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# MÉMOIRES ORIGINAUX

## ULTRASTRUCTURE OF THE MACROGAMONT OF *GOUSSIA CICHLIDARUM* LANDSBERG and PAPERNA, 1985, a coccidian parasite in the swimbladder of cichlid fish

I. PAPERNA*, J. H. LANDSBERG**, N. FEINSTEIN***

**SUMMARY.** Ultrastructural features of the macrogamont of *Goussia cichlidarum*, a coccidian parasite of cichlid fish are described. Host cells derived from the swimbladder lining epithelium are reduced to membranous sacs in early gamogony. These are displaced to the surface of the epithelium, but maintain their attachment until an early stage of oogony. An elaborate junction zone between the host cell and the epithelial cells suggests a nutritive function. Immature macrogamonts are bound by a double unit membrane with only a few micropores. Intravacuolar tubules are absent. Mature macrogamonts are bound by a single unit membrane and are surrounded by an additional two envelopes. Round organelles resembling wall-forming bodies of other coccidia, reach their maximum number and differentiation in mature macrogamonts.

### Ultrastructure du macrogamonte de *Goussia cichlidarum* Landsberg et Paperna, 1985, Coccidie parasite de la vessie natatoire d'un Poisson Cichlidé

**RÉSUMÉ.** Les caractères ultrastructuraux du macrogamonte de *G. cichlidarum*, Coccidie parasite d'un Poisson cychlidé, sont décrits. Les cellules-hôtes, qui proviennent de la bordure épithéliale de la vessie natatoire, sont réduites à des sacs membraneux au début de la gamogonie. Ceux-ci

* *Department of Animal Sciences, Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot 76-100 Israel.*
** *Laboratory for Research of Fish Diseases, Nir-David, 19150, Israel.*
*** *Electronmicroscopy unit, Life Science Institute, Hebrew University of Jerusalem, Israel.*

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sont repoussés à la surface de l'épithélium mais conservent leur point d'attache jusqu'au début de l'oogonie. La zone de jonction spécialisée entre la cellule-hôte et les cellules épithéliales suggère qu'elle exerce une fonction dans la nutrition. Les macrogamontes immatures sont limités par une double membrane-unitaire avec peu de micropores. Les tubules intra-vacuolaires sont absents. Les macrogamontes mûrs sont limités par une seule membrane unitaire et entourés par deux enveloppes supplémentaires.
Des organites arrondis ressemblant aux corps générateurs de la paroi des autres Coccidies atteignent leur nombre maximum et leur complète différenciation chez les macrogamontes mûrs.

## Introduction

*Goussia cichlidarum* Landsberg and Paperna, 1985 [(Barrouxiidae, Levine, 1983) Calyptosporidae, Overstreet, Hawkins and Fournie, 1984, Apicomplexa] parasitises the swimbladder epithelial lining of cichlid fish. Merogony and gamogony are completed within hypertrophic host cells which are displaced to the surface of the epithelial layer. The host cell degenerates during early parasite development and is reduced to a membranous sac enclosing the parasite. During oogony, the host cell membrane disintegrates and released sporonts complete their differentiation in the swimbladder lumen (Landsberg and Paperna, 1985). The ultrastructural features of the macrogamonts and their association with the host tissue are described herein.

## Materials and methods

Study material was obtained from hybrids of *Oreochromis aureus* (Steindachner) × *O. niloticus* (L.) and *Sarotherodon galilaeus* (L.) (Cichlidae) from fish ponds in the Jordan Valley, Israel. Infected swimbladders were fixed in either Karnovski or 3 % glutaraldehyde in pH 7.4, 0.1M cacodylate buffer for 4-12 h at 4° C. Some swimbladders were injected *in situ* with one or the other of these fixatives prior to their removal for processing. The latter method was no more advantageous than the first. Following extensive washing in cacodylate buffer, post fixation was in 1 % $OsO_4$ in the same buffer for 1h at room temperature (24° C). Part of the material was treated en bloc with aqueous uranyl acetate (in acetate-acetic acid buffer, pH 5). Following dehydration in ascending ethyl alcohols, the tissue was embedded in Araldite or in Spurr's medium. Thin sections (600-700A) were cut by diamond knife with an LKBIII ultratome. Sections were stained on the grid with 0.5 % uranyl acetate and lead acetate and examined in a Jeol JEM 100CX transmission electron microscope.

