HISTOCHEMISTRY OF THE SPOROCYST AND CERCARIA OF BRACHYLAIMUS FUSCATUS

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Abstract. The body and tail tegument of Brachylaimus fuscatus cercaria contains a great quantity of neutral mucousubstances and a small quantity of acidic mucousubstances with sulphogroups and proteins with tyrosine and cystine. The alkaline phosphatase activity is present in the tegument of very young cercariae, whereas in older cercariae, this enzyme can be detected only in collecting excretory canals. The digestive system of the cercaria is positive for neutral mucousubstances and proteins with tyrosine, tryptophan, cystine and cystine, and exhibits a high activity of acidic phosphatase. A system of four types of penetration gland cells was demonstrated in the cercaria of B. fuscatus. These are the dorsal, ventral and lateral gland cells containing a great amount of tryptophan, and postacetabular gland cells differing from the previous types in a high content of neutral and acid mucousubstances and in the absence of tryptophan.

This paper is a continuation of previous ones (Ždárská and Soboleva 1981a, b, Ždárská et al. 1984) dealing with the histochemistry of larvae of the superfamily Brachylaemoidae Allison, 1943. All of these papers are a part of complex investigations of the adaptation of these parasites to terrestrial conditions. The aim of these studies is to elucidate the functionally morphological peculiarities of these parasites in relation to other members of the order Strigeatoidea La Rue, 1926 (La Rue 1957) developing in water snails.

MATERIAL AND METHODS

The sporocysts with cercariae of Brachylaimus fuscatus (Bud., 1819) Babero, 1953 were obtained from naturally infected snails Ponsedienia duprionica (Martens, 1879) 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 mucousubstances, proteins and some enzymes are described in the papers by Ždárská and Panin (1977) and Ždárská et al. (1978).

RESULTS

A. SPOROCYST

The wall of the branched sporocyst consists of three layers — tegument with lamina basalis, muscle layer and inner limiting cellular layer. The tegument and inner limiting layer are strongly positive to PAS reaction even after saliva test and the inner limiting layer is also weakly positive to AB pH 2.6 and PAA-aldehyde fuchsin. The tegument and inner cellular layer in the narrowed part of sporocyst exhibit a low activity of alkaline phosphatase (Table I).

B. CERCARIA

The whole body of cercaria, including the cavities of oral and ventral suckers, and rudimental tail are covered with a tegument which is strongly PAS-positive even after saliva test (Plate II, Fig. 1) and exhibits a medium positive reaction to AB pH
2.6 which is negative after demethylation. It stains violet in AB-PAS method (Plate II, Fig. 2). Consequently, the tegument contains neutral mucopolysaccharides and acid mucopolysaccharides with sulphoglucours. The reactions for proteins (Table 1) are weak, stronger is the Moro-Sinley reaction for tyrosine and PAA-aldehyde fuchsin reaction for cystine. The tegument also the activity of alkaline phosphatase, but only in young, developing cercariae (Plate IV, Fig. 2). This enzyme is lacking in fully developed cercariae (Plate IV, Fig. 2).

The system of penetration gland cells opening in the oral sucker of B. fuscaus cercaria is very complex. Histochemical studies revealed four types of penetration gland cells, three of them (dorsal, ventral and lateral) containing a large amount of tryptophan. The dorsal gland cells (Plate II, Fig. 1) are localized on the dorsal side between pharynx and ventral sucker and open on the dorsal side of oral sucker, the ventral gland cells (Plate III, Fig. 2) occupy the ventral side immediately behind the oral sucker and open into the oral sucker on its ventral side, and the lateral gland cells are situated close to the oral sucker and open between the two above types. The fourth type of gland cells are the postacetal gland cells (Plate II, Fig. 2) localized behind the ventral sucker. Their long ducts run ventrally along intestinal branches (Plate I, Fig. 1) up to the pharynx and then they bend (Plate II, Fig. 2) to the dorsal side, run laterally and dorsally to prepharynx and penetrate into the oral sucker. They pass through its muscles up to its anterior margin where they open (Plate I, Fig. 1; Plate II, Figs. 1, 2). The postacetal gland cells histochemically differ from the other three types of gland cells. The dorsal, ventral and lateral gland cells contain a large amount of proteins with tryptophan, tyrosine and cysteine (Table 1), whereas the postacetal gland cells are strongly PAS-positive even after saliva test. There are also strongly positive to AB pH 2.6 and 0.6 reactions which are negative in the other three types of gland cells (Table 1). The bodies of all the four types of gland cells exhibit a weak activity of acid phosphatase, which is completely lacking in the ducts of dorsal, ventral and lateral gland cells. A strong activity of this enzyme, however, was detected in the ducts of postacetal gland cells (Plate I, Figs. 1, 2).

