

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

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**Abstract.** Five types of gland cells — ventral, lateral, suboesophageal, 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 holocrine 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, suboesophageal 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. Histochemical 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 suboesophageal 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 preencystation process, which has not yet been described in detail in this species. Also the electron microscopy used in the paper by Našincová (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 aconiatum* (Ždárská 1968) and *Moliniella anceps* (Ždárská 1971).

### MATERIAL AND METHODS

The rediae and cercariae of *Echinostoma revolutum* (Frölich, 1802) Dietz, 1909 were obtained from spontaneously infected snails, *Planorbis corneus*. For the detection of proteins and mucosubstances, the infected hepatopancreas of snail was fixed in Baker's solution (Pearse 1968) 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 appendices. 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. Participance 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 secretion 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 pre-formed 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.

Table 1. Histochemistry of the cercaria of *E. revolutum*

Reaction	Gland cells					Tegument		Nervous system	Tail	
	ventral	suboesophageal	lateral	dorsal	proper cystogenic	Young cercaria	Developed cercaria		Gland cells	Tegument
Van Gieson's method	red				yellow		red		+++	+++
PAS	-	-	+++	+++	-		+++		+++	+++
Schiff	-	-	+++	+++	-		+++		+++	+++
Saliva test + PAS	-	-	+++	+++	-		+++		+++	+++
Acetylation + PAS	-	-	+++	+++	-		+++		+++	+++
Desacetylation + PAS	-	rose	violet	red	-		violet		+++	violet
AB-PAS	-	-	+++	+++	-		+++		+++	+++
AB pH 2.6	-	-	+++	+++	-		+++		+++	+++
+ methylation	-	-	+++	+++	-		+++		+++	+++
+ demethylation	-	-	+++	+++	-		+++		+++	+++
Best's carmine	-	-	+++	+++	-		+++		+++	+++
Saliva test +	-	-	+++	+++	-		+++		+++	+++
Best's carmine	-	-	+++	+++	-		+++		+++	+++
Morel-Sisley	-	-	+++	+++	-		+++		+++	+++
DMAB	-	-	+++	+++	-		+++		+++	+++
DDD	-	-	+++	+++	-		+++		+++	+++
Thioglycolic acid +	-	-	+++	+++	-		+++		+++	+++
DDD	-	-	+++	+++	-		+++		+++	+++
PFA-AB	-	-	+++	+++	-		+++		+++	+++
AB pH 0.2	-	-	+++	+++	-		+++		+++	+++
PAA + aldehyde	-	-	+++	+++	-		+++		+++	+++
fuchsin	-	-	+++	+++	-		+++		+++	+++
Aldehyde fuchsin	-	-	+++	+++	-		+++		+++	+++
Alkaline phosphatase	-	-	+++	+++	-		+++		+++	+++
(α-naphthyl phosphate	-	-	+++	+++	-		+++		+++	+++
+ Fast blue BB)	-	-	+++	+++	-		+++		+++	+++
Acid phosphatase	-	-	+++	+++	-		+++		+++	+++
(α-naphthyl phosphate	-	-	+++	+++	-		+++		+++	+++
+ HPR)	-	-	+++	+++	-		+++		+++	+++
Non-specific esterase	-	-	+++	+++	-		+++		+++	+++
(α-naphthyl acetate	-	-	+++	+++	-		+++		+++	+++
+ HPR)	-	-	+++	+++	-		+++		+++	+++
Acetylcholinesterase	-	-	+++	+++	-		+++		+++	+++
(Karnovsky and Roots)	-	-	+++	+++	-		+++		+++	+++
Sudan Black B	-	-	+++	+++	-		+++		+++	+++
(paraffin)	-	-	+++	+++	-		+++		+++	+++

<sup>1</sup> only in fully developed cercaria, <sup>2</sup> only granules

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) Suboesophageal gland cells (Plate II, Fig. 2) are localized on the ventral side below the oesophagus. They form a medially situated longitudinal band between the oral sucker and ventral sucker. The narrowed 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 tyrosine, tryptophan, cystine and cystine (Table 1).

c) Lateral gland cells (Plate III, Fig. 1) are localized on the ventral side, around the oesophagus 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 Morel-Sisley'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 (Table 1, Plate III, Fig. 2).

e) Proper cystogenic 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 oesophagus and ceca are positive to PAS and Best's reactions and exhibit a high activity of acid phosphatase. The excretory granules are positive to Kóssa's test for calcium salts.

