**FINE STRUCTURE OF MEROZOITES
OF *EIMERIA? BEAUCHAMPI* LÉGER AND DUBOSCQ, 1917
IN *GLOSSOBALANUS MINUTUS* (KOW.) (ENTEROPNEUSTA)**

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SUMMARY. Merozoites of *Eimeria? beauchampi* Leger and Duboscq, 1917 from the digestive epithelium of the hepatic region of the enteropneust *Glossobalanus minutus* (Kow.) have been studied by electron microscopy for the first time. The merozoites show typical coccidian features with a trilaminate pellicle, an apical polar ring, a conoid with two accessory rings, micronemes, and two rhoptries whose ductules are directed along a central intra-conoidal microtubule, above which a spherical vesicle occurs. Approximately 50 subpellicular microtubules run from the apical end to the nuclear region of the parasites. Lipid droplets, amylopectin granules and a large mitochondrion also occur in the cytoplasm. The presence of a refractile-like body is a remarkable feature of the merozoites studied. The present data are discussed in the light of the available literature.

Key-words: Ultrastructure. Merozoites. *Eimeria*. *Coccidia*.

Étude ultrastructurale des mérozoïtes d'*Eimeria? beauchampi* Leger et Duboscq, 1917, Coccidie parasite de *Glossobalanus minutus* (Kow.) (Enteropneusta).

RÉSUMÉ. Les mérozoïtes d'*Eimeria? beauchampi* Leger et Duboscq, 1917 trouvés dans l'épithélium hépatique (digestif) de l'entéropneuste *Glossobalanus minutus* (Kow.) ont été étudiés pour la première fois au microscope électronique à transmission. Les mérozoïtes montrent des caractéristiques typiques des Coccidies, avec une paroi trilamellaire, un anneau polaire apical, un conoïde avec deux anneaux accessoires, micronèmes et deux rhoptries dirigées le long d'un microtubule central surmonté d'une vacuole sphérique. Environ 50 microtubules subpelliculaires partent de la région apicale vers la région du noyau. Des globules lipidiques, granules d'amylopectine et une grande mitochondrie sont aussi présentes dans le cytoplasme. La présence d'un corpuscule réfractile dans ces mérozoïtes constitue une caractéristique qui mérite d'être soulignée.

Mots-clés : Ultrastructure. Mérozoïtes. *Eimeria*. *Coccidia*.

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Introduction

In the course of our studies on the fine structure of the gut of the acorn worm *Glossobalanus minutus* (Kow.), two meronts with fully developed merozoites were observed in the digestive epithelium of the hepatic region.

In spite of the extensive literature available on Coccidia, studies on these parasitic protozoan of enteropneusts are very few, and mainly based upon isolated light-microscopical observations of different phases of the life cycle. Spengel (1893) found sporozoan forms in the digestive tract of two enteropneusts, but he did not give any information about their coccidian or gregarine nature. Later, Leger and Duboscq (1917) described two species, *Eimeria? epidermica* and *E.? beauchampi* in the epidermis and in the hepatic caeca of *Glossobalanus minutus*, respectively. More recently, Benito (1977) and Fernandez and Benito (1983) studied both sexual and asexual stages of *E.? beauchampi* found in the hepatic digestive epithelium of *G. minutus*. Furthermore the present authors have just studied the ultrastructure of the developing oocyst of *E.? beauchampi* in the same host (Fernandez *et al.*, in press).

Material and methods

Adult specimens of *Glossobalanus minutus* (Kow.) parasited by *Eimeria? beauchampi* Leger and Duboscq, 1917 were obtained from medium-coarse sand in the intertidal zone of the North coast of Spain.

Just captured they were dissected in order to obtain small tissue pieces which were immediately placed in fixative. Satisfactory fixation was obtained with: a) 4 % glutaraldehyde in sea water (6.5 g/l NaCl added) at 4° C, and b) 4% glutaraldehyde in Millonig phosphate buffer pH 7.4 made up with sea water (6.5 g/l NaCl added). Both methods were followed by postfixation in 1 % osmium tetroxide in sea water (6.5 g/l NaCl added) and in Millonig phosphate buffer respectively.

Following dehydration in graded acetone series, tissues were stained « in block » with uranyl acetate and embedded in Araldite via propylene oxide.

For light-microscopic observation semi-thin sections were stained with toluidine blue. Thin sections were cut in a LKBIII ultramicrotome, stained with lead citrate (10 min.) and photographed using a Philips EM201 electron microscope.

Results

Mature meronts of *Eimeria? beauchampi* with merozoites occurring in a common parasitophorous vacuole were found in the digestive epithelium of the hepatic region of their natural host *Glossobalanus minutus*. A residual body is sometimes present in the center of the meront.

