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# HISTOCHEMISTRY OF THE CYSTICERCOID OF HYMENOLEPIS ERINACEI (GMELIN, 1789)

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**Abstract.** The contents of mucosubstances, proteins and lipids were studied for the first time in a morphologically differentiated and invaginated larva of *H. erinacei* consisting of a tailed cyst with invaginated neck and scolex. The outer limiting layer of the cyst, neck and scolex gives a strongly positive reaction to PAA + AF and AB pH 0.2. The inner limiting layer of the cyst and neck shows no stain by any of the methods employed. The fine connective tissue fibres of the cyst tegument and subtegument of cyst, neck and scolex contain PAS-positive, ptyalin-resistant neutral mucosubstances and give a strong positive reaction to PFA + AB and PAA + AF. The microtriches of neck and scolex tegument contain tyrosine and tryptophan and show positive reaction to PFA + AB and PAA + AF. The amorphous substance in the tegument of cyst, neck and scolex contains tryptophan and proteins with SS groups, the amorphous substance of scolex gives a strong PAS-positive reaction to ptyalin-resistant neutral mucosubstances. The histochemical structure of the connective tissue fibres, muscle fibres and pyriform cells is identical in the cyst, neck and scolex. The connective tissue fibres contain acid mucosubstances and show a very weak reaction for proteins with SS groups and strong reaction with PAA + AF method. The muscle fibres contain arginine, tryptophan, tyrosine and proteins with SH groups and show a positive CT reaction. The pyriform cells contain glycogen, acid mucosubstances and a small amount of arginine and proteins with SS groups. The inner portion of calcareous bodies gives a positive reaction to AB pH 2.6 and Sudan black B. The inner zone of cyst shows a positive reaction to CT, PAA + AF, Luxol blue and Sudan black B.

The present paper is a continuation of morphological studies on the cysticercoid of *Hymenolepis erinacei* (Gmelin, 1789) (Valkounová and Prokopič 1980) dealing particularly with the histochemistry of cyst, neck and scolex tegument and subtegument.

## MATERIAL AND METHODS

The cysticercoids were obtained from spontaneously infected beetles *Geotrupes stercorarius* L., *Oeceoptoma thoracica* (L.), *Necrophorus humator* Goeze, *N. interruptus* Steph. and *N. vespilloides* Herbst. Isolated cysticercoids fixed in Baker's formaldehyde (Pearse 1968) were processed. The material for the detection of tryptophan was fixed for 24 h in Baker's fluid the pH of which was adjusted to 6.5 using 0.1N NaOH (Lojda 1965). Series of 5—7 μm thick paraffin sections were used for histochemical detection of mucosubstances, proteins and lipids. The methods were described in the paper by Valkounová and Prokopič (1979).

## RESULTS

The cysticercoid consists of a tailed cyst enclosing a neck and scolex in its cavity (scheme of invagination — Neradová-Valkounová 1971, Valkounová and Prokopič 1978).

**Cyst** — outer limiting layer
— tegument — outer part — circular and longitudinal fine connective tissue fibres
— inner part — amorphous substance
— basement layer
— subtegument — outer circular layer of connective tissue and muscle tissue fibres
— outer longitudinal layer of connective tissue and muscle tissue fibres
— pyriform cells, calcareous bodies
— inner longitudinal layer of connective tissue and muscle tissue fibres
— inner zone
— inner limiting layer
**Neck** — outer limiting layer
— tegument — proximal part — outer part — microtriches
— inner part — amorphous substance
— distal part — amorphous substance
— basement layer
— subtegument — outer circular and longitudinal layer of connective tissue and muscle tissue fibres
— pyriform cells, calcareous bodies
— parenchyma — only in proximal part of neck
— inner limiting layer
**Scolex** — outer limiting layer
— tegument — outer part — microtriches
— inner part — amorphous substance
— basement layer
— subtegument — same structure as subtegument of neck
— parenchyma — same structure as parenchyma of adult cestodes

## CYST

Tables 1,2

The outer limiting layer, which was considerably damaged and partly cut off, gave strongly positive reaction to PAA + AF and AB pH 0.2 (Plate I, Fig. 3), whereas the reaction to thioglycolic acid + DDD was negative. A weak positivity of ptyalin-resistant mucosubstances was observed after staining with Best's carmine and the PAS positivity was negligible. Numerous granules were situated on the surface of the outer limiting layer and showed the same reactions.

