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# *Cryptosporidium felis* sp. n. (Protozoa : Eimeriorina) from the Domestic Cat

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## Introduction

The genus *Cryptosporidium* is a member of coccidia of the family Cryptosporidiidae, suborder Eimeriorina. *Cryptosporidium muris* from the gastric glands of the laboratory mouse was first described by Tyzzer in 1907, and designated in 1910 as the type species of the new genus (Tyzzer, 1910). He described that the generic character was " an oocyst with four sporozoites and no sporocyst ", and the life cycle was extracellular. Another species, *C. parvum*, from the small intestine of the common mouse was described by Tyzzer in 1912. Although Tyzzer found cryptosporidia infected in rabbit (1912) and in chicken (1929), he considered both to be the same species, *C. parvum*. However, Levine (1961) separated the species in chicken as a distinct species from *C. parvum*, and named it *C. tyzzeri*. Slavin (1955) described *C. meleagridis* only on its endogenous stages found in the small intestine of turkey. Jervis *et al.* (1966) observed cryptosporidia in the small intestine of the guinea pig and considered them to be a new species or *C. parvum* adapted to a new host. After that, Vetterling *et al.* (1971 a) made several cross-transmission experiments with the species obtained from guinea pigs, and described it as a new species, *C. wrairi*. Barker and Carbonell (1974) described tow new species, *C. agni* from the small intestine of lambs and *C. vobis* from the small intestine of a calf. In the same year, Proctor and Kemp (1974) described *C. anserinum* from the large intestine of a domestic goose.

Recentry, several cryptosporidial infections were reported ; in calves (Panciera *et al.*, 1971 ; Meuten *et al.*, 1974 ; Schmitz and Smith, 1975), in pigs (Kennedy *et al.*, 1977), in chickens (Fletcher, 1975), in rhesus monkeys (Kovatch and White, 1972 ; Cockrell *et al.*, 1974) and in human patients (Nime *et al.*, 1976 ; Meisel *et al.*, 1976). Though these workers made the observations on the endogenous stages, the identification of the species was not done.

Some other species of *Cryptosporidium* were reported only on the characteristics of the oocyst found in the feces ; in rattlesnake (Triffitt, 1925), red fox (Wetzel, 1938), dingo (Bearup, 1954), Indian jungle cat (Dubey and Pande, 1963), kingsnake (Anderson *et al.*, 1968) and lizard (Duszynski, 1969). But these were dealt with non-valid species by Vetterling *et al.* (1971 a), for the reason that their endogenous stages were not known and each of these oocysts might be one of the sporulated sporocysts of the genus *Isospora*.

The cryptosporidial infection in the cat is hitherto unknown. And the oocyst has not yet been isolated from the feces of any hosts infected with cryptosporidian parasites.

In this report, the auther describes (a) the

oocyst in the feces, (b) the endogenous developmental stage, (c) the life cycle, (d) the host specificity, and (e) the pathogenicity of a cryptosporidian species found in the cat. Especially, it is revealed by electron microscopy that the oocyst formation and the sporogony are performed in the microvilli of epithelial cells of the small intestine of the host. The characters of the genus *Cryptosporidium* are also discussed.

## Materials and Methods

Thirteen cats obtained from the owners living in Osaka city were used ; 5 of them were found to be naturally infected with a species of *Cryptosporidium* by fecal examinations (3 one-month-old litter-mates and 2 adults, each weighing about 2 kg). Other 8 cryptosporidia-free adult cats were used for the transmission experiments. Each cat was kept in a separate cage and fed on the commercial cat food supplemented with canned fish. Cages and feeding utensils were cleaned and disinfected daily with boiling water.

*Fecal examinations.* Feces of the cat were examined daily by the modified zinc sulfate centrifugal-floatation technic ; 4 to 5 g of feces was emulsified into thick paste and then suspended in about 10 volumes of water. After sieving through stainless tea strainer into 50 ml tube, the suspension was centrifuged at 2,500 rpm by floating head type centrifuge for 5 min. The supernatant fluid was poured off, then 5 to 7 ml of zinc sulfate solution, sp. gr. 1.18, was added, the packed sediment was broken up by pipetting and again zinc sulfate solution was added enough to fill the tube. After centrifugation for 2 min. at 2,500 rpm, the surface of the floating film was touched gently by a cover glass, 18×18 mm, which was put onto a clean slide and then examined under ×1,000 magnification. When the oocysts were found, about 2 ml of supernatant was aspirated from the top layer with a pasteur pipette fitted with a rubber bulb, and after mixing up with 40 ml of water this was centrifuged at 2,500 rpm for 5 min. The sediment was washed 3 times by repeated centrifugations. A part of the sediment was smeared on slide glasses, dried, fixed in methanol and stained with Giemsa's solution. The other part of sediment was suspended in 2% potassium dicromate solution and stored in refrigerator for further use.

*Morphological observation of the endogenous stages.* Cats infected with cryptosporidia, naturally or experimentally, were necropsied. Small pieces of tissues cut off from each of upper, middle and lower parts of the small intestine were treated as follows : (1) The fresh mucosal scrapings of each level were examined by bright field and phase contrast microscopy ; (2) Stamp smears of mucosa from each level were fixed in methanol and stained with Giemsa's solution ; (3) The mucosal scrapings were stained with Lugol's solution to detect the iodo-philic granules in the parasites ; (4) The pieces from each level were fixed in 10% neutral buffered formaline with 7.5% sucrose, and they were routinely embedded in paraffin, sectioned and stained with hematoxylin and eosin ; (5) Small pieces from each level were fixed in Karnovsky's solution (Karnovsky, 1965), followed by 1% $OsO_4$ in 0.1 M sodium cacodilate buffer. After the block-staining in 2% uranyl acetate, the fixed tissues were washed, dehydrated and embedded in Epon 812 resin. Sections obtained from Epon block, cut at 0.5 to 1 $\mu$m, were stained with Giemsa after removing the resin (Inami *et al.*, 1968) and examined by light microscope ; (6) Ultra-thin sections from the Epon block were stained with uranyl acetate and lead citrate, and examined under a Hitachi HS-9 electron microscope.

Stomachs, caeca and colons of infected cats were also examined on the same procedures as described above.

*Transmission experiments.* (a) To determine the infectivity of the oocysts discharged in the feces, 4 coccidia-free cats were fed $5\times10^5$ oocysts mixed in canned fish ; each of them was sacrificed and examined on 4, 13, 21 and 25 days after feeding respectively. The fecal examination was carried out daily.

(b) To test the infectivity of the endogenous stages in naturally infected cats discharging the oocysts into their feces, about 2 g of mucosal scrapings obtained from the small intestine of the cats were given mixed with canned fish to 2 coccidia-free cats. Each of them was sacrificed and examined 5 and 21 days later respectively. The daily fecal examinations of these cats were also carried out. (c) Other 2 coccidia-free cats, not inoculated with oocysts, having served as the control were also sacrificed and examined at the end of the transmission experiments.

*Cross-transmission experiments.* 6 mice (ICR-strain, 7 weeks-old, female) and 6 guinea pigs (Hartley-strain, 180–200 g in body weight, male), all free from coccidial infection, were used. $3\times10^5$ oocysts, isolated from the naturally infected cat feces, were evenly divided into six parts and were inoculated *per os* into each of 3 mice and 3 guinea pigs respectively. Other each 3 mice and 3 guinea pigs were kept uninoculated as control. All of inoculated and uninoculated animals were kept in the separate cages. Each one inoculated and one uninoculated animal of both mouse and guinea pig were sacrificed at one week interval. The stomach, small intestine, caecum and colon of each animal were examined and the fecal examination was also made on all animals before sacrifice.

## Results

*Oocyst in the feces.* Numerous oocysts of *Cryptosporidium* were found in the feces of the naturally infected cats and they could be isolated from the feces by zinc sulfate centrifugal-floatation technic (Photo. 1). The oocysts are ellipsoidal or round with a smooth, colorless and thin wall, measuring 5 by 4.5 $\mu$m. They were observed already sporulated in the feces just discharged. Each oocyst contains 4 C-shaped sporozoites located around a residual body which is round and not granular, measuring 1 $\mu$m in diameter (Photos. 2, 3). Sporocyst is absent. There is not such a protrusion as "knob-like attachment organ", described by Tyzzer, on the surface of the oocyst. The sporozoites and the residual body of the oocyst, except the oocyst wall, can be stained by Giemsa's solution after methanol fixation (Photos. 4–6). The cytoplasm of the sporozoite and the residual body were stained blue, the nucleus of the sporozoite red. A sporozoite released from an oocyst is banana- or boomeran-shape with a nucleus near an extremity of the ooganism (Photo. 6).

*Sporozoite invasion into the host cell.* The life cycle is initiated by the entry of the sporozoite into a microvillus of an intestinal epithelial cell.

Electron microscopy shows that the sporozoite or the merozoite is entering into a microvillus of a goblet cell (Photo. 15). Such a microvillus is invaginated deeply to its bottom and an electron dense band is formed at the base (Photo. 15). Another electron dense zone is formed in the host cell cytoplasm near the first electron dense band too. A vacuolar area is located in the anterior region of the parasite. As the parasite enters the host cell, the microvillus of the host cell extend along the surface of the parasite, and finally covers all over the parasite, resulting in the formation of a parasitophorous vacuole (Photos. 16–18).

*Trophozoite.* To know the endogenous cycle of the parasite under the light microscope, the Giemsa stained sections from the epoxy resin were observed.

