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Biology of toxoplasmosis

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History

Toxoplasma gondii is a coccidium, with the domestic cat and other felids as its definitive host and a wide range of birds and mammals as intermediate hosts. It was first detected by Nicolle and Manceaux (1908, 1909) in a rodent *Ctenodactylus gondi*, and by Splendore (1908) in a rabbit. The name *Toxoplasma* is derived from the crescent shape of the tachyzoite (in Greek: toxo = arc, plasma = form). Knowledge of the full lifecycle of *T. gondii* was not completed until 1970, when the sexual phase of the lifecycle was identified in the intestine of the cat, by demonstrating oocysts in cat faeces and characterizing them biologically and morphologically (Dubey et al. 1970*a*, *b*).

Taxonomy

Toxoplasma gondii is placed in the phylum Apicomplexa (Levine 1970), class Sporozoasida (Leukart 1879), subclass Coccidiasina (Leukart 1879). Traditionally, all coccidia until 1970 were classified in the family Eimeriidae. After the discovery of the coccidian cycle of *T. gondii* in 1970, *T. gondii* has been placed by different authorities in the families Eimeriidae, Sarcocystidae or Toxoplasmatidae. Phylogenetic analysis of *T. gondii* and order Apicomplexa is shown in Figure 1.1.

Lifecycle

The definitive host is the domestic cat and other Felidae (Frenkel et al. 1970; Jewell et al. 1972), where the sexual cycle takes place in the intestinal epithelial cells. Infected cats excrete oocysts which are infectious to virtually all warm-blooded animals. There are three infectious stages of the parasite: the tachyzoite (the rapidly dividing form) in tissues, the bradyzoite (the slowly dividing form) inside cysts in tissues and the sporozoites in the oocyst in cat faeces (Figure 1.2).

