

DISTRIBUTION OF MUCOSUBSTANCES, PROTEINS, ENZYMEs AND LIPIDS IN THE TREMATODE *BRACHYLAIMUS AEQUANS*

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Dedicated to Prof. Dr. Zdeněk Lojda, Corresponding Member of the CAS, on the occasion of his 60th birthday

Abstract. The content of mucosubstances, proteins, lipids, and alkaline and acid phosphatases in *Brachylaimus aequans* has been determined by histochemical methods. The body tegument contains neutral mucosubstances, acid mucosubstances with carboxyl groups and proteins with tyrosine, tryptophan, cystine and cysteine. It does not exhibit any alkaline phosphatase and non-specific esterase activity. Acid phosphatase activity is present only in the tegument in the basal part of oral sucker cavity. The lining of the digestive system, i.e. the tegument of the basal part of oral sucker cavity and pharynx, and the basal layer of the caecal syncytium exhibit a high activity of acid phosphatase. The basal layer of caecal syncytium has a high accumulation of hydrophobic and hydrophilic lipids. The microvillous zone of caeca contains acid and neutral mucosubstances and proteins with tyrosine, tryptophan, cysteine and cystine. The metraterm and cell islets in testes have a high activity of acid phosphatase.

Brachylaimus aequans is the fourth of trematodes of the superfamily Brachylaimoidea subjected to histochemical studies, the previous ones being *Leucochloridiomorpha constantiae* (Harris and Cheng 1973a, b), *Hasstilesia ovis* (Soboleva and Ždárská 1983) and *Postharmostomum gallinum* (Nacheva and Osipovskaya 1985). Whereas *L. constantiae*, both larvae and adults, are still bound to water environment, the following three trematodes are connected with the terrestrial environment through their intermediate and definitive hosts. Adults of *L. constantiae*, *P. gallinum* and *B. aequans* develop from the metacercariae which grow but do not encyst inside the second intermediate host, whereas the adults of previously studied *H. ovis* develop from the metacercariae not leaving the sporocyst and the first intermediate host. The aim of the present paper is to elucidate what are the differences between the trematodes bound to terrestrial hosts and those bound to hosts living in water environment, to what extent the life cycle affects the structure of the adults. The studies are a part of complex investigations of the morphology, histochemistry and ultrastructure of trematodes performed in cooperation with the Academy of Sciences of the Kazakh S.S.R. It is a continuation of the paper by Soboleva and Ždárská (1983) and is intended to serve as a basis of further biochemical and ultrastructural studies of adult trematodes of the superfamily Brachylaimoidea.

MATERIALS AND METHODS

Adults of *B. aequans* at the age of 6—8 days were obtained from experimentally infected white laboratory mice. The used histochemical methods for the detection of mucosubstances, proteins, enzymes and lipids were described in a previous paper (Ždárská et al. 1978). More than 25 histochemical methods with the respective controls were used.

Table 1. Histochemistry of the trematode *Brachylaimus aequans*

Reaction	Tegument		Paren-chyma	Suckers	Intestine			Reproductive organs			Excre-tory system wall
	outer layer	inner layer			muscle layer	basal layer	micro-villi	testes	ova-rium	uterus	
PAS Schiff	+++++	+++	+++++ ¹	-	+	-	-	+++ ²	-	+	-
Saliva test + PAS	+++++	+++	-	-	-	-	-	+++ ²	-	-	-
Acetylation + PAS	-	-	+++++	rose/red	+	-/+	-	+++ ²	-	-	-
Desacetylation + PAS	+++++	violet	+++++	red	-	-	-	+++ ²	-	-	-
AB — PAS	+++	+++	+++++	rose/red	+	+++	violet	+++ ²	-	-	-
AB pH 2.6 + methylation	+++	+++	+++++	red	-	-	-	+++ ²	-	-	-
+ demethylation	+++	+++	+++++	+	++	++	++	+++ ²	-	-	-
Morel-Sisley	+++	+++	+++++	+	++	++	++	+++ ²	-	-	-
DMAB	+++	+++	+++++	+	++	++	++	+++ ²	-	-	-
DDD	+++	+++	+++++	+	++	++	++	+++ ²	-	-	-
Thioglycolic acid + DDD	+++	+++	+++++	+	++	++	++	+++ ²	-	-	-
PA + aldehyde fuchsin	++	+	-	-	-	-	-	+++ ²	-	-	-
Aldehyde fuchsin	-	-	-	-	-	-	-	+++ ²	-	-	-
Alkaline phosphatase (α -naphthyl phosphate + Fast blue BB)	-	-	-	-	-	-	-	+++ ²	-	-	-
Acid phosphatase (α -naphthyl phosphate + HPR)	-	-	-	-	-	-	-	+++ ²	-	-	-
Sudan Black B (glycerine jelly)	+++	+++	+++++	+	++	++	++	+++ ²	-	-	-
Sudan Black B (paraffin)	+++	-	+++	black ³	black ³	-	black ³	+++ ²	-	-	-
Luxol Fast blue	+++	-	+++	black ³	black ³	-	black ³	+++ ²	-	-	-
OTAN	rose	rose	blue ³	blue ³	blue ³	-	blue ³	blue ³	-	-	-
Acid haematein	blue	blue	blue ³	blue ³	blue ³	-	blue ³	blue ³	-	-	-

