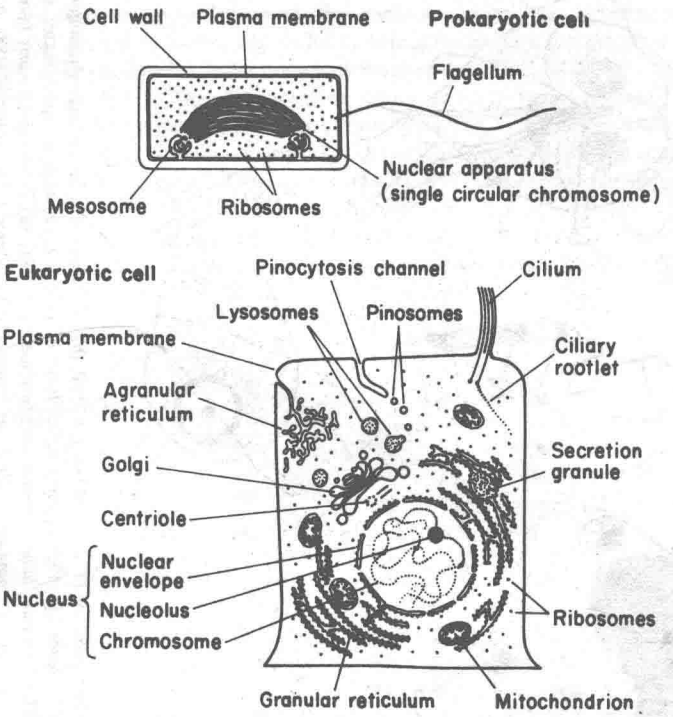

Fig. 1–6 Diagrams comparing structure of prokaryotic and eukaryotic cells; the former comprise bacteria and blue-green algae, the latter all other algae and higher plants, protozoa and multicellular animals: protozoa contain some or all of the structures shown – and others, including microfilaments and microtubules. (From Vickerman, K. and Cox, F.E.G., 1967, *The Protozoa*, Murray, London.)

Precise definition and discrimination is obviously impossible and probably unimportant; clearly there exists a continuum of life in which only division into more-or-less obvious levels of organization (virus, prokaryote, eukaryote) is strictly reasonable (even *Homo sapiens* is unicellular at one stage of the life cycle – the undivided egg). None-the-less, rather less comprehensive divisions, though increasingly arbitrary, are convenient and in general can be equated to more-or-less different levels or types of organization. Within the protozoa, these correspond to the phyla and lesser categories. All the protozoan phyla contain parasitic genera, the Apicomplexa, Microspora and Myxozoa consisting entirely of such forms. There are two major subdivisions of the Sarcomastigophora, depending mainly on the organism's method of locomotion – Mastigophora

