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# The Developmental Cycle of a Species of *Sarcocystis* Occurring in Dogs and Sheep, with Observations on Pathogenicity in the Intermediate Host

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*Summary.* Twelve dogs were fed mutton containing small sarcocysts, and killed 1, 3, 4, 6, 7, 10, 15, 16, 17 days after infection (DAI). Beginning 13–15 DAI sporocysts $14.7 \times 9.0$ μm were passed in the faeces of the dogs killed 15–17 DAI. Histological examination showed that developing stages were most numerous in the subepithelial tissue at the tips of villi in the proximal third of the small intestine. Macrogametes containing tiny PAS+ granules, and microgametocytes with peripheral developing microgametes were present 1 DAI. By 4 DAI oocysts, with a small nucleus and vacuolate cytoplasm were seen. Sporulation was observed 7–10 DAI. The first nuclear division resulted in 2 polar nuclei which divided laterally, resulting in 2 sporocysts each with 2 polar nuclei. This process was repeated once more to produce 4 nucleated sporozoites in each of 2 sporocysts. PAS+ granules were seen at the periphery of sporulating oocysts and sporocysts. There was a large PAS+ granule in the mid zone of sporozoites, with a smaller granule at one tip. Numerous sporulated sporocyst pairs were present beneath the epithelium at the tips of villi in dogs killed during patency.

Four lambs were inoculated orally with sporocysts passed by dogs following feeding of infected mutton. Fifteen DAI schizonts were seen in the endothelium of arteries and arterioles in many organs, but not brain. Twenty-four DAI, smaller schizonts were seen in capillary endothelium in many organs, including brain. The two other lambs died 42 and 104 DAI, after an illness characterized by anaemia and ill-thrift. Mature schizonts were found in cells in the brain 42 DAI, associated with nonsuppurative meningoencephalitis. Developing sarcocysts were found in muscle, associated with myositis. Sarcocysts in muscle 104 DAI were mature. In the brain there were degenerate cysts and mature sarcocysts, and non-suppurative meningoencephalitis.

## Introduction

In 1970 Fayer was able to demonstrate the probable coccidial nature of *Sarcocystis* by the use of tissue cultures, and later (Fayer, 1972) he studied gametogony of this organism *in vitro*. A number of workers have subsequently reported the relationship between the consumption of meat of cattle, sheep and pigs containing sarcocysts, and the appearance of *Isospora*-like oocysts or sporocysts in the faeces of dogs, cats and man. (Rommel, Heydorn and Gruber, 1972; Heydorn and Rommel, 1972a; Rommel and Heydorn, 1972; Mahrt, 1973).

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Heydorn and Rommel (1972b) described the development of oocysts in the lamina propria of villi in the small intestine of cats fed large sarcocysts isolated from bovine oesophagus, and recently the ultrastructure of oocysts and sporocysts, developing in the lamina propria of small intestinal villi of cats fed large sarcocysts from sheep oesophagus, has been studied (Mehlhorn and Scholtyseck, 1974). Fayer (1974) has illustrated the gamonts and the sporogony of oocysts developing in the small intestine of dogs fed *Sarcocystis*-infected beef.

Schizogony in a variety of organs, the development of sarcocysts in muscle, and the potential pathogenicity of heavy infections have been described in cattle fed sporocysts recovered from canine faeces. (Fayer and Johnson, 1973, 1974).

We have been involved in work defining the existence of separate cat-sheep and dog-sheep cycles of *Sarcocystis* (Ford, 1974; Munday and Corbould, 1974; Munday and Rickard, 1974). Descriptions of gametogony and sporogony of organisms in the intestine of dogs fed sheep meat containing sarcocysts, and of asexual stages and associated pathology in the tissues of sheep fed sporocysts of canine origin, are presented here.

## Materials and Methods

*Dogs.* Thirteen young dogs raised without access to raw meat were individually caged. Faecal flotation with 40% w/v sucrose solution or saturated sodium nitrate solution (specific gravity 1.36) revealed occasional oocysts of *Isospora rivolta* or *Isospora canis* in some dogs.

Eleven of these dogs were fed mutton (oesophagus, abdominal muscle, heart or diaphragm) from aged sheep slaughtered in abattoirs in the Launceston or Melbourne areas. Samples of mutton were usually fixed, sectioned and examined microscopically to confirm the presence of microscopic sarcocysts. In some cases macroscopic sarcocysts were also present. Mutton was coarsely minced and dogs were usually fed it over a period of 3–10 days or until prior killing. The twelfth dog was fed on mutton from two sheep obtained from Trefoil I. in Bass Strait, which has no cats. This meat was examined both grossly and microscopically and was found to have no macroscopic, but numerous microscopic, sarcocysts. The remaining (control) dog was fed dog pellets, and was kept in the same room as two of the dogs killed 15 days after infection (DAI).

