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HISTOCHEMISTRY OF THE LARVA OF DIPLOPYLIDIUM NOELLERI (SKRJABIN, 1924)

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Abstract. The larva of *D. noelleri* is surrounded by a capsule consisting of muscle and connective tissue fibres, which give positive reaction for acid mucosubstances with sulphogroups (connective tissue fibres), proteins with SH groups (muscle fibres) and positive CT reaction (fine connective tissue fibres and muscle fibres). The cells of the gelatinous matter filling the space between the inner wall of the capsule and the larva show strong positive reaction for acid mucosubstances with sulphogroups. The tegument of larva contains microtriches which are strongly positive for proteins with SS groups and arginine. Proteins with arginine and SH groups were detected in the amorphous substance of the tegument. Proteins with SS groups were found in the amorphous substance of rostellum and in the hooks. Fine connective tissue fibres of the subtegument contain neutral mucosubstances and are positive in CT reaction. The muscle fibres of subtegument give positive reaction for glycogen, proteins with arginine, tyrosine and SH groups. Acid mucosubstances with sulphogroups and proteins with SS groups were detected in the connective tissue fibres of the subtegument. The pyriform cells contain proteins with SH groups and are positive in CT reaction. The cells of parenchyma are positive for glycogen and proteins with SH groups. The muscle and connective tissue fibres of the parenchyma give the same reactions as those of the subtegument.

The present paper deals with the content of mucosubstances, proteins and neutral lipids in the tissue of *D. noelleri* larva. It is a continuation of a previous study of the morphological structure of these larvae (Valkounová 1982) and belongs to the series of papers dealing with larvae of cestodes of the families Hymenolepididae Fuhrmann, 1907 and Dilepididae Fuhrmann, 1907 (Valkounová and Prokopič 1978, 1979, 1980, 1981). The results will be used for comparative studies of the morphology of these larvae.

MATERIAL AND METHODS

The material was recovered from the mesentery and outer intestinal wall of snakes *Tarbophis obtusus*, fixed in Baker's neutral formaldehyde and stained with 30 histochemical methods (for details see Valkounová and Prokopič 1979).

RESULTS

The larva of *D. noelleri* is surrounded by a gelatinous matter and protected by a capsule consisting of muscles and connective tissues.

STRUCTURE OF THE CAPSULE AND LARVA

Capsule — outer layer — circular muscle and connective tissue fibres
— longitudinal muscle and connective tissue fibres
— middle layer — irregularly arranged muscle and connective tissue fibres, fibroblasts, nuclei and remnants of the plasma
— inner layer — longitudinal muscle and connective tissue fibres
— inner limiting layer

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

Reaction	Capsule				Gelatinous matter	Larva												
						Outer limiting layer	Tegument		Subtegument									
	Fine connective tissue fibres	Connective tissue fibres	Muscle fibres	Inner limiting layer			Microtriches	Amorphous substance	Basement layer	Fine connective tissue fibres	Connective tissue fibres	Muscle fibres	Pyriform cells	Parenchyma cells	Calcareous corpuscles	Hooks
PAS	+	|	+	±	+ / + +	+ / + +	±	+ +	|	+ +	|	+	±	+ + + +	|	|
Schiff	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|
Saliva test + PAS	+	|	±	±	+ / + +	+ / + +	|	+ +	|	+ +	|	+ / + +	|	|	|	|
Acetylation + PAS	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|
Desacetylation + PAS	+	|	+	±	+ / + +	+ / + +	|	+ +	±	+ +	|	+ +	|	+ + +	|	|
Best's carmine	+	|	+	±	+	+	|	+ +	|	+ +	|	+ + +	+ + [1]	|	± [1]	|
Saliva test + Best's carmine	+	|	+	±	+	+	|	+ +	|	+ +	|	+	|	|	± [1]	|
AB pH 2.6	|	+ + + +	|	|	+++/++++	|	+	+ +	|	|	+ + + +	|	+	±	|	++++
AB + PAS	rose	blue	rose	faint rose	violet	rose	light blue	violet	|	red	light blue	red	light blue	red	|	|
Methylation + AB pH 2.6	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|
Demethylation + AB pH 2.6	|	±	|	|	±	|	±	±	|	|	+	±	±	|	|	|
CEC (AB pH 2.6 + $MgCl_2$)	|	16 %[2]	|	|	16 %[2]	|	14 %[2]	14 %[2]	|	|	16 %[2]	|	|	|	|
MBE at pH	|	3.62	|	|	3.62	|	3.2	3.2	|	|	3.62	|	|	|	|
Aldehyde fuchsin	|	+ + + +	|	|	+ +	|	+ +	+ +	|	|	+	|	±	|	|	|
Hale	|	+	|	|	±	|	±	+ +	|	|	+ +	|	|	|	|	|
Hale + control	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|	|
Hale + PAS	red	light blue	rose	|	violet	rose	|	light violet	|	red	blue	red	light blue	red	|	|
Coloidal Fe^{3+}	|	±	|	|	±	|	+ / + +	+ / + +	|	|	±	|	+	|	|	|

