Parasitol Res (1990) 76:487–496

Parasitology Research

An ontogenetic study of the cholinergic and serotoninergic nervous systems in *Trilocularia acanthiaevulgaris* (Cestoda, Tetraphyllidea)

I. Fairweather [1], S. Mahendrasingam [1], C.F. Johnston [2], D.W. Halton [1], J.S. McCullough [1], and C. Shaw [2]

Departments of [1] Biology and [2] Medicine, The Queen's University, Belfast BT7 1NN, Northern Ireland

Accepted February 1, 1990

Abstract. The localisation and distribution of the cholinergic and serotoninergic components of the nervous system in the plerocercoid, adult and free proglottis stages of the tetraphyllidean tapeworm *Trilocularia acanthiaevulgaris* were determined by enzyme histochemical and immunocytochemical techniques. The central nerve ring (CNR) in the scolex contains two lateral ganglia and gives rise to five pairs of longitudinal nerve cords (LNC's; three lateral, two median). The nerve cords run posteriorly throughout the bodies of the plerocercoid and adult worms and the free proglottis. Nerves from the CNR and accessory lateral LNC's pass to the bothridia, where they give rise to extensive nerve plexuses. As the individual proglottides develop along the strobila, a small nerve ring forms at the anterior end of each proglottis; within the nerve ring, distinct bilateral ganglia develop prior to the release of the proglottis. All ten LNC's are present in the free proglottis. The genital atrium and cirrus sac are innervated by cholinergic and serotoninergic elements. The cholinergic nervous system predominates in the CNS within the scolex, whereas there is a larger population of 5-HT-immunoreactive nerve cells associated with the LNC's and segmental ganglia along the strobila and within the free proglottis.

The Tetraphyllidea are widely recognized as the oldest and most primitive group of the true tetrafossate tapeworms (Wardle et al. 1974). It is typical of many species that they undergo hyperapolysis – that is, the individual (or "free") proglottides are released in an immature condition and develop independently of the strobila and of each other. *Trilocularia acanthiaevulgaris*, a parasite of the spiny dogfish, *Squalus acanthias*, is one such hyperapolytic species, and previous studies have shown that its developmental sequence in the fish involves three forms. Thus, a plerocercoid-like juvenile form occurs in the stomach, migrating in the early summer months (May–July) to the spiral valve, where it develops into the strobilated adult form. Free proglottides are released from the strobila in June and July and undergo their cycle of reproductive development, shedding their eggs when gravid (McCullough and Fairweather 1984; McCullough et al. 1986). The rest of the life cycle is unknown (McCullough et al. 1986). Despite this, the presence of three distinct life cycle stages in the one host makes *T. acanthiaevulgaris* an ideal model for studies on the development of the nervous system.

Despite their strategic position in the evolution of cestodes, the Tetraphyllidea remain a neglected group, and this is also true for their nervous system. Histological studies have established the gross neuroanatomy of a number of species (Rees 1943, 1946, 1966; Rees and Williams 1965; Williams 1959). A number of electron microscopic studies have described certain aspects of the ultrastructural features of the nervous tissue (Fairweather et al. 1987a; Golubev and Kashapova 1975). However, the identity of transmitter substances in these species is not known. The present paper is the first of a two-part investigation into the range of potential transmitter substances in *T. acanthiaevulgaris*. It provides information on the localization and distribution of acetylcholine and 5-hydroxytryptamine (serotonin, or 5-HT) in the three developmental stages from the dogfish gut. A subsequent paper will deal with putative peptidergic transmitters (Fairweather et al. 1990).

Materials and methods

Specimens of *T. acanthiaevulgaris* were recovered from the gastrointestinal tract of the spiny dogfish, *Squalus acanthias*, caught in the Irish Sea and landed at Ardglass, County Down. Three life cycle stages of *T. acathiaevulgaris* were examined in the present study: the juvenile (plerocercoid-like) form that inhabits the stomach, and the adult and free proglottis that occur in the spiral valve.

Reprint requests to: I. Fairweather

Demonstration of the cholinergic nervous system

Two techniques were used: the indoxyl acetate method for non-specific esterases, and the acetylthiocholine iodide method for cholinesterases. For the indoxyl acetate technique (after Holt 1954, as described by Bancroft 1967), worms belonging to the three developmental stages were washed in elasmobranch saline (McCullough 1984, after Carvajal et al. 1982; Read et al. 1960), fixed for 2 h at 4° C in 4% (w/v) paraformaldehyde in 0.2 *M* TRIS-maleate buffer (pH 7.2), and then washed in several changes of 0.2 *M* TRIS-maleate buffer (pH 7.2) at 4° C over a 2-h period. Specimens were incubated in the indoxyl acetate substrate medium (pH 7.2) at 37° C for 15–60 min, followed by dehydration, clearing and mounting as whole-mount preparations. Controls included (1) incubation in the substrate medium lacking the indoxyl acetate substrate and (2) inclusion of the cholinesterase inhibitor eserine (physostigmine, 1×10^{-5} *M*) in the substrate medium.

The acetylthiocholine iodide technique used was essentially that of Wilson and Schiller (1969), a modification of the Bueding et al. (1967) method, which in turn is based on the original methods of Koelle and Friedenwald (1949) and Gomori (1952). Specimens of the three developmental stages were washed as described above, fixed for 30 min at 4° C in 10% (v/v) phosphate-buffered neutral formalin (pH 7.2), and washed in several changes of 0.2 *M* TRIS-maleate buffer (pH 7.2) at 4° C over a 45-min period. The worms were preincubated for 2 h at 37° C in Gomori's stock solution (pH 6.8), followed by incubation in the acetylthiocholine iodide substrate medium (pH 6.8) at 37° C for 15–60 min. The worms were then washed for 15 min at 37° C in a saturated solution of sodium sulphate and incubated in 70% (v/v) ethanol saturated with hydrogen sulphide for 30 min at room temperature, prior to hydration, clearing and mounting as whole-mount preparations. Controls included (1) incubation in the substrate medium lacking the substrate and (2) inclusion of the cholinesterase inhibitor eserine (1×10^{-5} *M*) in the substrate medium. 5-Bromo-4-chloroindoxyl acetate, acetylthiocholine iodide and eserine were obtained from Sigma Chemical Company Ltd (Poole, Dorset, England, UK).

Demonstration of the serotoninergic nervous system

For the visualization of 5-HT nerve elements in *T. acanthiaevulgaris*, the indirect immunofluorescence technique of Coons et al. (1955) was used. Whole-mount preparations of the three developmental stages were fixed for 4 h at 4° C in 4% (w/v) paraformaldehyde (PFA) in 0.1 *M* phosphate-buffered saline (PBS, pH 7.4), and then washed for 24 h at 4° C in PBS (pH 7.4) containing 0.3% (v/v) Triton X-100 and 0.2% (w/v) sodium azide. Incubation in primary antiserum was carried out for 2–4 days at 4° C, using either antiserum 43H2T (at a working dilution of 1:100) (Immuno Nuclear Corporation, Stillwater, Minn., USA) or antiserum 448(1) (at a working dilution of 1:500) (kindly donated by Dr. S.H. Mitchell, Department of Medicine, The Queen's University of Belfast; for details of immunogen, see Fairweather et al. 1987b). Specimens were subsequently washed for 24 h at 4° C in PBS, incubated for 24 h at 4° C in swine anti-rabbit IgG conjugated to fluorescein isothiocyanate (FITC) (Dako Ltd., High Wycombe, Buckinghamshire, England, UK), and washed again prior to mounting and examination with an Olympus BH-2 incident fluorescence microscope fitted with a supplementary EY 455 excitatory filter. Specimens were also imaged using an MRC-500 Confocal Scanning Laser Microscope (Bio-Rad Lasersharp Ltd., Abingdon, Oxfordshire, England, UK).

