NERVOUS SYSTEM OF *CLONORCHIS SINENSIS* AS REVEALED BY ACETYLCHOLINESTERASE ACTIVITY

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Abstract. The gross neuroanatomy of *Clonorchis sinensis* has been revealed by the localization of acetylcholinesterase, well known to be associated with the nervous system. The central nervous system is composed of two cerebral ganglia situated postero-dorsally to the pharynx and connected by a transverse commissure. These ganglia give off four pairs of nerves anteriorly and three pairs posteriorly. The anterior nerves contribute to the pharynx and to the formation of the circum-oral ring located in the oral sucker. The posterior nerves, of which the postero-ventral nerve cords are the most prominent, contribute to the innervation of the acetabulum, the gut, the reproductive organs and the excretory bladder. All the posterior nerve cords are connected by a number of transverse connections throughout their course forming a complicated nerve net. At least two types of nerve cells, bipolar and multipolar ones, were observed.

INTRODUCTION

It is well known that in digenetic trematodes there is an active nervous system which has a controlling influence on their motility. The occurrence of active acetylcholinesterase and a system catalyzing synthesis of acetylcholine from choline and acetic acid have been demonstrated in the nervous system of flatworms. However, information concerning the nervous system in digenetic trematodes is still inadequate. The basis of the present study was that *Clonorchis sinensis* not only is an important bile duct fluke of humans in China and East Asia, but also is a typical flattened, lanceolate distome, facilitating the localization of acetylcholinesterase as a means of demonstrating the nervous system *in toto*.

MATERIALS AND METHODS

Adult living specimens of *C. sinensis* were recovered from the bile ducts of cats, previously infected with *C. sinensis* cysts. Parasites were washed in 0.85% saline and immediately fixed in cold 10% neutral formalin for 20 minutes and repeatedly washed with distilled water. Then the specimens were treated with a solution containing 8 mg/ml acetylthiocholine iodide (AChI), the substrate of acetylcholinesterase (AChE), according to Koelle-Gomori as modified by Douglas (1966). After 3-4 hours of AChI treatment the specimens were washed in distilled water, mounted in polyvinylpyrolidone (PVP), then examined and photographed. Controls were established by either omitting the substrate from the working solution or adding eserine to a concentration of 1×10^{-4}M. AChE activity was observed in the ganglia, nerve cords, commissures and fibres innervating the trematode's whole body. Positive reaction to AChE was not observed in any of the controls. A total of 93 parasites were proceeded and observed.

RESULTS

The components of the nervous system of *C. sinensis* were clearly visualized using the method of AChI modified by Douglas (1966). The nervous system of *C. sinensis* is bilaterally symmetrical. The central nervous system is composed of two prominent swellings, the cerebral ganglia, situated postero-dorsally to the pharynx and connected by a thick dorsal commissure (Fig 1).

From the cerebral ganglia four pairs of longitudinal nerves proceed cephalad. Of these, the innermost pair are the stout pharyngeal nerves which, shortly beyond their origin, enter the pharynx at its postero-lateral margins. The remaining three pairs of anterior longitudinal nerves include the antero-dorsal, antero-ventral and antero-lateral nerves, named according to their emergence from the cerebral ganglia. The antero-dorsal nerves of either side arise from the dorsal side of the cerebral ganglia in between the pharygeal and antero-ventral nerve and proceed to the oral tip. The antero-ventral nerves are particularly well developed and arise from the ventro-anterior part of the cerebral ganglia; each of the antero-ventral nerves bifurcates into two trunks as soon as it leaves the cerebral ganglion. One is the antero-median ventral nerve, which enters into the oral sucker at its basal part; the other is the antero-outer ventral nerve, running parallel to the lateral body wall, which reaches to the oral extremity. The antero-lateral nerves are also two in number; each arises from the most lateral side of each cerebral ganglion; they run parallel to the margin of the body wall and finally connect with the antero-outer ventral nerves, entering into the oral sucker at its lateral side. The dorsal and the ventral cephalic stems embrace the oral sucker and, through numerous branching fibers, form a more or less complete ring surrounding the rim of the sucker.

The post-cephalic longitudinal nerves consist of 3 pairs of nerve cords, namely postero-dorsal, postero-ventral and postero-lateral. The postero-dorsal nerve cords originate from the middle facet of the cerebral ganglia, extend posteriorly and run more or less parallel to each other till they reach the level of the anterior testis where they combine together. On their way to the posterior, the postero-dorsal cords of the two sides are joined with each other by a number of thin transverse connections throughout their course (Fig 2); these connections in turn are further connected to one another by some still thinner longitudinal connections forming a complicated nerve net. Each postero-dorsal nerve cord is also joined with the postero-lateral nerve cord on the same side by means of another set of transverse connections.

