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HISTOCHEMISTRY OF THE CYSTICERCOID OF RODENTOTAENIA CRASSISCOLEX (LINSTOW, 1890)

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Abstract. The cysticercoid of *R. crassiscolex* is situated in a capsule consisting of muscles and connective tissue. The outer and inner layers of fine connective tissue fibres of the capsule contain neutral mucosubstances and proteins with SS groups. Muscle fibres of the middle layer of the capsule contain neutral mucosubstances and proteins with SH groups; connective tissue fibres of the same layer contain acid mucosubstances, proteins with SS groups. The inner limiting layer of the capsule and outer limiting layer of the cyst, neck and scolex contain neutral mucosubstances. The inner limiting layer of the cyst and neck did not stain by any of the methods used. The fine connective tissue fibres of the tegument of cyst have the same character as the fibres of the outer and inner layers of the capsule. Acid mucosubstances and proteins with arginine, tyrosine and SH groups were detected in the microtriches of the neck and scolex. The amorphous substance of the tegument of cyst, neck and scolex contains neutral mucosubstances, acid mucosubstances and proteins with SH groups. The amorphous substance of the tegument of rostellum, where the hooks grow, contains arginine and proteins with SS groups which were detected also in the hooks. The connective tissue fibres of the subtegument of cyst, neck and scolex and the connective tissue fibres of the parenchyma exhibit the same histochemical reactions as the connective tissue fibres of the middle layer of the capsule. The muscle fibres of the subtegument of cyst, neck and scolex, and the muscle fibres of the parenchyma contain glycogen and proteins with arginine and SH groups. Pyriform cells contain acid mucosubstances and proteins with SH groups. Glycogen was detected in bladder-like cells and in the cells of parenchyma neck and scolex.

The histochemistry of the cysticercoid of *R. crassiscolex* was studied in section material obtained from terrestrial snails, *Deroceras agreste* (Müller, 1774) and *Semilimax semilimax* (Férrusac, 1802). The present paper follows a morphological study of this cysticercoid (Valkounová and Prokopič 1978).

MATERIAL AND METHODS

Capsules with cysticercoids localized in the hepatopancreas, kidney and under the epithelium of respiratory and body cavities of snails were fixed in Baker's formaldehyde (Pearse 1968). The material for the detection of tryptophan was fixed at most for 24 h using Baker's fluid, the pH of which was adjusted to 6.5 by 0.1N NaOH (Lojda 1965). Histochemical methods for the detection of mucosubstances, proteins and lipids were applied on a series of 6 μm thick paraffin sections.

Neutral mucosubstances were demonstrated by PAS method (periodic acid — Schiff) combined with saliva test (Pearse 1968), Best's carmine combined with saliva test (Pearse 1968), acetylation (acetic acid anhydrine — pyridine 2 : 3, 48 h at 58 °C) and desacetylation (1 % KOH in 70 % ethyl alcohol, 20 min at 22 °C) (Pearse 1968). Acid mucosubstances were detected by Alcian blue (Alcianblau 8GS, Fluka) pH 2.6 (Quintarelli and Dellovo 1963, Quintarelli et al. 1964 a, b, Scott et al. 1964), Alcian blue pH 2.6 combined with methylation after Fischer and Lillie (1954) (methyl alcohol — concentrated HCl 100 : 1, 24 h at 60 °C) and demethylation (Spicer and Lillie 1959) (1 % KOH in 80 % ethyl alcohol, 30—45 min at 25 °C), Mowry's modification of Hale's method (Mowry 1958), coloidal Fe^{3+} after Müller (1955) (in Mowry 1958, 1963). Methylene blue extinction (MBE) after Pearse (1968) and critical electrolyte concentration (CEC) after Scott and Dorling (1965), Scott and Willett (1966), Scott and Stockwell (1967), Quintarelli and Dellovo (1963) were used for further differentiation of mucosubstances. Neutral and acid mucosubstances were differentiated by AB + PAS and Hale + PAS reactions after Mowry (1963).

