THE ULTRASTRUCTURE OF SOME ENDOGENOUS STAGES OF THE COCCIDIAN *EIMERIA BOVEROI* CARINI & PINTO, 1926 IN THE GUT EPITHELIAL CELLS OF THE GECKO *HEMIDACTYLUS MABOUIA* FROM BRAZIL

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Summary :

The ultrastructure of intracytoplasmic meronts and macrogamonts of *Eimeria* s.l. *boveri* in the small intestine of the gekkonid lizard *Hemidactylus mabouia* from Belem, Para north Brazil is described. Young meronts, and some of the fully grown macrogamonts, are coated with a glycocalyx which, in cross-section, has the appearance of a series of fine tubules. The wall of the parasitophorous vacuole (PV) is a single membrane, lined on the host-cytoplasm side with an endoplasmic reticulum (ER) which sometimes expands into large cisternae filled with electron-lucent, globular material. The membranal edges of the ER, canaliculi and cisternae have regularly spaced indentations filled with an electron-dense substance. Vesicular mitochondria are present in addition to those of the conventional type. Macrogamonts develop the characteristic type 1 and type 2 wall-forming bodies, and in some zygotes the cisternae containing the latter are grossly expanded. Both types of wall-forming bodies persist in young oocysts that have a distinct oocyst wall.

KEY WORDS : coccidia, *Eimeria boveri*, gekkonid lizard, *Hemidactylus mabouia*, Brazil, ultrastructure.

Résumé : ULTRASTRUCTURE DE QUELQUES STADES ENDOGÈNES D'*EIMERIA BOVEROI* CARINI & PINTO, 1926, COCCIDIE PARASITE DE L'ÉPITHÉLIUM INTESTINAL DU GECKO *HEMIDACTYLUS MABOUIA* DU BRÉSIL.

Description ultrastructurale des mérontes et macrogamontes intracytoplasmiques d'*E. boveri* de l'intestin grêle du gecko *Hemidactylus mabouia* de Bélém, Brésil. Les jeunes mérontes et certains macrogamontes totalement mûrs sont enveloppés d'un glycocalyx qui, en coupe transversale, est formé d'une série de fins tubules. La paroi de la vacuole parasitophore (PV) est une membrane simple doublée du côté du cytoplasme de la cellule hôte par un reticulum endoplasmique (ER) qui, parfois, se prolonge par de grandes citernes emplies d'un matériel globuleux peu dense. Les limites membranaires de l'ER des canalicules et des citernes présentent des indentations régulièrement espacées emplies d'une substance dense aux électrons. En plus des mitochondries du type habituel, des mitochondries vésiculaires sont présentes. Les précurseurs de la membrane des macrogamontes présentent les caractères des types 1 et II et chez certains zygotes les citernes qui les contiennent sont hypertrophiées. Les deux types de précurseurs de la paroi persistent chez les jeunes oocystes ayant une paroi bien définie.

MOTS CLÉS : coccidies, *Eimeria boveri*, gecko, *Hemidactylus mabouia*, Brésil, ultrastructure.

INTRODUCTION

Species of reptilian *Eimeria* (sensu lato) differ from those of mammals and birds in having sporocysts which are devoid of Stieda and substieda bodies (Cannon, 1967; Vetterling & Widmer, 1968). Paperna & Landsberg (1989) proposed the generic name of *Choleoeimeria* for some of those saurian parasites which develop in hypertrophied epithelial cells of the gall-bladder. The generic name *Acroeimeria* was given to another group which bulge invested within a displaced host-cell border on the surface of the intestinal mucosal epithelium. A third group of reptilian eimeriids exists, the members of which, like the *Eimeria* species (sensu stricto) of birds and mammals, have a simple intracytoplasmic development in the epi-

thelial cells, without major changes in the host cell or strange displacements. The only saurian intracytoplasmic coccidium ultrastructurally studied thus far is *E. gastroauris*, this species which develops in the stomach epithelium demonstrates a number of unique structural peculiarities (Paperna, 1993).

In a recent paper (Lainson & Paperna, 1999) we gave a redescription of *Eimeria boveri* Carini & Pinto, 1926 in the house-gecko *Hemidactylus mabouia*, and showed the endogenous stages do undergo this latter, simple type of intracytoplasmic development in the epithelial cells of the small intestine. The purpose of the present paper is to describe the ultrastructure of some of these stages: it would appear to be the first time that such a study has been made of a member of this group of saurian eimeriids.