The trophozoites are ovoid, about 2 by 1 $\mu$m large, each with a conspicuous nucleus (Photo. 7). Electron microscopy also shows that the parasite becomes an ovoid or a round trophozoite in the parasitophorous vacuole made after the invasion into the microvillus (Photos. 16–18). The trophozoite is surrounded by a pellicle and possesses a relatively large nucleus with an electron dense nucleolus. Within the cytoplasm there are rough endoplasmic reticulum, Golgi complex, electron dense granules like a fragment of microneme and many ribosomes. The attachment organ, composed of many membranous folds continuous to the pellicle of the parasite, developes in an end region of the tro-

phozoite. An electron dense band is formed near under the surface of the host cell contacted with the parasite. It is clearly shown that the membrane covering the parasite is originated from the microvillus itself of the epithelial cell, because the outer unit membrane of the parasite is apparently continuous to the same one of adjacent normal microvilli (pointed by arrows in Photo. 17).

*Schizogony*. The mature schizont or oocyst can be also observed in the Giemsa stained resin section (pointed by double arrows in Photo. 7). To differentiate between a mature oocyst and a mature schizont on the light microscopic sections is likely to be possible, since 4 sporozoites grow in the former and 8 merozoites in the latter. But, in practice, it seems to be a little difficult, because the oocyst is similar to the schizont both in size and in structure and also all sporozoites in each oocyst or all merozoites in each schizont will not appear ordinarily in only a microscopic section. Mature oocysts, gametes and schizonts are found easily and clearly in the fresh impression smear of the mucosal scraping under the phase contrast microscope (Photos. 8, 9). Mature schizonts, 4 to 5 $\mu$m in diameter, are round and contain 8 banana-shaped merozoites. In the Giemsa-stained stamp smears, each merozoite is about 5 $\mu$m long and 1 $\mu$m wide, and possesses an oval nucleus of about 1 $\mu$m in diameter (Photos. 13, 14). Nuclear division occurs three times in one schizogonic cycle. The binuclear (Photo. 10) and the 4-nuclear schizont are also found in the smear.

Electron microscopy of the developing schizont shows that the merozoites are formed by external budding (Photos. 19, 20). As the process of schizogony progresses, the pellicle of the schizont invaginate deeply and covers each one of the divided nuclei and the cytoplasmic mass containing well-developed rough endoplasmic reticula. Each merozoite is surrounded by a pellicle composed of three (outer, middle and inner) unit membranes (Photo. 23). The outer unit membrane are separated from the other two unit membranes by an electron-pale space. On the outer surface of the middle unit membrane, electron dense fibril-like structures, which distribute regularly at intervals of about 50 m$\mu$, around the organism are observed at the cross view of the merozoite. The anterior region of the merozoite is filled with numerous micronemes and electron dense bodies (or rhoptries), while the nucleus is present in the middle portion and the rough endoplasmic reticula are located in the posterior region (Photos. 21, 22, 24). A conoid is at the anterior end of each merozoite (Photo. 24). The cisternal endoplasmic reticula fill the cytoplasmic residuum of the developing schizont (Photos. 21, 22). When the process of merogony is nearly terminated, the parasitophorous vacuole comes to contain free merozoites, a small mass of residual cytoplasm of the schizont, a round body measuring 0.5 to 0.8 $\mu$m in diameter found in the residual cptoplasm, and an attachment organ left by parasites (Photos. 22, 24). After the release of merozoites from the parasitophorous vacuole, only the attachment organ remains adhered to the host cell in the vacuole (Photo. 29).

*Gametogony*. Developing macrogametes, 4 to 5 $\mu$m in diameter, having a large number of refractile granules in their cytoplasm are seen under the phase contrast microscope and in the Giemsa staind stamp smears under the light microscope (Photos. 9, 11). The refractile granules are stained dark brown by Lugor's solution.

Electron microscopy of the macrogamete shows that it contains a large number of polysaccharide granules, electron dense bodies, wall-forming bodies, membrane-bound vesicles and rough endoplasmic reticula in its cytoplasm (Photo. 25).

Fourteen to 16 microgametes develope from a single microgametocyte, 4 to 5 $\mu$m in diameter, and they have no recognizable flagella under the light microscope (Photo. 12).

*Oocyst formation and sporogony*. The oocyst formation and the sporogony also take place in the parasitophorous vacuole of the microvillus. Oocysts can be easily distin-

guished from the developing schizonts under the electron microscope by the formation of a conspicuous oocyst wall (Photos. 26, 28). Each sporozoite, C-shape, surrounded by a pellicle posessing the same construction as that of the merozoite, contains numerous micronemes, electron dense bodies, electron-pale vacuoles and highly condensed ribosomes in its cytoplasm. The fine networks of the debris are observed distributed in the vacant space of the oocyst. The attachment organ and some blebs of residuum remain outside of the oocyst wall. The sporozoite is occasionally found escaping from the mature oocyst in the parasitophorous vacuole (Photo. 28). An electron dense crystalline inclusion of nearly equilateral triangle, having the side length of 0.3 to 0.4 $\mu$m, is observed in the residual cytoplasm of the developing oocyst (Photos. 26, 27).

*Location.* The endogenous stages are found throughout the small intestine, but schizont and oocysts are more numerous in the lower levels than the upper and the middle levels. Though the parasites are distributed all over the surface of the intestinal villi, they are found more in the tips. The parasites are never found in the lamina propria or in the crypts. No parasite is found in the epithelial cells of the stomach, caecum and colon.

*Transmission and cross-transmission experiments.* The results of the transmission and cross-transmission experiments are summarized in Table 1. In all of cats fed the oocysts or the mucosal scrapings from the small intestine of the infected cat, the cryptosporidial infection was established; 3 out of 4 cats fed $5\times10^5$ oocysts and one of 2 cats fed the mucosal scrapings discharged oocysts in their feces, and in the remaining

Table 1 Results of Transmission Experiments

| Animals | | Inoculation (per os) | Discharge of oocysts | Endogenous stages in small intestine | Day of sacrifice (Days after inoculation) |
|---|---|---|---|---|---|
| Cat | No. 1 | $5\times10^5$ oocysts | − | + | 4 |
| | 2 | | + | + | 13 |
| | 3 | | + | + | 21 |
| | 4 | | + | − | 25 |
| | 5 | Mucosal scrapings from small intestine of infected cat | − | + | 5 |
| | 6 | | + | + | 21 |
| | 7 | None | − | − | 25 |
| | 8 | | − | − | 25 |
| Mouse | No. 1 | $5\times10^4$ oocysts | − | − | 7 |
| | 2 | | − | − | 14 |
| | 3 | | − | − | 21 |
| | 4 | None | − | − | 7 |
| | 5 | | − | − | 14 |
| | 6 | | − | − | 21 |
| Guinea pig | No. 1 | $5\times10^4$ oocysts | − | − | 7 |
| | 2 | | − | − | 14 |
| | 3 | | − | − | 21 |
| | 4 | None | − | − | 7 |
| | 5 | | − | − | 14 |
| | 6 | | − | − | 21 |

2 cats sacrificed in the early stages of infection, the developing parasites were found in their small intestines. The prepatent and patent periods were 5 to 6 days and 7 to 10 days respectively. The total number of oocysts discharged in the feces per one cat a day was extremely small in the case of the experimentally infected cat in contrast to that in the case of the naturally infected cat which was observed to discharge several millions of oocysts. None of 3 mice and of 3 guinea pigs fed $5\times10^4$ oocysts were found infected; no oocysts were discharged in their feces, nor any endogenous stages were found in their small intestines and stomachs.

*Pathogenicity*. The cats infected with the present species did not show any clinical signs of diarrhea or debilitation.

As the results of the morphological observations and the transmission experiments, the author believes that a cryptosporidian parasite found in domestic cats is a new species, and the scientific name, *Cryptosporidium felis* sp. n. is proposed for this parasite.

The concept of the life cycle of *C. felis*, including the information from the electron microscopy, is represented diagrammatically in Fig. 1.

## Description

*Oocyst*. Sporulated oocysts are observed in the feces of infected cats. They are ellipsoidal with a smooth, colorless and thin wall, measuring about 5 by 4.5 $\mu$m. Each oocyst contains 4 C-shaped sporozoites and

