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THE DISTRIBUTION AND ACTIVITY OF ENZYMES IN LARVAE OF TAENIA HYDATIGENA PALLAS, 1766

D. HULÍNSKÁ

Institute of Parasitology, Czechoslovak Academy of Sciences, Prague

Abstract. The present study has been carried out, using histochemical methods, to determine the distribution and activity of enzymes (alkaline phosphatase -AlP, acid phosphatase -AcP, non-specific esterase -NE) in *Cysticercus tenuicollis* from baby pigs with a massive experimental infection at days 7, 10, 13, 14, 16, 21 and 35 p.i. During the early migratory phase (7—14 days p.i.), AlP and AcP activity was intense in the bladder tegument, that of NE and AcP in differentiating cells. At days 16—35, enzymatic activity of cysticerci encysting on the omentum and the serous membranes of the abdominal cavity decreased until it was almost nonexistent in the bladder of a 35 day-old larva. This applied mainly to AlP and AcP. At day 10 p.i., when the scolex anlage started to differentiate, its cells and tegument displayed an intense activity of AcP and NE. Although AlP was not yet active in the scolex anlage at the initial stage of differentiation, it was detected later in the growing scolex anlage mainly in the transitory zone at the site of opening of the invaginated canal, and in the proliferating zone of the suckers and the rostellum. Concomitant with an increase in AlP activity in the growing scolex area was its decrease in the bladder at the time of its ontogenesis.

Although a certain amount of work has been carried out on the histochemistry of enzymes of various cestode species, this has mainly been concerned with a comparison of the activity of phosphatases in the adult and the invasive larva (Erasmus 1957a, b, Waitz 1963, Shield et al. 1973, and others). However, very little is known of the activity of enzymes at the time of larval development and the differentiation of their tissues. Arme (1966) maintained that differences in the distribution of enzymes should be ascribed not only to an active transport of substances, but also to tissue growth. Therefore the present study was designed primarily to determine differences in the activity of enzymes in the tissues of *C. tenuicollis* from baby pigs at different stages of their development, using histochemical methods.

MATERIALS AND METHODS

Larvae of *Taenia hydatigena* were obtained from white baby pigs with a massive experimental infection at different days p.i. (days 7, 10, 14, 21, 35). In addition, larvae were isolated at days 13 and 16 from minipigs of the same age (two months — 20 kg) with a massive experimental infection. Larvae obtained from the individual animals were different in size, their degree of differentiation and their location in the host. Pending on these facts we distinguished 6 types of larval phases:

Type I — Larvae inside migratory canals in the liver; in the lumen of the migratory canal, the space around and in front of the larva was empty, below the larva it was occupied by disintegrated liver tissue containing erythrocytes. In appearance, the larvae were elongate bladders, mostly yellowish in colour. Larvae which had remained for a prolonged period in the migratory canals, were at different phases of their differentiation.

Type II — Larvae isolated from migratory canals below the liver capsule were spherical, electron-dense, whitish bladders, at different degrees of differentiation after a prolonged stay in these canals.

Type III — partly arising above the liver surface having penetrated the capsule. Thin-walled, translucent, bladders with a whitish, differentiating, cellular, anlage at one pole.

Type IV — unattached in the ascitic flluid of the abdominal cavity. Ovoid translucent bladders with a cellular scolex anlage at one pole. In younger larvae, the tegument was starting to

Table 1. Larvae from white baby pigs at Days 7, 10, 14, 21, 35 p.i.

Characteristics of the larvae	type I*)	type II	type III	type IV	type V	type VI
Day 7 measurements in mm	0.8—1 × 0.3—0.4	0.5—0.7 × 0.7	0	0	0	
Degree of differentiation	b. elongate, yellowish	b. spherical, white	0	0		
Day 10 measurements in mm	0.8—0.9 × 1	1.2—1.5 × 0.9—1.2	2.4—2.6 × 0.9—1.5	0	0	0
Degree of differentiation	b. elongate, yellowish, with thin walls	b. spherical, white	b. oval, thinwalled, with scolex pole	0	0	0
Day 14 measurements in mm	3.5—3.9 × 1.8—1.9	3.9 × 3.8—3.9	0	4—4.3 × 1.8—2.3	4—4.3 × 1.8—2	4—4.5 × 2—2.3
Degree of differentiation	b. elongate, translucent with a scolex anlage	b. opaque, spherical, tegument invaginating into the scolex anlage	0	b. translucent, elongate, tegument invaginated into scolex anlage	b. translucent, ovoid, an opening of the invaginated canal at the site of atachment present	thin-walled, translucent cysts with bladders
Day 21 measurements in mm	4.5—5 × 3.5	0	0	5.6—6.5 × 3.4—4.5	6.5 × 4—4.5	6.7—7 × 3.9—4.5
Degree of differentiation	b. ovoid, translucent with a spiral canal in the scolex	0	0	b. ovoid, translucent, spiral canal and developed excretory system	folded zone at the opening of the spiral canal, rostellar cone forming the bulb	opaque cysts with thick walls, b. irregularly shaped, differentinating suckers
Day 35 measurements in mm	10—10.5 × 6—7	0	0	0	0	12—12.5 × 8.5—9
Degree of differentiation	b. oval, translucent folded wall, rostellar bulb and prebulb, small scolex	0 necrotic larvae	0	0	0	b. thick walled, folded, invasive larvae with suckers, hooks and rostellum

*) Characteristics of different types see p. 315, b—bladder.

Table 2. Larvae from minipigs at days 13 and 16

Characteristics of the larvae	type 1*)	type II	type IV
Day 13 measurements in mm	1.3 × 1.4	1.4—1.5 × 1.4	1.6—1.8 × 1.9
Degree of differentiation	b. elongate, yellow, scolex anlage	b. spherical, opaque	b. ovoid, translucent, tegument invaginating into cellular anlage
Day 16 measurements in mm	3.5—3.8 × 1.5—1.7	3.5—3.8 × 1.7	4.6—4.8 × 2.4—2.6
Degree of differentiation	b. elongate, invaginated canal and rostellar cone	b. ovoid, translucent, proliferating invaginated canal	b. ovoid, translucent, origin of excretory system formation, rostellar cone

*) characteristics of different types see p. 315, types III, V and VI absent, b. — bladder.

Table 3. Activity of enzymes in the bladder of a differentiating larva of *Taenia hydatigena*

Day p.i.	Reaction	Tegument	Muscle fibrils	Germ cells	Branching cells	Subtegumental cells	Protoplasmic band (parenchyma)	Differentiating excretory cells
Day 7	NE	++++	+++	+	+++		++++	
	AcP	+++	+	+++	++	0	+	0
	AlP	++++	−	+++	−		−	
Day 10	NE	++	+++	−	++++		++++	
	AcP	++++	+	+++	++	0	−	0
	AlP	++++	−	++	−		−	
Day 13	NE	++	+++	−	++++		+++	
	AcP	++++	+	+++	+	0	−	0
	AlP	+++	−	++	−		−	
Day 14	NE	++	+++	−	++++	++	−	
	AcP	++++	−	++	−	+++	−	0
	AlP	+++	−	+	−	+++	−	
Day 16	NE	+++	++	−	++++	+++		+++
	AcP	+++	−	+	+	+++	0	−
	AlP	++	−	+	−	++		−
Day 21 in the liver	NE	+++	++	−	+++	+++		+++
	AcP	+++	−	+	+	+++	0	−
	AlP	++	−	+	−	++		−
Day 21 in ascitic fluid	NE	++	+	−	+++	++		++++
	AcP	++	−	−	−	+	0	−
	AlP	−	−	−	−	−		−
Day 35	NE	+	+	−	++	−		++++
	AcP	++	−	−	−	++	0	−
	AlP	−	−	−	−	−		−

invaginate in the scolex pole of the larva, in older larvae the tegument was invaginated and formed an invagited canal.

