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HISTOCHEMISTRY OF THE CERCARIA AND SPOROCYST OF POSTHARMOSTOMUM GALLINUM (BRACHYLAIMIDAE)

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Abstract. The body and rudimentary tail of the cercaria of *Postharmostomum gallinum* Wittenberg, 1923 are covered with a tegument giving positive reaction for neutral and acid mucosubstances and tyrosin. Of the enzymes, non-specific esterase activity was detected only in tail tegument. The whole digestive system of the cercaria shows a strongly positive reaction for neutral mucosubstances and the internal covering of ceca also for acid mucosubstances. The praepharynx, tegument of pharynx and internal covering of ceca exhibit a high degree of acid phosphatase activity. The activity of non-specific esterase was demonstrated only in pharynx tegument in young cercaria. Histochemical methods demonstrated that the body of cercaria contains three types of penetration gland cells with a high content of tryptophane — dorsal, lateral and ventral, and one type of postacetabular gland cells with a high content of neutral and acid mucosubstances and acid phosphatase activity, particularly in the ducts. The secretion of lateral penetration gland cells seems to serve for the penetration of cercaria through the sporocyst wall which has no birth pore. The secretion of the dorsal gland cells enables the cercaria to pass through the tissues of the first intermediate host. The significance of the other types of penetration gland cells remains uncleared in this species. The branched sporocyst exhibits non-specific esterase activity only in the central part. The inner limiting layer of the sporocyst wall is strongly positive for acid and neutral mucosubstances.

The present paper is a further contribution to the knowledge of the histochemistry of larvae of the family Brachylaimidae Joyeux et Foley, 1930 the members of which adapted to the existence under terrestrial conditions. The most marked common feature in their life cycle is the fact that the cercaria moves freely in the terrestrial environment and enters the second intermediate host (terrestrial snail) through natural body openings. This study follows the previous papers dealing with the histochemistry of larval stages of *Brachylaimus aequans* (Žďárská and Soboleva 1980) and *B. fuscatus* (Žďárská and Soboleva 1984).

MATERIAL AND METHODS

The cercariae and sporocysts of *P. gallinum* were obtained from spontaneously infected terrestrial snails *Ponsadenia semenovi* (Martens, 1864) and *Monachoides candacharica* Pfeiff, 1846 collected in the vicinity of Alma-Ata. The material was fixed in Baker's fluid at 4 °C for 2—24 h. The histochemical methods used for the detection of mucosubstances, proteins and some enzymes were described in previous papers (Žďárská and Panin 1977 and Žďárská et al. 1978).

RESULTS

A. SPOROCYST

The sporocyst is branched. Long and thin sacs project from the central part and form several branches. The body wall of the sporocyst consists of three layers, tegument, muscle layer and inner limiting layer. The tegument is feebly positive for neutral

