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Daly, Thomas Joseph Michael

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CYST WALL ULTRASTRUCTURE OF *SARCOCYSTIS* (PROTOZOA: COCCIDIA) OF SOUTHERN AFRICAN MAMMALS

Thomas Joseph Michael Daly

A Thesis Submitted to the Faculty of Science,
University of the Witwatersrand, Johannesburg,
for the Degree of Doctor of Philosophy

Johannesburg 1987

DECLARATION

I declare that this thesis is my own, unaided work. It is being submitted for the degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.

(T.J.M. Daly)

This 1 day of September, 1987.

DEDICATION

In Memory of my Mother
Winnifred May
1913-1979

ABSTRACT

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DALY, Thomas Joseph Michael, Ph.D., University of the Witwatersrand, 1987.

The main part of this study, as reflected by the title, involved investigation of the occurrence and electron microscopic characterization of chronic Sarcocystis infection in both wild and domestic southern African mammals, mainly ungulates and carnivores. As judged by the ultrastructural morphology of the cyst wall, at least 21 species of Sarcocystis were found in 30 species of host animal. The fine structure of some sarcocysts, especially those in domestic animals, was identical to that of Sarcocystis species described previously from the same host species on other continents, while certain cyst wall characteristics exhibited by other sarcocysts in southern Africa were similar to those of cysts described from different host species in other parts of the world. It is concluded that the specificity for the intermediate host of at least some species of mammalian Sarcocystis is not as strict as has been thought. The occurrence of muscular Sarcocystis infection in carnivorous mammals (previously thought to be an unusual phenomenon) is considered to be further evidence of this.

A Sarcocystis muris-like organism in the black rat Rattus rattus and which proved to have the domestic cat as a final host, was isolated. Laboratory transmission experiments involving this parasite have shed further light on the important question of how specific Sarcocystis is for the intermediate host.

The indications are that some Sarcocystis species occurring in domestic meat animals in South Africa have similar effects, both physiologically and economically, to those reported in the same domestic hosts in other parts of the world. The veterinary and economic importance of Sarcocystis deserves greater recognition locally, particularly as natural outbreaks of acute bovine sarcocystosis have recently been reported in South Africa.

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Furthermore, many carcases are condemned at abattoirs in southern Africa because of "eosinophilic myositis" caused by Sarcocystis, resulting in considerable financial losses to the meat industry.

In overseas countries, certain Sarcocystis species of domestic animals have been found to be pathogenic for the intermediate host. Sarcocystis cruzi, which is prevalent in cattle in South Africa, likewise proved fatal to the intermediate bovine host in an experimental situation. This experimental material was used to develop a new technique which facilitates the location of schizonts in post-mortem tissue for diagnostic electron microscopy.

The possibility that the same species of Sarcocystis can occur in both wild and domestic intermediate hosts was considered. This is something which has not been investigated by previous authors. Ultrastructurally, cysts located in wild and domestic equine hosts were similar. Additional examples have also been discussed.

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ABBREVIATIONS USED IN ELECTRON MICROGRAPHS

A	Aggregates of fibrillar or microtubular elements which form plaques in the ground substance
AG	Amylopectin granule(s)
BL	Basal lamina
C	Conoid
CW	Primary cyst wall
F	Fibrillar element(s)
FL	Filamentous/fuzzy layer
G	Granule(s)
GS	Ground substance
HC	Host cell
I	Invaginations/indentations
LM	Limiting membrane
M	Merozoite
MC	Metrocyte
MI	Host cell mitochondrion
MN	Micronemc(s)
N	Nucleus of merozoite or metrocyte
OL	Osmiophilic layer
P	Parasite
PO	Pore in the primary cyst wall of the protrusion(s)
PR	Protrusion(s)
R	Filamentous or rod-shaped structure(s) in the ground substance of the macrocyst from the sheep
S	Septum
SCW	Secondary cyst wall
Z	Membrane in the stem of the mushroom-shaped protrusions of the cysts from the wildebeest and lechwe

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1. *INTRODUCTION*

Sarcocystis is a coccidian parasite of the protozoan Phylum Apicomplexa (Sporozoa). Species of Sarcocystis have been known for many years to characteristically occur as macroscopic or microscopic cysts in the cardiac and/or skeletal musculature of vertebrates. In 1972, an intestinal phase of the life-cycle was discovered in carnivorous mammals. Subsequently, it became apparent that the gut infection in man is not always subclinical. These findings, together with the fact that certain species of Sarcocystis proved to be pathogenic for the intermediate host, in particular for certain domestic hosts (see recent review by Herbert and Smith, 1987), helped to arouse a number of researchers' curiosity about the organism - which is in any case of basic protozoological interest.

It had been generally assumed, on the basis of some early transmission experiments, that Sarcocystis was strictly specific for the intermediate host. During the course of a survey of the prevalence of Sarcocystis in wild southern African vertebrates, infections were detected in several carnivorous mammals, suggesting a loose host specificity for at least some species of Sarcocystis. Extra-intestinal Sarcocystis infection in carnivores (presumably often a "dead-end" infection) had previously been regarded as rare.

Since each species of Sarcocystis has a characteristic morphology at the ultrastructural level (Mehlhorn et al., 1976), it was decided that the fine structure of the wall of sarcocysts occurring in southern African mammals (particularly ungulates and carnivores), should to be investigated. A study as wide-ranging as that reported here, has not previously been undertaken anywhere else in the world. The aim was:

- to determine the diversity, or otherwise, of Sarcocystis species which infect large mammals in the region;

- and to consider the results in relation to the question of host specificity of Sarcocystis (as far as can be judged by cyst wall types).

Rodents had been trapped in the same localities as certain carnivores with muscular infections, so those particular rodents found to be infected with Sarcocystis were included in the study. As a "sideline", the intermediate host specificity of a species of rodent Sarcocystis was investigated by means of transmission experiments in the laboratory. For the above two reasons, the literature on Sarcocystis of rodents (i.e. in addition to that in Chapter 4 on the cyst wall ultrastructure of large domestic and wild mammals) was chosen for historical review in this thesis.

Because of the large amount of lettering involved, one set of electron micrographs was lettered and then re-photographed for the purpose of producing the required number of copies of this thesis. It should be borne in mind that there has been some resultant loss of clarity in many instances (as compared with the original micrographs).

The reader who is studying the cyst wall ultrastructure of species of Sarcocystis will ideally need to have the papers (containing the electron micrographs) referred to in Chapter 4, spread out in front of him/her. In the absence of that visual comparative material, the results and discussion in this thesis will obviously be difficult to follow.

In Chapter 4, the "cyst membrane" is defined, after Gjerde (1985b), as the limiting or outer unit membrane of the cyst. The terms "limiting membrane", "outer unit membrane" and "outer cyst membrane" are used interchangeably. "Primary cyst wall" refers to the outer unit membrane plus the underlying osmiophilic layer. "Cyst wall" refers to the primary cyst wall (i.e. the outer unit membrane and the osmiophilic layer) plus the ground substance.

Cysts studied ultrastructurally were from naturally-infected animals, unless otherwise indicated in the electron micrograph captions. The fixative used for the cysts was formalin or formal-saline, unless otherwise stated in the captions (see also Chapter 3).

All measurements in the text are given as means, the derivation of which can be checked by reference to the original data in Table 5 at the end of Chapter 4; and to Appendix 7.

The scientific nomenclature employed in this thesis for wild mammalian hosts, is that of Smithers (1983).

2. HISTORICAL REVIEW: RODENT SARCOCYSTIS

RODENT SARCOCYSTIS SPECIES HAVING MAMMALS AS FINAL HOSTS

Sarcocystis muris, first described from a house mouse Mus musculus by Friedrich Miescher in 1843, was recognized by Ruiz and Frenkel (1976) as having an obligatory two-host life cycle. These authors failed to transmit S. muris from mouse to mouse or cat to cat. The sexual cycle took place in the definitive (final) host (cat); and asexual development into intramuscular cysts occurred in the intermediate host (mouse). Failure to infect rats, hamsters and guinea pigs with sporocysts suggested that S. muris was host specific (at least for the intermediate host).

Ruiz and Frenkel (1976) described the intestinal and extra-intestinal stages in the final and intermediate hosts respectively. Sporozoites from sporocysts ingested by outbred, Carworth-Webster-derived mice underwent schizogony in the liver in 11-17 days. Smooth-walled cysts, first seen in the skeletal muscle after 28 days, had mature merozoites (cystozoites) within 100 days post inoculation (d.p.i.) and became infective after 76 d.p.i. Merozoites penetrated through intestinal goblet cells of the final host and no schizogony was observed in the gut before gametogony took place. Micro- and macrogametes were found within 12 hrs, and at 24 hrs macrogametes were observed in the lamina propria of the intestine. Sporogony had commenced after 48 hrs, at which time oocysts contained sporoblasts. The sporocyst walls had developed after 96 hrs and 5-6 days later, fully sporulated sporocysts were found, each with four sporozoites. Sporocysts were usually shed separately in the faeces.

Immunity to S. muris at reinfection was not usually apparent as both mice and cats could be infected regularly. Mice could be superinfected with the result that both small and large cysts were present in muscle. However, a few mice in several experiments did not become reinfected for some unknown reason.

The ultrastructure of the cyst wall of S. muris in the intermediate host was described by Viles and Powell (1976). Non-pedigreed albino mice infected with Sarcocystis were used in the study. Both primary and secondary cyst walls were described, the

latter being part of the degenerate host muscle cell and the former having a distinct osmiophilic layer. The electron micrographs showed a simple cyst wall with interdigitations and an absence of any villus-like protrusions. The authors suggested that the cyst wall and interdigitations were formed from the host muscle cell. The ground substance was erroneously described as the parasitophorous vacuole.

Electron microscopic studies of cysts in the Australian rat Rattus fuscipes by Rzepczyk and Scholtyseck (1976) showed two types of sarcocysts, based on the morphology of the cyst walls. The two distinct species of Sarcocystis were suggested as coinhabiting the same intermediate host. Type A cysts had long, thin villus-like protrusions and Type B had irregular jagged protrusions. The morphology of both differed from the cyst wall described by Viles and Powell (1976) for S. muris. Type A cysts were septate and contained both metrocytes and merozoites. Metrocytes and merozoites had characteristic organelles, e.g. conoid, rhoptries and micronemes were seen in individual cells. Metrocytes undergoing endodyogeny were noted. Type B cysts had fibrillar elements in some protrusions and both metrocytes and merozoites were present. The authors indicated that the carpet snake Morelia spilotes variegata is probably the definitive host of one of the species of Sarcocystis occurring in R. fuscipes (Type A). Another predator of this rat is probably the final host of the other species.

A comparative study of the morphology of the cyst wall by Mehlhorn et al. (1976) revealed a similarity between sarcocysts in mice, whales, rhesus monkeys and baboons. Cysts from the skeletal muscles of laboratory mice, experimentally fed sporocysts from cats, had identical walls to those described by Viles and Powell (1976) for S. muris. The microscopic cysts from naturally infected baboons *Papio cynocephalus* and rhesus monkeys *Macaca rhesus* were in heart and skeletal muscle. Cysts from a naturally infected sperm whale Physeter catadon were macroscopic and were in skeletal muscle. The structure of the cyst wall of all these sarcocyst types was similar, there being many short, bleb-like protrusions up to 0.15 µm in length. Septa extended from the ground substance to divide each cyst into compartments. Mehlhorn et al. (1976) suggested that although the cyst wall ultrastructure is

characteristic for each species of Sarcocystis, it cannot be used on its own to identify species. However, fine structural morphology can be useful to differentiate between species within a single host. A study of the development of cyst wall from a unit membrane, as well as its characteristic morphology, led the authors to conclude that it is the parasites which determine the structure of the cyst wall and not the host cells.

The intramuscular development of S. muris and the ultrastructure of the cyst wall was examined by Sheffield et al. (1977) in experimentally infected mice. Metrocytes were observed dividing by endodyogeny at 40 d.p.i. and up to 325 d.p.i. Merozoites were seen after 53 d.p.i. but at that stage they were not infective to cats. Infectivity was found to be dependent on the production of mature merozoites which fill the cyst after 100 d.p.i. and are divided by septa into groups, each group probably originating from a single metrocyte. The organelles characteristic of the merozoites, e.g. conoid and rhoptries, function in the penetration of the host cell. Micrographs of the cyst wall suggest that this is the same species of Sarcocystis referred to as S. muris by Viles and Powell (1976).

Lai (1977), in a study on Sarcocystis in Malaysian field rats, found five different types of the parasite. The first, in R. exulans and R. jalorensis, was similar to S. muris. The second was S. singaporensis (similar to Rzepczyk's Type A organism) and found in R. exulans, R. jalorensis, R. annandeli and R. rattus diardii. The third and fourth cysts were thin-walled and the fifth had a striated wall.

Rifaat et al. (1978) studied the life cycle of S. muris using sporocysts recovered from cat faeces. Mature sporocysts (8-10 µm in diameter) were shed for a patent period of 20-25 days after a prepatent period of 4-5 days. Schizonts were first observed in the spleen 13 days after feeding sporocysts to laboratory-bred white mice (strain not given). Schizonts with 12 nuclei were observed after 21 days in the liver and pancreas of the intermediate host. Sarcocysts with smooth cyst walls were detected in skeletal muscle 50 days after inoculation. Merozoites in cysts 113 and 120 days after inoculation measured 3-4 µm x 10-12 µm. Twelve out of 22 kittens fed infected mice shed mature sporocysts in their stools. Mice that had a patent infection of Sarcocystis for 84 days and

longer initiated infection in the cats. Macrogametocytes (11 µm x 8 µm) and one microgametocyte were found in the subepithelial layer of the duodenum and upper jejunum. Fully developed sporocysts and oocysts were found in the intestinal lamina propria after 6 d.p.i. As neither the sarcocysts were found to be infectious to mice nor sporocysts infectious to kittens, it was concluded that S. muris has an obligatory two-host life cycle. No schizogony was found to take place in the gut of the final host.

Munday et al. (1978), in their survey of Sarcocystis in mammals in Australia, found eight species of rodents to be infected; five of them had sarcocysts of two different types in their musculature, as judged by light microscopy and examination for macrocysts. The morphology of the cyst walls and the "identification" of definitive hosts allowed the authors to equate some of the species with those described by other workers. The macroscopic sarcocysts found in R. fuscipes were similar to Rzepczyk's Type A cyst which has the python as its definitive host (see under the "snake" subsection of this review). The thick-walled microscopic cysts found in Mastocomys fuscus, Pseudomys higginsi, Rattus lutreolus, R. rattus and R. norvegicus were similar to the macrocyst from R. lutreolus found by Mehlhorn et al. (1976) and which has the black tiger snake Notechis ater as its definitive host (S. murinotechis Munday and Mason, 1980). Thin-walled sarcocysts with large zoites from R. rattus could not be related to any previously described Sarcocystis type (thin-walled cysts with large zoites were also found in three R. norvegicus by Munday et al., 1978 - see review below of the paper by Munday and Mason, 1981). Thin-walled sarcocysts with small zoites from Hydromys chrysogaster, R. lutreolus, R. rattus and R. norvegicus*, found in skeletal muscle and/or cardiac muscle, were suggested as being similar to Rzepczyk's Type B cyst (see "snake" subsection of this review) and Lai's Type III. Cysts found in Mus

* Munday et al. (1978) stated that sarcocysts with thin walls and small zoites were found in H. chrysogaster, R. lutreolus, R. rattus and R. fuscipes. In the discussion of the similarity of thin-walled cysts with small zoites found in rodents to Rzepczyk's Type B cyst, R. norvegicus is mentioned and R. fuscipes is not included.

musculus were of two types, one resembling a species of Sarcocystis which has the cat as a final host (Wallace's Type B) and the other similar to S. dispersa from the barn owl Tyto alba. The relationship of the first-mentioned cyst type to S. muris is not clear, because the cyst wall was not examined by electron microscopy.

Ashford (1978) described S. cymruensis from rats R. norvegicus. Infected meat was fed to cats, dogs and ferrets Mustela putorius. Only cats became infected and they shed sporocysts for more than 3 months. Sporocysts measured 10,5 µm x 7,9 µm. Cysts developed in Wistar white rats R. norvegicus but not in Swiss white mice M. musculus. Cysts measured up to 5 cm in length after 9 months and were found only in skeletal muscle. The internal surface of the cyst wall was suggested as being of parasite rather than host origin (the opposite had been suggested by Viles and Powell, 1976- vide antea). The morphology of the cyst wall was similar to that described by Viles and Powell (1976) for S. muris. Ashford (1978) made the distinction between S. cymruensis and S. muris because the former could not be transmitted to house mice.

Sarcocysts in rats R. norvegicus from Wales (Atkinson, 1978) were similar in appearance to S. muris (infective to Mus musculus) described by Sheffield et al. (1977) and Viles and Powell (1976) and to S. cymruensis (infective to R. norvegicus) described by Ashford (1978). Similar sarcocysts were also seen in R. rattus in Australia by B. L. Munday (pers. comm. to M. B. Markus, 1979). It can be assumed that Atkinson (1978) was working with S. cymruensis. Sporulated sporocysts (10.6 µm x 7,6 µm) were found in cat faeces 10 days after ingestion of infected rat muscle. Four out of 18 rats and one out of ten mice infected orally with sporocysts by Atkinson (1978) developed cysts in their muscles. A parasitaemia was detected in a rat at 11-15 days after infection by means of examination of blood smears. Schizonts (25 µm x 18 µm) were found in the lungs and liver of a rat killed 12 days after infection.

Rommel (1979) successfully infected two ferrets Putorius putorius furo by feeding them mice which had been experimentally infected with S. muris. Sporocysts were excreted by the ferrets after 7 days for a period of 9 days and were indistinguishable from S. muris sporocysts from cats. Mice inoculated with these

sporocysts developed cysts in their muscles after 4 months p.i.

Prevalence studies by Collins and Charleston (1979) showed a 100% infection rate for Sarcocystis occurring in rats R. norvegicus in rubbish dumps and 20% for rats in the rural areas of Palmerston North, New Zealand. The authors did not comment on the relationship of the organism to parasites such as S. cymruensis and S. muris. The prevalence of infection was attributed to the population of definitive hosts, as all dump areas had resident rat and cat populations. Muscle from these rats infected cats, which shed sporocysts measuring 10,6 µm x 7,9 µm. Sarcocysts found in mice were suggested as being similar to those studied by P. B. McKenna (pers. comm. to Collins and Charleston, 1979). Transmission of murine Sarcocystis to a definitive host was not attempted by Collins and Charleston (1979) (see review below of the paper by McKenna and Charleston, 1980).

Inoculation of calves with sporocysts of S. muris by Fayer and Frenkel (1979) produced no infection. Attempted transmission of the parasite to cats by feeding them organs and skeletal muscle from the experimental calves, did not result in infection.

Kan (1979) found three different kinds of Sarcocystis in five species of rodents in Malaysia. All three types of cyst (based on the ultrastructure of the cyst wall) were present in R. r. diardii and R. exulans. Rattus annandalei possessed only the Type II cyst and R. jalorensis only Type I. A bandicoot Bandicota indica had both Type I and Type II cysts in its skeletal muscles. The cysts in the rats were found in the skeletal muscles from the diaphragm, abdomen and limbs and just beneath the skin in the subcutaneous tissues. Type I cyst was microscopic and septate, with finger-like protrusions. Type II was macroscopic, with branched dendritic protrusions and Type III had rectangular protrusions with thick septa dividing the cyst into compartments.

Type I resembled the Type A sarcocyst described by Rzepczyk and Scholtyseck (1976) and Lai (1977) (Lai's Type II also found in R. annandalei). This is S. singaporensis. Type II was similar to Rzepcyk's Type B cyst and Lai's Type III cyst (= S. booliati). Macroscopic Type III cysts resemble sarcocysts reported from R. lutreolus (Mehlhorn et al. 1976) and by Munday et al. (1978). (As has been mentioned previously, the latter authors found this type

of cyst in Mastocomys fuscus, Pseudomys higginsi, R. lutreolus, R. rattus and R. norvegicus, with the black tiger snake being the definitive host - S. murinotechis: sporocysts from the black tiger snake were transmitted to experimental R. norvegicus intermediate hosts - Munday and Mason, 1980).

Kan (1979) concluded that species of rodent Sarcocystis do not have a very strict specificity for the intermediate host. Three types of cysts were considered by him to be three distinct species of Sarcocystis occurring in the various rodent intermediate hosts, as they were morphologically different and unlikely to represent developmental stages of one species.

Becker et al. (1979) reported on the development of S. muris merozoites in cultured cat lung cells and in cultured dog kidney cells. Complete sexual development occurred only in cultures from the known final host, and only a few gametocytes occurred in dog kidney cells. The authors suggested that the reason gametocytes of S. muris had been present in cultured dog kidney cells was because of the large number of passages of the cell line, which may have led to decreased host specificity. No schizogony took place before gametogony. After 6 hrs p.i. the spherical parasites lay within a parasitophorous vacuole. Micro- and macrogametocytes were indistinguishable after 12 hrs p.i. Microgametocytes gave rise to 20-30 microgametes (14 hrs p.i.), each of which possessed two or three flagella. Macrogametes were always spherical in form. No sexual stages were observed after 22 hrs p.i. At 18 hrs p.i., most of the parasitic stages were oocysts bounded by a wall consisting of a dense outer layer and four membranes. The oocyst cytoplasm was surrounded by two cell membranes. After 22 hrs p.i., sporulating oocysts were found in vivo in cats only in the lamina propria. The sporocysts possessed a bi-layered wall with rupture zones to facilitate excystation. Species differences between S. muris and other species of Sarcocystis could not be recognized from the morphology of the sporocyst wall.

Cysts in white mice infected with sporocysts of S. muris from cats were studied ultrastructurally by Fedoseyenko and Levit (1979). In addition to merozoites and dividing metrocytes, intermediate cells resembling those described for Frenkelia were found within the cysts. Both the metrocytes and intermediate cells

underwent endodyogeny, with the former giving rise to four daughter cells simultaneously.

Černá and Ally (1979) compared two species of Sarcocystis from the common vole Microtus arvalis. The first was S. cernae, whose definitive host is the kestrel Falco tinnunculus. The second was similar to S. putorii, described by Tadros and Laarman (1976). The definitive hosts of S. putorii are the ferret Mustela putorius furo, the European weasel M. nivalis and the stoat M. erminea. The morphology of the cyst walls differed between the two species. In addition to the thin cyst wall, formed by the membrane of the parasitophorous vacuole, the cyst wall of S. putorii had thin, closely adjoining processes. The muscle merozoites of S. putorii were longer (13-17 µm x 2-4 µm) than those of S. cernae (8-9 µm x 2-2,5 µm). The occurrance of two types of zoites in S. putorii suggested that sexual differentiation (such as between macro- and microgametocytes) may occur at the cystic stage of development of Sarcocystis.

McKenna and Charleston (1980) isolated sporocysts from naturally infected cats and succeeded in transmitting Sarcocystis to mice but not to laboratory rats. The sarcocysts were smooth, thin-walled cysts, divided into compartments by septa. These cysts resembled those of S. muris and the parasite was, therefore, considered to be this species. The sporocysts of naturally isolated S. muris were similar in size to those measured by Ruiz and Frenkel (1976). The prepatent period for the shedding of sporulated sporocysts by experimentally infected cats was 5-6 days p.i. McKenna and Charleston (1980) suggested that Sarcocystis species are highly host specific for their intermediate hosts, despite the morphological similarity of sarcocysts observed in rats and mice and their common definitive host. Sarcocysts, morphologically similar to S. muris, were found in both wild mice and rats R. norvegicus in New Zealand (Collins and Charleston, 1979). The species found in the rat was transmitted by the cat, although the definitive host for the sarcocyst located in the wild mouse was unknown.

A comparative study by Mehlhorn and Frenkel (1980), using S. muris in specific-pathogen-free (SPF) mice as a laboratory model, highlighted features unique to the genus of Sarcocystis. All cysts

were found within muscle fibers, irrespective of the age of the infection. The primary cyst wall of S. muris was composed of a membrane which formed numerous bleb- or vesicle-like structures on short stalks, which projected into the cytoplasm of the host cell. A dense layer of ground substance beneath the limiting membrane, filled the blebs. Mehlhorn and Frenkel (1980) found that although the primary cyst wall appeared wavy occasionally, no typical *protrusions were formed. A secondary cyst wall was not present* around the parasitized muscle fibre. Both metrocytes and merozoites were separated into clusters by septa of electron-lucent ground substance, which had the appearance of compartments in the interior of the cyst. The presence of metrocytes in the cyst, the osmiophilic layer of the primary cyst wall, the intrusion of the ground substance into the cyst as septa and the presence of species-distinct protrusions, are characteristic of the intramuscular cysts of Sarcocystis.

Collins (1981) reported on the prevalence of sarcocysts in R. rattus from Stewart Island near New Zealand. No ultrastructural examination of the intramuscular cyst walls was carried out for comparison with the microscopic thick and thin-walled sarcocysts found in R. rattus by Munday et al. (1978) in Australia. That the cat is the definitive host, was suggested by the large number of *feral cats on the island and by the studies of Ashford* (1978) and Collins and Charleston (1979), which showed that Sarcocystis in R. norvegicus is transmitted by cats.

A survey by Munday and Mason (1981), involving R. exulans from islands, showed the absence of Sarcocystis, despite the presence of a feral cat population. The idea of the cat being the final host of Sarcocystis of R. rattus found previously in Tasmania (Munday et al., 1978) was confirmed experimentally by the authors; but whether the Sarcocystis species found in black rats in Tasmania is the same as that which infected R. norvegicus and R. rattus in New Zealand (Collins and Charleston, 1979; Collins, 1981) could not be established. Sporocysts from cats infected with Sarcocystis of R. norvegicus (see Collins and Charleston, 1979) were similar in size to those from cats fed infected Tasmanian R. rattus by Munday and Mason (1981) (10,6 µm x 7,9 µm and 10,8 µm x 7,2 µm, respectively). *The cat/R. rattus sporocysts were* infective to R. norvegicus and

poorly infective to mice. It was suggested by Munday and Mason (1981) that this R. rattus species of Sarcocystis is distinct from both S. muris and S. cymruensis, which are possibly genus specific.

The enteric development of S. muris was described by Entzeroth and Chobotar (1982). Initially, gametocytes were located in a three-membranal-complex parasitophorous vacuole within the goblet cells of the intestine of the final host (7 hrs p.i.). At 12-14 hrs p.i., microgametes were seen to develop at the periphery of microgametocytes, which had characteristic, lobed nuclei. The flagella of the microgametes projected into the lumen of the parasitophorous vacuole. Spherical macrogametocytes had a central nucleus, endoplasmic reticulum and "wall forming" spherical bodies at 10 hrs p.i. Development of the oocyst wall seemed to be from a membrane complex beneath the epithelium of the intestine.

A study of the response of mice host to challenge infections of S. muris was carried out by Later et al. (1982). Mice immunized with irradiated sporocysts failed to become infected when challenged with 1000 sporocysts on days 40 and 77, respectively. Super-infection of mice with S. muris (Hofer et al., 1982) was possible after 60 d.p.i., but the number of cysts which developed was smaller than in the initial infection (cf. S. dispersa where complete immunity to reinfection was observed 100 d.p.i. by Hofer et al., 1982). Heterologous infections of mice with S. muris and S. dispersa were successful, without any reduction in the number of cysts that developed. Repeated homologous and heterologous infections were found to have no influence on antibody formation, which showed significant titre increases from day 40 in S. muris infections, and from day 20 in infections with S. dispersa. Cross NMRI mice and house mice were used in the study.

Tadros and Laarman (1982) discussed S. putorii, which they had described in 1978. The parasite completes its sexual cycle in mustelids. Sporocysts (10,5-12,8 µm x 7,5-9,5 µm) found in the faeces of European weasels Mustela nivalis produced intramuscular cysts in the European vole Microtus arvalis and the short-tailed vole M. agrestis. Based on morphological and transmission studies, Tadros and Laarman (1978) stated that M. arvalis and M. agrestis share at least one species of Sarcocystis. Asexual development in the intermediate host occurred in the lymph nodes, spleen and

particularly in the liver parenchymal cells. At least two generations of schizonts were observed in these organs at 12 and 37 d.p.i. Infection of M. arvalis with one million sporocysts of S. putorii from weasels caused a loss of condition, inappetance, dehydration, coma and death, which coincided with schizogony in the liver. Young cysts were first located in the muscle 37 d.p.i. The mature, macroscopic cysts had bristle-like cyst wall protrusions. *Ultrastructurally, the cyst wall protrusions were long and tongue-*like with narrow bases. The invagination of the limiting membrane into the thick underlying osmiophilic layer gave the protrusions a corrugated appearance. Fibrillar elements extended along the lengths of the protrusions into the ground substance (Tadros and Laarman, 1978).

In the weasel final host, the parasite had a prepatent and patent period of 7-8 days and 60 days, respectively. Ferrets M. putorius furo and polecats M. putorius also proved to be suitable final hosts for this parasite. Gametogony was observed in the lamina propria of the posterior third of the small intestine in experimentally infected ferrets. In naturally infected weasels, oocysts were detected in the lamina propria at the apeces of the villi. Sporocysts from the stoat M. erminea produced a similar parasite in voles. However, no infection occurred in the mink M. *lutreola as a final host, or in the bank vole* Clethrionomys glareolus as an intermediate host.

In the comprehensive review by Tadros and Laarman (1982), it is stated that foxes Vulpes vulpes and V. corsac serve as final hosts of S. citellivulpes of the yellow suslik Citellus fulvus.

A species of Sarcocystis from badgers Taxidea taxus, which has the Richardson's ground squirrel Spermophilus richardsonii as an experimental intermediate host, was described by Cawthorn et al. (1983) as S. campestris. The macroscopic cysts in the brain and skeletal muscle had finger-like protrusions with longitudinal striations. The cyst wall (6,4 µm) was thicker than that of S. citellivulpes. Cawthorn et al. (1983) pointed out that as the intermediate host specificities of S. campestris and S. citellivulpes are not known, and as the specificity for both intermediate and final hosts is highly variable for species of *Sarcocystis, cross-transmission experiments are needed to confirm*

that S. campestris and S. citellivulpes are indeed two distinct species. An initial schizogonic cycle takes place within the liver of the squirrel intermediate host 4 d.p.i., as in several other species of Sarcocystis that utilize rodents as intermediate hosts. The authors suggested that S. campestris may be an important pathogen for ground squirrels as four out of ten animals, infected with relatively few sporocysts, died. The deaths were associated with precystic stages of S. campestris.

Two new species of Sarcocystis were described by Dubey (1983b) from the meadow vole Microtus pennsylvanicus. Ultrastructural studies on the sarcocysts from one meadow vole, which harboured both parasites in skeletal muscle, showed differences in the morphology of the cyst wall. S. montanaensis were microscopic and thin-walled. They were less prevalent in the intermediate host (four out of 47 sarcocysts). The cyst wall had minute blebs with a distinct osmiophilic layer and lacked protrusions. Merozoites were 11,1 µm x 3,3 µm. S. microti had a thick wall with finger-like protrusions that contained fibrillar elements which extended into the ground substance. The merozoites were 15 µm x 3 µm. Both cyst types were divided into compartments by septa, in the interior of the cyst.

Four thin-walled cysts were found by Dubey (1983b) in one long-tailed vole M. longicaudatus and numerous thick-walled cysts were found in another. These cysts are similar to S. montanaensis and S. microti, respectively.

Dubey (1983b) considered the cysts he examined to be two new species of Sarcocystis, different from sarcocysts in "domestic" rats and mice. His decision was based on the fact that Sarcocystis is considered to be host specific (or at any rate was at the time, perhaps) and because cyst wall morphology is useful in distinguishing between species in a single host.

Attempted cross transmission of S. muris using voles Microtus pennsylvanicus and gerbils Merionas unguiculatus as potential intermediate hosts, was carried out by Woodmansee and Powell (1984). The attempts were unsuccessful, both in untreated and in dexamethasone-treated voles and gerbils. The authors suggested that differences in in vitro excystation rates of sporocysts in gerbils, voles and mice should not be ignored in the search for a

physiological basis for the host specificity of S. muris.

Entzeroth (1984) showed, ultrastructurally, the penetration of intestinal cells of the cat final host and cultured dog kidney cells by merozoites of S. muris from an experimentally infected mouse. The formation of the parasitophorous vacuole and the function of the rhoptries were described. Electron-dense granular material, thought to have been derived from the rhoptries, is excreted into the parasitophorous vacuole. This protein material may play a part in the cytochemistry of host-parasite membrane interaction.

Gill et al. (1986) studied the infectivity of sporocysts of S. muris when injected intraperitoneally into mice. As mice have been found to become infected when sporocysts whose walls are highly impermeable are injected into them, sporocysts were cultured in vitro with macrophages from uninfected mice. The authors found that more than 90% of the sporocysts had been phagocytosed within 8 hrs. Staining with Giemsa at intervals over 24 hrs showed that the proportion of sporozoites stained had increased. The authors suggested that the permeability of the sporocyst walls, possibly along suture lines, is affected by the antimicrobial oxidative mechanisms of the macrophages, thus allowing the sporozoites to escape.

Species of rodent Sarcocystis with mammalian definitive hosts, and which are listed in Levine and Tadros (1980) and Levine (1986) but not in the historical review, are:

Sarcocystis azevedoi Shaw and Lainson, 1969
Intermediate host: rice rat Oryzomys capito
Definitive host: unknown

Sarcocystis baibacinacanis Umbetaliev, 1979
Intermediate host: marmots Marmota baibacina, M. bobac, M. caudata
Definitive host: dog Canis familiaris, wolf C. lupus, fox Vulpes vulpes

Sarcocystis bozemanensis *Dubey, 1983*
Intermediate host: Richardson's ground squirrel Spermophilus richardsonii
Definitive host: unknown

Sarcocystis cr.iae de Almeida, 1928
Intermediate host: guinea pig Cavia porcellus
Definitive host: unknown

Sarcocystis cricetuli Patton and Hindle, 1926
Intermediate host: striped hamster Cricetulus griseus
Definitive host: unknown

Sarcocystis eutamias Tanabe and Okinami, 1940
Intermediate host: chipmunk Eutamias asiaticus
Definitive host: unknown

Sarcocystis oryzomyos Shaw and Lainson, 1969
Intermediate host: rice rat Oryzomys capito
Definitive host: unknown

Sarcocystis pitymysi Splendore, 1918
Intermediate host: vole Pitymys savii
Definitive host: unknown

Sarcocystis proechimyos Shaw and Lainson, 1969
Intermediate host: spring rat Proechimys guyannensis
Definitive host: unknown

RODENT SARCOCYSTIS SPECIES HAVING BIRDS AS FINAL HOSTS

In 1976, the life cycle of a species of Sarcocystis from the common vole Microtus arvalis and which has the kestrel Falco tinnunculus as the definitive host, was described (Černá and Loučková, 1976). Levine (1977) later named this species Sarcocystis *cernae (see Levine and Tadros, 1980).*

It was found that sporozoites of S. cernae (5-6 µm x 2 µm) excysted in the anterior portion of the small intestine of M. arvalis 2 hrs p.i. (Černá et al., 1978b). Schizogony, involving multiple synchronous endopolygeny, *was observed in the liver* parenchymal cells at 6 d.p.i. In a comparison of the asexual development with S. dispersa Černá, Kolářová and Šulc, 1978, multiple synchronous endopolygeny was also observed, by electron microscopy, in the liver of a house mouse infected with S. dispersa (see Senaud and Černá, 1978). S. dispersa was recovered from a barn owl Tyto alba by Černa et al. (1978a) and was found to produce intramuscular cysts in the mouse.

The formation of S. cernae cysts took place only in skeletal muscle. Inoculation of these cysts into the kestrel produced a patent infection after 7-8 days, with oocysts and sporocysts measuring 19-20 µm x 13-15 µm and 13-16 µm x 10-11 µm, respectively. Infective merozoites (cystozoites) were 8-9 µm x 2-2,5 µm. S. dispersa was shown to have stages of development, similar to those of S. cernae, in the liver (4-8 days p.i.); *formation of cysts in the skeletal muscle; and similar prepatency* (8-10 days p.i.). However, the dimensions of the oocysts (17-22 µm x 10-14 µm), sporocysts (11-14 µm x 8-12 µm) and cystozoic merozoites (8-9 µm x 2 µm) differed from those of S. cernae, according to Černá et al. (1978b) - see above.

Similarities in the developmental stages of two genera, Sarcocystis and Frenkelia, has prompted a suggestion by Černá et al. (1978b) that Frenkelia be made a sub-genus of Sarcocystis, based on a single difference between the two. In experiments with Sarcocystis species from birds, cysts were only found in muscle of intermediate hosts. Frenkelia on the other hand, has cystic stages in the brain of experimental animals.

Sporocysts of S. cernae inoculated into white SPF laboratory mice showed some degree of excystation at 3 and 3,75 hrs p.i.; most of the sporocysts did not excyst. No development of the parasite was observed in the liver. The authors (Černá et al., 1978b) expressed the opinion that the results confirm the considerable host specificity sarcosporidians have for their intermediate hosts. Two tawny owls Strix aluco which were fed skeletal muscle from *infected voles did not shed any oocysts/sporocysts of S. cernae in* their faeces.

The ultrastructural development of the asexual stages of S. dispersa in the liver of the house mouse was described by Senaud and Černá (1978). The parasite developed in the cytoplasm of hepatic cells, producing numerous merozoites by synchronous multiple endopolygenesis. After reaching the muscles, the merozoites reproduced in "xenoparasitones", the wall of which was said to be established by the host muscle cell and which isolates the parasites in a parasitophorous vacuole. The morphological arrangement of the cyst wall was described as being made up of numerous parallel folds. Both metrocytes and merozoites were identified, multiplying by endodyogeny. The authors suggested that differences in the morphology of the developmental stages, the structure of the cyst wall and the different definitive hosts (Tyto alba and *Asio otus for S. dispersa), show that S. dispersa and S.* muris (whose definitive host is the cat) are two different species of Sarcocystis.

