Chapter 7 / Physiology and Nutrition

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7.1 INTRODUCTION

In this chapter, a broad-brush view of parasite physiology is painted, a landscape rather than a portrait, in the school of Cézanne not Gainsborough; the aim is to leave the reader with an impression of this exceedingly broad topic at the inevitable expense of detail. Particular attention will be paid to the ways in which the physiological attributes of parasites impinge on applied aspects of the discipline and which may contribute ultimately to the regulation of parasitic diseases by human intervention.

Parasites possess unique physiological attributes that are a consequence of living in a hostile and ever-changing environment, the body of the host, with the associated necessity of passing through the external environment, possibly on more than one occasion in a single life cycle. It will become apparent to the student of parasitology that an understanding of parasite physiology can also make a fundamental contribution to parasite control.

7.2 SURVIVAL OUTSIDE THE BODY OF THE HOST AND TRANSMISSION

Many parasites release stages of their life cycles into the external environment, these include eggs, cysts and larvae. Such transmission phases are sometimes short-lived but they may possess considerable longevity and the ability to resist environmental degradation.

Parasite transmission between hosts is accomplished in one of three ways:

1 The host ingests eggs, cysts, larvae or an intermediate host containing infective stages of the parasite.

2 The host is inoculated with infective parasites during blood feeding activities of the intermediate (vector) host.

3 The host is actively sought and penetrated or settled by invasive parasite (see Table 7.1).

7.2.1 Cystic stages of parasites

Protozoa parasitic in the alimentary canal, such as *Entamoeba histolytica*, *Giardia duodenalis* and *Balantidium coli*, commonly produce cysts that contain quiescent, infective forms which are passed into the external environment with host faeces and await ingestion by the next individual host. *Naegleria fowleri* is a free-living organism which can cause human amoebic meningitis. It is acquired during bathing in, or by contact with, warm natural freshwaters. This organism, an opportunistic parasite, occurs in three forms, trophozoite, cyst and flagellate but in this case, the trophozoite and not the cyst is the infective stage, entering the human body via the nasal mucosa and migrating to the brain. Coccidian protozoans form resistant, infective cysts. Species of economic importance, such as *Eimeria* in poultry and *Toxoplasma* in cats, produce oocysts containing sporocysts which are voided in host faeces and are acquired by a new host during feeding.

Amongst the helminth parasites, encysted stages concerned with transmission are common. In the Digenea, cysts containing the metacercarial stage may be found either in the external environment (e.g. on vegetation in the liver fluke, *Fasciola hepatica*) or within the body of an intermediate host (e.g. *Opisthorchis sinensis* in freshwater fish). These cysts are complex in structure and serve as resistant hypobiotic stages in the parasite life cycle; the conditions for excystation

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Table 7.1 Transmission stages of parasites: a synopsis

	Parasite eaten by host	Arthropod vector transmits parasite	Parasite actively locates host
Protozoa	Coccidian cysts Amoebic cysts	Babesias Trypanosomes Malaria	–
Monogenea	–	–	Oncomiracidium
Digenea	Metacercaria Cercaria (some)	–	Miracidium Cercaria
Cestoda	Egg Cysticercoid Cysticercus Coenurus Hydatid cyst Coracidium Procercoid Plerocercoid	–	–
Acanthocephala	Egg Cystacanth	–	–
Nematoda	Egg Larvae (L_3) (ensheathed or free)	Larvae (microfilariae)	Larvae (e.g. hookworms)

are described later in this chapter (Section 7.3.2).

The encysted larvae of tapeworms are found only within the body of an intermediate host animal. These cysts may contain a single parasite larva (e.g. cysticercus) or contain an increasing number of larval worms produced by asexual internal budding of germinal tissue within the cyst itself (e.g. hydatid cyst of *Echinococcus granulosus*).

Several genera of nematode parasites form cysts involved in transmission. *Trichinella spiralis* larvae encyst in mammalian muscle and the bird gapeworm *Syngamus trachea* may encyst in the haemocoel of the invertebrate transport host. Plant parasitic nematodes produce cysts with remarkable capacity to avoid environmental desiccation: *Heterodera rostochiensis* can survive for up to 8 years within the cyst.

The life cycle of acanthocephalans typically includes an encysted cystacanth stage which resides within the haemocoel of the arthropod intermediate host; a second phase of encystment may take place if this host is ingested by a transport host in which development of the parasite to maturity cannot be achieved.

7.2.2 Helminth eggs

The shelled egg of helminth parasites is ideally suited as a transmission stage and, by virtue of the physico-chemical properties of its resistant shell, is able to withstand the rigours of the external environment for considerable periods of time.

The digenean egg is formed from 30 to 40 vitelline cells and a fertilized ovum surrounded by a protein shell. The phenol oxidase system of enzymes carries out complex cross-linking of these proteins in a process known as tanning. Early studies on *Fasciola* led to the suggestion that all digenean eggshells comprised tanned sclerotin formed by quinone tanning. More recent information, however, indicates that a variety of structural cross-linked proteins may confer upon the egg its rigidity and resistant properties, including sclerotin, keratin and elastin. In schistosomes, use of recombinant DNA techniques has identified female-specific gene products associated with eggshell production; vitelline proteins are rich in certain amino acids (glycine, tyrosine, aspartic acid, lysine and histidine) agreeing with

amino acid analysis data for the shell itself. The vitelline peptides of *Fasciola* are rich in dihydroxyphenylalanine (DOPA), tyrosine, aspartate or asparagine, glycine and lysine or arginine. Tyrosine is thought to be converted to DOPA at the post-translation stage before these vitelline proteins are exported from the vitelline glands. Therefore, the mechanical strength of the eggshell derives from tanning of the tyrosine-rich vitelline proteins, involving DOPA formation and subsequent quinone production by phenol oxidase to yield cross-linked proteins. DOPA-rich proteins are common components of biological 'glues' in a variety of free-living animals.

The eggshells of parasitic nematodes are different from those of platyhelminth parasites, being generally more complex in construction and containing non-proteinaceous structural components like chitin. This polymer of *N*-acetyl glucosamine is structurally important in fungi and in the exoskeleton of many invertebrates. Nematode eggshells are typically triple-layered comprising an inner lipid layer, a middle chitinous layer and an outer proteinaceous vitelline layer. Some nematodes, including the filarial nematodes, are ovoviviparous and do not release the egg from the uterus; even in these forms, with a much diminished eggshell, chitin is still present but its role is in doubt.

7.2.3 Mechanisms for locating the host

Active location of the intermediate or definitive host is carried out by a variety of larval helminth parasites including monogenean oncomiracidia, digenean miracidia and cercariae, cestode coracidia and infective L_3 nematode larvae. With the exception of the coracidium, all of these different larvae actively seek out and either attach to or penetrate the next host in the life cycle. The physiological mechanisms underlying the processes of finding a suitable host remain largely mysterious.

Monogenea

Chemotaxis is an essential feature of host finding in the external flukes. Data on a monogenean parasite of European marine flatfish, *Entobdella soleae*, reveals that the oncomiracidium displays a marked preference for the sole (*Solea solea*) which is mediated, in experimental conditions, by chemical recognition of the fish skin.

Digenea

Two distinct types of larva are involved in active location of the host in the Digenea – the miracidium and the cercaria. The miracidium emerges from the egg in water as a ciliated free-swimming stage of limited life expectancy whose endeavour is to locate and penetrate a suitable mollusc. The cercaria, also short-lived, is released from the snail and may either crawl or swim in water to locate and establish in the next host, as in the schistosomes, or it may encyst on vegetation and await ingestion (e.g. *Fasciola hepatica*).

Experimental studies on these larval stages have failed to reveal the nature of host-finding mechanisms. Many miracidia have sense organs, such as eye-spots and surface papillae, which may help to orientate the larva in its environment so as to bring it in close proximity to its host mollusc. Accordingly, miracidia react in various ways to environmental stimuli such as light, temperature, gravity, water currents and changes in carbon dioxide tension (Pco_2). Chemotactic recognition of the host may occur but is controversial and while some evidence supports miracidial attraction to chemicals released from snails, possibly in mucus or faeces, other data favours the hypothesis that miracidia locate snails by a random, trial-and-error process. Chemoattraction may also be important for host finding by cercariae but, again, data are both limited and controversial.

The ability of cercariae to establish in the next host is clearly affected by water flow, a fact that may have important medical and commercial implications. Cercariae of *Schistosoma mansoni* are adversely affected by water turbulence and they are unable to penetrate even under conditions in which their physical integrity remains unaltered. Whether it is host finding or attachment to the host that is altered remains to be discovered, but the data strongly suggest that one way of reducing the transmission of aquatic

parasites with free-swimming larvae is to increase water velocity where practicable.

Cestoda

Pseudophyllidean tapeworms possess a free-swimming, ciliated larva, the coracidium. This larva hatches from the egg in freshwater and is eaten by copepods; transmission here must be regarded as a passive process.

Nematoda

Chemical attraction is probably an important component of host-finding in many plant and animal parasitic nematodes. The infective L_3 larvae are well-provided with sensory structures and reveal a complex behavioural capacity associated with locating their hosts.

7.2.4 Entry mechanisms

Many parasites gain entry to their hosts by active penetration through the epidermis (cercariae, miracidia and some nematode larvae, such as hookworms).

Penetration of a suitable snail by the digenean miracidium is brought about by secretions from the complex of apical glands at the anterior end of the larva. These secretions contain both lubricants and lytic enzymes. During the process of snail penetration some miracidia shed their epidermal ciliated plates (e.g. *F. hepatica*) while in others cilia are retained.

The penetration of mammalian skin by schistosome cercariae is initiated by surface lipids of the host. Some non-essential fatty acids and complex skin lipids will induce the 'penetration response' in cercariae whereby the tail is shed and transformation to the schistosomulum stage commences. Experimentally, these effects can be inhibited by topical application of eicosanoids and eicosanoid-like substances, related to the prostaglandins. Both cercariae and mammalian skin can produce eicosanoids and these compounds may interact during the process of skin penetration (Fig. 7.1). Moreover, it is postulated that cercarial eicosanoids may act as immunomodulators and protect the penetrating larvae from host attack.

The infective larvae of hookworms and related nematodes actively penetrate host skin probably using lytic enzymes to aid in the process.

7.2.5 Heat shock proteins

A great many parasites experience, during the passage of their life cycle, dramatic changes in

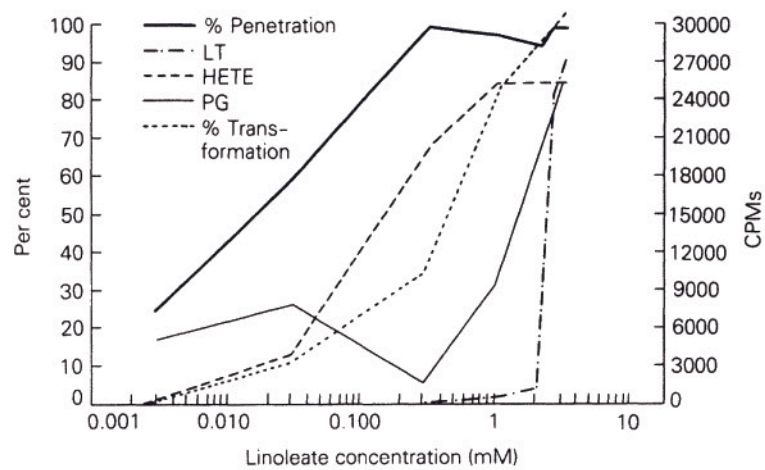

Fig. 7.1 Effects of linoleic acid on penetration through an artificial membrane and on transformation of cercariae of *Schistosoma mansoni*. The left-hand y axis depicts percentage penetration and percentage transformation of cercariae. The right-hand y axis depicts the concentration of cercarial prostaglandin (PG), leukotriene (LT) and hydroxyeicosatetraenoic acid (HETE) in response to elevated linoleic acid levels. (Data from Salafsky & Fusco, 1987.)

ambient temperature. Such changes may occur during the process of host entry, as when an egg or cyst is ingested, an active larva penetrates the skin or when an invertebrate or other cold-blooded animal introduces the infective parasite to the host bird or mammal. Such temperature changes will tend to be metabolically and physiologically harmful to the invading parasite. Accordingly, parasites can synthesize heat shock proteins (HSP) in response to temperature changes in much the same way as do free-living organisms. Heat shock protein production may be prolific, and in schistosomes HSP-70 represents more than 1% of the total protein synthesized by adult worms and is even more abundant in the transforming schistosomulum.

As a group, HSP are highly conserved molecules of diverse function, classified according to molecular weight and encoded by multigene families. In parasites, they are highly immunogenic and may not always be produced in response to temperature change. Increase in temperature induces HSP synthesis in *Leishmania* promastigotes, *Giardia* trophozoites, *Trypanosoma* trypomastigotes, *Naegleria* trophozoites, infective larvae of *Brugia* and schistosomula of *Schistosoma*. By contrast, schistosome cercariae placed in mammalian cell culture media at 23°C produce high levels of HSP when all other protein synthesis has been down-regulated, but they do not respond when placed in water at 37°C. The physiological function of the schistosome HSP-70 is unknown but it may be involved in the reshaping of the macromolecular architecture of the parasite as it moves from one environment to another. Heat shock proteins are also involved in the repair of faulty protein synthesis associated with the ageing process and in this way may be responsible for the considerable longevity typical of some parasites.

7.2.6 Circadian rhythms and parasite transmission

Transmission of some parasites to a new host may be associated with daily or circadian (i.e. around 24 hours) cycles. These can be classified as follows:

1 synchronous cell division (e.g. malaria parasites);
2 release of infective stages from:
  (a) final host (e.g. coccidia, pinworms),
  (b) intermediate host (e.g. schistosome, cercariae);
3 migratory patterns (e.g. trypanosomes, microfilariae).

Synchronous cell division

Asexual reproduction, termed schizogony, is profoundly periodic in occurrence in malaria parasites. Cell division within the red blood cell takes place every 24 hours in *Plasmodium knowlesi*, 48 hours in *P. vivax* or 72 hours in *P. malariae*. This periodicity is related to the production of gametocytes in the blood, which are infective to the mosquito vector. Maturation of gametocytes appears to occur at a time of day coincident with the feeding activities of the appropriate species of mosquito. Circadian rhythms of this type are possibly entrained to the daily cycle in body temperature of the homoiothermic hosts; experimentally induced hypothermia in monkeys will disrupt the circadian pattern of malarial development.

Circadian release of infective parasites

There are several examples of the circadian release of infective parasites, timed so as to optimize transmission. Amongst the Protozoa, *Isospora* oocysts are released from the gut of infected birds at roosting time each day (i.e. late afternoon). This may greatly increase the chances of uninfected birds acquiring the oocysts during feeding.