Type V — adhering to the serous surface of various organs inside the abdominal cavity. Large, translucent, ovoid bladders with a scolex anlage differentiated in a spirally coiled invaginated canal with a clearly visible opening at the surface of the modified, mammillary-like protruberating bladder wall. The larva showed first signs of differentiation of the scolex organs, i.e., a rostellar cone and the anlage of the suckers.

Type VI — encapsulated in the serous membrane. Larvae removed from the cyst were ovoid bladders with a compact whitish scolex in the modified bladder wall arising above the surface of the remaining part of the bladder wall. Scolex differentiated in a spiral canal, suckers and rostellum with hooks.

Our findings in baby pigs are shown in Table 1, those in minipigs in Table 2.

All larvae were measured before fixation. Characteristics of the individual cellular types used in our descriptions of histochemical reaction in larval tissues were essentially similar to those given in an earlier paper (Hulínská 1978).

Table 4. Activity of enzymes in the differentiating scolex of the *Taenia hydatigena* larva

Day p.i.	Reaction	Tegument	Muscle fibrils	Germ cells	Subtegumental cells	Cells with vesicles	Differenting excretory cells
Day 10	NE	+	+++	−			
	AcP	++++	+	++++	0	0	0
	AlP	−	−	−			
Day 13	NE	++	+++	−	+++	+++	
	AcP	++++	+	++++	−	−	0
	AlP	−	−	−	−	−	
Day 14	NE	++	++	−	++++	+++	
	AcP	+++	−	++++	−	−	0
	AlP	−	−	−	−	−	
Day 16	NE	++	++	++	++	+++	+++
	AcP	++++	+	++++	++++	−	−
	AlP	++++	−	+	+++	−	−
Day 21	NE	−	+	−	+	+	+++
	AcP	++	+	++	++	−	−
	AlP	+++	−	+	++	−	−
Day 35	NE	−	+		+	+	+++
	AcP	++	−	+	+	−	−
	AlP	+	−		−	−	−

Using histochemical methods, alkaline phosphatase (AlP), acid phosphatase (AcP) and non-specific esterase (NE) were detected in series of frozen and paraffin section either through organs containing larvae or through larvae isolated from organs and cysts. Tissues were fixed for 2 hr at 4 °C in Baker's neutral formaldehyde, dehydrated in acetone, cleared in benzene and embedded in paraffin. Tissues for frozen sections were fixed with Baker and embedded in gelatine. AlP was detected with α-naphthylphosphate and Fast blue BB (Pearse 1968), inhibited by 0.01 M NaF and incubated in the inhibitor for 5 min at 100 °C. AcP was detected with α-naphthylphosphate and hexasopararosaniline (HPR) (Barka and Anderson 1962), incubated at 100 °C (Lojda and Papoušek 1970) and incubated in a medium without substrate. NE was detected with a technique suggested by Davis and Ornistain and modified by Lojda and Papoušek (1970) using α-naphthylacetate and HPR. The results of the tests are shown in tables 3 and 4.

RESULTS

The activity of the enzymes AcP, NE and AlP was demonstrated in the liver at **day 7** of infection with *C. tenuicollis*, using histochemical methods. Differences were detected in the activity of enzymes in the liver parenchyma and in migratory canals occupied by the larva. It was intense in the undisturbed liver parenchyma, low in the haemorrhagic content of the migratory canal containing disintegrated parenchyma, granules and erythrocytes. In migratory canals still occupied by the larva, AcP and NE activity was intense in lymphocytes and eosinohpiles producing an enzymatically active defence zone around the larva (Plate I, Fig. 1). Migrating larvae were spherical or elongate bladders with a differentiating cavity traversed by protoplasmic bands distinguished by means of their NE reaction. The bladder wall composed of a multinucleate syncytium had a thickness of 23—26 μm. Its thin tegument (3.2—3.5 μm) displayed an intense NE activity (Plate I, Fig. 2) and a less pronounced AcP activity (Plate I, Fig. 1). AcP activity was found to be intense both in the larval tegument and in host cells adhering to the surface of the larva (Plate I, Fig. 4). Ne- and AcP activity was detected in basal, reticular fibers traversed by a network of longitudinal muscle fibres below the tegument. The fibrillar layer was 1.6—2 μm thick. A cellular layer underlying the fibrillar layer contained small spherical germ cells (Type A — see Hulínská 1978) measuring 3×3.5 μm, with large, spherical nuclei. We detected an intense AcP activity, a less intense NE activity in these cells. Ovoid nuclei of large cells measuring 5.2×5.6 μm (Type B — Hulínská 1978) could be seen among these cells. Their projecting cytoplasm displayed both AcP and NE activity. Protoplasmic extensions could be detected in migrating larvae on the basis of different reactions for NE. Structures which at first were regularly ovoid in shape (9.5×10.5 μm) were present below the cellular layer. These contained no remnants of degenerated nuclei, gave a reaction for NE and a faint reaction for AcP. Below them were larger structures lined with an intensely NE-positive membrane (0.05—0.6 μm) (Plate I, Fig. 3). In direction to the centre of the bladder, the structures changed into large, distinct cavities bound by a protoplasmic wall with fibrils which continued to enlarge.

On **day 10** of larval development, the haemorrhagies were larger and more numerous. Lesions inside the liver contained an occasional cysticercus occupying migratory canals close to the liver surface. The lesions gave negative reactions for AcP and AlP, weakly positive reactions for NE. The bladder tegument (thickness 3.8—4.2 μm) reacted intensively for AlP and AcP (Plate I, Fig. 5) with a maximum in the superficial border of the tegument consisting of microvilli and extensions of host cells. AcP activity was detected in ovoid cell nuclei (Type B) situated between longitudinal muscle fibres. The central layer of the bladder wall contained elongate cells. The contents of fibrils and vesicles in cytoplasmic cellular extensions gave a positive reaction for NE. Protoplasmic bands lining small cavities were present occasionally in the inner layer of the bladder wall. The tegument of larger, translucent, bladders protruding slightly above the liver surface after penetration of the capsule was thickened at one pole of the bladder (4.5 to 5 μm) to form the scolex anlage. This pole of the bladder gave a negative reaction for AlP. The thickened tegument was underlaid by multiplied cells, reticular fibres and muscle fibres. The cells representing germ cells of the scolex anlage measured 3.6×3.8 μm and contained a spherical nucleus lined with a thin cytoplasmic band. The cells displayed an intense AcP activity. They appeared at the time of the initial differentiation of cells of the scolex anlage. At the same time, i.e., at day 10 p.i., we isolated smaller, elongate larvae without a cellular scolex anlage from lesions in a heavily infested liver. Typical of this developmental phase was the presence of large, elongate cells differentiating in the parenchyma. The cells contained vesicles and communicated with structures

bound by a thin membrane. The structures contained glycogen, the plasma of the cells gave a positive reaction for AlP and NE.

At **day 13** p.i., small, spherical cysticerci appeared in migratory canals below the liver surface, ovoid to elongate bladders in haemorrhagic lesions in the liver and occasionally in the lung. Their histochemical reaction was similar to that of the foregoing developmental phase. Apart from cysticerci in organs, we recovered large unattached cysticerci from the ascitic fluid. They differed from those of the foregoing developmental phases in a starting invagination of the canal into the scolex anlage. Both in the liver and in haemorrhagies, the activity of enzymes was similar to that of the foregoing developmental phase. However, there was an increase in the number of defence cells both in the proximity to cysticerci in migratory canals and in lesions. This accounted for AcP and NE activity in several of these lesions. The thickness of the tegument of larger bladders from the ascitic fluid was 3.8—4.5 μm. The superficial rim of microvilli with adhering host cells on the bladder tegument gave an intense reaction for AlP, a less intense reaction for NE (Plate II, Fig. 1). AcP activity was detected in the bladder tegument. It was most intense at the scolex pole (Plate II, Fig. 2). The subtegumental muscle layer composed of circular and longitudinal fibres was NE-positive. NE-activity was highest in the scolex anlage of the larva in which the muscle layer was thickened (Plate II, Fig. 3). AcP and AlP activities were low to negative in the muscles. The thickness of the cellular layer was 19—22 μm in the bladder, 50—65 μm in the scolex anlage. The canal was invaginated to a depth of 30 μm in the scolex anlage, the width of the scolex base was 100—160 μm. AcP activity was most intense in small germ cells of the scolex anlage. The bladder wall contained an occasional germ cell (Type A) (Plate II, Fig. 2). The most intense NE-activity was detected in elongate, differentiating, subtegumental, cells measuring 14×3.5 μm (Plate II, Fig. 3) underlying the invaginated tegument of the scolex anlage. The cells were already in perpendicular arrangement to the surface. AcP activity was intense in the superficial layer (thickness 1 μm) of the invaginating tegument, less intense in the inner layer (thickness 3—4 μm) in which NE activity was high (Plate II, Fig. 4). The inner lining of the scolex anlage facing the bladder cavity contained cells with vesicles (Type D) which were NE-active. AlP activity was not detected in the scolex anlage and the invaginated tegument (Plate II, Fig. 5), but it was found in the tegument of the bladder where it was intense, and in small germ cells (Type A) where it was less pronounced. NE activity was detected in large, branching, cells (Type E) projecting into the bladder cavity.

