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Fine structure of epicytoplasmic stages of *Eimeria vanasi* from the gut of cichlid fish

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ABSTRACT: Fine structure of epicytoplasmic and hemi-epicytoplasmic merogony stages, merozoites dividing by endodyogeny, gamonts and oocysts of *Eimeria* (s.l.) *vanasi* from the intestine of the cultured cichlid fish *Oreochromis aurea* × *nilotica* is described. Parasites, which develop at the apical end of the host cell, are enclosed in a parasitophorous envelope formed by the coalescence of the parasitophorous vacuole wall with the host cell brush border. The envelope is void of microvilli and forms small projections or ridges. Deep pinocytotic-like invaginations and microfibrillar bundles extend from the parasitophorous vacuole membrane into the host cell cytoplasm. Invaginations become shallow and decrease in number as meronts or gamonts mature, or expand deeper into the host cell cytoplasm in hemi-epicytoplasmic forms. Macrogamonts contain 2 types of organelles which are structurally identical to the type 1 and type 2 wall-forming bodies characteristic of terrestrial vertebrate coccidians. In the process of wall formation, plasmalemma-bound oocysts become enclosed by a bilayered envelope overlaid by a layer of 10 lamellae. In the end of the process a bilayered firm oocyst wall is formed.

INTRODUCTION

Intracytoplasmic and epicytoplasmic coccidia occur simultaneously in the intestine of cichlid fishes *Tilapia* and *Oreochomis*, from Israel and southern Africa (Landsberg & Paperna 1987, Paperna 1991). The latter develop like members of *Epieimeria* Dykova & Lom, 1981, beneath the host cell brush border. The parasite within a parasitophorous vacuole (PV), which is apposed to the wall membrane of the apical end of the host cell, grows and bulges above the mucosal epithelium. Both types of infection are comprised of merogony and gamogony stages. Conspecificity has been suggested on the basis of established taxonomic criteria (e.g. oocyst and sporocyst sizes) (Landsberg & Paperna 1987). Subsequent ultrastructural studies (Paperna & Landsberg 1987, Paperna 1990, 1991) revealed further structural diversity, but at the same time hinted at a possible cyclic transition between epicytoplasmic and intracytoplasmic generations (Paperna 1990). A detailed follow-up study on the course of infection with intestinal coccidia in cultivated cichlids is now in progress, with one of its objectives being to determine the taxonomic relationships among the different forms of infection. The objectives of the present paper are restricted to a description of the fine structure of epicytoplasmic stages of *Eimeria* (s.l.) *vanasi* and of the forms intermediate to intracytoplasmic and epicytoplasmic stages.

MATERIALS AND METHODS

Study material was obtained from laboratory-infected and hatchery-infected fry (15 to 18 mm long, 3 wk to 1 mo old) of cultured tilapia *Oreochromis aurea* × *nilotica*.

Pieces from the anterior and posterior gut were fixed in Karnowski for 24 h at 4°C, rinsed repeatedly in cacodylate buffer (0.1M, pH 7.4) and post-fixed in 1% osmium tetroxide, in the same buffer, for 1 h. After rinsing in the same buffer, the material was dehydrated in graded ethanols and embedded in Epon. Thin sections cut on a Reichert Ultracut ultratome with a diamond knife were stained on grid with uranyl acetate and lead citrate and examined with a Jeol 100CX TEM.

RESULTS

Localization in the host cell

Observed infections were either epicytoplasmic (Figs. 2, 6, 8, 9 to 11, 13 & 17) or hemi-epicytoplasmic

