Fine structure of the gamonts of *Eimeria* (s. l.) *vanasi*, a coccidium from the intestine of cichlid fishes

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**ABSTRACT**: The fine structure of gamont-forming merozoites, macrogamonts and microgamonts of *Eimeria* (s. l.) *vanasi*, a coccidium found in the intestines of hatchery-raised fry of *Oreochromis aurea × nilotica*, is described. The apical ends of parasitophorous vacuoles containing young macrogamonts extend, together with the opposed host cell boundary, through the brush border above the epithelial layer. The macrogamont cytoplasm contains organelles characteristic of coccidian macrogamonts, including organelles which are structurally identical to Type 1 and 2 wall-forming bodies. Ribosome-lined ducts and Type 1 wall-forming-like organelles appear to be involved in the formation of the envelope around the macrogamonts. Microgamont fine structure conforms with that of coccidian microgamonts found in other vertebrates.

**INTRODUCTION**

*Eimeria* (s. l.) *vanasi* Landsberg & Paperna, 1987 parasitizes the intestine of juveniles of African cichlid fishes. Earlier communications report light microscopy (LM) studies of the endogenous developmental stages and of the oocyst (Landsberg & Paperna 1987), as well as an transmission electron microscopic (TEM) observation revealing numerous tubules in the host cell cytoplasm connected to funnels located at the rim of the parasitophorous vacuole (PV) (Paperna & Landsberg 1987). The fine structure of meronts and merozoites is described by the same author in a concurrent communication. In the present communication the fine structure of macrogamonts and microgamonts is reported and discussed.

**MATERIALS AND METHODS**

Infected fish, fry of *Oreochromis aurea × nilotica* (the cultured 'tilapia') were obtained from hatcheries in Beit Shaan valley (Landsberg & Paperna 1987). For TEM, pieces from the anterior gut were fixed in Karnowskifi for 24 h at 4 C, washed repeatedly in 0.1M cacodylate buffer at pH 7.4, and post-fixed in 1% osmium tetroxide in the same buffer, for 1 h. After rinsing in buffer, the material was dehydrated in ethanol and embedded in Epon. Thin sections, cut by an LKB III ultratome with diamond knife, were stained on a grid with uranyl acetate and lead citrate and examined with a Jeol 100CX TEM.

**RESULTS**

**Merozoites**

Merozoites developing into gamonts, 4.5 to 5 × 2 to 3 μm in size (Fig. 1), are bounded by 2 unit membranes. They have a large vesicular nucleus with homogeneously distributed chromatin and a distinct nucleolus, rhoptries exhibiting heterogeneous electron density, and faintly osmiophilic micronemes. The cytoplasm also contains a dense network of rough endoplasmic reticulum (ER), peripherally distributed mitochondria and a variable amount of lipid vacuoles. The wall of the PV consists of a pronounced membrane interrupted by a few tubular system funnels.

**Young macrogamonts**

PV containing the young macrogamonts (4 to 8 × 3 to 6 μm) occur beneath the brush border surface of the
Figs. 1 to 5. *Eunema* (s. l.) vanam. Fig. 1. Progeny of merozoites which will later develop into gamonts, within a PV (parasitophorous vacuole) connected to funnels (f-t) of the tubular system (x 13 900). Fig. 2. Young macrogamont located beneath the brush border zone (x 11 300). Figs. 3 and 4. Young macrogamonts within a PV extending into the brush border zone (E), PV boundary is connected to an extensive tubular system (x 8300 and 11 200, respectively). Fig. 5. Enlargement of the apical end of the PV extending into the brush border zone (x 25 500). Symbols are: (a) folded anterior end, (b) brush border; (c) anterior plaque; (d) thickened wall; (er) endoplasmic reticulum; (f) parasitophorous funnels; (fv) food vacuole; (L) lipid vacuole; (M) mitochondria; (m) micronemes; (N) nucleus; (NU) nucleolus; (r) rhoptries; (rr) residual rhoptries; (t) tubular system.
epithelial cells (Figs. 2 to 4). The PV is bounded by a
unit membrane interspersed with funnels connected to
an elaborate tubular system (Figs. 2 and 3). The apical
end of some of the gamont-containing PVs rises above
the epithelial host cell brush border surface along with
the apposed epithelial cell boundary membrane (Figs.
3 and 4). This surfacing portion is void of microvilli, and
the interior side of its wall is thickened by an overlay of
osmiophilic substance, to some distance below the
level of the microvillus boundary of the host cell (Figs.
3 and 5). The anterior end of the parasitophorous vacuole
contains granular debris (Figs. 3 and 5). The young
macrogamont is bounded by 2 unit membranes, its
anterior end is depressed (Figs. 3 and 4) or folded (Figs.
2 and 5), and a thick plaque is formed at the anterior
tip, on the inner boundary membrane (Fig. 3). Some
folds form pinocytotic-like depressions (Fig. 2). The
large nucleus contains a central, fragmented nucleolus.
The cytoplasm contains a network of rough ER, a few
food vacuoles at the anterior end (Fig. 2), peripherally
distributed mitochondria (Fig. 2), aggregates of faintly
osmiophilic micronemes (Figs. 2 and 4), a number
of small lipid vacuoles, and a few dense bodies (Figs. 2
and 3), presumably residues of rhoptries.

