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Endogenous development of two Coccidian parasites : Isospora arctopitheci and Sarcocystis hemionilatrantis

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ENDOGENOUS DEVELOPMENT OF TWO COCCIDIAN PARASITES: *ISOSPORA ARCTOPITHECI* AND *SARCOCYSTIS HEMIONILATRANTIS*

by

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Endogenous Stage of Two Coccidian Parasites:
Isospora arctopitheci and *Sarcocystis hemionilatrantis*

Director: C. A. Speer CAS

Development of *Isospora arctopitheci* and *Sarcocystis hemionilatrantis* was studied by light, and scanning (SEM) and transmission (TEM) electron microscopy.

Thirteen marmosets (*Saguinus geoffroyi*) were experimentally inoculated with sporulated oocysts of *Isospora arctopitheci*. Parasites were found at 1-7 days post inoculation (DPI) in epithelial cells of the villi of the small intestine, primarily the jejunum. No parasites were found in any extraintestinal tissue examined. Sporozoites invaded host cells and divided several times by endodyogeny to form as many as 16 zoites (3.7 x 1.5 μm) within a single parasitophorous vacuole. Usually, zoites escaped from host cells and entered other cells to undergo gametogony. Occasionally, zoites remained in the same parasitophorous vacuole and underwent gametogony. Microgamonts were 12.1 μm in diameter and contained about 50-100 microgametes. Macrogamonts were 11.7 x 14.9 μm. An eosinophilic body (EB) was associated with the surface of micro- and macrogamonts. In mature microgamonts, the EB usually remained with the centrally located residual body, whereas in zygotes it appeared to be incorporated into the oocyst wall. Oocysts were present at 7 DPI, measured 14.2 x 18.1 μm, and were passed unsporulated. Sloughing of the intestinal epithelium occured at 7 DPI. Four of the 13 marmosets died during the infection; one at 3 and 5 DPI, and 2 at 7 DPI.

Zygotes and oocysts of *Sarcocystis hemionilatrantis* were studied in the lamina propria of the small intestine of coyotes (*Canis latrans*) by SEM and TEM. Zygotes and oocysts did not occur in a parasitophorous vacuole but were only partially enveloped by host cell pseudopodia. Zygotes were spheroid, and had a thin oocyst wall. Wall forming bodies typical of Coccidia were not seen. Lipid bodies exocytosed into a space between the oocyst wall and the sporont pellicle, much of which adhered to the inner surface of the oocyst wall. Membrane layers and zones of granular material coalesced to form separate inner and outer layers of the sporont. These layers invaginated to separate the sporont into 2 sporoblasts. Mature oocysts contained 2 sporocysts, each with 4 sporozoites.

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ABBREVIATIONS

In Text

DPI	days post inoculation
DAI	days after inoculation
EB	eosinophilic body
M1-M6	membrane layers
NBR	National Bison Range
OW	oocyst wall
SEM	scanning electron microscopy
TEM	transmission electron microscopy
v/v	volume to volume
WFB	wall forming body
w/v	weight to volume
µm	micrometer, 10^{-6} meter

In Figures

Am	amylopectin
Bl	basal lamina
Bv	blood vessel
Cc	concavity
Cg	collagen
Db	dense body
Eb	eosinophilic body
En	endothelial cell

Fl	flagellum
Fx	finger-like extension of Eb
Gl	granular layer
Go	Golgi complex
Hn	host cell nucleus
Lb	lipid body
Ll	electron lucent layer between oocyst and sporocyst walls
Lu	lumen of intestinal tract
Ma	macrogamont
Mb	membrane fragment
Mi	microgamont in Isospora chapter
Mi	mitochondrion in Sarcocystis chapter
No	nucleolus
Np	nuclear pore
Nu	nucleus
Oo	oocyst
Ow	oocyst wall
Pd	pseudopodium
Pv	parasitophorous vacuole
Rb	residual body
Rr	rough endoplasmic reticulum
Sp	sporozoite
Sr	smooth endoplasmic reticulum

Sw1	outer sporocyst wall
Sw2	inner sporocyst wall
Zo	zoite

INTRODUCTION

Taxonomic Classification of *Isospora arctopitheci* Rodhain, 1933 and *Sarcocystis hemionilatrantis* Hudkins and Kistner, 1977

Kingdom PROTISTA

Subkingdom PROTOZOA

Phylum APICOMPLEXA Levine, 1970

Class SPOROZOEA Leuckart, 1879

Subclass COCCIDIA Leuckart, 1879

Order EUCOCCIDA Leger and Duboscq, 1910

Suborder EIMERIINA Leger, 1911

Family EIMERIIDAE Minchin, 1903

Genus *ISOSPORA* Schneider, 1881

Species *ARCTOPITHECI* Rodhain, 1933

Family SARCOCYSTIDAE Poche, 1913

Genus *SARCOCYSTIS* Lankester, 1882

Species *HEMIONILATRANTIS* Hudkins and Kistner, 1977

The families Eimeriidae and Sarcocystidae contain several genera of Coccidia which have considerable detrimental impact on domestic animals and wildlife. Life cycles of these genera are similar in general patterns yet for the few species whose life cycles have been studied, each organism displayed unique traits of development. Some of the more important genera are *Besnoitia*, *Eimeria*, *Frenkelia*, *Hammondia*, *Isospora*, *Sarcocystis*, and *Toxoplasma*. Life cycles of these genera are reviewed to provide a basis for comparison to the parasites studied in the present study.

Besnoitia

Besnoitia spp. are heteroxenous parasites which form cysts with unusually thick walls in connective tissues of their intermediate hosts (32) and gamonts and oocysts in felines which act as their final hosts. *Besnoitia besnoiti* is a parasite of bovines in which it forms a cyst in the subdermal connective tissue and produces areas of local necrosis, loss of hair, and high fever. Death may result in severe cases (15). *Besnoitia bennetti* causes a somewhat milder disease in equines characterized by thickened skin and mange-like symptoms (15). *Besnoitia jellisoni* forms cysts in the connective tissue in the viscera of mice which produce areas of necrosis (15).

Eimeria

In many domestic and wild animals Eimeria species cause the disease known as coccidiosis. Coccidiosis is transmitted from animal to animal by means of sporulated oocysts which are ingested in contaminated water or feed. Coccidiosis occurs primarily in young animals and especially in those animals housed or penned under crowded conditions. Economic losses due to coccidiosis are difficult to determine because of the lack of adequate records as well as the complex nature of the disease. The disease causes mortality, morbidity and weight loss in various domestic animals, but probably has its greatest economic impact in the poultry and cattle industries around the world. Fitzgerald (41) estimated that bovine coccidiosis alone caused a worldwide economic loss of $472 million.

Coccidiosis is still the most economically important parasitic disease of chickens (73). Because of the widespread use of anticoccidials by chicken growers, little economic loss due to mortality occurs in the United States. Losses due to coccidiosis in chickens are primarily due to morbidity which results in reduced weight gains, egg production and decreased food conversion. Ruff and Reid (73) estimated $60 to $120 million are lost each year in the United States due to weight loss plus another $35 million which are spent on anticoccidials.

Some Eimeria species are highly pathogenic, whereas other species are not, and certain strains of a given Eimeria species may be more pathogenic than others. During an Eimeria infection, the asexual stages cause little tissue damage to their host, whereas the gamonts and oocysts may cause extensive tissue destruction. Host cells infected with meronts are probably destroyed as the meront ruptures, releasing

merozoites into the lumen of the intestinal tract or into adjacent tissue. Gamonts and oocysts cause a marked alteration of their host cells which may become distorted, rupture, separate from adjacent cells and slough from the intestinal epithelium, exposing the deeper tissues. Since gamonts and oocysts are more numerous than the asexual stages, they cause more host cell destruction and, therefore, more tissue damage which may lead to a denuding of the intestinal epithelium.

In severe infections, the clinical signs usually include diarrhea with blood and portions of intestinal tissue in the feces. An enteritis develops which is complicated by secondary bacterial infection in denuded epithelial areas of the intestinal tract. Infected animals experience poor weight gain or weight loss, emaciation, and weakness and severely infected animals may die.

Frenkelia

Frenkelia spp. are obligatory heteroxenous parasites and are similar to *Sarcocystis* spp. in oocyst morphology, tissue cyst formation, and life cycle (15). Oocysts contain 2 tetrazoic sporocysts which are passed fully sporulated in the feces of the final host. Hosts in the *Frenkelia* life cycle are carnivorous birds and rodents. Two known species, *F. microti* and *F. buteonis*, use the buzzard, *Buteo buteo*, as a final host and mice and voles as intermediate hosts (15, 32).

Frenkelia is known to form thin-walled cysts in the brain of various intermediate hosts such as field voles, meadow mice, chinchillas, muskrats, and bank voles. The complete life cycle has been determined for only *F. buteonis* (71). Gamonts and oocysts develop in the lamina propria of the small intestine of the buzzard after ingestion of cysts from the brains of mice. In the intermediate host, meronts appear in the liver as early as 7 days after ingesting *F. buteonis* sporocysts and cysts appear in the brain beginning at 18 days (72).

Hammondia

Members of the genus Hammondia are morphologically identical to Toxoplasma gondii. Life cycles are extremely similar in that both use felines as final hosts and various other animals such as monkeys, mice, rats, dogs, and hamsters as intermediate hosts. However, Hammondia spp. differ from T. gondii by having an obligatory two-host cycle, by not being transmitted transplacentally, by not infecting extraintestinal organs of the cat, and by being relatively nonpathogenic to the intermediate host (15, 32, 44). Tissue cysts develop mainly in the muscle and occasionally in the brain of intermediate hosts. Oocysts are passed unsporulated in the feces of the felines that have ingested infected tissues (15).

Antibodies to Hammondia have been found to cross react with T. gondii antigens. Because of this, H. hammondi is currently being investigated as an immunoprophylactic against toxoplasmosis. Mice and hamsters infected with H. hammondi are protected against lethal doses of T. gondii oocysts (12). Humans have not been found to be hosts for any Hammondia species.

Isospora

The genus Isospora was originally believed to contain only parasites with a direct cycle similar to species of Eimeria in which asexual and sexual development occur in a single host. However, recent studies have shown that some isosporans have a facultative or obligatory two-host life cycle. Dubey (15) and Frenkel (43) described new genera Levineia and Cystoisospora, respectively, for organisms that may use two hosts in their life cycles, and suggested that those isosporans that have only a direct life cycle and no cyst-like stages should remain in the genus Isospora.