Faecal flotations were examined daily until the dogs were killed, with the exception of the control dog which was checked until the equivalent of 34 DAI. During the course of 5 separate experiments single dogs were killed 1, 3, 4, 6, 7, 10, 16 and 17 DAI while 4 dogs were killed 15 days after first consuming infected mutton.

Sections of small intestine were removed at 6 approximately equivalent intervals between the pylorus and ileocaecal valve immediately after the death of 5 dogs. In another 7 dogs tissues were recovered from the duodenum 20–30 cm below the pylorus. Brain, liver, kidney, lung, mediastinal and mesenteric lymph node and in some cases, stomach, were also taken. Tissues were fixed in Bouin's solution or 10% formal saline, embedded in paraffin wax and sectioned at 6 μm. Sections were subjected to routine procedures for staining by haematoxylin and eosin or periodic acid-Schiff (PAS). Scrapings of fresh mucosa were also examined on a microscope fitted with a warming stage (37°C).

*Lambs.* Three lambs were removed from their dams at birth and fed an artificial milk replacer for 48 hours, after which they were returned to the ewes and allowed to suck. A further three lambs were allowed to suck their dams from birth. All lambs were free of *Sarcocystis* antibodies immediately prior to dosing the four inoculated animals.

When they were 28 and 42 days old respectively, two of the lambs reared entirely on their mothers were infected with 500000 and 200000 sporocysts passed by a dog which had been fed mutton from a sheep originating on Trefoil I. They received intramuscular dexamethasone injections[1] (2 mg/day) for 11 days before and 10 days after infection. One of these lambs

1 Dexadreson, Intervet (Aust.) Pty.Ltd.

died of nutritional muscular dystrophy 15 DAI. The second was killed 24 DAI. The third lamb was a negative control used in other work, held in association with the other two animals, and killed 30 days after they were infected.

Two of the 3 colostrum-deprived lambs, 7 and 11 days of age, were fed large numbers (unquantified) of sporocysts passed by a dog which had been fed mutton containing macroscopic and microscopic sporocysts. One animal died 42 DAI. Muscle biopsies were taken monthly from the uninoculated control and the other inoculated lamb, which died 104 DAI.

Blood samples were taken from experimental lambs at birth, and subsequently weekly, for detection of antibodies against *Sarcocystis* and *Toxoplasma gondii* (Munday and Corbould, 1971, 1974). Muscle biopsies and tissues obtained from lambs at death (skeletal muscle, myocardium, diaphragm, oesophagus, brain, lung, liver and kidney) were fixed in 10% formal saline and processed for light microscopy as described above.

Measurements were made with a calibrated eyepiece micrometer on a Reichert Zetopan microscope using an oil immersion objective.