Macrogamonts

Macrogamonts (8 to 14 × 7 to 10 μm) (Figs. 6 to 15) are
bounded by a thin, single-layered wall and enclosed in a
bilaminated envelope (Figs. 12 to 15) which is attached
to points on the macrogamont surface at apparently
regular intervals (Figs. 6 and 7). The space between the
macrogamont outer envelope and the wall of the PV
contains a flocculent substance (Figs. 8 and 15). The
wall of the PV, which consists of a single membrane, is
interspersed with funnels from the tubular system (Fig.
15). The nucleus is usually centrally located (Fig. 7) and
contains a large nucleolus (Fig. 9). Adnuclear bodies
occur at the periphery of the nucleus and seem to be
enclosed in extensions of the nucleolemma (Figs. 7 and
8). Canaliculi form a few aggregates at the periphery of
the nucleus (Figs. 7 and 11). Mitochondria occur
immediately beneath the cell wall (Figs. 6 and 15).
Ducts or canals lined with ribosomes (Figs. 6 and 8),
together with a small vesicle containing electron-dense
material (Figs. 12 to 14), open to the point of attach-
ment between the outer envelope and the cell wall
(Fig. 8). Similar small vesicles of electron-dense
material occur in the internal cytoplasm (Figs. 8 and 9).
These vesicles resemble Type 1 wall-forming bodies
(WF1) of higher vertebrate coccidia. An aggregation of these
bodies is seen in some of the large cisternae (Fig. 10).
The rough ER forms a dense network of parallel bran-
ches in the peripheral cytoplasm (Figs. 5, 6 and 9) and
a winding network of ducts and cisternae in the
perinuclear zone (Figs. 7 and 8). In some macro-
 gamonts small amylopectin granules surround large,
partly-extracted lipid vacuoles (Fig. 9), while in others
the granules are larger and are distributed independ-
ent of highly osmiophilic lipid vacuoles (Figs. 7
and 8).

Microgamonts

Microgamonts were only found at an early stage of
microgametogenesis. The microgamonts (10 to 11 × 8
to 9 μm) (Figs. 16 to 19), are bounded by a single
membrane. The walls of PVs containing microgamonts
are fringed with funnels from the tubular system (Fig.
16). Nuclei with peripherally concentrated hetero-
chromatin aggregate beneath the surface of the micro-
Figs. 16 to 19. *Eimeria* (s. l.) vanasi. Premature microgamonts and microgamonts, adjacent to microgamete primordia with emergent flagella arising from their basal bodies (Fig. 17). A small mitochondrion accompanies each nucleus (Figs. 17 and 18). Clusters of rounded mitochondria were seen at some sites in the peripheral cytoplasm (Fig. 19). Golgi apparatuses and ER tubules occur in the cytoplasm beneath the nuclei (Figs. 17 and 19). The internal cytoplasm contains many amyllo-
pectin granules, a few larger lipid vacuoles, multembranous vesicles and fragmented residues of nuclei (Figs. 17 and 18).

**DISCUSSION**

*Eimeria* (s. l.) *vanasi* merozoites developing into macrogamonts are readily distinguishable from those developing into meronts by LM (Landsberg & Paperna 1987) as well as by TEM (Paperna unpubl.). The latter are attenuated, have a condensed nucleus with heterogeneous chromatin, electron-dense rhoptries and micronemes, and lack lipid vacuoles. Merozoites developing into microgamonts could not be identified either by LM or by TEM.