The classical life cycle of Isospora is represented by I. belli, an uncommon parasite of man. Infection is initiated by ingestion of sporulated oocysts. Sporozoites excyst from oocysts in the gut and parasitize epithelial cells of the upper small intestine where asexual and sexual development occurs. Zygotes form oocysts which may sporulate before passage in the feces. It is speculated that mature oocysts may even excyst before leaving the single host and re-infection occurs. This contained life cycle may explain the chronic nature of I. belli infections in man. Oocysts of most isosporans are passed unsporulated in the feces of the final host.

In contrast to the classical scheme, some isosporans are now known to have a two-host cycle. Such parasites are I. felis (cat-rodent), I. rivolta (cat-bird), I. canis (dog-mice), and I. ohioensis (dog-mice). Intermediate hosts become infected by ingesting oocysts. Sporozoites excyst from the oocysts and then penetrate through the intestine and

develop extraintestinally, usually in the viscera (16, 18, 56, 74). Tissue cysts are formed after several merogonous generations. These cysts differ from cysts of other coccidians as they contain a single zoite rather than numerous ones. A thick PAS positive wall encompasses the cyst. The single zoite is infective to the final host upon ingestion and sexual development takes place within the cells of the intestinal epithelia or lamina propria.

Though strict host specifity is not required by isosporans as it is by sarcocystans, the host range of the former usually includes relatively few intermediate hosts and a single final host. An exception is I. arctopitheci which has been found to use 6 genera of primates, 4 families of carnivores, and even a marsupial as a final host and various mammals and birds as intermediate hosts (50, 51). Due to the varied life cycles within Isospora, the genus is apt to be redefined by taxonomists.

Sarcocystis

Species of Sarcocystis are obligate 2-host parasites which infect humans and various animals. These protozoan parasites have a predator/prey cycle in which predators (carnivores or omnivores) act as final hosts and prey animals (omnivores or herbivores) act as intermediate hosts. Gametogony, oogony, and sporogony occur in the final host. Individual species of Sarcocystis have been found to be so specific for their final and intermediate hosts that taxonomists often use composite names for these parasites such as S. bovicanis, S. capracanis, and S. hemionilatrantis. Carnivores or omnivores that serve as final hosts become infected by ingesting meat containing sarcocysts and omnivores or herbivores that serve as intermediate hosts become infected by ingesting sporocysts that contaminate food and water. Sporocysts and occasionally oocysts are released from the intestinal mucosa of the final host and are passed in the feces. Oocysts and sporocysts of Sarcocystis spp. are usually fully sporulated when they are shed in the feces of the final host. Each sporocyst contains 4 sporozoites each of which is infective to the appropriate intermediate hosts.

In the intermediate host, Sarcocystis has a vascular and a muscular phase. The vascular phase usually lasts 4-5 weeks and precedes the muscular phase. During the vascular phase, sporozoites escape from the oocysts or sporocysts, penetrate the intestinal mucosa, and multiply in the endothelial cells of the blood vessels in nearly all organs of the body. After 2-3 generations of merogony, merozoites

enter muscle cells and develop into cysts called sarcocysts which are usually visible to the unaided eye. This encystment is the muscular phase. After several weeks, bradyzoites within the sarcocysts are infective to the appropriate final host which becomes infected by ingesting infected meat. When final hosts ingest infected meat, bradyzoites escape from the sarcocysts in the gut of the final host and invade the mucosa of the small intestine. Bradyzoites develop rapidly into gamonts, fertilization occurs, and zygotes develop into oocysts. Oocysts and sporocysts escape from the intestinal mucosa and are shed in the feces.

Within the bovine, an intermediate host, 3 distinct asexual generations have been described (14, 22, 29, 34, 35). Sporozoites develop into meronts at 15-16 DAI in the endothelial cells of small to medium-sized arteries. Cecum, large intestine, kidneys, pancreas, and cerebrum are the organs primarily involved. Meronts are usually relatively large and can contain as many as 350 merozoites (22). Some meronts appear to occlude small arteries. At 26-33 DAI, merozoites escape from meronts, mature, and invade endothelial cells of the capillaries and arterioles of various tissues where they develop into second generation meronts. Brain, heart, lung, liver, kidneys, skeletal muscles, tongue, intestine, and esophagus, as well as other organs, are parasitized with the kidneys being the most heavily infected (35). A third generation apparently occurs free in the blood stream and in circulating leucocytes (31). Sarcocysts are believed to persist in various muscles during the lifetime of the bovine. Sarcocysts usually cause no obvious cellular response in infected tissues.

Two other species of bovine Sarcocystis have been described, S. bovifelis (syn. S. hirsuta) and S. bovihominis which use cats and humans as final hosts, respectively (32).

Sheep also serve as an intermediate host for various Sarcocystis spp. (14). Sarcocystis tenella (syn. S. ovicanis) is considered to be the most pathogenic species for sheep in which it may cause abortion, anorexia, ataxia, myelomalacia, and death.

Sarcocystis has also been reported in mule deer, white-tailed deer, elk, and bison (68). Mule deer fawns inoculated with sporocysts of S. hemionilatrantis died 27 to 63 DAI after an acute phase of anorexia, weight loss, pyrexia, and weakness (52). Meronts were found near blood vessels in skeletal muscles as well as tongue, heart, esophagus, and diaphragm. Mature cysts did not appear until 60 DAI. Speer et al (86) found that at 3 to 5 days after coyotes ingested infected mule deer meat zygotes and oocysts were present in the coyote small intestine, and sporocysts and oocysts were released in the feces 9-13 DAI. They also found that coyotes could be reinfected with the same meat used to establish the initial infection. A marked cellular response consisting of lymphocytes, macrophages, mast cells, and plasma cells occurred in the small intestines of coyotes killed at 14 days after the initial feeding of infected meat and at 3, 5, and 7 days after the second feeding. The mucosa was hyperemic with distended blood vessels. Blood vessels also had thickened basement membranes. Edema occurred between the lamina propria and the intestinal epithelium.

Man may serve as an intermediate or final host for some species of Sarcocystis (2, 14, 15, 32, 36, 53, 61). Various morphological types

of sarcocysts have been found in biopsy of human tissue which indicate that there may be several Sarcocystis species infecting man. Pathogenicity of the sarcocyst stage in man has not yet been investigated thoroughly.

Toxoplasma

Toxoplasma gondii is the etiologic agent of toxoplasmosis. *Toxoplasma* was first discovered in 1908 in a North African rodent by Nicolle and Manceaux (66). It is an unusual coccidian parasite because it has little specificity for intermediate hosts. Evidently, it can infect any warm-blooded animal, bird or mammal, as an intermediate host, but uses wild and domestic felids only as final hosts. *Toxoplasma* can be transmitted congenitally, by carnivorism, or by fecal contamination (45). Felids and intermediate hosts become infected by ingesting tachyzoites in pseudocysts, bradyzoites in cysts, or sporozoites in oocysts. Merogony, gametogony, and oocyst formation occur in the intestinal epithelium of felids that previously ingested oocysts, tachyzoites, or cysts. Extraintestinal merogony also occurs in felids which may be lethal, depending upon the number of organisms ingested, as well as the age and immune status of the host. Only asexual reproduction by endodyogeny occurs in various tissues in intermediate hosts which have ingested *Toxoplasma* pseudocysts, cysts, or oocysts.

World-wide, the prevalence of *Toxoplasma* infection in humans and various wild and domestic animals, as determined by antibody tests, has been shown to vary between 7% to 94% (55). Even though infection is usually very common, clinically evident infections are relatively rare. Concomitant infections, extremes of age, exposure, poor nutrition, pregnancy, lactation, blood diseases, concomitant malignancy and immunosuppressive drugs may cause *Toxoplasma* infection to become sufficiently high to cause clinical symptoms. In order for congenital toxoplasmosis

to occur, a woman must experience an acute infection during pregnancy. The earlier during pregnancy that maternal infection occurs, the greater the likelihood of severe disease in the fetus. Toxoplasma may cause death of the fetus or varying degrees of central nervous system damage which may be manifested by retinochoroiditis, epilepsy, or mental deficiency associated with cerebral necrosis and calcification (5).

Toxoplasma also causes serious disease in domestic animals such as sheep, swine, dogs, and cats (6, 7, 42, 48, 55). It can cause abortion in sheep which is of considerable economic importance in New Zealand and Great Britain (6, 7).

In all of the aforementioned life cycles, only general schemes of invasion and parasite location have been studied. Exact host-parasite interactions, such as penetration into the gut, location within a tissue, and development in the host cell, are not well understood. Though few of the very numerous species within the Coccidia group have been investigated, each new species studied has revealed unique and characteristic aspects of development.

Therefore the purpose and intent of this project has been to study the life cycles of two previously uninvestigated coccidian parasites, determine their unique characteristics, and compare and contrast their development with that known of other species.

ENDOGENOUS DEVELOPMENT OF *ISOSPORA ARCTOPITHECI*

Materials and Methods

Thirteen titi marmosets, *Saguinus geoffroyi*, were purchased from animal vendors who had trapped the animals in the Republic of Panama. Animals were housed in separate cages which were cleaned daily and were fed commercial canned animal foods and fresh fruits from the United States. Feces of each animal, which were collected for a 10-14 day period before parasite inoculation and examined for parasites isolated by the formalin-ether technique (69) or by flotation in Sheather's sugar solution, were found to be negative for coccidia.

Oocysts of *I. arctopitheci* were collected from the feces of naturally infected marmosets, suspended in a 2.5% (w/v) $K_2Cr_2O_7$ solution and allowed to sporulate at 24°C for 5-7 days. Each of the 13 marmosets was inoculated by gavage with a 2ml aqueous solution containing 1-2 x 10^5 sporulated oocysts of *I. arctopitheci*. Animals died or were killed and necropsied at 1, 2, 3, 5, 6, 7, and 10 days post-inoculation (DPI). Tissues, obtained from the duodenum, jejunum, ileum, cecum, colon, liver, lungs, kidneys, pancreas, spleen, diaphragm, and mesenteric lymph nodes, were fixed in Bouin's fluid, embedded in paraffin, sectioned and stained with hematoxylin-eosin. Specimens were examined and photographed by a model 18 Zeiss photomicroscope with planapochromatic lens. Size ranges are expressed in micrometers (μm) followed by the mean in parentheses.