MATERIALS AND METHODS

Material for transmission electron microscopy (TEM) was from the same specimen of *H. mabouia* used in our light microscope

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description of the oocysts and endogenous stages of *Eimeria* (s.l.) *boveri* (Lainson & Paperna, 1999). The intestine was cut longitudinally and one half used to pinpoint the site of development of the parasite by examination of the gut segment between a slide and a coverslip. Appropriate portions were fixed in 2.5 % glutaraldehyde in cacodylate buffer (0.1M, pH 7.4) for 24 hours at 4°C, rinsed repeatedly in the same buffer, post-fixed in 1.0 % osmium tetroxide in the same buffer for one hour and, after rinsing in the buffer, dehydrated in graded ethyl alcohol dilutions and embedded in Agar 100 medium (Agar Scientific Ltd., U.K.). Thin sections, cut on a Reichert Ultracut microtome with a diamond knife, were stained on grids with uranyl acetate and lead citrate, and examined with a Jeol 100CX TEM.

RESULTS

Infection in the epithelium of the small intestine was scanty, and only young meronts (Figs 1-4) and young and mature macrogamonts (Figs 5-13) were traced on the grids examined. Parasitophorous vacuoles (PV) with young meronts and macrogamonts (Figs 1, 5) contained aggregates of globular particles, which could be also traced in the host-cell cytoplasm adjoining the PV (Fig. 7). The globular particles were also present in variable quantities in PV's containing later stage macrogamonts (Figs 6, 7) and sometimes these also contained an electron-dense precipitate (Figs 8, 11). The PV possessed a single membrane (Figs 4, 6A) bordered on the side adjoining the host cytoplasm by an endoplasmic

Figs. 1-4. Fig. 1. Young meront (x and arrowhead marks the areas enlarged in Figs. 2 & 3) ($\times 13,640$) – Figs. 2 and 3. Enlarged view of the meronts rim showing the glycocalyx-like coating of the pellicle and the large vesicular mitochondrion ($\times 32,735$). Fig. 4. Further differentiated young meront ($\times 10,000$).

Abbreviations to figures 1-13: A. Amylopectin granule – ad. Adnuclear organelles – dM. Mitochondrion filled with electron dense substance – C. Cisterna – Cn. Canaliculi – f. Food vacuole – Gb. Granular bodies – g. Intravacuolar globular substance – gx. consolidating granular bodies – L. Lipid vacuole – M. Mitochondrion – p. Cell wall membrane – n. Nucleus – R. ER layer beneath the PV wall, with regularly spaced electron-dense dots – VC. Expanded cisterna containing type 2 wall-forming body – vM. vesiculate mitochondrion – x. Glycocalyx-like layer – w. Consolidating wall-forming body – W. WFB – W1. Type 1 wall-forming body – W2. Type 2 wall-forming body. Scales = 1 μ m.

Figs. 5-8. Fig. 5. Young macrogamont (desmosome-arrowed) $\times 10.830$ – Fig. 6. Maturing macrogamont ($\times 6.364$) – Fig. 7. Maturing macrogamont, enlarged view, star: convoluted mitochondrion ($\times 14.780$) – Fig. 8. Maturing macrogamont already with both type 1 and 2 WFBs ($\times 12.852$).

reticulum (ER) which at times expanded into very large cisternae filled with electron-lucent globular material. The membrane edges facing the PV wall of both the ER canaliculi and the cisternae contained regularly spaced indentations filled with an electron-dense substance (ribosomes?) (Figs 1, 4, 11, 12). The host cells are epithelial, as evident by the presence of desmosomes (Fig. 5): the cytoplasm of some infected cells was of a denser nature than that of the surrounding, non-infected host cells (Fig. 5).

Young meronts were already bounded by a single unit membrane. The first one we studied (Fig. 1) was covered with what appeared to be a glycocalyx which, in cross-section, was seen as a tubular grid (Fig. 2). Figure 3 gives a detailed view of this glycocalyx-like coat over the cell wall. It was absent from the second, somewhat more developed meront that we examined (Fig. 4), but was again seen covering the plasmalemma of a fully developed macrogamont (Fig. 12). In both of the meronts the cytoplasm was fringed by elongate, vesicular mitochondria, with a single array of tubules at their periphery (Fig. 2). A very few micronemes were present, a variable number of electron-dense spheres (which were possibly the residue of rhoptries), and amylopectin granules.

