Esterases in trematodes: 1. Nerve arrangement and distribution of esterases in *Singhiatrema longifurca* and *Paradistomoides orientalis*

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MS received 26 March 1977; revised 5 September 1977

Abstract. Location of esterases and nerve arrangement is studied in *Singhiatrema longifurca* and *Paradistomoides orientalis*. Both the trematodes possess a pair of cerebral ganglia connected by cerebral commissure. From here three pairs of nerves proceed to anterior and posterior sides. Anterior pairs, interconnected by transverse commissures and connectives innervate the oral sucker and the anterior tip. Of the posterior pairs, ventrolaterals are well developed in both. In *S. longifurca* nervous organization is highly complicated with many branches. *P. orientalis* also sends a number of branches to various organs. Both parasites possess many presumptive neuro-secretory and sensory cells. Various degrees of esterase activity in both worms is located in excretory vesicle and ducts, suckers, genital pore, gonads, oviducts and eggs.

Keywords. Esterases; presumptive neurosecretory cells; receptors; *Singhiatrema longifurca*; *Paradistomoides orientalis*.

1. Introduction

A number of references are available on esterases in vertebrates and invertebrates including their role in the synaptic or neuromuscular transmission of nerve impulse (Giese 1973). But only a few publications are available in helminths as to their nature, distribution and functions. Notable among them are those of Beuding (1952), Chance and Mansour (1953), Lee et al (1963), Lee and Tatchel (1964), Schordein and Waitz (1965), Fraday and Knapp (1967), Hart (1967) and Bruckner and Voge (1974). Halton and Jennings (1964) have demonstrated acetyl cholinesterase activity by using o-acetyl-5-bromoindoxyl. This has also helped in tracing the nervous system of the species. In *Hymenolepis*, acetyl cholinesterase activity was studied biochemically by Graff and Read (1967). Nervous system in some common helminths was also demonstrated but not in detail by Ramanaiah and Agarwal (1977).

The authors of the present study have taken up biochemical and histochemical studies of esterases in digenetic trematodes. To start with, o-acetyl-5-bromoindoxyl has been employed in an attempt to locate the esterases in the trematodes *Singhiatrema longifurca* Simha (1958) and *Paradistomoides orientalis* Narain and Das (1929) after modifying the method of Holt and Withers (1952) to make it convenient for trematodes. In the present study the authors not only demonstrate the distribution of esterases but also successfully studied the fine nerve arrangement, presumptive neurosecretory cells and sensory receptors. The results of these studies are reported in the present communication.
2. Materials and methods

Trematodes of the present study, freshly collected from the host, were immediately flattened and fixed in 10% neutral formalin, for 4-6 hr. They were washed in several changes of distilled water at 10°C and incubated at 30°C for 12-24 hr in the standard bromoindoxyl acetate incubation medium of the following composition: 1.2% Alcoholic 5-bromoindoxyl acetate (0.1 ml); 0.1 M tris buffer of pH 6-8 (2.0 ml); 0.5 M potassium ferro and ferricyanide (1.0 ml each); 0.1 M calcium chloride (1.0 ml) and made to 14 ml. Specimens were washed in distilled water, dehydrated in ascending grades of alcohols, cleared in Beachwood creosote and mounted in DPX mountant. The sites containing esterases are stained in deep indigo blue.

3. Results

Of the two species studied, the nervous system is well developed in *S. longiforma* (figure 1) and is of less complexity in *P. orientalis* (figures 7 and 8). As there are various grades of nervous organization, the disposition is described separately.

3.1. Nervous disposition

3.1.1. *S. longiforma* (figure 1): For convenient study of the distribution of nerves, the body of the worm is divided into the following regions:

**Oral sucker region**: Area anterior to the pharynx, mainly occupied by the oral sucker.

**Preacetabular region**: Area from the anterior margin of the pharynx to the anterior margin of the acetabulum.

**Acetabular region**: Area mainly covered by the acetabulum.

**Postacetabular region**: Area between the posterior margin of the acetabulum and the hind end of the body.

3.1.2. **Oral sucker region**: A pair of cerebral ganglia (figure 2) occur one on each side of the anterior margin of the pharynx and both of them are connected by the cerebral commissure lying between the oral sucker and the pharynx. From each cerebral ganglion three nerves viz. antero-dorsal, antero-inner ventral and antero-outer ventral (named according to their position) proceed to the anterior side (figure 1).