CEC — critical electrolyte concentration; MBE — methylene blue extinction; [1]) only inner part; [2]) At this concentration of $MgCl_2$ the affinity to AB pH 2.6 disappears

Gelatinous matter — cells of irregular shape, in the section 4—5 sides, tightly adjacent to one another

Larva — outer limiting layer
- tegument — microtrichs
  - amorphous substance
- basement layer
- subtegument — circular muscle and connective tissue fibres
  - longitudinal muscle and connective tissue fibres
  - pyriform cells
  - calcareous corpuscles
- parenchyma — same structure as parenchyma of adult cestodes

CAPSULE

Tables 1, 2

The muscle and connective tissue fibres of all layers of the capsule give the same histochemical reactions. The fine connective tissue fibres are weakly positive in PAS reaction (Plate I, Figs. 1, 2), in staining with Best's carmine, in these methods combined with saliva test and in Sudan black B staining in paraffin sections. A stronger positivity was demonstrated in CT reaction. The connective tissue fibres give a strong reaction for acid mucosubstances in the method with AB pH 2.6 (Plate II, Fig. 1) or AF. After demethylation, the reaction with AB pH 2.6 is negative. Consequently, the connective tissue fibres contain acid mucosubstances with sulphogroups as it is indicated by the results of CEC and MBE tests. Muscle fibres were found to contain an almost negligible amount of glycogen and other neutral mucosubstances and a higher amount of proteins with arginine and SH groups. The CT reaction was the strongest. The outer limiting layer was not detected. The inner limiting layer gave negative results in staining methods, including almost negligible reactions for neutral mucosubstances (±).

The gelatinous matter contains a small amount of neutral ptyalin-resistant mucosubstances (Plate I, Figs. 1, 2) and proteins with tyrosine, SH and SS groups. More pronounced was only the reaction with AB pH 2.6 (Plate II, Fig. 1), which in combination with MBE and CEC methods revealed acid mucosubstances with sulphogroups.

LARVA

Tables 1, 2

The outer limiting layer contains neutral ptyalin-resistant mucosubstances. The microtriches of tegument contain a very small amount of acid mucosubstances and proteins with tyrosine (+). The reaction for proteins with arginine (Plate II, Fig. 2) and SS groups was stronger. Positive reactions were obtained if the methods of PAA + AF, Sudan black B (Plate III, Fig. 2) and Luxol blue were used. The amorphous substance of the tegument contains neutral ptyalin-resistant mucosubstances, acid mucosubstances, proteins with arginine (Plate II, Fig. 2), tyrosine and SH groups. Positive were also the CT reaction, and reactions with Sudan black B (Plate III, Fig. 2) and Luxol blue (Plate III, Fig. 1). The amorphous substance of rostellum and hooks was found to contain proteins with SS groups. The basement layer could not be stained by any of the used methods.

The fine connective tissue fibres of the subtegument contain neutral ptyalin-resistant mucosubstances and show positive CT reaction. They envelope the thick connective tissue fibres of the subtegument (Plate IV, Fig. 1) which contain acid mucosubstances with sulphogroups, proteins with SS groups and give positive reaction to Luxol blue staining. The muscle fibres of the subtegument contain a small amount

