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LOCALIZATION OF SOME ENZYMES IN POLYCEPHALIC LARVAE OF TWO SPECIES OF THE GENUS MULTICEPS (CESTODA)

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Abstract. The histochemical distribution of alkaline phosphatase (AlP), acid phosphatase (AcP) and nonspecific esterase (NE) has been determined in polycephalic larvae of the species *Multiceps multiceps* Leske, 1870 and *M. endothoracicus* Kirschenblat, 1948. In the scolex anlages of both species, an activity of these enzymes has been determined in sites associated with growth and with the transport of metabolites. In a young larva of *M. multiceps*, the activity of AlP, AcP and NE was strong in the entire proliferating bladder wall surrounding groups of scoleces, but absent in the remaining part of the bladder wall outside the area of these scolex groups. In young larvae of *M. endothoracicus*, AlP- and NE activity was highest in the modified bladder wall forming a verrucose mound, and at the site of the opening of the invaginated canal. No activity of these enzymes was demonstrated in mature and aging larvae.

The histochemistry of enzymes in various cestode species has been studied by numerous authors. Yamao (1952 a) found phosphatase activity in *Anoplocephala perfoliata*, *A. magnia*, *Moniezia benedeni*, *M. expansa*, *Hydatigera taeniaeformis*. In another paper (Yamao 1952 b) he demonstrated acid phosphatase activity in *Cysticercus bovis*, and alkaline phosphatase in the hydatid cyst of *Echinococcus granulosus* and *Cysticercus fasciolaris*. Lefevre (1952) studied the distribution of alkaline phosphatase in adult cestodes of the species *Moniezia benedeni*, *Taenia saginata*, *Multiceps serialis* and others. Pennoit-de Cooman and van Grembergen (1974) and Erasmus (1957 a) demonstrated acid phosphatase activity in the cysticercus, and alkaline phosphatase in the adult of *Taenia pisiformis*. Erasmus (1957 b) reported alkaline and acid phosphatase for *Cysticercus tenuicollis* and *Moniezia expansa*. Kilejian et al. (1961) demonstrated alkaline phosphatase in *Echinococcus granulosus*. Waitz (1963) found alkaline and acid phosphatase activity in larvae and adults of *Hydatigera taeniaeformis*, similarly Bogitsh (1963) in larvae of *Hymenolepis microstoma* and *H. diminuta*, and Waitz and Schardein (1964) in the adult of *H. nana*, *H. diminuta* and others. Nonspecific esterase and cholinesterase were demonstrated by Žďárská (1973) in *Cysticercus bovis;* nonspecific esterase by Shield et al. (1973) in the cysticercus of *T. pisiformis*. The aim of the present investigation was to demonstrate the presence of alkaline phosphatase, acid phosphatase and nonspecific esterase in larvae of *M. multiceps* and *M. endothoracicus*.

MATERIAL AND METHODS

Our material consisted of developmental stages of a larva of *M. multiceps* (Fig. 1) obtained from the brain of a sheep at the abattoir in Alma-Ata. A group of differently differentiated scoleces was seen on a bladder measuring 4 × 6 mm. The length of the primary scolex was 121 μm, its width 62,1 μm; the scolex with the developing rostellum measured 80.6 μm in length, 24.5 μm in width, scolex anlages with an invaginated canal were 46 μm long and 20.6 μm wide. Larvae of *M. endothoracicus* were

obtained from the thoracic cavity of *Rhombomys opimus* Lichtenstein, 1923. Younger stages (Fig. 2) were obtained on day 42 of infection. The bladder measured 10 × 15 mm. The verrucose mound of the bladder wall measured 0.4—0.5 in width, 0.7—0.8 mm in height. In young scoleces we distinguished 4 sucker anlages and the anlage of the rostellar cone. In an older stage obtained on day 60 of infection (Fig. 3), the bladder measured 15 × 20 mm. The width of the verrucose mound was 1.2—1.3 mm The length of the verrucose mound in which the scolex was submerged, was 1—1.2 mm. The bottom of the differentiated spiral canal harboured 4 definitive suckers and a rostellum with 59 hooks.

Alkaline (AlP) and acid (AcP) phosphatase and nonspecific esterase (NE) were demonstrated histochemically in frozen and paraffin-embedded sections. Paraffin sections: Fixation in Baker's neutral formaldehyde for 2 hr at 4 °C, rinsing in 5 % saccharose (2 hr at 4 °C), dehydration in acetone (4 °C), clearing in benzene. Frozen sections: Fixation in Baker's neutral formaldehyde (2 hr at 4 °C).

AlP was demonstrated with α-naphthyl phosphate and Fast blue BB and Fast red TR (Pearse 1968). The specificity of the reaction was tested by means of an inhibition with 0.01 NaF, incubation of sections in a medium from which the substrate was ommitted. Inactivation time at 100 °C was 5 minutes.

