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# Distribution of acetylcholinesterase activity during the development of cysticercoids of *Hymenolepis diminuta* (Cestoda)

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## Abstract

The distribution of acetylcholinesterase (AChE) in oncospheres and developing cysticercoids of *Hymenolepis diminuta* was examined. The enzyme was localized in the nervous system and in some non-nerve cells of these larvae. In oncospheres AChE was detected in hook muscles and in the binucleated medullar center that is known to enclose two neurons. At early developmental stages of the cysticercoids the enzyme was localized in the post-oncospheral hook muscles and in subtegumental muscle fibers of the cercomer. At medium and late stages of development the activity of AChE was detected in the developing nervous system and in two and, subsequently, in four populations of cells, which gradually spread over the whole internal wall of the cyst, thus forming a thin multilayer AChE-positive lining of the cyst cavity. Following withdrawal of the scolex the lining separates the parenchyma of the turned neck from the cyst tissues and remains AChE-positive during the whole life of the parasite, i.e. up to the death of the infected host. The role played by non-neural AChE associated with the cyst cavity lining is unknown, but seems to regulate both the transport of nutrients and minerals into the scolex and waste substances in the opposite direction.

## Key words

Cestoda, *Hymenolepis diminuta*, cysticercoids, acetylcholinesterase, histochemistry

**Abbreviations:** BW284C51 – 1,5-*bis*-(4-allyldimethylammoniumphenyl)-pentane-3-one dibromide; Fast Blue RR salt – 4-benzoylamino-2,5-dimethoxybenzenediazonium chloride hemi(zinc chloride) salt; GPI – glycosylphosphatidylinositol; *iso*-OMPA – tetraisopropylpyrophosphoramide; PBS – phosphate-buffered saline.

## Introduction

When ingested by the yellow mealworm (*Tenebrio molitor*), the oncosphere of the cestode *Hymenolepis diminuta* actively penetrates the wall of the intestine to reach the hemocoel of the beetle. Here the oncosphere develops into a cysticercoid. Development takes from 15 to 25 days depending on the temperature and intensity of the infection. During larval development the germinative cells divide and form a mass of mesenchyma cells that gradually differentiate into cells of the cysticercoid (Ogren 1962). In some of these cells a cholinesterase activity was detected by Bogitsh (1967). Rybicka (1967) observed hydrolysis of acetylthiocholine and butyrylthiocholine already within oncospheres in unfixed cryostat sections from gravid proglottids of *H. diminuta*. However, the product of the histochemical reaction was not associated with any recognizable structure, due to the complete disintegration of the cells as a result of freezing of the specimen and then thawing of the sections on microscope slides. Samii and Webb (1990) found acetylcholine-like immunoreactivity in 2–4 loci of the shelled hexacanths occupying the uterus of the gravid proglottids, but the immunoreactive structures were unrecognizable. There is, however, no information on the distribution of AChE in the developing cysticercoids. Our preliminary histochemical tests revealed the presence of both a neural and a non-neural AChE in these larvae and, therefore, we decided to make a thorough study of the expression of the activity of the enzyme in the differentiating cells and tissues of the cysticercoids.

Invertebrates synthesize only globular forms of acetylcholinesterases: those of the T ("tailed") type (amphiphilic $G_1^a$ $G_2^a$, $G_4^a$ forms) and that of the H ("hydrophobic") type (an

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amphiphilic GPI-anchored form GPI-$G_2^a$) and do not synthesize butyrylcholinesterases (Massoulié and Bon 1982, Massoulié *et al*. 1999, Massoulié 2002). We have shown previously (Moczoń and Świetlikowska 2005) that AChE from the mature cestode *H. diminuta* is a globular amphiphilic enzyme of the T type, the monomeric form of which has molecular mass of 66 kDa. The present study opened a good opportunity to swiftly compare the electrophoretic mobilities of AChEs extracted from the three developmental stages of the cestode, i.e., from oncospheres, cysticercoids and mature tapeworms and to examine whether some nonspecific esterase of the parasite is capable of hydrolyzing thiocholine esters during a prolonged incubation of polyacrylamide gel strips in appropriate histochemical media.

## Materials and methods

*Acetylcholinesterase histochemistry*

Approximately 2 $cm^3$ of a dense suspension of invasive oncospheres isolated from feces of infected Wistar rats were artificially hatched by mechanical breaking of their eggshells, followed by a treatment with 0.3–0.5% solution of papain in saline (Moczoń 1993). The larvae were separated from the shell debris by centrifugation in a triphasic gradient of polyvinylpyrrolidone (Moczoń 1993). Some hexacanths were fixed in a 2% formalin-0.1 M cacodylate buffer mixture at pH 7.3 for 30 min and processed for histochemistry of AChE, while the remaining larvae were homogenized and used for electrophoresis of esterases as mentioned below.

