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The ultrastructure of The Parasite and The Mucus Cell Relationship and Endogenous Stages of *Cryptosporidium muris* in The Stomach of Laboratory Mice

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Abstract: The ultrastructure of endogenous stages of *Cryptosporidium muris* in the stomach of laboratory mice was studied by transmission electron microscopy. Electron microscopy showed the various developmental stages as free in the gastric gland lumina and attached to the mucus cells. The changes in organelle composition and structure of various stages from the sporozoite to young trophozoites and matured oocysts were demonstrated in detail. Two types of the meront were discriminated. The finding of a microgamete on the parasitophorous vacuolar membrane surrounded a macrogamont was interesting for the stage of fertilization in *C. muris.* Types 1 and 2 wall-forming bodies were present in fertilized macrogamete or zygote. During sequential development of the endogenous stages, the zygote was separated from the attachment organelle by the clefts, the sporogony process simultaneously appeared when the wall formation progressed, and the sporoblasts were enveloped by budding from the sporont. As a result, thick and thin walled oocysts were found in the parasitophorous vacuole.

The interaction of *C. muris* to mucus cells were quite different from those of other protozoans including *Crytosporidium parvum.* Each developmental stages of the parasite contained a unique "attachment organelle" which is characteristic of the base of their parasitophorous vacuole. This organelle consisted of a well-developed feeder organelle and the filamentous process. In the free stage of the parasite, it was observed that the zoites developed a surface membrane complex. It was thought that this membrane complex is involved in the building of the attachment organelle.

Key Words: Laboratory mice, Stomach, *Cryptosporidium muris,* Ultrastructure

Laboratuvar Farelerinin Midesinde *Cryptosporidium muris*'in Endojen Evreleriyle Parazit-Mukus Hücre İlişkisinin Ultrastrukturu

Özet: Laboratuvar farelerinin midesine yerleşen *Cryptosporidium muris*'in endojen gelişim evrelerinin ultrastrukturu transmission elektron mikroskop (TEM)'la incelendi. *C. muris*'in bilinen endojen gelişim evrelerinin tamamının mide bez lumenlerinde serbest veya mukus hücrelere tutunmuş olarak bulunduğu görüldü. Sporozoitlerden genç trophozoit ve olgun oosistlere gelişen parazitlerin yapısı ve organel kompozisyonundaki değişiklikler ayrıntılarıyla demonstre edildi. *C. muris* infeksiyonunda ilk olarak biri 6-8 merozoitli ve diğeri 4 merozoitli olmak üzere iki tip meront ayırımı yapılabileceği gösterildi. Makrogamontu çevreleyen parazitofor vakuoler membran üzerinde mikrogamet bulgusu, fertilizasyon aşamasının demonstrasyonu açısından ilgi çekiciydi. Birbirini izleyen gelişim evlerinde tip 1 ve 2 duvar şekillendirici cisimciklerin fertilize makrogamet veya zigotta ortaya çıktığı belirlendi. Zigotun yarıklanmalarla birleşme organelinden ayrılarak sporonta geliştiği ve ince kalın duvarlı olmak üzere iki tip oosistin oluştuğu saptandı.

Mukus hücre-parazit ilişkisi *C. parvum* dahil diğer protozoonlarınkinden heryönüyle farklıydı. Mukus hücreye tutunmuş her parazit, parazitofor vakuollerinin karekteristiği olan farklı bir birleşme organeli içeriyordu. Bu organel, iyi gelişmiş bir gıda organeli ile filamentöz yapıdan ibaretti. Mide bez lumenlerinde serbest olarak bulunan zoit'lerin yüzey membran kompleksi geliştirdiği gözlendi. Bu membran kompleksinin mukus hücreye bağlanmada, parazitofor vakuolar membran ve birleşme organeli gelişiminde büyük rolü olduğu düşünüldü.

Anahtar Sözcükler: Laboratuvar faresi, mide, *Crytosporidium muris,* ultraktruktur.

Introduction

Protozoan parasites of the genus *Cryptosporidium* were first reported from the stomach of laboratory mice at the beginning of this century by Tyzzer (1, 2) who proposed *Cryptosporidium* as a new genus and *C. muris* as a new species. *C. parvum* as a second species in the genus *Cryptosporidium* was described from the intestine of laboratory mice later by the same author (3). Under light microscopy, Tyzzer (1, 2) provided complete description of *C. muris* in the stomach of mice. He reported asexual and sexual modes of reproduction followed by spore (oocyst) formation, noting the presence of an attachment organelle at all stages and, demonstrated its transmissibility through feeding trials.

