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ULTRASTRUCTURE OF THE CERCARIA AND SPOROCYST OF BRACHYLAIMUS FUSCATUS (RUD., 1819)

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Abstract. The paper deals with the ultrastructure of the developing definitive tegument of *Brachylaimus fuscatus* cercaria. The structure of excretory and digestive systems of cercaria and structure of the sporocyst wall are described in detail. The characters differentiating this species from other members of the superfamily Brachylaimoidea are discussed.

The present work is a part of a complex investigation of the genus *Brachylaimus* and continuation of preceding papers by Žďárská (1983) and Žďárská and Soboleva (1980 a, b, 1984 b). The aim of this study is the elucidation of morphological changes which have occurred during the adaptation of the triheteroxenous life-cycle of these trematodes to the existence under terrestrial conditions.

MATERIAL AND METHODS

The cercariae were obtained from naturally infected snails *Ponsadenia duplocincta* (Martens, 1879) collected in the vicinity of Alma-Ata. Parts of hepatopancreas containing *B. fuscatus* sporocysts were fixed at 4 °C in 3 % glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) for 2 h and postfixed in 1 % OsO_4 for 2h. Then they were dehydrated through alcohol series and embedded through acetone into Epon and Araldit. Ultrathin sections were cut by Reichert OM-U2 ultramicrotome, contrasted with 20 % uranyl acetate and Reynold's solution of lead acetate and examined with JEM 100 B electron microscope.

RESULTS

A. CERCARIA

The whole surface of young developing cercaria is covered with a thin tegument with single microvilli (Plate II, Figs. 1, 2) which disappear during the development. The cytoplasm of tegument contains nuclei with nucleolus (Plate II, Fig. 1, Plate III, Fig. 2), single mitochondria, and parts of rough endoplasmic reticulum, but no rod-shaped, electron-dense granules. The muscle layer under lamina basalis is not yet developed.

The tegument of a fully developed cercaria (Plate III, Fig. 1) is slightly folded and covered with glycocalyx. Its basal plasmalemma forms numerous invaginations reaching 1/6 of tegument height near the lamina basalis. The tegument cytoplasm contains mostly light vacuoles, rod-shaped, electron-dense granules arranged perpendicularly to the surface, and single mitochondria. Well developed circular and longitudinal muscles are situated under lamina basalis (Plate III, Fig. 1) and below them are the bodies of subtegumental cells containing rod-shaped, electron-dense granu-

les, light vacuoles and Golgi complex. The nuclei of these cells contain finely dispersed chromatin.

The tegument contains bulbous, sensitive, aciliary receptors (Plate IV, Fig. 1), which do not communicate with its surface. They are surrounded by a plasmalemma which is connected with the plasmalemma of tegument by a desmosome. The apical part of the receptor is reinforced with a strongly electron-dense, disc-shaped structure under the plasmalemma. A modified ciliary rootlet is present under this structure.

The excretory system of cercaria consists of typical terminal flame cells and tubules. Numerous lamellae project into the lumen of larger tubules ducts and collecting ducts (Plate IV, Fig. 2, Plate V, Figs. 1, 2, Plate VI, Fig. 1). Larger ducts contain single lateral (non-terminal) flames (Plate IV, Fig. 2) with typical ciliae. These excretory ducts consist of a single cell spirally wrapped around the lumen and joined to itself by a desmosome (Plate IV, Fig. 2, Plate V, Fig. 1). Their cytoplasm contains basal bodies of cilia, mitochondria, microtubules, and ribosomes. The flame cells, tubules and ducts are surrounded by connective tissue layer at the surface.

The digestive system of cercaria up to the pharynx is covered with syncytial tegument. The intestinal branches (Plate III, Fig. 3) are lined with cells with long, thin microvilli at the sides turned into the lumen. The basal parts of cells are surrounded by lamina basalis under which a layer of longitudinal and circular muscles is situated.