Mammalian pinworms (e.g. *Syphacia muris* in rats and *Enterobius vermicularis* in humans) migrate diurnally from the rectum to the perianal region to lay their eggs. This migration by female worms is related to a lowering of rectal temperature during sleep and it enables the inadvertent 'hand-to-mouth' transmission of parasite eggs to occur without faecal contamination.

The cercariae of many digeneans are released into water from snails at specific times of the day coinciding with the presence of the appropriate new host. This phenomenon has been well-

studied for schistosomes, where each species exhibits unique characteristics in the chronology of cercarial shedding. The majority of schistosome species are strictly circadian in their behaviour having a single peak of release each day. *Schistosoma mansoni*, *S. haematobium*, and certain strains of *S. japonicum* and *S. bovis*, all release cercariae during the day, while *S. rodhaini* and other strains of *S. japonicum* release cercariae at night. Of the factors that may influence the periodicity of cercarial shedding from snails, the most important are environmental light and temperature cycles. This has been confirmed by experimental manipulations of light period and temperature: reversal of photoperiod rapidly causes a reversal of the pattern of cercarial shedding and, while alterations of the relative lengths of light and dark periods may be influential, the emission wavelength of light is unimportant. Thermoperiod plays a less significant role than does photoperiod. Human schistosomes tend to shed cercariae during the day, *S. rodhaini*, a parasite of wild rodents, sheds cercariae at night and *S. margrebowiei*, infecting antelope which drink at dawn and dusk, has two peaks of cercarial emission which coincide with the appearance of the potential host animals.

Circadian migratory activities

Some parasites that inhabit deep internal tissues of the host and are transmitted by surface-biting invertebrate vectors are faced with the problem of enabling their infective stages to reach superficial tissues at the time when the vector is present and feeding. As a consequence, we see amongst certain parasites elegant physiological adaptations to facilitate transmission under such circumstances. The microfilarial larvae of the filarial nematodes are well-researched examples of this phenomenon. The adult worms reside in the lymphatic system (e.g. *Wuchereria bancrofti*) or in thick nodules in the skin and subdermal regions (e.g. *Onchocerca volvulus*) while their larvae are transmitted by mosquitoes or simuliid blackflies respectively, both of which feed on peripheral blood. To optimize infection of the vector, many filarial nematodes have developed diurnal rhythms in migration of their larvae. These patterns can be classified accordingly and are related to the feeding activities of the insect species concerned with transmission:

1 microfilariae numerous in host peripheral blood at night only, absent by day (e.g. *W. bancrofti*, *Brugia malayi*);

2 microfilariae numerous in peripheral blood by day only, absent by night (e.g. the eye worm, *Loa loa*);

3 microfilariae more numerous in peripheral blood during the evening (e.g. the heart worm, *Dirofilaria immitis*);

4 microfilariae present in peripheral blood for entire 24 hour period, but more numerous in the afternoon (e.g. Pacific form of *W. bancrofti*).

When the microfilariae are not in the peripheral blood they accumulate within the pulmonary circulation at the capillary junctions of arterioles and venules. The difference in oxygen tension (Po_2) between arterial and venous blood at these junctions is the initiating trigger for the diurnal migration of the larvae. When the Po_2 is in excess of 55 mmHg the larvae of *W. bancrofti* accumulate in the pulmonary circulation; they emerge and migrate to the peripheral circulation when the Po_2 decreases to 47 mmHg or below (see Fig. 7.2 & Table 7.2).

7.3 ESTABLISHMENT AND SURVIVAL WITHIN THE HOST

After locating and gaining entry into a suitable host, the parasite has to become established in a physiologically suitable microhabitat in order to grow, either to sexual maturity or to an intermediate stage, such as another larva, whose continued development occurs in the next host. Parasite establishment and growth require that a complex series of physiological conditions are met. These are summarized in Fig. 7.3.

7.3.1 Transformation of digenean cercariae

During attachment and subsequent penetration of the host epidermis, cercariae undergo a physiological transformation in which they rapidly become adapted to the conditions within the host body. Transformation has been studied in some detail in schistosomes but in few other digenean

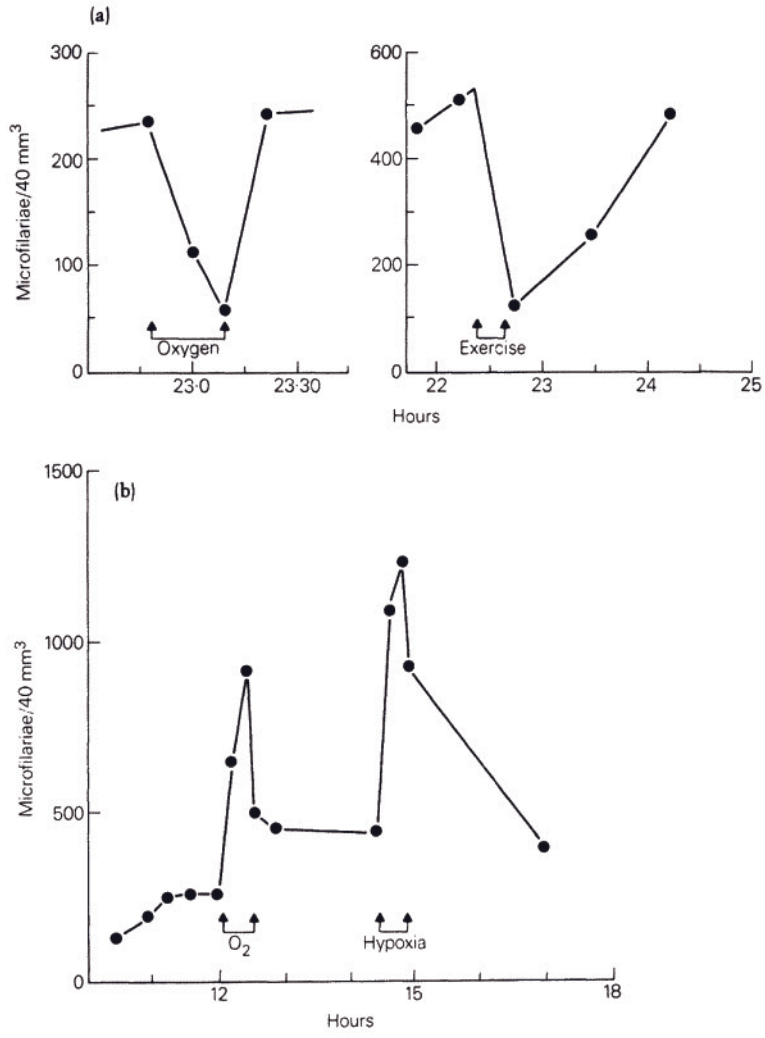

Fig. 7.2 The distribution of microfilariae in the host according to the state of oxygenation. (a) *Wuchereria bancrofti* in humans – both breathing oxygen and vigorous exercise produce a high venous-arterial difference in blood oxygen tension and microfilariae migrate away from the peripheral circulation. (b) *Dirofilaria repens* in dogs. In all graphs, the curves represent the microfilarial count in peripheral blood obtained by conventional skin-shipping and direct counting. (Data from Hawking, 1975).

Table 7.2 The effects of oxygen tension in human venous and arterial blood on the distribution of *Wuchereria bancrofti* microfilariae. (Based on data in Hawking, 1975.)

Activity of host	Arterial Po_2 (mmHg)	Venous Po_2 (mmHg)	Venous–arterial difference (mmHg)	Microfilarial distribution
Resting (day)	95	40	55	Lungs
Sleeping (night)	85–90	43	42–47	Peripheral blood
Breathing 100% O_2	640	53	587	Lungs
Breathing 14% O_2	51	32	19	Peripheral blood (partial release)
Vigorous exercise	91	20	71	Lungs

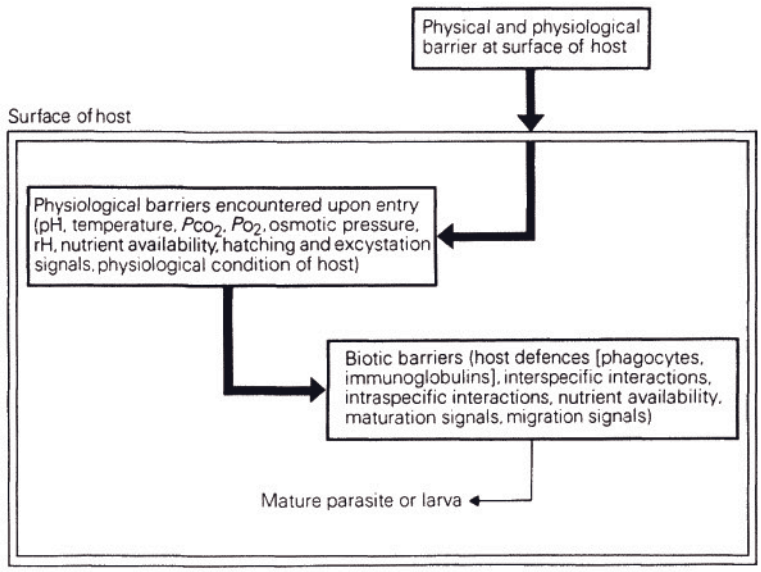

Fig. 7.3 Barriers to establishment encountered by invading parasites.

parasites. The schistosome cercaria is adapted for an existence in freshwater at 25°C in which it swims vigorously, gaining motility from its tail. Once attached to mammalian skin, the tail is lost and penetration commences. It was thought that tail-loss signalled cercarial transformation to the schistosomulum but cercariae with their tails intact will also transform in an isotonic medium at 37°C. On skin contact, the cercaria secretes a powerful protease from its preacetabular glands and morphological changes in its tegument are initiated. Over a period of just a few hours the schistosome cercaria transforms into a migratory larva, the schistosomulum. These two larvae are metabolically distinct, the cercaria is aerobic and the schistosomulum produces lactate anaerobically; they possess morphologically and antigenically diverse surfaces; and they show osmotic and thermal restriction in their tolerance. The dramatic modifications to the worm surface reflect the need for the parasite to defend itself against host immune attack, to which end it can bind host molecules to effect immunological disguise, and also acquire the ability to transport nutrients transtegumentally (see Section 7.8.3). The major features that accompany schistosome transformation are summarized in Table 7.3 and it is probable that these events are common to many species of digenean. Differences that occur may depend on whether the host invaded is a poikilotherm or a homoiotherm.

7.3.2 Hatching and excystation

Many parasites enter their hosts encapsulated either within cysts or within egg membranes (see Sections 7.2.1 & 7.2.2). Such parasites inevitably enter the host via the alimentary canal, within which they become activated and then liberated from these capsules prior to further development. Not all parasites that gain entry to their host by being eaten are encapsulated; for instance, many helminth larvae reside in the tissues of an intermediate host free from a cyst (e.g. metacercariae of some strigeid digeneans, pseudophyllidean pro- and plerocercoids and many nematode larvae). It is not clear why some intermediate stages of parasites form cysts and others do not; in part, it may reflect the nature of the host response to the parasite since many cysts are made up of contributions from both parasite and host.

Protozoa

Activation and excystation of protozoan cysts has been examined *in vitro* for a relatively small number of species (Table 7.4).

Optimum experimental conditions include tem-

Table 7.3 Transformation of the schistosome cercaria. (Data from Wilson, 1987.)

Condition	Cercaria	Schistosomulum
Habitat	Freshwater	Body of bird or mammal
Temperature	25°C	37–41°C
Motility	Swims using tail	Crawling or burrowing
Glands	Pre-, postacetabular and head glands full: secrete contents on skin contact	Glands empty
Tegument	Trilaminate surface membrane; extensive glycocalyx	Heptalaminate surface membrane: reduced glycocalyx
Permeability	Impermeable to nutrients	Permeable to nutrients
Osmotic tolerance	Survives in water, dies in complex media	Intolerant of freshwater
Energy metabolism	Oxidative, cyanide-sensitive	Anaerobic, cyanide-insensitive
End-products	$CO_2 + H_2O$	Lactate (after 24 hours)
Surface immunochemistry	Antigenically simple	Antigenically complex

Table 7.4 Conditions for *in vitro* excystation of some protozoan parasites. (Data from Lackie, 1975.)

Species	Temperature (°C)	pH	Gas phase	Enzymes added	Bile	Host
Entamoeba histolytica	37	–	Air or anaerobic	Reducing agents	–	Man
E. invadens	8–24	–	–	–	–	Reptiles
Eimeria bovis	40	7.5–8.5	Air or 50% Air/CO_2	Trypsin + reducing agents	1%	Cattle
E. tenella	37–41	7.6	Air or CO_2	Trypsin, HCO_3^-, pancreatin	Present	Poultry
Cystoisospora canis	22–37	–	Air or CO_2	Trypsin	0.5%	Dogs

perature increase, if the parasite is invading a homoiotherm, neutral pH, low P_{O_2}, high P_{CO_2} and the presence of reducing potential. Activation of the parasite within the cyst may be distinct from excystation, the former depending upon high P_{CO_2} and the latter requiring proteolytic enzyme action. In the Coccidia, for example *Eimeria* and *Isospora*, excystation of the sporocyst after its release from the oocyst can involve the breakdown of a localized region of the cyst wall – the Stieda body – by the action of bile salts and trypsin.

Digenea

The eggs of the majority of digeneans hatch in water under suitable environmental conditions of light, salinity and temperature. The eggs of some other digeneans are ingested by molluscs and hatch in the snail gut.

The operculate eggs of *Fasciola hepatica* hatch under the influence of light, and in fact, specific wavelength may be important. Light appears to stimulate activity in the miracidium resulting in permeability changes to the viscous cushion just

below the operculum, the hydration of which may force off the operculum allowing the parasite to escape. Schistosome eggs do not have an operculum and the larval parasite emerges on rupture of the eggshell; osmotic pressure is the major physiological effector for the hatching of schistosome eggs, such that a rapid decrease in osmotic pressure, as would be experienced when the egg is passed into freshwater, triggers hatching. The influence of light and ambient temperature are of less significance. In some digeneans, activation may involve the production of lytic enzymes that contribute to the process of excystation.

The metacercariae of many digeneans are enclosed within cysts whose walls vary in specific architecture and dimension. For those species that invade birds or mammals and whose cysts are thin-walled, excystation is initiated by the elevation of ambient temperature alone. Excystation in the laboratory of the more complex metacercarial cysts requires the action of serial treatment with pepsin followed by trypsin as well as temperature changes; bile salts may also play an important role in this process (e.g. *F. hepatica*). Within the Digenea, excystation initiators vary according to species; often certain of these factors activate the encysted larva itself and then a combination of external and internal factors contribute to the final emergence of the parasite. The initiators for digenean excystation include temperature, pH, redox potential, P_{O_2}, P_{CO_2}, osmotic pressure, bile salts and inorganic ions.