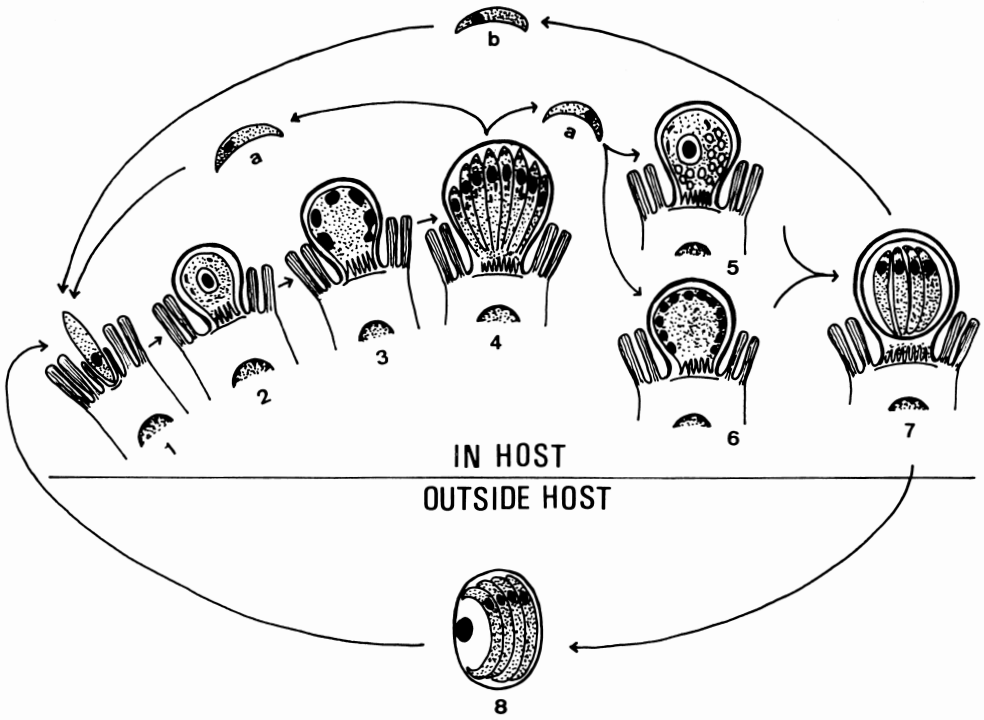


Fig. 1 Diagrammatic representation of the life cycle of *Cryptosporidium felis*. 1-4. Asexual cycle of the endogenous stage: 1. Sporozoite or merozoite invading into a microvillus of a small intestinal epithelial cell; 2. A fully grown trophozoite; 3. A developing schizont with 5-6 nuclei; 4. A mature schizont with 8 merozoites. 5 and 6. Sexual cycle: 5. Macrogametocyte; 6. Microgametocyte with many nuclei. 7. A mature oocyst containing 4 sporozoites without sporocyst. 8. Oocyst discharged in the feces. a. Merozoite released from mature schizont. b. Sporozoite released from mature oocyst.

a residual body. Sporocyst is absent. The oocyst has no protrusion on its surface.

*Sporogony and Sporozoite.* Sporogony is performed in the microvilli of the intestinal epithelial cells. Sporozoites are of banana-shape, 4 to 5 $\mu$m long and 1 $\mu$m wide. The prominent nucleus is seen in the anterior or posterior portion of the sporozoite by Giemsa staining.

*Trophozoite.* After the penetration into the microvilli, sporozoites or merozoites become rather oval or round trophozoites, 2.0 to 2.5 $\mu$m in diameter. The trophozoite possesses a relatively large nucleus, 1.0 to 1.3 $\mu$m in diameter, with a conspicuous nucleolus in it. The trophozoite forms an attachment organ composed of many membranous folds at the site on which the parasite comes in contact with the host epithelial cell cytoplasm.

*Schizogony and Merozoite.* The schizogony also takes place in the microvilli. Following thrice nuclear divisions, 8 banana-shaped merozoites are formed in a schizont, 4 to 5 $\mu$m in diameter. Each merozoite, about 5 $\mu$m long and 1 $\mu$m wide, possesses an oval nucleus, about 1 $\mu$m in diameter.

*Gametogony.* Macrogamete, 4.0 to 5.5 $\mu$m in diameter, possesses a large number of refractile granules in the cytoplasm. Plenty of iodo-philic granules are observed in the cytoplasm by the Lugor's staining. These iodo-philic granules disappear as the sporogony progresses. Fourteen to 16 microgametes develope from a single microgametocyte, 4.0 to 5.0 $\mu$m in diameter.

*Life cycle.* The sporulated oocysts discharged in the feces, are infective to cats. No intermediate hosts are necessary. Sporozoites released from the ingested oocysts at the upper small intestine enter into the microvilli of the epithelial cells. The microvillus invaded by the sporozoite swells, expands, covers all over the surface of the parasite, and finally, forms a parasitophorous vacuole surrounding the parasite. The parasite always remains in the vacuole, never enters more deeply into the cytoplasm of the epithelial cell, nor into the lamina propria. The vermiform sporozoite developes into the ovoid or round trophozoite there, and then, schizogony takes place. Merozoites formed in schizonts, are released from the microvilli and then invade into another microvilli again. How often the schizogonic stage is repeated is unknown. The gametogony occurs following several schizogonic stages, and micro- and macrogametes are formed. The oocyst-wall formation and the sporogony also takes place in the parasitophorous vecuole of the microvilli. Thus, *C. felis* is the " intramicrovillar " parasite like other cryptosporidial species. Oocysts were first detected in the feces 5 to 6 days after inoculation of oocysts, and were discharged continuously for 7 to 10 days in experimentally infected cats. But in one of the naturally infected cats, the oocysts could be observed in the feces for several months, at least 5 months, though finally in very small number and discontinuously. Occasionally, some of the sporozoites were found escaping from the mature oocysts in the microvilli. And, therefore, the auto-infection may occur in the small intestine.

*Type host.* Domestic cat, *Felis domestica*

*Location.* Small intestine

*Geographical distribution.* Osaka, Japan (insofar as known)

*Prevalence.* Unknown

*Pathogenicity.* No diarrhea nor other overt clinical signs

*Type specimens.* The holotype and paratype specimens are provisionally preserved in the laboratory at the Department of Medical Zoology, Osaka City University Medical School, Osaka, Japan.

## Discussion

There are several reports on *Cryptosporidium* spp. from various hosts. Some investigators found the oocyst-like organisms in which contained four naked sporozoites in the feces, and identified them as the oocysts of *Cryptosporidium* without any observations of their endogenous stages. But, at present, it has been considered that some of these oocysts might be the sporulated sporocysts

broken out from the oocysts of *Isospora* or *Sarcocystis*.

Nowadays, it is indispensable for the identification of cryptosporidia to observe their endogenous stages and to make transmission and cross-transmission experiments.

Up to now, the following points on *Cryptosporidium* have been remained obscure; (a) Whether the oocyst is discharged in the feces of the infected animals or not, (b) Whether the oocyst comes to mature inside the host or not, (c) How the transmission occurs in the natural condition and (d) Whether the description of the genus character is adequate or not.

In this report, the author revealed these four points on *C. felis*.

The author found the sporulated oocysts in the feces of cat, and identified them to be those of cryptosporidian parasites on the basis of the light- and electron microscopic findings of their endogenous stages in the small intestine and from the results of the transmission experiments using both oocysts and the endogenous stage of parasites.

The size of this oocyst, 5 by 4.5 $\mu$m, is quite different from that of each of all other cryptosporidian oocysts reported; 11 by 7 $\mu$m from the Indian jungle cat (Dubey and Pande, 1963), 17 by 11 $\mu$m from the dingo (Bearup, 1954), 13.5 by 8 $\mu$m from the red fox (Wetzel, 1938), 10.9 by 8.1 $\mu$m from the kingsnake (Anderson *et al.*, 1968) and 21.7 by 11.5 $\mu$m from the lizard (Duszynski, 1969). At present, all species related to these descriptions on the cryptosporidian oocysts are considered to be non-valid.

Tyzzer (1910) described that the stomach contents and gastric mucosa of mice infected with *C. muris* were infective to normal mice but uninfective to rats. Vetterling *et al.* (1971 a) also reported that the small-intestinal mucosa of guinea pigs infected with *C. wrairi* were infective to normal guinea pigs but not to mice, rabbits, chickens and turkeys. In the present study, both the small-intestinal scrapings of cats infected with *C. felis* and the oocysts isolated from the feces were infective to the normal cats but not to mice and guinea pigs. So that, the host specificity of *Cryptosporidium* is considered to be quite strict. Although the morphological findings of *C. felis* resemble those of *C. parvum* (Tyzzer, 1912; Hampton and Rosario, 1966), *C. meleagridis* (Slavin, 1955) and *C. wrairi* (Vetterling *et al.*, 1971 a, b), the results of the transmission experiments reveal that these are distinct species each other.

Numerous mature oocysts of *C. felis* were found in the feces of naturally infected cats and they could be isolated from the feces by the zinc sulfate centrifugal-floatation technic. Vetterling *et al.* (1971 a) also attempted the fecal examinations on guinea pigs infected with *C. wrairi* to detect the oocyst using a cverslip floatation technic and the FTE sedimentation technic, but they could not find any oocyst. Any other investigator who observed the endogenous stages of cryptosporidia did not perform the fecal examination.

Tyzzer (1910, 1912) made exallent observations in detail on *C. muris* and *C. parvum* under a light microscope. He considered that all developmental stages of both species occured in the gastric glands in the case of *C. muris* and in the small intestine in the case of *C. parvum* respectively, and each life cycle of both species would be repeated in the each host. The other hand, Slavin (1955) observed the endogenous stages of *C. meleagridis* by a light microscope, but he could not find any sporulated oocyst both in the small intestine or in rectal smears. Vetterling *et al.* (1971 a, b) observed the endogenous stages of *C. wrairi* in detail by light- and electron microscopes, but they could not find any oocyst too. They found, however, the facts that a 1st generation schizont formed 8 merozoites and a 2nd generation schizont only 4 merozoites in the case of *C. wrairi*. Then, they concluded that the oocyst with 4 sporozoites which was reported by Tyzzer (1910, 1912) might be the 2nd generation schizont. Barker and Carbonell (1974) found, however, the oocyst of *C. bovis* developing in the parasitophorous vacuole of a microvillus by the electron microscopy.

Also in the present report, it was revealed that the oocyst of *C. felis* matured in the microvillus of the small intestinal epithelial cell and each oocyst possessed 4 sporozoites by the electron microscopy.

The mode of transmission of *Cryptosporidium* in the natural condition has not yet been made clear, although Tyzzer (1907, 1910, 1912) suggested the fecal transmission. Vetterling *et al.* (1971 a) attempted to infect the normal guinea pig with the fecal materials collected from *C. wrairi*-infected one, but the trial was unsuccessful. In the case of *C. felis*, normal cats could be infected with the oocysts isolated from the infected cat feces. Therefore, the occurrence of the fecal transmission from cat to cat was proved in the species.

