

HISTOCHEMISTRY OF THE HAPTOR SCLERITES FOR SOME SPECIES OF THE GENUS *TETRAONCHUS* DIESING, 1850 (MONOGENOIDEA)

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Abstract. Histochemical methods disclosed the presence of proteins in sclerites of the haptor in three species of the genus *Tetraonchus* Diesing, 1850. The basal part of the anchors, and the connecting bar, contained proteins with tyrosine, tryptophan, SH and SS groups. The marginal hooks contained proteins with SH and SS groups, while tyrosine was present in one part of these hooks only, i.e., in the shaft. Histochemical reactions for neutral and acid mucosubstances were negative.

In addition to other species, three species of the genus *Tetraonchus* Diesing, 1850, namely *Tetraonchus rogersi* Ergens, 1971, *T. roytmani* Strelkov, 1963 and *T. spasskyi* Strelkov, 1963, were obtained from the gills of *Brachymistax lenok* (Pall.) (Salmonidae, Clupeiformes) caught in 1966 on the territory of the Mongolian People's Republic. The sclerites of their haptors were studied with histochemical methods.

MATERIALS AND METHODS

The parasites together with the tissue of the fins were fixed in neutral 4% formaldehyde and embedded in paraffin; serial sections were stained with standard histological and specific histochemical methods for the demonstration of proteins and mucosubstances. Of the proteins, SH groups were detected histochemically with the DDD test (2,2-dihydroxy-6,6-dinaphthylsulphide) using control blocking with N-ethylmaleimide. SS groups were detected with peracetic acid and aldehyde fuchsin in a modification of the PAA-Schiff test (Pearse 1968). Tyrosine was detected with Morel-Sisley's diazotization method modified by Lillie (1957), tryptophan with DMBA (dimethylamino-benzaldehyde). The group test for tyrosine, histidine and tryptophan was performed with the coupled, tetrazonium reaction.

For the histochemical detection of mucosubstances, we used the PAS reaction combined with acetylation and desacetylation for neutral mucosubstances, and Alcian blue (Alcianblau 8 GS, Fluka) pH 2.6, for acid mucosubstances. For the contemporary confirmation of neutral and acid mucosubstances, we used Mowry's modification of the AB-PAS reaction. All methods were performed as suggested by Pearse (1968).

RESULTS

1. Anchors and connecting bar. Both the ventral and dorsal anchors consist of two parts, i.e., the point and the basal part (Ergens 1971). We found considerable differences in the histochemical reactions of both parts (Table 1). The point did not react for either proteins or mucosubstances in any of the histochemical tests used. It was stainable only with picric acid in van Gieson's method. The basal part was positive

for proteins. The reaction for tyrosine was highly positive (Plate I, Fig. 1), that for tryptophan feebly positive. The entire basal part gave a highly positive reaction for SS groups (PAA-aldehyde fuchsin) (Plate II, Fig. 1); the reaction for SH groups (DDD-test) was highly positive (di-coupling) inside the basal part only, while no reaction was given by the thin superficial layer covering the basal part.

This indicates that the basal part of the anchors contains basic proteins with tyrosine and tryptophan, and proteins with SH and SS groups. The same histochemical composition was disclosed in the connecting bar (Table 1).

On the surface of the basal part of the anchors and the connecting bar it was possible to distinguish histochemically superficial areas for the attachment of the muscles. These areas reacted positively for PAS and aldehyde fuchsin.

2. Marginal hooks. The marginal hook is composed of two parts, i.e., the hook proper and the shaft. Histochemical reactions of both parts were similar except for tyrosine as disclosed with Morel-Sisley's diazotization method (Table 1). The shaft was tyrosine-positive, the hook proper negative (Plate I, Fig. 2). Reactions for proteins with SH and SS groups were positive in both parts (Plate II, Fig. 2).

