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Electron Microscopy of Stages of *Isospora felis* of the Cat in the Mesenteric Lymph Node of the Mouse

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Abbreviations. A Polysaccharide granules (? Amylopectin); C Conoid; CR Crystalloid body; DK Dark bodies; ER Endoplasmic reticulum; FI Fibrillar elements; G Golgi apparatus; GM Granular material within the parasitophorous vacuole; IT Intravacuolar tubules; LP Limiting membrane of the parasitophorous vacuole; MI Mitochondrion; MIH Mitochondrion of the host cell; MN Micronemes; MP Micropore; MT Subpellicular microtubules; N Nucleus; NH Nucleus of the host cell; NHN Nucleus of the adjacent host cell; NM Nuclear membrane; NU Nucleolus; P Polar ring; PE Pellicle; PV Parasitophorous vacuole; R Rhoptries; UM Unit membrane; WZ Host cell

Summary. Stages of *Isospora felis* of the cat in the mesenteric lymph node of the mouse 25 days after oral inoculation with oocysts, have been described at the ultrastructural level. The organisms occurred singly within parasitophorous vacuoles in host cell cytoplasm and were sporozoite-like, having a large crystalloid body up to 5.5 μm in length posterior to the nucleus. The size and appearance of the parasitophorous vacuole varied. Some vacuoles contained numerous, small, electron dense granules about 30 nm in diameter. Because of the aggregation of granules and their arrangement within the parasitophorous vacuole, the impression was sometimes gained by light microscopy that parasites were surrounded by a sheath or cyst wall. However, a cyst wall was not present. In host cells, spherical, membrane-bound bodies with a homogeneous, electron dense core and a maximum diameter of 0.25 μm were filed along the limiting membrane of the parasitophorous vacuole.

These extra-intestinal parasites were considered to be *waiting stages*, with a biological function similar to that of the tissue cyst stage of other genera of isosporan coccidia.

Introduction

Isospora felis, the largest of the coccidia of domestic cats, has been thought to develop exclusively within the intestinal epithelium of this host. Dubey and Frenkel

(1972) and Markus (1975a), however, demonstrated that *I. felis* may additionally invade extra-intestinal tissues of cats. Frenkel and Dubey (1972) found, furthermore, that rodents may serve as potential vectors or intermediate hosts of *I. felis*. These authors saw stages of the parasite in the mesenteric lymph nodes of mice but showed indirectly by transmission experiments that *I. felis* also invades other extra-intestinal tissues. Markus (1975a, b) observed *I. felis* both in mesenteric lymph nodes of mice and in other organs; and he saw extra-intestinal forms in experimental birds. Markus (1975a) considered that limited division of sporozoites took place in both mice and chickens within a few days of oocysts being fed to these hosts. Frenkel and Dubey (1972), Frenkel (1974) and Markus (1975a, b) thought that these extra-intestinal stages in chronically infected non-feline hosts were monozoic cysts.

If cysts of *I. felis* occur in non-feline hosts, this could indicate that the parasite is awaiting transmission, for example through a cat capturing an infected mouse. In order to ascertain whether or not *I. felis* has a tissue cyst formed in internal organs during the first month of the infection, we studied by means of electron microscopy the mesenteric lymph node of one of a series of mice which had been infected with sporulated oocysts.

