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Histochemical demonstration of esterase in certain freshwater larval trematodes with a note on neuroanatomy

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Abstract. Histochemical method for non-specific esterases has been applied to cercariae belonging to 4 different trematode groups viz Monostome, Amphistome, Xiphidio and Echinostome. Various structures containing esterases revealed positive reactions to the standard method and are visualized in simple whole mount preparation. Different degrees of non-specific esterase activities were observed in the nervous and the excretory systems, suckers and sub-tegumentary cells. In general, nervous systems of cercariae are composed of a pair of cerebral ganglia connected to each other by a transverse commissure to form a cerebral complex. These give off 3 pairs of anterior longitudinal and 3 pairs of posterior longitudinal nerve cords. Simultaneously nervous systems of redia of monostome cercaria have also been focused in the present study.

Keywords. Esterase; trematode; cercariae; redia; nervous system.

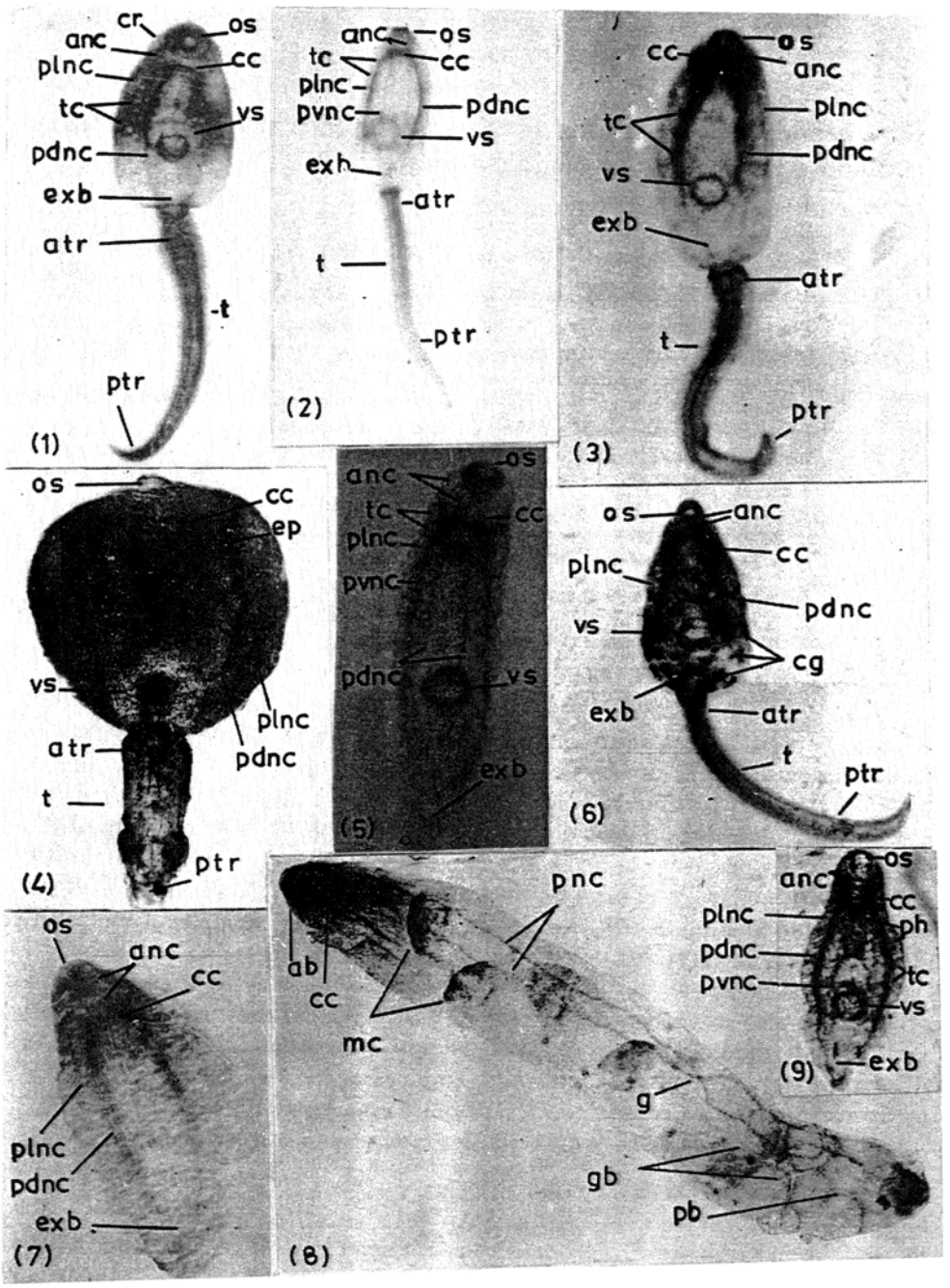
1. Introduction

Several workers have studied the nervous system by localization of either non-specific esterase (NSE) or acetylcholinesterase in different adult (Simha and Rao 1977; Rao *et al* 1981; Choubisa *et al* 1982; Mishra and Tandon 1984) as well as in certain larval digeneans (Dixon and Mercer 1965; Fripp 1967; Jennings and Leflore 1972; Bruckner and Voge 1974; Diconza and Basch 1975; Krishna and Simha 1979; Leflore 1979; Leflore *et al* 1980; Grabda and Moczon 1981; Venkatanarsaiah 1981; Choubisa and Sharma 1983b). None of these workers have attempted a comparative study on the nervous system of different larval trematode groups.

In the present study an attempt has been made not only to trace the nervous systems but also to observe the activity of NSE in different structures of cercariae and compare the time periods required for optimum visualization of esterases in 8 different cercarial species. The study also emphasises the nervous system of redia of monostome cercaria, *Cercaria buckleyi* (Pandey and Agrawal 1977).

2. Materials and methods

