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Mots-clés : Ultrastructure. *Eimeria gastrosauris.* Geckos. Australie. Muqueuse gastrique. Mérogonie. Gamogonie.

ULTRASTRUCTURAL STUDY OF *EIMERIA GASTROSAURIS* A COCCIDIUM FROM THE STOMACH EPITHELIUM OF AUSTRALIAN GECKOES

I. PAPERNA

SUMMARY

The fine structures of trophozoites, merozoite formations, micro and macrogamonts and young occysts of *Eimeria gastrosauris* are described. This parasite undergoes endogenous development in the gastric mucosa epithelium of the Australian geckoes *Heteronotis binoei* and *Oedura monilis.* The wall of the parasitophorous vacuole is densely lined with intravacuolar folds on the inside; outside, microfibrils often accumulate into a distinct layer in the host cytoplasm surrounding the vacuole. Microgamonts, as well as macrogamonts, are deeply invaginated. Type 1 wall-forming bodies in the macrogamonts vary in texture, being either lamellar or granular. Type 2 wall-forming bodies are comprised of two layers of different-density matrices. Only early stages of wall formation were available for study.

RÉSUMÉ : **Étude ultrastructurale d'*Eimeria gastrosauris*, coccidie de l'épithélium stomacal de Geckos australiens.**

Description de la structure fine des trophozoïtes, de la formation des mérozoïtes, des micro et macrogamétocytes et des jeunes oocystes d'*Eimeria gastrosauris.* Son développement tissulaire s'effectue dans l'épithélium de la muqueuse gastrique des Geckos australiens *Heteronotia binoei* et *Oedura monilis.* La face interne de l'enveloppe de la vacuole parasitophore présente de très nombreux replis intravacuolaires. A l'extérieur, des microfibrilles s'accumulent fréquemment, formant, dans le cytoplasme de la cellule hôte, une couche individualisée autour de la vacuole parasitophore. Microgamontes et macrogamontes présentent de profondes invaginations. Les *wall forming bodies* de type 1 sont tantôt lamélaires, tantôt granuleux chez le microgamonte. Ceux de type 2 sont formés de deux couches de densité différente. La formation de l'enveloppe n'a pu être étudiée que chez les jeunes stades. Les auteurs suggèrent que les replis intravacuolaires, particulièrement développés chez cette espèce, jouent un rôle dans la nutrition et pourraient représenter une adaptation à sa localisation particulière dans l'estomac.

INTRODUCTION

Eimeria gastrosauris Paperna (in press) undergoes endogenous development in the gastric epithelial lining of the Australian geckoes *Heteronotia binoei* and *Oedura monilis.* In this paper the ultrastructure of merogonic and gamogonic stages is described. *E. gastrosauris* is the first Eimerian coccidian species found to have its endogenous development in the stomach of a vertebrate host.

MATERIALS AND METHODS

Stomach mucosa with endogenous coccidian stages was obtained from naturally infected *Heteronotia binoei* (type host specimen of *E. gastrosauris,* from the Mt. Isa region, NW Queensland) and *Oedura monilis* (from Mt. Speke, NE Queensland). Infection in the collected animals was detected by microscopic examination of stool samples. Following necropsy, pieces of stomach mucosa were fixed in Karnowski for 24 h at 4°C, rinsed repeatedly in cacodylate buffer (0.1 M, 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 serially dehydrated in ethanol and embedded in Epon. Thin sections cut on a Reichert-Jung Ultracut ultratome with a diamond knife were stained on grid with uranyl acetate and lead citrate and examined with a Jeol 100 CX TEM.

RESULTS

In both gecko host species, endogenous development took place in cells loaded with mucus droplets from the gastric epithelial lining *(Figs. 1, 4, 9, 16).* The fine structural details of merogony stages and gamonts from the two host species were identical *(Figs. 4, 7, 11, 12).* The parasitophorous vacuoles were always rounded *(Figs. 1, 4, 8, 9)* and the inside of their wall was lined with numerous intravacuolar folds, some of which had been broken off *(Figs. 1, 5, 6).* On the outer perimeter, in the host cytoplasm, the parasitophorous vacuole was surrounded by numerous microfibrils *(Fig. 6)* which had, in older infections, either

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consolidated into a distinct layer *(Figs. 2, 5)* or disaggregated *(Figs. 7, 13-15)*.