## Results

### 1 - The host cell *(Fig. 1-4)*.

The host cells for all stages of gamogony were reduced to an envelope consisting of a single unit membrane. Membranes of adjoining host cells were tightly packed,

but never fused into a common membrane *(Fig. 1)*. The entire volume of the host cell was filled with a dense reticular or granular substance of varying density. Host cells displaced to the surface were each attached to the perimeter of a single lining epithelial cell at interrupted junction zones. These interruptions were caused by folds in the attachment surface as confirmed by SEM (Paperna and Cross, 1985). The points of attachment were marked by an electron dense junction layer *(Fig. 1, 2, 3)* and by the absence of microvilli which characteristically lined non-infected surface cells *(Fig. 2, 3)*. In the junction area, the thick electron dense membrane of the host cell was covered by a layer of rounded electron dense globules. This layer was superimposed over the fine superficial unit membrane of the epithelial cell, leaving a narrow, but distinct, middle stratum of medium electron density between the adjoining membranes *(Fig. 3, 4)*. A spherical, thick-walled, 1nm in diameter structure was seen (in many cross sections) within the host cell lumen immediately above *(Fig. 2, 3)* or interconnected with the adjoining layer *(Fig. 4)*.

**2 - Early gamonts** *(Fig. 1)*

Early gamonts (9.0 × 6.3 μm) were oval with a considerably invaginated surface and bound by two distinct unit membranes separated from each other by an electron lucent zone *(Fig. 1)*. The prominent nucleus was enclosed in a distinct envelope and contained an electron dense nucleolus. The cytoplasm contained : varying numbers of amylopectin-like bodies forming an electron dense perimeter ; one or two large opaque vacuoles with an homogenous margin of medium electron density ; a few food vacuoles in the marginal zone ; outlines of endoplasmic reticulum ; Golgi apparatus, and several tubular mitochondria all of which were indistinct due to the high density of the cytoplasm *(Fig. 1)*. In a few early gamonts, a well-defined micropore was observed.

**3 - Immature macrogamonts** *(Fig. 5-9)*.

Cells with an undulating perimeter were oval-to-round (in cross section — 7-10 × 15-22 μm) *(Fig. 5)*. The cell was bound by two distinct unit membranes. Micropores were few. The large nucleus, bound by a distinct (bilaminated) envelope (*Fig. 9)*, contained an electron dense nucleolus *(Fig. 5, 9)*. Occasionally, the nuclear perimeter was lobate *(Fig. 9)*. Distinct food vacuoles, containing an aggregated, electron dense floccular substance, were seen in the subpellicular zone, especially near deep pellicular invaginations *(Fig. 6)*, and in close proximity to elements of the Golgi apparatus *(Fig. 5, 9)*. Electron transparent large vacuoles (possibly outlines of lipid vacuoles), occurred in the sub-pellicular zone alongside the amylopectin-like bodies *(Fig. 9)*. One or two, large opaque vacuoles with a central electron lucent core *(Fig. 5, 7, 8)* were enclosed by a circular network of endoplasmic reticulum *(Fig. 8)*. The endoplasmic reticulum (often ribosome-lined) formed networks throughout the cytoplasm. These networks were interconnected by radial trunks, usually of two parallel tubuli *(Fig. 5)*. Numerous cisternae occurred along the endoplasmic reticulum especially in the subpellicular zone *(Fig. 5, 6)*. Canaliculi were rare, occurring as elongated