Table 1. Histochemistry of the cercaria and sporocyst of *P. gallinum*

Reaction	Cercaria										Sporocyst		
	Penetration gland cells				Tegument	Praepharynx	Pharynx	Intestinal wall	Suckers	Genital anlage	Tegument	Muscle layer	Limiting layer
	ventral	lateral	dorsal	postacetabular									
PAS	–	–	–	+++++	+++++	+++++	+++++[6]	+++++	++	+	+	+++	+++++
Schiff	–	–	–	–	–	–	–	–	–	–	–	–	–
Saliva test + + PAS	–	–	–	+++++	+++++	+++++	+++++[6]	+++++	–	–	+	–	+++++
Acetylation + + PAS	–	–	–	–	–	–	–	–	–	–	–	–	–
Desacetylation + PAS	–	–	–	+++++	+++++	+++++	+++++[6]	+++++	++	+	+	+++	+++++
AB — PAS	–	–	–	blue 1 violet 2	violet	–	red	red/ violet	rose	rose	rose	rose	violet
AB pH 2.6	–	–	–	+++++	+++	–	–	++	–	–	–	–	+++
+ methylation	–	–	–	–	–	–	–	–	–	–	–	–	–
+ demethylation	–	–	–	+++++	+++	–	–	++	–	–	–	–	+++
Morel — Sisley	+++++	+++++	+++++	+++++	++	–	+++	+	++	+++	+	+	+
DMAB	+++++	+++++	+++++	–	+	–	++	+	+	+	+	+	–
DDD	+	+	+	–	+	–	++	+	++	++	+	+	–
Thioglycollic acid + DDD	+	+	+	–	+	–	++	+	++	++	+	+	–
PFA — AB	–	–	–	+++++	+	–	+	+	+	+	+	+	+++
AB pH 0.2	–	–	–	+++++	+	–	–	+	–	–	–	–	+++
PAA + aldehyde fuchsin	+++++	+++++	+++++	+++++	+++	++	++[6]	+++	–	–	–	–	++
Aldehyde fuchsin	–	–	–	+++	+++	–	–	++	–	–	–	–	–
Alkaline phosphatase (α-naphthyl phosphate + + Fast blue BB)	–	–	–	–	–	–	–	–	–	–	–	–	–
Acid phosphatase (α-naphthyl phosphate + + HPR)	–	–	–	+++/[3]	–	+++++	+++++[6]	+++++	–	–	–	–	–
Non-specific esterase (α-naphthyl acetate + + HPR)	–	–	–	–	+++++[5]	–	++[7]	–	–	–	++/[4] +++	–	–

1 — openings, 2 — cell bodies, 3 — openings are more strongly positive, 4 — central part of sporocyst is more strongly positive, 5 — only tail tegument, 6 — only tegument, 7 — only in young cercariae

mucosubstances and proteins and only in the central part of the sporocyst it exhibits the activity of non-specific esterase. The inner limiting layer is strongly positive to PAS (Plate III, Fig. 1) even after saliva test and to AB pH 2.6 after demethylation (Table 1). This demonstrates that it contains mostly neutral mucosubstances and acid mucosubstances with carboxyl groups.

B. CERCARIA

Fully developed cercariae are elongate. The anterior part of body is covered with small spines arranged in a chessboard form. The suckers are almost equal. The pharynx is large. The ceca extend up to the posterior margin of the ventral sucker. Two collecting excretory canals open into a well developed excretory bladder. Four types of penetration gland cells (ventral, lateral, dorsal and postacetabular) opening in oral sucker were demontrated in the body of cercaria. The tail is short and oval. The measurements of cercaria are given in the paper by Soboleva(1975).

The tegument of body and rudimentary tail is strongly positive to PAS (Plate III, Figs. 1, 2) even after saliva test and stains with medium intensity in the methods with AB pH 2.6 and with aldehyde fuchsine (Table 1). It is feebly positive for tyrosine. Only the tegument of the rudimentary tail shows a high activity of non-specific esterase (Plate IV., Fig. 2). In the excretory system the collecting excretory canals exhibit a high activity of alkaline phosphatase (Plate IV, Fig. 1).

The praepharynx, pharynx tegument and internal covering of ceca (Plate III, Fig. 2) are strongly PAS-positive even after saliva test, but only the internal covering of ceca could be stained with AB pH 2.6. A high activity of acid phosphatase was demonstrated in praepharynx, pharynx, tegument and internal covering of ceca (Plate I., Figs. 1, 2). Non-specific esterase activity was present in pharynx tegument, but only in young cercariae. The suckers give a weak positive reaction to PAS, which is negative after saliva test. Consequently, they contain glycogen. The genital anlage is positive for proteins with tyrosine, tryptophan and cysteine.

The dorsal, lateral and ventral penetration gland cells (Table 1) are strongly positive for tryptophan, tyrosine and cystine (Plate II, Fig. 1). The postacetabular penetration gland cells (Plate II, Fig. 2) are strongly PAS-positive even after saliva test and stain with AB pH 2.6. At the simultaneous detection of neutral and acid mucosubstances by the AB-PAS method the ducts of these cells stain blue and the body stains violet. The ducts of postacetabular gland cells exhibit the activity of acid phosphatase (Plate I., Figs. 1, 2), but in their bodies the activity of this enzyme is lower. They are also positive in the methods with aldehyde fuchsine.