The fine connective tissue fibres of tegument contained PAS-positive, ptyalin-resistant neutral mucosubstances and lipids (Plate II, Fig. 1) and gave a strong positive reaction to PFA + AB and PAA + AF. The reaction to thioglycolic acid + DDD was again negative. The amorphous substance of tegument was found to contain tryptophan and proteins with SS groups and gave positive CT reaction. The basement layer was positive only to Sudan black B.

The connective tissue fibres of the subtegument contained acid mucosubstances and gave very weak reaction for proteins with SS groups and stronger reaction for lipids as determined by staining with Luxol blue. The connective tissue fibres showed also positive reaction to PFA + AB and PAA + AF, though the reaction to thioglycolic acid + DDD was very weak. The connective tissue fibres of the outer circular and longitudinal connective tissue and muscle layer are regularly but rather sparsely distributed, whereas the connective tissue fibres of the inner longitudinal connective tissue and muscle layer are dense (Valkounová and Prokopič 1980). For this reason the latter layer stained more intensively with the above methods. The muscle fibres contained PAS-positive, ptyalin-resistant neutral mucosubstances, arginine, tryptophan, tyrosine and proteins with SH groups and showed a positive CT reaction. The pyriform cells contained glycogen (Plate I, Figs. 1, 2) and acid mucosubstances, gave a positive CT reaction and were weakly positive for arginine, proteins with SS groups and lipids as detected by Sudan black B and Luxol blue staining.

**Table 1.** Results of histochemical reaction for the detection of mucosubstances in the wall of cyst

| Reaction | Outer limiting layer | Tegument | | Subtegument | | | |
|---|---|---|---|---|---|---|---|
| | | Fine connective tissue fibres | Amorphous substance | Connective tissue fibres | Muscle fibres | Pyriform cells | Calcareous corpuscles |
| PAS | ± | ++ | ± | − | ++ | ++/+++ | − |
| Schiff | − | − | − | − | − | − | − |
| Saliva test + PAS | ± | ++ | ± | − | ++ | − | − |
| Acetylation + PAS | − | − | − | − | − | − | − |
| Desacetylation + PAS | − | + | − | − | ± | ++ | − |
| Best's carmine | + | + | ± | − | + | +++ | ++[1] |
| Saliva test + Best's carmine | + | + | ± | − | + | ± | ++[1] |
| AB pH 2.6 | − | − | ± | ++ | − | ++ | ++[1] |
| AB + PAS | − | rose | − | blue | rose | violet | − |
| Methylation + AB pH 2.6 | − | − | − | − | − | − | − |
| Demethylation + AB pH 2.6 | − | − | ± | +/++ | − | ++ | ++[1] |
| CEC (AB pH 2.6 + $MgCl_2$) | − | − | 10 %[2] | 14 %[2] | − | 6 %[2] | − |
| MBE at pH | − | − | 7,25 | 3.62 | − | 6.12 | − |
| Aldehyde fuchsin | − | ± | + | + | − | + | − |
| Hale | − | ± | ± | ± | − | ± | − |
| Coloidal $Fe^{3+}$ | − | ± | +/++ | ± | − | + | − |
| Hale + control | − | − | − | − | − | − | − |
| Hale + PAS | − | rose | − | light blue | rose | violet | − |