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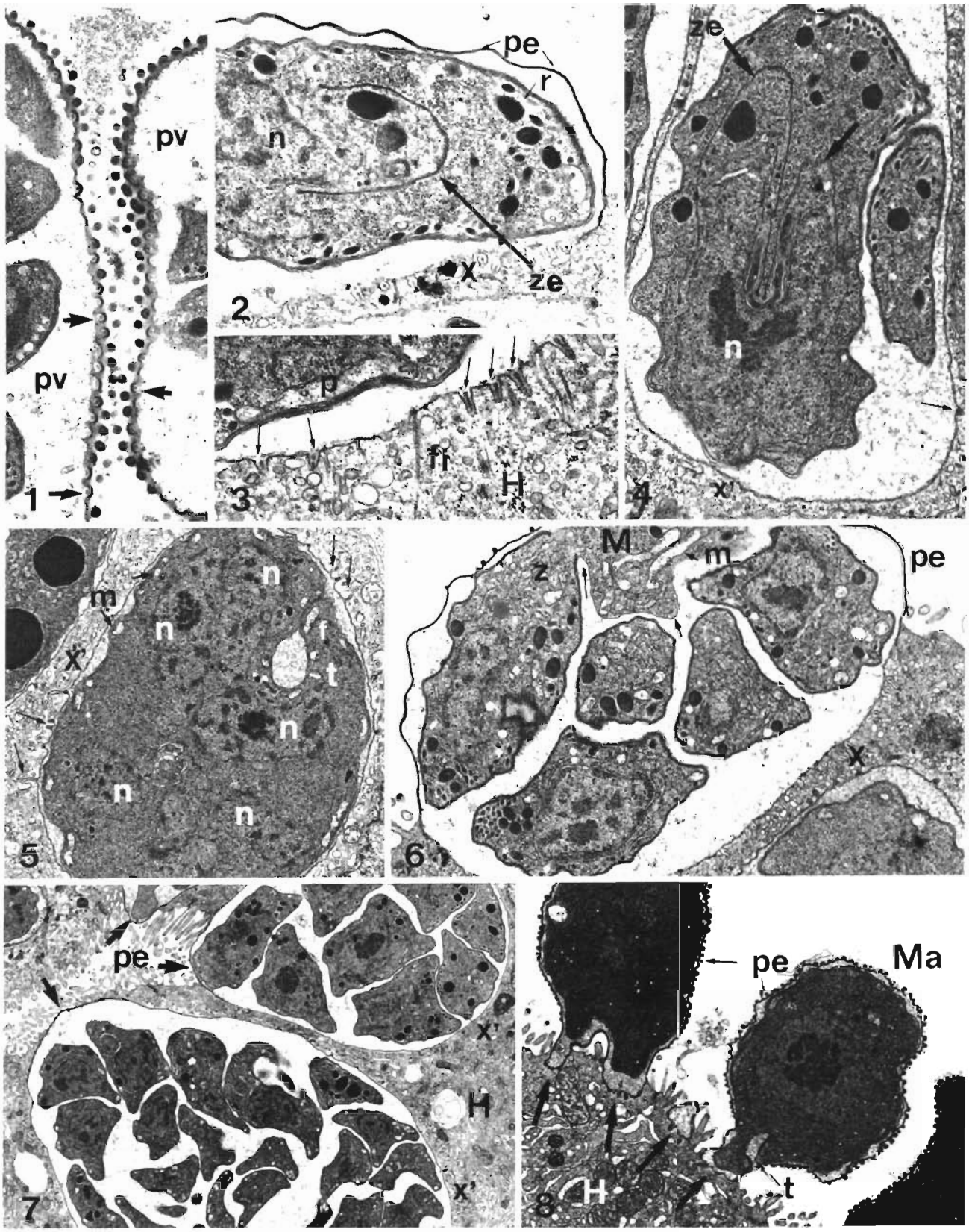
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(i.e. with the PV located deeper within the host cell cytoplasm and its apical end remaining epicytoplasmic, apposed to or displacing the host cell brush border wall; see Figs. 4, 5, 7 & 14 to 16). Both forms of infection included merogony and gamogony stages, whereas all observed oocysts were epicytoplasmic. The parasitophorous envelope (PE) enclosing the parasite as it bulges into the gut lumen consisted of the PV wall closely adherent or merged with the host cell brush border boundary, which was itself void of microvilli (Figs. 3 & 6). Tight folds in the apposed membranes formed small projections or ridges enclosing small axial cords or fibrils (Fig. 1). The number of folds was variable, and they were sometimes altogether absent (Figs. 2 & 6). Folds were fewer or absent on the PE of hemi-epicytoplasmic stages (Figs. 5 & 12).