On **day 14** p.i., all cysticerci, i.e., those unattached in the abdominal cavity, those still inside the liver or on the surface of serous membranes, possessed a developed scolex anlage with a distinctly visible canal formed by the invaginating tegument. Several cysticerci were starting to produce translucent cysts on the omentum or serous membranes of the intestine or the spleen. The value of enzymatic activity was identical in these differently distributed cysticerci, whether in the liver or unattached in the abdominal cavity. In all these, the bladder tegument displayed an intense activity of AcP and AlP, a low NE activity. Host cells adhering to the bladder tegument of cysticerci in the liver were intensely AlP-active, less active were cells of the bladder (Plate III, Fig. 1). Even the bladder tegument of a cysticercus enclosed in a translucent cyst on the omentum, was AlP active (Plate III, Fig. 2). NE activity was low in the tegument, more intense in differentiating subtegumental cells. The scolex anlage gave a positive reaction for NE, the subtegumental muscle layer was less NE active (Plate III, Fig. 3). The thickness of the bladder tegument (4.6—5 μm) increased to 7—8 μm at the site of its passage into the invaginated canal of the scolex anlage, where NE-activity was most intense, AcP activity less pronounced. In comparison with the foregoing phase, the scolex anlage was larger (120—180 μm), and contained an increased number of germ

cells under the layer of elongate subtegumental cells. The germ cells displayed a more intense AcP activity, NE activity was detected in subtegumental cells, and in large cells with vesicles (Type D) in the inner layer of the scolex anlage. There was no AlP activity in the scolex anlage. AcP activity was intense in the tegument of cysticerci enclosed in cysts and attached to the serous membranes of various organs; it was less intense in cells of the bladder. Host cells adhering to microvilli of the bladder and at different degrees of lysis displayed little enzymatic activity (Plate III, Fig. 4).

On **day 16** p.i., the scolex anlage both of cysticerci inside haemorrhagies in the liver and those unattached in the abdominal cavity continued in its development. Enzymatic activity detected in haemorrhagies in the liver and in the bladder wall of cysticerci was identical to that of the foregoing stage. The thickness of the bladder tegument of larger cysticerci from the ascitic fluid of the abdominal cavity was 6—7 μm. AcP and NE activity were detected in the tegument and, to a lesser degree, in microvilli which by now measured 7—7.5 μm in length (Plate IV, Fig. 1). NE activity was low in damaged host cell adhering to the microvilli, but was most intense in cells of the parenchyma (Type E) protruding into the bladder cavity, and in cells of the differentiating excretory system (Type D). NE and AcP activity were intense both in subtegumental cells and germ cells of the scolex anlage which measured 210—460 μm (Plate IV, Fig. 2). AcP activity was most intense in the tegument of the invaginated canal and in cells of the rostellar conus, less pronounced in the muscle layer and in subtegumental cells (Plate IV, Fig. 3). Cells of type C and fibrils of the differentiating receptaculum displayed an intense activity both of NE and AcP. Cells with vesicles (Type D) were present in the receptaculum. The tegument of the invaginated canal, and subtegumental cells of the scolex anlage displayed an intense AlP activity (Plate IV, Fig. 4) which was not detected in the foregoing developmental phases.

On **day 21** p.i., enzymatic activity was detected in cysticerci still persisting in the disintegrated liver parenchyma. AlP activity was intense in the tegument, in subtegumental cells and host cells in close proximity to the cysticercus (Plate V, Fig. 1). AcP activity was intense in the tegument, less intense in cells (Plate V, Fig. 2). In comparison with AcP activity in unattached cysticerci from the ascitic fluid of the abdominal cavity and those encysted on the omentum, AcP activity was lower in cysticerci from the liver. The bladder tegument possessed already long microtriches with an NE activity similar to that of the bladder tegument (Plate V, Fig. 3). No enzymatic activity was detected in damaged host cells adhering to the microthrix border. Of intense NE activity was the parenchyma of the bladder particularly in the proximity to excretory canals and flame cells projecting into the bladder cavity. AlP and AcP activity was detected in the inner layer of the tegument forming the spiral canal in the differentiating scolex (Plate V, Fig. 4). A low AlP activity was detected even in cells of the wall of the spiral canal, in cells of the rostellar bulb and differentiating suckers. AcP active were cells of the bulb and those in the basal part of the tegument of the spiral canal. In contrast to the scolex, AlP activity was not detected in the bladder tegument, and was found to be low in cells of the bladder.

On **day 35** p.i., the majority of cysticerci were encysted on serous membranes of the abdominal cavity. Cysticerci recovered at that time from the liver were less advanced in their development than those obtained from cysts. The differentiation of the scolex was not yet complete, the rostellum was divided in a bulb and prebulb, its tegument was bearing young hooks and granules. The bladder was big with a folded wall. The activity of all enzymes tested was considerably less intense than that detected in foregoing developmental phases. Both the folded tegument (4.5 — 5 μm thick) and subtegumental muscles gave a weak reaction for NE, which was stronger only in cells and canals of the excretory system (Plate VI, Fig. 1). AcP activity was detected in the

bladder tegument and in subtegumental cells where it was less intense (Plate VI, Fig. 2). In a scolex with a less advanced differentiation, the rostellar bulb and prebulb were AlP active. AlP activity was low in the tegument of the basal part of the spiral canal, more intense at the site of its opening (Plate VI, Fig. 3). In older cysticerci encysted on the omentum, the scolex was fully developed bearing a;rostellum and hooks. Enzymatic activity in this scolex was confined to the nerve endings (Plate VI, Fig. 4). At sites where vesicles and damaged host cells adhered to the microthrix border, AcP activity was detected in the rostellar tegument.