Trophozoites, already almost completely bound by a single unit membrane (11.0 × 4.6 μm), contained a large lipid vacuole, exhausted rhoptries (a processing artifact?), numerous micronemes and several mitochondria *(Fig. 1)*. The only other merogony stages found were merozoite formations from divided meronts *(Fig. 2)*. These (7.8 × 2.2 μm) contained numerous micronemes, a few rhoptries and mitochondria, some amylopectin granules, and a large Golgi apparatus in the prenuclear zone *(Figs. 2, 3)*.

Premature microgamonts (33 × 24 μm; *Fig. 4)* were lobate or folded; their numerous nuclei were distributed along the outer and infolded rims, accompanied by mitochondria and a globular organelle: a medium density granular enclave loaded with translucent minute globules. The cytoplasm also contained several lipid vacuoles and some amylopectin granules *(Fig. 4)*. The cell boundary membrane, near the nuclei was interrupted by a micropore *(Fig. 5)* and centrioles appeared between the nuclei and the surface of the gamont *(Figs. 6, 7)*. Some nuclei contained a centrocone *(Fig. 6)*. Mature microgamonts with microgametes were not found in the ultrathin sections.

Elongated merozoites destined to develop into macrogamonts (10.0 × 3.5 μm) contained a nucleus with a distinctly large nucleolus, many small mitochondria and a globular organelle *(Fig. 8)*. These organelles also occurred in young and mature macrogamonts. Young, rounded macrogamonts (13.0 × 9.5 μm) were bound by a single unit membrane, but still retained many rhoptries and micronemes *(Fig. 9)*. Their cytoplasm contained a large granular organelle, in addition to the globular one described above *(Fig. 9)*. Mature macrogamonts (17-23 × 14-19 μm; *Figs. 10-13*) were bound by a two-layered wall, sometimes with an additional incomplete inner lamella *(Figs. 14, 15)*. Similar to the microgamonts, they were lobate or deeply invaginated. The cytoplasm contained rough endoplasmic reticulum and many globular organelles *(Figs. 10-12)*. During macrogamont maturation, lipid vacuoles *(Figs. 10,11)* were replaced with amylopectin granules *(Figs. 12, 13)*. The small, type 1 wall-forming bodies (WF1) demonstrated various textures: lamellar, concentric or granular *(Figs. 13-16)*. The large type 2 wall-forming bodies (WF2) were located within endoplasmic reticulum cisternae, often surrounded by concentric rough endoplasmic reticulum. They consisted of a medium-dense foamy substance apposed to a central, homogeneously electron-dense core *(Figs. 13-15)*. Small aggregates of electron-dense granules were seen, which seemed to be the precursors for WF1 or for both types of wall forming bodies. WF2 anlagen contained a medium-density homogeneous substance *(Figs. 10-12)*, which turned into a homogeneously electron-dense body before reaching their final compound shape.

In the presumed zygote (39 × 28 μm), the cytoplasm became loaded with amylopectin granules *(Fig. 13)*, while WF1 and WF2 remained unchanged *(Figs. 13-15)*. Canaliculi, characteristic of mature macrogamonts and zygotes, were absent.

Wall formation began by thickening and subsequent duplication of the oocyst wall *(Figs. 16, 17)*, the latter process starting with the aggregation of ribosomes (and accompanying endoplasmic reticulum?) beneath the outer wall *(Fig. 16)*. Outlines of another developing membrane appeared beneath the inner wall. Granular material aggregating between the two walls caused the inner one to bulge inwards *(Fig. 17)*.

