HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON THE CERCARIA AND REDIA OF ECHINOSTOMA REVOLUTUM

Z. ŽDÁRSKÁ and V. NAŠINCOVÁ
Institute of Parasitology, Czechoslovak Academy of Sciences, České Budějovice

Abstract. Five types of gland cells—ventral, lateral, subossephagal, dorsal, and proper cystogenic—were revealed in the body of developing cercaria of Echinostoma revolutum. The tail contains one type of gland cells. Three types of holostric gland cells in the body of cercaria (ventral, lateral, and dorsal) release their secretion into the body tegument still during their development inside the redia. The tegument of cercaria represents a preformed outer layer of the cyst wall of metacercaria. Two types of gland cells, subossephagal and proper cystogenic, are preserved in the body of cercaria leaving the first intermediate host. The tail is already without caudal gland cells which disappeared at the time when the secretion was released into its tegument. Histochimical studies revealed proteins with tyrosine, tryptophan, cysteine and cystine in the secretion of ventral gland cells, acid phosphatase activity, proteins and a small amount of neutral mucosubstances in subossephagal gland cells, neutral and acid mucosubstances with sulpho- and carboxy-groups and tyrosine in lateral gland cells, mostly neutral mucosubstances in dorsal gland cells, and mostly proteins with cysteine and cystine and phospholipids in proper cystogenic gland cells. In the redia, a nerve ganglion is well developed immediately behind the pharynx and exhibits a high activity of acetylcholinesterase. The intestine of redia exhibits acid phosphatase activity and contains cystine and phospholipids. Alkaline and acid phosphatase activity was demonstrated in the tegument.

After twenty years, we again return to the studies on larval stages of Echinostoma revolutum, namely the histochemistry of redia and cercaria. Our previous papers dealt with the morphology of larvae of this trematode (Ždárská 1964a) and partly with the histochemistry of its metacercaria (Ždárská 1964b). This paper presents the results obtained by means of more modern histochemical methods which enabled us to study in detail the participation of secretions of different gland cells of cercaria in the precystation process, which has not yet been described in detail in this species. Also the electron microscopy used in the paper by Našincova (in press) supplied more detailed information on the surface structures of both body and tail of this cercaria.

E. revolutum is the third species dealt with in our studies on the functionally morphological peculiarities of echinostome cercariae. Our previous papers concerned the larvae of Echinoparyphium acornatum (Ždárská 1968) and Molinellia anceps (Ždárská 1971).

MATERIAL AND METHODS

The redia and cercariae of Echinostoma revolutum (Frölich, 1802) Diets, 1909 were obtained from spontaneously infected snails, Planorbarius corneus. For the detection of proteins and mucosubstances, the infected hepatopancreas of snail was fixed in Baker’s solution (Pearse 1944) at laboratory temperature for 24 h. The same solution (at 4 °C for 2 h) was used for the fixation for the detection of enzymes. Serial sections were used for the histological and histochemical methods. The methods for the detection of mucosubstances and proteins were described in the paper by Ždárská and Panin (1977).
RESULTS

A. REDIA

The body of redia has a muscular pharynx (Plate I, Fig. 2), short intestine, collar, birth pore and ventral locomotory appendages. The body wall consists of the tegument, circular and longitudinal muscles and layer of parenchyma. Subtegumental cells are localized in the layer of parenchyma. The tegument, particularly in the anterior part of body, gives positive reactions for tyrosine and tryptophan and exhibits a high activity of acid and alkaline phosphatases. The subtegumental cells contain also tyrosine and tryptophan, but only acid phosphatase was found in them. The parenchymal part of body wall is filled with glycogen and the inner margin of the body cavity is covered with a substance containing neutral and acid mucosubstances. The tegument of pharynx has a high activity of acid and alkaline phosphatases and gives positive reactions for tyrosine and tryptophan. The posterior part of pharynx is surrounded by a large nerve ganglion (Plate IV, Fig. 2). Short branches terminating at the anterior end of pharynx project from the ganglion. Both the ganglion and branches exhibit non-specific esterase and acetylcholinesterase activity. Several gland cells containing tyrosine, tryptophan and cystine and exhibiting a high activity of acid phosphatase are localized around the pharynx. The intestine contents has a high activity of acid phosphatase and gives intensive positive reaction for phospholipids and cystine.