DISCUSSION

The data on the penetration gland cells in *P. gallinum* cercaria are almost lacking in the literature. Alicata (1940) and Yamaguti (1975) did not mention these gland cells at all. Soboleva (1975) recorded 5 pairs of penetration gland cells the bodies of which were localized behind the ventral sucker. Their position corresponds to the postacetabular gland cells. Most probably the dorsal, ventral and lateral penetration gland cells previously have not been observed in native preparations of this cercaria because of their small sizes. However, due to the high content of tryptophan, they can be selectively demonstrated histochemically using the method with DMAB. The postacetabular gland cells contain a great amount of neutral and acid mucosubstances with COOH groups and exhibit acid phosphatase activity.

Like in the cercaria of *B. fuscatus* (Žďárská and Soboleva 1984), there are 4 types of penetration gland cells in *P. gallinum* cercaria. The postacetabular penetration gland cells contain a great amount of neutral and acid mucosubstances, whereas the dorsal, lateral and ventral penetration gland cells contain a great amount of tryptophan. The acid phosphatase activity was also demonstrated in both species. The third studied species of the family Brachylaimidae — *B. aequans* does not contain acid phosphatase in these gland cells and only two types of penetration gland cells, postacetabular and dorsal, are developed in it (Žďárská and Soboleva 1980).

It remains unclear what are the secretions of individual gland cells in Brachylaimidae used for. With regard to the fact that the gland system of these cercariae corresponds in its morphology, localization and histochemistry to that of the cercariae of the order Strigeatoidea La Rue, 1957, particularly of the genus *Schistosoma* (Ebrahimzadeh 1970, Dorsey 1974), their function may be analogical. However, it is very difficult to elucidate this problem in Brachylaimidae, since the life cycle of the members of this family is different from the life cycle of *Schistosoma*. The cercariae of Brachylaimidae move in the terrestrial environment and enter into the body of the second intermediate host through natural openings, whereas the cercariae of *Schistosoma* penetrate actively through the skin using the secretion of prae- and postacetabular gland cells. It remains to be elucidated for which purpose is used the secretion of postacetabular gland cells in the cercariae of Brachylaimidae if they do not penetrate into the tissue of the second intermediate host. The secretions of the other types of gland cells seem to perform the same function as in *Schistosoma*. The lateral gland cells, which are considered to be identical with the escape gland in *S. mansoni* cercaria, enable the cercaria to penetrate through the sporocyst wall which has no birth pore in the members of Brachylaimidae, similarly as in *Schistosoma* (Dorsey 1974).

The dorsal gland cells found by us in *B. aequans*, *B. fuscatus* (Žďárská and Soboleva 1980, 1984) and *P. gallinum*, are considered to be identical with praeacetabular gland cells in *S. mansoni*. This means that their secretion should be used during the penetration into the second intermediate host. The ventral gland cells of *B. fuscatus* and *P. gallinum* cercariae might be identical with the head glands of *S. mansoni* cercaria which are preserved even in young schistosomulae.

Like in the cercariae of the other species of Brachylaimidae — *B. fuscatus* and *B. aequans* — also the cercaria of *P. gallinum* exhibits a high activity of acid phosphatase in the digestive system and a high activity of alkaline phosphatase in the collecting excretory canals.

Detailed studies of the cercariae of some species of the family Brachylaimidae revealed that many of their morphological characters are identical with those of higher members of the order Strigeatoidea developing in water animals and give evidence that their systematical position in the superfamily Brachylaimoidea behind the family Leucochloridiomorphidae and in front of the family Leucochloridiidae and Hasstilesiidae is justified (Gvozdev and Soboleva 1978).