## DISCUSSION

The histochemistry of *Echinostoma revolutum* redia is almost identical with that of *Neophasis lageniformis* (Køie 1971), *Sphaeridiotrema globulus* (Reader 1972) and *Fasciola hepatica* (Moore and Halton 1975). The basic structure of the wall of *E. revolutum* redia conforms in its morphology to that of the rediae of *Parorchis acanthus* (Rees 1971, 1980), which was studied in detail, and to those of *Acanthoparyphium spinulosum* (Bils and Martin 1975), echinostome rediae described by Vercammen-Grandjean (1960) and rediae of *Petasisiger neocommensis* (Ginetsinskaya 1968), *Cryptocotyle lingua* (Krupa et al. 1967, 1968, Irwin et al. 1978), *Aporocotyle simplex* (Køie 1982), *Philophthalmus rhionica*, and *Metagonimus yokogawai* (Ginetsinskaya 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 *Sphaeridiotrema globulus* (Reader 1972), confirms the transport of substances 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 LeFlores et al. (1984) and Fried et al. (1984). Individual types of gland cells of echinostome cercariae have been studied only occasionally (Lie 1966).

The main goal 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 — *Echinoparyphium aconiatum* (Žďárská 1968) and *Moliniella anceps* (Žďárská 1971). The cercaria of *E. revolutum* is most closely related to that of *M. anceps* in the number of types of gland cells participating in the formation of body 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 suboesophageal) are completely preserved. In *M. anceps*, three types (suboesophageal, dorsal proper cystogenic and caudal proper cystogenic) are preserved completely and one type incompletely (a part of the secretion of dorsal gland cells remains localized in their bodies). The tegument of *E. revolutum* and *M. anceps* 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. aconiatum* cercaria, like in that of *E. revolutum*, are completely emptied. No caudal proper cystogenic gland cells (present in *M. anceps* cercaria) have been demonstrated in *E. revolutum* cercaria, though Gulka 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. anceps* metacercaria. It consists of the secretion of caudal 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 suboesophageal gland cells which contain, in addition to them, neutral mucosubstances and acid phosphatase activity at the early phase of development. The secretions of lateral gland cells of *E. revolutum* and *M. anceps* cercariae contain neutral and acid mucosubstances with carboxy- and sulphogroups. The secretion of dorsal gland cells in *E. revolutum* cercaria differs from that in *E. aconiatum* and *M. anceps* cercaria in that it does not contain 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 cercariae of *E. aconiatum* and *M. anceps* (Žďárská 1969). 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 in case of the detection of plug in the cyst wall of *E. revolutum* metacercaria which cannot be detected histochemically, but is distinctly visible in the electron microscope, as reported by Gulka and Fried (1979). It remains unclear at what site of the cyst the plug is formed. Is this predilection site the caudal pocket of cercaria like in *M. anceps*? 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. revolutum* cercaria.

# ГИСТОЛОГИЯ И ГИСТОХИМИЯ ЦЕРКАРИИ И РЕДИИ *ECHINOSTOMA REVOLUTUM*

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**Резюме.** В теле развивающейся церкарии *Echinostoma revolutum* встречается 5 типов железистых клеток — вентральные, латеральные, подпшцеводные, дорзальные и настоящие цистогенные. В хвосте только один тип. Три типа голокринных железистых клеток церкарии — вентральные, латеральные и дорзальные — выделяют секрет в tegument тела еще в течение развития в редии. Tegument церкарии представляет собой преформированный внешний слой стенки цисты метациркрии. В теле церкарии, выходящей из первого промежуточного хозяина, сохранены два типа железистых клеток — подпшцеводные и настоящие цистогенные. Хвост уже без каудальных железистых клеток, которые исчезли во время выделения секрета в его tegument. Гистохимическими методами были обнаружены в секрете вентральных железистых клеток белки с тирозином, триптофаном, цистеином и цистином; в подпшцеводных железистых клетках обнаружена активность кислой фосфатазы, белки и небольшое количество нейтральных мукосубстанций; в латеральных железистых клетках — нейтральные и кислые мукосубстанции со сульфо- и карбоксигруппами и тирозин; в дорзальных железистых клетках — большей частью нейтральные мукосубстанции и в настоящих цистогенных клетках — большей частью белки с цистеином и цистином и фосфолипиды. У редии, непосредственно за глоткой, находится хорошо развитый нервный ганглий, обладающий высокой активностью ацетилхолинэстеразы. Кишечник редии обладает активностью кислой фосфатазы и содержит цистин и фосфолипиды, тогда как в tegumente встречаются кислая и щелочная фосфатазы.