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

Reaction	Capsule			Larva														
					Tegument			Subtegument										
	Fine connective tissue fibres	Connective tissue fibres	Muscle fibres	Gelatinous matter	Micro-triches	Amorphous substance	Basement layer	Fine connective tissue fibres	Connective tissue fibres	Muscle fibres	Pyriform cells	Parenchyma cells	Hooks	Calcareous corpuscles
Sakaguchi			+/++	++	++	++++			++	+++	++	+++/+		
DMAB			+	++	++	+			++	+	++			
Morel—Sisley			++	+	+	+/++			+	++	+	+		
CT		+	+	+	+	+	++	+	+	+++	+++	+		
DDD			+	+	++	+				+++	+	+		
Thioglycollic acid + DDD			+	+	+	++++ [2]			+	+++	+++	+	+	
N-ethylmaleimid + DDD						+++					++			
AB pH 0.2		+			+	++++ [2]			+		+	++		
PFA + AB					+	++ [2]			+		+		+	
PAA + AF		+			+	++++ [2]			+		+		++++	
Sudan black B (in paraffin sections)		+		+	++++	++++			+		+	+		+ [1]
Luxol blue		+		++	+/++	++++			+++		+	+		+

CT — coupled tetrazonium reaction; [1]) only inner part; [2]) numerator: reaction in the rostellum, denominator: reaction in the remaining parts of larva

of neutral ptyalin-resistant mucosubstances, acid mucosubstances and a detectable amount of glycogen and proteins with arginine, tyrosine and SH groups. They give a positive CT reaction. The pyriform cells contain proteins with arginine and SH groups and they are positive to CT reaction, Sudan black B (Plate III, Fig. 2) and Luxol blue. The parenchymal cells contain glycogen (Plate I, Figs. 1, 2), proteins with arginine and SH groups and are positive to CT method. The muscle and connective tissue fibres of the parenchyma show the same histochemical reactions as those of the subtegument. The inner part of calcareous bodies situated in the subtegument and parenchyma was slightly stained with Best's carmine, AB pH 2.6 and Sudan black B (±).

DISCUSSION

In the description of the morphology of *D. noelleri* larva (Valkounová 1982) some deviations in its structure were mentioned in comparison with the cysticercoids the scolex of which is invaginated into posterior portion of larva — cyst. While comparing the histochemical structure of *D. noelleri* and previously studied cysticercoids of *Rodentotaenia crassiscolex* (Linstow, 1890) and *Hymenolepis erinacei* (Gmelin, 1789). (Valkounová and Prokopič 1979, 1981) it may be assumed that in case of *D. noelleri* completely developed larvae are concerned, which have lived for some time in the host body. The histochemistry of these larvae is described in the present paper.

Earlier phases of larval development could be observed in *R. crassiscolex*, *H. erinacei* and also in some sections of *D. noelleri* (Plate IV, Fig. 2). Compared to fully developed larvae, even twice as much acid mucosubstances were demonstrated in the tegument, connective tissue fibres and pyriform and parenchymal cells of the younger phases. This difference was still more marked in the granules on the scolex surface in *H. erinacei*, in the fluid filling the capsule cavity in *R. crassiscolex* and in parenchymal cells of *D. noelleri* (compare Plate II, Fig. 1, Plate IV, Fig. 2). On the other hand, the amount of glycogen in parenchymal cells and muscle fibres was low and increased gradually with decreasing amount of acid mucosubstances during the development of the larva. It is possible that there is a certain relation between the occurrence of acid mucosubstances and glycogen as it is known that the glycogen can arise by splitting of acid mucosubstances in worms. In the earlier phases of larval development, the reserve substances are probably deposited also in form of acid mucosubstances.

ГИСТОХИМИЯ ЛИЧИНКИ *DIPLOPYLIDIUM NOELLERI* (SKRJABIN, 1924)

Й. Валкоунова

Резюме. Личинка *D. noelleri* окружена капсулой, состоящей из соединительно-тканевых и мышечных волокон, дающих положительную реакцию на кислые мукосубстанции с сульфогруппами (соединительно-тканевые волокна), белки с SH группами (мышечные волокна) и положительную CT реакцию (тонкие соединительнно-тканевые волокна и мышечные волокна). Клетки желатинозного вещества, выполняющие пространство между внутренней стенкой капсулы и личинкой, содержат кислые мукосубстанции с сульфогруппами. В тегументе личинки встречаются микротрихи, дающие выразительно положительную реакцию на белки с SS группами и аргинином. Основное вещество тегумента содержит белки с аргинином и SH группами. Белки с SS группами были обнаружены в основном веществе хоботка и в крючьях. Тонкие соединительно-тканевые волокна субтегумента содержат нейтральные мукосубстанции и положительны при CT реакции. В мышечных волокнах субтегумента обнаружен гликоген, белки с аргинином, тирозином и SH группами. Соединительно-тканевые волокна субтегумента содержат кислые муко-

субстанции с сульфогруппами и белки с SS группами. В грушевидных клетках обнаружены белки с SH группами и СТ реакция оказалась положительной. В клетках паренхимы обнаружен гликоген и белки с SH группами. Мышечные и соединительно-тканевые волокна паренхимы личинки дают одинаковые реакции как волокна субтегумента.