Specimens for scanning electron microscopy (SEM) were obtained from intestinal tissues which had been embedded previously in paraffin

and sectioned for light microscopy. After the paraffin was removed by toluene, the tissues were placed in absolute ethanol, critical-point dried in a Tousimis Samdri Critical-point dryer, mounted on metal studs with the sectioned surface upward, coated with 25 nm gold:palladium (60:40) in a SPI sputter, and examined by a Zeiss Novascan 30 scanning microscope.

Results

Location and Incidence

Endogenous stages of I. arctopitheci were seen in epithelial cells of the upper 2/3 of villi in the duodenum, jejunum and upper ileum. The jejunum contained the greatest density of parasites. Parasites were usually situated beneath or along side the host cell nucleus within a parasitophorous vacuole (Figs. 2-15, 19). As the parasites developed and increased in size, the host cell nucleus became flattened against the margin of the cell (Fig. 4, 7-10, 12, 15). Some epithelial cells were multiply infected (Fig. 4). Parasites were first seen at 1 DPI, were most numerous at 7 DPI, and were not seen at 10 DPI. At 7 DPI, a single young macrogamont was seen within the lumen of a blood vessel in the lamina propria of the jejunum. Parasites were not seen in any of the other tissues examined.

Asexual development

Intracellular sporozoites, which were seen only at 1 and 3 DPI (Fig. 1), were crescent-shaped with a blunt posterior and a pointed anterior end, measured 8.5-10.5 x 2.5-3.5 (9.4 x 3.3; n = 4), and contained a spheriod nucleus, and nucleolus which were 3.2 and 2 in diameter, respectively. Asexual stages which were present at 3-7 DPI appeared to multiply by endodyogeny (Figs. 3, 4) which resulted in 2 daughter zoites (Fig. 2). Zoites in pairs each measured 6.5-10 x 2.5-5.5 (7.6 x 2.3; n = 24) and had a nucleus and nucleolus which were 2.3 and 1.5 in diameter, respectively. Usually, zoites remained within

their original parasitophorous vacuole and underwent one to three additional generations of endodyogeny to form as many as 16 zoites (Fig. 5, 6). Zoites became progressively smaller with each generation of endodyogeny. Zoites in a single parasitophorous vacuole containing 12-16 zoites measured 3-4 x 1.5 (3.7 x 1.5; n = 50).

Sexual development

Immature gamonts were present at 5-7 DPI; mature gamonts at 6 and 7 DPI. Immature macrogamonts were recognized easily by their prominent nucleus and nucleolus (Figs. 7, 8, 11). Immature micro- and macrogamonts could also be differentiated from asexual stages by an eosinophilic body (EB) which was present at the surface of each gamont (Figs. 7, 9, 11, 13). In intermediate gamonts, the EB was hemispherical, measured 3.5-9.6 x 0.9-3.5 (5.7 x 2.3; n = 15) and was located at one end of the parasite (Figs. 7, 13). The EB increased in size coincidentally with an increase in the size of the gamont. In zygotes, the EB became flattened and spread over the surface of the organism (Figs. 10, 23-24) to form a uniform eosinophilic layer, 0.5-1 thick, which eventually appeared to become part of the outer layer of the oocyst wall. By SEM, the surface of the EB was continuous with the surface of the macrogamont (Figs. 23, 24). Finger-like processes were present on the surfaces of relatively small EBs and some processes appeared to contact the inner surface of the parasitophorous vacuole (Fig. 24). Such finger-like processes were smaller or absent on the surfaces of larger EBs (Figs. 23).

Intermediate macrogamonts were elongate and measured 10-17.5 x 6-12 (13.2 x 8.1; n = 20) (Fig. 7), whereas mature macrogamonts were ovoid and measured 12-18.5 x 9-15 (14.9 x 11.7; n = 26).

Several nuclear divisions occurred in young microgamonts while the organism was still elongate (Figs. 13, 14). As nuclear division progressed, the nuclei became smaller and indistinct (compare Figs. 13 and 14). The nucleolus was prominent in young microgamonts (Figs. 13, 14), but was not visible in the nuclei of intermediate and nearly mature microgamonts (Fig. 15). Intermediate microgamonts had numerous nuclei distributed randomly throughout their cytoplasm, whereas the nuclei were arranged at the periphery of nearly mature gamonts (Fig. 15). Folding or segmenting of the microgamont was not seen. The EB was usually located within the central residual body or occasionally at the margin of the gamont. Mature microgamonts measured 9.5-14 (12.1; n = 14) in diameter and contained about 50-100 microgametes which were arranged peripherally about a central residual body (Fig. 16, 21). By SEM, microgametes were found to be biflagellate and measured 2-2.7 x 0.5-0.7 (2.3 x 0.6; n = 20).

In some specimens, macro- or microgamonts were present within a host cell parasitophorous vacuole which also contained several merozoites or 1-3 other micro- or macrogamonts (Figs. 11, 12, 19, 22). In a few specimens, a micro- and macrogamont were seen within the same parasitophorous vacuole (Fig. 19). Occasionally, young and intermediate macrogamonts each with 2-4 prominent nuclei were seen (Fig. 18).

Oocysts

Oocysts, which were present only at 7 DPI, measured 15.7-19.1 x 10.4-18.3 (18.1 x 14.2; n = 20), had an indistinct oocyst wall and a granular, eosinophilic sporont (Fig. 17).

Pathology

Four of the 13 marmosets died during the infection; one at 3 and 5 DPI, and 2 at 7 DPI. The two marmosets that died at 7 DPI showed bloody diarrhea for 2 days before death, whereas the animals that died at 3 and 5 DPI showed no noticeable signs of intestinal disorder.

Host cells containing oocysts showed various degenerative changes such as loss of the brush border and lysis (Fig. 17). Destruction of several contiguous epithelial cells caused sloughing of the intestinal epithelium, especially at the tips of the villi, which exposed the lamina propria to the intestinal lumen (Fig. 20).

EXPLANATION OF ILLUSTRATIONS

Plate 1. Endogenous stages of Isospora arctopitheci in the small intestine of the marmoset.

Figure 1. Sporozoite in intestinal epithelial cell. X2000

Figure 2. Two zoites in the same parasitophorous vacuole (Pv). X2200

Figure 3. Portions of 3 organisms in same parasitophorous vacuole (Pv). Note the 2 nuclei (Nu) in 1 organism which is undergoing endodyogony. X1700

Figure 4. Three organisms in same host cell. Note that 2 organisms are undergoing endodyogony (double arrows) and that a membrane (single arrows) separates each organism. X2200

Figure 5. Three zoites (Zo) in the same parasitophorous vacuole (Pv). X2200

Figure 6. Group of about 10 relatively small zoites (Zo) in same parasitophorous vacuole. X2400

Figure 7. Intermediate macrogamont with prominent nucleolus (No) and eosinophilic body (Eb). X2000

Figure 8. Intermediate macrogamont. X2100

Figure 9. Mature macrogamont with centrally located nucleus and nucleolus, and eosinophilic body (Eb). X2000

Figure 10. Zygote. Note eosinophilic body (Eb) and flattened host cell nucleus (Hn). X2200

Figure 11. Two macrogamonts (Ma) in same parasitophorous vacuole (Pv). X2100

Figure 12. Several zoites (Zo) and a macrogamont (Ma) in same parasitophorous vacuole. X2200

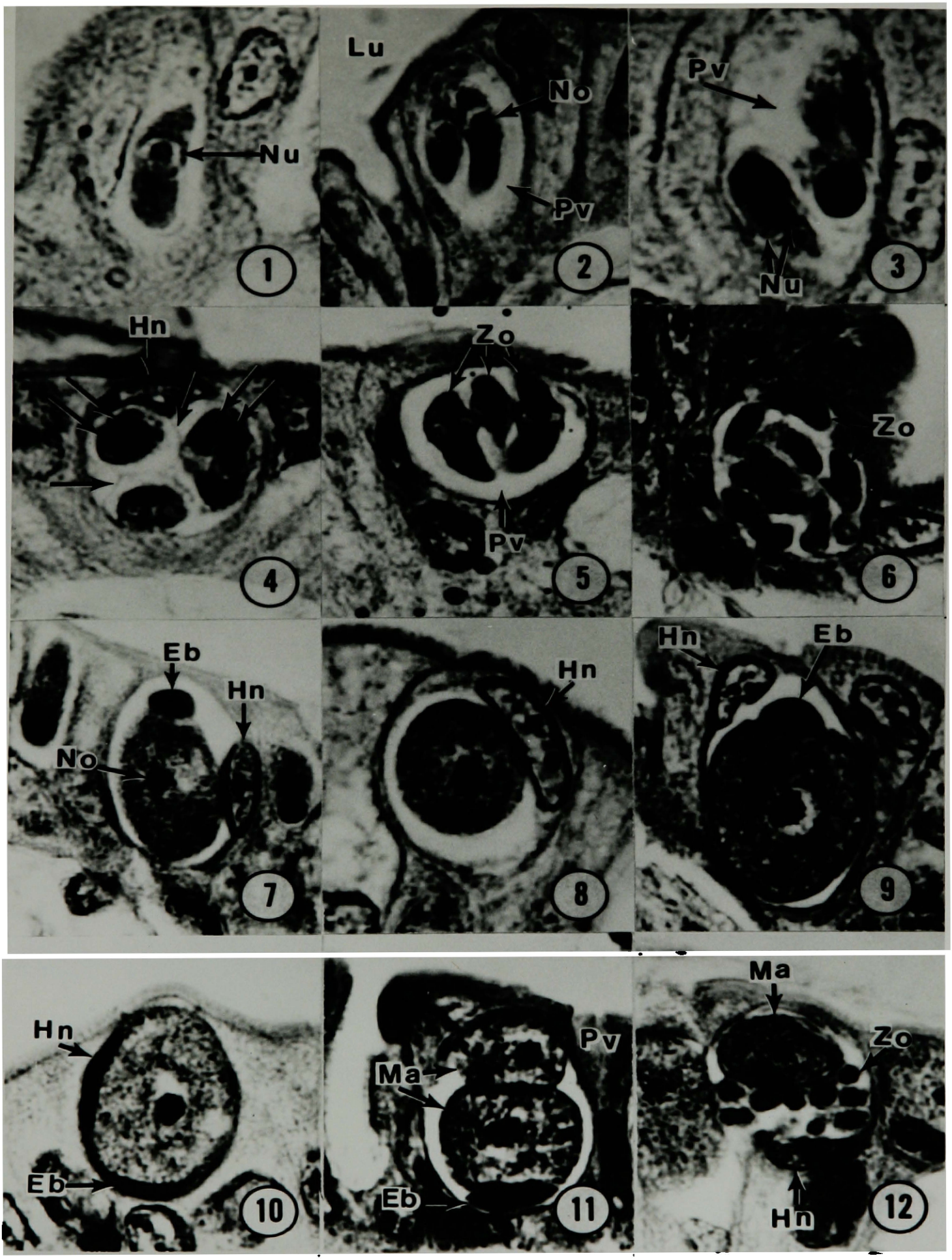

Nu
1
Lu
No
Pv
2
Pv
Nu
3
Hn
4
Zo
Pv
5
Zo
6
Eb
Hn
No
7
Hn
8
Hn
Eb
9
Hn
Eb
10
Pv
Ma
Eb
11
Ma
Zo
Hn
12

Plate 2. Endogenous stages of *Isospora arctopitheci*.