DISCUSSION

The distribution and intensity of enzymatic activity in differently developed larvae of *T. hydatigena* was shown to be dependent on host-parasite relationships. The defence reaction of the host was most intense during the early (migratory) phase of development of *T. hydatigena* in the liver, and so was the activity of AlP, AcP and NE. It decreased gradually after the completion of the migratory phase and the release of larvae from the liver to the ascitic fluid of the abdominal cavity. After the emergence of larvae from the liver, this was damaged and traversed by numerous migratory canal mostly without larvae. If an occasional larva remained in the liver, its development was less advanced than that of unattached or encysted larvae. This accounted for differences in the enzymatic activity of larvae recovered from different sites, i.e., from the liver, the ascitic fluid and from cysts. The activity of AlP changed in intensity in the different parts of the cysticercus body during different developmental stages in dependence on their functions. AlP activity was detected in the tegument of the younger differentiating bladder functioning in absorption, locomotion and defence. Even in older larvae (10—14 days) AlP activity was restricted to the bladder tegument and was not present in the cellular scolex anlage, because the differentiation of the scolex anlage was not advanced enough as to take on nutritive and defence functions. Yamao (1952) maintained that the tegument of scoleces of *Echinococcus granulosus* and *Cysticercus fasciolaris* became AlP active as late as the phase of rostellar development. Also Kwa (1972) observed an increased AlP activity in the tegument of a completely differentiated scolex at the site of secretion of vesicles in the sparganum of *Spirometra erinacei*. In our material, AlP activity was first detected in a 16 day-old larva at the time of the proliferation of the spiral canal into the scolex anlage which at that time was at a more advanced state of differentiation. The scolex tegument and especially that part covering the opening of the developing spiral canal functioned in the attachment and encystment on serous membranes, because hooks and suckers were not yet developed. According to earlier suggestions, the presence of phosphatases in the superficial layer of the cestode tegument facilitated the uptake of nutrients (Rothman 1966, Lumsden 1965, Lumsden et al. 1968, Kwa 1972) and protected the larva against host cells (Shield et al. 1968, Ubelaker et al. 1973, Hulínská 1977, 1978, and others). In general, host cells were most numerous at the site of the opening of the spiral canal where also AlP activity was most intense as demonstrated for *Cysticercus tenuicollis* and also, e.g., for *C. bovis* (Žďárská 1973). A different activity of AcP and NE in cells detected in the initial phases of development of *C. tenuicollis* was shown to be associated with the differentiation, multiplication and growth of cells. In a 7 day-old larva, an intense activity of AcP and a low activity of NE were detected in small germ cells (Type A) distributed under the tegument. An intense NE activity and a less pronounced AcP activity were detected in branching cells (Type C) present in the middle layer of the bladder. In an occasional elongate cell (differentiating subtegumental cells) (Type B) numerous in

older larvae (14—16 days), both NE and AcP activity were intense. The growth and multiplication of subtegumental cells especially in the scolex anlage caused a general increase in the size of the larva. Our observation of an increacing NE activity in differentiating subtegumental cells which became more numerous as the larva developed agreed with the demonstration of an increased NE activity in subtegumental cells of a growin *C. pisiformis* made by Shield et al. (1973). We also agree with Takahashi (1958), Erasmus (1957 a, b, 1968), Arme (1966), Erasmus and Öhman (1965) in that a different distribution and a different intensity of enzyme activity were directly related to the potentiality of growth of a certain part of the larval body. In support of this opinion was the demonstration of a decreased activity of both AcP and NE in subtegumental cells of an older *C. tenuicollis* (aged 34 days) in which the number, shape and size of subtegumental cells was unchanged. The only site of NE-activity in the bladder was the excretory system, where it served in waste disposal and was still active in the final phase of larval development.

ЛОКАЛИЗАЦИЯ И АКТИВНОСТЬ ФЕРМЕНТОВ В ЛИЧИНКЕ *TAENIA HYDATIGENA* PALLAS, 1766

Д. Гулинска

Резюме. При помощи гистохимических методов исследовали локализацию и активность ферментов (щелочная фосфатаза — AlP, кислая фосфатаза — AcP, неспецифическая эстераза — NE) у *Cysticercus tenuicollis* от экспериментально зараженных поросят. Исследования проводили на 7-й, 10-й, 13-й, 14-й, 16-й, 21-й и 35-й день после заражения. В период ранней стадии, стадии миграции (7—14 дней после заражения), обнаружена высокая активность AlP и AcP в тегументе пузыря и активность NE и AcP в дифференцирующихся клетках. На 16—35-й день ферментативная активность цистицерков, энцистирующихся на сальнике и серозных оболочках брюшной полости, понижается, особенно активность AlP и AcP, которая почти исчезает в пузыре 35-дневних личинок. В зачатке сколекса, дифференцирующемся с 10-го дня после заражения, высокая активность AcP и NE в клетках и тегументе зачатка. AlP в зачатке сколекса в начале дифференциации не активна, но ее активность повышается одновременно с ростом зачатка, особенно в тегументе переходной области отверстия инвагинированного канала и в пролиферирующей зоне присосок и хоботка. Одновременно с повышением активности AlP в растущей области сколекса понижается активность в пузыре в течение его онтогенеза.

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D. H., Parasitologický ústav ČSAV, Flemingovo n. 2, 166 32 Praha 6, ČSSR

EXPLANATIONS

A microtriches
B tegument
C subtegumental muscles
D subtegumental cells
E excretory cells of excretory system
F excretory canals
G defence reaction
H host cells
I branching cells
J protoplasmic bands and cavities
K scolex anlage
L germ cells of the scolex
M cyst
N cells with vesicles in the receptaculum
O nerve endings
P hooks
R rostellum
S calcareous corpuscles

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G. V. Kolonin: Mirovoye rasprostranenie iksodovykh kleshchey (rod Haemaphysalis). (World distribution of ixodid ticks. Genus Haemaphysalis). *Publ. House Nauka, Moskva 1978, 71 pp., 3 Tables, 11 maps. Price 0.75 R.*

Since the publication of the monograph by Nuttall and Warburton (1915) devoted to the genus *Haemaphysalis* over 100 species of this genus have been described and a wealth of new information has been accumulated on its representatives. The author set a task to demonstrate in this book the geographic distribution of all known species to date. His taxonomic classification adheres to the Hoogstraal's concept. The first section constitutes a survey of species arranged in subgenera, each species provided with a note on its distribution or biotope of occurrence, spectrum of hosts and main literature. A total of 146 species belonging to 13 subgenera is thus presented, three species not being classified in subgenus. Unlike Camicas and Morel (1977) the author does not list *Paraphysalis* Hoogstraal as a distinct subgenus. The subsequent part of the book deals with taxonomic analysis of the fauna of the *Haemaphysalis* genus, geographic regionalization of the genus range, a survey of species, selected synonymy and a list of literature including 197 references. The book is supplemented with 11 maps depicting the distribution of species in nine regions recognized by the author. Accuracy could be improved here by indicating the reference number of relevant map with each of the 140 species recorded. The list of selected synonymy on page 59, particularly its arrangement, also deserve some criticism. E.g. *H. leachi*, in fact a distinct species, is listed as a synonym of four other species. Neither the synonymy of *H. parva* appears to be clear enough to the reader who is not an experienced specialist. On the whole, however, it may be said, that this publication, though small in volume but rich in data, is a highly valuable ixodological handbook.

Dr. V. Černý, C.Sc.

