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HISTOCHEMISTRY OF CERCARIAE AND SPOROCYSTS OF DICROCOELIUM LANCEATUM

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Abstract. The cercariae of *D. lanceatum* Stiles et Hassal, 1896 belonging to the group of nonvirgulate xiphidiocercariae possess three types of gland cells — proper cystogenic, penetration and ventral. The proper cystogenic gland cells are well developed and contain neutral mucosubstances, acid mucosubstances with carboxyl groups and sulphogroups, proteins with SH-groups and tyrosine. They have a high activity of acid phosphatase and non-specific esterase. The penetration gland cells contain mainly proteins with tryptophan and neutral mucosubstances. Feebly developed ventral gland cells give positive reaction for acid mucosubstances and proteins with SS-groups. Sporocyst walls contain glycogen and hydrophobic lipids and exhibit an activity of alkaline phosphatase.

This paper is a part of complex investigations of *D. lanceatum* larvae following the histochemical study published by Žďárská and Panin (1977).

MATERIAL AND METHODS

The material was obtained from naturally infected snails *Bradybaena lantzi* in pastures in the vicinity of Alma-Ata. The collection was carried out in cooperation with the Zoological Institute of the Academy of Sciences of the Kazakh S. S. R. After removal of the shell the snails were fixed in Baker's solution at 4 °C for 24 h for the detection of proteins, mucosubstances and lipids. The material was then washed in distilled water and embedded in paraffin or gelatine. For the detection of enzymes the material was fixed in Baker's solution at 4 °C for 2 h and washed in 5 % sucrose at 4 °C for 2 h. A part of the material was then put into Holt's sirup and cut with freezing microtome. The other part was embedded using a shortened method through cold acetone and benzene to paraffin. Histochemical detection of mucosubstances, proteins and lipids was carried out by the methods given in the paper by Žďárská et al. (1978) and enzymes were detected by the methods given in the paper by Žďárská and Panin (1977).

RESULTS

A. Cercaria. A major part of body of a fully developed cercaria is filled with proper cystogenic gland cells (Plate I, Fig. 2; Plate II, Fig. 1). Their content stains orange with Mallory's phosphotungstic haematoxylin, blue with Mallory's trichrome and yellow with Van Gieson's method.

At the histochemical detection of mucosubstances these cystogenic gland cells are strongly PAS-positive and the PAS-reaction is resistant to salivary test. Also the reaction with AB is strongly positive. After demethylation, and β-glucuronidase pretreatment this reaction is much weaker but not quite negative (Table 1). Consequently, acid mucosubstances both with sulphogroups and carboxyl groups are involved. At simultaneous detection of neutral and acid mucosubstances using AB-PAS method (Plate II, Fig. 1), the contents of ducts stain differently from those in the bodies of these cells. The ducts give PAS-positive, whereas the bodies AB-positive reaction. This indicates that neutral mucosubstances prevail in the ducts and acid mucosubstances in the bodies of cells. Of the reactions for proteins, strongly

positive were the methods for the detection of SH-groups and the coupled tetrazonium method for simultaneous detection of tyrosine, tryptophan and histidine. The contents of cystogenic gland cells were negative at the detection of hydrophobic lipids, and at the detection of hydrophilic lipids only walls and nuclei of these gland cells were positive. The bladder-like nucleus of each gland cell gives a feeble positive Feulgen's reaction. The contents of proper cystogenic gland cells both in the bodies and ducts exhibit a high activity of acid phosphatase and non-specific esterase.

Table 1. Results of histochemical reactions of gland cells of cercaria

Reactions	Gland cells		
	proper cystogenic	penetration	ventral
PAS	+++++	++	–
Schiff	—	–	–
Saliva test + PAS	+++++	++	–
Acetylation 58 °C, 48 h + PAS	–	–	–
Desacetylation + PAS	+++++	++	–
AB – PAS	blue – red	–	blue
AB pH 2.6	+++++	–	+++
+ methylation	–	–	–
+ demethylation	++	–	–
Hyaluronidase + AB	+++++	–	+++
β-glucuronidase + AB	+	–	–
Neuraminidase + AB	+++++	–	+++
Morel-Sisley	++	++	–
DMAB	–	+++++	–
Coupled tetrazonium reaction	+++++	++	–
DDD	red	pink	–
Thioglycollic acid + DDD	red	pink	pink
PFA-AB	+++++	–	+++++
AB pH 0.2	+++++	–	+++++
PAA—AF	+++++	+/++	+++++
Aldehyde-fuchsin	++	–	–
Fettrot	–	–	–
Sudan black B	–	–	–
OTAN	–	–	–
Alkaline phosphatase	+	–	–
Acid phosphatase	+++++	–	–
Nonspecific esterase	+++++	–	–

Penetration gland cells contain mainly proteins with tryptophan (Plate I, Fig. 1). The reactions for tyrosine and SH-groups are feebly positive. Of the methods for the detection of mucosubstances, only PAS reaction was positive. No activity of any of the above-mentioned enzymes was found in penetration gland cells. The ventral gland cells, present only in young cercariae, give a strongly positive reaction with AB pH 0.2 and peracetic acid-aldehyde fuchsin (Plate I, Fig. 2). They are AB negative after demethylation and β-glucuronidase pretreatment.