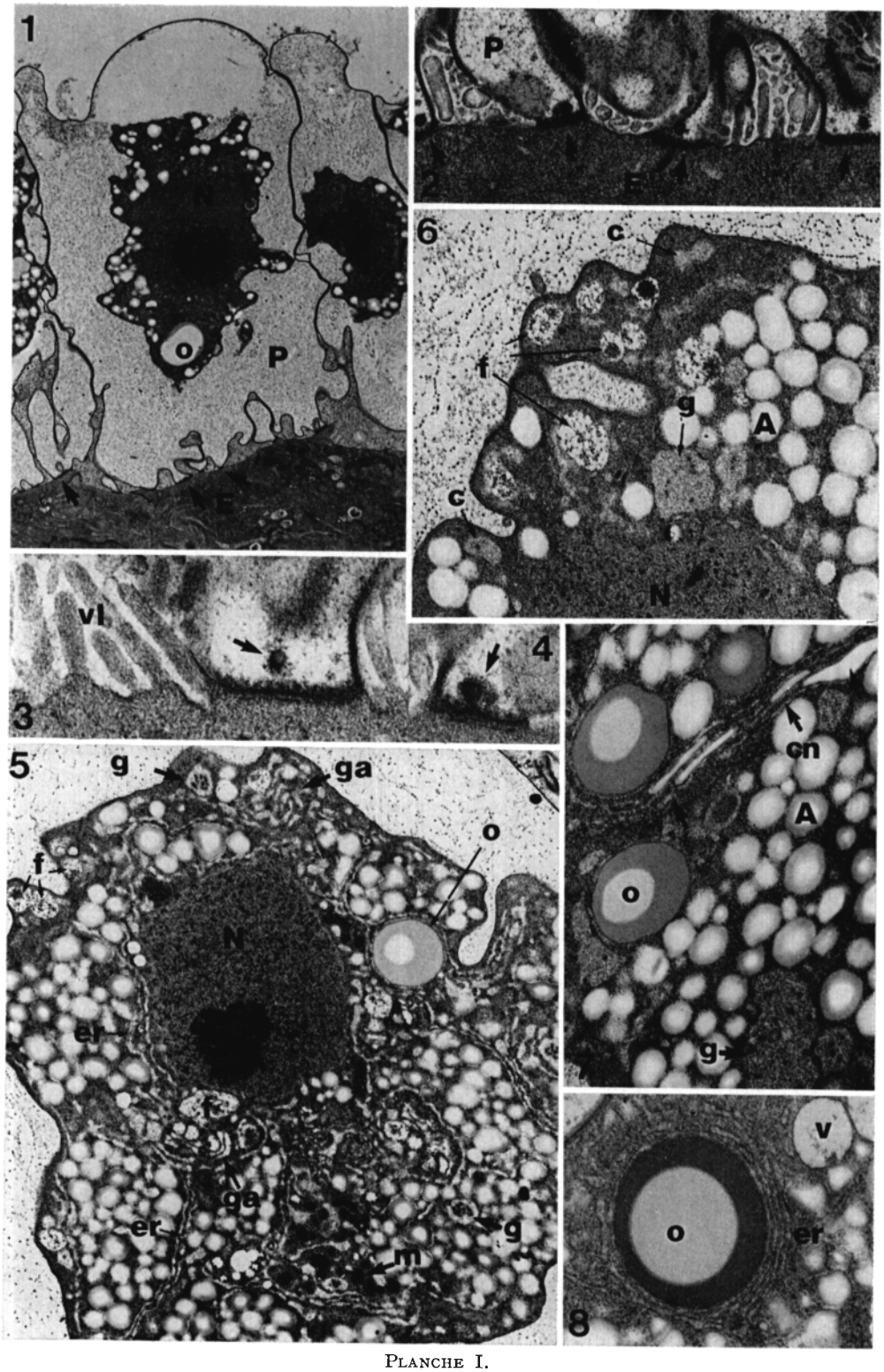


PLANCHE I.

electron dense inclusions within a dense network of the endoplasmic reticulum and in close proximity to opaque vacuoles *(Fig. 7)*. Varying numbers and sizes of mitochondria were present *(Fig. 5, 9)*. A few granular bodies (characteristic of mature macrogamonts), made an early appearance as rounded inclusions containing a variable number of large electron dense granules on a background of fine granulation *(Fig. 5, 6, 7)*.