Figure 13. Young microgamont with an eosinophilic body (Eb) and 2 nuclei, each with a prominent nucleolus (No). X2200

Figure 14. Zoite (Zo) and microgamont with several nuclei (Nu) in same parasitophorous vacuole. X2200

Figure 15. Nearly mature microgamont with peripheral nuclei (Nu). X2200

Figure 16. Mature microgamont with numerous microgametes (Mg) and a risidual body (Rb). X2200

Figure 17. Oocyst (Oo). Host cell appears to have lysed. X2200

Figure 18. Macrogamont with 2 nuclei (Nu). X2200

Figure 19. Macrogamont (Ma) and microgamont (Mi) in the same parasitophorous vacuole (Pv). X2100

Figure 20. Tip of villus showing sloughed epithelium (arrows). Note numerous parasites in the epithelium, and the blood vessel (Bv) in the lamina propria. X650

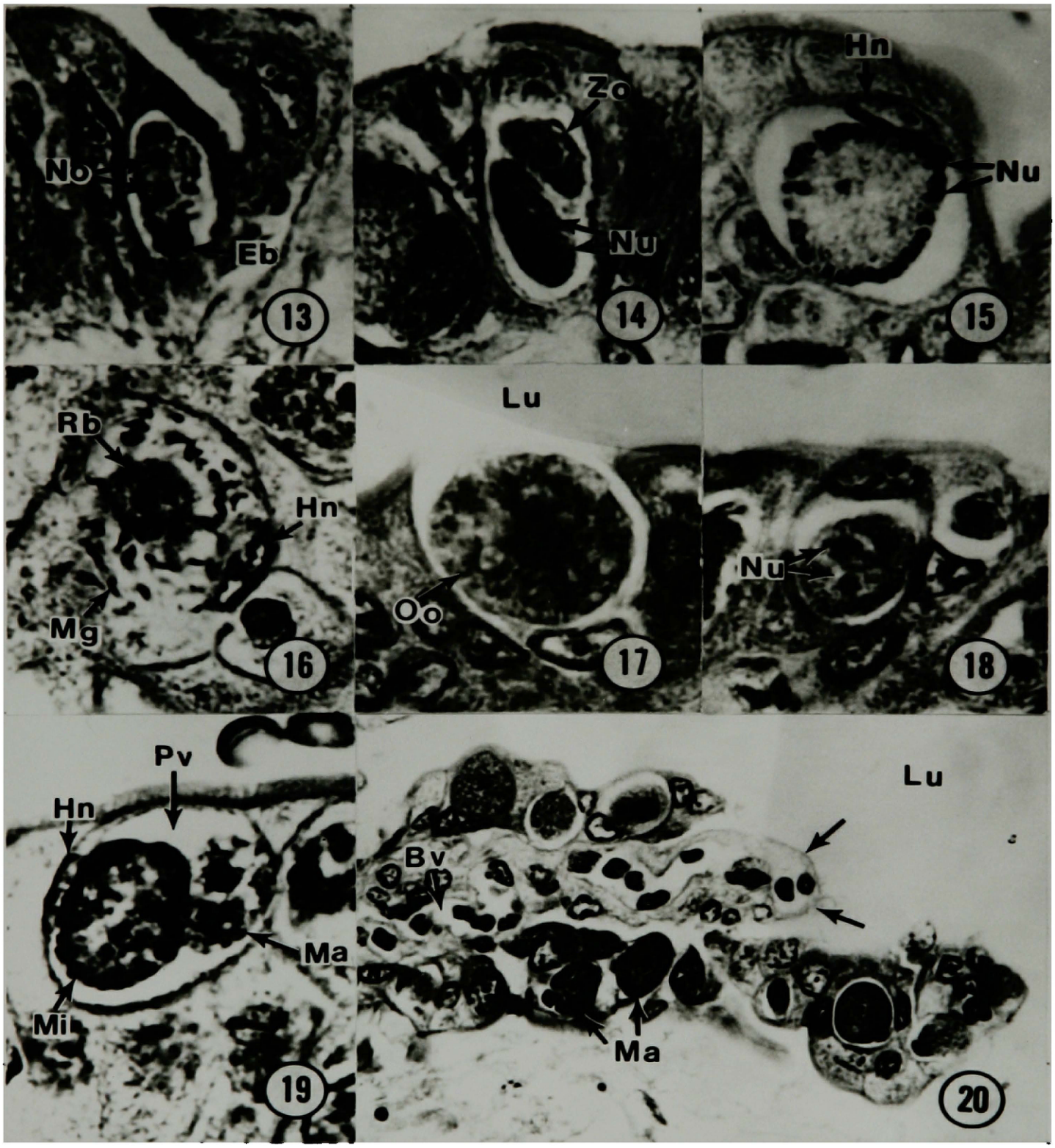

No
Eb
13
Zo
Nu
14
Hn
Nu
15
Rb
Hn
Mg
16
Lu
Oo
17
Nu
18
Pv
Hn
Ma
Mi
19
Lu
Bv
Ma
20

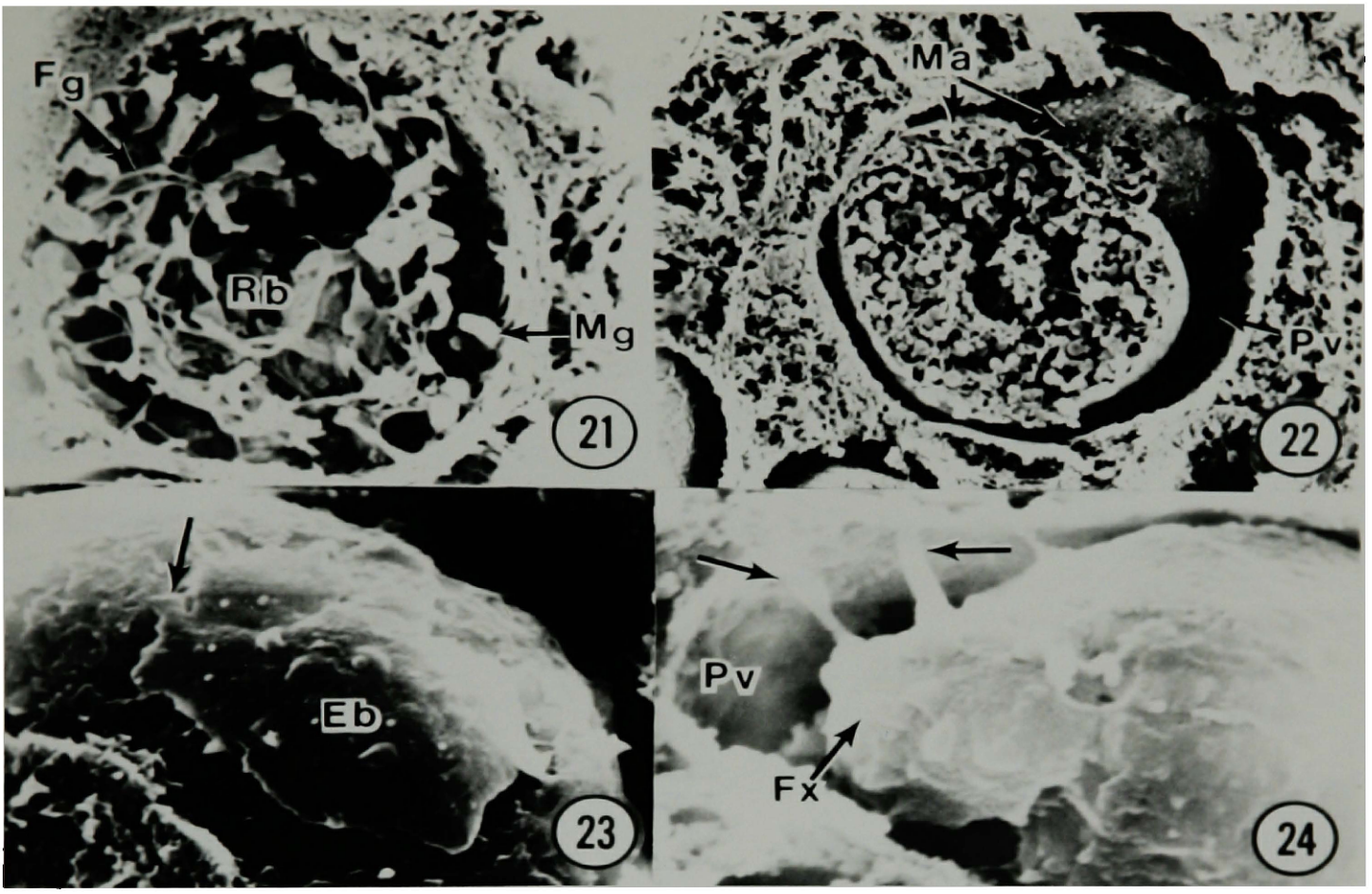
Plate 3. Scanning electron micrographs of the sexual stages of *Isospora arctopitheci*.