Controls included (1) omission of primary antiserum, (2) substitution of primary antiserum with non-immune rabbit serum (Dako Ltd., High Wycombe, Buckinghamshire, England, UK) and (3) liquid-phase preabsorption of primary antiserum with 5-HT-HCl (Sigma Chemical Company Ltd., Poole, Dorset, England, UK) at 500 µg/ml diluted antiserum.

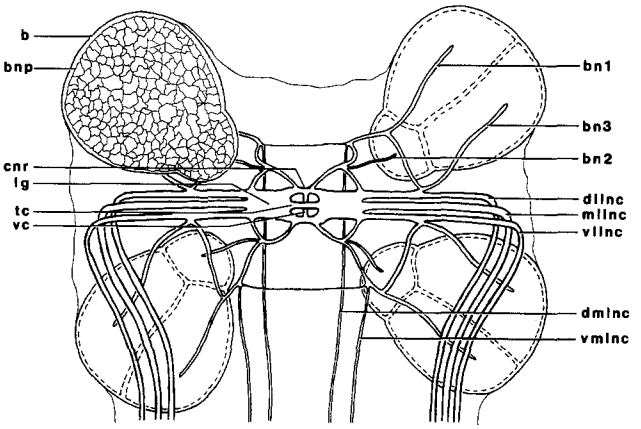


Fig. 1. Diagram of the nervous system in the scolex of *T. acanthiaevulgaris*, viewed from the ventral surface. *b*, bothridium; *bn1, bn2, bn3*, bothridial nerves 1, 2 and 3; *bnp*, bothridial nerve plexus; *cnr*, central nerve ring; *dllnc*, dorso-lateral longitudinal nerve cord; *dmlnc*, dorsal median longitudinal nerve cord; *lg*, lateral ganglion; *mllnc*, main lateral longitudinal nerve cord; *tc*, transverse connective in central nerve ring; *vc*, vertical connective in central nerve ring; *vllnc*, ventro-lateral longitudinal nerve cord; *vmlnc*, ventral median longitudinal nerve cord

Fig. 2. Plerocercoid. Whole-mount preparation, showing the distribution of the CNS in the scolex. Indoxyl acetate method. *B*, bothridium; *BN1, BN2, BN3*, bothridial nerves 1, 2 and 3; *LG*, lateral ganglion; *LNC*, lateral longitudinal nerve cords; *MNC*, median longitudinal nerve cords; *arrows*, nerve plexus innervating the musculature of the bothridium. Bar, 50 µm

Fig. 3. Plerocercoid. Whole-mount preparation, showing the origin of bothridial nerve 1 (*BN1*) from the lateral ganglion (*LG*) and bothridial nerve 3 (*BN3*) from the accessory lateral longitudinal nerve cord (*ALNC*). Indoxyl acetate method. Bar, 25 µm

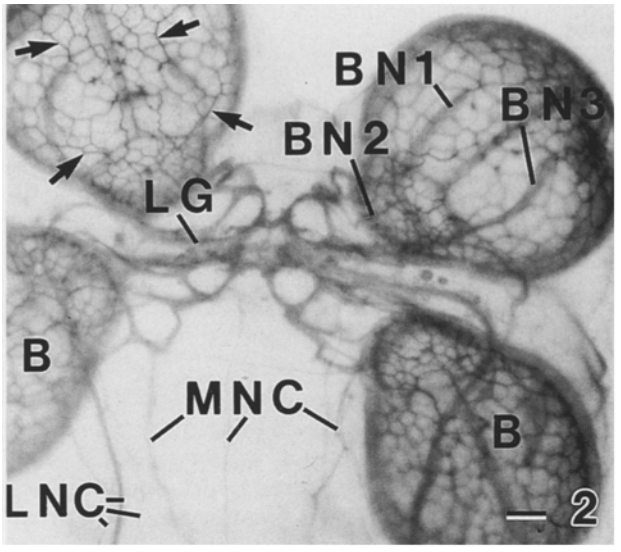
Fig. 4. Plerocercoid. Whole-mount preparation, showing the nerve cell bodies (*arrows*) associated with the lateral ganglia and other elements of the central nerve ring in the scolex. Indoxyl acetate method. Bar, 25 µm

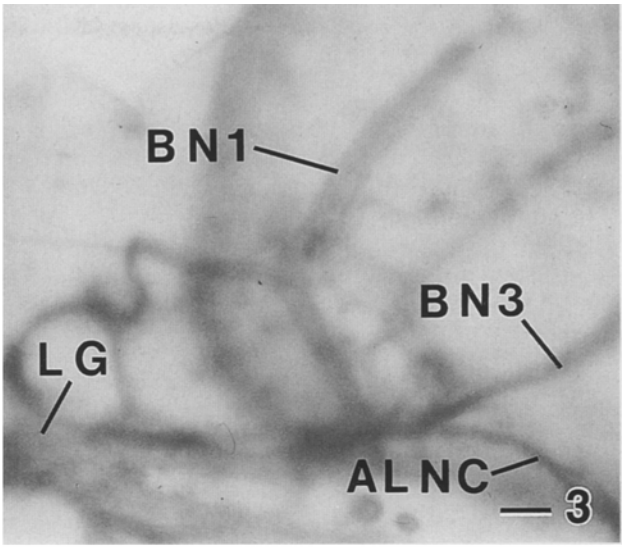
Fig. 5. Plerocercoid. Whole-mount preparation, showing two large nerve cell bodies (*arrows*) lying alongside the main lateral longitudinal nerve cord (*MLNC*) near its origin from the lateral ganglion (*LG*). Indoxyl acetate method. *ALNC*, accessory lateral longitudinal nerve cord. Bar, 25 µm

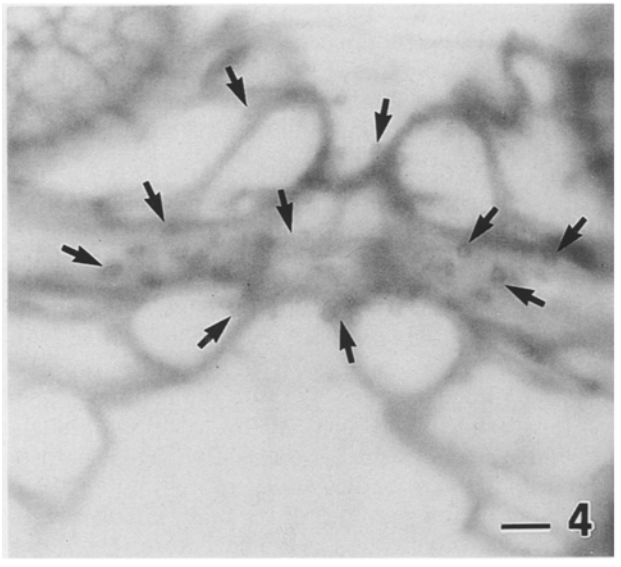
Fig. 6. Adult worm. Whole-mount preparation, showing the formation of nerve rings (*arrows*) in the developing proglottides in the strobila. Acetylthiocholine iodide method. Bar, 100 µm