ГИСТОХИМИЯ ЦЕРКАРИИ И СПОРОЦИСТЫ *POSTHARMOSTOMUM GALLINUM* (BRACHYLAIMIDAE)

З. Ждярска и Т. Н. Соболева

Резюме. Тело и рудиментарный хвост церкарии *Postharmostomum gallinum* Wittenberg, 1923 покрыты тегументом, проявляющим положительную реакцию на нейтральные и кислые мукосубстанции и тирозин. Неспецифическая эстераза встречается только в тегументе хвоста. Пищеварительная система церкарии положительна на нейтральные

мукосубстанции и внутренняя выстилка кишки также на кислые мукосубстанции. Предглотка, тегумент глотки и внутренняя выстилка стенки кишки обладают высокой активностью кислой фосфатазы. Активность неспецифической эстеразы проявляется только в тегументе глотки у молодых церкарий. При помощи гистохимических методов в теле церкарии были обнаружены три типа пенетральных железистых клеток — дорзальные, латеральные и вентральные, содержащих большое количество триптофана, и один тип — постацетабулярные железистые клетки, содержащие большое количество нейтральных и кислых мукосубстанций и обладающие активностью кислой фосфатазы, особенно в протоках. Секрет латеральных пенетральных железистых клеток вероятно служит для проникания церкарии через стенку спороцисты, у которой нет родильной поры и секрет дорзальных железистых клеток — для проникания церкарии тканями первого промежуточного хозяина. Значение других типов пенетральных железистых клеток у этого вида пока не ясно. Разветвленная спороциста проявляет активность неспецифической эстеразы только в центральной части. Граничный слой стенки спороцисты сильно положителен на кислые и нейтральные мукосубстанции.

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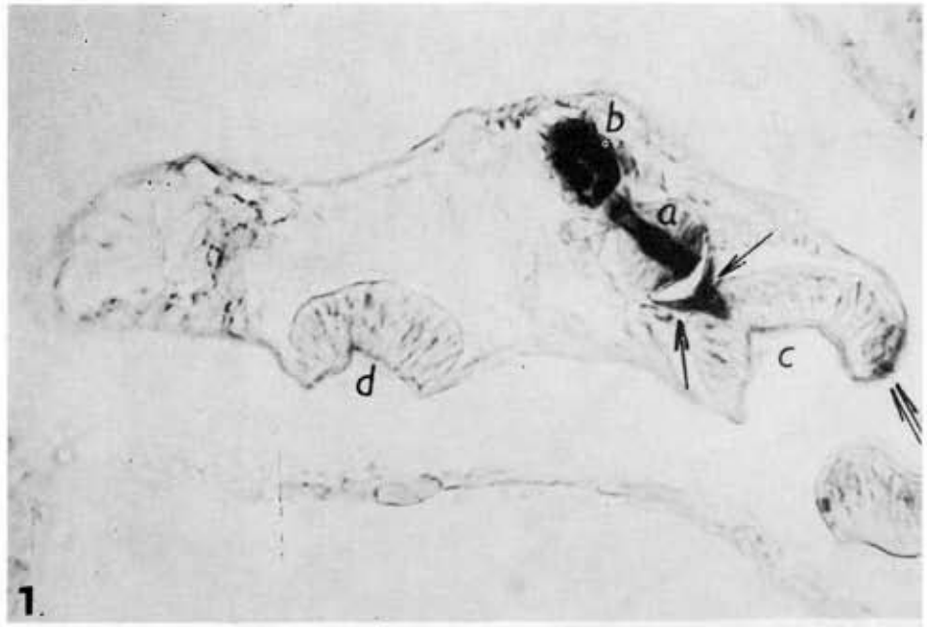