The beetles *Tenebrio molitor* infected with oncospheres of *H. diminuta* were maintained in the laboratory at approximately 20°C. Under these conditions the development of cysticercoids within heavily infected beetles took from 18 to 20 days. The hosts were dissected everyday and the extracted parasites were washed with PBS. Following fixation in a 0.1 M sodium cacodylate-buffered 2% formalin at pH 7.3 (2–4 h at room temperature) and washing with PBS, the larvae were processed for histochemical detection of AChE activity.

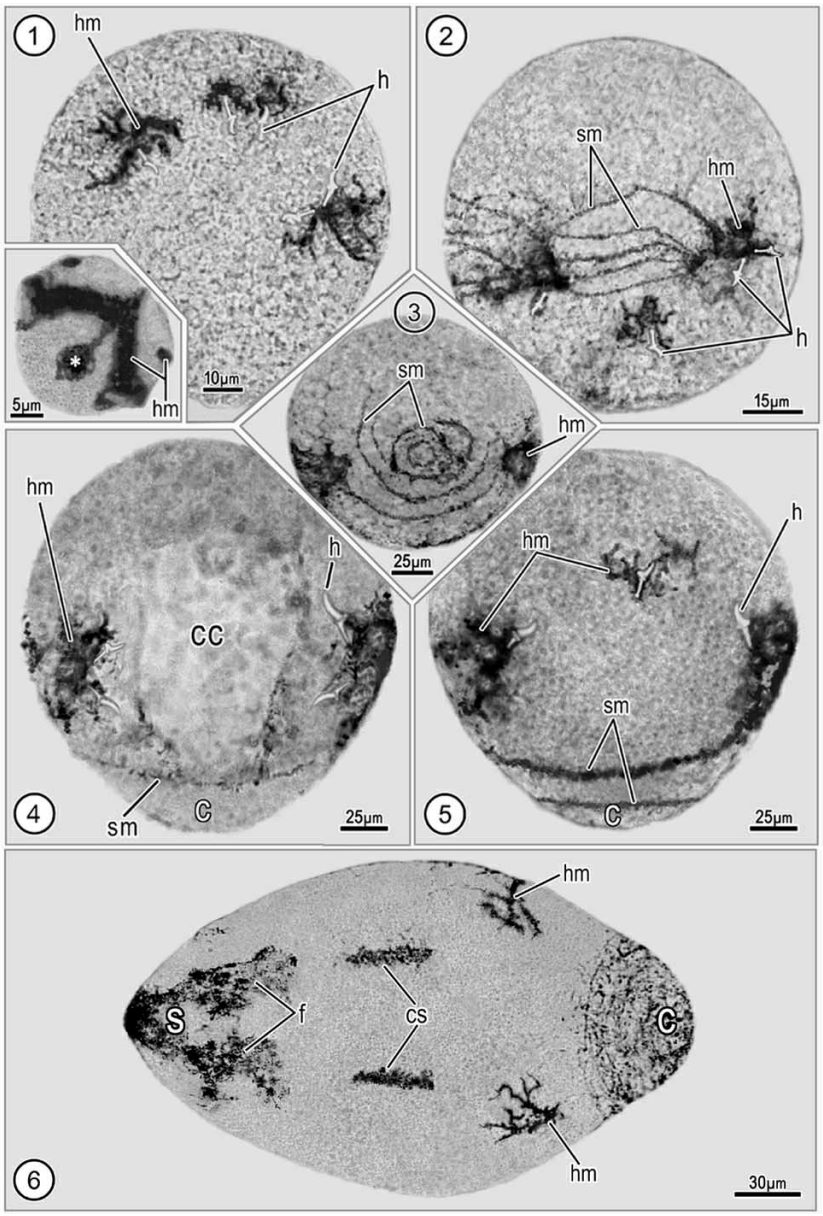
Two histochemical methods were employed with the use of acetylthiocholine iodide, butyrylthiocholine iodide, and *N*-acetyl-β-methylthiocholine iodide as substrate. The direct-coloring thiocholine method of Karnovsky and Roots (1964) proved to be useful for the detection of AChE activity in the oncospheres. For studying cysticercoids the incubation medium recommended by Tsuji (1974) was chosen, although it produced rather coarse precipitates. The medium comprised 10 mM copper sulfate, 50 mM glycine, 0.1 M acetate buffer at pH 6.0, and 5 mM thiosubstrate. Following incubation, the primary product of the reaction was converted to cupric sulfide by means of a diluted solution of ammonium sulfide. Additionally, the myristoylcholine method of Gomori (1948) was applied to pre-encysted and encysted cysticercoids to examine, whether the enzyme is capable of hydrolyzing esters of choline and long-chain fatty acids. The medium comprised 0.03 M Tris-maleate buffer at pH 7.6, 0.0125 M cobalt acetate, and 20 mM myristoylcholine chloride. The white primary product of the enzymic reaction (cobalt myristate) was converted into cobalt sulfide by means of a diluted ammonium sulfide solution. Two control reactions were employed: (1) incubation of the specimens in the absence of a substrate, and (2) preincubation for 30 min in buffered solutions of the following cholinesterase inhibitors: physostigmine hemisulfate ($10^{-5}$ M), BW 284C51 ($10^{-5}$ M), and *iso*-OMPA ($10^{-5}$ M). A stock solution of the latter compound was prepared in anhydrous isopropanol just before use. The processed material was washed in saline, coverslipped and examined under a microscope without prior dehydration.