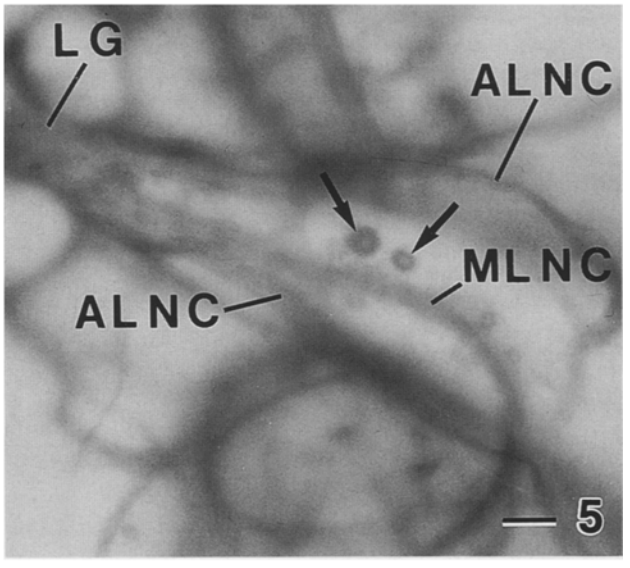
Fig. 7. Free proglottis, showing the level of cholinesterase activity in the main lateral longitudinal nerve cord (*MLNC*) decreasing posteriorly along the proglottis. Acetylthiocholine iodide method. Bar, 25 µm

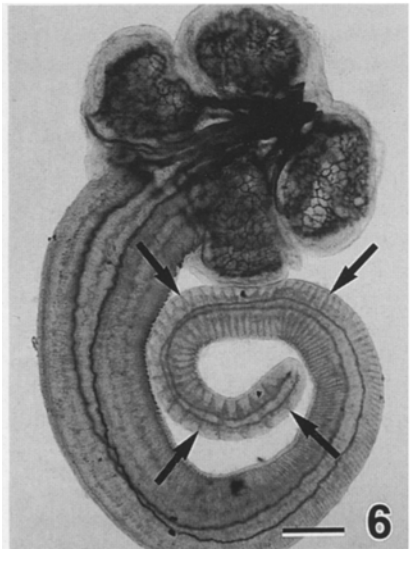
Fig. 8. Free proglottis. An intense reaction for esterase activity is present in the spiny anterior region (*SA*) of the proglottis, in the genital atrium (*GA*) and in the cirrus sac (*CS*). Esterase activity is also present in the lateral (*LNC*) and median (*MNC*) longitudinal nerve cords and their cross-connectives (*arrows*). Indoxyl acetate method. *E*, eggs in uterus. Bar, 50 µm


BN1
BN3
BN2
LG
B
MNC
B
LNC
2

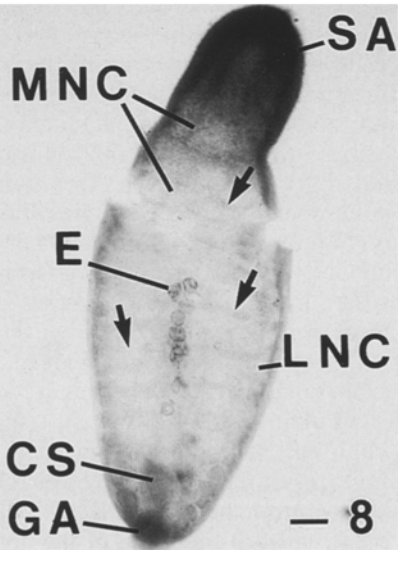

BN1
BN3
LG
ALNC
3


4


LG
ALNC
MLNC
ALNC
5


6


MLNC
7


SA
MNC
E
LNC
CS
GA
8

Figs. 2–8

Results

Cholinergic nervous system

There was no difference in the distribution of staining with the two esterase techniques used; therefore, the results are described together. No staining was evident in any of the control material.

The CNS in the scolex of the juvenile and adult worms is well developed and is represented diagrammatically in Fig. 1. It consists of a relatively small, squared central nerve ring (CNR), which is crossed by a prominent transverse connective and a more delicate vertical connective (Fig. 2). From the lateral margins of the CNR arise three pairs of nerve cords, which are fused at their origin to form paired lateral ganglia (Fig. 2). The nerve cords extend laterally for a short distance before curving backwards to pass down into the neck region (Fig. 2); they become the lateral longitudinal nerve cords (lateral LNC's) that run posteriorly throughout the bodies of the juvenile and adult worms and the free proglottis. Of the three nerve cords, the central one is the most well developed and is hereafter referred to as the main lateral LNC. The other two are more delicate and form the latero-dorsal and latero-ventral LNC's (also referred to collectively as the accessory lateral LNC's) (Fig. 2). The three lateral LNC's are joined by cross-connectives.

A nerve tract from each corner of the CNR joins with a similar nerve tract arising from the origin of one of the accessory lateral LNC's to form a triangular structure (Fig. 3). From the apex of the triangle, a nerve extends to the centre of one of the posterior loculi of a bothridium: this is bothridial nerve 1 (Fig. 3). A separate nerve passes to the centre of the anterior loculus: this is bothridial nerve 2 (Fig. 2). The other posterior loculus is innervated by a nerve arising further along the accessory lateral LNC: this is bothridial nerve 3, which is joined to bothridial nerve 1 by a cross-connective (Fig. 3). The three bothridial nerves give rise to a well-developed nerve plexus innervating the musculature of each bothridium (Fig. 2). The plexus appears to ramify throughout the bothridium and is joined to a nerve ring running around the margin of the bothridium; it is not sub-divided into plexi within the individual loculi (Fig. 2). A number of nerve cell bodies are evident within the plexus (Fig. 2). The two bothridial nerves 1 on either side of the CNR are joined by a transverse connective, and from the junctions so formed arises one of the two pairs of median LNC's that run down the centre of the worm in all three life cycle stages (Figs. 2, 3).

Numerous small nerve cell bodies are associated with the CNR and main nerve tracts in the scolex. More than 50 cells could be distinguished on each of the dorsal and ventral faces of the CNR: they were clustered mainly over the common root of the lateral LNC's, whereas others were situated at the bases of the bothridial nerves (Fig. 4). Typically, two or three larger cell bodies lay along the shoulder of each main lateral LNC before it curved backwards down the neck region (Fig. 5). Nerve cells were associated with the lateral LNC's in the neck region but became less frequent with increasing distance from the CNR.

The ten LNC's evident in the neck region continue backwards throughout the body of the juvenile worm and the strobila of the adult. As the individual proglottides develop along the strobila, so a small nerve ring develops at the anterior end of each proglottis (Fig. 6). Thickenings of nervous tissue develop at the junction between the nerve ring and the lateral LNC's; these thickenings develop into distinct ganglia by the time the proglottides detach, although individual cell bodies could not be distinguished with the esterase techniques (Fig. 6).