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

**Table 2.** Results of histochemical reactions for the detection of proteins and lipids in the wall of cyst

| Reaction | Granules on the surface | Outer limiting layer | Tegument | | Subtegument | | | | Inner zone |
|---|---|---|---|---|---|---|---|---|---|
| | | | Fine connective tissue fibres | Amorphous substance | Connective tissue fibres | Muscle fibres | Pyriform cells | Calcareous corpuscles | |
| Sakaguchi | − | − | − | ± | − | ++ | +/++ | − | +/++ |
| DMAB | − | − | − | +/++ | − | ++ | ± | − | − |
| Morel — Sisley | − | − | − | ± | − | +/++ | − | − | − |
| CT | − | − | − | ++ | − | ++ | ++ | − | +++ |
| DDD | − | − | − | ± | − | ++ | ± | − | − |
| Thioglycollic acid + DDD | − | − | − | ++ | + | +++ | +/++ | − | − |
| N-ethylmaleimid + DDD | − | − | − | − | − | − | − | − | − |
| AB pH 0.2 | +++ | +++ | ± | + | ± | − | − | − | − |
| PFA + AB | − | − | ++/+++ | + | ++ | − | − | − | − |
| PAA + AF | ++++ | ++++ | ++++ | +/++ | +++ | ± | +/++ | − | +++/++++ |
| Sudan black B | − | − | ++ | − | + | − | + | ++[1] | ++++ |
| Luxol blue | − | − | +/++ | − | ++ | − | +/++ | − | ++++ |

[1]) Reaction only in the inner part; CT — coupled tetrazonium reaction

The inner portion of calcareous bodies showed a positive reaction for ptyalin-resistant mucosubstances if stained by Best's carmine, positive reaction to AB pH 2.6 and weak reaction to Sudan black B. The surface portion of calcareous bodies occupied about 1/8 of the diameter and did not stain by any of the methods used. Only in some sections the surface portion of calcareous bodies of cyst, neck and scolex stained very slightly with AB pH 2.6, whereas the inner portion showed negative reaction. In some cases the wall of cyst, neck and scolex contained calcareous bodies which did not stain with Best's carmine after ptyalin digestion.

All components of the cyst subtegument, i.e., the connective tissue and muscle fibres, pyriform cells and in some sections also calcareous bodies, were densely covered with fine fibres showing the same histochemical reactions as the fine connective tissue fibres of the tegument. Different density of these fibres in the subtegument and the resulting different intensity of stain, particularly with PAS following ptyalin digestion or with Luxol blue, could lead to an erroneous assumption that there is a greater amount of neutral mucosubstances and lipids in the fine connective tissue fibres of the subtegument than in those of the tegument.

The inner zone contained a small amount of arginine and lipids (Plate II, Figs. 3, 4) and was positive to CT reaction (Plate II, Fig. 1) and PAA + AF. The inner limiting layer showed no stain by any of the methods employed.

## NECK AND SCOLEX

Tables 3, 4

The outer limiting layer, like the outer limiting layer of the cyst, gave positive reaction to AB pH 0.2 and PAA + AF; the reaction for PAS-positive, ptyalin-resistant mucosubstances was negligible. The outer limiting layer of the neck and scolex is usually more damaged than that of the cyst, so that in most sections the surface of the neck and scolex is formed by microtriches. This phenomenon is quite common in larvae of cysticercoid type, in which the neck and scolex are actively and intensively moving inside the cyst. Numerous granules on the surface of the outer limiting layer and in the cyst cavity showed the same reactions as the outer limiting layer (Plate I, Fig. 3) and in addition to it they contained glycogen and a small amount of acid mucosubstances (demonstrated only by AB pH 2.6).

The microtriches contained tyrosine and tryptophan and were positive to CT reaction (Plate II, Fig. 1), PFA + AB and PAA + AF, but the reaction to thioglycolic acid + DDD was negative. The amorphous substance of the tegument contained ptyalin-resistant neutral mucosubstances which were strongly PAS-positive in the scolex and weakly PAS-positive in the neck (Plate I, Fig. 2), protein with SS groups and a small amount of tryptophan. It showed positive reaction to CT (Plate II, Fig. 1). The basement layer stained by Sudan black B, like that of the cyst wall.

The connective tissue fibres in the subtegument showed a very weak reaction for acid mucosubstances and proteins with SS groups, as determined by thioglycolic acid + DDD method, and gave a strong reaction to PAA + AF. They stained intensively with Luxol blue (Plate II, Fig. 4) and faintly with Sudan black B. The muscle fibres contained arginine, tryptophan, tyrosine and proteins with SH groups and were positive to CT reaction.