Merogony stages

Epicytoplasmic trophozoites still bound by a merozoite pellicle, e.g. newly established merozoites (Fig. 2), were described previously (Paperna 1991). The portion of the PV wall adjoining the host cell cytoplasm in the epicytoplasmic trophozoites, meronts and merozoite formations consisted of pinocytotic-like invaginations, each containing an electron-dense (ED) axis or core. Between each invagination, the PV wall was connected to a microfibrillar bundle (Fig. 3). The portion of the PV wall in hemi-epicytoplasmic merogony stages adjoining cytoplasm further inside the host cell lacked the microfibrillar bundles, and had fewer, shallower and often deformed invaginations filled with a dense substance (Fig. 5). Epicytoplasmic and hemi-epicytoplasmic newly established merozoites (trophozoites) divided by endodyogeny prior to their differentiation to meronts (Figs. 2 & 4).

Meronts in the process of nuclear division were not found in the ultrastructurally studied material.

Both epicytoplasmic and hemi-epicytoplasmic meronts divided into a variable number of merozoites (Figs. 6 & 7). Merozoites of larger progenies showed correspondingly smaller dimensions in cross section indicating an overall smaller size (Fig. 7). PV size corresponded to merozoite progeny size.

Microgamont stages

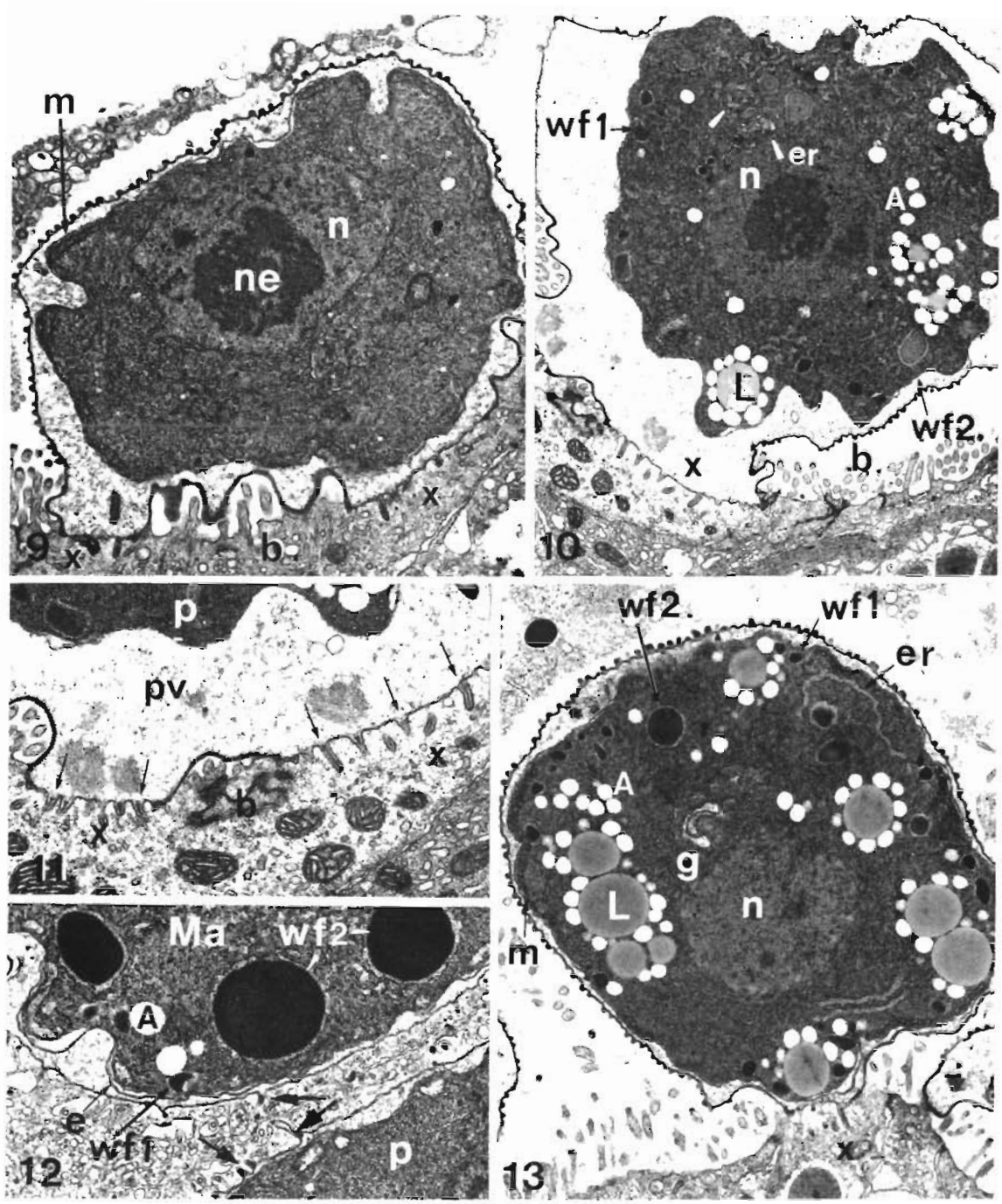
Only young, dividing hemi-epicytoplasmic microgamonts were found in the material studied. They were 10 to 12 × 7 to 8 µm in size, and contained several dividing nuclei, many peripherally located mitochondria, food vacuoles, deep trophic invaginations and nuclei, each with a distinct nucleolus (Fig. 5).