Two types of gland cells — proteinaceous and mucinous — can be differentiated at ultrastructural level on the basis of the type of secretion. The proteinaceous gland cells are localized in the anterior part of body and contain electron dense granules of irregular shape (Plate VI, Fig. 1). The mucinous secretion is contained in postacetabular gland cells, which are localized around the ventral sucker (Plate VI, Fig. 2). The gland apparatus of this cercaria will be dealt with in more detail in a separate paper.

B. SPOROCYST

The body surface of sporocyst is covered with a tegument with a large number of thin microvilli, often surrounded by amoebocytes of the host (Plate I, Fig. 1). The tegument (Plate I, Fig. 2) on the outer side, even on lamina basalis, is bordered by trilaminal unit membrane. The cytoplasm of tegument contains numerous mitochondria, light vacuoles, and single membranous bodies. A thin lamina basalis covering a thin layer of circular and longitudinal muscle fibres is situated under the tegument. The subtegumental cells contain a nucleus with finely dispersed chromatine, a large number of mitochondria, rough endoplasmic reticulum, and Golgi apparatus. They are connected with the tegument through processes. The parenchymal cells have a large nucleus with finely dispersed chromatine, and the cytoplasm contains a large number of α- and β-particles of glycogen and single lipid vacuoles.

DISCUSSION

The tegument in both developing and fully formed cercariae of *B. fuscatus* has the same structure as that of *B. aequans* cercaria (Žďárská 1983). It is covered with a thick glycocalyx protecting the cercaria against the dehydration at the time when it freely moves on the land searching for the second intermediate host. Like in *B. aequans* and *Hasstilesia ovis* (Žďárská and Soboleva 1982), the calcium salts do not deposit in the glycocalyx of *B. fuscatus* cercaria.

The body, like in *B. aequans* and *Postharmostomum gallinum* cercariae (Žďárská and Soboleva 1985), is first covered with a primitive epithelium which disappears as soon as the definitive tegument starts to develop. At the beginning, the nucleated syncytial definitive tegument is low and single microvilli project from it. These microvilli later disappear, the glycocalyx develops, and electron dense, rod-shaped granules produced by the subtegumental cells start to appear in the tegument cytoplasm. Our findings of primitive epithelium and developing definitive tegument in the cercaria of *B. fuscatus* corroborate those of Meuleman and Holzmann (1975) in the cercaria of *Schistosoma mansoni*.

The cercaria of *B. fuscatus* contains in the tegument the same aciliated sensory endings as the cercaria of *Leucochloridium perturbatum* (Žďárská and Soboleva 1984), namely the type without basal body, but with a modified rootlet, very similar to one of the three types of receptors described by Allison (1980) in *Gyrocotyle rugosa* and to aciliated sensory ending described by Fujino et al. (1979) in *Clonorchis sinensis*.

The ultrastructure of the excretory system in *B. fuscatus* cercaria corresponds to the general scheme of excretory system in trematodes, as reported by Gallagher and Threadgold (1967), Wilson (1969), Bennett (1977), and Smyth and Halton (1983). It begins with a terminal flame cell, which continues by a thin tubule with microvilli consisting of a single cell spirally wrapped around the lumen and joined to itself by a desmosome. Larger tubules and ducts possess numerous lamellae in their lumen. At some sites, lateral flames (non-terminal) project into larger ducts and transport the fluid towards the excretory bladder. Even these ducts consist of one cell the cytoplasm of which contains basal bodies of cilia, mitochondria, microtubules, and ribosomes. The cell with lateral flame is spirally wrapped and joined to itself by a desmosome. Similar cells were described by Rohde (1971) in *Multicotyle purvisi*. The excretory bladder is covered with a tegument which is substantially identical with the body tegument. The same was observed in other cercariae by Powel (1972, 1977), Gibson (1973) and Popiel (1977).

The digestive system of *B. fuscatus* cercaria, like that of other members of Brachylaimoidea (Žďárská et al. 1982, Žďárská 1983, Žďárská and Soboleva 1984 a), is lined with the tegument from the oral sucker up to the pharynx and the intestine is lined with cells with a large number of long, thin microvilli.