DISCUSSION

The fine structure of *Eimeria gastrosauris,* which undergoes endogenous development in the gastric mucosa, conforms to the general scheme characteristic of reptilian, as well as avian and mammalian intestinal *Eimeria*. At the same time however, it demonstrates a few of its own structural peculiarities: lobate or deeply invaginated macrogamont and microgamont, the unique structures of type 1 and 2 wall-forming bodies, the presence of structures such as the globular and granular organelles and the absence of canaliculi. The structural details of the parasitophorous vacuole are characteristic, being lined from the inside with numerous intravacuolar folds and enclosed on the host cytoplasmic side by a fibrillar layer.

Wall-forming bodies of all eimerian coccidia demonstrate interspecific structural variation (Scholtyseck *et al.,* 1971) and are apparently characteristic for each eimeria species.

Microgamonts of many eimerian coccidia become, with maturity, folded or deeply invaginated (Hammond *et al.,* 1969; Ferguson *et al.,* 1977). However, folds or invaginations in macrogamonts are exceptional among *Eimeria* spp. When they do occur, they are shallow and few (Hammond *et al.,* 1967).

Structures reminiscent of the globular organelles seen here are the adnuclear bodies seen in merogony stages of reptilian gall bladder coccidia (Paperna and Landsberg, 1989*a*); the adnuclear bodies seen in *Caryospora colubris* (Paperna, 1991) are different organelles. The nature of the granular organelle is unknown. It seems to be a differently structured Golgi apparatus. Canaliculi have been found in all other reptilian coccidia macrogamonts studied to date (Ostrovska and Paperna, 1987; Paperna, 1989; Paperna and Landsberg, 1989*a*; Paperna, 1991).

Intravacuolar folds are comprised of two closely apposed unit membranes (Ferguson *et al.,* 1977) and their numbers vary in the parasitophorous vacuoles of many eimerian coccidia at all stages of development (Michael, 1975). The breaking off of intravacuolar folds which is often observed (Hammond *et al.,* 1967) seems to be either a wearing pro-