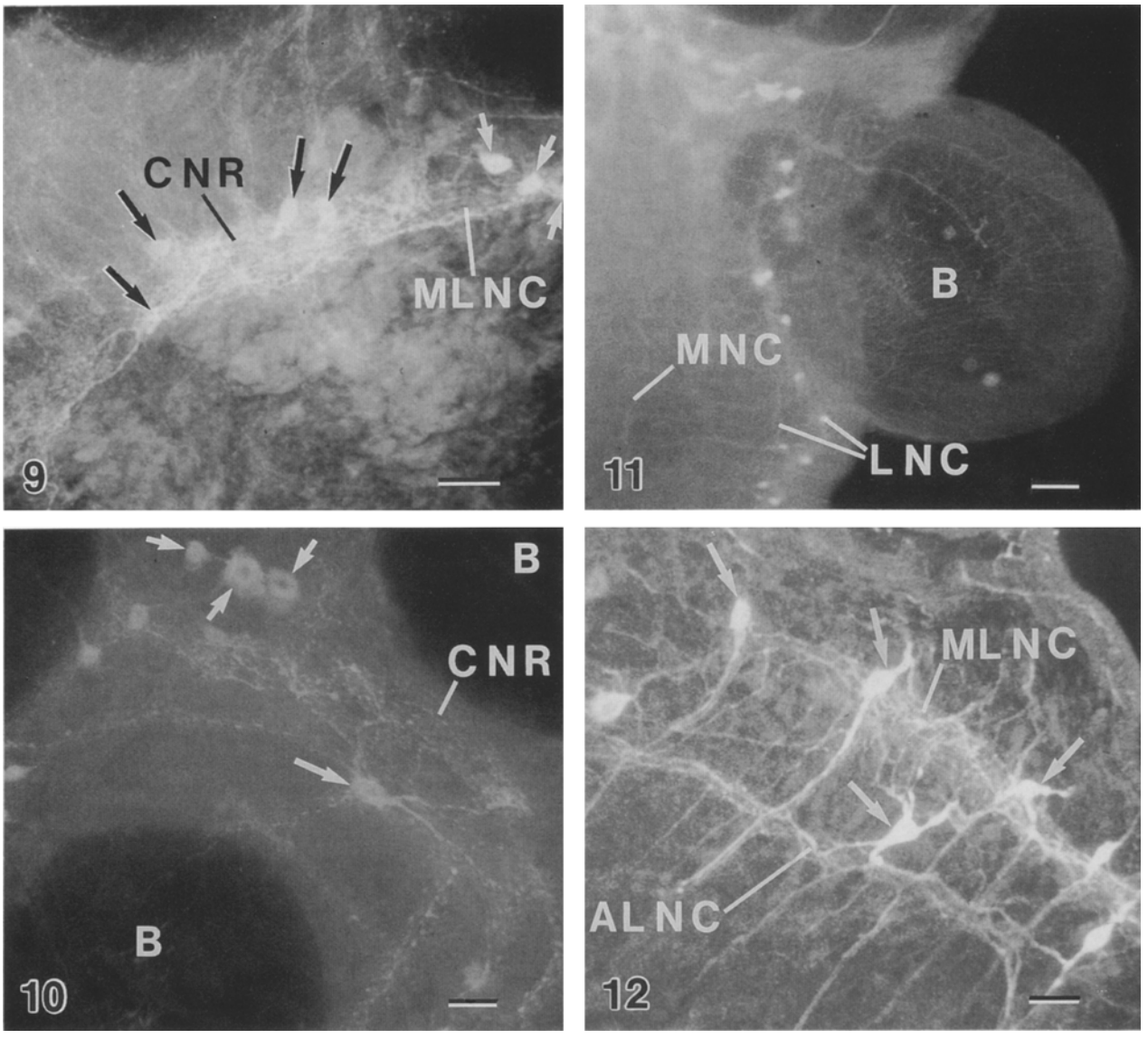
All ten LNC's were present in the free proglottis, with the main lateral LNC being the most prominent (Fig. 7). There appears to be a swelling of the nerve cords at the anterior end of the proglottis, and the intensity of cholinesterase activity is greatest within the spiny anterior end; it declines as the nerve cord passes further backwards along the main body of the proglottis (Fig. 7). Transverse connectives ramify between the LNC's (Fig. 8). An intense reaction was evident with the indoxyl acetate technique in the region of the genital atrium and in the muscular lining of the cirrus sac (Fig. 8).

5-HT

5-HT-immunoreactive nerve fibres were seen to contribute to the CNR in the scolex of juvenile and adult worms (Fig. 9). Up to six nerve cell bodies were associated with the CNR: they were multipolar in type and approximately 17×10.5 μm in size (Fig. 9). Typically, a single, large, stellate multipolar nerve cell (size, approximately 19×12 μm) lies either side of the CNR (Fig. 10). Also, three multipolar cell bodies (size, approximately 17×11 μm) lie along the shoulder of the main lateral LNC's near their origin from the CNR and before they curve backwards down the neck region of the worm (Figs. 9, 10). Immunoreactive nerve fibres extend from the CNR to the bothridia, where they give rise to a delicate nerve net (Fig. 11).

In the neck region of both stomach and spiral valve worms, immunoreactive nerve fibres were present in all the LNC's (Fig. 12). The LNC's appeared to be fairly delicate structures, consisting of only a few nerve fibres, and no one nerve cord was more prominent than any other; they were joined by several transverse connectives (Fig. 12). A series of regularly spaced nerve cell bodies were associated with the main lateral LNC's, although their nerve processes extended to the accessory lateral LNC's as well (Fig. 12). The cells were predominantly multipolar in type (size, approximately 16×11 μm), although smaller bipolar cells were also evident.

The LNC's and their associated cell bodies extended posteriorly along the strobila of the adult worms. As individual proglottides began to develop, a regular pattern of one or two immunoreactive nerve cell bodies was evident along the anterior margin of each proglottis (Fig. 13). With the growth of the proglottides (due largely to their elongation), the numbers of cells increased



Figs. 9–20. Whole-mount preparations showing immunoreactivity to 5-HT

Fig. 9. Plerocercoid. Confocal scanning laser micrograph (CSLM), showing 5-HT-immunoreactive nerve fibres and nerve cell bodies (*large arrows*) in the central nerve ring (*CNR*) in the scolex. Three immunoreactive nerve cell bodies (*small arrows*) are associated with the main lateral longitudinal nerve cord (*MLNC*) near its origin from the central nerve ring. Bar, 25 μm

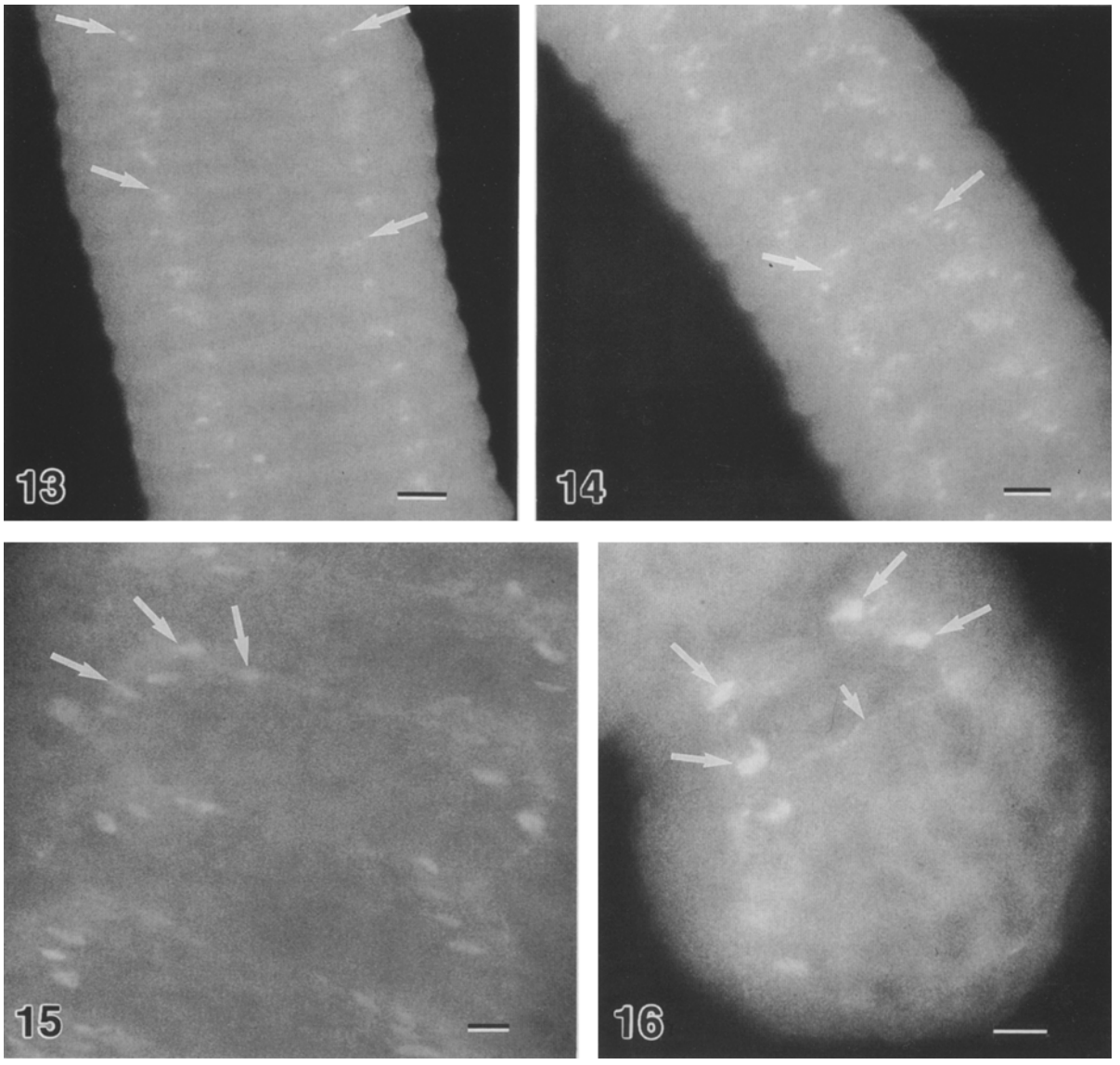
Fig. 10. Adult worm, showing one of the large, stellate multipolar nerve cell bodies (*large arrow*), immunoreactive to 5-HT, that lie on either side of the central nerve ring (*CNR*). Three immunoreactive nerve cell bodies (*small arrows*) are associated with the main lateral longitudinal nerve cord near its origin from the central nerve ring. *B*, bothridium. Bar, 25 μm

