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Ultrastructure of *Sarcocystis* sp. from the Malaysian House Rat, *Rattus rattus diardii*

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Summary. The ultrastructure of *Sarcocystis* sp. from the Malaysian house rat, *Rattus rattus diardii*, was studied with the electron microscope. The thin, uniformly-dense primary cyst wall had a row of vesicular invaginations which were also seen along the wall of the villi-like projections or cytophaneres. Within the villi were spherical bodies and hollow, curled structures. The ground substance beneath the primary cyst wall extended into the cyst as thin septa or trabeculae separating the tightly-packed zoites into compartments. Merozoites had a double-layered membrane, a conoid, 2 conoidal rings, 22 subpellicular microtubules, 6 rhoptries, 80–100 micronemes, scattered lipid droplets, a sac-like mitochrondrion, beside which was a Golgi apparatus. A micropore was occasionally seen at the anterior third of the zoite whereas the nucleus occupied the posterior third. Metrocytes were few in number and peripheral in location.

Introduction

Compared to *Sarcocystis* from herbivorous and other domestic animals, relatively little is known about the ultrastructure of rodent *Sarcocystis*.

In the Southeast Asian region, *Sarcocystis* was reported from various species of house and field rats (Holz and Leim, 1965; Brown et al., 1974; Cross et al., 1974; Carlos and Schafer, 1972). However, the above studies were mainly reports of incidence of rodent *Sarcocystis*, with little or no description of the parasite. In the instances where some description was given, this was only at light microscopic level. Some light microscopic studies of *Sarcocystis* spp. from Brazilian rodents were carried out by Shaw and Lainson (1969). Rzepczyk (1974) reported a *Sarcocystis* sp. from *Rattus fuscipes*, an indigenous rat of Australia. She also demonstrated a rat-snake life-cycle between this rodent *Sarcocystis* and the carpet python, *Morelia spilotes variegata*. A similar rat-snake cycle was demonstrated between *S. singaporensis* in experimentally infected *Rattus norvegicus* and the Malaysian reticulated python *Python reticulatus* (Zaman and Colley, 1975, 1976).

The recent detailed ultrastructural studies of *Sarcocystis* from rodents and other small mammals include that of *S. singaporensis* (= *S. orientalis*, Zaman and Colley, 1975) found in experimentally infected *R. norvegicus*. This species was previously described as *S. orientalis* (Zaman and Colley, 1975). However, as this name was later discovered to be occupied by a *Sarcocystis* species reported in goats, *S. orientalis* in infected *R. norvegicus* was replaced by *S. singaporensis* (Zaman and Colley, 1976). Rzepczyk and Scholtyseck (1976) described two distinct morphological types of *Sarcocystis* from *R. fuscipes*. Viles and Powell (1976) observed that the cyst wall of *Sarcocystis* from *Mus muscularis* consists of many invaginations. The ultrastructure of *S. booliati* from the Malaysian moonrat, *Echinosorex gymnurus*, was described by Kan and Dissanaike (1976).

The present paper is a detailed ultrastructural study of a *Sarcocystis* sp. found naturally in *Rattus rattus diardii*, the Malaysian house rat. This is the commonest house rat in Malaysia, found generally in towns and houses (Harrison and Quah, 1962). The particular rat from which *Sarcocystis* was reported was trapped in an oil palm plantation in Klang, Selangor, Malaysia.

Materials and Methods

The diaphragm, gastrocnemius and abdominal muscles of the naturally infected rat were cut into small pieces (about 1 cu. mm) and processed in the standard way for electron microscopy (Kan and Dissanaike, 1976). Thin sections were viewed with a Hitachi HS-8 electron microscope at 50 kW.

Results

Sarcocysts were microscopic in size and found only in the skeletal muscles. None was found in the subcutaneous muscles. The features of the cyst wall and zoites are tabulated in Table 1.