In 1976, S. sebeki was described by Tadros and Laarman (Levine and Tadros, 1980). The successful intraperitoneal inoculation of pre-cystic schizonts of S. sebeki from donor to recipient intermediate hosts Apodemus sylvaticus, was reported by Tadros and Laarman (1979). In the study, laboratory-bred long-tailed fieldmice A. sylvaticus were inoculated orally with sporocysts from the tawny owl. Schizonts which contained numerous merozoite. ere found in the livers 8 days p.i. Fieldmice which were then inoculatad intraperitoneally with the homogenized liver suspension, developed sarcocysts in the abdominal wall, diaphragm, hindlimbs, forelimbs and thorax, 4 months after inoculation.

As these experimental infections resulting from intraperitoneal *injection of pre-cystic merozoites were light compared with those*

in animals fed sporocysts, and the development of sarcocysts took place in muscular tissue in close contact with the peritoneal cavity, it was suggested that the merozoites from schizonts are capable of invading muscular tissue directly.

In a review of the Sarcosporidia of carnivorous birds by Černá et al. (1982), it was reported that a Sarcocystis species in Tengmalm's owl Aegolius funereus produced intramuscular cysts in the bank vole C. glareolus. S. cernae occurs in the kestrel F. tinnunculus; S. dispersa in owls T. alba, A. otus and Tyto novaehollandiae; and S. scotti and S. sebeki in tawny owls S. aluco. The authors found in addition, that sporocysts from the goshawk Accipter gentilis produced intramuscular cysts in the house mouse. Sporocysts were also found in the eagle owl Bubo bubo, sparrowhawk Accipter nisus and marsh harrier Circus aeruginosis, but the identities and intermediate hosts of these sarcosporidians were not established.

In a comparative review of Sarcosporidia of rodents having birds of prey as definitive hosts, Tadros (1981) discussed the research on four species of Sarcocystis: S. cernae of the vole M. arvalis with the kestrel F. tinnunculus as the definitive host; S. sebeki of the long-tailed fieldmouse A. sylvaticus and which has the tawny owl S. aluco as a definitive host; and two species from mice which complete their sexual cycles in owls, namely S. scotti in the tawny owl and S. dispersa in the barn owl.

Ultrastructural examination of the asexual stages of the four rodent/bird of prey Sarcocystis species revealed merozoites in schizonts in parenchymal liver cells, arranged around a residual body in the form of a rosette (Tadros, 1981). Asexual multiplication was by synchronous endopolygeny, as described by Černá et al. (1978b) for S. cernae and S. dispersa. The parasites did not develop a parasitophorous vacuole (according to Tadros, 1981), and damage to the liver, occurring 5-7 d.p.i. when infection was probably at its peak, often had fatal results.

Muscle cysts contained metrocytes 12 d.p.i. and cystozoic merozoites as early as 30 d.p.i. The early maturation of cysts of rodent/bird Sarcocystis species differs from those species having rodent/mammal hosts. Ruiz and Frenkel (1976) reported that cysts of S. muris, which has the cat as a final host, were only infective

after 76 d.p.i. The size of the rodent/bird cystic stages also differed from Sarcocystis species having mammalian final hosts - the elongate and slender merozoites being less than 10 µm in length, in contrast to those of S. muris, which are 14-16 µm long. All four species of Sarcocystis discussed by Tadros (1981) had smooth cyst walls without conspicuous protrusions (which Tadros (1981) stated are generally found in Sarcocystis species with *mammalian final hosts*).

Sexual development of S. cernae took place in the epithelial cells of the duodenum and in the small and large intestine of the host. Gametogonic stages were observed specifically in the epithelium of the villi and the crypts. Developing oocysts had moved to the lamina propria, where mature oocysts were found 7 d.p.i.

Excystation of the sporocysts of S. cernae was observed by the author (Tadros, 1981) after exposure to carbon dioxide (CO_2). A specific bile-trypsin solution activated the sporozoites, which were released when the sporocyst wall shattered into four hinged plates.

Sarcocystis and Frenkelia are parasites having identical obligatory, heteroxenous life cycles, with asexual development taking place by schizogony and endodyogeny within a tissue cyst of *a specific intermediate host. Sexual development involves* gametogenic and sporogonic stages in the intestine of a definitive host, without an initial schizogonic phase. In addition, both parasites have similarities in the morphology of their tissue cysts, and physiologically similar cystozoic merozoites. In view of this, Tadros (1981) assumes that Frenkelia is phylogenetically closely related to other avian/rodent sarcosporidians. The hypothesis that obligatorily heteroxenous parasites are derived from heteroxenous coccidians of carnivores - through increasing degrees of heteroxenity - is said to be supported by the fact that sarcosporidian species with phylogenetically related avian final hosts have morphologically similar tissue cysts.

Tadros and Laarman (1982) gave details concerning a species of Sarcocystis of A. sylvaticus which was originally recovered from the faeces of a tawny owl. This species of Sarcocystis was named S. sebeki by Tadros and Laarman in 1976. The species was found to be

pathogenic to the rodent intermediate host following administration of 500 000 sporocysts. Six to 7 days after infection, schizogony was observed in the parenchymal cells of the liver. The intramuscular sarcocyst had an invaginated cyst wall, but lacked protrusions. S. sebeki has been successfully transmitted to laboratory mice, but not to bank voles C. glareolus or the European vole M. arvalis.

S. dispersa, a species recovered from the barn owl, produced intramuscular sarcocysts in laboratory mice. The species was described by Černá and Senaud (1977*), according to Tadros and Laarman (1982). In confirmation of the life cycle of S. dispersa, Tadros and Laarman (1982) observed immature and mature schizonts in parenchymal cells of the liver, 4 and 7 days, respectively, after oral inoculation of a laboratory mouse with sporocysts of S. dispersa. The asexual schizogonic development of this parasite by synchronous multiple endopolygeny was first investigated by Černá and Senaud (1977). Senaud and Černá (1978) described the ultrastructural morphology of the cyst wall. Černá et al. (1978a) reported that S. dispersa could not be successfully transmitted to the vole M. arvalis. The long-eared owl A. otus proved to be a suitable final host.

Sporocysts from a naturally infected tawny owl S. aluco produced intramuscular cysts in labo-atory mice but not in the long-tailed fieldmouse A. sylvaticus, the European vole M. arvalis or the bank vole C. glareolus. Levine and Tadros (1980) named this parasite S. scotti.

Černá and Loučková (1976) found that oocysts from the kestrel F. tinnunculus produced macroscopic sarcocysts in skeletal muscles of the vole M. arvalis. The life cycle of this parasite, named S. cernae by Levine (1977), was discussed by Černá et al. (1978b). Tadros and Laarman (1982) isolated the same organism from kestrels

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* Černá (1983) points out that S. dispersa was named in a paper by Černá et al. (1978a), containing a description of the life cycle in the intermediate and final hosts. The paper by Černá and Senaud (1977) dealt with the electron microscopy of part of the asexual cycle of S. dispersa.

in the Netherlands and confirmed the multiplication by synchronous endopolygeny in schizonts found in the liver. They reported the excystation of the sporocysts in Microtus bile-trypsin solution (Tadros, 1981 - see above).

Tadros and Laarman (1982) stated that species of Sarcocystis of rodents with birds of prey as final hosts, have five features in common:

1. *Rapid growth of the sarcocysts.*
2. Small, elongate merozoites (cystozoites).
3. Sarcocyst walls that lack protrusions.
4. *Schizonts do not have a parasitophorous vacuole* and schizogony takes place in liver parenchymal cells by synchronous endopolygeny.
5. The schizogonic stage is pathogenic *to the host in heavy* infections.

On the subject of the pathogenicity of Sarcocystis for the final host, Tadros and Laarman (1982) claim that intensive intestinal infection with S. sebeki caused the deaths of young tawny owls. Juvenile kestrels with natural massive infections of oocysts and sporocysts of S. cernae in their small intestines, were found dead or dying. In experimental infections of adult kestrels and tawny owls with S. cernae and S. sebeki, respectively, blood-stained tissue strands packed with sporocysts were extruded in the feaces.

Sarcocysts from the skeletal muscle of a European weasel were similar to S. sebeki from A. sylvaticus. An owl fed infected mustelid muscle shed scanty sporocysts.

Tadros and Laarman (1982) commented that the appearance of muscular sarcocysts in carnivores that are not normally prey, may be the result of sporocysts (shed by other carnivores) being accidentally ingested, making the carnivore a *dead-end host. There* is also evidence that Sarcocystis is less host specific for the intermediate host than has previously been believed. Tadros and Laarman (1982) reiterated Markus and Daly's (1980) comment that if some species of Sarcocystis are found to form extra-intestinal stages in the same host in which the intestinal development takes place, then fundamental revision of the current concept of the life cycle of Sarcocystis would become necessary.

The gametogonic development of Sarcocystis species within the subepithelial lamina propria in the intestine of avian final hosts, has far reaching effects on the epizootiology of infection. Tadros and Laarman (1982) suggest that this developmental site, which allows the endosporulation of oocysts, protects them from adverse environmental conditions during sporulation, and allows the prolonged patency of mature sporocysts in the faeces. These mechanisms provide for the widespread dissemination of sporocysts over vast areas of terrain and migratory routes. In addition, the sporocysts are resistant to environmental influences. Sporocysts of S. cernae and S. dispersa were still infective to their respective intermediate hosts after having been recovered from deep-frozen barn owls and kestrels (-20°C for 24-48 hrs).

Černá (1982) reported on the usefulness of S. dispersa in the mouse as a laboratory model. By using the model, it was established that multiplication during schizogony takes place by multiple synchronous endopolygeny (Senaud and Černá, 1978), and that further asexual multiplication takes place in the bloodstream before the parasites reach muscle cells. Infections of S. dispersa could be established in one-day-old mice which recieved injections of 1000 sporocysts. The model showed the use of Giemsa-stained smears of infected organs or muscle homogenates in the diagnosis of sarcosporidiosis.

The degree of immunity to challenge infection of the intermediate mouse host with S. dispersa was described by Hofer et al. (1982). The authors observed the reduction in the number of sarcocysts during the course of infection and the destruction of cysts through cellular reaction. There was no reduction in the number of cysts in heterologous infections, using S. muris. Homologous reinfections of S. dispersa resulted in complete immunity after 10C d.p.i. The formation of antibodies was not influenced by the repeated homologous or heterologous infections. Cross NMRI mice and house mice were used in the study.

Cervá and Černá (1982) found that with the use of antigen prepared from S. dispersa cystozoic merozoites, the indirect haemagglutination test (IHA) could be used mainly for detecting homologous antibodies in mice with muscle cysts. The use of the test with sera from other animal species indicated that the antigen

of S. dispersa and that from Sarcocystis species infecting rabbits, sheep, pigs and horses, is related. SPF mice of ICR strain were used in the study. A total of 31 males and females (weighing between 18-20 g) were orally inoculated with a single dose of 100 000 sporocysts of S. dispersa. At given time intervals, their blood was used in the serological examination. Specific antibodies were detected on day 20 p.i., and the highest titre level was recorded from a mouse which had survived for 125 days p.i.

Cervá and Černá (1982) have suggested that either antibody formation may be stimulated only when the parasites pass from the reticuloendothelial system to the muscle or that the antigen is unable to detect antibodies against the merozoites produced at the early stage of mouse infection.

Gut (1982) attempted to induce immunity in mice by injecting formalinized zoites of S. dispersa intraperitoneally and subcutaneously. This artificial immunization had the same result as regards the formation of specific antibodies as a real infection with S. dispersa; and provided no protective immunity to further infection with the same parasite. Mice were infected with up to 100 000 sporocysts of S. dispersa on day 48 and muscle cysts were found in five out of 17 animals on day 70 after inoculation with 100 000 zoites. Antibody levels reached their maximum 67 days after experimental infection of mice with S. dispersa zoites, and repeated infection had no influence on their formation. SPF mice of ICR strain were used in the study.

Černá (1983) detected dividing merozoites (division occurred by a process similar to endodyogeny) in the bloodstream of intermediate hosts of S. dispersa and S. cernae. Two types of merozoites of S. dispersa were located in blood smears and impression smears from lungs, liver and spleen. Slender merozoites measured 7-9 μm x 2 μm and broad, dividing merozoites (8-9 μm x 4-5 μm) were found free or in macrophages. Dividing merozoites of S. cernae were seen on days 5 and 6 p.i. in blood smears and impression smears from liver, lungs and spleen.

Recipient SPF white mice became infected when inoculated with blood (from 7 days p.i.) containing dividing merozoites from donor mice. Recipient mice continued to become infected from blood taken from donors, 9 and 14 d.p.i., after circulating merozoites could no

longer be detected microscopically in the peripheral blood of donors. Černá (1983) suggested that multiplying merozoites may circulate in the blood at the same time that others are producing *cysts in muscles of the intermediate host.* Zoites of S. dispersa were found in muscles of experimentally infected mice from 10 d.p.i., and their presence in circulating blood was confirmed up to 14 d.p.i. by experimental intraperitoneal transmission.

The existence of multiplicative stages in the blood of the intermediate host, or the production and presence of antibodies in juveniles, indicates probable transplacental transmission in female mice if they become infected with Sarcocystis during pregnancy.

Munday (1983) isolated three isosporan parasites, typical of *Sarcocystis species, from* naturally infected masked owls Tyto novaehollandiae. The first was isolated in mice and was indistinguishable from S. dispersa (see Černá et al., 1978a). The second (it is not clear on what basis this was distinguished from the other two) was not infective to either rats R. norvegicus or mice, but may prove to be transmissible to other species of prey hosts. Oocysts (15-17,5 µm x 11,9-13 µm) and sporocysts (11,5-13 µm x 7,25-8,7 µm) from intestinal scrapings of a masked owl produced an infection in laboratory rats R. norvegicus, but not in mice. *This is the third parasite* isolated by Munday (1983).

Sarcocysts of the third isolate were found in the diaphragms of two inoculated rats but elsewhere in the skeletal musculature of one. The cysts in the diaphragms were twice the diameter of those in skeletal muscle. Ultrastructurally, the cysts had a simple invaginated wall, without protrusions. The cyst wall morphology was quite different to that of S. dispersa, in that it lacked extensive folding. Munday (1983) pointed out that with regard to cyst wall morphology and/or definitive hosts, it is possible to distinguish the *organism from* other Sarcocystis species described in rats e.g. S. cymruensis (see Ashford, 1978), S. murinotechis (see Munday and Mason, 1980), S. singaporensis (see Zaman and Colley, 1976) and various unnamed Sarcocystis species (Munday, 1983).

Munday (1983) suggested that this parasite may be the same as the Sarcocystis species in wild rats (H. chrysogaster, R. lutreolus, R. rattus and R. norvegicus) in Tasmania (Munday et al., 1978), which were thin-walled and similar to Rzepczyk's Type B

and Lai's Type III sarcocysts (Rzepczyk and Scholtyseck, 1976; Lai, 1977). The cyst wall was similar to that of S. boolfati of the moonrat Echinosorex gymnurus (Dissanaike and Poopalachelvam, 1975). Life cycle studies using moonrats and owls would perhaps clarify the relationship of Munday's parasite to S. boolfati.

Černá (1984) summarized the situation as regards Sarcocystis-like organisms having birds of prey as final hosts. Sarcocystis-like sporocysts and oocysts had at that time been found in four species of owls and in five species of predatory birds. In addition to S. cernae, S. dispersa, S. scotti and S. sebeki from avian final hosts (Table 1), oocysts and sporocysts had also been found in the eagle owl B. bubo, common sparrowhawk A. nisus and the marsh harrier C. aeruginosus. Whether these were new or described species of Sarcocystis and what their intermediate hosts were, was unknown. Černá (1984) also reported the recovery of sporocysts from the intestine of a raven Trypanocorax frugileus.

Recently, a new species of Sarcocystis from snowy owls Nyctea scandiaca has been reported (Cawthorn et al., 1984). This species, named S. rauschorum by the authors, was isolated from intestinal scrapings of a snowy owl and passed through experimental varying lemmings Dicrostonyx richardsonii as the intermediate host. Laboratory rats R. norvegicus, house mice, white-footed mice Peromyscus leucopus, red-backed voles Clethrionmomys gapperi and brown lemmings Lemmus sibiricus, could not be infected.

Sporocysts from the intestinal scrapings measured 11,6 µm x 9,2 µm. After recycling of the parasite through experimentally infected varying lemmings, the prepatent period in snowy owls was found to be 7 days and sporocysts were shed for 12-19 days. Sporocysts recovered from the faeces were significantly shorter and narrower (10,6 µm x 8,2 µm) than those from the intestinal scrapings, and had compact residual bodies within the first 4 days of patency. Sporogony was observed throughout the length of the small intestine in the lamina propria, where gametogony was also reported to take place.

Schizogony occurred within hepatocytes. Both immature schizonts (17,2 µm x 13,7 µm) and a single mature schizont (23 µm x 20 µm) were observed at 7 d.p.i. Later, precystic merogonous stages developed in numerous tissues, particularly in the lungs.

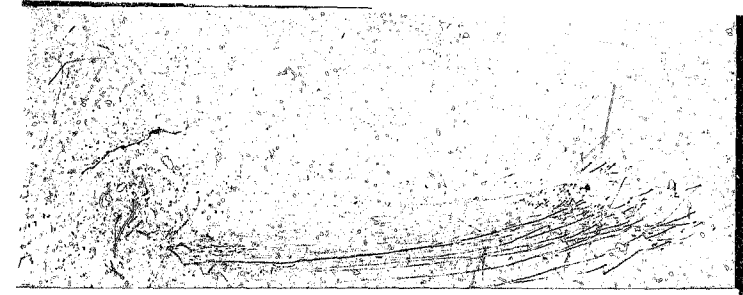
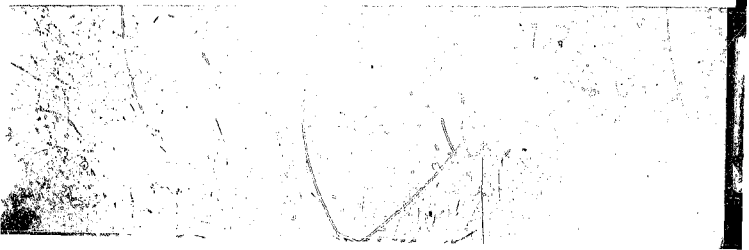


TABLE I: *Described species of rodent* Sarcocystis with birds of prey as final hosts.

SPECIES	*INTERMEDIATE HOST*	FINAL HOST	AUTHORS OF LIFE CYCLE
S. cernae Levine, 1977	Microtus arvalis	*Falco tinnunculus*	Černá and Loučková, 1976
S. dispersa Černá, Kolářová and Šulc, 1978	Mus musculus	Tyto alba	Černá, 1976
		Asio otus	Černá, Kolářová and Šulc, 1978
		Tyto novaehollandiae	Munday, 1977
S. scotti Levine and Tadros, 1980	Mus musculus	Strix aluco	Tadros and Laarman, 1980
S. sebeki Tadros and Laarman, 1976; Levine, 1978	Apodemus sylvaticus Mus musculus	Strix aluco	Tadros and Laarman, 1976
S. rauschorum Cawthorn, Gajadhar and Brooks, 1984	Dicrostonyx richardsonii	Nyctea scandiaca	Cawthorn, Gajadhar and Brooks, 1984



Microscopic cysts were present in skeletal muscle, tongue and ocassionally in the cardiac muscle of the intermediate host at 14 d.p.i. Cystozoic merozoites (6 µm x 2 µm) appeared at 28 d.p.i. Metrocytes, located peripherally in older cysts, and merozoites contained organelles charactaristic of the Sarcocystidae.

After 57 days, cysts were infective to the owl final host. The cyst wall (0,35-0,70 µm thick) morphology is described as simple and without protrusions, similar to that of S. muris in mice (Viles and Powell, 1976), and that of S. cernae, S. sebeki, S. scotti and S. dispersa from raptorial birds. Cawthorn et al. (1984) state that although species of Sarcocystis may have similar cyst walls, species determination should also take into consideration the final and intermediate host specificities, descriptions of the endogenous and exogenous stages and the patent and prepatent periods. S. rauschorum is quite distinct from the other four species of S.rcocystis listed above and which have raptorial birds as final hosts. However, like these other species, S. rauschorum appears to have a narrow range of intermediate hosts.

Sporocysts from the goshawk A. gentilis were transmitted to *white SPF mice by* Kolářová (1986) *and gave rise to* sarcocysts morphologically identical to S. dispersa. Oocysts and sporocysts from coprological examinations of 34 goshawks were found to be similar to those of S. dispersa from barn owls and long-eared owls. As birds (including long-eared owls) constitute the major part of the goshawk's diet, rather than mice, it was suggested that goshawks, as hosts, are (accidentally) utilized only for the passive passage and distribution of S. dispersa in nature*.

All species of Sarcocystis with rodent intermediate hosts and avian definitive hosts and which are listed in Levine and Tadros (1980) and Levine (1986), have been mentioned in this historical review.

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* The goshawk A. gentilis is also the final host of at least one species of avian Sarcocystis, namely S. accipitris (see Černá and Kvašňovská, 1986). This paper is not relevant to this historical review (which is concerned with species of Sarcocystis of rodents).

RODENT SARCOCYSTIS SPECIES HAVING SNAKES AS FINAL HOSTS

The existence of a species of Sarcocystis that had a rat-snake life cycle, was reported by Rzepczyk (1974). Naturally-infected carpet snakes Morelia spilotes variegata produced sporocysts (9,6 µm x 6,6 µm) and oocysts (13,8 µm x 9,6 µm) in their faeces, and scrapings of the duodenal wall revealed fully sporulated oocysts. Rzepczyk (1974) subsequently found a high prevalence (95%) of Sarcocystis in southern bush-rats Rattus fuscipes from regions of S. E. Queensland. At least two types of cysts were found microscopically, in skeletal muscle: those with villous walls and those without. Infected muscle fed to a carpet snake resulted in the production of sporocysts which were found in the duodenum and in the faeces in the cloaca when the snake was killed (18 weeks p.i.). Both the carpet pythons and R. fuscipes were collected from the same geographical area, and the evidence suggests that they are naturally associated in a food chain. Sporocysts from carpet snakes were successfully transmitted to laboratory-bred R. norvegicus but no infection occurred in laboratory-bred mice.

A subsequent study by Rzepczyk and Scholtyseck (1976) of the ultrastructure of the two types of cysts found in R. fuscipes, revealed sarcocysts with thick striated walls (Type A) which were more numerous than the Type B cysts, which had thin, non-striated walls. The Type A cysts had a primary cyst wall with regularly arranged villus-like protrusions (6,8-8,7 µm x 0,8-1,4 µm), which were smooth but punctuated by small invaginations (the authors suggested that these were sites of pinocytosis). The cyst membrane was greatly invaginated to form narrow, neck-like regions at the bases of the protrusions. Clusters of thread-like structures occurred in the granular material of the protrusions. The ground substance extended into the interior of the cyst as septa. Both metrocytes (5,5 µm x 3,2 µm) and merozoites (4,7 µm x 1,8 µm) were present, with their characteristic organelles, e.g. conoid, rhoptries and micronemes were seen in individual cells.

Type B cysts had an irregularly-folded primary cyst wall which gave rise to branched and unbranched protrusions of variable sizes and shapes. In some broad protrusions, fibrillar elements were

observed and the ground substance extended as fine strands into the interior of the cyst. Metrocytes (4,1 µm x 2,7 µm) without deep invaginations of the pellicle were observed but micropores and characteristic organelles were present. Like those in Type A cysts, most metrocytes were in some stage of endodyogeny. Elongate merozoites (4,6 µm x 1 [illegible]) with characteristic organelles were also found.

Rzepczyk and Scholtyseck (1976) considered that two distinct species of Sarcocystis were involved, as judged by morphological differences. From the previous study (Rzepczyk, 1974), it was known that the carpet python is definitive host of at least one of the species of Sarcocystis concerned. The remaining species probably has a final host among other predators of R. fuscipes, which include reptiles, birds and mammals.

In a comparative study of the ultrastructural cyst wall morphology of sarcocysts from various hosts, Mehlhorn et al. (1976) described macroscopic cysts from the skeletal musculature of a naturally-infected, wild, eastern swamp rat Rattus lutreolus from Australia. The primary cyst wall was folded into broad invaginated protrusions that were both long (6 µm) and wide (4 µm). Fibrils were not present in the protrusions.

Kan and Dissanaike (1977) studied the ultrastructure of a Sarcocystis species found in a naturally infected Malaysian house rat Rattus rattus diardii. The microscopic sarcocysts from the skeletal muscle had a thin primary cyst wall with long villus-like protrusions (5,7 µm in length). The proximal ends of the protrusions were narrow, and the cyst wall was highly invaginated in this area. The remainder of the walls of the protrusions was smooth and punctuated by invaginations 306-625 nm apart. The ground substance extended into the cyst as thin septa, dividing the zoites into compartments. Both merozoites (5,3 µm x 1,8 µm) and peripheral metrocytes (5,2 µm x 2,5 µm) were observed.

This species of Sarcocystis was very similar in appearance to S. singaporensis from experimentally infected brown rats R. norvegicus, but with slight differences in the size of the merozoites, which were larger than those of S. singaporensis (5,6 µm x 1,4 µm). The protrusions were also longer than those of S. singaporensis (4,0 µm) and the cysts contained fewer metrocytes.

The structure and appearance of the cyst wall was also similar to Type A cysts described by Rzepczyk and Scholtyseck (1976), although the protrusions were shorter than those from R. fuscipes (8,7 µm x 6,8 µm), (Kan and Dissanaike, 1977).

Lai (1977), in a light microscopic study, reported five types of cysts in skeletal (including subcutaneous) musculature, oesophagus and diaphragm of four species of Malaysian field rats. The first type of cyst from the little house rat R. exulans and the Malaysian wood rat R. jalorensis was "identified" as S. muris. The second was considered to be S. singaporensis (similar to Rzepczyk's Type A organism) and was found in the Singapore rat R. annandalei, and in R. exulans, R. jalorensis and R. r. diardii. The third and fourth cysts were thin-walled and the fifth had a striated wall.

In their survey of Sarcocystis in Australian mammals, Munday et al. (1978) found eight species of rodents infected. Microscopic sarcocysts from R. lutreolus, R. rattus, R. norvegicus, long-tailed rats Pseudomys higginsi and broad-toothed rats Mastacomys fuscus were found in skeletal muscle and appeared to be similar, having thick, striated walls and small zoites. The primary cyst wall was folded into protrusions that were long (6 µm) and wide (4 µm). They were similar in ultrastructure to the cyst described by Mehlhorn et al. (1976) from R. lutreolus. This species of Sarcocystis (S. muriniotechis) has the black tiger snake Notechis ater as its definitve host. Thin-walled cysts containing large zoites were also found in a single R. rattus and three R. norvegicus.

Muscle cysts with thin walls and small zoites were located in the water rat Hydromys chrysogaster, R. lutreolus, R. rattus and R. norvegicus. These cysts were similar to Rzepczyk's Type B cyst and Lai's Type III cyst - (see footnote on p. 23).

Macroscopic cysts found in R. fuscipes and which had thick, striated walls appeared similar to Rzepczyk's Type A cyst and S. singaporensis, both of which have pythons as their definitive hosts. Two types of cyst were found in the house mouse. One resembled a species of Sarcocystis which has the cat as a final host (Wallace's Type B) and the other was similar to S. dispersa from barn owls Tyto alba.

Brehm (1979) studied the development of S. singaporensis in Wistar Rattus norvegicus after infection with sporocysts (9,3 µm x

7,3 µm) from the reticulated python Python reticulatus. Two stages of schizogony were observed in endothelial cells of blood vessels in the liver, spleen, kidney, intestine, lymph nodes, diaphragm and brain at 6 and 15 d.p.i., respectively. From 40 d.p.i., skeletal muscle cysts with metrocytes and merozoites were found. The mature muscle cysts in rats measured 1226 µm x 184 µm (Brehm and Frank, 1980). Infected muscle fed to a python resulted in the production of sporocysts, shed on the 8th and 13th day after infection, with a minimum patency of 73 days. Gametogony and sporogony were observed below the epithelium in the duodenum and the anterior portion of the middle intestine. Unsporulated oocysts were first seen in situ in the gut wall at 4 days p.i.

Kan (1979) described three different species of Sarcocystis, based on the ultrastructure of the cyst wall, from five rodent species in Malaysia. Type I was microscopic, with finger-like protrusions. It resembled Rzepczyk's Type A cyst and Lai's Type II (found in R. annandalei). This species, also similar to S. singaporensis, was found in R. r. diardii, R. exulans and R. jalorensis.

Type II was macroscopic, with branched dendritic protrusions. It resembled Rzepczyk's Type B and Lai's Type III. This cyst type was found in R. r. diardii, R. exulans and was the only kind of cyst in R. annandalei. Macroscopic Type III cysts had rectangular protrusions with thick septa. Although the Type III cysts were only found in R. r. diardii and R. exulans in Kan's (1979) study, similar sarcocysts had been reported from R. lutreolus by Mehlhorn et al. (1976); and Munday et al. (1978) had located this type of cyst in M. fuscus, P. higginsi, R. lutreolus, R. rattus and R. norvegicus. It was named S. muriniotechis by Munday and Mason (1980).

As the three types of cyst described by Kan (1979) were morphologically distinct, they were considered to be three different species of Sarcocystis, which have a less than strict specificity for the rodent intermediate host.

Sporogonic development of Sarcocystis idahoensis in the intestine of the gopher snake Pituophis melanoleucas, 9 to 26 days after being fed infected deer mice Peromyscus maniculatus, was

described by Bledsoe (1979)*. Oocysts were located in parasitophorous vacuoles in epithelial cells adjacent to the basement membrane and often protruded into the lamina propria of the intestine. Sporogony took place throughout the small intestine and the anterior portion of the large intestine from days 9 to 23 p.i. The formation of sporozoites started at 23 days p.i. Fully sporulated oocysts and sporocysts were found in the intestinal lumen 26 days p.i.

The prepatent periods for two gopher snakes fed infected deer mice, were 31 and 39 days respectively; however, it should be noted that the snakes only defaecated once every one to three weeks. Under natural conditions, the prepatent period may be more variable, such as 4-5 months if the hosts hibernate. The rate of sporogony may also vary according to the environmental temperature, as the hosts are poikilothermic. Bledsoe (1979) suggested that the high prevalence of S. idahoensis infection in deer mice (40,8%) is probably due to the extended prepatent period through the winter hibernation of the host, which provides a source of infection for young deer mice in the spring.

In a subsequent publication, Bledsoe (1980a) reported the isolation of S. idahoensis from a Great Basin gopher snake Pituophis melanoleucus deserticola in deer mice P. maniculatus. Pacific gopher snakes P. m. catenifer and San Diego gopher snakes P. m. annectens were used to maintain the parasite by feeding them deer mice infected with sporocysts from the Great Basin gopher snake.

Schizogony in the deer mice occurred in hepatocytes, in contrast to the striated muscle capillaries reported for the Sarcocystis species discussed by Rzepczyk (1974) and S. singaporensis, schizogonous development of which took place in the lungs of Rattus norvegicus. Sarcocysts of S. idahoensis (4,6 µm x 0,3 µm) in skeletal muscle had smooth or slightly irregular cyst walls with small, villus-like protrusions. Septa divided the cyst into

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* S. idahoensis from a Great Basin gopher snake and which has deer mice as intermediate hosts, was named in a subsequent publication by Bledsoe (1980a).

compartments which contained merozoites and peripherally located metrocytes. Asexual schizogonic multiplication in the intestine was not observed prior to gametogony in infected gopher snakes. Microgametocytes were located above the gut epithelial host cell nucleus and macrogametocytes were usually found below. Oocysts were situated adjacent to the basement membrane, often protruding into the lamina propria of the intestine. Fully sporulated oocysts (13,6 µm x 22,3 µm) and sporocysts (13,4 µm x 11,1 µm) were located in the intestine and faeces of the gopher snake. Bledsoe (1980a) suggested that S. idahoensis is a species of Sarcocystis distinct from Isospora roudabushi, found in bullsnakes P. m. sayi. I. roudabushi is, however, considered to be a species of Sarcocystis by Bledsoe (1980a), since fully sporulated sporocysts are present in faeces. The two definitive hosts are geographically separated and the oocysts are found in different locations in the intestine. Both deer mice and white-footed mice are found in the geographical range of the bullsnake, while only deer mice are present in the gopher snake's range. Transmission experiments have shown that S. idahoensis does not reach patency in white-footed mice or in laboratory mice.

The attempted transmission of S. idahoensis sarcocysts by Bledsoe (1980b) to dogs and cats as potential definitive hosts proved unsuccessful. Inoculation of sporocysts into laboratory white mice and white-footed mice did not produce an infection beyond the stage of excystation, which occurred in two white-footed mice 4 hrs p.i. Oral and intraperitoneal inoculation of deer mice P. maniculatus with endozoic merozoites from the liver, did not initiate schizogony in the liver of the recipient, but sarcocysts were found in their skeletal muscles. Deer mice infected orally and intraperitoneally with cystozoic merozoites were negative for *sarcocysts and schizonts after 17 days p.i.*

Although Bledsoe (1980b) considered Sarcocystis species to have a narrow intermediate host range, which exists even within the genus for S. singaporensis, the explanation for the specificity for deer mice as intermediate hosts excluding white-footed mice, is attributed to the latter being found outside the geographic range of the gopher snake in Idaho. It is suggested that cannibalism among deer mice and other populations may provide an alternative

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mode of transmission of the parasite, due to the ingestion of merozoites from schizonts in the tissues of infected animals.

Munday and Mason (1980) introduced the name S. murinotechis for the species of Sarcocystis infecting black rats R. rattus, brown rats R. norvegicus, long-tailed rats P. higginsi and the broad-toothed rat M. fuscus. The parasite was also found in the eastern swamp rat R. lutreolus by Mehlhorn et al. (1976) and Munday et al. (1978), and has the black tiger snake Notechis ater as the definitive host. Ultrastructurally, the cyst wall had short, broad protrusions (6 µm x 4 µm) with invaginations, but lacking narrow neck-like bases; making it quite distinct from S. singaporensis and Rzepczyk's Type A organism. The sarcocysts of S. murinotechis are ultrastructurally identical to those found in R. fuscipes. (In Munday et al. (1978) a macroscopic, thick, striated-walled sarcocyst from the southern bush rat R. fuscipes was reported to be similar to S. singaporensis and to Rzepczyk's Type A cyst)*.

Sporocysts from tiger snakes measured 11,0 µm x 7,25 µm. Schizogony occurred in the heart, skeletal muscles, lungs and kidneys of experimental R. norvegicus. At 12 weeks p.i., sarcocysts measuring 60-90 µm in diameter and up to 300 µm in length were present in the skeletal muscles. Large doses of sporocysts proved pathogenic to the rat intermediate host and to experimental white mice. The occurrence of a single, thick-walled sarcocyst in the skeletal muscle of an experimental white mouse suggests that the parasite may develop to some extent in this rodent, and in wild mice.

Beaver and Maleckar (1901) isolated three species of Sarcocystis from laboratory rats R. norvegicus, infected with sporocysts from a

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* Presumably the macroscopic cyst described in Munday et al. (1978) from R. fuscipes as being similar to S. singaporensis and Rzepczyk's Type A organism is different from the cyst described as S. murinotechis from R. fuscipes. Should this be the case, then R. fuscipes is the intermediate host of at least three species of Sarcocystis. In addition to the two species mentioned above, R. fuscipes is host to a sarcocyst with thin walls and small zoites, described by Munday et al. (1978) - see above.

naturally-infected python P. reticulatus from Singapore. Sporocysts of different sizes initially produced schizonts in the lungs (7 d.p.i.) and in endothelial cells in the heart, skeletal muscle, brain, eye, kidney, omentum and lungs at day 12 p.i. At 15 d.p.i., large schizonts were found in the lungs, and merozoites were located in cardiac and skeletal muscle. Sarcocysts in skeletal muscle were detected as early as 22 d.p.i., and three species of Sarcocystis could be differentiated at 9 weeks p.i. The thick-walled cysts with large, stalked villi (5,4 µm x 1,5 µm) were described as S. singaporensis, similar to Rzepczyk's Type A organism. The merozoites were small (6,0 µm x 1,5 µm) and the presence of septa was indicated by the groupings of zoites. The sarcocyst type, which ultrastructurally possessed short villi (1,6 µm x 0,5 µm) with microvilli radiating from the primary cyst wall) was named S. villivillosi. The merozoites were small (5,0 µm x 1,0 µm) and grouped into compartments formed by septa. Macroscopic cysts (1-2 mm x 0,3-0,5 mm) in the skeletal muscles had thin walls with thin protrusions that formed branched, anastomosing structures. Both the protrusions and the cyst membrane between the *protrusions were invaginated. The compartmentalized merozoites were* small (7-8 µm x 1,5 µm) and bannana-shaped. This Sarcocystis species was named S. zamani.

Beaver and Maleckar (1981) suggested that despite differences in the dimensions of the cyst, sarcocysts of S. zamani appear similar to those of Rzepczyk's Type B cysts in R. fuscipes, Lai's Type III cysts, cysts described by Kan (1979) (Type 2 from R. annandalei, R. exulans and R. r. diardii) and cysts from R. rattus, R. norvegicus, M. chrysogaster and R. lutreolus found by Munday et al. (1978) - (see footnote on p. 23).