On the generic character of *Cryptosporidium*, Tyzzer (1910) described simply as follows: "Sporocyst absent or united with oocyst so that the entire organism becomes a single spore with four sporozoites". As the result, some investigators may mistakenly identify some oocyst-like organisms containing 4 naked sporozoites found in the feces of some animals as the oocysts of *Cryptosporidium*. These instances were already mentioned above. Vetterling *et al.* (1971 a) and Lainson and Shaw (1973) also described them in their reports. For the reasons mentioned above, Vetterling *et at.* (1971 a) emended the Tyzzer's description on the genus character, by adding their findings as follows; "Endogenous development takes place on the surface of the host cells or within their striated borders. The microgametes have no visible flagella. Sporulated oocyst are unknown". Yet, in the present examination, the author could find and isolate the oocyst from the feces of cats as already described. And it was also revealed by the electron microscopy that the oocyst formation and the sporogony takes place in the epithelial cells of the small intestine, and the oocyst contains 4 sporozoites without sporocyst. These results support the Tyzzer's description concerning the oocyst, although the observed species are different from each other. Therefore, the present author proposes to supplement and emend the genus character for *Cryptosporidium* by Vetterling *et al.* as follows: "Endogenous development takes place on the surface of the host cells or within their striated borders. The oocyst with 4 sporozoites is formed in the host and discharged in the feces. Sporocyst is absent".

Tyzzer considered that both *C. muris* and *C. parvum* were "extracellular" throughout their life cycle. But, Humpton and Rosario (1966) investigated the parasitic feature of *C. parvum* with electron microscopy and suggested the parasite was "intracellular", and Vetterling *et al.* (1971 b) confirmed it with the electron microscopy of *C. wrairi*. In the present study on *C. felis* the author also could reconfirm it to be "intracellular" under the electron microscopy. It can be assumed now that *Cryptosporidium* parasitizes only in the microvilli of the host epithelial cells throughout the endogenous stages of the life cycle.

Vetterling *et al.* (1971 a) described that the 2nd generation schizont of *C. wrairi* contained 4 merozoites while the 1st generation schizont 8 merozoites. But, in the present observation on *C. felis*, it could not be verified how many merozoites the 2nd generation schizont has.

Other ultrastructural findings on the cell components of *C. felis* were almost same as those of the other species, such as *C. parvum* (Humpton and Rosario, 1966) and *C. wrairi* (Vetterling *et al.*, 1971 b), while the fibrillike structures in the pellicle of the merozoite and the crystalline inclusion in the cytoplasmic residuum of the developing oocyst were found in the present species *C. felis* for the first time. The author, occasionally, observed the swallen and empty microvilli possessing only the attachment organ, and considered them to be the ruptured ones which were formed as the result of escaping of the merozoites or sporozoites. Kovatch and White (1972) observed the same feature in the small intestine of the rhesus monkey infected with *Cryptosporidium* sp., and they considered it

to be a artifact.

On the pathogenicity of cryptosporidia, Slavin (1955) reported the diarrhea and a low death rate in 10 to 14 day-old turkey poult. Kovatch and White (1972) and Panciera *et al.* (1971) observed the diarrhea and enteritis in a juvenile rhesus monkey and in a calf respectively. On the human case, Nime *et al.* (1976) reported a 3-year-old child with severe acute self-limited enterocolitis associated with the *Cryptosporidium*, and Meisel *et al.* (1976) also reported an intestinal cryptosporidiosis in an immunologically suppressed 39-year-old man who developed overwhelming watery diarrhea. In other species such as *C. muris*, *C. parvum* and *C. wrairi* neither diarrhea nor other overt clinical signs were observed in the infected animals (Tyzzer, 1910, 1912; Vetterling *et al.*, 1971 a). Also in the cats infected with *C. felis* in the present work, any clinical sign of diarrhea or debilitation was not observed. However, loss of microvilli, degeneration of host epithelial cells and atrophy of villi in heavy infection, especially in young cats, would suggest the possibility of the malabsorption.

## Summary

*Cryptosporidium felis* sp. n. was described from the domestic cat. The fecal examination of infected cats, light and electron microscopic observations of the endogenous stages, and the transmission and cross-transmission experiments were carried out. This is the first report on the oocyst discharged in the feces of animals infected with *Cryptosporidium*. Oocysts are ellipsoidal, about 5 by 4.5 $\mu$m, with a smooth, colorless and thin wall. Each oocyst contains 4 C-shaped sporozoites measuring about 5 by 1 $\mu$m, and a round residual body measuring 1 $\mu$m diameter. Oocysts in the feces have no "knob-like attachment organ" on their surface. There is no sporocyst in oocyst. Oocysts discharged in the feces are already sporulated. All stages of this protozoon, the trophozoite, the schizont, the gamont, the oocyst and the sporozoite are found in the parasitophorous vacuole in the microvillus of the epithelial cell of small intestine. The parasite never enters into the epithelial cell cytoplasm near the nucleus, nor into the lamina propria, that is to say, the parasite is "intramicrovillar". Occasionally, some of sporozoites were observed releasing from the mature oocyst in the microvillus. Therefore, it is suggested that the autoinfection may occur in the small intestine. Oocysts collected from the feces by zinc sulfate centrifugal-floatation technic are infective to normal cats, but not to mice and guinea pigs. The whole aspect of the life cycle was represented diagrammatically. The pathogenicity and the generic and the specific character were also discussed.

### Acknowledgements

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# Coccidia の新種 *Cryptosporidium felis* sp. n. について

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Genus *Cryptosporidium* は *Eimeria*, *Isospora*, *Toxoplasma* などと同じく，Sporozoa の中の Coccidia に属する寄生性原虫である．Tyzzer (1907) によつて実験用マウスの胃から初めて発見され，その後，マウス，モルモット，ウサギ，ヒツジ，ウシ，ニワトリおよびシチメンチョウの小腸から，それぞれ独立種が記載されており，近年，ブタ，アカゲザルおよびヒトからも感染が報告されている．

今回，ネコの小腸に初めてこの原虫の寄生を認め，その形態を光学顕微鏡ならびに電子顕微鏡によつて観察し，腸管内での発育環を明らかにするとともに，感染実験によつてその感染経路および他種動物への感染性についても検討した．

その結果，本種はネコを固有宿主とする独立種であると考えられたので *Cryptosporidium felis* という新種名を提唱した．

本種はネコの小腸上皮細胞の微絨毛内に形成された Parasitophorous vacuole の中に寄生して，そこで schizogody, gametogony を行ない oocyst を形成する．さらに sporogony もそこで行なわれて成熟 oocyst が糞便とともに排出される．

Oocyst は約 5×4.5 μm の楕円形で，oocyst wall は薄く無色で，表面は平滑．内部にはC字状に彎曲したバナナ状の sporozoite を4コと1コの residual body を包蔵する．sporocyst は無い．

ネコに経口的に摂取された oocyst から小腸上部で sporozoite (5×1 μm) が遊離し，それらは上皮細胞の1本の微絨毛に侵入し，そこに parasitophorous vacuole を形成して trophozoite となる．虫体は終始そこに留まり，上皮細胞の核の近くの細胞質や lamina propria に達することはなく，寄生された微絨毛は大きく拡張し，虫体の全表面を覆う．

Trophozoite は上皮細胞の細胞質表面に接した部位に，この属に特徴的なヒダ状の attachment organ を形成する．その後 trophozoite は大きく丸く成長し，schizogony の過程で3回核分裂し，5×1 μm のバナナ状の merozoite を8コ形成する．merozoite は parasitophorous vacuole から遊離して他の正常微絨毛に再度侵入し，あるものは schizogony を繰り返し，あるものは gametogony に移行して gamete を形成する．zygote は parasitophorous vacuole 内で oocyst wall を形成し，oocyst となる．引き続き sporogony がおこなわれ，4コの sporozoite が発育して oocyst は成熟する．

