Z. Parasitenkd. 57, 107–116 (1978)

Zeitschrift für
Parasitenkunde
Parasitology Research

Studies on *Sarcocystis* in Malaysia

II. Comparative Ultrastructure of the Cyst Wall and Zoites of *Sarcocystis levinei* and *Sarcocystis fusiformis* from the Water Buffalo, *Bubalus bubalis*

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Summary. The two species of *Sarcocystis* – *S. levinei* and *S. fusiformis* from the water buffalo, *Bubalus bubalis*, show some ultrastructural similarities in their cyst wall and zoites. The zoites of both species are of about the same size, banana-shaped and have 22 subpellicular microtubules, numerous micronemes, eight rhoptries, a micropore in the region of the micronemes, an elongated mitochondrion, and a nucleus. *S. levinei* has 200–300 micronemes and *S. fusiformis* has about 400. The sarcocysts of both species are trabeculated and their cyst walls have cytophaneres containing annulated fibrils and coarse, electron dense granules. The cytophaneres of *S. levinei* are sloping, with irregular, wavy outlines, whereas *S. fusiformis* has the cauliflower-type of cytophaneres. This difference in the appearance of the cytophaneres, together with the difference in size of the sarcocysts and their definitive hosts, further confirms that *S. levinei* and *S. fusiformis* are two distinct species in the water buffalo.

Introduction

There has been considerable confusion over the nomenclature and hosts of various species of *Sarcocystis* reported from cattle and buffalo so far. Levine (1977) has clarified that *S. fusiformis* (Railliet 1897) Bernard and Bauche, 1912, is the large sarcocyst-forming species from the water buffalo. We have recently reported another species, *S. levinei*, producing much smaller, microscopic sarcocysts in the same host (Dissanaike et al., 1977; Dissanaike and Kan, 1978). Feeding experiments demonstrated that the definitive host of *S. fusiformis* is the cat and that of *S. levinei* is the dog (Dissanaike and Kan, 1978). Some aspects of the ultrastructure of *S. levinei* had been described in the same paper and the zoites of *S. fusiformis* had been partly described by Zaman and Colley (1972). The present paper is a detailed comparative study of the ultrastructure of the cyst wall and zoites of *S. levinei* and *S. fusiformis*.

0044-3255/78/0057/0107/$02.00

Materials and Methods

Both species of *Sarcocystis* were obtained from the oesophageal muscle of an infected buffalo. Measurements of the sarcocysts and zoites of both species were made from fresh saline preparations. In the case of *S. fusiformis*, the dimensions of various structures of the cyst wall and zoites were averages of measurements made from sarcocysts ranging from 1 mm to 25 mm in length. For electron microscopy, the large sarcocysts of *S. fusiformis* (length: 2–25 mm) were dissected from the muscle and immersed immediately in ice-cold 5% buffered glutaraldehyde for $^1/_2$ h, after which the cyst wall was pricked at various places with a pair of sharp scissors to ensure quick penetration of the fixative into the zoites within the sarcocysts[1]. At the end of 1–2 h, the large sarcocysts were cut into small pieces (1 mm^2) and fixed for another 2 h in a fresh change of 5% glutaraldehyde at 0° C. The smaller sarcocysts (length: 1–2 mm or smaller) were dissected under the dissecting microscope and fixed separately in ice-cold 5% glutaraldehyde for $^1/_2$ h, after which they were cut into 2–3 pieces and fixed for another 1–2 h in a fresh change of 5% glutaraldehyde at 0° C. The sarcocysts thus fixed were processed in the usual way for electron microscopy (Kan and Dissanaike, 1976) and sections were examined with a Hitachi HS-8 electron microscope at 50 kV.