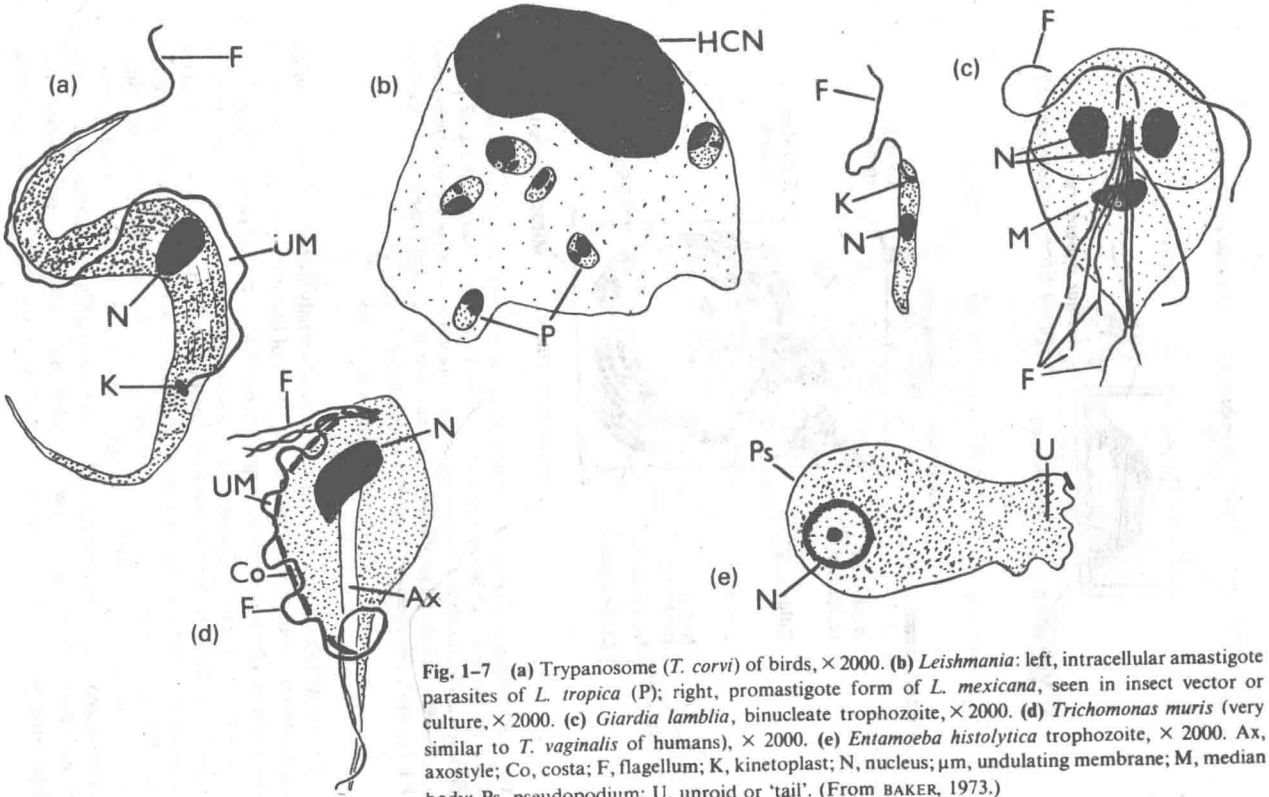

Fig. 1–7 **(a)** Trypanosome (*T. corvi*) of birds, × 2000. **(b)** *Leishmania*: left, intracellular amastigote parasites of *L. tropica* (P); right, promastigote form of *L. mexicana*, seen in insect vector or culture, × 2000. **(c)** *Giardia lamblia*, binucleate trophozoite, × 2000. **(d)** *Trichomonas muris* (very similar to *T. vaginalis* of humans), × 2000. **(e)** *Entamoeba histolytica* trophozoite, × 2000. Ax, axostyle; Co, costa; F, flagellum; K, kinetoplast; N, nucleus; μm, undulating membrane; M, median body; Ps, pseudopodium; U, unroid or 'tail'. (From BAKER, 1973.)

(flagellates) and Sarcodina (amoebae): the former includes important parasites of blood and other tissues (the trypanosomes and leishmanias, Figs. 1–7a and b) and others of the gut (*Giardia*, Fig. 1–7c) or urinogenital tract (*Trichomonas*, Fig. 1–7d), while the latter includes the notorious dysentery amoeba (*Entamoeba histolytica*, Fig. 1–7e). The Apicomplexa comprise three main groups – Gregarinia (Fig. 1–8a) (parasites mainly of invertebrates), Coccidia (including malaria parasites, Fig. 1–8b, and many other important forms) and Piroplasmia (Fig. 1–8c). The Ciliophora contains a vast number of species, some very highly organized, but relatively few parasitic. One (*Balantidium coli*, Fig. 1–9) can cause dysentery in man, and another interesting group lives in the rumen of ruminant, herbivorous mammals (Fig. 1–1); they are commensals which considerably benefit their hosts (see Table 1).

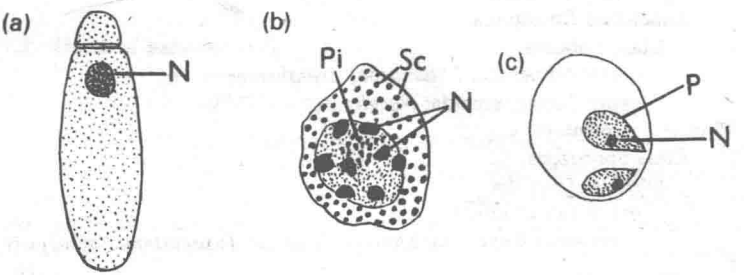

Fig. 1–8 (a) *Gregarina ovata*, a gregarine from the gut of an earwig, × 2000. N, nucleus. (b) Schizont (division stage) of *Plasmodium vivax* within human red blood cell, × 2000. N, nuclei; Pi, malarial pigment (undigested residue of host cell's haemoglobin); Sc, Schüffner's dots (lesions on surface of infected red cell). (c) Two piroplasms (*Babesia canis*) within red blood cell of dog, × 2000. N, nucleus of piroplasm, P. (From BAKER, 1973.)