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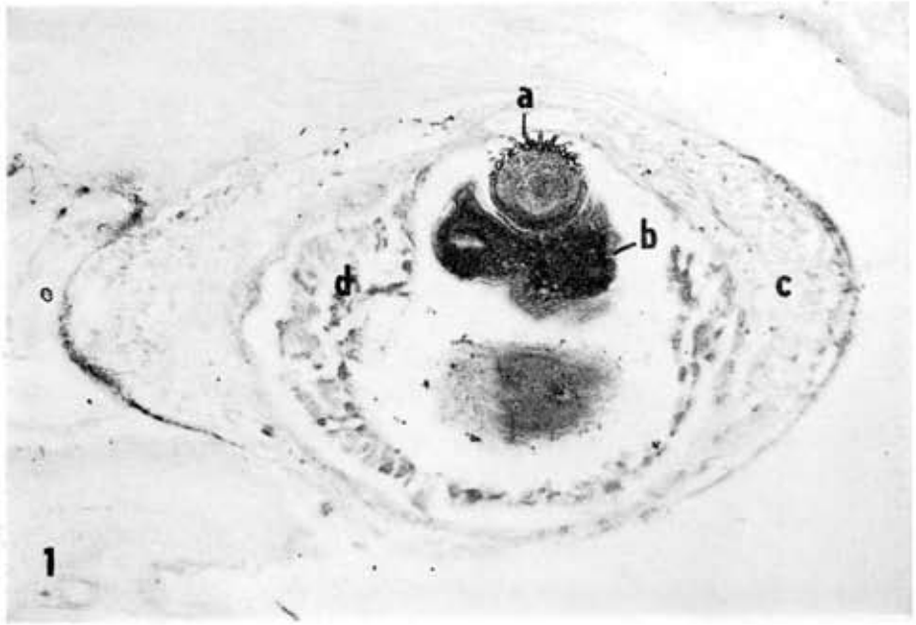