Results

The features of the sarcocysts, cyst wall, and zoites (merozoites) of *S. levinei* and *S. fusiformis* are tabulated in Table 1. Both species of *Sarcocystis* from the water buffalo are spindle-shaped and occur in the oesophageal muscle, but the cyst of *S. fusiformis* is 10–25 times larger than that of *S. levinei.*

Cyst Wall

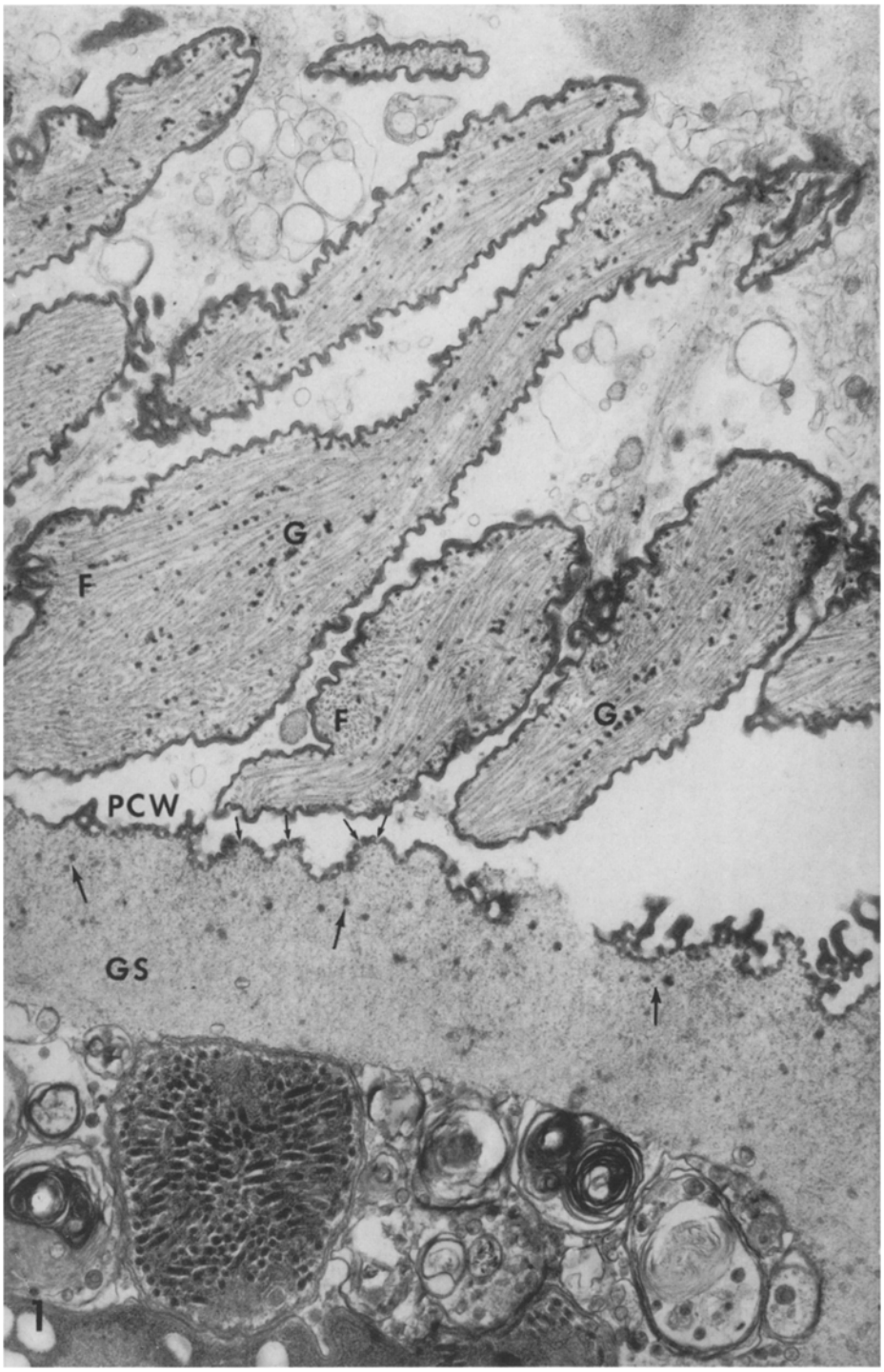
The cyst wall of both species of *Sarcocystis* has cytophaneres or projections from the primary cyst wall, and both species have trabeculae or septa with no limiting membrane. However, the appearance of the cytophaneres of these two species are strikingly dissimilar. In *S. levinei*, the cytophaneres are sloping, with irregular, highly folded wavy outlines (Fig. 1). The walls of these projections do not have invaginations, these being confined to the primary cyst wall (PCW) between the projections (Fig. 1). These invaginations are about 26 nm deep (18–33) and 78 nm (56–89) apart. The cytophaneres are about 7.4 μm high (6.4–10.0 μm). In *S. fusiformis*, the cytophaneres have the characteristic 'cauliflower' appearance, with many dendritic branches (Figs. 2 and 3). The walls of these cauliflower-like branches also do not have invaginations, which are confined to the primary cyst wall (Fig. 3). The PCW of *S. fusiformis* is slightly thicker (48 nm) than that of *S. levinei* (39 nm). The height of these cauliflower-like projections is about 5.4 μm (2.9–11.1 μm), but the height of these projections shows a wider range of variation than that of *S. levinei.*