Since Tyzzer's original descriptions, the first ultrastructural study of the genus *Cryptosporidium* was performed with *C. parvum* from the intestine of mice (4). In the following years, comprehensive studies have revealed the ultrastructural features of the endogenous stages of *C. parvum* in various host animals (see for reviews 5, 6). Natural infections with *C. muris* have only ben reported from mice (7, 8), rats (9), cattle (10-12), mountain gazelles (13) and a camel (14). Transmission experiments with *C. muris* oocysts isolated from the feces of mice and rats appeared similar features with oocyst morphology and the site specifity for infection in various laboratory animals (15, 16). Similar findings were obtained by directly isolated oocysts from the murine stomach (17). Up to now, however, comprehensive studies concerning the ultrastructure of *C. muris* are inadequate. In a single study, ultrastructural observations of *C. muris* were presented in experimentally infected mice (18), although development of *C. muris* in the stomach have been reported with a few electron micrographs from wild house mice (7) and mountain gazelles (13).

The purpose of this study is to present the results of the ultrastructural observations in detail for *C. muris* from the naturally infected laboratory mice.

Materials and Methods

The stomachs of randomly-breed, conventionally reared laboratory mice (6-week-old, five male and five female) were used in the present study. They were obtained from the animals of the colonies of the Hıfzısıhha Intitute's animal unit in Ankara, Turkey. The animals of these colonies had been found to be naturally infected by endogenous stages of *C. muris* (8). Samples of 1 mm^3 from the stomachs of selected animals from same colonies were put into 4% glutaraldehyde fixative prepared by cacodilate buffer for transmission electron microscopy. Then they were postfiexd with 1% osmium tetroxide; dehydrate in graded alcohols and blocked in Epon 812. Ultrathin sections were obtained and stained with uranyl acetate and lead citrate and examined in a Carl-Zeiss EM 9 S electron microscopy.

Results

Endogenous developmental stages of *C. muris* were enclosed by the parasitophorous vacuolar membranes with two layers, and the gastric gland lumina were completely filled by them. They were attached to the apical surface of the mucus cell of the gastric glands with

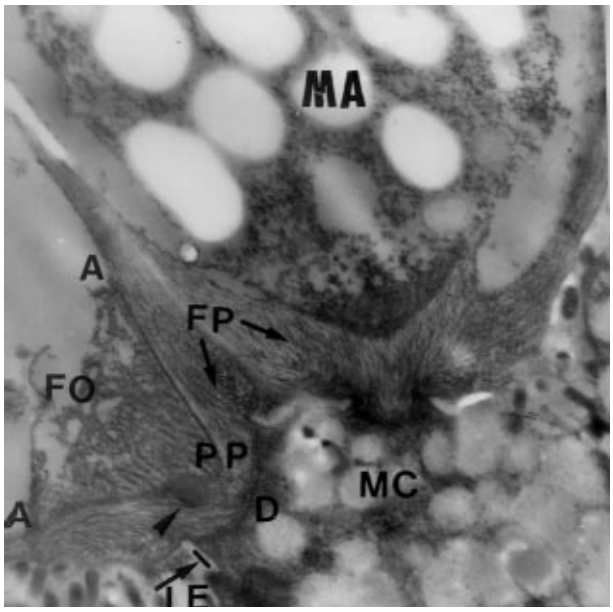

Figure 1. Transmission electron micrograph showing unique attachment organelle associated with a developing thin-walled oocyst (left) and fertilizated macrogamete or early zygote (right, MA) of *C. muris*. Both parasites attached to the apical surface of the mucus cell (MC) and developing oocyst has separated from the attachment organelle (oocyst wall not shown). Note a well-developed feeder organelle (FO) and filamentous process (FP), the annular ring (A), electron-dense band (D), anterior projection of the parasite (PP), local elevation of the mucus cell (LE), food vacuole (arrowhead) in the base of anterior projection of the parasite. x17.000.

their conoidal projection. This projection was relatively blunt in young trophozoites, whereas it was sharply ended in young macrogamonts. The parasites contained a unique attachment organelle which is characteristic of the base of each parasitophorous vacole. This organelle consisted of a well-developed feeder organelle with membranous lamellae and the filamentous process with thin regular filaments; both were integral parts of the attachment organelle during the entire endogenous development of *C. muris* (Figure 1). The parasite plasmalemma immediately behind the projecting anterior end of the parasite formed an annular ring as connected with the inner layer of the parasitophorous vacuole. There was an electron-dense line at the border of the feeder organelle, which was formed by the projecting anterior end of the parasite and connected to the annular ring. The feeder organelle was extended between the projecting end of the parasite and the annular ring. The outer membrane of the parasitophorous vacuole was closely connected with the plasma membrane of the mucus cell at the attachment organelle border. The