Young macrogamonts, measuring $7.0\text{--}8.0 \times 4.0\text{--}5.0$, were recognizable by their nucleus containing a very large, central nucleolus: only a few organelles could be traced in the cytoplasm, including marginally positioned food vacuoles and vesicular mitochondria, segments of the ER and a few micronemes (Fig. 5). The mature macrogamonts (Fig. 6) reach from $13.0\text{--}15.0 \times 8.0\text{--}11.0$ in size. In the developing macrogamont the type 1 wall-forming bodies (WFB1) appear first: they are double-walled, round bodies which are filled either homogeneously or with clumps of an electron-dense substance (Figs 7, 8). The type 2 wall-forming bodies (WFB2) develop subsequently and are larger, homogeneously electron-dense spheres formed within ER cisternae (Figs 6, 8, 9). Growing macrogamonts also contain a few large, low-density granular bodies which are similar in size to the WFB2, and enclosed in a concentric formation of rough ER (Fig. 7). Small, granular bodies, seen emerging from a similar rough ER concentric formation, seem to be anlagen of either WFB2 or the low-density granular bodies. Vesicular mitochondria, with a single layer of peripheral tubules, occur together with regular tubular mitochondria which sometimes appear to be convoluted (Fig. 7). Also present in both the pre-mature and mature macrogamonts were a few large lipid vacuoles, amylopectin granules and canaliculi (Figs 6, 8). The nucleus of these parasites is accompanied by dense, granular adnuclear bodies (Fig. 6).

Images seen in Figures 9 and 10 are apparently zygotes. There is no sign, however, of the onset of oocyst wall

formation, and the parasites still contain both WFB1 and WFB2. WFB1 content is either homogenous or in a variable stage of disintegration: the WFB2 remain intact (Fig. 9). In one macrogamont (Fig. 10) the cisternae containing the WFB2 are expanded, and the electron-dense wall-forming body appears to be floating inside: part of the mitochondrion, in such specimens, becomes filled with electron-dense deposit. Young oocysts were generally resistant to processing for TEM, and only a few images (Fig. 13) enabled one to see formation of the oocyst wall which, superimposed by a thin membrane, covered a similarly thin plasmalemma. Such zygotes still contain a few WFB1 and a larger number of WFB2 within their cisternae.

DISCUSSION

Position of the young meronts and macrogamonts within their host cells, and the general arrangement of internal structures, is similar to that usually shown by avian and mammalian eimeriid parasites. There are, however, some noteworthy peculiarities at the host/parasite interphase, as well as in the parasites themselves. ER and large cisternae lining the PV wall have, till now, been found only in the piscine coccidium *E. vanasi* (Paperna, 1995), and in mouse macrophages infected with *Toxoplasma gondii* (Jones & Hirsch, 1972). In the latter case the ER edges, dotted at regular intervals with electron-dense material described as ribosomes, are reminiscent of the regularly spaced indentations filled with electron-dense material seen along the ER and cisternae in the host cells of *E. (s.l.) boveroi*, described above. In *E. vanasi* the ER structure is dependent on structural changes which take place as the parasite develops (Paperna, 1995). It has been suggested (Mauel, 1996) that in macrophages infected with *Toxoplasma gondii* the ER lining either provides protection against the macrophage's lysosomal activity or is involved in providing the PV with 'factors necessary for parasite multiplication'.

The tubule-like layer (apparently a glycocalyx) coating the young meront pellicle and the wall of mature macrogamonts is an uncommon feature, but it has been seen in a few coccidians such as *Acrooimeria pintoii* (Lainson & Paperna, 1999; Paperna & Lainson, unpublished). Again, vesicular mitochondria are unusual, but have been seen in meronts of the saurian coccidian *Isospora cannoni* (Paperna & Finkelman, 1998). Although wall-forming bodies are found universally in all terrestrial-host species of *Eimeria*, *Isospora* and related eimeriids so far examined, the relative sizes of WFB1 and WFB2, and their configuration in the parasite, do vary in different species. The expansion of the cisternae holding the WFB2 (Fig. 10) could have been

Figs 9-13. Fig. 9. Mature macrogamont (zygote), enlarged view ($\times 14.882$) – Fig. 10. Zygote with expanded cisternae ($\times 14.890$) – Fig. 11. Enlarged sector from Figure 8, of a macrogamont, and PV wall associated structures (dense deposit arrowed) ($\times 26.861$) – Fig. 12. Enlarged sector of Figure 9, showing macrogamont's wall coated with a glycocalyx-like layer and the PV with the associated ER system ($\times 39.883$) – Fig. 13. Young oocysts with consolidating wall: 1. outer membrane; 2. the thick wall; 3. the plasmalemma ($\times 18.571$).

the result of a processing fault, for such a fault is evident in the parasite's nucleus; all membranes and other ultrastructural configurations in the parasite cell were, however, intact. A similar process involving cisternae expansion has also been seen in *Schellackia ptyodactyli* (Paperna & Finkelman, 1996). The observed three layered oocyst was apparently beyond the five membrane formation stage, characteristic to the wall formation process of *Eimeria* spp. of avian and mammalian hosts (Chobotar *et al.*, 1980: the outermost membrane appears to be comprised of oocyst wall membranes 1 and 2, the thick middle layer as in other coccidia is wall 3, while wall 4 merges with 5 into the oocyst plasmalemma.

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