Cestoda

The eggs of many tapeworm species hatch in the external environment upon receipt of, and in response to, suitable stimuli. By contrast, the eggs of the Cyclophyllidea hatch in the gut after ingestion by the invertebrate or vertebrate host. The cyclophyllidean egg has a thin outer capsule but the oncosphere larva is enclosed within a thick, protective embryophore. Hatching of the eggs of taeniid tapeworms is a biphasic process whereby the hexacanth larva is first activated, bringing about disruption of the onchospheral membrane, and digestion of the outer capsule is completed by the action of host proteolytic enzymes. In non-taeniid cyclophyllideans, hatching is largely a mechanical process due to the action of the host mouthparts on the eggshell. Hatching of these eggs can be accomplished *in vitro* in simple physiological saline. In *Hymenolepis* species, however, onchosphere activation requires high P_{CO_2} and the presence of bicarbonate ions and digestive enzymes. Hatching of the taeniid egg requires exogenous pepsin (e.g. *Taenia saginata*) or pancreatin (e.g. *T. pisiformis*) and activation of the encapsulated larva is influenced by host bile salts.

Acanthocephala

Activation of the cystacanth larva takes place on temperature elevation (if a parasite of homoiotherms) and bile salts may also play a role (e.g. *Moniliformis dubius*). Excystation is due either to mechanical (e.g. *Polymorphus minutus*) or enzymic (e.g. *M. dubius*) disruption of the thin outer capsule. The egg (or shelled acanthor larva) hatches in the midgut of arthropods. Hatching *in vitro* depends upon ionic strength of the medium employed, the concentration of bicarbonate ions and pH. Enzymes, such as insect chitinases are without effect.

Nematoda

The eggs of many nematodes hatch in the external environment to release either infective larvae or larvae that develop to the infective stage (e.g. *Ancylostoma*, *Nippostrongylus*). Upon receipt of the appropriate environmental stimuli, including water, and changes in temperature or oxygen, the enclosed larva liberates lytic hatching enzymes whose action may increase water uptake into the egg. Hatching may be induced therefore by increased turgor pressure within the egg (e.g. *Trichostrongylus*).

The eggs of other parasitic nematodes hatch only after ingestion by a suitable host (e.g. *Ascaris*, *Toxocara*). *Ascaris* eggs hatch *in vitro* at 37 °C in media with high P_{CO_2}, and neutral pH containing bicarbonate ions and reducing factors. The larva within the egg is activated to produce a hatching fluid comprising enzymes capable of digesting the ascaroside and chitinous layers of the eggshell.

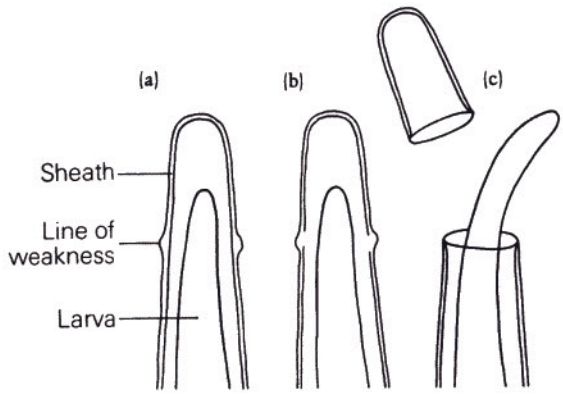

Fig. 7.4 Exsheathment in infective trichostrongyle larvae (e.g. *Haemonchus*). (a) A line of cuticular weakness is developed by localized anterior swelling of sheath. (b) Digestion of inner layers of line of weakness. (c) Rupture of sheath along line of weakness and release of larva. (After Lee & Atkinson, 1976.)

Trichostrongyle L_3 larvae are enclosed within a sheath formed from the cuticle of the second larval ecdysis; exsheathment takes place within the gut of a suitable host under the influence of carbon dioxide, bicarbonate ions, reducing agents at neutral pH and at the appropriate temperature. The larval parasite is induced, by these environmental stimuli, to produce an exsheathing fluid that contains enzymes for disruption of the sheath to allow the infective larva to emerge (Fig. 7.4).

7.3.3 The role of bile salts in parasite establishment

Bile, a complex mixture of organic acids, is released into the upper duodenum of vertebrates via the bile duct. Bile contains bile salts, which are steroid-like molecules based on cholic acid, bile acids, which are the degradation products of red blood cells, and bicarbonate ions. Bile salts can be of considerable significance in determining host specificity of many parasites. They affect parasites in a number of ways including: (1) membrane permeability; (2) activation of encysted forms; (3) lysis of parasite surface membranes; (4) synergism with host digestive enzymes; and (5) metabolic action.

One way in which bile salts may act as determinants of host specificity in parasitism is exemplified by studies on the hydatid organism, *Echinococcus granulosus* (Table 7.5). Larval protoscoleces, removed from the hydatid cyst, respond in various ways to bile from different animals *in vitro*: bile rich in deoxycholic acid is lytic to the protoscoleces whereas bile from dogs or other carnivores, the natural hosts for *E. granulosus*, is low in this particular bile acid. The lytic action of bile salts from unsuitable hosts provides a possible mechanism of host specificity at the physiological level.

Table 7.5 *In vitro* survival of protoscoleces of *Echinococcus granulosus* in 10% bile from various vertebrates. (Data obtained from curves by Smyth & Haslewood, 1965.)

Survival time of protoscoleces (days)	Percentage survival (source of bile)				
	Dog	Sheep	Pig	Ox	Fish
1	100	100	100	100	100
2	100	100	100	100	75
3	100	95	88	92	–
4	100	80	52	36	0

Additionally, bile salts can affect both establishing and established parasites. Experimental cannulation of the bile duct of rats infected with the tapeworm *Hymenolepis diminuta* brings about a reduction in size and fecundity of the worm whereas cannulation prior to infection inhibits establishment.

Bile salts activate and initiate excystation in many parasites including protozoans (e.g. *Eimeria*), digeneans (e.g. *Fasciola*), cestodes (e.g. *Taenia pisiformis*), and acanthocephalans (e.g. *Moniliformis*, *Polymorphus*). The physiology of these events is not well-understood.

7.3.4 Hypobiosis

The condition under which animals may become quiescent during their development is termed hypobiosis. Periods of arrested development may occur in free-living animals that inhabit arid or otherwise intemperate regions and the hypobiotic state is therefore adopted in response to climatic extremes. In the case of parasites, hypobiosis

occurs not infrequently; immature stages, encapsulated within eggs or cysts or free within host tissues, are hypobiotic states whereby the parasite becomes arrested in its development and awaits a suitable trigger to initiate the continuation of its development to adulthood. Hypobiosis may represent a serious problem in commercial terms when we consider some of the nematode parasites of cattle.

Arrested larval development (ALD) is a feature of many of the major parasitic diseases of cattle caused by trichostrongyles including *Dictyocaulus viviparus* (husk or lungworm), *Ostertagia ostertagi*, *Haemonchus contortus*, *Trichostrongylus axei*, *Oesophagostomum radiatum* and *Cooperia* species (collectively referred to as the agents of 'parasitic gastro-enteritis'). Sheep or cattle become infected with these parasites by ingesting the L_3 larvae during grazing; the larvae enter the gut and develop to adults within 21 days, either in the lungs, abomasum or small intestine, depending upon the species. This pattern of development can become disrupted under certain conditions and populations of arrested early L_4 larvae occur, usually within the abomasal glands of the stomach. The factors that trigger ALD in these parasites have not been identified but, host immunity, season of the year and parasite population size may all be involved. Arrested larvae will begin to develop in conditions of experimental or natural immunodepression (e.g. during parturition) and following anthelmintic treatment to remove crowded adult worm infections. There is a wealth of evidence that ALD in trichostrongyles occurs on a seasonal basis, being prevalent in autumn in the northern hemisphere and spring in the southern hemisphere. Arrested larvae resume their development and become adult worms at the start of the next grazing season. Artificial induction of ALD has been accomplished by conditioning parasite L_3 larvae in a climatic chamber; a large proportion of these 'autumn conditioned' L_3s become hypobiotic in naive calves. Chilling parasite larvae to 4°C for 5–10 weeks achieves similar effects. The factors that trigger the resumption of development of arrested nematode larvae are unknown.

Arrested larval development (Fig. 7.5) may be seen, therefore, as a response by L_3 larvae to external environmental stimuli, such as lowering of ambient temperature or the onset of a hot dry summer period, or to host-mediated stimuli, such as immunocompetence. Moreover, these hypobiotic larvae may also be drug resistant and many anthelmintic drugs are ineffective against them. Thus, hypobiosis in trichostrongyles represents a sophisticated adaptation to environmental adversity through physiological responses to complex stimuli.

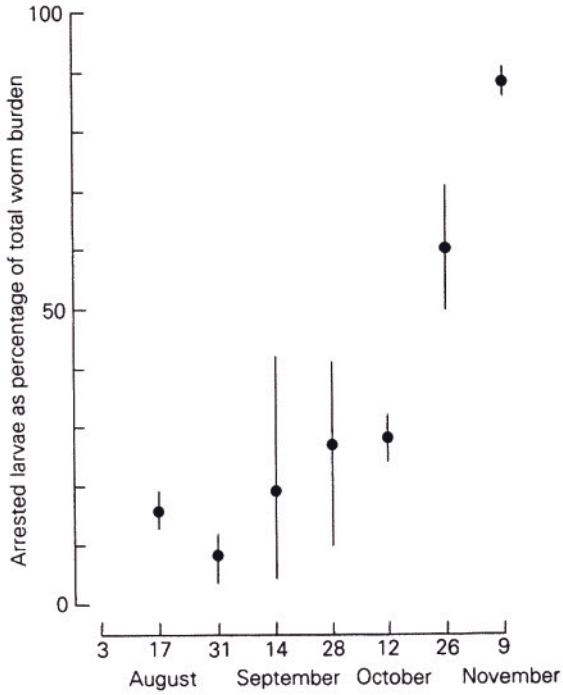

Fig. 7.5 Change in proportion of arrested larvae of *Ostertagia* in calves from the west of Scotland between late summer and autumn. Data are mean and ranges of percentage of arrested L_4s detected at autopsy 7–14 days after removal from contaminated pasture. (Data from Armour & Duncan, 1987.)

Nematodes also exhibit a variety of additional quiescent states that are responses to particular stress factors of environmental origin. Anabiosis is an extreme state of arrest which may last for considerable periods of time: some nematodes stored dry for over 20 years can then develop normally. In the anabiotic state the parasite has no detectable metabolism and the process of ageing is suspended. Desiccation, low temperatures,

osmotic stress and low Po_2 can all induce specific anabiotic repsonses in stages of nematode parasites that occur external to the body of the host.

7.3.5 Migration and site selection

From the point of entry into the host body, the majority of parasites undergo a migration of varying complexity that will take them to their final site of residence. This may be highly specific, as in human schistosomes, cattle lung worms or fish eye- or brain-flukes, or be rather more generalized, as with hydatid cysts that settle in a variety of different sites. Migrations of this type are commonly accompanied by growth and development of the parasite and are thus referred to as *ontogenetic migrations*. These migratory events, which may be of short or long duration, may culminate in a sexually mature parasite or a larval or cystic stage that exhibits hypobiosis, as described above, until ingested by the final host. Usually the migration will take place over a fixed route if normal parasite development is to occur. The physiological triggers and determinants that orchestrate these migrations are not understood but are clearly important facets of host specificity.

Aberrant migratory patterns can occur if a parasite enters an unsuitable host in which normal and complete development cannot be accomplished. This has been widely documented in strongyloid and ascaridoid nematodes where larval worms invading the wrong host, in this case humans, can cause serious damage. These are the migratory *larva migrans* which are typical of hookworms, *Ancylostoma braziliense* and some ascaridoids, such as *Toxocara canis*. On invasion, these parasite larva undertake what is presumably an inadequately signposted migration either in the superficial tissues (e.g. *cutaneous larva migrans, Ancylostoma*) or in deeper tissues (e.g. *visceral larva migrans, Toxocara*). Since the physiological triggers for normal migration and development are lacking in the incorrect host, these parasites eventually die in an ectopic site; therefore they can cause disease that is often difficult to diagnose. Some common patterns of ontogenetic migration are depicted in Fig. 7.6, but in no case do we understand the physiological mechanisms that are involved.

Some parasites undergo migrations within the host body that are not related to growth and development. These include diurnal migrations associated with transmission (see Section 7.2.6) and diurnal migrations related to the nutritional cycle of the host (e.g. *Hymenolepis diminuta* in the rat intestine).

The complex and varied nature of site selection and pattern of migration seen within parasites, especially helminths, argue compellingly that these parasites possess sophisticated sensory capacity to enable them to recognize and respond to the appropriate stimuli emanating from the tissues of the host body. This aspect of parasite physiology has proved to be a difficult area of research to establish and the sensory biology of parasites remains a topic of ignorance and neglect.

7.3.6 Invasion of tissues

During the process of establishment many parasites invade specific cells in host tissues where they reside either temporarily or for long periods of time. The physiology of cell recognition and adherence in parasitology is of interest since many intracellular parasites are responsible for causing major diseases of both humans and animals. Examples of tissue-invading parasites include malarias and babesias (red blood cells), leishmanias (macrophages), coccidians, cestodes and nematodes (*muscularis mucosa* of the gut), schistosomes (circulatory system), trypanosomes (nervous system) and larval digeneans, cestodes and nematodes (body musculature). The physiology of cell recognition, cell adhesion and penetration is complex and poorly understood and is best described for malaria parasites.

Cell invasion in malaria

Two essentially unrelated features of the cell biology of malaria parasites have attracted considerable attention: red blood cell recognition and invasion and cytoadherence of the parasitized red cell to the endothelial lining of host blood vessels.