Fig. 11. Adult worm, showing the extensive plexus of 5-HT-immunoreactive nerve fibres innervating the musculature of a bothridium (*B*). *LNC*, lateral longitudinal nerve cords; *MNC*, median longitudinal nerve cords. Bar, 50 μm

Fig. 12. Plerocercoid. CSLM. 5-HT-immunoreactive nerve fibres are present in the main (*MLNC*) and accessory (*ALNC*) lateral longitudinal nerve cords and their cross-connectives in the neck region. A number of immunoreactive nerve cell bodies (*arrows*) are associated with the nerve cords, their processes extending to more than one nerve cord. Bar, 10 μm

to four or five, and they formed a series of cells arching towards the centre of the proglottis (Figs. 14, 15). This process continued such that by the time the terminal proglottis was reached, there were two distinct aggregations of nerve cells on either side and in the anterior half of the proglottis (Fig. 16). These aggregations of nerve cells form the presumptive ganglia of the free proglottides, and each ganglion contained approximately eight or nine cells.

In the mature free proglottis, the ganglia lie at the junction between the spiny anterior end and the main body of the proglottis and are joined by three or four transverse connectives (Fig. 17). From the ganglia, immunoreactive nerve fibres pass forwards to innervate



Figs. 13–16. Adult worm, showing stages in the formation of ganglia in the developing proglottides in the strobila.

Fig. 13. Early stage, with one or two nerve cell bodies (*arrows*) coming together. Bar, 25 μm

Fig. 14. Later stage, showing aggregations of 3 or 4 nerve cell bodies (*arrows*) on either side at the anterior margin of each proglottis. Bar, 25 μm

Fig. 15. Developing ganglia at higher magnifications, showing the nerve cell bodies (*arrows*) arching towards the centre of the proglottis. Bar, 10 μm

Fig. 16. Terminal proglottis, showing the paired ganglia comprising several nerve cell bodies (*large arrows*); the ganglia are united by transverse connectives (*small arrows*). Bar, 10 μm

the anterior attachment organ, in which they ramify to form a nerve plexus, which contains a number of small multipolar cell bodies. Nerve cords extend posteriorly from the ganglia; essentially, they are continuations of the ten LNC's evident in the strobila of the worm (Fig. 18). A regular series of multipolar nerve cell bodies (size, approximately 9×6 μm) were associated with the lateral LNC's, their nerve processes extending to more than one nerve cord (Fig. 18). A few 5-HT-immunoreactive nerve cells were associated with the genital atrium (Figs. 19, 20), and immunoreactive nerve fibres were evident in the muscular lining of the cirrus sac (Fig. 20). No immunostaining was evident in any of the 5-HT control material.

Discussion

With the exception of a study on a possible and unidentified catecholamine/amino acid transmitter in the primitive cestodarian, *Gyrocotyle fimbriata* (Keenan and Koopowitz 1982), the present investigation is the first attempt to identify neurotransmitter substances in cestodes other than the Cyclophyllidea and Pseudophylli-

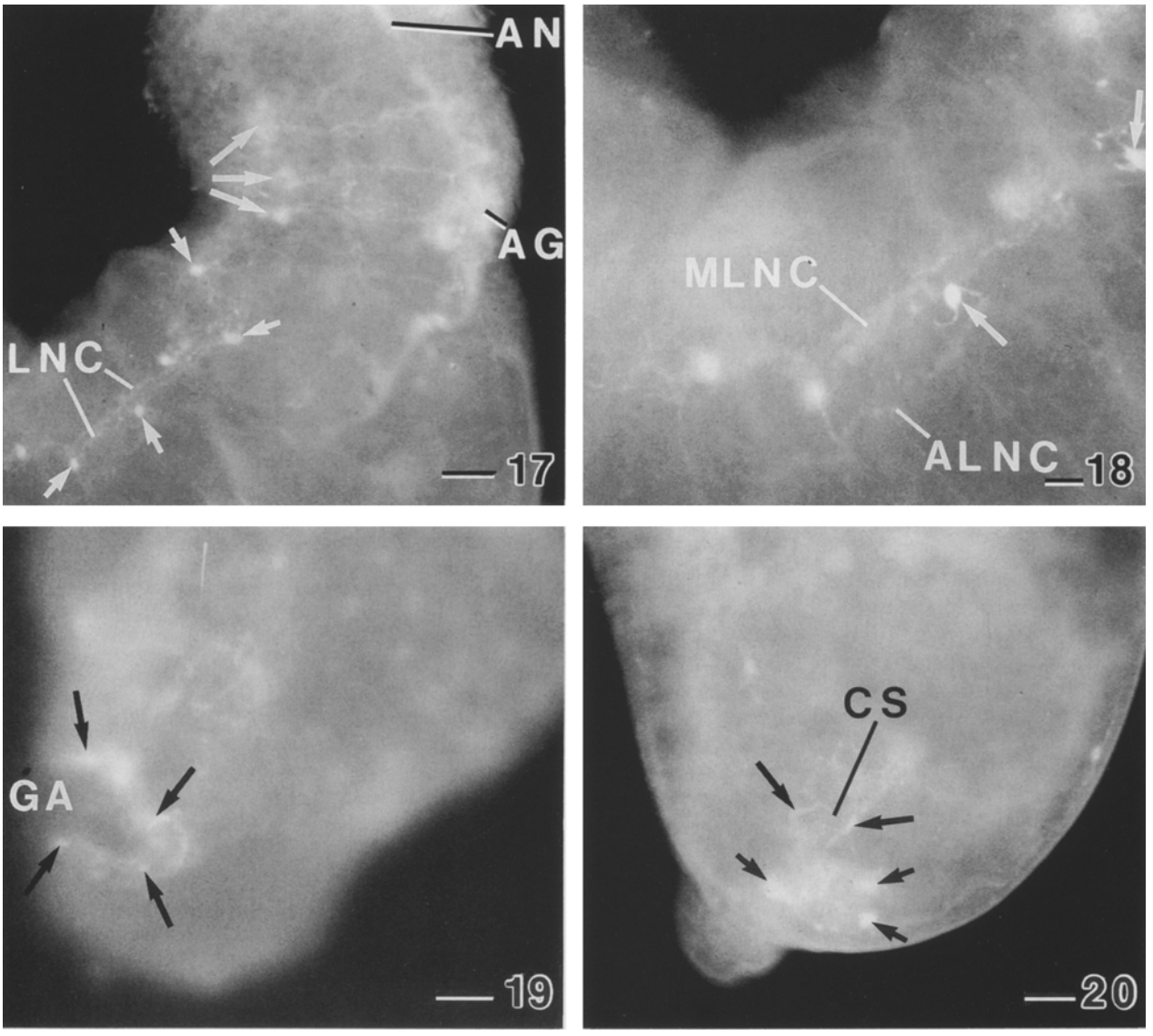


Fig. 17. Free proglottis. 5-HT-immunoreactive nerve cell bodies (*large arrows*) are present in the anterior ganglia (*AG*). An anterior nerve (*AN*) extends forwards from each ganglion into the anterior attachment organ. Immunoreactive nerve fibres are also present in the lateral longitudinal nerve cords (*LNC*) that extend posteriorly from the ganglia. A number of immunoreactive nerve cell bodies (*small arrows*) are associated with the nerve cords. Bar, 25 μm