Cyst Wall. The primary cyst wall is a thin, electron-dense layer, about 76 nm thick (Fig. 3). At regular intervals of about 200 nm, invaginations of about 27 nm deep are seen in the primary cyst wall.

This primary cyst wall forms long, villi-like projections (Fig. 1). These projections or villi are 5.7 μm in length and about 55 nm apart. The proximal end of the villus where it is attached to the primary cyst wall is narrow and circular in cross-section, with a diameter of about 0.09 μm (Figs. 1 and 2). The main portion of the villus is hexagonal in cross-section, measuring approximately 0.91 $\times$ 1.56 μm (Fig. 2), whereas the distal or terminal end of the villi are again roughly round in cross-section, with a diameter of 1.09 μm (Fig. 2). The wall of the villi, which is a continuation of the primary cyst wall, is very thick at the proximal end, measuring about 106 nm (Figs. 2 and 3), but much thinner at the side and distal end, with a thickness of only 35–38 nm (Figs. 3 and 5). Invaginations are also seen regularly along these villi-like projections, being quite deep (57 nm) and very close together (191 nm) at the proximal end (Fig. 3), whereas those invaginations at the side and terminal portion of the villi are much further apart – between 306–625 nm (Figs. 3 and 5). Within the villi are moderately electron-dense spherical bodies and hollow, curled structures (Figs. 3 and 5). These hollow, curled elements appear to be most frequently seen in the proximal and distal ends of the villi.

Between the proximal end of the villi, just above the ground substance beneath the primary cyst wall, are mitochondria and vesicular structures (Fig. 3). The mitochondria-like structures within this thin layer (about 100 nm thick) are quite unlike the intact mitochondria found scattered regularly between the muscle fibrils above the primary cyst wall (Figs. 1 and 2).

The ground substance just beneath the primary cyst wall is about 0.33 μm thick (Figs. 1–3). This layer is composed of fine, fibrillar-like elements and electron-dense granules (Fig. 3). The ground substance continues into the cyst as very thin septa or trabeculae which divide the zoites within the cyst into compartments (Fig. 4).

Zoites (= *Merozoites*). The zoites (1.8 $\times$ 6.3 μm) are tightly packed within the cyst and separated into groups by very narrow septa which have no limiting membrane

Table 1. Measurements of cyst wall and structures of zoites of *Sarcocystis* sp. from *R. r. diardii*

A. Cyst Wall

a. Primary cyst wall (PCW)
   1. Thickness of PCW: 76 nm (69–83)
   2. Height of villi-like projections from PCW: 5.7 μm (5.3–5.9)
   3. Intervillous space: 55 nm (44–77)
   4. Diameter of villi (circular) at proximal end: 0.09 μm (0.08–0.10)
   5. Measurements of hexagonal cross-section of villi: 0.91 × 1.56 μm (0.88–0.96 × 1.50–1.62)
   6. Diameter of villi (circular) at distal end: 1.09 μm (1.00–1.11)
   7. Thickness of PCW at proximal end of villi: 106 nm (97–117)
   8. Thickness of wall of villi at side: 38 nm (33–42)
   9. Thickness of wall of villi at distal end: 35 nm (33–36)

b. Invaginations of PCW and villus wall

	Depth of invaginations	Distance bet. invaginations
1. PCW	27 nm (17–39)	208 nm (167–250)
2. Proximal end of villus	57 nm (44–69)	191 nm (111–222)
3. Side of villus	68 nm (61–75)	625 nm (555–778)
4. Distal end of villus	61 nm (56–69)	309 nm (278–333)

c. Spherical bodies and curled structures in villi
   1. Diameter of spherical bodies: 23 nm (17–28)
   2. Diameter of coiled tubular structures: 14 nm

d. Ground substance
   1. Thickness of ground substance: 0.33 μm (0.25–0.42)