Tab. 1. Histochemistry of haptor sclerites in the genus *Tetraonchus*

Reaction	Anchors			Connecting bar		Marginal hooks	
	Point	Basal part	Surface attachment areas of muscles	Connecting bar proper	Surface attachment areas of muscles	Hook proper	Shaft
Morel-Sisley	—	+++++	—	+++++	—	—	++
Coupled tetrazonium reaction	—	+++	—	+++	—	—	—
DMAB	—	+	—	+	—	—	—
DDD	—	violet*)	—	violet*)	—	red	red
Peracetic acid + aldehyde fuchsin	—	+++++	+++++	+++++	+++++	+++++	+++++
Aldehyde fuchsin	—	—	+++++	—	+++++	—	—
PAS	—	—	++	—	++	—	—
Schiff	—	—	—	—	—	—	—
Acetylation + PAS	—	—	—	—	—	—	—
Desacetylation + PAS	—	—	++	—	++	—	—
AB pH 2.6	—	—	—	—	—	—	—
AB-PAS	—	—	red	—	red	—	—

*) except for the surface layer

DISCUSSION

The only histochemical studies available on members of the genus *Tetraonchus* Diesing, 1850 are those made by Lyons (1966) for the species *T. monenteron*. This author examined the sclerites of the haptor for the presence of SS groups. Her results are essentially similar to ours except for the fact that she did not mention the two parts composing the anchor of *T. monenteron*, i.e., the basal part and the point. With none of the methods employed did we succeed to demonstrate SS or SH groups in the anchor point. This part appears to be a most intact scleroprotein which receives picric acid only. Histochemical reactions of the haptor sclerites in members of the genus

Tetraonchus exclude the possibility that the protein involved may be similar to the clamp-protein reported by Ramalingam (1973) for higher monogeneans.

It may be assumed that the substance forming the main parts of the haptor sclerites in evidently all species of the genus *Tetraonchus* is a scleroprotein with SS groups similar to keratine.

The substance forming the sclerites of the haptor in monogeneans is in histochemical accord with the substance forming the embryonic and rostellar hooks of cestodes (Dvorak 1969a, b, c, d). Basically, it is a scleroprotein with SS groups similar to keratine. Also Lyons (1966) concluded on the basis of a comparison of the sclerites of monogeneans with those of trematodes and cestodes that the substance forming the monogenean sclerites is closer to that forming the sclerites of cestodes than to that forming the sclerites of trematodes. On the basis of this knowledge this author suggested that monogeneans are closer to cestodes than to trematodes as regards their systematic position.

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ГИСТОХИМИЯ СКЛЕРИТОВ ПРИКРЕПИТЕЛЬНОГО АППАРАТА У НЕКОТОРЫХ ВИДОВ РОДА *TETRAONCHUS* DIESING, 1850 (MONOGENOIDEA)

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Резюме. В склеритах прикрепительного аппарата у трех видов рода *Tetraonchus* Diesing, 1850 гистохимическим путем обнаружили протеины. Основная часть срединных крючьев и соединительная пластинка содержат протеины с тирозином, триптофаном, SH и SS группами. Краевые крючья содержат протеины с SH и SS группами и лишь одна их часть — рукоятка также содержит тирозин. Гистохимические реакции по отношению к нейтральным и кислым мукосубстанциям были отрицательны.

REFERENCES

- DVORAK J. A., *Hydatigera taeniaeformis*: Strobilocerci hooks. I. Collection and preparation; elemental, amino acid, and infrared spectrophotometric analyses. Exp. Parasitol. 26: 111—121, 1969 a.
- , *Hydatigera taeniaeformis*: Strobilocerci hooks. III. Molecular weight and N-terminal amino acid determinations. Exp. Parasitol. 26: 128—133, 1969b.
- , *Hymenolepis microstoma*: Interference microscopy of embryonic lateral hooks. I. Effects of temperature and reagent concentration on reaction with 2-mercaptoethanol. Exp. Parasitol. 26: 92—100, 1969 c.
- , *Hymenolepis microstoma*: Interference microscopy of embryonic lateral hooks. II. Structure and reaction to 2-mercaptoethanol. Exp. Parasitol. 26: 101—110, 1969 d.
- ERGENS R., The species of the genus *Tetraonchus* Diesing, 1850 (Monogenoidea) recovered from fishes of Mongolia. Folia parasit. (Praha) 18: 139—147, 1971.
- LYONS K. M., The chemical nature and evolutionary significance of monogenean attachment sclerites. Parasitology 56: 63—100, 1966.
- PEARSE A. G. E., Histochemistry: theoretical and applied. Vol. I. London: J. and A. Churchill Ltd., 1968.
- RAMALINGAM K., Chemical nature of monogenean sclerites I. Stabilization of clamp-protein by formation of dityrosine. Parasitology 66: 1—7, 1973.