S. singaporensis is reported to occur in R. r. diardii, R. exulans, R. jalorensis and the bandicoot Bandicota indica, as well as experimentally-infected R. norvegicus. If Rzepczyk's Type A organism is found to be the same as S. singaporensis, then R. fuscipes should be included in the host list (Rzepczyk and Scholtyseck, 1976; Munday et al., 1978). S. villivillosi appears to be a new species of Sarcocystis, and has not been reported in any naturally-infected host.

Sarcocystic infections did not develop in any of the laboratory

mice fed sporocysts from the python. Three strains of laboratory-bred R. norvegicus were used in the study. They were agouti Curtis-Dunning Irish, hooded Long Evans and albino Sprague-Dawley.

When sporocysts of S. singaporensis were fed to Malaysian field rats R. tiomanicus, R. exulans, R. rattus, long-haired rats R. villosissimus and R. colletti* by Frank and Häfner (1981), merozoites and/or cysts could be found in either tissue smear preparations (not given) or in sections of the muscles, of all the experimental rodents. R. norvegicus is recognized as an intermediate host of S. singaporensis, but Frank and Häfner (1981) considered this rat to be a secondary invader of East Asian regions and, therefore, unlikely to be the only host for this Sarcocystis species in nature. R. rattus, naturally infected with S. singaporensis in Thailand, produced sporocysts in the faeces of P. reticulatus (13 and 15 d.p.i.), but not in the Indian python P. molurus bivattatus. The authors suggested that R. rattus is a natural host of S. singaporensis, which is considered to be genus-specific for the intermediate hosts and species-specific for the definitive hosts (but see the account below of the work of Häfner and Frank, 1984).

Tadros and Laarman (1982) reviewed the finding by Zaman and Colley (1975) of S. singaporensis (S. orientalis) in the faeces of a Malaysian reticulated python P. reticulatus. Sporocysts (7-11 µm x 7-10 µm) fed to laboratory R. norvegicus proved to be pathogenic to the host 5-12 days after the administration of heavy doses (Zaman and Colley, 1975). Symptoms of pyrexia, lymph node enlargement, increased respiration or dyspnoea and diarrhoea occurred in the intermediate rodent host. Anaemia and petechial bleeding were also reported (Brehm and Frank, 1980). Pathogenicity is due to the schizogonous stages that occur in the various tissues and organs of the intermediate host. In R. norvegicus, merozoites of S. singaporensis, probably from an initial schizogonic cycle, were detected in blood smears, either free or within leucocytes, 4 to 9 d.p.i. Schizonts were seen in the lungs and macroscopic cysts

* The common names of some species of rodents mentioned here and elsewhere in the text, are not given in standard reference works.

were found in skeletal muscle 2 months after sublethal doses of sporocysts had been administered (Zaman and Colley, 1975).

Tadros and Laarman (1982) commented that gametogony of *Sarcocystis* takes place at a slower rate within epithelial intestinal cells (> 18 to 24 hrs) in reptiles than in sub-epithelial intestinal tissues in non-reptilian final hosts.

Studies by Tadros and Laarman (1982) on S. idahoensis from imported gopher snakes P. melanoleucus, confirmed the findings of Bledsoe (1980a) with regard to pathogenicity, schizogony and sarcocystic stages in deer mice P. manículatus.

Dubey (1983a) reported the finding of two distinct kinds of sarcocyst in the skeletal muscles of d r mice P. manículatus from Montana. *Thin-walled* sarcocysts were identified as S. idahoensis. Macro- and microscopic sarcocysts (38-1875 µm x 27-81 µm), which by light microscopy had thick walls with hair-like protrusions, were considered to be a new species of Sarcocystis, which was named S. peromysci. Septa were present in the cysts. Merozoites (cystozoites) of S. peromysci were 11,2 µm x 3,1 µm in size. While 13 out of 25 infected deer mice were positive for S. peromysci, mixed infections of both types of Sarcocystis were found only in two deer mice. Ultrastructural studies on the cyst wall were not carried out.

Studies by Häfner and Frank (1984) on the host specificity and host range of three species of Sarcocystis, which have rodent-snake life cycles, have shown that S. singaporensis and S. villivillosi have intermediate hosts which are members of the related genera Bandicota and Rattus. Naturally-infected spiny rats Maxomys surifer from Thailand, which had sarcocysts similar to S. muriniotechis, could not be infected with either S. singaporensis or S. villivillosi. Maxomys surifer had at one time been included in the genus Rattus.

Snake species belonging to the family Boidae proved to be suitable definitive hosts for S. singaporensis and S. villivillosi. These included the Asian python P. timorensis, the Australian black-headed python Aspidites melanocephalus and, to a lesser extent, the African rock python P. sebae.

A third Sarcocystis species, from Kenyan river-jacks Bitis nasicornis, proved infective for a wide range of rodents, including

white mice, multimammate mice Praomys (Mastomys) natalensis, jirds Meriones unguiculatus, gerbils Gerbillus perpallidus and a golden hamster Mesocricetus auratus. These rodents *acted as poor* intermediate hosts, and, as they are not found within the distribution area (i.e. rain forest) of the definitive host, at least one natural intermediate host can be anticipated. Reports that other Bitis species (African adders) from savanna areas (inhabited by some of the above intermediate hosts) serve as final hosts for this species of Sarcocystis, suggests a loose specificity of this parasite for its hosts.

Häfner and Frank (1984) suggested that host specificity may depend upon the phylogenetic ages of both/all host types or the numbers of available hosts, as the genus Rattus includes an *extensive spectrum of host species* and individuals. Alternatively, Sarcocystis species may develop in closely related "groups" of hosts from the same geographical area, but animals from other, less closely related genera *may nevertheless be suitable physiologically* to act as incidental hosts.

Häfner and Matuschka (1984) reported on a Sarcocystis species which was isolated from river-jacks B. nasicornis and transmitted experimentally through several hosts (Mesocricetus, dwarf hamsters Phodopus, Gerbillus, Meriones, Mastomys and Mus). The parasite had four different Bitis species as experimental definitive hosts. The snakes were the puff adder B. arietans, the horned adder B. cauvalis, the gaboon viper B. gabonica and B. nasicornis; and they excreted sporocysts (11,1 µm x 8,1 µm) which produced macroscopic intramuscular sarcocysts in the rodents. These cysts were up to 2,5 cm in length and 100-400 µm in width. The cyst walls appeared smooth, and septa grouped the cystozoic merozoites (8-9 µm x 2 µm) into compartments.

Häfner and Matuschka (1984) considered the Sarcocystis species isolated from river-jacks as being the same parasite originally isolated from the puff adder B. arietans. Sporulated sporocysts from the puff adder were *first reported as Isospora dirumpens* by Hoare in 1933. Following elucidation of the life cycle of Sarcocystis, I. dirumpens is considered to be a species of Sarcocystis and has been re-named S. dirumpens. S. *dirumpens* is presumably the same species from river-jacks discussed by Häfner

and Frank (1984) - see above.

Sporulated oocysts (17,9 µm x 11,0 µm) and sporocysts (10,8 µm x 7,9 µm) from intestinal scrapings of a rattlesnake Crotalus scutulatus scutulatus were fed to Swiss Webster white mice by Entzeroth et al. (1985a). Sarcocysts were found in the skeletal muscles of the laboratory mice from 67 d.p.i. The simple primary cyst wall formed bleb-like vesicles on short stalks. In younger cysts, the cyst wall had an undulating appearance similar to the cyst from Acomys spinosissimus in the present study (see Figure 1). In older cysts, the cyst wall had a more even appearance. The granular ground substance had a thickness of approximately 1,0 µm, and formed septa in the interior of the cyst which divided the merozoites and the metrocytes into compartments. Metrocytes at the periphery of the cyst were seen to divide by endodyogeny.

The simple morphology of the cyst wall was considered by the authors to be similar to that of S. muris and, based on light photomicrographs, to S. idahoensis (Bledsoe, 1980a). The Sarcocystis species in the rattlesnake/mouse life cycle was named S. crotali by the authors.

Häfner and Frank (1986) studied the ultrastructure of S. dirumpens from experimentally infected Mastomys natalensis, Phodopus sungorus, Meriones unguiculatus, Mus musculus and Rattus norvegicus. The primary cyst wall was invaginated, with minute bleb-like evaginations. The ground substance was 0,5-1 µm thick and extended into the interior of the cysts. Merozoites measured 7-8 µm x 1,6-1,9 µm. Cysts were considered to be very similar to S. sebeki, which has a rodent/bird life cycle. The primary cyst wall of S. crotali (rodent/rattlesnake life cycle), described as "spherical vesicles on short stalks", is considered by the authors in contrast to that of S. dirumpens. They observed metrocytes dividing by endodyogeny in the more mature central parts of the cyst and metrocytes undergoing multiple synchronous endopolygeny at the cyst poles. It was, therefore, suggested that there might be several generations of metrocytes, the first dividing by endodyogeny and subsequent ones multiplying by endopolygeny. Two other rodents from East Africa, woodland mice Grammomys caniceps and striped grass mice Lemniscomys barbarus, were found to be experimental, and therefore possibly natural, intermediate hosts.

Sporocysts (11,1-12,3 µm x 8,2-9,4 µm) shed by a wild-caught Aesculapian snake Elaphe longissima in southern Germany were found by Matuschka (1986) to be infective to species of voles. Through repeated transmissions, it was found that all snakes of the genus Elaphe used in the study were susceptible to infuction by the parasite. The earliest that sporocysts were shed in their faeces was after 20 d.p.i. Sarcocysts (1 200-4 500 µm x 70-170 µm) which developed in bank voles Clethrionomys glareolus, common voles Microtus arvalis, root voles M. oeconomus and Günther's voles M. guenther1 had invaginated cyst walls with protrusions that were thin, invaginated and close to the body of the cyst. Cystozoic merozoites measured 10-12 µm in length and were 2 µm in width. Like S. dirumpens, this Sarcocystis species is presumed to be genus specific with regard to the snake definitive host, and has been named S. clethrionomyelaphis by the author.

O'Donoghue et al. (1987b) found macroscopic and microscopic sarcocysts of S. singaporensis in the skeletal muscles of seven different species of rodent in North Sulawesi and West Java in Indonesia. S. singaporensis with thick, radially-striated cyst walls and broad, spatula-like protrusions were found in Bunomys sp., B. adsperusu, Maxomys bartensi, Maxomys musschenbroekii, Parauromys dominator, Rattus sp. and R. exulans. Ultrastructurally, the cyst wall protrusions were 3,5-5,0 µm in length, with bulbous, distal regions (3,0-4,5 µm x 1,0-2,5 µm) joined to the cyst wall by short, narrow stalks (0,5-1,5 µm x 0,1-0,7 µm). The stalks had highly invaginated walls and the bulbous protrusions were filled with loosely-packad ground substance. Microscopic cysts with smooth cyst walls were detected in four different species of rodent (Bunomys sp., B. adsperusu, P. dominator and Rattus sp.) from North Sulawesi. Ultrastructurally, the protrusions (0,5-2,5 µm x 0,05-0,2 µm) were slender and hair-like, with invaginated walls. The protrusions extended away from the surface of the cyst. The primary cyst wall was invaginated and a thin band of ground substance (less than 0,2 µm) lay beneath it. Septa separated the merozoites (3,5-6,0 µm x 1-2 µm) and metrocytes into compartments in the interior of the cyst. This species of Sarcocystis was named S. sulawesiensis, based on its morphology, host range and limited geographic distribution. Mixed infections of S. singaporensis and

S. sulawesiensis were found in only three rodent species: Bunomys sp., B. adsperusu, and F. dominator. As the definitive hosts for S. singaporensis and S. sulawesiensis were not identified, the authors concluded that extensive research is needed on the various *Sarcocystis species found in rodent hosts*, not only to elucidate their life cycles but also to determine the pathogenicities of the respective parasites and their significance with regard to host disease.

Sporocysts from the faeces of a Palestinian viper Vipera palaestinae were orally inoculated into mice, Günther's voles, jirds (Meriones unguiculatus and M. tristrami), multimammate mice, and rabbits Oryctolagus cuniculus, by Matuschka et al. (1987). Only laboratory mice became infected. Schizonts were located in liver cells of laboratory mice 9-10 days after infection with sporocysts (12,2 µm x 9,6 µm) shed by naturally and experimentally infected vipers. Mice given 60 000 sporocysts became ill between 9 and 11 d.p.i., and high dosages ($5x10^5$) proved fatal to some. Macroscopic *sarcocysts were located in the skeleta*l muscle of mice on day 36 p.i. and measured 5 000-8 000 µm x 150-400 µm on day 165 p.i. Ultrastructurally, the cyst wall had cauliflower-like protrusions which were 3,5 µm long. The septate cysts contained merozoites which were 12-13 µm long. The prepatent period in the vipers was 27 days p.i. Fully sporulated oocysts were located in the submucosa or lamina propria of the intestine of a viper killed 19 d.p.i. The authors stated that this Sarcocystis species differed from S. dirumpens with regard to the structure of its cyst wall and the sizes of the sporocysts and sarcocysts. All other snake/rodent Sarcocystis species have different intermediate and final hosts and geographical distributions from the species described. It was, therefore, considered a new species of Sarcocystis and named S. muriviperae by the authors. *Matuschka et al. (1987) suggested that* because representatives of rodent genera other than Mus failed to become infected, this may indicate strict host specificity on the *part of S. muriviperae*. It should, however, be noted that some of the white mice (strain not given) also failed to become infected when inoculated with 60 000 or $5x10^5$ sporocysts.

The species of Sarcocystis that have rodent intermediate hosts and snake definitive hosts and which are listed in Levine and Tadros (1980) and Levine (1986), but not in this historical review, are:

Sarcocystis lampropeltis (Anderson, Duszynski and Marquardt, 1968) Levine and Tadros, 1980
Intermediate host: unknown
Definitive host: king snake *Lampropeltis c. californiae*

Sarcocystis roudabushi (Pellérdy, 1974) Levine and Tadros, 1980
Intermediate host: unknown
Definitive host: gopher snake Pituophis s. sayi

3. MATERIALS AND METHODS

PROCESSING OF MUSCLE TISSUE FOR TRANSMISSION ELECTRON MICROSCOPY

Muscle fixed in buffered formal-saline, glutaraldehyde or Karnovsky's fixatives before processing.

METHOD:

1. Washed in 0,2M cacodylate buffer for 30 mins at 4°C.
2. 1% osmium tetroxide in 0,15M cacodylate buffer for 1 hr at 4°C.
3. Rinsed in 0,1M sodium acetate 2x10 mins at 4°C.
4. 0,25% aqueous uranyl acetate for 1 hr at 4°C.
5. Rinsed in 0,1M sodium acetate 2x10 mins at 4°C.
6. 35% acetone for 5 mins at 4°C.
7. 50% acetone for 5 mins at 4°C.
8. 70% acetone/1% uranyl acetate for 3 hrs at 4°C.
9. 90% acetone for 10 mins at room temperature (r.t.).
10. 95% acetone for 10 mins at r.t.
11. 100% acetone 3x15 mins at r.t.
12. Araldite/acetone (25:75) for 2 hrs at r.t.
13. Araldite/acetone (50:50) for 2 hrs at r.t.
14. Araldite/acetone (75:25) for 2 hrs at r.t.
15. Araldite for 24 hrs at r.t.
16. Polymerized for 2 days at 60°C.
17. Sectioned on a Reichert OMU-3 ultramicrotome.

SOLUTIONS:

Cacodylate buffer 0,2M (pH 7,46)

Sodium cacodylate $[Na(CH_3)_2AsO_2.3H_2O]$	42,8 g
Hydrochloric acid 1N	6,9 ml

- made up to 1000 ml with distilled water.

Sodium acetate 0,1M

Sodium acetate	1,36 g

- made up to 100 ml with distilled water.

Aqueous uranyl acetate 0,25%

Uranyl acetate	0,25 g

- made up to 100 ml with distilled water.

Uranyl acetate 1% in 70% acetone

Uranyl acetate	1 g

- made up to 100 ml with 70% acetone.

FIXATIVES:

Glutaraldehyde

Glutaraldehyde 25%	5 ml
Cacodylate buffer 0,2M	15 ml
Water	20 ml

- fixation times 15 mins-24 hrs, followed by post-fixation in osmium tetroxide.

Osmium tetroxide in 0,15M cacodylate buffer

Osmium tetroxide [OsO_4] 4%	2 ml
Cacodylate buffer 0,2M	6 ml

Neutral-buffered formal-saline 10,9%

Formaldehyde 35%	250 ml

Distilled water	1750 ml
Sodium chloride	17 g
Sodium dihydrogen orthophosphate [$NaH_2PO_4.H_2O$]	8 g
Disodium hydrogen orthophosphate (anhydrous) [Na_2HPO_4]	13 g

- fixation time 2-10 days.

Note: Most of the material for this study was contributed as muscle samples by various field workers. Consequently, it was not normally feasible to have muscle fixed in a more suitable (for electron microscopy) fixative than formal-saline, particularly considering the large numbers of muscle samples involved. However, fixation in formal-saline has been found to be perfectly adequate for study of the "gross" ultrastructural features of the sarcocyst wall. Even material which has been preserved in formalin for 15 years is suitable (M. B. Markus, personal communication). As an aside, deep freezing of muscle prior to fixation in formal-saline also has little effect on the "gross" fine structural morphology of the sarcocyst wall (Kaiser and Markus, 1981), a finding which is not, however, relevant to the present study.

Formaldehyde/glutaraldehyde (Karnovsky, 1965)

Paraformaldehyde	1,2 g
Cacodylate buffer 0,2M	15 ml
Water	20 ml
Glutaraldehyde 25%	5 ml
Calcium solution	0,4 ml

- paraformaldehyde was heated (80°C) in the water with 2 drops of sodium hydroxide (1N NaOH) in order to disolve it. The solution was allowed to cool and then the other components were added. The fixative was used within 24 hrs. The fixation time was 2 hrs at 0°C.

Solutions for formaldehyde/glutaraldehyde fixative:

Stock calcium solution

Calcium chloride [$CaCl_2.6H_2O$]	10 g
Water	1000 ml

- *1 ml added to each 100 ml of fixative.*

Sodium hydroxide 1N

Sodium hydroxide [NaOH]	4 g

- made up to 100 ml with distilled water.

Note: A 1ON solution is 40 g made up to 100 ml with distilled water, or 4 g made up to 10 ml.

EMBEDDING RESIN:

Araldite

*Araldite M (CY212)**	*500 g*	*10 ml*
DDSA (hardener)*	500 g	10 ml
DMP 30 (accelerator)*	15 ml	0,3 ml
Dibutyl phthalate (plasticiser)*	50 ml	1 ml

- The liquids are highly viscous and require thorough mixing. The mixed resin can be dispensed into small containers and stored in the deep freeze for up to 6 months.

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* Available from TAAB Laboratories Equipment Ltd, England.

STAINING OF SECTIONS FOR TRANSMISSION ELECTRON MICROSCOPY

A. ULTRA-THIN SECTIONS

Stained with aqueous uranyl acetate 7% (saturated) and lead citrate 0,4%.

METHOD:

1. Both solutions centrifuged before use at 1224 g* (2700 rpm) for 3 mins.
2. Drops of aqueous uranyl acetate 7% placed on a wax surface. Grids floated on the drops for 10 mins (dull surface downwards if this was the side with the sectioned material).
3. Grids rinsed in 3 beakers of distilled water (30 rapid dips in each).
4. Staining procedure repeated with lead citrate 0,4%.
5. Grids rinsed in 3 beakers of distilled water (30 rapid dips in each).
6. Grids stored in grid holder or anti-static box and allowed to air dry.
7. Sections were examined in a Jeol JEM-100S or a Jeol JEM-100C electron microscope.

STAINING SOLUTIONS:

Aqueous uranyl acetate 7% (saturated)

Uranyl acetate	0,8 g
Distilled water	10 ml

- mixed well by shaking.

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* g was used in this thesis to denote the standard acceleration due to gravity and to distinguish it from g denoting grams.

Lead citrate 0,4%

Lead citrate	0,04 g
Distilled water	10 ml
Sodium hydroxide 1ON	0,1 ml

- *shaken vigorously until all lead citrate was disolved.*

(From Venable, J.H. and Coggeshall, R., 1965, Journal of Cell Biology 25: 407-408).

B. *SEMI-THIN SECTIONS*

Stained with Toluidine blue.

METHOD:

1. Sections placed in water on a glass slide.
2. Dried on a hot plate (60°C) so that sections adhered to the slide.
3. Sections covered with stain.
4. Left on hot plate for 30 secs (until the edge of the stain turned gold in colour).
5. Rinsed in distilled water.
6. Left to dry.
7. Examined under light microscope.

STAINING SOLUTIONS:

Toluidine blue stain

Pyronin B 1% in distilled water	1 part
Toluidine blue 1% in borax 1%	4 parts

PREPARATION OF ULTRA-THIN SECTIONS FOR THE EXAMINATION OF SCHIZONTS IN BOVINE BRAIN TISSUE BY TRANSMISSION ELECTRON MICROSCOPY

METHOD 1: ULTRA-THIN SECTIONS FROM HISTOLOGICAL SECTIONS

This method was adapted from Federman and Vallespir (1984), but modified.

1. Formalin-fixed sections of wax-embedded brain tissue (7-20 µm thick) were dewaxed, hydrated and stained with haematoxylin and eosin in order to locate the schizonts.
2. After staining the sections were mounted in glycerine.
3. Schizonts were identified by light microscopy and their positions marked (the positions were marked by examining the slide upside down and circling the schizonts by means of a diamond marker fitted to the microscope).
4. The coverslip was loosened by immersing the slide in warm water.
5. All excess tissue was scraped away, leaving a broad band of tissue surrounding each schizont.
6. Plastic moulds* used for embedding material for light microscopy, with holes (2 mm x 2 mm) cut in their bases, were placed over each schizont and surrounding tissue (a thin layer of vacuum grease around the edge of the hole kept the mould steady on the slide and prevented leakage of the respective fluids during processing).
7. By means of Pasteur pipettes, the tissue was processed for transmission electron microscopy by exposure to the following solutions (made up as described earlier in this chapter):

Cacodylate buffer 0,2M	2x1 min.
1% osmium tetroxide	5 mins
Cacodylate buffer 0,2M	3x1 min.
50% acetone	1 min.

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* Plastic embedding moulds S-22 and T-8 available from Peel-a-way Scientific, California, U.S.A.

70% acetone/1% uranyl acetate	5 mins
90% acetone	1 min.
100% acetone	3x1 min.
Araldite/acetone (50:50)	15 mins
Araldite	see below

8. The slide and attached moulds were placed in an oven and the resin polymerized at 60°C for 48 hrs.
9. After removal from the oven, the slide was allowed to cool to room temperature.
10. The slide was then placed on a hot plate at 100°C for 5 mins, after which time the moulds, together with the (now) resin-embedded tissue, was snapped off.

METHOD 2: ULTRA-THIN SECTIONS FROM SEMI-THIN SECTIONS

This method was adapted from Crocker et al. (1982), but modified.

1. Semi-thin sections (2-3 µm thick) of araldite-embedded brain tissue were mounted on a slide, stained with toluidine blue and allowed to dry.
2. Schizonts were identified by light microscopy and marked (schizonts were circled directly, using a diamond marker fitted to the microscope).
3. After marking, the sections were covered with a layer of solvent-based adhesive (Durofix*) and incubated at 37°C for 30 mins.
4. The slide was then placed in water and the adhesive layer containing the sections was allowed to float off, overnight.
5. By means of a dissecting microscope, the circular places of tissue containing the schizonts ("punched-out" as a result of circling of the schizonts with a diamond marker) were lifted, after the Araldite resin and adhesive had been dissolved in ethyl acetate.

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* Available from Rawlplug Co. Ltd, England.

6. Each piece of tissue containing a schizont was placed in a capsule (B.E.E.M.)* of fresh Araldite resin and polymerized at 60°C for 48 hrs.

METHOD 3: ULTRA-THIN SECTIONS FROM SEMI-THIN SECTIONS LIFTED DIRECTLY FROM SLIDES

1. Semi-thin sections (2-3 µm thick) of araldite-embedded brain tissue were mounted on a slide, stained with toluidine blue and allowed to dry.
2. Schizonts were identified by light microscopy and marked (schizonts were circled directly, using a diamond marker fitted to the microscope).
3. A surplus, polymerized resin block was glued over the semi-thin section on the slide (Araldite** epoxy adhesive was used for this purpose).
4. The adhesive was allowed to dry overnight.
5. The slide was then placed on dry ice for 10-15 mins, after which time the resin block, together with the section, was snapped off the slide.

Note: Ultra-thin sections were cut on a Reichert Ultracut-E microtome at a setting of 65 nm. Back illumination of the tissue in the resin block allowed the schizonts to be easily seen when mounted on the ultramicrotome. Sections were placed on copper grids, stained with uranyl acetate and lead citrate and examined in a Jeol JEM-100S transmission electron microscope.

The treatment of the histological sections with various solutions in method 1 and the initial sectioning by means of a stainless-steel microtome knife, damaged the tissue. Score-marks and holes were evident in tissue when it was examined by transmission electron microscopy. Sections processed by method 2 were of better quality, probably because of minimal handling of the

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* Available from Better Equipment For Electron Microscopy Inc., New York, U.S.A.

** Available from Ciba-Geigy (Pty) Ltd.

tissue and the use of glass knives for cutting the semi-thin sections. The lifting of semi-thin sections directly from slides (method 3) provided good quality ultra-thin sections for transmission electron microscopy. In addition, the method proved to be simple and less time-consuming than the other two. The electron micrograph in this thesis (Figure 115) of a schizont in formalin-fixed bovine brain (from an experimental animal which had died at *least eight, and probably more, hours* previously) is of a semi-thin section processed as outlined in method 3 and sectioned at 65 nm. The animal died on day 28 post (oral) inoculation with sporocysts.

MEASUREMENTS OF SARCOCYST COMPONENTS FROM MICROGRAPHS OF VARIOUS MAGNIFICATIONS

Details of the procedure followed as regards the measurement of sarcocyst components from electron micrographs, are given in the preamble to Table 6.

PROCESSING OF MUSCLE TISSUE FOR LIGHT MICROSCOPY

METHOD 1: IN AN AUTOMATIC TISSUE PROCESSOR (Shandon-Elliott)

1. 70% alcohol	1 hr
2. 95% alcohol (1)	1,5 hrs
3. 95% alcohol (2)	1,5 hrs
4. Abs. alcohol (1)	1,5 hrs
5. Abs. alcohol (2)	2 hrs
6. Abs. alcohol (3)	2 hrs
7. Chloroform (1)	1,5 hrs
8. Chloroform (2)	1,5 hrs
9. Paraffin wax*(1)	2 hrs
10. Paraffin wax (2)	3,5 hrs
11. Embedded in fresh paraffin wax	-

METHOD 2: MANUALLY

1. 70% alcohol	1,5 hrs
2. 95% alcohol	1 hr
3. Abs. alcohol (1)	1 hr
4. Abs. alcohol (2)	1 hr
5. Chloroform (1)	0,5 hr
6. Chloroform (2)	0,5 hr
7. Paraffin wax (1)	1 hr
8. Paraffin wax (2)	2 hrs
9. Paraffin wax (3)	1 hr
10. Embedded in fresh paraffin wax	-

Note: After embedding, the tissue was sectioned with a Leitz wedge-shaped microtome knife on a Leitz 1512 rotary microtome.

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* Paraplast available from Monoject Scientific Inc., Ireland.

PROCESSING OF LIVER AND GUT EPITHELIAL TISSUE FOR LIGHT MICROSCOPY

METHOD:

1. 50% Alcohol	0,5 hr
2. 70% Alcohol	1 hr
3. 95% Alcohol	1 hr
4. Abs. Alcohol (1)	1 hr
5. Abs. Alcohol (2)	1 hr
6. Methyl benzoate (1)	10 min.
7. Methyl benzoate (2)	10 min.
8. Paraffin wax (1)	1,5 hrs
9. Paraffin wax (2) (in vacuum oven)	overnight
10. Paraffin wax (3)	1,5 hrs
11. Embedded in fresh paraffin wax	

Note: To prevent inversion of the gut epithelial tissue while in the fixative, the tissue was sometimes pinned to flat pieces of wax or cardboard before fixation.

The pieces of liver were washed *in 30% alcohol* several times (0,5 hr) *before processing.*

After embedding, the tissue was sectioned with a Leitz wedge-shaped microtome knife on a Leitz 1512 rotary microtome.

STAINING OF TISSUE SECTIONS FOR LIGHT MICROSCOPY

Tissue mounted on glass slides with albumen adhesive, and stained with haematoxylin and eosin.

METHOD:

1. Dewaxed in 2 changes of xylene.
2. Hydrated through 2 changes of absolute alcohol and 1 change of 95% alcohol to running water.
3. Stained in modified Mayer's haematoxylin for 5 mins.
4. Washed in running tap water.
5. Differentiated in 1% acid alcohol (4 rapid dips).
6. "Blued" in running alkaline tap water for 10 mins.
7. Stained in eosin/phloxine solution for 30 secs.
8. *Washed well in running tap water.*
9. Dehydrated from running tap water through 95% alcohol (1 change) to absolute alcohol (2 changes).
10. Cleared in 2 changes of xylene.
11. Mounted with entellan.

STAINING SOLUTIONS:

Modified Mayer's haematoxylin

Haematoxylin	4 g
Distilled water	1000 ml
Sodium iodate	0,3 g
Aluminium ammonium sulphate	50 g
Citric acid	1,5 g
Chloral hydrate (preservative)	75 g

- aluminium ammonium sulphate dissolved in water (heat not used). Haematoxylin, sodium iodate, citric acid and chloral hydrate added, in that order. Haematoxylin solution filtered through coarse filter paper.

Eosin/phloxine

Eosin 1%	500 ml
Phloxine 1%	250 ml
Distilled water	750 ml

SOLUTIONS:

Acid alcohol

Hydrochloric acid (concentrated)	10 ml

- made up to 1000 ml with 70% alcohol.

Mayer's glycerol albumen adhesive

Fresh egg white	50 ml
Glycerol	50 ml
Sodium salicylate	1 g

- the ingredients were mixed and filtered through coarse filter paper.

STAINING OF IMPRESSION SMEARS FOR LIGHT MICROSCOPY

Impression smears fixed in 100% methanol for 1,5 mins before staining with Giemsa.

METHOD:

1. Stained in Giemsa 10% (with slide lying flat and upside down on ridges in flat staining dish obtained from the London School of Hygiene and Tropical Medicine) for 1 hr (sometimes more; sometimes less, depending on parasite stage or tissue concerned).
2. Rinsed momentarily in tap water.
3. Allowed to air dry.

STAINING SOLUTIONS:

Giemsa's stain 10%

Phosphate buffer	1 part
Distilled water	4 parts
Then:	
Giemsa's solution*	1 part
Phosphate buffer/water solution (above)	9 parts

Solutions for Giemsa's stain:

Phosphate buffer (pH 7,1-7,2)

Disodium hydrogen orthophosphate (anhydrous) 5 g
[Na_2HPO_4]
Potassium dihydrogen orthophosphate (anhydrous) 2,1 g
[KH_2PO_4]

- made up to 1000 ml with distilled water

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* Giemsa's stain (Gurr's New Improved R66): from BDH Chemicals Ltd.

PROCEDURE FOR POST MORTEM FIXATION OF TISSUE

A. BODY ORGANS

Applicable mainly to rats/mice used for transmission experiments. After death the specimen was weighed and the time of death noted.

METHOD:

1. Blood smears were prepared and allowed to dry.
2. Touch preparations were made from the peritoneal cavity and allowed to dry.
3. The liver was cut and impression smears were prepared. Pieces (not larger than 5 mm^3) were placed in formal-saline at 0^oC. Pieces (not larger than 1 mm^3) were placed in glutaraldehyde or Karnovsky's fixative at 0^oC.
4. The lungs were removed. After impression smears had been obtained, pieces (up to 5 mm^3) were placed in formal-saline *fixative at 0^oC. This procedure was repeated for kidney, brain,* adrenal gland, pancreas, heart, spleen and mesenteric lymph node.
5. After dissection, impression and blood smears were fixed in 100% methanol and stained with 10% Giemsa. Some fixed spares were kept.
6. Tissues fixed in glutaraldehyde or Karnovsky's fixative were removed from the ice after 2 hrs and placed in the refrigerator (4^oC) for 4-5 hrs before being processed for transmission electron microscopy. Formal-saline-fixed tissues were stored at room temperature after being removed from the ice and were processed for light microscopy several days/weeks later.

B. SKELETAL MUSCLE

METHOD:

1. *During the course of laboratory autopsies,* pieces of skeletal muscle (not larger than 5 mm^3) were placed in formal-saline at

room temperature and stored for at least several days (and often several months) before being processed for light microscopy.

2. Skeletal muscle sent to the laboratory in bottles by field collectors, usually arrived in formal-saline. These samples were often large. Consequently, smaller pieces (5 mm^3 in size or less) were dissected off the edge and processed for light microscopy.
3. Following light microscopic examination, smaller pieces (1 mm^3 in size or less) of "positive" skeletal muscle were processed for transmission electron microscopy.
4. Fresh skeletal muscle received was immediately placed in formal-saline (pieces not larger than 5 mm^3) for subsequent light microscopic examination. Thin strips of fresh muscle in phosphate buffered saline (P.B.S.) were squashed between two glass slides and examined under the light microscope. "Positive" muscle was then cut (1 mm^3) and fixed in glutaraldehyde or Karnovsky's fixative for subsequent transmission electron microscopy.

SOLUTIONS:

Phosphate buffered saline (P.B.S.) (pH 7,4)

Sodium chloride [NaCl]	8,5 g
Potassium chloride [KCl]	0,2 g
Potassium dihydrogen orthophosphate (anhydrous) [KH_2PO_4]	0,2 g
Disodium hydrogen orthophosphate [$Na_2HPO_4.12H_2O$]	2,9 g
Calcium chloride dihydrate [$CaCl_2.2H_2O$]	0,13 g
Magnesium chloride [$MgCl_2.6H_2O$]	0,10 g

- the components were added in the above order (never until the previous component had dissolved). The calcium chloride was dissolved in 30 ml of distilled water and then added slowly to the buffer solution. This was repeated with the magnesium chloride. Made up to 1000 ml with distilled water.

DETECTING SPOROCYSTS/OOCYSTS IN FAECAL MATERIAL

METHOD:

1. A small sample (5-10 g) of faecal material was mixed with distilled water (10 ml) and sieved through fine gauze in order to remove large particulates, etc.
2. The filtrate was centrifuged in a 10 ml tube at 1224 g (2700 rpm) for 3 mins.
3. The supernatant was removed and the pellet was broken up and thoroughly mixed in the centrifuge tube with a saturated sodium chloride solution.
4. After centrifugation at 1224 g for 3 mins, the solution was left standing for 5 mins, to allow the sporocysts/oocysts to accumulate at the surface.
5. A bacteriological loop was inserted into the surface fluid. The fluid drawn across the loop (film) was deposited on to a clean microscope slide.
6. Repeating this procedure resulted in there being a drop of fluid on the slide. A coverslip was placed over the fluid. This was followed by scanning under the light microscope for the presence of sporocysts/oocysts.
7. The coverslip which was placed over the fluid was usually sealed with Vaseline (petroleum jelly) to prevent evaporation of the sodium chloride solution before examination of the preparation had been completed.

HARVESTING OF SPOROCYSTS/OOCYSTS FROM FAECAL MATERIAL

METHOD:

1. Faecal material collected from individual animals was placed in 2,5% potassium dichromate (made up with tap water) in labelled plastic containers and stored at 4°C.
2. The faeces were sieved through fine gauze (with potassium dichromate or distilled water added, if necessary, to the top of the mesh while sieving) in order to separate the large particulate matter.
3. The liquid containing the sporocysts/oocysts was centrifuged in 50 ml tubes at 1224 g (2700 rpm) for 3 mins.
4. After removal of the supernatant, the pellet was broken up and thoroughly mixed in the centrifuge tube with a saturated sodium chloride solution.
5. After centrifugation at 1224 g for 3 mins, the solution was left standing for 5 mins, to allow the sporocysts/oocysts to accumulate at the surface.
6. The surface liquid was pipetted (Pasteur pipette) into 50 ml centrifuge tubes containing clean distilled water.
7. These tubes were centrifuged at 1224 g for 3 mins, after which the supernatants were removed and replaced with clean distilled water.
8. The above washing procedure was repeated again in order to eliminate the sodium chloride from the concentrate.
9. The concentrates were removed from the tubes and placed in a small glass bottle containing distilled water or potassium dichromate.
10. After allowing the concentrate to settle in the bottle for 1 day, the supernatant was removed and replaced with fresh concentrate.
11. Large numbers of sporocysts/oocysts could be accumulated in this way over a period of days.

DETERMINATION OF THE NUMBER OF SPOROCYSTS/OOCYSTS IN A FLUID

Determination of the number of sporocysts/oocysts in an experimental inoculum was carried out using a haemocytometer (improved Neubauer).

METHOD:

1. The supporting ridges of the haemocytometer were moistened and the coverslip pressed firmly in place (until Newton's rings *appeared*).
2. The sporocysts/oocysts in suspension were mixed by inverting the bottle and then pipetting the fluid up and down, before transfering a drop to the haemocytometer chamber.
3. Four large counting squares were used (each consists of sixteen *smaller squares*), *i.e. one* large counting square in each corner of the grid. All the sporocysts/oocysts in each large square were counted, omitting those on the top and left hand lines but including those on the bottom and right hand lines.
4. After counting, the mean of the four large squares was *multiplied* by 10 000. This gave the number of sporocysts/oocysts per ml.
5. (Adjustments to the sporocyst/oocyst concentrations were made if necessary).