Explanations:

¹ — particularly around the excretory system and caeca² — only the metraterm³ — droplets⁴ — substance between the droplets⁵ — only eggs in the vicinity of ootype⁶ — only cell islets⁷ — only the basal part of tegument of oral sucker cavity and around pharynx.

RESULTS

The tegument of *B. aequans* (Table 1) contains a great amount of PAS-positive, saliva test-resistant substances, acid mucosubstances with carboxyl groups, and proteins with tyrosine (Plate I, Fig. 1), tryptophan, cystine and cysteine. Among the methods for detection of hydrophilic lipids, the reactions with Sudan black B (Plate I, Fig. 2), acid haematein, Luxol blue and OTAN were strongly positive (see Table 1). The spines in the tegument were positive for hydrophilic lipids and proteins with tyrosine, cysteine and cystine. The body tegument of the trematode did not contain any of the detected enzymes (Table 1). A high acid phosphatase activity was demonstrated only in the tegument of the basal part of oral sucker cavity.

The parenchyma and suckers of this trematode contain mostly glycogen and hydrophobic and hydrophilic lipids. The strongly developed muscles of suckers are weakly positive for tyrosine, tryptophan, cysteine and hydrophilic lipids (Plate I, Fig. 2). A great amount of hydrophobic lipids is situated between the muscle fibres. The walls of canals of the excretory system exhibit a high activity of alkaline phosphatase. Glycogen is concentrated in the parenchyma around the excretory canals and around intestinal branches.

The digestive system consisting of the oral sucker cavity, pharynx and caeca exhibits a high activity of acid phosphatase in the tegument and gland cells in the basal part of oral sucker, in the tegument of pharynx and in the basal layer of caecal syncytium (Plate II, Fig. 1). The basal layer of intestinal epithelium contains a large number of droplets of hydrophobic lipids. The substance between these droplets is strongly positive for hydrophilic lipids (Plate I, Fig. 2) and tyrosine (Plate I, Fig. 1). The microvillous zone of caecal syncytium contains a great amount of neutral mucosubstances (PAS-positive reaction after saliva test) and acid mucosubstances, and shows a medium positive reaction for proteins with tyrosine and tryptophan.

Of the reproductive organs, the metraterm exhibits most pronounced histochemical reactions (Table 1), whereas the remaining part of uterus wall does not stain in the used histochemical methods. The metraterm exhibits acid phosphatase activity and contains PAS-positive, saliva test-resistant neutral mucosubstances, tyrosine and tryptophan. The testes and ovary give reactions with a low degree of intensity for proteins with tyrosine and tryptophan. Only single cell islets in the testes exhibit a high activity of acid phosphatase. The ovary shows a low activity of alkaline phosphatase. The vitelline cells and egg shell (only immediately behind the ootype) stain intensively with aldehyde fuchsin, in the methods for the detection of tyrosine, cystine and cysteine, and in the methods with Sudan black B for the detection of lipids. The shell of older eggs cannot be stained by any of the used histochemical methods.

DISCUSSION

The histochemistry of *B. aequans* adults has not yet been studied. In contrast to other members of Strigeatoidea La Rue, 1957, in which alkaline and acid phosphatases were demonstrated in the tegument Erasmus and Öhman 1963, Öhman 1965, 1966a, b, Bogitsh 1966a, b, Wheater and Wilson 1976), the body tegument of *B. aequans* and *H. ovis* (Soboleva and Žádorská 1983) does not contain any of these enzymes. Only the tegument of the basal part of oral sucker cavity, which is regarded as a part of the digestive system, exhibits acid phosphatase activity. Since also the cells in this part of oral sucker contain acid phosphatase, we assume that they perform a similar function as the holdfast organ of Strigeidae. The absence of alkaline phosphat-

ase activity in the tegument of the two trematode species may be explained by the fact that, in relation to their body size, their intestinal branches are enormously developed and therefore the transport of nutritive substances through the tegument is unnecessary.