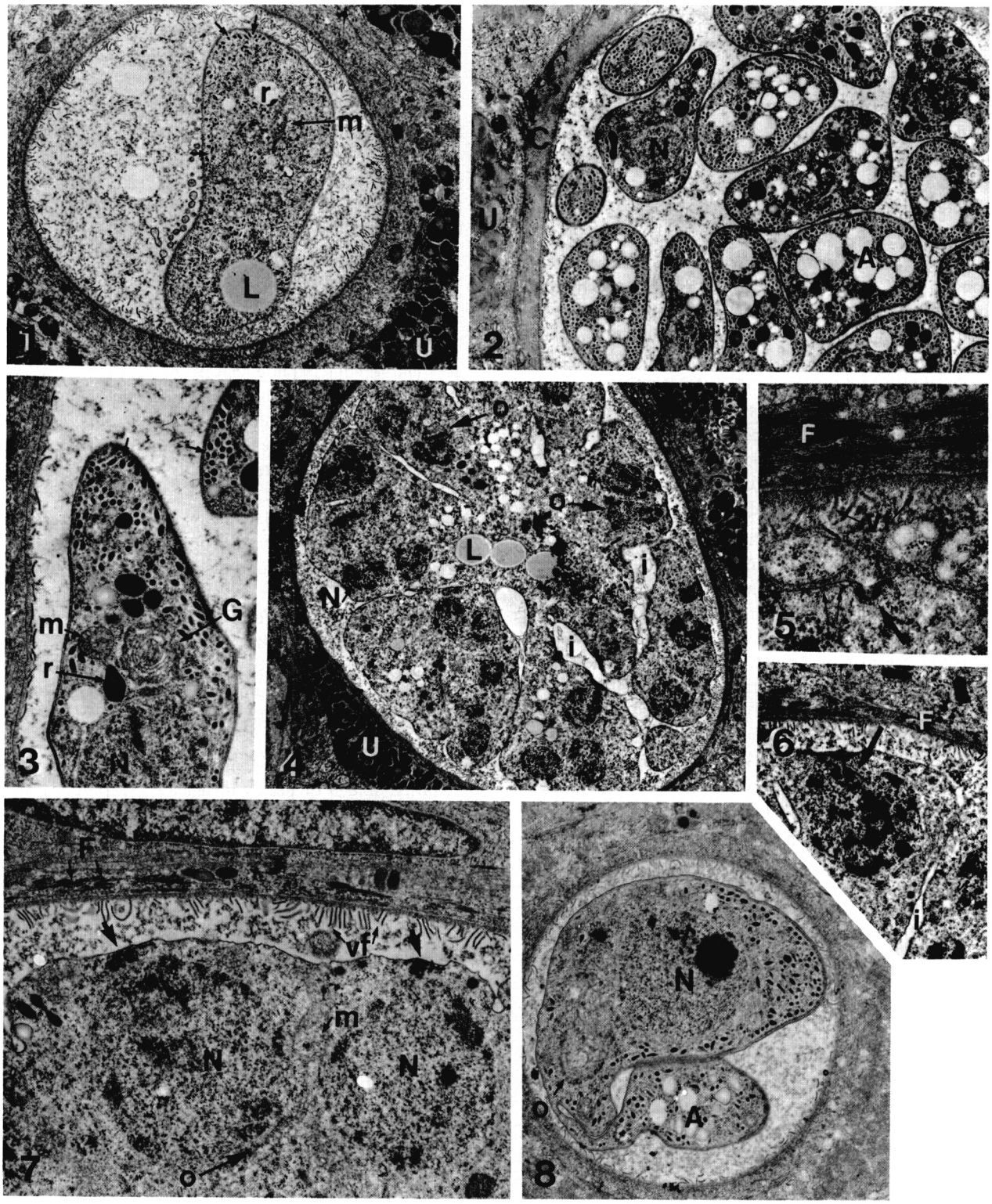

PLATE I.

cess or a processing artifact. In none of the studied coccidians, however, are the intravacuolar folds so numerous, so evenly distributed over the parasitophorous wall and so persistently present throughout endogenous development, until oocyst formation. The extreme extension of the parasitophorous wall surface resulting from the increased number of vacuolar folds, could have been evolved to accelerate nutrient transport, thereby compensating for the probable inferior nutritive conditions inherent to living in the gastric mucosa. This latter assumption is supported by the paucity of coccidian species found in the stomach mucosa of vertebrates.

The aggregation of granular substance between the outer and inner wall of the young oocyst is reminiscent of the process by which material from disaggregated WF1 is deposited within the developing oocyst wall membranes in avian and mammalian *Eimeria* (Pittilo and Ball, 1984). Only initial stages of oocyst wall formation were, however, observed. At these stages, the majority of WF1 as well as WF2 remain seemingly unchanged. The process of wall formation among sauriam eimeriam coccidia purportedly demonstrating greater taxonomic diversity (Paperna and Landsberg, 1989*b*), is apparently less uniform than in *Eimeria* spp. of avian and mammalian hosts (Paperna, 1989; Paperna and Landsberg, 1989*a*).

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PLATE I. — *Eimeria gastrosauris* from the stomach of *Heteronotia binoei* (Figs. 2, 3, 5, 7, 8) and *Oedura monilis* (Figs. 1, 4. 6).

FIG. 1. — Trophozoite with exhausted rhoptries, and numerous micronemes (small arrows) within a rounded parasitophorous vacuole lined with intravacuolar folds (fine arrows) (× 4,700).

FIG. 2. — A formation of merozoites; note the fibrous encapsulation at the periphery of the parasitophorous vacuole (C) (× 7,000).

FIG. 3. — Enlarged view of a merozoite from a formation with prominent Golgi apparatus (small arrows, micronemes) (× 14,400).

FIG. 4. — Differentiating, invaginated microgamont (× 2,700).

FIG. 5. — Microgamont cell wall (thin arrows) with a micropore (bold arrow) and view of the parasitophorous wall with the apposed fibrillar layer (× 15,000).

FIG. 6. — Microgamont nucleus with a centrocone (arrow) (× 6,200).

