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ULTRASTRUCTURE OF THE EXCRETORY SYSTEM OF BRACHYLAIMUS AEQUANS (TREMATODA: BRACHYLAIMOIDEA)

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Abstract. The ultrastructure of the flame cell, excretory capillaries, ducts, collecting ducts, excretory bladder, and excretory pore of *Brachylaimus aequans* was studied 6—8 days p.i. The excretory ducts, collecting ducts and excretory bladder are provided with numerous lamellae on the luminal side. The cilia of lateral flames in the excretory ducts have a triated rootlet. The excretory pore is covered by a tegument identical with the body tegument. The syncytium of the excretory bladder is connected with the tegument of the excretory pore by means of a septate desmosome. No lipid or excretory corpuscles have been demonstrated in the excretory system.

The present study is a continuation of a previous paper dealing with the ultrastructure of the digestive system of this trematode (Žďárská et al. 1988). Its aim was to provide a deeper knowledge of the functional morphology of trematodes adapted at all their developmental stages to the existence in terrestrial animals.

MATERIALS AND METHODS

Adult specimens of *Brachylaimus aequans* (Loose, 1899) at the age of 6—8 days were obtained from experimentally infected laboratory mice with metacercariae from the molluscs *Macrochlamys schmidti*, originating from the vicinity of Alma-Ata. The trematodes were released from the host intestine, washed with saline and fixed in 3 % glutaraldehyde on 0.1 M cacodylate buffer (pH 7.2) for 2 h at 4 °C, and then postfixed in 1 % OsO_4 for 2 h at 4 °C. After dehydration through alcohol series the material was embedded into Durcupan through acetone. Ultrathin sections cut with Reichert's Om U2 ultramicrotome were contrasted by 20 % uranyl acetate and Reynold's solution of lead citrate and examined in Philips EM 420 and Philips EM 300 electron microscopes.

RESULTS

1. The flame cell (Pl. I, Fig. 2) comprises a cup cell, the cytoplasm of which is concentrated mostly in the proximal part, whereas in the distal part, only a thin rim of the cytoplasm surrounds the cavity in which a bundle of cilia (the "flame") is situated. In the proximal part of the cell, there is a nucleus with chromatin concentrated below the nuclear membrane, granular endoplasmic reticulum, mitochondria, and electron-lucid vesicles. The plasma membrane in this part of cell forms gap junctions with parenchymal cells. The thin rim of cytoplasm surrounding the basal part of the flame projects into processes on the inner side. These processes resemble the microvilli and are termed internal leptotriches (Pl. I, Fig. 1). The most distal part of this rim does not form a coherent layer, but protrudes into internal ribs between which fit the external ribs (Pl. I, Fig. 1) formed by the cytoplasm of the first cell of the excretory capillary. The cilia of the flame are anchored by means of basal bodies and short rootlets in the distal part of the cytoplasm near the nucleus. The cilia have a typical

structure of 9 + 2 (Pl. II, Fig. 2). The proximal ribbed part of the first cell of excretory capillary is attached to the distal ribbed part of the flame cell.

2. **The excretory capillary** (Pl. I, Fig. 2) consists of spirally wrapped cells. Each cell forms an extracellular duct coiling spirally around the lumen. The margins of cells are connected with one another by a dosmosome and interdigitations (Pl. I, Fig. 2). The first cell of the excretory capillary is widened in form of a cup and its proximal end protrudes into the external ribs which fit between the internal ribs of the flame cell. The ribs are interconnected by a fine fibrous layer. This connection is well visible in the transverse sections through this part (Pl. I, Fig. 1). No pores have been seen between the ribs. The widened proximal part of the first excretory capillary projects, in addition to external ribs, also into microvillus-like external leptotriches anchored in the intersticial material. The cytoplasm of the cells of excretory capillaries contains numerous mitochondria, granular endoplasmic reticulum, and electron-lucid vesicles. The chromatin in the nucleus is situated below the nuclear membrane and contains a nucleolus. The excretory capillaries open into wider excretory ducts.