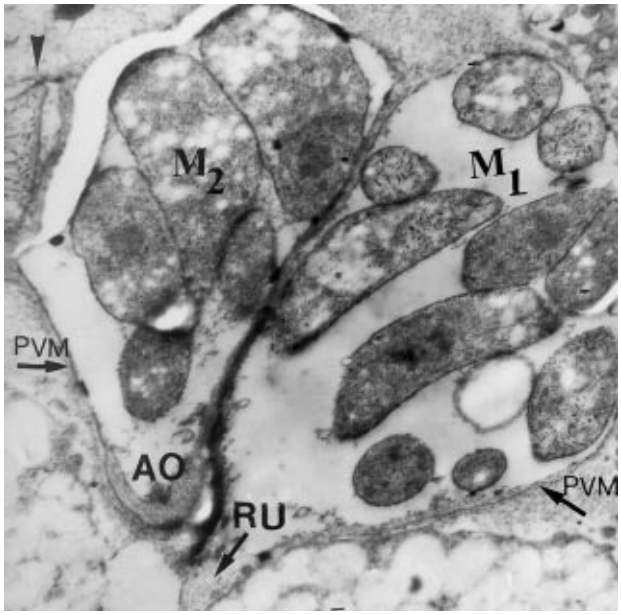

Figure 2. Matured type 1 (M1) and 2 (M2) meronts and a section of curved apical end of freed merozoite (above left) in the lumen of the gastric gland. Note rupture of maturated type 1 meront (RU), dislodgement from original attachment of type 2 meront as intact with attachment organelle (AO) and the parasitophorous vacuolar membrane (PVM), and the apical end of free merozoite is protruded anteriorly and an electron-dense fibrillar-membranous structure extruded from the anteriormost end (arrowhead). x13.500.

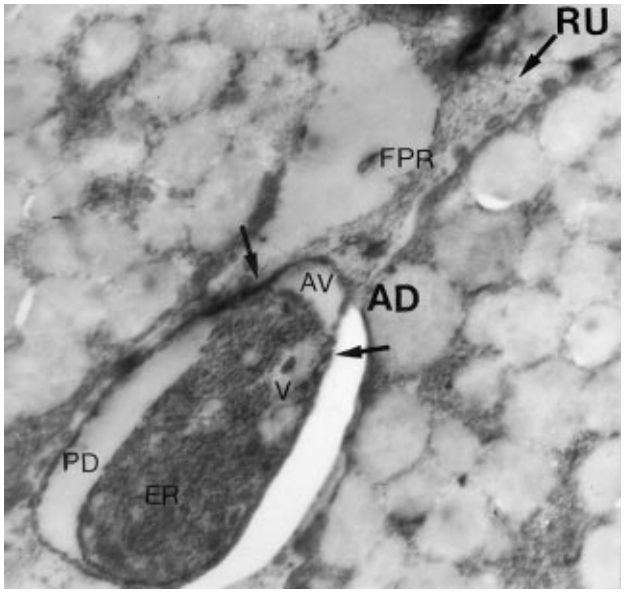

Figure 3. The appearance of a freed and attaching merozoite with an interesting aspect immediately after rupture (RU) of the parasitophorous vacuolar membrane (PVM) of type 1 meront as a sequential micrograph of Figure2. Note the filamentous process remnants (FPR), adhesion between parasite-mucus cell plasma membrane (AD), dissociation of the parasite plasmalemma (PD), apical vacuole (AV), apical rings (arrows) including local membranous elevations and folds toward the exterior, electron-lucent vacuolations (V), rough endoplasmic reticulum (ER) in the cytoplasm of the parasite. x28.500.

projecting anterior end of the parasites was densely covered by the filamentous process in accordance with its shape from the attachment base to near the anterior third of the parasites. An electron-dense band with two layers distinctly separated the filamentous process from the cytoplasm of the mucus cell. There were also seen local elevations or indentations of the mucus cell surface which is located across from the projecting end of the parasites. In both conditions, the mucus cell exhibited a crater-like appearance opposite to the inhabitant parasite.

It was observed that the most of the parasites firmly attached to the mucus cells. Some of the parasites, especially matured forms, however, were losely attached to the mucus cells or dislodged from their original attachment as intact with the filamentous process, parasitophorous vacuolar membrane (PVM), and even electron-dense band. It was also clearly seen that the zoites were freed from fully matured forms by rupture of the PVM, probably from the annular ring line (Figure 2). Figure 3 shows freed and attaching merozoite with interesting aspect immediately after rupture of the PVM of type 1 meront. Soon after the merozoite released from the meront, its plasmalemma became dissociated and showed several folds, its anterior region vacuolated, and its anterior cytoplasm included several electron-lucent vaculoations. Such vacuolations were not seen in those of the parasitophorous vacuole. In addition, the rough endoplasmic reticulum appeared in the posterior cytoplasm. The cytoplasmic vacuolations fused with the anterior limiting membrane of the merozoite and produced the membranous folds and an opening to the exterior on the point of the apical rings. Simultaneously, the apical end of the parasite was protruded anteriorly and an electron-dense fibrillar-membranous structure extruded from the anteriormost end (Figures 2 and 3). Indeed, there was clearly evidence of a surface membrane material organization produced by the parasites. At the same time, thin wisps of irregular filaments with adhesion zones extended between the parasite and the mucus cell at this stage (Figure 3). They accumulated in front of the anterior vacuole of the parasite during initial mucus cel-parasite contact. It was clearly seen that these thin filaments were leaved from the filamentous process by the rupture of PVM of fully matured meronts and that they served to enable to freed parasites to adhere to the