The suckers contain large amount of glycogen. The glycogen content in body parenchyma is lower. Positive reactions for hydrophilic lipids and cholinesterase activity were found in cerebral ganglion and main nerve trunks. The stilet contains proteins with SH-groups and tryptophan.

B. Sporocyst. The sporocyst wall contains a large amount of glycogen both in its narrowed

thick-walled and widened thin-walled portion. In younger sporocysts, the hydrophobic lipids are present in the whole wall, in old sporocysts only in the narrowed thick-walled portion (Plate II, Fig. 2). The reactions for hydrophilic lipids and proteins are very feeble. The wall of the whole sporocyst exhibits a high activity of alkaline phosphatase. In addition to it, the narrowed thick-walled portion has acid phosphatase activity in the tegument and non-specific esterase activity in large cells situated under the muscle layer.

DISCUSSION

The gland cells of *D. lanceatum* cercariae differ considerably in their morphology from other non-virgulate xiphidiocercariae of the order Plagiorchiata. The non-virgulate xiphidiocercariae possess usually four types of gland cells, namely penetration, ventral, dorsal and proper cystogenic gland cells (Dubois 1929, Kruidenier 1953, Kruidenier and Mehra 1957, Žďárská 1969, Erasmus 1972, Ginetsinskaya and Bhutta 1976). Each type has different histochemical properties. In the cercariae of *D. lanceatum*, there are two types of gland cells, penetration and proper cystogenic, which are fully developed. The third type, ventral gland cells, are rudimentary and are present only in young cercariae. They have never been described in this cercaria. The fourth type, dorsal gland cells, are completely lacking. The difference in the number of types of gland cells, i.e., the presence of only two types of fully developed gland cells in *D. lanceatum* cercariae, separates this species from the xiphidiocercaria of *Plagiorchis laricola* studied earlier (Žďárská 1969).

Histochemical studies revealed that the contents of proper cystogenic gland cells of *D. lanceatum* cercariae differ from those of *P. laricola* only in that they contain acid mucosubstances in addition to neutral mucosubstances and proteins with tyrosine and SH-groups. The enzymatic activity cannot be compared, because it was not studied in the cercariae of *P. laricola*. The secretion of the penetration gland cells differs in that it contains proteins only with tryptophan, tyrosine and SH-groups and not with SS-groups like cercaria of *P. laricola*.

The gland cells participating in the formation of the tegument of cercaria are reduced or completely lacking in *D. lanceatum* cercariae. The ventral gland cells are reduced and the dorsal gland cells are absent. In *P. laricola* cercariae, these two types of gland cells participate in the formation of the tegument of cercaria. At first the secretion of large ventral gland cells is released and then the secretion of the small dorsal gland cells which both are detectable in the tegument by histochemical methods. In the cercariae of *D. lanceatum*, the small amount of the secretion released from rudimentary ventral gland cells of young cercariae cannot be detected histochemically in the tegument using light microscopy. Consequently, the conclusions of Ginetsinskaya and Bhutta (1976) are valid only in that oligomerization of ventral gland cells (after Ginetsinskaya and Bhutta "mucoid glands") associated with their enlarging concerns only those species of the order Plagiorchiata which develop in water snails. Their secretion seems to perform a protective function in the tegument of free-swimming cercariae. In the other species of Plagiorchiata, which develop in terrestrial snails, the ventral gland cells do not increase but decrease in size. The oligomerization and decrease in size of ventral gland cells and complete absence of dorsal gland cells in the cercariae of *D. lanceatum* seem to be related with the fact that the cercariae do not move freely in the outer environment, but are relased from the terrestrial snail in the form of slime balls which are swallowed by ants. The secretion has thus lost its protective function during the evolution. At the time when *D. lanceatum* cercariae leave their first intermediate host the penetration gland cells remain preserved and

enable the cercariae to penetrate into the body cavity of the second intermediate host — the ant. Also the proper cystogenic gland cells, the secretion of which forms the wall of the cyst of metacercaria, are preserved.

It may be concluded that the gland cells participating in the formation of tegument, i.e. dorsal and ventral gland cells, which remained preserved in cercariae of Plagiorchiata developing in water snails gradually disappeared from the cercariae developing in terrestrial snails.

ГИСТОХИМИЯ ЦЕРКАРИЙ И СПОРОЦИСТ *DICROCOELIUM LANCEATUM*

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Резюме. Церкарии *D. lanceatum* Stiles et Hassal, 1896 относящиеся к группе ксифидиоцеркарий, имеют железистые клетки трех видов — настоящие цистогенные, клетки проникновения и вентральные клетки. Хорошо развитые настоящие цистогенные железистые клетки содержат нейтральные мукосубстанции, кислые мукосубстанции с карбоксильными группами и сульфогруппами, белки с SH-группами и тирозином и проявляют высокую активность кислой фосфатазы и неспецифической эстеразы. Клетки проникновения содержат большей частью белки с триптофаном и нейтральные мукосубстанции. Слабо развитые вентральные железистые клетки содержат кислые мукосубстанции и белки с SS-группами. Стены спороцист содержат гликоген и гидрофобные липиды и проявляют активность щелочной фосфатазы.