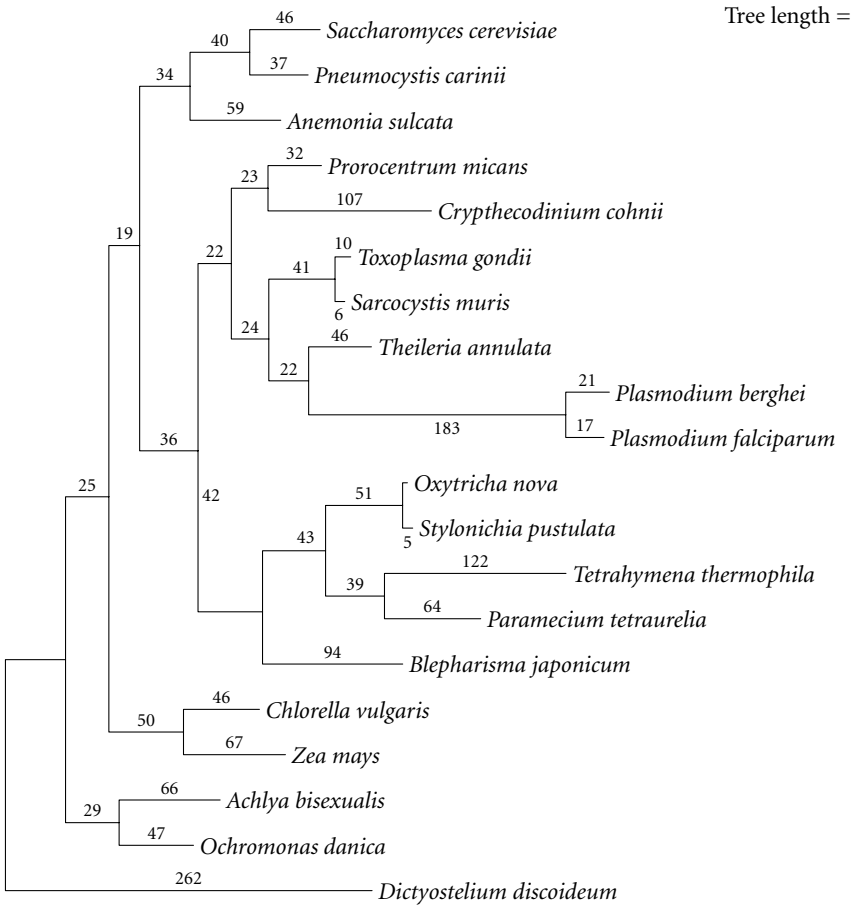

Figure 1.1 Phylogenetic analysis of *Toxoplasma gondii* and other Apicomplexa. Numbers represent character changes between organisms on the branch of the tree. The tree length represents the overall change of characters that result in the most parsimonious tree obtained. (From Gagnon, S., Levesque, R. C., Sogin, M. L., & Gajadhar, A. A. (1993). Molecular cloning, complete sequence of the small subunit ribosomal RNA coding region and phylogeny of *Toxoplasma gondii*. *Molecular and Biochemical Parasitology*, **60**, 145–8. With permission from the authors and the publisher.)

The enteroepithelial cycle in the definitive host – the cat

Five morphologically distinct asexual stages (types A–E) of *T. gondii* develop in enterocytes before gametogony begins (Dubey & Frenkel 1972). The origin of the gametes has not been finally established, but it is believed that merozoites (stages D and E) develop into gametes. Gametes occur throughout the small intestine, but are most prevalent in the ileum, where they are found 3–15 days after infection.

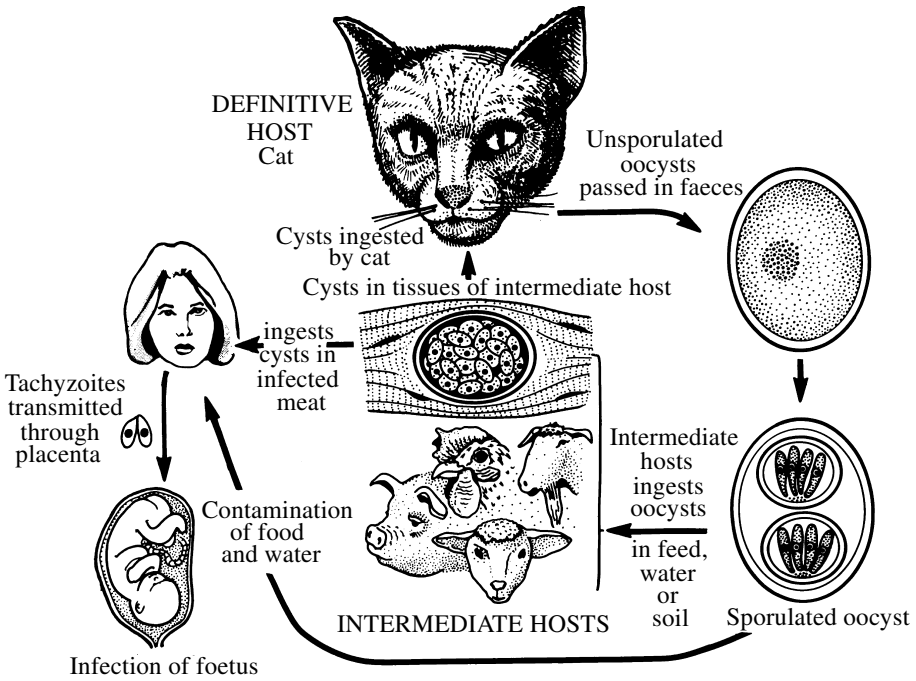

Figure 1.2 Lifecycle of *Toxoplasma gondii*.

The microgamete (the male gamete) is biflagellate and fertilizes the macrogamete (the female gamete) within the enterocyte. Oocysts are formed when a wall is laid around the fertilized gamete (zygote). Oocysts are expelled into the intestinal lumen after the rupture of enterocytes and are unsporulated when excreted in faeces.

The prepatent period (interval between ingestion and shedding of oocysts) after the ingestion of tissue cysts is 3–10 days, with peak oocyst production between 5 and 8 days after a patent period varying from 7 to 20 days (Dubey & Frenkel 1972, 1976). Cats not previously infected with *T. gondii* shed oocysts after ingesting each of the infective stages of the parasite: the tachyzoite, the bradyzoite and the sporozoite (Frenkel et al. 1970; Dubey & Frenkel 1976). The prepatent period varies according to the stage of *T. gondii* with which the cat is infected, with a short (3–10 days) prepatent period when the oral inoculum contains bradyzoites and a long prepatent period (>13 days) when the inoculum contains tachyzoites (Dubey 1998*b*) or sporozoites (Freyre et al. 1989; Dubey 1996). Cats previously infected with *T. gondii*, and which produced oocysts during the previous infection, are generally immune to renewed oocyst shedding, but immunity is not life long (Dubey & Frenkel 1974; Frenkel & Smith 1982; Dubey 1995).