Antero-inner ventral which is thick and ganglionated, arises from the place where the commissure and the ganglion join and travels at the inner margin of the lateral rim of oral sucker. As these nerves proceed to the anterior tip, they become thinner and finally meet with one another at the rim of the anterior margin of oral sucker. Each nerve of this pair consists of three ganglia of which the posterior one is well developed, anterior least and the middle of intermediate development. The corresponding ganglia of antero-inner ventrals are connected by three separate commissures; which in turn are connected with one another by a pair of longitudinal connections extending from the first to the third.

Each nerve of the antero-outer ventral pair that is ganglionated, arises from the outer margin of the cerebral ganglion and proceeds anteriorly outer to the oral
sucker. There are two ganglia on each nerve. At the posterior ganglion this nerve is connected with the postero-ventral nerve by a short connective. At the anterior ganglion it is connected with the antero-dorsal nerve by a connective. From here this nerve proceeds anteriorly and innervates the antero-lateral margin of the oral sucker.

Each nerve of the antero-dorsal pair arises from the cerebral ganglion in between the other two and proceeds on the dorsal side of the lateral rim of the oral sucker. This is least developed of the three and possesses a single ganglion. At this ganglion this nerve is connected with the anteriormost ganglion of antero-inner ventral by a connective. Two similar connectives, join the antero-dorsal with antero-inner ventral at the latter’s middle and posterior ganglia.

3.1.3. Preacetabular region (figure 1): This region mainly consists of three pairs of longitudinal nerves viz. ventral pair, dorsal pair and ventrolateral pair. Of these the ventrolateral pair is well developed, dorsal pair moderately and the ventral pair least developed.

The ventrolateral pair consists of two thick nerves originating from the posterior
side of each cerebral ganglion. The nerves of the ventral pair arise from the outer side of each ganglion and travel to the posterior side on the outer side of the ventrolateral pair to terminate in the middle of the acetabular region. The nerves of the dorsal pair arise from the inner lateral side of each ganglion and proceed to terminate into the anterior rim of the ventral sucker. All these nerves are connected by four transverse ring connectives.

In addition, the ventral and dorsal nerves of each side excluding the ventrolateral, are connected to each other, antero lateral to acetabulum. There is a pair of short nerves connecting the second and fourth ring connectives. These run parallel on the inner side of each ventrolateral nerve. At each margin of the oesophagus occurs a nerve, 'oesophageal nerve' that arises from the cerebral ganglion and proceed to the end of this region. These possess few sensory cells. The dorsal, ventral and ventrolaterals also supply connectives to the sensory cells.

In addition to these major nerves 6-10 thin and delicate nerves arise, directly from the cerebral ganglion and traverse parallel to and in the vicinity of the ventrolateral nerves. All these extend into the postacetabular region.

3.1.4. Acetabular region: All the three pairs of nerves extend into this region and the ventrals terminate in the middle of this area. Ventral and ventrolaterals are connected by short connectives.

To the inner side of each ventrolateral 6-10 nerve cells are connected. A number of nerves arise from the inner side of the ventrolaterals and also from the nerve cells. These diverge to the centre and innervate the acetabulum on its ventral and lateral surfaces (acetabular nerves). Few of these connections numbering 4-6 traverse along the posterior border of the acetabulum and join the ventrolaterals to form postacetabular nerve band (figure 3). At these joinings and at the region of acetal- bular nerves the ventrolaterals are ganglionated.

3.1.5. Postacetabular region: Each of the ventrolateral nerves soon after entering this region gives rise to two branches, one lying externally and the other internal to it (figures 1 and 2). The internal one, originating a little anteriorly at a ganglion extends to the posterior side and terminates at the end of the third quarter of this area. The outer one originating a little posterior, travels to the posterior side immediately below the body wall and meets its fellow on the posterior side (figure 2). This outer nerve consists of a number of well developed sensory cells (figures 1 and 4), which may be of tactile nature. The outer, the inner and the ventrolateral nerves are connected by 8 transverse ring connectives. The ventrolaterals proceed to the posterior side and they meet with one another (figure 2).

In addition to these, this region consists of other two sets of nerves.

One set of these consists of thin nerves, starting directly from the cerebral ganglion (figure 1). These nerves enter this region and immediately diverge to proceed to the opposite side obliquely. While doing so, they cross their fellows from the opposite side, thereby forming a network like structure (figure 5).