B. CERCARIA

1. Participation of gland cell secretion in the formation of tegument

Five types of gland cells develop inside the body of cercaria and their contents gradually increase with the growth of the cercaria. In a fully formed cercaria, the ventral gland cells are the first to release their secretion into the tegument on the ventral side from where it spreads also to the dorsal side. As soon as the ventral gland cells are empty, the lateral gland cells start to release their secretions into the tegument on the ventral side. The secretion also spreads in the tegument of the whole cercaria body. Finally the dorsal gland cells start to release their secretion on the dorsal side and it again spreads on the whole body surface. At that time also the caudal gland cells release their secretion into the tail tegument. Consequently, the body tegument of cercaria before leaving the redia contains the secretions of ventral, lateral and dorsal gland cells and the tail tegument contains the secretion of caudal gland cells. The mentioned types of cells disappear during holocrine secretion. In the body of a fully developed cercaria, the proper cystogenic gland cells and suboesophageal cells are preserved and the tail is without gland cells. The tegument of cercaria with different secretions inside represents a preformed outer layer of the cyst wall. The inner layer of the cyst wall arises during the encystation from the secretion of proper cystogenic gland cells, the outer layer arises from the secretions contained in the tegument of cercaria.

2. Histology and histochemistry of individual gland cells

a) Ventral gland cells (Plate I, Fig. 1; Plate II, Fig. 1) are the smallest of all and are localized on the ventral side immediately below the body wall from pharynx up to posterior end, except for the sites where the suboesophageal and lateral gland cells, ventral nerve trunks (see Plate I, Fig. 1 and Plate IV, Fig. 1) and suckers are situated. They are club-shaped and their narrowed end is directed to the fibres of body wall muscles.
Their homogeneous cytoplasm stains red with Van Gieson's method and gives positive reactions (Table 1) for proteins with tyrosine, tryptophan, cysteine and cystine.

b) Subesophageal cells (Plate II, Fig. 2) are located on the ventral side below the osophagus. They form a medially situated longitudinal band between the oral sucker and ventral sucker. The narrow end of their club-shaped body touches the muscles of the body wall. Their cytoplasm is finely granulated, exhibits acid phosphatase activity and gives slightly positive saliva test-resistant PAS reaction and reaction for proteins with tryrosine, tryptophan, cysteine and cystine (Table 1).

c) Lateral gland cells (Plate III, Fig. 1) are localized on the ventral side, around the osophagus and oral and ventral suckers. Their oval to spherical bodies contain a large amount of vacuolized secretion. The secretion is strongly positive in PAS and Best's reactions (even after saliva test) and in the reaction with AB pH 2.6 weakened after demethylation. Consequently, these glands contain neutral and acid mucosubstances with carboxy- and sulphogroups. The secretion is also strongly positive in Molè-Sieley's reaction for tyrosine (Table 1). The tegument, in which the secretion of these cells was released, reacts in the same way to the histochemical tests (Plate III, Fig. 2).

d) Dorsal gland cells (Plate III, Fig. 1) are localized on the dorsal side immediately below the body wall. They are star-shaped and their processes penetrate into intercellular spaces between the other cells. Their finely granulated cytoplasm gives strongly positive saliva test-resistant PAS and Best's reactions. Their secretion released into the tegument reacts in the same way (Plate I, Plate III, Fig. 2).

e) Proper cestogenic gland cells (Plate III, Fig. 2) fill the middle part of body along its whole length. They are club-shaped and their narrowed part is directed towards the body wall on the dorsal side. The contents of these cells stain yellow by Van Gieson's method. The granules contain mostly proteins with cysteine and cystine and phospholipids (Table 1).

f) Caudal gland cells are distributed along the longitudinal axis of tail and form processes directed towards tegument. At the later phase of cercaria development, their secretion is pressed through the processes into the tegument. The caudal gland cells fill the inner part of tail except for the narrowed end. They are strongly positive for neutral mucosubstances and acid mucosubstances with carboxy- and sulphogroups. The secretion released into the tail tegument reacts in the same way (Table 1).