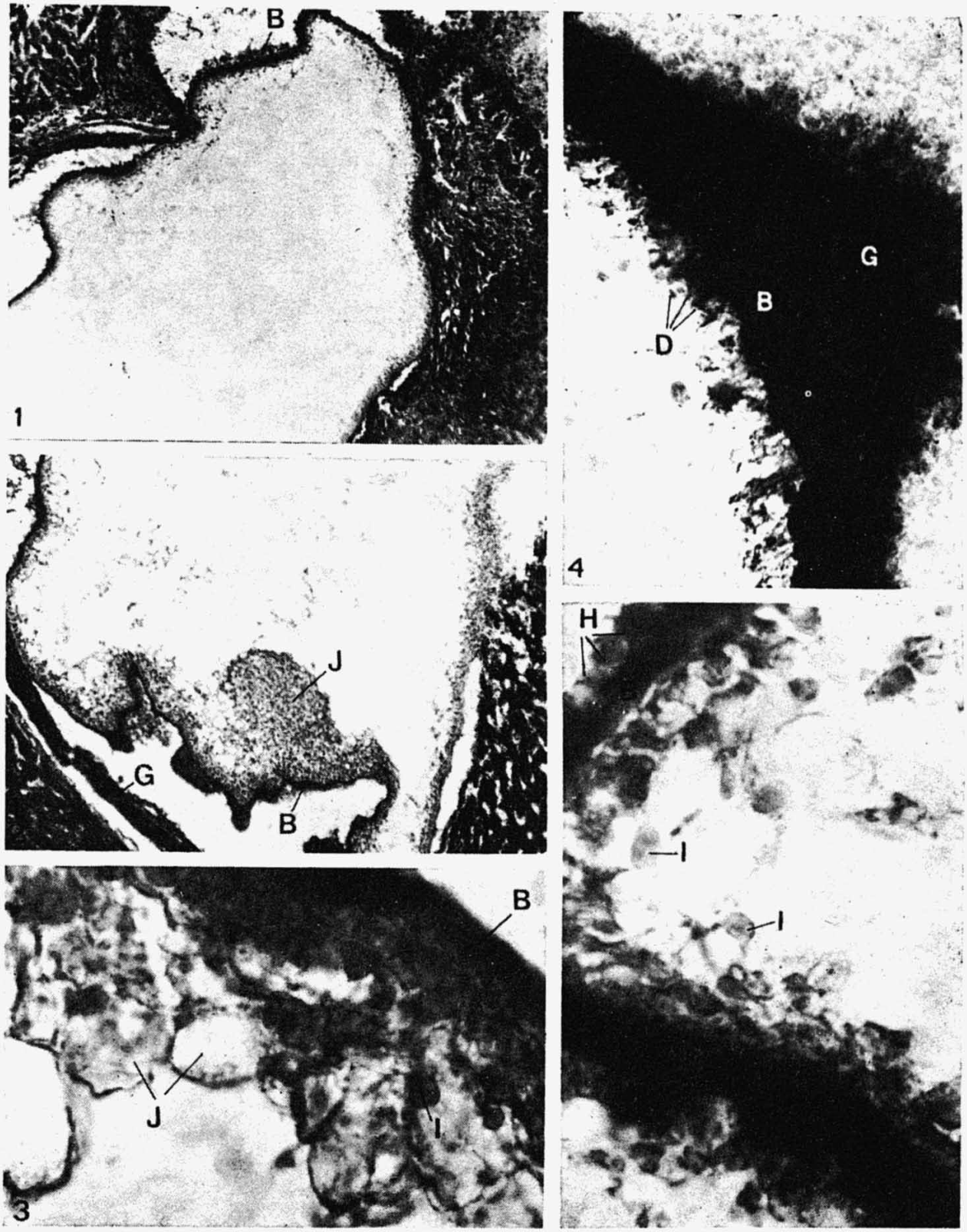


Fig. 1. Migratory phase of a 7 day-old *C. tenuicollis* in the liver of a baby pig. AcP activity in close proximity to the migratory canal with the larva inside it. Intense AcP activity in the larval tegument and adhering host cells. Weak AcP activity in differentiating cells of the bladder (α-naphthyl-phosphate + HPR, × 193).

Fig. 2. Intense NE activity in the bladder tegument and in protoplasmic bands occupying part of the bladder cavity; also in host cells in the migratory canal (α-napthylacetate + HPR, × 193).

Fig. 3. A differentiating bladder isolated from a migratory canal below the capsule: Intense NE activity in the tegument and the inner part of the bladder wall composed of cells and protoplasmic bands partly filling the bladder cavity (α-naphthylacetate + HPR, × 769).

Fig. 4. Intense AlP activity in close proximity to the migratory canal in the liver, and in the larval tegument, less intense in differentiating cells, negative in the liver parenchyma outside the larva (α-naphthylphosphate — Fast blue BB, × 500).

Fig. 5. Larva released from the liver surface at day 10 p.i.: little NE activity in the tegument; host cells and branching cells of the bladder (Type B) NE-active, also differentiating cells of the parenchyma in the inner layer of the bladder wall (α-naphthylacetate + HPR, × 769).

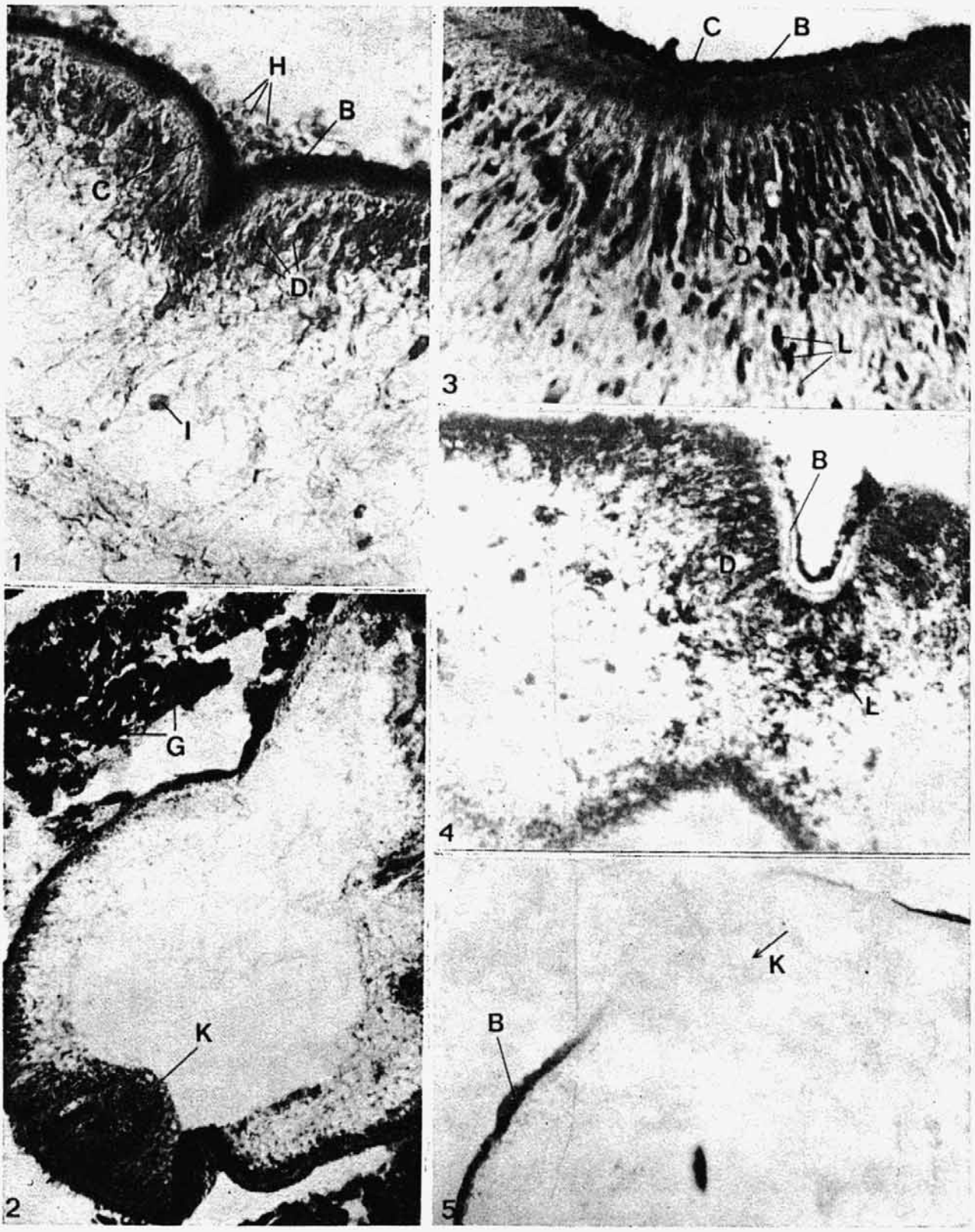


Fig. 1. Intense NE activity in the tegument of a 13 day-old *C. tenuicollis* from a mirgatory canal below the liver capsule; NE activity less intense in the muscle layer of the bladder and in differentiating subtegumental cells (Type B). More intense NE activity in parenchymal cells at the site facing the bladder cavity (α-naphthylacetate + HPR, × 320).
Fig. 2. Intense AcP activity in proximity to the migratory canal below the capsule, and in the tegument of a 13 day-old larva. Scolex anlage, differentiating subtegumental cells and germ cells most AcP active (α-naphthylphosphate + HPR, × 180).
Fig. 3. Intense NE activity in the tegument of the scolex anlage and in elongate, subtegumental cells, less intense in the thickened layer of ubtegumental muscles (α-naphthylacetate + HPR, × 320).
Fig. 4. AcP activity in the invaginated canal of the scolex anlage of a 13 day-old larva from the ascitic fluid detected in the superficial layer of the tegument, in cells of the scolex anlage, in cells of the bladder (α-naphthylphosphate + HPR, × 180).
Fig. 5. The scolex pole of a bladder from the ascitic fluid AlP negative, the bladder tegument AlP positive (α-naphthylphosphate + Fast blue BB, × 180).