e. Trabeculae present as thin septa with no limiting membrane

B. Zoites (=Merozoites)

a. Size of zoites: 1.8 × 6.3 μm (1.5–2.0 × 6.2–6.5)
   Size of dividing zoites: 1.0 × 2.4 μm

b. Cell membrane
   1. Thickness of outer membrane: 11.1 nm
   2. Thickness of inner double membrane: 22.2 nm
   3. Thickness of intermembraneous space: 13.8 nm
   4. Total thickness of cell membrane: 57.1 nm

c. Conoid
   1. Height of conoid: 254 nm (222–267)
   2. Diameter at apex of conoid: 225 nm (222–227)
   3. Diameter at base of conoid: 273 nm (250–295)
   4. Number of spiral elements: 8
   5. Number of conoidal rings: 2

d. Microtubules
   Number of microtubules: 22
   Diameter of microtubules: 25 nm (22–28)

e. Rhoptries
   Number of rhoptries: 6
   Diameter of ductules of rhoptries: 52 nm (41–61)
   Structure of rhoptries: uniformly dense, with no limiting membrane

f. Micronemes
   Number of micronemes: 80–100
   Diameter of micronemes: 82 nm (80–89)

g. Micropore
   Inner diameter: 125 nm
   Outer diameter: 208 nm
   Position: Anterior half of zoite

h. Nucleus
   Size: 1.1 × 2.4 μm (0.9–1.4 × 1.9–3.1)
   Position: Posterior half of zoite

C. Metrocytes
   Size of metrocytes: 2.5 × 5.2 μm (2.4–2.8 × 5.0–5.4)
   Size of nucleus: 2.2 × 2.9 (2.0–2.4 × 2.8–2.9)
   Position in cyst: peripheral
   Number: few
   Micropore: present