Fertilization initiates oocyst wall formation. The oocyst is the developed zygote, which is the product of sexual reproduction through fertilization of the macrogamete by the microgamete. The oocysts are discharged into the intestinal lumen by rupture of the epithelial cells, and thereafter excreted in cat faeces. The oocysts sporulate within 1–5 days after excretion, depending on aeration, humidity and temperature, by dividing into two sporocysts. Each sporocyst contains four sporozoites. Thus, there are eight sporozoites in one oocyst. The sporulated oocyst can remain infectious in the environment for months even in cold and dry climates (reviewed in Dubey 1977).

The asexual cycle in the definitive host – the cat

As the entero–epithelial cycle progresses, bradyzoites penetrate the lamina propria below the epithelial cell in the intestine of the cat and multiply as tachyzoites. The tachyzoites are disseminated throughout the body within a few days, eventually encysting in tissues. The extra-intestinal cycle in the cat differs from the similar cycle in nonfeline intermediate host in two aspects: (1) tachyzoites have not been demonstrated in feline intestinal epithelial cells, whereas they do occur in nonfeline intermediate hosts (Dubey & Frenkel 1973), and (2) the entero–epithelial types of *T. gondii* are noninfectious to mice by any route (Dubey & Frenkel 1976), which suggests that the feline entero–epithelial forms do not give rise to tachyzoites.

Intermediate host

Toxoplasma gondii tachyzoites are disseminated throughout the body of the intermediate host in macrophages and lymphocytes as well as free in the plasma. Tachyzoites continue to divide within the host cell by endodyogeny (internal division into two) until the host cell is filled with parasites. At a given time the dividing tachyzoites cannot be contained within the host cell, which bursts. The tachyzoites are released and seek new host cells to repeat the process. Depending on the strain of *T. gondii* and the host resistance, tachyzoites may be found for days or even months after acute infection. For example, tachyzoites persist in foetal membranes for weeks after infection of the mother or the dam, and are nearly always present in placentas of mothers at the time of parturition, if the foetus was infected *in utero*.

At some time after infection the tachyzoites transform to bradyzoites in tissue cysts. The signals responsible for the transformation are not known, and the debate continues as to whether signals from the host immune system are needed. Bradyzoites also divide by endodyogeny. Bradyzoites are enclosed in a thin cyst wall. Tissue cysts may be found as early as 3 days after infection but are usually not numerous until 7 weeks after infection (Dubey & Frenkel 1976; Derouin & Garin 1991; Dubey et al. 1998). Intact tissue cysts probably do not cause any

inflammation and may persist for life. It has been suggested that tissue cysts may switch from the bradyzoite stage to the tachyzoite stage during the life of the tissue cysts, producing new tachyzoites which may give rise to new tissue cysts thus ensuring a prolonged infective stage (Hérion & Saavedra 1993). If the intermediate host is eaten by another warm-blooded animal, tissue cysts are able to infect a new host.

Fewer than 50% of cats shed oocysts after ingesting tachyzoites or oocysts, whereas almost all cats shed oocysts after ingesting tissue cysts (Dubey & Frenkel 1976). Cats infected with oocysts and tachyzoites probably give rise to bradyzoites, which after a variable period of time may disseminate to the intestinal mucosa and start the entero–epithelial cycle with the resulting production of oocysts (Freyre et al. 1989). For comparison, the lifecycles of major coccidian genera are shown in a simplified form in Figure 1.3.

Morphology, ultrastucture and antigens

The tachyzoite

The tachyzoite (previously called trophozoite) is crescent shaped and is approximately 2×6 μm in size (Figure 1.4). The tachyzoite has a pellicle, subpellicular microtubules, a polar ring, a conoid, rhoptries, micronemes, mitochondria, endoplasmatic reticulum, Golgi apparatus, ribosomes, rough surface endoplasmatic reticulum, micropores and a well-defined nucleus (Figure 1.5).