DETERMINATION OF THE DIMENSIONS OF SPOROCYSTS AND OOCYSTS

A Union vernier ocular micrometer and a Leitz SM-Lux microscope were used to measure all sporocysts (at a magnification of X100).

FREEZE-DRYING RAT/CAT SARCOCYSTS

The cysts were dissected out into cold P.B.S. They were then concentrated by centrifugation at 500 g (1725 rpm) for 5 mins, after which the saline was removed and the pellet freeze-dried. The freeze-dried material was stored in a dessicator at 4°C.

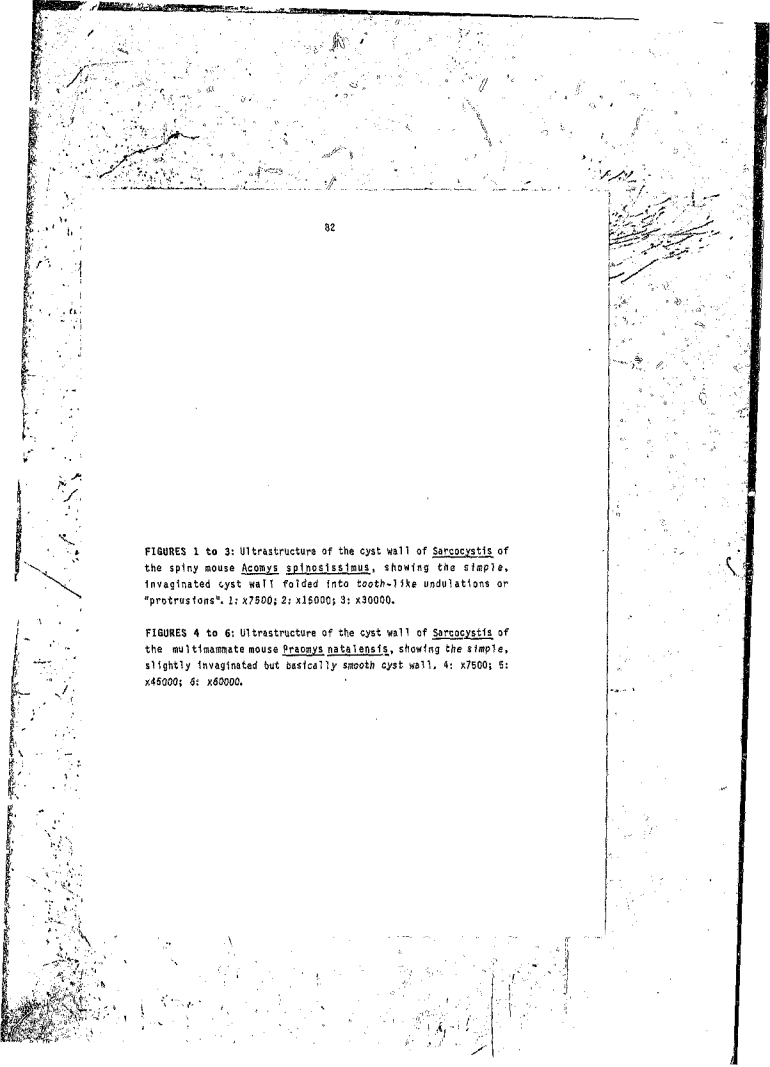
4. RESULTS AND DISCUSSION: ULTRASTRUCTURAL CYST WALL MORPHOLOGY OF SARCOCYSTIS IN SOUTHERN AFRICAN WILD AND DOMESTIC MAMMALS

SARCOCYSTS WITH SIMPLE WALLS

I. RODENTS

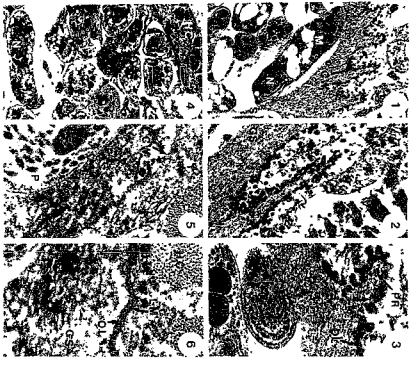
All sarcocysts in the skeletal muscles of wild rodents from the eastern Transvaal were simple-walled cysts. The wall of a cyst in the spiny mouse Acomys spinosissimus was highly invaginated and formed bleb-like structures which were similar to but appeared larger than those of Sarcocystis muris in mice (Viles and Powell, 1976; Sheffield et al., 1977; Mehlhorn and Frenkel, 1980). The *entire cyst wall, including the ground substance, had an undulating* or peg-like appearance, with the peg-like "protrusions" measuring 1,50 µm in height and 0,50 µm in width at their bases (Figures 1-3). *This undulating appearance was also evident in the* S. muris micrographs of Sheffield et al. (1979). The wall of the cyst in A. spinosissimus, including the ground substance, was 0,90 µm thick and the bleb-like structures were *0,2 µm in length. The maximum* length of the blebs of S. muris was 0,1 to 0,15 µm (Mehlhorn and Frenkel, 1980). Merozoites of S. muris were 11-16 x 4,5 µm (Mehlhorn and Frenkel, 1980), and thus were larger than those found in Acomys (5,88 µm x 1,64 µm).

In contrast to the invaginated cyst wall of the spiny mouse, that of a sarcocyst in the multimammate mouse Praomys natalensis, appeared smooth (Figures 4-6). The thick osmiophilic layer was interrupted at short intervals along its length by indentations of the outer unit membrane. The width of the cyst wall, including the thin underlying layer of ground substance, was 0,48 µm. The ground substance extended into the cyst as septa (Figure 4). Merozoites were 5,09 µm in length and 1,62 µm wide and were, therefore, of a similar size to those found in Acomys. The (morphology of the) cyst *from the multimammate mouse may represent a stage in the* development of the cyst wall. Older cysts might have the highly invaginated appearance seen in the cyst from the spiny mouse. This possibility is mentioned because *it has been found that the cyst* wall morphology of S. muris varies slightly, according to the age



FIGURES 1 to 3: Ultrastructure of the cyst wall of Sarcocystis of the spiny mouse Acomys spinosissimus, showing *the simple*, invaginated cyst wall folded into *tooth-like* undulations or "protrusions". *1:* x7500; *2;* x15000; 3: x30000.

FIGURES 4 to 6: Ultrastructure of the cyst wall of Sarcocystis of the multimammate mouse Praomys natalensis, showing *the simple*, slightly invaginated but *basically smooth* cyst wall. *4:* x7500; *5:* x45000; *6: x60000.*


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of the cyst (W. A. G. Charleston, 1986, personal communication to M. B. Markus).

Sarcocysts in the namaqua rock mouse Aethomys namaquensis and the red veld rat Aethomys chrysophilus both have similar, highly invaginated cyst walls (Figures 7-9). The cyst membrane forms spherical, stalked structures that are similar to those described for S. muris (see Viles and Powell, 1976; Sheffield et al., 1977; Mehlhorn and Frenkel, 1980) and for the parasite in the spiny mouse. The cyst wall in the red veld rat was thinner (0,40 µm) than that in the namaqua rock mouse (0,69 µm) and the spiny mouse (0,90 µm), and the bleb-like structures were shorter (0,13 µm). The lengths of the bleb-like structures in the namaqua rock mouse were similar to those of the spiny mouse (0,2 µm) and the merozoites were 5,78 µm x 1,62 µm. The osmiophilic layer of the cyst in the red veld rat was less complete (due to the invaginations of the outer cyst membrane), and the underlying ground substance was thinner than that of the cyst in the namaqua rock mouse. It should be noted that the differences between these two cysts may be a reflection of differences in their maturity. As the cyst develops, the osmiophilic layer may become pinocytosed or incorporated into the vesicle-like structures of the outer cyst membrane. The underlying ground substance may also become thinner in older or mature cysts.

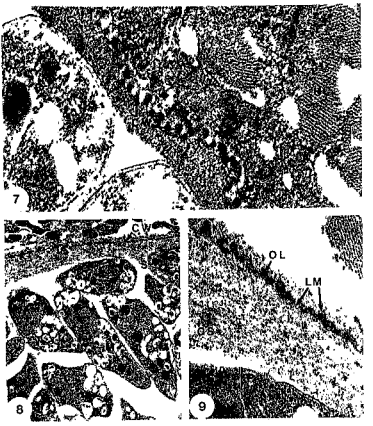
In summary, ultrastructural examination of cysts in the infected, wild southern African rodents revealed that they are similar and may, in fact, be the same species of Sarcocystis - which may or may not be S. muris.

In a series of transmission experiments, a macroscopic Sarcocystis species of the black rat Rattus rattus was fed to cats in the laboratory. Sporocysts shed by the cats after a prepatent period of 7-8 days were then inoculated, by stomach tube, into laboratory-bred black rats R. rattus, white rats R. norvegicus and white mice M. musculus. Only black rats and white rats developed sarcocysts in their skeletal muscles (details are given elsewhere in this thesis).

Cysts taken from the rats were fixed and processed for transmission electron microscopy. The ultrastructural morphology of the wall of sarcocysts from black and white rats was identical

FIGURE 7: Ultrastructure of the simple, invaginated cyst wall of Sarcocystis of the red veld rat Aethomys chrysophilus. x45000.

FIGURES 8 and 9: Ultrastructure of the simple, invaginated cyst wall of Sarcocystis of the namaqua rock mouse Aethomys *namaquensis*. 8: x9000; 9: x37500.


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(Figures 10-13). The cyst membrane was invaginated to form stalked, vesicle-like structures. The width of the cyst wall (including the ground substance) was 0,60 µm and 0,48 µm, in black and white rats respectively. The sarcocysts from both rat species were similar to S. muris from mice (Sheffield et al., 1977), both as regards cyst wall morphology and the size of the bleb-like structures (0,14 µm in the rats).

It was apparent, from the fine structural examination of the sarcocysts in the black and white rats, that the outer unit membrane and the osmiophilic layer form two or more layers of stalked protrusions, one superimposed upon the other (Figures 12 and 13). This lattice-work of protrusions is also found in S. muris (see Sheffield et al., 1977) and, to a lesser extent, in sarcocysts from the other wild rodents.

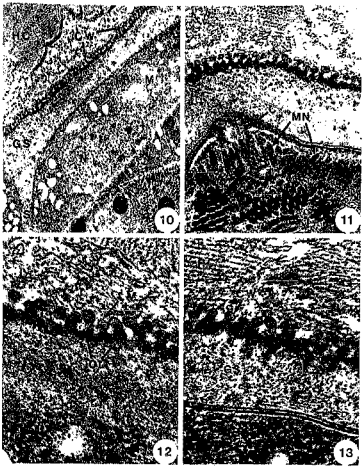
The difference in appearance of the cyst wall between the black and white rats and that from the wild rodents may be due to the thickness of sections or the density of the protrusions. If the protrusions branch and form a close lattice-work, as is vident in the rats and S. muris, then the cysts concerned may either be older or different types of cysts from those found in the wild rodents, whose lattice-work forms a wider, less dense pattern of branching protrusions.

As the cysts examined ultrastructurally were derived from rats and not mice, the question remains whether the sarcocysts were those of S. muris or not. A number of authors have reported on the infectivity for rats of S. muris derived from mice, and the strict specificity that this species of Sarcocystis appears to have for its intermediate host (see historical review of Sarcocystis in rodents).

In this present study, the electron microscopic structure of sarcocysts from black and white rats showed a greater similarity to that of S. muris derived from mice (Viles and Powell, 1975; Sheffield et al., 1977; Mehlhorn and Frenkel, 1980) than to cysts from the wild rodents. However, the ages of the cysts in the wild rodents were not known, so it is not possible to make accurate comparisons. Sarcocystis species that have related intermediate hosts and simple cyst wall structures are difficult or impossible to identify on the basis of cyst wall morphology alone.

FIGURES 10 to 12: Ultrastructure of the simple, invaginated cyst wall of Sarcocystis of the black rat Rattus rattus, showing stalked, vesicular, bleb-like structures extending into the host cell cytoplasm. 10: x15000; 11: x37500; 12: x75000. Experimental infection, 6 months post inoculation. Fixative: glutaraldehyde.

FIGURE 13: Ultrastructure of the simple, invaginated cyst wall of Sarcocystis of the white rat Rattus norvegicus albinus, showing stalked, vesicular, bleb-like structures extending into the host cell cytoplasm. x60000. Experimental infection, 5 months post inoculation.


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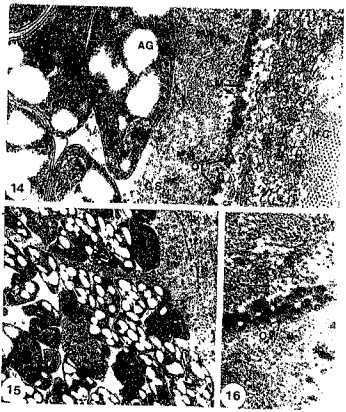
Properly controlled transmission experiments to determine the final and intermediate hosts could lead to an understanding of the host specificity of rodent species. However, interpretation of the results of transmission experiments is complicated by the fact that inoculum size and mouse strain influence the development, or otherwise, of a detectable, patent S. muris infection in a mouse (and probably, therefore, in a rat as well) (M. B. Markus, 1987, personal communication).

II. WILD CARNIVORES

A simple-walled sarcocyst type in the skeletal muscle of a lion Panthera leo from the eastern Transvaal was not unlike certain Sarcocystis species of rodents and reptiles. The distinct electron dense, osmiophilic layer was interrupted by invaginations of the outer unit membrane (Figure 14). The invaginations were highly developed in places so that a lattice-type appearance of the cyst wall with bleb-like structures was evident (Figure 16). The cyst wall was 0,72 µm wide, and included the ground substance, which extended as septa into the interior of the cyst (Figure 15). The merozoites, which were sectioned obliquely, measured 2,90 µm x 0,79 µm.

The wall of the sarcocyst from the lion appeared to be less invaginated than that of S. muris in mice (Viles and Powell, 1976; Sheffield et al., 1977; Mehlhorn and Frenkel, 1980), and seemed similar to cysts from wild rodents. The cyst wall in the lion shows some indication of the development of a lattice-type structure, as was evident in the namaqua rock mouse. The lion cyst was structurally similar to Sarcocystis montanaensis from the meadow vole Microtus pennsylvanicus (see Dubey, 1983b), a Sarcocystis species in a marsupial Antechinus sp. (see Munday et al., 1978) and a Sarcocystis species from R. norvegicus, which has the masked owl Tyto novaehollandiae as its final host (Munday, 1983). Cysts from the eastern chipmunk Tamias striatus (see Entzeroth et al., 1983b) and Sarcocystis bozemanensis from Richardson's ground squirrel Spermophilus richardsonii (see Dubey, 1983c) had cyst walls not unlike those in the lion and the red veld rat. A sarcocyst from the mouse M. musculus which has the rattlesnake

FIGURES 14 to 16: Ultrastructure of the simple, invaginated cyst wall of *Sarcocystis* of the lion *Panthera leo*. Figure 16 shows the lattice-type arrangement which was evident in the wall of the same cyst. 14: x45000; 15: x12000; 16: x45000.


AG
14
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16

Crotalus s. scutulatus as its final host (Entzeroth et al., 1985a) also had a simple cyst wall, but appeared more highly invaginated than the cyst from the lion.

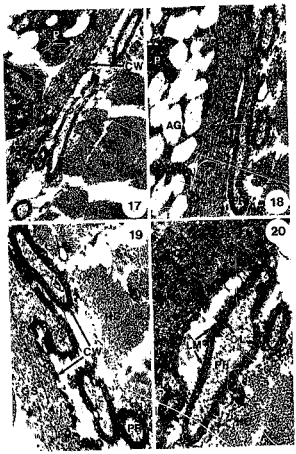
A Sarcocystis species in cardiac and skeletal muscle of domestic cats (Kirkpatrick et al., 1986) had a simple, invaginated cyst wall with vesicle-like protrusions (0,42 µm x 0,39 µm) at irregular intervals. The sarcocyst wall was 0,80 µm thick. The morphology of the cyst wall of the Sarcocystis species in the cat was not unlike that of a cyst found in the skeletal muscle of a lioness from Zimbabwe. In this lioness, the invaginated cyst wall had small protrusions that were thread-like in longitudinal section (1,26 µm x 0,26 µm) and circular or vesicle-like in cross section (0,46 µm x 0,25 µm) (Figure 17). Where the protrusions joined the cyst, they turned sharply and lay immediately adjacent to the cyst wall (Figure 18). In addition, the limiting membrane of the protrusions had a spiny appearance (Figure 20) which was also apparent in micrographs of protrusions of the cyst in the cat (Kirkpatrick et al., 1986). The osmiophilic layer became discontinuous at intervals along the protrusions and the limiting membrane was invaginated, creating pore-like structures (Figure 19). The width of the cyst wall was 0,53 µm. The merozoites were separated into clusters by septa and contained numerous amylopectin granules. The merozoites measured approximately 12,4 µm x 1,5 µm.

Cysts examined ultrastructurally (in the Department of Zoology, University of the Witwatersrand) from another lion from Zimbabwe and one from the Kruger National Park, South Africa, also showed cyst wall structures similar to those of the lioness referred to above (J. B. Bush, 1986, personal communication).

The cyst from the domestic cat shown in the electron micrographs of Kirkpatrick et al. (1986) may be illustrative of a stage in the development of a more complex cyst wall. Other than lacking long, thread-like protrusions in longitudinal section, the cyst from the domestic cat was similar, both as regards morphology and size, to that of the lioness discussed here.

Sarcocysts in four individuals of two species of genet from the eastern Transvaal had identical, simple-walled cysts, differing from the thick-walled cyst found in the African civet, to which they are related. The cyst wall of the sarcocyst from the small-

FIGURES 17 to 20: Ultrastructure of the cyst wall of Sarcocystis of a lioness Panthera leo. Both the protrusions and the cyst wall between the protrusions are invaginated. Figure 20 shows the stalked appearance of the limiting membrane of the protrusions. 17: x22500; 18: x45000; 19: x45000; 20: x45000.


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spotted genet Genetta genetta was 0,50 µm wide (Figure 21), with a thin layer of ground substance, and was grossly similar to the simple-walled cyst found in the slender mongoose Galerella sanguinea (Figure 26). Merozoites in the cyst from the small-spotted genet were 3,97 µm x 1,19 µm (Figure 22). The large-spotted genet Genetta tigrina had a cyst wall 0,43 µm wide and merozoites measuring 3,63 µm x 1,13 µm (Figures 24 and 25).

Although the cysts from both species of genet were not unlike those described from rodents (e.g. *Wiles and Powell*, 1976; Sheffield et al., 1977; Mehlhorn and Frenkel, 1980), the walls did not appear to be as deeply invaginated. The genet cysts were similar to the sarcocyst found in the lion and to the Sarcocystis species in R. norvegicus which has the masked owl as a final host (Munday, 1983). In the sarcocyst from G. genetta (Figure 23), a lattice-type appearance of the cyst wall was evident in places.

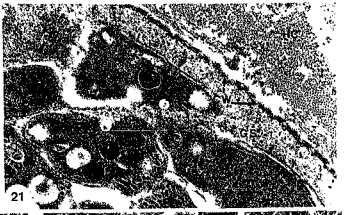
As far as predator/prey relationships are concerned, birds of prey have a specialised diet. Raptors have been reported as final hosts of some Sarcocystis species of rodents (see historical review of work on Sarcocystis of rodents). Markus et al. (1984) found, likewise, that vultures produced sporocysts after being fed infected impala Aepyceros melampus meat. Raptorial birds, such as certain eagles, eat mongooses and genets. Consequently, they may even be potential final hosts of Sarcocystis in these carnivores. However, *the range of potential final hosts of Sarcocystis of small* mammalian carnivores may not be restricted to meat-eating birds. Large mammalian carnivores could be involved. Small mammalian predators which act as intermediate hosts of Sarcocystis could be dead-end hosts, but not necessarily so.

Two slender mongooses G. sanguinea from the eastern Transvaal were hosts to two different types of Sarcocystis, one of which has already been referred to above. This sarcocyst type, recovered from the first mongoose (TM 30565) (the second was TM 30566 - see below), had an indented cyst membrane without protrusions (Figure 28). The osmiophilic layer was distinct and repeatedly interrupted by invaginations of the cyst membrane. The underlying layer of ground substance was thin, and formed thin membranous septa between groups of merozoites. The cyst wall was narrower (0,31 µm) than similar cysts in G. genetta and the merozoites were longer (5,21 µm

FIGURE 21: Ultrastructure of the simple, invaginated cyst wall of Sarcocystis of the small-spotted genet Genetta genetta. x30000.

FIGURE 22: Merozoites grouped by septa, in the cyst from the small-spotted genet. x12000.

FIGURE 23: Lattice-type structure of part of the wall of the same cyst (as in Figure 21) from the small-spotted genet. x31250.


21


22

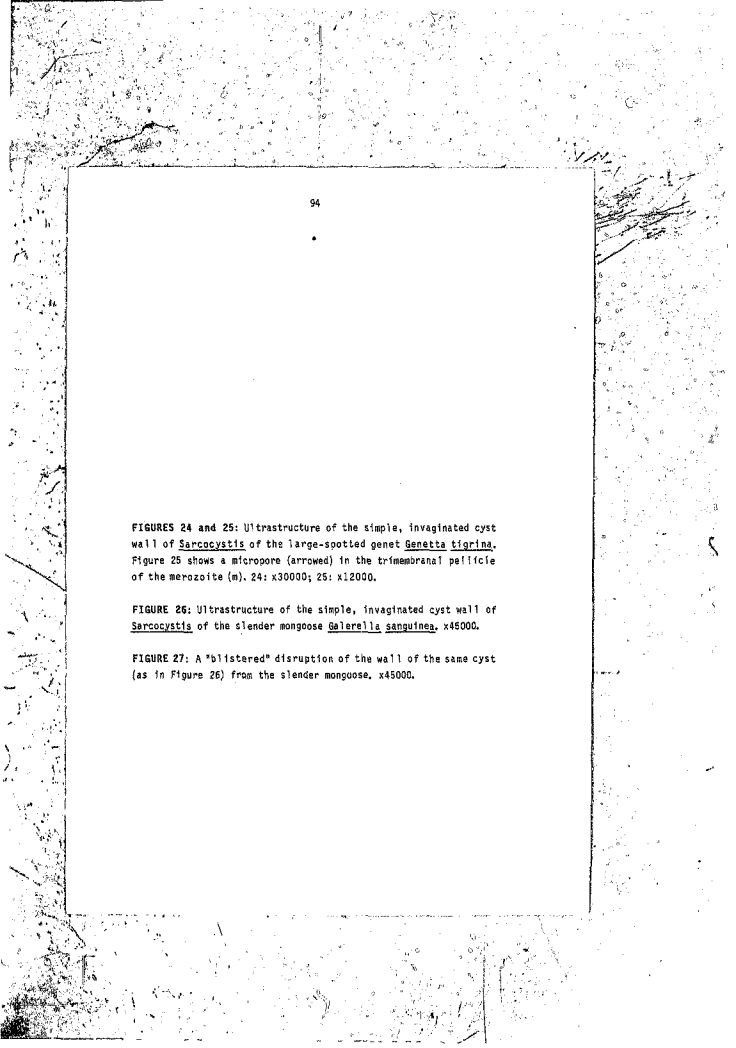

23

x 0,97 µm).

In some sections, a "blistered" appearance of the cyst wall seemed to indicate localized disruption of the host tissue, the adjacent cyst membrane and the underlying ground substance (Figure 27). This disruption may have been the result of some cellular response. It did not seem to be caused by mechanical damage, as the remaining host tissue was intact.

The morphology of the cyst wall and the thin layer of ground substance forming membranous septa, is reminiscent of the simple cyst walls of a Sarcocystis species in the rhesus monkey Macaca mulatta (Mehlhorn et al., 1976). Simple-walled cysts in rodents, e.g. S. muris in M. musculus (see Viles and Powell, 1976; Sheffield et al., 1977; Mehlhorn and Frenkel, 1980), and cysts of the rattlesnake-mouse cycle (Entzeroth et al., 1985a), appeared more invaginated than the cyst from the slender mongoose. Sarcocysts from the Malaysian long-tailed monkey Macaca fascicularis (see Kan et al., 1979), had cyst walls not unlike those of the mongoose, but the presence of fibrillar elements in the ground substance of the monkey cyst precludes any similarity between the two. Cysts located in the brain of a porcupine Erethizon dorsatum were identified as a species of Frenkelia by Kennedy and Frelier (1986). Ultrastructural examination of the cyst wall revealed an invaginated cyst membrane reinforced by osmiophilic material. The thin layer of ground substance and the thin, membranous septa which compartmentalized the cyst were features similar to those of sarcocysts of the rhesus monkey (Mehlhorn et al., 1976) and slender mongoose.

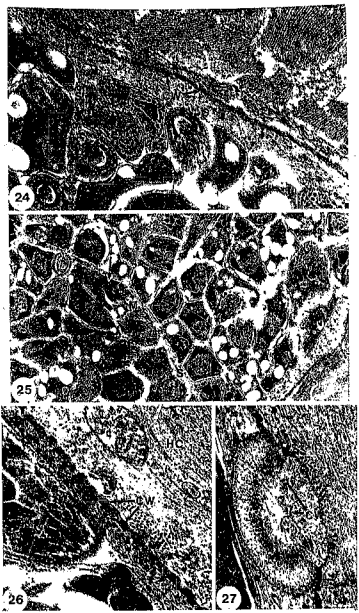
With reference to the above discussion, it should be emphasized that Sarcocystis species having a simple cyst wall structure have been documented from a wide variety of intermediate hosts: sarcocysts (diameter 80,0 µm) from the red squirrel Tamiasciuris hudsonicus (see Entzeroth et al., 1983c) had highly invaginated cyst walls. The cyst wall was 0,39 µm wide (this measurement included the ground substance) and was similar to S. bozemanensis (diam. 30-112 µm) from Richardson's ground squirrel Spermophilus richardsonii (see Dubey, 1983c), the cyst wall of which was 0,43 µm wide. Entzeroth et al. (1983b) described smaller cysts (diam. 19,8-29,6 µm) with walls approximately 0,26 µm wide (0,32 µm including



FIGURES 24 and 25: Ultrastructure of the simple, invaginated cyst wall of Sarcocystis of the large-spotted genet Genetta tigrina. Figure 25 shows a micropore (arrowed) in the trimembranal pellicle of the merozoite (m). 24: x30000; 25: x12000.

FIGURE 26: Ultrastructure of the simple, invaginated cyst wall of Sarcocystis of the slender mongoose Galerella sanguinea. x45000.

FIGURE 27: A "blistered" disruption of the wall of the same cyst (as in Figure 26) from the slender mongoose. x45000.


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25
26
27

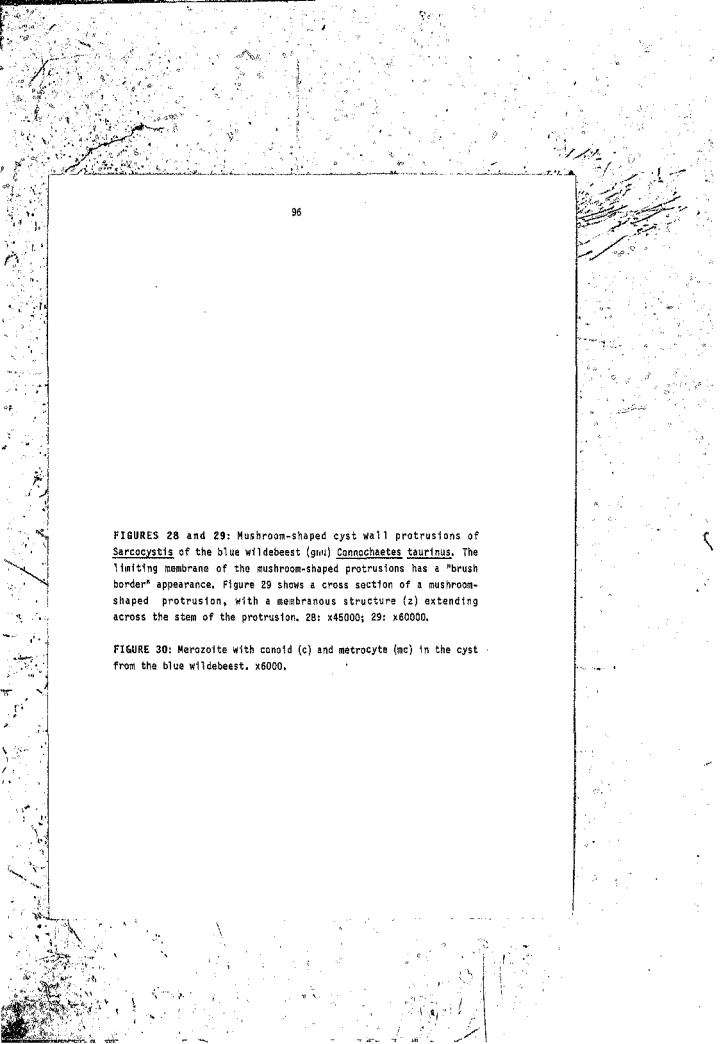
the protrusion lengths of 60 nm) from the eastern chipmunk Tamias striatus. These cysts were morphologically similar to both S. bozemanensis and the Sarcocystis species from the red squirrel.

Sarcocysts with walls that were morphologically similar to S. muris in mice have been described in papers reporting the results of life cycle studies. Entzeroth et al. (1985a) found cysts with simple cyst walls (1,0 µm) in mice M. musculus (diam. 0,5 mm). This species of Sarcocystis had the Mojave rattlesnake Crotalus s. scutulatus as its final host.

Simple-walled sarcocysts were found in the lizard Alabuta multicarinata by Tadros and Laarman (1978). This cyst (diam. 1,0 mm) was highly invaginated, with some branches of the protrusions extending short distances into the granular layer adjacent to the host tissue. S. podarcicolubris from Tyrrhenian wall lizards Podarcis tiliguerta, S. gallotiae from Canarian lizards Gallotia galloti and S. dugesii from Madeiran lizards Lacerta dugesii (see Matuschka, 1981; Matuschka and Mehlhorn, 1984) have protrusions which distinguish them from the cyst described by Tadros and Laarman (1978).

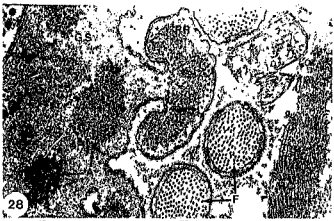
Ultrastructurally, sarcocysts from the Malaysian long-tailed monkey Macaca fascicularis (see Kan et al., 1979) had cyst walls (0,47-0,8 µm) that were similar to those in the *babaon Papio* cynocephalus and rhesus monkey Macaca mulatta (see Mehlhorn et al., 1976; Mehlhorn and Heydorn, 1978). However, bundles of fibrillar elements in the ground substance of the cyst from the long-tailed monkey were not evident in cysts from the rhesus monkey.

In addition to S. muris in mice and the sarcocysts referred to above, cysts with a simple wall structure have also been located in other small mammals, for example in the meadow vole Microtus pennsylvanicus (and named S. montanaensis) (see Dubey, 1983b); and in, for example, R. norvegicus by Ashford (1978) and Munday (1983). (See also historical review of Sarcocystis in rodents). Sarcocysts with simple, invaginated walls have, likewise, been found in diverse vertebrate hosts such as an Australian marsupial Antechinus sp. (see Munday et al., 1978), a sperm whale (Mehlhorn et al., 1976) and a white-rumped swift Apus caffer (see Kaiser and Markus, 1983a).



FIGURES 28 and 29: Mushroom-shaped cyst wall protrusions of Sarcocystis of the blue wildebeest (gnu) Connochaetes taurinus. The limiting membrane of the mushroom-shaped protrusions has a "brush border" appearance. Figure 29 shows a cross section of a mushroom-shaped protrusion, with a membranous structure (z) extending across the stem of the protrusion. 28: x45000; 29: x60000.

FIGURE 30: Merozoite with conoid (c) and metrocyte (mc) in the cyst from the blue wildebeest. x6000.


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F


LM
OL
29


30

From the foregoing, it can be seen that the ultrastructure of the cyst wall can frequently be used to distinguish between species of Sarcocystis. However, it is difficult to compare the ultrastructural morphology of simple-walled cysts; and investigation of the final and intermediate host specificity by means of transmission experiments is needed in order to facilitate determination of the species of Sarcocystis concerned.

SARCOCYSTS WITH MUSHROOM-SHAPED PROTRUSIONS, IN WILD UNGULATES

Four blue wildebeest (gnus) Connochaetes taurinus (M82-86; M82-204; M82-206; M82-207) from Natal harboured sarcocysts that had mushroom-shaped protrusions (Figure 28). These cysts were morphologically identical to some found in impala Aepyceros melampus from Zimbabwe (M80-132; M80-133; M80-135) (Figure 31) and the eastern Transvaal (M83-100; M83-101) (Figure 33). A lechwe Kobus leche (M83-34) from Namibia also had identical cysts in its skeletal musculature (Figure 34). These cysts are ultrastructurally similar to one found in the diaphragm of a chamois Rupicapra rupicapra by Cornaglia et al. (1980).

The protrusions have a thick osmiophilic layer and are filled with fibrils that originate in the ground substance beneath the indented cyst membrane. In the wildebeest, the height of the mushroom-shaped protrusions was 0,79 µm and the measurement across was 1,14 µm. In "circular" cross section, the mean longest x shortest measurement was 0,99 µm x 0,58 µm. The protrusions in the impala were wider than those of the wildebeest but were similar in height (see Table 2). In all specimens examined, small cilia-like structures extended from the cyst membrane surrounding the protrusions, imparting a "brush border" appearance to the outer cyst membrane (Figures 28, 32 and 36).

In the cyst from the lechwe (Figure 36), small club-shaped structures were found at intervals, interspersed between the cilia-like structures along the outer cyst membrane surrounding the protrusions. The function of these structures is not known. Perhaps they serve to increase the absorptive area, thereby facilitating molecular transportation between the parasite and the host cell cytoplasm.

TABLE 2: Comparison of ultrastructural *features of mushroom*-shaped cyst wall protrusions in ungulates (µm).

HOST	DIAMETERS	PROTRUSIONS (M)		PROTRUSIONS (C)		CYST WALL	MEROZOITES
		LONGEST	SHORTEST	LONGEST	SHORTEST		
Wildebeest	35,0 x 53,8	1,34	0,79	0,99	0,58	0,70	7,56 x 3,22
Impala (E.T.)	63,8 x 76,3	2,40	0,76	1,16	0,60	0,79	8,73 x 2,31
Impala (Zim.)	45,0 x 68,5	1,59	1,11	1,08	0,75	0,93	5,50 x 2,06
Lechwe	30,0 x 40,0	1,19	0,56	0,88	0,60	0,52	8,10 x 2,70

E.T.= Eastern Transvaal
Zim.= Zimbabwe

Note: Protrusions (M) refers to *mushroom-shaped cross sections* of the protrusions visibly attached to the body of the cyst. *Protrusions* (C) refers to "circular" cross sections of protrusions *visibly* unattached to the body of the cyst. The terms M and C conform to the standard used in computing the data for all animals examined in this study.

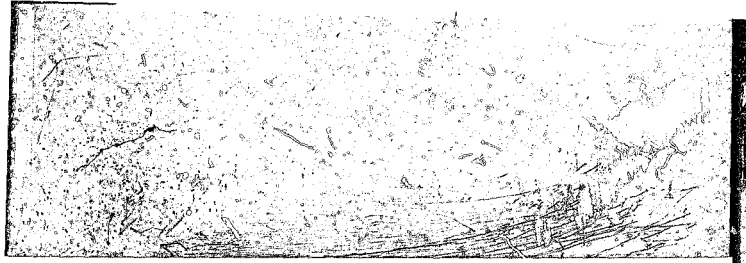
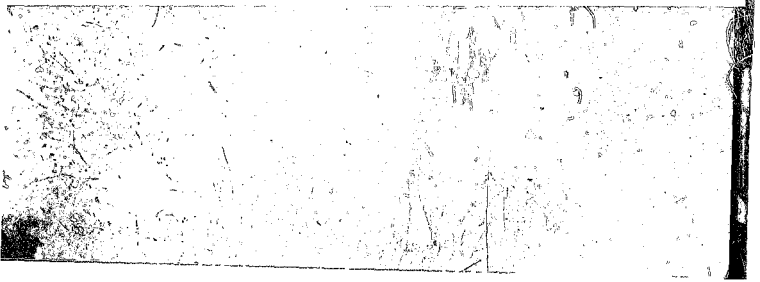


TABLE 2: Comparison of ultrastructural features of mushroom-shaped cyst wall protrusions in ungulates (μm).

HOST	DIAMETERS	PROTRUSIONS (M)		PROTRUSIONS (C)		CYST WALL	[illegible]
		LONGEST	SHORTEST	LONGEST	SHORTEST		
Wildebeest	35,0 x 53,8	1,34	0,79	0,99	0,58	[illegible]70	7,56 x 3,22
Impala (E.T.)	63,8 x 76,3	2,40	0,76	1,16	0,60	[illegible]	8,73 x 2,31
Impala (Zim.)	45,0 x 68,5	1,59	1,11	1,08	0,75	[illegible]	5,90 x 2,05
Lechwe	30,0 x 40,0	1,19	0,56	0,88	0,60	[illegible]	8,10 x 2,70

E.T.= Eastern Transvaal
Zim.= Zimbabwe

Note: Protrusions (M) refers to mushroom-shaped cross sections of the protrusions visibly attached to the body of the cyst. Protrusions (C) refers to "*circular*" *cross* sections of protrusions *visibly unattached* to the body of the cyst. The terms M and C conform to the standard used in computing the data for all animals examined in this study.