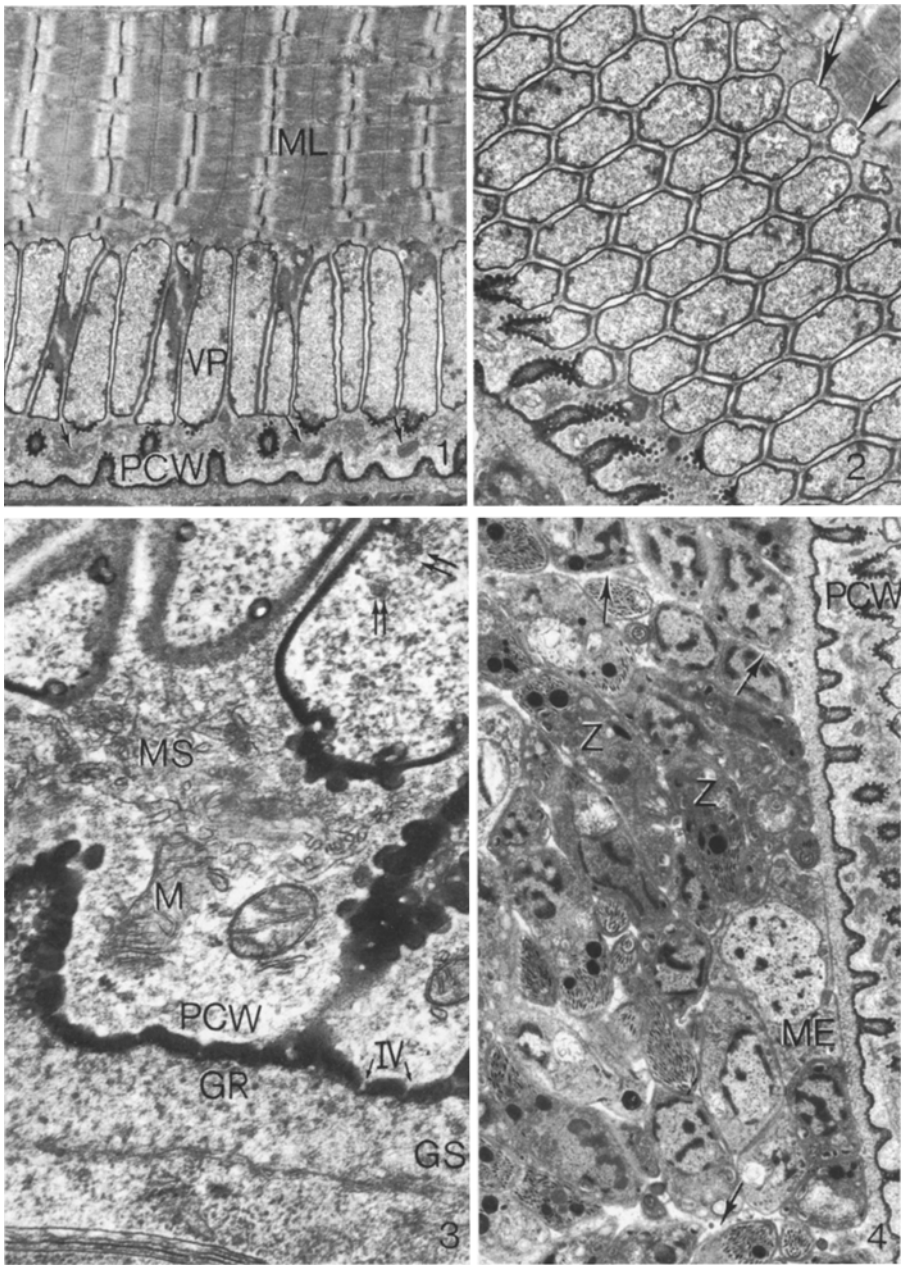

Fig. 1. *Sarcocystis*: Cysts wall showing villi-like projections (*VP*) or cytophaneres from the primary cyst wall (*PCW*). At the base of the projections just above the *PCW* is a space filled with mitochondria-like structures (*arrowed*). Above the villi-like projections is a band of muscles (*ML*) which forms the secondary cyst wall (*SCW*). × 5,450

Fig. 2. *Sarcocystis*: Cyst wall showing villi-like projections with hexagonal cross-sections and roughly round cross-sections at distal end (*arrowed*). Note narrow connections of the cytophaneres to the *PCW* at the proximal end. × 6,400

(Fig. 4). These zoites have the characteristic features of most *Sarcocystis* species. Each zoite is covered by a double-layered membrane or pellicle. The outer membrane is thick and electron-dense whereas the inner membrane is a thinner double-layered membrane (Figs. 6 and 9). The total thickness of the cell membrane is about 5.7 nm. The conoid at the anterior end of the zoite is composed of 8 spiral elements and is surrounded by 2 conoidal rings. Twenty-two subpellicular microtubules originate from the polar ring of the conoid (Fig. 9). Into the conoid open the ducts of the 6 rhoptries (Fig. 9). These rhoptries are uniformly dense, with no limiting membrane and has a diameter of about 360 nm at the distal end (Fig. 6). Among the rhoptries are about 80–100 micronemes (Figs. 6 and 8). Like other *Sarcocystis* spp. the rhoptries and micronemes occupy the anterior third of the zoite (Fig. 6). Behind the rhoptries is a sac-like mitochondrion, with vesicular internal cristae lying within a relatively clear matrix (Fig. 6). Adjacent to the mitochondrion is a system of vesicular and tubular structures which may be the Golgi apparatus (Fig. 6). Behind the mitochondrion is the nucleus which occupies the posterior third of the zoite (Fig. 6). The nucleus measures about 1.1 × 2.4 μm and has masses of dense chromatin within it. Lipid droplets are scattered within the zoite. A micropore is occasionally seen at the anterior third of the zoite, around the region of the rhoptries and micronemes.