In both species, the cytophaneres are filled with characteristic hollow annulated fibrils which run along the longitudinal axis of the projections and scattered

[1] These cysts were divided into batches according to their lengths: 2–5 mm, 5–10 mm, and 10 mm and above, and these batches were processed separately for electron microscopy

Table 1. Comparison of *S. levinei* (Dissanaike and Kan, 1977) and *S. fusiformis* (Railliet, 1897) Bernard and Bauche, 1912 from *Bubalus bubalis*

I.	*Sarcocysts*	*S. levinei*	*S. fusiformis*
(i)	Size of sarcocyst	0.9 × 0.1 mm (0.80–1.15 × 0.09–0.14)	1–25 × 0.5–5 mm
(ii)	Shape of sarcocyst	Narrow, spindle shape	Spindle shape
(iii)	Location of sarcocyst	oesophageal muscle	oesophageal muscle
(iv)	Contents of sarcocyst	Few, large, irregular compartments filled with zoites	Numerous, honey-comb compartments filled with an empty space in centre of older cysts
II.	*Cyst Wall*		
(a)	Primary cyst wall (PCW)		
(i)	Thickness of PCW	39 nm (33–44)	48 nm (30–61)
(ii)	Depth of invaginations of PCW	26 nm (18–33)	49 nm (32–60)
(iii)	Distance between invaginations	78 nm (56–89)	63 nm (41–72)
(b)	Cytophaneres		
(i)	Appearance	Sloping, with irregular highly-folded, wavy outlines	'Cauliflower' with dendritic branches
(ii)	Height of cytophaneres	7.4 μm (6.4–10.0)	5.4 μm (2.9–11.1)
(iii)	Annulated fibril within cytophaneres:		
	Outer diameter of fibrils	25 nm (22–27)	35 nm (30–41)
	Inner diameter of fibrils	12 nm (9–16)	16 nm (14–17)
(iv)	Granules within cytophaneres	Coarse, electron-dense scattered between annulated fibrils	Coarse, electron-dense scattered between annulated fibrils
(c)	Ground substance		
(i)	Thickness of ground substance	1.90 μm (1.57–2.07)	2.5 μm (1.0–4.0)
(ii)	Trabeculae	Present with no limiting membrane	Present with no limiting membrane
III.	*Zoites* (Merozoites)	*S. levinei*	*S. fusiformis*
(i)	Size (from saline preparations)	17.8 × 4.2 μm (17.0–18.2 × 3.8–4.6)	17.6 × 3.8 μm (16.0–19.4 × 3.4–4.3)
(ii)	Number of subpellicular microtubules	22	22
(iii)	Number of micronemes	200–300	±400
(iv)	Number of rhoptries	8	8
(v)	Structure of rhoptries	Uniformly dense with no limiting membrane	Uniformly dense with no limiting membrane
(vi)	Micropore	Anterior half of zoite	Anterior half of zoite
(vii)	Mitochondrion	Anterior to nucleus	Anterior to nucleus
(viii)	Nucleus	Just posterior to midline of zoite	Towards posterior end of zoite
IV.	*Metrocyte*		
(i)	Location	peripheral,	peripheral,
(ii)	Micropores	several	present
V.	Definitive host	Dog	Cat


G
F
F
G
PCW
GS

between these fibrils are coarse, electron-dense granules (Fig. 1 and 3). In both species, electron-dense granules (Fig. 1 and 3), are scattered within the ground substance which extends into the cysts to form trabeculae with no limiting membrane.

