HISTOCHEMISTRY OF THE TREMATODE HASSTILESIA OVIS (TREMATODA: HASSTILESIIDAE)

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Abstract. Trematodes H. ovis collected from infected sheep were studied by means of 20 histochemical methods and the contents of mucous substances, proteins and enzymes were demonstrated. The body tegument contains neutral mucous substances, acid mucous substances and proteins with tyrosine, tryptophane and SH and SS groups. It does not exhibit any activity of alkaline and acid phosphatase and non-specific esterase. A large amount of tyrosine is present in the esca, vitellaria and gonada. Hydrophilic lipids were detected in body tegument and vitellaria, hydrophobic lipids in the basal layer of intestine, audhca, pharynx and gonada. The muscular and basal layers of intestine contain proteins with tyrosine, tryptophan, cystine and cysteine. The microvilli exhibit a strong reaction for alkaline phosphatase and acid mucous substances and a weaker reaction for neutral mucous substances. In contrast to the metacercaiae of H. ovis, which are poor in proteins, carbohydrates and enzymes, adult trematodes contain all substances necessary for parasitizing in the intestine of the definitive host.

The trematodes H. ovis parasitize in the small intestine of sheep and goats in mountainous pasturelands in Kazakhstan and Kirghizia. The intensity of infection is even 50—60 % in some of the sheep-breeding farms. In spite of the fact that the trematodes are very small, about 1.00 mm, they are so numerous that the mucosa of the small intestine, particularly its middle part, is completely covered with the parasites. The presence of 10—12 thousand worms necessarily evokes a question of the negative effect on the organism of the host.

The pathogenicity of H. ovis and the changes in the sheep intestine caused by this trematode had not been studied until recently. In all manuals available only a catarrhal inflammation of intestine was mentioned. As it has been recently demonstrated, the changes induced by this parasite are very specific. Vsevolodov and Gvоздев (1976) described pathomorphological changes in the intestine of Ochotona rutillus caused by a relative species H. ochotoneae. It was found that the parasites penetrating among the villi of the mucosa induce hyperplastic changes manifesting themselves in the increase and proliferation of cells in the epithelium of villi and crypts.

Vsevolodov and Soboleva (1981), who studied the pathomorphology of intestine in sheep infected with H. ovis and compared it with that in Ochotona rutillus, came to the conclusion that a common feature is the tendency to the development of a productive type of inflammatory reaction in the mucosa. The reactions in sheep, however, are much stronger. The pathological process induced by H. ovis in the sheep intestine is characterized by desquamative and atrophic changes in the intestinal epithelium with marked infiltration and proliferation, which prevail. Even these preliminary results suggest the pathogenic role of the trematodes and the disease caused by them — hasstilesiasis.

This paper is a part of complex studies on hasstilesiasis. The elucidation of histochemical peculiarities of H. ovis is significant for the analysis of the parasite-host relationship and for recommendation of measures in the control of this disease.
## MATERIAL AND METHODS

The trematodes were collected from infected sheep in the collective farm “Barneveld” in the Aza region. The histochemical methods used for the demonstration of mucous substances, proteins and enzymes were described in a previous paper (Zdarská et al. 1978). About 20 histochemical tests were used.

## RESULTS

It was demonstrated that the body tegument of the trematodes contains neutral mucous substances, acid mucous substances and proteins, tyrosine, tryptophan and SH and SS groups (Table 1). The body wall consists of three layers: tegument with basement membrane, muscular layer and inner cellular layer. The tegument does not exhibit alkaline phosphatase activity (Plate II, Fig. 2). It is PAS positive, salivary test resistant and gives a strong reaction for tyrosine (Plate I, Fig. 2) and tryptophan and weak reaction for SH and SS groups. The layer of circular and longitudinal muscles contains glycogen and is positive for proteins, mainly for tyrosine. The oral and ventral suckers were found to contain proteins with tyrosine, tryptophan, cysteine and cystine. A large amount of tyrosine was found in the eggs and in vitellaria (Plate I, Fig. 2) and gonads. Hydrophilic lipids were detected in the body tegument, intestinal wall and vitellaria (Plate II, Fig. 1), hydrophobic lipids were found in the basal layer of intestine, in suckers, pharynx and gonads. The muscular and basal layers of intestinal branches contain proteins with tyrosine (Plate I, Fig. 2), tryptophan, cysteine and cystine (Table 1). The layer of microvilli gives a strong reaction for acid mucous substances and a weaker one for neutral mucous substances and exhibits a high activity of alkaline phosphatase (Plate II, Fig. 2) and a lower activity of acid phosphatase. The body parenchyma contains the largest amount of glycogen, mainly between the oral and ventral suckers and around the gonads (Plate I, Fig. 1). It is characteristic that the egg shell of trematodes gave no positive reaction in any of the methods used.