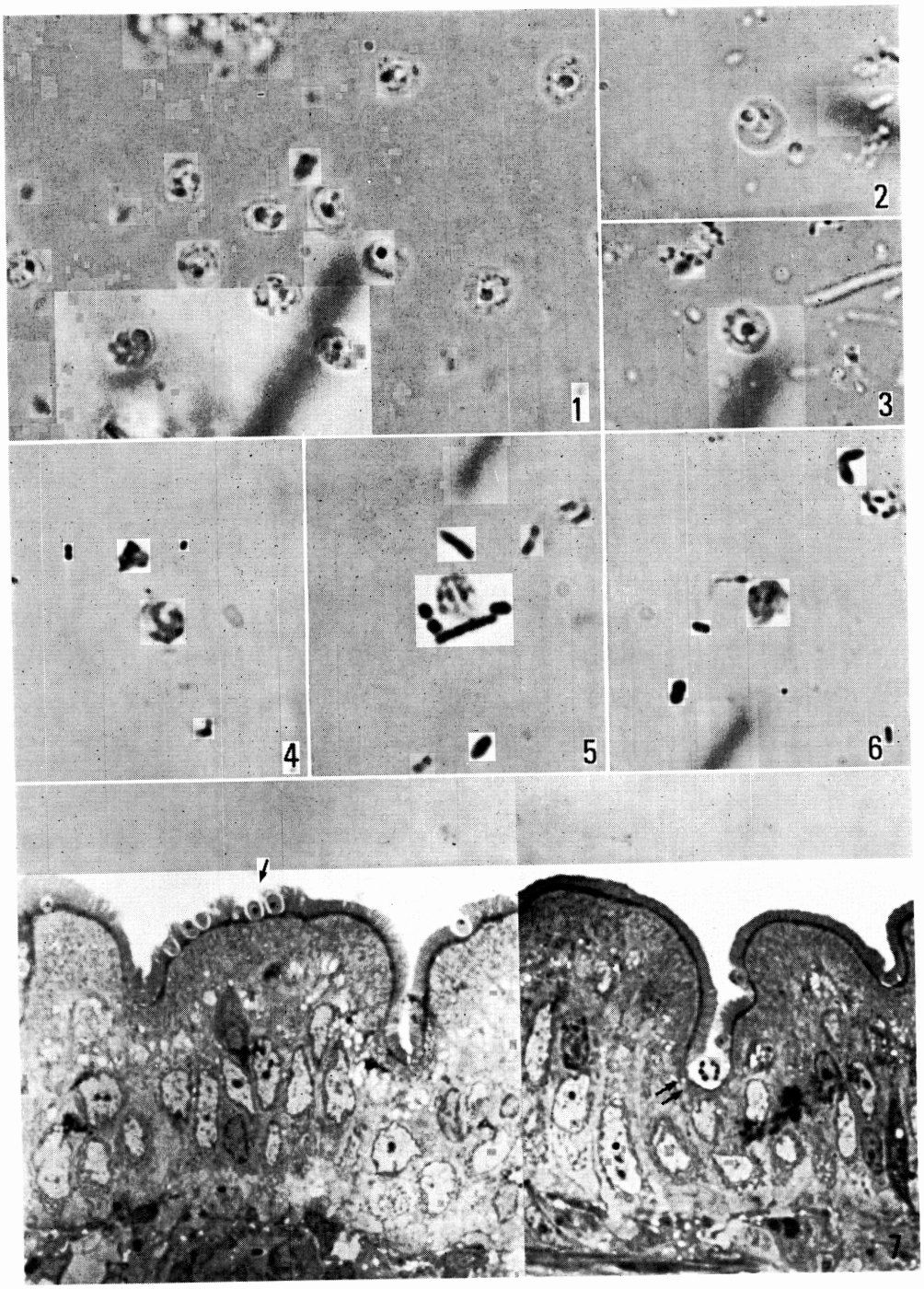
Trophozoite が形成した attachment organ は merozoite が放出されたあとも，また，oocyst が排出されたあとも parasitophorous vacuole 内で宿主細胞に付着したまま残される．

Parasitophorous vacuole 内で成熟した oocyst からは，時に，sporozoite が遊離しつつある像が観察されるので，腸管内での自家感染の存在も示唆される．

本種は中間宿主なしで直接ネコに経口感染するが，マウス，モルモットには感染しなかつた．

病原性はあまりなく，下痢その他の臨床症状は認められなかつた．

従来，*Cryptosporidium* 属原虫の特徴の記載には不十分な点があり，多くの研究者達によつて誤まつた種の同定がなされてきたが，今回この生活史を明確に示すことができたことによつて，これらの不備な点を補うことができた．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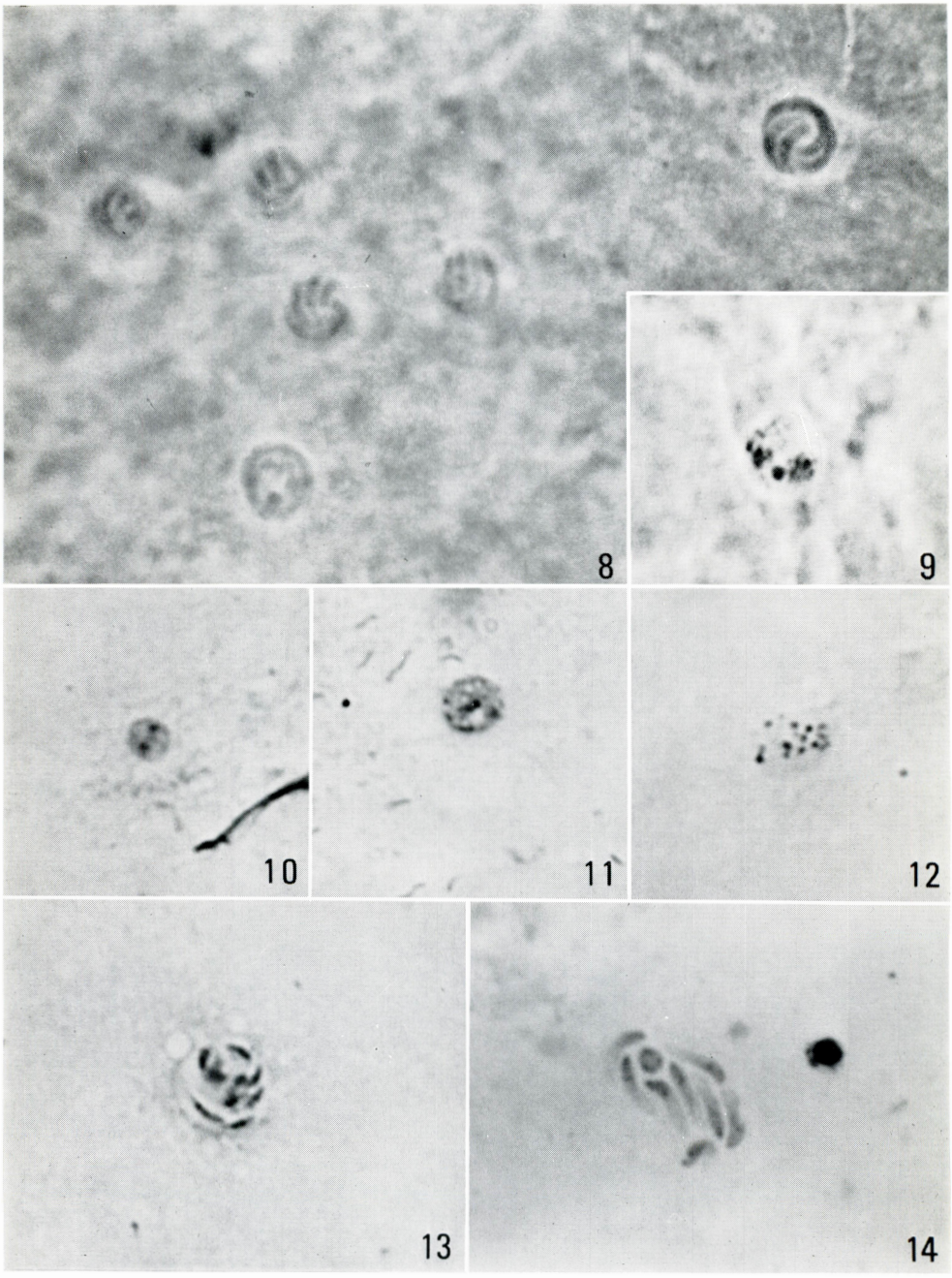
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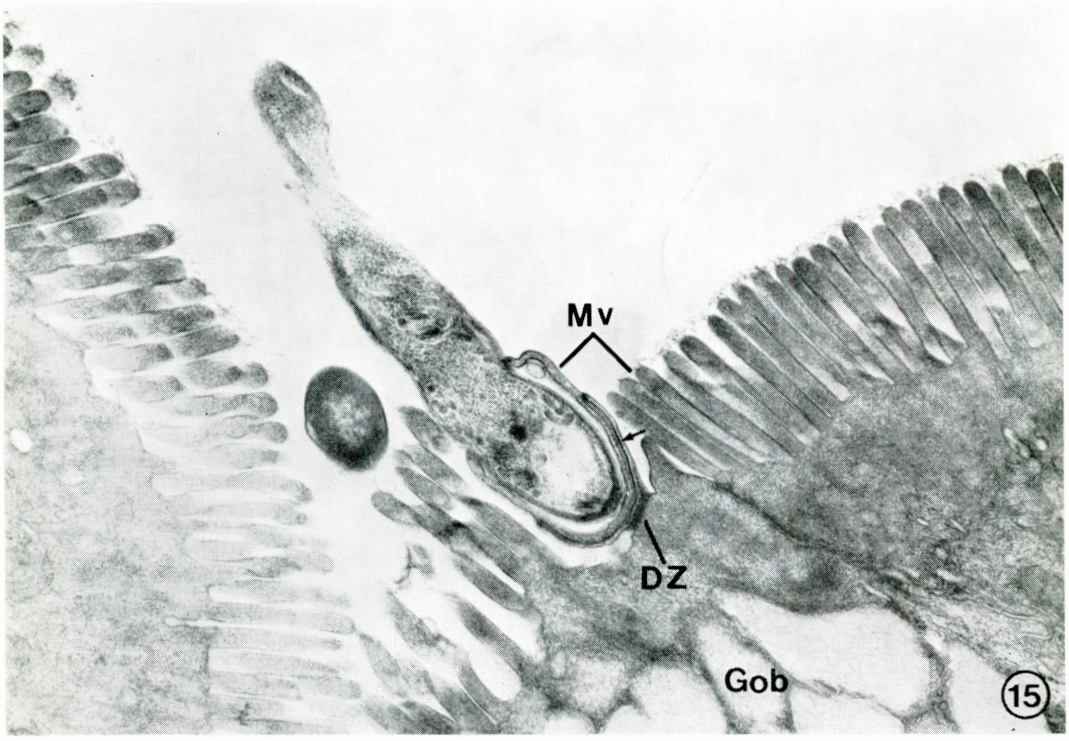
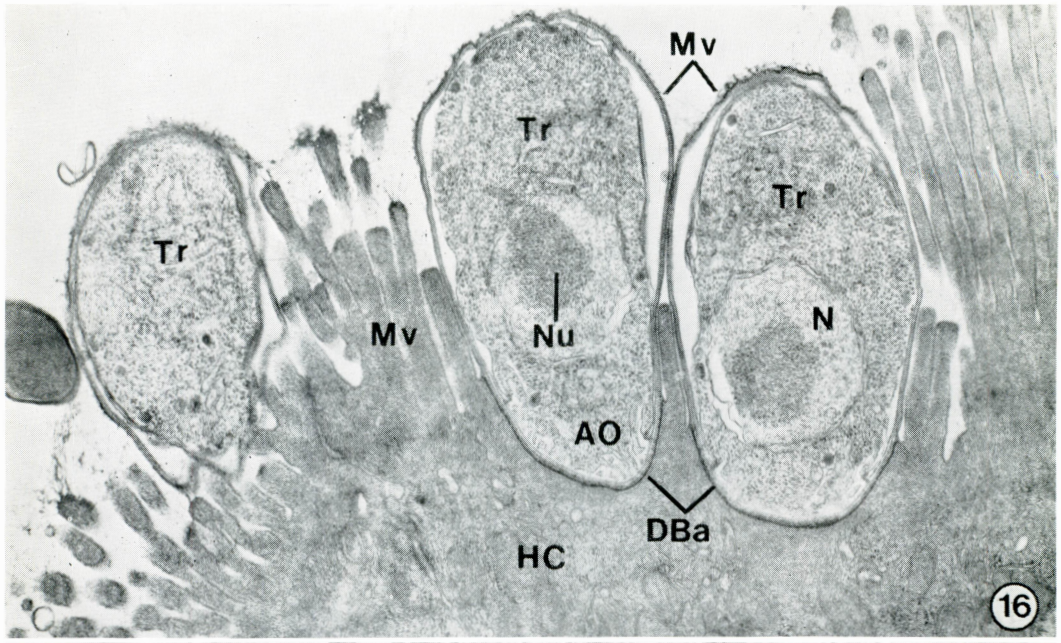