3. **The excretory ducts** (Pl. II, Figs. 1, 3, Pl. III, Figs. 1, 2) consist of flat epithelial cells, with numerous long lamellae protruding into the lumen (Pl. III, Figs. 1, 3). The cells are interconnected by septate desmosomes (Pl. III, Fig. 2). The cytoplasm contains mitochondria and electron-lucid vesicles. Single lateral flames consisting of long cilia are situated in excretory ducts. In the cytoplasm of cells forming the lateral flames, the cilia are anchored by means of short striated rootlets and basal bodies (Pl. III, Fig. 1). The lamellae are lacking at the site where the lateral flame is situated, but they surround it on its whole periphery. The lateral flames serve for the flow of the filtrate to the excretory bladder. The epithelium of excretory ducts lies on a thin lamina basalis. The basal plasma membrane does not form invaginations in these ducts. The excretory ducts are covered by a layer of connective tissue. Processes of parenchymal cells penetrate this layer and lamina basalis and form gap junction with the epithelial cells of the excretory ducts (Pl. II, Fig. 3).

4. **The collecting excretory ducts** represent the last but one part of the excretory system of this trematode. They have the form of two long, wide ducts running along the trematode body from the ventral sucker up to the excretory bladder. Their syncytial wall forms numerous long lamellae protruding into the lumen. These lamellae are identical with the lamellae in smaller excretory ducts. The syncytium of the collecting excretory ducts is surrounded by lamina basalis, connective tissue layer, and thin muscle layer. The processes of parenchymal cells penetrate through basalis and their plasma membrane forms gap junctions with the plasma membrane of the syncytium. The cytoplasm contains single mitochondria and electron-lucid vesicles.

5. **The excretory bladder** (Pl. IV, Figs. 1, 2) has essentially the same structure as the collecting excretory ducts. Its syncytium is thicker than that of collecting excretory ducts and its basal plasma membrane forms numerous invaginations. The apical part of syncytium cytoplasm forms numerous lamellae, which are shorter than that in the collecting excretory ducts. The syncytium cytoplasm of the excretory bladder contain numerous mitochondria, electron-lucid vesicles and single nuclei. No lipid droplets or excretory corpuscles have been observed in it. Below the syncytium lies lamina basalis and below it a well-developed muscle layer (Pl. IV, Figs. 1, 2). The plasma membrane of the syncytium and the plasma membrane of tegument are interconnected by a septate desmosome at the site where the excretory pore begins (Pl. IV, Fig. 2).

6. **The excretory pore** (Pl. IV, Figs. 1, 3) is lined with a tegument essentially identical with the body tegument. It differs from the body tegument only in a smaller

number of dense rod-shaped granules. In addition to these granules, the teguments contain also electron-lucid vesicles, granular endoplasmic reticulum, and mitochondria. The apical part of the syncytium forms high folds and the basal plasma membrane has numerous invaginations. Below the tegument lies lamina basalis and below it, a thick muscle layer. The tegument of the excretory pore is connected by a septate desmosome to the syncytium of the excretory bladder.

DISCUSSION

The flame cells of a *B. aequans* adult have a typical structure, which occurs in both larvae and adults of trematodes (Senf et al. 1961, Reisinger and Graack 1962, Pantelouris and Threadgold 1963, Wilson 1969, Rohde 1971, 1972). The cilia of the flame are anchored by means of basal bodies and short rootlets into the cytoplasm of the flame cell. The flame cell forms a rim around the flame which is connected by the internal ribs to the first cell of the excretory capillary. The proximal end of the capillary projects into the external ribs behind which the external leptotriches project. The internal and external ribs are connected by a thin fibrous layer in which no pores were demonstrated. On the luminal side, the cytoplasmic rim of the flame cell projects into microvillus-like internal leptotriches. The flame cell is separated from the surrounding parenchymal cells by a layer of interstitial material. This layer is lacking only at the sites of gap junction. Bennett (1977) described a pinocytotic activity in the flame cells of *Fasciola hepatica*. However, this activity was not demonstrated by us in the flame cell of *B. aequans*, but it was evidently present in the excretory capillaries and ducts.

The apical processes of ciliary membranes, which have been demonstrated by Rohde (1971, 1972) only in Aspidogastrea, are not developed in the flame cells of *B. aequans* or other parasitic platyhelminthes — Digenea, Monogenea, and Cestoda. Like those of Digenea, Aspidogastrea, and Monogenea (but not Cestoda), the flame cells of *B. aequans* possess the external leptotriches.