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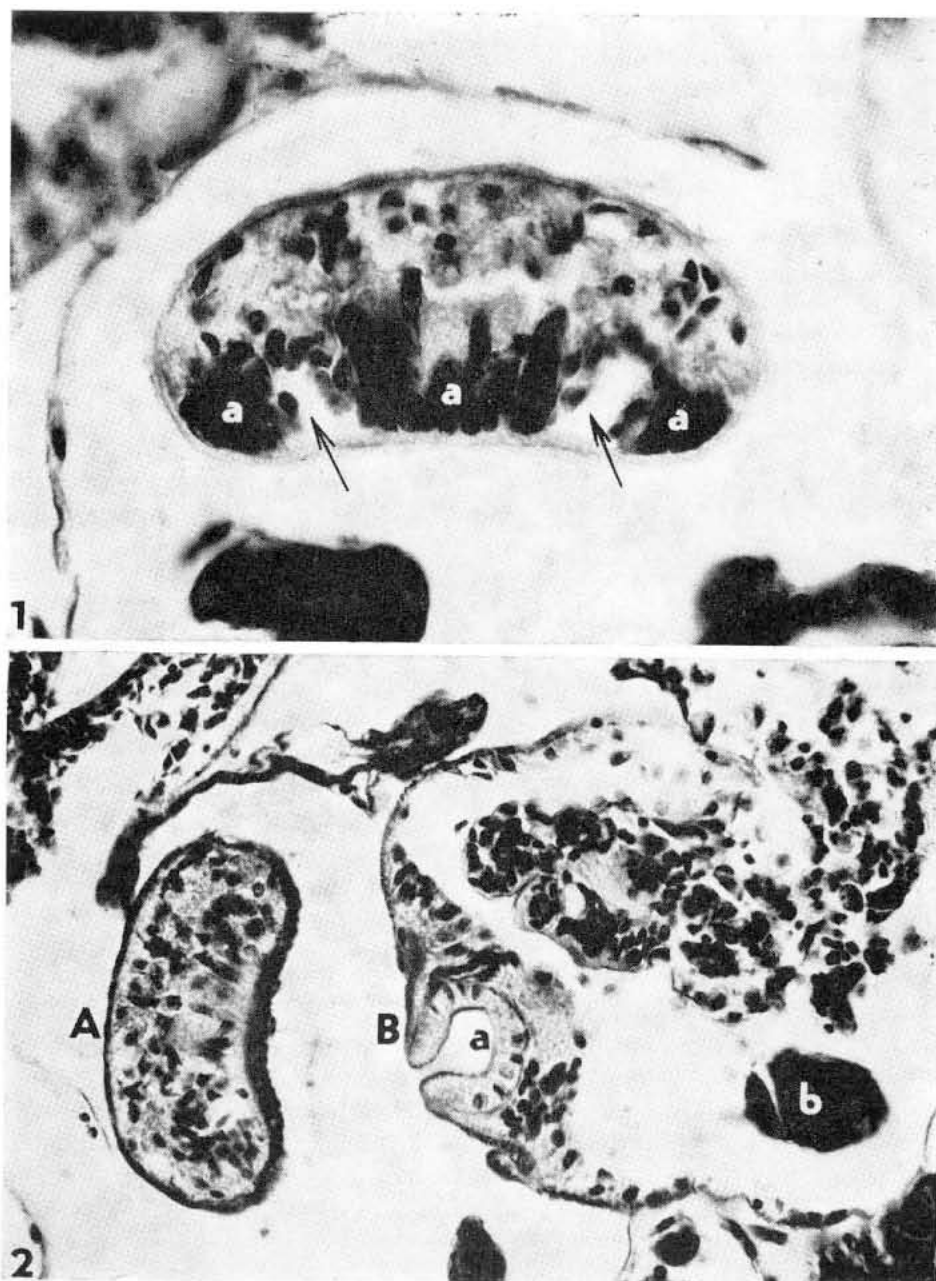