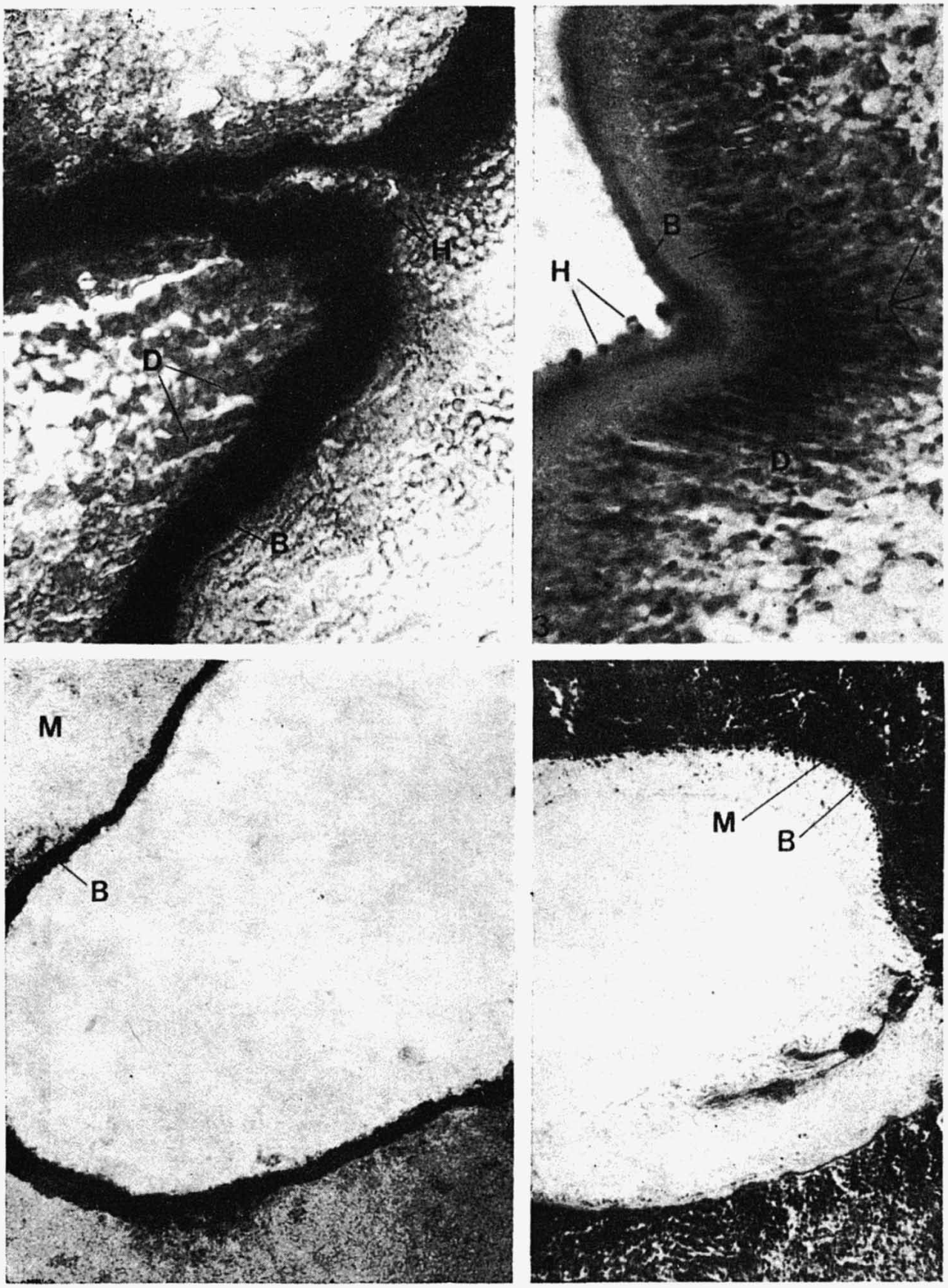


Fig. 1. A 14 day-old larva from the liver of a baby pig: Intense AlP activity in the bladder tegument, less pronounced in subtegumental cells and host cells adhering to the surface of the tegument (α-naphthylphosphate + Fast blue BB, × 830).
Fig. 2. A 14 day-old larva encysted on the omentum: Intense AlP activity in the tegument of the larva, none in the cyst (α-naphthylphosphate + Fast blue BB, × 190).
Fig. 3. Intense NE activity in the tegument of the invaginated canal, in elongate subtegumental cells, branching cells and germ cells of the scolex anlage of a 14 day-old larva; low NE activity in subtegumental cells (α-naphthylacetate + HPR, × 150).
Fig. 4. AcP activity in the tegument and subtegumental cells of a larva encysted on the serous membrane of the intestine (α-naphthylphosphate + HPR, × 208).