*Non-denaturing electrophoresis of esterases*

Artificially hatched, naked hexacanths were pelleted, resuspended in a 10 mM sodium phosphate buffer at pH 7.0 and disrupted by application of three 5-sec ultrasonic bursts at 100 W (Braun sonifier, model Labsonic L), with intervening cooling at 0°C. Following centrifugation (60 min, 60,000 *g*, 4°C), the supernatant was used as a source of esterases. Using a whole-glass homogenizer, fully developed 27–30-day-old cysticercoids and an anterior fragment of the strobila of the mature tapeworm were homogenized in a 10 mM sodium phosphate buffer at pH 7.0 and centrifuged as above. Samples of the extracts were electrophoresed according to Laemmli (1970) in a polyacrylamide gel (T6, C2) in the presence of either 1% (w/v) Triton X-100 or a mixture of 0.5% (w/v) Triton X-100 and 0.2% sodium deoxycholate (Triton was believed to protect the enzyme from denaturation by deoxycholate). Following electrophoresis, the gel that contained Triton X-100 was washed with a 0.05 M maleate buffer at pH 6.0. The gel that contained both Triton X-100 and deoxycholate was extensively washed with the maleate buffer supplemented with 0.5% (w/v) Triton X-100 and, subsequently, with the buffer alone. Both gels were then cut into pieces and processed for the detection of esterase activities. For acetylcholinesterase activity, a medium comprising a 0.05 M maleate buffer at pH 6.0, 5 mM $CuSO_4$, 25 mM glycine and 10 mM acetylthiocholine iodide was employed. Following 4 h incubation, the gel strips were extensively washed with tap and distilled water, transferred to tight capped plastic boxes and sealed with a "developing" mixture comprising 0.25 M 2-amino-2-methyl-1,3-propanediol-HCl buffer at pH 9.0, to which a small volume of ammonium sulfide was added just before use. The gels were kept in this slightly yellow mixture until white bands of the product of the histochemical reaction became perfectly black. To examine whether or not, nonspecific esterases of the cestode are capable of splitting acetylthiocholine, some gel strips were kept for up to 24 h in the acetylthiocholine-containing incubation medium that was changed every six hours. Following incubation the reaction pattern was compared with that obtained in gels processed for the detection of the activities of nonspecific esterases.

For nonspecific esterase activities a medium comprising 0.05 M sodium phosphate buffer at pH 7.2 saturated with 1-naphthyl acetate (substrate) and supplemented with Fast

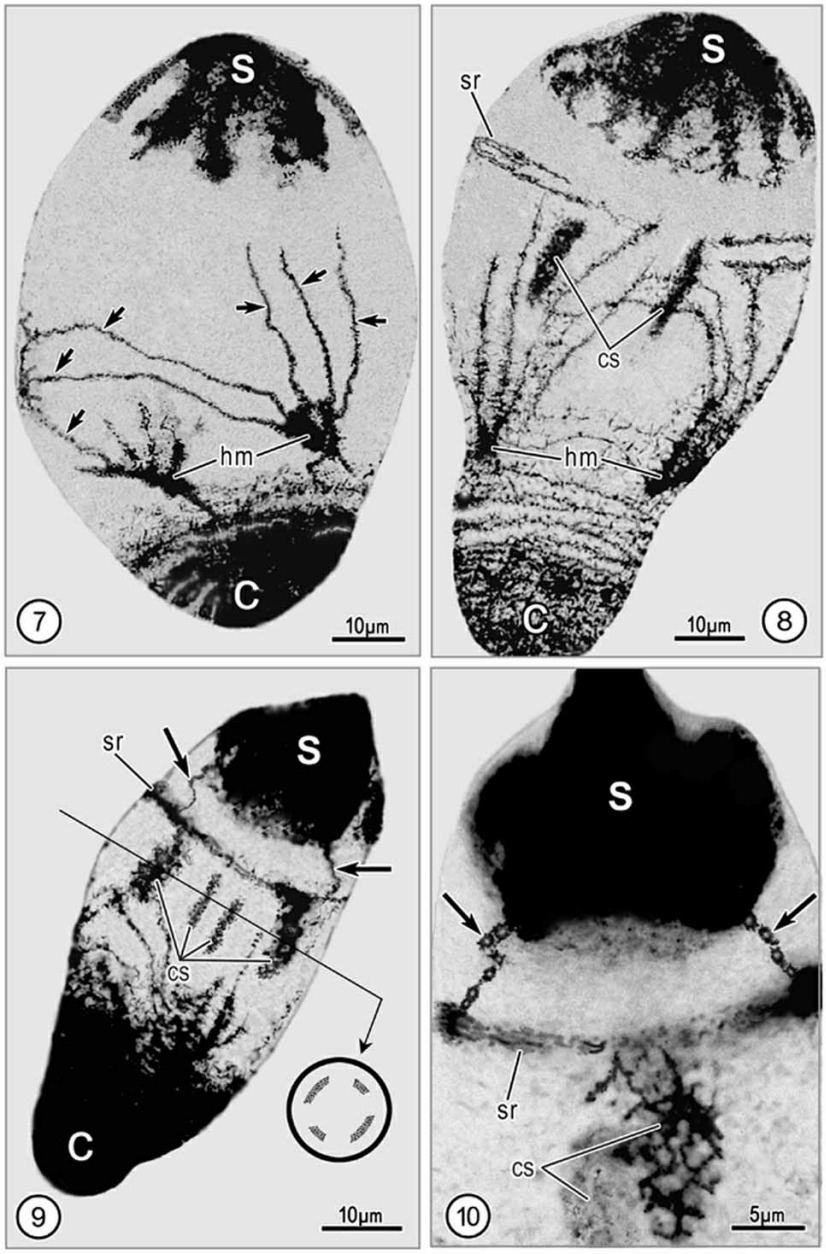
Blue RR salt (1 mg $ml^{-1}$) was used (a stock solution of the substrate was initially prepared in *N*,*N*-dimethylformamide). In order to avoid an excessive background staining, resulting from a spontaneous hydrolysis of the substrate in the presence of the relatively unstable diazonium salt, the medium had to be changed every hour for a freshly prepared one. Physostigmine ($10^{-5}$ M) was used to discriminate between nonspecific esterases and acetylcholinesterase. Finally, the gel strips were washed with 7% acetic acid, the reaction patterns were recorded and copied onto graphically prepared backgrounds.