The structure of the sporocyst is identical in the basic features with that of the branched sporocyst of *Postharmostomum gallinum* (Žďárská and Soboleva 1985), but substantially differs from the branched sporocysts of other members of Brachylaimoidea. It differs from the sporocysts of *Leucochloridium* in the structure of muscle and parenchyma layers (the muscles are less developed and the parenchymal part does not contain any pigment cells - Žďárská et al. 1982, Žďárská and Soboleva 1984 a), and from the sporocyst of *Hasstilesia ovis* in more developed muscles and in the structure of tegument. In *H. ovis* sporocysts, the tegument does not form microvilli, but a special lacunal system is developed in it (Žďárská and Soboleva 1982).

All morphological changes in *B. fuscatus* cercaria, like in all other previously studied cercariae of Brachylaimoidea, lead in general to the decrease of dehydration in the terrestrial environment and to the reduction of the organ of movement — the tail. In addition, there occur some special changes, which are specific for the life-cycles of triheteroxenous or secondarily diheteroxenous type.

УЛЬТРАСТРУКТУРА ЦЕРКАРИИ И СПОРОЦИСТЫ *BRACHYLAIMUS FUSCATUS* (RUD., 1819)

З. Ждярска и Т. Н. Соболева

Резюме. В работе описана ультраструктура развивающегося дефинитивного тегумента церкарии *Brachylaimus fuscatus*. Подробно описаны также структура экскреторной и пищеварительной систем церкарии и структура стенки спороцисты. Обсуждаются отличительные признаки *B. fuscatus* по сравнению с другими видами надсемейства Brachylaimoidea.

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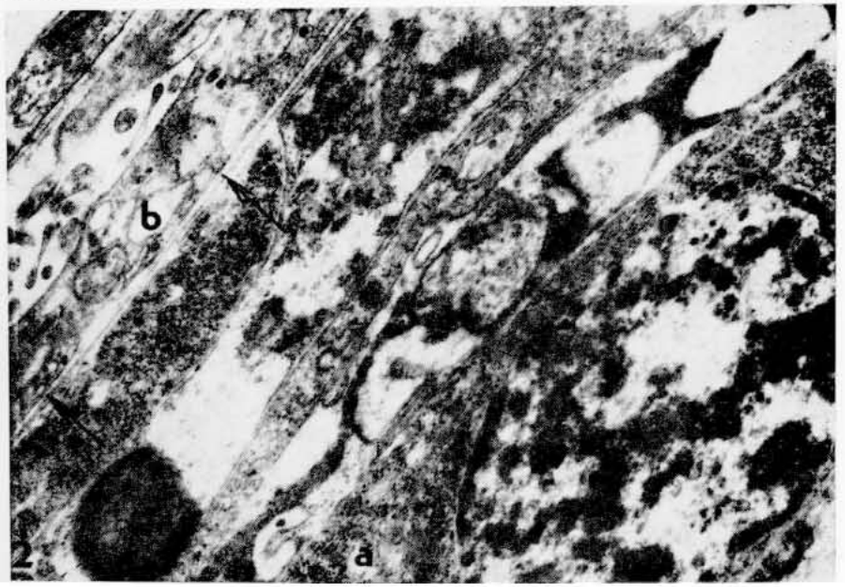
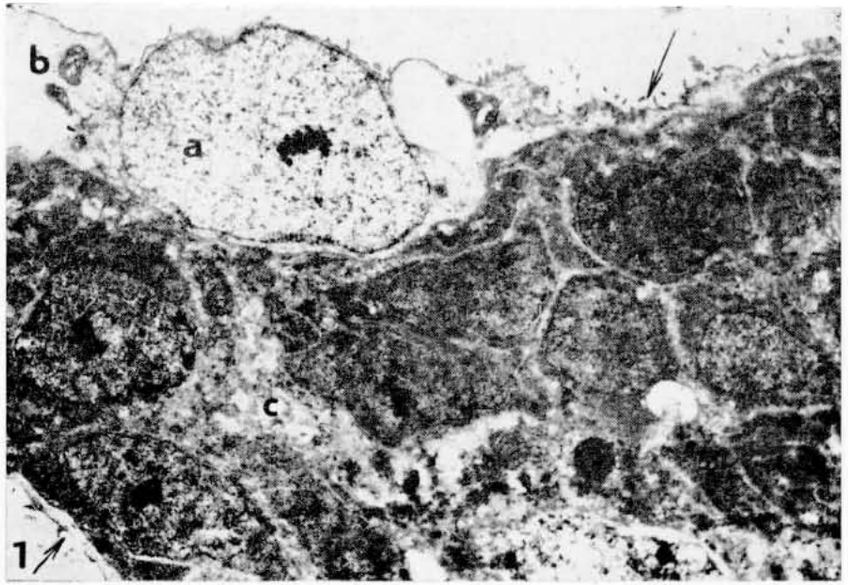