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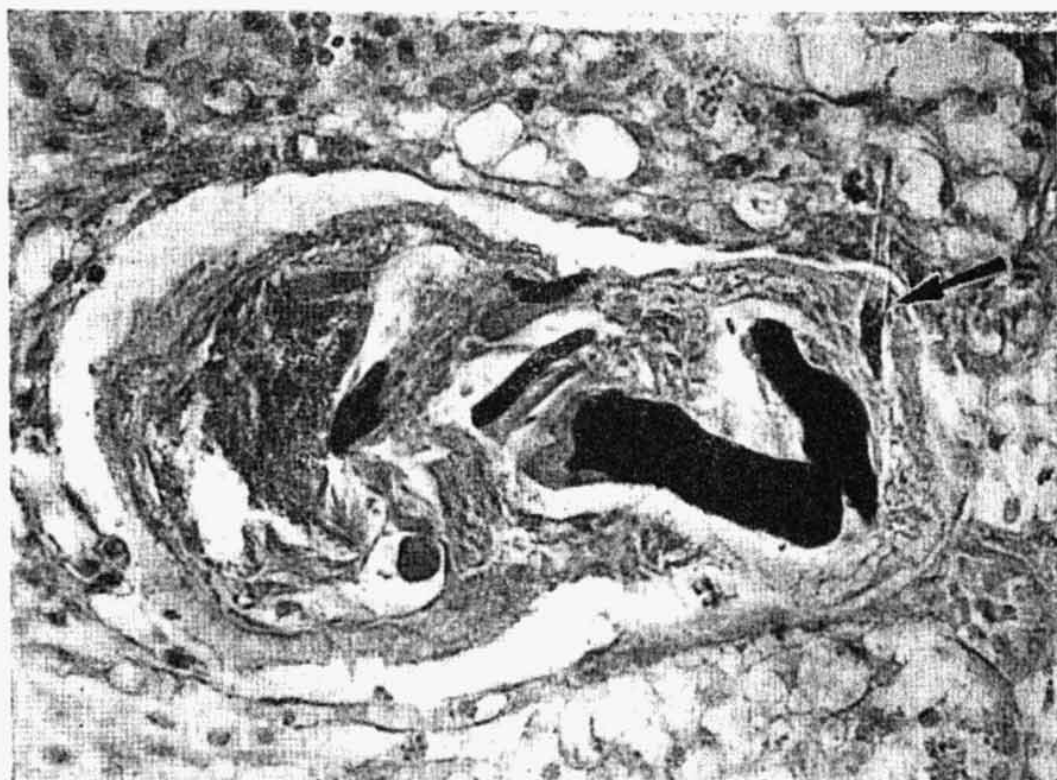


Plate I

Fig. 1. Oblique section through the haptor with sections through the anchors and connecting bar; arrow — clearly visible point of anchor (tyrosine-negative) passing into the basal part (tyrosine-positive). Morel-Sisley's diazotization method ($\times 790$).