In contrast to *H. ovis* (Soboleva and Ždárska 1983), the tegument of *B. aequans* is strongly positive to PAS reaction for neutral mucusubstances. This was observed also in other trematodes (Harris and Cheng 1973a, Threadgold 1976).

The microvillous zone of caeca in *B. aequans*, like in *H. ovis* and other trematodes (Reznik 1962, Sharma 1979), contains neutral mucusubstances and acid mucusubstances with carboxyl groups, but does not exhibit any activity of alkaline phosphatase. The basal layer of caecal syncytium in *B. aequans*, in contrast to *H. ovis*, shows a high activity of acid phosphatase. The activity of this enzyme in intestinal branches was demonstrated in all hitherto studied trematodes (Reznik 1963, Erasmus and Öhman 1963, Öhman 1965, 1966a, b, Threadgold 1968, Bogitsh 1968, 1972, Bogitsh et al. 1968, Dike 1969, Gupta 1970, Davis and Bogitsh 1971, Trimble et al. 1971, Ernst 1975, Nizam et al. 1975, Srivastava and Gupta 1975, Nacheva 1977, Sharma 1977), with the exception of *Schistosomatium douthitti* (Bogitsh 1975, Bogitsh and Shannon 1971).

The basal layer of caecal syncytium in *B. aequans* is characterized not only by a high acid phosphatase activity, but also by a large amount of proteins with tyrosine, tryptophan, cysteine and cystine, and, particularly, by a large amount of both hydrophobic and hydrophilic lipids. A high lipid content in the intestinal wall was described also by Harris and Cheng (1973b) in the trematode *L. constantiae* of the superfamily Brachylaimoidea and in other trematodes (Parshad and Guraya 1976, Yusufi and Siddiqui 1976, Frayla and Smyth 1983, Haseeb et al. 1984). These hydrophobic lipids seem to be the end products of glycolysis and are excreted from the organism (Smyth and Halton 1983) in form of the so-called excretory lipids.

The histochemistry of trematode genital organs is relatively little known. Attention has been paid particularly to vitelline cells and formation of eggs and their shells (Burton 1963, Guraya 1970, Sharma 1976, Kanwar and Agrawal 1977, Ginetinskaya and Lyukshina 1979, Smyth and Halton 1983). Further detailed studies of the spermatogenesis at the cytochemical level will be necessary for elucidating the presence of acid phosphatase in cell islets in the testes of *B. aequans*.

The main difference between the histochemistry of *B. aequans* and *H. ovis* is the higher activity of acid phosphatase and absence of alkaline phosphatase in the intestine of *B. aequans*, and lower concentration of neutral mucusubstances in the tegument, absence of acid phosphatase in the anterior part of digestive system and very low activity of acid phosphatase in the caeca of *H. ovis* (Soboleva and Ždárska 1983).

A comparison of the histochemistry of individual developmental stages of *B. aequans* reveals that the excretory system in all developmental stages, i.e. in the cercaria (Ždárska and Soboleva 1980), metacercaria (unpublished) and adult exhibits a high alkaline phosphatase activity. In the tegument and intestine, however, the activity of this enzyme is present only in very young cercariae. Acid phosphatase activity in the digestive system occurs first in the young cercaria, only in the anlage of intestine. In the fully developed cercaria and metacercaria, like in the adult trematode, this enzyme is present already in the whole digestive system, i.e. in the lining of the oral sucker cavity, pharynx and caeca. As to the glycogen, its amount gradually increases in individual developmental stages, particularly in the parenchyma and suckers. The tegument of fully formed cercaria, metacercaria and adult contains a large amount of neutral and acid mucusubstances.

РАСПРЕДЕЛЕНИЕ МУКОСУБСТАЦИЙ, БЕЛКОВ, ФЕРМЕНТОВ И ЛИПИДОВ У ТРЕМАТОДЫ *BRACHYLAIMUS AEQUALIS*

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Резюме. С помощью гистохимических методов изучали содержание мукосубстанций, белков, липидов, щелочной и кислой фосфатаз и неспецифической эстеразы у trematodes *Brachylaimus aequans*. Тегумент тела содержит нейтральные мукосубстанции, кислые мукосубстанции с карбоксильными группами и белки с тирозином, триптофаном, цистином и цистеином. Активность щелочной фосфатазы и неспецифической эстеразы не обнаружена. Активность кислой фосфатазы установлена только в tegumente базальной части полости ротовой присоски. Выстилка пищеварительной системы, т. е. tegument базальной части ротовой присоски с глоткой и базальный слой синцития ветвей кишечника проявляют высокую активность кислой фосфатазы. Базальный слой синцития ветвей кишечника содержит также большое количество гидрофобных и гидрофильных липидов. В зоне микроворсинок кишечника выявлены кислые и нейтральные мукосубстанции и белки с тирозином, цистеином и цистином. В метратерме и в островках клеток в семенниках отмечали высокую активность кислой фосфатазы.