Zoites (Merozoites)

The zoites from both species of *Sarcocystis* from the water buffalo are banana-shaped and are of about the same size, though *S. levinei* appears to be slightly bigger than *S. fusiformis* (see Table 1, Figs. 4 and 5). Both zoites have an anterior conoid, 22 subpellicular microtubules, eight rhoptries, which are uniformly dense with no limiting membrane, a micropore at the anterior half of the zoite and a mitochondrion anterior to the nucleus. In both species, the mitochondrion is elongated and highly developed. The nucleus of *S. levinei* appears to be more anteriorly situated (just posterior to the midline of the zoite) than that of *S. fusiformis*, which is nearer to the posterior end of the zoite. (Figs. 4 and 5). This characteristic is more obvious in whole zoites stained with Giemsa than in electron microscopic sections. *S. levinei* has less micronemes (200–300) than *S. fusiformis* (± 400).

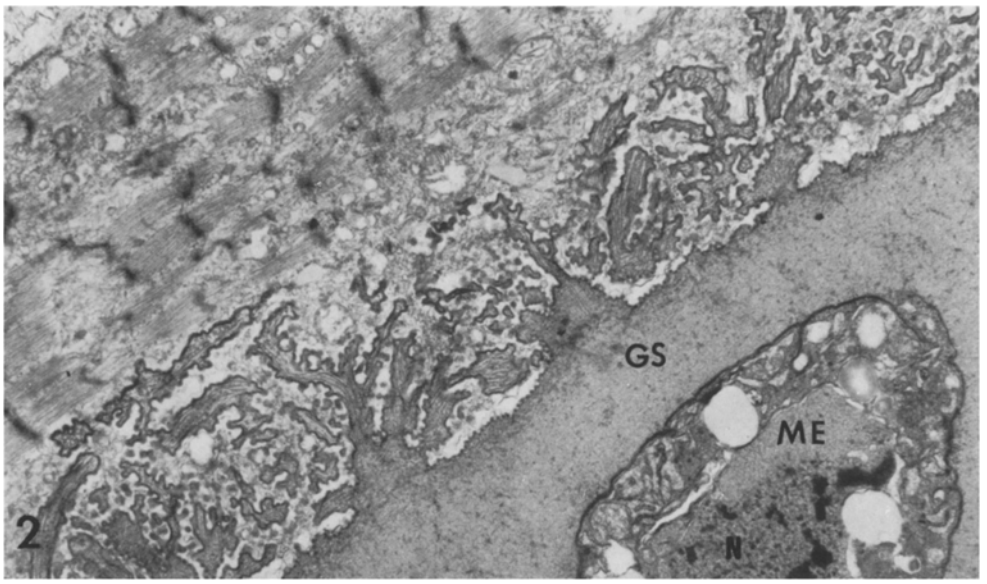
Metrocyte

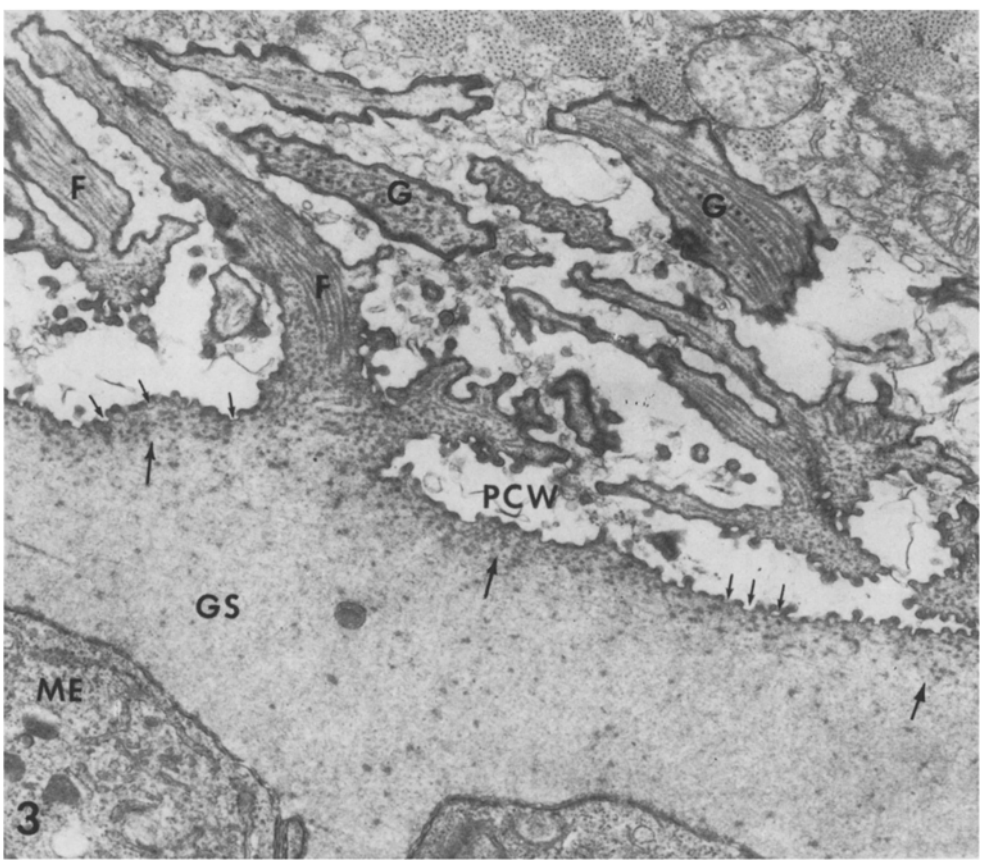
Both species have metrocytes which are located peripherally. In *S. fusiformis*, these have double cell walls and large nuclei with scattered chromatin granules (Fig. 2). The metrocytes of *S. levinei* have been described in an earlier paper (Dissanaike and Kan, 1978). However, dense, osmiophilic granules are not seen within the projections in *S. ovifelis* (Heydorn et al., 1975; Mehlhorn et al., 1976; Kan, personal observation).