The postero-ventral nerve cords are a pair of very stout, long nerves, the most prominent of the nervous system. They arise from the postero-lateral sides of the cerebral ganglia. They run posteriorly parallel to each other till they reach the tail where they give off nervous branches supplying the excretory bladder (Fig 3). On their way to the posterior, the postero-ventral cords of the two sides are joined with each other by some thin transverse connections throughout their course; these connections in turn are further connected to one another by two or three thinner longitudinal connections forming a nerve net in the middle part of the body. On the anterior half of the worm body, each postero-ventral nerve cord is also joined with the postero-lateral nerve cord on the same side by means of another set of transverse connections. On the posterior half of the worm body, owing to the disappearance of postero-lateral nerve cords a number of nervous branches arising from the postero-ventral nerve cords are joined with each other by some slender branches from the combination of the two postero-dorsal nerve cords, forming a loose nerve net in the hind body.

The ventral sucker is innervated by nerve fibers stemming directly from the postero-ventral cords. Around the border of the ventral sucker there is a loose ring formed by nerve fibers and slightly deeper, and around the opening of the sucker there is a complete ring. The nerves arising from the branches supplying the ventral sucker form concentric rings surrounding the rim of the sucker and these rings are joined by several radial anastomoses which are clearly visible (Fig 4). The muscles of the ventral sucker are innervated by the nerves supplying the sucker. In addition, the genital organs, including ovary, seminal receptacle, uterus, ootype, vitelline glands and testes are also innervated by fine nerves arising from the main postero-ventral nerve cords.

The postero-lateral nerve cords are also two in number, less prominent than the postero-ventral nerve cords. They are extremely marginal in position, lying just beneath the body wall (Fig 1-2). They arise from the most lateral sides of the cerebral ganglia and also extend posteriorly where they join the postero-dorsal nerve cords, just anterior to the seminal reseptacle. All the postero-lateral nerve cords of the two sides are joined with both postero-dorsal and postero-ventral nerve cords on the same side by a number of transverse connections, forming a complicated nerve net on both sides in the anterior half of the worm body.

In the present study two major types of nerve cells were recognized (Fig 5). Bipolar and multipolar nerve cells were observed in the whole mounts of the enzymatic preparations. The cell bodies are prominent with an oval to more spindle-like shape. Bipolar nerve cells are large in number but small in size with average of 15.6 × 8.3 µm, whereas the multipolar cells are smaller in number but larger in size, being 27.3 × 15.8 µm.

DISCUSSION

First of all, attention should be called to the cholinergic character of the nervous system in digenetic trematodes. The occurrence of active acetylcholinesterase was demonstrated in all parts of the nervous system of *C. sinensis*. By means of the method employed it was possible to determine the distribution and structure of the central ganglia, commissure, the main nerve cords, as well as those of minute nerves and nerve cells.

Hitherto, studies on the nervous system of digenetic trematodes with enzymatic techniques have been carried out in several species belonging to the following families: Schistosomatidae, Paramphistomatidae, Diplostomatidae, Fasciolidae and Dicrocoeliidae (Bueding *et al,* 1967; Halton, 1967; Ramisz and Szankowska, 1970; Xia and He, 1982; Mishra and Tandon, 1984, 1986; Niewiadomska and Moczon, 1987). As flukes have more or less similar body shape, especially in the former three families, the neuroanatomy of these species shows some specific characters. *C. sinensis,* belonging to Opisthorchiidae is a typically flattened, lanceolate distome, its nervous system is clearly visualized by using the enzymatic technique *in toto* and may act as a basic pattern representative of digenetic trematodes.

A comparison of the nerve pattern in *C. sinensis* with that in paramphistomes, schistosomes and diplostomes shows some general similarities and several differences. The occurrence of three pairs of posterior longitudinal nerve cords, with the most prominent being postero-ventral, is the most constant feature of the digenetic species investigated so far. The presence of a pair of pharyngeal nerves, in addition to the usual three pairs of anterior longitudinal nerves has been described in paramphistomes and *Clonorchis,* but not in schistosomes. It suggests, therefore, that the number of anterior longitudinal nerves proceeding from the cerebral ganglia may be three or four pairs, depending on the species of flukes which are provided with or without pharynx. For example, there is no pharynx in schistosomes and their cerebral ganglia give off 3 pairs of nerves anteriorly (Xia and He, 1982), while species with a pharynx have 4 pairs of anterior longitudinal nerves (Niewiadomska and Moczon, 1987). However, Ramisz and Szankowska (1970) reported that the pharyngeal nerves in *Fasciola hepatica* and *Dicrocoelium dendriticum* proceed from the post-cephalic ones from the cerebral ganglia, and then they enter into the pharynx. Accordingly, there are 4 pairs of post-cephalic longitudinal nerve cords in these two species.