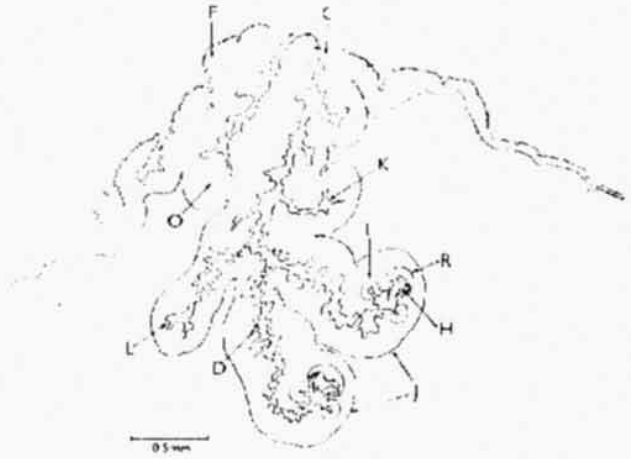

Fig. 1. Schematic illustration of part of the proliferating bladder of a *M. multiceps* larva with scolex anlages at the site of the opening of the invaginated canal of the primary scolex.

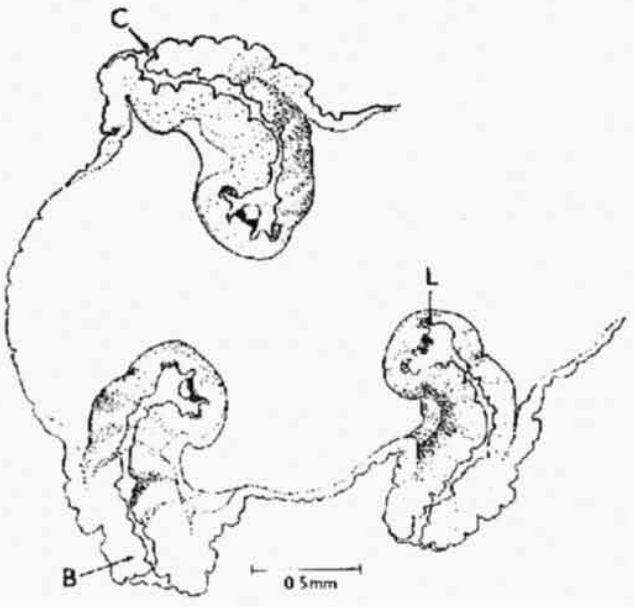

Fig. 2. Reconstruction of part of the bladder with scoleces of a young larval stage of *M. endothoracicus*. A verrucose mound formed by the modified bladder wall surrounds the opening of the invaginated canal.

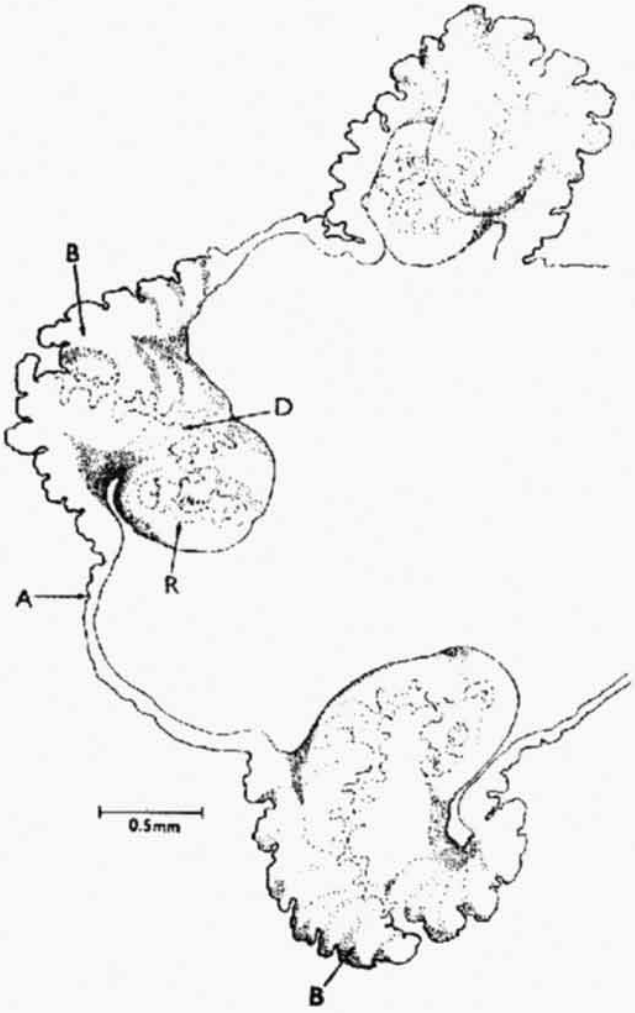

Fig. 3. Reconstruction of part of the bladder of an older larval stage of *M. endothoracicus* with three scoleces submerged in the changed bladder wall except for the rostellar part surrounded by a large mound. A — bladder, B — verrucose mound, C — opening of the invaginated canal, D — spiral canal, E — tegument, F — proliferating area of the bladder, G — granules, H — hooks, I — invaginated canal, J — mature scoleces, K — rostellar cone, L — suckers, M — excretory canal, N — nuclei of subtegumental cells, O — scolex anlages, P — rostellar pad, R — rostellum;

AcP was demonstrated with α-naphthyl phosphate and hexasopararosaniline (HPR) (Bárka and Anderson 1962). The reaction was controlled by inactivation at 100 °C (Lojda and Papoušek 1967) and incubation of sections in a medium without substrate.