Invasion of the red cell by *Plasmodium* is a specific, sequentially-defined process which involves: (1) cell recognition by the merozoite; (2) orientation of the parasite with respect to the red

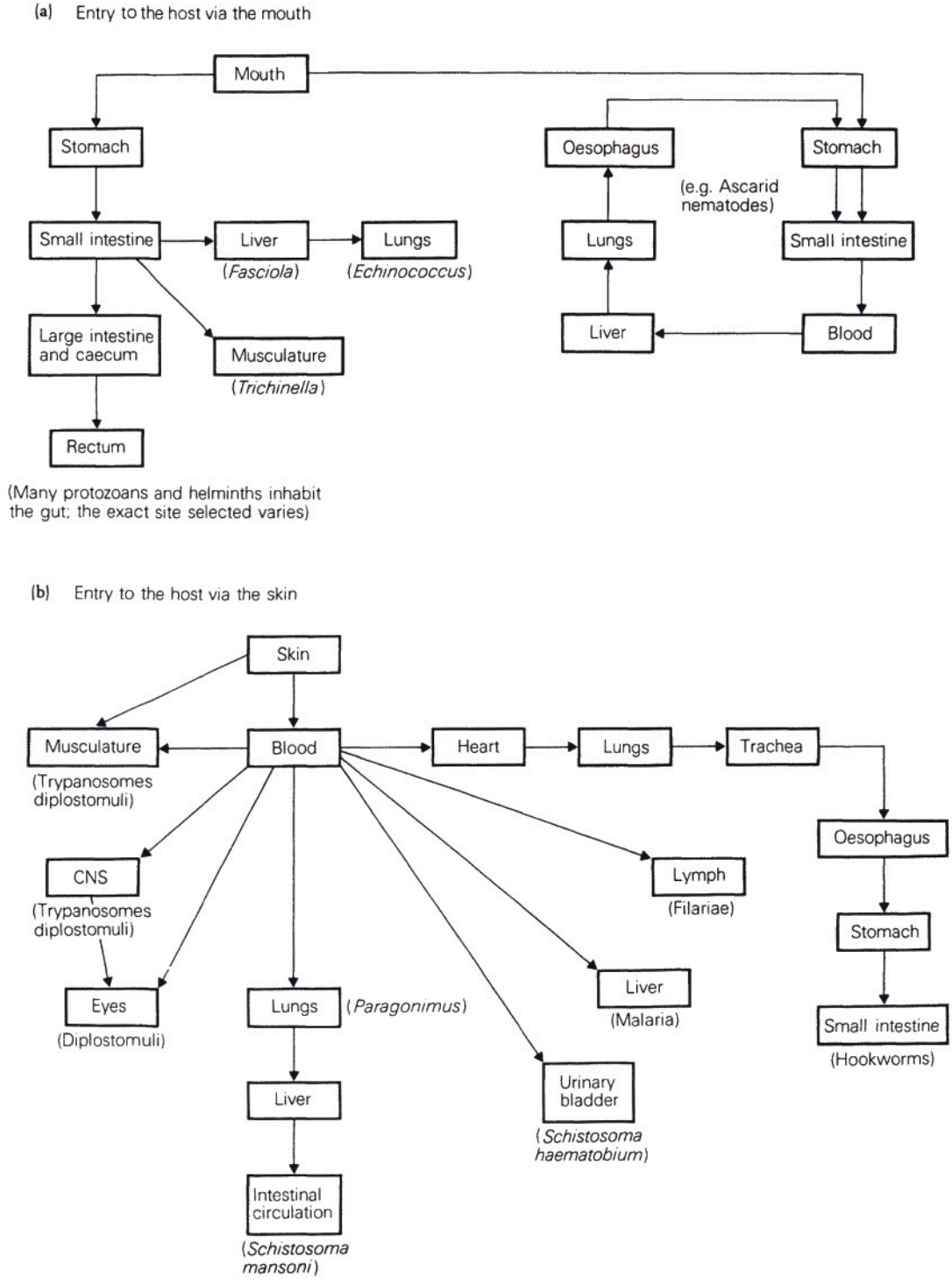

Fig. 7.6 Patterns of ontogenetic migrations carried out by parasites within the body of the vertebrate host.

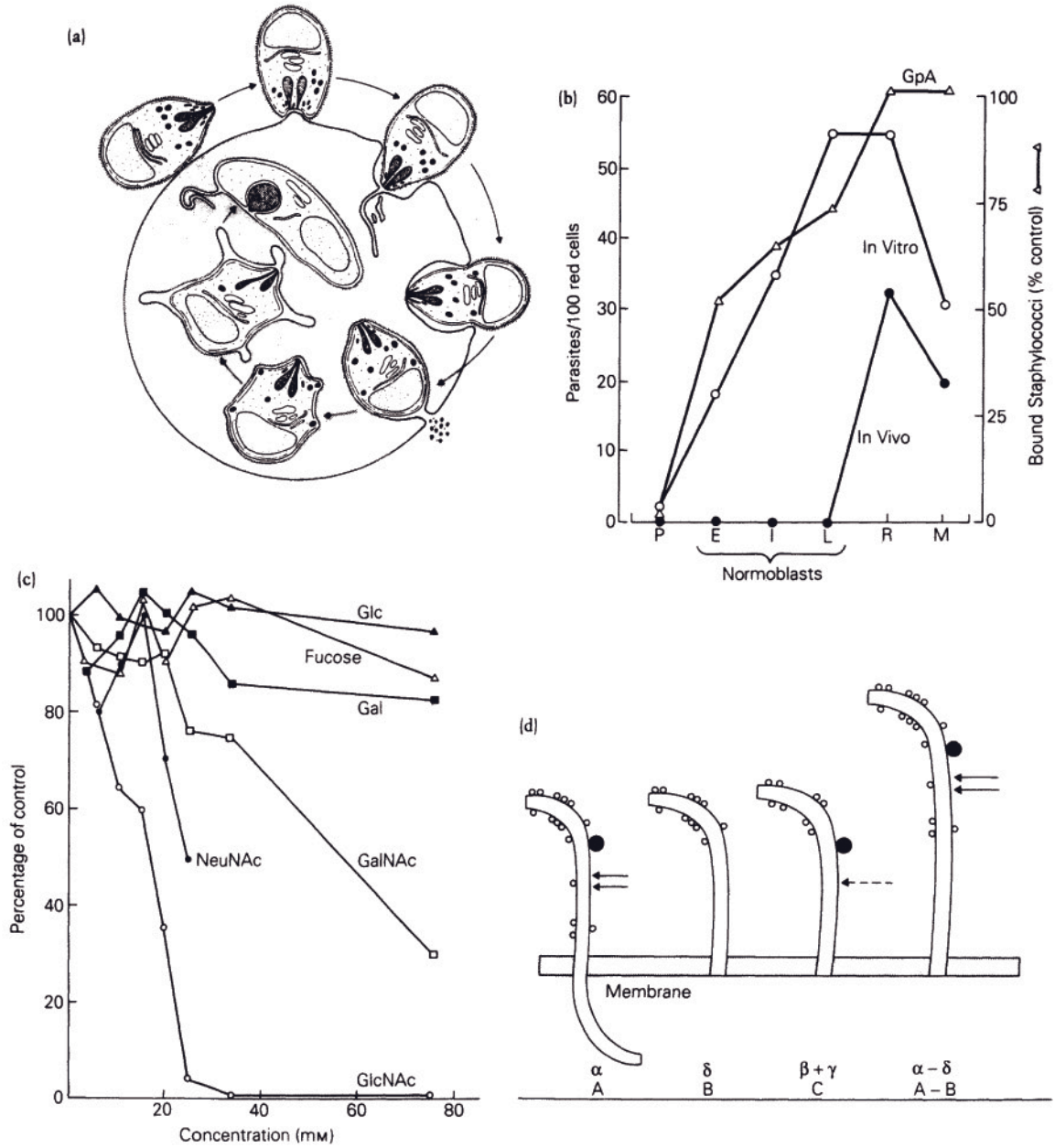

Fig. 7.7 Red cell invasion by *Plasmodium*. (a) Invasion sequence by merozoites of *P. knowlesi* (after Bannister, 1977). (b) Invasion of red blood cells by *P. falciparum in vitro* (○–○) and *in vivo* (●–●) relative to appearance of glycophorin A (GpA): P, pronormoblasts; E, early; I, intermediate; L, late normoblasts; R, reticulocyte; and M, mature erythrocyte. (c) Red cell invasion by *P. falciparum* related to the presence of various sugars. Data expressed as a percentage of control invasion: Glc, glucose; Gal, galactose; GalNAc, *N*-acetyl-D-galactosamine; NeuNAc, *N*-acetyl-neuraminic acid; GlcNAc, *N*-acetyl-D-glucosamine. (d) Glycophorins A, B and C schematically represented on the red cell surface. The small circles are *O*-glycosidically linked oligosaccharides and the large circles are *N*-glycosidically linked oligosaccharides. Arrows indicate sites of tryptic cleavage. A-B is a hybrid molecule. ((b)–(d) from Pasvol & Jungery, 1983.)

cell surface and apposition of the apical complex; (3) formation of a junction between the invading merozoite and red cell surface at the point of contact; (4) induction of invagination of the red cell membrane by secretions from the merozoite; and (5) entry of the parasite through extensive invagination of the red cell surface membrane forming the parasitophorous vacuole (Fig. 7.7a). Recognition of the red cell by the merozoite depends on specific surface receptors and varies according to age of cell, blood group antigens, and host specificity. Invasion of human red cells is diminished in races lacking Duffy antigens (e.g. in *P. vivax*); treatment with *N*-acetyl-D-glucosamine, trypsin or neuraminidase blocks cell invasion by *P. falciparum*. Recent evidence implicates surface glycophorins as major determinants of invasion. Red cell surface glycophorins are sialic acid-rich glycoproteins comprising four subgroups (a, b, c, and d) and their role as surface receptors for *P. falciparum* merozoites is now well-established. Cells lacking either glycophorin a (En(a-)-cells) or glycophorin d (S-s-U-cells) resist merozoite invasion to a significant extent and this resistance can be enhanced by tryptic removal of remaining glycophorin molecules. Glycophorins represent a significant component of the red cell membrane and yet they have no clear role, since they can be absent without any red cell dysfunction. *Plasmodium falciparum* will develop normally within glycophorin-deficient cells and will invade young red cells, in which it cannot develop, relative to the progressive appearance of glycophorins on the cell surface (Fig. 7.7b, c & d).

Although the exact role of red cell glycophorins as receptors for merozoites is unknown and the evidence that they contribute to the initiation of erythrocyte invasion is convincing, other factors are also involved. Once recognition of the cell is accomplished, the invasion continues by the formation of a junction of about 10 nm, containing fine fibrils that extend between the thickened red cell membrane and the apical protruberance of the merozoite: Duffy-negative, invasion-resistant cells do not form this junction but trypsin treatment of these cells renders them permissive to invasion by *P. knowlesi* merozoites and a typical host cell–parasite junction is formed. The apical organelles of the merozoite, the rhoptries and micronemes, initiate the actual invasion of the red cell by releasing secretions which include a histidine-rich protein. During invasion, the red cell membrane invaginates progressively to enclose the merozoite and the junction moves so that its position is maintained at the mouth of the developing parasitophorous vacuole. This mobility of the junction may be related to membrane fluidity, since treatment of merozoites with cytochalasin B inhibits invasion despite the formation of a junction on attachment of the merozoite; here no junctional migration occurs. On completion of normal invasion, the junction seals up behind the merozoite which now lies completely enclosed within the parasitophorous vacuole. This vacuole is made of original red cell membrane which has undergone molecular reorganization. Not only does the intracellular malarial parasite induce molecular changes within the membrane of the parasitophorous vacuole, but it also affects the red cell surface itself. Under certain conditions, surface electron-dense knobs appear on the red cell membrane and caveola-vesicle complexes may also be formed.

One major feature of *P. falciparum* infections is the sequestration of infected red cells through cytoadherence to endothelial cells of the host circulatory system, mediated through erythrocyte surface knobs. Sequestration is an important adaptation by which the parasite may avoid circulation through the spleen where host defence is active. Pathologically, sequestration may contribute to obstructed blood flow typical of cerebral malaria. Falciparum malaria differs from other human malarias in that only red cells containing young ring stages of the parasite circulate freely, while cells infected with more mature parasites become sequestered.

The surface knob, which is the functional unit of cytoadherence of *P. falciparum*-infected red cells, comprises a cup-shaped membranous structure and associated protrusion of the red cell surface membrane (Fig. 7.8). These knobs contain a unique protein of Mr 80 000, rich in histidine and proline which is lacking in knobless strains of the parasite. Its role in mediating cytoadherence remains unexplained but, since antibody has

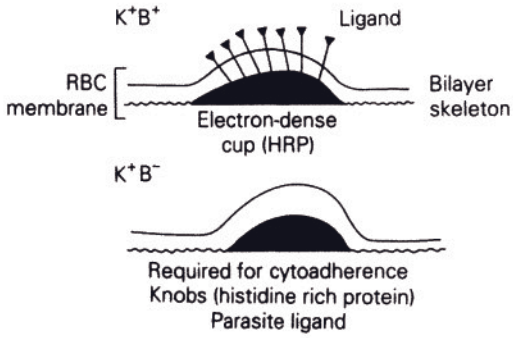

Fig. 7.8 Model for knobs on the red cell surface induced by infection with *Plasmodium falciparum*. K^+B^+, knobby parasite able to bind to blood vessel endothelium; K^+B^-, knobby parasite incapable of endothelial binding due to absence of putative cytoadherence factor. (After Leech *et al.*, 1984.)

been shown to inhibit cytoadherence in a strain-specific way, it suggests that additional molecules may also be involved.

Cell invasion in other parasites

Many other protozoan parasites adopt an intracellular habit and therefore invade host cells. Like malaria parasites, these also may reside within a parasitophorous vacuole and enter by invagination of the cell surface rather than by penetration of the membrane itself. This pattern of invasion occurs in the Coccidia (e.g. *Eimeria* invading gut cells and *Toxoplasma* invading macrophages). The parasitophorous vacuole may be temporary (e.g. *Babesia*) and regresses shortly after entry is accomplished. *Toxoplasma*, *Eimeria* and *Leishmania* all invade host cells by inducing their own phagocytosis. Treatment of host cells with drugs that inhibit phagocytosis, such as colchicine or cytochalasin B, alters the pattern of invasion. *In vitro* studies on cell invasion by *Leishmania* parasites suggests a degree of induction and specificity in the mechanisms involved. Promastigotes of various species of *Leishmania* induce phagocytosis by host macrophages and these cells respond to the presence of live parasites by producing pseudopodial whorls or lamellar sheaths; killed parasites are also taken up but at a reduced rate. Cytochalasin B inhibits macrophage invasion by these promastigotes, which will also invade non-phagocytic cells in culture. Cautious interpretation must be placed on these *in vitro* studies and it is not certain that the same mechanisms necessarily function *in vivo*. After invasion, *Leishmania* parasites reside within a parasitophorous vacuole formed from host cell membranes. Survival of the parasite within a cell, whose role is to ingest and kill invaders, is both of considerable interest and applied significance (see Chapter 8, Section 8.7).

Trypanosoma cruzi, the causative agent of Chagas disease in the Americas, is an obligate intracellular protozoan. In cell culture, trypomastigotes invade fibroblasts by mechanisms that involve interaction between the surface membranes of the host cell and the parasite. The parasite produces a lectin-like protein responsible for cytoadherence aided by a proteolytic activating system; penetration is effected by a tunicamycin-sensitive glycoprotein. The host cell contributes to these events by producing glycoproteins that are active in adherence and also penetration. By contrast, entry of *T. cruzi* into macrophages is by phagocytosis; trypomastigotes survive within the macrophage by escaping the confines of the lysosomal vacuole and replicating within the cytoplasm.