Materials and Methods

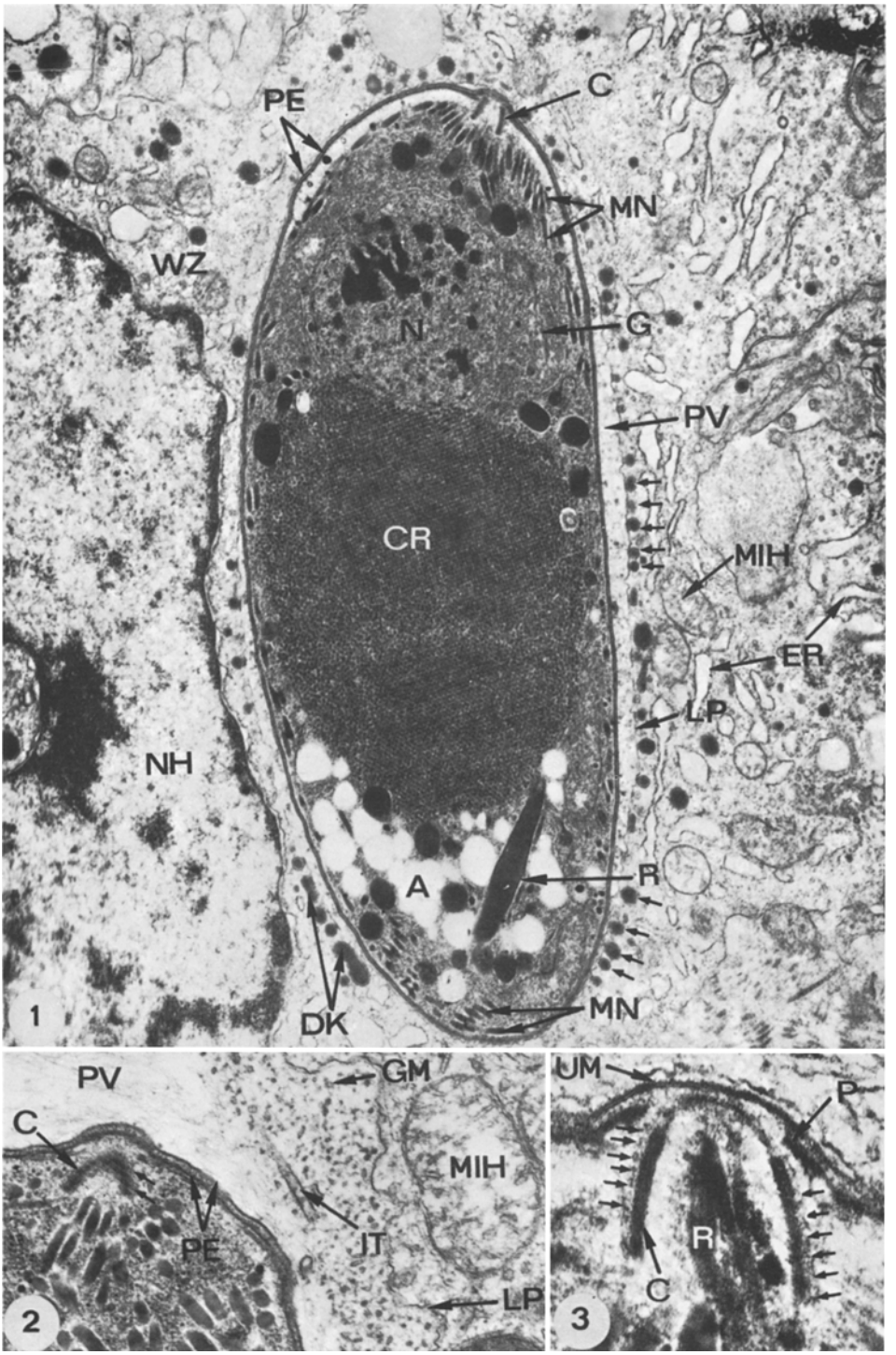
Oocysts of *Isospora felis* were recovered by centrifugal flotation, using a saturated NaCl solution, from faeces of a naturally infected kitten that did not have a concomitant *I. rivolta* or any other detectable coccidial infection. *I. felis* was passaged four times in four coccidia-free, conventionally reared cats respectively and, finally, in a specific pathogen-free (SPF) cat. After each passage, oocyst samples obtained by centrifugal flotation were very carefully screened. At no time were coccidia other than *I. felis* seen. Oocysts harvested from fresh SPF cat faeces were washed in water after flotation, sporulated at room temperature in 2.5% w/v $K_2Cr_2O_7$ and stored at 4° C in potassium dichromate solution. Five weeks later the oocysts were washed again in water and used to infect mice.