Fig. 1. Horizontal section through microvillous zone (a) of tegument of *B. fuscatus* sporocyst surrounded by host amoebocytes (b). c — tegument. G. Os, UAc, Pb (×10 800). **Fig. 2.** Longitudinal section through dilated sac wall of *B. fuscatus* sporocyst with degenerating parenchymal cell (a) bordering the sporocyst cavity. b — tegument, arrows — thin lamina basalis. G, Os, UAc, Pb (×22 500).



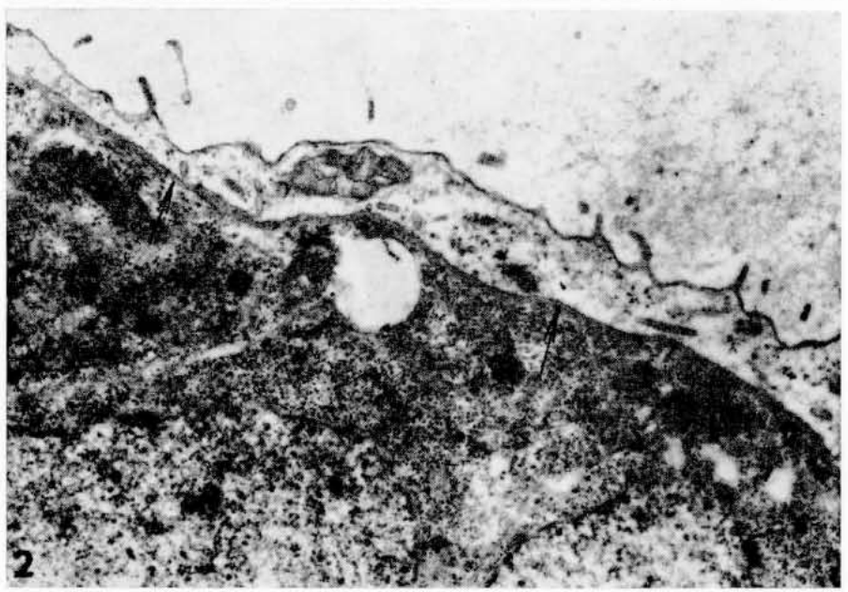


Fig. 1. Horizontal section through posterior part of body of *B. fuscatus* cercaria at the beginning of development of the definitive tegument. Nucleus with nucleolus (a) and mitochondria (b) are well visible in the horizontal section (left at the top) and short microvilli (arrows) in the perpendicular section (right at the top and left at the bottom) through the tegument. c — posterior end of cercaria body. G, Os, UAc, Pb (×10 000). **Fig. 2.** Detail of developing definitive tegument (at the top) with single short microvilli on the surface and thin lamina basalis (arrows) under the tegument; the muscle layer below it is not yet developed. G, Os, UAc, Pb (×18 000).