**Figs 1–6.** Acetylcholinesterase activity in the invasive oncosphere and in the cysticercoids at early stages of their development. **1.** AChE activity in post-oncospheral hook muscles of the 4-day-old cysticercoid. Insert: AChE activity in oncospheral hook muscles and in the binucleated medullar center (asterisk) of the invasive oncosphere. **2.** AChE activity in post-oncospheral hook muscles and adjacent subtegumental muscle fibers of a 5-day-old cysticercoid. **3.** AChE-positive hook muscles and subtegumental muscle fibers at the tip of the prospective cercomer of the 5-day-old larva. **4** and **5**. AChE activity in the 6–7-day-old cysticercoids at a blastula-like stage. **6.** AChE activity in post-oncospheral hook muscles and circular muscle fibers in the cercomer anlage of the 9-day-old larva. A bifurcated fan-shaped population of myoblasts and/or neuroblasts (f) occurs in the scolex anlage. Two AChE-positive cell strands are visible at the midbody of the larva. **Abbreviations to all figures:** c – cercomer, cav – cyst cavity, cc – central body cavity, ccl – cyst cavity lining, cs – cell strands, cw – cyst wall, h – hook, hm – hook muscles, ln – longitudinal nerve cord, n – neck, s – scolex, sm – subtegumental muscles, sr – subscolex ring, suc – suckers

## Results

Acetylcholinesterase detected in the invasive oncospheres and in the developing and fully developed cysticercoids preferred acetylthiocholine over butyrylthiocholine and split *N*-acetyl-β-methylthiocholine, albeit at a relatively low rate. Myristoylcholine was hydrolyzed within the pre-encysted and encysted cysticercoids at a very low rate, since almost an overnight incubation of these larvae in the myristoylcholine-containing medium was necessary to obtain results comparable with those observed for acetylthiocholine hydrolysis. Physostigmine ($10^{-5}$ M) and BW284C51 ($10^{-5}$ M) inhibited




**Figs 7–10.** Cysticercoids at middle stages of their development. **7.** In the 10-day-old larva AChE activity occurs in the scolex anlage, in the circular muscle fibers of the future cercomer, and in the post-oncospheral hook muscles, from which the histochemical reaction runs locally along and across some subtegumental muscle fibers (arrows). **8.** AChE activity associated with the developing suckers in the scolex, with the subscolex ring, with the first pair of the cell strands, hook muscles, and muscle fibers in the cercomer of the 11-day-old cysticercoid. **9.** AChE activity in the subscolex ring, in the two pairs of cell strands, and in neurons of the future longitudinal nerve cords (arrows) in the 12-day-old larva. Insert: Position of AChE-positive cell strands in the transversal section through the cysticercoid body. **10.** Anterior body part of the 12-day-old cysticercoid. AChE activity is associated with the migrating neurons (arrows), with the subscolex ring and with two cell strands, one of which is out of focus

AChE activity in all specimens examined and $10^{-5}$ M *iso*-OMPA had no inhibitory effect. These observations proved that the examined enzyme was "true" AChE.