NE was demonstrated with the method α-naphthyl acetate + HPR (Davis and Ornstein 1958) in the modification suggested by Lojda and Papoušek (1967).

RESULTS

A. ENZYME ACTIVITY IN LARVAE OF MULTICEPS MULTICEPS

Activity of AcP, AlP and NE was demonstrated histochemically in a polycephalic larva of *M. multiceps* with a bladder measuring 4×6 mm. Typical of this larva was the formation of new scoleces on the proliferating bladder in the area of the opening of the canal (C) of the primary scolex, causing a local grouping of scoleces (Fig. 1) on the bladder wall. Scoleces of the same group were at different stages of differentiation and also enzyme activity was differently high. That of AlP and AcP was highest in the youngest scolex anlages (O), lower in scoleces with a differentiating rostellum and a spiral canal (D), absent in aging, primary scoleces. In scolex anlages, AlP activity was highest in both homogeneous layers of the tegument of the invaginated canal (Plate I, Fig. 1), AcP activity was higher in the inner homogeneous layer of the tegument of the canal, and in subtegumental cells (Plate I, Fig. 2). AcP activity was high in the tegument covering the bottom of the canal, and in dividing cells of the scolex anlage (Plate I, Fig. 3) and in cells of the rostellar cone (K) (Plate I, Fig. 4). AlP activity was high in interstitial cells and fibres of the suckers (Plate I, Fig. 5). AcP activity was lower in the sucker anlages (L). NE activity was demonstrated mainly in subtegumental cells and in cells of the cone. It was lower in the tegument of the invaginated canal.

In young, differentiating scoleces with a developed rostellum (R) and hooks, AlP and AcP activity was lower in the rostellar pad (Plate II, Fig. 1). In the hook organ at the base of the developing handle of the hook, papillar extensions contained AlP-positive granules. The tegument of the spiral canal gave a negative reaction for these enzymes except for roughly one third of length of the canal below the suckers where AlP, AcP and NE activity could be demonstrated in both homogeneous layers of the tegument of this canal (Plate II, Fig. 2). In this area, AcP and NE activity was disclosed in the subtegumental cells and in the wall of the excretory canals (Plate II, Fig. 3).

In the bladder, AlP, AcP and NE activity was highest in the area around the opening of the invaginated canal (C) of the primary scolex. In the tegument of this area, AlP and AcP activity was higher at the base of the microtriches and in the basal fibrillar layer than in the homogeneous layer proper. AcP and NE activity was high in subtegumental cells and excretory canals (Plate II, Fig. 4). The bladder wall (F) covering the group of scoleces was heavily folded throughout. In proportion with an increasing distance from the opening of the canal of the primary scolex, enzyme activity diminished, the bladder wall farther away from the scolex group was smooth, enzyme activity was absent in it. The results of histochemical reactions are shown in Tables 1 and 2.

B. ENZYME ACTIVITY IN LARVAE OF MULTICEPS ENDOTHORACICUS

AlP, AcP and NE activity was demonstrated in two developmental stages of *M. endothoracicus*, i.e. in a younger stage with a starting differentiation of the rostellum and verrucose mound of the bladder (Fig. 2), and in an older stage with the proximal part of the scolex with a developed rostellum partly submerged in the modified wall of the verrucose mound (Fig. 3).

In the young scolex anlage, AlP activity (Plate III, Fig. 1) was highest in the inner homogeneous layer of the tegument of the invaginated canal, in the tegument and cells

Table 1. Results of histochemical tests demonstrating enzymes in the scoleces of a *M. multiceps* larva

Reactions	Young scolex anlages									Maturing scoleces							
	Suckers		Rost. cone		Tegument of invaginated canal					Suckers	Rostellum		Tegument of proliferating area of spiral canal				
	fibres	cells	tegument	cells	microtriches	homogeneous layer		basal layer	subtegumental cells		granules in hook organ	rostellar pad	microtriches	homogeneous layer		basal layer	subtegumental cells
						outer	inner							outer	inner		
Alkaline phosphatase (α-naphthyl phosphate + Fast Red TR)	++-	++++	+++	++++	-	++-	+++	-	-	-	++	-+	-	+++	-++	-	-
100 °C/5 min. + test medium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Test medium without substrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid phosphatase (α-naphthyl phosphate - HPR)	-	++	+++	++++	-	++	++++	-+	+++-	-	-	+	-	+++	-++	-	+-+
NaF + test medium	-	-	-	-	-	-	++	-	-		-	-	-	-	-	-	-
100 °C/5 min. + test medium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Test medium without substrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nonspecific esterase (α-naphthyl acetate + HPR)	-	-	+++	+++	-	-	+	+	+++	-	-	-	-	+-	++	+	+++

Table 2. Results of histochemical tests for the demonstration of enzymes in the bladder of a *M. multiceps* larva