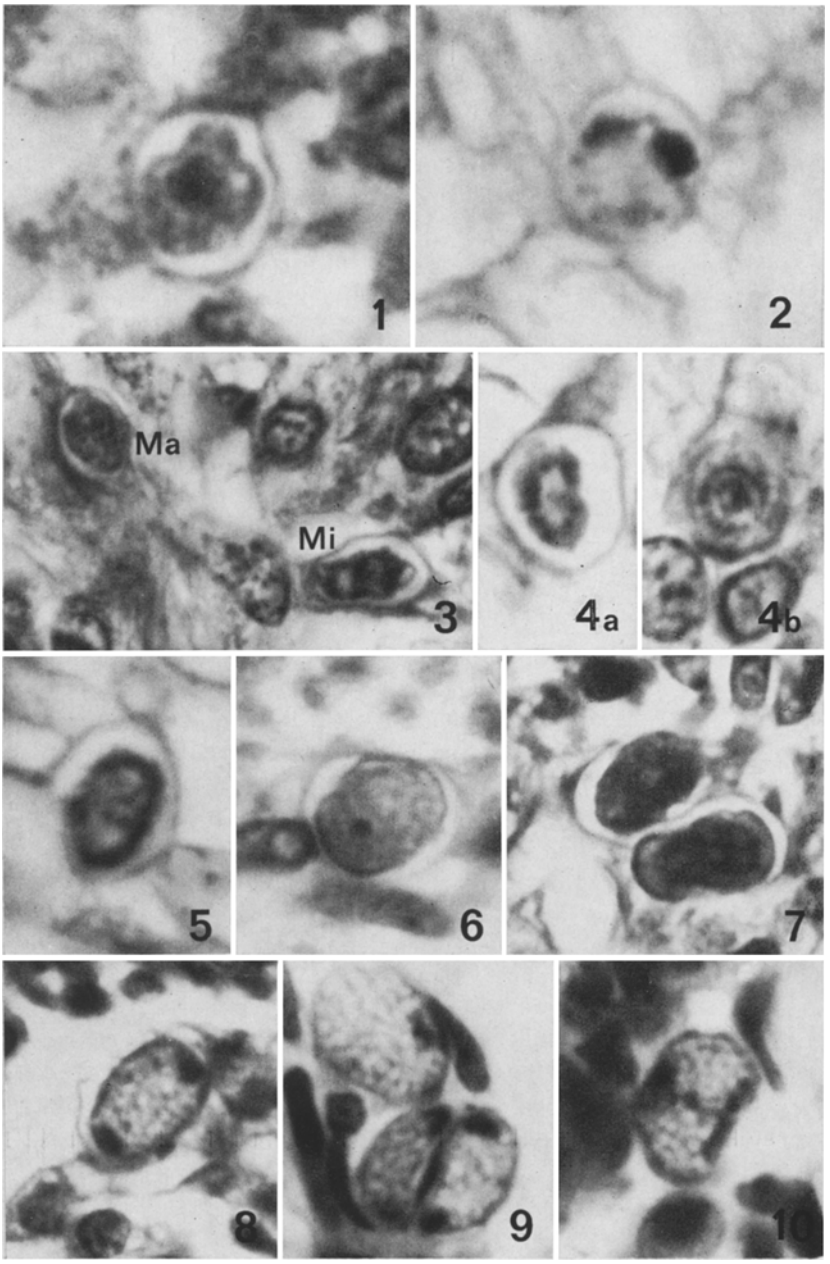
## Results

*Dogs Infected with Mutton Containing Sarcocysts.* Beginning 13–15 DAI all 6 dogs killed more than 10 days after the first feed of infected mutton passed sporocysts which were about $14.7 \times 9.0$ μm and contained 4 sporozoites and a compact granular residual body. There was no stieda body, and sporocysts were often slightly flattened on one side. The one control dog did not pass oocysts or sporocysts of any type up until observations ceased 34 DAI.

Parasites were found in the immediately subepithelial lamina propria in the distal half of villi in sections of small intestine of all dogs fed mutton except for the animal killed 3 DAI. In the 5 animals in which distribution along the intestine was examined, organisms were most common in tissue from the anterior third of the intestine, although they were seen in the 4 more distal samples.

In the dog killed 1 DAI, gamonts were observed in parasitophorous vacuoles at the level of the basal lamina of the epithelium near the tips of villi. It was difficult to determine if they were within host cells, and organisms in PAS-stained sections occasionally appeared to be on the lumenal side of the basal lamina, between enterocytes. Others were definitely within the lamina propria, adjacent to a crescent-shaped host-cell nucleus. Macrogametes varied from about 4.2 μm in diameter up to bodies about $5.6 \times 4.2$ μm (Fig. 1). They were irregular in shape, and had a basophilic nucleus lying within a moderately basophilic cytoplasm. Some had pale-stained areas or vacuoles in the cytoplasm, particularly near the periphery (Fig. 1). Extremely small periodic acid-Schiff positive (PAS+) granules or masses of granules were scattered throughout the cytoplasm of some macrogametes at this time (Fig. 2).

Microgametocytes were very difficult to identify with certainty. However, a proportion of the gamonts formed a population distinct from macrogametes in that they were larger, and more elongate (about $7.0 \times 4.2$ μm) with basophilic material scattered irregularly throughout and around the edge of the cytoplasm (Fig. 3). Rare gamonts had a small pale central area with a number of basophilic bodies around its margin, considered to be nuclei peripheralizing around the central residual body of a microgametocyte (Figs. 4a, 4b). Occasionally protrusions from the margin of such organisms were observed, possibly the flagella of microgametes (Figs. 4b, 5). In a fresh mucosal scraping from this dog a single spherical




Figs. 1—12. Stages in small intestine of dogs fed *Sarcocystis*-infected mutton

Fig. 1. Macrogamete at the base of the epithelium in dog small intestine 1 DAI. Note the central nucleus and several peripheral vacuoles in the cytoplasm. H & E. ×3800

Fig. 2. Macrogamete with PAS+ granules in the peripheral cytoplasm 1 DAI. PAS. ×3600

Fig. 3. Mononuclear gamont, probably macrogamete (Ma) and more elongate gamont with irregular basophilic material throughout and around periphery of cytoplasm, possibly a microgametocyte (Mi) 1 DAI. H & E. ×2100

body about 8 μm in diameter with peripheral granules and numerous rapidly undulating flagella was observed and interpreted as a microgametocyte.

In the dog killed 4 DAI more advanced macrogametes, with a central densely basophilic nucleus and pale basophilic cytoplasm containing numerous marginal vacuoles, were observed, and these evolved to oocysts. Oocysts were ovoid, and about 11.2×9.8 μm with a small basophilic nucleus and vacuolate cytoplasm (Fig. 6). They were surrounded by a distinct eosinophilic wall, which frequently was crenated and wrinkled in sections. Oocysts which were forming an oocyst wall often appeared to have eosinophilic polar "caps" (Fig. 7). Vacuoles containing oocysts were up to 14×9.8 μm and oocysts may have shrunk during processing. In PAS-stained preparations, developing macrogametes had tiny PAS+ granules scattered among peripheral vacuoles. PAS+ granules were more numerous in oocysts. Microgametocytes resembling those described 1 DAI were seen, but were considered to be related to the continuous ingestion of infectious material.