Other set of nerves that are restricted only to this region arise from the postacetabular nerve band (figure 1) lying immediately behind the acetabulum. They proceed parallel to the ventrolateral nerves (figure 5) to the posterior side and few of them unite with the synapse of the ventrolateral nerves. These are more in number at the lateral regions and they decrease in the middle of the body. They are thick at the
Figures 2-6. Microphotographs of *S. longifurca*. 
2. Nervous disposition. 3. Postacetabular nerve band from ventrolateral nerves on the posterior side of the acetabulum. 4. Sensory cells at the integument from the outer nervous branch in the postacetabular region. 5. Postacetabular region. Two types of nerve bundles—one forming network-like structure and other in margins proceeding to the posterior side can be seen clearly. 6. Sensory receptor.
origin and gradually become thinner as they travel to the posterior. These nerves also possess number of sensory cells (figure 6).

3.1.6. *Paradistomoides orientalis*: The nervous system consists of a pair of cerebral ganglia (figures 7 and 8), each one located at the posterior corners of pharynx. These two ganglia are connected by a commissure, the cerebral commissure lying posterior to pharynx (figure 8).

From each cerebral ganglion three nerves proceed to the anterior side of which the middle proceeds from the dorsal side and the other two from the ventral side. Of the ventrals, one pair is median in position and the nerves of the other lie outer to the dorsal pair. Hence, they are named as antero-inner ventral and antero-outer ventral (figure 7).

The antero-inners proceed to the middle of the oral sucker, where both of them are connected by the sixth transverse connective (there are seven transverse connectives in the oral sucker region). From here they diverge to the periphery where they join with the antero-outer ventral, at a ganglion.
The antero-dorsal nerves joined by means of seventh transverse connective proceed to the middle of the oral sucker where they are ganglionated. From this ganglion arise two connectives of which one joins the antero-inner ventral and the other joins the seventh transverse connective. The second connective of each side is also connected by the fifth transverse connective.

The antero-dorsal from its ganglion extends inwardly bears a single ganglion and gets united with the antero-outer ventral. Between its two ganglia, the antero-dorsals are united by third and fourth transverse connectives. The second transverse connective occurs uniting both the antero-dorsals at their anterior ganglion. In addition to this there are diagonal nerve connections also that connect the anterior ganglion in one antero-dorsal with the posterior ganglion on the other.

The antero-outer ventral nerve of each side after its origin proceeds a little outwards and travels forwards. It possesses a ganglion where it is joined by antero-inner ventral and a little distance further is joined by antero-dorsal. Then it curves to the median side and at the anterior rim of the oral sucker joins with its fellow. This connection could be considered as the first transverse connective. The ganglion on it is connected by the anterior ganglion of the antero-dorsal and from the first transverse connective a number of minor diagonal connections arise uniting with the second transverse connective and the anterior ganglion of antero-dorsal.

To the posterior side from the cerebral ganglion arise three pairs of nerves viz. the dorsal, the ventral and the ventrolateral. Of all these, the ventrolateral pair is well developed, dorsal pair moderately and the ventral is least developed of all (figure 7).

In addition, from the outer side of each cerebral ganglion arises one fine nerve that proceeds to the surface of the body in the anterior one fourth and establishes connection with a receptor (figure 7).

Each nerve of the dorsal pair arises from the dorsal side of each cerebral ganglion and proceeds on either side of the acetabulum to terminate in the middle of the latter half of the body. At the acetabular region, each dorsal nerve is united with the ventral nerve by a short nerve.

Each nerve of the ventral pair arises from the outer margin of the cerebral ganglion and proceed outer to the ventrolaterals to terminate in the vitelline region.

Each nerve of the ventrolateral pair arises from the posterior side of each cerebral ganglion and they extend into the posterior 1/8th of the body where they unite with one another (figure 7). On their way, they supply a number of nerves to the various parts of the body. At the region of the acetabulum arise three nerves to the inner side and two to the outer side. Of the inner one, the first gives a number of branches; the anterior of which from both sides are united with one another. The two branches from its posterior side are connected with the second main branch. All the three branches innervate the acetabulum (figure 7). A little posterior to the sucker, two more branches from each ventrolateral innervate the testis of their respective side. At the region of the ovary also there is a branch from it innervating the ovary and the oviduct. In the posterior half of the body there is a nerve from ventrolateral to the vitellaria and two nerves to the excretory vesicle. On the posterior side where both are united, arise and proceed to the posterior margin a pair of nerves that join the presumptive receptors (figure 7).