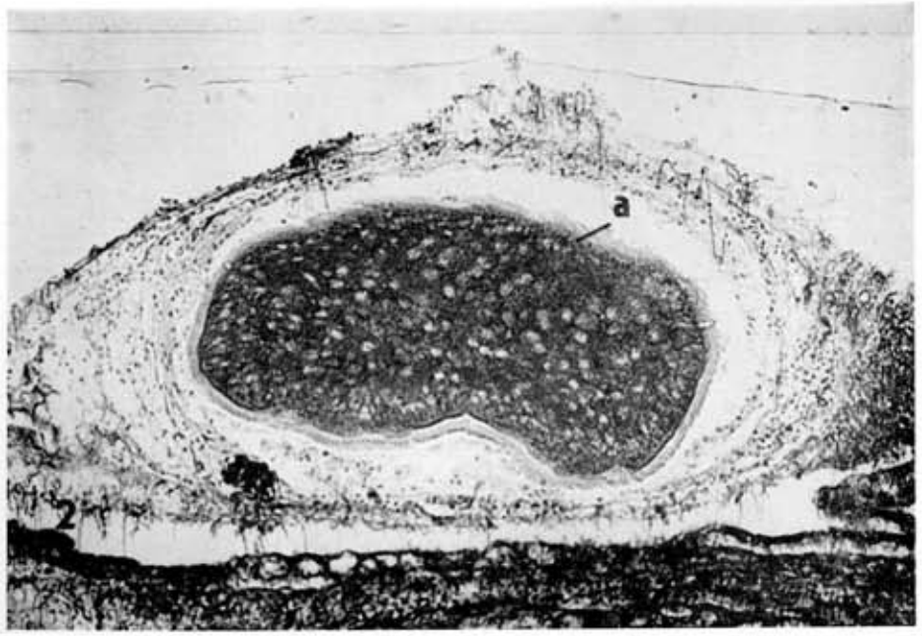
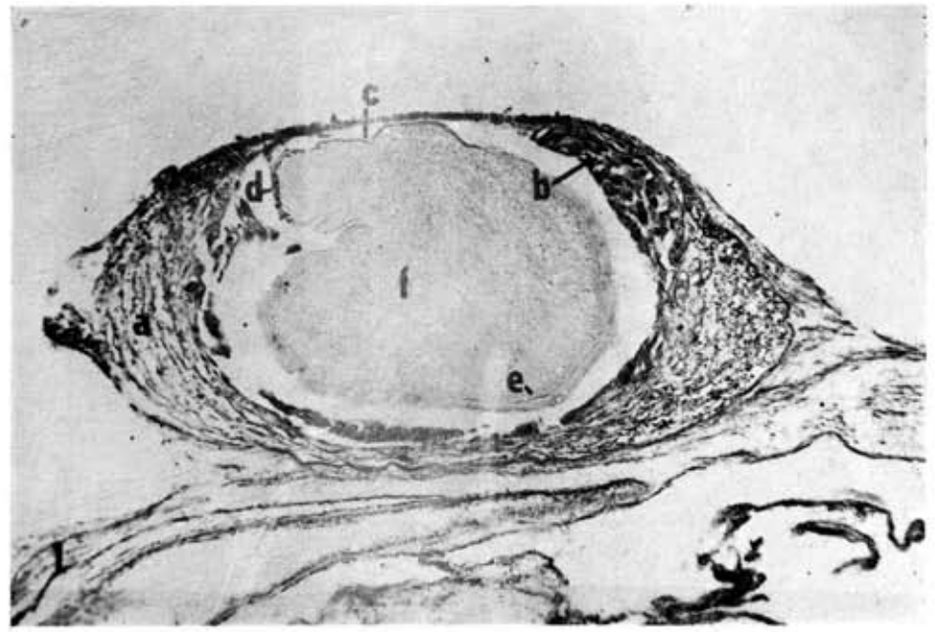


Fig. 1. Difference in the content of neutral mucosubstances in the body of larva — in a section through rostellum (a) and sucker (b) compared to capsule wall (c) and cells of gelatinous matter (d). **PAS** reaction (×120). **Fig. 2.** Positive reaction for neutral mucosubstances in the parenchyma of larva (a). Best's carmine (×130).



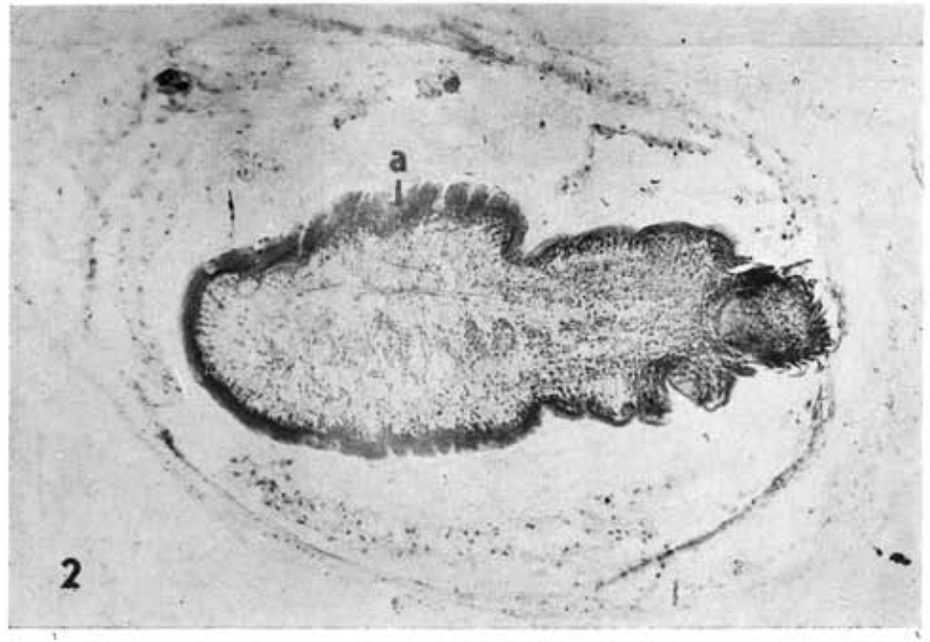


Fig. 1. Acid mucosubstances in the capsule wall (a), cells of gelatinous matter (b), tegument (c), hooks (d) and subtegument (e) of fully developed larva. Parenchymal cells (f) contain a negligible amount of acid mucosubstances. AB pH 2.6 (×100). **Fig. 2.** Arginine in the tegument of larva (a). Sakaguchi (×100).