The pyriform cells contained glycogen (Plate I, Figs. 1, 2) and acid mucosubstances and showed positive reaction to CT (Plate II, Fig. 1). In addition to it, small amounts of arginine, tyrosine, proteins with SS groups and lipids were demonstrated. The calcareous bodies gave the same reactions as those in the cyst wall. The connective tissue and muscle fibres, pyriform cells and calcareous bodies were covered with fine

**Table 3.** Results of histochemical reaction for the detection of mucosubstances in the wall of neck and scolex

| Reaction | Granules on the surface | Outer limiting layer | Tegument | | Subtegument | | | Parenchyma cells | Calcareous corpuscles |
|---|---|---|---|---|---|---|---|---|---|
| | | | Microtriches | Amorphous substance | Connective tissue fibres | Muscle fibres | Pyriform cells | | |
| PAS | ++ | ± | ± | +/++[3] / +++ | − | +/++ | ++/+++ | ++++ | − |
| Schiff | − | − | − | − | − | − | − | − | − |
| Saliva test + PAS | − | ± | − | +/++[3] / +++ | − | +/++ | − | − | − |
| Acetylation + PAS | − | − | − | − | − | − | − | − | − |
| Desacetylation + PAS | − | − | − | +/++ | − | ± | ++ | +++ | − |
| Best's carmine | +++ | + | − | − | − | + | +++ | ++++ | +++[1] |
| Saliva test + Best's carmine | − | + | − | − | − | + | ± | + | +++[1] |
| AB pH 2.6 | +/++ | − | ± | ± | +/++ | − | ++ | ++ | ++[1] |
| AB + PAS | light violet | − | − | red | blue | rose red | violet | violet | − |
| Methylation + AB pH 2.6 | − | − | − | − | − | − | − | − | − |
| Demethylation + AB pH 2.6 | +/++ | − | − | ± | +/++ | − | ++ | ++ | ++[1] |
| CEC (AB pH 2.6 + $MgCl_2$) | − | − | 10 %[2] | 10 %[2] | 14 %[2] | − | 6 %[2] | 4 %[2] | − |
| MBE at pH | − | − | 7.25 | 7.25 | 3.62 | − | 6.12 | 6.12 | − |
| Aldehyde fuchsin | − | − | ± | ± | +/++ | − | ++ | ++ | − |
| Hale | − | − | ± | ± | ± | − | ± | − | − |
| Coloidal $Fe^{3+}$ | − | − | ++ | +/++ | ± | − | + | +++ | − |
| Hale + control | − | − | − | − | − | − | − | − | − |
| Hale + PAS | − | − | − | rose red | light blue | rose | violet | violet | − |

[1]) Reaction only in the inner part.
[2]) At this concentration of the $MgCl_2$ the affinity to AB pH 2.6 disappears.
[3]) Numerator: reaction in the neck, denominator: reaction in the scolex.

**Table 4.** Results of histochemical reactions for the demonstration of proteins and lipids in the wall of neck and scolex

| Reaction | Granules on the surface | Outer limiting layer | Tegument | | Subtegument | | | Parenchyma cells | Calcareous Corpuscles |
|---|---|---|---|---|---|---|---|---|---|
| | | | Microtriches | Amourhous substance | Connective substance tissue fibres | Muscle fibres | Pyriform cells | | |
| Sakaguchi | − | − | ± | ± | − | ++[1] / ++++ | +/++ | ++ | − |
| DMAB | − | − | ++/+++ | +/++ | − | ++ | ± | − | − |
| Morel — Sisley | − | − | +++ | ± | − | ++[1] / +++ | + | +[1] / +/++ | − / − |
| CT | − | − | +++ | ++ | − | ++++ | ++ | +++ | − |
| DDD | − | − | − | ± | − | +++ | ± | ++ | − |
| Thioglycollic acid + DDD | − | − | − | +++ | + | +++ | +/++ | +++ | − |
| N-ethylmaleimid + DDD | − | − | − | − | − | ± | − | ± | − |
| AB pH 0.2 | +++ | +++ | +/++ | ± | ± | − | − | − | − |
| PFA + AB | − | − | +++ | − | + | − | − | − | − |
| PAA + AF | ++++ | ++++ | ++++ | ++ | ++++ | ± | +/++ | ++ | − |
| Sudan black B | − | − | − | − | + | +/++ | + | +++ | +/++[2] |
| Luxol blue | − | − | − | − | +++ | − | +/++ | ++/+++ | − |

[1] Numerator: reaction in the neck, denominator: reaction in the scolex.
[2] Reaction only in the inner part.

connective tissue fibres giving the same histochemical reactions as the fine connective tissue fibres in the tegument and subtegument of cyst.

