ULTRASTRUCTURAL STUDY OF THE GLANDULAR SYSTEM AND ITS PARTICIPATION IN TEGUMENT FORMATION IN ECHINOSTOMA REVOLUTUM CERCARIA

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Abstract. Six types of gland cells occurring in the body of developing Echinostoma revolutum cercaria are described at ultrastructural level. Three types of the gland cells, lateral, ventral and dorsal, release their secretion into the tegument of cercaria during its development in the redia. Another three types, paraoesophageal, penetration and proper clyptomeric gland cells are preserved in the body of the free-swimming cercaria. The paraoesophageal gland cells open on the tegument surface. Secretory granules of all types of gland cells and stratification of the body tegument arising by gradual release of the contents of the above mentioned three types of gland cells are characterized in detail.

The present paper is a continuation of preceding histochemical studies of gland cells (Ždárská and Naíncová 1985), ultrastructural studies of sensory receptors (Ždárská et al. 1987) and tail (Ždárská et al. 1989) of this cercaria. It aims at contributing to a complex elucidation of the morphogenesis of E. revolutum cercaria.

MATERIALS AND METHODS

Developing and free-swimming cercariae of Echinostoma revolutum (Frebel, 1892) were obtained from naturally infected snails Planorbis corneus collected in the locality České Vrbno (Czechoslovakia).

The material for transmission electron microscopy was fixed in 3% glutaraldehyde at 4°C in 0.1 M cacodylate buffer (pH 7.2) for 2 h and postfixed in 1% OsO4 for 2 h, dehydrated through an alcohol series and embedded through acetone into Durcupan and Vestopal. Ultrathin sections were cut with Reichert’s OM-U2 ultra microtome, contrasted with 20% uranyl acetate and Reynolds’s solution of lead citrate, and examined in Philips 420 and JEM 100 B electron microscopes.

RESULTS

A. INVOLVEMENT OF THE GLAND CELL SECRETION IN THE FORMATION OF THE BODY TEGUMENT

Six types of gland cells develop in the cercaria body and their contents increase with the growth of the cercaria. In a fully formed cercaria, the lateral gland cells are the first to release their secretion into the tegument on the ventral side from where it spreads also to the dorsal one. Then the contents of the ventral gland cells is released into the tegument of the whole body with the exception of suckers. Eventually the dorsal gland cells release their secretion on the dorsal side from where it again spreads on the whole body surface except for the suckers.

Consequently, secretions of ventral, lateral and dorsal gland cells are deposited in the body tegument of the cercaria before it leaves the redia. These three types of cells disappear after the holocyclic secretion. Only the proper clyptomeric, paraeso-
phagocytic and penetration gland cells are preserved in a fully formed cercaria. The body tegument of the cercaria represents a preformed outer layer of the cyst wall. The inner layer of the cyst wall is formed during the encystation and consists of the secretion of the proper cystogenic gland cells.

B. GLAND CELLS

1. Lateral gland cells (Pl. II, Fig. 2) are localized on the ventral side along the oesophagus and around the oral and ventral suckers. They are preserved only in a developing cercaria. Fully developed lateral gland cells are filled with dimorphic granules. The granules of the first type are larger and irregularly foamed, those of the second type are small, dense granules. The rough endoplasmic reticulum is pressed to the plasma membrane. Thin muscle fibres surround the bodies of these gland cells. The nucleus has a nucleolus and the chromatin is concentrated in clusters below the nuclear membrane. The secretory granules of these cells are the first to be released into the tegument (Pl. II, Fig. 1). They spread in its cytoplasm from the ventral side over the whole body. In the tegument of a free-swimming cercaria they form a layer under its distal plasma membrane.

2. Ventral gland cells (Pl. III, Fig. 2) are the smallest of all gland cells. They are localized on the ventral side immediately under the body wall from pharynx up to the posterior end, except for the sites where the paraschosophageal and lateral gland cells, ventral nerve strains and suckers are situated. They are club-shaped and their narrowed ends reach the muscle fibres of the body wall. Their cytoplasm contains mainly transversely striated spindle-shaped secretory bodies. Wide electron-dense

![Fig. 1. Detail of tegument of free-swimming cercaria. A — transversely striated granules of ventral glands cells, B — granules of lateral gland cells, C — granules of dorsal gland cells (G, Os, UAe, Ph) (O3570×).](image)

and electron-light bands alternate on the bodies. Each of the bands is divided in the middle by a thin, electron-dense strip (Fig. 1). In addition to these bodies, the cytoplasm contains rough endoplasmic reticulum and its cisternae. These gland cells are preserved only in the body of a developing cercaria. Their secretion is released from the cell body into the tegument as the second, i.e. after the secretion of the lateral gland cells, with which it mixes and in a free-swimming cercaria it is localized in the distal part of the tegument (Fig. 1; Pl. III, Fig. 1).