The miracidia inside the eggs contain glycogen, proteins and phospholipids.

## DISCUSSION


The tegument of *H. ovis*, in contrast to other trematode species (Reznik 1962, Harris and Cheng 1973, Panin and Zdarská, in press), gives a very weak PAS reaction, salivary test resistant and gives a weak reaction with alcin blue.

More distinct was the reaction for tyrosine and tryptophan. The reactions for acid and alkaline phosphatase and non-specific esterase were negative. The parenchyma contains a large amount of glycogen, particularly in the anterior part of body between the two suckers and in posterior part around the gonads. A smaller amount of glycogen was demonstrated in suckers. The intestinal branches give positive reactions for tyrosine, cysteine and cystine, contain a large amount of hydrophobic lipids and exhibit a high activity of alkaline phosphatase and lower activity of acid phosphatase, which plays a role in the transport of nutrients through the wall of intestine into the body parenchyma.

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### Table 1. Histochemistry of the trematode Heterobothia ovis

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1) only in distal part of body round the reproductive organs.
The high lipid content in the intestinal wall corresponds to the literary data (Harris and Cheng 1973 a). Most probably they are the endproducts of glycolysis and are excreted from the organism as excretory fat.

The activity of some enzymes in the digestive system of trematodes was demonstrated by several authors (Bogitsh 1972, Bogitsh et al. 1968, Bogitsh and Shannon 1971, Davis and Bogitsh 1971 a, b). Our results confirm it.

Comparing the adult specimens of H. ovis with the cercariae and metacercariae of the same species, which are very poor in proteins, carbohydrates and enzymes (Zdarska and Soboleva 1981), it may be concluded that the adult trematodes H. ovis possess all substances necessary for the parasitism in the organism of the definitive host.

ГИСТОХИМИЯ ТРЕМАТОД HASTILIESIA OVIS (ТРЕМАТОДА: HASSTILESIIDAE)
T. N. Soboleva and Z. J. Jaraska

Резюме. Трематоды H. ovis собранные от зараженных овец были использованы для гистохимического исследования. При помощи 20 гистохимических тестов выявлено содержание мукобицинов, белков и энзимов. Замечено, что тегумент тела содержит высокую концентрацию мукобицинов, кислое мукобицинов, протеин с тирозином, триптофаном и SH и SS группами. Тегумент тела не проникает скелетной фосфатдной активность. Тиранм в значительном количестве находится в лихим тегумент, более в железнича и в нейонках. Гидрофильные и гидрофобные — в базальном слое кишечника, в присосках, фаринкс и гонадах. Мышечный и базальный слой кишечника содержат протеин с тирозином, триптофаном, цистином и цистеином. Микроволна имеет селена реакцию на железы, более на нейонках. Выстилающий слой кишечника показывает высокую активность скелетной фосфатдной активности. Таким образом, по сравнению с метацеркарпами H. ovis, которые без белков, углеводов и ферментов, макро содержат все необходимые вещества для паразитирования в кишечнике овечьего хозяина.

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Fig. 1. Oblique section through posterior part of body of *H. oesi*. The parenchyma around the gonads contains a large amount of glycogen. PAS (×170). Fig. 2. The tegument, vitellaria (right and left) and intestine (middle) of *H. oesi* are strongly positive for tyrosine in Merel-Svoboda's test. (× 400).

Fig. 1. The intestinal wall (at the bottom) and vitellaria (right and left) are most strongly positive for hydrophilic lipids in Sudan Black B (paraffin) method. (×350). Fig. 2. Longitudinal section through a part of *H. oesi* body. Only intestinal branches exhibit a high activity of alkaline phosphatase: x - naphthyl phosphate + Fast blue BB (×270).