**4 - Mature macrogamonts** *(Fig. 10-14)*.

Mature macrogamonts were rounded, 15 um in diameter (in cross section) *(Fig. 10)* and bound by a single unit membrane with the inner membrane reduced to an electron lucent narrow belt adjacent to the outer unit membrane *(Fig. 11, 13)*. Externally, two additional envelopes, each consisting of a one unit membrane, were present. One (mI) was closely adjacent, but not attached, to the cell's unit membrane and thus did not include the micropore *(Fig. 13)* ; the other, (mII) was more peripheral *(Fig. 10, 11)*. Granular substance filled the spaces between the macrogamonts, their envelopes and the host cell wall *(Fig. 10, 11)*.

Fully differentiated granular bodies, up to 1 um in diameter, contained distinct electron dense granules within a matrix of fine granules. Stages in the differentiation of the granular bodies were distinguished by an increase in size and number of large granules *(Fig. 12)*. Granular bodies were limited by electron lucent narrow margins lined externally by aggregations of ribosomes. At some points they were interconnected with cisternae of the endoplasmic reticulum *(Fig. 12)*. The numerous granular bodies were localized in patches, intermittent with zones occupied by the amylopectinlike bodies *(Fig. 10)*. Some were encircled by concentric layers of endoplasmic reticulum *(Fig. 14)*.

The endoplasmic reticulum lined by ribosomes formed a tubular plexus in the subpellicular zone *(Fig. 11)*. A few small vacuoles and mitochondria were observed. Elements of the Golgi apparatus were common in the perinuclear zone ; they appeared to be delicate and thin (less active ?) and contrary to those found previously, were accompanied only by very few vacuoles and vesicles *(Fig. 12)*. Opaque vacuoles were present and once, were seen interconnected with an amylopectin-like body *(Fig. 11)*.

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PLANCHE I.

*Fig. 1.* Young gamont in the parasitophorus sac (× 4000).
*Fig. 2.* Junction zone of the host cell (o the pa asitophorus sac) to the epithelial cell (× 20000).
*Fig. 3.* Intravascuolar-like tubule (arrow) located above the junction zone (× 44000).
*Fig. 4.* Intravacuolar-like tubule seen connected to the junction zone (× 42000).
*Fig. 5.* Premature macrogamont, general view (× 6500).
*Fig. 6.* Pellicular and subpellicular zone showing pellicular invaginations, food vacuoles and early granular bodies (× 11000).
*Fig. 7.* Canaliculi and opaque vacuole (× 10800).
*Fig. 8.* Opaque vacuole with concentric network of endoplasmatic reticulum (× 15000).

