

HISTOCHEMISTRY OF THE CYSTICERCOID OF CORONACANTHUS INTEGER (HAMANN, 1891) (HYMENOLEPIDIDAE)

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Abstract. The content of mucosubstances, proteins and lipids was studied in lens-shaped cysticercoids older than 20 days. Compared to smaller, oval cysticercoids younger than 20 days, the older cysticercoids contain a smaller amount of neutral and acid mucosubstances and proteins with SH-groups in the tegument and tegument-forming cells, and acid mucosubstances in connective tissue fibres. Calcareous bodies were regularly detected in the subtegument of oval cysts, whereas in the subtegument of lens-shaped cysts, they occurred only occasionally.

The studied type of cysticercoid forms a tailed cyst containing the neck and scolex of the cestode. Up to approximately 20 days after invagination the cyst is oval, the neck surrounds the scolex and is coalesced with the cyst at the site of invagination. Older cysticercoids grow and get the shape of a lens. The neck is usually torn off from the cyst wall and contracted in its cavity (Valkounová 1983). The present paper deals with the content of mucosubstances, proteins and lipids in the lens-shaped cysticercoids, which were found most frequently. The differences in the histochemical structure between the lens-shaped and oval, i.e. younger cysts are discussed.

MATERIAL AND METHODS

The cysticercoids were recovered from the body cavity of spontaneously infected *Gammarus* (*Rivulogammarus*) *fossarum* Koch, 1835 from the localities Pyšely, Kamenný Újezd, Písek and Vimperk.

Individual stages of cysticercoid development, starting with the mobile oncosphere, were previously studied in the laboratory using the material from Copepods and Ostracods (Neradová-Valkounová 1971). These studies revealed that the oncospheres start to grow on the 3rd day of larval development in the body cavity of the intermediate host. In the case of *C. integer* we also considered the growing oncospheres in *Gammarus* to be 3 days old. Oval cysticercoids from these oncospheres were then observed until days 18–22 after invagination in the laboratory. Older cysticercoids got gradually the shape of a lens.

Isolated cysticercoids were fixed in Baker's neutral formaldehyde (Pearse 1968). The material for the detection of tryptophan was fixed by Baker's fixation method adjusted by 0.1 N NaOH to pH 6.5 (Lojda 1965). A series of 6 µm thick paraffin sections was used for the histochemical detection of neutral and acid mucosubstances, proteins and lipids. The methods used were described in a previous paper (Valkounová and Prokopič 1979).

RESULTS

The cyst of *C. integer* is the first of the histologically studied cysts in the tegument of which the microvilli can be distinguished in the light microscope (for details see Valkounová 1983).

Table 1. Results of histochemical reactions for the detection of mucosubstances

Reaction	Cyst						Neck and scolex							
	Tegument		Subtegument				Tegument		Subtegument				Hooks	
	Microvilli	Amorphous substance	Fine connective tissue fibres	Connective tissue fibres	Muscle fibres	Pyriiform cells	Microtriches	Amorphous substance	Fine connective tissue fibres	Connective tissue fibres	Muscle fibres	Pyriiform cells		
PAS	±	-	++	-	+	-	+	+	+	-	+++	+++ +++ +++ +++ +++ (Calcareous corpuscles	-
Schiff	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Saliva test + PAS	±	-	++	-	-	-	+	+	+	-	+	+++	-	-
Acetylation + PAS	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Desacetylation + PAS	±	-	++	-	+++	-	+	+	++	-	+++	+++ +++ +++ +++ +++ (-	-
Best's carmine	+	-	+	+	+	±	-	-	+	-	+++	+++	+	-
Saliva test + Best's carmine	+	-	+	-	±	-	+	+	+	-	+	+	-	-