LM studies of *Eimeria vanasi* revealed the existence of both epicytoplasmic ('epiepithelial') and intracytoplasmic gamonts (Landsberg & Paperna 1987). The conspicuousness of the epicytoplasmic and intracytoplasmic stages has been discussed elsewhere (Landsberg & Paperna 1987). Our TEM studies revealed only intracytoplasmic mature gamonts. The PVs containing the young gamonts, and extending beyond the brush border level of the host cell, may have been in the process of emerging to, or submerging from the epicytoplasmic position. If the first interpretation is correct, then the young gamonts represent epicytoplasmic gamonts.

All gamont stages, as well as meronts dividing into gamont-forming merozoites, were enclosed in PV connected to a tubular system extending into the host cell cytoplasm. This system was described and discussed in a previous communication (Paperna & Landsberg 1987). The tubular system was vestigial or absent in the walls of PVs containing asexual stages (Paperna & Landsberg 1987, Paperna unpubl.).

Most piscine coccidia lack a hard oocyst wall, and the sporocyst wall assumes the cardinal protective function (Dykova & Lom 1981). The mature macrogamonts of *Eimeria vanasi* were enclosed in a superficial envelope, suggesting that they were already macrogametes or zygotes. One or 2 envelopes were seen enclosing macrogametes or zygotes of *Goussia iroquoina* (Paterson & Dessir 1984) and *E. laureleus* (Desser & Li 1984), in *G. aculeati* (Jastrzebski 1984) and in *G. zarnowskii* (Jastrzebski & Komorowski 1990), or a granular or condensed body within the ER cisternae reminiscent of WF2 in [G. iroquoina (Paterson & Dessir 1981a, 1984); in *E. laureleus* (Desser & Li 1984), in *G. aculeati* (Jastrzebski 1989) and in *G. zarnowskii* (Jastrzebski & Komorowski 1990) or a granular or condensed body within the ER cisternae reminiscent of WF2 in [G. iroquoina (Paterson et al. 1986) and *E. sardinae* (Morrison & Hawkins 1984)]. Dense or granular bodies were thought to be involved in sporocyst wall formation in *Sarcocystis tenella* (Mehlhorn & Scholtyseck 1974) and *Aggregata eberthi* (Heller 1969). Material from the 'dense bodies' of *G. iroquoina* and *E. laureleus* macrogametocytes was re-aggregated in the sporoblasts and sporocysts, but did not seem to participate in sporocyst wall formation (Paterson & Dessir 1981b, Dessir & Li 1984). In *G. zarnowskii* 'dense bodies' located beneath the macrogamont boundary released their contents into the spaces between the forming envelopes (Jastrzebski & Komorowski 1990). WF2-like bodies of *G. cichlidarium* seem to contribute their granular contents to the formation of the sporocyst wall (Paperna & Landsberg 1985).

Box et al. (1980) commented on the similarities of *Sarcocystis* sp. and related genera to piscine coccidia, noting the similar development of a fragile, transitory oocyst wall with hard wall formation only at the sporocyst stage, and the lack of a stieda body in the sporocyst. This apparent relationship is only superficial: both types of WF, or at least one (WF1), have been found in macrogamonts of all *Sarcocystis* sp. studied to date and...
the final, fragile oocyst wall is nevertheless formed by the same or similar process to that observed in *Eimeria* species, which form a hard wall (Vetterling et al. 1973). Adnuclear bodies were seen before in macrogamonts of *Schellackia cf. agamae*, their nature and function are unknown (Ostrovská & Paperna 1987).

The process of microgametogenesis in *Eimeria vanasi* was similar to that which occurs in the saurian coccidium *E. turricus* (Paperna & Landsberg 1989), in *Isospora* sp. from passerine birds (Milde 1979) and possibly in the piscine species *Calyptospora fungduli* (as may be evident from Fig. 3 of Hawkins et al. 1983), e.g. the flagellum emerges at the surface of the microgamont before the body of the microgamete, while the microgamont's nucleus does not undergo subdivision prior to microgamete formation. This form of microgametogenesis diverges somewhat from the general coccidian pattern described for many *Eimeria* spp. (Scholtyseck et al. 1972), including piscine spp. (Paton & Desser 1981b, Jastrzębski 1989, Jastrzębski & Komorowski 1990).

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**LITERATURE CITED**


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