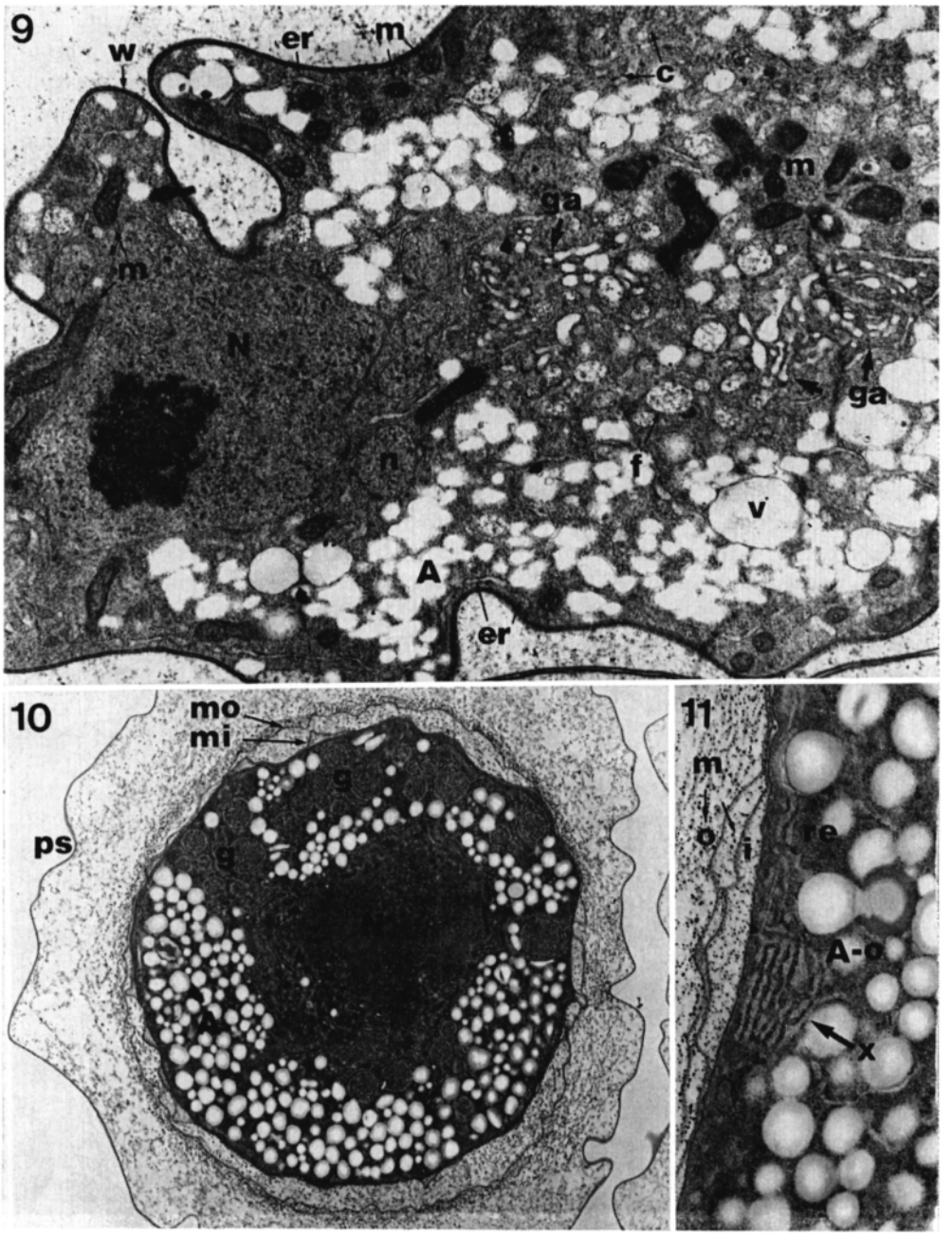


PLANCHE II.

*Fig. 9.* Detailed view of the cytoplasmic organelles of a premature macrogamont (× 17700).
*Fig. 10.* General view of mature macrogamont (× 3750).
*Fig. 11.* Plexus of rough endoplasmic reticulum in the sub pellicular zone (× 11400).