The whole digestive system of B. fuscaus cercaria, i.e., the tegument of oral sucker, prepharynx, pharynx and epithelium of intestinal branches, show a strong activity of acid phosphatase (Plate I, Figs. 1, 2). The tegument of pharynx and epithelium of intestinal branches are strongly positive to PAS even after saliva test (Table 1) (Plate II, Fig. 1), negative to AB pH 2.6 and weakly positive for proteins with tyrosine, tryptophan, cystine and cysteine (Table 1).

The genital anlage is strongly positive for tyrosine, tryptophan and cysteine and exhibits a weak activity of acid phosphatase (Table 1).

The suckers contain glycogen (PAS reaction negative after saliva test) and are weakly positive for tyrosine, tryptophan and cysteine. The collecting excretory canals exhibit a strong activity of alkaline phosphatase (Plate IV, Fig. 2).

**DISCUSSION**

In contrast to B. aequana (Zdárská and Soboleva 1980), the cercaria of B. fuscaus contains four types of penetration gland cells. The penetration gland cells in B. aequana cercaria are identical with the postacetal and dorsal penetration gland cells in B. fuscaus and exhibit identical histochemical properties. A strong positive reaction to PAS and AB pH 2.6 is characteristic of postacetal gland cells and the high content of tryptophan is characteristic of dorsal gland cells. The histochemical properties of the penetration gland cells of the two species differ in the
activity of acid phosphatase which was not detected in *B. aegypti* cecaria either in cell bodies or in their ducts.

A comparison of the glandular system in cecaria of *B. fusca* as a member of this order *Schistosoma mansoni*, which was studied in detail in the last time (Stirewalt and Krudenier 1961, Robson and Erasmus 1970, Ebrahimzadeh 1970, Ebrahimzadeh and Kraft 1971, Dorsey 1974, 1975, 1976) shows that the postacetabular penetration gland cells of the two species are identical in their histochimical properties and localization. The localization of the dorsal gland cells corresponds to the localization of preacetabular gland cells in *S. mansoni* cecaria, lateral gland cells of *B. fusca* cecaria correspond to escape glands in *S. mansoni* cecaria and ventral gland cells might correspond to head glands. The electron microscopic studies of the glandular system of *B. fusca* cecaria will certainly help elucidate this hypothesis. The secretion of the lateral gland cells is probably used, like the secretion of escape glands in *S. mansoni* cecaria, for the penetration through the sporocyst wall (Dorsey 1974). Like in *B. aegypti* and *S. mansoni* sporocysts, the birth pore has not yet been demonstrated in *B. fusca* sporocyst. The cecaria leaving the sporocyst must penetrate through its wall.

If the ventral penetration gland cells in *B. fusca* cecaria are identical with the head gland in *S. mansoni* cecaria, they should be preserved in the young metacercaria. They were demonstrated in the schistosomula of *S. mansoni* (Dorsey 1976). A definitive explanation of the function of individual gland cells in *B. fusca* cecaria requires further detailed studies of the cecariae leaving their first intermediate host and metacercariae developing in the second intermediate host. It might be interesting to detect what is the secretion of postacetabular gland cells used for, if the cecariae do not actively penetrate through the tissues of further host, as it is the case with *S. mansoni* schistosomulae. As it is known, the cecariae of the genus *Brachylaimus* enter the second intermediate host through natural openings and develop to metacercariae in the lumen.

**ГИСТОХИМИЯ СПРОКСИТИ И ПЕРКАРИИ BRACHYLAIMUS FUSCA**

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Резюме. Тип губка и хвостовая перкария Brachylaimus fusca содержит больший процент нуклеозидов и нуклеотидов по сравнению с более мелкими муксубутинами со значительно большей активностью фосфатазы С. В. Р. В. в просвечивающей световой микроскопии. Активность фосфатазы в исследованной поросятке составляет 25-30 усл. ед./хол/гл. Некоторые результаты обсуждены в свете предыдущих работ, проведенных в этой области.