Caesarian derived female T.O. mice supplied by A. Tuck and Son Limited, Rayleigh, England were maintained in the laboratory on an ad lib. diet of water in drinking bottles and mouse pellets. In one of several experiments, 470,000 or 630,000 sporulated oocysts of *I. felis* in water were fed by stomach tube to each of 16 normal and 16 immunosuppressed mice. Sixteen mice were used as uninfected controls. Control and infected mice, which weighed 30–40 g at autopsy, were sacrificed at the following intervals: 24, 48, 72 and 96 h and 5, 10, 25 and 45 days (Markus, 1975a). The samples of mesenteric lymph node used for electron microscopy in the present study were from one of the normal mice killed on day 25 post inoculation. Examination under the light microscope of Giemsa-stained impression smears of the mesenteric lymph node, liver and spleen of this individual showed typical extra-intestinal isosporan stages in all three tissues.

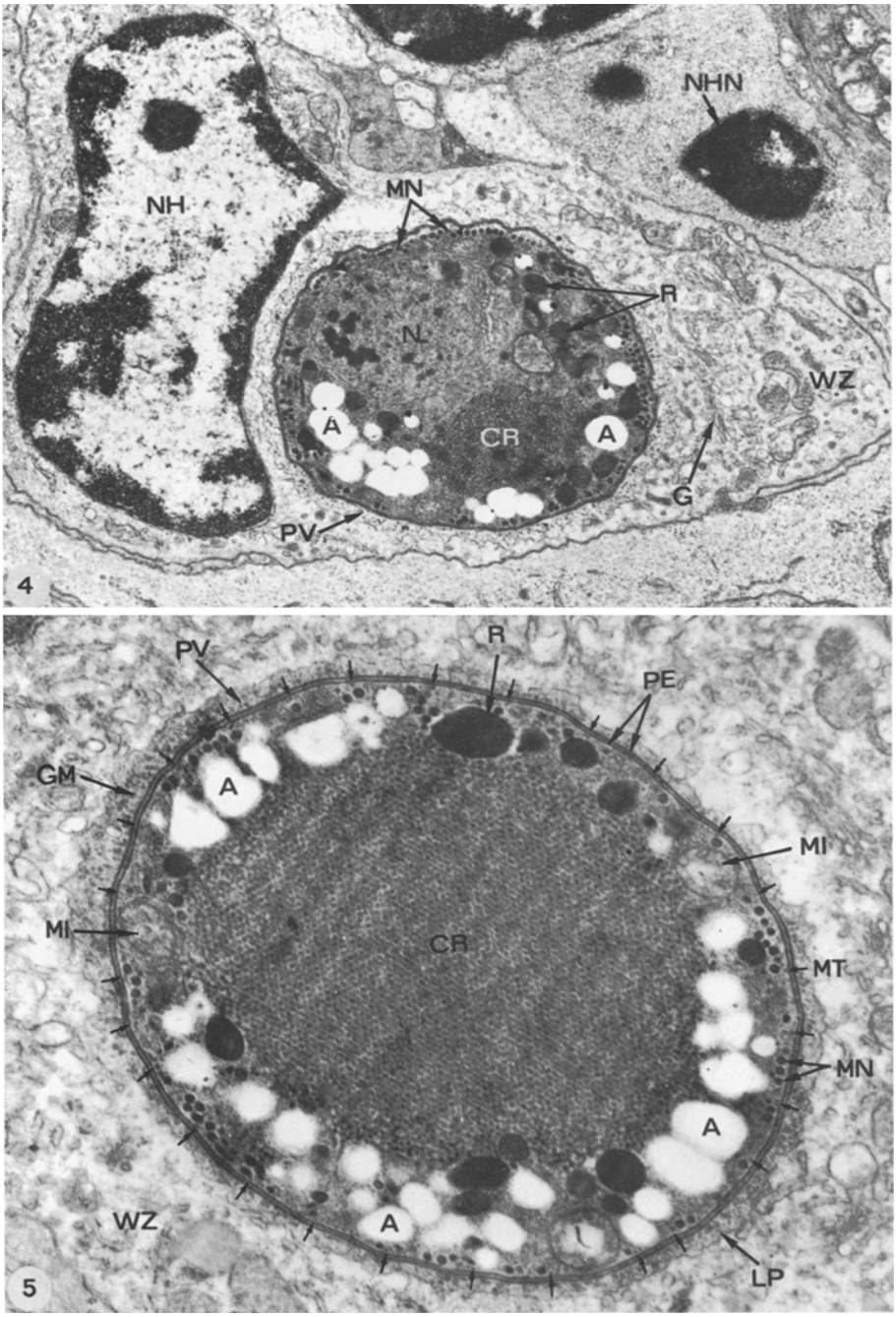
Small pieces of mouse mesenteric lymph node up to about 1 mm cubes were placed in Karnovsky's fixative (Karnovsky, 1965), buffered at pH 7.4, for 15 min at room temperature, followed by 1 h at 0° C. The tissue was washed in 0.12 M cacodylate buffer at 0° C for 30 min, then postfixed with 2.5% OsO_4 in 0.1 M cacodylate buffer at 0° C for 1 h, after which it received two 10 min rinses in 0.1 M sodium acetate at 4° C. Following this, the pieces were placed in 0.25% aqueous uranyl acetate at 4° C for 1 h, rinsed twice in 0.1 M sodium acetate at 4° C for 10 min each time and dehydrated in 35% and 50% acetone for 5 min each. While in 70% acetone, the tissue was prestained in 1% uranyl acetate and 1% phosphotungstic acid at 4° C for 20 h. After being dehydrated further at room temperature in 95% acetone for 10 min and in three changes of 100% acetone for 15 min each, the pieces of lymph node were kept in an acetone/araldite mixture at room temperature for 6 h and then embedded in araldite. Ultra-thin sections were cut on a Reichert OM U 3 ultramicrotome and contrasted with uranyl acetate and lead citrate, for about 15 min in each. Sections were examined with a Zeiss EM 9 S 2 electron microscope.