Reactions	Transition of the bladder in the invaginated canal of a primary scolex								Tegument of folded bladder wall in the area of the scoleces				Tegument of the bladder
	Tegument					Parenchyma							
	microtriches		homogeneous layer	basal layer	subtegumental cells	muscles	excretory canal	connective tissue of receptaculum	microtriches	homogeneous layer	basal layer	subtegumental cells	
	apex	base											
Alkaline phosphatase (α-naphthyl phosphate + Fast Red RT)	–	+++	++	++	++	–	++++	–	–	+++	–	–	–
100 °C/5 min. + test medium	–	–	–	–	–	–	–	–	–	–	–	–	–
Test medium without substrate	–	–	–	–	–	–	–	–	–	–	–	–	–
Acid phosphatase (α-naphthyl phosphate + HPR)	–	+++	+++	+++	+++	–	++++	+	–	+++	+	++	–
100 °C/5 min. + test medium	–	–	–	–	–	–	–	–	–	–	–	–	–
Test medium without substrate	–	–	–	–	–	–	–	–	–	–	–	–	–
Nonspecific esterase (α-naphthyl acetate + HPR)	+	++	–	+	+++	–	++	+	–	–	+	+++	
100 °C/5 min. + test medium	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 3. Results of histochemical tests for the demonstration of enzymes in scoleces of a *M. endothoracicus* larva

Reactions	Young scolex anlages								Scolex submerged in the modified wall of the verrucose mount							
	Suckers	Rostellar cone		Tegument of invaginated canal					Suckers	Rostellum			Tegument of the spiral canal above rostellum			
		tegument	cells	microtriches	homogeneous layer: outer	homogeneous layer: inner	basal layer	subtegumental cells		granules in hook organ	rostellar pad	lateral prominence	microtriches	homogeneous layer: outer	homogeneous layer: inner	basal layer	subtegumental cells
Alkaline phosphatase (-naphthyl phosphate + Fast Red TR)	+++	++	++	–	–+	++++	–	+	–	+	++	++	–	++	++++	–	+
100 °C/5 min. + test medium	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
NaF + test medium	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–
Acid phosphatase (—naphthyl phosphate + HPR)	+++	++	++	–	+++	++++	++	+++	+	+	++	++	–	+++	++++	+	+++
NaF + test medium	–	–	–	–	–	–	–	–		–	–	–	–	–	–	–	–
Test medium without substrate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Nonspecific esterase (-naphthyl acetate + HPR)	–	+	+++	–	–	+	+	++	–	++	–	–	–	++	–	–	+++
Test medium without substrate	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Table 4. Results of histochemical tests in the bladder of a *M. endothoracicus* larva

Reactions	Tegument of the folded wall of the verrucose mound at the opening of the spiral canal				Tegument of the modified wall of the verrucose mound on scolex surface			
	microtriches	homogeneous layer	basal layer	subtegumental cells	microtriches	homogeneous layer	basal layer	subtegumental layer
Alkaline phosphatase (α-naphthyl phosphate + Fast Red TR)	–	++++	–	++	–	++	–	+
Test medium without substrate	–	–	–	–	–	–	–	–
100 °C/5 min. + test medium	–	–	–	–	–	–	–	–
Acid phosphatase (α-naphthyl phosphate + HPR)	+	+++	++	++++	+	++–	+	+++
NaF + test medium	–	–	–	–	–	–	–	–
Test medium without substrate	–	–	–	–	–	–	–	–
Nonspecific esterase (α-naphthyl acetate + HPR)	–	++	+	+–+	–	+i	+	+++
Test medium without substrate	–	–	–	–	–	–	–	–

of the rostellar cone, in the sucker anlages (L) and in the proliferating cells forming the scolex anlage. AcP activity was higher than that of AlP in the rostellar cone (K), in the tegument and subtegumental cells of the invaginated canal (Plate III, Fig. 2). The only sites of NE-activity were the cells of the rostellar cone and subtegumental cells. (Table 3).

In the older stage with a scolex submerged in the modified wall of the verrucose mound (B), a low activity of AlP and AcP was demonstrated in the rostellar pad, and an occasional activity in granules of the hook base, the praebulbus and cells of lateral extensions of the canal wall at the level of the rostellum. Apart from a short strip above the rostellum, and around the opening of the spiral canal, enzyme activity could not be demonstrated. In the tegument of the differentiating spiral canal above the rostellum (Plate III, Fig. 3), and at the site of its opening into the suckers, activity of AlP, AcP and NE was found in the homogeneous layers. An AcP and NE activity was demonstrated in the subtegumental cells. In the tegument of the canal AlP activity was high at the site of its passage in the folded wall of the verrucose mound (Plate III, Fig. 4), lower in subtegumental cells. A low AcP activity was found in the tegument of the folded wall of the canal at the site of its opening, a high AcP and NE activity in the excretory canals and subtegumental cells. The modified wall of the bladder (B) covering the scolex was less folded and the tegument was thickened; in it AcP activity was higher (Plate IV, Fig. 1), AlP and NE activity lower (Table 4). In subtegumental cells, activity of AcP and NE was higher (Plate IV, Fig. 2). The modified wall of the bladder covering the scolex passed first in a short, proliferating zone(F) of the folded bladder wall and then thinned as the distance from the scolex increased until it became completely smooth. AcP activity was demonstrated in the subtegumental cells of the short zone of proliferation, and in the excretory canals (Plate IV, Fig. 3). NE activity was high in the subtegumental cells. Enzyme activity could not be demonstrated in the smooth, thin wall of the bladder outside the scolex area (Table 4).