Metrocyte. Metrocytes are large (2.5 × 5.2 μm) but few in number and occupy a peripheral position within the cyst (Fig. 4). Each metrocyte contains a nucleus (2.2 × 2.9 μm) which contains scattered, electron-dense granular masses within a relatively clear matrix (Fig. 7). The cytoplasm of the metrocyte is filled with electron-dense granules and membraneous structures. A micropore is also seen in the double-layered membrane of the metrocyte (Fig. 7).

Discussion

The validity of *S. singaporensis* from experimentally infected *R. norvegicus* as a new species had been established by Zaman and Colley (1975). They had also compared the morphology of this parasite with other known species of rodent *Sarcocystis*. It may be relevant at this point to compare the ultrastructure of the 3 species of *Sarcocystis* from Malaysian small mammals that have been studied with the electron microscope.

As far as can be compared *Sarcocystis* sp. from *R. r. diardii* is very similar to *S. singaporensis* from experimentally infected *R. norvegicus* with some slight differences. In *Sarcocystis* sp. from *R. r. diardii*, the cytophaneres are longer (5.7 μm), the zoites are larger (6.3 × 1.8 μm) and metrocytes are fewer, whereas *S.*

Fig. 3. *Sarcocystis*: Primary cyst wall (*PCW*) with regularly spaced invaginations (*IV*) beneath which is the ground substance (*GS*) with granules (*GR*) and fibrillar elements (*arrowed*). The proximal ends of projections have a small diameter, the *PCW* is thicker and the invaginations are very much closer together. The invaginations on the wall of the projections are further apart. The space between the proximal ends of the projections is filled with degenerating mitochondria (*DM*) and membraneous structures (*MS*). Spherical bodies (*arrowed*) and curled tubular structures (*double arrow*) are seen within the projections. × 29,000

Fig. 4. *Sarcocystis*: Part of cyst showing primary cyst wall (*PCW*), ground substance (*GS*) and zoites (*Z*) being separated into compartments by thin septae or trabeculae (*arrowed*). A metrocyte (*ME*) is seen at the periphery of the cyst wall. × 5,540