**Abbrebiations :** AO, attachment organ ; B, blebs of residuum ; Co, conoid ; Cr, crystalline inclusion ; DB, dense body ; DBa, dense band ; DZ, dense zone ; ER, rough endoplasmic reticulum ; Go, Golgi complex ; Gob, goblet cell ; HC, host cell ; Ma, macrogamete ; Mn, microneme ; Mv, microvillus ; Mvi, inner membrane of microvillus ; Mvo, outer membrane of microvillus ; Mz, merozoite ; N, nucleus ; Nu, nucleolus ; Oc, oocyst ; OW, oocyst wall ; P, parasite pellicle ; PG, polysaccharide granule ; Pi, inner nuit membrane of parasite pellicle ; Pm, middle unit membrane of parasite pellicle ; Po, outer unit membrane of parasite pellicle ; PV, parasitophorous vacuole ; RB, round body ; Sp, sporozoite ; Sz, schizont ; Tr, trophozoite ; V, membrane-bound vesicles ; WB, wall-forming body .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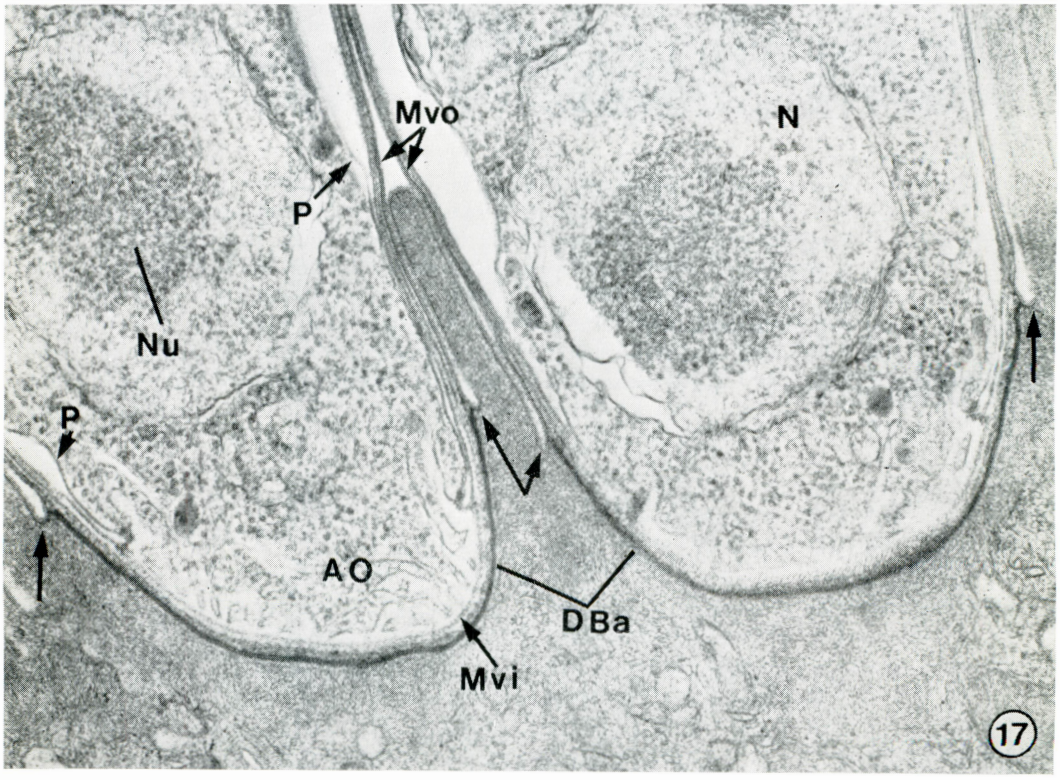
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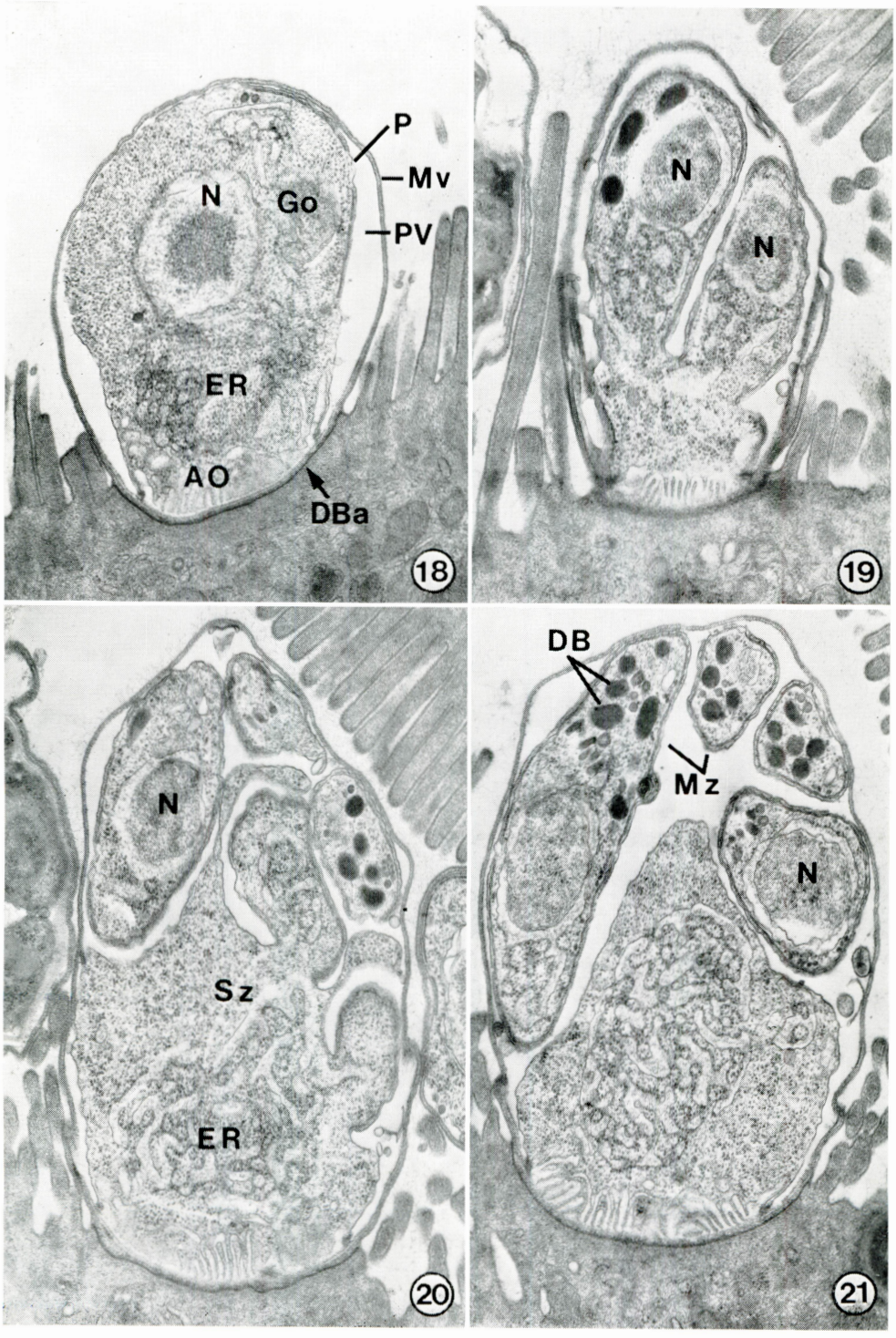
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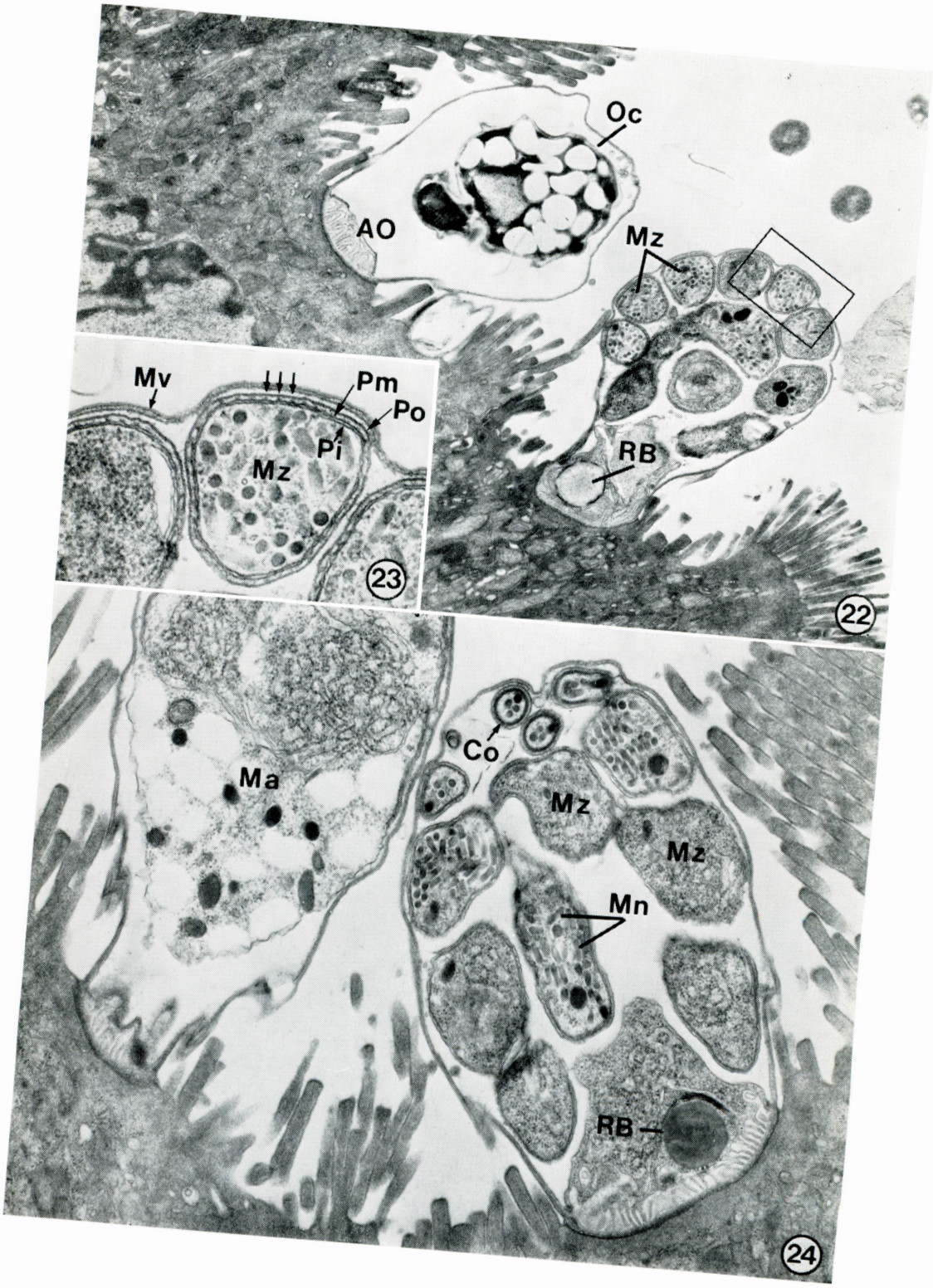
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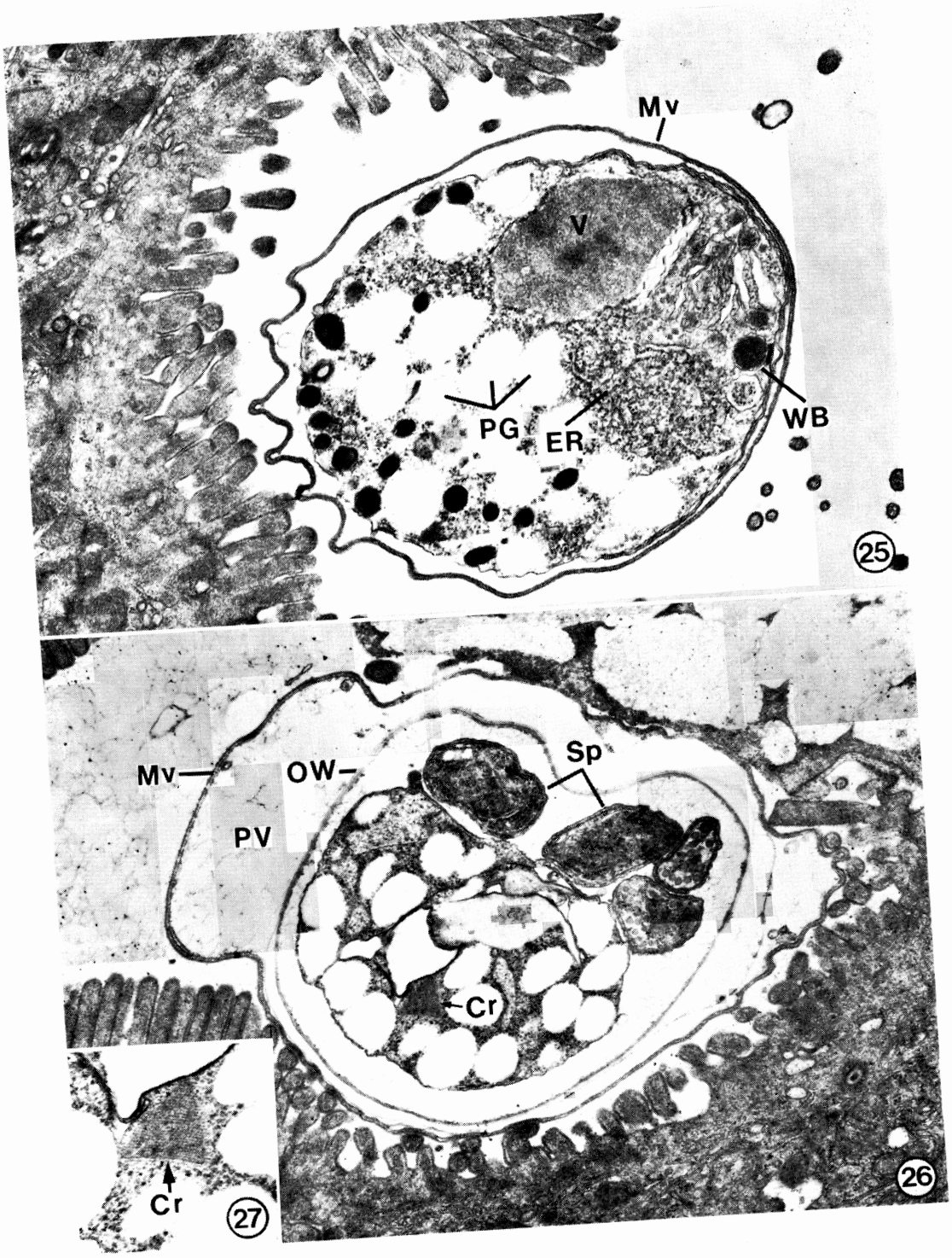
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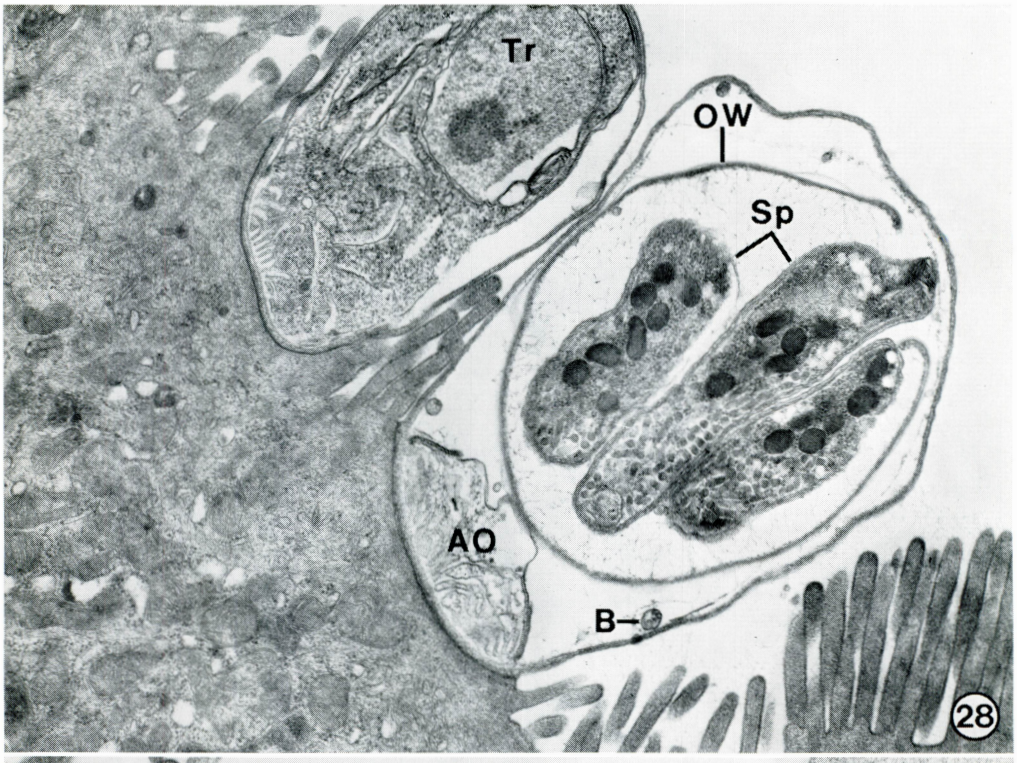
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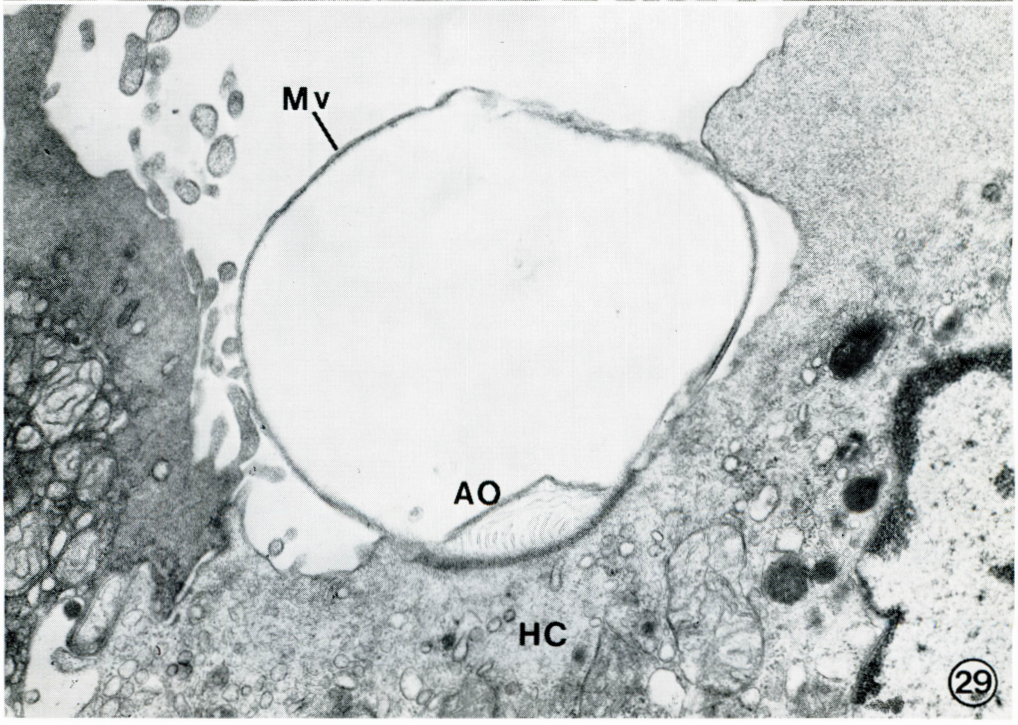
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## Explanation of Photographs