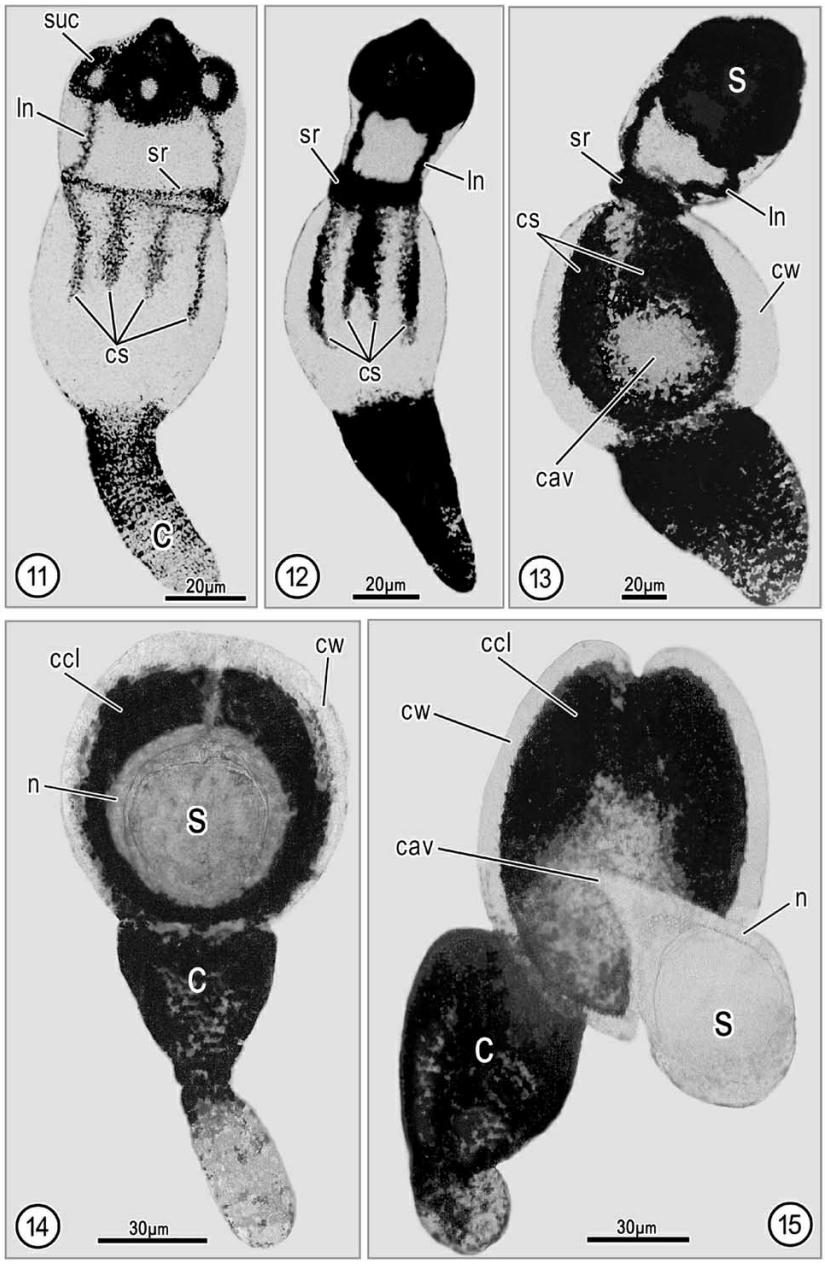
Acetylcholinesterase activity in the oncospheres was very low, particularly with *N*-acetyl-β-methylthiocholine iodide as substrate. Consequently, the larvae had to be incubated in the substrate-containing mixtures for up to 4 h to allow the enzyme to produce an appreciable quantity of the reaction product. The enzyme was localized around the oncospheral hooks, apparently within and around the hook muscles which have lost their anchorages in the depth of the larval bodies due to the deleterious influence of the incubation media, but remained still attached to the hooks with their opposite ends (Fig. 1, insert). Therefore, the distribution of AChE activity in the hook muscles does not reflect the true organization of the hook muscle system of intact or well-fixed oncospheres (cf. Ogren 1972). AChE activity was also detected in the posterior part of the larval body, where two neurons were localized by Hartenstein and Jones (2003). The reaction product that diffused out of the damaged nerve cells screened the binucleated medullar center, i.e., the perikaryon of the oncospheral tegument that was described by Rybicka (1973) in *H. diminuta* oncospheres and by Świderski and Tkach (2002) and Swiderski (1997) in oncospheres of other cestode species.

At the very early morphogenetic stage, i.e., on the 3rd and 4th day of the post-embryonic development, the young cysticercoid was composed of a number of the undifferentiated mesenchymal cells (Fig. 1) surrounding a small blastocoele-like cavity. At this stage AChE activity was limited to the post-oncospheral hook muscles. No cholinergic neurons were detected at this stage of the development, although they are known to occur in the invasive oncosphere.