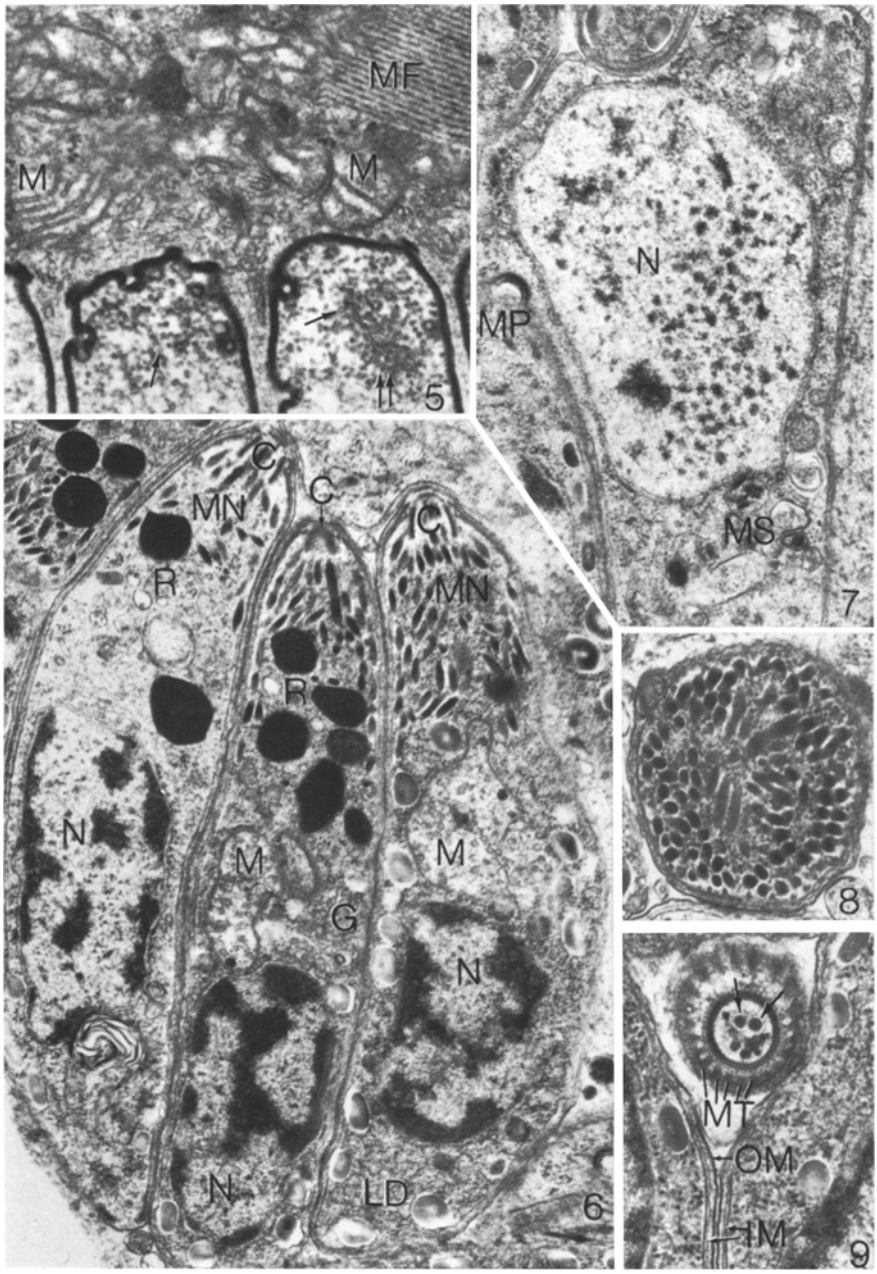

Fig. 5. *Sarcocystis*: Distal end of villi-like projections showing invaginations. Above these projections are groups of degenerating mitochondria (*DM*) and muscle fibres (*MF*). Note spherical bodies (*single arrows*) and curled tubular structures (*double arrows*) within distal end of projections. × 29,000

Fig. 6. *Sarcocystis*: Group of 3 zoites showing anterior conoid (*C*) with 2 conoid rings (*CR*), spiral elements (*arrowed*), rhoptries (*R*), micronemes (*MN*), a sac-like mitochondrion (*M*) with vesicular internal cristae lying within a relatively clear matrix, a Golgi apparatus (*G*) adjacent to it and below this

singaporensis appears to have more metrocytes, shorter cytophaneres (4.0 μm) and smaller zoites (5.5 × 1.4 μm). However, these differences in the parasite from *R. r. diardii* possibly only indicate that the particular parasite is older or at a later stage of development than the parasite described in *R. norvegicus* as such differences have also been observed in developing stages of *Sarcocystis* sp. from the Indian water buffalo, *Bubalus bubalis* (Kan, personal observation). Besides, the same parasite may show slight morphological differences in different hosts. Zaman and Colley (1975) observed the presence of "microtubules" in the reservoir-like space above the inner layer of the cyst wall of *S. singaporensis*. From our present study, these "microtubules" appear to be superficial cross-sections of the invaginations of the primary cyst wall at the proximal ends of the villi-like projections.