AB pH 2.6	++	+	-	++	-	±	+++	++	-	+	+++	-	+	+
Methylation + AB pH 2.6	-	-	-	-	-	±	-	-	-	-	+	-	-	-
Demethylation + AB pH 2.6	++	+	-	±	-	±	++	light violet	red	-	+	red violet	+	+
AB + PAS	blue	light blue	red	blue	red	light blue	light violet	4 %	8 %	4 %	7.25	3.62	8 %	blue
CEC (AB pH 2.6 + MgCl ₂)	4 % ²⁾	4 %	-	8 %	-	4 %	7.25	4 %	3.62	4 %	7.25	3.62	8 %	blue
MBE at pH	7.25	7.25	-	3.62	-	7.25	7.25	4 %	3.62	4 %	7.25	3.62	8 %	blue
Aldehyde fuchsin	++	+	-	+	-	++	+++	+	+	+++	+++	+	+	+
Hale	+++	+	+	++	-	+	+++	++	++	+	+	+	+	+
Hale + control	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hale + PAS	blue	light blue	light violet	blue	red	light blue	blue	light violet	red	light blue	red violet	+	+	+
Coloidal Fe ³⁺	++	+	-	++	-	+	+++	+	++	+	+	+	+	+

CEC — critical electrolyte concentration, MBE — methylene blue extinction

¹⁾ Numerator: reaction in the neck and scolex without rostellar sac; denominator: reaction in the rostellar sac

²⁾ At this concentration of the MgCl₂ the affinity to AB pH 2.6 disappears

STRUCTURE OF THE CYSTICERCOID

Cyst

- outer limiting layer
- tegument — microvilli
 - amorphous substance
- basement layer
- subtegument — circular layer of muscle and connective tissue fibres
 - longitudinal layer of muscle and connective tissue fibres
 - pyriform cells forming the tegument are surrounded by muscle and connective tissue fibres, calcareous corpuscles occur only in some cysticercoids
 - longitudinal layer of muscle and connective tissue fibres

Neck and scolex

- outer limiting layer
- tegument — microtriches
 - amorphous substance
- basement layer
- subtegument — circular and longitudinal layer of muscle and connective tissue fibres
 - pyriform cells forming the tegument are surrounded by muscle and connective tissue fibres
- parenchyma — parenchyma cells, calcareous corpuscles, muscle and connective tissue fibres

CYST

The outer limiting layer is not positive in any of the used methods. The microvilli contain acid mucosubstances, proteins with arginine, tryptophan, sulphhydryl and disulphide groups. They are positive also in the copulation tetrazonium reaction (CT) and reactions with luxol blue and Sudan black B. They contain only a small amount of neutral mucosubstances. The amorphous substance of the tegument contains a small amount of acid mucosubstances and proteins with SS-groups and it was positive to CT reaction.

Fine argyrophilic fibres of subtegument contain neutral mucosubstances and proteins with tyrosine. The thick connective tissue fibres contain acid mucosubstances, proteins with tyrosine, SS-groups and neutral lipids. The muscle fibres are positive for neutral mucosubstances and proteins with arginine, tyrosine and SH-groups. They contain a small amount of neutral lipids. The tegument-forming pyriform cells stain with aldehyde-fuchsine and alcian blue (AB) at pH 0.2, but they are negative to AB at pH 2.6. They contain a small amount of proteins with arginine and they are positive to CT. Also neutral lipids were detected. Calcareous bodies were found only in some sections and could be stained with Best's carmine.

NECK AND SCOLEX

The outer limiting layer does not stain by any of the used methods. The microtriches and amorphous substance of tegument are positive to PAS reaction even after saliva test. They contain acid mucosubstances, proteins with arginine, tryptophan, tyrosine and SS-groups. Proteins with SH-groups occurred in a smaller amount. Also neutral lipids were detected.