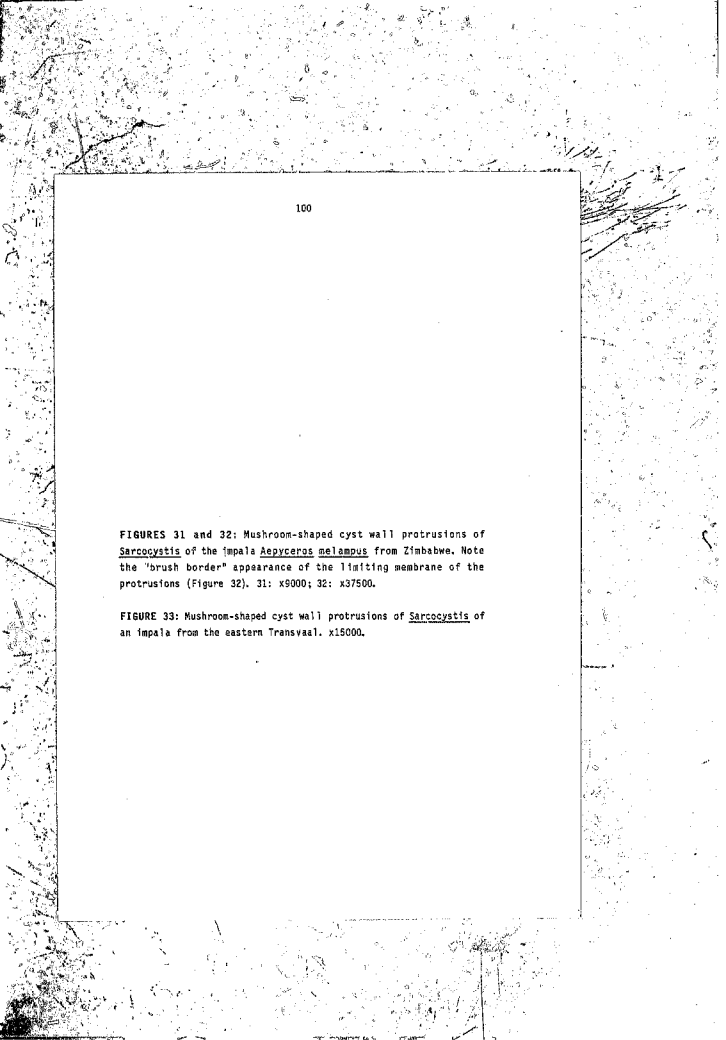


In the wildebeest and the lechwe cysts, a thin membrane was seen to extend across the stem of the mushroom-shaped protrusion, separating the ground substance from the less *dense material in the* protrusion (Figures 29 and 35). A pale-staining metrocyte in the cyst from the wildebeest is shown in Figure 30.

Recent ultrastructural evidence (longitudinal sections of protrusions which I cut recently) suggests that the fibrils extend from the ground substance and along the backs of the protrusions in longitudinal section. The protrusions are, therefore, long and spatula- or spine-like in shape when viewed longitudinally (photomicrographs not included here). This explains why the fibrils are never seen to penetrate the membrane in the stems of the mushroom-shaped protrusions, i.e. when the protrusions are viewed in cross section.

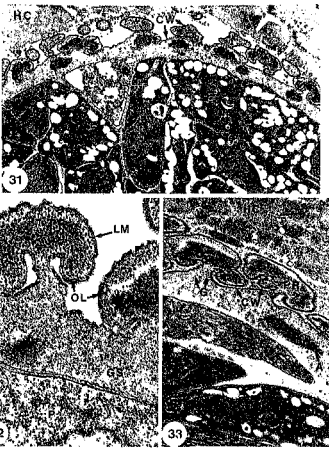
The biggest cysts, with diameters of up to 120 x 55 µm (i.e. the longest diameter x shortest diameter of a cyst in K.S.), were found in impala from Zimbabwe, and may have been the most mature. The dimensions of the merozoites (Table 2) from Zimbabwean impala are not related to *the maturity of the cysts, as the merozoites were* not cut in perfectly longitudinal planes. The orientation of the fibrils suggests that the protrusions, in cross section, are oval in shape and that the cysts in the impala from the eastern Transvaal were sectioned obliquely rather than transversely. This would explain the large widths of the protrusions recorded from this cyst (Figure 33).

Macroscopic cysts were found by O'Donoghue et al. (1987a) in the muscularis externa and the submucosa of the forestomach, small intestine and colon (and sometimes in the muscularis externa of the oesophagus and caecum) of unadorned wallabies Petrogale assimilis and Bennett's wallabies Macropus rufogrisies. The authors did not state whether or not they examined skeletal muscles in general, for cysts. Ultrastructurally, *the thick primary cyst wall was* invaginated and folded at intervals to form mushroom-shaped protrusions. The bulbous protrusions measured 1,5-1,8 µm in height and 1,2-1,8 µm in width. The stalks (0,5-0,8 µm thick) were filled with microfibrils which extended along their lengths, and the bulbous protrusions were filled with amorphous ground substance. Slivers of electron dense material extended downwards into the



FIGURES 31 and 32: Mushroom-shaped cyst wall protrusions of Sarcocystis of the impala Aepyceros melampus from Zimbabwe. Note the "brush border" appearance of the limiting membrane of the protrusions (Figure 32). 31: x9000; 32: x37500.

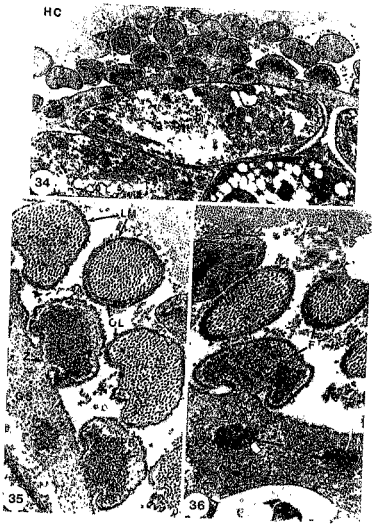
FIGURE 33: Mushroom-shaped cyst wall protrusions of Sarcocystis of an impala from the eastern Transvaal. x15000.


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33

protrusions from the cyst wall. Externally, the cysts were surrounded by collagen fibres and connective tissue cells. There was no evidence of the original host cell. Internally, septa divided the merozoites (8,5-12,0 µm x 1,5-2,8 µm) and metrocytes (10-20 µm x 8-18 µm) into compartments. The merozoites contained organelles typical of apicomplexan protozoan parasites, having the apical complex and 22 subpellicular microtubules. Merozoites divided by endodyogeny. Although the definitive host is unknown, this Sarcocystis species was designated S. mucosa by O'Donoghue et al. (1987a). Sarcocystis mucosa, having been originally described by Blanchard in 1885, was tentatively assigned to the genus Eimeria by Levine (1979), until further studies were carried out. The species is listed as a synonym of Eimeria (?) mucosa by Levine and Tadros (1980). However, Levine (1979) states that the organism had previously been assigned to the genus Sarcocystis by Labbé in 1899 (not, incidentally, in 1889 as indicated by O'Donoghue et al., 1987a). The species should, therefore, be known as Sarcocystis mucosa (Blanchard, 1885) Labbé, 1899.

Whether S. mucosa is the same species of Sarcocystis found in wild African ungulates during the course of the present study, is unknown. The bulbous region of the protrusions of the cysts found in wild ungulates did contain fibrillar elements when viewed in cross section, in addition to the granular material. Fibrillar elements were never seen extending along the stems of the protrusions of the cysts in the ungulates. However, their presence in the ground substance beneath the primary cyst wall was suggested, as plaque-like structures were visible when the cyst was viewed in cross section. The cyst walls of the protrusions appeared wavy and invaginated at intervals, and were not as smooth as those of the protrusions of S. mucosa. Slivers of electron-dense material which protruded from the apical cyst wall into the ground substance of the protrusions were not evident in the protrusions of the cysts from the southern African ungulates. The cysts in the ungulates were microscopic and located in the skeletal muscles of the hosts. The muscular layers of the gastrointestinal tract were not examined.

FIGURES 34 to 36: Mushroom-shaped cyst wall protrusions of Sarcocystis of the lechwe Kobus leche from Namibia. Figure 35 shows a membranous structure (z) extending across the stem of a mushroom-shaped protrusion. Figure 36 shows the presence of small, club-shaped structures on the limiting membrane around the protrusions, in addition to the cilia-like structures. 34: x12000; 35: x37500; 36: x37500.


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35
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SARCOCYSTS WITH BEAN- OR BAR-SHAPED PROTRUSIONS, *IN WILD UNGULATES*

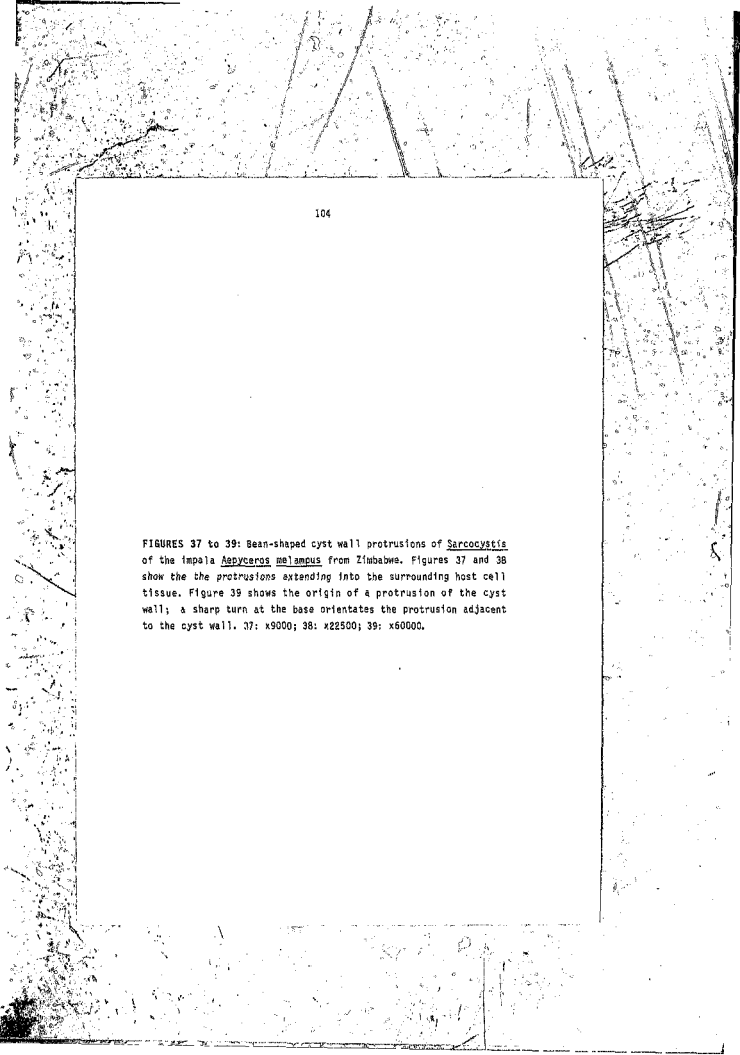
A second type of cyst found in an impala (M83-48) from Zimbabwe possessed long, bean-shaped protrusions (0,51 µm x 0,12 µm in X.S.) which extended into the surrounding host tissue (Figures 37-39). These protrusions were smooth-walled, with a thin osmiophilic layer, and were filled with a granular substance. The primary cyst wall had an invaginated appearance and there was a thin layer of ground substance layer beneath it. The cyst wall was 0,25 µm wide.

This sarcocyst (diameters 85,0 x 80,0 µm, i.e. longest x shortest diameter of the cyst in X.S.) was morphologically similar to *the Type 5 cyst found in roe deer* Capreolus capreolus by Entzeroth (1982) and not unlike Sarcocystis cruzi in an ox-coyote life cycle (Dubey, 1982). *The Type 5 cyst from* the diaphragm of roe deer had short, stubby protrusions (0,4-0,8 µm x 0,3 µm) that could give rise to bean-shaped protrusions. *The cyst wall was 0,5 µm wide* and similar to that of S. cruzi.

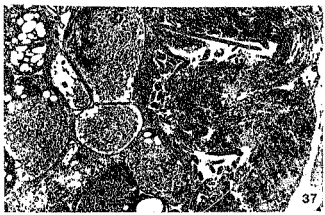
A sarcocyst (Type A) found in a moose Alces alces by Colwell *and* Mahrt (1981), had bean-shaped protrusions that originated at intervals from the invaginated primary cyst wall. The protrusions described by Colwell and Mahrt (1981) as membranous extensions are actually tubular processes. These protrusions are approximately 0,2 µm wide in cross section. The morphology of the sarcocyst in the moose (Type A = Sarcocystis alcestatrans) is not unlike the cyst *from* the impala.

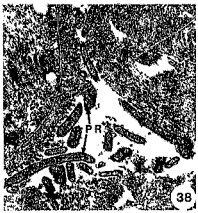
Long, bean-shaped protrusions were evident in the Type 6 cyst *found in roe deer by Entzeroth* (1982). They were 5-8 µm in length and 0,3 µm in width, and were similar to the protrusion lengths for S. cruzi (5 µm) *in cardiac muscle* in the ox-coyote cycle (Dubey, 1982), as well as those from the impala, which were up to 4 µm in length. The impala cyst *had protrusions that* originated from the cyst wall at intervals and which were not immediately juxtaposed as in the Type 6 cysts from roe deer.

Sarcocysts that had bean-shaped protrusions which penetrated the host tissue but which also had closely associated *bases* where they joined the cyst wall (Sarcocystis sybillensis), have been found in the North American elk Cervus elaphus (see Dubey et al., 1983). S. sybillensis from heart, tongue, oesophagus and skeletal muscle of



FIGURES 37 to 39: Bean-shaped cyst wall protrusions of Sarcocystis of the impala Aepyceros melampus from Zimbabwe. Figures 37 and 38 show the the protrusions extending into the surrounding host cell tissue. Figure 39 shows the origin of a protrusion of the cyst wall; a sharp turn at the base orientates the protrusion adjacent to the cyst wall. 37: x9000; 38: x22500; 39: x60000.


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elk had a thin layer of ground substance and protrusions that were 6,16 µm in length and 0,3 µm in width. The sarcocyst from the elk is morphologically similar to the Type 6 cyst in roe deer found by Entzeroth (1982), in which the ground substance was virtually absent.

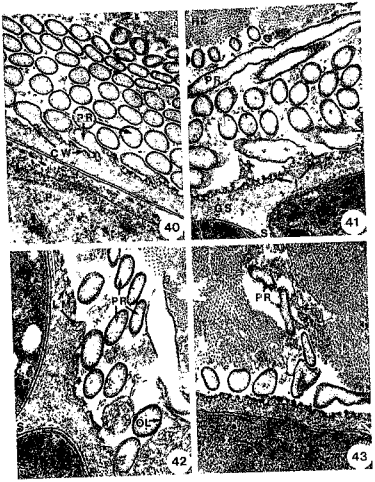
S. sybillensis from the elk (Dubey et al., 1983) has a developmental stage that is very similar to a stage in the development of the cyst wall of S. cruzi, as is evident in micrographs of Pacheco et al. (1978).

The cyst in the impala may be a stage in the development of a more mature cyst wall, in which the protrusion bases are more closely associated. If this proves to be the case, then both the impala cyst and S. cruzi are morphologically similar to S. sybillensis from the North American elk (Dubey et al., 1983) and to the Type 6 sarcocyst in roe deer (Entzeroth, 1982).

Strip-like protrusions have been found in sarcocysts from numerous cervid hosts. Although they are not unlike the Sarcocystis species from the impala, their protrusions appear narrower. S. wapiti (see Speer and Dubey, 1982) from the North American elk C. elaphus, and which has the coyote Canis latrans and the domestic dog C. familiaris as final hosts, has a highly invaginated cyst wall with strip-like protrusions similar to S. gruenerí from cardiac muscle of reindeer Rangifer t. tarandus (see Gjerde, 1985a). S. wapiti is also similar to a Sarcocystis species from red deer C. elaphus (see Entzeroth et al., 1983a) and to S. cervicanis from naturally-infected red deer (see Hernández-Rodríguez et al., 1981). Entzeroth et al. (1983a) consider S. gruenerí, S. wapiti and S. cervicanis from the red deer to be synonyms. Although S. gruenerí is a name which has been applied specifically to sarcocysts of reindeer (Entzeroth et al., 1985b), the recent ultrastructural investigation of this species in reindeer by Gjerde (1985a), which revealed striking similarities to other sarcocyst types in cervids, may indeed give S. gruenerí priority as a specific name for this Sarcocystis species in all cervid hosts. This would only apply if S. gruenerí described from reindeer by Yakimoff in 1936 is the same as that described by Gjerde and Bratberg (1984) and cross-transmission experiments show that morphologically similar sarcocysts from cervids belong to the same

FIGURES 40 and 41: Bean-shaped cyst wall protrusions of Sarcocystis of the kudu Tragelaphus strepsiceros from Namibia. After the protrusions originate from the cyst wall, they turn and lie immediately adjacent to it. Figure 40 shows peripheral protrusions cut in cross section. They have a flattened, bean-shaped appearance. Cross sections of the protrusions at their basal regions, close to the cyst, appear circular. 40: x30000; 41: x30000.

FIGURE 42 and 43: Bean-shaped cyst wall protrusions of Sarcocystis of the kudu from the eastern Transvaal. Figure 42 shows the cyst wall ultrastructure of a cyst in skeletal muscle. Figure 43 illustrates the electron microscopic structure of the wall of a cyst from cardiac muscle of the same animal. 42: x45000; 43: x37500.


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species of Sarcocystis (see Gjerde, 1985a). S. gruneneri in cardiac muscle of reindeer has the dog, silver fox Vulpes vulpes and blue fox Alopex lagopus as final hosts (Gjerde. 1984c; Gjerde and Bratberg, 1984).

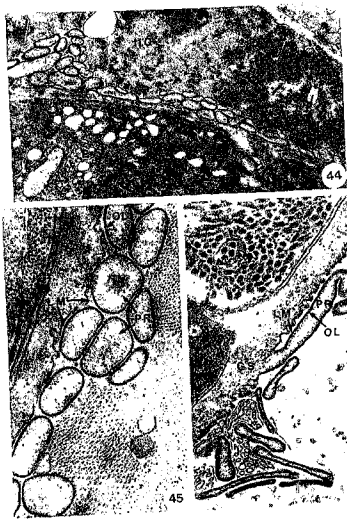
The Type 4 cysts from roe deer (Entzeroth, 1982), and which have strip-like protrusions (1,2 µm x 20-30 nm), are also morphologically similar to S. wapiti (see Speer and Dubey, 1982) and S. gruneneri (see Gjerde, 1985a), the latter having had longer protrusions (than the roe deer cysts), namely 4,5 µm in length and 30-40 nm wide (thick). Protrusions of S. cervicanis (see Hernández-Rodríguez et al., 1981) were 1,4 µm x 32 nm and those of a Sarcocystis species described from the red deer by Entzeroth et al. (1983a) were 0,5-3 µm long and 20 nm wide. There is a remarkable similarity between the Type 4 cysts described by Entzeroth (1982) from roe deer and a section through a S. cruzi cyst illustrated by Böttner (1984), which had rod-like protrusions. In longitudinal section, the S. cruzi cyst of Böttner (1984) had wide-based protrusions that narrowed to form strip-like extensions, similar to those of a Sarcocystis species from the fallow deer Cervus dama (see Entzeroth et al., 1985b).

Entzeroth et al. (1985b) stated that the protrusions of the cyst in the fallow deer appeared as flattened sacs, rather than hair-like, as they were never seen in circular form when cut in cross section. However, cyst wall protrusions from the fallow deer and roe deer are similar to the protrusions of thin-walled cysts from sheep (S. tenella sensu lato), illustrated by O'Donoghue et al. (1986), and which were described as being hair-like. The bi-concave, strip-like nature of the cyst wall protrusions of the sheep cysts were also similar to the protrusions of cysts in the gemsbuck Oryx gazella and waterbuck Kobus ellipsiprymnus in the present study (to be discussed later on). The protrusions of the cyst from the gemsbuck (Figure 48) were circular at their bases, and strip-like or flattened at their extremeties in cross section.

The species of Sarcocystis in red deer does not appear to be distinct from Type 4 cysts in roe deer (Entzeroth et al., 1983a). This reinforces the belief that species of Sarcocystis are not as host specific for their intermediate hosts as has been thought. The morphological similarity and similar canid life cycle suggest that

FIGURES 44 and 45: Bean-shaped cyst wall protrusions of Sarcocystis of the bushbuck Tragelaphus scriptus. 44: x15000; 45: x60000.

FIGURE 46: Bean-shaped cyst wall protrusions of Sarcocystis of the waterbuck Kobus ellipsiprymnus. x45000.


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the cervids discussed above are intermediate hosts of at least one common species of Sarcocystis (see Gjerde, 1985a).

Macrocysts of S. hardangeri, from skeletal muscle of wild and domestic reindeer (Gjerde, 1984b; Gjerde, 1984d; Gjerde, 1985d), had a diffuse, invaginated appearance, not unlike a sarcocyst (Type B) in the moose Alces alces (see Colwell and Mahrt, 1981). Both are similarly surrounded by a thickened basal lamina and a layer of connective tissue (Gjerde, 1985d).

Sarcocysts morphologically similar to the second type of cyst in the impala from Zimbabwe were found in the skeletal muscle and the heart muscle of the same kudu Tragelaphus strepsiceros from the eastern Transvaal (Figures 42 and 43). Protrusions of the cyst wall in a kudu from Namibia (M83-50) appeared to be circular at their bases in cross section, and became more flattened towards the periphery where they infiltrated the host tissue. The flattened apeces of the protrusions appeared bean-shaped in cross section (Figures 40 and 41). Cysts in the waterbuck from the eastern Transvaal and the gemsbuck from Namibia had similar cyst walls to those of the kudu sarcocysts, with protrusions that became extremely flattened or strip-like further away from the cyst (Figures 47-49). The cyst from the waterbuck had fewer protrusions (Figure 46). Protrusions that were more circular in cross section, but similar to those of sarcocysts in the kudu, were found on a cyst in skeletal muscle of a bushbuck Tragelaphus scriptus from the eastern Transvaal (Figures 44 and 45).

The morphology of the walls of sarcocysts from the kudu and gemsbuck from Namibia are structurally similar to S. rangi from the skeletal muscle of reindeer (Gjerde, 1985f). As in S. rangi, the lengths of the protrusions were difficult to ascertain because the protrusions turned sharply sideways immediately above their bases and extended alongside the surface of the cyst (Figure 49). In cross section, the protrusions were circular, oval or bean-shaped and measured 0,33-0,53 µm x 0,12-0,25 µm (i.e. the longest x shortest measurements, see Table 3). The thickness of the cyst walls for all ruminants with morphologically similar sarcocysts (Table 3) were somewhat narrower than that of S. rangi (see Gjerde, 1985f). The mean range was 0,17-0,66 µm, compared to 0,5-1,3 µm for S. rangi. However, the cysts from ruminants other than reindeer had

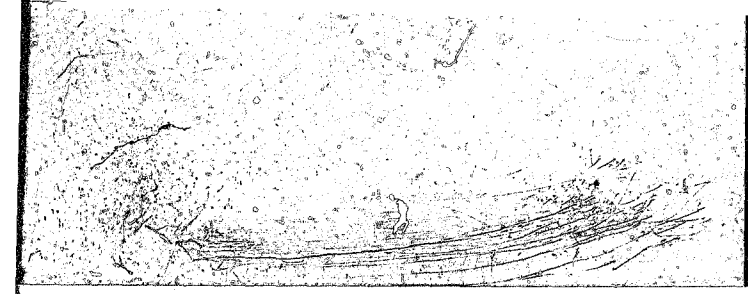


TABLE 3: Comparison of ultrastructural features of bean-shaped cyst wall protrusions in ungulates (µm).

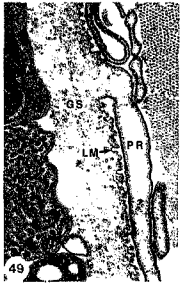
HOST	DIAMETERS	PROTRUSIONS (X.S.) LONGEST	PROTRUSIONS (X.S.) SHORTEST	CYST WALL	MEROZOITES
Impala (Zim.)	80,0 x 85,0	0,51	0,12	0,25	7,92 x 2,19
Kudu (E.T.)	44,2 x 55,0	0,33	0,15	0,21	8,73 x 2,17
Kudu (Nam.)	52,5 x 55,0	0,33	0,25	0,48	10,67 x 3,13
Waterbuck (E.T.)	45,0 x 52,5	0,50	0,15	0,24	6,92 x 1,95
Gemsbuck (Nam.)	42,5 x 52,5	0,51	0,20	0,66	10,67 x 2,36
Bushbuck (E.T.)	52,5 x 57,5	0,53	0,17	0,17	-

E.T.= Eastern Transvaal
Nam.= Namibia
Zim.= Zimbabwe

FIGURES 47 to 49: Bean-shaped cyst wall protrusions of *Sarcocystis* of the gemsbuck *Oryx gazella*. Figure 49 shows a protrusion lying adjacent to the cyst wall and the sharp turn at the base of the protrusion where it originates from the cyst. 47: x4500; 48: x37500; 49: x45000.


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a mean diameter of 64,1 µm and were smaller than those from skeletal muscle of reindeer (180 µm) (Gjerde, 1985f).

The cyst protrusions in all the animals were generally spaced at intervals along the cyst and had smooth walls reinforced by a thin osmiophilic layer. The cyst membrane was invaginated between the bases of the protrusions. In some hosts, the cyst membrane between the protrusions had distinct vesicular invaginations, while in others, such as in cysts from the bushbuck, the cyst membrane appeared smooth and less invaginated. The origin of the protrusions of the cyst wall was not evident in these smooth areas, as the bases were not included in the ultra-thin section (Figure 45). The thin underlying layer of ground substance filled the protrusions (less electron dense) and extended into the interior of the cyst as septa.

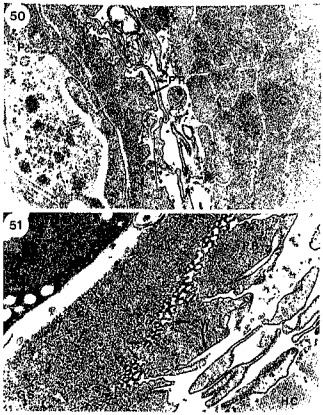
The size of merozoites, which were difficult to measure as they were not always sectioned in a perfect plane, was up to 10,67 µm x 3,13 µm (Table 3). The host skeletal muscle tissue remained structurally unaltered in all specimens examined, despite host cell infiltration by the protrusions of the sarcocysts.

A possible third type of sarcocyst found in impala from Zimbabwe had "flower-shaped" protrusions (Figures 50-53). Of three individuals, one had cysts of the first and third type (M80-135); one had the second and third type (M83-48); and the third individual harboured only the third type of cyst (M83-1A).

The protrusions of the third cyst type were broad-based (1,52 µm), with smooth walls, and were up to 3,27 µm in length (Figure 52). The cyst wall was 0,58 µm wide, and highly invaginated between the protrusions and at their bases. The protrusions appeared to join laterally at their bases and tapered into flat, ribbon-like extremities, which were strip-like in cross section (Figure 52). No fibrillar elements were present in the granular substance. In longitudinal section, the protrusions were flower-shaped. The thin, ground substance layer continued into the interior of the cyst as septa. Merozoites measured 7,64 µm x 2,46 µm.

The "flower-shaped" cyst wall protrusions of one of the three kinds of Sarcocystis in the impala were not unlike protrusions found in the present study in sheep microcysts from the Transvaal (Figures 110 and 111). The ribbon-like nature of the flower-shaped

FIGURES 50 and 51: Broad-based cyst wall protrusions ("flower-shaped") of Sarcocystis *of the impala* Aepyceros melampus *from Zimbabwe. The highly invaginated, broad bases of the protrusions and the thin protrusions in cross section suggests that the protrusions are wide but flat and ribbon-like in their entirety. Figure 51 shows that the protrusions are joined laterally. 50: x15000; 51: x12500.*


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protrusions was also not unlike that of the protrusions of thick-walled microscopic cysts of S. tenella from sheep and the protrusions of thick-walled, microscopic S. capracanis cysts from goats (O'Donoghue et al., 1986). Ultrastructurally, both S. tenella and S. capracanis have palisade-like protrusions that appear to be broad, flat and ribbon-like in longitudinal section.

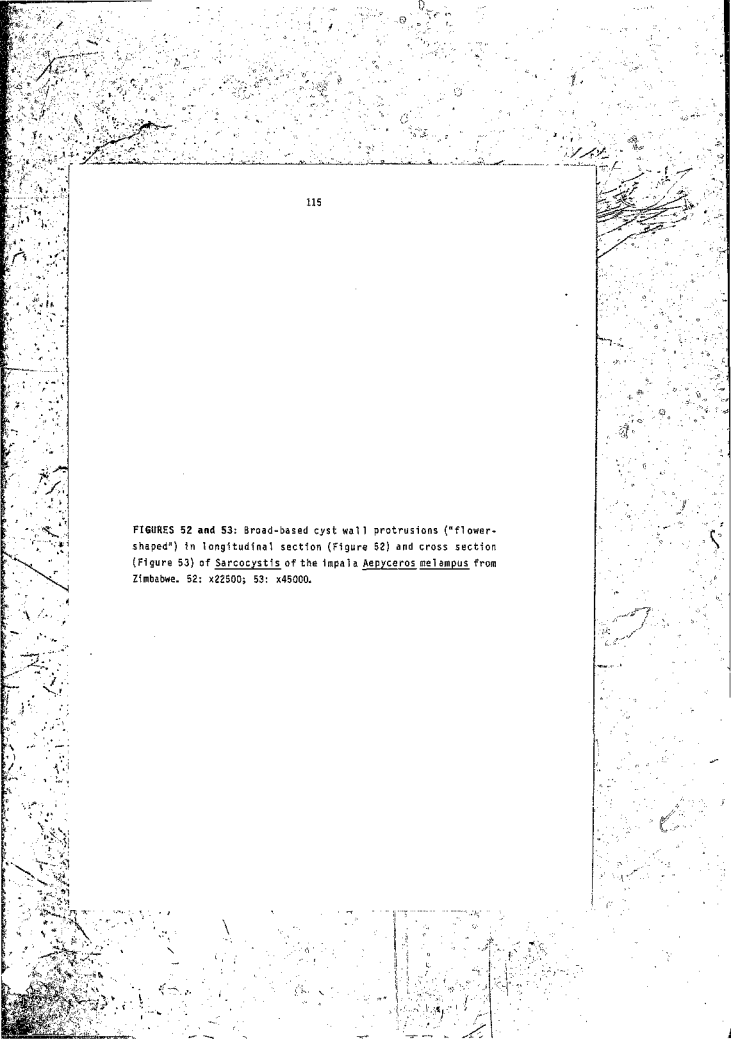
SARCOCYSTS WITH LONG PROTRUSIONS

I. WILD CARNIVORES

A sarcocyst from a second slender mongoose (TM 30566) was reminiscent of S. putorii from the vole Microtus arvalis (see Tadros and Laarman, 1978). The invaginated primary cyst wall had long (6,67 µm x 0,67 µm), slender protrusions that were square or rectangular in cross section (1,08 µm x 0,60 µm) (Figures 54 and 55). Beneath the cyst membrane was a thick, electron-dense osmiophilic layer. The protrusions contained numerous microtubular elements that extended into the ground substance. The ground substance formed a layer 1,00 µm thick beneath the primary cyst wall, in which bundles of microtubular elements were found. Septa separated the merozoites (7,88 µm x 2,25 µm) into groups or clusters in the interior of the cyst (Figure 56). The protrusions of S. putorii appeared wider (than those of the slender mongoose) in longitudinal section; and circular in cross section. In addition, they contained fibrils rather than microtubular elements.

The definitive host of the Sarcocystis species found in the slender mongoose is not known. It may be one of the various predators of the subfamily Herpestinae. As the mongoose is known to eat insects which can act as transport hosts of sporocysts, the appearance of cysts in the musculature of mongooses may represent a (fortuitous) dead-end event.

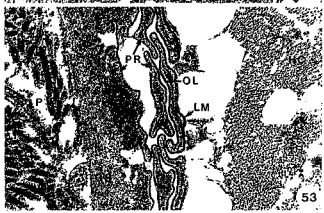
The cyst wall of S. putorii is similar in morphology to that of S. micoti in the meadow vole Microtus pennsylvanicus (see Dubey, 1983b). Both of these Sarcocystis species from voles are not unlike cysts located in avian intermediate hosts (Kaiser and Markus, 1981). Similar cysts were found in the Cape dikkop Burhinus capensis, the laughing dove Streptopelia senegalensis, a lilac-



FIGURES 52 and 53: Broad-based cyst wall protrusions ("flower-shaped") in longitudinal section (Figure 52) and cross section (Figure 53) of Sarcocystis of the impala Aepyceros melampus from Zimbabwe. 52: x22500; 53: x45000.



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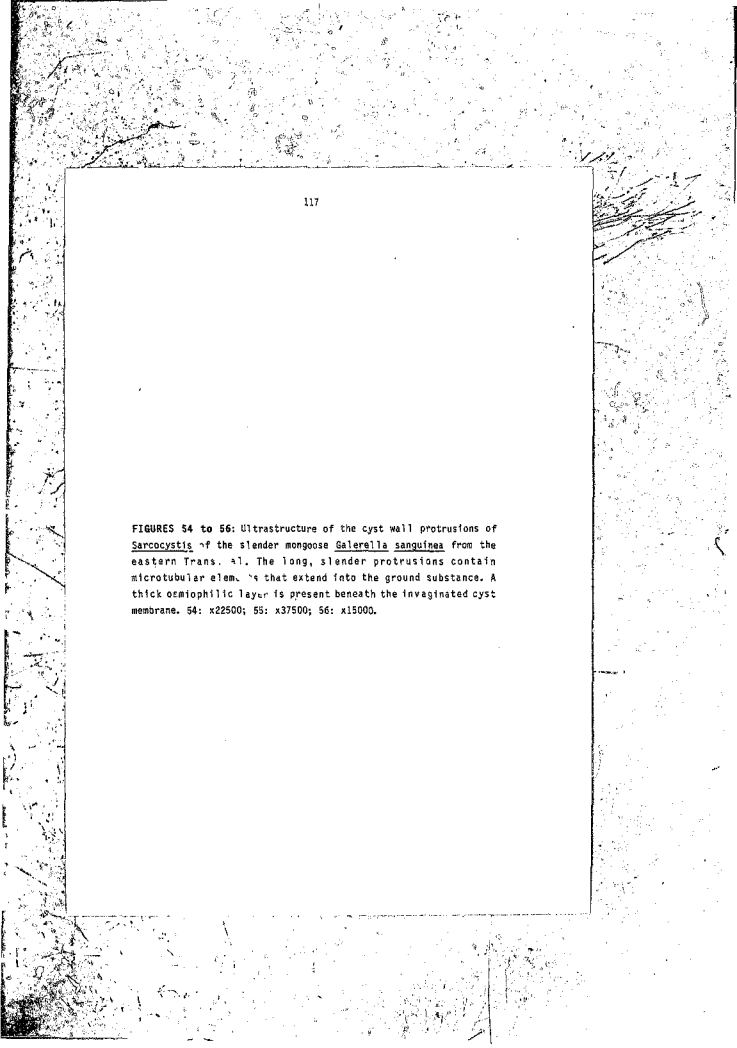
breasted roller Coracias caudata, a red-billed hornbill Tockus erythrorhynchus and in a yellow-billed hornbill Tockus flavirostris (Kaiser and Markus, 1983b).

A sarcocyst in skeletal muscle of a spotted hyaena Crocuta crocuta from the eastern Transvaal had broad, splayed, palisade-like protrusions which were rounded at their apeces (Figure 57). No fibrillar elements were found in the granular material of the protrusions and the osmiophilic layer was thick and quite distinct. This layer showed the presence of pores by the invagination of the cyst membrane into the osmiophilic layer (Figure 58). A fine, filamentous material formed a layer 0,15 µm thick, covering the outer surface of the protrusions and between their bases. The protrusions were 1,58 µm long and 1,07 µm wide. The cyst membrane was highly invaginated at the protrusion bases. The cyst wall was 0,94 µm wide and the underlying ground substance formed septa in the interior of the cyst. Merozoites measured approximately 9,5 µm x 1,75 µm.

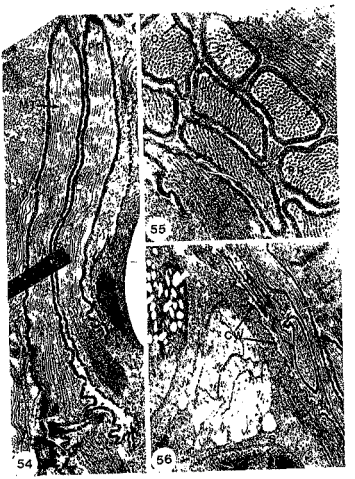
Although the protrusions from the cyst in the spotted hyaena bore some resemblance to the protrusions of S. odocoileocanis from *white-tailed deer (Dubey and Lozier, 1983)*, which has invaginated bases, the latter has a characteristic, electron-dense layer in the protrusions, below the primary cyst wall; and which lacks the "fuzzy" filamentous covering. The shape of the protrusions is similar to that in a domestic hen (Mehlhorn et al., *1976). However*, the hen cysts have fibrillar elements which were not found in the protrusions of the cyst from the spotted hyaena.