Abbreviations

F	fibrils	*ME*	metrocyte
G	granules	*MN*	micronemes
GS	ground substance	*N*	nucleus
LD	lipid droplets	*PCW*	primary cyst wall
M	mitochondrion	*R*	rhoptries

Fig. 1. *S. levinei*, showing the primary cyst wall (*PCW*) with irregularly spaced invaginations (*small arrows*). The villi-like projections of this *PCW* have irregular, wavy outlines and contain hollow, annulated fibrils (*F*) with coarse, electron-dense granules (*G*) scattered between the fibrils. The ground substance (*GS*) beneath the *PCW* contains coarse, electron-dense granules (*large arrows*). $\times 14{,}000$


GS
ME
N
2


F
G
F
G
PCW
GS
ME
3

Discussion

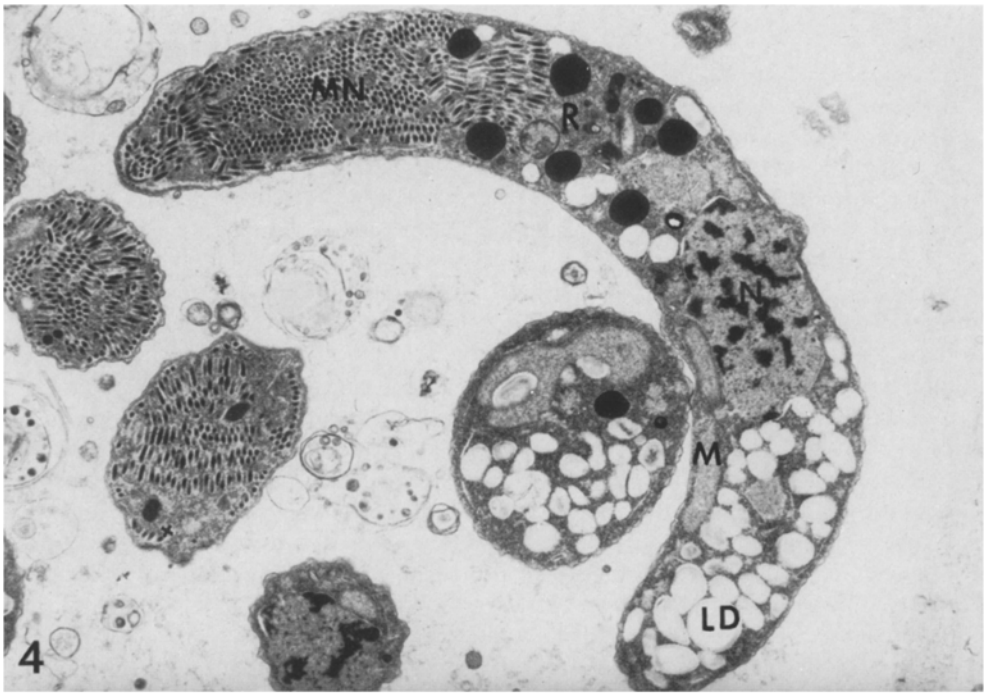
The ultrastructure of the cyst wall of *S. fusiformis* has never been described. Simpson (1966) described long, curved, tongue-like or Y-shaped villi interspersed between blunt, thin projections from the cyst wall of '*S. fusiformis*' from the myocardium obtained from beef animals. He was apparently describing the cyst wall of *S. cruzi*, later designated as *S. bovicanis* by Heydorn et al. (1975) in their proposal for a new nomenclature of the Sarcosporidia.