The argyrophilic fine connective tissue fibres of subtegument contain a small amount of glycogen and tyrosine and they are positive to luxol blue. The thicker connective tissue fibres of subtegument contain acid mucosubstances and proteins with tryptophan, tyrosine and SS-groups. They are positive to CT, luxol blue and Sudan black B. Muscle fibres of subtegument contain glycogen and proteins with arginine, tyrosine

Plates I, II; Tables 1, 2

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Table 2. Results of histochemical reactions for the detection of proteins and lipids

Reaction	Cyst				Neck and scolex			
	Tegument		Subtegument		Tegument		Subtegument	
	Microvilli	Amorphous substance	Fine connective tissue fibres	Connective tissue fibres	Muscle fibres	Pyriform cells	Parenchyma cells	Hooks
Sakaguchi	+	+	+	+	+	+	+	+
DMAB	+	+	+	+	+	+	+	+
Morel-Sisley	+	+	+	+	+	+	+	+
CT	+/++	+/++	+	+	+	+	+	+
DDD	+/++	+/++	+	+	+	+	+	+
Thioglycolic acid + DDD	+/+++	+/++	+	+	+	+	+	+
N-ethylmaleimid + DD	-	+	+	+	+	+	+	+
AB pH 0.2	+/++	+	+	+	+	+	+	+
PFA + AB pH 0.2	+	+	+	+	+	+	+	+
PAA + AF	++	+	+	+	+	+	+	+
Sudan black B (in paraffin section)	++	+	+	+	+	+	+	+
Luxol blue	++	+	+	+	+	+	+	+

* numerator: reaction in the neck and scolex without rostellar sac-denominator: reaction in the rostellar sac

and SH-groups. The tegument-forming pyriform cells contain a small amount of acid mucosubstances and proteins with arginine and a great amount of proteins with SS-groups. They are positive to CT, luxol blue and Sudan black B. The parenchyma cells of neck and scolex contain a small amount of glycogen and a greater amount of neutral ptyalin-resistant mucosubstances, acid mucosubstances, and proteins with SS-groups. They markedly differ from the parenchyma cells of the rostellar sac which are arranged in palisade and contain a great amount of neutral and acid mucosubstances and proteins with SH- and SS-groups. The cavity of sucker is filled with PAS-positive ptyalin-resistant gelatinous matter (++). The hooks contain acid mucosubstances and a great amount of proteins with SS-groups. Calcareous bodies stain with Best's carmine. The connective tissue and muscle tissue fibres of parenchyma of neck and scolex exhibit the same reactions as in the subtegument, only the muscle fibres of suckers contain approximately double amount of glycogen.

DISCUSSION

The cysticercoids of *C. integer* do not cease to grow after invagination of the scolex and neck into the cyst cavity, as it is the case of hitherto described species from beetles, snails and planktonic crustaceans, but they keep growing and turn from oval to lens-shaped. Their size increases from $417-461 \times 272-329 \mu\text{m}$ to $564-667 \times 272-329 \mu\text{m}$ and the microvilli differentiate in their length from original $1 \mu\text{m}$ up to $18 \mu\text{m}$. The lens-shaped cysts are evidently adapted in their shape to the host body and its body cavity where they are situated in such a way that their longer axis runs in the direction of flattening. The growing of the tegument and subtegument of cyst in lens-shaped cysticercoids (Valkounová 1983) seems to be related to the life span of cysticercoids conditioned by the life cycle of *Gammarus* (they survive the winter season). Long and dense microvilli seem to perform a nutritional function in the cysticercoid and the increased connective tissue and muscle fibres of subtegument make it firm and elastic.

The oval cysticercoids differ in some components of their histochemical structure from the lens-shaped ones. The tegument and tegument-forming cells of oval cysticercoids contain a greater amount of neutral (+ + + / + + +) and acid (+ + + +) mucosubstances and proteins with SH-groups (+ + +). The connective tissue fibres contain a greater amount of acid mucosubstances (+ + + +). The tegument of oval cysticercoids is strongly positive to AB pH 2.6 even after demethylation, it stains strongly with aldehyde-fuchsine and methylen blue at pH 7.25 (MBE method) and the affinity to AB pH 2.6 disappears at 4% concentration of MgCl_2 in the CEC method. The connective tissue fibres of oval cysticercoids are negative to AB pH 2.6 after demethylation, stain with methylene blue at pH 3.62 and the affinity to AB pH 2.6 disappears at 8% concentration of MgCl_2 . Consequently, in oval cysticercoids, acid mucosubstances with carboxyl groups can be detected in the tegument and acid mucosubstances with sulphogroups in connective tissue fibres.