Results

All stages of *I. felis* observed here were situated singly within a host cell. The parasites measured approximately 11–14 μm in length and were about 4–5.5 μm in



Figs. 1–3. *Isospora felis*. Electron micrographs. **1.** Longitudinal section through a parasite within a lymphoreticular cell of a mouse on the 25th day post inoculation. Note that numerous, dense bodies (DK: arrows) are filed along the limiting membrane (LP) of the parasitophorous vacuole (*PV*). ×14,000. **2.** Tangential section through the conoid (*C*). Note the construction by microtubules (arrows). ×22,000. **3.** Longitudinal section through a conoid (*C*) at high magnification. There are regularly arranged fibrillar elements at the outer sides of this conical structure (arrows). × 60,000



Figs. 4–5. *Isospora felis*. Electron micrographs of cross sections through parasites within small parasitophorous vacuoles (*PV*). Note in Fig. 5 that the subpellicular microtubules (*MT*) are regularly arranged around the margin of the organism (arrows). **4.** ×15,000. **5.** ×33,750

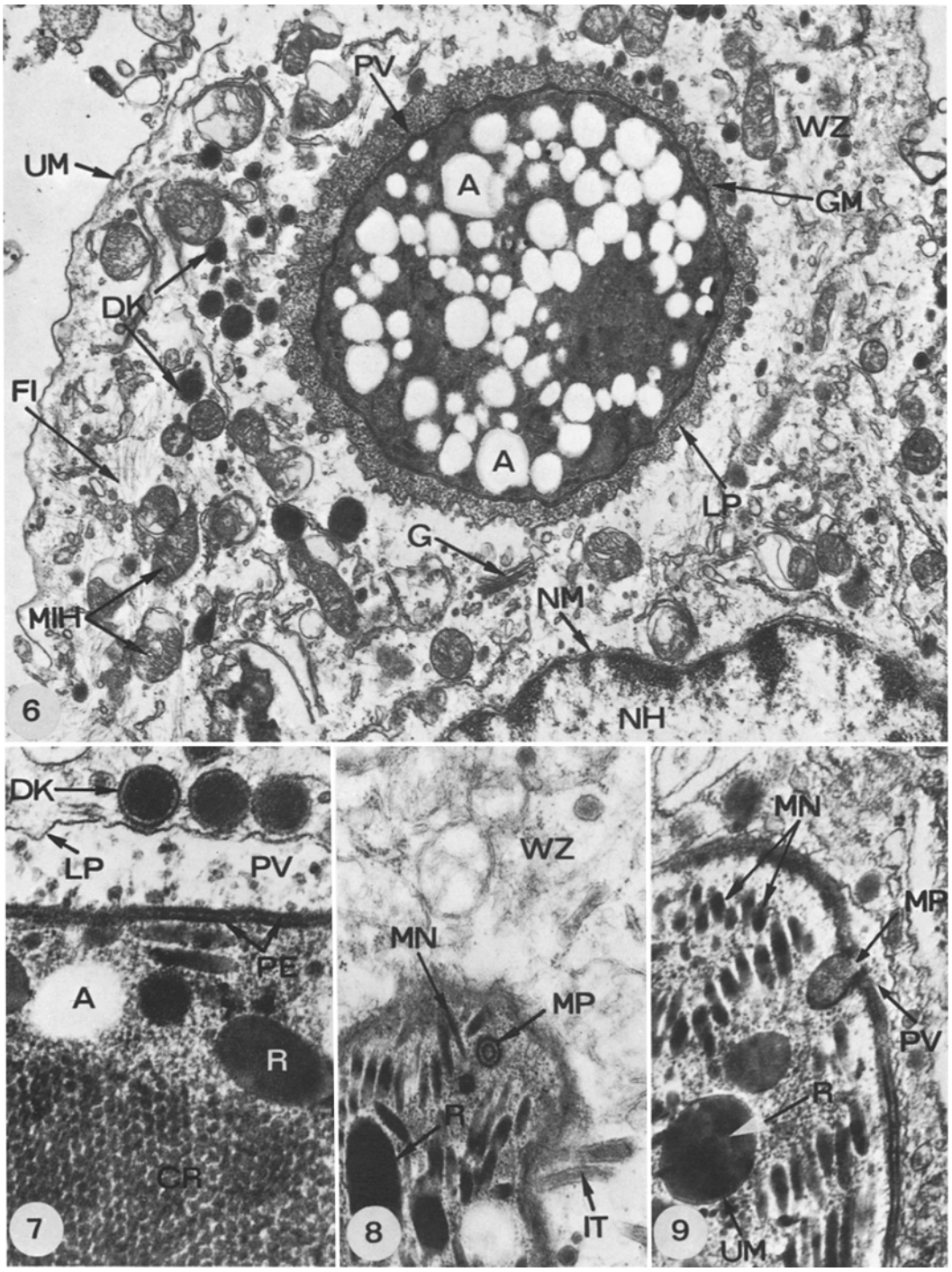
diameter at their mid-region. Their typical appearance is depicted schematically in Fig. 10. Spherical forms were not observed. The organisms were always limited by a typical, three-layered coccidian pellicle (Figs. 1, 2, 5, 7; PE). The inner layer of the pellicle was interrupted only at the apical and posterior poles, where it formed polar rings (Figs. 3, 10; P), and at the micropores (Figs. 8, 9; MP). The outer unit membrane (Figs. 3, 10; UM) surrounded the whole parasite. The micropores occurred singly whenever seen and were always situated in an anterior third of the cell. They had a diameter of about 80 nm and the invagination often measured approximately 0.3 μm (Fig. 9; MP). About 27 subpellicular microtubules were located, mostly regularly arranged beneath the margin of the pellicle. They extended from the apical polar ring for about