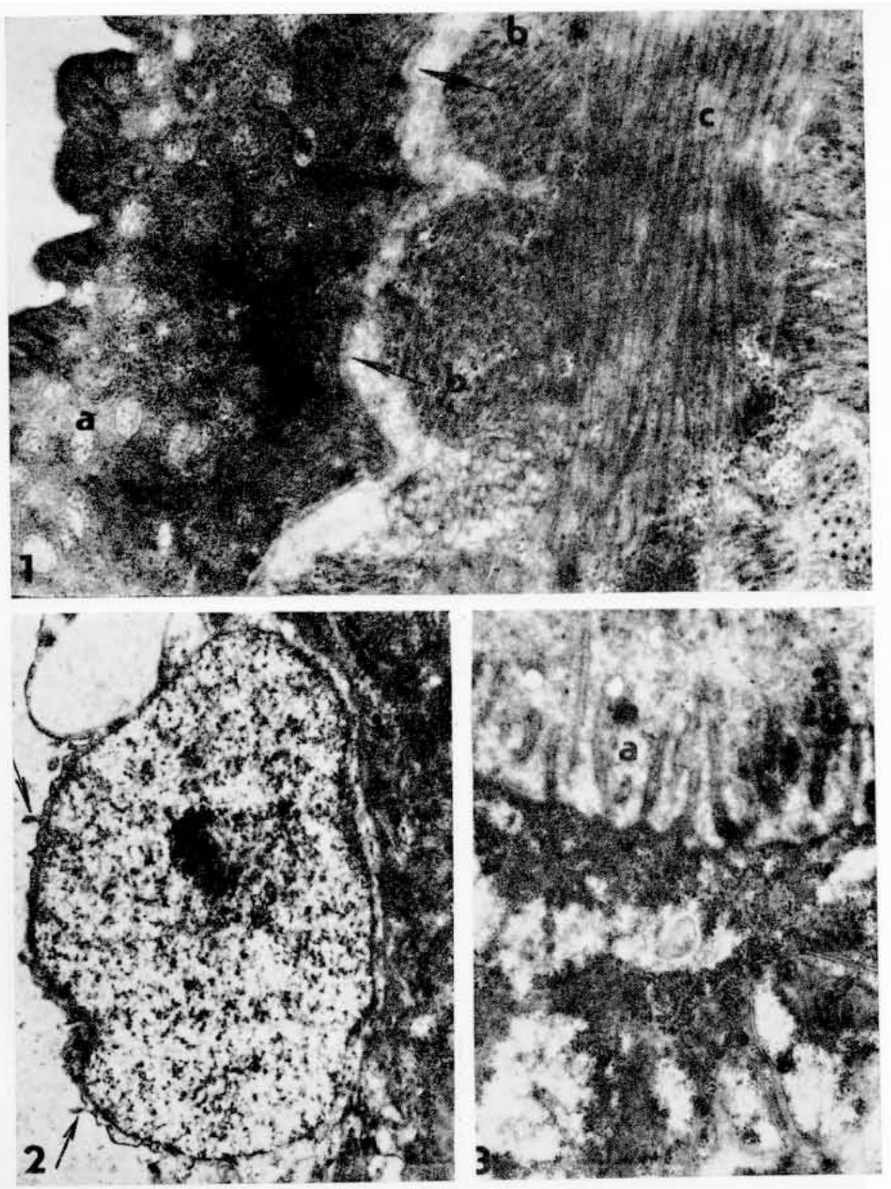


Fig. 1. Fully developed definitive tegument (at the left) of *B. fuscatus* cercaria. Numerous light vacuoles are visible in the tegument (a) and electron-dense rod-shaped granules orientated perpendicularly to the surface are situated immediately below the outer plasmalemma. The basal plasmalemma of tegument forms numerous invaginations. Thick lamina basalis (arrows) is visible between the tegument and circular muscles (b). c — longitudinal muscles. G, Os, UAc, Pb (×27 000). **Fig. 2.** Detail from Plate II, Fig. 1. The nucleus in the syncytial layer of tegument is bordered by a narrow band of cytoplasm with single microvilli (arrows). G, Os, UAc, Pb (×12 250). **Fig. 3.** Transverse section through the wall of developing intestine of *B. fuscatus* cercaria. Numerous thin microvilli project from the apical part of intestinal cell (a). G, Os, UAc, Pb (×14 400).