In some cases, host cell invasion by protozoan parasites is the major pathophysiological event. In *Entamoeba histolytica*, secreted products, such as 'amoebapore' (a parasite-derived, pore-forming protein), cytotoxin and proteolytic enzymes all contribute to invasion of the host intestinal cells and to the severe intestinal and liver pathology associated with this phase of amoebiasis.

While several species of helminth parasite invade host cells (e.g. *Trichinella spiralis* in muscle cells) little information on mechanisms of entry is available.

7.4 REPRODUCTIVE PHYSIOLOGY

Many parasites have complex life cycles which involve stages that reproduce by sexual processes in one host and by asexual proliferation in another host. These mechanisms ensure that genetic information is varied and becomes widely

disseminated and also that infective parasite stages are produced in sufficient numbers to favour successful transmission in a hostile environment.

7.4.1 Asexual mechanisms

Asexual splitting, or budding, occurs in many parasitic Protozoa, all of the Digenea and in some Cestoda.

Asexual reproduction in the Protozoa involves binary or multiple fission, schizogony, endodyogeny or single budding, while in helminth parasites individual numbers are dramatically increased by internal budding (polyembryony). During the life cycle of the Digenea, an asexual phase occurs exclusively within the molluscan intermediate host. Normally two distinct asexual generations are passed within the snail, mother sporocyst to daughter sporocysts or sporocyst to rediae, the net result being that from a single miracidium entering a snail many hundreds of thousands of cercariae may subsequently emerge. Each of these individuals will be genetically identical.

The majority of cestodes produce only a single larva from the egg but proliferative external budding occurs in the urocystis and urocystidium larvae and internal budding occurs within the polycercus, coenurus and hydatid larvae of the Taeniidea. The hydatid cyst of *Echinococcus* can generate several million protoscoleces by asexual budding, while the coenurus rarely produces more than a few hundred larval tapeworms.

7.4.2 Sexual reproduction

Many protozoans reproduce by a form of sexual reproduction but it is not always easy to distinguish between the fusion of individual parasites and the fusion of gametes. In some species the gametes are morphologically distinct, such as male microgametes and female macrogametes, and sexual processes may alternate with asexual reproduction, each taking place in a different host. In malaria parasites, asexual schizogony increases numbers of merozoites in the host blood but sexual gametocytes are also formed which are transmitted to mosquitoes in which host gamete fusion and sexual proliferation takes place. The stimulus which controls the formation of gametocytes has interested malariologists for a long time; current thinking favours the view that trophozoites are directed towards sexual reproduction by environmental factors associated with host cell lysis or degeneration. Here gametocytogenesis can be regarded as an escape mechanism from unfavourable conditions by means of the genomic variation conferred by random fusion of gametes in the mosquito.

The sexuality of trypanosomes is a topic of current interest and the traditional opinion that these parasites reproduce only by asexual binary fission has been challenged. Sexual stages in the life cycle of trypanosomes may occur either within the mammalian host in an extravascular location or within the tsetse fly vector. In support of the latter contention, giant forms of trypanosomes, which are capable of liberating large numbers of new trypanosomes, have been isolated from midgut cells of flies; these giant forms are apparently the product of fusion of two individuals and this represents a mechanism by which genetic interchange might occur. Natural populations of African trypanosomes demonstrate considerable electrophoretic variability which perhaps indicates the importance of sexual mechanisms in increasing genomic variability in the wild.

All monogeneans are hermaphrodites and asexual mechanisms are unknown. Cross-fertilization usually takes place between adjacent individuals but self-fertilization may also occur.

The majority of digeneans are hermaphrodite and both self- and cross-fertilization have been recorded; the schistosomes have separate sexes and cross-fertilization is therefore mandatory. The physiology of egg production is well-understood; eggs are released from the mature ovary and enter the oviduct. Spermatozoa from the partner are stored, after copulation, in a seminal receptacle. These are then released along with a small number of vitelline cells and they make their way to the ootype where the ova are fertilized. During development the eggshell becomes tanned *in utero* and on release the egg is fully protected by the rigid shell (see Section 7.2.2).

Almost all tapeworms are hermaphrodites and each proglottis (segment) contains a full com-

plement of male and female apparatus. Both cross- and self-fertilization occur. Cestodes mature posteriorly such that the terminal segments are the oldest and, when gravid, contain ripe eggs that are either shed independently or within the liberated proglottis itself. The tapeworm egg is not tanned like that of the Digenea, but is surrounded by a capsule comprising various constituent layers.

Acanthocephalans have separate sexes and are sexually dimorphic. Spermatozoa are introduced into the pseudocoelom of the female at copulation; ova within the female are associated with ovarian balls of germinal tissue. Eggs are fertilized and subsequently liberated from the ovarian balls to complete their development free in the pseudocoelom. Female acanthocephalans release large numbers of eggs which are protected by a covering of protein and chitin.

The majority of parasitic nematodes are sexually dimorphic and reproduce sexually; a small number of species reproduce either hermaphroditically or parthenogenetically but no somatic asexual processes have been described. The male nematode generally possesses a single testis and accessory structures, such as a copulatory bursor or paired spicules, which are used during copulation. The female nematode may have one or two sets of gonads; sperm are stored in a seminal receptacle and these fertilize mature oocytes *in situ*. Eggshell formation is initiated by the process of fertilization and continues during egg maturation. In some groups, such as the filarial nematodes, egg hatching takes place *in utero* and the eggshell is accordingly reduced in size and chemical complexity.

Almost all parasites are characterized by their enormous reproductive capacity and they produce, either by sexual or asexual mechanisms, or sometimes both, extremely large numbers of offspring. Physiologically this strategy is demanding in terms of nutrients and energetic commitment to reproduction.

7.4.3 Reproductive synchrony

Reproductive events in a small number of parasite species are synchronized to host sexual cycles and breeding patterns; this relationship serves to liberate infective parasites into the environment simultaneously with susceptible juvenile hosts. The best known examples come from the flagellated protozoans of amphibians and arthropods.

The release of opalinid gametes from the amphibian gut is initiated by host sex hormones. The Hypermastigina that inhabit the gut of arthropods can be stimulated to reproduce sexually under the influence of host moulting hormones. Flagellates of termites are lost with each successive moult since they inhabit the insect hindgut which is lined with cuticle. Here, synchrony of sexual processes in the parasite with moulting in the host ensures reinfection, which in this example is a mandatory phenomenon since the parasite is the source of essential digestive cellulases.

7.5 CHEMICAL COMMUNICATION

Chemical communication between animals of the same species or of different species has long been the subject of intensive research interest. Among the insects, the topic of communication via pheromones has had considerable commercial significance in the field of insect pest control. Surprisingly, little is known about chemical communication between parasites, yet this information could prove to be invaluable in the quest for novel strategies with which to control the world's major parasitic diseases.

Pheromones are probably produced by many helminth parasites and they may serve as sexual attractants: there is considerable indirect, but little direct, evidence to support this contention.

Laboratory identification of parasite pheromones is normally made using *in vitro* bioassays whereby movement of individual worms in aqueous or semi-solid media is assessed within choice chambers of various design. Less frequently, supporting data has been obtained from *in vivo* observations on mate location by parasitic worms, but such information has proved more difficult to interpret unambiguously.

Solubility, enzyme and chromatographic studies on putative pheromones from helminths have all provided indirect evidence for the types of chemical messengers involved in sexual

attraction, but no parasite pheromone has yet been identified or characterized chemically. Sterols and peptides have both been implicated as candidate pheromones in parasitic nematodes (e.g. *Nippostrongylus brasiliensis*) and digeneans (e.g. *Echinostoma*). However, detailed biochemical analyses of pheromones of *N. brasiliensis* suggest a more complex picture in which the worms produce a mixture of lipidoidal and hydrophilic substances which can variously attract either the same or the opposite sex. The site of pheromone production appears to be highly varied in helminth parasites. In nematodes, the copulatory organs themselves and the body surface are the apparent source of attractant molecules, while in the digeneans, the tegument, alimentary canal and excretory system all produce unidentified chemoattractants. Studies on sensory receptors of helminths that may detect chemical messengers released into the environment have failed to provide unequivocal evidence on location and precise function of these organelles.

Schistosomes present an interesting and somewhat unusual picture in terms of sexual attraction: they possess separate sexes that show varying degrees of interdependence for growth and development and in which processes chemoattractants must play an important but undisclosed role. Males of *Schistosoma mansoni* will grow and develop to maturity in the absence of females whereas female worms lacking males grow poorly and never reach sexual maturity although they have the potential to do so if males are experimentally introduced even as much as a year later. Other schistosome species show a lesser degree of sexual dependence. Laboratory studies reveal that adult male and female schistosomes attract one another by chemical means involving lipid-based pheromones; homosexual attraction and pairing can occur and under these conditions partial sex-reversal of the smaller male partners has been observed. Females of *S. mansoni* will only grow to sexual maturity after a period of residence within the gynaecophoral groove of the male, implying tactile as well as chemical stimuli as controlling mechanisms. The receptors involved in interpreting this complex array of signals have not been described. In a parasitic disease whose pathogenesis is directly related to sexual activity of the parasite, pheromones that initiate worm pairing, growth and maturation would represent ideal and novel drug targets.

Aside from chemical aspects of parasite reproductive biology, the general endocrinology of helminths is little understood. Hormones concerned with regulation of parasite growth and development have been examined for only a small number of species and their role remains a matter for some speculation. Parasitic nematodes, like their free-living relatives and arthropods, grow by a series of moults in which the old cuticle is shed and replaced by a new structure. These events are controlled by juvenoid, ecdysteroid and neuropeptide hormones in insects and, by analogy, they should also function similarly in nematodes. Biochemical identification of these hormones from parasite tissues has been confirmed but experimental verification of their biological function has not yet been made, nor has their biosynthesis been demonstrated unequivocally. Therefore, despite the presence of ecdysteroids (ecdysone and 20-hydroxyecdysone) in nematodes and platyhelminths, their function remains elusive, particularly since moulting only occurs in the nematodes. It may be that the complex surface biology of helminth parasites is regulated by conserved families of developmental hormones, but this has yet to be established (Table 7.6).

7.6 NEUROPHYSIOLOGY OF HELMINTH PARASITES

Parasite neurobiology has long been a 'Cinderella' topic with parasitologists but it should be apparent that, with their relatively sophisticated patterns of host-finding, site-location, mate-finding and reproductive biology, parasites are more complex than many textbooks would admit and their neurobiology will be accordingly complicated. Furthermore, a great many antiparasite drugs act on the neuromuscular system of helminths. Thus parasite neurophysiology is gaining in importance as a topic of considerable applied significance.

Table 7.6 Ecdysteroid hormones in helminth parasites. (Data from Mercer, 1985; Rees & Mercer, 1987.)

DIGENEANS	
Schistosoma mansoni	Ecdysone in schistosomula, ecdysone + 20(OH)ecdysone in eggs and adults
Fasciola hepatica	Ecdysone + 20(OH)ecdysone in adults
CESTODES	
Echinococcus granulosus	Ecdysteroids in hydatid cyst fluid
Hymenolepis diminuta	Free and conjugated ecdysteroids in eggs and adults
Moniezia expansa	Ecdysone, 20(OH)ecdysone + 20,26di-(OH)ecdysone in adults; no conjugates
NEMATODES	
Ascaris suum	Free and conjugated ecdysteroids in adults
Anisakis simplex	Ecdysone, 20(OH)ecdysone + 20,26di-(OH)ecdysone in L_3 larvae
Dirofilaria immitis	Ecdysone, 20(OH)ecdysone, free and conjugated ecdysteroids in adults
Oncocerca gibsoni	Ecdysone + 20(OH)ecdysone in adults
Parascaris equorum	Metabolizes exogenous ecdysone

7.6.1 Helminth nervous systems

The nervous system of platyhelminth parasites comprises an anterior complex of cerebral ganglia with, posteriorly, a bilaterally symmetrical series of nerves serving the body. The system is formed of a nerve network containing unmyelinated fibres, the majority of which have motor function. The acanthocephalan nervous system is comparably undeveloped and contains a cerebral ganglion from which arise single and paired nerves; the male worm has a second ganglion associated with the reproductive system.

Information of the nervous system of parasitic nematodes derives from early studies on ascarids and more recent work on free-living forms such as *Caenorhabditis elegans*. A nerve ring, containing the main concentration of neurones, is associated with the oesophagus, from which motor nerves are directed to the head; sensory nerves connect the nerve ring to anterior sense organs. A paired, ganglionated, ventral nerve cord extends posteriorly and connects with a dorsal nerve via a series of commissures. There is a posterior nerve ring which contains ganglia connecting with both motor and sensory neurones. The pattern of the nervous system tends to be consistent throughout the phylum.

7.6.2 Sense organs and sensory biology

The functioning of helminth sense organs has been determined primarily from morphological and behavioural studies and little direct information has been obtained. The major areas of sensory physiology that have been examined are photoreception in free-living larval helminths, chemoreception in host-, site- and mate-location, and sensory recognition of and responses to temperature gradients and gravity.

Many larval helminths respond to light in their environment, utilizing these responses to come into juxtaposition with a suitable host for invasion. Eye-spots are present in some helminth larvae, including most monogenean oncomiracidia and some digenean miracidia. Larval forms lacking such sense organs may nevertheless respond either positively or negatively to light but

it is often difficult to distinguish between one stimulus and another in an experimental arena. Photosensitivity is undoubtedly important for orientation of invasive helminth larvae with respect to host finding and often also for the initiation of hatching of helminth eggs.

Response to a thermal gradient may be necessary to accomplish infection of a warm-blooded host (e.g. larval hookworms in a terrestrial environment and schistosomes in an aquatic environment). It is not known what sensory apparatus is involved in these responses. Similarly, parasites may respond to gravity, chemical gradients including oxygen or carbon dioxide, and to the presence of other individual parasites, but the sensory organelles involved here have also yet to be identified. Surface receptors include ciliated sensillae (Monogenea), ciliated pits and papillae (Digenea), tegumental protrusions (Cestoda) and amphids, papillae and ciliated pits in the Nematoda.