FIG. 7. — Microgamont peripheral zone containing nuclei and centrioles (bold arrows) (× 15,000).

FIG. 8. — Merozoites with a nucleus containing a large nucleolus, destined to become a macrogamont (× 10,000).

Abbreviations: A, amylopectin granules; B1: type 1 wall-forming body; B2, type 2 wall-forming body; F, apposed fibrillar layer; G, Golgi apparatus; i, invagination (or fold); L, lipid vacuoles; m, mitochondria; N, nucleus; o, globular organelle; r, rhoptries; U, mucus droplets of the gastric epithelial cell; vf, intravacuolar folds.

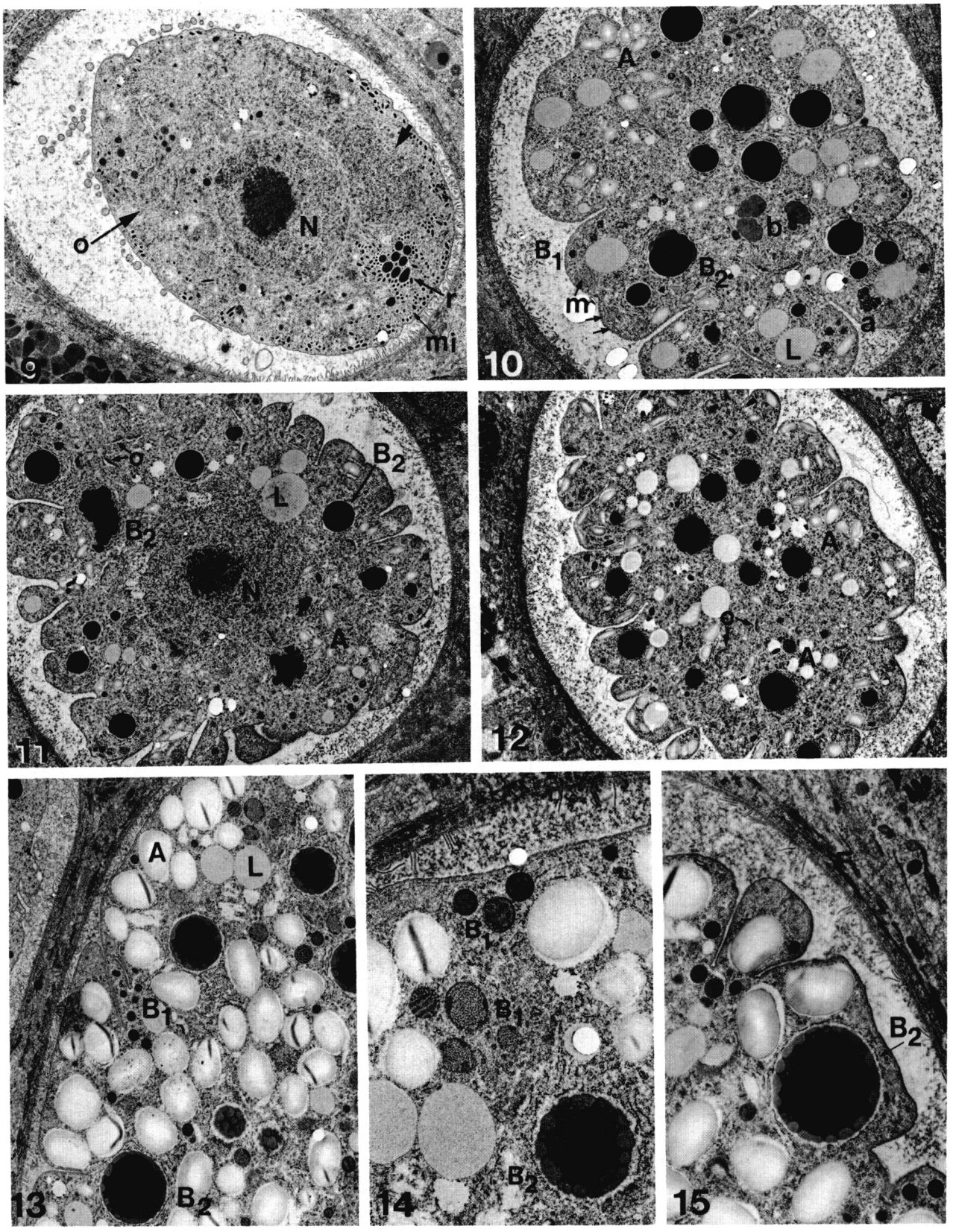

PLATE II. — *E. gastrosauris* from *H. binoei* (Figs. 9-11, 13-15) and *O. monilis* (Fig. 12).

FIG. 9. — Young macrogamont with globular and granular organelles (bold arrow),rhoptries and micronemes (× 6,000).