Figure 21. Mature microgamont containing several microgametes (Mg) with 2 flagella (Fg), and a central residual body (Rb). X3300

Figure 22. Two macrogamonts (Ma) within the same parasitophorous vacuole (Pv). X2400

Figure 23. Eosinophilic body (Eb) which appears to have spread over the surface of the macrogamont. Note small finger-like extensions of the Eb and that the Eb is continuous (single arrow) with the thin outer layer of the organism. X12,800

Figure 24. Eosinophilic body (Eb) on the surface of a young gamont. Note finger-like extensions (Fx) of the Eb and that one Fx contacts the inner surface of the parasitophorous vacuole (Pv) membrane (single arrow). X9500


Fg
Rb
Mg
21
Ma
Pv
22
Eb
23
Pv
Fx
24

DISCUSSION

The endogenous development of I. arctopitheci in the small intestine of titi marmosets was similar to that described for other isosporids (16, 18-20, 56-59, 74) except that asexual multiplication occurred exclusively by endodyogeny. Merogony, in addition to endodyogeny, has been found to occur in I. neorivolta (20) and I. ohioensis (16) in dogs, I. rivolta (18) and Toxoplasma gondii (19) in cats, and I. suis (57) in pigs. In cell cultures, sporozoites of I. canis (37), I. felis (38), and I. rivolta (26) multiply by endodyogeny only. As far as I know, I. arctopitheci is the first coccidian found to undergo asexual multiplication exclusively by endodyogeny. This characteristic may account, in part, for the ability of I. arctopitheci to infect a wide range of hosts (50, 51).

Zoites of I. arctopitheci usually remained within the same parasitophorous vacuole in which they had developed and underwent several generations of endodyogeny to form as many as 16 zoites. Such development in which several generations of merogony or endodyogeny occur within the same parasitophorous vacuole is known to occur in vivo or in vitro in other coccidia such as various Eimeria species (81), I. canis (56), I. felis (74), I. ohioensis (16), I. neorivolta (20), I. rivolta (18), T. gondii (19), and Hammondia hammondi (76).

Certain merozoites of E. magna (82), E. nieschulzi (77), I. suis (57), and zoites of I. arctopitheci (present study) developed into macro- or microgamonts without leaving their original parasitophorous vacuole. In the present study of I. arctopitheci, usually macro- or microgamonts

only occurred in the same parasitophorous vacuole. However, in I. suis (57), as well as I. arctopitheci, a macro- and microgamont were occasionally seen in the same parasitophorous vacuole. Such observations indicate that a single zoite is capable of giving rise to both macro- and microgamonts or that two merozoites penetrated the same host cell and were somehow incorporated within the same parasitophorous vacuole.

In the present study, I occasionally observed multinucleate macrogamonts of I. arctopitheci. Multinucleate macrogamonts have been reported for Eimeria magna (80, 81) and E. tenella (13) which were considered to be oddities of in vitro cultivation. Speer (80) found that multinucleate macrogamonts of E. magna were formed by the fusion of several organisms which were in direct surface contact within the same parasitophorous vacuole of the host cell. Multinucleate macrogamonts of I. arctopitheci probably form by a similar mechanism since in the present study several macrogamonts were often found to occupy the same parasitophorous vacuole. Some multinucleate macrogamonts of E. magna were found to form unusually large multinucleate oocysts in cultured cells (81). Such multinucleate oocysts were not observed in I. arctopitheci.

The eosinophilic body seen in gamonts of I. arctopitheci resembles the crescent body associated with intracellular sporozoites, meronts and/or gamonts of E. auburnensis (11), E. bovis (33, 46), E. leuckarti (1), E. nieschulzi (78), E. ninakohlyakimovae (54, 88), and E. zuernii (86). Both bodies are eosinophilic. However, in contrast to the homogeneous eosinophilic body of I. arctopitheci, crescent bodies are granular (54, 78) and are seldom associated directly with the surface of the

parasite. Also, crescent bodies do not appear to be incorporated into the developing parasite, whereas the eosinophilic body appeared to become part of the outer layer of the oocyst wall in I. arctopitheci.

Histologic lesions caused by I. arctopitheci are similar to those described for I. neorivolta (20) and I. ohioensis (17) in dogs and I. rivolta in cats (18), in which necrosis and desquamation of the tips of villi occurred in those areas of the intestine occupied by the parasite. Isospora ohioensis (17) and I. rivolta (18, 23) are pathogenic to newborn pups and kittens, respectively, but are nonpathogenic to weaned hosts. In contrast, I. arctopitheci is pathogenic to adult marmosets.

ENDOGENOUS DEVELOPMENT OF *SARCOCYSTIS HEMIONILATRANTIS*

Materials and Methods

Eight coyote pups (*Canis latrans*) were taken from dens on the National Bison Range (NBR) near Moiese, Montana, and housed in individual cages. After the pups had been weaned on canned evaporated goat milk, they were given commercial dry dog food and water *ad lib*. Fecal specimens were collected for 21 days prior to experimentation, floated in Sheather's sugar solution, and examined for parasites. A mule deer (*Odocoileus hemionus*) was taken from the NBR which had macroscopic cysts of *Sarcocystis* in its esophagus and microscopic cysts in skeletal muscles. To provide inocula for the coyotes, skeletal muscle from the deer was ground in a commercial meat grinder, divided into 500 g portions, wrapped in plastic, and stored at 4°C. A 500 g portion of meat was fed to each of 8 coyotes once on each of 2 consecutive days. Three coyotes were each fed an additional 500 g portion of meat each day at 16 and 17 days after the original feeding.

Five of the 8 coyotes were euthanized by an overdose of Serylan (phencyclidine hydrochloride) on various days: 1 pup on day 12 and 1 pup on day 14 after initial feeding with infected meat, and 1 pup on each of days 3, 5, and 7 after the second feeding (19, 21, and 23 days after the initial feeding). Tissues were taken at 6 inch intervals along the small and large intestines, and cecum, and from the heart, liver, lung, spleen, diaphragm, adrenals, and mesenteric lymph nodes. Tissues for scanning electron microscopy (SEM) were fixed in 10% (v/v) neutral buffered formalin, embedded in paraffin, and sectioned with a

steel knife. After the paraffin was removed by toluene, the tissues were dehydrated in alcohol, critical-point dried, coated with 35 nm gold:palladium (60:40), and examined with a Zeiss Novascan 30 scanning electron microscope. For transmission electron microscopy (TEM), tissues were fixed in 3% (v/v) glutaraldehyde in cacodylate buffer (pH 7.2), rinsed in cacodylate buffer, post-fixed in 2% (w/v) osmium tetroxide, partially dehydrated in ethanol and prestained in 1% (w/v) uranyl acetate and 1% (w/v) phosphotungstic acid in 70% (v/v) ethanol for 12 to 18 hours. Tissues were then completely dehydrated in ethanol, placed in 2 changes of propylene oxide, and embedded in Epon 812 or Spurr's medium. Thin sections were cut with glass knives on MT-2 Porter-Blum ultramicrotome, stained with lead citrate, and examined by a Zeiss EM9S-2 transmission electron microscope.

Results

During the 21 days before feeding Sarcocystis-infected meat, the coyotes were found to be negative for coccidia.

Transmission Electron Microscopy

Zygotes and oocysts of S. hemionilatrantis were found in the lamina propria of the small intestines of coyotes killed at 3, 5, 7, 12 and 14 days after ingesting (DAI) mule deer meat. Zygotes (young oocysts) were present at 3 and 5 DAI, whereas mature oocysts, some of which were partially or fully sporulated, were present at 3, 5, 7, 12 and 14 DAI. Macrogamonts, microgamonts and fertilization were not found in any of the sections examined by transmission electron microscopy.

Zygotes and oocysts were extracellular being only partially surrounded by pseudopodium-like processes from fibroblasts and macrophages (Figs. 25, 27, 28, 31). Collagen fibers of host origin were often present near the outer surface of the oocyst and occasionally were located between the oocyst and pseudopodia of enveloping host cells (Figs. 25, 26, 28). Zygotes were usually situated near small blood vessels (Fig. 27).

Zygotes were spherical, 8.5 - 10.8 µm in diameter, and contained several organelles such as a single nucleus and nucleolus, several Golgi complexes, lipid and amylopectin bodies, mitochondria, free ribosomes, and smooth and rough endoplasmic reticulum (Figs. 25, 27). The nucleus was relatively large, 0.5 - 0.7 µm, and contained a peripherally located nucleolus, 0.1 - 0.2 µm in diameter. Amylopectin and lipid bodies arose

in the cytoplasm near the margin of the parasite (Fig. 25). Zygotes had a relatively thin oocyst wall (63-84 nm thick; n = 10) at their margin, indicating that fertilization had already occurred (Figs. 25-28, 30, 31). The oocyst wall consisted of a membrane (9.8 nm; 9-10.5 nm; n = 10) on its inner most margin, an electron-dense, granular outer layer (147.7 nm; 49-216 nm; n = 10), and an electron-lucent layer (17.4 nm; 10-29.5 nm; n = 10) interposed between the membrane and the outer layer (Fig. 30).

Four membranes (M1-M4) and electron-dense material appeared at the margin of the zygote (sporont). Some of these structures eventually formed the inner and outer layers of the sporocyst wall. Some of the lipid bodies within the cytoplasm of the zygote exocytosed into a space between the surface of the sporont and the oocyst wall (Figs. 25, 26, 28). In advanced stages, the exocytosed lipid adhered to the inner surface of the oocyst wall (Fig. 31). The outer layer of the sporocyst wall began to accumulate as moderately electron-dense granular material in a space between M2 and M3. Initially, the sporocyst wall consisted of loosely aggregated electron-dense granules, which later coalesced to form a homogeneous electron-dense layer. Development of the oocyst beyond this stage could not be studied by TEM because the sporocyst wall became highly resistant and impermeable to the fixative and embedding medium. However, further oocyst development and sporulation could be followed by scanning electron microscopy.

Scanning Electron Microscopy

Scanning electron microscopy was used to study parasites in tissue

specimens that had been embedded in paraffin and sectioned by a steel knife. After the paraffin was removed, the specimens were critical-point dried, coated with gold:palladium and examined by the SEM. Young to fully sporulated oocysts were found on the cut surfaces of the tissue specimens. Young oocysts had a homogenous protoplasm and were surrounded by an oocyst wall (Fig. 32). A space occurred between the inner surface of the oocyst wall and the outer surface of the developing sporocyst wall (Figs. 32-35). As the parasite increased in size, lipid and amylopectin bodies appeared within the cytoplasm (Figs. 33-40). During preparation some of the lipid and amylopectin bodies had fallen out of some specimens leaving behind concavities within the parasite cytoplasm (Figs. 34-36, 40). The outer surface of the sporocyst wall appeared relatively smooth and slightly wrinkled (Fig. 35). At various points about the surface of some specimens, the outer surface of the sporocyst wall and the inner surface of the oocyst wall appeared connected together (Fig. 35). These may represent points of lipid exocytosis as discovered by TEM. The earliest stages of sporogony observed were those specimens that contained two sporoblasts (Figs. 36, 40). Each sporoblast was completely surrounded by a thin perforated layer of material which was destined to become the inner layer of the sporocyst wall (Figs. 36-38). The outer layer of the sporocyst wall surrounded both sporoblasts completely. The sporoblast cytoplasm was nearly completely filled with lipid and amylopectin bodies. Each sporoblast had a nucleus located at each pole (Fig. 12). In fully sporulated oocysts, each of the two sporocysts within fully sporulated oocysts was completely surrounded by outer and

inner layers of the sporocyst wall, indicating that the outer layer had invaginated between the two sporocysts in order to envelope them completely. Each sporocyst contained four sporozoites (Fig. 41).