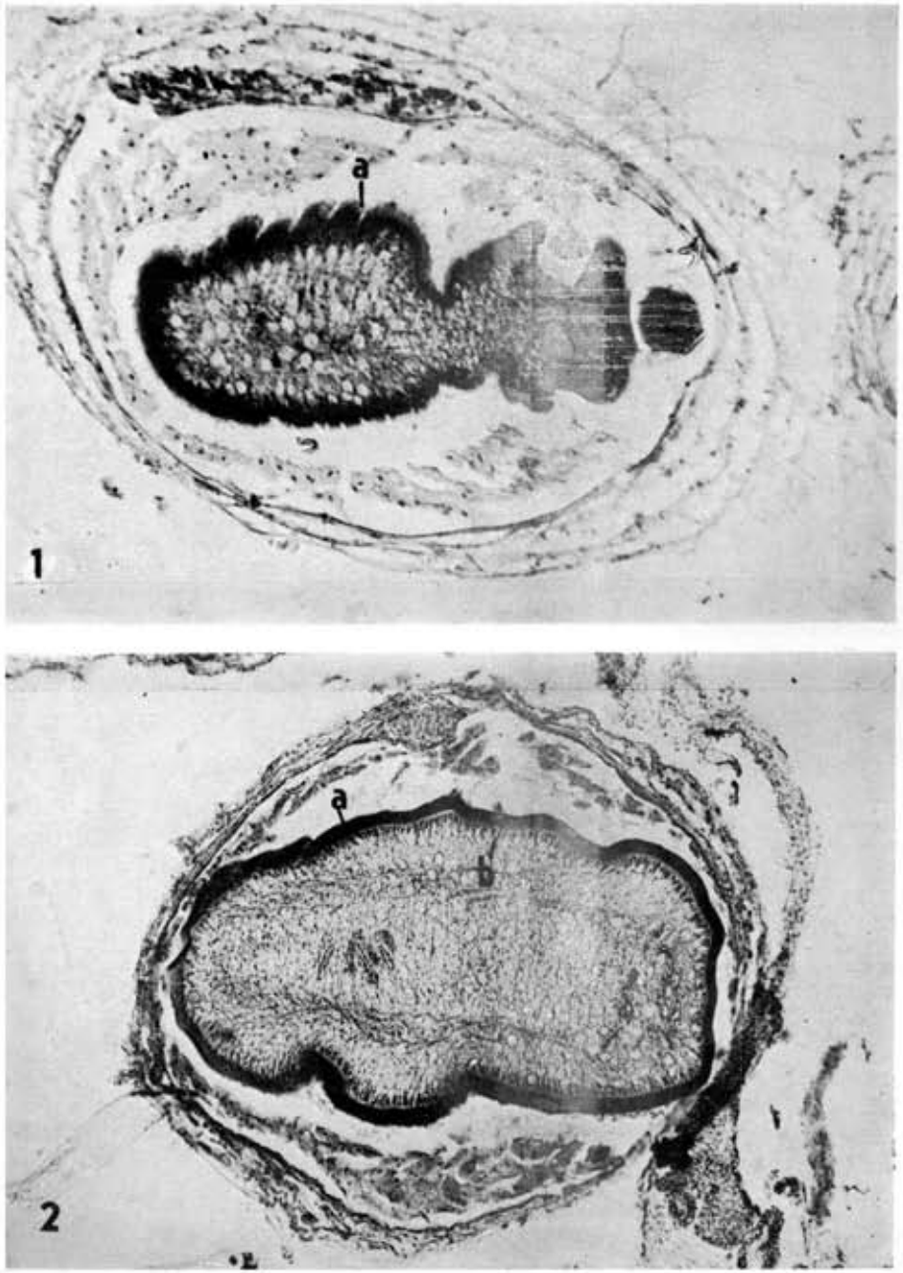


Fig. 1. Intense reaction with Luxol blue in the tegument and subtegument of larva (a) (×140). **Fig. 2.** Positive reaction for neutral lipids in the tegument (a) and pyriform cells of subtegument (b) of larva. Sudan black B (×120).