The nerve system of the cercaria exhibits a high activity of acetylcholinesterase. The cells of osophagus and oes are positive to PAS and Best's reactions and exhibit a high activity of acid phosphatase. The excretory granules are positive to Köss's test for calcium salts.


discussion

The histochemistry of Echinostoma revolutum redia is almost identical with that of Neophallus longicornis (Kile 1971), Sphaerodirum globulosa (Reiser 1972) and Fasirina dolichura (Halton 1973). The basic design of the tegument of E. revolutum redia conforms in its morphology to that of the redia of Parorchis aequinuchus (Rees 1971, 1980), which was studied in detail, and to those of Acaenopharyngophorus sp., sp. unpaucit. (Bils and Martin 1975), echinostome rediae described by Vercammen-Grandjean (1960) and rediae of Pescher neocommumens (Ginetinskaya 1968), Cryptocotyle lingua (Krupa et al. 1987, 1988, Irwin et al. 1978), Aporocotyle simplex (Klie 1983), Mammolaimus rhabdosus, and Metagonimus yokogawai (Klie 1983). Like in other rediae, also in E. revolutum redia the body muscles and muscles of pharynx are strongly developed. Both the pharynx and body wall contain a large amount of glycogen. The body cavity is covered with a substance containing neutral and acid mucosubstances. The presence of alkaline phosphatase in the tegument of E. revolutum redia, like in that of Spharodirea globulosa (Reiser 1972), confirms the participation of the tegument in the transport of nutrients through the body wall, though the receipt of nutrients takes place in the intestine.

The histochemistry of developing E. revolutum cercaria has not yet been dealt with. Only the enzyme activity in the free-swimming cercaria was studied by LeFlore et al. (1984) and Fried et al. (1984). Individual types of gland cells of echinostome cercariae have been studied only occasionally (Lio 1968).

The aim of this work was to elucidate the participation of the secretion of individual types of gland cells present in the body and tail of cercaria in the formation of tegument and to compare the mode of their gradual emptying with that of earlier studied echinostome cercariae — Echinorhynchus acutus (Zdarski 1988) and Malmiella aniceps (Zdarski 1971). The cercaria of E. revolutum is most closely related to that of M. aniceps in the number of types of gland cells participating in the formation of tegument. Three types of gland cells, ventral, lateral and dorsal, participate in the formation of tegument in the cercariae of these two species, whereas in E. aconiatum cercaria, only two types, ventral and dorsal, are involved in it. In the cercariae of E. revolutum leaving the redia, only two types of gland cells (proper cystogenic and subesophageal) are completely preserved. In M. aniceps, three types (ventral, dorsal and cystogenic) are completely preserved and one type incompletely (a part of the secretion of dorsal gland cells remains localized in its bodies). The tegument of E. revolutum and M. aniceps cercariae contains the secretions of three types of cells, whereas that of E. aconiatum cercaria contains the secretion of two types (ventral and dorsal) of gland cells. The dorsal gland cells in E. revolutum, on the contrary, is completely emptied. No caudal proper cystogenic gland cells (present in M. aniceps cercariae) have been demonstrated in E. revolutum cercaria, though Guikkaia and Fried (1979) in their ultrastructural studies observed a different structure at a site in the inner layer of the cyst wall, which seems to correspond to the plug in the cyst wall of M. aniceps metacercaria. It consists of the secretion of proper cystogenic gland cells. However, we have not managed to detect histochemically different cystogenic gland cells in the caudal part of the body of E. revolutum cercaria.