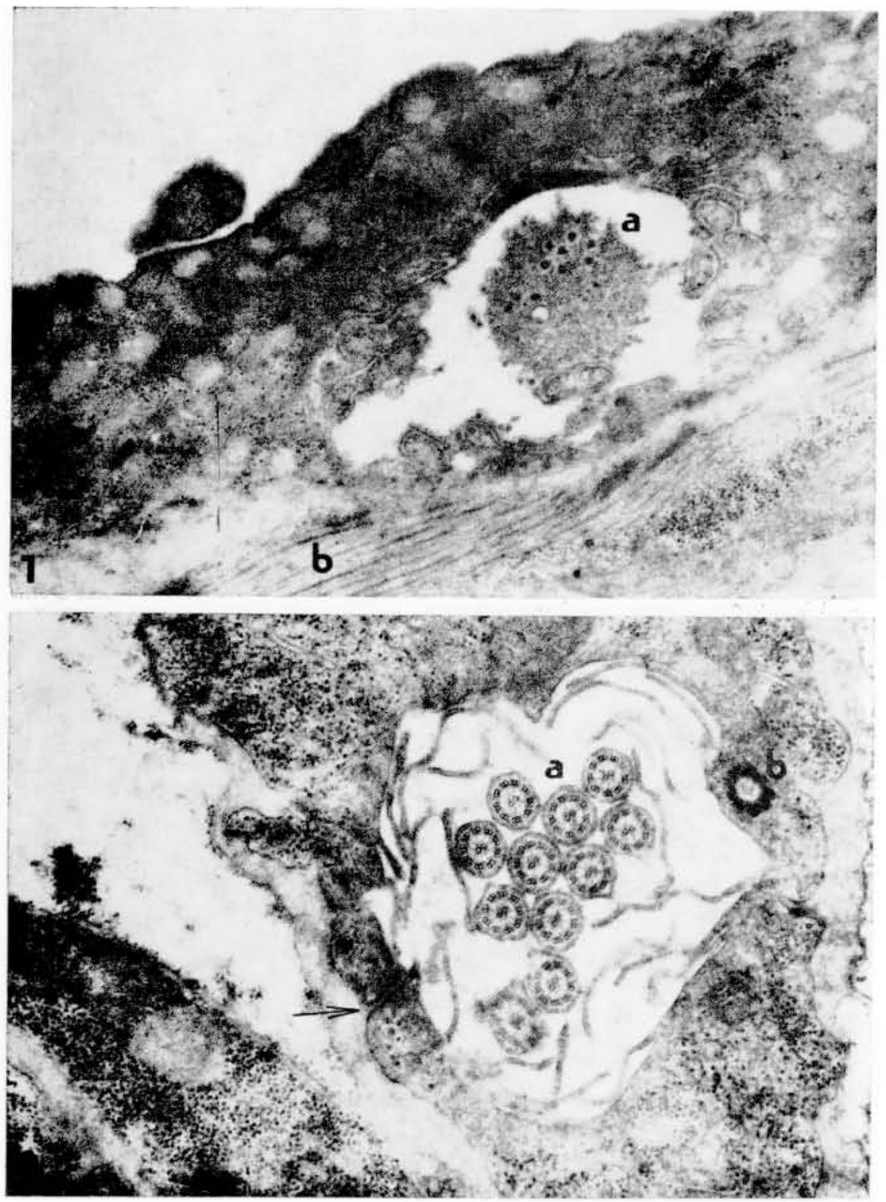


Fig. 1. Bulbous sensory ending (a) in the tegument of *B. fuscatus* cercaria. b — circular muscles. G, Os, UAc, Pb (×22 500).
Fig. 2. Transverse section through excretory tubule with lateral flame (a) in *B. fuscatus* cercaria. Basal body of a cilium (b), mitochondria and microtubules are visible in the tubule wall. The lumen is bordered by numerous laminae. The cell forming the excretory tubule is joined to itself by a desmosome (arrow) and surrounded by a fibrous layer (b). G, Os, UAc, Pb (×27 000).