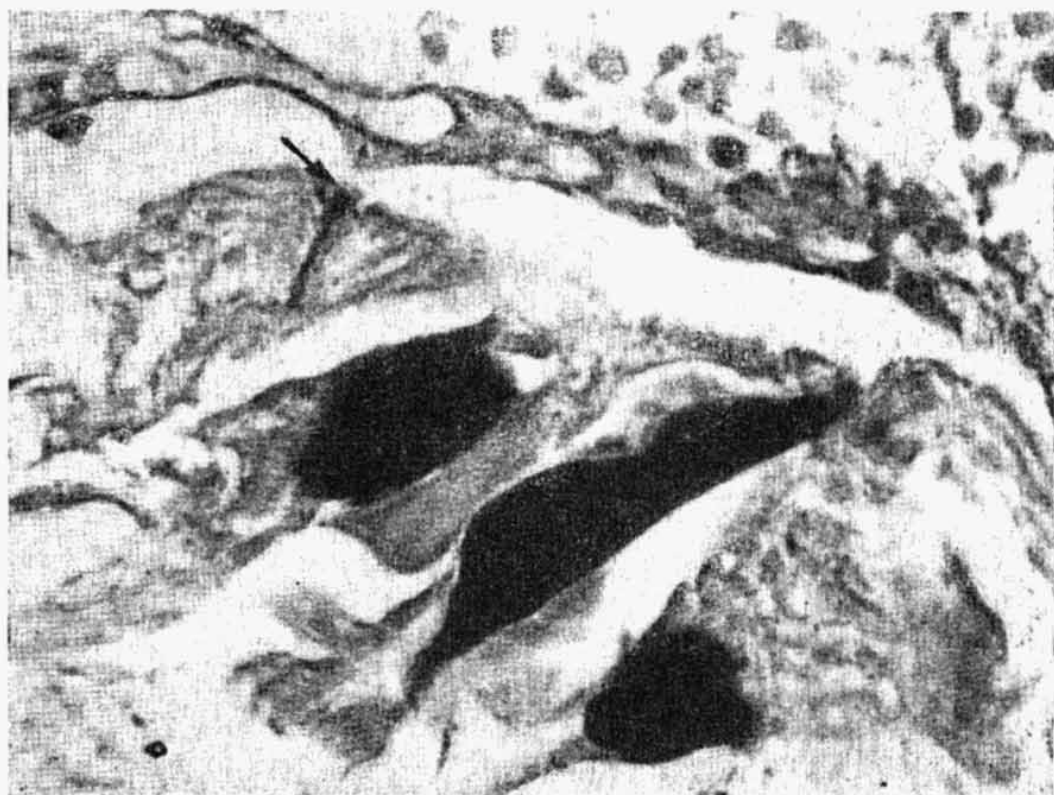


Fig. 2. Oblique section through the haptor. Note the marginal hook (arrow) with clearly visible shaft (tyrosine-positive) passing into the hook proper (tyrosine-negative). Morel-Sisley's diazotization method ($\times 1\,300$).

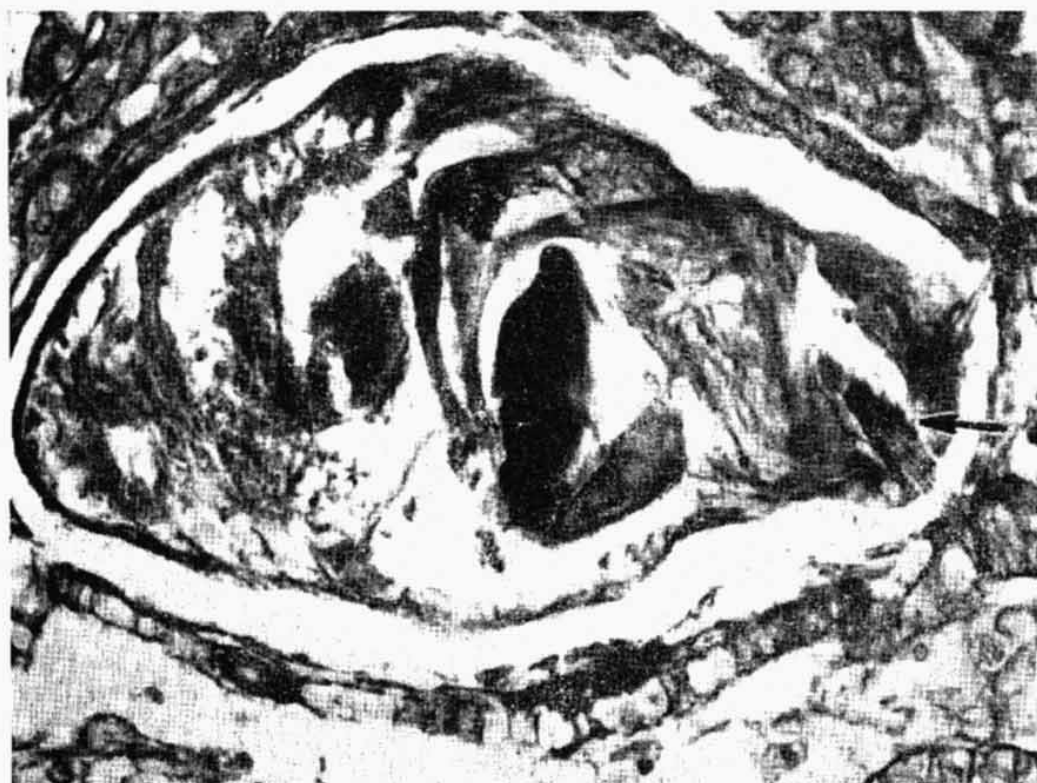


Plate II

Fig. 1. Clearly visible transition of the point of anchor (unstained) into the basal part stained intensively with the method for proteins with SS groups (arrow). PAA — aldehyde fuchsin ($\times 790$).



Fig. 2. Marginal hook (arrow) containing proteins with SS groups. PAA — aldehyde fuchsin ($\times 1\,300$).