The excretory capillaries are composed of cells spirally wrapped around a lumen which is therefore extracellular. No lamina basalis is developed. The cells of the excretory capillary are surrounded by interstitial material. Single microvilli are situated on the luminal side of these cells, like in *Fasciola hepatica* (Bennet 1977).

The lateral flames in larger excretory ducts of *B. aequans* differ from hitherto described lateral flames of Digenea (Bennet 1977), Aspidogastrea, and Monogenea (Rohde 1971, 1972, 1973) in that their cilia have a striated rootlet. According to Bennet (1977) the lateral flames are significant only in the metacercaria, where they pump the liquid toward the excretory bladder. The author does not consider them to be important in the adult specimens. In our opinion, the lateral flames are equally important in both adults and metacercaria. The accumulation of lipid droplets recorded in other trematodes (Bennet 1977, Smyth and Halton 1983) was not observed in collecting excretory ducts and excretory bladder of *B. aequans*. The excretion of lipids from the exretory bladder has been observed in *Fasciola hepatica* (Burren et al. 1967). *Cyathocotyle bushiensis* (1967), and *Echinostoma revolutum* (Fried and Appel 1977). Although Smyth and Halton (1983) state that the characteristic inclusion in the excretory system of Digenea are lipid droplets, we have not managed to demonstrate them in *B. aequans*, either histochemically (Žďárská and Soboleva 1987) or in ultrastructural studies. We assume that in this trematode the lipid droplets are removed from the body only through the digestive tract, by the caecal syncytium, and the pharynx tegument (Žďárská et al. 1988), in which an accumulation of lipids has been demonstrated. The fate of lipid droplets excreted into

the lumen of the digestive system remains uncleared. The lipids excreted by the excretory system leave the body of the trematode. Lipid droplets are considered to be the waste products, which in the form of molecules passed through the parenchymal cells into the syncytium of the excretory or digestive system, either through the gap junctions or through the interstitial material.

Not even the excretory corpuscles, demonstrated in adults of *Podocotyle staffordi* and *Hemiurus ocreatus* (Gibson 1973) and in very young adults of *Cyathocotyle bushiensis* (Erasmus 1967, 1972) and *Fasciola hepatica* (Bennett 1977), could be demonstrated in the excretory bladder of *B. aequans*.

Our observations showed that the lining of the excretory bladder is distinctly separated from that of the excretory pore. The plasma membrane of the excretory bladder syncytium is connected by a septate desmosome to the plasma membrane of the excretory pore tegument.

The epithelium of excretory ducts and syncytium of collecting ducts are connected with parenchymal cells by gap junctions. In this way a potential circulating system arises (Gallagher and Threadgold 1967). Numerous lamellae of the epithelium of excretory ducts and syncytium of collecting ducts and excretory bladder protruding into the lumen enlarge the resorption area (like the microvilli in the digestive system).

It may be concluded that the excretory system of *B. aequans* differs significantly from the excretory system of hitherto studied trematodes in that no lipid droplets have been found in its wall or lumen. Lipid droplets are excreted only in the digestive system in this species (see Žďárská et al. 1988).

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УЛЬТРАСТРУКТУРА ВЫДЕЛИТЕЛЬНОЙ СИСТЕМЫ *BRACHYLAIMUS AEQUANS* (TREMATODA: BRACHYLAIMOIDEA)

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Резюме. Изучали ультраструктуру мерцательной клетки, капилляров, протоков, собирательных каналов, экскреторного пузыря и экскреторной поры трематоды *Brachylaimus aequans* на 6—8 день после заражения. Экскреторные протоки, собирательные каналы и экскреторный пузырь снабжены многочисленными ламеллями на стороне просвета. Жгутики латеральных пучков в экскреторных протоках имеют исчерченный корень. Экскреторная пора покрыта тегументом, который соответствует тегументу тела. Синцитий экскреторного пузыря соединяется с тегументом экскерторной поры через септированную десмосому. В экскреторной системе не были обнаружены ни липидные капельки ни экскреторные тельца.