Photos. 1–3 Oocysts of *Cryptosporidium felis* isolated from fresh feces of the infected cats by zinc sulfate cetrifugal-floatation method. ×1,500.
Photo. 1 Numerous sporulated oocysts in floating materials.
Photo. 2 C-shaped sporozoites and a residual body in an oocyst.
Photo. 3 Cross view of 4 sporozoites in an oocyst.
Photos. 4–6 Oocysts isolated from feces. Giemsa's staining after methanol fixation. The cytoplasm of sporozoite is stained light, the nucleus of sporozoite and residual body are stained dark, while the oocyst wall is not stained. ×1,500.
Photo. 4 C-shaped sporozoites surrounded a residual body.
Photo. 5 Four sporozoites are seen logitudinally in an oocyst.
Photo. 6 A banana-shaped sporozoite is released from a broken oocyst.
Photo. 7 Numerous trophozoites (single arrow) and a mature schizont or oocyst (double arrows) in microvillus border of epithelial cells of small intestine. 0.5-micron section of epoxy-resin-embedded tissue. Giemsa's staining. ×1,000.
Photos. 8, 9 The fresh impression smears of the mucosal scrapings of small intestine. Phase contrast micrographs. ×2,000.
Photo. 8 Mature schizonts containing C-shaped merozoites.
Photo. 9 A developing microgametocyte.
Photos. 10–14 Mucosal stamp smears of the small intestine. Giemsa's staining. ×2,000.
Photo. 10 A binuclear schizont.
Photo. 11 A macrogamete with a large number of refractile granules in its cytoplasm.
Photo. 12 A developing microgametocyte.
Photo. 13 A mature schizont containing 8 merozoites and a residual body.
Photo. 14 Eight banana-shaped merozoites and a round residual body.