Macrogamont stages

All epicytoplasmic macrogamonts found were premature (Figs. 8 to 10 & 13). Very young (Fig. 14), as well as mature (Fig. 15) macrogamonts were hemi-epicytoplasmic; their PV was mostly intracellular with only the tips extending through the host cell brush border. The PV membrane of the epicytoplasmic stage adjoining the host cell cytoplasm contained either numerous invaginations, or fewer, shallow depressions (Figs. 9 & 11). Invaginations, like in hemi-cytoplasmic merogony stages, were shallow and fewer along the PV membrane of hemi-epicytoplasmic stages adjoining interior portions of the host cell cytoplasm (Figs. 12 & 14). The portion of the PV wall of hemi-epicytoplasmic mature macrogamonts (Figs. 15 & 16) extending through the brush border, the PE, was thickened and exhibited characteristic folds. These thickenings and folds were absent in PE of hemi-epicytoplasmic young macrogamonts (Fig. 14). Thickening of the PV wall adjoining the host cell cytoplasm (including its invaginations) occurred too, in both epicytoplasmic (Fig. 9) and hemi-

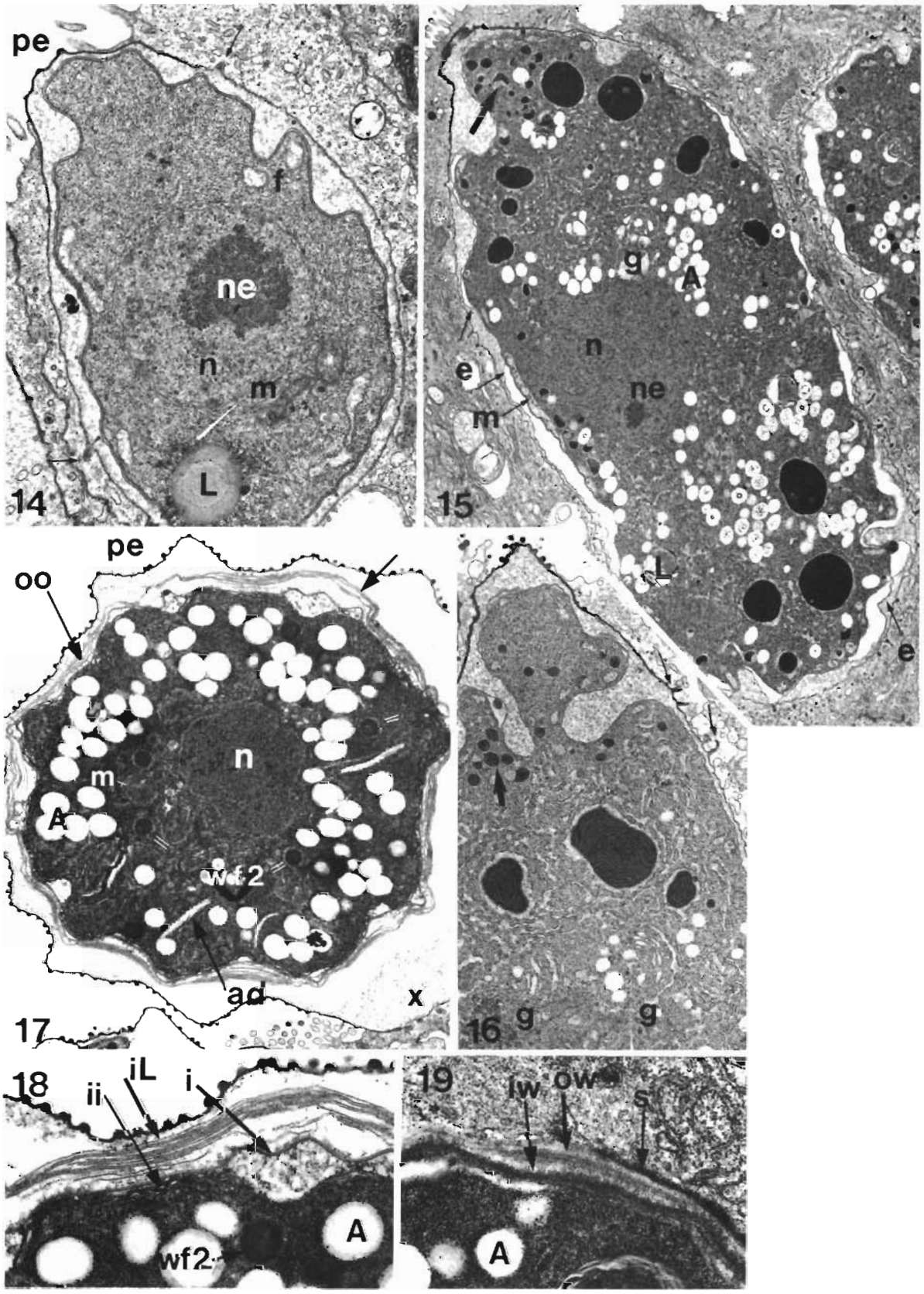
Figs. 1 to 8. *Eimeria* (s.l.) *vanasi*. Fig. 1. Parasitophorous envelope (PE) of *E. vanasi* epicytoplasmic stages. Arrows: axial cords within the folds (×18 850). Fig. 2. Endodyogeny in an epicytoplasmic newly established merozoite (trophozoite) (×16 600). Fig. 3. Parasitophorous vacuole (PV) of epicytoplasmic trophozoite adjoining the host cell cytoplasm. Arrows: pinocytotic-like invaginations (×30 000). Fig. 4. Endodyogeny in a hemi-epicytoplasmic newly established merozoite (trophozoite) (×14 800). Fig. 5. Hemi-epicytoplasmic young microgamont