In the parenchyma of neck and scolex, the connective tissue fibres and calcareous bodies gave the same reactions for mucosubstances (Plate I, Figs. 1, 2), proteins (Plate II, Fig. 2) and lipids (Plate II, Figs. 3, 4) as the corresponding components of the subtegument. The muscle fibres in the parenchyma, in contrast to those in cyst, neck and scolex subtegument, contained glycogen. The parenchymal cells contained glycogen (Plate I, Figs. 1, 2), acid mucosubstances, arginine, proteins with SH groups (Plate II, Fig. 2) and lipids, and gave CT-positive reaction (Plate II, Fig. 1).

## DISCUSSION

For better understanding of the histochemical reactions a scheme of the structure of *H. erinacei* cysticercoid is given in the Results (Valkounová and Prokopič 1980 — detailed description).

The cysticercoid of *H. erinacei*, like adult specimens of this cestode, possesses a rostellum without hooks. A comparison with species possessing rostellum with hooks reveals a distinct difference in the structure of rostellum and in the contents of proteins with SS groups in rostellar tegument. The circular and longitudinal connective tissue and muscle fibres in the rostellum and rostellar sac of *H. erinacei* are sparse and the connective tissue fibres are thinner than those in the cysticercoids with developed rostellum, like in *Rodentotaenia crassiscolex* (Linstow, 1890) (Valkounová and Prokopič 1978). The rostellar tegument in *H. erinacei* cysticercoid does not differ in the contents of proteins with SS groups from the tegument of the remaining parts of scolex and neck, whereas in *R. crassiscolex* (Valkounová and Prokopič 1979) the rostellar tegument, like the hooks, is strongly positive for proteins with SS groups (twice as much as in the remaining portions of getument of scolex and neck). Differences in the morphology and histochemistry of rostellum were observed also in larvae of cysticercus type with hookless rostellum (*Cysticercus bovis*) compared to other cestodes of the family Taeniidae (Haldeman, 1851), the rostellum of which is armed with hooks (Schaaf 1905, Rees 1951, Baron 1968, Bilquees and Freeman 1969, Mount 1970, Žďárská 1973).

Calcareous bodies were found in the subtegument of cyst and in the subtegument and parenchyma of neck and scolex. They showed a different rate of positivity to some histochemical reactions related with the age of larva. We have studied histochemical reactions in fully developed larvae; younger larval stages will be the subject of further studies. The inner portion of calcareous bodies in completely developed larvae of various age was found to contain acid mucosubstances. In some cases, a negligible amount of acid mucosubstances was detected also at the periphery of bodies, whereas the inner portion was negative for acid mucosubstances. The occurrence of acid mucosubstances in calcareous bodies was reported also by Nieland and von Brand (1969) and Žďárská (1975). In contrast to Žďárská (1975), who stated that calcareous bodies stained intensively with Luxol blue, we observed positive reaction inside calcareous bodies stained by Sudan black B.

The calcareous bodies were PAS-negative and again only their inner portion was stained by Best's carmine or Best's carmine combined with saliva test. In some sections were calcareous bodies which did not stain by Best's carmine after saliva test. Chowdhury et al. (1962) and von Brand et al. (1960) detected glycogen-like polysaccharides in the calcareous bodies, but Žďárská (1975) does not agree with this finding. Complementary studies on the reaction for neutral mucosubstances in calcareous bodies in differentiating and differentiated larva before invagination (see

Neradová-Valkounová 1971 for individual phases of larval development) are necessary for a definitive solution of these problems. However, the histological and histochemical studies of larvae of cysticercoid type and their younger developmental stages are very laborious due to the small size and subtleness of the material. Also collecting and compiling of the necessary quantity of material, as well as processing isolated cysticercoids, is difficult.