Organisms were few in the dog examined 6 DAI, and were developing macrogametes and oocysts like those seen 4 DAI.

At 7 DAI oocysts in the subepithelial tissues were in the process of sporulation. Oocysts with a single basophilic nucleus, and a cytoplasm packed with a mass of vacuoles in an eosinophilic matrix, were about 12.6–14.0 μm×8.4–9.8 μm. They completely filled the parasitophorous vacuole. The first sporulation division resulted in an oocyst with 2 densely basophilic nuclei, at opposite poles of the cytoplasm (Fig. 8). These nuclei then appeared to divide, accompanied by schism of the cytoplasmic mass into two portions. The result was two sporocyst progenitors, each with 2 polar U-shaped nuclei (Fig. 9). In some cases this second nuclear division appeared to be followed almost immediately by a third division (Fig. 10) followed by a separation of the cytoplasm within sporocysts. This stage was the most advanced seen 7 DAI, and such organisms had 2 distinct sporocysts, each containing 2 separate elongate masses of vacuolate cytoplasm with 2 nuclei at opposite poles.

Fig. 4a. Probable early microgametocyte with small basophilic nuclei surrounding a central pale-staining mass in the cytoplasm 1 DAI. H & E. ×2300

Fig. 4b. Probable microgametocyte, with central residuum, peripheral nuclei and projections, possibly flagella, into the parasitophorous vacuole. 1 DAI. H & E. ×1700

Fig. 5. Probable microgametocyte with basophilic margin and projections, possibly microgametes, into the parasitophorous vacuole 1 DAI. H & E. ×2300

Fig. 6. Developing oocyst with eosinophilic wall, vacuolate cytoplasm and single nucleus 4 DAI. H & E. ×1700

Fig. 7. Developing oocysts with eosinophilic polar caps 4 DAI. H & E. ×1600

Fig. 8. Oocyst after first nuclear division of sporogony. 7 DAI. H & E. ×1600

Fig. 9. Two oocysts; the lower one has undergone the second nuclear division of sporogony and the cytoplasm has divided to produce 2 sporocysts, each with 2 polar U-shaped nuclei. H & E. ×1600

Fig. 10. Oocyst containing 2 sporocysts. Nuclei in the lower pole of each sporocyst appear to be undergoing the third nuclear division of sporogony. H & E. ×1600

In some instances the sporocyst wall appeared as a clear, yellow, crinkled membrane. PAS+ granules, in some cases large and tending to obscure the cytoplasm, were scattered throughout the cytoplasm and at the margin of unsporulated oocysts, and around the periphery of sporocysts in oocysts undergoing sporulation (Fig. 11). Similar stages were observed in the dog killed 10 DAI.

In dogs with patent infections (15–17 DAI) all stages of oocyst formation and sporulation were seen. Thirty fully sporulated sporocysts in 15 pairs in villi in fresh mucosal scrapings from one animal were 14–15.4 × 7.7–11.2 (mean 14.7 × 9.0 μm). Sporocysts now contained 4 falciform sporozoites each with a small polar and larger central mass of PAS+ granules (Fig. 12). These was also a central basophilic nucleus. Sporocysts usually seemed related in pairs, but it was difficult to determine if an oocyst wall was still intact or if the organisms were intracellular. They usually appeared not to be. In some animals sporocysts were extremely numerous, particularly beneath the epithelium at the extreme tips of the villi.

There appeared to be no cellular reaction to the presence of the parasites in the intestine. No schizonts were found in the intestine, nor were any protozoon organisms observed in extraintestinal tissues.

*Sheep Infected with Sporocysts from Dog Faeces.* In the lamb which died 15 days after ingesting sporocysts, schizonts containing submature merozoites were observed in the endothelium of small arteries and arterioles and occasionally capillaries, in mesenteric lymph node, skeletal muscle and heart (Fig. 13). These schizonts were about 17–35 μm in diameter. No schizonts were seen in brain. In the endothelium of a vessel in the mesenteric lymph node, a large basophilic mononuclear organism resembling a metrocyte was seen (Fig. 14).

Twenty-four days after infection small schizonts containing submature or mature merozoites were seen in the endothelium of terminal arterioles near the glomerulus, or in glomeruli (Fig. 15), in hepatic sinusoids, in lymph node, capillaries in skeletal muscle, and in small vessels and capillaries in the spinal cord, meninges and brain (Fig. 16). Most schizonts in glomeruli were small, about 6–10 μm in diameter, but several immature schizonts were elongate, up to 30 × 10 μm. In capillaries in muscle, schizonts were about 10 μm in diameter, while in brain they measured 11–14 μm. No organisms were found at 30 DAI in the control lamb.