During the survey of larval digeneans and their snail hosts from freshwater habitats of Udaipur region (Rajasthan), different kinds of cercariae such as Monostome, Amphistome, Echinostome and Xiphidio were collected (Choubisa and Sharma 1983a; Choubisa 1985). The rearing of snails and method for collection of their trematode larvae have already been described (Choubisa and Sharma 1983a, 1985). For the present study 8 cercarial species viz *Cercaria komiyai* (figure 1), *Cercaria* sp. VII Kerala (figure 2), *C. granulosa* (figure 3), *C. pigmentata* (figure 4), *C. talensis* (figure 5), *Cercaria* sp. VIII Kerala (figure 6), *C. buckleyi* (figure 7) and *C. indicae*



Figures 1–9. 1. *C. komiyai.* 2. *Cercaria* sp. VII Kerala. 3. *C. granulosa.* 4. *C. pigmentata.* 5. *C. talensis.* 6. *Cercaria* sp. VIII Kerala. 7. *C. buckleyi.* 8. Redia of *C. buckleyi.* 9. *C. indicae* XVII (× 100).

(ab, Anterior body; anc, Anterior nerve cord; atr, Anterior tail region; cc, Cerebral complex; cg, Cystogenous glands; cr, Collar region; exb, Excretory bladder; g, Ganglion; gb, Germ balls; mc, Mature cercaria; os, Oral sucker; pdnc, Posterior dorsal nerve cord; ph, Pharynx; plnc, Posterior lateral nerve cord; pnc, Posterior nerve cord; ptr, Posterior tail region; pvnc, Posterior ventral nerve cord; t, Tail; tc, Transverse connections; vs, Ventral sucker).

XVII (figure 9) and redia (figure 8) of monostome cercaria, *C. buckleyi* have taken. These larvae belonging to different groups of trematode have been listed in table 1. The standard techniques were used for the localization of NSE in cercariae and rediae as described by Holt and Withers (1952), and Jennings and Leflore (1972). Control whole mounts were also made out according to Choubisa and Sharma (1983b). Simultaneously, time periods were also noted for optimum visualization of NSE for each cercarial species.

3. Observations

NSE activity was seen in the form of deep blue microcrystals in nervous and excretory systems, sub-tegumentary cells, suckers, pharynx and in tail region of all 8 cercarial species. Not all but few cercariae, *C. indicae* XVII (figure 9), *C. komiyai* (figure 1) and *Cercaria* sp. VIII Kerala (figure 6) revealed the mild activity of NSE in their intestinal caeca. Cystogenous gland cells also showed strong activity of esterase in the *Cercaria* sp. VIII Kerala only. In general, the maximum activity of NSE was found on oral and ventral suckers and their adjoining areas, nervous system and in tail region, whereas excretory and digestive systems and genital rudiments showed moderate to mild activity of NSE. The various degree of enzyme activity in different regions of cercariae have been shown in table 1.

