Schizogony in *Toxoplasma gondii*: An Electron Microscope Study

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**ABSTRACT.** Schizonts of *Toxoplasma gondii* develop within vacuoles in the intestinal epithelial cells of the cat. The schizont is surrounded by a pellicle consisting of an inner and an outer membrane. There is abundant endoplasmic reticulum and ribosomes but organelles characteristic of endodyogeny are absent in the two-nucleate stage. After two or more nuclear divisions merozoites begin to form adjacent to the nuclei in a manner similar to that of endodyogeny. However, schizogony differs from endodyogeny by formation of more than two offspring per parent cell. It also differs from schizogony in other coccidia by the formation of merozoites adjacent to the nucleus rather than at the schizont surface. The adaptability of *T. gondii* to survive in a variety of hosts may be related to its unusual method of reproduction.

Nicolle and Manceaux (1909) and later workers reported that *Toxoplasma gondii* divided by binary fission. However, Goldman et al. (1958) recognized that division was accomplished by a unique method which they termed endodyogeny. Electron microscope studies by Ludvik (1958), Gavin et al. (1962), Ogino and Yoneda (1966), Wildfahr (1964), van der Zypen and Piekarski (1967), and Sheffield and Melton (1968) have confirmed the findings of Goldman et al. and have suggested that endodyogeny may be the only type of division taking place in *T. gondii*.

Hutchison et al. (1970) and Frenkel et al. (1970) have reported the presence of schizogenic stages of *T. gondii* in the intestinal epithelium of cats. These stages appeared to be similar to those seen in *Eimeria* and *Isospora* infections. The present work depicts the fine structure of the developing schizont and describes a type of division unlike that previously seen in schizogony and having some of the characteristics of endodyogeny.

**Materials and Methods**

Precautions were taken to obtain kittens which were coccidia-free. A pregnant queen which had been born, raised and bred in a closed breeding colony at the NIH Animal Center was obtained and housed in an isolation cage where she delivered and nursed four kittens until they were killed. Numerous examinations of her feces did not reveal any *Isospora* oocysts. No stages of *I. felis* or *I. rivolta* were seen in histological sections of small intestine from the kittens.

Two of these kittens were infected two days after birth by feeding them emulsified mouse brains which contained cysts of *T. gondii* (strain C-56). Seven days after feeding, they were anesthetized and perfused via the descending aorta with physiological buffered saline followed by 5% glutaraldehyde in phosphate buffer at pH 7.2. The small intestine of each kitten was removed, cut into 6 equal lengths and then stored in the glutaraldehyde fixative at 4°C until needed. A portion from each length of intestine was prepared for histological examination to determine which areas were suitable for electron microscopy. Portions of heavily infected segments were then post-fixed for 2 hours with 2% OsO₄ in phosphate buffer, dehydrated and embedded in Epon. Thin sections were subsequently cut and stained with lead citrate and uranyl acetate prior to examination with the electron microscope.

**Results**

Histological sections of small intestine from kittens revealed various endogenous stages of *Toxoplasma gondii*. An area of heavy infection was found in the lower small intestine of one animal and a portion of that tissue was processed for electron microscopy.

An early schizont stage is shown in Figure 1. The organism is somewhat oval-shaped and lies within a vacuole near the apical edge of the host epithelial cell. An unusually thick vacuolar
wall separates the parasite from the host cell cytoplasm. The organism has the typical two-layered pellicle seen in other stages of *T. gondii* as well as other sporozoans but no subpellicular fibrils have been seen. There is an abundance of ribosomes, both free and attached to the membranes of the endoplasmic reticulum. The endoplasmic reticulum is more highly developed than in proliferative forms or sporozoites. Several mitochondria with typical tubular cristae are present. The nuclei are each surrounded by a nuclear envelope and have scattered clumps of chromatin around the periphery and in the central area. A conoid (not illustrated) is present at the anterior end. In the two-nucleate stage there are no structures in the parasite which resemble the membranes and organelles typically seen during endodyogeny.

Formation of merozoites may proceed after the second nuclear division. Three developing merozoites are seen adjacent to 3 of the 4 nuclei shown in Figure 2. Each has a cone-shaped, thickened membrane which represents the inner membrane of the mature merozoite. A well-formed conoid is seen in the central merozoite and is probably present in the others although out of plane of this section. The round dense body within the membrane is the precursor of the paired organelle of the mature merozoite. The polar areas (arrows) of 2 nuclei can be seen extending into the forming merozoites. The cytoplasm of the schizont contains many ribosomes, rough surfaced endoplasmic reticulum, mitochondria and a Golgi adjunct. The outer and inner membranes are also present and associated with them is a micropore.

It has not been determined whether more than two merozoites can form from a single nucleus (as in endodyogeny) nor how many nuclei are present in the schizont prior to the initiation of merozoite formation.

In Figure 3, a section of a schizont passes through eleven forming merozoites. Two of the merozoites are completely separated from the remainder at this level of section. Separation of merozoites appears to take place by invagination of the outer membrane of the schizont and, perhaps, vesicle formation between the merozoites with subsequent fusion as occurs in endodyogeny. The outer and inner membrane complex remains intact except where a merozoite lies at the outer edge of the schizont in which case the schizont inner membrane is absent and the two-layer complex is made up of the schizont outer membrane and the inner membrane of the forming merozoite.