Histochemical properties of the secretions of individual types of gland cells in the studied cercariae of the three echinostome species are substantially identical. In E. revolutum cercaria, like in the other two species, the ventral gland cells contain mostly proteins with tyrosine, cysteine and cystine and the same amino acids are present in the secretion of subesophageal gland cells which contain, in addition to them, neutral mucosubstances and acid phosphatase activity. At the early stage of development, the secretions of lateral gland cells of E. revolutum and M. aniceps cercariae contain neutral and acid mucosubstances with carboxy- and sulphogroups. The secretion of dorsal gland cells in E. revolutum cercaria differs from that of E. aconiatum and M. aniceps cercariae in that it contains neither acid mucosubstances, but only neutral mucosubstances. The proper cystogenic gland cells in all the three species contain proteins with cysteine and cystine and phospholipids. Large caudal gland cells are developed in the tail of E. revolutum cercaria, like in earlier studied cercaria of E. aconiatum and M. aniceps (Zdarski 1988). The secretion of these cells in all the three species is identical — it contains mainly neutral mucosubstances and acid mucosubstances with carboxy- and sulphogroups.

The ducts of gland cells opening on the dorsal side of anterior end of oral sucker, which have often been described in morphological studies of echinostome cercariae, could not be demonstrated in E. revolutum cercaria by any of the histological and histochemical
methods used. This may be due to the fact that the ducts are not filled with the secretion or the amount of secretion is so small that it cannot be detected histochemically. A similar situation is observed in case of detection of plug in the duct wall of E regulator miracercaria which cannot be detected histochemically, but is distinctly visible in the electron microscope, as reported by Gulk and Fried (1979). It remains unclear at what site of the cyst the plug is formed. Is this predilection site the caudal portion of cercaria like in M. aeropis? This site does not seem to be fortuitous, but it is related to a certain part of the body tegument of cercaria or to the localization of somewhat different proper cystogenic gland cells, which cannot be histochemically differentiated from the others. The solution of this question requires detailed ultrastructural studies of the tegument and gland cells of E. regulator miracercaria.

REFERENCES


—, Ultrastructure of the redia of Neoplasia acuta (Forbes) (Meromyzophyidae) and release of cercaria. Parasitology 76: 193-199, 1976.

Fig. 1. Longitudinal section through young cercaria of *E. revolutum* with developing ventral gland cells (arrows). B. Longitudinal section through younger cercaria in which the gland cells are not yet developed. a — oral sucker, b — ventral sucker, c — tail. Van Gieson (*x* 370). Fig. 2. Sections through *E. revolutum* cercariae with well visible cystogenic gland cells intensively stained by PAA-alkaline fuchsin method for the detection of cystine. Suboesophageal gland cells (arrow) are well visible in the transverse section right at the top. a — ventral sucker, b — pharynx of redia (*x* 200).

Fig. 1. Transverse section through anterior part of *Echinostoma revolutum* cercaria with ventral gland cells (a) intensively stained red by Van Gieson's method; arrow — unstained ventral nerve trunk (*x* 750). Fig. 2. A. Transverse section through a fully developed cercaria of *E. revolutum*. The secretion of ventral gland cells is already in the tegment. B. Longitudinal section through anterior end of redia. a — pharynx, b — intestine. Van Gieson (*x* 480).
Fig. 1. Longitudinal (A) and transverse (B) sections through a young cercaria of *E. revolutum*. Dorsal (arrows) and lateral (a) gland cells are intensely stained by Best's method. b — oral sucker, c — ventral sucker (× 350). Fig. 2. Longitudinal section through a fully developed *E. revolutum* cercaria. The secretion of dorsal and lateral gland cells is already in the tegument. a — oral sucker, b — ventral sucker, c — tail, d — oesophagus. Best's method (× 250).

Fig. 1. Transverse (A) and longitudinal (B) sections through *E. revolutum* cercaria stained with the method for the detection of acetylcholinesterase (Karnovsky and Roots). a — ventral nerve trunks, b — ganglion, c — tegument of oral sucker (× 350). Fig. 2. Longitudinal section through redia stained by the same method as in Fig. 1, with well-visible ganglion (a) and processes of nerve fibres into anterior part of pharynx (arrows). b — pharynx, c — intestine (× 670).