The other features of taxonomic importance, such as the thickness of the primary cyst wall, the absence or presence of trabeculae, the number of subpellicular microtubules, rhoptries and micronemes have not been observed in *S. singaporensis* from experimentally infected *R. norvegicus*. However, one may at this point hazard the opinion that *S. singaporensis*, Zaman and Colley, 1976, is apparently not strictly intermediate host-specific and may have a wide host range among various species of rodents and small mammals. This is further supported by the observation of *Sarcocystis* in *R. fuscipes* by Rzepczyk and Scholtyseck (1976) who described two morphological distinct types of sarcocysts from *R. fuscipes*. One of these – Type A sarcocysts – has regularly arranged, villus-like projections with straight, non-wavy walls of uniform thickness (50 nm), punctuated by numerous small invaginations. The length of the projections ranged from 6.8 to 8.7 μm and their widths from 0.8 to 1.4 μm. Although these villus-like projections of the cyst wall of Type A sarcocysts from *R. fuscipes* are longer compared to those of *Sarcocystis* sp. from *R. r. diardii* (5.7 μm) and *S. singaporensis* from experimentally infected *R. norvegicus* (4.0 μm), the structure and appearance of the cyst wall of these parasites are basically similar. Like *Sarcocystis* sp. from *R. r. diardii*, sarcocysts Type A from *R. fuscipes* also have fine septa or trabeculae and metrocytes are present but few in numbers.[1]

However, when compared with *S. booliati* from the Malaysian moonrat, *Echinosorex gymnurus*, which is an insectivore, *Sarcocystis* sp. from *R. r. diardii* shows many more differences. It has a thicker primary cyst wall (76 nm) which has shallower invaginations (27 nm), the zoites are slightly larger (6.3 × 1.8 μm), the pellicle of these zoites is thicker (57.1) nm), it has fewer rhoptries (6) but more micronemes (80–100). In contrast, *S. booliati* has a thinner primary cyst wall (53 nm) with deeper invaginations (76 nm), slightly smaller zoites (6.0 × 1.7 μm) which have a thinner pellicle (36.1 nm), more rhoptries (8), less micronemes (50–60) and lacks cytophaneres and trabeculae.

[1] Since the completion of this study, the Type B *Sarcocystis* described by Rzepczyk and Scholtyseck (1976) has been observed in various species of Malaysian rodents. Details of this species will be published later.

is the nucleus (*N*) with masses of dense chromatin. Lipid droplets (*LD*) are scattered throughout the zoites. × 20,200

Fig. 7. *Sarcocystis*: Metrocyte showing a large nucleus (*N*) with small chromatin granules within a clear nuclear matrix. Membraneous structures (*MS*) are seen within the cytoplasm of the metrocyte (*ME*). A micropore (*MP*) is seen along the cell wall of the metrocyte. × 14,800

Fig. 8. *Sarcocystis*: T.S. of anterior portion of zoite showing 80–100 micronemes. × 29,000

Fig. 9. *Sarcocystis*: T.S. of conoid showing ductules of rhoptries (*arrowed*) and 22 subpellicular microtubules (*MT*). Note thicker outer membrane (*OM*) and less, dense, double-layered, inner membrane (*IM*) of surrounding zoites. × 29,000

A dense network of microtubules was observed within the villus-like projections of *Sarcocystis* from the wild grackle, *Quiscalus quiscula* (Zeve et al., 1966) and the parallel, finger-like projections of the cyst wall of *S. miescheriana* were shown to have many fine double fibrils or "capillary tubes" (Ludvik, 1960). Parallel fibrils were also seen in longitudinal sections of the palisade-like protrusions of the cyst-wall in *S. fusiformis* (Gestrich et al., 1975; Mehlhorn et al., 1975). But the villi-like projections of *Sarcocystis* sp. from *R. r. diardii* contained mainly spherical bodies and small clumps of hollow, curled elements. The latter appear to be very simple structures and are not suggestive of having a microtubular type of structure or function.

Abbreviations

C	conoid	ML	muscle layers
CR	conoidal rings	MN	micronemes
G	Golgi apparatus	MP	micropore
GS	ground substance	MS	membraneous structures
GR	granules	MT	microtubules
IM	inner membrane	N	nucleus
IV	invaginations	OM	outer membrane
LD	lipid droplets	PCW	primary cyst wall
M	mitochondrion	R	rhoptries
ME	metrocyte	VP	villi-like projections
MF	muscle fibres	Z	zoite

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