At completion of schizogony, the merozoites lie free in the vacuole and are still surrounded by the thick vacuolar wall. Sections of 24 mature merozoites are seen in Figure 4.

**Discussion**

After the reports of Hutchison et al. (1970) and Frenkel et al. (1970) demonstrating endogenous stages of *Toxoplasma gondii* in cat intestine it became obvious that *T. gondii* could not divide exclusively by endodyogeny. Schizogony was reported in *T. gondii* by Gavín et al. (1962) as one of several means by which the organism could divide. However, Sheffield and Melton (1968) demonstrated that the rosettes which Gavín et al. had interpreted as schizonts were actually the products of repeated divisions by endodyogeny with delayed separation of the offspring. Sheffield and Melton (1968) also compared schizogony in *Eimeria* and *Plasmodium* with endodyogeny and concluded that the processes were similar with the exceptions of there being only two offsping in endodyogeny and the site of their formation was internal rather than at the surface of the parent cell.

Schizogony in *T. gondii*, as described here, begins with one or more nuclear divisions followed by formation of merozoites. Merozoite formation is initiated by the development of anterior and organelles within a cone-shaped

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**Figures 1–2.** Electron micrographs of schizonts of *Toxoplasma gondii*. 1. Young schizont. 20,200×. 2. Dividing schizont. Note polar regions (arrows) of 2 nuclei extending into the forming merozoites. 16,500×.

Abbreviations for all figures: Conoid (C), endoplasmic reticulum (ER), merozoite (M), mitochondrion (MI), micropore (MP), nucleus (N), pellicle (P), vacuolar wall (VW).

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membrane near the nucleus. Elongation of the membrane and inclusion of a nucleus results in immature merozoites which have only an inner membrane and are all surrounded by the schizont membrane complex. Invagination of the schizont outer membrane and new membrane formation completes the merozoite development and separation.

Schizogony in *T. gondii* differs from endodyogeny as seen in the proliferative or cyst forms by simultaneously having more than two offspring formed within the parent organism and by having two or possibly more nuclear divisions prior to the formation of the offspring. Formation of merozoites in *Eimeria bovis* was reported by Sheffield and Hammond (1967). The developing merozoites form at the surface of the schizont and later appear as buds. Development of the merozoites and regression of the schizont cytoplasm terminates with the separation of merozoites at the posterior end from the schizont residuum. Similar results were reported by Colley (1968) in *E. nieschulzi* and by Senaud and Cerna (1969) in *E. magna* and *E. tenella*.

Scholtyseck (1965) described a somewhat different method of division by schizogony in *E. perforans* and *E. stiedae*. In these species, merozoites separated by the formation of concentric rings of endoplasmic reticulum which isolated the merozoites from each other. Development of the conoid or other anterior end organelles was not reported.

Schmidt et al. (1967) observed merozoite formation in *Isospora* sp. and noted that buds develop at the schizont surface similar to sporozoite formation in *Plasmodium gallinaceum*.

The recognition of a type of division by schizogony which is different from those previously recorded in the literature may have some bearing on our understanding of the lack of host specificity of *T. gondii*. In cell cultures, experimental animals and humans endodyogeny is the only verified form of division. The present study has shown that endodyogeny may be a variation of schizogony as it occurs in the cat. Perhaps the ability to modify its normal pattern of division and instead repeatedly multiply by endodyogeny in abnormal hosts may account for the successful adaptation and pathogenicity of this parasite.

**Acknowledgments**

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**Literature Cited**


Figures 3-4. Electron micrographs of schizonts of *Toxoplasma gondii*, cont’d. 3. Late schizont. Note 2 separated merozoites and the invagination of the schizont membrane (arrow). 17,700×. 4. Mature merozoites. 10,750×.


Announcement—New Editor

Dr. Harley G. Sheffield, Laboratory of Parasitic Diseases, National Institutes of Health, Bethesda, Maryland, USA, Zip Code 20014, has been elected Editor of the *Proceedings of the Helminthological Society of Washington* starting with Volume 38 (1971).

Effective immediately, all manuscripts and any other correspondence on Editorial Matters on Volume 38 (1971) and beyond should be addressed to Doctor Sheffield.

I wish to thank all the contributors to the Proceedings, and the staff of Allen Press for their cooperation. To my colleagues on the Editorial Board and the many others who graciously reviewed manuscripts, my deep appreciation. I am sure that you all will give Doctor Sheffield the same cooperation that you have given me in the past.

Francis G. Tromba
Immediate Past Editor

60th Anniversary Banquet

The Helminthological Society of Washington will hold a banquet commemorating its 60th anniversary during the Second International Congress of Parasitology in Washington, D. C.

The banquet is scheduled for Tuesday, September 8, 1970 at 6:00 p.m. and will feature a distinguished parasitologist as guest speaker.

All participants in the Congress are invited to attend. Tickets will be available at the Registration Desk.