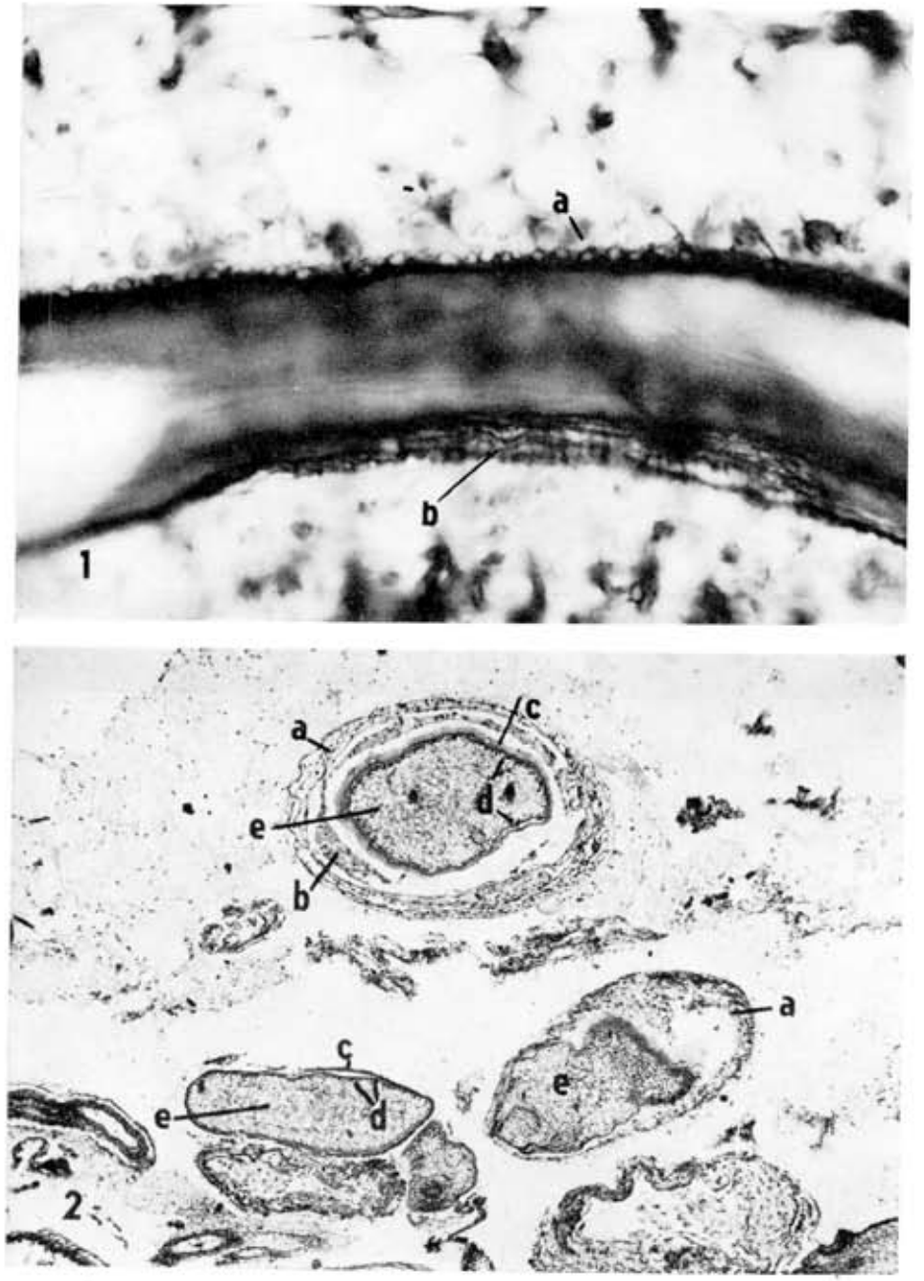


Fig. 1. Transversely (a) and longitudinally (b) arranged connective tissue fibres of the subtegument of larva. Gomori (×1,000). **Fig. 2.** Content of acid mucosubstances in the capsule wall (a) and cells of gelatinous matter (b) is approximately the same as in the body of earlier phase of larva — in tegument (c), connective tissue fibres and pyriform cells of subtegument (d) and parenchymal cells (e). AB pH 2.6 (×50).