Baron (1971) in his histochemical study of the cysticercoid of *Raillietina cesticillus* (Molin, 1858) found a strong PAS-positive reaction for ptyalin-resistant mucosubstances in the outer limiting layer of the cyst ("hyaline coat") and in the scolex tegument. In our material we have detected PAS-positive, ptyalin-resistant mucosubstances not only in the outer limiting layer of cyst, but also in the outer limiting layer of neck and scolex, in different quantity in individual species. The cysticercoid of *R. crassiscolex* (Valkounová and Prokopič 1979) showed a strong positivity, whereas in *H. erinacei* a small amount of PAS-positive mucosubstances occurred only in the outer limiting layer of cyst, neck and scolex. In agreement with Baron (1971), we have demonstrated PAS-positive and ptyalin resistant mucosubstances in the scolex tegument and, in addition to it, also in the tegument of cyst and neck. Baron (1971) did not give a more exact localization of positive reaction in the scolex tegument. In our material of *R. crassiscolex* cysticercoids (Valkounová and Prokopič 1979) the positive reaction was found in the amorphous substance of tegument of cyst, neck and scolex and in fine connective tissue fibres in the outer portion of cyst tegument. Baron (1971) did not describe fine connective tissue fibres in the cyst tegument. In the cysticercoids of *H. erinacei*, the ptyalin-resistant mucosubstances gave a very weak PAS-reaction in the amorphous substance of cyst tegument and a stronger PAS-positive reaction in the amorphous substance of neck and scolex tegument and in fine connective tissue fibres of cyst tegument. No ptyalin-resistant neutral mucosubstances were detected in microtriches of tegument of neck and scolex.

## ГИСТОЛОГИЯ ЦИСТИЦЕРКОИДА ЦЕСТОДЫ *HYMENOLEPIS ERINACEI* (GMELIN, 1789)

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**Резюме.** В первый раз изучено содержание мукосубстанций, белков и липидов у морфологически дифференцированной и инвагинированной личинки *H. erinacei*, состоящей из хвостатой цисты, в полости которой инвагинированы шейка и сколекс цестоды. Наружный предельный слой цисты, шейки и сколекса дает очень сильную положительную реакцию на PAA + AF и AB pH 0,2. Внутренний предельный слой цисты и шейки не окрашивался ни одним из применяемых методов. Тонкие волокна соединительной ткани в тегументе цисты и субтегументе цисты, шейки и сколекса содержат PAS—положительные, резистентные к птиалину нейтральные мукосубстанции и дают сильную положительную реакцию при использовании методов PFA + AB и PAA + AF. Микротрихи тегумента шейки и сколекса содержат тирозин и триптофан и дают положительную реакцию при PFA + AB и PAA + AF. Аморфное вещество тегумента цисты, шейки и сколекса содержит триптофан и белки с SS-группами, аморфное вещество сколекса положительно к PAS пробам на резистентные к птиалину нейтральные мукосубстанции. Гистохимическая структура соединительно-тканевых и мышечных волокон и грушевидных клеток одинакова в цисте, шейке и сколексе. В соединительно-тканевых волокнах выявлены кислые мукосубстанции, очень слабая реакция на белки с SS-группами и сильная реакция к PAA + AF. Мышечные волокна содержат аргинин, триптофан, тирозин и белки с SH-группами и дают положительную реакцию к CT. Грушевидные клетки содержат гликоген, кислые мукосубстанции и небольшое количество аргинина и белков с SS-группами. Во внутренней части известковых телец обнаружена положительная реакция при методу AB pH 2,6 и судана черного B. Внутренняя зона цисты положительна к CT, PAA + AF, люксоловому синему и судану черному B.