A number of similar receptors have been noticed by the authors, occurring
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<th>Pharynx</th>
<th>Ventral sucker</th>
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<th>Ovary</th>
<th>Ootype</th>
<th>Excretory chamber</th>
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VL—Very less;  L—Less;  M—Moderate  MA—More activity
surrounding the genital pore (figure 9) attached to main nerves and nerve branches and also in the margins of the body.

3.2. Localization of esterases in the body (figures 2 and 8)

When the worms of the present study were incubated for esterases with indoxyl acetate various densities of colour development were observed in various parts of the body. Density of the colour development is taken as the degree of enzyme activity. The distribution and the degree of activity is shown in a tabular form in table 1.

4. Discussion

The location of acetyl cholinesterase in trematodes is of much help in tracing the nervous system. This is because the indoxyl substrate can easily penetrate the nervous sheath and the ester indoxyl is hydrolysed by esterase to liberate the free indoxyl. The indoxyl is oxidized to bright blue indigo III. The hydrolysis of the ester of indoxyl can take place by non-specific esterases, specific and non-specific cholinesterases and lipases. Hence, unless the inhibitors are used it is not possible to identify the esterase that is responsible for hydrolysing the indoxyl ester in the present worms.

However, the application of indoxyl esters selectively brings out the nervous system from other systems. To some extent the excretory ducts also become evident, but there are species variations as the excretory ducts in Paradistomoides are quite evident and are not so in S. longifurca.

An interesting aspect observed in the present study is the innervation of various organs. In S. longifurca, the integument is heavily innervated, especially so in the posterior region and in P. orientalis there are separate nerves to the excretory vesicle and ducts. This innervation suggests the nervous control on osmoregulation. Moreover, there is evidence of the involvement of esterases with permeability (Schwabe 1959 and 1961). Further, the innervation of pharynx, oesophagus, ventral sucker and the genital pore indicate the nervous control over these organs in S. longifurca. P. orientalis is also no exemption to this as various organs like pharynx, oesophagus, suckers and gonads are richly innervated indicating the control of the nervous system which has not been thoroughly investigated by Ramanaiah and Agarwal (1977).

With regard to various degrees of esterase activity in different organs of these trematodes, it is difficult to interpret the results unless the specific esterases are identified.

Acknowledgements

The authors gratefully acknowledge the help rendered by P Venkat Reddy, M Kameswari and G Raghu Ramulu, at various stages of this work.
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References

Beuding E 1952 Acetyl cholinesterase activity of Schistosoma mansoni; Brit. J. Pharmacol. 7 563-566
Bruckner A David and Voge Marietta 1974 The Nervous system of larval Schistosoma mansoni as revealed by acetyl cholinesterase staining; J. Parasitol. 60 437-446
Chance M R A and Mansour T E 1953 A contribution to the pharmacology of movement in the liver fluke; Brit. J. Pharmacol. 8 134-138
Fraday C H and Knapp S E 1967 A radiosotopic assay of acetyl cholinesterase in Fasciola hepatica; J. Parasitol. 53 298-302
Graff D J and Read C P 1967 Specific acetyl cholinesterase in Hymenolepis diminuta; J. Parasitol. 53 1030-1031
Halton D W and Jennings J B 1964 Demonstration of the nervous system in the monogenetic trematode Diplodoon paradoxum Nordmann by the indoxyl acetate method for esterases; Nature 202 510-511
Hart J L 1967 Studies on the nervous system of Tetrathyridia (Cestoda; Mesocestoides), J. Parasitol. 53 1032-1039
Narain D and Das R S 1929 On the anatomy of the new trematodes of the genus Dicrocoelium with a key to the species of the genus; J. Bombay Nat. Hist. 33 250-261
Ramanaiah B V and Agarwal S M 1977 Studies on the nervous system of certain adult and larval digeneans; First National Convention of Indian Helminthologists p. 48
Schardein J L and Waitz J A 1965 Histochemical studies of esterases in the cuticle and nerve cords of four cyclophyllidean cestodes, J. Parasitol. 51 356-363
Schwabe C W, Kousa M and Acra A N 1961 Host parasite relationships in Echinococcus. IV. Acetyl cholinesterase and permeability regulation in the hydatid cyst wall; Comp. Biochem. Physiol. 2 161-172
Simha S S 1958 Studies on the trematode parasites of reptiles found in Hyderabad state; Z. Parasitenkunde 18 161-218

Key to numbers (figures 1 and 7)