FIGS. 10-12. — Maturing, invaginated macrogamonts with already differentiated type 1 and 2 wall-forming bodies, precursors of wall-forming bodies (a) and anlagen of type 2 wall-forming bodies (b) (× 5,000; × 3,800; × 4,100).

FIG. 13. — Zygote-young oocysts loaded with amylopectin granules. with still intact wall-forming bodies of the two types (× 4,600).

FIGS. 14-15. — Enlarged view of the cell boundary and wall forming bodies of a mature macrogamont (× 11,800).

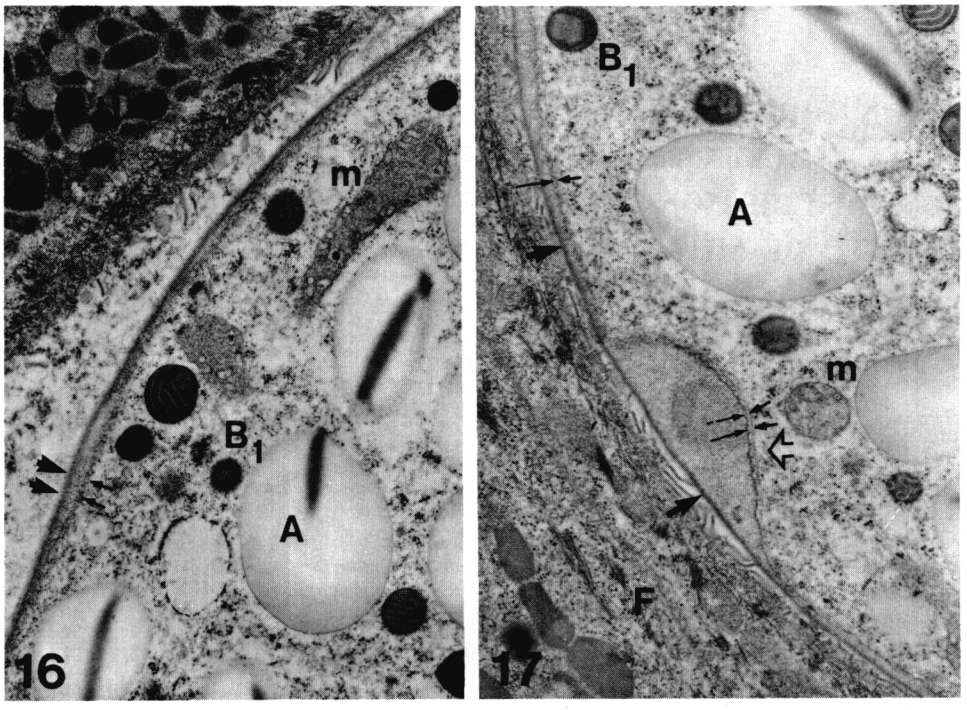

PLATE III. — *E. gastrosauris* from *H. binoei:* stages of wall formation in young oocysts.

FIG. 16. — Thickening and duplication of the oocyst wall (fine arrows, aggregation of rhibosomes) (× 19,500).

FIG. 17. — Formation of a third wall (fine arrows) and deposition of granular substance (open arrow) between the outer (bold arrow) and the inner (fine arrow) layer (× 18,700).

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