DISCUSSION

Although numerous authors have studied various enzymes in numerous cestode species (Cyclophyllidea, Pseudophyllidea), the activity and localisation of enzymes in polycephalic larvae have not been demonstrated as yet. Enzymatic activity has been studied mainly on the adult cestode and a few authors only have studied it on larvae (Yamao 1952a, b, Erasmus 1957a, b, Pennoit-de Cooman and van Grembergen 1947, Bogitsh 1963, Morris and Finnegan 1968, Arme 1966, Žďárská 1973, Shield et al. 1973).

In our study on a polycephalic larva of *M. multiceps* and two developmental stages of *M. endothoracicus*, we have given attention mainly to the localisation of enzyme activity in developing scoleces (from a young scolex anlage to a completely developed scolex). Our results indicated that the activity of AlP, AcP and NE diminished as the development advanced. The highest activity of phosphatases and NE was demonstrated in sites of growth, i.e., in proliferating cells of the scolex anlage, the rostellar cone and the anlage of the rostellum developing from the cone, in the anlages of the suckers, in subtegumental cells of the invaginated canal and in the proliferating zone of the bladder which, in *M. endothoracicus* forms a modified wall on the surface of the scolex and the wall of the verrucose mound. In larvae of *M. multiceps*, the proliferating wall of the bladder passes into the wall of the invaginated canal of the primary scolex at the site of its opening.

Arme (1966) pointed out that the different localisation of AlP and AcP activity in the various developmental stages of *Ligula intestinalis* was not only associated with the active transport of metabolites, but also with growth. He agreed with Pennoit-de Cooman, van Grembergen (1947) and Erasmus (1957) in that AcP activity was higher in larvae than in adult cestodes. Öhman-James (1968) demonstrated AlP and AcP activity in the tegument and subtegument of the plerocercoid and adult of *Diphyllobothrium dendriticum:* enzyme activity was higher in the apical end of the scolex and in the bothria; in the excretory canals, acid phosphatase only could be demonstrated. Ždárská (1973) demonstrated a high activity of AlP and AcP in *C. bovis* at the site where the bladder passed in the invaginated canal. Shield et al. (1973) found a high AlP activity in the tegument and "secretory" cells of a young *C. pisiformis*. NE activity was observed in the tegument mainly at the site of its invagination. Takahashi (1959) demonstrated both acid and alkaline phosphatase in the tegument and subtegumental cells of the scolex of a sparganum of *Spirometra erinacei*, while Kwa (1972) found alkaline phosphatase only in the tegument of the scolex of this sparganum. Lumsden et al. (1968) observed in their electron microscopic study that the presence of phoshatases on the surface of the tegument facilitates the absorption of nutrients. Rothman (1966) demonstrated with the electron microscope AlP and NE activity on the surface of the tegument of *Hymenolepis citelli*. It has been confirmed by our investigation of two species of polycephalic larvae that AlP and AcP activity was higher, that of NE lower, in the tegument of the invaginated canal of young scolex anlages. in the tegument of the proliferating zone of the spiral canal and the bladder. In subtegumental cells, activity of AcP and NE was high, that of AlP low.

In young scolex anlages of *M. endothoracicus* and *M. multiceps* requiring an increased transport of metabolites for the intensive growth of, e.g., the rostellum, suckers and the wall of the invaginated canal, AlP and AcP activity was high. In older scoleces in which the development of these parts had been completed, the activity of these enzymes was either greatly reduced or nonexistant. Differences in the rate of activity of these enzymes in different developmental stages of these cestodes are in complete agreement with the role ascribed generally to alkaline and acid phosphatase in the organism, i.e., that of enzymes responsible for the transport of metabolites.

ЛОКАЛИЗАЦИЯ НЕКОТОРЫХ ФЕРМЕНТОВ В ПОЛИЦЕФАЛЬНЫХ ЛИЧИНКАХ ДВУХ ВИДОВ РОДА *MULTICEPS* (CESTODA)

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Резюме. Гистохимическим методом выявлена активность ферментов, щелочной фосфатазы (AlP), кислой фосфатазы (AcP) и неспецифической эстеразы (NE) у полицефальных личинок *Multiceps multiceps* Leske, 1780 и *M. endothoracicus* Kirschenblat, 1948. В закладках сколекса обоих видов выявлена активность этих ферментов в тех местах, которые соединены с ростом и активным транспортом веществ. У молодой личинки *M. multiceps*, обнаружена также высокая активность AlP, AcP и NE в целой пролиферующей стене пузыря, окружающей группы сколексов, тогда как в остальной части стены эта активность отсутствовала. У молодых личинок *M. endothoracicus* самая высокая активность AlP, AcP и NE в модифицированной стене пузыря, образующей бородавчатую выпуклость и в отверстии инвагинированного канала. У половозрелых и стареющих личинок обоих видов активность этих ферментов не обнаружена.