The nucleus is situated in the central or posterior part of the cell (Sheffield & Melton 1968). The pellicle consists of three membranes. The inner membrane is discontinuous in three areas: at the polar ring (anterior), at the micropore (lateral) and towards the posterior end. The polar ring is an osmiophilic thickening of the inner membrane at the anterior end of the tachyzoite. The polar ring encircles the conoid, a cylindrical cone which consist of six to eight fibrillar elements arranged like a compressed spring. The 22 subpellicular microtubules originate from the polar ring and run longitudinally for almost the entire length of the cell (Sulzer et al. 1974) and probably provide a frame for the parasite.

The rhoptries are four to ten club-shaped, gland-like structures with an anterior narrow neck and posterior-sac-like end reaching as far as the nucleus. The rhoptries contain a honey-combed structure and are thought to have a secretory function associated with host cell penetration. When the parasite has attached to the host cell, the contents of the rhoptries are discharged through the conoid (Nichols et al. 1983). The micronemes are rice-grain-like structures, usually fewer than 100 in number, situated at the conoidal end of *T. gondii* without any defined function, but they may participate in invasion of the host cell (Joiner & Dubremetz 1993). In addition to the rhoptries and the micronemes, the parasite contains dense granules which also appear to have a secretory function (Charif et al. 1990).

