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ULTRASTRUCTURE OF THE "PALETOT" OF EURYTREMA PANCREATICUM DAUGHTER SPOROCYSTS

Z. ŽĎÁRSKÁ

Institute of Parasitology, Czechoslovak Academy of Sciences, České Budějovice, Czechoslovakia

Abstract. A "paletot" formed by amoebocytes of the host — terrestrial snail *Bradybaena lantzi* — was demonstrated by ultrastructural studies of the surface of daughter sporocysts of *Eurytrema pancreaticum*.

The present study deals with the ultrastructure of the nucleated tunica outside the tegument of *Eurytrema pancreaticum* daughter sporocyst. The relationship between *Bradybaena lantzi* and *E. pancreaticum* sporocysts has been described in a previous paper (Žďárská 1979a). Detailed ultrastructural studies showed that the envelope of young *E. pancreaticum* sporocysts is identical with one type of "paletot" described on the surface of sporocysts of the order Plagiorchiata.

MATERIALS AND METHODS

The sporocysts were obtained from a spontaneously infected snail *Bradybaena lantzi* collected in the vicinity of Alma-Ata. The material was fixed for 2 h in 3% glutaraldehyde (G) in 0.1 M cacodylate buffer (pH 7.2) and postfixed for 2 h in 1% OsO_4 (Os), dehydrated through an alcohol series and transferred through acetone to Araldite. Ultrathin sections were stained with uranyl acetate (UAc) and lead citrate (Pb) and examined in a JEM 100B transmission electron microscope.

RESULTS AND DISCUSSION

The tegument of a developing daughter sporocyst localized on the intestinal wall of the snail *B. lantzi* is densely covered with microvilli to which a layer of host amoebocytes adheres. The cellular "paletot" consists of cylindrically arranged cells in some parts (Pl. II, Fig. 2) or of flat cells in others (Pl. I, Figs. 1, 2; Pl. II, Fig. 1). The amoebocyte layer remains best preserved on the narrowed proboscis-like anterior part and of the zone with verrucose formations (Pl. I, Fig. 1; Pl. II, Fig. 1). On the widened posterior part of the sporocyst, this layer disappears rather quickly (for the morphology see Žďárská 1979b).

The snail amoebocytes surrounding the sporocysts have a large nucleus with chromatin clusters concentrated below the nuclear membrane (Pl. I, Figs. 1, 2; Pl. II, Figs. 1, 2). The cytoplasm contains rough endoplasmic reticulum, microtubules, glycogen particles, various types of vacuoles, electron-dense inclusions, mitochondria, and very often a larger number of lipid droplets (Pl. I, Fig. 2; Pl. II, Fig. 2). The pseudopodial processes of the amoebocytes are directed towards the microvillous zone of the sporocyst tegument. Extracellular transversely striated fibrils (Pl. II, Fig. 3) and also single muscle fibres occur between the tegument and amoebocytes. These fibrils and muscle fibres seem to represent the superficial part of the degenerating intestinal wall below which the sporocyst started to develop.

Schell (1961, 1962a, b, 1965) used the term "paletot" for the envelopes surrounding the daughter sporocysts. According to him the "paletot" arises either from the cells

and fibrils of the host connective tissue layer, like in *Glypthelmins quieta* and *Haplometra intestinalis* (Schell 1961, 1962a) or from the amoebocytes (haemocytes) of host, like in *Telorchis bonnerensis* (Schell 1962b). Another group of authors (Dobrovolskii 1969, 1971, Dobrovolskii and Raikhel 1973, Dobrovolskii et. al. 1983, Ginetsinskaya et al. 1976, Ginetsinskaya and Dobrovolskii 1983) dealing with the "paletot" in members of the order Plagiorchiata also confirmed that the paletot was a product of the host and not of the parasite and that it should be regarded as the host's defence against the parasite, i.e. the daughter sporocyst. Of the same opinion was Køie (1987) who considered the cellular layers on the sporocyst surface to be of host origin.

Rather few studies have treated this problem at the ultrastructural level (Dobrovolskii et al. 1983, Køie 1987). Some authors regard at the ultrastructural level the cellular layer on the daughter sporocyst surface as its part (James et al. 1966, James and Bowers 1967, Popiel 1978, Popiel and James 1978a, b) or as a primitive epithelium (Nesterenko 1978). The primitive epithelium on the daughter sporocyst surface is known to disappear before leaving the mother sporocyst (Meuleman et al. 1980). Therefore in the so-called double-layered daughter sporocysts developing outside the mother sporocyst the outer nucleated layer does not originate from the parasite, but from the host, and evidently represents a product of the host's effort to wall off the parasite. In addition to the ultrastructural studies of this simple cellular layer on the sporocyst surface there are also papers concerning multilayered or compact cysts around the daughter sporocysts (Yoshimo 1976, Krupa et al. 1977) which are quite clearly the product of the host's defence mechanism.