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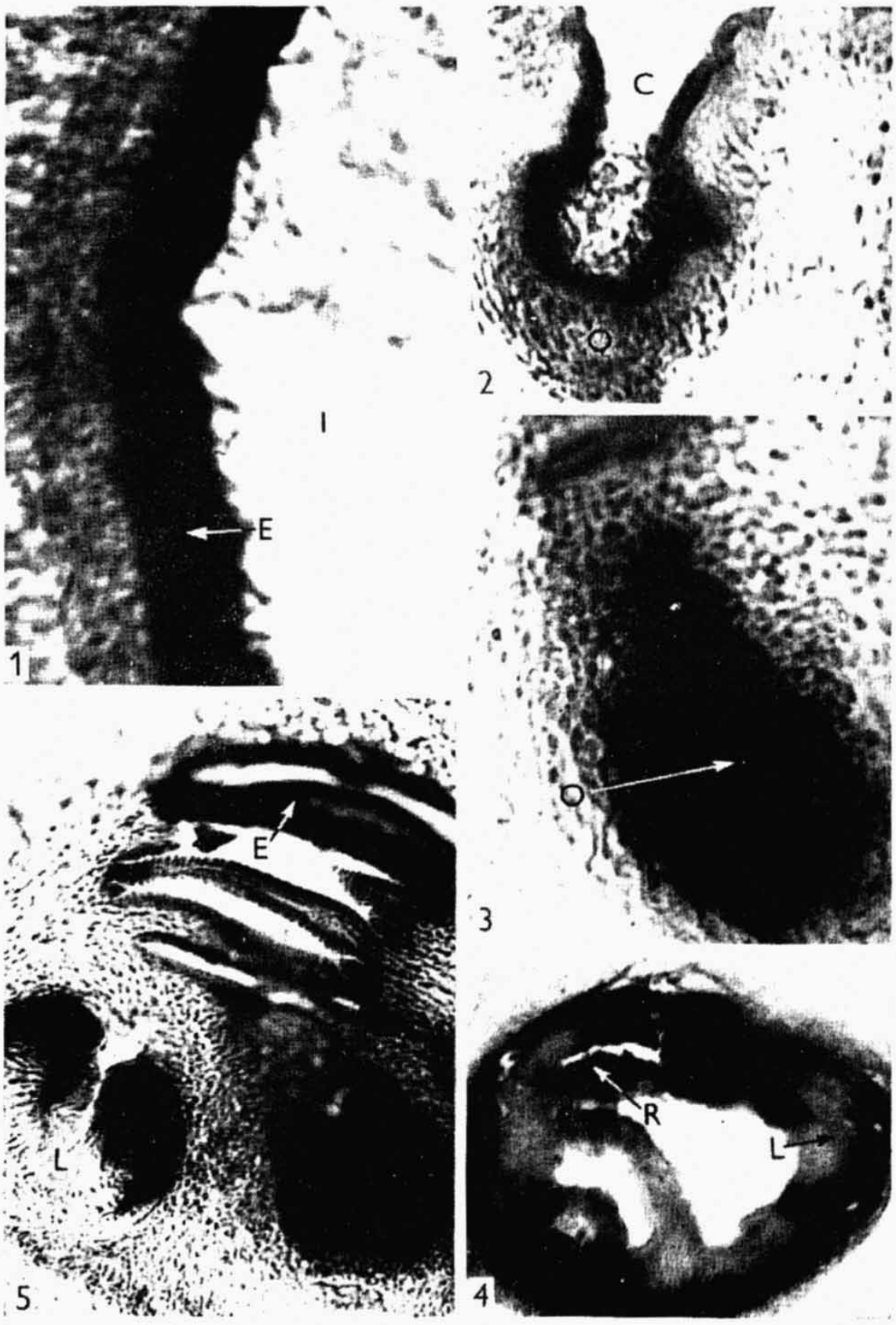

Fig. 1. Tegument of the investigated canal of the scolex anlage of *M. multiceps* with AlP activity (α-naphtyl phosphate + Fast red TR, × 1,200).
Fig. 2. Section through the invaginated canal of the primary scolex anlage with NE-activity in the tegument and cells (α-naphthyl acetate + HPR, × 350).
Fig. 3. Tangential section through the scolex anlage with a high activity of AcP in the cells (α-naphthyl phosphate + HPR, × 675).
Fig. 4. Transverse section through the proximal part of a young scolex with a differentiating rostellum and suckers showing AcP activity (α-naphthyl phosphate + HPR, × 150).
Fig. 5. Tangential section through an older scolex anlage displaying AlP activity in the suckers and tegument of the proliferating invaginated canal (α-naphthyl phosphate + Fast red TR, × 450).