3. Dorsal gland cells (Pl. IV, Figs. 1, 2) are localized on the dorsal side immediately below the body wall. They are star-shaped and their processes penetrate into the intercellular spaces between other cells.

In a developing cercaria, the Golgi apparatus, rough endoplasmic reticulum, single forming secretory granules and nucleus with dispersed chromatin and nucleolus are well developed in these cells (Pl. IV, Fig. 1). Before the contents of these cells are discharged into the tegument, the cell body is usually packed with finely foamed granules (Pl. IV, Fig. 2) and the cellular organelles are restricted. The secretion is pressed out into the tegument on the dorsal side, from where it spreads over the whole body. The granules remain mostly in the basal part of tegument and form the thickest layer on the dorsal side of the cercaria.

4. Proper cystogenic gland cells (Fig. 2, Pl. IV, Fig. 2) fill the dorsal part of body along its whole length. They are club-shaped and their narrowed part is directed towards the dorsal body wall. In a developing cercaria, these gland cells possess only single granules, the rough endoplasmic reticulum and its cisternae prevail. There are single mitochondria and a nucleus with nucleolus and chromatin clusters under the nuclear membrane. In a later phase of development of these cells, red-shaped granules

![Fig. 2. Detail of granules of proper cystogenic gland cells. A — longitudinal section, B — transverse sections (G, Os, UAe, Ph) (7800×).](image)
restrict all the above structures. The rod-shaped granules have a characteristic structure. It is a protein in a shape of a rolled up band (electron-dense layer), as it is evident from the longitudinal and transverse sections (Fig. 2). During the encystation of the cercaria, the bands roll out and lie on one another forming thus the inner sclerotin layer of the cyst wall.

5. Paraesophageal gland cells (Pl. I, Fig. 2) are filled with large light granules of irregular shape which fuse in the opening. The ducts of these cells (Pl. I, Fig. 2), which are reinforced by microtubules, penetrate through the muscle layer and lamina basalis. In the basal part of tegument, the plasma membrane of the ducts is connected with the plasma membrane of tegument through a septal desmosome possibility. The constrictor muscles of these ducts are reinforced by microtubules localized below the plasma membrane (Pl. I, Fig. 1). The ducts open on the tegument surface of the dorsal side of oral sucker.

6. Penetration gland cells are localized on the ventral side near the osophagus. In the free-swimming cercaria, their bodies and long ducts are filled with irregular, strongly electron-dense granules restricting the other organelles. In a developing cercaria, rough endoplasmic reticulum and its cisternae prevail. There occur also single mitochondria. The long ducts of penetration gland cells are reinforced by microtubules localized below the plasma membrane (Pl. I, Fig. 1). The ducts open on the tegument surface of the dorsal side of oral sucker.

**Discussion**

Only few papers dealing with the ultrastructure of gradually developing gland apparatus in the cercaria have been published (Dorsey 1975, Rees and Day 1976). In the majority of them, only completely developed gland cells, representing cell bodies packed with secretory granules, are described. The structures which participate in the process of secretion were neglected. In our studies, we had the opportunity to observe in detail the development of the gland cells, which contain more Golgi apparatuses, rough endoplasmic reticulum with cisternae, mitochondria and nuclei with nucleolus at the beginning of their development. At the final stage of development, i.e., before the secretion is discharged into the tegument, the cellular organelles in these gland cells are restricted and the cell bodies are packed with secretory granules.