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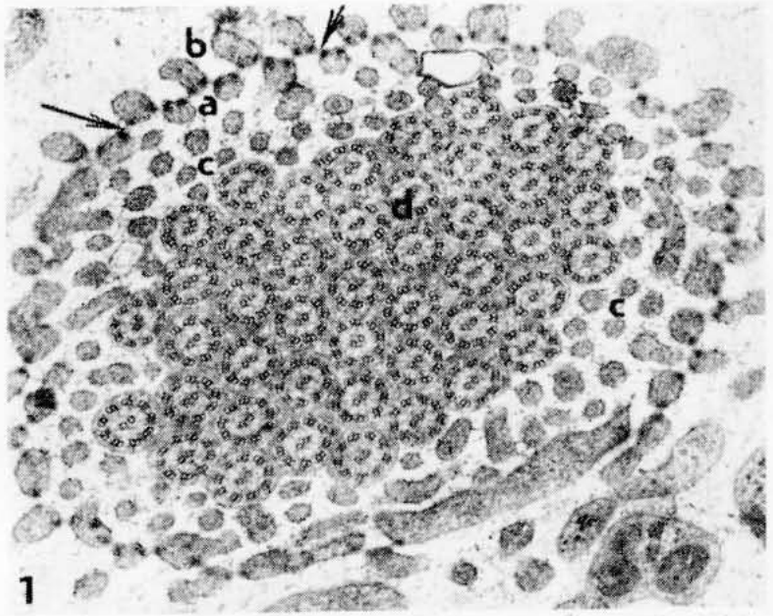


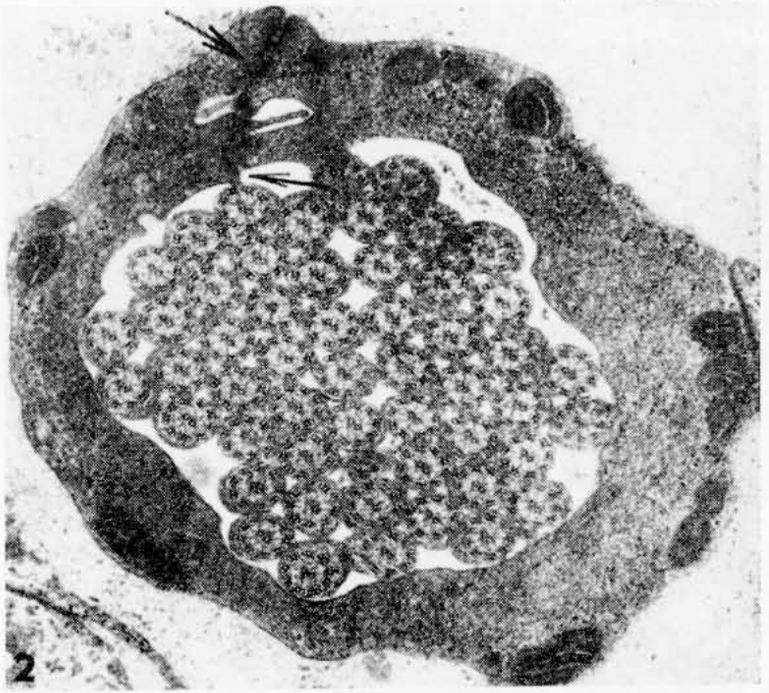
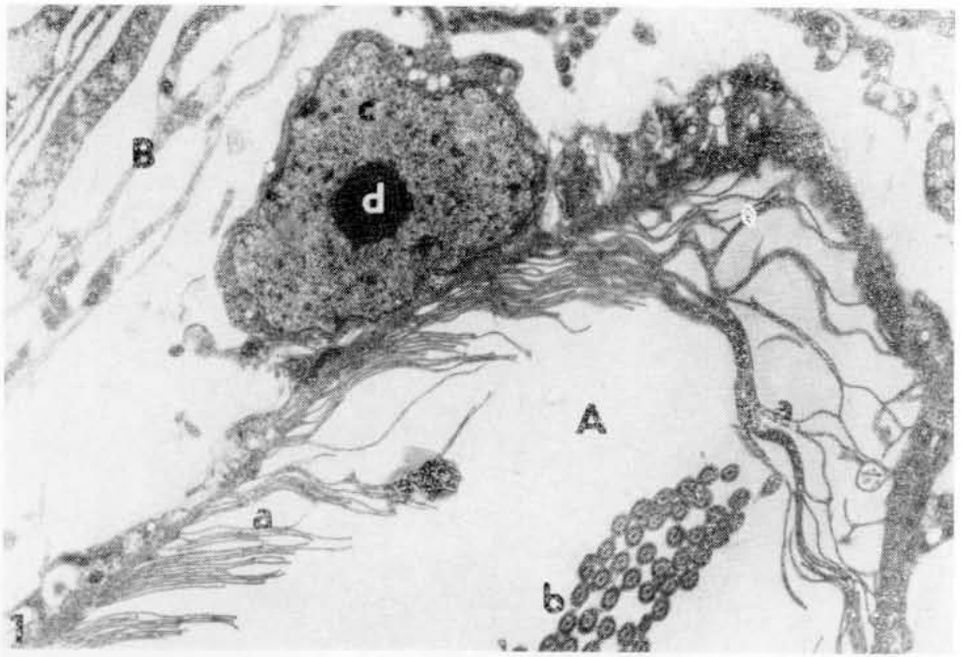
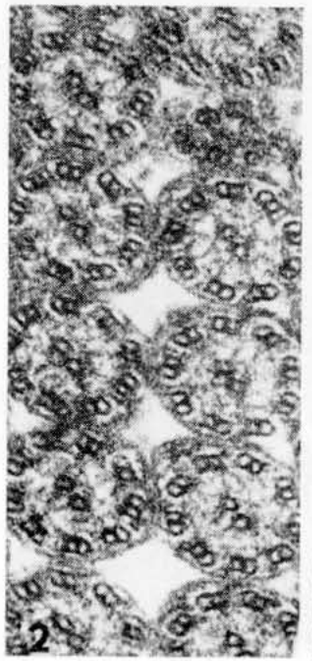