Fig. 18. Free proglottis. 5-HT-immunoreactive nerve fibres are present in the main (*MLNC*) and accessory (*ALNC*) lateral longitudinal nerve cords in the main body of the proglottis. Multipolar immunoreactive nerve cell bodies (*arrows*) are associated with the nerve cords. Bar, 10 μm

Fig. 19. Free proglottis. 5-HT-immunoreactive nerve cell bodies and nerve fibres form a plexus (*arrows*) surrounding the genital atrium (*GA*). Bar, 25 μm

Fig. 20. Free proglottis. 5-HT-immunoreactive nerve fibres (*large arrows*) are present in the nerve plexus innervating the muscular lining of the cirrus sac (*CS*). A number of immunoreactive nerve cell bodies (*small arrows*) lie around the genital atrium. Bar, 25 μm

dea. Certainly, it is the first description of transmitters in a tetraphyllidean species. Previous studies on cholinesterase activity in cestodes have concentrated on cyclophyllideans: descriptions of gross neuroanatomy based on the distribution of cholinesterase activity in adult worms are available for a variety of species (Kralj 1967; Lui et al. 1964; Ramisz 1967; Shield 1969). Several larval forms have also been studied (Eränkö 1968; Hart 1967; Leflore and Smith 1976; Rees 1973). In *Hymenolepis diminuta*, cholinesterase activity has been demonstrated in the oncosphere (Rybicka 1967), cysticercoid (Bogitsh 1967) and adult (Douglas 1966; Fairweather 1978; Wilson and Schiller 1969). Fewer studies have been carried out on the Pseudophyllidea (Kotikova and Kuperman 1977, 1978). In *Triaenophorus nodulosus*, cholinesterase activity has been localized in the oncosphere, procercoid, plerocercoid and adult (Kotikova and Kuperman 1977).

Inhibitor studies have shown that the cholinesterase activity in cestode nervous tissue is predominantly due to an acetylcholinesterase enzyme (Bogitsh 1967; Eränkö

kö et al. 1968; Fairweather 1978; Hart 1967; Schardein and Waitz 1965; Shield 1969). Cholinesterase and acetylcholinesterase activity have also been demonstrated biochemically in a number of adult and larval cestodes (e.g. Eränkö et al. 1968; Graff and Read 1967; Pylkkö 1956). The association between acetylcholinesterase and the nervous system of cestodes, and its established role in cholinergic neurotransmission, suggests that acetylcholine functions as a neurotransmitter in these organisms. This idea is supported by pharmacological evidence (see below). Acetylcholine or acetylcholine-like substances have been demonstrated in a number of species (Artemov and Lur'e 1941; Hariri 1974; Pylkkö 1956).

5-HT has been demonstrated by formaldehyde-induced fluorescence in a number of cestode species (Gustafsson and Wikgren 1981; Lee et al. 1978; Shield 1971). More specific immunocytochemical techniques have been applied to *H. diminuta* (Fairweather et al. 1988; Webb and Mizukawa 1985) and to *Diphyllobothrium dendriticum*; in the latter species, 5-HT has been demonstrated in three life cycle stages, namely, procercoid, plerocercoid and adult (Gustafsson et al. 1985, 1986; Wikgren 1986). The presence of 5-HT throughout the CNS of *Trilocularia acanthiaevulgaris* suggests a role in neurotransmission and/or neuromodulation; in the peripheral plexuses associated with muscular organs such as the bothridia and cirrus sac, 5-HT may also be involved in neuromuscular transmission. Amongst cestodes, it is only in *H. diminuta* that there is sufficient evidence to consider 5-HT for transmitter status. Thus, there is evidence for neuronal localization (see above); for synthetic pathways (including intermediates) and inactivation mechanisms (reuptake and metabolism) (Ribeiro and Webb 1983, 1984; Webb 1985), pharmacological evidence in the form of motility responses to 5-HT agonists and antagonists (see below), and evidence for the existence of 5-HT receptors and their operation via an adenylate cyclase, cAMP system (Ribeiro and Webb 1986, 1987). Quite distinct from its transmitter role are the hormone-like effects that 5-HT has on various aspects of carbohydrate metabolism in the worm (Rahman et al. 1983; Sangster and Mettrick 1987).

The parallel distribution of cholinesterase activity and 5-HT immunoreactivity in *T. acanthiaevulgaris* suggests that acetylcholine and 5-HT may act as antagonistic transmitters in the control of locomotor activity in this species. The application of exogenous acetylcholine, 5-HT and their agonists and antagonists to neuromuscular preparations of *H. diminuta* and the trypanorhynch, *Grillotia erinaceus*, has indicated an inhibitory role for acetylcholine and an excitatory role for 5-HT (Sukhdeo et al. 1984; Thompson and Mettrick 1984; Thompson et al. 1986; Ward et al. 1986). These roles are true for platyhelminths in general; amongst the trematodes, they have been demonstrated for *Schistosoma mansoni* (Hillman 1983; Pax et al. 1984) and for *Fasciola hepatica* (Holmes and Fairweather 1984). Such a dual mechanism for the control of motility in *T. acanthiaevulgaris* (and, indeed, other platyhelminths) may be simplistic because other neurotransmitters (particularly peptides) may be involved (see Fairweather et al. 1990).

Previous histological studies on tetraphyllideans have described four "cerebral" ganglia, connected by a broad median commissure and more delicate dorsal and ventral commissures, and from which arise a variable number (2–10) of LNC's that extend posteriorly along the strobila (Rees 1943, 1946; Rees and Williams 1965; Williams 1959). The complexity of the nervous system in the scolex, as in other cestode groups, reflects the complexity of the adhesive apparatus (Fairweather and Threadgold 1983); *T. acanthiaevulgaris* is no exception to this rule. The present study provides evidence, based on cholinesterase histochemistry and 5-HT immunostaining, for a well-developed nerve ring in the scolex, which was confirmed by the three-dimensional imaging available with the confocal scanning laser microscope. In *Acanthobothrium coronatum*, nerve cells were said to be concentrated in the median commissure and absent from the "cerebral" ganglia (Rees 1966). Indeed, Rees (1946) describes the median commissure in *Phyllobothrium dohrnii* as being "most probably the true nerve centre". However, such statements on the presence of nerve cells and other details of the neuroanatomy need carry little weight in view of the non-selective nature of the staining techniques used and the problems of differentiating nervous tissue from the surrounding parenchyma. The histochemical and immunocytochemical methods used in the present study provide more reliable information and have shown that a substantial population of nerve cells is associated with the CNR and LNC's. This has been confirmed by a separate study on peptidergic nerve cells (Fairweather et al. 1990).