7.6.3 Neurotransmission and neurosecretion

Helminth parasites synthesize a number of putative neurotransmitter substances including adrenalin, noradrenalin, acetylcholine, DOPA, dopamine, GABA (gamma-aminobutyric acid) and serotonin (5-HT). Cholinergic synapses are widely distributed throughout the helminths and acetylcholinesterase has been located histochemically in many species. The neuromuscular junctions of nematodes are cholinergic: acetylcholine decreases muscle resting potential while substances like physostigmine increase sensitivity to acetylcholine; piperazine, a widely used nematocidal drug, inhibits the stimulatory effects of acetylcholine. The major inhibitory neurotransmitter in nematodes is GABA (Fig. 7.9) and its action at the synapse is thought to be the focus of activity of the anthelmintic ivermectin. Platyhelminth neurotransmitters include acetylcholine, 5-HT, noradrenalin and dopamine; GABA is

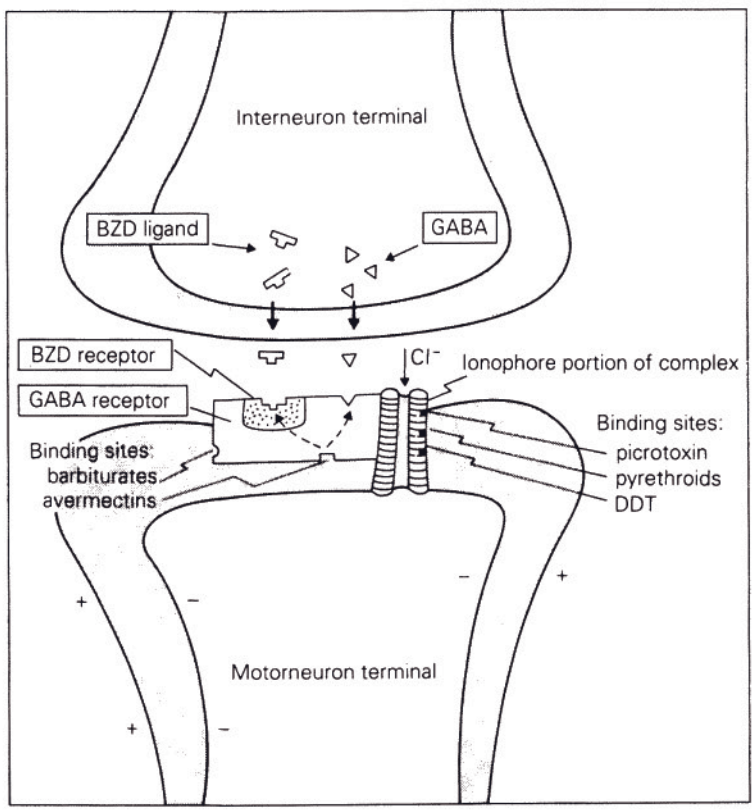

Fig. 7.9 Interaction between the nematode synapse and the drug ivermectin. Potentiation of GABA and benzodiazepine binding (dotted lines) cause Cl^- influx and motorneurone hyperpolarization. Ivermectin (avermectins) also potentiate GABA release, which may explain their anthelmintic mode of action in causing worm paralysis. (After Campbell, 1985.)

Fig. 7.10 Aminergic and peptidergic nervous elements in the proglottid of a mature cestode (*Diphyllobothrium dendriticum*). All four types of immunoreactivity are present in the two main nerve cords (n). (After Gustafsson, 1985.)

, fibres immunoreactive to peptide histidine isoleucine surrounding testicular follicles; , immunoreactivity to FMRF- amide (Phe–Met–Arg–Phe–NH_2) in cirrus sac (c); , immunoreactivity to growth hormone releasing factor in vaginal wall (v) and vitelline glands (y); ●, immunoreactivity to serotonin in vaginal wall, uterine pore and cirrus sac.

presumed to be unimportant since ivermectin has no effect on platyhelminth parasites.

Chemical messengers within the nervous system, such as neuropeptides, amines, amino acids and acetylcholine, are the focus of growing attention since they may differ in parasite and host and thus present chemotherapeutic potential. The complexity of this topic has been revealed in recent studies; for instance, in tapeworms, aminergic, cholinergic and peptidergic neurones have been identified (Fig. 7.10). At least 29 neuropeptides have been detected by immunoreactivity with specific antisera to mammalian peptide hormones including bovine pancreatic polypeptide, growth hormone releasing factor, peptide histidine isoleucine, gastrin, gastrin releasing peptide, leu-encephalin, neurotensin, vasotocin, oxytocin and FMRF-amide. Nothing is known of the function of this complex of neuropeptides in cestodes but once disclosed the possibility exists for the development of novel drug targets at the neurophysiological level.

The products of some parasites are capable of causing what appear to be endocrinological lesions in their hosts via the production and release of substances that mimic host hormone action. This occurs in larval helminth infections of snails, such as *Trichobilharzia ocellata*, a bird schistosome which induces host gonadal regression via a substance called 'schistosomin'; this substance is synthesized by the snail itself under the influence of the parasite. Many larval digeneans cause profound growth and sexual changes in molluscs, some of which may be mediated endocrinologically. Plerocercoids of the tapeworm, *Spirometra mansonoides*, release a platelet growth factor (PGF) which is a remarkable mimic of mammalian growth hormone (Table 7.7). The plerocercoid stage shows little host specificity, infecting a wide range of animals, including humans, causing the condition known as sparganosis. The similarities between PGF and human growth hormone have led to speculation that this parasite has acquired the human gene for growth hormone which it is able to express in the plerocercoid stage. Viral transduction is one possible method by which this proposed genetic exchange may have occurred.

7.6.4 The neuromuscular junction in helminths

The muscle cells of nematodes, as revealed by studies on *Ascaris*, are unusual in that they contain both nervous and contractile elements: the

Table 7.7 Comparison of plerocercoid growth factor (PGF) of *Spirometra mansonoides* with mammalian growth hormone (MGH). (Data from Phares, 1987.)

Parameter	PGF	MGH
Weight gain	Increase	Increase
Skeletal growth	Increase	Increase
Somatomedin activity	Increase	Increase
Endogenous GH	Decrease	Decrease
Insulin-like in:		
Normal rats	Yes	No
Hypophysectomized rats	Yes	Yes
Antiinsulin-like in: Diabetogenic	No	Yes
Binding to rabbit and rodent GH and Prolactin receptors	Yes	Yes
Lactogenic in pigeon crop-sac assay	Yes	Yes
Primate GH-activity	Yes	Yes
Molecular weight	24 000	22 000
Isoelectric point	pH 4.7	pH 4.9
Reaction with monoclonal antibody to human GH (%)	61	100

muscle arm synapses with motoneurones of the nerve cord. The contractile portion contains regular arrangements of thick and thin myofilaments, typical of striate muscle, in which H, A and I, but not Z bands are apparent. The neuromuscular junction of other helminth parasites is poorly understood.

7.7 LOCOMOTORY PHYSIOLOGY

Almost all parasites are capable of movement and some stages in the life cycle, especially those concerned with active transmission in the external environment, are highly motile. Even adult parasites can be highly active when viewed after host autopsy, but this may not necessarily reflect their natural state *in vivo*.

Information in locomotory physiology is largely restricted to observations on the effects of various external stimuli upon activity and speed of movement. Amongst the Protozoa, ciliary, flagellar and amoeboid movement have all been described; parasites appear to be no different in this regard from free-living forms. Members of the Apicomplexa, such as *Eimeria* and *Plasmodium*, exhibit gliding movements during which the parasite undergoes no alteration in body shape. The physiological mechanisms underlying this process are unknown but the notion of a 'linear motor', powered by interactions between actin and myosin filaments in the parasite surface membranes, has been proposed (Fig. 7.11). Ciliary locomotion occurs in some helminth parasites, including oncomiracidia, miracidia and coracidia. In other larval stages and adult helminths, locomotion takes the form of swimming, crawling or burrowing brought about by muscular action. Nematode parasites, like their free-living relatives, move in a characteristic undulatory manner which is the net product of their cylindrical body shape, with opposing dorsal and ventral musculature and a fluid-filled pseudocoelom which acts as a hydrostatic skeleton. Additionally, the pattern of muscle innervation aids the generation of sine waves in the body shape to bring about movement. The resting pseudocoelomic hydrostatic pressure of *Ascaris* is approximately 70 mmHg and it can vary between 16 and 225 mmHg during wave-form production by posteriad contraction of the body musculature; these waves are dorsoventral in plane and the animal lies on its side during locomotion. The three underlying mechanisms associated with nematode locomotion, spontaneous myogenic depolarization, neuromuscular coordination and local changes of hydrostatic pressure, are controlled by serotonin and epinephrine.

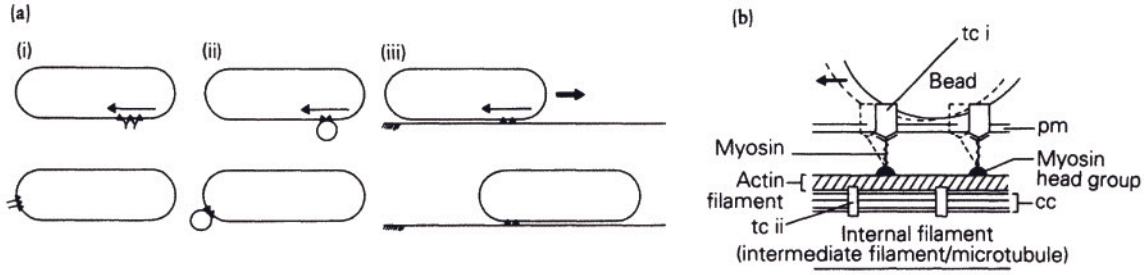

Fig. 7.11 Biomechanics of locomotion in sporozoan (apicomplexan) protozoans. (a) Gliding movement with associated capping and bead translocation; (i) antibody (Y) interacts with cell surface components forming a cluster which may activate the proposed 'linear motor' sweeping complexes to rear of cell; (ii) beads and cell surface interact to initiate 'linear motor' that moves bead to end of cell; (iii) substrate interacts with cell surface to activate motor, since substrate cannot move, cell is moved forwards. (b) Model for proposed 'linear motor', based on interactions of surface actin and myosin: tc i and tc ii, transmembrane components; cc, cortical cytomembranes; pm, plasma membrane. Interaction of myosin head group with ATP and actin filaments leads to conformational changes associated with release of ADP. Addition of further ATP would cause release of myosin head group from actin filament and the bead illustrated would move to the left. (After King, 1988.)

7.7.1 Helminth locomotion for drug assays

One of the most common determinants of the efficacy of antiparasite drugs in laboratory screening tests concerns the action of the compound on parasite motility. While this is perhaps a debatable criterion for *in vitro* drug screening, it does have a proven track record in distinguishing potentially useful drugs from those of little value. Naturally, effects on motility can only be one of many parallel facets of compound evaluation.

The so-called 'micro-motility meter' has been devised as a relatively simple instrument for determining the effects of putative drug substances on helminth movement *in vitro* and it provides an attempt at objectivity in this potentially highly subjective assay procedure (Fig. 7.12). Many drugs will depress parasite motility in this system which is an inexpensive and rapid primary screen. However, the majority of new compounds are still tested on conventional model parasite systems as the motility screen has a predilection for drugs that influence the parasite neuromuscular system and may not identify compounds that are specific for alternative targets.

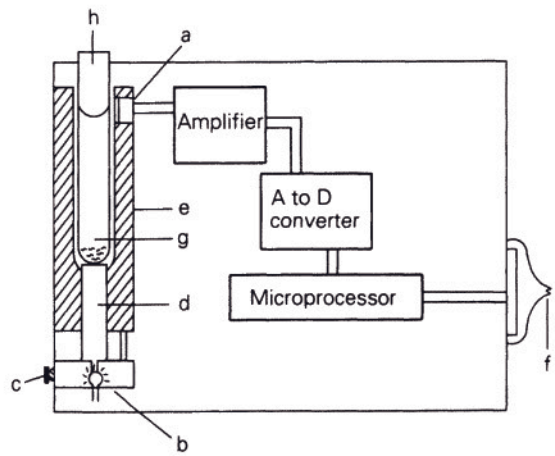

Fig. 7.12 Diagram of a micromotility meter for quantification of helminth movement in the presence of drugs and other substances. Medium is placed in a tube (g) and light from a lamp (b) passes to the meniscus (h) through a plastic light pipe (d); part of the light is deflected to the photodiode (a), the signal amplified, digitized and microprocessed. Addition of a motile worm will result in fluctuations in the digitized signal and worm movement can be compared statistically under different conditions. (After Bennett & Pax, 1987).

7.8 NUTRITION OF PARASITES

The popular concept that parasites feed at the expense of the host may have little foundation in reality. There are certain conditions under which the nutritional demands of a parasite may result in physiological stress to the host, such as the acquisition of vitamin B_{12} by *Diphyllobothrium*

latum in the gut of humans and anaemia in hookworm disease, but in general there is little evidence to support the notion that parasites cause disease by their nutritional activities, although the act of feeding itself may be physically damaging. In truth, we are rather ignorant of the nutritional physiology of most parasites and are not in a position to define the nutrient requirements of more than the small handful of species investigated. We must be careful not to imply a lack of metabolic dependence of the parasite on its host, but within the established, stable host–parasite relationship, physiological excesses by the parasite tend to be eliminated in favour of activities that lead to a benign association. Moreover, most parasites inhabit sites in the host body where nutrients are themselves often in excess supply.

7.8.1 Nutrient requirements and *in vitro* culture

A widely adopted approach to the study of parasite nutrition involves either short-term maintenance or long-term culture *in vitro*. It is important to distinguish between these two, since the former is used primarily to examine methods of nutrient acquisition by parasites that have been removed from their hosts and held, relatively briefly, in physiologically simple media, while the latter approach employs complex media often to determine the precise nutrient requirements of the parasite. Whichever approach is adopted, the questions about the comparability of data obtained from *in vitro* studies with the events that occur *in vivo* must be addressed, and of necessity, nutritional information obtained from *in vitro* studies must be cautiously interpreted. However, since it is virtually impossible to gather meaningful nutritional facts from *in vivo* studies due to the complexity of the host–parasite interaction, we must continue to rely on *in vitro* cultivation to provide us with answers to fundamental questions on parasite nutrition.

Relatively few parasites have been cultured *in vitro* under axenic and chemically-defined conditions, and many culture media contain complex, undefined additions, such as serum, which are necessary for parasite survival. Defined media have been developed for some protozoans (e.g. *Crithidia fasciculata*, *Leishmania donovani* and *L. brasiliensis*, *Trypanosoma cruzi*, *Trichomonas vaginalis*) but this has not yet proved to be possible for helminth parasites.

7.8.2 Nutrient acquisition by parasites

In addition to feeding in the conventional sense, employing mouth and associated alimentary apparatus, many parasites obtain both dissolved and macromolecular nutrients by uptake across the body surface (membrane transport). In some groups of parasite (e.g. Protozoa, Cestoda, Acanthocephala) no mouth or alimentary canal is present so that the body surface forms the major system for molecular exchange.