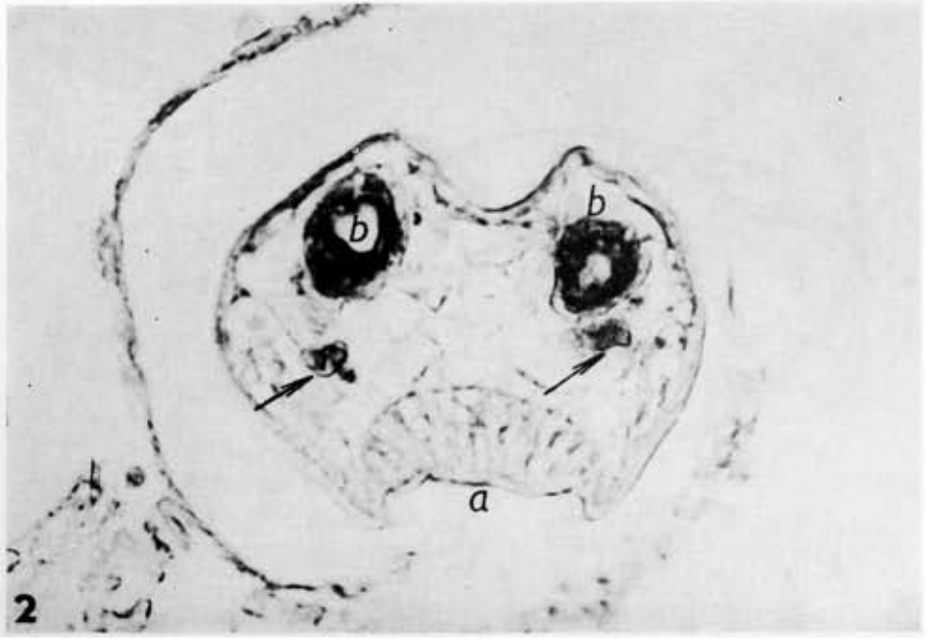
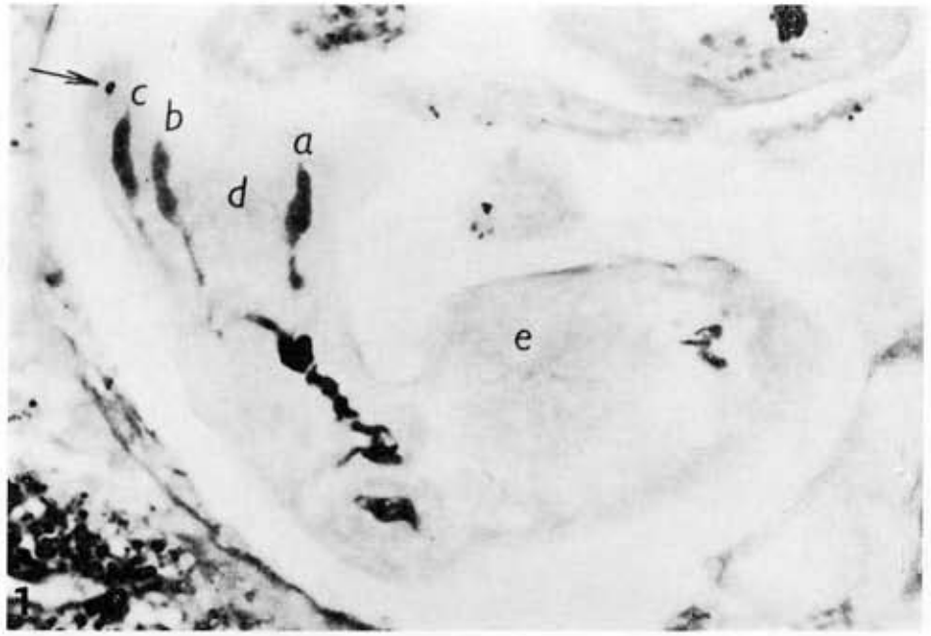


Fig. 1. Longitudinal dorsoventral section through *P. gallinum* cercaria. Acid phosphatase activity is present in the wall of prepharynx (arrows), tegument of pharynx and internal covering of ceca (a), intestine (b) and ducts of postacetabular glands (double arrow); c — oral sucker, d — ventral sucker (α-naphthyl phosphate + HPR) (×550). **Fig. 2.** Transverse section through *P. gallinum* cercaria at level of ventral sucker (a). Acid phosphatase activity is present in ducts of postacetabular gland cells (arrow) and internal covering of ceca (b) (α-naphthyl phosphate + HPR) (×700).