From the fourth or fifth day of cysticercoid development, AChE activity appeared in some subtegumental circular muscle fibers (Fig. 2). A day later, the AChE-positive circular muscles were also visible at the tip of the prospective cercomer (Fig. 3). Six to seven days after infection the parasite assumed the shape of a late blastula with a broad central body cavity (Fig. 4) and the cercomer anlage was well recognizable (Figs 4 and 5). On the 8–9th day following infection, the hook muscles, which were still AChE-positive, appeared to spread over greater areas of the body when compared to the earlier stages (Fig. 6). At those days AChE activity was also associated with the process of neurogenesis and myogenesis that gradually led to the development of the central nervous system and the suckers. First, two fan-shaped AChE-positive areas appeared in the scolex anlage. They represented a bifurcated population of the mesenchymal cells, differentiating toward muscular and neural tissues. In the 10–11-day-old cysticercoids, the population bifurcated again and hence, two pairs of AChE-positive cell bands, joined together at the rostellar region, were observed (Fig. 7). A day or two days later a further bifurcation led to the formation of four pairs of AChE-positive cell bands, the distal rims of each pair tending to approach each other (Fig. 8). The differentiation of the mesenchymal cells into myocytes of the suckers runs probably in parallel to the development of the innervation of the suckers. The post-oncospheral hook muscles appeared as if they had proliferated and expanded (Figs 7 and 8). However, we did not find any two cysticercoids showing an identical pattern of such a strange reaction originating from the hook muscles. In some larvae such expanded structures were short, whereas in others they were long and branched. Moreover, following incubation in the medium of Karnovsky and Roots (1964), which poorly penetrates multicellular structures and produces a soft, gel-like reaction product, the expanded structures did not appear, although oncospheral hook muscles were highly reactive. They also did not appear when an indigogenic reaction for both cholinesterase and nonspecific esterase activities (Holt and Withers 1952) was applied.

Another AChE-positive structures, which were detected in the 11-day-old cysticercoids, were two short subtegumental strands of cells occupying the area of the prospective cyst (Fig. 8). These strands, which were sporadically observed even in the younger, 8–9-day-old larvae (Fig. 6), indicated the beginning of formation of the cyst cavity lining. AChE activity also appeared in a number of circular muscle fibers arranged in a subscolex ring (Figs 8 and 9). This ring apparently represented the developing sphincter that constricts the edges of the cyst just after the withdrawal of the scolex is completed. In the 12-day-old cysticercoids another two AChE-positive cell strands appeared beneath the ring (Fig. 9). These, and the former two, strands lay in pairs in two perpendicularly-oriented planes (Fig. 9, insert). At the same time, a number of other cells, which also displayed AChE activity, migrated in file from the differentiating central nervous system toward the subscolex muscular ring (Figs 9 and 10). These cells can be considered as neurons, which will soon form the longitudinal nerve cords in the parasite neck.

On the 14th day post infection, four O-shaped outlines of the prospective suckers were already observed in the scolex (Fig. 11). Unfortunately, the reaction product generated by AChE that was associated with the developing innervation of the suckers and, possibly, with their myoblasts as well, screened the central nervous system situated in the interior of the scolex. Further development of the cysticercoids was accompanied by a gradual increase in number of the AChE-positive strand-forming cells (Figs 12 and 13), which spread over the whole inner surface of the prospective cyst (Figs 14 and 15). There was also an increase in number of the muscle fibers constituting the subscolex ring, as judging from the increase in the intensity of the reaction in the ring (Figs 12 and 13). On the 18–19th day post infection, the withdrawal of the scolex into the cyst cavity was observed (Fig. 14). Following encystment, the AChE-positive cells gradually concentrated at the inner surface of the cyst, thus forming a thin AChE-positive lining that prevented unhydrolyzed thiocholine substrates from penetrating the withdrawn body part (Figs 15 and 16). This apparently protective structure exhibited AChE activity over the rest of the post-developmental life of the cysticer-