Abbreviations used in the figures: A, amylopectin granules. Av, apical vesicle. C, conoid. IM, inner membrane complex. L, lipid droplet. MN, micronemes. MT, mitochondrion. N, nucleus. OM, outer membrane. PM, subpellicular microtubules. PV, parasitophorous vacuole. R1, R2, preconoidal rings. RB, refractile-like body. RH, rhoptries. RM, residual material. TV, thick-walled vacuoles.

PLANCHE I

Fig. 1: General view of mature meront in a parasitophorous vacuole with merozoites. Note the disgregated, residual material probably from the meront. Scale bar, 1 μ m.

Fig. 2: Partial view of a group of merozoite sections in the parasitophorous vacuole. Some organelles: nucleus, mitochondrion, micronemes (arrows). Scale bar, 0.5 μ m.

The merozoites were seen in transverse and longitudinal sections having a long, slender shape (6-7 μm length and 1-1.3 μm with) (Figs. 1, 2, 8). They are bounded by a typical apicomplexan pellicle made up of an outer membrane and an inner membranous complex which is composed of two unit membranes closely

PLANCHE II

Fig. 3: Longitudinal section through the apical pole of a merozoite showing rhoptries, the single, central microtubule (white arrow), the apical vesicle (black arrow), the two pre-conoidal rings and the subpellicular microtubules. Scale bar, 0.200 μm .

Fig. 4: Oblique section of the same region. The conoid, the three-membranous pellicle and the subpellicular microtubules can be seen. Scale bar, 0.250 μm .

Fig. 5: Cross section through the anterior part of a merozoite with the refractile-like body and the small osmiophilic granules strongly packed (arrow). Scale bar, 0.5 μm .

Fig. 6: Thick-walled vacuole lying close to the refractile-like body. Note the GER cistern surrounding the refractile-like body (arrow). Scale bar, 0.250 μm .

attached to one another. This inner membrane complex is interrupted at the apical zone of the parasite to form the polar ring. Internally to the outer pellicle membrane there are two preconoidal rings and a conoid showing the typical hollow cone-like structure (0.10-0.13 μm in length, with the lower and upper diameters of 0.16-0.22 and 0.30-0.35 μm respectively) with a central, intraconoidal microtubule (Figs. 3, 4). Small structures identified as micronemes (Fig. 2) and two homogeneous osmiophilic rhoptries whose ductules extend into the conoid (Fig. 3) were found in the anterior region of the merozoites. The intraconoidal microtubule and rhoptries are located immediately below an electron-dense apical vesicle (about 0.48 μm in diameter) (Fig. 2).

Aproximately 50 subpellicular microtubules are regularly distributed around the periphery of the parasite (Figs. 4, 7) running from the anterior end to the nuclear region. The nucleus, with the chromatin peripherally distributed in discontinuous clumps, is placed nearer the posterior end (Fig. 2).

A patent (about 0.6 μm in diameter) electron-dense and homogeneous body

PLANCHE III

Fig. 7: Transverse sections at the middle region of the parasite. The mitochondria of the two merozoites have been cut at different levels, and both of them appear surrounded by a GER cistern (large arrow). Amylopectin granules, a vesicle attached to the inner membranous complex (fine arrow) and peripheral microtubules (arrowheads) can be seen. Scale bar, 0.250 μm .

(resembling the refractile bodies of sporozoites) is located at the anterior region of the parasites. Also, small groups of closely packed osmiophilic particles are found near the refractile-like body (Figs. 4, 5).

Fig. 8: Schematic diagram of a merozoite showing its general organization.

A large mitochondrion (*Fig. 2*) with a dense matrix and tubular cristae extends in the vicinity of the nucleus; sometimes and as could be seen from transverse sections, it shows a ring-like appearance which may suggest that it is glass-shaped (*Fig. 7*). A cistern of rough endoplasmic reticulum surrounded the mitochondrion and it might be observed clearly connected with the perinuclear envelope.

Lipid droplets and amylopectin granules were common cytoplasmic inclusions. Vesicles with contents of moderate density were also found in the cytoplasm; some of them were attached to the inner membranous complex (*Fig. 7*). Vacuoles containing osmiophilic and -light substances were also seen. Some of these vacuoles were thick-walled and occurred beside the refractile-like body (*Fig. 6*).

Unfortunately, no images of micropores and Golgi apparatus have been obtained from our preparations.

Discussion

The cellular organization of *Eimeria? beauchampi* merozoites is similar to that observed in other coccidian motile stages (Porchet-Henneré and Vivier, 1970; Scholtyseck, 1973; Scholtyseck, 1979; Elwasila, 1981). Although extraintestinal development has been recorded in several studies, Eimeriid coccidian are usually thought to be organisms that infect epithelial tissues of the intestinal tract. *E.? beauchampi* merozoites occur in the epithelium of the gut of the enteropneust *Glossobalanus minutus* and develop in a parasitophorous space which is lined by a unit membrane like the developmental stages of *Eimeria* sp. (Scholtyseck, 1984). It is generally accepted that the parasitophorous vacuole may function as reservoir of nutrients, and that the limiting membrane has a great importance as a protective structure (Chobotar and Scholtyseck, 1982).