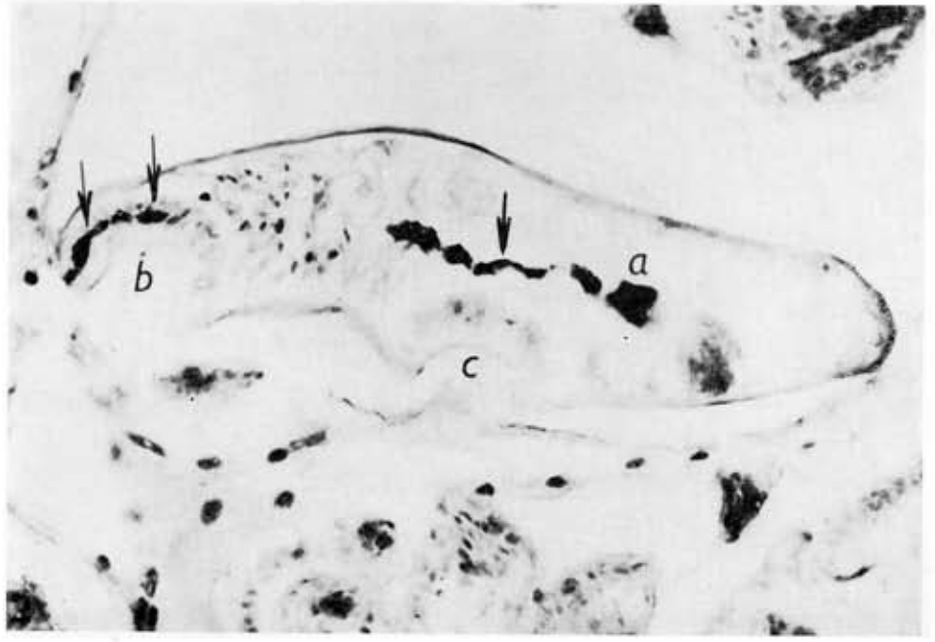
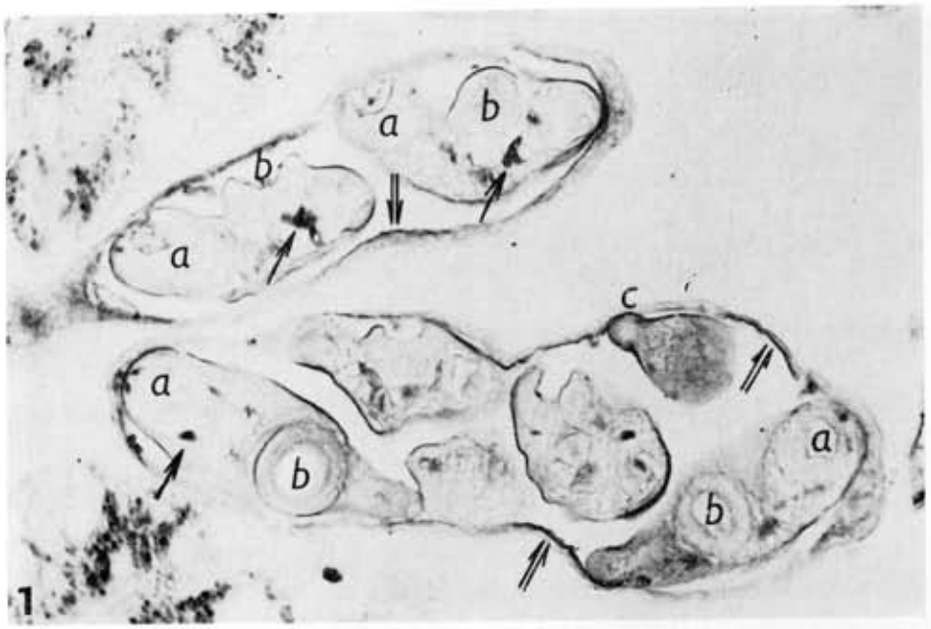