two-thirds of the length of the cell and were not seen in the posterior third (Fig. 5; MT). A typical conoid consisting of microtubules was encircled by the apical polar ring (Figs. 1, 2, 3, 10; C). The conoid was approximately 0.37 μm in length and had a basic diameter of about 0.32 μm. The outer surface of this conical structure was covered by fine fibrillar material (Fig. 3; arrows). Within the interior of the conoid, the ductules of the club-shaped rhoptries were seen. These organelles were observed throughout the whole parasite but occurred predominantly at the apical and posterior poles (Figs. 1, 4, 5, 9, 10; R). Their club-shaped parts measured approximately 0.14–0.4 μm in diameter, whereas the width of the ductules was in the region of only 55 nm. Small, rod-like micronemes of about 0.3 μm by 60 nm were found in larger numbers at the apical and posterior poles of the organism than elsewhere (Figs. 1, 2, 4, 8, 9; MN). The most prominent structures within the parasites were the spherical nucleus, about 2–3 μm in diameter, and a large, ovoid body with a crystalline appearance. The latter measured up to 5.5 μm in length. The nucleus was always situated a little anterior to the middle of the cell and was always in close contact with the crystalloid body (Figs. 1, 10; N, CR). A spherical nucleolus was usually positioned near the end of the nucleus closest to the crystalloid body. The crystalloid body was composed of numerous, regularly arranged, electron dense, spherical granules about 30 nm in diameter (Figs. 5, 7; CR). Along the outer surface of the crystalloid body, which was not bounded by a membrane, numerous polysaccharide granules brilliant white in appearance, were observed (Figs. 5, 6, 10; A). The largest of these granules measured 0.6–0.7 μm. The structure of other organelles like the tubular mitochondria, the Golgi apparatus anterior to the nucleus and the rough and smooth endoplasmic reticulum, were as in other coccidia.