Fig. 1. Transverse section through the flame cell of *B. aequans* at the level of ribs. a — internal ribs, b — external ribs, c — internal leptotriches, d — cilia, arrows — fibrous layer (G, Os, UAc, Pb) (× 42 300). **Fig. 2.** Transverse section through the first cell of the excretory capillary at the level of distal end of flame. Arrows — septate desmosome and interdigitation (G, Os, UAc, Pb) (× 35 000).





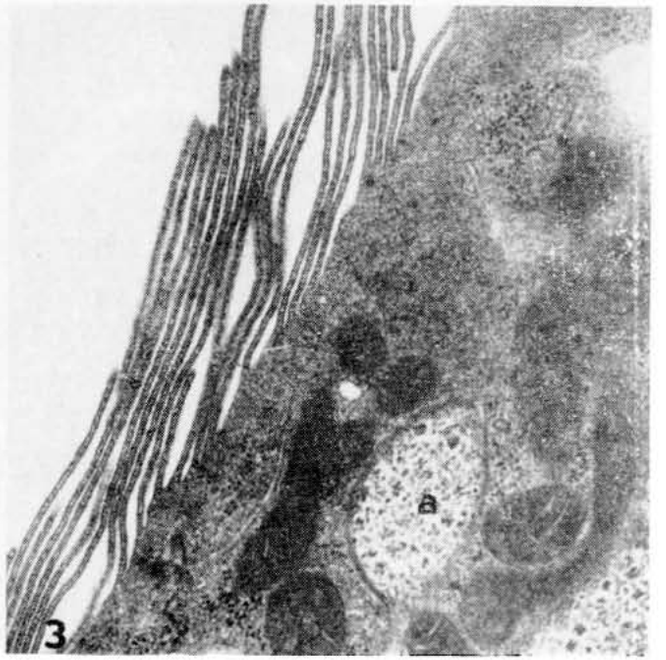
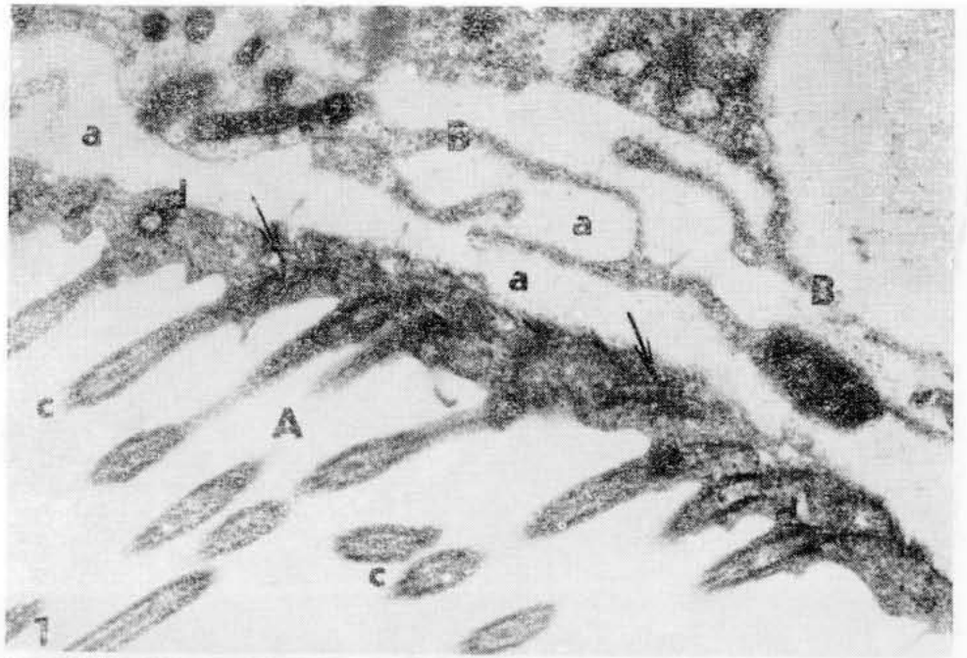