The two lambs infected for longer periods died 42 and 104 DAI, following an illness characterized by anaemia, weakness and poor growth. At no stage did their sera contain antibody to *Toxoplasma gondii*, but both developed complement-fixing antibody against *Sarcocystis* 5 and 6 weeks after infection respectively. Developing sarcocysts were present in muscle of the lamb which died 42 DAI. They were about 50 × 10 μm, and contained ovoid young zoites. The cyst wall was extremely thin, being barely discernable under the light microscope. There was non-suppurative myositis in skeletal muscle, myocardium, and oesophageal and tongue muscle. Small schizonts, containing mature merozoites, were present in swollen host cells (possibly glial cells or capillary endothelium) in the brain (Fig. 17). There was a non-suppurative meningoencephalitis characterized by meningitis, perivascular cuffing and focal gliosis.

In a muscle biopsy taken from the fifth lamb 60 DAI, developing sarcocysts similar to those observed 42 DAI were seen (Fig. 18), whereas no sarcocysts were found in a biopsy taken from the control lamb at the same time. At death, 104

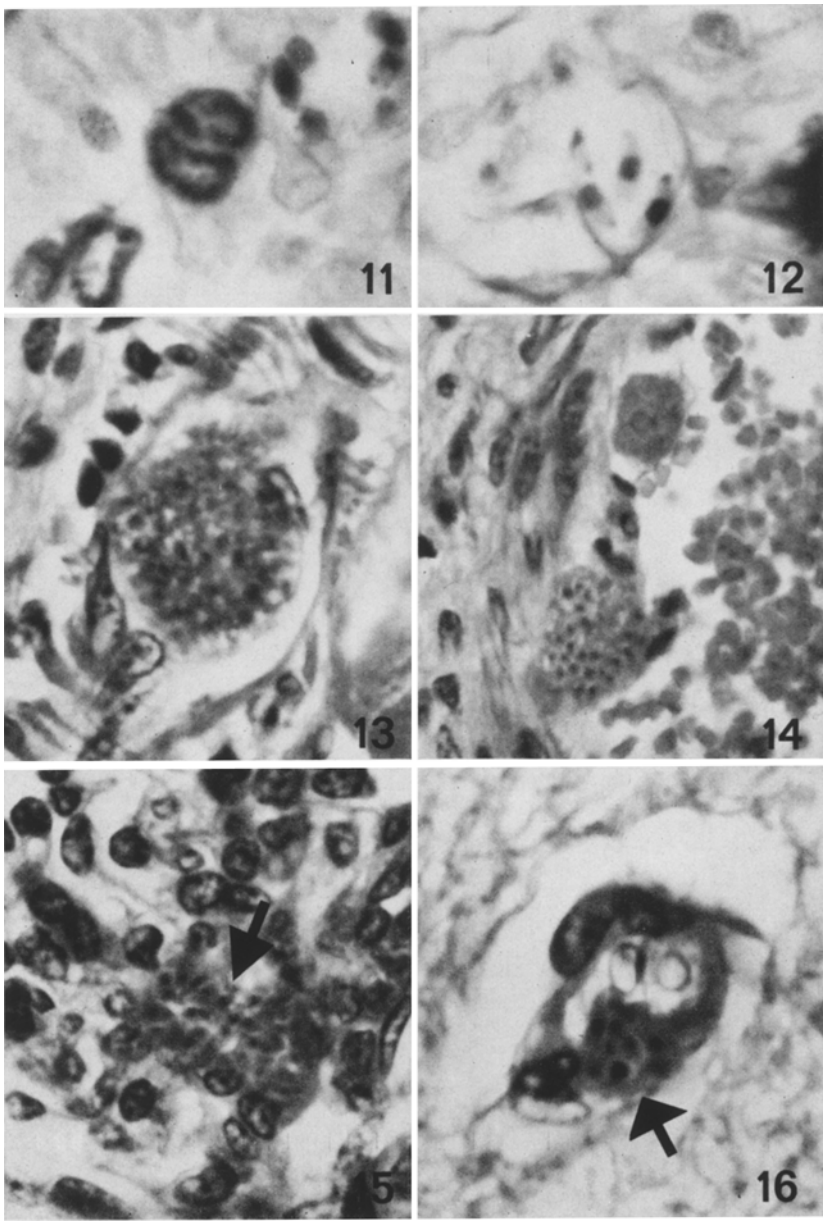



Fig. 11. PAS+ material around the periphery of sporocysts and in sporozoites in sporulating oocysts. PAS. ×1600

Fig. 12. Large PAS+ aggregate in middle and small PAS+ granule at tip, of sporozoites in fully sporulated sporocyst. 17 DAI. PAS. ×1600

Figs. 13—22. Organisms in sheep inoculated with sporocysts of canine origin. H & E

Fig. 13. Developing schizont in endothelium of a small artery in skeletal muscle 15 DAI. ×1300

Fig. 14. Developing schizont (lower) in endothelium of an arteriole in mesenteric lymph node. Above is a large basophilic mononuclear organism resembling a metrocyte. 15 DAI. ×930