All parasites observed in the mesenteric lymph node on the 25th day p.i. were situated intracellularly. They were never in direct contact with the cytoplasm of the host cell but were always within a parasitophorous vacuole (Figs. 1, 4, 5, 6, 9; PV), which was often near the host cell nucleus (Figs. 1, 4; NH). This vacuole was always limited by a typical unit membrane (Figs. 2, 5, 6, 7; LP). The size and appearance of the parasitophorous vacuole varied considerably:

1. Some parasites lay within a small parasitophorous vacuole, the interior of which appeared mostly electron pale and contained only a few small granules about 30 nm in diameter (Figs. 1, 7; PV).

2. Other parasites were situated within a parasitophorous vacuole which was completely filled with these electron dense granules (Fig. 6; PV, GM). Because of the proximity of the granules to each other, the parasitophorous vacuole gave an impression of denseness.

3. Many parasites were situated within a relatively large parasitophorous vacuole. Here the granular elements were restricted to a zone at the periphery of the


PV
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vacuole, the parasite being immediately surrounded by an electron pale space (Figs. 2, 10; PV, GM). In such cases the limiting membrane of the parasitophorous vacuole formed large, intravacuolar tubules about 50–60 nm in diameter and often with a length of 2 μm (Figs. 2, 8, 10; IT). A parasite in a parasitophorous vacuole of the third type is schematically depicted in Fig. 10, together with surrounding host cell structures.

In all instances, spherical granules in the host cell were filed along the limiting membrane of the parasitophorous vacuole (Figs. 1, 7; DK, LP). These granules were membrane-bound and had a homogeneous, electron dense core. Their maximum diameter was 0.25 μm and they were not as prominent in cells that were not parasitized. Numerous host cell mitochondria of the cristae-type were always scattered around the parasitophorous vacuole (Figs. 2, 10; MIH) and it seemed that parasitized cells were richer in these structures than those which did not contain parasites.

Discussion

The parasites described here had the typical appearance of coccidian sporozoites (Roberts and Hammond, 1970; Roberts et al., 1970). The crystalloid body might be homologous with the refractile body of sporozoites of species of *Eimeria*. In the present study, this body was found to be composed of numerous, small, electron dense granules as in sporozoites of *Isospora canis* (Roberts et al., 1972), sporoblasts of the large form of *I. bigemina* from cats[1] (Mehlhorn and Scholtyseck, 1974), *Aggregata eberthi* (Porchet-Henneré and Richard, 1969), *Eucoccidium dinophili* (Bardele, 1966), *Hepatozoon domerguei* (Vivier et al., 1972) and ookinetes of Haemosporina (Garnham et al., 1969; Desser, 1970; Canning and Sinden, 1973; Trefiak and Desser, 1973). In the eimerian sporozoites, however, the refractile body appeared homogeneous. This might be the result of the method of fixation, a different functional state and/or a generic difference.