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Fig. 1. Penetration gland cells (arrows) in horizontal (a) and oblique dorso-ventral (b) section stained intensely using the method for the detection of tryptophan. DMAB method. (440 ×).
Fig. 2. Ventral gland cells (arrows) and proper cystogenic gland cells (a) in longitudinal dorso-ventral section through a young cercaria (right). b — oral sucker, c — ventral sucker. PAA-aldehydei fuchsin. (400 ×).

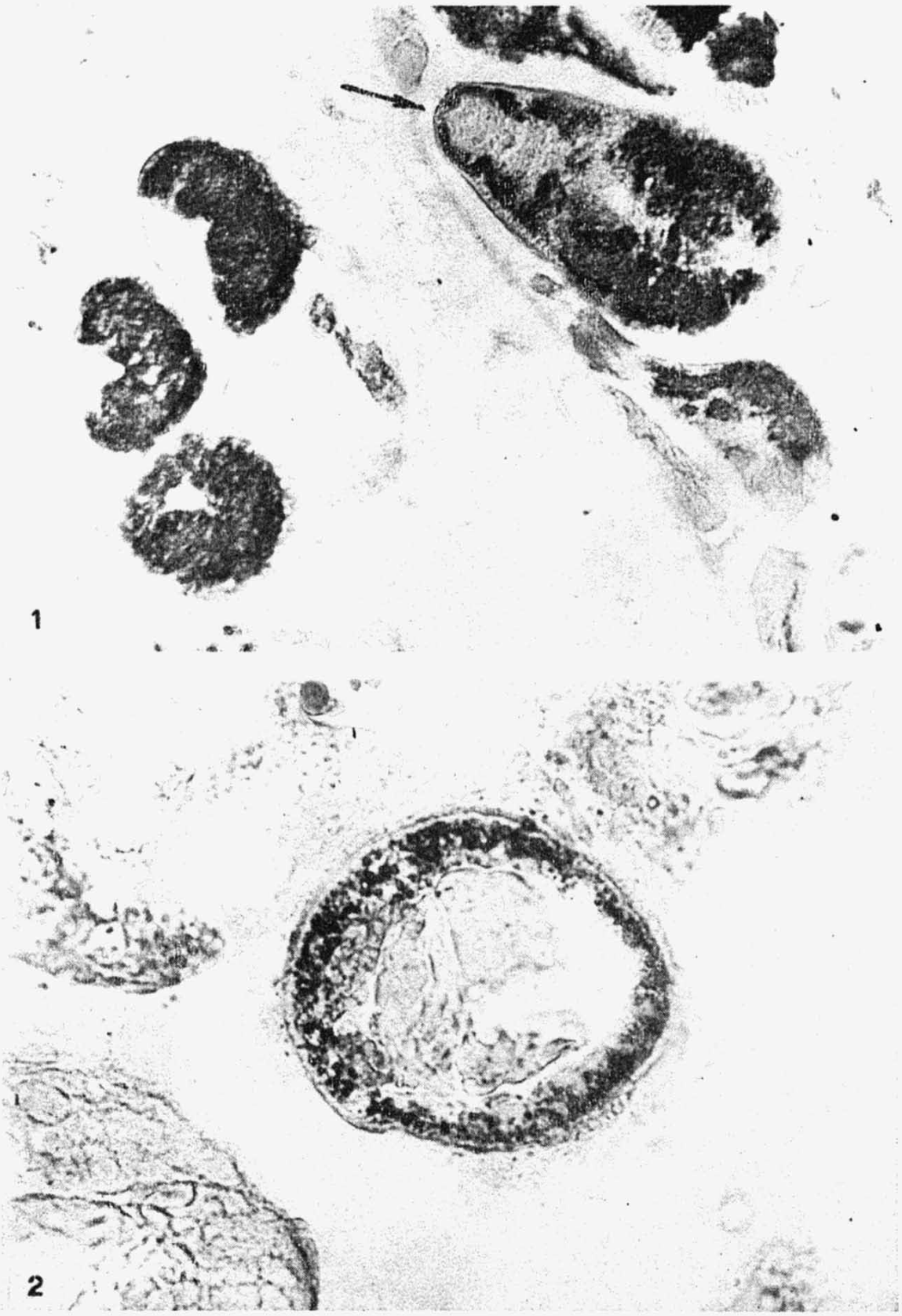

Fig. 1. Horizontal section (right at the top) through a cercaria with well visible proper cystogenic gland cells the ducts of which open into stilet capsule (arrow). On the left transverse section through bodies of cercariae with intensely stained content of proper cystogenic gland cells. AB-PAS. (200×.)
Fig. 2. Transverse section through narrowed thick-walled portion of sporocyst containing a large number of hydrophobic lipids. OTAN. (460×.)