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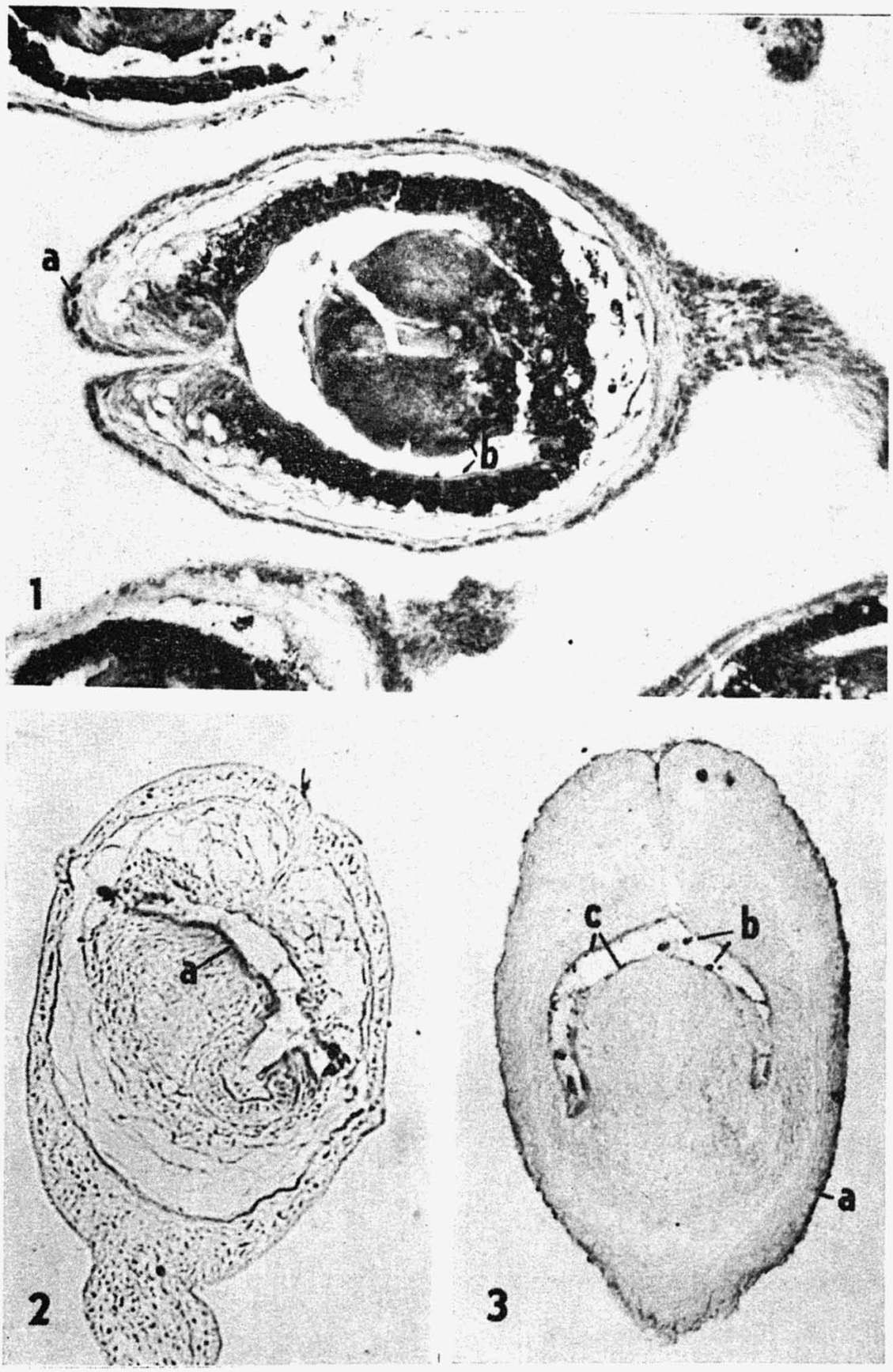


**Fig. 1.** Glycogen contents in pyriform cells of cyst (a), pyriform and parenchymal cells of neck and scolex (b). PAS reaction (× 240). **Fig. 2.** PAS-positive, ptyalin-resistant neutral mucosubstances in amorphous substance of scolex tegument (a). Saliva test + PAS (× 190). **Fig. 3.** Strong positivity to AB ph 0.2 in outer limiting layer of cyst (a), in granules in cyst cavity (b). Outer limiting layer of scolex and neck (c) very damaged due to their active movement, so that the positivity to AB pH 0.2 is not distinct (× 240).