In contrast to histochemical methods (Zďarská and Naščinová 1985), by means of which we have not managed to differentiate the paraesophageal gland cells from the lateral and dorsal ones, the paraesophageal gland cells can be well distinguished from the other gland cells in the electron microscope on the basis of the structure of secretory granules. While the lateral and dorsal gland cells open into the tegument, the paraesophageal gland cells open on its surface. The electron-microscopic studies showed that the lateral gland cells are the first to release their contents and release of the secretion of the ventral gland cells follows. Due to the gradual discharge of secretion of lateral, ventral and dorsal gland cells, the secretions, particularly on the dorsal body surface, are preserved in layers. Secretory granules of lateral gland cells are localized under the distal plasma membrane of the tegument, then follows the layer of ventral gland cell secretion and then that of dorsal gland cell secretion.

The ultrastructure of the glandular apparatus of echinostome cercariae has been studied only incompletely in 4 species, namely Himasthla quinquevis (Lautenschlager and Cardell 1959, Cardell 1962, Laurie 1974), H. rhigedana and Acanthoparyphium species (Bils and Martin 1966), and Echinostoma paraensei (Stein and Basch 1977). The cercaria of Echinostoma revolutum has not yet been studied by means of electron microscopy.

E. revolutum cercaria belongs to the cercariae of primitive type which penetrate into the second intermediate host through natural openings and not through its tissues. Also its cyst, due to the presence of a plug (Gulka and Fried 1979), is close to the cysts of free in nature encysting adolescarae. Consequently, from the phylogenetic point of view this cercaria is closely related to the cercarae the subsequent developmental stage of which is adolescarae. Among them belong Fasciola hepatica (Dixon and Mercer 1964, Mercer and Dixon 1967) and Notocotylus attenuatus (Southgate 1971), the glandular apparatus of which has been studied at the ultrastructural level. A comparison of the ultrastructure of individual gland cells of these cercariae with our echinostome cercaria led us to the conclusion that they are very similar.

The glandular cells of Echinostoma revolutum possess a distinct transverse striation visible in the electron microscope both inside the cell bodies and after the discharge into the tegument. Similar transversely striated granules were observed also in the gland cells localized on the ventral side of cercaria of Fasciola hepatica (Mercer and Dixon 1967) and Notocotylus attenuatus (Southgate 1971). Of a similar structure are also the granules of dorsal and proper ostation gland cells of E. revolutum cercaria and of the above two species. The character of secretory granules of E. revolutum cercaria has many common features also with phylogenetically higher species (Leong and Howell 1971, Stein and Lumsden 1971a, b, Strong and Cable 1972, Rees 1974, Rees and Day 1976, Halton and Johnston 1985).

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Fig. 1. Tegument (A) of dorsal side of oral sucker of cercaria with ducts of penetration gland cells (b) filled with electron-dense granules. The ducts below the plasma membrane are reinforced by microtubules (arrows). B — muscles of oral sucker (G, Os, UAc, Pb) (18840 ×). Fig. 2. Opening (arrow) of paracerebral gland cell on the surface of tegument. The body of this gland cells is filled with electron-dense granules (A). B — muscles of body wall (G, Os, UAc, Pb) (12000 ×).

Fig. 1. Definitive tegument of a young cercaria with remains of embryonal epithelium (A). The tegument contains only granules of lateral gland cells B — spine, C — circular muscles (G, Os, UAc, Pb) (22380 ×). Fig. 2. Detail of irregularly foamed secretory granules in a lateral gland cell (A) surrounded by single muscle fibres (B) (G, Os, UAc, Pb) (27000 ×).
Fig. 1. Tegument of free-swimming cercaria with transversely striated granules of ventral gland cells, irregularly formed granules of lateral gland cells (A) and regularly finely foamed granules of dorsal gland cells (B). C — muscles of body wall (G, Os, UAc, Pb) (2100 x). Fig. 2. Central gland cell (A) with transversely striated granules localized below the muscle layer (B) of body wall of cercaria (G, Os, UAc, Pb) (11800 x).

Fig. 1. Detail of dorsal gland cell of a very young cercaria with well visible Golgi apparatus (A) and developing granules, mostly still unfoamed. B — nucleus with melanin (G, Os, UAc, Pb) (11100 x). Fig. 2. Detail of dorsal gland cell (A) in a completely developed cercaria. All granules are finely foamed. The cell adheres to the muscles of the dorsal body wall (B). C — proper cystogenic gland cell (right at the bottom) (G, Os, UAc, Pb) (x 24000).