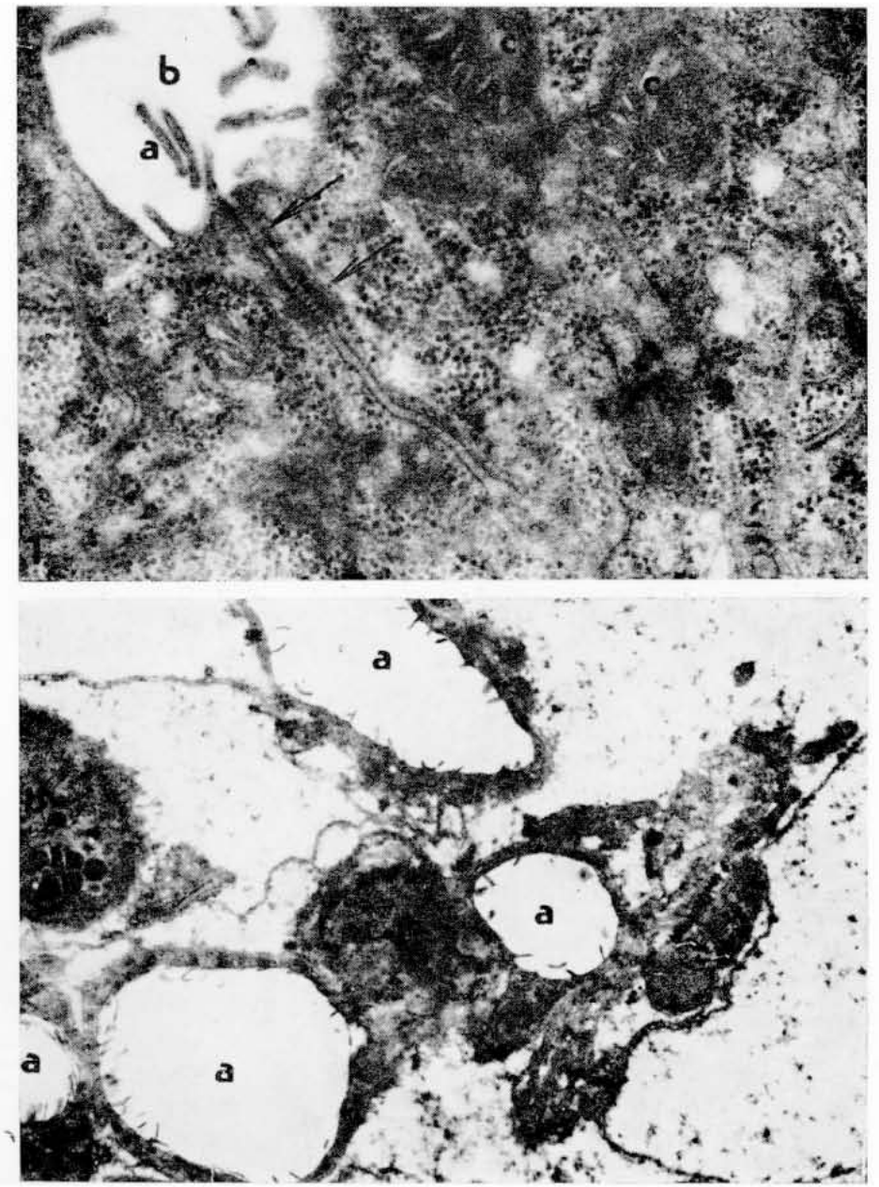


Fig. 1. Detail of the desmosome (arrows) connecting the spirally wrapped cell which forms the excretory tubule (b) in *B. fuscatus* cercaria. Numerous laminae (a) project from the surface of the cell forming the lumen. The cytoplasm contains mitochondria (c) and a large number of ribosomes. G, Os, UAc, Pb (×45 000).
Fig. 2. Transverse section through larger excretory ducts (a) with laminae. b — part of gland cell with protein granules. G, Os, UAc, Pb (×10 800).