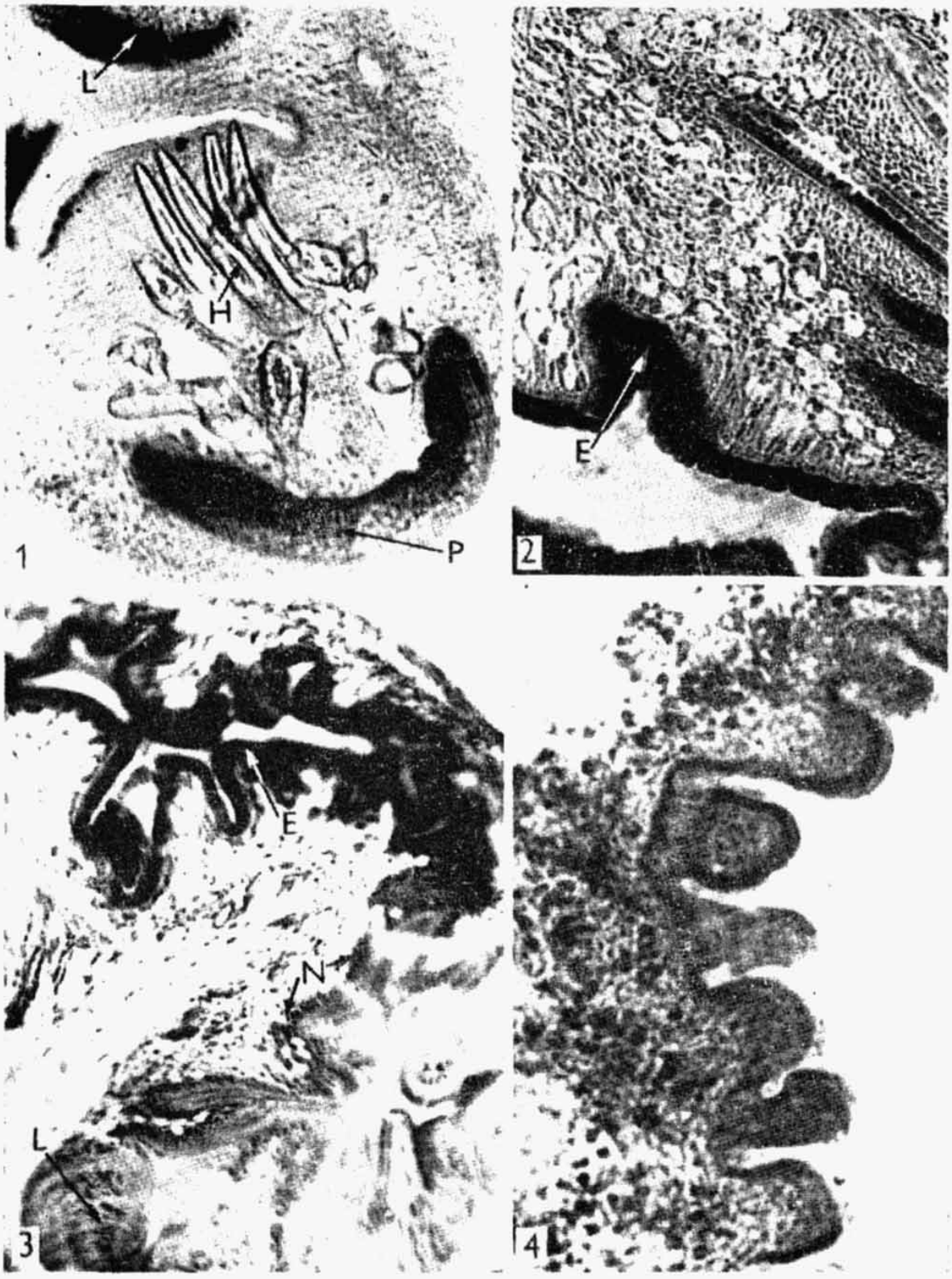

Fig. 1. Oblique section through part of the scolex of *M. multiceps* with a developing rostellum and hooks. AlP activity is in the rostellar pad, suckers and granules around hooks (α-naphthyl phosphate + Fast red TR. × 150).

Fig. 2. Oblique section through the proliferating area of the canal covered with a thickened tegument, showing AlP activity. No AlP activity in the thin tegument covering the remaining part of the canal (α-naphthyl phosphate + Fast red TR. × 375).

Fig. 3. In an almost longitudinal section through the scolex, AcP activity visible in the tegument and subtegumental cells of the proliferating area in the canal above the rostellum and below the suckers (α-naphthyl phosphate + HPR, × 150).

Fig. 4. Oblique section through the folded bladder wall in the area of a group of scoleces. AcP activity visible in the tegument, the subtegument, subtegumental cells and excretory canals (α-naphthyl phosphate + HPR, × 225).

Fig. 1. Section through the scolex anlage of a young *M. endothoracicus*. AlP activity in the inner layer of the tegument of the invaginated canal, in cells of the scolex anlage and in the differentiating suckers (α-naphthyl phosphate + Fast red TR, × 350).
Fig. 2. Longitudinal section through part of the rostellar cone. AcP activity visible in the tegument of the canal, subtegumental cells and cells of the cone (α-naphthyl phosphate + HPR × 900).
Fig. 3. At a great magnification AlP activity visible in the folds of the differentiating spiral canal (α-naphthyl phosphate + Fast red TR, × 1,350).
Fig. 4. Oblique section through the opening of the differentiating canal in the folded wall of the verrucose mount. AlP activity visible in the tegument (α-naphthyl phosphate + Fast red TR, × 150).