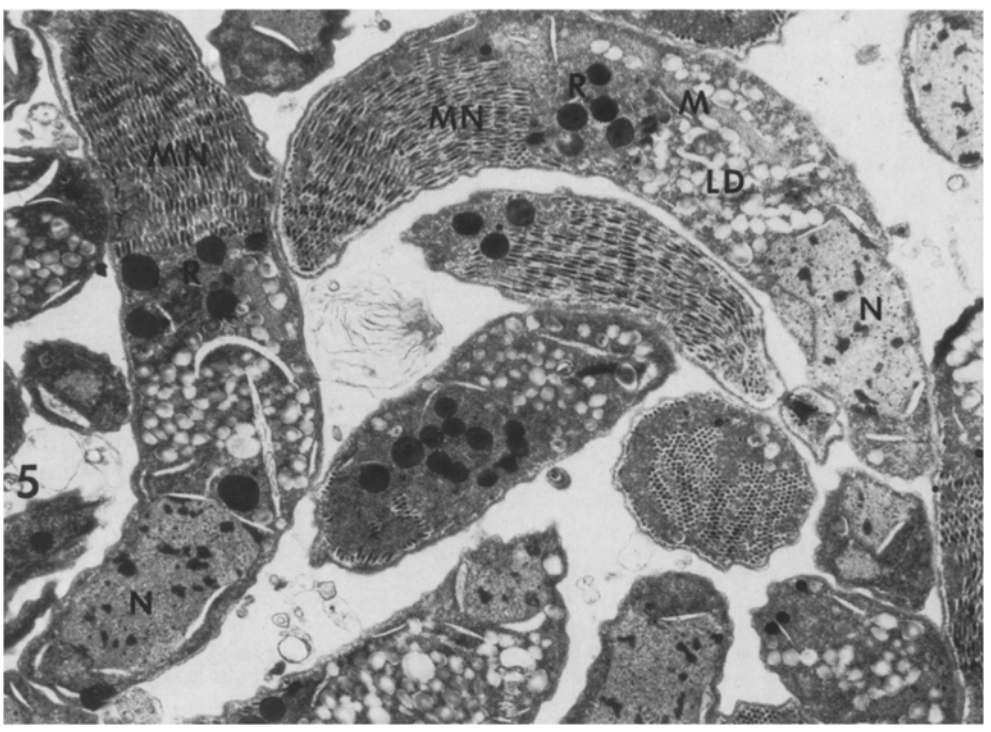
Except for the obvious difference in the size of the sarcocysts, the present study revealed that *S. levinei* and *S. fusiformis* from the water buffalo are ultrastructurally similar in many aspects. The most striking differences between these two species are the height and appearance of the cytophaneres. It is interesting to note at this point that the larger species (*S. fusiformis*) has a cyst wall which has generally shorter projections or cytophaneres than the smaller species (*S. levinei*). The cytophaneres of *S. levinei* are characteristically sloping, with irregular, wavy outlines, whereas those of *S. fusiformis* are the typical 'cauliflower' type, with dendritic branches. The cyst wall of *S. fusiformis* thus bears some resemblance to that of *S. ovifelis* from the domestic sheep, *Ovis aries*, which also has cauliflower-like protrusions containing numerous fibrils;