Arginine in proteins was detected by Sakaguchi's method modified by Baker (1947) (in Pearse 1968), tryptophan DMAB (dimethylaminobenzaldehyde) by the method of Adams (1957) (in Pearse 1968), tyrosine by Morel-Sisley's diazotization method modified by Lillie (1957) (in Pearse 1968). The coupled tetrazonium reaction (CT) after Müller and Chytil (1962) was used for the group test for tyrosine, histidine and tryptophan. SH groups were detected by the method with DDD (2,2-dihydroxy-6,6-dinaphthyldisulphide) (Barrnett and Seligman 1954). N-ethylmaleimide (Pearse 1968) was used for the blockade of SH groups. SS groups were detected by the method with DDD combined with thioglycolic acid (Pearse 1968), PFA + AB (performic acid + Alcian blue) method after Pearse (1968), controlled with AB pH 0.2 (Pearse 1968) and PAA + AF (peracetic acid + aldehyde fuchsin) (Pearse 1968).

Lipids were detected only in paraffin sections using Sudan black B, and Luxol blue (Luxol Fast Blue) (Pearse 1968).

RESULTS

The cysticercoid of *R. crassiscolex* consists of a cyst containing neck and scolex of the cestode. The cyst is surrounded by a muscle and connective tissue capsule which is formed from the host tissue of the snail. In some places the cyst adheres to the wall of the capsule, in other places the cyst is separated from the wall of the capsule by a space with remainders of a dense fluid which surrounded the whole non-invaginated larva in earlier stages of development.

STRUCTURE OF THE CAPSULE AND CYSTICERCOID

Capsule — outer layer — connective tissue fibres
— middle layer — muscle and connective tissue fibres
— inner layer — connective tissue fibres
— inner limiting layer

Fluid filling the capsule cavity — granules, muscle and connective tissue fibres

Cyst — outer limiting layer
— tegument — outer part — circular and longitudinal connective tissue fibres
— inner part — amorphous substance
— basement layer
— subtegument — fibrous part — outer circular and inner longitudinal muscle and connective tissue fibres
— cellular part — pyriform and bladder-like cells*)

Neck — outer limiting layer
— tegument — proximal part — outer part — microtriches
— inner part — amorphous substance
— distal part — amorphous substance
— basement layer
— subtegument — same structure as in cyst wall
— parenchyma — only in proximal part of neck
— inner limiting layer

Scolex — outer limiting layer
— tegument — outer part — microtriches
— inner part — amorphous substance — hooks
— basement layer
— subtegument — same structure as in cyst wall
— parenchyma — same structure as parenchyma of adult cestodes

CAPSULE (Tables 1—3)

Connective tissue fibres of the outer and inner layers and inner limiting layer stained most intensively by PAS method and Best's carmine. Since no glycogen was demonstrated in these layers by ptyalin digestion, all substances belonged to the group of

*) Pyriform and bladder-like cells are surrounded by muscle and connective tissue fibres projecting from the fibrous part of the tegument and subtegument

Table 1. Results of histochemical reactions for the detection of mucosubstances in the wall of capsule

Reaction	Outer and inner layer	Middle layer		Inner limiting layer	Fluid filling the cavity between capsule and cyst		
	Connective tissue fibres	Connective tissue fibres	Muscle fibres		Granules	Connective tissue fibres	Muscle fibres
PAS	+++	±	+++	+++	++++	±	++
Schiff	—	—	—	—	—	—	—
Saliva test + PAS	+++	—	+++	+++	+	—	++
Acetylation + PAS	—	—	—	—	—	—	—
Desacetylation + PAS	+++	—	++	+++	+++	—	++
AB + PAS	red	blue	red	red	violet	blue	red
AB pH 2.6	—	++	—	—	++++	++	—
Methylation + AB pH 2.6	—	—	—	—	—	—	—
Demethylation + AB pH 2.6	—	+++	—	—	++++	++	—
CEC (AB pH 2.6 + $MgCl_2$)	—	8 %*	—	—	4 %*	8 %*	—
MBE at pH	—	3.62	—	—	4.66	3.62	—
Best's carmine	++++	—	++	+++++	+++++	—	++
Saliva tests + Best's carmine	++++	—	++	++++	+	—	++
Aldehyde fuchsin	—	+++/++++	—	—	+++	+/++	—
Hale	—	blue	—	—	blue	blue	—
Coloidal Fe^{3+}	—	blue	—	—	blue	blue	—
Hale + control	—	—	—	—	—	—	—
Hale + PAS	red	blue	red	red	violet	blue	red