Fig 1—Anterior part of *Clonorchis sinensis,* showing two central ganglia which are connected by a thick commissure give off 4 anterior pairs of short but distinct nerves and 3 posterior pairs of long nerve cords. ×40

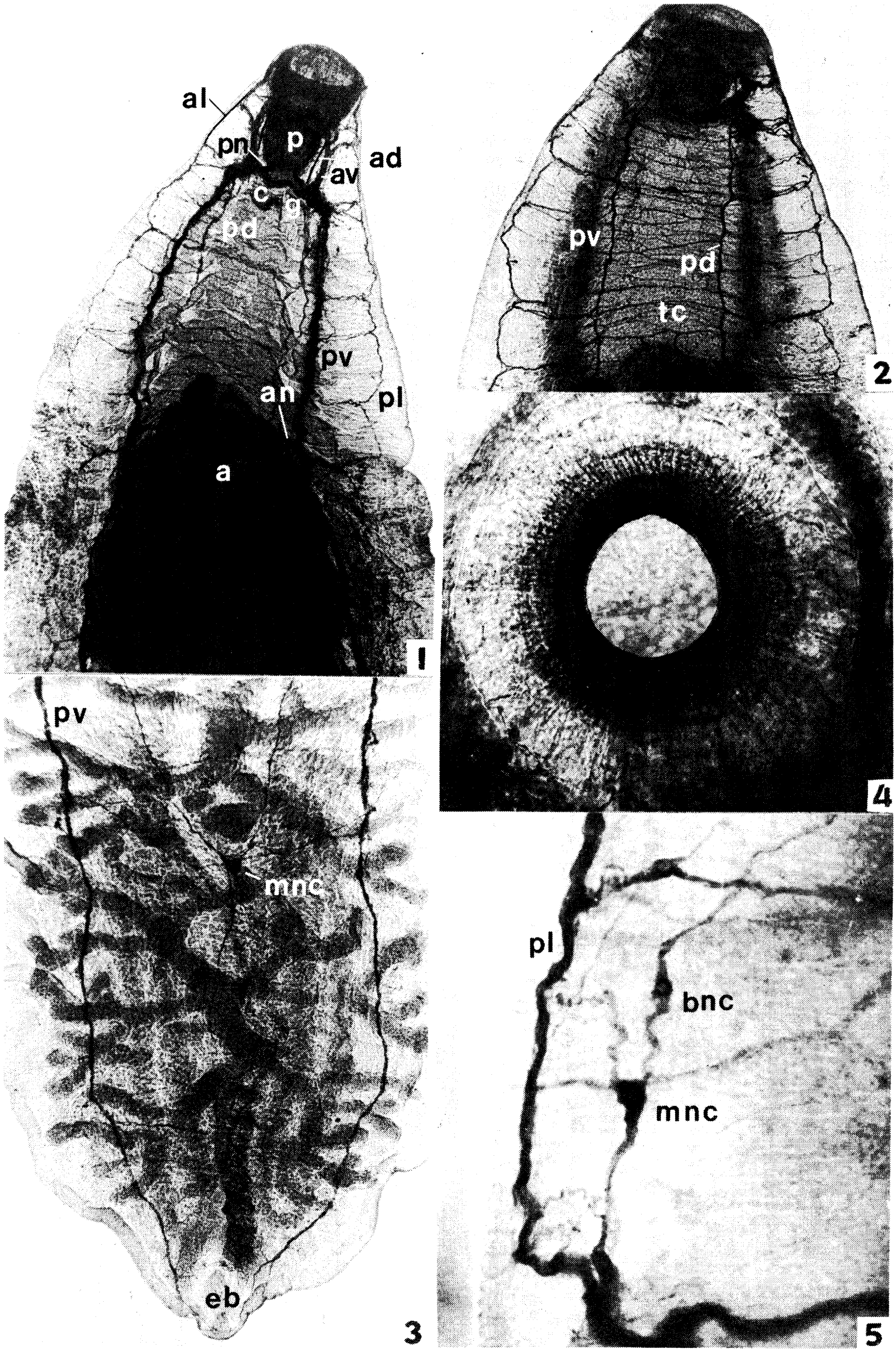
Fig 2—Three pairs of posterior nerve cords join with a number of thin transverse connections forming a nerve net in the anterior part of the worm body. ×40

Fig 3—Two postero-ventral nerve cords run posterior parallel to each other and their nerve net in the posterior part of worm body. ×40

Fig 4—Innervation by the nerve supplying the acetabulum. ×200

Fig 5—A magnified part of anterior region, showing the bipolar and multipolar nerve cells. ×4000

a = acetabulum; ad = antero-dorsal nerve; al = antero-lateral nerve; an = acetabular nerve; av = antero-ventral nerve; bnc = bipolar nerve cell; c = commissure; eb = excretory bladder; g = ganglion; mnc = multipolar nerve cell; p = pharynx; pd = postero-dorsal nerve cord; pl = postero-lateral nerve cord; pn = pharyngeal nerve; pv = postero-ventral nerve cord; tc = transverse connections.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Several types of neurosecretory cells in different morphology and function have been reported in digenetic trematodes, such as: unipolar, bipolar and multipolar nerve cells, or A, B, C types of neurons (Shyamasundari and Hanumantha-Rao, 1975; Bhatnagar *et al,* 1980). In our results, two major types of nerve cells are recognized. Many bipolar and multipolar nerve cells were observed in the parynchyma, in close vicinity to the various organs of the body. Under high resolution these cells revealed very fine connections with the nerve net or the main nerves. In order to throw some light on the physiological role of the two types of nerve cells in the nervous system of *C. sinensis,* detailed ultrastructural studies and more histochemical tests are further required.

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