Fig. 1. Longitudinal dorsoventral section through *P. gallinum* cercaria with well visible ducts of ventral (a), lateral (b), dorsal (c) and postacetabular penetration gland cells (arrow); d — oral sucker, e — ventral sucker (PAA + aldehyde fuchsin) (×850). **Fig. 2.** Longitudinal dorsoventral section through *P. gallinum* cercaria. Ducts (arrows) of postacetabular gland cells stain blue and their bodies (a) stain violet in AB-PAS; b — oral sucker, c — ventral sucker (×450).



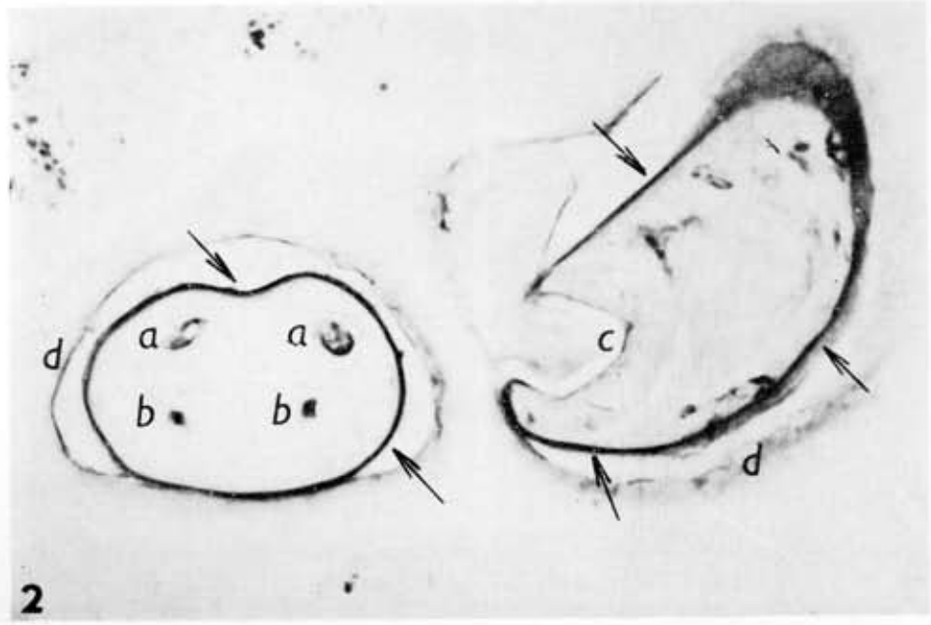
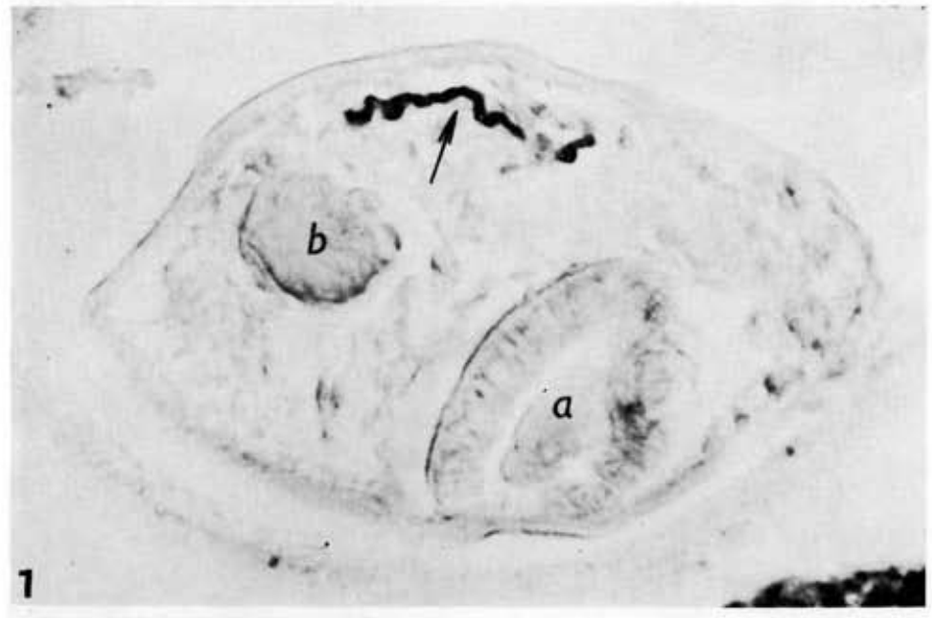