* At this concentration of $MgCl_2$ the affinity to AB pH 2.6 disappears

Table 2. Results of histochemical reactions for the detection of proteins in the wall of capsule

Reaction	Outer and inner layer	Middle layer		Fluid filling the cavity between capsule and cyst		
	Connective tissue fibres	Connective tissue fibres	Muscle fibres	Granules	Connective tissue fibres	Muscle fibres
Sakaguchi	—	—	+	++++	—	+
DMAB	—	—	±	—	—	±
Morel — Sisley	—	—	+	—	—	+
CT	—	—	++	—	—	++
DDD	—	±	++	++	±	++
Thioglycollic acid + DDD	+++	++	+	+++	++	+
N — ethylmaleimide + DDD	—	—	—	—	—	—
AB pH 0.2	—	++	—	+++	++	+
PFA + AB	+++	+++	—	++++	+++	—
PAA + Aldehyde fuchsin	++++	+++++	—	+++	++	—

Table 3. Results of histochemical reactions for the detection of lipids in the wall of capsule, cyst, neck, and scolex

Reaction	Outer and inner layer of capsule	Middle layer of capsule		Fluid filling the cavity between capsule and cyst		Tegument of cyst	Subtegument of cyst neck and scolex			Parenchyma of neck and scolex	
	Connective tissue fibres	Connective tissue fibres	Muscle fibres	Connective tissue fibres	Muscle fibres	Connective tissue fibres	Connective tissue fibres	Muscle fibres	Bladder-like cells	Connective tissue fibres	Muscle fibres
Sudan black B	—	++	+	++	+	—	++	+	+/++	+	+
Luxol blue	+/++	++	±	++	+/++	+/++	+++/++++	+/++	+	+++/++++	+/++

Table 4. Results of histochemical reactions for the detection of mucosubstances in the wall of cyst

Reaction	Outer limiting layer	Tegument		Subtegument			
		Connective tissue fibres	Amorphous substance	Connective tissue fibres	Muscle fibres	Pyriform cells	Bladder-like cells
PAS	+++	+++/++++	++	±	++	+	++++
Schiff	—	—	—	—	—	—	—
Saliva test + PAS	+++	+++/++++	++	±	—	+	+
Acetylation + PAS	—	—	—	—	—	—	—
Desacetylation + PAS	+++	+++/++++	+/++	—	++	+	++++
AB + PAS	red	red	light violet	blue	red	light violet	red
AB pH 2.6	—	—	++	+++	—	++	—
Methylation + AB pH 2.6	—	—	—	—	—	—	—
Demethylation + AB pH 2.6	—	—	++	+++	—	++	—
CEC (AB pH 2.6 + $MgCl_2$)	—	—	4 % *	12 % *	—	4 % *	—
MBE at pH	—	—	6.75	3.88	—	6.75	—
Best's carmine	+++	++++	+	—	++	+	+++++
Saliva test + Best's carmine	+++	++++	+	—	—	+	+
Aldehyde fuchsin	—	—	++	++	—	++	—
Hale	—	—	light blue	blue	—	light blue	—
Coloidal Fe^{3+}	—	—	light blue	blue	—	light blue	—
Hale + control	—	—	—	—	—	—	—
Hale + PAS	red	red	light violet	blue	red	light blue	red

* At this concentration of $MgCl_2$ the affinity to AB pH 2.6 disappears

ptyalin-resistant neutral mucosubstances. A reaction of medium intensity for ptyalin-resistant neutral mucosubstances was found in muscle fibres of the middle layer. Acid mucosubstances containing sulphogroups were present in connective tissue fibres of the middle layer (Table 1, MBE and CEC methods). Arginine and tyrosine (feeble reaction) were demonstrated in muscle fibres of the middle layer. Positive reaction to SH groups was found in muscle fibres of the middle layer, to SS groups in connective tissue fibres of outer, middle and inner layers. Lipids were detected in muscle and connective tissue fibres of the middle layer using Sudan black B and in connective tissue fibres of the outer, middle and inner layers using Luxol blue.