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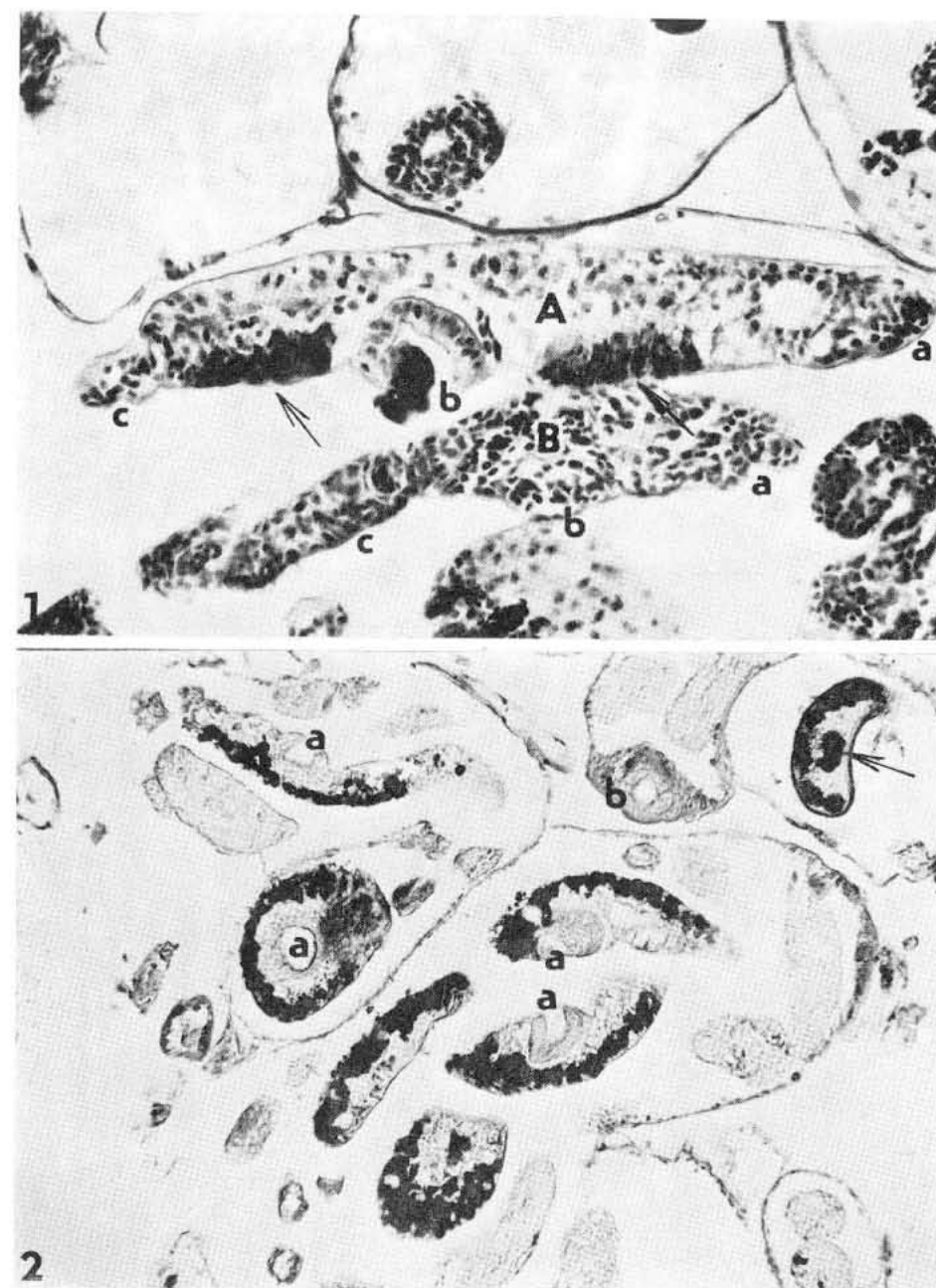