The trilaminar pellicle enveloping the merozoites of *E.? beauchampi* is a typical feature of the Apicomplexa motile stages (Vivier and Petitprez, 1969; Vivier *et al.*, 1970; Porchet-Henneré and Vivier, 1970; Porchet and Torpier, 1977; Dubremetz and Torpier, 1978; Vivier, 1979; Porchet *et al.*, 1982). In the present study the outer membrane of the pellicle shows infoldings which probably are artifacts. Sometimes, the inner membrane complex appears to be connected with the limiting membrane of vesicles which are similar to the ones described by Schrevel (1971).

The number of subpellicular microtubules varies considerably in the different groups of the apicomplexan and it is genus—or species—specific (Scholtyseck, 1979). Although 22-26 microtubules have been described in Eimeriidae merozoites, we have found approximately 50. This feature may be interpreted as an adaptational modification of the parasite.

Among other features highly specific, the sporozoan invasive stages possess two sets of apical organelles which have been described as micronemes and rhoptries. Their origin and function have not been clearly established although both

micronemes and rhoptries are generally regarded as secretory organelles. Porchet-Henneré and Nicolas (1983) suggested that rhoptries represent the extrusomes of other protist, and Steward *et al.* (1985) indicated that the rhoptry material could be involved in the recognition and penetration of the host cells as well as in the motility of the invading parasite. In this study, the endogenous merozoites have two osmiophilic rhoptries as happens in most of eimerian species (Senaud and Cerna, 1969; Scholtyseck and Mehlhorn, 1970; Scholtyseck, 1979). Furthermore, we have seen that rhoptries are directed along a central microtubule inside the conoid, similar to that described in *Eimeria vermiformis* (Adams and Tood, 1984). Two joined intraconoidal microtubules were also reported in other coccidian invasive stages (Porchet-Henneré, 1972; Porchet-Henneré and Nicolas, 1983; Barta and Desser, 1986; Nichols and Chiappino, 1987). It has been suggested that these internal microtubules play an important role in the extension and retraction of the conoid and its associated structures, positioning of rhoptries, and potentiating secretion and invasion (Scholtyseck, 1982; Porchet-Henneré and Nicolas, 1983; Nichols and Chiappino, 1987). On the other hand, we have seen an electron dense vesicle near and above the central microtubule, similar to those described in numerous coccidian merozoites (Scholtyseck and Pierkaski, 1965; Sheffield and Hammond, 1966; Roberts *et al.*, 1970; Dubremetz, 1975). Porchet-Henneré and Nicolas (1983) observed this apical vesicle connected with the two intra-conoidal microtubules. Small apical vesicles were also seen during early stages of the formation of merozoites in *Eimeria vermiformis* (Adams and Todd, 1984).

A prominent feature of the *E.? beauchampi* merozoites is the presence of a homogeneous osmiophilic body resembling those already described in sporozoites and so-called « refractile bodies ». Previous ultrastructural studies differentiated merozoites from sporozoites by the absence of refractile bodies. However, some eimerian merozoites have been reported to possess these structures (Cooley, 1968; Hammond *et al.*, 1970). Furthermore, Sampson and Hammond (1972) in *Eimeria alabamensis*, Pacheco *et al.* (1975) in *E. tenella*, and Lindsay and Blagbun (1984) in *E. deblickei* indicated the presence of refractile bodies in first-generation merozoites developing in cell cultures. The occurrence of these structures in merozoites developed in natural host was also described by Scholtyseck and Ghaffar (1981) in *Eimeria falciformis*. The functional significance of the refractile bodies is not well understood although Vivier (1979) points out that they are proteinic inclusions which may function as a reserve to be used at the beginning of the development of the trophozoite.

The small-spherical osmiophilic particles often observed near the refractile-like body of merozoites here studied may also be considered a reserve of proteinic material.

In common with other member of Sporozoa, the principal glucid reserve of merozoites of *Eimeria? beauchampi* is the paraglycogen granules whose amylopectin nature is well known from the works of Ryley *et al.* (1969) and Shrevel (1970); however, the elaboration process is still not fully understood.

The thick-walled vacuoles which occur near the refractile-like body are similar

to those described in the sporozoan and mainly, in the coccidian (Vivier and Henneré, 1965; Vivier and Petitprez, 1969; Porchet-Henneré, 1972; Porchet-Henneré and Ormieres, 1973; Boulard *et al.*, 1982) where functional relationship with the nucleus has been attributed to these structures.

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