In the nervous system of all 8 cercarial species, the reaction of NSE was strong particularly on cerebral ganglia and pair of anterior and posterior dorsal longitudinal nerve cords. Lateral and ventral nerve cords revealed comparatively low activity. However, oral and ventral suckers also showed strong activity of esterase. It was clear from the variety of structures visualized by this reaction that a number of types of esterase were involved. Further, it was confirmed that use of inhibitor (10^{-4}M eserine sulphate) in the incubation medium, most of the cholinesterase activity in the nervous system was eliminated in the control cercariae and only cerebral ganglia were found slightly positive. No effect of the inhibition could be seen

Table 1. Activities of non-specific esterases in various structures of certain trematode larvae.

Types of larval trematode/ Larval species	OS	VS	Ph	Oes	C	CGC	GR	EB	NS	T	Incubation time (min)
Monostome											
Cercaria buckleyi	++	0	+	−	−	−	−	±	+++	+	5
Amphistome											
C. pigmentata	+	+	+	+	±	++	−	+	+++	+	12
Echinostome											
C. komiyai	++	++	+	++	±	++	−	+	+++	++	8
C. granulosa	+++	++	±	−	−	+	−	−	+++	+++	8
Cercaria sp. VII Kerala	++	++	+	−	−	±	−	+	+++	++	10
Cercaria sp. VIII Kerala	+++	++	+	+	+	+++	+	++	+++	++	7
Xiphidio											
C. talensis	++	++	++	−	−	+	−	±	+++	+	5
C. indicae XVII	+++	+++	++	+	+	±	+	±	+++	+	6

+++, Very intense; ++, moderate; +, mild; ±, faint; −, no activity; O, organ absent; C, caeca; CGC, cystogenous gland cells; EB, excretory bladder; GR, genital rudiments; NS, nervous system; OS, oral sucker; Ph, pharynx; T, tail; VS, ventral sucker.

in the other structures of cercariae such as alimentary canal, subtegumentary cells which appear to be scattered throughout the mesenchyme, posterior part of the excretory bladder, cystogenous cells, those cells in the oral and ventral suckers and central core of the tail. It has proved that number of esterase involved in these structures. Cercariae incubated in substrate free medium did not reveal the esterase activity in any tissues.

The nervous systems of present cercariae are found to be almost similar in pattern and composed of two cerebral ganglia connected with a thick band like transverse commissure to form a cerebral complex and lies immediately posterior to oral sucker, 3 pairs of anterior longitudinal and 3 pairs of posterior longitudinal nerve cords that arise from the lateral sides of each cerebral ganglia (figures 1–7 and 9). Three pairs of nerve cords could be differentiated according to their position, lateral, ventral and dorsal in the anterior and posterior regions, respectively. The anterior dorsal, ventral and lateral nerves and their numerous fine nerve branches encircle and innervate the oral sucker, pharynx and prepharynx regions. The posterior dorsal longitudinal nerve cords are the largest. They are stout, prominent and deeply stained compared with the posterior, ventral and lateral nerve cords. These, on their way towards the ventral sucker innervate the gut, genital rudiments, excretory bladder and also gives fine branches to the tegument. Each posterior lateral nerve cord units with its fellow, dorsal and ventral nerves by transverse connectives to form 6–12 transverse commissures in ring form immediately after commencement of the posterior region of cercarial body. Each commissure give off several fine branches of nerves distributed all over the body in the form of a net work.

In the tail region, all cercaria revealed strong activity of NSE particularly in the central core of the tail. Except *C. granulosa* (figure 3) none of the other cercarial species showed a pair of nerves, dorsal and ventral originating from the ganglion situated at near the base of tail and running posteriorly. 12–15 transverse connections in ring forms were also found in tail of this echinostome cercaria.