In spite of the fact that *E. pancreaticum* sporocysts differ from the other sporocysts of the order Plagiorchiata in that they actively leave their snail host, their early development on the intestinal wall and the reaction of their host are similar to those of the sporocysts not leaving their host. This concerns the sporocysts possessing the amoebocyte paletot. However, the cylindrically arranged amoebocytes and flattened amoebocytes on the surface of *E. pancreaticum* sporocyst were not found to fuse with the tegument surface, as it was described by Dobrovolskii et al. (1983) in the sporocyst paletot of *Xiphidiocercaria* sp. 7, Odening. The amoebocyte pseudopodia of *B. lantzi* penetrate only into the vicinity of sporocyst microvilli. Only rarely there occur amoebocyte pseudopodia surrounding lipid droplets prominating above the tegument surface. The amoebocyte pseudopodia were separated from the lipid droplet only by the apical plasma membrane of tegument (see Pl. I, Fig. 1). At some sites, there were lipid droplets spontaneously releasing from the tegument and others situated freely between the microvillous zone of tegument and paletot. We assume therefore that the lipid droplets accumulated in the amoebocytes are phagocytized lipid droplets released from the sporocyst tegument.

The fact that the host of *E. pancreaticum* sporocysts belongs to terrestrial snails does not influence the form of the paletot. All available papers dealing with the defence reaction against sporocysts have concerned the aquatic snails. No terrestrial snails have yet been studied in this relation.

УЛЬТРАСТРУКТУРА „МАНТИИ„ ДОЧЕРНЕЙ СПОРОЦИСТЫ *EURYTREMA PANCREATICUM*

З. Ждярска

Резюме. На поверхности дочерней спороцисты *Eurytrema pancreaticum* была с помощью электронной микроскопии обнаружена „мантия„ состоящая из амебоцитов хозяина — наземного моллюска *Bradybaena lantzi*.

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Z. Ž., Parazitologický ústav ČSAV,
Branišovská 31,
370 05 České Budějovice, ČSFR