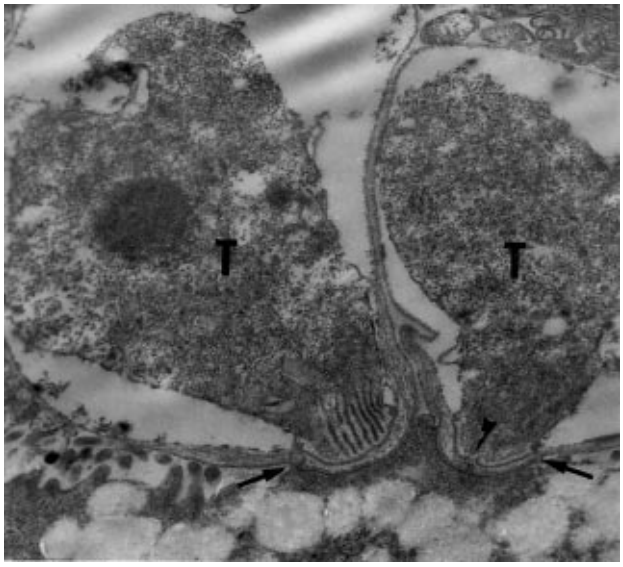

Figure 4. Developing (right) and more completely developed (left) two trophozoites attached to the same mucus cell. Note differences in size, the organelle composition, and the attachment organelle with development stage of the feeder organelle (arrowhead) and the annular rigs (arrows). x13.500.

mucus cell surface after they have become dislodged from their original attachment.

Following attachment, the free stages remained elongated for some time and subsequently transformed into an ovoid shape, and underwent differentiation. In the early attachment state, the annular ring was contacted to the inner and outer layers of the PVM's and the cytoplasmic membrane of the mucus cell with the margins of the anterior projection of the parasite (Figure 4). It was clear that this structure was corresponding to the region of the apical rings which were seen to produce structural changes in freed forms. Thin wisps of the filaments, which at first accumulated in front of the anterior vacuole of the parasite during initial mucus cell-parasite contact, developed to the filamentous process with regular thin filaments. The filamentous process became prominent as the parasite matured, wholly occupied the base of the parasitophorous vacuole, and extended between the outer and the inner layers of the parasitophorous vacuole to near the anterior third of the parasites. The shape of the filamentous process varied according to the development time and the shape of the apical end of the parasite. It was prominently depressed in direction of the mucus cell from margins outside of local elevations of the mucus cell surface by pressure of maturating forms (Figure 1), but not in the early stages. There were also found membrane-bound vacuoles in the

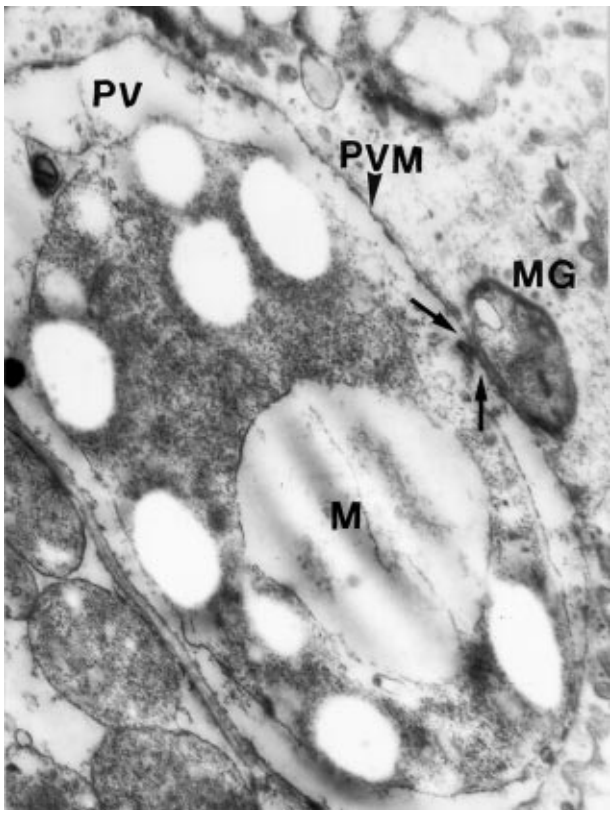

Figure 5. Transverse section of the fertilization process. A microgamete (MG) is situtated on the parasitophorous vacuolar membrane (PVM) of the macrogamont (M) and its nucleoplasm is entering the cytoplasm of the macrogamont through a ring or chanelle-like structure (arrows). PV is parasitophorous vacuole. x16.000.

filamentous process of some of the parasites (Figure 1). These vacuoles were interpreted as food vacuoles taken from the mucus cell cytoplasm to the parasite. The inner part of the anterior projection of the matured parasites consisted of the feeder organelle lamellae which are associated with other in various direction and including minute intermembrane particles.