On the other hand, the cytophaneres of both *S. levinei* and *S. fusiformis* have characteristic annulated fibrils, scattered amongst which are coarse, electron-dense granules. These annulated fibrils and granules have not been observed in the cytophaneres of goats, monkeys, and various species of Malaysian rodents, including bandicoots (Kan, personal observation). Mehlhorn et al. (1975a) observed fibrils and osmiophilic granules in the cytophaneres of 'old cysts (98th day and more') from calves infected with sporocysts of *Isospora hominis*. The size of these fibrils (40 nm) are similar to those of *S. fusiformis* (30–41 nm) but smaller than those in *S. levinei* (22–27 nm). Mehlhorn et al. did not elucidate the function and origin of the granules. The species was later designated as *S. bovihominis* by Heydorn et al. (1975). Gestrich et al. (1975) later also described fibrils in the folded, palisade-like protrusions of *Sarcocystis* from calves infected with oocysts and sporocysts of the large form of *I. bigemina* from cats (*S. bovifelis*). No granules were observed. Mehlhorn et al. (1975b, 1976) later concluded that *S. bovifelis* and *S. bovihominis*, both microscopic cysts in muscles of cattle, showed no fundamental difference in the structures of their cyst wall, which has regularly-folded, palisade-like protrusions containing 200–300 fibrils or tubular elements of 15–18 nm in diameter. No function was ascribed to these fibrillar elements. Mehlhorn et al. only observed osmiophilic granules

Fig. 2. *S. fusiformis*, showing the highly dendritic 'cauliflower' type of projections from the primary cyst wall. Part of a metrocyte (*ME*) with a large nucleus (*N*) is seen within the ground substance (*GS*) of the sarcocyst. ×8,000

Fig. 3. *S. fusiformis* showing the dendritic branches of the cauliflower type of projections from the *PCW* which has irregularly spaced invaginations (*small arrows*). Within the dendritic branches are hollow, annulated fibrils (*F*) and coarse, electron dense granules (*G*). Smaller, less dense granules (*big arrows*) are seen at the base of the projections and in the ground substance (*GS*) just beneath the *PCW*. Part of a metrocyte (*ME*) is seen in the ground substance. ×18,500


MN
R
N
M
LD
4


MN
R
M
LD
MN
R
N
N
5

in older cysts. However, in our study with *S. levinei* and *S. fusiformis*, osmiophilic granules appear to be present in both old and young cysts (Kan, personal observation).

A dense network of 'microtubules' with an annulated appearance has been described in the cytophaners of the wild grackle, *Quiscalus quiscula* by Zeve et al. (1966). They suggested an absorptive and/or conductive function to these 'microtubules'. Very fine double-fibrils have also been observed within the finger-like projections of the cyst wall of *S. miescheriana* by Ludvik (1960). He suggested that they are probably 'capillary tubes' for the transport of nutritive materials into the cyst. We have also seen such tubular fibrils in the cyst wall of some sarcocysts from cattle and sheep (Kan, personal observation). But these appear simpler in structure than those in *S. levinei* and *S. fusiformis* and coarse electron-granules are absent. Thus, while fibrillar or tubular elements are a common feature in the cytophaneres of various species of *Sarcocystis* from buffaloes, cattle and sheep, electron-dense granules appear to be characteristic of *Sarcocystis* species from the water buffalo and cattle only. The significance of these granules cannot be clearly understood at this juncture.

The zoites of *S. levinei* and *S. fusiformis* only differ slightly in their size range, the number of micronemes, and the position of the nucleus. Both have eight rhoptries and an elongated mitochondrion. An elongated mitochondrion was also seen in the zoites of *S. fusiformis* described by Zaman and Colley (1972), but they did not count the number of micronemes and suggested that there appeared to be at least ten rhoptries in the zoite.

Acknowledgements. We are grateful to the technical staff of the Department of Parasitology for their assistance and to Mr. Chong Kok Leong and the staff in the Electron Microscope Room, Department of Pathology, Faculty of Medicine, University of Malaya, for their assistance in the preparation of the materials for electron microscopy and the processing of the electron micrographs.