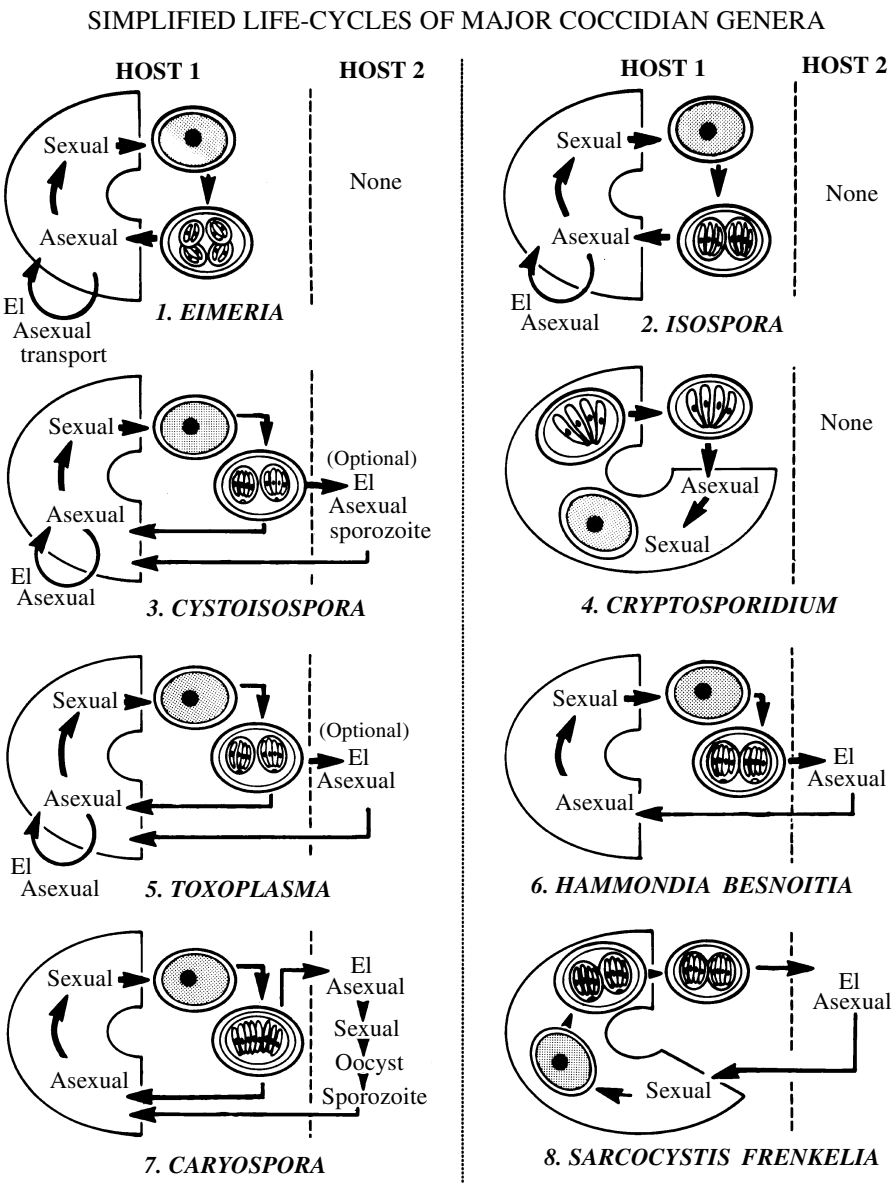

Figure 1.3 Simplified lifecycles of major coccidian genera. For each genus, a diagrammatic representation of the intestinal tract appears on the left of the dotted line, under 'HOST 1' (the definitive host), where oocyst morphology is also shown. On the right, under 'HOST 2', the extraintestinal stages that develop in the intermediate host are listed in order of development. (From Fayer, R. & Dubey, J. P. (1987). *Int J Parasitol*, **17**, 615. With permission.)

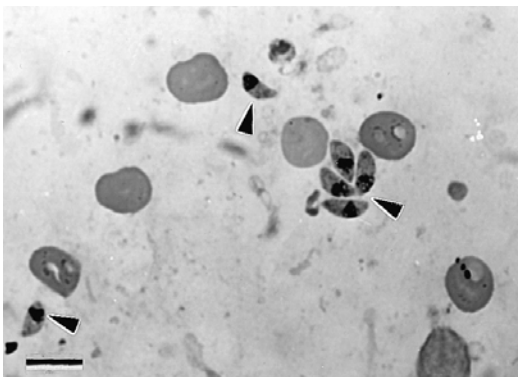

Figure 1.4 *Toxoplasma gondii* tachyzoites (arrowheads) in smear. Mouse peritoneum. Giemsa stain. ×750. Bar = 10 μm.

The functions of the conoid, rhoptries and micronemes are not fully known. The conoid can rotate, extend and retract and is important when the parasite searches for an attachment site at the host cell, as the parasite can rotate, glide and twist. Myosin has been found in the apical end of the parasite (Schwartzman & Pfefferkorn 1983), and actin has been found both at the apical end and distributed throughout the cytoplasm (Endo et al. 1988). The motion observed during parasite entry corresponds to the orientation of the subpellicular microtubules, and it is likely that the microtubules are the basis of the motility system. The microphores are sites specialized for the uptake of nutrients through endocytosis (Nichols et al. 1994).

After entry into the host cell, the parasite is surrounded by a parasitophorous vacuole membrane (PVM). The PVM contains numerous intravacuolar tubules (Sheffield & Melton 1968; Sibley et al. 1985; Sibley & Krahenbuhl 1988; Sibley et al. 1995; Dubey et al. 1998). The intravacuolar tubules appear to be connected to the parasite plasmalemma and consist of host cell vimentin-type intermediate filaments (Halonen & Weidner 1994). *Toxoplasma gondii* enters the host cell by active invasion (Werk 1985).

Endodyogeny is a process in which two progeny form within *T. gondii*, and consume it from within (Sheffield & Melton 1968). The Golgi apparatus divides first, and the anterior cell membranes of the progeny are formed at the anterior end. The nucleus of the parent cell becomes horseshoe-shaped, and part of the nucleus moves towards the anterior end of the developing cells. The nuclear membranes remain intact and the chromosomes do not condense at metaphase. The progeny move towards the cell membrane of the parent parasite as they continue to grow,