Trophozoites, first examples of the attachment organelle development stage between the mucus cell and the parasite, contained a large nucleus with a prominent nucleolus, various ribosomes, rough endoplasmic reticulum, Golgi complex, lipid body, and a well-developed attachment organelle in various stages of development. During early development of the trophozoites, there was observed an invagination or dissolution in the anterior projection of the parasite, and an elevated membranous structure from the inner layer of the electron-dense band between mucus cell and the filamentous process (Figure 4). This growth clearly was in agreement with the feeder

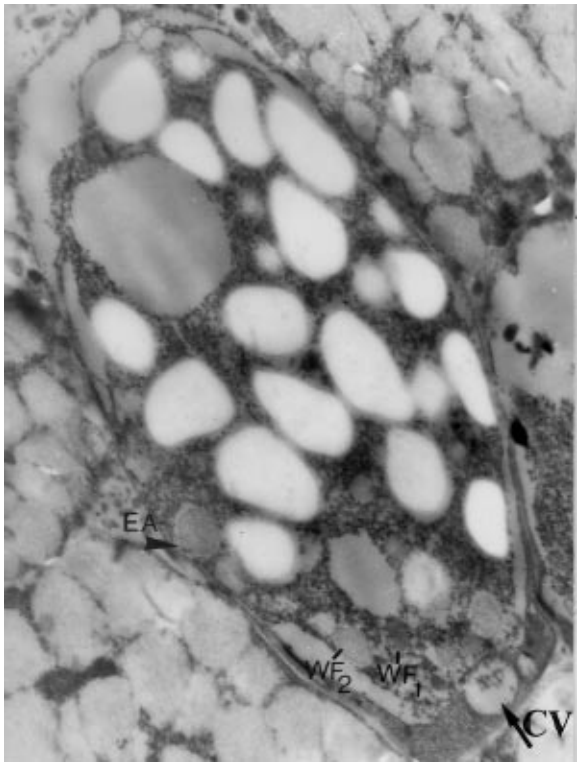

Figure 6. A fertilizated macrogamete or early zygote showing two types of the wall-forming bodies (WF1 and WF2) which are clustered at the periphery of the parasite and the base of the attachment organelle. Note some of the wall-forming bodies show tendency to discharge their contents as an electron-dense arch (EA) towards the outside. The feeder organelle disappear and the filamentous process contains central vacuolations (CV). x14.500.

organelle development.

Two types of the meronts were seen in the present study (Figure 2). One of these contained six-eight merozoites and was therefore in the first generation; the other contained four merozoites and was in the second generation. When both types of the meronts matured, all of their cytoplasm was incorporated into the merozoites. Type 1 merozoites were arranged randomly within the parasitophorous vacuole, whereas type 2 merozoites were arranged regularly with their posterior ends broadly rounded and directed away from the base of the attachment organelle. Type 1 merozoites showed several appearances such as banana, crescent, or comma-shaped with a rounded posterior end that tapered to a pointed anterior end. Nucleus with prominent nucleolus was located in the central third of the parasite. Type 2 merozoites were cudgel-shaped; location of nucleus and their internal cytoarchitecture were similar to those of type 1 merozoites, but they were shorter and wider.

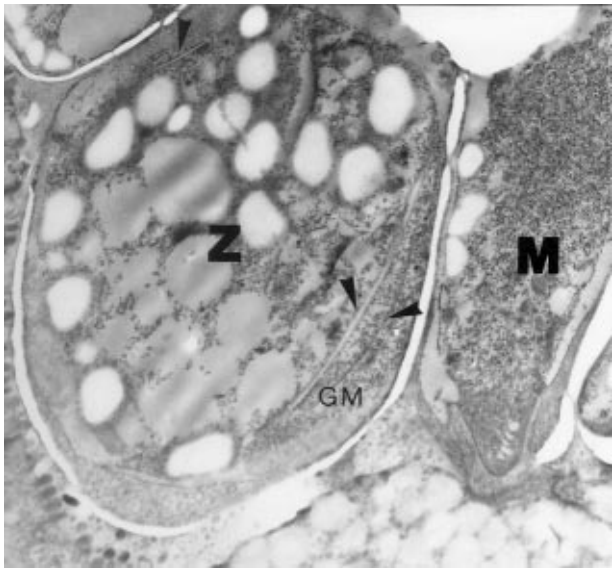

Figure 7. An early macrogamont (M) with the amylopectin granules (periphery located), and a zygote (Z) which is developing to the sporont. Note that the clefts (arrowheads) appeared at the periphery of the zygote as circumscribed and supported by external membranes. They are filled diffusely by very thin granular material (GM). x11.000.