**REFERENCES**


**NOTE TO THE LIFE CYCLE OF ORNITHODOROS DENMARKI**

On 4 April 1980, during our stay in Cuba, we collected 1 400 specimens of the argasid tick *Ornithodorus dammini* Kohls, Sonnenhine et Clifford, 1955 at Cayo Mono Grande, the province of Matanzas. The little island is the only known locality in the Cuban territory where this species was already found in 1972 (Cruz de la J., Foyoraya No. 129—3, 1974). Fifty adults were transported to Prague.

Out of 1 400 specimens available for virological testing 13 strains of Hughes' virus were isolated by C. D. H. D., V. V. P. et al., Acta virol. 29: 186—189, 1982. Apart from the said virus isolation 10 strains of *Soldanella* and *Rass* viruses (serogroup *Hughes*), *Johnston Toil* (serogroup *Quarantii*) and *Midway* (serogroup *Nyananki*) were isolated by other laboratories from this tick species (Clifford C. M., in: E. Kurstak (Ed.), *Arctic and Tropical Arboviruses*, Academic Press, New York—San Francisco—London, pp. 83—109, 1979). Due to these facts and insufficient knowledge of the taxonomy of this tick species we consider it expedient to present some data on its development, although obtained from scanty material.

Larvae were allowed to feed on one-day-old chicks, nymphs on chickens aged one week and adults — on a cock. The ticks were kept in glass tubes with gauze taupols at 25 ± 0,5 °C and relative humidity 75—80 % under technical parameters of the rearing box, with an additional short photo period (4 hours of light and 16 hours of darkness). The larvae had been fed on chickens 1—10 days, nymphs 1 after 10—14 days. These nymphs I metamorphosed into nymphs II without feeding (after 27—31 days), then after feeding for 30—60 minutes and following 17 to 20 days moulting into nymphs III. Thirty three out of 50 unfed larvae had hatched from egg batches laid by collected females during the transport, engorged at the beginning of May. Out of these unfed larvae, 10 nymphs II and 3 nymphs III were graduated. Further development could not be accomplished. Out of 8, 5, 7, allowed to feed at the end of July,
Fig. 1. Horizontal section through *B. fuscomaculatus* cercaria showing high activity of acid phosphatase in the tegument of prepharynx (a) and pharynx (b), in intestinal branches (c) and in ducts of postacetable penetration gland cells (arrow) opening at the anterior margin of oral sucker (d). (p-naphthylphosphate + HPR) × 900. Fig. 2. Transverse section through *B. fuscomaculatus* cercaria at level of oral sucker (a). Acid phosphatase activity is present in intestinal branches (b) and ducts of postacetable penetration gland cells (arrows) (p-naphthylphosphate + HPR) × 970.

Fig. 1. Longitudinal dorsoventral section through *B. fuscomaculatus* cercaria demonstrating a high content of neutral mucosubstances in body tegument, oral (a) and ventral (b) suckers, inner layer of intestinal branches (c), bodies (d) and ducts (arrow) of postacetable gland cells, and limiting layer of sporocyst wall (double arrow) (PAS) × 530. Fig. 2. Longitudinal dorsoventral section through *B. fuscomaculatus* cercaria stained by AB-PAS with well visible ducts of postacetable gland cells (arrows). a — oral sucker, b — ventral sucker (× 600).
Fig. 1. Horizontal section through body of *B. fuscatus* cercaria at level of dorsal penetration gland cells (arrows) showing the high content of tryptophan. a — oral sucker [DMAB method] (×250).

Fig. 2. Longitudinal dorsoventral section through *B. fuscatus* cercaria with well visible ventral gland cells (a) and their ducts (arrows) and two bodies of dorsal penetration gland cells (b). a — oral sucker, d — ventral sucker. [DMAB] (×600).

Fig. 1. Longitudinal section through *B. fuscatus* cercaria with well visible ducts of dorsal, lateral and ventral penetration gland cells (arrows). a — oral sucker, b — ventral sucker, c — genital anlage, d — rudimentary tail. [DMAB] (×600).

Fig. 2. Section through *B. fuscatus* cercariae of different age. Very young cercariae (a) exhibit high activity of alkaline phosphatase in the tegument, fully developed cercariae only in collecting excretory canals (arrows) and in anterior part of pharynx (b). c — oral sucker, d — ventral sucker. [σ-naphthylphosphate + Fast blue BB] (×400).