Fig. 1. Transverse section through the excretory duct (A) of *B. aequans*. B — processes of parenchymal cells, a — lamellae, b — cilia of lateral flame, c — nucleus, d — nucleolus (G, Os, UAc, Pb) (× 11 500). **Fig. 2.** Detail of cilia of flame cell (× 88 800). **Fig. 3.** Detail of lamellae and cytoplasm of epithelial cell of the excretory duct with gap junction (a) of parenchymal cell (G, Os, UAc, Pb) (× 34 350).



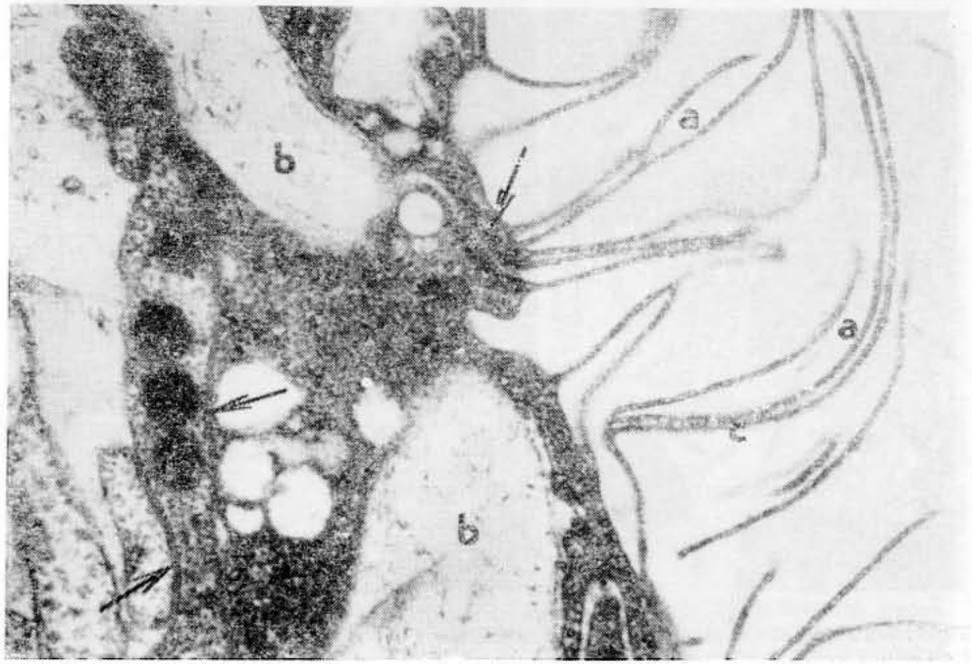
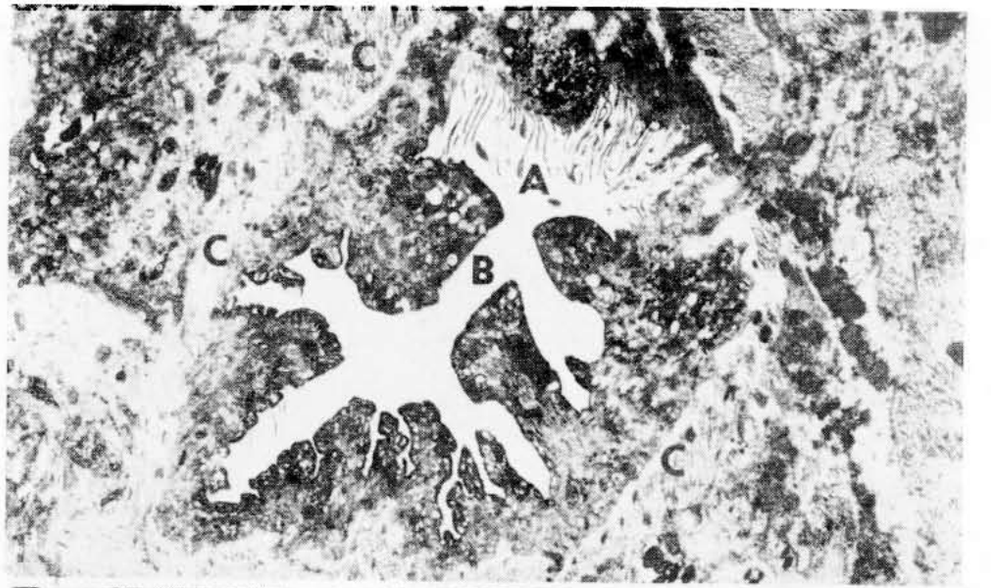