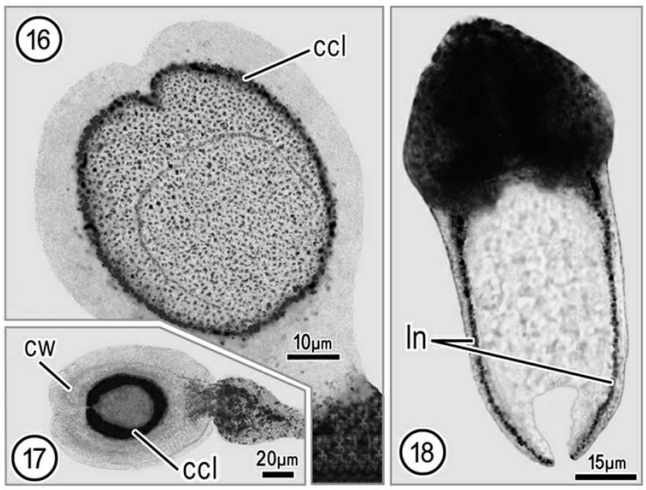



**Figs 11–15.** Cysticercoids at later stages of their development. **11.** AChE activity associated with the suckers, longitudinal nerve cords, subscolex ring and two pairs of cell strands in the 14-day-old larva. **12.** An increase in number of AChE-positive strand-forming cells, which tend to spread around the cavity of the prospective cyst in the 15-day-old cysticercoid. **13.** AChE activity in the 18-day-old cysticercoid just before scolex withdrawal. Almost the whole cavity of the cyst is surrounded by the lining-forming cells (cs). Note the untimely contracted subscolex muscular ring. **14.** AchE-positive cyst cavity lining that surrounds the withdrawn scolex and inverted neck of the 19-day-old larva. **15.** Following a prolonged histochemical reaction, the scolex and the neck were extracted from the cyst cavity to show that AChE associated with the cyst cavity lining prevented acetylthiocholine from penetration into the extracted body parts of the 20-day-old parasite

coids (Fig. 17), i.e., up to the death of the infected hosts. It must be stressed that the black O-shaped line around the encysted body parts (Figs 16 and 17) was due to the activity of AChE associated with the cellular lining of the cyst cavity and not to the activity of the enzyme located in the neurons of the longitudinal nerves of the turned neck. Manipulation with the focus of the microscope clearly showed the spherical shape of the mass of cupric sulfide crystals deposited around the whole body of the future adult cestode.

In scoleces, which were experimentally excysted by a sequential treatment of the 32-day-old cysticercoids with appropriate pepsin and trypsin solutions, AChE activity was asso-

ciated with the central nervous system, with the innervation of the suckers, and with two longitudinal nerve cords in the neck (Fig. 18).




**Figs 16–18.** Premature and mature cysticercoids. **16.** Activity of AChE in the cyst cavity lining of the premature 20-day-old cysticercoid after a shortened duration of the histochemical reaction. The "upper surface" of the lining is in clear focus to show AChE-positive cells. **17.** AChE activity in the cyst cavity lining of the mature 27-day-old cysticercoid (compare the thickness of the cyst wall with that visible on Fig. 15). **18.** AChE activity in the artificially excysted scolex. The central nervous system is unrecognizable (overstained), as the histochemical reaction was prolonged to demonstrate the less reactive longitudinal nerve cords in the parasite neck

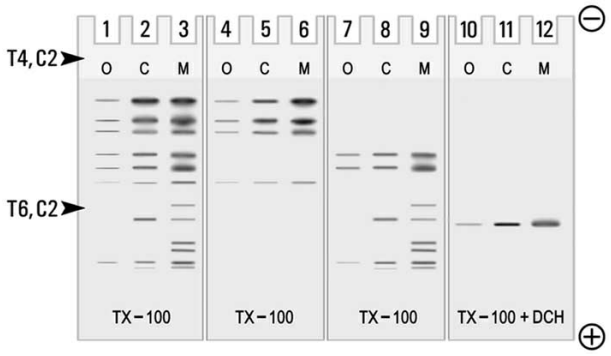



**Fig. 19.** Comparative electrophoresis of esterases extracted from the invasive oncospheres (O), fully developed cysticercoids (C), and the mature cestode *H. diminuta* (M). Samples of the extracts supplemented either with 1% (w/v) Triton X-100 (TX-100) or with a mixture of 0.5% (w/v) Triton X-100 and 0.2% sodium deoxycholate (TX-100 + DCH) were electrophoresed in the presence of these detergents in the gels. **1–3.** Hydrolysis of 1-naphthyl acetate by AChE and nonspecific esterases. **4–6.** Hydrolysis of acetylthiocholine by AChE that was partly deaggregated by Triton X-100. **7–9.** Hydrolysis of 1-naphthyl acetate by nonspecific esterases (AChE was inhibited with $10^{-5}$ M physostigmine). **10–12.** Hydrolysis of acetylthiocholine by AChE that was fully deaggregated by deoxycholate; an identical pattern of the separation was obtained when deoxycholate was occasionally replaced by 0.1% sodium dodecyl sulfate

The number of nonspecific esterases detected in gels after electrophoresis varied depending on the developmental stage of the parasite (Fig. 19, lanes 7–9). None of these enzymes was capable of hydrolyzing acetylthiocholine either during 4 or 24 h of incubation of gels in the acetylthiocholine-containing histochemical medium. AChE from the oncospheres, cysticercoids and mature tapeworms showed a strong tendency to aggregation, which made electrophoresis in the absence of any detergent unsuccessful. When partly deaggregated by Triton X-100, each of the three AChEs generated four bands in the polyacrylamide gel (Fig. 19, lanes 4–6). When fully deaggregated by deoxycholate, each AChE generated a single band in the gel (Fig. 19, lanes 10–12). No differences in electrophoretic mobility between AChE from the three sources were observed.