The nervous system of redia (figure 8) was found simple and composed of cerebral complex which lies immediately behind the pharynx. Pair of nerves originates from cerebral complex, proceeds posteriorly and unite to form a ganglion near three-fourth of the hind region of the body (figure 8). From this ganglion, again a pair of nerves runs to the posterior end of the redia. Cerebral complex also gives rise to 2–3 nerve branches which innervate the pharynx. No transverse connections were observed in these redia as found in its cercaria, *C. buckleyi.*

Different time periods were observed for optimum visualization of NSE activity in trematode larvae (table 1), 12 min for amphistome (*C. pigmentata*), 7–10 min for echinostome cercariae (*C. komiyai, C. granulosa* and *Cercaria* sp. VII and VIII Kerala), 5–6 min for xiphidio cercaria (*C. talensis* and *C. indicae* XVII), 5 min for monostome cercaria (*C. buckleyi*) and 15 min for redia of *C. buckleyi.*

4. Discussion

The localization of NSE in the larval trematodes is very helpful in tracing of many structures besides the nervous system. Jennings and Leflore (1972) have shown the

various organ systems of cercaria of *Himasthala quissetensis* by localizing NSE in whole mount preparation. Similarly, Choubisa and Sharma (1983b) and Choubisa (1985) have also localized NSE in *Tetracotyle metacercaria*, *T. lymnaei* and in different cercarial groups viz *C. chauhani* (Monostome), *C. itoi* (Echinostome), *C. johrii* (Gymnocephalous), *C. thapari* (Xiphidio), and *C. udaipuriensis* and *C. gurayai* (Furcocercous) and discussed the various morphological features and their nervous systems. Simultaneously Choubisa (1985) has also recorded the various time periods for optimum visualization of NSE. The maximum incubation time, (14 min) was recorded for gymnocephalous cercaria, *C. johrii* and minimum (5 min) for *C. chauhani* (Monostome) and interpreted that former species showed a number of incipient adult features, such as the presence of immature gonads, and thus the occurrence of a more impermeable tegument. Similarly, in the present study amphistome cercaria, *C. pigmentata* revealed the maximum incubation time (12 min) and it can corroborate the findings of Choubisa (1985).

Earlier workers reported the presence of a nervous system in cercarial body but they could not define the arrangement of nerves in the tail region. However, Choubisa (1985) traced out the fine arrangement of nerves in the tail region of furcocercous cercaria, *C. udaipuriensis* and *C. gurayai*: two ganglia one situated at the anterior and other at posterior region where the tail is bifurcated. Two nerves, ventral and dorsal, originated from the anterior ganglion proceeded posteriorly and finally connected to the posterior ganglion. Further, both main nerves connected by 8–10 fine transverse connections of nerves in ring forms. Similarly, experimental cercaria, *C. granulosa* (figure 3) also revealed the nerve arrangement in its tail stem but other cercarial species did not show this clearly. However, the nervous system of these cercariae is basically similar but their other fine branches of nerves are differently distributed and it is related to activity of particular part of the body.

The nervous system of the redia in these studies (figure 8) is simple and composed of two cerebral ganglia united with each other by a transverse commissure to form a mass of cerebral complex situated behind the pharynx. One posterior nerve from each cerebral ganglion proceeds to three-fourth of redial body where both nerves are united to form a small ganglion and again a pair of nerves originate from it to run to the posterior end of the body. However, the findings of Krishna (1981) reveal that the nervous system of the redia *Echnistoma revolutum*, is composed of only a cerebral mass located behind the pharynx but no other nerve arrangement in redia could be observed. Other workers, Bruckner and Voge (1974) have also demonstrated the nervous system of miracidia of *Schistosoma mansoni* by means of ASChI and composed of a thick neural mass in the anterior region and 3 pairs of longitudinal nerve trunks, connected to the transverse commissure and it was almost similar to its cercaria. But nervous system of present redia is different from its monostome cercaria, *C. buckleyi* (figure 7).

In the present study, all different species of cercariae revealed the nervous system basically similar but arrangement and maximum of nerves in particular region of larvae were found to be different and it is correlated with the activity of particular regions such as oral and ventral suckers and also tails of certain cercariae, echinostome species (figures 1, 2, 3 and 6). Difference in the incubation time periods for optimum visualization of esterase activity in various cercariae depend on the permeability of tegument and stage of their development.

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