Macrogamonts were easily recognizable by a relatively large nucleus with nucleolus, lipid bodies, amylopectin granules, and a well-developed attachment organelle. They accumulated food reserves in the form of lipid bodies and amylopectin granules, and their size was enlarged and relatively rounded up at the mature stage.

Although microgamonts were not seen, microgamete was observed on the parasitophorous vacuolar membrane covering the macrogamont (Figure 5). At the point of fusion of the macrogamont and the microgamete a narrow ring or channel-like structure associated with the projection of plasmalemma of the macrogamont was observed. This was interpreted as the fertilization process. A centrally located large refractile globule in the cytoplasm of the macrogamont was also observed. At the serial sections of the fusion, microgamete was bound by unite membrane and included various tubules extended to the fusion side, a mitochondrion-like structure and a membrane bound vacuole. It was possible to follow that the majority of nucleus of the microgamete entered the cytoplasm of the macrogamont. Typical flagella could not be found. The actual penetration of a microgamete into a macrogamont was not observed.

After fertilization of the macrogamont by the

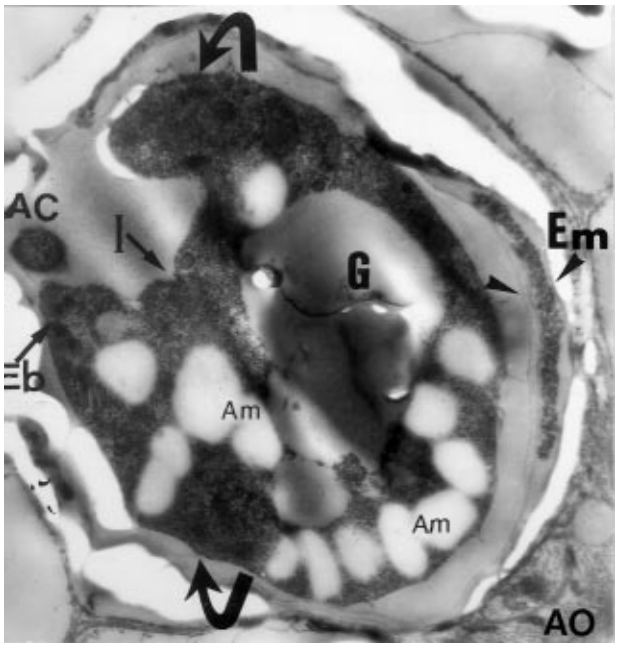

Figure 8. Early stage of sporogony showing separation of the sporoblasts from the sporont. The sporont is wholly separated from the attachment organelle (AO), covered by external membranes (Em) containing granular material segments, and include a centrale globule (G) and amylopectin granules (Am). The sporoblasts are budding and there is a small invagination (1) on the middle of the sporont side where the sporoblasts are budding. Note development of the apical comlex (AC) organelles, electron-dense bodies (Eb), and prominent nuclei (curved arrows) of the budding sporoblasts. x14.500.

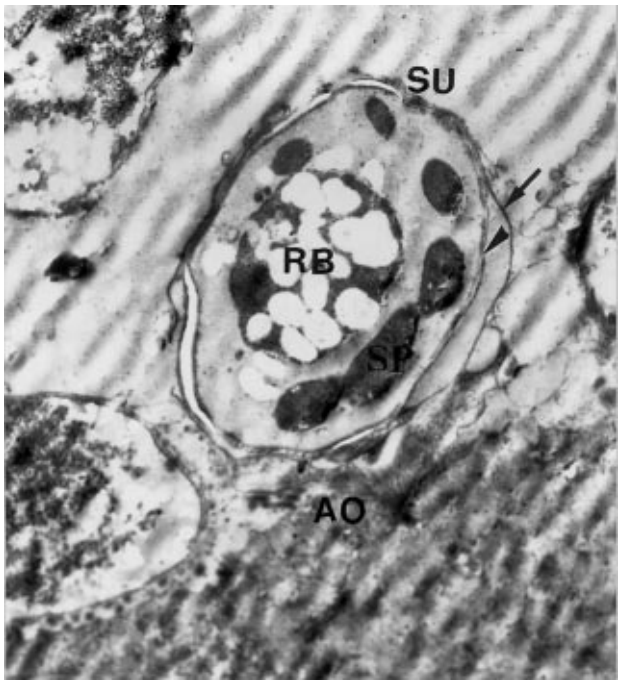

Figure 9. A fully sporulated thick-walled oocyst within a parasitophorous vacuole. The parasite is just surrounded by the parasitophorous vacuolar membrane (arrow), the feeder organelle disappeared and the filamentous process regressed. Note the suture (SU) in the inner oocyst wall, the residual body (RB) with the amylopectin granules, and naked sporozoites (SP) within the oocyst wall with two layers (arrowheads) which have separated from the attachment organelle (AO. x8.500.