We are also thankful to the Director, Shah Alam Abattoir and his staff for their cooperation with the materials from buffaloes.

This work is carried out with the assistance of a Research Grant from the University of Malaya.

References

Dissanaike, A.S., Kan, S.P., Retnasabapathy, A., Baskaran, G.: Demonstration of the sexual phases of *Sarcocystis fusiformis* (Railliet, 1897) and *Sarcocystis* sp. of the water buffalo (*Bubalus bulalis*) in the small intestines of cats and dogs. Trans. R. Soc. Trop. Med. Hyg. **71**, 273 (1977)

Dissanaike, A.S., Kan, S.P.: Studies on *Sarcocystis* in Malaysia. I. *Sarcocystis levinei* n. sp. from the water buffalo *Babalus bubalis*. Z. Parasitenkd. **55**, 127–138 (1978)

Fig. 4. Zoites of *S. levinei* with micronemes (*MN*) at the anterior end, behind which are rhoptries (*R*), an elongated mitochondrion (*M*), and a nucleus (*N*) posterior to the midline of the zoite. Lipid droplets (*LD*) are seen at the posterior end of the zoite. ×8,000

Fig. 5. Zoites of *S. fusiformis* with micronemes (*MN*), rhoptries (*R*), an elongated mitochondrion (*M*), nucleus (*N*), and lipid droplets (*LD*) at the region of the mitochondrion. Note the posterior position of the nucleus. ×8,000

Gestrich, R., Mehlhorn, H., Heydorn, A.O.: Light and electron microscope studies on cysts of *Sarcocystis fusiformis* in the muscles of calves infected experimentally with oocysts and sporocysts of the large form of *Isospora bigemina* from cats. Zentralb. Bakteriol. [Orig. A.] **233**, 261–276 (1975)

Heydorn, A.O., Gestrich, R., Mehlhorn, H., Rommel, M.: Proposal for a new nomenclature of the Sarcosporidia. Z. Parasitenkd. **48**, 73–82 (1975)

Kan, S.P., Dissanaike, A.S.: Ultrastructure of *Sarcocystis booliati* Dissanaike and Poopalachelvam, 1975 from the moonrat, *Echinosorex gymnurus*, in Malaysia. Int. J. Parasitol. **6**, 321–326 (1976)

Levine, N.D.: Nomenclature of *Sarcocystis* in the ox and sheep and of fecal coccidia of the dog and cat. J. parasitol. **63**, 36–51 (1977)

Ludvik, J.: The electron microscopy of *Sarcocystis miescheriana* Kühn, 1865. J. Protozool. **7**, 128–135 (1960)

Mehlhorn, H., Heydorn, O., Gestrich, R.: Light and electron microscope studies on cysts of *Sarcocystis fusiformis* in the muscles of calves infected experimentally with oocysts and sporocysts of *Isosopora hominis* Railliet et Lucet, 1891. 1. The development of cyst and cyst wall. Zentralb. Bakteriol. [Orig. A] **231**, 301–322 (1975a)

Mehlhorn, H., Senaud, H., Heydorn, A.O., Gestrich, R.: Comparaison des ultrastructures des kystes de *Sarcocystis fusiformis* Railliet, 1897 dans la musculature du bœuf, après infection naturelle et après infection expérimentale par des sporocystes d'*Isospora hominis* et par des sporocystes des grandes formes d'*Isospora bigemina* du chien et du chat. Protistologica **11**, 445–455 (1975b)

Mehlhorn, H., Hartley, W.J., Heydorn, A.O.: A comparative ultrastructural study of the cyst wall of 13 *Sarcocystis* species. Protistologica **12**, 451–467 (1976)

Simpson, C.F.: Electron microscopy of *Sarcocystis fusiformis*. J. Parasitol. **52**, 607–613 (1966)

Zaman, V., Colley, F.C.: Fine structure of *Sarcocystis fusiformis* from the Indian water buffalo (*Bubalus bubalis*) in Singapore. Southeast Asian J. Trop. Med. Public Health **3**, 489–495 (1972)

Zeve, V.H., Price, D.L., Herman, C.M.: Electron microscope study of *Sarcocystis* sp. Exp. Parasitol. **28**, 338–346 (1966)

Received September 3, 1977