with dividing nuclei. Arrows: pinocytotic-like invaginations (×6650). Fig. 6. Epicytoplasmic dividing meront. Arrows: portion of the meront cytoplasm (M) bound by single membrane (×12 300). Fig. 7. Hemi-epicytoplasmic dividing meronts with their PV extending to the brush border surface of the epithelial host cells (arrows) (×3960). Fig. 8. Epicytoplasmic young macrogamonts. Bold arrows: adjoining zone of the PV membrane to the host cell cytoplasm (×6600)

Abbreviations for figures. A: amylopectin granules; ad: axial ducts; b: brush border; e: outer wall membrane; er: ER, endoplasmic reticulum; f: food vacuole; fi: fibrillar bundle; g: Golgi apparatus; H: host cell cytoplasm; L: lipid vacuole; M: meront cytoplasm; m: mitochondria; Ma: macrogamont; n: nucleus; ne: nucleolus; oo: oocyst wall; p: parasite; pe: PE, parasitophorous envelope; pv: PV, parasitophorous vauole; r: rhoptries; t: deep invagination; wf1: WF1, wall-forming body 1; wf2: WF2, wall-forming body 2; x: epicytoplasmic PV membrane adjoining host cell cytoplasm; x': hemi-epicytoplasmic PV membrane adjoining host cell cytoplasm; ze: daughter cell



Figs. 9 to 13. *Eimeria* (s.l.) *vanasia*. Fig. 9. Epicytoplasmic young macrogamont. Note the thickened PV membrane facing host cell cytoplasmic side (×10 760). Fig. 10. Differentiating epicytoplasmic macrogamont (×7000). Fig. 11. Enlarged view of PV membrane of macrogamonts shown in Fig. 10 adjoining host cell cytoplasm (×11 620). Fig. 12. Enlarged ends of hemi-epicytoplasmic mature macrogamont (top) and young microgamont (p). Arrows: pinocytotic-like invaginations (×10 000). Fig. 13. Premature epicytoplasmic macrogamont (×8300)