Alimentary systems

The Monogenea, Digenea and Nematoda all possess a recognizable alimentary system and mouth with which are often associated structures that relate to the particular pattern of feeding. In some protozoan parasites (e.g. sporozoans) a permanent or sometimes temporary oral structure is developed, the cytostome. This is a specialized region concerned with nutrient uptake, such as haemoglobin acquisition by intracellular forms of *Plasmodium*, at which location food vacuoles, surrounded by cytostomal membrane, are formed. Digestion takes place entirely within the vacuole by chronologically distinct acid and alkaline phases.

The Monogenea and Digenea have well-developed alimentary systems comprising a mouth surrounded by a sucker, a muscular and often glandular pharynx, an oesophagus which may have associated glands, and two blind-ending digestive caeca (Fig. 7.13); only rarely is an anus present. The morphological complexity of the platyhelminth gut varies considerably but in a way unrelated to feeding activity. Among the Monogenea, two patterns of feeding predominate: blood feeding and feeding on tissues and mucus. There are physiological adaptations associated with these different types of nutrition: in the majority of blood feeders, the gastrodermal lining of the digestive caeca is shed following each meal, whereas the gastrodermis is non-deciduous

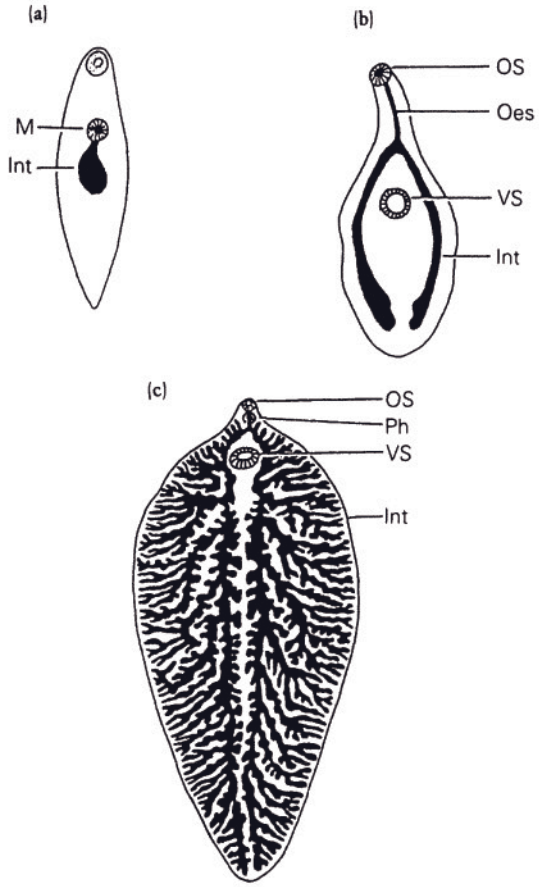

Fig. 7.13 Morphological types of digenean alimentary canal: Int, intestinal caeca; M, mouth; Oes, oesophagus; OS, oral sucker; Ph, pharynx; VS, ventral sucker. (From Chappell, 1980: after Dawes, 1968.)

in tissue feeders. Similarly, amongst the Digenea, blood and tissue feeders are found. Different species of digenean have evolved distinct approaches to the physiological problems associated with haematophagy: schistosomes are exclusively sanguivorous and they digest haemoglobin extracellularly, removing waste metallic iron by periodic regurgitation through the mouth. The liver fluke, *Fasciola hepatica*, by contrast, feeds on both tissue and blood and completes digestion of the blood meal intracellularly in the gastrodermis, passing waste iron to the excretory canals to be voided. In *Fasciola*, a curious gastrodermal cell cycle has been identified, which is related to the various phases of ingestion and digestion of food.

The gut of parasitic nematodes differs little from that of free-living relatives. Significant morphological modifications are developed anteriorly around the mouth and these reflect the diet, taking the form of teeth, jaws and penetrating stylets for engaging host tissues during feeding. The alimentary canal of nematodes comprises an anterior mouth and associated structures, a muscular and sometimes glandular pharynx, oesophagus, intestine in which both digestion and nutrient absorption take place, and a posterior rectum and anus. In a small number of insect–parasitic nematodes, the alimentary canal can be lost completely, under which conditions the exposed hypodermis of the cuticle becomes the site for nutrient acquisition (e.g. *Mermis*, *Bradynema*).

7.8.3 The parasite surface and its role in nutrition

Morphological adaptations

The surface (plasma) membrane and its associated glycoprotein coat forms, in all parasites, at least one facet of the nutritional interface with the host, and in some parasites, where the surface assumes a major nutritional function, there are marked morphological adaptations at this interface. These are most clearly seen in the platyhelminths and acanthocephalans, in which the surface architecture typifies a digestive–absorptive epithelium with its enormous increase in surface area. This is achieved by the development of surface folds and microvilli (e.g. Monogenea, Digenea, Cestoda), tubercles and spines (e.g. Digenea) or surface pores with branching invaginated canals (e.g. Acanthocephala), all of which are illustrated in Fig. 7.14. In the Protozoa, apart from the cytostome referred to above, the surface of parasites differs little from free-living forms in its nutrition-related morphology. However, in the intracellular Protozoa, the nutritional interface additionally includes the surface membrane of the host cell, perhaps in the form of the parasitophorous vacuole, and thus there may be two or more distinct barriers to nutrient acquisition.

Early light microscopy suggested that platyhelminth parasites were enclosed in a protective cuticle, presumed to be a defensive structure against host digestive and immunological attack. Electron microscopy has revealed the true picture and we now know that these parasites possess a metabolically active, non-cuticular surface, termed the tegument. This entire tissue system includes the external glycoprotein glycocalyx, the surface membrane, and beneath it, but above the basement membrane, the anucleate syncytium. The cell bodies, which form the epidermal covering in free-living platyhelminths, are sunken in parasites and are located interior to the basement

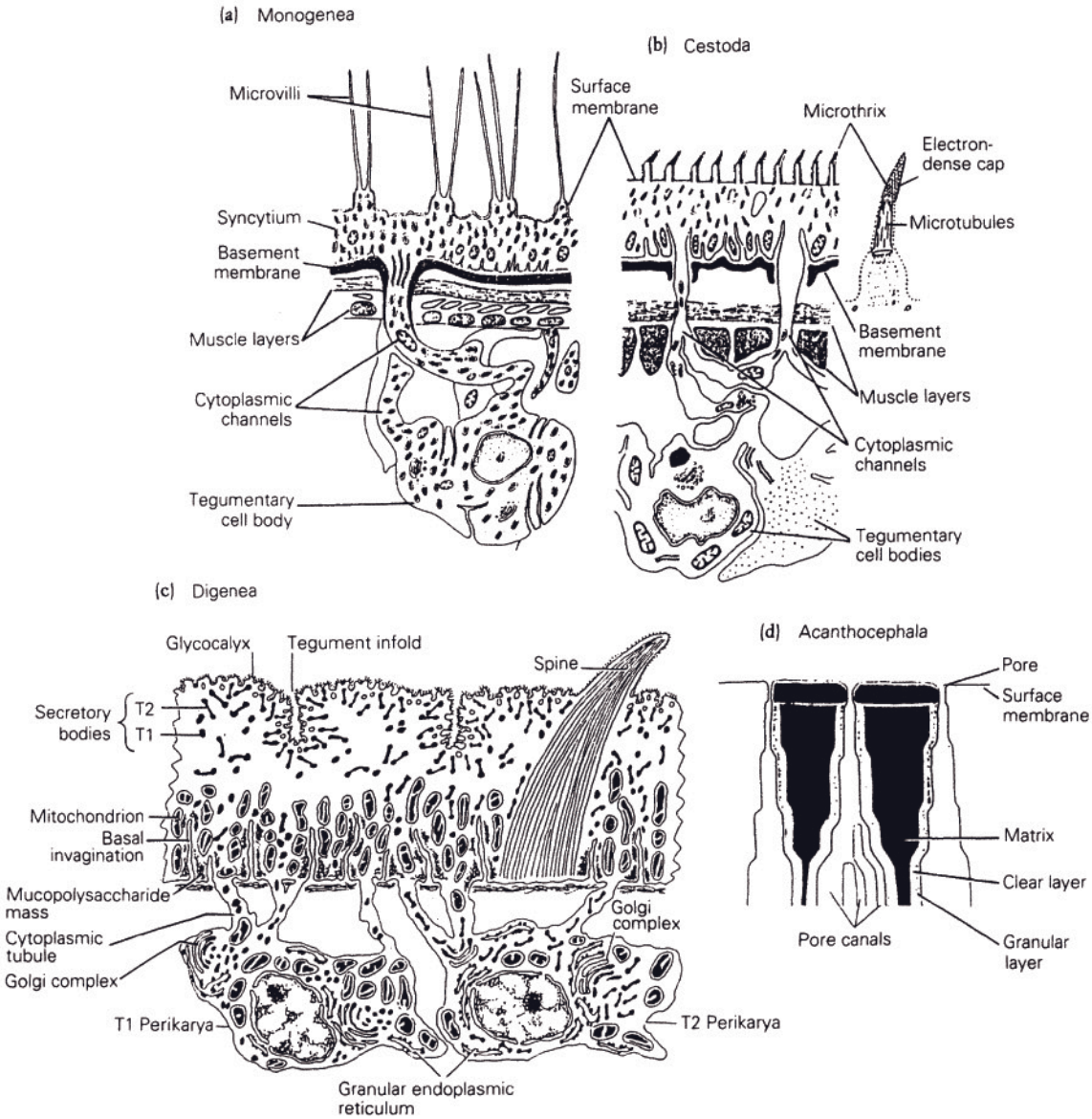

Fig. 7.14 Surface morphology in platyhelminth and acanthocephalan parasites. (From Chappell, 1980: after Lyons, 1970; Smyth & Halton, 1983; Threadgold, 1984; Crompton, 1970.)

membrane within the muscle layers (Fig. 7.14a, b, c). The syncytial layer is replete with organelles of metabolic function and replacement membrane material. Morphological study thus implicates the helminth surface as an active participant in nutrition as well as its apparent role in defence by active surface renewal. The acanthocephalan surface is physiologically comparable although morphologically quite different from the platyhelminths (Fig. 7.14d). Only in the nematodes does a true cuticle occur, but even in this group recent evidence suggests that the cuticle may be permeable to low molecular weight nutrients and to water.

Membrane transport mechanisms in parasites

Transport of nutrient molecules into parasites occurs by one, or a combination of simple diffusion, carrier-mediated transport (facilitated diffusion, active transport, exchange diffusion) or macromolecular transport (endo- or exocytosis). These transport mechanisms can be distinguished kinetically and biochemically and their occurrence may be determined in laboratory studies using *in vitro* methods.

Simple diffusion obeys Fick's law, which states that the rate of solute movement is directly related to the concentration difference of that solute

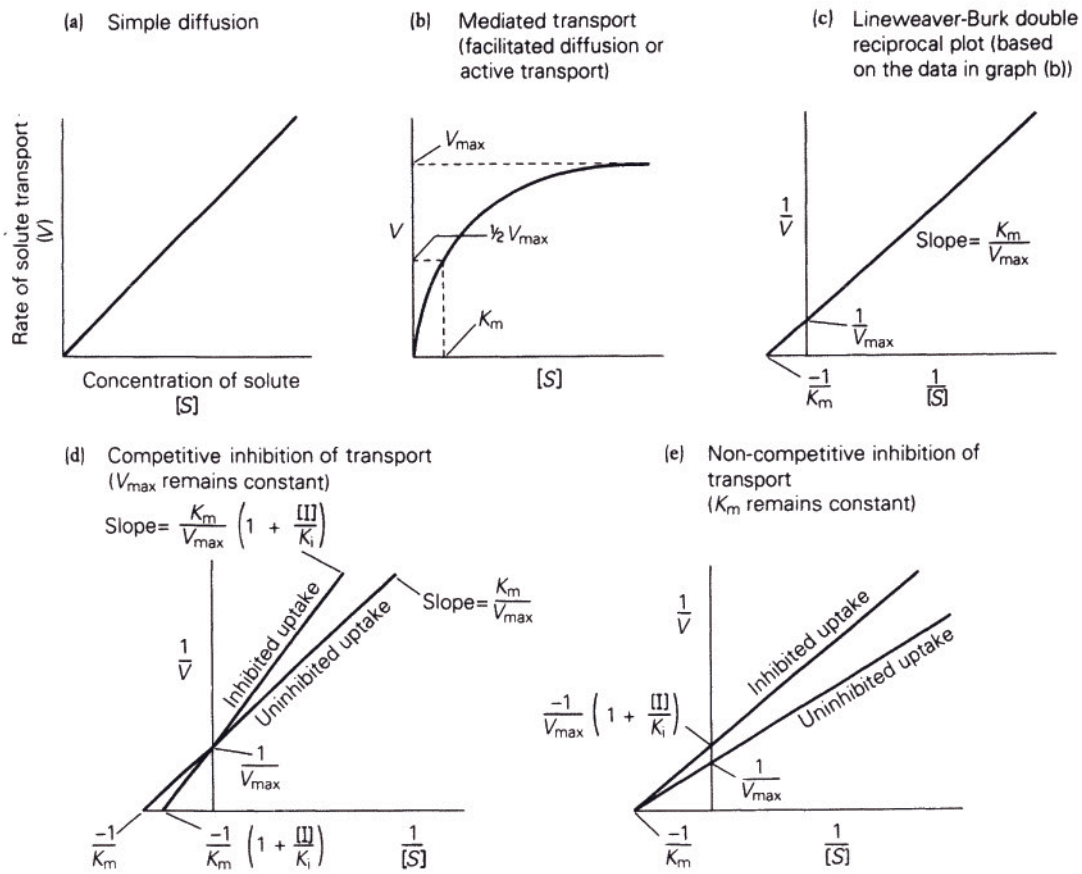

Fig. 7.15 Kinetics of membrane transport mechanisms. Often, nutrient uptake patterns follow a combination of (a) and (b) in which mediated transport is more important at low solute concentrations with diffusion assuming greater significance at higher values. The inhibitor constant (K_i) is determined experimentally as shown in (c) and (d); [I] concentration of inhibition; K_m, transport or Michaelis constant; V_{max}, maximum rate of transport; NB ($K_m = 1/2\ V_{max}$).

on either side of a semi-permeable membrane; it therefore displays linear kinetics (Fig. 7.15a), is independent of metabolic energy and temperature and does not respond to interference by competitive inhibitors. Simple diffusion involves movement of molecules down a concentration gradient. Carrier-mediated transport requires that the nutrient molecules bind specifically to, and complex with, carriers (permeases, transporters, transport sites or loci) in the membrane; these complexes have intramembrane mobility and translocate nutrients to the opposing side of the membrane where complexes are dissociated and nutrients released. This type of transport displays saturation kinetics (Fig. 7.15b, c), is temperature- and energy-dependent (e.g. active transport but not facilitated diffusion), responds to inhibition both competitively and non-competitively (Fig. 7.15d, e) and can be either uphill in terms of solute concentration (e.g. active transport) or down an electrochemical gradient (facilitated diffusion). Saturable transport systems (i.e. carrier-mediated) can be examined kinetically by application of the Michaelis–Menten (enzyme) equations from which can be derived the transport constant, K_t, defining the affinity of the substrate for the carrier, and V_{max}, which is the maximum velocity of the transport process.