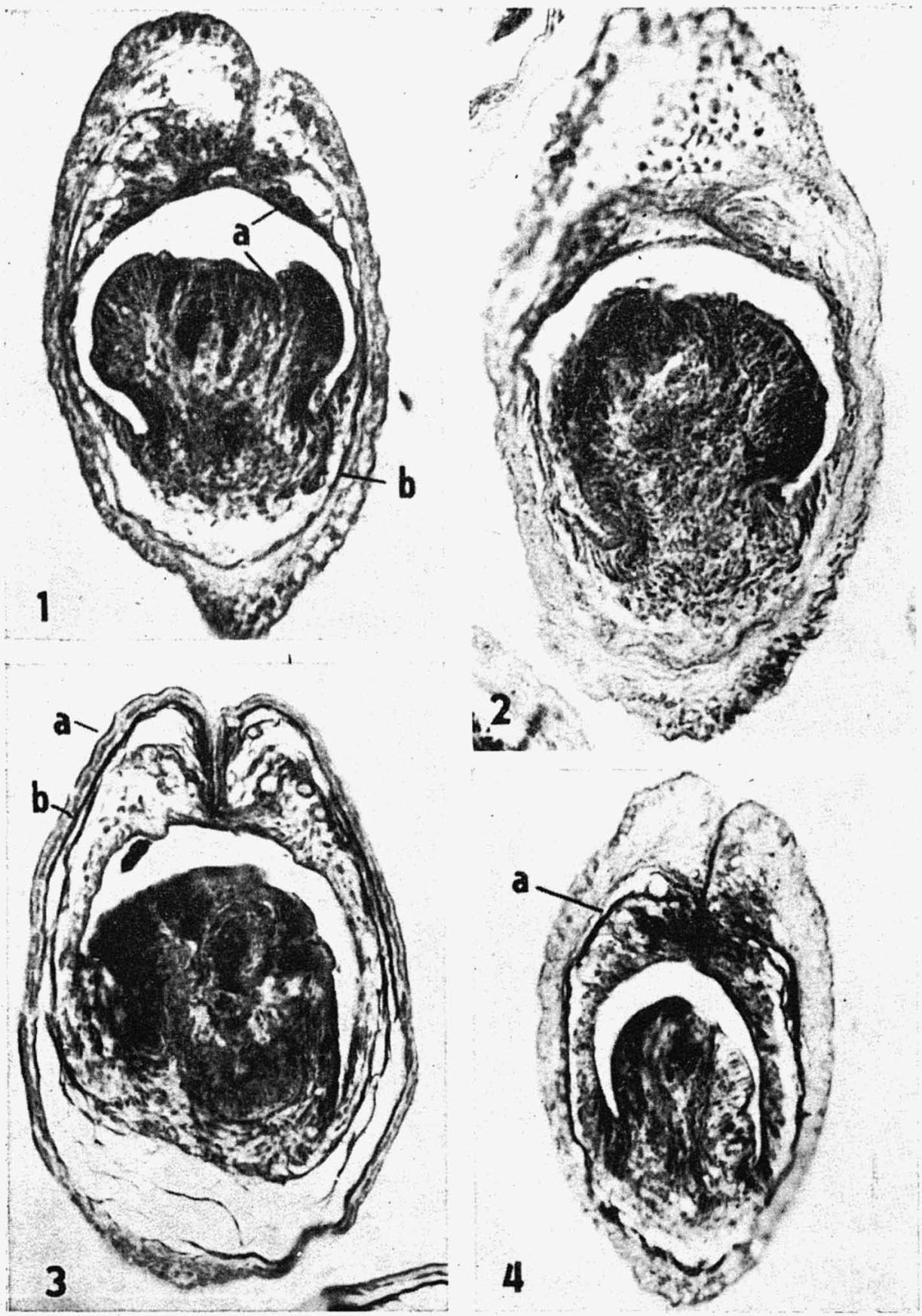


**Fig. 1.** Strong positivity to CT-reaction in neck and scolex (a) present in microtriches, amorphous substance of tegument, muscle fibres, pyriform and parenchymal cells. Inner zone of cyst (b) also gives positive reaction. (× 240). **Fig. 2.** Proteins with SH groups in muscle fibres and parenchymal cells of scolex and neck. DDD (× 303). **Fig. 3.** Fine connective tissue fibres in cyst tegument (a) stained by Sudan black B. Stronger reaction was found in inner zone of cyst (b), in neck and scolex (× 240). **Fig. 4.** Intensive Luxol blue staining in inner zone of cyst (a), connective tissue fibres, and pyriform and parenchymal cells of neck and scolex (× 240).