In the oval cysts, calcareous bodies staining characteristically by Kóssa's method are regularly distributed in the subtegument. In lens-shaped cysts, these bodies were not detected at all or they occurred only in some parts of subtegument. Their number markedly decreased in the parts of cyst where the muscle and connective tissue fibres increased during the growth of the subtegument. They stained very faintly with Kóssa's method and more markedly only with Best's carmine.

In addition to common muscle and connective tissue fibres, which are arranged in a typical manner, a great number of cells having the character of palisade-arranged

parenchyma cells were observed in the rostellar sac of *C. integer*. These cells contain a great amount of reserve substances which are probably related to the number of hooks on the rostellum (65—75).

ГИСТОХИМИЯ ЦИСТИЦЕРКОИДА ЦЕСТОДЫ *CORONACANTHUS INTEGER* (HAMANN, 1891) (HYMENOLEPIDIDAE)

И. Валкоунова

Резюме. Изучали содержание мукосубстанций, белков и липидов в чечевичеобразных цистицеркоидах в возрасте более 20 дней. В отличие от меньших, овальных цистицеркоидов, которые моложе, чем 20 дней, старшие цистицеркоиды содержат небольшое количество нейтральных и кислых мукосубстанций и белков с SH-группами в тегументе и тегумент-образующих клетках и кислые мукосубстанции в волокнах соединительной ткани. Известковые тельца регулярно встречались в субтегументе овальных цист, тогда как в субтегументе чечевичеобразных цист они встречались только временами.

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FIRST RECORD OF THE TREMATODE TYLODELPHYS PODICIPINA KOZICKA ET NIEWIADOMSKA, 1960 IN CZECHOSLOVAKIA

During studies on the endohelminths of fishes of the Mácha Lake fishpond system in northern Bohemia, Czechoslovakia, carried out in the years 1975—77 and 1981—82, a different type of diplostomatid metacercariae was found, in addition to those of *Tylodelphys clavata* (Nordmann, 1832), in the vitreous body of the eyes of perch (*Perca fluviatilis* L.) and ruffe (*Gymnocephalus cernuus* (L.)); these were noted, in contradistinction to *T. clavata*, for a markedly larger size of body and an inverse size ratio of suckers. In the paper by Moravec (Scripta Fac. Sci. Nat. UJEP Brun., Biol. 2, 8: 77—80, 1978) these metacercariae were included into the species *T. clavata*; however,

subsequent studies have indicated that they belong to the species *Tylodelphys podicipina* which has been recognized as a distinct species only relatively recently (Kozicka J., Niewiadomska K., Acta Parasit. Polon. 8: 25—35, 1960). Although a large number of fishes belonging to 13 species was examined from the locality, *T. podicipina* was recorded only from *P. fluviatilis* (prevalence 58 %, intensity 1—14 specimens) and *G. cernuus* (prevalence 50 %, intensity 1—6); on the other hand *T. clavata* was found here in 7 fish species, including *P. fluviatilis* (prevalence 80 %, intensity 1—244) and *G. cernuus* (prevalence 67 %, intensity 1—26).