Fig. 1. Section through sporocyst and cercariae of *P. gallinum*. Tegument and postacetabular gland cells of cercaria (arrow) and inner border layer (double arrows) of sporocyst wall are intensively stained with PAS; a — oral sucker, b — ventral sucker, c — tail (×250). **Fig. 2.** Section through *P. gallinum* cercariae with intensively stained tegument (arrows), ceca (a) and ducts of postacetabular gland cells (b); c — oral sucker, d — sporocyst wall (PAS) (×550).



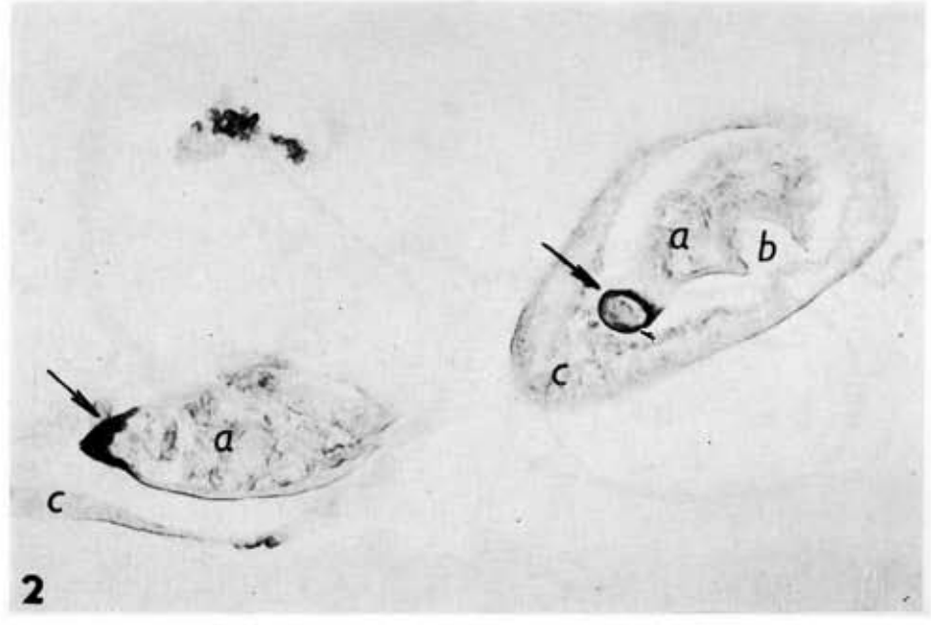


Fig. 1. Fully formed *P. gallinum* cercaria exhibits alkaline phosphatase activity only in collecting excretory canals (arrow); a — ventral sucker, b — genital anlage (α-naphthyl phosphate + Fast blue BB) (×900). **Fig. 2.** In *P. gallinum* cercaria only tail tegument exhibits the activity of non-specific esterase (arrows); a — body of cercaria, b — ventral sucker, c — sporocyst wall (α-naphthyl acetate + HPR) (×280).