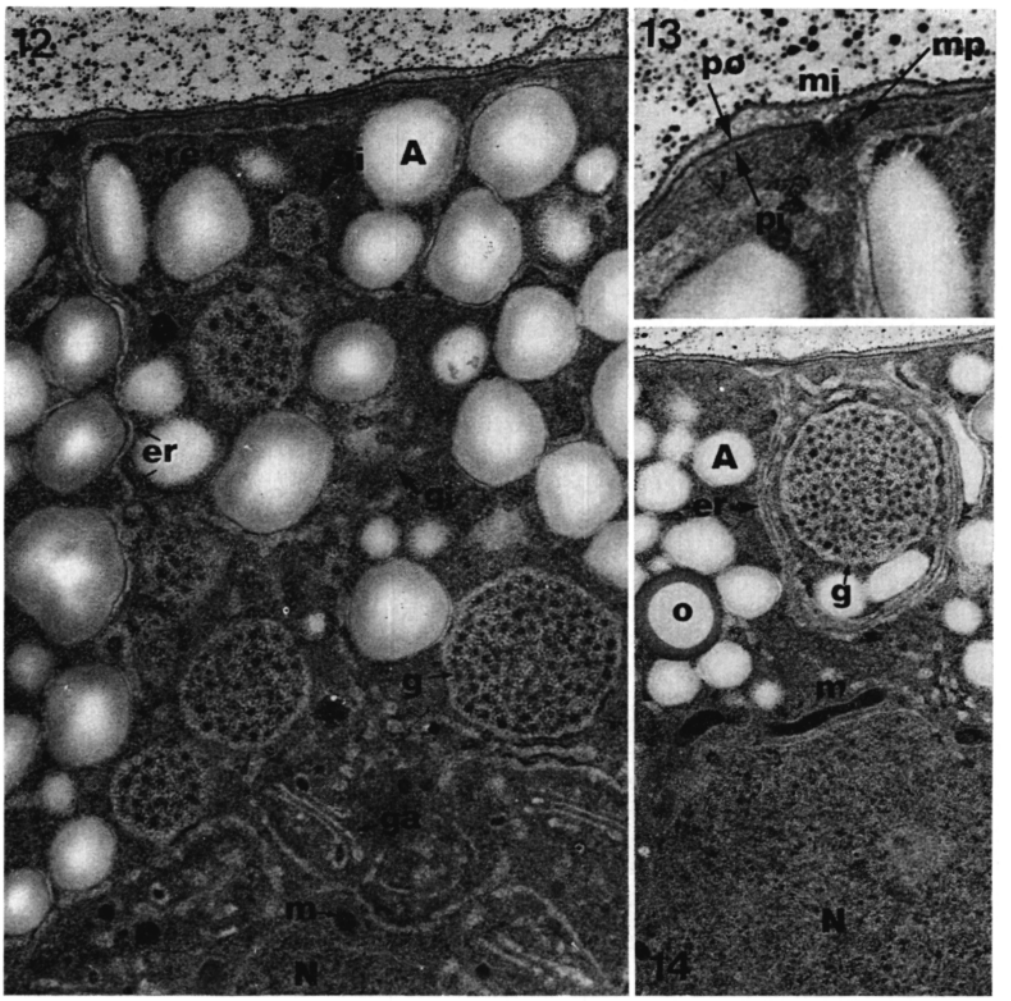


PLANCHE III.

*Fig. 12.* Cytoplasmic organelles of mature macrogamont, stages in the differentiation of the granular bodies (× 28800).
*Fig. 13.* Pellicular zone in vicinity of a micropore (× 43200).
*Fig. 14.* Granular body concentrically enclosed by endoplasmic reticulum (× 21100).

## Discussion

During early gamont differentiation, the host cell is reduced into a membranosu sac which remains attached to the surface epithelium of the swimbladder until an early stage of oogony. It disintegrates during parasite oogony, which is completed in the swimbladder lumen (Paperna and Landsberg, 1985). The junction zone between the host/epithelial cell interface appears to have a nutrititve function

which implies that the membranous sac acts as a nursing parasitophorous cell and is not just an inert envelope. This feature emphasises the intracellular nature of *G. cichlidarum* as also appears to be the case in other "epiepithelial" coccidia of the genera *Cryptosporidium* (Vetterling, Takeuchi and Madden, 1971) and *Epieimeria* (Boulard and Blanc, 1985 ; Daoudi, Marques and Bouix, 1985). However, the true nature of the relationship of these parasites with their host cells still remains uncertain.