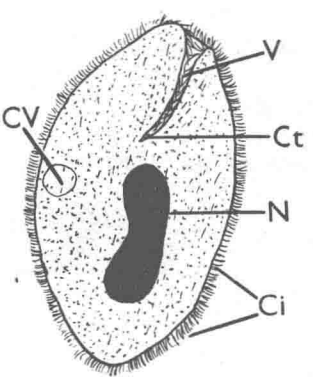

Fig. 1–9 *Balantidium coli* trophozoite, × 650. Ci, cilia; Ct, cytostome ('mouth') at base of vestibule (V); CV, contractile vacuole; N, macronucleus. (From BAKER, 1973.)

Table 1. Outline classification of some parasitic protozoa.

SUBKINGDOM PROTOZOA
- **Phylum Sarcomastigophora**
  - Subphylum Mastigophora
    - Class Zoomastigophorea
      - ORDER Kinetoplastida: *Leishmania, Trypanosoma*, etc.
      - ORDER Diplomonadida: *Giardia*, etc.
      - ORDER Trichomonadida: *Trichomonas, Histomonas*, etc.
      - ORDER Hypermastigida: *Lophomonas, Trichonympha*
  - Subphylum Opalinata
    - Class Opalinatea
      - ORDER Opalinida: *Opalina*
  - Subphylum Sarcodina
    - Superclass Rhizopoda
      - Class Lobosea
        - ORDER Amoebida: *Entamoeba, Acanthamoeba*, etc.
        - ORDER Schizopyrenida: *Naegleria*
- **Phylum Apicomplexa**
  - Class Sporozoea
    - Subclass Coccidia
      - ORDER Eucoccidiida
        - SUBORDER Eimeriina: *Eimeria, Isospora, Toxoplasma, Sarcocystis*, etc.
        - SUBORDER Haemosporina: *Plasmodium*, etc.
    - Subclass Piroplasmia
      - ORDER Piroplasmida: *Babesia, Theileria*, etc.
- **Phylum Microspora**
  - Class Microsporea
    - ORDER Microsporida: *Nosema*, etc.
- **Phylum Myxozoa**
  - Class Myxosporea
    - ORDER Bivalvulida: *Myxobolus*, etc.
- **Phylum Ciliophora**
  - Class Kinetofragminophorea
    - Subclass Vestibuliferia
      - ORDER Entodiniomorphida: *Entodinium*, etc. ('rumen ciliates')

2 Finding and Infecting the Host

This vital problem has been solved in several ways. The least specialized are the contaminative methods adopted by parasitic protozoa having only a single host species (*monoxenous* parasites), though these, involving a sojourn in the external environment, are the most hazardous solutions. Other parasites, with hosts of two different species (*dixenous* parasites), have developed more sophisticated means of transport from one host to another.

2.1 One-host parasites

2.1.1 Infection by ingestion

Most one-host parasitic protozoa enter the host by being swallowed. This entails protection from desiccation while in the external environment and from destruction by the acidic gastric juice while passing through the stomach. These two problems have been overcome by the development of resistant cysts enclosing a quiescent stage of the parasite. Cysts may be relatively simple proteinaceous structures such as those surrounding parasitic amoebae (e.g. *Entamoeba invadens*, Fig. 3–1) and flagellates (e.g. *Giardia lamblia*), or more complex many-layered structures like those protecting intestinal coccidia such as *Eimeria* species and *Toxoplasma gondii* (Fig. 2–1). Coccidians have a double

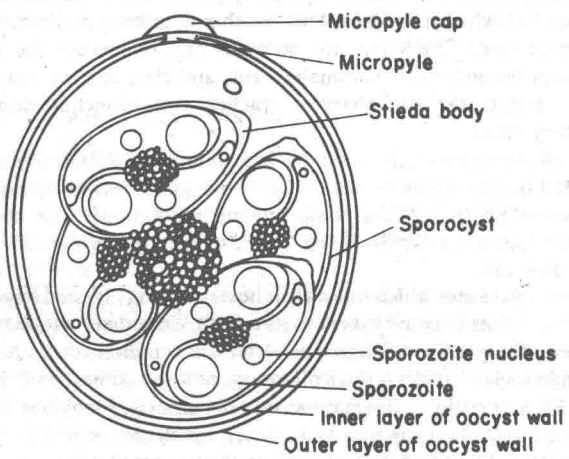

Fig. 2–1 Diagram of structure of oocyst of *Eimeria* (after Levine, 1973, *Protozoan Parasites of Domestic Animals and of Man*, 2nd ed. Burgess Publishing Co., Minneapolis.)