microgamete, fertilized macrogamete or the zygote developed (Figure 6). The type 1 and 2 wall-forming bodies appeared in the cytoplasm of these forms. With membrane-bound and electron-dense or electron-lucent granular material contents, they were clustered at the periphery of the parasites and often at the base of the attachment organelle. Some of the wall-forming bodies at the periphery showed tendency to discharge their contents as an electron-dense arch towards the outside (Figure 6). Meanwhile, sporont was simultaneously separated from the attachment organelle as the clefts appeared at the periphery of the parasite. The clefts were circumscribed and supported by external membranes. These clefts were filled diffusely by very thin granular material similar to those of the wall-forming bodies (Figure 7). Coincident with this growth, the wall-forming bodies disappeared in the cytoplasm of the parasite. These clefts extended, then were completely separated from the zygote plasmalemma, and subsequently, appeared as segmental external layers bearing granular material particles with regular granular pattern in the intermembranous spaces. They especially became prominent when sporogony process started (Figure 8). The newly formed external layers were irregular in appearance and thickness coincident with the granular material content in segments. In subsequent development, however, they underwent condensation and became prominent as a wall consistig of two layers separated by electron-lucent space except for thin-walled oocysts which are surrounded only by a single membrane.

When the oocyst wall formation progressed, it was found that the sporogony process simultaneously appeared (Figure 8). The feeder organelle either remained intact during thin-walled oocyst development (Figure 1), or disappeared during thick-walled oocyst development (Figure 9). Similarly, the filamentous process either remained intact, or relatively regressed, became more loose, and included central vacuolations and some structures similar to the wall-forming bodies which are surrounded by a distinct membrane and exhibiting

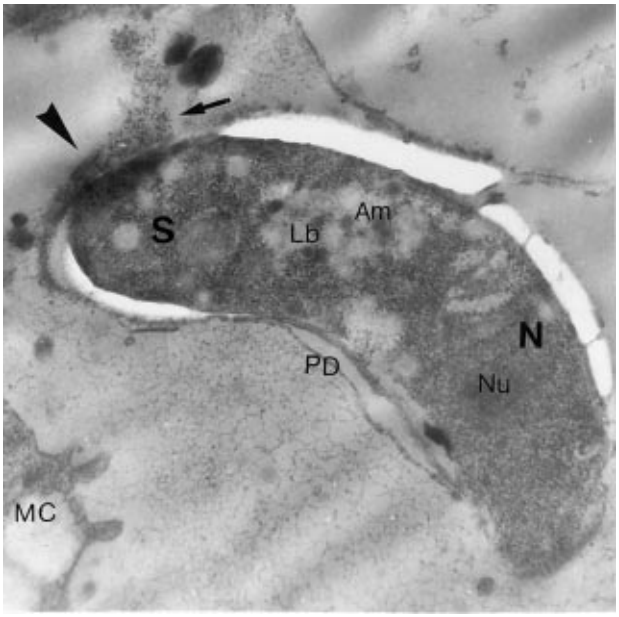

Figure 10. A free sporozoite in the gastric gland lumen. Note partial flexing, dissociation of the plasmalemma (PD), conoid-like framework with regular diagonal stripes (arrowhead) associated with releasing fibrillar-membranous material (arrow) from the apical end or the protruded conoid. Its visible internal organelles include small amylopectin granules (Am), the lipid bodies (Lp), one anterior spherical structure (S), and a nucleus (N) with prominent nucleolus (Nu) near the posterior end. x21.000.

chanelle-like opening toward the parasite. There was clearly evidence of the feeder organelle and the filamentous process involvement in oocyst wall formation, probably by association with wall-forming body production. This possibility was agree with development of the thick-walled oocysts which are passed unaltered in the feces.

At the early stage of the sporogony process (Figure 8), the sporont included a central globule and amylopectin granules. It was observed that the sporoblasts were developed by budding from the sporont. There was a small invagination on the middle of the sporont side where the sporoblasts are budding (Figure 8). Each of the two budding sporoblasts included a prominent nucleus with nuclear pores, intranuclear particles and even nucleolus. The development of the apical complex organelles of budding sporoblast at this stage could be clearly seen (Figure 8). Sporogony process involved in formation of a central oocyst residuum and division of the sporont into sporoblasts by budding, which then developed into sporozoites. No sporocyst membranes or walls were observed within the oocyst wall. In addition to numerous thick-walled oocysts that enclosed with the outer and inner walls, several oocysts surrounded by a single membrane were observed in parasitophorous vacuole. Both could easily be distinguished from the developing meronts by the formation of a conspicuous oocyst wall and residual body (Figure9), but it was difficult to observe interior of oocyst forms, since the majority collapsed, some ruptured, and were not well fixed and electron-dense. However, it was possible to find that the sporozoites released from fully formed thin-walled oocysts and attached to the mucus cells. The sporozoites contained small amylopectin granules and lipid vacuoles near the center of the cell, one spherical structure including granular material, an a nucleus with prominent nucleolus near the posterior end. They showed the structural schanges similar to merozoites before the attachment to the mucus cell (Figure 10).