## Discussion

The results of the histochemical reactions presented here show the occurrence of AChE in neurons as well as in some non-neural cells of the examined larvae, i.e., in the hook muscles of the oncospheres and both in the subtegumental muscle system and the cyst cavity lining of the cysticercoids. The hook muscles of the oncospheres do not atrophy just after their promoting role during the invasion is finished, but they remain viable and exhibit AChE activity for at least 12 days in the course of the development of the cysticercoid. The most reasonable explanation for the appearance of the AChE-positive structures outgrowing the hook muscles (Figs 7 and 8) is that during the incubation in Tsuji's medium an increasing number of needle-shaped crystals of the primary reaction product, deposited in and around hook muscles, produced lesions to the subtegumental muscle fibers, thereby opening the way for the substrate, which, when diffusing along and across some muscle fibers, was locally split by their AChE.

The occurrence of AChE in myoblasts of the developing suckers is uncertain, since it was impossible to recognize whether the enzyme was located within the myoblasts or was associated with the developing neuroblasts (Figs 6 and 8). It is also not clear why the subtegumental muscle system was AChE-positive only at the posterior end of the parasite body. One might suppose this could result from better permeability of this body area to thiocholine esters. However, in the tripartite cysticercoid bodies the activity of AChE was easily detectable in the cell strands situated beneath the subtegumental muscle system of the prospective cyst (Figs 11–13), thus indicating that the permeability of muscle plasma membranes, rather than that of the tegument, was a problem.

The formation of the cyst cavity was found to be accompanied by the differentiation of some AChE-negative mesenchymal cells into AChE-positive cells, which adhered to the internal wall of the cyst. The morphology of these "fiber-like" or "spindle-shaped" cells at the light microscope level was first described by Voge and Heyneman (1957) and by Voge (1960). Allison *et al*. (1972) described the ultrastructure of

these "bipolar squamous epithelial cells", which at the end of cysticercoid morphogenesis became arranged in a multilayer cyst cavity lining composed of forty to fifty layers of thin cytoplasmic strands.

AChE hydrolyzes the neurotransmitter acetylcholine at cholinergic synapses in the central and peripheral nervous systems. About fifty years ago, the term acetylcholinesterase made biologists think of the nervous system only. However, in an early embryogenesis of vertebrates and invertebrates the activity of non-neural AChEs appears in various differentiating and actively migrating cells (Karczmar 1963, Koelle 1963, Silver 1974). In adult animals non-neural AChEs are also associated with glandular cells and with many membranes across which transport of water and ions is taking place. These enzymes operate in such animal non-neural structures as amniotic and allantoic membranes of birds and mammals (Cuthbert 1963, Burt *et al*. 1970), mammalian nephrons (Fourman 1967), salt-gland of the duck (Fourman 1969), epithelial cells of frog skin (Koblick *et al*. 1962), mammalian liver (Berninsone *et al*. 1989), corneal epithelium (Williams and Cooper 1965), keratinocytes (Grando *et al*. 1993), various types of migratory cells, placenta (in primates only), suprarenals, thyroid and parathyroid glands, sweat, mammary and salivary glands, blood cells, spermatocytes, and many others. A number of parasitic nematodes and their larval stages secrete AChE that may facilitate their survival within host organisms (Lee 1996). According to Nizami *et al*. (1977) some mature trematodes also secrete AChE to their environment. Non-neural AChEs operate in the tegument, testes, and ovary of *Fasciola hepatica* (Probert and Durrani 1977), in the tegument of schistosomes (Levi-Schaffer *et al*. 1984, Espinoza *et al*. 1991, Jones *et al*. 2002), and in the tegument of the mature tapeworms *Echinococcus granulosus*, *Hydatigera taeniaformis* (Shield 1969) and *Raillietina johri* (Roy 1980). However, the true role played by AChEs in these structures is generally unknown. Therefore, it is hard to speculate about a physiological role of AChE associated with the cyst cavity lining in the cysticercoids of *Hymenolepis diminuta*. Nevertheless, as the parenchymal cells of the turned neck of the cysticercoid adhere closely to the cyst cavity lining, it seems obvious that the lining regulates both the transport of nutrients and minerals into the scolex and waste substances in the opposite direction. Judging from the relatively high activity of two enzymes of oligosaccharide metabolism, i.e., β-D-glucosidase and α-D-galactosidase (Moczoń 1981), the lining is physiologically highly active, at least in terms of carbohydrate metabolism. It is likely that, beside the activity of AChE, the cyst cavity lining bears a number of other membrane-bound, but still unknown enzymes, which regulate the passage of metabolites between the larval and the post-larval body of the cysticercoid.

AChEs from the oncospheres, cysticercoids and mature tapeworms were indistinguishable by electrophoresis in terms of electrophoretic mobility and number of bands generated in the gel. This evidences the molecular identity of these enzymes and the existence of a single AChE gene in the genome of the examined parasite. More information on AChE from *H. diminuta* can be found in our previous publication (Moczoń and Świetlikowska 2005).

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