The fact that only sporozoite-shaped stages containing a crystalloid body were found in the mesenteric lymph node on the 25th day p.i. indicates that few or no divisions had taken place. The sporozoite-like nature of these parasites, which in the mesenteric lymph node of the mouse are still of similar appearance by light microscopy 15 months p.i. (Frenkel, 1974), suggests that they represent *waiting stages*. Such forms of *I. felis* in mice have been shown to be infectious to cats from less than 14 to at least 67 days p.i. (Frenkel and Dubey, 1972; Markus, 1975a). Powell and McCarley (1975) saw macroscopic sarcocysts in muscles of mice from about 100 days p.i. However, Markus (unpublished data) did not at any time find sarcocysts in experimental mice given *I. felis* oocysts. The inocula used for mice by

[1] This parasite was given the name *Sarcocystis ovifelis* by Heydorn et al. (1975)

Figs. 6–9. *Isospora felis*. Electron micrographs. **6.** Cross section on the 25th day post inoculation through a parasite within a parasitophorous vacuole (*PV*) which gives an impression of denseness because of numerous granular elements (*GM*) which closely surround the organism. Note the electron dense bodies (*DK*) and the numerous fibrillar elements (*FI*) within the host cell (*WZ*). ×17,000. **7.** High magnification of the margin of a parasite. Note the crystalloid body (*CR*), composed of small, spherical granules, and the dense bodies (*DK*) filed along the limiting membrane of the parasitophorous vacuole (*LP, PV*). ×50,000. **8** and **9.** Cross section (**8**) and longitudinal section (**9**) of micropores of the parasite (*MP*), situated at the apical pole. **8.** ×14,000. **9.** ×30,000