Discussion

With the exception of freed zoites in the gastric gland lumina, Uni et al. (18) have identified the various endogenous stages of *C. muris* attached to the mucus cells of the gastric glands by transmission electron microscopy. They reported that the entire development of the parasite occurred in the surface mucus cells of the gastric glands and that the ultrastructual features of the attachment size of *C. muris* to the host cell differed remarkably from those of *C. parvum* and its closely related species. These observations was confirmed in the present study. They observed a projection of the parasite at the site of attachment to the mucus cell, which was originally called a "knob-like projection" by Tyzzer (2). In the present study, it was also found that this projection of the parasite, which is conoidal, was relatively blunt in young trophozoites, whereas it was sharply ended in young macrogamonts.

With the original light microscopic observations, Tyzzer (1-3) considered that *C. muris* and *C. parvum* were extracellular throughout their life cycle. In a transmission and scanning electron microscopic study of *C. parvum* in calves, Pohlenz et al. (19) proposed a theory of extracellular development as they suggested that the parasitophorous envelope develops independently as the result of parasite-directed synthesis of membrane material and glycocalyx. However, the most investigators (see for reviews 5, 6) suggested intracellular development for *C. parvum* and closely related species. Goebel and Brandler (20) described the site of development of this protozoan parasites as intracellular

and extracytoplasmic. Uni et al. (18) assessed the attachment organelle development and the mucus cell-parasite interaction by the observation of the parasites attached to the mucus cells. They mentioned that the parasites were enclosed by a parasitophorous vacuolar membrane of host microvillous origin and that the filamentous process was derived from the host cell. As a result, they believed that the parasites grew intracellularly in the microvilli of the mucus cell. On the contary, the present observations suggest that the parasites were wholly responsible from the building of the attachment organelle and the PVM's.

It is well known that active substances result from the interaction between some coccidia and the host cell. Most authors (21-24) suggested that rhoptries and/or micronemes as the apical complex organelles may be secretory organelles which produce some substances like enzymes or proteins, then used for penetration in host cells by discharged from the apical pole. In infection with *C. parvum* in experimentally infected mice, vacuolation of the anterior region of invading merozoites has also been observed and suggested that the material in the rhoptries and/or micronemes is released during host cell attachment (25). The electron micrographs of the present study show that an electron-dense substance extruded from the anteriormost end of the zoites and that extrusion is possible for the first time in *cryptosporidium* species positively. The present observations also provide strong evidence that the apical complex organelles in *C. muris* may be secretory organelles that aid in cellular attachment. These secretory products excellently may be incorporated into the host cell membrane, may function in a manner to produce changes in the surface characteristics of the cell, or lyses the plasma membrane. As a result, it could be considered that the attachment organelle is incorporated by excretory products or components from both host cell and the parasite, instead it is only derived from intact host microvillous membrane.

Although Uni et al. (18) reported that it was not possible to discriminate generation types of the merozoites, present observations discriminate both of meronts with two types merozoites. The merozoites were similar to those of *C. parvum* and other coccidia and possessed most of the organelles typical of the phylum Apicomplexa (5, 6).

Little is known about the fertilization in the *cryptosporidium* species. Although portions of microgametes have been observed within macrogamonts, actual penetration has not been observed (5). It has been established that only the nucleus and closely associated microtubules of the microgamete entered the macrogamont during fertilization (5). The present observations agree with these findings.

Uni et al. (18) reported that the wall of the oocysts at the early developmental stages was composed of three layers and that the outermost layer was fragile and mostly disintegrated as the oocyst matured. They also considered to be a true oocyst wall of the outermost layer whereas the middle and innermost layers assumed to develop into the sporocyst wall. However, the present observations showed that the outermost layer was in fact a layer developing by the formation of the clefts which are circumscribed and supported by additional external membranes at the periphery of the zygote. This layer then produced the outer layer of the fully sporulated thick-walled oocyst. The middle and innermost layers consisted of only two unit membranes, and it could be considered that they produced the outer and the inner zone of the inner layer of fully sporulated thick-walled oocysts. Similar observations have been reported for oocyst wall formation of *C. parvum* in experimentally infected mice (25). A similar process is possible for *C. muris.*

There have been found two kinds of oocysts in genus *cryptosporidium* (5, 6, 25). In the present study, two kinds of oocysts were also found. Ultrastructurally, they were similar to those of *C. parvum* and other coccidia with the oocyst wall consisting of distinct inner and outer layers. It is definitely clear that thick-walled forms transmitted the infection to another host and that thin-walled forms are autoinfective.

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