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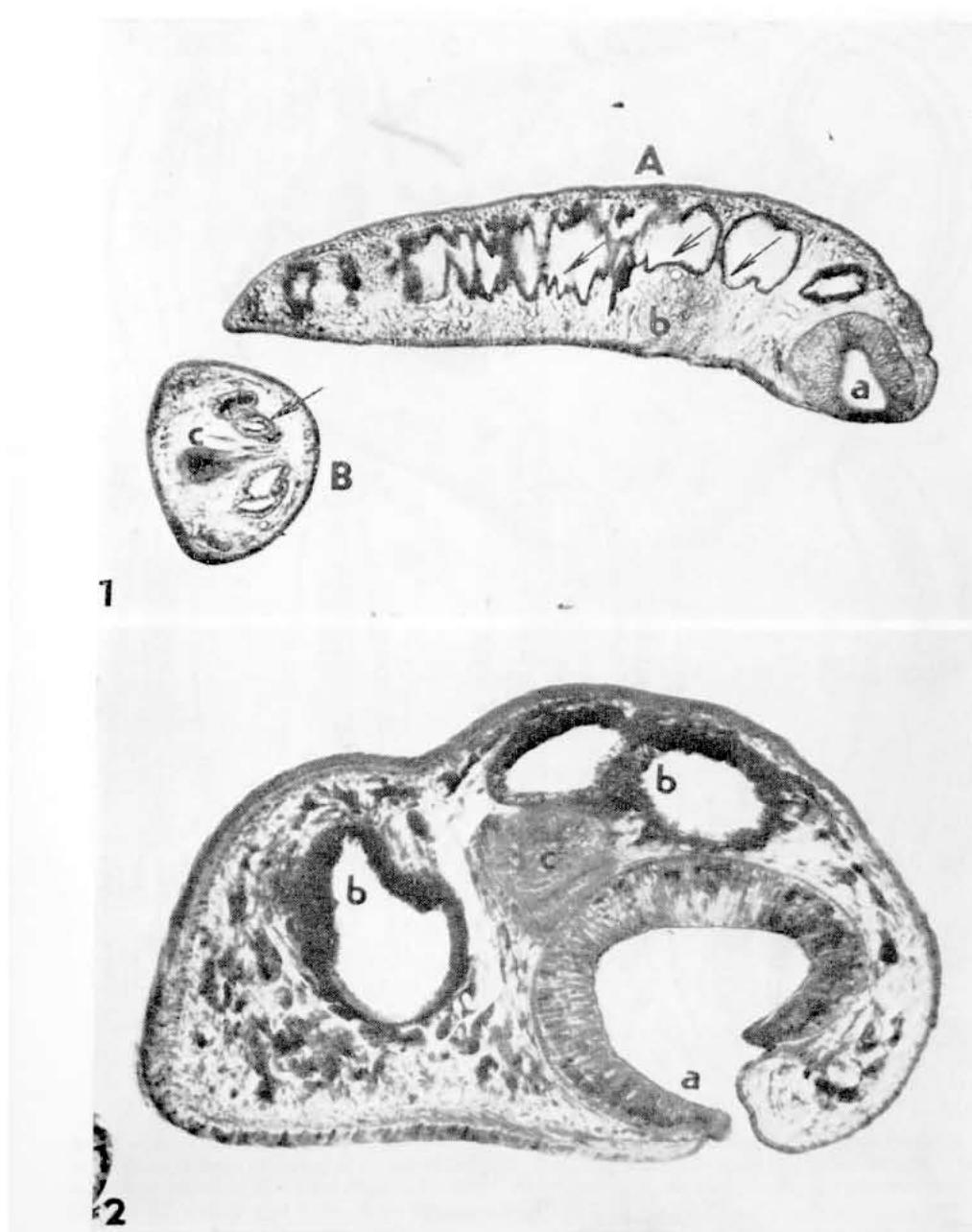


Fig. 1. Longitudinal dorso-ventral (A) and transverse (B) section through the body of *B. aequans* demonstrating the high content of tyrosine in the syncytium of caeca (arrows); a — oral sucker, b — ventral sucker, c — pharynx. Morel—Sisley ($\times 150$). Fig. 2. Section through the anterior part of body of *B. aequans*. Tegument, oral sucker (a) and particularly the syncytium of caeca (b) contain a large amount of phospholipids; c — pharynx. Sudan black B (paraffin) ($\times 280$).