The fluid filling the space between the wall of capsule and the cyst was found to contain glycogen in granules, PAS-positive ptyalin-resistant neutral mucosubstances in muscle fibres. Acid mucosubstances containing carboxyl groups were identified in granules, acid mucosubstances characterized by sulphate groups in connective tissue fibres (Table 1). Granules were found to contain arginine (strong reaction), muscle fibres contained arginine and tyrosine (feeble reaction). Proteins with SH groups were detected in granules and muscle fibres, proteins with SS groups in connective tisssue fibres. Lipids were detected in muscle and connective tissue fibres using Sudan black B and Luxol blue.

CYST (Tables 3—5)

Ptyalin-resistant neutral mucosubstances were detected in the outer limiting layer using PAS method and Best's carmine. The outer limiting layer stained only with these two methods. Otherwise it was discernible by its refractility. The inner limiting layer of the cyst did not stain by any of the methods used. PAS-positive ptyalin-resistant neutral mucosubstances were also demonstrated in connective tissue fibres and in the amorphous substance of the tegument and in pyriform cells (feeble reactions). Glycogen was detected in muscle fibres of the subtegument and in bladder-like cells at ptyalin digestion which preceded staining with PAS method and Best's carmine. Acid mucosubstances were detected in the amorphous substance of the tegument, in connective tissue fibres of the subtegument and in pyriform cells. The amorphous substance of the tegument and pyriform cells contain acid mucosubstances with carboxyl groups, connec-

Table 5. Results of histochemical reactions for the detection of proteins in the wall of cyst

Reaction	Tegument		Subtegument		
	Connective tissue fibres	Amorphous substance	Connective tissue fibres	Muscle fibres	Pyriform cells
Sakaguchi	—	+/++	—	+++	+
DMAB	—	±	—	±	±
Morel — Sisley	—	+/++	—	+	+/++
CT	—	+	—	++	+
DDD	—	++	±	++	++
Thioglycollic acid + DDD	+++	++	++	++	++
N — ethylmaleimide + DDD	—	—	—	—	—
AB pH 0.2	+	++	++	—	++
PFA + AB	+++++	++	+++/++++	—	++
PAA + Aldehyde fuchsin	++++	++	+++	—	++

Table 6. Results of histochemical reactions for the detection of mucosubstances in the wall of neck and scolex

Reaction	Outer limiting layer	Tegument		Subtegument				Parenchyma		
		Micro-triches	Amorphous substance	Connective tissue fibres	Muscle fibres	Pyriform cells	Bladder-like cells	Connective tissue fibres	Muscle fibres	Cells
PAS	+++	—	++	±	+++	+	+++	±	++++	+++
Schiff	—	—	—	—	—	—	—	—	—	—
Saliva test + PAS	+++	—	++	±	—	+	+	±	—	+
Acetylation + PAS	—	—	—	—	—	—	—	—	—	—
Desacetylation + PAS	+++	—	+/++	—	++	±	++	—	+++	++
AB + PAS	red	blue	light violet	blue	red	light blue	red	blue	red	red
AB pH 2.6	—	++	++	+++	—	++	—	+++	—	±
Methylation + AB pH 2.6	—	—	—	—	—	—	—	—	—	—
Demethylation + AB pH 2.6	—	++	++	+++	—	++	—	+++	—	±
CEC (AB pH 2.6 + $MgCl_2$)	—	4 % *	4 % *	12 % *	—	4 % *	—	12 % *	—	—
MBE at pH	—	6.75	6.75	3.88	—	6.75	—	3.88	—	—
Best's carmine	++++	—	++	—	+++	+	+++	—	++++	+++
Saliva test + Best's carmine	++++	—	++	—	—	+	+	—	—	+
Aldehyde fuchsin	—	+/++	++	++	+	++	—	++	—	—
Hale	—	light blue	light blue	blue	—	light blue	—	blue	—	—
Coloidal Fe^{3+}	—	light blue	light blue	blue	—	light blue	—	blue	—	—
Hale + control	—	—	—	—	—	—	—	—	—	—
Hale + PAS	red	light blue	light violet	blue	red	light blue	red	blue	red	red