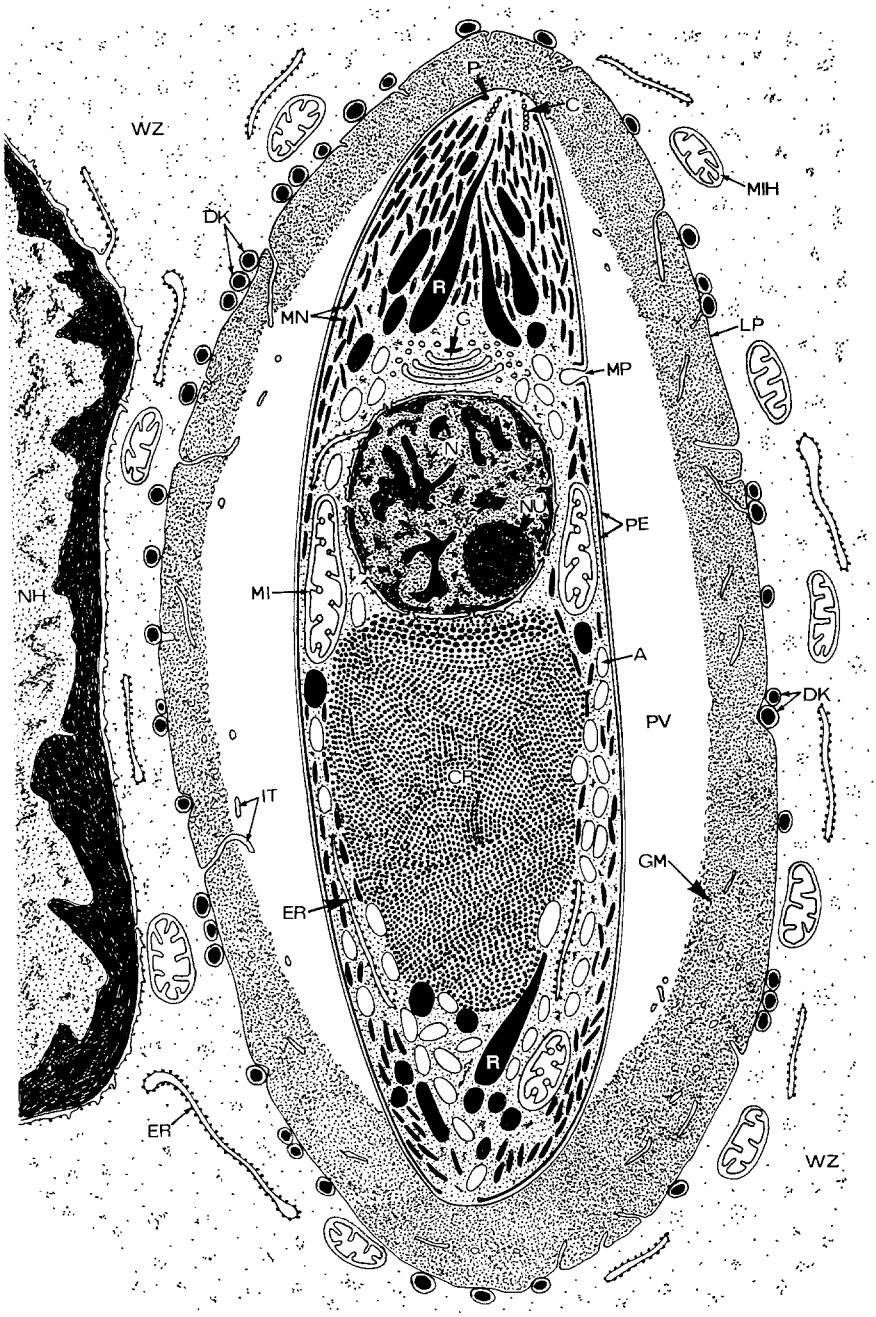

Fig. 10. *Isospora felis.* Diagrammatic representation of a longitudinal section through a parasite within a lymphoreticular cell of a mouse, 25 days post inoculation

Powell and McCarley (1975) probably contained sporocysts of *Sarcocystis* in addition to *I. felis* oocysts.

The possibility that sporozoites had divided in vivo prior to day 25 p.i. cannot be excluded, for refractile bodies – typically present in sporozoites – have been found in first generations of some *Eimeria* species (Hammond et al., 1970) and in paired, sporozoite-like *I. felis* daughter organisms in cultured cells (Fayer and Thompson, 1974). The latter parasites were unlike the products of sporozoite-initiated schizogony of *I. felis* in the intestine of the cat and resembled the forms seen in mice. That some division of sporozoites may take place in mice is suggested by a shorter prepatent period in cats fed mouse tissues containing *I. felis* than in cats fed oocysts (Frenkel and Dubey, 1972; Markus, 1975a). Two organisms have occasionally been seen by light microscopy in, apparently, the same parasitophorous vacuole in cells of mouse mesenteric lymph node (Markus, 1975a). While these could have been daughter organisms, it is also possible that growth of two sporozoites that have invaded the same cell may take place without the sporozoites dividing.

Actual tissue cysts comparable with those of other isosporan coccidia such as cysts of *Sarcocystis* in muscle cells (Mehlhorn et al., 1975a, b) were not observed here. The *I. felis* parasites were always situated within a parasitophorous vacuole typical for intracellular coccidian parasites (Scholtyseck and Piekarski, 1965) but in no case was the limiting membrane of the parasitophorous vacuole thickened. The electron dense granular material, which was in close contact with the parasite (vacuole type 2, above), or which was distributed in a broad zone along the margin of the parasitophorous vacuole (type 3, above), may under the light microscope look like a "sheath" or "cyst", as seen by Frenkel and Dubey (1972) and Markus (1975a, b). This is not a typical parasitophorous vacuole because no obvious developmental process had taken place within it. Normally coccidia grow considerably within a vacuole by ingesting its interior but the *I. felis* parasite had retained its basic shape. It is evident from this and the fact that at day 25 p.i. the parasites were not dividing, that these organisms should be regarded as waiting stages.

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