Based on the ultrastructural morphology of the cyst wall, the species of Sarcocystis from the spotted hyaena represents a "new" type. It is not known which (if any) final host completes the life cycle. The intermediate host is a carnivorous scavenger and may be a dead-end host for this species of Sarcocystis which, in all probability, normally completes its life cycle in some other host(s). If the spotted hyaena is the true intermediate host for this species of Sarcocystis, then the definitive host or hosts must be one or more of the predators or scavengers that might, on occasion, feed on it.

The cyst wall protrusions of a sarcocyst found in the African civet Civettictis civetta from the eastern Transvaal, were broad,



FIGURES 54 to 56: Ultrastructure of the cyst wall protrusions of Sarcocystis of the slender mongoose Galerella sanguinea from the eastern Trans. al. The long, slender protrusions contain microtubular elem. s that extend into the ground substance. A thick osmiophilic layer is present beneath the invaginated cyst membrane. 54: x22500; 55: x37500; 56: x15000.

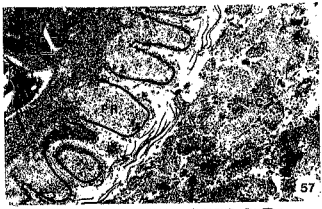

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columnar and palisade-like, with blunt apeces (Figures 59-61). The fibrillar elements appeared to be peripherally located and grouped into bundles centrally. The coarse granular material in the protrusions was different to that of the ground substance and appeared less dense in places. The protrusions were 4,04 µm in length and 1,00 µm in width, with smooth walls which had a distinct osmiophilic layer. The cyst membrane was deeply invaginated at the bases of the protrusions and the underlying ground substance formed thin septa that penetrated the interior of the cyst. These layers formed the 0,80 µm wide cyst wall. Merozoites were 7,67 µm x 2,18 µm in size.

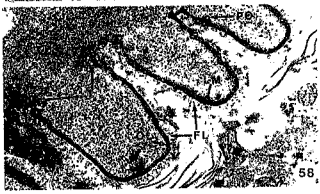
The shape of the protrusions of the *Sarcocystis species from the* African civet is not unlike that of S. odoi of the white-tailed deer (Dubey and Lozier, 1983). However, differences in certain *characteristics, such as the density of the granular material* (which appears coarser in the civet), and the number of fibrils and their arrangement, are evident from a comparison of the micrographs. The presence of an electron-dense layer beneath the protrusion wall and the indented appearance of the protrusion wall of S. odoi are not features of the smooth wall of the protrusions of the civet sarcocyst. The smooth-walled protrusions of S. hominis (syn. S. bovihominis), as illustrated by Mehlhorn et al. (1976), and of S. gracilis from the roe deer C. capreolus (see Entzeroth, 1985), have fibrillar elements that extend into the ground substance. S. gracilis is probably the same as the Type I cyst in roe deer (Entzeroth, 1982). The micrographs of S. hominis (see Tadros and Laarman, 1978) have protrusions which have a serrated appearance. *Type II sarcocysts in mule deer Odocoileus hemionus*, as reported by Dubey and Speer (1985), have fibrillar elements at the bases of the protrusions and which do not extend along the length of the protrusions as in the cyst from the civet. S. leporum from the cottontail rabbit Sylvilagus floridanus (see Elwasila et al., 1984) has fine, fibrillar elements grouped in the protrusions, similar to fibrils in the civet cyst. The serrated or invaginated appearance of the protrusion wall in S. leporum is quite different from the smooth- walled protrusions of the civet sarcocyst.

The African civet feeds on small mammals, insects, reptiles and is also partial to carrion. Final hosts (if any) for the

FIGURES 57 and 58: Ultrastructure of the cyst wall protrusions of Sarcocystis of the spotted hyaena Crocuta crocuta. A filamentous or "fuzzy" layer (FL) lies adjacent to the limiting membrane surrounding the protrusions (Figure 58). 57: x16000; 58: x30000.



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Sarcocystis species described from [illegible] "intermediate" host may be one or more of the larger mammalian carnivores or a large bird(s) of prey.

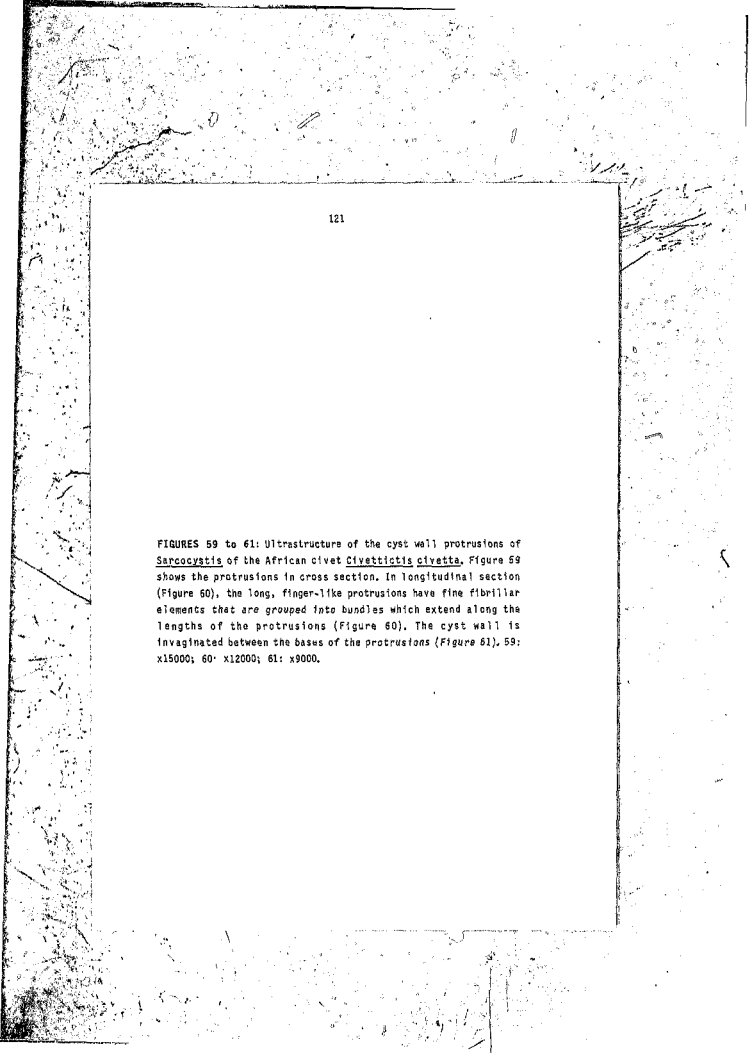
II. WILD UNGULATES

The walls of sarcocysts in warthog Phacochoerus aethiopicus from Namibia (Figure 66), Natal (Figures 62-64) and Zimbabwe (Figure 65) are morphologically similar to S. miescheriana from swine and wild boar, as described and illustrated by Tadros and Laarman (1978), and S. suicanis from pigs (Gobel et al., 1978). The protrusions were broad (2,17-3,06 µm x 1,00-1,40 µm) and smooth- walled, with *invaginations of the cyst wall at the bases. Fibrils appeared* to be grouped in bundles within the granular substance of the protrusions (Figures 63 and 65). In cross section, the fibrils were arranged peripherally around the inner circumference of the wall and also in groups which were centrally placed (Figure 64). The osmiophilic layer was very thin. In some micrographs (Figure 66), the protrusions appeared short, due to the plane of section. The granular material of the protrusions was less electron dense than the thin underlying layer of ground substance.

The cyst wall was 0,62-0,75 *µm* wide. The merozoites measured 7,50 µm x 2,31 µm. Figure 67 shows a possibly dividing merozoite with two nuclei, in the cyst from a warthog from Natal.

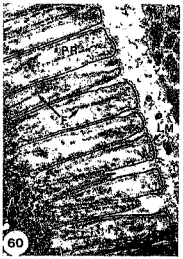
Sarcocystis suicanis has protrusions which are 2,7-3,2 µm x 0,4-0,7 µm and appear to be thinner than those of the warthog, although the cyst wall morphology in the two hosts is very similar, as was mentioned above. The cyst wall is also thicker (0,9 µm) and the osmiophilic layer also appears thicker. In a comparative review by Tadros and Laarman (1978), S. miescheriana was reported to have *protrusions 3,5 µm* long with microtubular elements, each of which was composed of 3 parallel fibrils. A sarcocyst from a wild pig had protrusions which measured 2,5-3,5 µm x 1,5 µm (Mehlhorn et al., 1976).

A macroscopic cyst (diameters 1,07 x 0,85 mm) in a warthog from the eastern Transvaal had broad-based, cauliflower-like protrusions, with a distinct osmiophilic layer and short, straight, filamentous elements (Figures 68 and 69). Unlike the macroscopic



FIGURES 59 to 61: Ultrastructure of the cyst wall protrusions of Sarcocystis of the African civet Civettictis civetta. Figure 59 shows the protrusions in cross section. In longitudinal section (Figure 60), the long, finger-like protrusions have fine fibrillar elements that are grouped into bundles which extend along the lengths of the protrusions (Figure 60). The cyst wall is invaginated between the bases of the protrusions (Figure 61). 59: x15000; 60: x12000; 61: x9000.


59
HC


60
P.P.C.
L.M.


61

cysts of S. hirsuta found by Böttner (1984), the protrusions were short, with very irregular outlines; and they did not arise from narrow bases. The protrusions measured 3,82 µm x 6,12 µm and the cyst wall was 1,67 µm wide. The dendritic appearance of the macrocyst found in sheep oesophagus was unlike the broad-based protrusions of this warthog cyst. The ground substance of the latter formed a thin layer beneath the primary cyst wall. Between the bases of the protrusions, the cyst membrane had the same irregular outline as that of the protrusions (Figure 70). Although a secondary cyst wall could not be identified with certainty, myofibrils and remnants of the host muscle cell were evident between the protrusions and adjacent to the cyst. The disruption of the host muscle tissue is probably due to the large size of the cyst. Merozoites measured 10,60 µm x 2,36 µm (Figure 71).

A cyst with finger-like protrusions (5,10 µm x 0,66 µm) and fibrillar elements that extended into the ground substance was found in a hippopotamus Hippopotamus amphibius from the eastern Transvaal (Figures 72-74). The protrusions were longer and narrower than those of a sarcocyst found in a kudu from Zimbabwe. The diameters of the kudu cyst were 90,0 x 85,0 µm and it was larger than that from the hippopotamus (60,0 x 57,5 µm). The cyst wall of the sarcocyst in the hippopotamus was 1,21 µm wide and the ground substance extended as thin septa into the interior of the cyst and divided the merozoites (10,53 µm x 2,17 µm) into groups.

This sarcocyst was not unlike S. gracilis in roe deer (Entzeroth, 1985) which has finger-like protrusions (2,5-5,0 µm x 0,5-0,7 µm) with slightly wavy walls (as in the hippopotamus), and fibrillar elements that bunch in the ground substance.

Morphologically similar sarcocysts were found in giraffe Giraffa camelopardalis from Namibia (Figures 75 and 76) and the eastern Transvaal (Figures 77 and 78).

The cyst walls were invaginated and had regularly spaced protrusions. The mean distance between protrusions of the cyst (diam. 85,0 x 80,0 µm) in the giraffe from the eastern Transvaal was 1,26 µm and the protrusions appeared to be more widely spaced than in the giraffe from Namibia (mean distance between protrusions: 1,01 µm; cyst diam. 100,0 x 75,0 µm). The thin, elongated protrusions, with wavy fibrils, appeared flat and ribbon-

FIGURES 62 to 64: Ultrastructure of the cyst wall of Sarcocystis of the warthog Phacochoerus aethiopicus from Natal. In longitudinal section (Figure 63) and in cross section (Figure 64), the fibrillar elements lie underneath the osmiophilic layer (separated by an electron-lucent layer) and are also grouped in the granular *material of the protrusions. 62: x9000; 53: x30000; 64: x30000.*


62


63
PR


64
PR

like in cross section. The wall of the smaller Transvaal cyst was thinner (0,35 µm) and the protrusions were longer (1,75 µm x 0,19 µm) than those in the giraffe from Namibia (cyst wall 0,67 µm; protrusions 1,26 µm x 0,23 µm).

Protrusions of the cyst in the giraffe from Namibia, cut in cross section near their bases, appeared to have microtubular rather than fibrillar elements in the granular material (Figure 75). Whether this is also the case for the cyst in the giraffe from the eastern Transvaal, is unknown as the protrusions (and, consequently, these elements) were sectioned obliquely rather than transversly.

Below the osmiophilic layer of the primary cyst wall, a thin layer of ground substance was present, which formed septa in the interior of the cyst from both giraffes.

The Sarcocystis species in the giraffe was not unlike sarcocysts in the fallow deer Cervus dama (see Entzeroth et al., 1985b) and Type 4 cysts of roe deer Capreolus capreolus (see Entzeroth, 1982), which have strip-like protrusions. From the orientation of fibrils in the giraffe cyst, it appeared that the protrusions had broad, flat bases that gave rise to flattened extensions. The extensions were not as narrow or strip-like as those from the fallow deer. The sarcocyst in fallow deer has short, wide bases and a region of constriction from which the strip-like protrusions arise. No fibrillar elements are present in either the roe deer or the fallow dear protrusions. Morphologically, the sarcocysts from roe deer (Entzeroth 1982) and the fallow deer (Entzeroth et al., 1985b) appear similar.

A second type of sarcocyst in a kudu from Zimbabwe (M83-3) had long, finger-like protrusions (4,18 µm x 1,24 µm) with fine fibrillar elements along their lengths (Figure 79). Fibrillar attachments were also present between adjacent protrusions and between transverse sections of the same protrusion (Figure 80).

This cyst was not unlike the Type 1 cyst in roe deer C. capreolus (see Entzeroth, 1982) or S. gracilis in roe deer (Entzeroth, 1985). The protrusion walls in the kudu were wavy or lightly indented. The cyst wall was 0,94 µm wide and the ground substance compartmentalized the merozoites (8,54 µm x 2,42 µm) (Figure 81) in the interior of the cyst by forming thin septa. The

FIGURE 65: Ultrastructure of the cyst wall protrusions of Sarcocystis of the warthog Phacochoerus aethiopicus from Zimbabwe. x22500.

FIGURE 66: Ultrastructure of the cyst wall protrusions of Sarcocystis of the warthog from *Namibia*. x15000.

FIGURE 67: A possibly dividing merozoite with two nuclei, in a sarcocyst in the warthog from *Namibia*. x6000.


CW
65
66
N
Dividing
merozoite
67

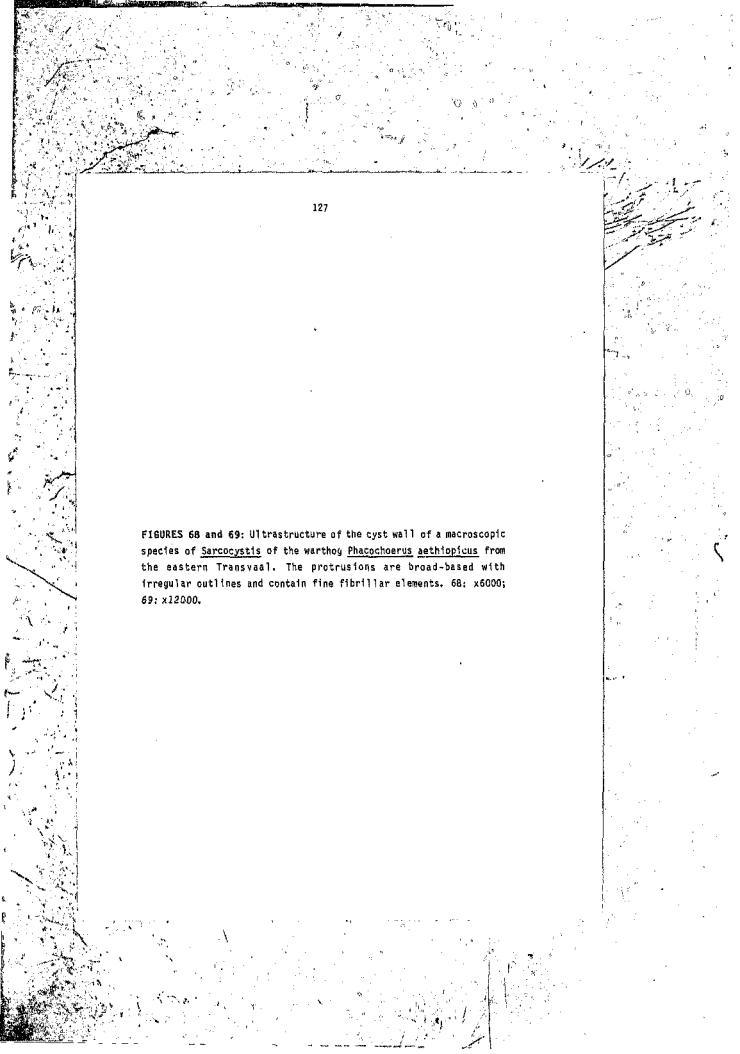
fibrillar elements of the protrusions from the cyst found in the kudu did not bunch in the ground substance. The fact that the *fibrillar elements of the Type 1* cyst and S. gracilis do bunch in the ground substance, indicates that those sarcocysts, described by Entzeroth (1982; 1985), are one and the same species of Sarcocystis. *The finger-like protrusions in the kudu were broader* than those of S. gracilis (protrusions of S. gracilis measured 2,5-5,0 µm x 0,5-0,7 µm) and were diagonally juxtaposed rather than linearly arranged.

The cysts from the kudu also appeared morphologically similar to S. hominis (syn. S. bovihominis), as described by Tadros and Laarman (1978). This is of interest because domestic cattle are kept on the farm where the material from the kudu was obtained.

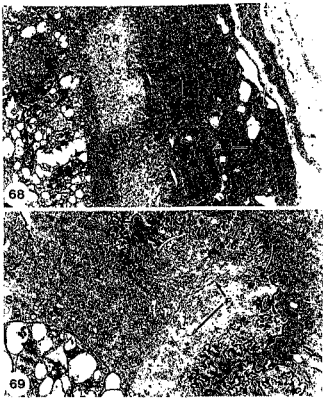
In the study of S. gracilis from roe deer (Entzeroth, 1985), three types of Sarcocystis found in roe deer in a previous investigation (Entzeroth, 1982) were reported to represent cysts of different ages belonging to the same species.

If Entzeroth's (1982) Types 1, 2 and 3 are cysts of the same species, with finger-like protrusions and clumped fibrillar *elements, then it should be noted* that the *Type 2* age-cyst bears some similarity to a developmental stage of S. rangiferi and to S. tarandi in reindeer (Gjerde, 1985c; 1985e). However, the protrusions of S. rangiferi were *much longer (12 µm) and broader* (3 µm) than those of cysts in roe deer, and the cyst wall was 1-2 µm wide (these are the dimensions of a young sarcocyst of S. rangiferi (see Gjerde, 1985c)). Cysts of Types 2 and 3 *in roe deer* (Entzeroth, 1982) had protrusions which are 5-8 µm in length and 1,2-1,5 µm in width. The protrusions of sarcocysts of S. tarandi (see Gjerde, 1985e) were also longer and wider (10,5 µm x 2,5 µm) than those of the roe deer but similar to S. rangiferi.

A Sarcocystis species from white-tailed deer Odocoileus virginianus, which has protrusions that are 5,0-8,4 µm long and 3,2-4,4 µm wide (Dubey and Lozier, 1983), is similar in respect of the protrusions to a cyst in mule deer O. hemionus (Type III), with indented walls and few fibrillar elements (Dubey and Speer, 1985). The Type III cyst in mule deer had protrusions 8,1-9,0 µm long and *3,5-5,6 µm wide, with a cyst* wall 1,57 µm wide. Both of these cysts (i.e. the type III cyst in mule deer and the cyst in white-tailed



FIGURES 68 and 69: Ultrastructure of the cyst wall of a macroscopic species of Sarcocystis of the warthog Phacochoerus aethiopicus from the eastern Transvaal. The protrusions are broad-based with irregular outlines and contain fine fibrillar elements. 68: x6000; 69: x12000.


68
69

deer) are morphologically similar to the mature macrocysts (836-4740 µm x 135-810 µm) of S. rangiferi in the skeletal muscle of *reindeer (Gjerde, 1985c), which are encapsulated by* thickened endomysial connective tissue (Gjerde, 1984a; 1985c).

Of the six species of Sarcocystis found in domestic reindeer (Gjerde, 1984d), S. tarandi and S. rangiferi have sarcocysts with finger-like protrusions. The protrusions have wavy or indented cyst membranes distally, reinforced by a thin osmiophilic layer. Fine microtubular elements are orientated longitudinally in the electron-lucid granular material of the protrusions. Dense granules or plaques, common in sarcocysts of both S. tarandi and S. rangiferi, are present in the granular material at the tips of the protrusions. In cross section, the protrusions form a hexagonal pattern.

Differences in fine structure and in the length of protrusions of S. tarandi and S. rangiferi (10,4 µm x 2,5 µm vs 12-14 µm x 8 µm), are given by Gjjerde as evidence that these are different species. However, micrographs of a young cyst of S. rangiferi (see Gjerde, 1985c) are similar to those of S. tarandi (see Gjerde, *1985e). Although the protrusions of S. tarandi may be shorter and* narrower than the mature protrusions of S. rangiferi, young cysts of the latter had protrusions that measured 12 µm x 3 µm (Gjerde, 1984e). Cysts of S. tarandi were also shorter and more slender (999 µm x 75 µm vs 2106 µm x 403 µm) and lacked the fibrillar layer surrounding the sarcocysts of S. rangiferi (see Gjerde, 1984a).

If species of Sarcocystis are considered to have a characteristic cyst wall which distinguishes them from other species occurring in the same intermediate host, and also from most other species occurring in other hosts (Gjerde, 1985a), then the morphological similarity between S. tarandi and S. rangiferi makes it possible that both belong to the same species of Sarcocystis; and that S. tarandi reflects a developmental stage of the mature cyst shown by Gjerde (1985c) for S. rangiferi.

S. odocoileocanis in white-tailed deer (Dubey and Lozier, 1983) has finger-like protrusions (1,9-4,0 µm x 1,0-3,2 µm) which are morphologically similar to those of S. hemionilatrantis in mule *deer (Dubey and Speer, 1985). However, S. hemionilatrantis in the* deer-coyote cycle appears to have shorter protrusions (1,78-2,42 µm

FIGURE 70: Ultrastructure of the invaginated cyst wall between the broad-based protrusions of the macrocyst in the warthog from the eastern Transvaal. x12000.

FIGURE 71: Peripherally located merozoite (M) in the macrocyst in the warthog from the eastern Transvaal. The nucleus (N) is situated posteriorly. x7500.


LM
BB
GS
70
MN
71

x 1,95-2,78 µm).

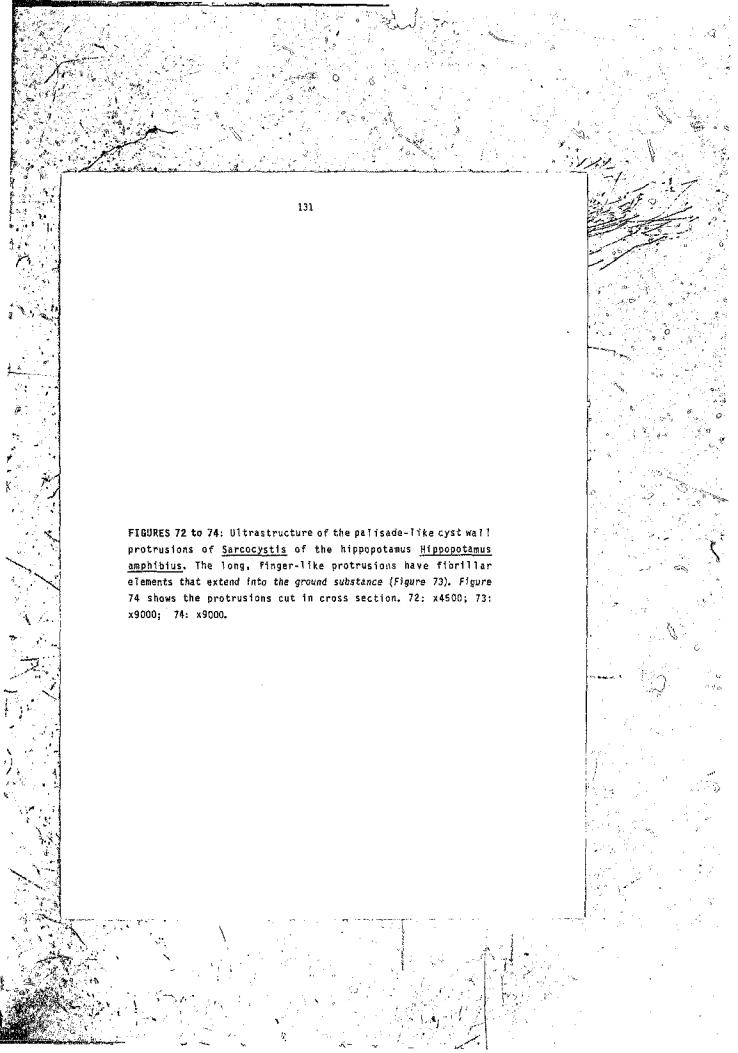
Both of these cyst types resemble S. tarandivulpes of reindeer (Gjerde, 1985b; 1985f), the protrusions of which are morphologically similar but mensurally shorter and broader (0,6-1,2 µm x 2,7-3,7 µm). The dog C. familiaris and foxes V. vulpes and A. lagopus are definitive hosts for S. tarandivulpes (see Gjerde, 1984c). Grey foxes, coyotes and dogs are the final hosts of S. odocoileocanis. Deer muscle infected with S. odocoileocanis, did not produce sporocysts in the faeces when fed to red foxes V. vulpes (Dubey and Lozier, 1983).

A Sarcocystis species found in white-tailed deer by Entzeroth et al. (1982) is morphologically similar to, and is probably the same species as S. odocoileocanis of Dubey and Lozier (1983). The cyst wall thickness of 0,4-1,0 µm (Entzeroth et al., 1982) was also similar to that of S. odocoileocanis (cyst wall 0,4-0,6 µm wide). The protrusions of Entzeroth et al.'s (1982) cyst were 1-1,45 µm long and 1,18 µm wide, with characteristic invaginated bases, and with an electron-dense layer separated from the primary cyst wall by an electron-lucid layer. The protrusions were filled with a granular material and lacked fibrillar elements.

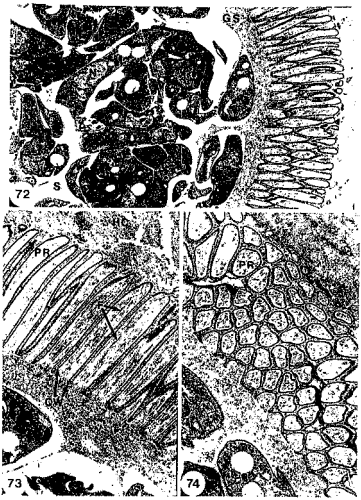
The morphological similarity between S. odocoileocanis and another Sarcocystis species also found in white-tailed deer (Dubey and Lozier, 1983), to S. hemionilatrantis and the Type III cyst, respectively, both from mule deer (Dubey and Speer, 1985), may indicate the presence of the same two species of Sarcocystis in different deer hosts. The possibility also exists that only a single species of Sarcocystis is present in both deer, and it has morphologically similar developmental stages. The Type III cyst found in mule deer was recently named S. youngi by Dubey and Speer (1986).

The Type II cysts in mule deer found . Dubey and Speer (1985) had finger-like protrusions (6,2-7,1 µm x 0,7-1,3 µm) filled with a granular material and fibrillar elements that extended along the lengths of the protrusions. The protrusion walls appeared smooth. This species of Sarcocystis was later named S. hemioni by Dubey and Speer (1986).

Sarcocystis odoi from white-tailed deer (Dubey and Lozier, 1983) also has finger-like protrusions (4,2-5,6 µm x 0,8-1,8 µm), shorter



FIGURES 72 to 74: Ultrastructure of the palisade-like cyst wall protrusions of Sarcocystis of the hippopotamus Hippopotamus amphibius. The long, finger-like protrusions have fibrillar elements that extend into the ground substance (Figure 73). Figure 74 shows the protrusions cut in cross section. 72: x4500; 73: x9000; 74: x9000.


GS
S
72
PR
73
74

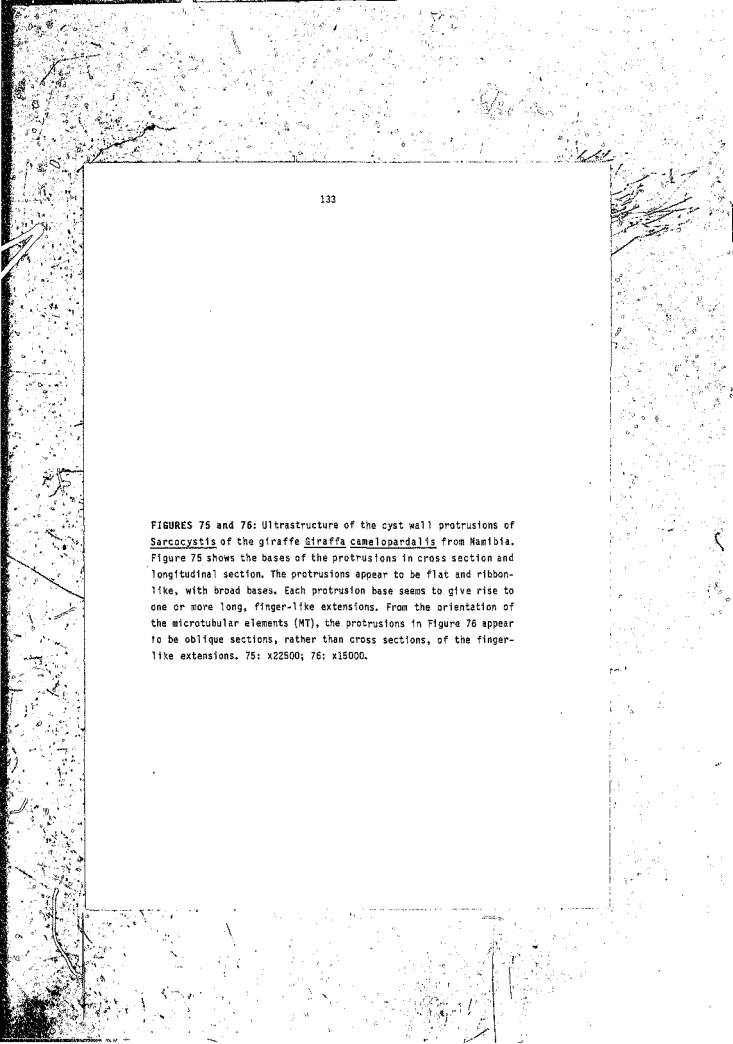
than those in mule deer. The fibrillar elements are grouped or bundled in the granular material of the protrusions, and an electron-dense layer is evident below the protrusion walls.

Whether the three Sarcocystis species found in mule deer by Dubey and Speer (1985) (which are S. hemionilatrantis and Types II and III - S. hemioni and S. youngi, respectively) are the same or developmental stages similar to S. odocoileocanis, S. odoi and a Sarcocystis species from white-tailed deer (Dubey and Lozier, 1983), respectively, and/or are related to Sarcocystis spp. in other cervids, can only be determined by cross- transmission studies. Whether new species should have been named in the absence of such studies, is debatable.

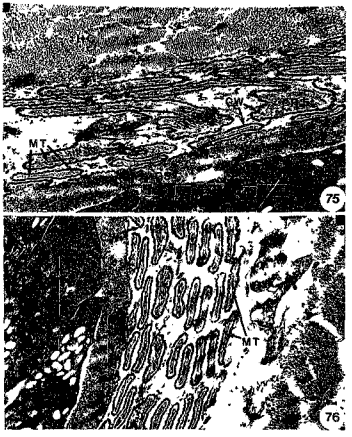
High magnification electron micrographs of the protrusions of S. hemionilatrantis, recovered from mule deer by Speer and Dubey (1986), revealed the presence of plaque-like structures. These specialized structures in the primary cyst wall consisted of six layers with microfilaments extending from the innermost layer into the central core of the protrusions. It is thought that the plaques and their associated microfilaments may provide some rigidity or motility to the protrusions or, alternatively, may be sites of molecular exchange between the parasite and the host cell cytoplasm. These structures have also been seen by those authors in published electron micrographs of S. odocoileocanis.

Dubey and Speer (1986) described a fourth species of Sarcocystis from mule deer. By light microscopy, the sarcocyst wall was 2-10 µm thick and had fine, hair-like protrusions. The species was named S. americana by Dubey and Speer (1986).

A sarcocyst in a tsessebe Damaliscus lunatus (M85-72) from the northern Transvaal had broad, closely arranged, palisade-like protrusions with wavy, smooth walls (Figure 82). No fibrils were present in the granular substance of the protrusions. The protrusion zone was 3,1 µm thick. The cyst membrane was invaginated between the bases of the protrusions; and below the primary cyst wall, a thin (0,85 µm) granular (electron-dense granules) ground substance layer extended into the cyst as septa. The merozoites measured 11,17 µm x 2,34 µm. The protrusions were more or less rectangular in cross section (1,37 µm x 1,54 µm) and appeared to be flat (Figure 84). The invaginated bases indicated the thin, ribbon-



FIGURES 75 and 76: Ultrastructure of the cyst wall protrusions of Sarcocystis of the giraffe Giraffa camelopardalis from Namibia. Figure 75 shows the bases of the protrusions in cross section and longitudinal section. The protrusions appear to be flat and ribbon-like, with broad bases. Each protrusion base seems to give rise to one or more long, finger-like extensions. From the orientation of the microtubular elements (MT), the protrusions in Figure 76 appear to be oblique sections, rather than cross sections, of the finger-like extensions. 75: x22500; 76: x15000.

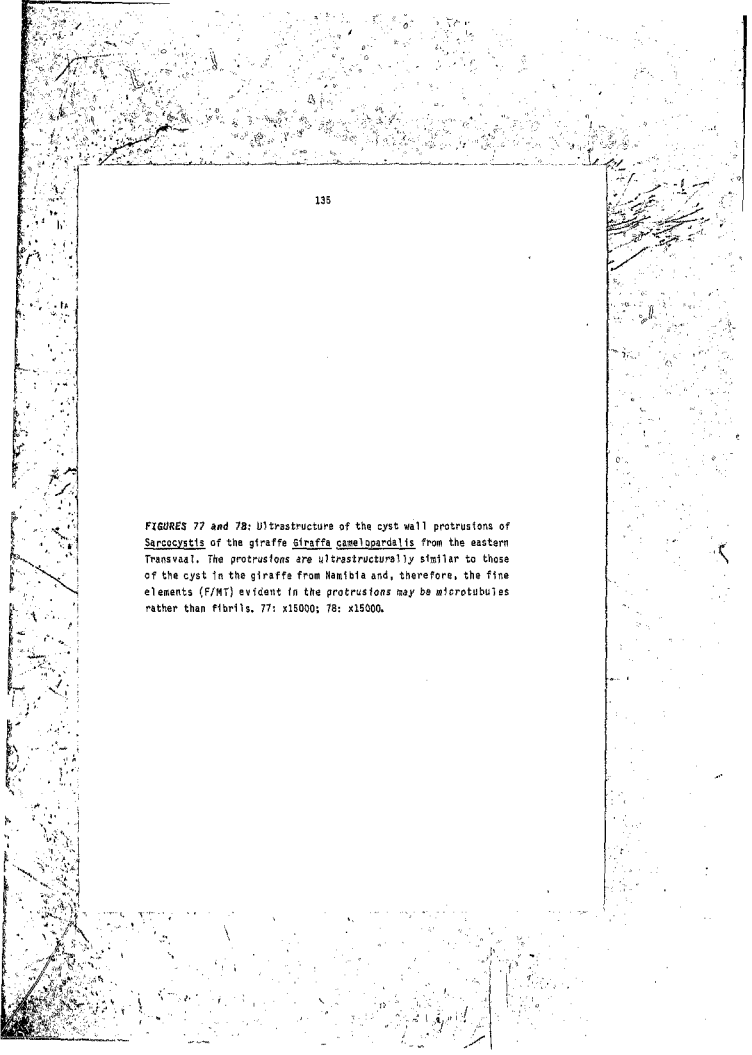

GW
MT
75
MT
76

like nature of the protrusions (Figures 82 and 83). No electron-dense layer was seen below the protrusion walls, as is evident in sarcocysts of S. odocoileocanis from white-tailed deer (see Entzeroth et al., 1982; Dubey and Lozier, 1983) and S. hemionilatrantis in mule deer (see Dubey and Speer, 1985). Ultrastructurally the protrusions of the cyst from the tsessebe were similar to those of microscopic cysts found in sheep in the present study (Figures 110 and 111). The protrusions of the cysts from the sheep were flat and ribbon-like; and were of similar length (2,5 µm), but narrower than those of the cyst in the tsessebe.