Fig. 15. Schizont (arrow) in glomerulus of lamb killed 24 DAI. ×1200

Fig. 16. Schizont (arrow) in endothelium of a small vessel in the brain 24 DAI. ×1700

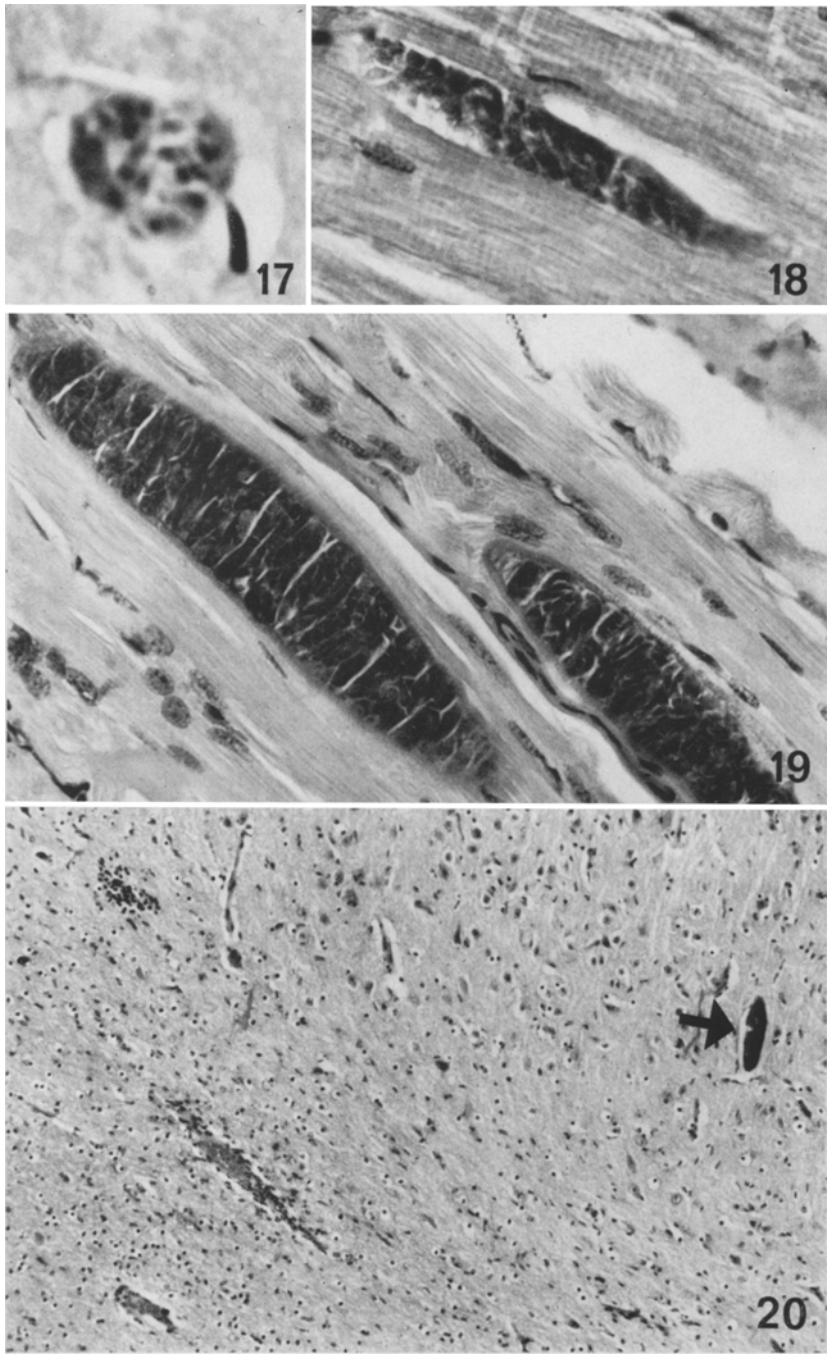



Fig. 17. Schizont, containing mature merozoites, in endothelium of a cerebral capillary 42 DAI. ×1600

Fig. 18. Sarcocyst developing in skeletal muscle 60 DAI. A cyst wall is not discernable. ×620

Fig. 19. Mature sarcocysts in muscle 104 DAI. Note the transversely striated basophilic cyst wall. ×425

Fig. 20. Non-suppurative encephalitis in the brain of the lamb which died 104 DAI. There is perivascular cuffing, gliosis, and a large sarcocyst (arrow) visible. ×110