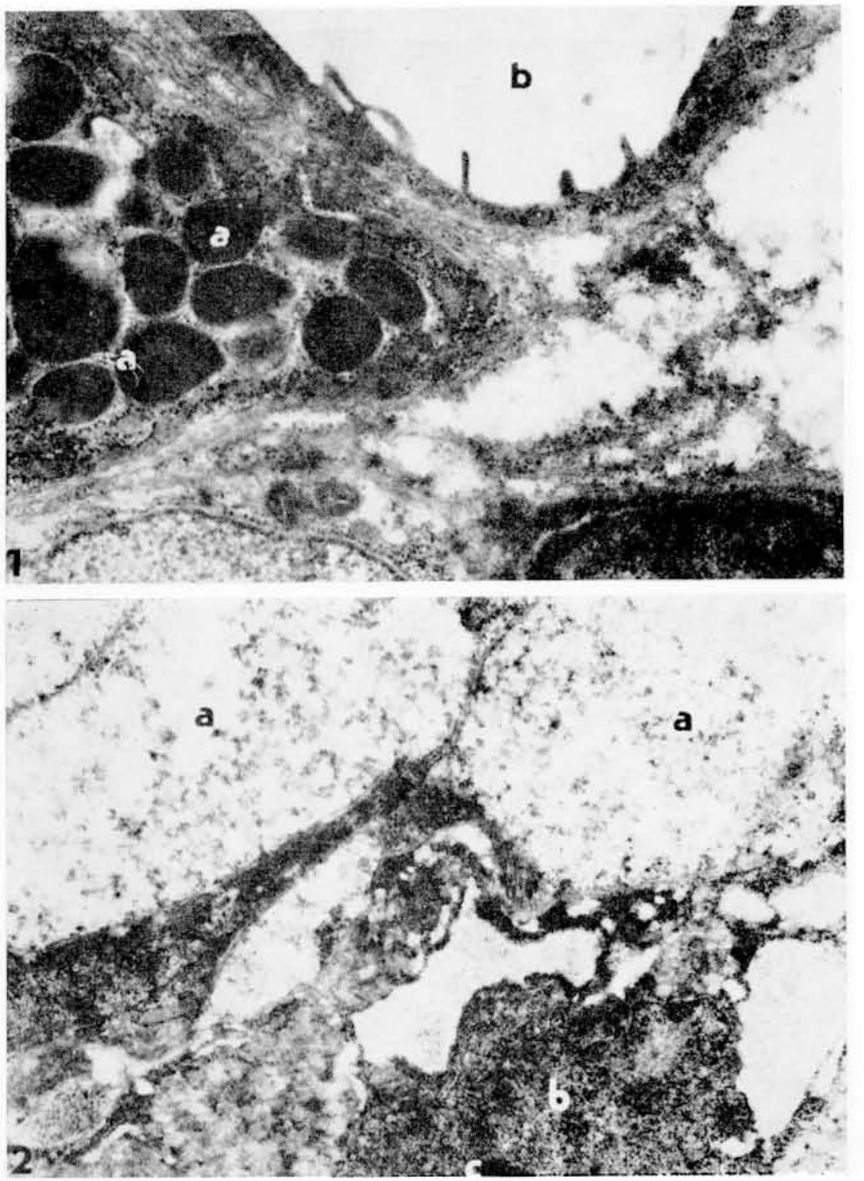


Fig. 1. Section through a part of gland cell with protein granules (a) and excretory duct in *B. fuscatus* cercaria. G, Os, UAc, Pb (×15 300). **Fig. 2.** Section through mucinous gland cells (a) in *B. fuscatus* cercaria. b — nucleus, c — nucleolus. G, Os, UAc, Pb (×15 300).