Figs. 14 to 19. *Eimeria* (s.l.) *vanasia*. Fig. 14. Hemi-epicytoplasmic young macrogamonts showing the PE portion of the PV (pe) extending above the surface of the epithelial host cell. Arrows: pinocytotic-like invaginations (×13 200). Fig. 15. Hemi-epicytoplasmic mature macrogamont. Arrow: WF1 (×6000). Fig. 16. Front portion of hemi-epicytoplasmic mature macrogamonts with large laminated WF2 and small ED WF1 (arrow). Note thickened PV membrane and its invaginations, as well as the PE, compared with the partly erroded host cell cytoplasm (×9000). Fig. 17. Epicytoplasmic oocyst (×8000). Fig. 18. Forming oocyst wall: (iL) layer of lamellae, (i) outer envelope, (ii) inner membrane (Plasmalemma) (×20 000). Fig. 19. Consolidated outer (ow) and inner (iw) oocyst wall, with (s) surface ED precipitate (×20 000)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cytoplasmic forms. In the latter, such thickening was confluent with that of the PE (Fig. 16).

Young macrogamonts (6 to 7 × 4 to 5 µm in size; Figs. 8, 9 & 14) were bound by a bilayered cell membrane. One macrogamont revealed a deep invagination (Fig. 8). The cytoplasm contained a nucleus, with a large nucleolus, a few food vauoles, mitochondria located predominantly at the periphery, endoplasmic reticulum (ER) with a variable number of small cisternae (Figs. 8 & 9) and one or a few lipid vacuoles (Fig. 14). As macrogamonts grew and differentiated (8 to 10 × 7.5 to 8.5; Figs. 10 & 13), round amylopectin granules appeared, often surrounding the larger lipid vacuoles. Type 1 wall-forming bodies (WF1) appeared as irregularly spherical ED bodies. Type 2 wall-forming bodies (WF2) aggregated within ER cisternae, forming laminate granular depositions (Figs. 10 & 13). In some macrogamonts a single Golgi apparatus was seen. The ER also formed large elongated cisternae, or expanded ducts (Fig. 13).

Mature macrogamonts (18 to 20 × 6 to 9 µm; Fig. 15) had an increasing number of amylopectin granules and a decreasing number of lipid vacuoles, a dense network of ER and a number of Golgi complexes (Fig. 16). WF1 were present, containing ED material (Fig. 15). The large WF2 were located within the ER cisternae and consisted of a laminated ED substance (Figs. 12 & 16). The macrogamonts' bilayered wall membrane was separated and adjoined the PV boundary, while its cytoplasm remained bound by a newly formed plasmalemma. The flocculent substance filling the PV remained between the detached wall and the PV wall (Figs. 12 & 15).

Oocysts

Epicytoplasmic oocysts (7.5 to 8.5 µm in diameter; Fig. 17) were enclosed within the same type of folded PE and invaginations occur at its PV juncture with the host cell cytoplasm. In the dense cytoplasm loaded with amylopectin granules, it was possible to trace a dense ER network, particularly around the nucleus, mitochondria in the peripheral cytoplasm, several WF2, some of which were adhering to the forming wall, and several axial ducts, similar to the ribosome-lined ducts reported from intracytoplasmic macrogamonts (Paperna 1990).

The oocyst was bound by a bilayered membrane which was gradually separating from the plasmalemma-bound cytoplasm overlaid by a second layer consisting of about 10 lamellae (Fig. 18). In later-stage oocysts the multilamellated layer disappeared and a bilayered wall has been formed, consisting of an inner, denser, slightly dentated layer and outer laminated layers; in some places an ED substance had precipitated on the surface of the formed oocyst wall (Fig. 19).

Hemi-epicytoplasmic oocysts were not found in the ultrastructurally studied material.