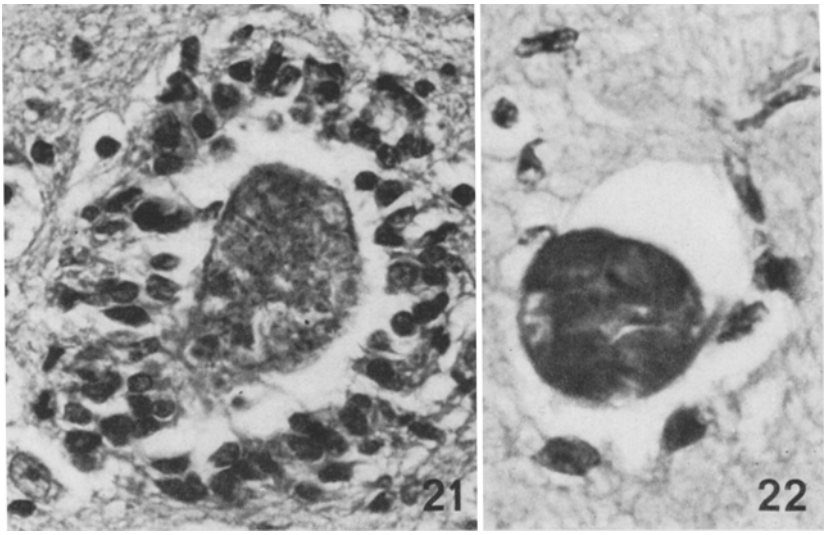



Fig. 21. Degenerate cyst surrounded by glial cells in the brain 104 DAI. ×600

Fig. 22. Sarcocyst developing in ovine brain 104 DAI. Note the peripheral cells resembling metrocytes. ×870

DAI, there was myositis resembling that seen 42 DAI, but the sarcocysts, which were numerous throughout the skeletal muscle and myocardium, were mature (about 200×40 μm) with closely packed zoites, a transverse lamellar pattern and a thick, transversely striated, basophilic cyst wall (Fig. 19). There was extensive non-suppurative meningoencephalitis in this lamb (Fig. 20), associated with the presence of large (up to 50 μm) degenerate cysts, surrounded by a halo of glial cells (Fig. 21), and sarcocysts up to 100×25 μm, with peripheral metrocytes and central zoites (Fig. 22), and in some cases, a transversely striated cyst wall.

## Discussion

Work delineating the definitive-intermediate host relationships of the sarcocysts found in sheep has been reported elsewhere (Munday and Rickard, 1974). This demonstrated the independent existence of a cat-sheep cycle of *Sarcocystis* involving large slow-growing macroscopic cysts in the sheep, and a dog-sheep cycle producing microscopic, more rapidly infective sarcocysts in ovine muscle. It is this latter cycle previously demonstrated by Ford (1974) and Munday and Corbould (1974) which we describe here. There is no evidence that the ovine *Sarcocystis* with a feline definitive host is capable of producing sporocysts in dogs, and we did not eliminate the possibility of feeding such cysts to dogs in the current experiments.

The location and general morphogenesis of stages we have described in the intestinal villi of the dog resemble those found in cats fed cysts in bovine muscle (Heydorn and Rommel, 1972b), cats fed cysts from ovine muscle (Mehlhorn and Scholtyseck, 1974) and dogs fed bovine cysts (Fayer, 1974). However, neither

Heydorn and Rommel (1972b) nor Fayer (1974) observed microgametocytes, in the latter case perhaps because the first observation made was 48 hours after administration of a single inoculum. Fayer (1972) had previously demonstrated that microgametocytes in tissue culture were identifiable 24 hours after infection and mature at 30 hours. The organisms which we consider to be microgametocytes (Figs. 3, 4a, b, 5) were present 24 hours after infection, and the most advanced stages observed (Figs. 4b, 5) may not have been mature, although their small size renders interpretation of sections difficult. The dog killed 1 DAI had never passed oocysts of any type, so it is improbable that these organisms were other than *Sarcocystis*.

The morphology and development of macrogametes resembles, except for minor differences in size, that observed by Heydorn and Rommel (1972b) and Fayer (1974). However, we saw small PAS+ granules in the cytoplasm of macrogametes at 1 DAI (Fig. 2) while in the cat such granules were first observed 3 DAI (Heydorn and Rommel, 1972b) and in the dog infected with cysts of bovine origin they were seen first 5 DAI.

Bodies considered to be oocysts, in that they had a complete eosinophilic wall, were present 4 DAI. The eosinophilic "caps" seen here, and by Heydorn and Rommel (1972b), but not by Fayer (1974), are thought to be associated with the formation of the oocyst wall. Oocysts were first found in cats 4 DAI (Heydorn and Rommel, 1972b) but in dogs infected with bovine cysts, macrogamonts comparable to those termed "oocysts" here were not seen by Fayer (1974) until 6 DAI. He thought that the PAS+ granules in the cytoplasm of the developing macrogamete were associated with formation of the oocyst wall, but our observation of such granules as early as 1 DAI and their persistence in sporozoites in fully sporulated organisms, may indicate that they are polysaccharide granules involved in energy storage. Vetterling *et al.* (1973) observed a few such granules during ultrastructural examination of *Sarcocystis* macrogametes grown in tissue culture, but did not comment on their presence in oocysts. Electron microscopic examination will be needed to define the genesis of the oocyst wall in organisms in the intestine.