Plate 4. Transmission electron micrographs of young oocysts of S. hemionilatrantis in lamina propria of small intestine of coyotes.

Figure 25. Oocyst with large nucleus (Nu) and nucleolus (No), and various other organelles. Note lipid bodies (Lb) near periphery of organism and lipid (arrows) exocytosing into space between oocyst wall (Ow) and outer sporocyst wall (Swl); Ap, amylopectin; Cg, collagen; Db, dense body; Go, Golgi complex; Pd, pseudopodia of host cells; Swl and Sw2, enveloping sporocyst walls. X16,000

Figure 26. Lipid body (Lb) exocytosing into space between oocyst wall (Ow) and outer sporocyst wall (Swl). X47,500

Figure 27. Enveloping host cell pseudopodium (Pd) around oocyst in close proximity to blood vessel (Bv); sporont is surrounded by membranes 1 and 2 (not clearly visible) and membranes 3 and 4 (arrows); Bl, basal lamina of blood vessel; Bv, lumen of blood vessel; Db, dense body; En, endothelial cell of blood vessel; Ll, electron lucent layer between sporont and oocyst wall; Ow, oocyst wall; Sr, smooth endoplasmic reticulum; Swl and 2, developing sporocyst walls; Rr, rough endoplasmic reticulum. X38,000

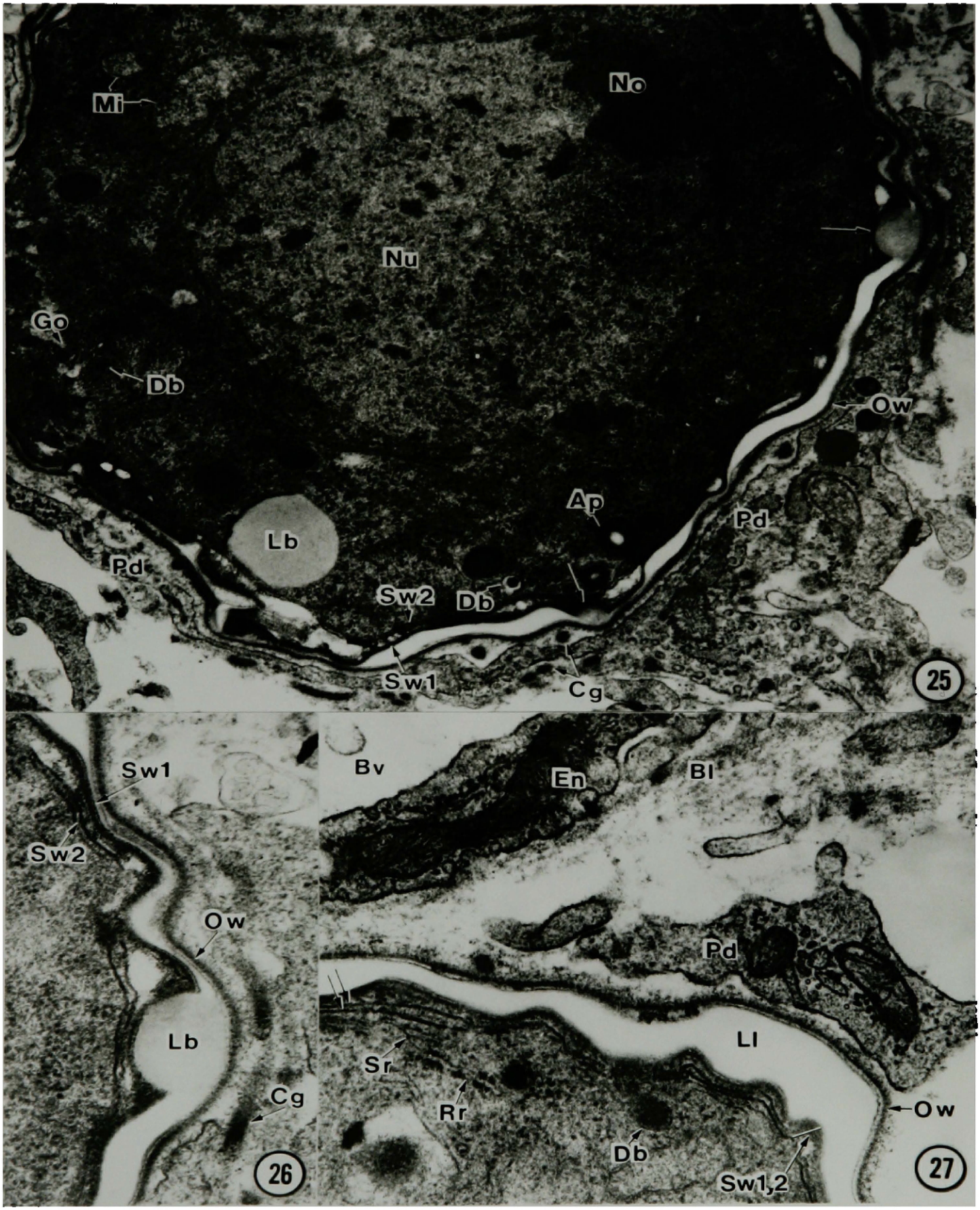

Mi
No
Nu
Go
Db
Ow
Ap
Pd
Lb
Pd
Sw2
Db
Sw1
Cg
25
Sw1
Sw2
Ow
Lb
Cg
26
Bv
En
Bl
Pd
Sr
Rr
Ll
Ow
Db
Sw1,2
27

Plate 5. Transmission electron micrographs of the oocyst wall and the formation of the sporocyst walls of *S. hemionilatrantis*.

Figure 28. Thin oocyst wall (Ow) and forming sporocyst walls (Sw1 and Sw2). Note zone of granular material bound by membranes (double arrows) that comprise the outer sporocyst wall (Sw2). Exocytosed lipid (single arrows) occupying the space between the oocyst wall (Ow) and outer sporocyst wall (Sw1). X42,675

Figure 29. Amylopectin (Ap) granules and lipid body (Lb). X138,000

Figure 30. Oocyst wall (Ow) consisting of a granular outer layer and an inner membrane that are separated by an electron-lucent zone. Note zone of granular material bound by membrane that comprises outer sporocyst wall (Sw1) and membranes of inner sporocyst wall (Sw2). X101,600

Figure 31. Margin of more advanced sporont. Note lipid body (Lb) adhering to inner surface of oocyst wall (Ow) and membrane fragments (Mb) prior to sporocyst wall assembly during sporulation. X38,000

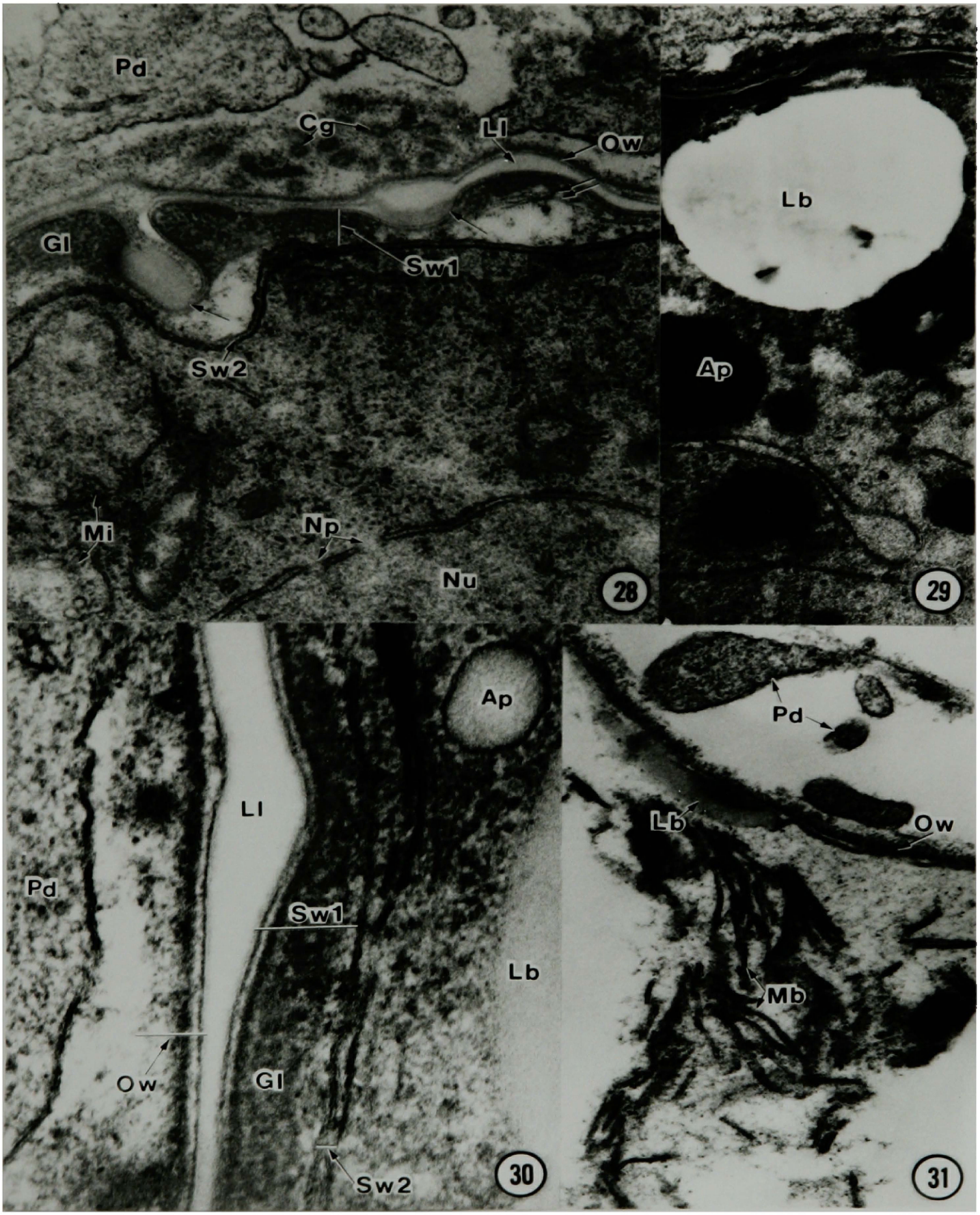

Pd
Cg
Ll
Ow
Gl
Sw1
Sw2
Mi
Np
Nu
28
Lb
Ap
29
Ap
Ll
Pd
Sw1
Lb
Ow
Gl
Sw2
30
Pd
Lb
Ow
Mb
31

Plate 6. Scanning electron micrographs of zygotes and sporulating oocysts of *S. hemionilatrantis*.