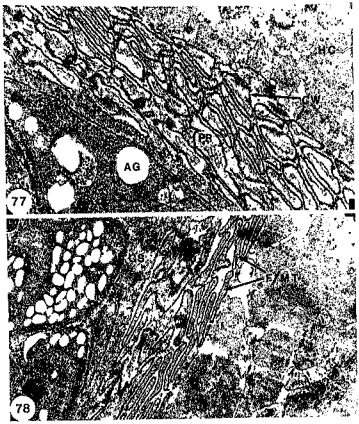
A sarcocyst in a springbuck Antidorcas marsupialis (M83-58) from Namibia was remarkably similar to S. sybillensis from the North American elk Cervus elaphus (see Dubey et al., 1983), which has the dog as an experimental definitive host. The slender protrusions in both intermediate hosts had a smooth outer limiting membrane with highly invaginated bases (Figure 85). The central arrangement of osmiophilic granules along the lengths of the protrusions in the springbuck cyst is also a feature of S. sybillensis. The protrusions are long and did not appear in their entirety in section. The few that were most complete, measured 7,54 µm x 0,30 µm. The protrusions of S. sybillensis were up to 6,15 µm long and were 0,30 µm wide at their bases. As in the cysts from the elk, the cyst wall in the springbuck was thick (1,40 µm). The ground substance continued as septa into the cyst and divided the merozoites (8,67 µm x 2,67 µm) into groups (Figure 86).

SARCOCYSTS IN DOMESTIC AND WILD EQUINE HOSTS

Ultrastructurally, the wall of a sarcocyst found in the oesophagus of a horse Equus caballus in the Transvaal, had protrusions which were long and thin (3,46 µm x 0,77 µm), extending away from the cyst (Figure 87). Their walls were highly invaginated and osmiophilic granules were present amongst the fibrillar elements. The fibrillar elements were centrally situated and ran into the ground substance. At the bases of the protrusions, the fibrillar elements became tightly packed and formed bundles or plaques in the ground substance. In cross section (Figure 88), the



FIGURES 77 and 78: Ultrastructure of the cyst wall protrusions of Sarcocystis of the giraffe Giraffa camelopardalis from the eastern Transvaal. *The protrusions are ultrastructurally similar to those of the cyst in the giraffe from Namibia and, therefore, the fine elements (F/MT) evident in the protrusions may be microtubules rather than fibrils.* 77: x15000; 78: x15000.


AG
BR
77
78

protrusions measured 1,57 µm x 0,45 µm. The cyst wall, which comprised a thin layer of ground substance and the invaginated primary cyst wall, was 0,91 µm thick.

Skeletal muscle cysts in two species of zebra from various localities in Namibia, had similar cyst walls. Sarcocysts in a mountain zebra Equus zebra hartmannae from northern Namibia (Figure 89) were identical to those found in four mountain zebra from central Namibia (Figures 90 and 91). The width of the cyst walls was 0,62 µm and 0,87 µm, respectively. The protrusion lengths were shorter than those of the horse, and measured 1,93 µm x 0,40 µm and 2,28 µm x 0,47 µm, respectively. The protrusions from the mountain zebra sarcocysts lay adjacent to the cyst wall and the fibrillar elements were less condensed in the ground substance than those in the domestic horse. In addition to being present in the protrusions, electron-dense granules were also evident elsewhere in the ground substance of the mountain zebra cysts. The osmiophilic layer became thicker and was less invaginated towards the apical or distal ends of the protrusions (Figure 90). Merozoites were 8,40 µm x 2,55 µm (Figure 95), and, in Figure 96, a metrocyte with two nuclei is evident in a cyst from a mountain zebra. The micrographs of the cyst wall in a Burchell's zebra Equus burchelli from northern Namibia (Figures 93 and 94) show the centrally located fibrillar elements of the protrusions in cross section, and extending into the ground substance in longitudinal section. The mean width of the cyst wall of E. burchelli was 0,95 µm and the protrusions measured 2,51 µm x 0,47 µm.

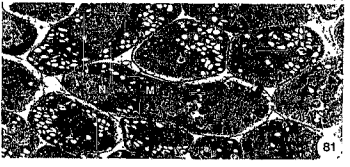
The ultrastructural morphology of these sarcocysts in domestic and wild equines was similar to S. bertrami (syn. S. equicanis) in European horses (Gobel and Rommel, 1983), which had protrusions 2-3 µm in length. The cysts were not unlike S. bertrami in British horses, found by Edwards (1984), although the horse and zebra protrusions in the present study were longer than those of S. bertrami (0,8-1,5 µm). From an examination of the micrographs of S. bertrami (see Edwards, 1984), it is evident that the protrusions were sectioned obliquely; and their length measurements, as given by Edwards (1984), were similar to the longer cross sectional (and oblique) measurements of the protrusions of the cyst in the Transvaal horse and the cysts in the zebras in this study (horse

FIGURES 79 and 80: Ultrastructure of the cyst wall protrusions of Sarcocystis of the kudu Tragelaphus strepsiceros from Zimbabwe. Figure 79 shows the protrusions, which appear to be diagonally juxtaposed, in longitudinal section. The fibrillar elements are grouped in the granular material. In cross section (Figure 80), fine fibrillar elements (F) appear to link adjacent protrusions. 79: x15000; 80: x30000.

FIGURE 81: A merozoite (M) with a posteriorly located nucleus in a cyst from the Zimbabwean kudu. x9000.


79


80

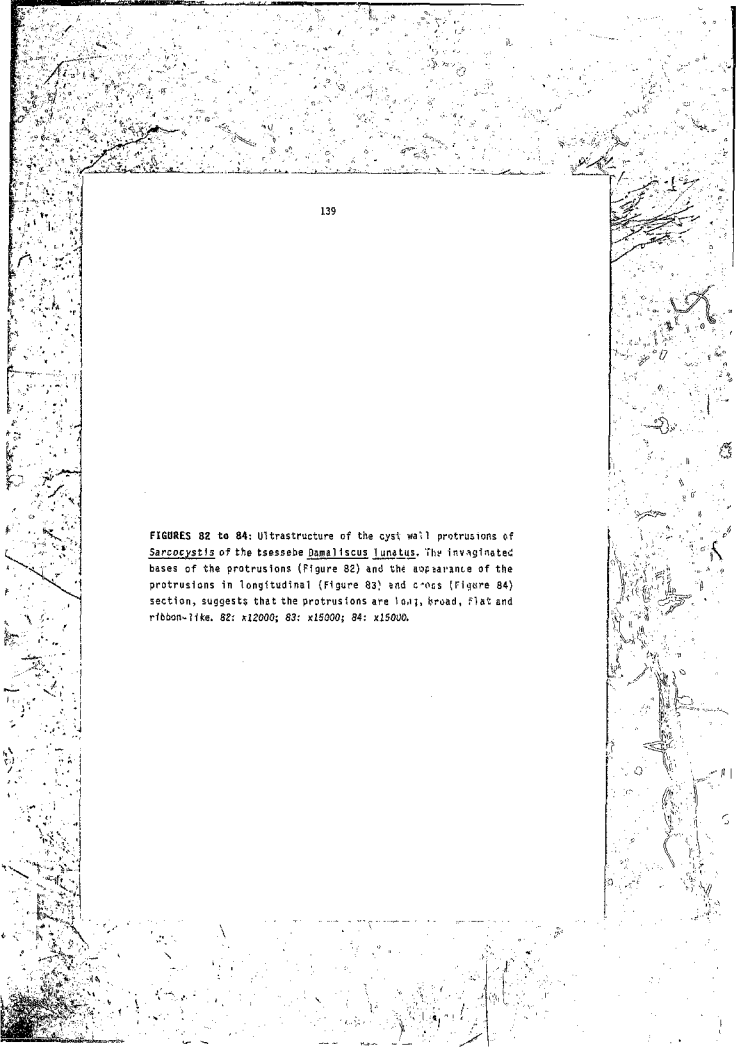

81

and zebras: range 0,63-2,20 µm; mean 1,21 µm). Edwards (1984) found donkeys infected with what may be S. bertrami.

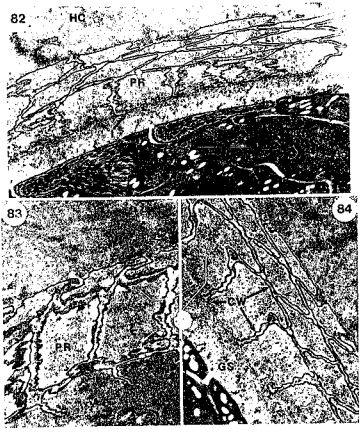
Sarcocysts from the domestic horse and wild zebras in South Africa resemble a cyst type in a horse in the United States of America (Daly et al., 1983). Tinling et al. (1980) found that this American horse cyst had features like those of S. fayeri. The thick-walled, radially-striated cyst of S. fayeri is similar to that of the domestic horse cyst in the present study, which also had protrusions that extended away from the cyst, thereby imparting a striated appearance by light microscopy. The most obvious difference between S. fayeri and S. equicanis, as stated by Tinling et al. (1980), is the presence of microtubular elements in the former species. Micrographs of cross sections through the protrusions of the horse sarcocyst in the present study show that some fibrillar elements do appear to be tubular (Figure 88), but they did not form junctions with the pellicles of the peripheral merozoites or metrocytes. A high magnification electron micrograph of the fibrillar elements in the protrusions of a cyst found in the mountain zebra, also revealed that microtubular elements are possibly present (Figure 92). However, as the majority of the elements appeared to be solid structures, the presence of a single possible microtubular element is considered a dubious criterion for differentiation.

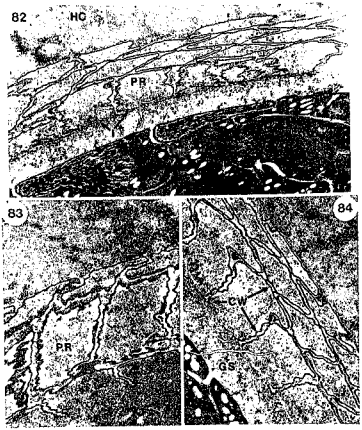
If the differences between S. fayeri and S. equicanis are based on light microscopical examination of the cyst wall, then the Sarcocystis species in the domestic horse could be different from that of the zebras. Sarcocysts found in the zebras had protrusions that lay adjacent to the cyst wall, giving it an unstriated or thin-walled appearance, as described for S. equicanis (see Erber and Geisel, 1981). The number of protrusions in the zebra cysts appeared to be less numerous than those in the South African horse, which may influence the light microscopic appearance of the cyst wall (Tinling et al., 1980). However, whether this is an age-related phenomenon or a basic difference in the structure of the cysts, is unknown.

Ultrastructurally, the protrusions from the cyst in the South African horse appeared to have fewer fibrillar elements than were present in the zebra cysts, and, although the dimensions of the



FIGURES 82 to 84: Ultrastructure of the cyst wall protrusions of *Sarcocystis* of the tsessebe *Damaliscus lunatus*. The invaginated bases of the protrusions (Figure 82) and the appearance of the protrusions in longitudinal (Figure 83) and cross (Figure 84) section, suggests that the protrusions are long, broad, flat and ribbon-like. *82: x12000; 83: x15000; 84: x15000.*


82
HC
PR
83
84
BR
CW
GS


82
HC
PR
83
PR
84
CW
GS

merozoites found in the zebra cysts compared favourably with those of S. equicanis (see Göbel and Rommel, 1980; Tinling et al., 1980), the lengths of the merozoites in the sarcocyst of the horse could not be determined. The diameters of the cysts from the zebras had a range of 55,0-115,0 µm and were smaller than the diameters of the cysts found in the South African horse (82,5-150,0 µm).

Based on certain specific features, such as the diameters of the cysts, the number of fibrillar/microtubular elements, the arrangement of the protrusions and the presence or absence of microtubular elements, it is possible to make a distinction between the cysts from the horse and those from the zebras. If cysts of S. fayeri have microtubular elements, as described by Tinling et al. (1980), together with smaller diameters, and fewer microtubules and protrusions that extend away from the cyst, then the sarcocyst from the horse is most likely this species.

The features of the sarcocysts from the zebras are more in line with those features attributed to S. equicanis. However, as the diameters of the cysts from the zebras tended to be smaller than those from the horse, and as the possibility exists that microtubular elements may occur in the protrusions, the species of Sarcocystis in the zebras fulfils at least two of the four criteria for identification as S. fayeri. Studies of variation in equine sarcocyst morphology, correlated with cyst age, are clearly needed.

Although the cysts from domestic and wild equines are morphologically similar, it is uncertain how many species of Sarcocystis occur in the domestic horse. At least three species of Sarcocystis have been named from horses: S. bertrami, S. equicanis and S. fayeri. While S. bertrami has become synonymized with S. equicanis (see Edwards, 1984), some authors are of the opinion that all cyst types found in the horse, i.e. including S. fayeri, are of one Sarcocystis species only (Hinaidy and Loupal, 1982). R. Fayer recently commented that he thinks that probably there is only one species of Sarcocystis in horses (M. B. Markus, 1987, personal communication).

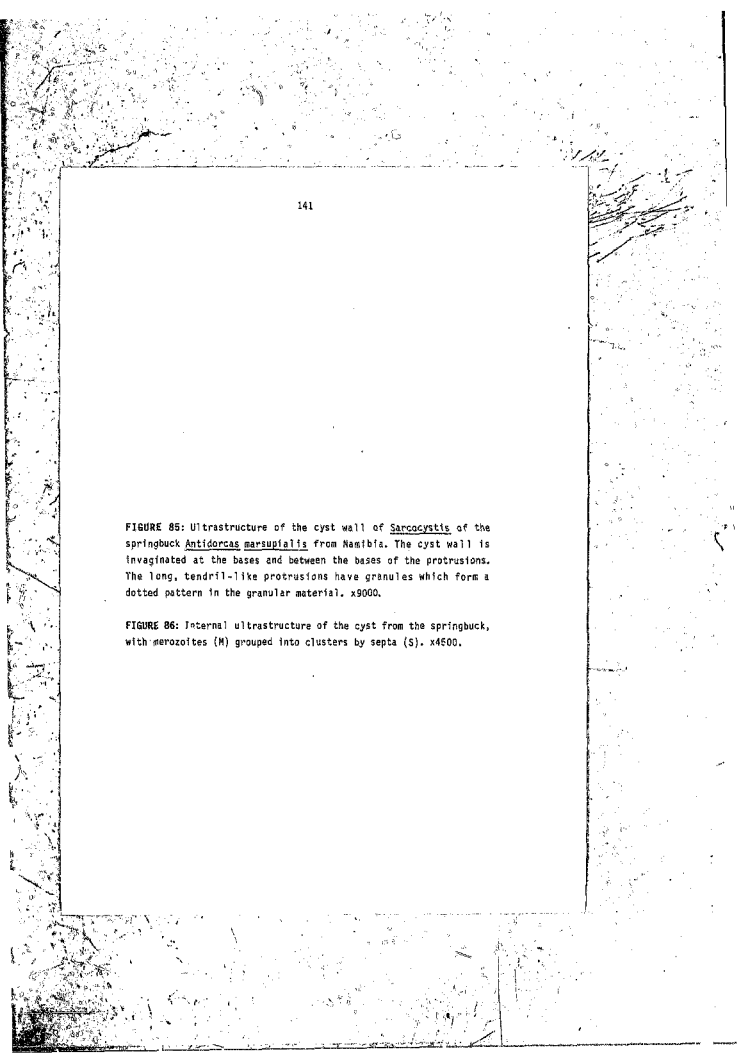
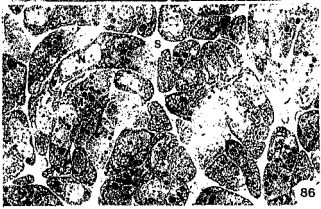


FIGURE 85: Ultrastructure of the cyst wall of Sarcocystis of the springbuck Antidorcas marsupialis from Namibia. The cyst wall is invaginated at the bases and between the bases of the protrusions. The long, tendril-like protrusions have granules which form a dotted pattern in the granular material. x9000.

FIGURE 86: Internal ultrastructure of the cyst from the springbuck, with merozoites (M) grouped into clusters by septa (S). x4500.



85



86

SARCOCYSTS IN DOMESTIC UNGULATES

I. CATTLE

The nomenclature used in the present study for species of Sarcocystis in cattle is that of Levine and Tadros (1980) and Levine (1986) (see Table 4). However, the macroscopic cyst type is referred to in this thesis as the cat/bovine Sarcocystis species. S. hirsuta has been used in the present study for microscopic, thick-walled cysts found in cattle, and which have the cat as a definitive host. These cysts are morphologically similar to "S. bovifelis" (see Mehlhorn et al., 1976), which is considered to be a synonym.

Microscopic Thick-walled Cysts

In the micrographs presented by Böttner (1984), three different types of microscopic, thick-walled cyst could be distinguished.

The first had protrusions that were palisade-like with smooth walls and which exhibited vesicle-like invaginations of the unit membrane at their bases. Fine, fibrillar elements filled the protrusions, and extended into the ground substance where they had (collectively) a bulbous appearance. These protrusions were not unlike those of "S. bovifelis" (syn. S. hirsuta) as described by Mehlhorn et al. (1976) - which has palisade-like protrusions with fibrillar elements that extend into the ground substance. The fibrillar elements are fine and form tubular patterns in cross section.

A second cyst type illustrated by Böttner (1984) had thin, finger-like protrusions with very irregular primary cyst walls. The outer unit membrane surrounding the protrusions was deeply invaginated into a thick osmiophilic layer. The protusions had narrow bases where they joined the body of the cyst, and electron-dense granules were dispersed between fine fibrillar elements.

The third cyst type illustrated by Böttner (1984) had palisade-like protrusions with slightly invaginated cyst walls and fibrillar elements that did not fill the entire protrusions. These

TABLE 4: The taxonomy of Sarcocystis species of domestic ungulates.

From Levine (1986). Only synonyms used in the present study are listed.

S. cruzi (Hasselmann, 1923) Wenyon, 1926
Synonym: S. bovicanis Heydorn, Gestrich, Mehlhorn, and Rommel, 1975.
Definitive hosts: dog Canis familiaris, coyote C. latrans, wolf C. lupus, red fox Vulpes vulpes and probably racoon Procyon lotor.
Intermediate hosts: ox Bos taurus, gaur B. gaurus (?), bison Bison bison.

S. hirsuta Mouléé, 1888
Synonym: S. bovifelis Heydorn, Gestrich, Mehlhorn, and Rommel, 1975.
Definitive hosts: cat Felis catus, wild cat F. silvestris.
Intermediate host: ox Bos taurus.

S. hominis (Railliet and Lucet, 1891) Dubey, 1976
Synonym: S. bovihominis Heydorn, Gestrich, Mehlhorn, and Rommel, 1975.
Definitive hosts: man Homo sapiens, rhesus monkey Macaca mulatta, baboon Papio cynocephalus, chimpanzee Chimpansee troglodytes (?).
Intermediate host: ox Bos taurus.

S. miescheriana (Kühn, 1865) Labbé, 1899 (Type species)
Synonym: S. suicanis Erber, 1977.
Definitive hosts: dog Canis familiaris, wolf C. lupus, red fox Vulpes vulpes, racoon Procyon lotor.
Intermediate host: pig Sus scrofa.

S. porcifelis Dubey, 1976
Definitive host: cat Felis catus.
Intermediate host: pig Sus scrofa.

S. suihominis (Tadros and Laarman, 1976) Heydorn, 1977
Synonym: S. porcihominis Dubey, 1976.
Definitive hosts: man Homo sapiens, chimpanzee Chimpansee troglodytes, rhesus monkey Macaca mulatta, cynomolgus monkey M. fascicularis.
Intermediate host: domestic pig Sus scrofa.

S. bertrami Doflein, 1901
Synonyms: S. asinus Gadaev, 1978; S. equicanis Rommel and Geisel, 1975; S. fayeri Dubey, Streitel, Stromberg, and Toussant, 1977 (?).
Definitive host: dog ..nis familiaris.
Intermediate hosts: horse Equus caballus, ass E. asinus.

S. fayeri Dubey, Streitel, Stromberg, and Toussant, 1977
Definitive host: domestic dog Canis familiaris.
Intermediate host: horse Equus caballus.
Remark: This may turn out to be a synonym of S. bertrami.

S. gigantea (Railliet, 1886) Ashford, 1977
Synonyms: S. ovifelis Heydorn, Gestrich, Mehlhorn, and Rommel, 1975; S. tenella (Railliet, 1886) Moulé, 1886, in part.
Definitive host*: cat Felis catus.
Intermediate host: sheep Ovis aries.

S. medusiformis Collins, Atkinson, and Charleston, 1979
Definitive host: cat Felis catus.
Intermediate host: sheep Ovis aries.

S. tenella (Railliet, 1885) Moulé, 1886
Synonym: S. ovicanis Heydorn, Gestrich, Mehlhorn, and Rommel, 1975.

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* Levine (1986) lists (presumably in error) the red fox Vulpes vulpes as a final host of S. gigantea. However, if the red fox is indeed a final host of S. gigantea, I have not seen the paper which reports this.

Definitive hosts: dog Canis familiaris, coyote C. latrans, dingo C. dingo (?), red fox Vulpes vulpes.
Intermediate host: sheep Ovis aries.

S. arieticanis Heydorn, 1985
Definitive host: dog Canis familiaris.
Intermediate host: sheep Ovis aries.

S. ferovis Dubey. 1983
Definitive host: coyote Canis latrans.
Intermediate host: bighorn sheep Ovis canadensis.

S. capracanis Fischer, 1979
Synonym: S. capricanis El-Rafaii, Abdel-Baki, and Selim, 1980, lapsus calami.
Definitive hosts: dog Canis familiaris, coyote C. latrans, fox Vulpes vulpes.
Intermediate host: domestic goat Capra hircus.
Remark: The relationship of this species to S. orientalis is unknown.

S. caprifelis El-Rafaii, Abdel-Baki, and Selim, 1980
Definitive host: cat Felis catus.
Intermediate host: goat Capra hircus.

S. hircicanis Heydorn and Unterholzner, 1983
Definitive host: dog Canis familiaris.
Intermediate host: goat Capra hircus.

S. orientalis Machul'skii and Miskaryan, 1968
Definitive host: unknown.
Intermediate host: wild goat Capra sibirica.
Remark: This may be a synonym of S. moulei.

S. moulei Neveu-Lemaire, 1912
Synonym: S. orientalis Machul'skii and Miskaryan, 1968 (?).
Definitive host: dog Canis familiaris.
Intermediate host. goat Capra hircus.

protrusions were similar in appearance to those of S. hominis, as illustrated by Tadros and Laarman (1978). However, whether this cyst type was S. hominis could not be deduced by Böttner et al. (1987b). Scanning electron microscopy showed these cyst protrusions to be flattened and tongue-like, and no features were observed under either TEM or SEM that were suggestive of distinctive morphologies of S. hirsuta and S. hominis, respectively (Böttner et al., 1987b).

As regards thick-walled cysts, Böttner et al. (1987b) concluded that the cyst wall (its development and cyst wall widths) and the protrusion widths are a meagre basis for distinguishing cysts of indeterminate age, as cyst wall morphology could change significantly with age.

Macroscopic Cysts

Macroscopic cysts (mean diameters 170,8 µm x 123,3 µm) in some beef bought from a butcher in Natal were identified as cat/bovine Sarcocystis (Figures 97-100). The protrusions were broad, with short microtubular elements that radiated from narrow bases (Figure 98). Macroscopic cat/bovine sarcocysts, described by Böttner (1984) from cattle, likewise have protrusions with narrow bases where they join the body of the cyst. The outlines of the protrusions of the bovine cyst from Natal were irregular, with a thick osmiophilic layer present and osmiophilic (electron-dense) granules scattered between the microtubular elements (Figure 97). The protrusions were 2,28 µm wide and were up to 7,0 µm in length in longitudinal section (Figure 99). The cyst wall was 1,41 µm thick and the ground substance (which had electron dense granules) beneath the invaginated cyst membrane formed septa in the interior of the cyst. Merozoites measured 7,17 µm x 2,56 µm.

A longitudinal section of the macroscopic cat/bovine cyst found by Böttner (1984) is identical to S. levinei in water buffalo Bubalus bubalis, described by Dissanaike and Kan (1978). However, S. levinei, unlike the cat/bovine Sarcocystis species, is transmitted by the domestic dog in an experimental situation. Microscopic cysts of S. levinei (see Kan and Dissanaike, 1978) had diameters of 90-140 µm, protrusions 6,4-10,0 µm long, cyst walls

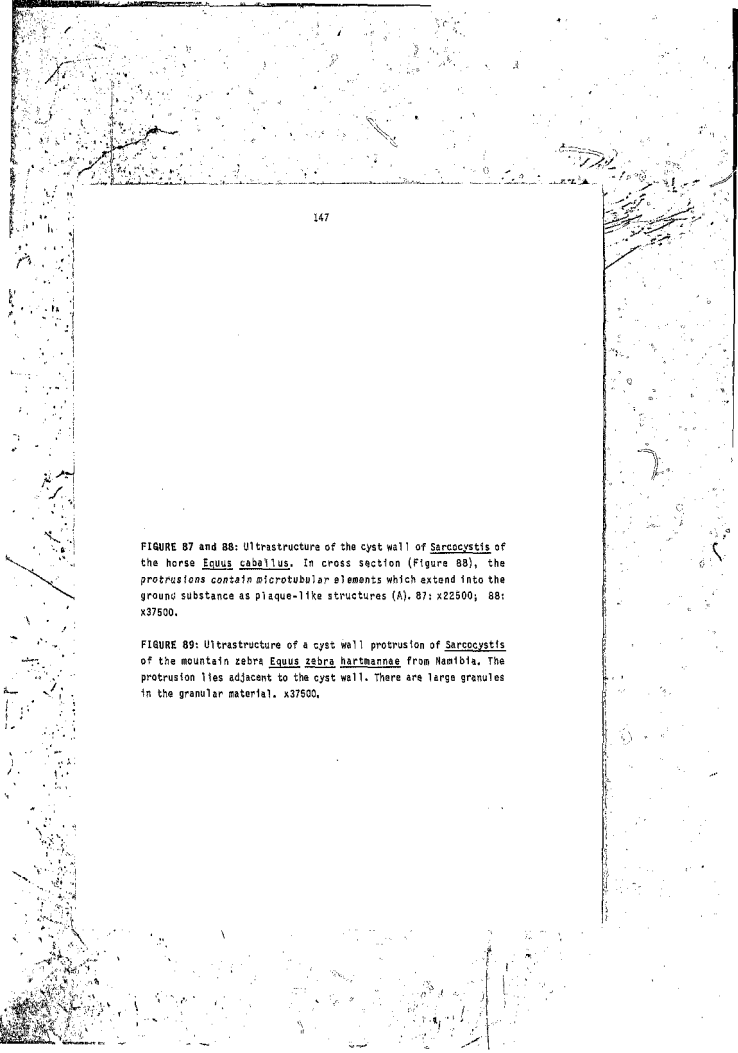


FIGURE 87 and 88: Ultrastructure of the cyst wall of Sarcocystis of the horse Equus cabellus. In cross section (Figure 88), the *protrusions contain microtubular elements* which extend into the ground substance as plaque-like structures (A). 87: x22500; 88: x37500.

FIGURE 89: Ultrastructure of a cyst wall protrusion of Sarcocystis of the mountain zebra Equus zebra hartmannae from Namibia. The protrusion lies adjacent to the cyst wall. There are large granules in the granular material. x37500.

89
88
87

1,57-2,07 µm wide and merozoites which measured 17,0-18,2 x 3,8-4,6 µm.

In an investigation concerned with the identity of macroscopic cysts (similar to those examined in the present study) in cattle in New Zealand (Böttner et al., 1987a), cysts could be transmitted only to cats and not to dogs or a human volunteer. By light microscopy, the cyst wall ranged from 3,3 µm to 7,0 µm in thickness (mean 4,81±1,03 µm) and the widths of the protrusions from 1,2 µm to 2,6 µm (mean 1,66±0,39 µm). Ultrastructurally, the protrusions were large, with irregular outlines and narrow bases. In longitudinal section, the finger-like protrusions were narrow, and similar to those described in some microscopic, thick-walled cysts by Böttner et al. (1987b) and to the protrusions of the thick-walled cyst (referred to in the present study as the second type) illustrated by Böttner (1984). On the basis of their transmission and scanning electron microscopic studies of the macroscopic cat/bovine cysts in cattle, Böttner et al. (1987a) suggested that with increasing age and growth of young microscopic S. hirsuta cysts, the cyst wall protrusions become larger and develop a highly irregular surface, although the basal attachments of the protrusions to the cyst wall remain the same. These authors were of the opinion that the narrow bases suggest that only growth and enlargement of the protrusions occurs, and that no new protrusions are formed. Where the protrusions lie against the host tissue, they become flattened and mushroom-like (Charleston and Böttner, 1986). The fibrillar elements are thought to become disorganised and reorientated into small elements that radiate from the narrow bases. Böttner et al. (1987a) concluded that because the ultrastructure of the cyst changes with age, the identification of cysts based on morphology alone is something to be approached with caution.

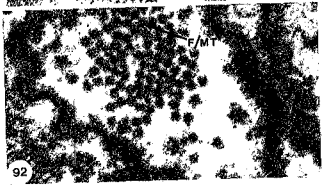
However, only macroscopically visible cysts were used in the study by Böttner et al. (1987a), and no developmental (younger cysts from experimental infections) stages of the cyst wall were described. Electron microscopy of the macroscopic cysts revealed considerable differences from published descriptions of microscopic S. hirsuta cysts, and from the microscopic, thick-walled cysts referred to in the present study as the first type and third type

FIGURES 90 and 91: Ultrastructure of the cyst wall protrusions of Sarcocystis of the mountain zebra Equus zebra hartmannae from Namibia. *In Figure 90, a protrusion lying adjacent to the cyst wall* is shown in longitudinal section. It has fibrillar elements (F) and granules (G) in the granular material and ground substance. In Figure 91, the fibrillar elements in the protrusions and ground substance are seen in cross section. 90: x45000; 91: x45000. Fixative: glutaraldehyde.

FIGURE 92: A high magnification micrograph of a cross section through a protrusion of a cyst in a mountain zebra from Namibia. Most of the fibrillar elements appear as solid structures while a few seem to be tubular (F/MT) in cross section. x150000. Fixative: glutaraldehyde.


GS
90


PR
91


F/MT
92

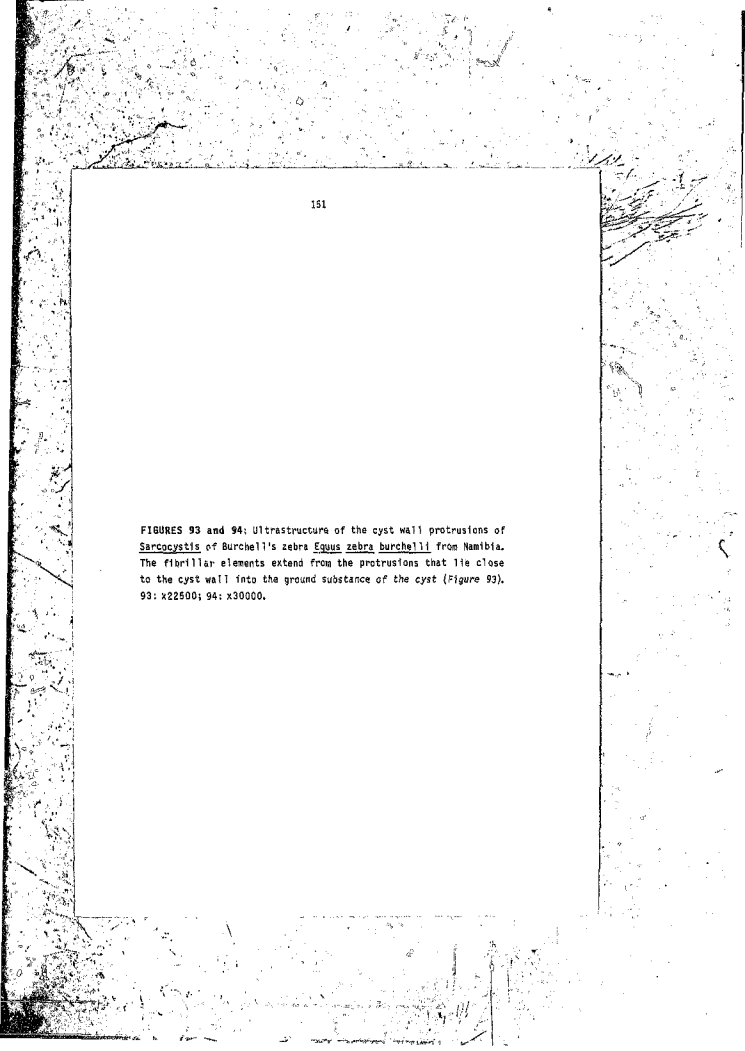
described by Böttner et al. (1987b).

My own view is that, although it is possible that minor changes can occur in cysts during growth, major changes such as those suggested by Böttner et al. (1987a), seem unlikely. On examination of the micrographs in the doctoral thesis of Böttner (1984), cyst wall morphologies for two microscopic, thick-walled cysts are to be seen (Böttner, 1984; Figs 16 and 17) at the same magnification. One has a fine structure similar to that described for S. hirsuta, having densely-packed palisade-like protrusions (referred to in the present study as the first type). The other has long, irregularly-spaced protrusions with irregular walls, narrow bases and electron-dense granules (referred to in the present study as the second type). The latter is probably a young cyst of the macroscopic cat/bovine Sarcocystis species.

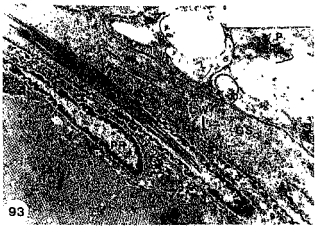
The possibility remains, therefore, that two species of Sarcocystis, both having the cat as a definitive host, occur in cattle. Both the macroscopic and microscopic species are, at present, known as "S. hirsuta".

Microscopic Thin-walled Cysts

Sarcocystis cruzi (syn. S. bovicanis) was examined in cardiac muscle of cows from the Transvaal. The electron micrographs showed a cyst wall with few protrusions and which were circular or bean-shaped in cross section (0,32 µm x 0,13 µm) (Figures 101-103). The cyst type shown in Figures 101 to 103 was transmitted to puppies in my laboratory by feeding them infected, minced heart muscle. Subsequently, sporocysts in gut mucosal scrapings from these dogs were fed to a calf. On day 28 p.i., the calf died. Schizonts were found in endothelial cells in most organs (see details of transmission experiments). The cyst type examined ultrastructurally has, therefore, been correlated with a particular final host (it is unlikely that the Sarcocystis species which I examined by electron microscopy was a different one). The protrusions had smooth walls and no fibrillar elements in their granular substance. The cyst membrane was highly invaginated in places, between the bases of the protrusions. A thin layer of ground substance was apparent beneath the primary cyst wall. The cyst wall had a mean thickness of



FIGURES 93 and 94: Ultrastructure of the cyst wall protrusions of Sarcocystis of Burchell's zebra Equus zebra burchelli from Namibia. The fibrillar elements extend from the protrusions that lie close to the cyst wall into the ground substance of the cyst (*Figure 93*). 93: x22500; 94: x30000.



93



94

0,31 µm.

Sarcocysts in bovine heart muscle in South Africa were identical to "S. bovicanis", i. e. S. cruzi (see Mehlhorn et al., 1976), which had a cyst wall 0,3-0,4 µm thick, and protrusions 3,0 µm in length and 0,2-0,3 µm in width. Thin-walled cysts (S. cruzi) in the oesophagi and, to a lesser extent, in the diaphragms of cattle in New Zealand (Böttner, 1984), as well as S. cruzi as described in an ox-coyote cycle by Dubey (1982), were also identical to the cysts described and illustrated in the present study. The shape of the protrusions at their origin on the cyst wall (Figure 102 in this thesis) was similar to the stubby protrusions of the Type 5 cyst found by Entzeroth (1982) in roe deer.

Pacheco et al. (1978) studied the fine structure of immature cysts of S. cruzi and showed the formation of the cyst wall at various stages of development. It should be noted that an immature sarcocyst of S. sybillensis from the North American elk (Dubey et al., 1983) had a cyst wall that is similar to a stage in the development of S. cruzi (see Pacheco et al., 1978). A S. cruzi cyst described by Böttner (1984), which has rod-like protrusions, is remarkably similar to a sarcocyst from the roe deer (Type 4) (see Entzeroth, 1982), which has strip-like protrusions in cross section. A longitudinal section of the rod-like protrusions of S. cruzi (see Böttner, 1984) is also similar to a Sarcocystis species from the fallow deer (Entzeroth et al., 1985b), which has strip-like protrusions with wide bases. Therefore, there is a possibility that the Sarcocystis species from the fallow deer and the Type 4 cyst from the roe deer are the same species. The Type 5 cyst from roe deer (Entzeroth, 1982) may be a stage in the development of this Sarcocystis species from roe deer (Type 4) and fallow deer. Gjerde (1985f) considers one of the types of cyst found in cattle in New Zealand by Böttner (1984) (referred to in the thesis by Böttner (1984) as a thin-walled S. cruzi cyst with rod-like protrusions - it is also similar to both S. cervicanis (syn. S. wapiti) and S. gruaneri, which occurs in reindeer) as a new species of bovine Sarcocystis in addition to S. cruzi, S. hirsuta and S. hominis.

FIGURE 95 and 96: Internal ultrastructure of the cyst in the mountain zebra Equus zebra hartmannae from Namibia. Merozoites (M) are shown in Figure 96. A dividing metrocyte (MC), with two nuclei, is to be seen in Figure 96. 95: x4500; 96: x4500.

95
96

Conclusion

Other than the microscopic S. cruzi, which has the dog as a final host, and S. hominis (syn. bovihominis), there appear to be at least two additional Sarcocystis species in cattle: Sarcocystis hirsuta, i.e. "S. bovifelis", which is microscopic and has a cat definitive host (Mehlhorn et al., 1976), and the microscopic/macroscopic cat/bovine cyst type (Böttner, 1984 and present study).