## Explanation of Photographs

Photos. 15-29 Electron micrographs of *Cryptosporidium felis*. Fixation in 2 % paraformaldehyde-2.5 % glutaraldehyde followed by 1.0 % osmium tetroxide, each fixative buffered with ice-cold 1.0 M cacodylate buffer (pH 7.4); embedding in Epon 812; block-staining with uranyl acetate; ultra-thin section staining with uranyl acetate and lead citrate.

Photo. 15 Sporozoite or merozoite entering into a microvillus of a goblet cell of small intestine. The microvillus invaginates deeply to its bottom, and an electron dense band is formed at the base of the microvillus (arrow) and another electron dense zone is formed on the host cell side in the cytoplasm near the first dense band too. ×20,000.

Photo. 16 Three young trophozoites covered with microvilli of host cell. Two of them possess a large nucleus with a nucleolus each. An attachment organ is located in part of the trophozoite touching to the dense band of the host cell. The electron dense zone has already disappeared. ×20,000.

Photo. 17 A higher magnified micrograph of the same parasite as those shown in Photo. 16. The parasite pellicle is distinct from the covering membranes, of which the most exterior membrane is apparently continuous to the adjacent normal microvilli (pointed by 4 arrows). Therefore, the outmost membrane covering parasite is nothing but that of a swollen microvillus. Electron dense bands are seen lying on the surface of the cytoplasm of the host cell, bordering the inner membrane of microvillus. An attachment organ is composed of membranous folds and continuous to the parasite pellicle. ×50,000.

Photo. 18 A developing trophozoite having a rough endoplasmic reticulum, a Golgi complex and a nucleus with a nucleolus, in the parasitophorous vacuole made of a microvillus. ×20,000.

Photo. 19 Two budding merozoites, each having a nucleus without nucleolus, dense bodies and rough endoplasmic reticula, etc. The pellicle of each merozoite is continued with that of the cytoplasmic residuum of a shoizont. ×20,000.

Photo. 20 A developing schizont. ×20,000.

Photo. 21 A more developed schizont than that shown in Photo. 20. Within anterior portion of a merozoite there are found several dense bodies (rhoptries), micronemes and membrane-bound electron-pale vacuoles. A nucleus without nucleolus is located in the middle portion, while the posterior portion is filled with rough endoplasmic reticula. The cytoplasmic residuum of schizont is also filled with the cisternal rough endoplasmic reticula. ×20,000.

Photo. 22 An almost mature schizont (right) and an oocyst (left) in each microvillus. Ten cross or oblique sections of 8 merozoites are seen in one schizont. ×5,000.

Photo. 23 A boxed area in Photo. 22. is shown in a higher magnification. The pellicle of each merozoite is composed of three (outer, middle and inner) unit membranes. The outer membrane are separated from the other two membranes by electron-pale space. On the outer surface of the middle nuit membrane, electron dense fibril-like structures distributing regularly at a distance of 50 m$\mu$ are seen (arrows). ×40,000.

Photo. 24 A fully mature schizont (right), and a developing macrogamete (left). A conoid is seen within the anterior portion of a merozoite. An attachment organ still remains in each parasitophorous vacuole, adhering to the host cell. A round body is seen in the cytoplasmic residuum of the schizont. ×20,000.

Photo. 25 A developing macrogamete. Many characteristic polysaccharide granules, dense bodies, wall-forming bodies, membrane-bound vesicles and rough endoplasmic reticulum are seen in the cytoplasm. ×20,000.

Photo. 26 A developing oocyst. An oocyst wall covering the developing oocyst and 4 sporozoites are seen in the parasitophorous vacuole of a microvillus. A crystalline inclusion is found in the cytoplasmic residuum of oocyst. ×20,000.

Photo. 27 The crystallin inclusion is shown in a higher magnification. It is nearly equilateral triangle, having a side length of 0.3 to 0.4 $\mu$m. ×40,000.

Photo. 28 A fully developed oocyst (lower) and a developing trophozoite (upper). The sporozoite covered with a pellicle contains numerous micronemes, dense bodies and electron-pale vacuoles in the cytoplasm. The oocyst wall is broken in a part at an opposite side to the attachment organ, and a sporozoite is leaving there. Attachment organ remains outside of the oocyst wall. ×20,000.

Photo. 29 A swollen and empty microvillus having a large parasitophorous vacuole in which only an attachment organ still remains after the escaping of merozoites or oocyst. The host cell is almost degenerated. All of the microvilli except the parasitized one are lost and the host cell cytoplasm is vacuolated. ×20,000.