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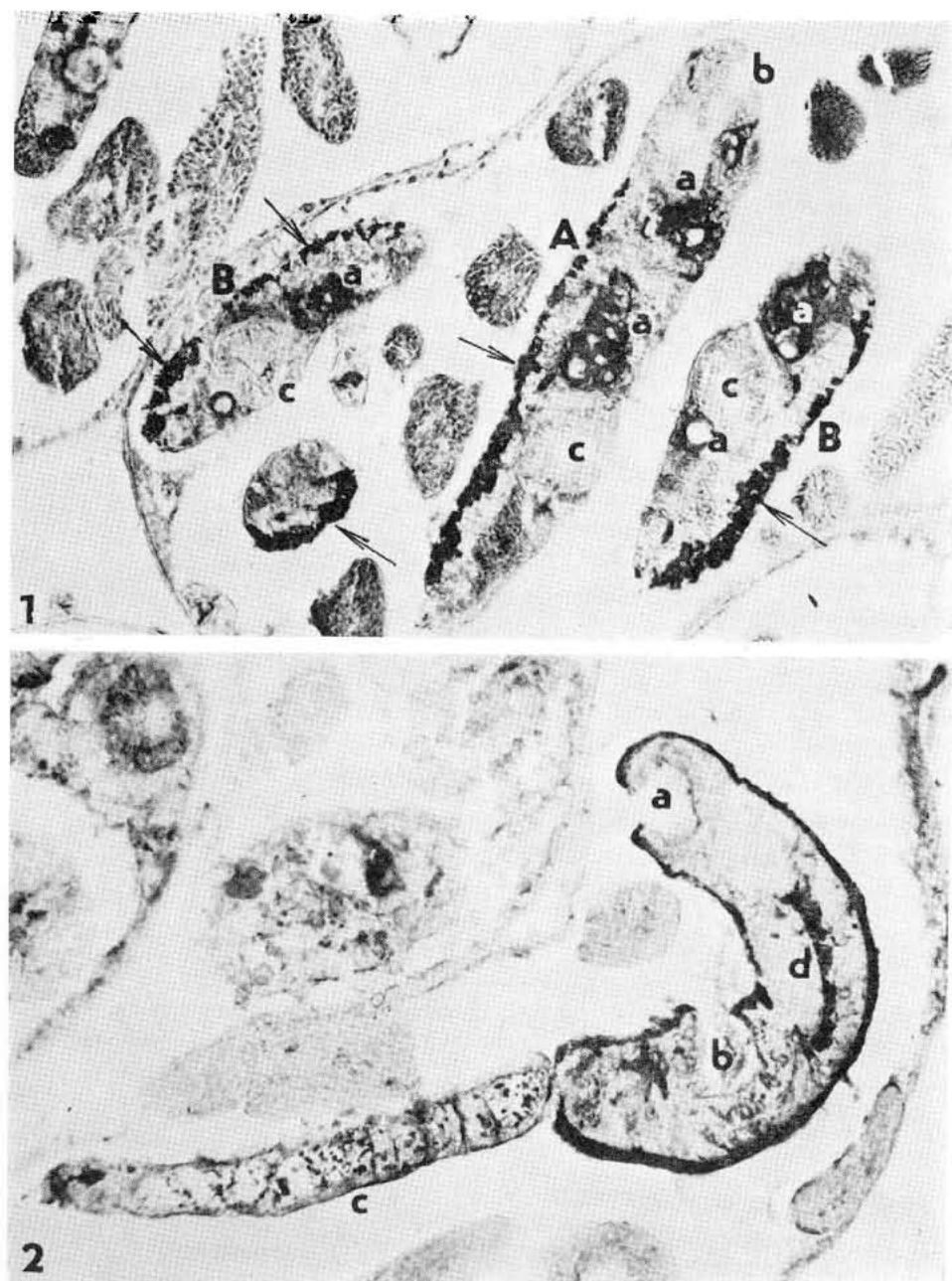