II. HORSES (see "SARCOCYSTS IN DOMESTIC AND WILD EQUINE HOSTS")

III. PIGS

A sarcocyst found in oesophageal muscle of a domestic pig Sus scrofa from the Transvaal, had long, broad-based protrusions (Figure 104). The cyst membrane at the bases of and between the protrusions was highly invaginated. Distally, the protruded walls were smooth, with a thin osmiophilic layer. Microtubular elements that appeared circular in cross section, extended along the lengths of the protrusions. The protrusions appeared to be wide and flattened at their bases (2,80 µm x 0,80 µm in X.S.) and narrower more distally (1,49 µm x 0,84 µm in X.S.). This sarcocyst was remarkably similar to S. suihominis in the diaphragm of a domestic pig (Tadros and Laarman, 1978), and which had irregularly-shaped protrusions and possessed microtubular elements.

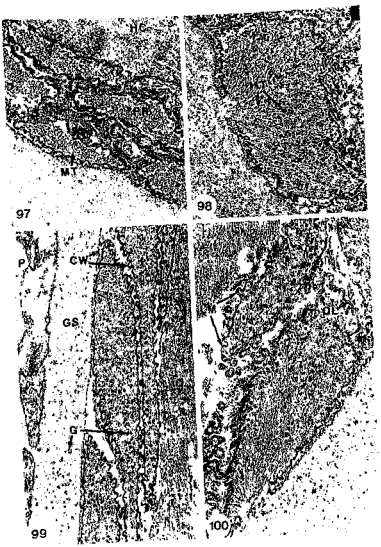
IV. SHEEP

The haphazard use by authors of scientific names for different species of ovine Sarcocystis and also the use of a single name for different species, has resulted in a great deal of confusion as far as nomenclature is concerned. It is difficult to compare scientific work done on a single species of Sarcocystis when that species is referred to by two or more names.

According to Levine and Tadros (1980) and Levine (1986), "S. ovifelis" is a synonym of S. gigantea (see Table 4). Ultrastructurally, S. gigantea is identical to "S. ovifelis" as described by Mehlhorn et al. (1976). However, the same cyst and in

FIGURES 97 to 100: Ultrastructure of cyst wall protrusions of the macroscopic cat/bovine Sarcocystis species in Bos taurus. In Figure 97, the microtubular elements (MT) are viewed in cross section. Figure 98 shows microtubular elements radiating from the narrow bases of the protrusions. Figures 99 and 100 are longitudinal/oblique sections of protrusions. In addition to the microtubular elements, granules (G) are also present in the granular material of the protrusions. 97: x30000; 98: x15000; 99: x16000; 100: x22500. Fixative: glutaraldehyde.

Note: The meat from which these cysts were recovered, had been frozen at 20^{0}C for an unknown period prior to thawing of the meat and fixation of the cysts in glutaraldehyde.


97
98
99
100
P
CW
GS
G

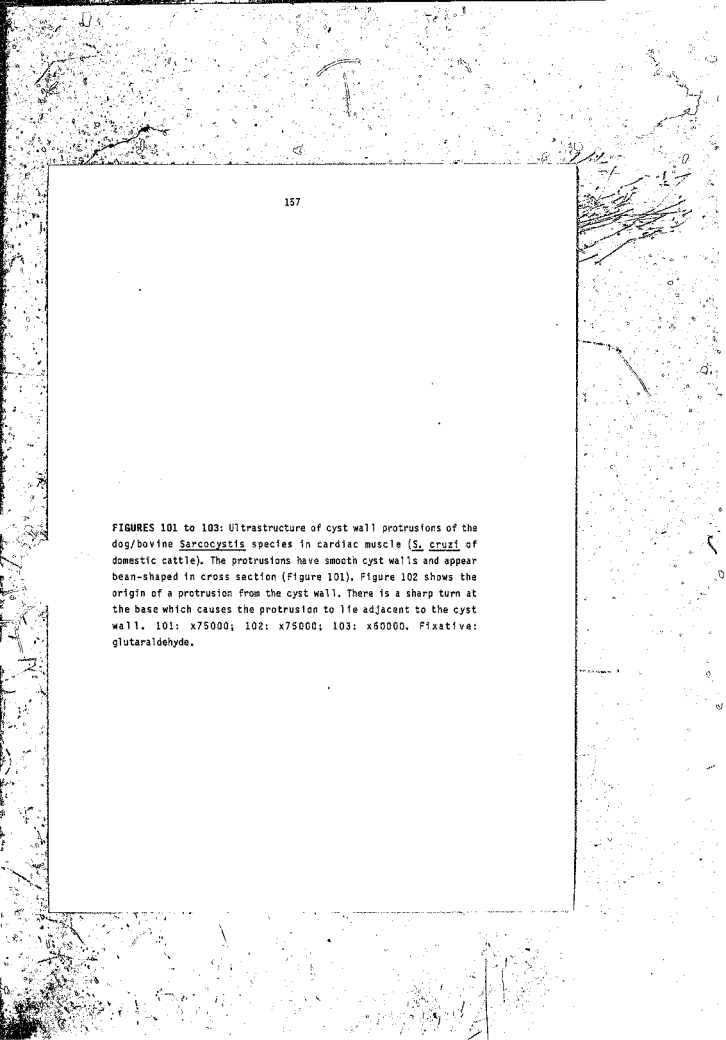
fact the identical micrograph is an illustration for "S. tenella" in a study of the Sarcosporidia by Mehlhorn and Heydorn (1978). The thick-walled, microscopic cyst from sheep is referred to by Mehlhorn et al. (1976) and Mehlhorn and Heydorn (1978) as "S. ovicanis". "S. ovicanis" is listed by Levine (1986) as a synonym of S. tenella which, in turn, is in part a synonym of S. gigantea.

Regarding the nomenclature used with reference to Sarcocystis species of sheep, Ashford (1977) pointed out the validity of the names given by Railliet in 1886. The Sarcocystis species which produces microscopic sarcocysts in sheep and which has the domestic dog C. familiaris, coyote C. latrans, the red fox Vulpes vulpes and probably the dingo C. dingo as definitive hosts, is S. tenella (Railliet, 1886). The species producing macroscopic sarcocysts in sheep and having the domestic cat F. catus as a definitive host, is S. gigantea (Railliet, 1886). S. ovicanis and S. ovifelis are synonyms of S. tenella and S. gigantea, respectively (Ashford, 1977). Ashford's (1977) paper effectively reduces confusion as regards the nomenclature of Sarcocystis species in sheep. Nevertheless, Britt and Baker (1983) reported the finding of macroscopic, ovoid cysts in the oesophageal muscle of sheep and erroneously called this cyst type S. tenella. Ashford and Britt (1983) subsequently pointed out that the correct specific name to use is S. gigantea.

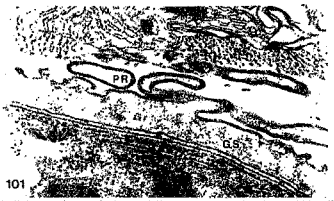
Species of Sarcocystis in sheep are either referred to in the present study by the names given in Ashford (1977), and listed by Levine and Tadros (1980) and Levine (1986) (see Table 4); or in general terms such as "dog/ovine Sarcocystis species".

Macroscopic Cysts

Bergmann and Kinder (1975) found macrocysts in sheep oesophageal muscle. The cauliflower-like protrusions were similar to those of the macroscopic S. gigantea cysts from sheep oesophageal and abdominal muscle found later by Collins et al. (1979). Collins et al. (1979) discussed three macroscopic cyst types from sheep. The oesophageal and "fat" macrocysts from skeletal muscle both had cauliflower-like protrusions and were considered to be S. gigantea (syn. S. ovifelis). The third macrocystic species, also



FIGURES 101 to 103: Ultrastructure of cyst wall protrusions of the dog/bovine Sarcocystis species in cardiac muscle (S. cruzi of domestic cattle). The protrusions have smooth cyst walls and appear bean-shaped in cross section (Figure 101). Figure 102 shows the origin of a protrusion from the cyst wall. There is a sharp turn at the base which causes the protrusion to lie adjacent to the cyst wall. 101: x75000; 102: x75000; 103: x60000. Fixative: glutaraldehyde.



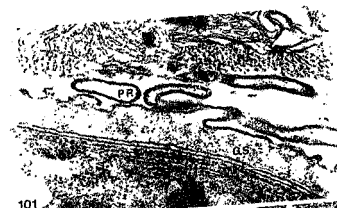
101



102



103



101



102



103

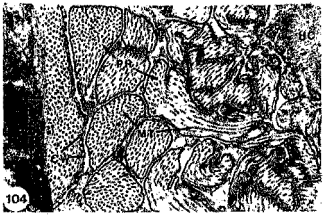
transmissible to cats, had characteristic, snake-like extensions of rounded protrusions; hence the name S. medusiformis. The development of S. medusiformis in sheep was investigated by Obendorf and Munday (1987). Mature cysts (487 d.p.i.) were located in the laryngeal, abdominal and diaphragmal muscles of a dosed lamb. A collagenous secondary cyst wall was evident in the cysts of S. gigantea studied by Collins et al. (1979), but was absent in S. medusiformis. Bergmann and Kinder (1975) interpreted the secondary cyst wall of the macroscopic cyst from sheep (S. gigantea) as being basal membrane material. The suggestion that there is a reduplication of the basal membrane as the inner component of the secondary cyst wall of S. gigantea, has been supported by Gjerde (1985c).

Sarcocystis gigantea is identical to S. ovifelis of Mehlhorn et al. (1976). The diffuse protrusions were 4,5 µm long and the cyst wall was 2,0 µm thick (Mehlhorn et al., 1976). This cyst was referred to as S. tenella in a review of the fine structure of Sarcosporidia by Mehlhorn and Heydorn (1978).

A macrocyst from sheep oesophagi (from an experimental infection in which sporocysts from cats were used) developed the characteristic S. gigantea cyst wall at 10 months p.i. (Munday and Obendorf, 1984). At 25 months p.i., the cysts were ultrastructurally identical to those described previously (S. ovifelis: see Mehlhorn et al., 1976; S. tenella: see Mehlhorn and Heydorn, 1978; S. gigantea: see Collins et al., 1979). A secondary cyst wall of connective tissue was 2-3 µm thick and enclosed the parasitized host muscle cell (Munday and Obendorf, 1984). A reduplication of the basal lamina, as described by Gjerde (1985c), could not be seen in the micrographs presented by Munday and Obendorf (1984). Immature (pre - 119 d.p.i.) cysts of S. gigantea were superficially similar to S. tenella (syn. S. ovicanis), but could be distinguished by the thick (1-2 µm) sarcocyst wall. The presence of immature sarcocysts at 6 weeks p.i. and the occurrence of encephalitis in the sheep suggested that schizogony occupied the first 5 weeks after infection.

Macroscopic cysts (mean diameters 1068,2 µm x 862,4 µm) in oesophageal muscle of sheep from the Transvaal were ultrastructurally identical to S. gigantea (see Mehlhorn et al.,

FIGURE 104: Ultrastructure of the cyst wall protrusions of *Sarcocystis* of the domestic pig *Sus scrofa*. The protrusions have invaginated cyst walls at their bases and contain microtubular elements. The creased appearance of the protrusions at the periphery suggests that the protrusions taper and flatten towards their apeces. x22500.

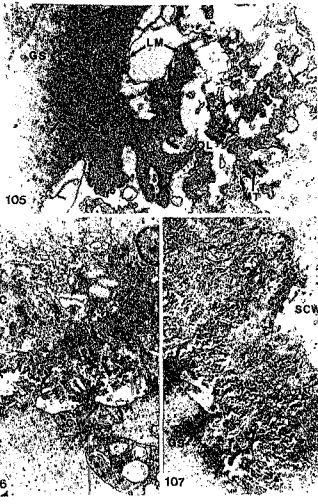

104

1976; Mehlhorn and Heydorn, 1978; Collins et al., 1979; Munday and Obendorf, 1984). The cauliflower-like protrusions had irregular, invaginated outlines, and contained microtubular elements at various orientations (Figures 105 and 106). A thick layer of ground substance (3,26 µm) beneath the invaginated cyst membrane formed broad septa which separated the merozoites (10,52 µm x 2,58 µm) into clusters (Figure 108). In addition to electron-dense granules, rod-like structures were also present in the ground substance. These rod-like structures appeared filamentous, with a central core of linearly arranged granules (Figure 109). A secondary fibrous cyst wall was evident and enclosed remnants of the parasitized muscle sarcoplasm and myofibrils (Figure 107). The fibrous secondary cyst wall may be a reduplication of the external (basal) lamina, formed by the parasitized fibre (Bergmann and Kinder, 1975; Gjerde, 1985c). An additional external layer derived from the endomysium, which is characterized by the presence of fibroblasts, was not evident around the macrocysts.

Microscopic Cysts

Microcysts in the oesophageal and cardiac muscle of sheep from the Transvaal and Natal had protrusions that were long, wide and flattened (ribbon-like) (Figure 113). In cross section, the protrusions were 1,02 µm wide and 0,16 µm thick. They were highly folded with smooth, irregularly-shaped walls and were filled with a granular material. In some micrographs of the wall (Figure 112), the protrusions were fewer, unfolded and appeared bar-shaped - being similar to S. fenovis from bighorn sheep Ovis canadensis (see Dubey, 1983d). The cyst membrane was invaginated at intervals along the length of the wall; seemingly in areas that did not give rise to protrusions. The cyst wall was 0,21 µm wide and the thin layer of ground substance formed septa which extended into the interior of the cyst. Merozoites, which were not sectioned perfectly along their length, measured approximately 6,83 µm x 2,50 µm. Sarcocyst protrusions in a sheep from the Transvaal appeared more numerous and measured 0,76 µm x 0,27 µm in cross section (Figure 110). In cross section, the protrusions from the sheep cyst are not unlike the flower- and bean-shaped protrusions of cysts from the impala A.

FIGURES 105 to 107: Ultrastructure of the cyst wall of the macroscopic *Sarcocystis* species of the sheep *Ovis aries*. The cauliflower-like protrusions have invaginated cyst walls and contain microtubular elements. A secondary cyst wall (SCW) adjacent to the remnants of the host tissue, is evident in Figure 107. 105: x22500; 106: x6000; 107: x3000. Fixative: glutaraldehyde.


LM
GS
PL
105
HC
106
SCW
GS
107

melampus. In longitudinal section, however, the protrusions appeared to be flattened and arranged in palisade-like formation (*Figure 111*). Their morphology was characteristic of S. tenella (syn. S. ovicanis), as illustrated and described by previous authors. Therefore, it is likely that the cysts found in sheep in the Transvaal and Natal are S. tenella (syn. S. ovicanis).

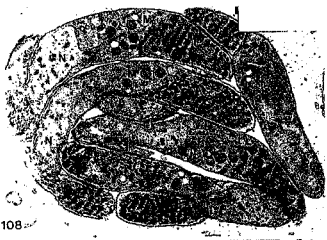
S. tenella ("ovicanis"), according to Mehlhorn et al. (1976), had palisade-like protrusions which were closely adjacent and approximately 3,5 µm in length. The close arrangement of the protrusions gave the wall a striated appearance by light microscopy. The protrusions had no fibrillar elements in their granular material and had slightly invaginated bases. The ground substance beneath the cyst wall formed a narrow band 0,2-0,3 µm in thickness. Merozoites were 14-17 µm in length (Mehlhorn and Heydorn, 1978). Thick-walled sarcocysts in skeletal muscle of sheep, examined by Bergmann and Kinder (1975), were like S. tenella ("ovicanis") as illustrated by Mehlhorn et al. (1976). In a study by Dubey et al. (1982) of the development of S. tenella in a sheep-canid cycle, the cyst wall was found to be 1,5-0,2 µm wide and the protrusions measured 2,0-0,9 µm x 0,5-0,3 µm. The merozoites were 7,22 µm x 1,67 µm. Although the protrusion lengths as stated by Dubey et al. (1982) are shorter than those given by Mehlhorn et al. (1976), the cysts are ultrastructurally similar. The lengths of the protrusions for the cyst from sheep referred to as S. tenella in the present study, are approximately 2,5 µm.

The protrusions of young cysts of S. tenella ("ovicanis") appeared circular in cross section whereas older cysts had flattened or polygonal protrusions in cross section (Mehlhorn et al., 1976). This would suggest that the cysts from sheep in the present study were mature, because if the protrusions become flattened or polygonal in older cysts, then in cross section they would appear bar-shaped as illustrated in Figure 112.

These cross-sectional protrusions should not be confused with the protrusions of a second type of dog/sheep Sarcocystis species (thin-walled), which are hair-like (see Bergmann and Kinder, 1975). The hair-like protrusions appear bar-shaped in cross section but are much smaller at the same magnification, are much more numerous and are irregularly arranged along the cyst wall. The dog/ovino

FIGURE 108: Ultrastructure of a peripheral cluster of merozoites in the macroscopic sarcocyst from the sheep Ovis aries. The merozoites (M) have prominent micronemes and posteriorly located nuclei (N). x7500. Fixative: glutaraldehyde.

FIGURE 109: Ultrastructure of the ground substance of the macroscopic cyst from the sheep. The ground substance contains numerous, short, filamentous or rod-like structures (R) with granules which form a dotted pattern along their lengths. x6000. Fixative: glutaraldehyde.



108



109

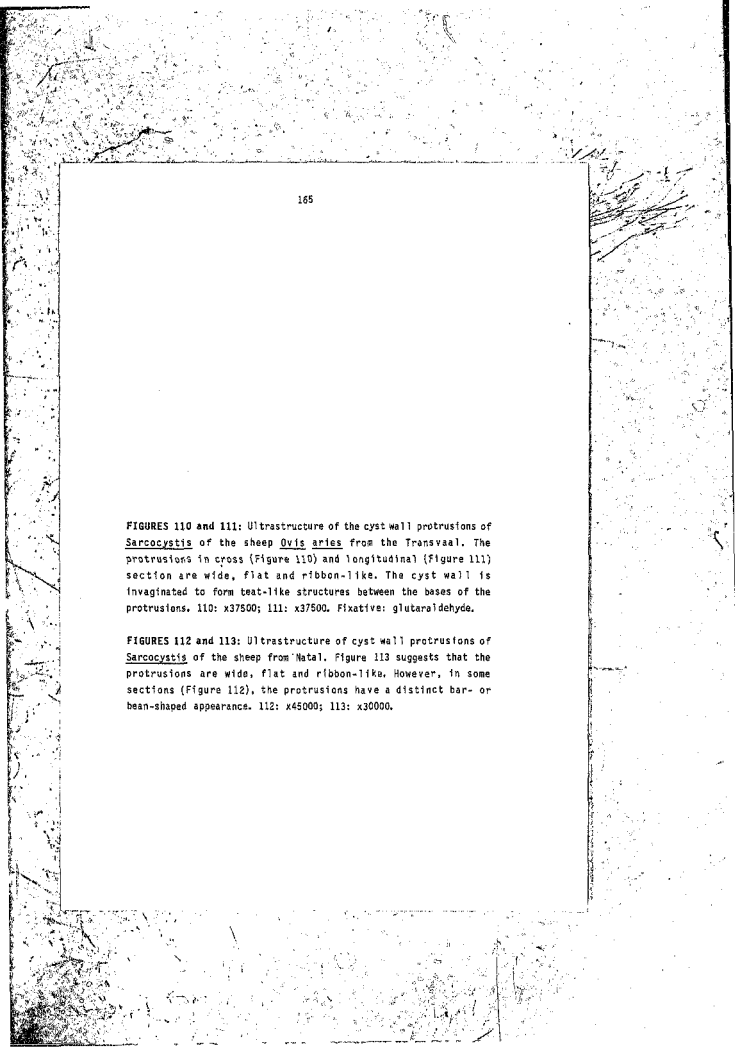
Sarcocystis species with hair-like protrusions occurs in South Africa (J. B. Bush, 1987, personal communication). O'Donoghue et al. (1986) illustrated both thick-walled and thin-walled dog/sheep Sarcocystis species. The palisade-like protrusions of the thick-walled cysts were smooth, and appeared to be flattened in longitudinal section.

Myositis associated with degeneration of muscle cysts in sheep was studied by O'Toole (1987). Thick-walled cysts present from 42 days p.i., developed cylindrical palisade-like protrusions that were 1,55 µm x 0,45 µm at 60 days p.i. At 81 days p.i., the cyst wall protrusions of non-degenerating cysts became associated with numerous host cell mitochondria. Although the thick-walled sarcocyst illustrated by the author is similar to the thick-walled Sarcocystis species in the present study, no association between protrusions and host cell mitochondria was apparent in the latter.

The life cycle and host specificity of a second dog-transmitted species of sheep, namely S. arieticanis, was reported by Heydorn (1985). Sporocysts were isolated and inoculated into sheep. Two febrile reactions were recorded in sheep: at 14-18 d.p.i. and at 23-33 d.p.i. These peaks of high temperature were associated with schizogony. First generation schizonts were found in mesenteric arteries and in the arteries of the mesenteric lymph nodes. Second generation schizonts were present in endothelial cells in different organs, particularly in cardiac and skeletal muscular tissue, on days 26, 27 and 31 p.i. However, no schizonts were found in the kidneys. Large doses of sporocysts proved fatal to lambs and sheep. Cyst walls with hair-like protrusions (seen by light microscopy) were found after 56 d.p.i. Sarcocysts were located in cardiac, tongue, oesophageal and skeletal muscles. Merozoites were found within cysts on day 70 p.i. In mature cysts, the protrusions measured 4,5-9,0 µm in length.

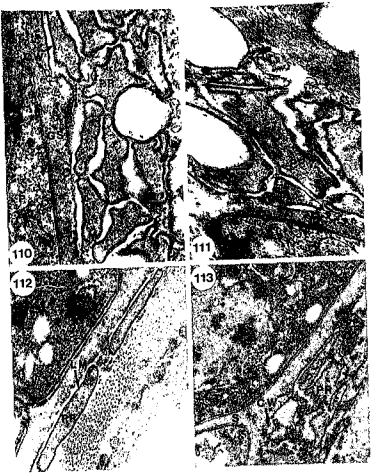
In the dog final host, microgametocytes and macrogametocytes were found in epithelial cells and under the epithelium, respectively, of the posterior half of the small intestine, 15-20 hrs after infection. Sporulated sporocysts of S. arieticanis (9,8-10,5 µm x 15,0-16,5 µm) were shed in the faeces after 12 days p.i. Sporocysts were found not to be infective for cats or dwarf goats.

Heydorn (1985) states that the results show that two different



FIGURES 110 and 111: Ultrastructure of the cyst wall protrusions of Sarcocystis of the sheep Ovis aries from the Transvaal. The protrusions in cross (Figure 110) and longitudinal (Figure 111) section are wide, flat and ribbon-like. The cyst wall is invaginated to form teat-like structures between the bases of the protrusions. 110: x37500; 111: x37500. Fixative: glutaraldehyde.

FIGURES 112 and 113: Ultrastructure of cyst wall protrusions of Sarcocystis of the sheep from Natal. Figure 113 suggests that the protrusions are wide, flat and ribbon-like. However, in some sections (Figure 112), the protrusions have a distinct bar- or bean-shaped appearance. 112: x45000; 113: x30000.


110
111
112
113

species of Sarcocystis, both having the dog as a final host, are found in the musculature of sheep. Although electron micrographs of the ultrastructural morphology of the cyst wall of S. arieticanis were not presented in the study by Heydorn (1985), the light microscopic description given of the protrusions sounds like that of the hair-like protrusions of the thin-walled cyst in sheep illustrated by O'Donoghue et al. (1986) and Bergmann and Kinder (1975) and which has also been found in South Africa (J. B. Bush, 1987, personal communication). It is possible that S. arieticanis is the same species of Sarcocystis as that having hair-like cyst wall protrusions at the electron microscopic level.

Two morphologically distinct types of microcyst in British sheep were reported by O'Toole et al. (1986). One was described as a thin-walled cyst (1-2 µm) with widely spaced biconcave protrusions that were 10-12 µm long. As the hair-like protrusions of thin-walled cysts do appear biconcave in section, it is likely that the thin-walled cyst type may represent the second dog-transmitted species of sheep Sarcocystis i.e. S. arieticanis.

Erber (1982) studied the life cycle of a thin-walled microscopic Sarcocystis species (which he referred to as "S. tenella") in sheep. Infected mutton (oesophagus and diaphragm) was fed to dogs, which shed sporulated sporocysts (13,75-15,8 µm x 9,7-10,8 µm) after a prepatent period of 8-13 days, depending on the number of cysts ingested. Conventionally-reared lambs (4-6 months old), inoculated orally with sporocysts, developed a high fever on day 20 p.i., which was followed by anaemia, anorexia and paresis. At death, on days 28 and 31 p.i. in two lambs, petechial haemorrhages of the internal body surface and seromucous fluid were apparent in the abdominal and thoracic cavities. Immature sarcocysts were located at 50 d.p.i. On day 70 p.i., the characteristic hair-like protrusions of the thin-walled dog/sheep cyst measured up to 8 µm in length. Fully mature cysts with numerous merozoites and a few metrocytes were found at 117 d.p.i. However, thick-walled cysts of S. tenella ("ovicanis") were also found in the tongue, oesophageal, heart and skeletal muscles at 60 d.p.i. Although the size of the sporocysts and the course of infection of the thin-walled microscopic dog/sheep Sarcocystis species in lambs is almost the same as that of thick-walled S. tenella ("ovicanis"), Erber (1982)

concluded that the former is a separate species as the hair-like protrusions clearly distinguish it from S. tenella ("ovicanis") from day 60 p.i. onwards. Although there are differences in the stages of parasitaemia and sporocyst size between the thin-walled microscopic dog/sheep Sarcocystis species studied by Erber (1982) and S. arieticanis (see Heydorn, 1985), both are described as having cysts with hair-like protrusions, and may be the same species. If this is found to be the case, then the thin-walled dog/sheep Sarcocystis species referred to by Erber (1982) would be S. arieticanis. However, Erber (1982) suggested that the name S. tenella should be retained for this second microscopic dog/sheep Sarcocystis species, as it is well-suited to the morphological properties of the cyst. In that case, S. ovicanis would then have to be considered a separate species of Sarcocystis and not a synonym of S. tenella. However, the matter, inter alia, of priority in regard to scientific names would need to be taken into account (in the unlikely event of the suggested name changes proving to be desirable).

Isoenzyme electrophoretic studies were carried out by O'Donoghue et al. (1986) on macroscopic cysts from sheep, cattle and mice, and on microscopic cysts from sheep, cattle and goats. Based on 15 enzymes, genetic and phenotypic differences were found between four broad categories of cysts examined; almost 87% fixed differences were scored between the macrocysts from sheep, microscopic cysts (i.e. "S. tenella"*, S. capracanis and S. cruzi), macroscopic S. hirsuta and S. muris. Differences between the microscopic cysts from sheep and goats (i.e. both thick- and thin-walled dog/sheep Sarcocystis species, and S. capracanis) were less than 25%, but both differed from S. cruzi in almost 50% of the scores. The macroscopic S. gigantea cysts and the macroscopic S. medusiformis cysts had less than 80% fixed differences between them. The authors stated that electrophoretic techniques can be used to characterize and estimate the degree of genetic divergence between various

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* Both thin- and thick-walled microscopic dog/sheep Sarcocystis species were provisionally referred to as "S. tenella" by O'Donoghue et al. (1986).

Sarcocystis species, and to provide genetic confirmation of the taxonomic classification of species. The technique provides more evidence to justify the separation of morphologically dissimilar species of Sarcocystis, in addition to use of (more laborious) cross-transmission studies as criteria. Although the seven genetic types found in the study correlated well with morphotypic classifications, the degree of difference between the microscopic dog/sheep Sarcocystis species and S. capracanis, being less than 25%, *was considered insufficient evidence for their being separate* biological species. Thin-walled cysts from sheep, having been pooled for electrophoresis with thick-walled cysts of S. tenella (syn. S. ovicanis) for the study, were found to have thin, hair-like protrusions when examined ultrastructurally by the authors. This cyst was ultrastructurally similar to thin-walled cysts from sheep found by Bergmann and Kinder (1975) i.e. the second microscopic dog/sheep Sarcocystis species.

Ford (1986) reported differences in electrophoretic mobility for *seven out of 12 enzymes, correlated with* "fat" *macrocysts* (S. gigantea) found mainly in the oesophagus and "thin" macroscopic cysts (S. medusiformis) found mainly in the skeletal muscle of sheep. This degree of difference validates the separation of "fat" and "thin" macroscopic cysts from sheep as distinct species. Both of these cyst types showed isoenzyme differences from microscopic cysts from cardiac muscle of sheep.

Conclusion

There appear to be at least four species of Sarcocystis in sheep: S. gigantea and S. medusiformis, both with macroscopic cysts; the thick-walled microcyst, S. tenella ("S. ovicanis") and a thin-walled microcyst, S. arieticanis.

V. GOATS

Thin- and thick-walled sarcocysts of "S. capracanis" from goats *were examined by Aryeetey* et al. (1980).

The thick-walled cysts in heart, tongue, skeletal muscle, diaphragm and brain had a similar structure to S. tenella

("ovicanis") (see Mehlhorn et al., 1976), but were bigger in width (1,2 µm).

Sarcocysts found in the tongue had thin cyst walls (0,5-0,7 µm), and protrusions (0,5 µm x 0,5 µm) similar to those of thin-walled cysts described from sheep by Bergmann and Kinder (1975). The thin-walled goat cyst was considered a second caprine Sarcocystis species by Aryeetey et al. (1980). If this is indeed the case, then "S. capracanis" is not the only Sarcocystis species in goats which is morphologically similar to a species found in sheep. Whether the thin-walled cysts from goats are an identical species to the thin-walled cysts from sheep (Bergmann and Kinder, 1975) requires further investigation, including cross-transmission experiments.

Another complication is the fact that both of these species are ultrastructurally similar to the cyst wall of S. cruzi of cattle (see illustrations in Böttner, 1984), and to the bean-shaped protrusions of cysts from the impala (second type) found in the present study.

A dog-transmitted Sarcocystis species of goats was investigated by Heydorn and Unterholzner (1983) which they named S. hircicanis. Although no ultrastructural study of the cyst wall was carried out by the authors, it was described as having filiform protrusions. From light micrographs, the protrusions appeared hair-like and measured 5,0-3,5 µm in length. Cysts developed by about day 42 p.i. in the heart, tongue, oesophagus and striated muscle, and after 77 days p.i., filiform protrusions appeared on the cyst wall.

Although no ultrastructural comparison of S. hircicanis can be made with the thin-walled cysts from goats illustrated by Aryeetey et al. (1980) (and no comparison can be made with regard to development as the thin-walled cyst was located by Aryeetey et al. (1980) in a single animal on day 118 p.i.), it may turn out that these two thin-walled cyst forms are both S. hircicanis.

Despite the ultrastructural similarity between S. capracanis (or one of the possible two species for which this name is currently used) and S. tenella (syn. S. ovicanis), transmission experiments have suggested that they may not be identical (see Aryeetey et al., 1980). Heydorn (1985), in fact, stated that despite the morphological similarity between Sarcocystis species of goats and sheep, they are specific for their intermediate hosts. He found

that sporocysts of S. capracanis and S. hircicanis were not infective for sheep.

Nevertheless, an electron micrograph of the developing cyst wall of S. capracanis at 56 d.p.i. in the dissertation by Aryeetey (1979) (identical to a micrograph in Aryeetey et al. (1980) for S. capracanis on day 43 p.i.) is similar to the developing cyst of S. tenella on day 58 p.i. (Dubey et al., 1982). An electron-dense core at the proximal end of the protrusions in immature cysts of S. tenella was also present in immature sarcocysts of S. capracanis (see Dubey et al., 1982). Palisade-like protrusions of S. capracanis, examined ultrastructurally by Dubey et al. (1984) from a goat killed 64 d.p.i., were 2,47 µm x 0,5 µm. in size. The cyst wall was 16,2 nm thick and the protrusions contained a core of microfilaments. From the micrographs it appears that the protrusions are thin, and similar to the longitudinal sections of protrusions of the microcyst found in sheep in the present study (Figure 111). Dubey et al. (1984) stated that their cyst was structurally similar to those found by Fischer (1979) and Aryeetey et al. (1980). Except for minor variations, the protrusions were similar in size at 64 d.p.i. to the palisade-like protrusions observed by Aryeetey et al. (1980) at 56, 92 and 118 d.p.i.

TABLE 5: Measurements of sarcocyst components from micrographs of various magnifications.

In most cases, the measurements were taken from micrographs of a single cyst from a single animal.

Where more than one cyst of the same species of Sarcocystis was measured from the same animal, this is indicated by the number of measurements given for cyst diameters for that specimen in Table 5.

In cases where more than one animal of the same species from a particular region had the same kind of sarcocyst (based on morphological structure), the measurements for the components were *pooled - as indicated* in Table 5, where more than one specimen number precedes a group of measurements.

Cysts were measured randomly and no selection was made on the basis of maturity and/or size. The number of measurements for each component varies, *and was dependent on the number and suitability* of the micrographs. It is assumed that the measurements follow an approximately "normal" distribution.

In Table 5, the number of measurements* (NO.) for each component (see component code key: C.) is stated and the sample mean (S.MN) is the mean value of all the measurements for that component. Minimum (MIN.) and maximum (MAX.) values indicate the range over which the measurements extended. The sample standard deviation (S.S.D.) indicates the interval on both sides of the mean within which 68% of the measurements lie.

Statistical analysis of the data, using Student's-t test based on the sample mean, allowed determination of a confidence interval for the population mean (P.MN) at a 95% confidence level. The population mean is the theoretical mean which would be obtained from all possible measurements of one component, with a 95% *probability that its value would lie* within the confidence interval. In cases where mathematical calculations yielded a negative value for the lower limit of the confidence interval, it was set equal to 0,00, as a 0% probability of the population mean

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* The individual measurements for all the components are listed in Appendix 7.

being a negative number was assumed. A confidence interval for the population standard deviation (P.S.D.), based on the sample standard deviation, was determined at a 95% confidence level using the Chi-square test. The population standard deviation is the theoretical standard deviation which would be obtained from all possible measurements of one component, with a 95% probability that its value would lie within the confidence interval.

The components of the cysts were measured in millimeters, from which the equivalent dimensions in micrometers (μm) were calculated, according to the magnifications of the micrographs. All measurements for the cyst components in Table 5 are stated in micrometers (μm).

In order to measure the sarcocyst diameters from semi-thick sections on microscope slides, a magnification factor for an ocular micrometer (graticule in eyepiece) was used. The magnification factor was determined by calibration of a SM-Lux Leitz microscope with the aid of a 0,01 interval stage scale.

KEY:

C. Component code
NO. Number of measurements
MIN. Minimum value
MAX. *Maximum value*
S.MN Sample mean
S.S.D. Sample standard deviation
P.MN Population mean at a 95% confidence level
P.S.D. Population standard deviation at a 95% confidence level

COMPONENT CODE KEY (C.):

a Width of cyst wall including ground substance
b Length of protrusions (longitudinal section: L.S.)*
c Width of protrusions (L.S.)*
d Longest measurement for protrusions (cross section: X.S.)**
e Shortest measurement for protrusions (X.S.)**
f Length of merozoites (L.S.)
g Width of merozoites (L.S.)
h Distance between the bases of protrusions at cyst wall
i Longest diameter of cyst in semi-thick sections (X.S.)**
j Shortest diameter of cyst in semi-thick sections (X.S.)**
k Length of cyst in semi-thick sections (L.S.)
l Width of cyst in semi-thick sections (L.S.)

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* Complete longitudinal sections of the protrusions were not obtained in cases where they were convoluted and/or not lying perfectly in the plane of section (see also Figure 116 in Appendix 7). Measurements for b and c refer to the parts of the protrusions visibly attached to the cyst body in the micrographs. A longitudinal section of a protrusion which was not connected at its base to the body of the cyst, was never measured. The reader should refer to the appropriate Figures (micrographs) when consulting the data in Table 5.

** In cases of perfectly circular cross sections of protrusions, measurements for d and e would be identical. Similarly, in cases of perfectly circular cross sections of cysts, measurements for i and j would be identical.

ORDER: RODENTIA
FAMILY: MURIDAE
SUBFAMILY: MURINAE
Spiny mouse Acomys spinosissimus
Region: Transvaal (Eastern)
Cyst Wall Morphology: Simple, invaginated cyst wall.

SPECIMEN NUMBER(S): TM 30578

C.	NO.	MIN.	MAX.	S.MN	S.S.D.	P.MN	P.S.D.
a	5	0,70	1,10	0,90	0,18	0,68 - 1,12	0,11 - 0,52
b	10	0,68	3,20	1,50	0,84	0,90 - 2,10	0,58 - 1,53
c	5	0,45	0,55	0,50	0,04	0,45 - 0,56	0,03 - 0,12
f	3	5,00	6,40	5,88	0,77	3,97 - 7,79	0,40 - 4,81
g	3	1,50	1,80	1,64	0,15	1,26 - 2,02	0,08 - 0,95
i	1			325,0			
j	1			175,0			

Note: Measurements for the lengths (b) and widths (c) of protrusions are for the tooth-like undulations or "protrusions" of the primary cyst wall and ground substance.

Multimammate mouse Praomys natalensis
Region: Transvaal (Eastern)
Cyst Wall Morphology: Simple, invaginated cyst wall.

SPECIMEN NUMBER(S): TM 30557

C.	NO.	MIN.	MAX.	S.MN	S.S.D.	P.MN	P.S.D.
a	5	0,46	0,50	0,48	0,02	0,46 - 0,49	0,01 - 0,04
f	5	4,67	5,40	5,09	0,34	4,67 - 5,52	0,20 - 0,98
g	5	1,30	2,00	1,62	0,26	1,30 - 1,94	0,16 - 0,74
i	1			42,5			
j	1			42,5			

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