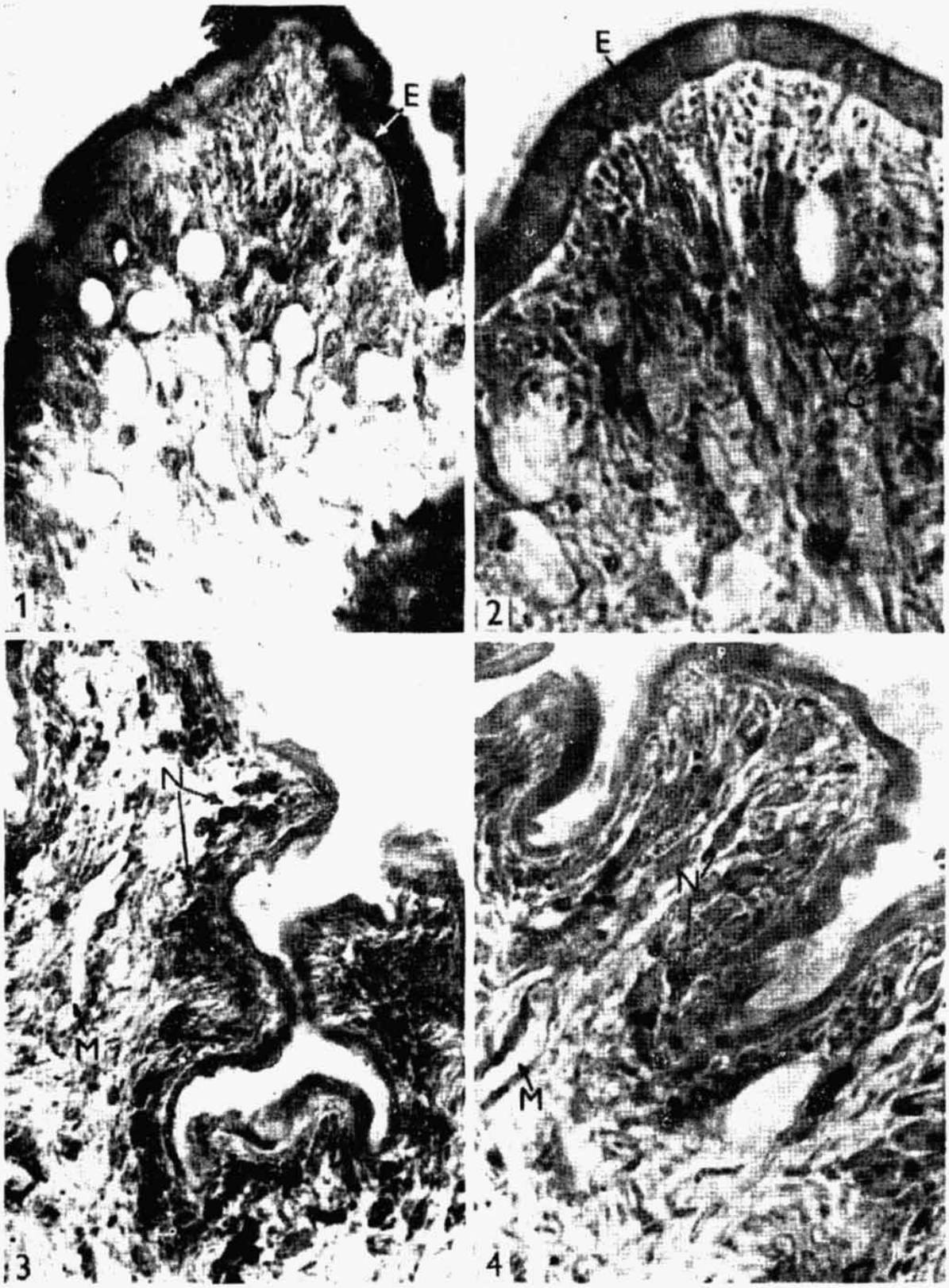

Fig. 1. Oblique section through the modified wall of the verrucose mound in *M. endothoracicus*. AcP activity in the tegument and subtegumental cells (α-naphthyl phosphate + HPR, × 300).
Fig. 2. Section through the modified wall with NE activity at the base of the microtriches and in the basal layer of the tegument. Positive granules in subtegumental cells. (α-naphthyl acetate + HPR, × 300).
Fig. 3. Section through the proliferating area and the bladder below the verrucose mound. AcP activity in subtegumental cells and excretory canals, in lower parts in the tegument (α-naphthyl phosphate + HPR, × 300).
Fig. 4. Section through the proliferating area in the bladder showing NE activity in subtegumental cells, excretory canals and lower in the tegument (α — naphthyl acetate + HPR, × 300).