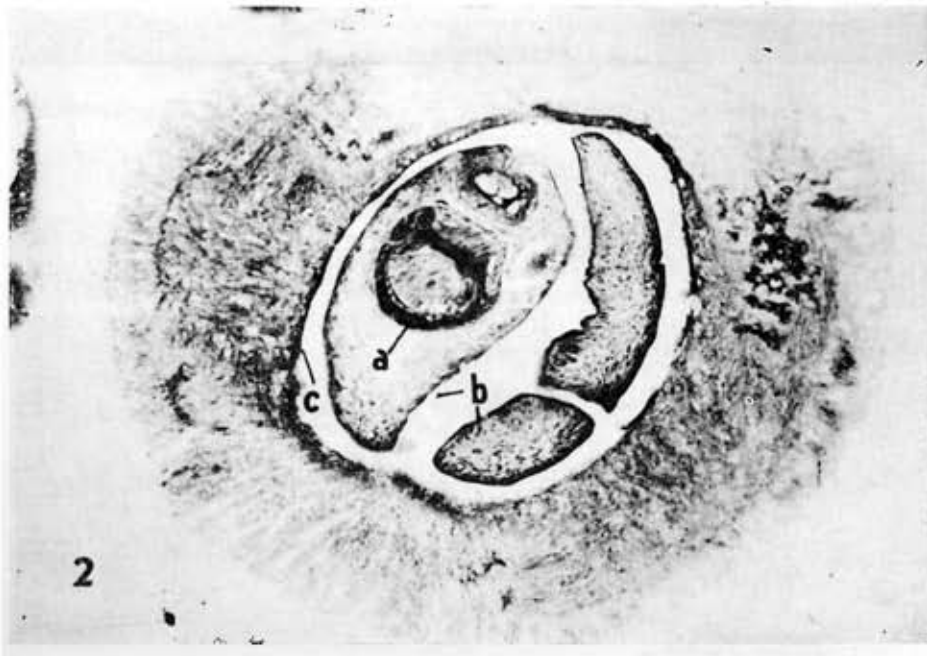
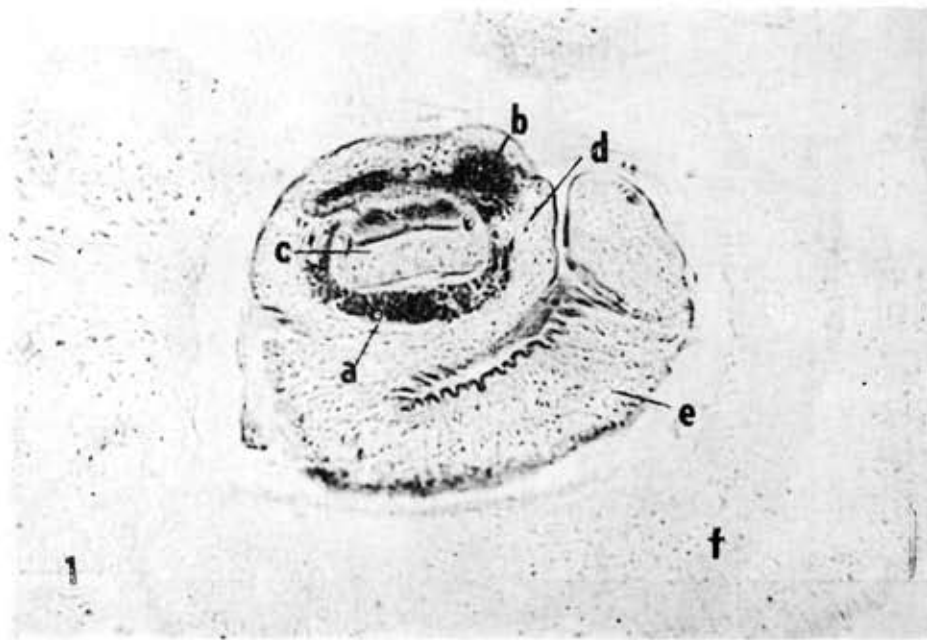


Fig. 1. Distribution of neutral mucosubstances in the tegument, muscle fibres and parenchyma cells of cysticercoid. Rostellar sac (a), gelatine matter filling the sucker cavity (b), rostellum (c), scolex (d), neck (e) and cyst (f). PAS ($\times 240$). **Fig. 2.** Occurrence of acid mucosubstances in the tegument, connective tissue fibres and parenchyma cells of the rostellar sac (a), scolex and neck (b) and cyst (c). AB pH 2.6 ($\times 200$).