Fig. 1. Detail of bases of cilia of lateral flame (A). B — processes of parenchymal cells, a — interstitial material, b — cytoplasm of epithelial cell, c — cilia, d — basal bodies, arrows — striated rootlets of cilia. (G. Os, UAc, Pb) (× 26 300). **Fig. 2.** Detail of junction of epithelial cells by septate desmosome (arrow) and gap junction with parenchymal cells (double arrows). a — lamellae, b — interstitial material (G, OS, UAc, Pb) (× 48 400).



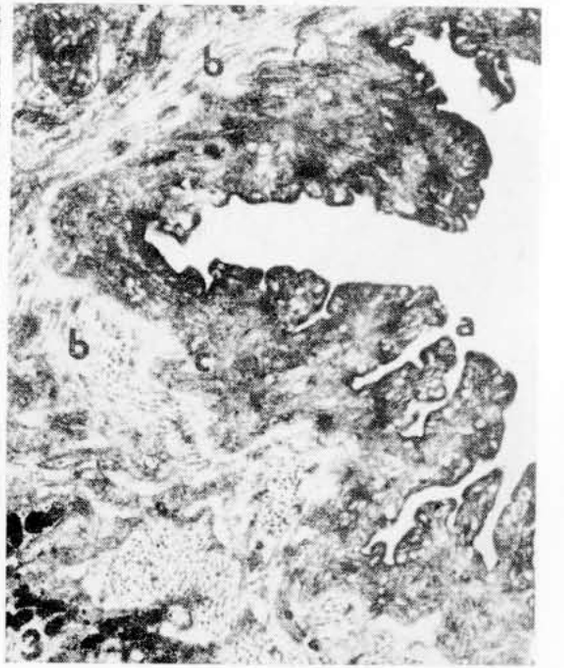


Fig. 1. Transition of excretory bladder (A) to excretory pore (B). C — muscles (G, Os, UAc, Pb) (× 9 000). **Fig. 2.** Detail of excretory bladder from Fig. 1. a — lamellae of excretory bladder, b — muscles, c — excretory pore, arrow — desmosome connecting the tegument with the syncytium of excretory bladder (G, OS, UAc, Pb) (× 15 100). **Fig. 3.** Detail of the tegument (a) of excretory pore from Fig. 1. b — muscles, c — invagination of basal plasma membrane of tegument (G, Os, UAc, Pb) (× 15 100).