* At this concentration of $MgCl_2$ the affinity to AB pH 2.6 disappears

Table 7. Results of histochemical reactions for the detection of proteins in the wall of neck and scolex

Reaction	Tegument		Subtegument			Parenchyma			Hooks
	Micro-triches	Amorphous substance	Connective tissue fibres	Muscle fibres	Pyriform cells	Connective tissue fibres	Muscle fibres	Cells	
Sakaguchi	+++++	* +/++ / ++	—	+++	+	—	+++	+++	—
DMAB	+	* ± / ±	—	+	±	—	±	+	—
Morel — Sisley	+++	+/++	—	+	+/++	—	+	—	—
CT	++++	+	—	++	+	—	++	+++	—
DDD	—	++	—	++	++	—	++	—	—
Thioglycollic acid + DDD	++	* ++ / ++++	++	+/++	++	++	++	—	+++
N — ethylmaleimide + DDD	—	—	—	—	—	—	—	—	—
AB pH 0.2	—	++	++	—	++	++	—	—	—
PFA + AB	—	* ++ / ++++	+++	—	++	+++	—	—	++++
PAA + Aldehyde fuchsin	+++++	* ++ / +++++	+++	—	++	+++	—	—	+++++

* Numerator = raection in amorphous substance of tegument of neck and scolex. Denominator = reaction in amorphous substance of tegument of rostellum where the hooks grow

tive tissue fibres contain acid mucosubstances with sulphate groups (Table 4). Arginine and tyrosine were demonstrated in the amorphous substance of the tegument, in muscle fibres of the subtegument and in pyriform cells. Proteins with SH groups were identified in the amorphous substance of the tegument, in muscle fibres of the subtegument and in pyriform cells. Proteins with SS groups were detected in connective tissue fibres of the tegument and subtegument. Lipids were found in connective tissue fibres of the tegument using Sudan black B, in bladder-like cells, in muscle and connective tissue fibres of the subtegument and parenchyma using Sudan black B and Luxol blue.

NECK, SCOLEX (Tables 3, 6, 7)

Histochemical reactions for the detection of mucosubstances revealed neutral ptyalin-resistant mucosubstances in the outer limiting layer, in the amorphous substance of the tegument and in pyriform cells (feeble reactions) of the scolex and neck, using PAS method and Best's carmine. Glycogen was demonstrated at ptyalin digestion combined with PAS method and Best's carmine in muscle fibres of the subtegument, in bladder-like cells, in muscle fibres and in the cells of parenchyma. Acid mucosubstances were detected in microtriches, in amorphous substance of tegument, in connective tissue fibres of subtegument, in pyriform cells and in connective tissue fibres of parenchyma. Microtriches, amorphous substance of tegument and pyriform cells contain acid mucosubstances with carboxyl groups, connective tissue fibres contain acid mucosubstances with sulphate groups. Arginine, tryptophan and tyrosine were detected in microtriches, and in muscle fibres of the subtegument, arginine and tryptophan in the cells of parenchyma. Arginine and tyrosine were detected in the amorphous substance of tegument, in pyriform cells and in muscle fibres of parenchyma. Proteins with SH groups were demonstrated in the amorphous substance of tegument, in muscle fibres of subtegument, in pyriform cells and in muscle fibres of parenchyma. Proteins with SS groups were detected in microtriches, in amorphous substance of tegument of rostellum where the hooks grow, in hooks, in connective tissue fibres of subtegument and in parenchyma. Lipids were found in muscle and connective tissue fibres of subtegument and parenchyma and in bladder-like cells using Sudan black B and Luxol blue.

DISCUSSION

The almost identical morphology and structure of the tegument of cyst, neck and scolex, as well as the identical morphology and structure of the subtegument of all parts of the cysticercoid, result from the mode of development of larvae when the younger stages are not invaginated and the tegument of cyst, neck and scolex forms a confluent layer. It is therefore necessary to regard the tegument of the cyst, neck and scolex as a single layer. By invagination of the neck and scolex into the cyst cavity this layer is shifted in such a way that it seemingly forms three layers (on the surface of cyst, neck and scolex). The same concerns also the subtegument.