Figure 32. Young oocyst (zygote) prior to formation of inclusion bodies yet surrounded by the oocyst wall (Ow) and outer sporocyst wall (Swl). X11,800

Figure 33. Oocyst slightly more advanced than one in figure 32; note lipid bodies (Lb) in cytoplasm. X7080

Figure 34. Oocyst containing numerous amylopectin granules (Ap) and lipid bodies, one of which is exocytosing (arrow). Concavities (Cc) have been left by displaced lipid bodies. X5000

Figure 35. Oocyst surrounded by outer sporocyst wall (Swl); note numerous lipid bodies (Lb), one which may be exocytosing (arrow). X11,900

Figure 36. Oocyst containing 2 sporoblasts, one of which is binucleate; note that the inner sporocyst wall surrounds each sporoblast and the outer sporocyst wall surrounds both sporoblasts. X5300

Figure 37. Oocyst containing 2 sporoblasts, one of which exhibits the porous nature of the inner sporocyst wall (Sw2) in contrast to the continous outer sporocyst wall (Swl). X11,800

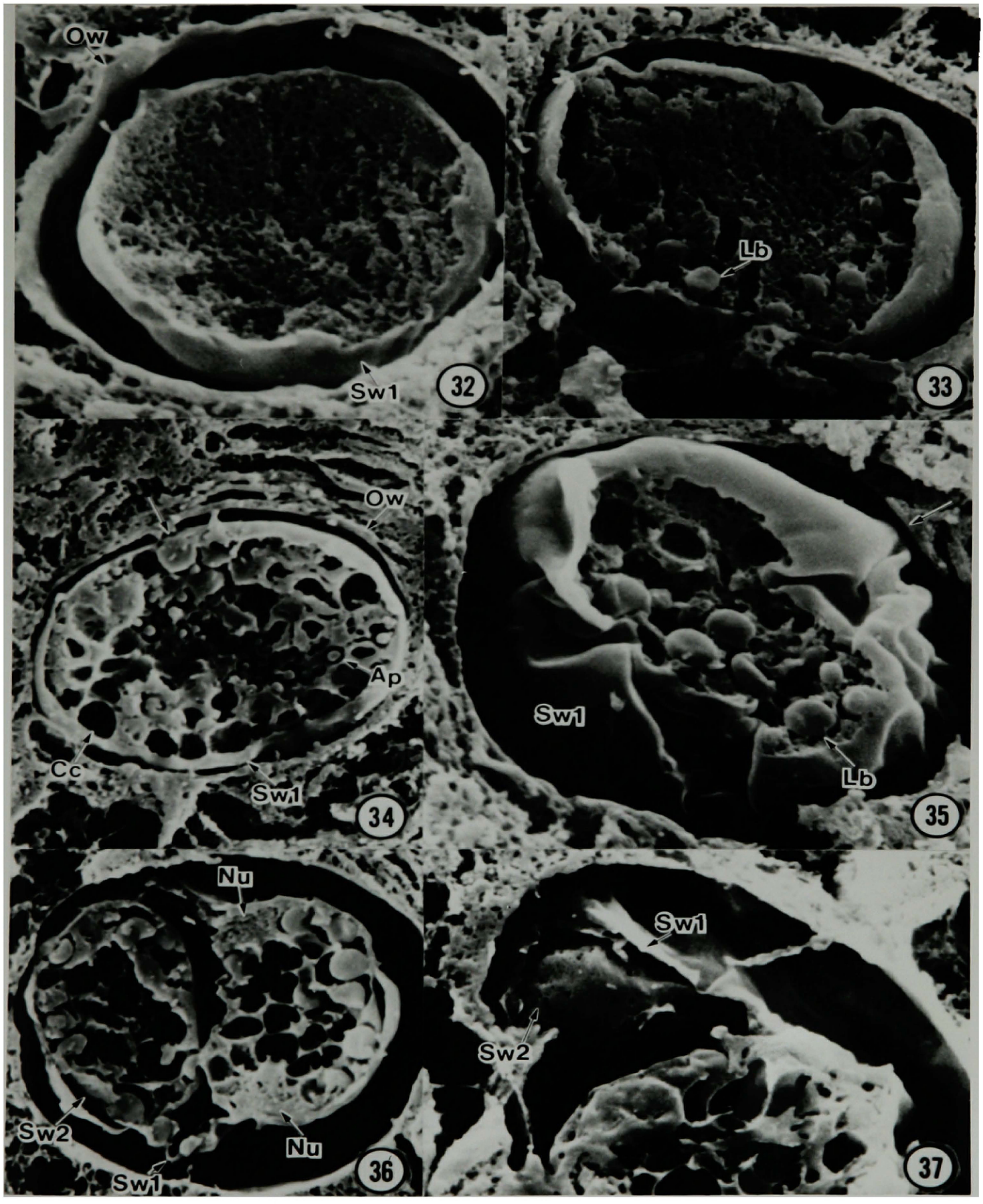

Ow
Sw1
32
Lb
33
Ow
Ap
Cc
Sw1
34
Sw1
Lb
35
Nu
Sw2
Nu
Sw1
36
Sw1
Sw2
37

Plate 7. Scanning electron micrographs of partially and fully sporulated oocysts of S. hemionilatrantis.

Figure 38. Higher magnification of the 2 layers (Sw1 and Sw2) of the sporont wall; note porous inner wall (Sw2) and continous outer wall (Sw1). X18,000

Figure 39. Fully sporulated oocyst with 2 sporocysts, each surrounded by inner (Sw2) and outer (Sw1) sporocyst walls. X5300

Figure 40. Partially sporulated oocyst with 2 equal sporoblasts; Mb represents membrane material as determined by TEM. X9800

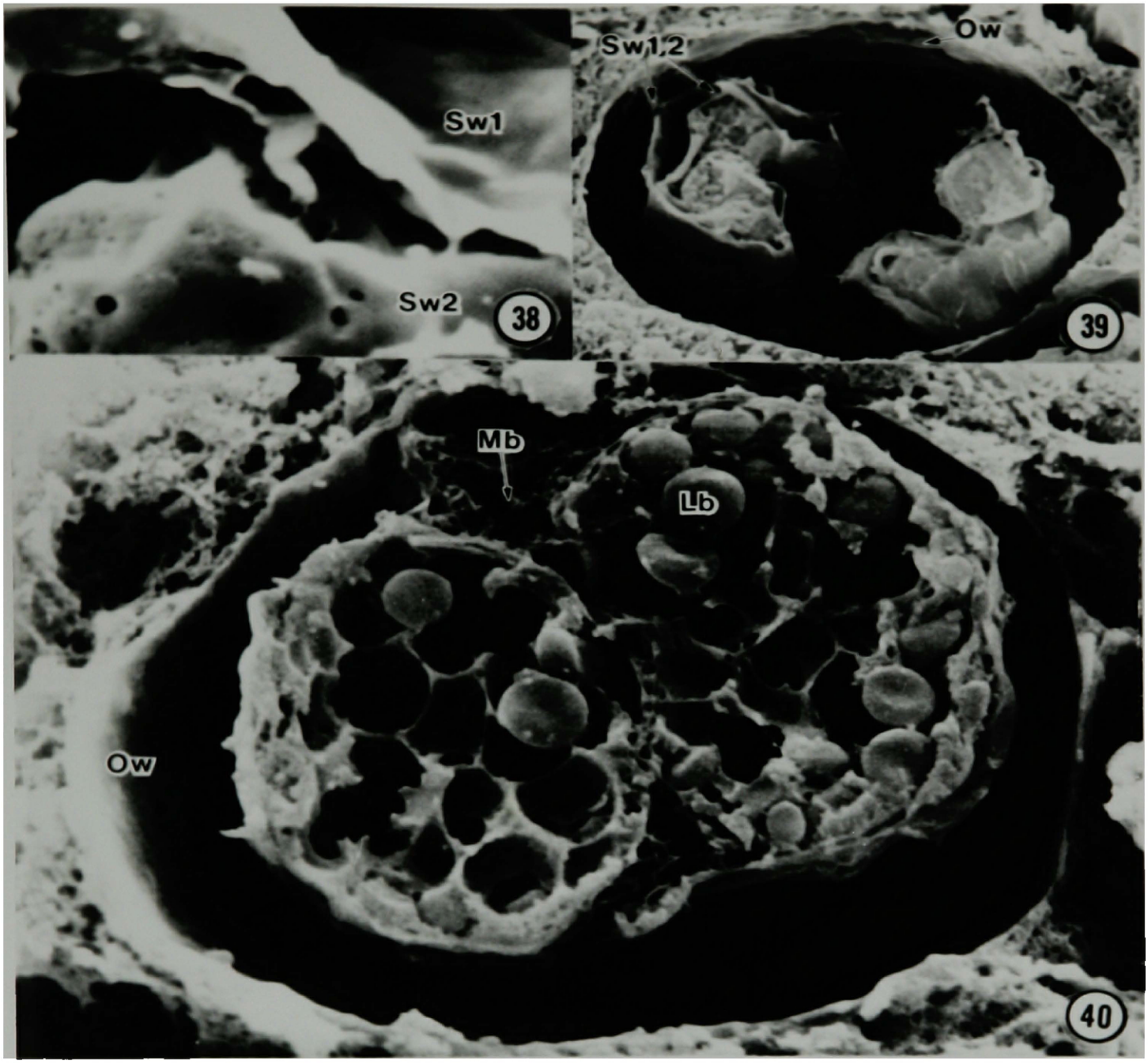
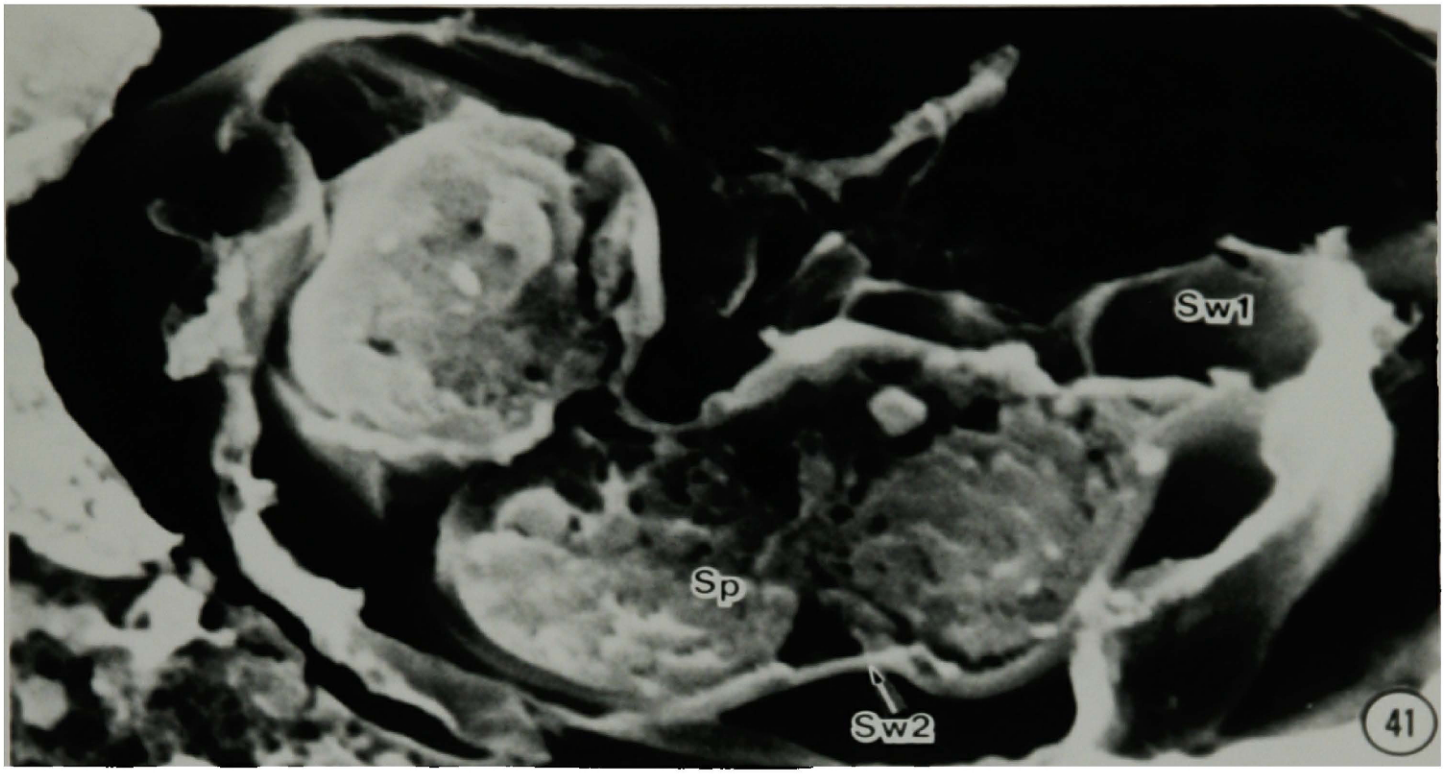