Fish coccidia lack the intravacuolar tubules and folds which are present in vertebrate terrestrial host cells (Michael, 1975 ; Scholtyseck, 1979). However, in *G. cichlidarum*, a small organelle reminsicent of an intravacuolar tubule was repeatedly found near the junction zone of the host/epithelial cell interface. Micropores were fairly common in *G. cichlidarum* yet they were far less numerous than those present in coccidia of terrestrial hosts. The opaque vacuoles seen in all developmental stages, appear to contain a lipid substance reminiscent of the lipid inclusions common to all coccidia (Scholtyseck, 1979). Their limited occurrence and their localization within plexuses of endoplasmic reticulum, are suggestive of their being active metabolic organelles rather than nutrient storing inclusions. Organelles reminiscent of the wall-forming bodies of terrestrial host coccidia (Scholtyseck, Mehlhorn and Hammond, 1971 ; Chobotar and Scholtyseck, 1982) have been described in piscine macrogamonts (Paterson and Desser, 1981 ; Hawkins, Solangi and Overstreet, 1983 ; Desser and Li, 1984 ; Morrison and Hawkins, 1984 ; present study). In terrestrial coccidia, wall-forming bodies participate in the formation of the solid, dessication resistant, oocyst wall (Scholtyseck *et al.*, 1971 ; Scholtyseck, 1979). In fish coccidia, the oocyst wall is usually membranous, thin and fragile (Lom, 1971 ; Molnar, 1977). Paterson and Desser (1981, 1984) concluded that the electron dense inclusions (named Mv-Mx) in *G. iroquoina* are not homologous with wall-forming bodies of terrestrial vertebrate coccidia. The inclusions described in *C. funduli* also apparantly differ (Hawkins *et al.*, 1983). The granular bodies presently described (although unique in their granular content) are more reminiscent of the wall forming bodies found in terrestrial coccidia. Similar, but particulate bodies were also found in *E. sardinae* (Morrison and Hawkins, 1984). In *G. cichlidarum*, the granular bodies reached their full differentiation (into round heavy granulated bodies) in mature (apparently fertilized) macrogamonts and disintegrated during oogenesis (Paperna and Landsberg, 1985). Although they seem to be associated with the final phase of gamogony, their actual function and homology with the wall forming bodies of terrestrial coccidia remains obscure.

Granular bodies have also been described in macrogamonts of coccidia in invertebrate hosts (Heller, 1969). Their presence seems to be more related to the formation of the sporocyst wall than to that of the oocyst wall (Heller, 1969 ; Porchet-Hennere and Richard, 1971). This situation might similarly apply to *G. cichlidarum* (Paperna and Landsberg, 1985) as well as to other piscine coccidia (Morrison and Hawkins, 1984).

The granular bodies in *G. cichlidarum* appear to form within the endoplasmic reticulum, as do some wall-forming bodies of other piscine coccidia, *C. funduli* (Hawkins *et al.*, 1983), *G. iroquoina* (Paterson and Desser, 1981) and *E. sardinae*

(Morrison and Hawkins, 1984). In this respect, they are similar to coccidia of terrestrial vertebrates (Chobotar, Senaud, Ernst and Scholtyseck, 1975 ; Ferguson, Birch-Andersen, Hutchison and Siim, 1977). Mature macrogamonts were enclosed by two loose additional unit membranes. Duplication of the wall membranes generally marks the end of fertilization and the onset of oocyst formation in coccidia of terrestrial hosts (Speer, Hammond, Youssef and Danforth, 1973 ; Vetterling, Pacheco and Fayer, 1973). This suggests that the mature macrogamonts of *G. cichlidarum* are already zygotes. Other fine structural changes which support this view are : a) the predominance of granular bodies ; b) reduction in size and number of mitochondria (as similarly observed in fertilized macrogametes of other coccidia (Speer *et al.*, 1973)) ; c) reduced volume of the Golgi apparatus ; d) formation of an extensive subpellicular endoplasmic reticulum plexus, also seen in *G. iroquoina* (Paterson and Desser, 1981).

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*Abréviations utilisées pour l'illustration :* A- amylopectin-like granules ; A-o, Amylopectin like body merges into opaque vacuole ; c-cisterna ; cn- canaliculi ; E-host epithelium ; er- endoplasmic reticulum ; f- food vacuoles ; g- granular bodies ; ga- golgi apparatus ; gi- forming granular body ; m- mitochondria ; mi- inner macrogamont's additional membrane ; mo- outer macrogamont' additional membrane ; mp- micropore ; N- nucleus ; n- extensions or lobes of the nucleus ; o- opaque vacuole ; P- parasitophorus sac ; pi- subpellicular unit membrane ; po- pellicular unit membrane ; ps- parasitophorus membrane ; re- rough endoplasmic reticulum ; v- empty vacuole ; w- parasite cell wall (pellicle) ; x- subpelicular rough endoplasmic reticulum plexus.

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