DISCUSSION

There are no fundamental differences between epicytoplasmic and hemi-epicytoplasmic stages. Unlike intracytoplasmic stages, entirely enclosed within a PV in the host cell cytoplasm, the PV wall of both epicytoplasmic forms partially coalesces with the apical wall of the host cell to form a distinctly structured PE. Based on the available data it is difficult to decide whether the hemi-epicytoplasmic stages precede or follow the epicytoplasmic stages in the parasite's development. The projections seen on PV membrane deep within the host cell cytoplasm are undoubtedly derived from the pinocytotic-like invaginations of the epicytoplasmic PV which adjoins the host cell cytoplasm. Hemi-epicytoplasmic mature macrogamonts had the same type of WF2 with laminated texture as the younger stage epicytoplasmic macrogamonts, and they seemed to be emerging from, rather than regressing into the host cell.

The relationship between epicytoplasmic and intracytoplasmic stages cannot be resolved on the basis of morphological or ultrastructural data. The main difference between the presently described epicytoplasmic and hemi-epicytoplasmic stages and the intracytoplasmic stages of a previously described *Eimeria vanasi* (Paperna 1990, 1991) lies in the structure of the PV wall. A prominent tubular system with large funnels or their vestiges is connected to the PV wall of intracytoplasmic gamonts and merogony stages (Paperna & Landsberg 1987). The PV wall of epicytoplasmic and hemi-epicytoplasmic stages contains projections derived from pinocytotic-like invaginations or their derivatives. A previous observation (Paperna 1990) of young macrogamonts located within a PV connected to a tubular system but with its apical end extruding through the host cell brush border further complicates the issue. The latter young macrogamonts differ in some morphological details from the presently reported young macrogamonts.

There are several features common to both epicytoplasmic/hemi-epicytoplasmic and intracytoplasmic macrogamonts: cytoplasmic organization in the epicytoplasmic oocyst is very similar to that of previously described (Paperna 1990) intracytoplasmic mature macrogamonts: both have axial or 'ribosome-lined' ducts which are absent in the hemi-epicytoplasmic mature macrogamonts. In all forms, amylopectin granules form around the lipid vacuoles. Epicytoplasmic, hemi-epicytoplasmic and intracytoplasmic macrogamonts all have WF1 and WF2, but they differ in the

way of depositing their ED contents (laminate deposition in the first and second and concentric deposition in the third). The adnuclear bodies and canaliculi which are prominent in intracellular forms were absent in the epicytoplasmic and hemi-epicytoplasmic stages.

Our ultrastructural data confirm the occurrence of endodyogeny in epicytoplasmic newly-established merozoites (trophozoites) as suggested previously from observations of epicytoplasmic trophozoite couples (Landsberg & Paperna 1987, Paperna 1991). Endodyogeny has been previously reported in merozoites during or immediately after their differentiation from the intracytoplasmic meronts, or in intracytoplasmic trophozoites before their development into dividing meronts (Paperna 1991). Characteristic to asexual generations of *Toxoplasma, Sarcocystis* and related genera (Dubey 1977) endodyogeny therefore appears to be a regular form of asexual division in successive generations of merozoites, in the life cycle of *Eimeria vanasi*. It has been found to occur in merozoites of *Goussia cichlidarum* (Kim & Paperna unpubl.), but is unknown in other piscine coccidia. Endogenous division in merozoites occurs in a few species of *Eimeria* from mammalian hosts (Roberts et al. 1970, Danforth & Hammond 1972).

The peculiarities of *Eimeria vanasi* epicytoplasmic trophozoites as compared to other epicytoplasmic piscine coccidia were discussed in detail in a previous communication (Paperna 1991). The finger-like protrusions found in epicytoplasmic species such as *Epieimeria anguillae* (Molnar & Baska 1986) or *Goussia zarnowskii* (Jastrzebski & Komorowski 1990) are not necessarily homologous with the structures described from epicytoplasmic generations of *Eimeria vanasi*. The 2 former epicytoplasmic species also lack the folds found on the PE of *E. vanasi*. WF1 and WF2 seen in both epicytoplasmic and intracytoplasmic macrogamonts are lacking. The dense bodies found in *G. zarnowskii* are similar to those found in many intracytoplasmic piscine coccidia [such as *G. iroquoina*, Paterson & Desser (1981) or *G. carpelli*, Steinhagen (1991)], but they are not involved in wall formation. In fact the wall formation process in these species as reported by Desser & Li (1984) and Lom et al. (1991) is entirely different from that seen in the epicytoplasmic oocysts of *E. vanasi*.

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