Sw1
Sw2
38
Sw1,2
Ow
39
Mb
Lb
Ow
40

Plate 8. Scanning electron micrograph of a single sporocyst of *S. hemionilatrantis*.

Figure 41. Sporocyst containing sporozoites (Sp) surrounded by inner (Sw2) and outer (Sw1) sporocyst walls. X14,600


Sw1
Sp
Sw2
41

DISCUSSION

The life cycle of coccidian parasites (i.e. Eimeria, Isospora, Toxoplasma, Sarcocystis and others) of animals and humans includes both asexual and sexual stages of multiplication. Sexual stages of nearly all coccidian species develop intracellularly in the intestine of the final host. Bradyzoites or merozoites of the parasite differentiate into microgametes (male) or macrogamonts (female) which is followed by fertilization and development of the zygote into an oocyst. Although thousands of species of Coccidia have been described from numerous hosts and the endogenous development of a few of these species have been studied by light and electron microscopy, the act of fertilization has been observed only once (75). Eimerian microgametes have been observed on the surface of macrogamonts by light microscopy (62), and SEM (58) and within a macrogamont by TEM (47). Sheffield and Fayer (75) found that fertilization in S. cruzi (syn. S. bovicanis) resembled that reported for other invertebrates and mammals (4, 25), where membrane fusion occurs rather than penetration of the male gamete into the female gamete as previously assumed for the Coccidia.

Gamonts have been observed only in a few species of Sarcocystis (3, 21, 28, 63, 65, 87, 89), in which they were found to develop to maturity in cultured cells or within cells of the final host within 18-24 hours after inoculation. In the present study, stages of S. hemionilatrantis which appeared similar to macrogamonts of S. cruzi (28) as determined by light microscopy were actually found to be zygotes and developing oocysts when examined by TEM. Such stages occurred at

3 and 5 days after ingestion of infected meat but not at 7 days. At 7, 12 and 14 days, oocysts of S. hemionilatrantis were in the process of sporulating or had completed sporulation. Thus, gametogony and fertilization of S. hemionilatrantis must evidently occur within the first 3 days of development of this parasite in coyotes.

Mechanisms of bradyzoite penetration of the intestinal mucosa and the types of host cells used for sexual development of Sarcocystis species have not been thoroughly studied. Gamonts and/or oocysts of S. cruzi (28), S. hemionilatrantis (present study) and S. tenella (21) develop to maturity in the lamina propria of the small intestine, whereas those of S. orientalis develop within enterocytes (89). Dubey et al. (28) found that bradyzoites of S. tenella entered goblet cells of the small intestine of coyotes or dogs where they developed into gamonts. Zygotes and oocysts were later found only in the lamina propria. Thus, it appears that gamonts of most of those species of Sarcocystis studied thus far begin to develop in enterocytes but later become displaced beneath the epithelium.

Gamonts and oocysts of S. suihominis (63) develop intracellularly, whereas gamonts of S. cruzi (75) and zygotes and oocysts of S. hemionilatrantis (present study) develop extracellularly within the lamina propria. Ultrastructural studies with other coccidian genera have shown that gametogony and oogony occur while the parasites are still intracellular and that sporogony usually occurs extracellularly outside of the host. Sarcocystis and Frenkelia are the only genera of coccidia known to undergo sporogony within the final host.

The ultrastructure of the oocyst wall has been described for mature

oocysts of Isospora canis (84), I. canaria and I. serini (83), S. tenella (64), Toxoplasma gondii (40), and various Eimeria species (24, 39, 60, 67, 70, 77, 85). These reports have shown that the oocyst wall of various species of Coccidia consists of one to four prominent layers. Among the species studied, the innermost layer appears to be relatively consistent in thickness and appearance, being composed of fine granular electron-lucent material. The oocyst wall of S. hemionilatrantis consisted of three distinct layers (present study), whereas that of S. tenella was found to consist of a single electron-lucent layer (64) similar in appearance to the innermost layer of the oocyst walls of other coccidian species which have two or more layers. Two prominent layers occur in the wall of immature oocysts of a Sarcocystis species from grackles which developed in cell culture (87). The oocyst wall of T. gondii has an inner electron-lucent and an outer electron-dense layer (40). Oocysts of Eimeria species have been found to have two prominent layers, an innermost electron-lucent and an outermost electron-dense layer (24, 39, 60, 67, 70, 77), whereas those of Isospora species have three or four layers (83, 84). The oocyst walls of S. hemionilatrantis and other Sarcocystis species appear much thinner and fragile than the oocyst walls of other coccidia. The fragility of the oocyst walls of Sarcocystis may account for the fact that sporocysts and seldom oocysts are passed in the feces of final hosts infected with Sarcocystis species (86), whereas oocysts only are shed by hosts infected with species of other coccidian genera. Evidently, the oocyst wall of Sarcocystis species breaks down releasing the sporocysts which are then passed in the feces of the final host.

Oocyst wall formation was not observed in the present study of S. hemionilatrantis, because the earliest stages observed already had a fully formed oocyst wall. Since the oocyst wall was already formed, this may account for the lack of wall forming bodies in the cytoplasm of zygotes of S. hemionilatrantis which are typically present in gamonts and oocysts of other Sarcocystis and coccidian species. Two types of wall forming bodies have been found in macrogamonts of S. suihominis (63) and a single type in a Sarcocystis species from grackles (87). In the present study, lipid bodies gradually increased in number and size within the cytoplasm of zygotes of S. hemionilatrantis. Some of the lipid bodies were exocytosed into a space between the zygote and the oocyst wall, and some of these appeared to adhere to the inner surface of the oocyst wall.

Certain aspects of sporocyst wall formation in S. hemionilatrantis were found to be similar to oocyst wall formation in other Coccidia. Four membranes (M1-M4) appeared at the margin of S. hemionilatrantis zygotes and an electron-dense granular material accumulated between M2 and M3 which eventually formed the outer layer of the sporocyst wall. The inner layer of the sporocyst wall was evidently formed by M3 and M4. The actual formation of the inner layer of the sporocyst wall was not observed by TEM because by this stage in development the parasite had become impermeable to the fixative and embedding medium. No exocytosis of material across M3 and M4 was observed in the present study. Therefore, submicroscopic molecules of sporocyst wall forming material were evidently transported across M3 and M4 into a space between M2 and M3 where they reaggregated to form the outer layer of the sporocyst

wall. A similar mechanism involving the disaggregation of wall forming bodies, transport of the material across membranes at the margin of the zygote and the reaggregation of wall forming material was proposed as a mechanism of oocyst wall formation in E. nieschulzi (77).

Sporulation of S. hemionilatrantis oocysts occurred by a process similar to that described for other species of Sarcocystis (8, 28, 86), but differed markedly from that described for S. suihominis (63). During sporulation, the cytoplasm of S. suihominis oocysts was observed to flow through an opening in the sporocyst wall into a space between the oocyst and sporocyst walls (63). Presumably, the extruded cytoplasm formed one sporoblast and the remaining protoplasm formed the other one. However, the extruded cytoplasm lacked a nucleus which would have to be present to give rise to the sporozoite nuclei. Cytoplasmic extrusion was not seen in the present study. Sporoblasts were formed by invagination of the parasite protoplasm into two equal halves by the sporocyst wall.

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