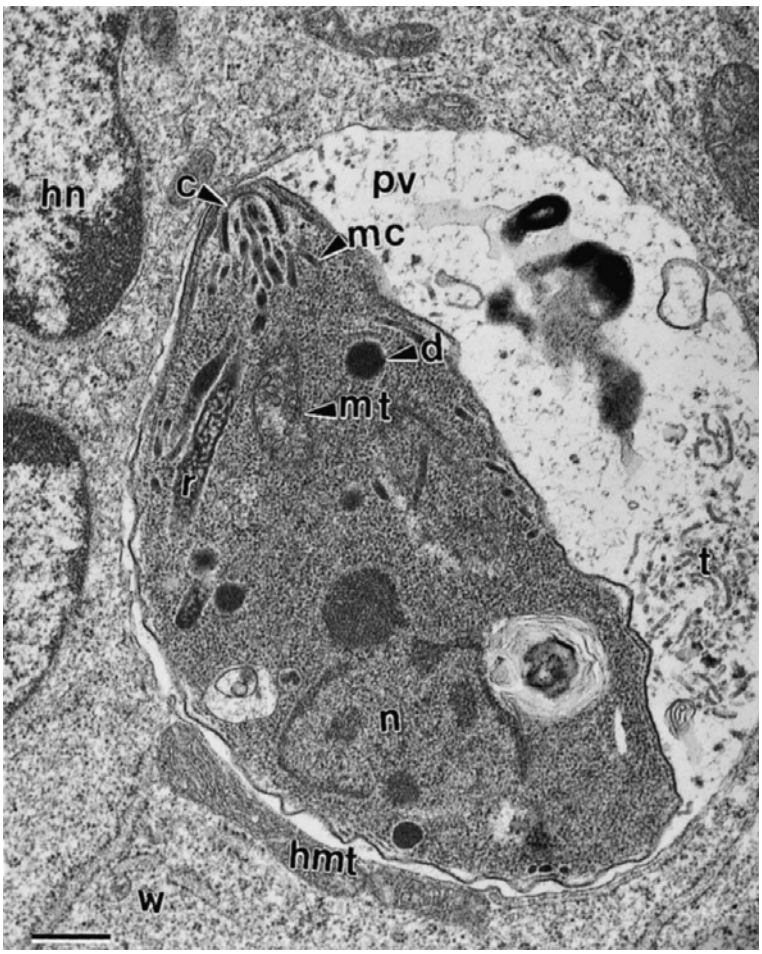

Figure 1.5 Electron micrograph of a *T. gondii* tachyzoite in the parasitophorous vacuole (pv) in the cytoplasm of a human foreskin fibroblast, second day *in vitro* culture after inoculation with GT-1 strain tachyzoites. Note the conoid (c), micronemes (mc), rhoptries (r), dense granules (d), mitochondrion (mt), nucleus (n) in the tachyzoite and numerous intravacuolar tubules (t) inside the parasitophorous vacuole (pv). hmt refers to host cell mitochondrion and hn is host cell nucleus. ×28,300. Bar = 0.35 μm (Courtesy of Dr D. S. Lindsay, Auburn University, Alabama, USA.)

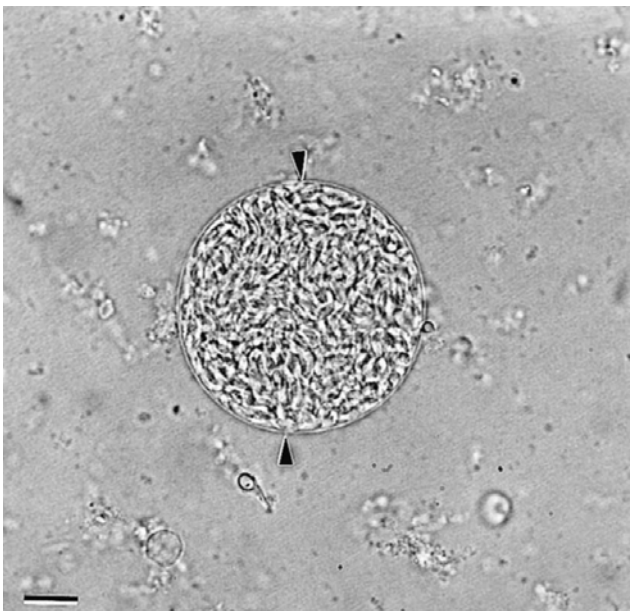

Figure 1.6 *Toxoplasma gondii* tissue cyst in the saline homogenate of mouse brain. Note the thin cyst wall (arrowheads) enclosing hundreds of bradyzoites. Unstained ×750. Bar = 10 μm.

and finally the inner membrane of the parent parasite disappears and the outer membrane fuses with the inner membrane of the progeny, and two new tachyzoites are formed.