Thus transport systems of different parasites may be compared kinetically and their substrate

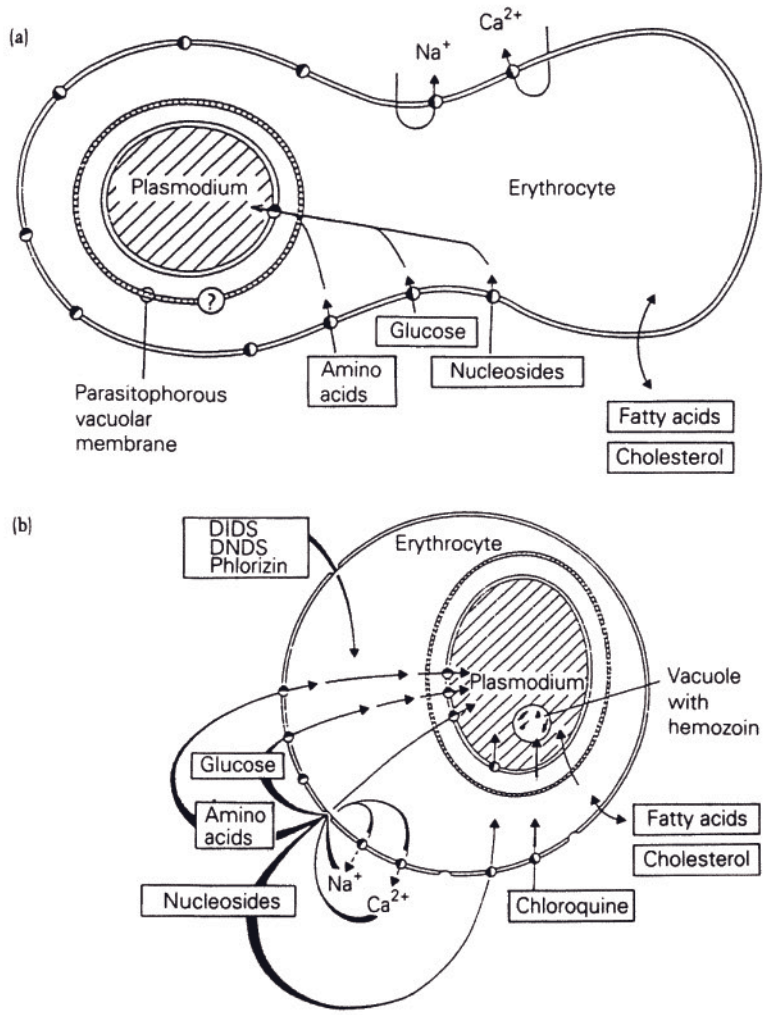

Fig. 7.16 Nutrient transport in the malaria-infected red cell before (a) and after (b) parasite-induced changes in membrane permeability. Carriers are represented as circles, exchange is shown as a double-headed arrow. After induction of permeability changes, the red cell membrane becomes leaky towards the disulphonic stilbenes DIDS and DNDS, and phlorizin. The drug Chloroquine accumulates via a membrane carrier and becomes concentrated within food vacuoles. (After Sherman, 1988.)

specificities determined by inhibitor studies. The applied importance of such work lies in the potential for the development of drugs that target specific transport systems in parasites thereby denying entry of essential nutrients. At present, very few antiparasite drugs operate in this way, but the area is rich with potential for rational chemotherapy.

The nutrient transport systems of relatively few parasites have been examined in critical detail. Below are described, in summary, data collected for four groups of parasite about which a reasonable quantity of information is available.

Plasmodium. Malaria parasites transport carbohydrates, amino acids, purine nucleosides, fatty acids, complex lipids, anions and cations and the presence of the parasite confers upon the infected red cell pathological alterations in nutrient transport that may favour the development of the parasite (Fig. 7.16). The asexual stages of the parasite within the red cell lack stored carbohydrate but require considerable quantities of glucose to fuel their active metabolism and division. Infected erythrocytes use between 10 and 50 times more glucose than uninfected cells and the parasite appears to induce permeability changes in the red cell membrane which facilitate the passage of host glucose and amino acids into the erythrocyte.

The nutritional source of amino acids for intracellular stages of malaria is not fully understood; haemoglobin digestion undoubtedly provides significant amounts, but the infected red cell also shows increased transport of free amino acids in culture. In *P. falciparum*, these changes in amino acid transport rates are first seen 15 hours after invasion and the infected erythrocyte loses energy-coupled transport systems in favour of diffusion; whether these amino acids enter the parasite itself by carrier-mediated transport or by diffusion remains to be determined and awaits the development of methods permitting culture of the asexual stages of the parasite outside the red cell.

Malaria parasites transport exogenous purine nucleosides but not pyrimidines. This may be related to their inability to synthesize the purine ring *de novo*. Parasites liberated from red cells may accumulate certain purines (e.g. adenosine, guanosine and hypoxanthine) and can incorporate radioactivity from labelled adenosine, AMP and ATP. Once again, however, studies on liberated parasites produce questionable data. Several studies have demonstrated that lipids (i.e. free fatty acids, cholesterol and phospholipid) are readily incorporated into malaria parasites resident within the red cell but the transport processes involved are unknown.

Trypanosomes. The extracellular habit of trypanosomes makes it relatively easy to examine the transport of nutrients. Carbohydrates are transported by specific carriers, some of which are capable of moving more than one species of nutrient molecule. *Trypanosoma lewisi* has two carriers that transport glucose, mannose, fructose, galactose and glucosamine; *T. equiperdum* has three carriers for monosaccharide transport, a glucose transporter, and two distinct systems for glycerol. These carriers have been defined by the use of inhibitors of transport *in vitro*. Amino acid transport into trypanosomes is complicated and several distinct carriers have been described using kinetic and inhibitor studies. *Trypanosoma cruzi* transports basic amino acids (e.g. arginine, lysine) by multiple carriers that possess unusual specificities in terms of substrates carried. Here, both neutral and acidic amino acids inhibit basic amino acid transport, a situation not encountered in mammalian transport systems. By contrast, arginine transport in *T. equiperdum*, a species with at least four distinct amino acid carrier systems, is remarkably substrate specific. *Trypanosoma brucei gambiense* transports amino acids by a mixture of carrier-mediated and non-specific (diffusion) mechanisms. Similarly, in *T. equiperdum* and *T. lewisi*, simple diffusion may assume greater significance than saturable processes when amino acids are present at higher concentrations, although it is not easy to extrapolate from these *in vitro* studies to what may happen in the blood stream of a mammal. Lipid acquisition by trypanosomes is complex and is associated with membrane-bound enzymes (acetyltransferase and phospholipase A_1), while 3′-nucleotidase/nuclease has been implicated in the uptake of purines.

Tapeworms. Much of the pioneering work on membrane transport of low molecular weight nutrient molecules in parasites was carried out on tapeworms, in particular *Hymenolepis diminuta*, the rat tapeworm, at Rice University (Texas) (see Pappas & Read, 1975). This parasite has proved to be an ideal model since it can be obtained readily in large quantities in the laboratory, is non-pathogenic both to the rat and to humans, and having no gut, absorbs all of its nutrients across the tegument. The tapeworm transports carbohydrates by both carrier-mediated systems and by diffusion – glycerol and glucose enter by separate carriers but both depend on sodium ion concentration. Amino acid and purine/pyrimidine transport are complex processes: there are six separate amino acid carriers; four transporting neutral amino acids, one for acidic and one for basic amino acids; and at least three purine/pyrimidine carriers with multiple-binding capacity. Fatty acid transport is similarly complicated and separate systems, transporting short-chain and long-chain moieties, have been described.

Digenea. Transport of nutrients across the digenean tegument is complicated by the presence of an alimentary canal and undoubtedly, *in vivo*, the gut plays a major role in nutrition, aided to an unknown extent by tegumental transport systems. Study of the latter can be carried out *in vitro* either by ligation of the pharynx or by using short-term studies in which oral ingestion of nutrients assumes an insignificant role. Both *Schistosoma mansoni* and *Fasciola hepatica* transport monosaccharides by carrier-mediated mechanisms of varying substrate specificities and while schistosomes transport amino acids also by tegumental carriers, *Fasciola* appears to lack these, absorbing amino acids by simple diffusion only. No explanation for this difference is available but it might relate to the differential dependence on the worm gut as a source of nutrients.

Digestive enzymes

The digestive enzymes of most parasites have been little studied, thus there is a paucity of information on their characteristics, substrate specificities, pH optima, secretory regulation and location. Digestive enzymes occur in the food vacuoles of protozoan parasites and in both the alimentary canal and the body surface of helminths.

The alimentary protease of *S. mansoni* has become a topic of considerable research interest, ironically primarily because of its serodiagnostic potential rather than because of its digestive role. Schistosomes ingest large quantities of host blood via the mouth and digest haemoglobin readily; the schistosome gut is typically delineated by the presence of the black pigment, haematin, which is the result of this digestion. A single proteolytic enzyme (haemoglobinase) occurs in the gut of *S. mansoni*, this being a thiol-protease with a pH optimum of around 3 and which hydrolyses globin to peptides but not to individual amino acids. It is not known whether these peptides are further hydrolysed intracellularly within the gastrodermis or if the tegumental carriers provide the major source of free amino acids from host serum. Other alimentary peptidases have recently been located histochemically, suggesting that haemoglobin digestion could be completed in the schistosome gut. The proteases of schistosomes vary in their occurrence during the life cycle and may thus have stage-specific functions; the haemoglobinase itself is only expressed in the developing schistosomulum and adult worm, in which stages haematophagy becomes physiologically important (Fig. 7.17). This adult protease is highly antigenic and is useful in the diagnosis of schistosomes in subclinical human cases as a prelude to chemotherapy.

Several helminths possess surface enzymes, either of parasite origin or derived from the host, that may have a digestive function. *Hymenolepis diminuta* synthesizes digestive phosphohydrolases, hydrolysing phosphate esters, monoglyceride hydrolases and ribonucleases, all of which function in a digestive capacity at the tegumental surface. The tapeworm can also bind host digestive enzymes, such as amylases, whereupon enzyme activity may become enhanced, although the mechanism of this so called 'contact digestion' is open to interpretation. Conversely, tapeworms can bind and inhibit host enzymes (e.g. trypsin, chymotrypsin) and this is possibly one adaptation for parasite survi-

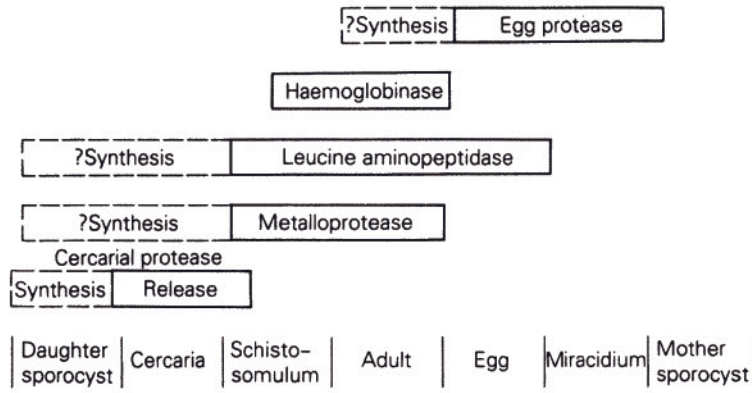

Fig. 7.17 Schistosome proteases may be expressed in a stage-specific manner. (After McKerrow & Doenhoff, 1988.)

val in an enzymatically hostile environment.

Surface membrane-bound enzymes have also been described in some protozoans. *Leishmania* spp, and other trypanosomatids, for instance, possess a 3′-nucleotidase/nuclease complex in their surface membranes which can hydrolyse 3′-nucleotides and nucleic acids. The hydrolytic activity of this complex is implicated in the acquisition of purines associated, perhaps, with the inability of the parasite to synthesize the purine ring *de novo*. The membrane-bound acyltransferase of African trypanosomes may also play a part in nutrient acquisition, in this case in the uptake and internalization of lipid, particularly phopholipids.

7.8.4 Excretory physiology

Since the life histories of many parasites are complex, involving alternation of free-living and parasitic stages, it might be expected that the processes of regulation of water and ion content and removal of toxic excretory products would reflect such complexity. Little information, however, is available.

In parasites, two types of excretory system are found: (1) the contractile vacuole of protozoans; and (2) the protonephridial system of platyhelminths. Contractile vacuoles are present in many ciliates, but are absent from amoebae and sporozoans. It seems likely that these vacuoles are involved in both osmoregulation and excretion of nitrogenous waste.

The protonephridial system of platyhelminth parasites comprises numerous blind-ending tubules that interconnect and open to the outside at a single nephridiopore. Each tubule has at its terminus a flame cell or cluster of cells, so called because the wave-like beating of the flagella is reminiscent of a flickering candle. Each 'flame' contains between 50 and 100 flagella whose beat regulates fluid flow in the excretory tubule and possibly draws solutes into the terminal organ of the protonephridial system from the surrounding parenchyma. Ultrafiltration may occur at this stage and it has been demonstrated experimentally that a sufficiently high filtration pressure could be developed by the flagellar beat of the flame cell.

Excretion of nitrogenous waste (e.g. ammonia) may take place via diffusion or via the protonephridial system. Detailed analysis of the protonephridial canal fluid of the tapeworm *H. diminuta* reveals the occurrence of inorganic ions (sodium, potassium, chloride, carbonate), ammonia, amino acids, urea and lactic acid. This analysis accounts for 90% of dry matter and the pH of the canal fluid is 4.5 with a P_{CO_2} of 120 mmHg. These data imply an excretory role but the key elements of ultrafiltration and active transport within the system have yet to be demonstrated.

The excretory systems in acanthocephalans and nematodes are poorly understood and there is little physiological evidence for either group to substantiate what is inferred on morphological grounds alone.

7.9 PARASITE PHYSIOLOGY IN A WIDER CONTEXT

There are many areas of physiology which clearly reflect the unique and often remarkably complex life styles adopted by parasites. The inherent multi-faceted nature of the parasitic life cycle suggests an enormous adaptability, ultimately residing within the genome, which must surely convince the student of the sophisticated status of the parasitic animal. As more information accrues, we come to realize that there are more targets for chemical, immunological or environmental attack upon which we may effect parasite control, but at the same time we discover that the subtleties of parasite evolution have conferred upon these organisms a considerable buffer against the types of onslaught we can currently mobilize.

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