The present study has also provided unequivocal evidence for the formation of ganglia in the developing proglottides in the strobila, supplying each free proglottis with its own independent nerve centre. The existence of ring commissures connecting the LNC's in the strobilar region of the body has been described in *Anthobothrium auriculatum* and *A. coronatum*, but none of these develops into distinct accumulations of nervous tissue (Rees 1943; Rees and Williams 1965). Indeed, Rees and Williams (1965) clearly state that "there is no evidence of the formation of a new nerve centre" (in the detached proglottis). The difference between these two species and *T. acanthiaevulgaris* is probably due to the fact that they are euapolytic, whereas *T. acanthiaevulgaris* is hyperapolytic. This means that in *A. auriculatum* and *A. coronatum* the proglottides are shed when they are mature and eggs are beginning to pass into the uterus; consequently, they have only a short independent existence. In contrast, the free proglottis of *T. acanthiaevulgaris* detaches from the strobila when it is immature and lives a much longer (up to 2–3 months) free existence in the gut (McCullough et al. 1986). An independent nerve centre is essential for its survival and to control its reproductive development. The presence of a true nerve centre in the free proglottis suggests that the latter should be regarded as the sexual form of this worm species.

In conclusion, then, the present study extends current knowledge of the neurochemistry of cestodes beyond the Cyclophyllidea and Pseudophyllidea, and this theme is continued in a separate paper dealing with

peptidergic neurotransmitters (Fairweather et al. 1990). The work concentrated on a tetraphyllidean species and, more importantly, on a hyperapolytic species. Consequently, it was possible to obtain information on the development of the strobilar and proglottidal nervous systems in one of the most primitive of tapeworms, at a stage before delayed autotomy led to the segmented condition evident in advanced tapeworms such as the Cyclophyllidea and, perhaps, to a reduction in the complexity of the nervous "centres" in individual proglottides.

Acknowledgements. This investigation was supported in part by a Queen's University of Belfast Visiting Studentship (S.M.) and a Postgraduate Studentship from the Department of Education of Northern Ireland (J.S.M.). The authors would like to thank Mr. Angus Cochrane of Ardglass, County Down, for the supply of dogfish, and Anna McCollum for expert technical assistance.

References

Artemov NM, Lur'e RN (1941) Content of acetylcholine and cholinesterase in tapeworm tissues. Izv Akad Nauk SSSR [Biol] 2:278–282

Bancroft JD (1967) An Introduction to Histochemical Technique. Butterworth and Co Ltd, London

Bogitsh B (1967) Histochemical localization of some enzymes in cysticercoids of two species of *Hymenolepis*. Exp Parasitol 21:373–379

Bueding E, Schiller EL, Bourgeois JG (1967) Some physiological, biochemical, and morphologic effects of tris (*p*-aminophenyl) carbonium salts (TAC) on *Schistosoma mansoni*. Am J Trop Med Hyg 16:500–515

Carvajal J, Barros C, Santander G (1982) In vitro culture of *Rhodobothrium mesodesmatum* (Cestoda: Tetraphyllidea), parasite of a Chilean clam. Proc Helminthol Soc Wash 49:226–230

Coons AH, Leduc EH, Connolly J (1955) Studies on antibody production. I. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit. J Exp Med 102:49–60

Douglas LT (1966) Thiocholine technique: I. Modification for demonstration of acetylcholinesterase in *Schistosoma mansoni* (Trematoda) and in *Hymenolepis diminuta* (Cestoda). Acta Histochem 24:301–306

Eränkö O, Kouvalainen K, Mattila M, Takki S (1968) Histochemical and biochemical observations on cholinesterases of cat's tapeworm *Taenia taeniaformis*. Acta Physiol Scand 73:226–233

Fairweather I (1978) Studies on the nervous system of *Hymenolepis nana* (Cestoda: Cyclophyllidea). PhD Thesis, University of London

Fairweather I, Threadgold LT (1983) *Hymenolepis nana*: the fine structure of the adult nervous system. Parasitology 86:89–103

Fairweather I, Mahendrasingam S, Halton DW (1987a) The fine structure of the nervous system of the tapeworm *Trilocularia acanthiaevulgaris* (Cestoda, Tetraphyllidea) (abstract). Proc R Microsc Soc 23 [Suppl]:2

Fairweather I, Maule AG, Mitchell SH, Johnston CF, Halton DW (1987b) Immunocytochemical demonstration of 5-hydroxytryptamine (serotonin) in the nervous system of the liver fluke, *Fasciola hepatica* (Trematoda, Digenea). Parasitol Res 73:255–258

Fairweather I, Macartney GA, Johnston CF, Halton DW, Buchanan KD (1988) Immunocytochemical demonstration of 5-hydroxytryptamine (serotonin) and vertebrate neuropeptides in the nervous system of excysted cysticercoid larvae of the rat tapeworm, *Hymenolepis diminuta* (Cestoda, Cyclophyllidea). Parasitol Res 74:371–379

Fairweather I, Mahendrasingam S, Johnston CF, Halton DW, Shaw C (1990) Peptidergic nerve elements in three developmental stages of the tetraphyllidean tapeworm, *Trilocularia acanthiaevulgaris*; an immunocytochemical study. Parasitol Res 76:497–508

Golubev AI, Kashapova LA (1975) Some ultrastructural peculiarities of neurons in the cestode *Pelichnibothrium speciosum* (Monticelli, 1889) (Cestoda: Tetraphyllidea). Parazitologiya 9:439–442

Gomori G (1952) Microscopic Histochemistry. Principles and Practice, ch 8. University of Chicago Press, Chicago, pp 137–221

Graff DJ, Read CP (1967) Specific acetylcholinesterase in *Hymenolepis diminuta*. J Parasitol 53:1030–1031

Gustafsson MKS, Wikgren MC (1981) Peptidergic and aminergic neurons in adult *Diphyllobothrium dendriticum* Nitzsch, 1824 (Cestoda, Pseudophyllidea). Z Parasitenkd 64:121–134

Gustafsson MKS, Wikgren MC, Karhi TJ, Schot LPC (1985) Immunocytochemical demonstration of neuropeptides and serotonin in the tapeworm *Diphyllobothrium dendriticum*. Cell Tissue Res 240:255–260

Gustafsson MKS, Lehtonen MAI, Sundler F (1986) Immunocytochemical evidence for the presence of "mammalian" neurohormonal peptides in neurones of the tapeworm *Diphyllobothrium dendriticum*. Cell Tissue Res 243:41–49

Hariri M (1974) Quantitative measurements of endogenous levels of acetylcholine and choline in tetrathyridia of *Mesocestoides corti* (Cestoda) by means of combined gas chromatography-mass spectrometry. J Parasitol 60:227–230

Hart JL (1967) Studies on the nervous system of tetrathyridia (Cestoda: *Mesocestoides*). J Parasitol 53:1032–1039

Hillman GR (1983) The neuropharmacology of schistosomes. Pharmacol Ther 22:103–115

Holmes SD, Fairweather I (1984) *Fasciola hepatica*: the effects of neuropharmacological agents upon *in vitro* motility. Exp Parasitol 58:194–208

Holt SJ (1954) A new approach to the cytochemical localization of enzymes. Proc R Soc Lond [Biol] 142:160–169

Keenan L, Koopowitz H (1982) Physiology and *in situ* identification of putative aminergic neurotransmitters in the nervous system of *Gyrocotyle fimbriata*, a parasitic flatworm. J Neurobiol 13:9–21

Koelle GB, Friedenwald JS (1949) A histochemical method for localizing cholinesterase activity. Proc Soc Exp Biol Med 70:617–622