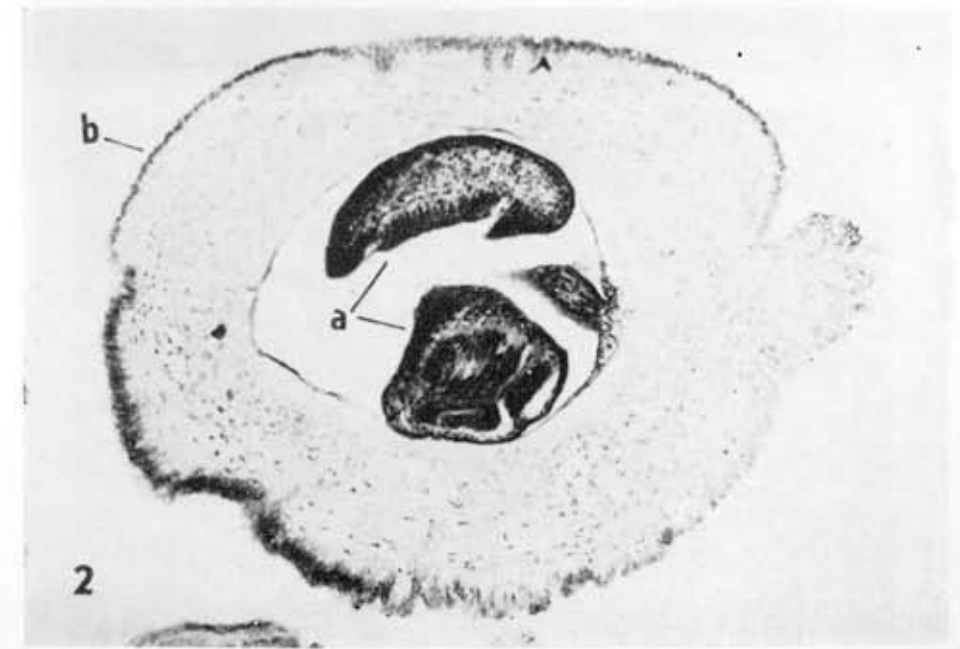
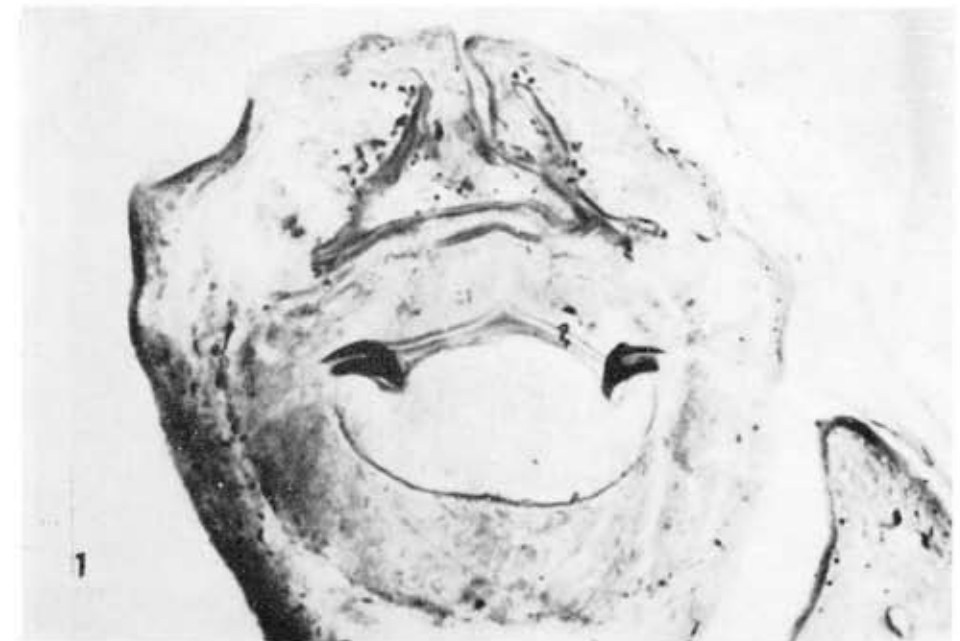


Fig. 1. High content of proteins with SS-groups in hooks. PAA-AF ($\times 770$). **Fig. 2.** Proteins with SH-groups are abundant in the tegument, muscle fibres and parenchyma cells of neck and scolex (a), less in cyst tegument (b). DDD ($\times 170$).