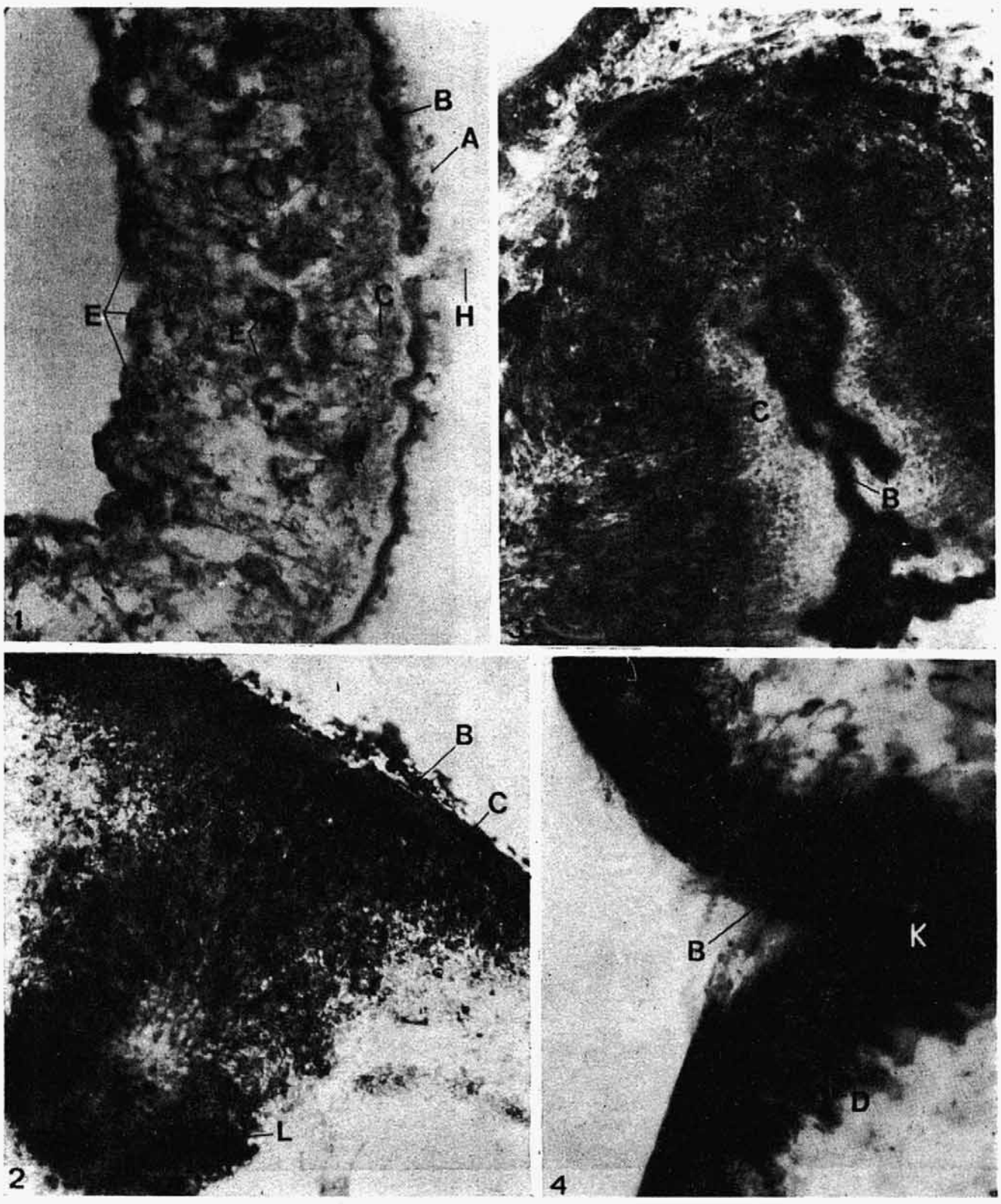
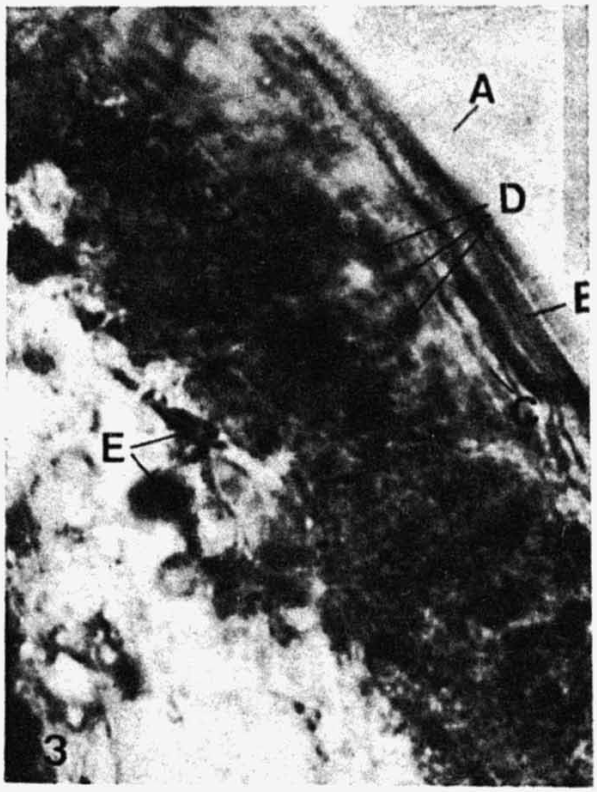
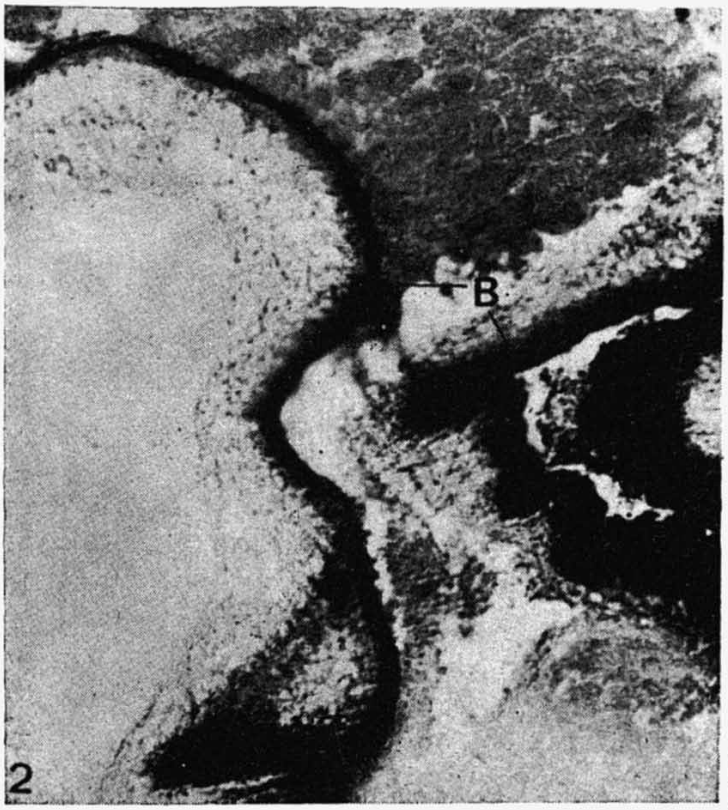


Fig. 1. 16 day-old larva from the ascitic fluid: Intense NE activity in the tegument and subtegumental ce ls of the bladder, most intense in branching cells (Type E) and cells of the excretory system (Type D) (α-naphthylacetate + HPR, × 346).
Fig. 2. 16 day-old larva: Scolex anlage — NE activity intense in subtegumental and germ cells (α-anphthylacetate + HPR, × 270).
Fig. 3. Invaginated canal of the scolex anlage: Intense AcP activity in the tegument, low activity in the muscle layer, intense in cells with vesicles (Type D) at the site of the differentiating receptaculum (α-anphthylphosphate + HPR, × 346).
Fig. 4. Differentiating scolex anlage of a 16 day-old larva from the liver: Intense A1P activity in the tegument covering the opening of the invaginated canal, low in the subtegumental muscle layer, intense in subtegumental cells, low in cells of the parenchyma (α-naphthylphosphate + Fast blue BB, ×460).