The bradyzoite and tissue cysts

The bradyzoite (brady = slow) is the organism dividing slowly within a tissue cyst (Frenkel 1973) and is a synonym of cystozoite. A tissue cyst is a collection of bradyzoites surrounded by a well-defined host cell membrane (Figures 1.6, 1.7). The bradyzoites also multiply by endodyogeny. Tissue cysts are from 5 μm to 60 μm in size in the brain and 100 μm in other tissues (Dubey 1993) and contain four to several hundred bradyzoites. Tissue cysts may develop in any tissue but are most prevalent in neural and muscular organs such as the eye and brain, skeletal and cardiac muscles (Figure 1.8). The cyst wall is thin (<0.5 μm). The tissue cyst develops in the host cell cytoplasm and its wall is intimately associated with the host cell endoplasmic reticulum (ER); indeed the cyst wall is partly of host origin (Ferguson & Hutchison 1987*a*,*b*; Sims et al. 1988). Mature cyst walls are lined with a granular material which is also found between the bradyzoites (Figure 1.9). In older cysts, degenerating bradyzoites may occasionally be found (Pavesio et al. 1992). The

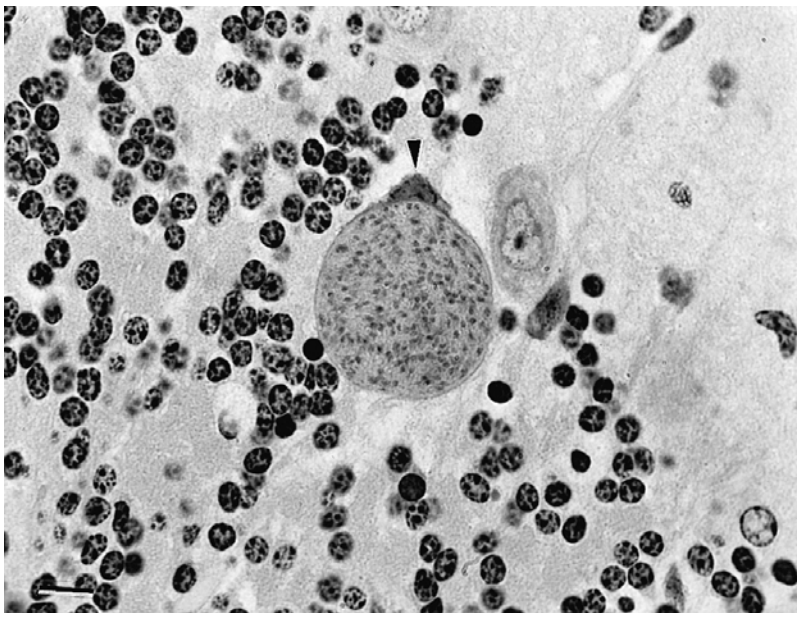

Figure 1.7 Intracellular *T. gondii* tissue cyst in a section of mouse cerebellum. Note the host cell nucleus (arrowhead). Haematoxylin and eosin stain. ×750. Bar = 10 μm.

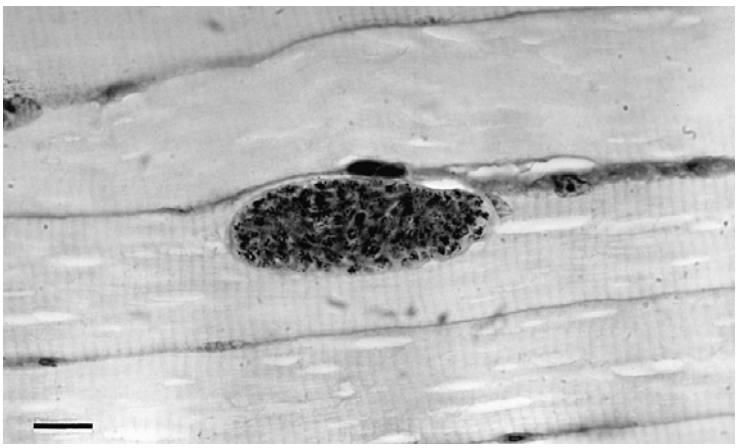

Figure 1.8 *Toxoplasma gondii* tissue cyst in a section of mouse skeletal muscle. Note the cyst is elongated and contains dark-staining polysaccharide granules in bradyzoites. Periodic acid Schiff haematoxylin. ×750. Bar = 10 μm.