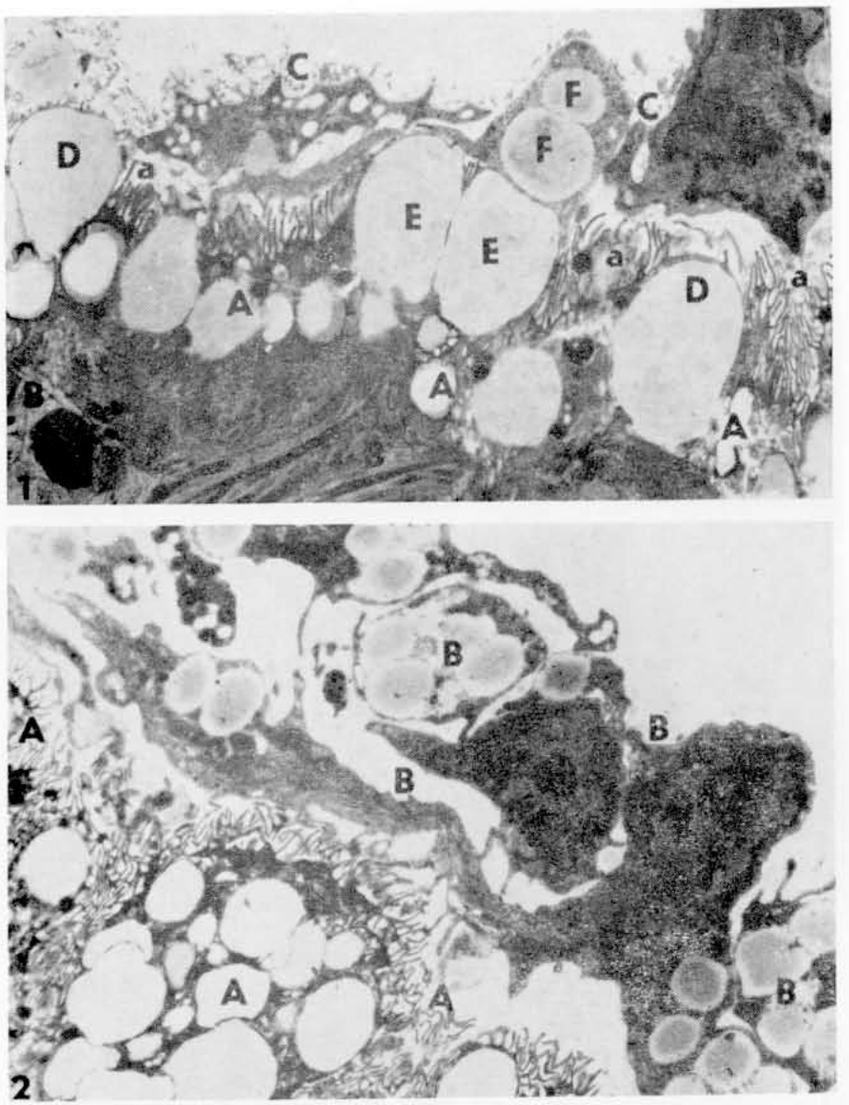

Fig. 1. Tegument (A) of *E. pancreaticum* daughter sporocyst at the site of verrucose formatiors (B) surrounded by amoebocytes (C). The tegument contains large lipid droplets, some of which (D) elevate the apical plasma membrane above the level of microvillous zone of tegument (a). Two of these lipid droplets (E) are surrounded by pseudopodia of an amoebocyte which has already two lipid droplets (F) inside its body. G, OS, UAc, Pb (× 9 400). **Fig. 2.** Oblique section through tegument (A) outside the verrucose formation and paletot of amoebocytes (B) whose cytoplasm contains lipid droplets (C). G, Os, UAc, Pb (× 7 900).

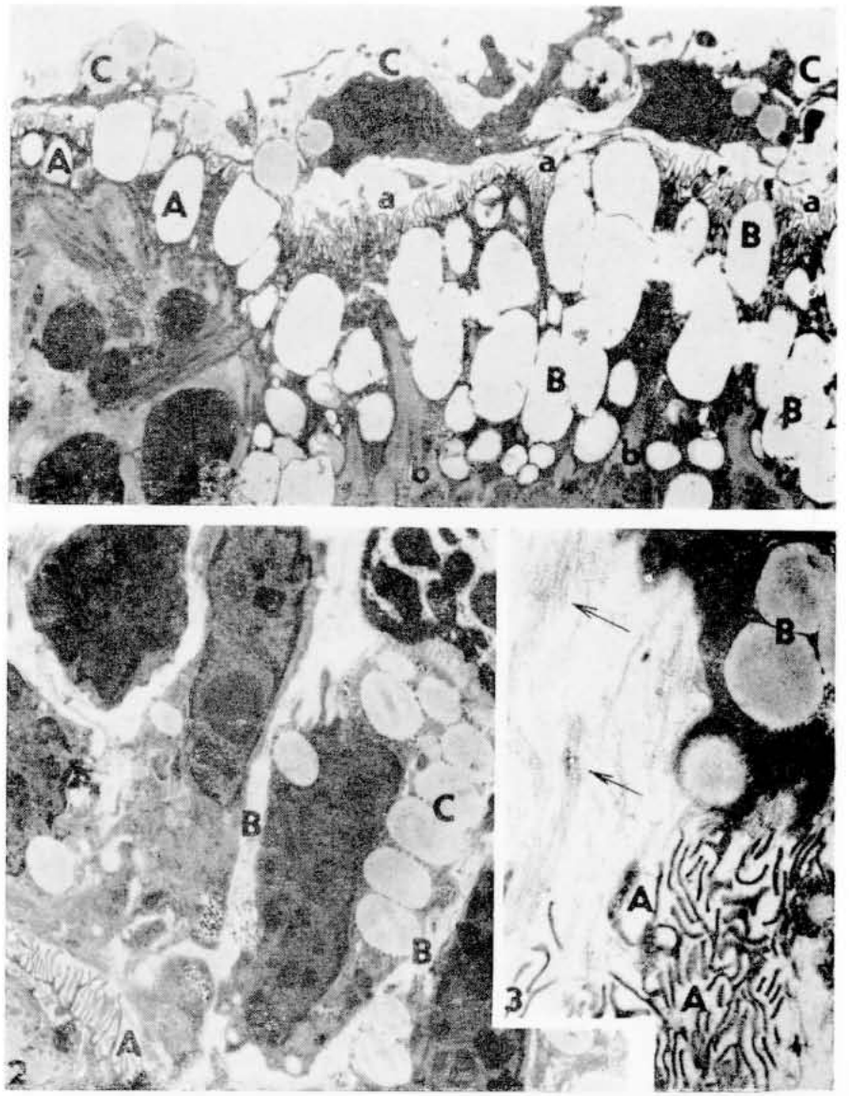

Fig. 1. Thin tegument of the verrucose formation (A) and thick tegument of sporocyst body (B) of *E. pancreaticum* is covered with a row of flattened amoebocytes (C). a — microvillous zone of tegument, b — connective tissue layer, c — circular muscles. G, Os, UAc, Pb (× 7 900). **Fig. 2.** Microvillous zone of sporocyst tegument (A) covered with cylindrically arranged amoebocytes (B). One of them (C) contains a large number of lipid droplets. G, Os, UAc, Pb (× 7 900). **Fig. 3.** Transversely striated fibrils (arrows) between microvillous zone of sporocyst (A) and amoebocyte (B). G, Os, UAc, Pb (× 13 300).