**Fig. 1.** Transverse section through anterior part of *Echinostoma revolutum* cercaria with ventral gland cells (a) intensively stained red by Van Gieson's method; arrow — unstained ventral nerve trunk ( $\times 750$ ). **Fig. 2.** A. Transverse section through a fully developed cercaria of *E. revolutum*. The secretion of ventral gland cells is already in the tegument. B. Longitudinal section through anterior end of redia. a — pharynx, b — intestine. Van Gieson ( $\times 480$ ).

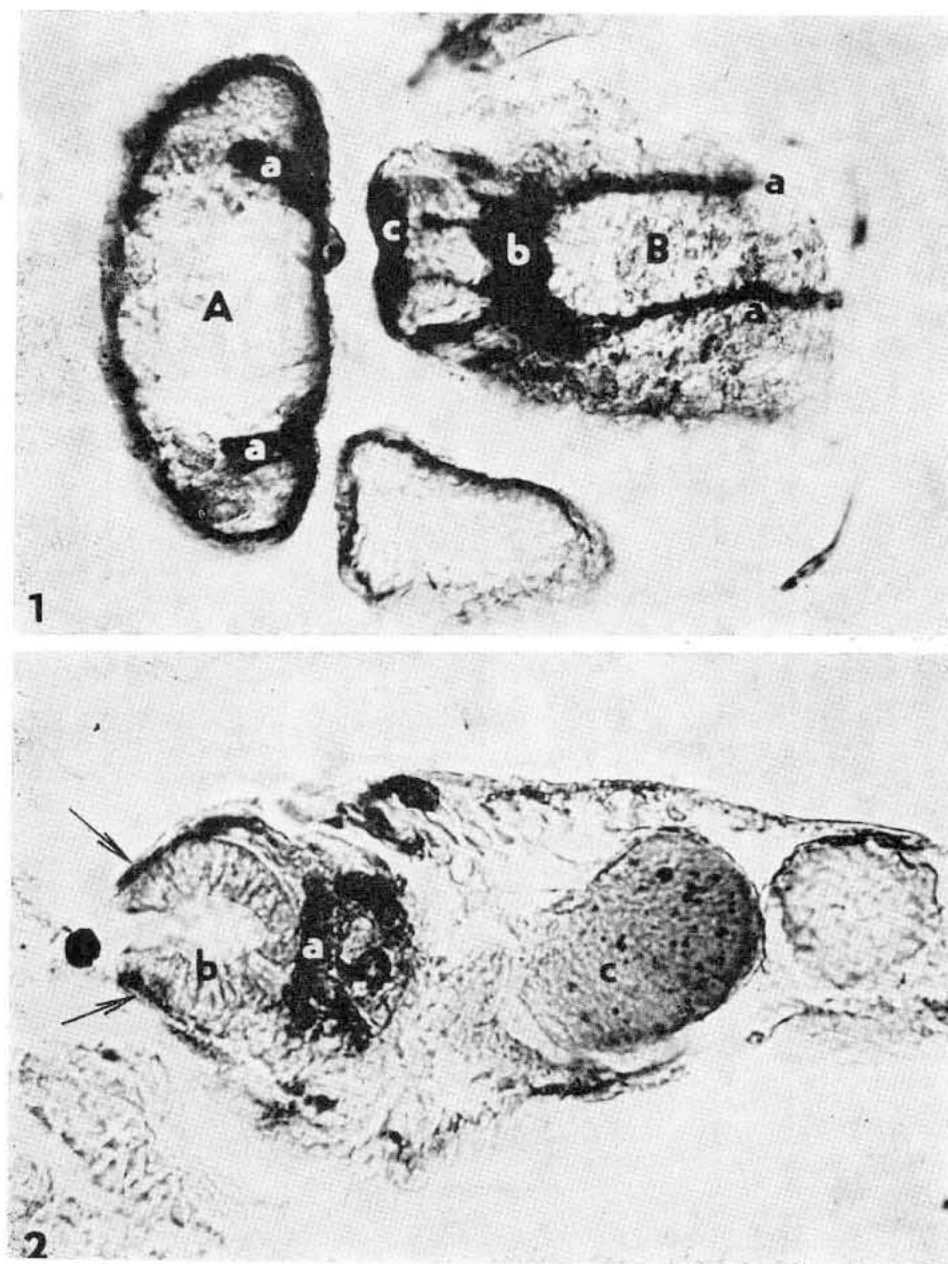


**Fig. 1.** A. Longitudinal section through young cercaria of *E. revolutum* with developing ventral gland cells (arrows). B. Longitudinal section through younger cercaria in which the gland cells are not yet developed. a — oral sucker, b — ventral sucker, c — tail. Van Gieson ( $\times 370$ ). **Fig. 2.** Sections through *E. revolutum* cercariae with well visible cystogenic gland cells intensively stained by PAA-aldehyde 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 ( $\times 200$ ).





**Fig. 1.** Longitudinal (A) and transverse (B) sections through a young cercaria of *E. revolutum*. Dorsal (arrows) and lateral (a) gland cells are intensively stained by Best's method. b — oral sucker, c — ventral sucker ( $\times 380$ ). **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 ( $\times 350$ ).



**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 ( $\times 550$ ). **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 ( $\times 670$ ).