Kotikova EA, Kuperman BI (1977) The development of the nervous apparatus of *Triaenophorus nodulosus* (Cestoidea, Pseudophyllidea) during ontogenesis. Parazitologiya 11:252–259

Kotikova EA, Kuperman BI (1978) The anatomy of the nervous apparatus in cestodes of the families Amphicotylidae and Diphyllobothriidae (Pseudophyllidae). Parazitologiya 12:210–217

Kralj N (1967) Morphologic and histochemical studies on the nervous system of tapeworms revealed by the cholinesterase method (*Taenia hydatigena*, *Dipylidium caninum* and *Moniezia expansa*). Vet Arh 37:277–286

Lee MB, Bueding E, Schiller EL (1978) The occurrence and distribution of 5-hydroxytryptamine in *Hymenolepis diminuta* and *H. nana*. J Parasitol 64:257–264

Leflore WB, Smith BF (1976) The histochemical localization of esterases in whole mounts of *Cysticercus fasciolaris*. Trans Am Microsc Soc 95:73–79

Lui A, Kralj N, Krvavica S, Bečejac S (1964) A contribution to the study of the nervous system of some Anoplocephalidae. Vet Arh 34:112–118

McCullough JS (1984) Studies on the ecology, histochemistry and ultrastructure of *Trilocularia acanthiaevulgaris* (Cestoda; Tetraphyllidea) from the spiny dogfish. PhD Thesis, The Queen's University, Belfast

McCullough JS, Fairweather I (1984) A comparative study of *Trilocularia acanthiaevulgaris* Olsson 1867 (Cestoda, Tetraphyllidea) from the stomach and spiral valve of the spiny dogfish. Z Parasitenkd 70:797–807

McCullough JS, Fairweather I, Montgomery WI (1986) The seasonal occurrence of *Trilocularia acanthiaevulgaris* (Cestoda: Tetraphyllidea) from spiny dogfish in the Irish Sea. Parasitology 93:153–162

Pax RA, Siefker C, Bennett JL (1984) *Schistosoma mansoni*: differences in acetylcholine, dopamine and serotonin control of circular and longitudinal parasite muscle. Exp Parasitol 58:314–324

Pylkkö OO (1956) Cholinesterase in *Diphyllobothrium latum* and *Taenia saginata*. Ann Med Exp Biol Fenn 34:328–334

Rahman MS, Mettrick DF, Podesta RB (1983) Effects of 5-hydroxytryptamine on carbohydrate metabolism in *Hymenolepis diminuta* (Cestoda). Can J Physiol Pharmacol 61:137–143

Ramisz A (1967) Studies on the nervous system of nematodes and cestodes by means of histochemical method for active acetylcholinesterase. III. Nematodes of the genus *Capillaria* and cestodes of the genera *Dilepis* and *Choanotaenia*. Acta Parasitol Pol 14:365–379

Read CP, Simmons JE Jr, Campbell JW, Rothman AH (1960) Permeation and membrane transport in parasitism: studies on a tapeworm – elasmobranch symbiosis. Biol Bull 119:120–133

Rees G (1943) The anatomy of *Anthobothrium auriculatum* (Rud.) (Cestoda) from *Raja batis* L. Parasitology 35:1–10

Rees G (1946) The anatomy of *Phyllobothrium dohrnii* (Oerley) from *Hexanchus griseus* (Gmelin). Parasitology 37:163–171

Rees G (1966) Nerve cells in *Acanthobothrium coronatum* (Rud.) (Cestoda: Tetraphyllidea). Parasitology 56:45–54

Rees G (1973) Cysticercoids of three species of *Tatria* (Cyclophyllidea: Amabiliidae) including *T. octacantha* sp. nov. from the haemocoele of the damsel-fly nymphs *Pyrrhosoma nymphula*, Sulz and *Enallagma cyathigerum*, Charp. Parasitology 66:423–446

Rees G, Williams HH (1965) The functional morphology of the scolex and the genitalia of *Acanthobothrium coronatum* (Rud.) (Cestoda: Tetraphyllidea). Parasitology 55:617–651

Ribeiro P, Webb RA (1983) The synthesis of 5-hydroxytryptamine from tryptophan and 5-hydroxytryptophan in the cestode *Hymenolepis diminuta*. Int J Parasitol 13:101–106

Ribeiro P, Webb RA (1984) The occurrence, synthesis and metabolism of 5-hydroxytryptamine and 5-hydroxytryptophan in the cestode *Hymenolepis diminuta*: a high performance liquid chromatographic study. Comp Biochem Physiol [C] 79:159–164

Ribeiro P, Webb RA (1986) Demonstration of specific high-affinity binding sites for [^{3}H] 5-hydroxytryptamine in the cestode *Hymenolepis diminuta*. Comp Biochem Physiol [C] 84:353–358

Ribeiro P, Webb RA (1987) Characterization of a serotonin transporter and an adenylate cyclase-linked serotonin receptor in the cestode *Hymenolepis diminuta*. Life Sci 40:755–768

Rybicka K (1967) Embryogenesis in *Hymenolepis diminuta*. V. Acetylcholinesterase in embryos. Exp Parasitol 20:263–266

Sangster NC, Mettrick DF (1987) Effects of 5-hydroxytryptamine, cyclic AMP, AMP, and fructose 2,6-bisphosphate on phosphofructokinase activity in *Hymenolepis diminuta*. Comp Biochem Physiol [B] 88:317–321

Schardein JL, Waitz JA (1965) Histochemical studies of esterases in the cuticle and nerve cords of four cyclophyllidean cestodes. J Parasitol 51:356–363

Shield J (1969) *Dipylidium caninum, Echinococcus granulosus* and *Hydatigera taeniaeformis*: histochemical identification of cholinesterases. Exp Parasitol 25:217–231

Shield JM (1971) Histochemical localization of monoamines in the nervous system of *Dipylidium caninum* (Cestoda) by the formaldehyde fluorescence technique. Int J Parasitol 1:135–138

Sukhdeo MVK, Hsu SC, Thompson CS, Mettrick DF (1984) *Hymenolepis diminuta*: behavioural effects of 5-hydroxytryptamine, acetylcholine, histamine and somatostatin. J Parasitol 70:682–688

Thompson CS, Mettrick DF (1984) Neuromuscular physiology of *Hymenolepis diminuta* and *H. microstoma* (Cestoda). Parasitology 89:567–578

Thompson CS, Sangster NC, Mettrick DF (1986) Cholinergic inhibition of muscle contraction in *Hymenolepis diminuta* (Cestoda). Can J Zool 64:2111–2115

Ward SM, Allen JM, McKerr G (1986) Neuromuscular physiology of *Grillotia erinaceus* metacestodes (Cestoda: Trypanorhyncha) *in vitro*. Parasitology 93:121–132

Wardle RA, McLeod JA, Radinovsky S (1974) Advances in the Zoology of Tapeworms, 1950–1970. University of Minnesota Press, Minneapolis

Webb RA (1985) The uptake and metabolism of 5-hydroxytryptamine by tissue slices of the cestode *Hymenolepis diminuta*. Comp Biochem Physiol [C] 80:305–312

Webb RA, Mizukawa K (1985) Serotonin-like immunoreactivity in the cestode *Hymenolepis diminuta*. J Comp Neurol 234:431–440

Wikgren MC (1986) The nervous system of early larval stages of the cestode *Diphyllobothrium dendriticum*. Acta Zool (Stockh) 67:155–163

Williams HH (1959) The anatomy of *Phyllobothrium sinuosiceps* sp. nov. (Cestoda: Tetraphyllidea) from *Hexanchus griseus* (Gmelin), the six-gilled shark. Parasitology 49:54–69

Wilson VCLC, Schiller EL (1969) The neuroanatomy of *Hymenolepis diminuta* and *H. nana*. J Parasitol 55:261–270