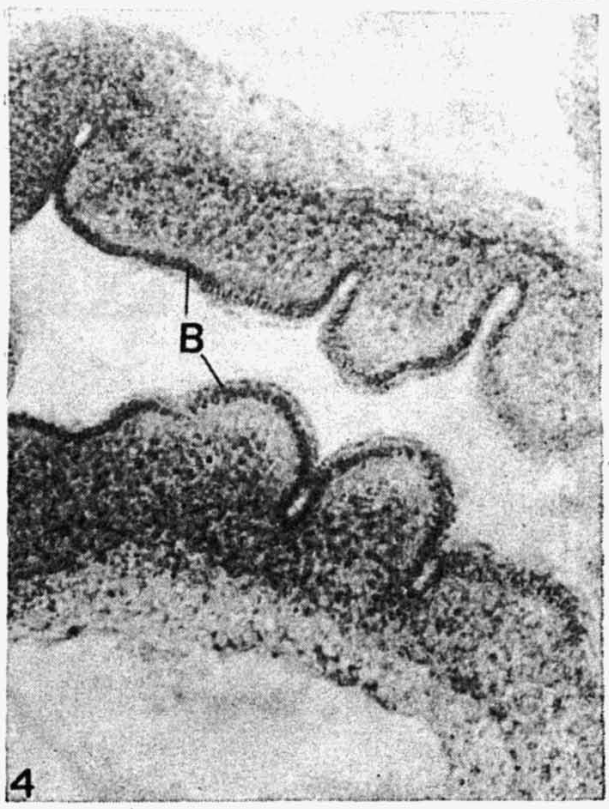
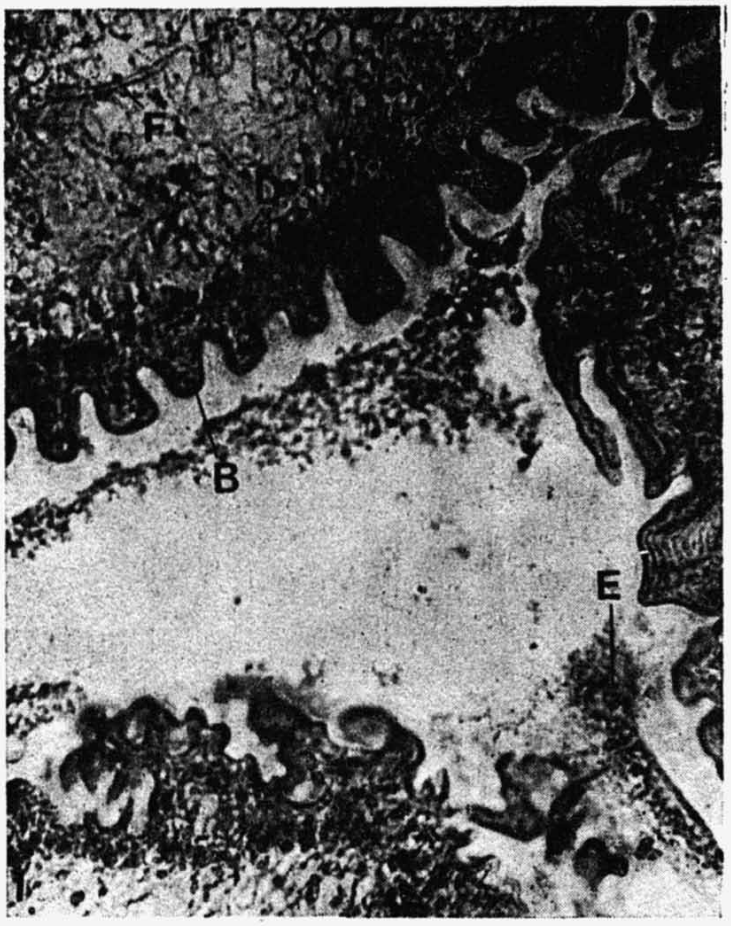
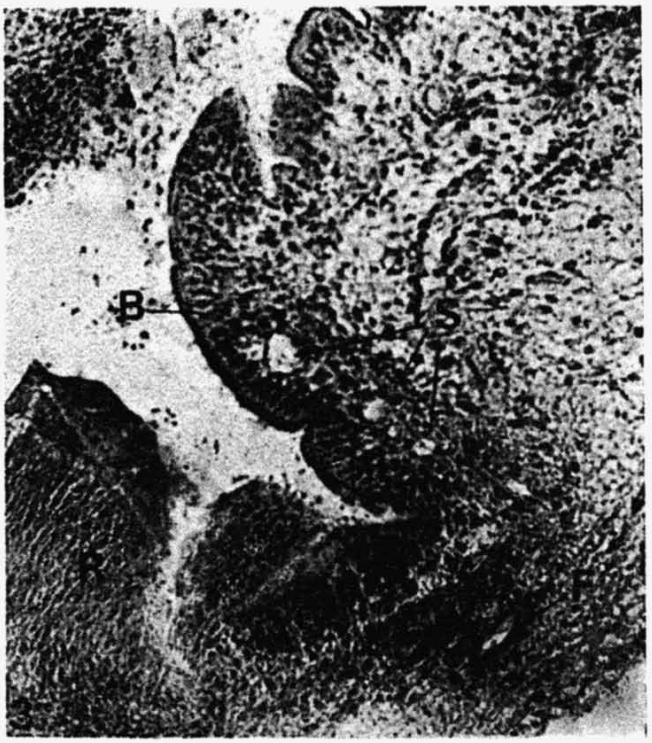
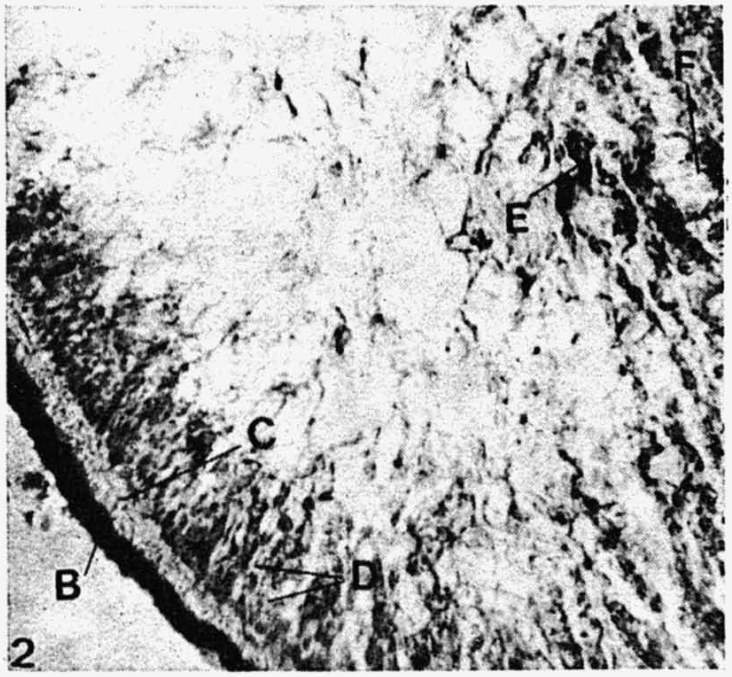


Fig. 1. A 21 day-old larva from the liver: A1P activity intense in the tegument, host cells and subtegumental cells (α-naphthylphosphate + Fast blue BB, ×340).
Fig. 2. A 21-day old larva from the liver: Intense AcP activity in the tegument, low AcP activity in subtegumental cells and parenchymal cells (α-naphthylphosphate + HPR, ×230).
Fig. 3. The bladder of an unattached larva from the ascitic fluid: NE activity in the mitrothrix border; less pronounced NE activity in the tegument and subtegumental layer of muscles and cells, most intense in cells and canals of the excretory system (α-naphthylacetate + HPR, ×770).
Fig. 4. A 21 day-old larva: A1P activity low in the inner layer of the tegument and in cells of the wall of the differentiating spiral canal in the scolex of the larva (α-naphthylphosphate + Fast blue BB, ×272).







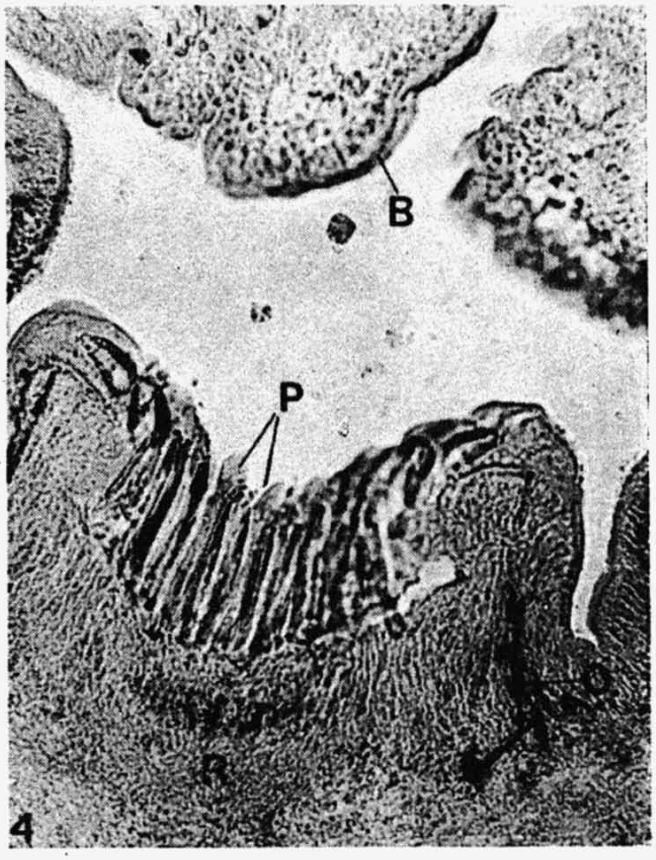


Fig. 1. A 34 day-old larva from the liver: Low NE activity in coarse folds of the bladder tegument. Subtegumental cells and excretory canal NE-active (α-naphthylacetate + HPR, × 175).
Fig. 2. A 34 day-old larva from the liver: AcP activity intense in the tegument of the bladder, low cells and in the parenchyma (α-naphthylphosphate + HPR, × 250).
Fig. 3. A1P activity in the tegument of the spiral canal above the rostellar bulb, in cells of the bulb and cells of the wall of the canal (α-naphthylphosphate + Fast blue BB, × 83).
Fig. 4. A 34 day-old larva from a cyst on the serosa of the intestine: Definitive rostellum with hooks — A1P activity in nerve endings and weak A1P activity in cells of the rostellum (α-naphthylphosphate + Fast blue BB, × 183).