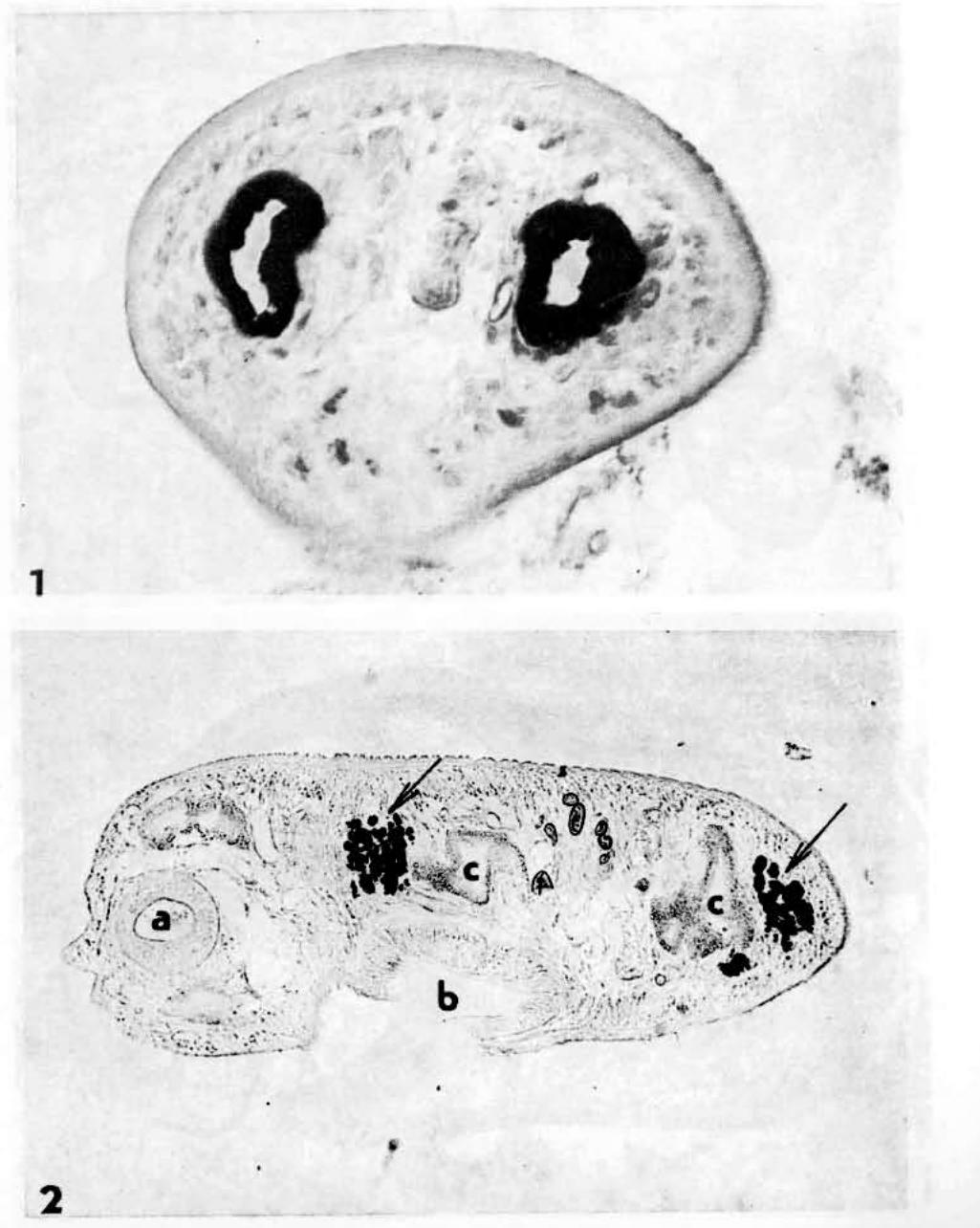


Fig. 1. Transverse section through the body of *B. aequans* demonstrating the high acid phosphatase activity in the syncytium of caeca. α -naphthyl phosphate + HPR ($\times 280$). Fig. 2. Longitudinal dorso-ventral section through the body of *B. aequans* demonstrating the high affinity of aldehyde fuchsin to the vitelline cells (arrows); a — pharynx, b — ventral sucker, c — intestine. Lugol + aldehyde fuchsin ($\times 180$).