Our interpretation of sporogony may differ slightly from that implied by Fayer (1974), who reported the existence of uninucleate sporocysts. This may be an artefact as a result of plane of section, since we consider that the cytoplasmic division resulting in two sporocysts occurs after a primary nuclear division producing an oocyst with 2 polar nuclei (Fig. 8) and is simultaneous with a cleavage of these nuclei, resulting in 2 sporocysts each with 2 polar nuclei (Fig. 9). These sporocyst nuclei are frequently U-shaped (Fig. 9) as observed by Mehlhorn and Scholtyseck (1974) and in some cases they appear to be dividing again shortly after sporocyst formation (Fig. 10). At 7 DAI some sporocysts in pairs contained 2 cytoplasmic bodies with a nucleus at each pole, and presumably the cytoplasm in each of these subsequently divides to form uninuclear sporozoites.

It was not possible to determine if organisms were within host cells at all stages, though the ultrastructural study of Mehlhorn and Scholtyseck (1974) suggests that they may be, and the identity of host cells is unknown. In mucosal scrapings 15 DAI sporocysts always appeared to be in pairs, unlike Fayer's

(1974) observation of sporocysts in separate vacuoles or as widely separated individuals.

Our observations on lambs infected with sporocysts passed by dogs after mutton feeding indicate that two cycles of schizogony may exist, since the schizonts found in the lamb 15 DAI had a different distribution (mainly endothelium of arterioles, not in brain) than the smaller, more numerous, schizonts found 24 DAI (mainly in capillaries, including brain). During the early phases of infection after inoculation with faecal forms of *Sarcocystis*, other workers have found schizonts in muscles and brain of mice (Wallace, 1973) in capillaries and sinusoids in numerous organs of cattle (Fayer and Johnson, 1973, 1974) and capillaries in muscle of rats (Rzepczyk, 1974). Whether stages in arterioles are present early in infection of species other than sheep is unknown.

Fayer and Johnson (1974) also observed metrocyte-like cells in endothelium at the same time that schizonts were present.

Developing sarcocysts seen in muscle 42 and 60 DAI differed from those observed at about the same time in calves (Fayer and Johnson, 1974) in that they lacked a distinct cyst wall. The infection in sheep also differed during the later stages from that in cattle, since degenerate cysts and bodies considered to be sarcocysts resembling those in muscle (Figs. 20, 22) were found in the brain 104 DAI. In calves after 40 DAI no schizonts or sarcocysts were found in non-muscular tissues (Fayer and Johnson, 1974). Even so, this finding is not unexpected as mature sarcocysts are found in the brains of a small percentage of sheep and cattle examined by veterinary pathologists (Munday, unpublished; Hartley and Hooper, pers. com.).

We believe that lambs which died at 42 and 104 DAI were suffering from a disease induced by sarcosporidial infection localized mainly in the muscles and central nervous system. Wallace (1973) observed myositis in mice before the appearance of sarcocysts in muscle, 8 mice became overtly ill, and 4 died. The signs described by Fayer and Johnson (1973) in calves dying after inoculation of sporocysts included anaemia, anorexia and cachexia, similar to those we saw in the lambs. Also 3 rats infected by Rzepczyk (1974) with sporocysts from a carpet python died or were killed when ill.

The syndromes observed in mice, cattle and rats seem to be associated with schizogony and one of our lambs died at the end of this phase (42 DAI). Lymphadenopathy was a feature of the disease in calves (Fayer and Johnson, 1973) and rats (Rzepczyk, 1974), but neither author commented on myositis and encephalitis, the predominant findings in fatal infection in the lambs. It seems possible that Dalmeny disease in cattle (Corner *et al.*, 1963) and the disease affecting a calf described by Lainson (1972) were due to *Sarcocystis* infection. The condition produced here in lambs had certain features in common with naturally-occurring protozoan infections described in sheep by Hartley and Blakemore (1973) and McErlean (1974), especially in regard to the involvement of the central nervous system and the presence of schizonts and stages resembling sarcocysts, which were present in the affected tissues described in both these reports (W. J. Hartley, pers. com.).

The development of stages, in both the definitive and intermediate hosts, of the *Sarcocystis* species involved in dog-sheep and dog-cattle cycles is similar,

sporocysts are about the same size, and the prepatent period is approximately the same. The possibility of the existence of a single species of *Sarcocystis* with sexual stages in the dog and asexual stages in the sheep and ox is attractive. The main difference between Fayer's (1974) observations on sexual stages in dogs, and ours is that gamonts and oocysts in dogs infected using mutton were most numerous in the anterior small intestine, while in dogs infected with beef, they were more common in the posterior small intestine. However, detailed cross infection trials, currently underway (Rickard and Munday, unpublished) will be required to define possible interrelationships among cycles of *Sarcocystis* involving dogs and cats as definitive hosts and sheep and cattle as intermediate hosts.

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