cyst – an outer oocyst containing two or more sporocysts; within the latter lie the infective sporozoites. The oocyst wall has two layers: the outer is a quinone-tanned protein and the inner is protein plus lipid. The resistance of this wall is shown by the fact that it is impermeable to aqueous solutions such as 2.5% potassium dichromate, a disinfectant in which the oocysts are often stored for many months for subsequent experimental use; the dichromate kills the bacteria present in the faeces containing the oocysts but, as it does not penetrate the oocyst wall, has no effect on the contained sporocysts or oocysts. Lipid solvents and gases such as ammonia, oxygen and carbon dioxide can, however, penetrate the wall. The oocyst has an aperture (micropyle), normally secured by a plug. After being swallowed, the cyst is either mechanically broken, for example, in the gizzard of a bird, or the plug is dissolved, perhaps by an enzyme secreted from within after stimulation by prolonged exposure to a high concentration of carbon dioxide in an animal's gut, at about 37°C. The liberated sporocysts are then unplugged (the plug is called the Stieda body) by a series of events involving bile salts and trypsin, in an alkaline environment at about 37°C, and the sporozoites actively wriggle out.

The cyst wall of *Entamoeba* is simpler, about 0.5 μm thick and made of a carbohydrate-protein complex. Excystation (hatching) seems to depend on active movement by the enclosed amoeba, stimulated by an alkaline environment deficient in oxygen such as occurs in a mammal's duodenum. Excystation of the flagellate *Giardia* occurs when the cyst, having been exposed briefly to very acid conditions (pH about 0.5 to 2, for 5 to 30 minutes), subsequently enters a neutral environment.

Thus none of these parasites would emerge from the cyst after being swallowed until the relatively friendly environment of the small intestine had been reached. The amoeba *Acanthamoeba castellanii*, which sometimes lives within human throats, secretes an alkaline protease to aid in its emergence from the cyst, but what stimulates it to do this is unknown. Presumably, as the amoeba is often free-living, the stimulus cannot involve the physiological conditions found in the mammalian gut; and this, in turn, may be why the parasite infects the nasopharynx, trachea and bronchus rather than the alimentary canal.

Not all parasites transmitted by ingestion need a cyst: *Pentatrichomonas hominis*, a harmless commensal of the human gut, does not encyst but succeeds in infecting up to 32% of some human populations. The usual textbook statement that it survives by being 'resistant' merely disguises our ignorance of how it does this.

One-host parasites which infect their hosts by being ingested rely on the host's unhygienic habits to convey them to its mouth, since their cysts leave the host in the latter's faeces. Food, fingers or water are common routes to a new host; many mammals also clean their offspring, or one another, by licking the anal region. Flies or cockroaches may assist in the process. Many one-host parasites have invertebrate hosts (insects and others), which they infect by being ingested, but even less is known of their physiology than our rather meagre knowledge of those with vertebrate hosts.

2.1.2 Infection by other routes

This is rare among one-host parasites. Noteworthy examples include *Trichomonas vaginalis*, which inhabits the human vagina and urethra, and *Tritrichomonas foetus*, which lives in the same organs of domestic cattle. (The spelling of the latter's generic name is not a misprint: most students of the group place the two organisms in separate genera, though both used to be referred to as *Trichomonas*.) Both these species are transmitted during coitus and (like the related *P. hominis*, referred to in section 2.1.1.), neither forms a cyst. *Trypanosoma equiperdum*, a parasite of horses in South America, Soviet Russia, Iran and probably North and South Africa, also relies on its hosts' sexual behaviour for transmission. The parasites live mainly in fluid-filled patches beneath the skin of the penis and vulva and are freed by abrasion of the skin during sexual intercourse. The gregarine *Monocystis* is also, probably, transmitted during sexual union of its earthworm hosts. Location of a new host is made easy for such venereally transmitted parasites. Two protozoa living harmlessly in the human mouth, *Trichomonas tenax* and *Entamoeba gingivalis*, also achieve transmission without needing a cyst, presumably during direct mouth-to-mouth contact (i.e., kissing).