The differentiation of the outer part of tegument occurs on the basis of various functions. In our light microscopical studies the cyst was found to contain circular and longitudinal connective tissue fibres making the cysticercoid firm, and the proximal part of neck and scolex contained microtriches serving for nutrition. Ubelaker et al. (1970) in their electron microscopical studies of cysticercoid of *Hymenolepis diminuta* (Rudolphi, 1819) described fine projections termed "microvilli" in the outer part of cyst tegument and microtriches on the whole surface of the outer part of neck tegument. Similar projections on the surface of cyst tegument were observed also by Baron (1971) in the

cysticercoid of *Raillietina cesticillus* (Molin, 1858). The author named them "microtrix-like projection". We shall be able to differentiate the surface of tegument of cysticercoid of *R. crassiscolex* only after further studies by means of electron microscope.

Histochemical reactions in the amorphous substance of tegument and in pyriform cells of subtegument support the opinion of the authors (Valkounová and Prokopič 1978) that the pyriform cells participate in the formation of the tegument. Both the amorphous substance and the pyriform cells contain neutral mucosubstances, acid mucosubstances, arginine, tyrosine and proteins with SH groups. In a non-invaginated cysticercoid, the PAS reaction on the ptyalin-resistant mucosubstances is feeble in the tegument and strong in pyriform cells. In the invaginated cysticercoid this reaction is contrary and the pyriform cells gradually degenerate.

Histochemical studies of other authors gave similar results, though larvae or adult cestodes of different species were described. For instance, Baron (1971) studied the histochemistry of the cysticercoid of *R. cesticillus*, Heyneman and Voge (1957) described glycogen distribution in cysticercoids of *Hymenolepis citelli* McLeod, 1933, *H. diminuta* and *H. nana* (Siebold, 1852). Mayberry and Tibbitts (1972) studied the localization of glycogen and neutral lipids in *H. diminuta* using histochemical methods. Hendrich and Daugherty (1957) reported the occurrence of glycogen in *H. diminuta* and *R. cesticillus*, Howells and Erasmus (1969) described histochemical structure of tegument and interproglottidal glands in *Moniezia expansa* (Rudolphi, 1805).

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ГИСТОХИМИЯ ЦИСТИЦЕРКОИДА ЦЕСТОДЫ *RODENTOTAENIA CRASSISCOLEX* (LINSTOW, 1890)

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Резюме. Цистицеркоид цестоды *R. crassiscolex* находится в капсуле состоящей из мышц и соединительной ткани. Наружный и внутренний слой тонких волокон соединительной ткани капсулы содержит нейтральные мукосубстанции и белки с SS группами. В мышечных волокнах среднего слоя капсулы обнаружены нейтральные мукосубстанции и белки с SH-группами. В волокнах соединительной ткани среднего слоя капсулы выявлены кислые мукосубстанции, белки с SS группами. Внутренний лимитирующий слой капсулы и наружный лимитирующий слой цисты, шейки и сколекса содержат нейтральные мукосубстанции. Внутренний лимитирующий слой цисты и шейки нельзя было окрасить ни одним из примененных методов. Тонкие волокна соединительной ткани тегумента цисты оказывают одинаковый характер как волокна наружного и внутреннего слоев капсулы. В микротрихах шейки и сколекса обнаружены кислые мукосубстанции, белки с аргинином и тирозином и SH группами. Основное вещество тегумента цисты, шейки и сколекса содержит нейтральные мукосубстанции, кислые мукосубстанции и белки с SH группами. В основном веществе тегумента хоботка, где растут крючья, обнаружены аргинин и белки с SS группами, которые были найдены также в крючьях. Волокна соединительной ткани субтегумента цисты, шейки и сколекса и волокна соединительной ткани паренхимы проявляют одинаковые гистохимические реакции как волокна соединительной ткани среднего слоя капсулы. В мышечных волокнах субтегумента цисты, шейки и сколекса и в мышечных волокнах паренхимы обнаружен гликоген, белки с аргинином и SH группами. Грушевидные клетки содержат кислые мукосубстанции и белки с SH группами. В пузыровидных клетках и в клетках паренхимы, шейки и сколекса обнаружен гликоген.

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