2.2 Two-host parasites

Host location is made easier for these protozoa than it is for most of the one-host parasites, since they usually rely on the activity of one host (the annelid or arthropod vector) to find the other (vertebrate). However, the complexity of adaptation required to adjust their life cycles to those of two very different hosts must make them vulnerable to behavioural changes by either host – or to artificial human interference such as control of vector insects. It is this complexity which is largely responsible, also, for making these parasites such fascinating objects of study.

2.2.1 Infection by ingestion (carnivorism)

Probably quite a common route of entry to the vertebrate host is by the latter eating an infected vector. The parasites are released from the crushed prey in the predator's mouth and penetrate the buccal mucosa – sometimes perhaps through small scratches caused by the hard, chitinous exoskeleton of an ectoparasitic arthropod.

Many species of *Trypanosoma* can be transmitted in this way. Examples of those for which it is the normal route of infection include *T. lewisi* (of *Rattus* spp. and their fleas *Xenopsylla cheopis* and *Ceratophyllus fasciatus*) and probably other trypanosomes of the same subgenus (*Herpetosoma*), *T. corvi* of Corvidae (rooks, etc.) and louse flies (*Ornithomyia avicularia*), and *T. grayi* of African crocodiles (*Crocodilus niloticus*), transmitted by *Glossina palpalis*, a tsetse fly which lives dangerously by sucking blood from within the wide-open mouth of crocodiles basking in the sun and 'panting', like dogs, to cool themselves. The

human pathogen *T. cruzi* in South and Central America may well be transmitted thus to some of its rodent and other reservoir hosts, if they eat infected vectors (hemipteran bugs of the family Reduviidae). All these examples are trypanosomes which complete their development in the vector's hind gut (stercorarian development); probably, except for some trypanosomes of fish which are transmitted by ingestion of leeches (Hirudinea), this kind of development occurs in all groups of trypanosomes normally transmitted by ingestion of the vector. Other parasites, not normally transmitted carnivorally, may sometimes be. *Trypanosoma brucei* and *T. congolense*, of African ungulates, are normally transmitted by the bite of infected tsetse flies (*Glossina*: see section 2.2.4), but lions (*Panthera leo*) and other carnivores may become infected by eating infected vertebrate prey. *Toxoplasma gondii* may gain access to its hosts if they ingest infected prey (see sections 3.2.2 and 6.2).

2.2.2 Infection by ingestion (coprophagy)

This is really an adjunct of the previous type. *Rattus* species and other rodents may ingest faeces of fleas (Siphonaptera) containing infective metacyclic trypomastigotes of the trypanosome subgenus *Herpetosoma* while cleaning their fur, instead of eating the insects themselves. Birds can become infected similarly with *T. corvi* while cleaning their feathers. *Crocodilus niloticus* can presumably become infected if a defaecating *Glossina palpalis* deposits trypomastigotes of *T. grayi* while, or just after, feeding in the reptile's mouth. Other examples may await discovery.

2.2.3 Infection by ingestion (haematophagy)

Almost all two-host parasites infect their invertebrate vectors in this way, by being ingested with the vector's meal of blood taken from an infected vertebrate. The few exceptions include parasites such as *Leishmania tropica* which, as far as is known, does not circulate in the blood of its vertebrate host (man and other mammals), but is confined to a single sore on the skin. *L. tropica* presumably therefore relies, in order to get into its vector (*Phlebotomus* sp.), on a hungry insect probing through an infected area of skin when seeking its blood meal. Some parasitic protozoa have special stages in the vertebrate's blood destined to infect the vector. These may be sexual stages (gametocytes) in malaria parasites (*Plasmodium* spp.) and adeleine haemogregarines. Morphologically and physiologically differentiated asexual individuals play this role in some, if not all, species of *Trypanosoma* and, probably, some eimeriine haemogregarines (*Lankesterella, Schellackia*), the sporozoites of which are destined for passive transfer to a new vertebrate host by an ectoparasitic mite (Acarina). Other parasites are not known to produce special forms to infect vectors (e.g. *Leishmania* and *Babesia*). However, this assertion may merely reflect current ignorance (especially with *Babesia*).

Protozoa have not generally developed the degree of synchronization between their life cycles and their vector's feeding behaviour that certain filarial