STUDIES ON THE NERVOUS SYSTEM OF SOME CYCLOPHYLLIDEAN CESTODES

by

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The work reported in this thesis, except where specifically mentioned, was performed by me.

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The animal world, more particularly that of higher animals, is distinctively signalized by the presence of a nervous system. Most features of animals are shared with other forms of life - for example, metabolism, growth, and inheritance. But the behavior of animals endowed with a nervous system sets them apart by its complexity. The organization and function of the machinery of behavior are surely the highest achievements in the natural world.

- Bullock & Horridge, 1965.

Frontispiece: Dipylidium caninum scolex.
The whole mount is stained by the
5-bromoindoxyl acetate technique
for esterases and much of the
nervous system is evident.
(Photograph taken by Mr I. Fox).

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CHAPTER 1

GENERAL INTRODUCTION

THE PROBLEM

The importance of the nervous system, in controlling physiological and developmental processes by the mediation of neurosecretory substances, has become widely recognised in recent years, and neurosecretory cells have been described in most major animal groups. In the Platyhelminthes, there is some evidence that neurosecretory cells occur in turbellarians (Ude, 1964) and trematodes (Ude, 1962; Gresson & Threadgold, 1964), and this may indicate that a neuroendocrine system exists in this group.

It has been established (Smyth, 1964) that the rostellar gland cells of 35-day adult Echinococcus granulosus are secretory. Smyth speculated (p.523) that 'the secretion could be hormonal in nature and its release may be related to the regulation and maturation of the strobila'. This stimulated the idea that the rostellar gland in E. granulosus might be part of a neurosecretory mechanism. Since this study was commenced, neurosecretory cells have been described in the rostellum of the cestode Hymenolepis diminuta (Davey & Breckenridge, 1967).

Thus, the present study was originally directed towards elucidating the morphological features of the nervous system of E. granulosus, with special reference to any nerve cells which might be neurosecretory. With this aim in view, the paraldehyde fuchsin and chrome-

haematoxylin techniques, which typically stain neurosecretory cells (Gabe, 1966) were applied, but no cells stained satisfactorily. Moreover, less striking and less selective stains were not satisfactory owing to the difficulty in distinguishing the ganglion cells in the small scolex of this species. Several other techniques for nervous tissue were also applied, but none of these stained the nervous system satisfactorily.

It was early realised that E. granulosus, in view of its small size and its refractoriness to selective histological staining, was not very suitable as an experimental organism for this particular problem and that a broader and more basic approach to the problem of the physiology of the nervous system was required.

Accordingly Dipylidium caninum, a species common in local dogs, was chosen as the main experimental organism, but some other cestode species, including E. granulosus, were also examined as they became available.

Using a histochemical approach supplemented by other techniques, it has been possible to describe the morphology of the nervous system in some detail, and to broach some of the problems in understanding the physiology of the cestode nervous system.

MATERIALS

The cestode species used, together with their hosts and method of collection, are listed in Table 1.1. The following details supplement this list.

Most of the adult Dipylidium caninum and Echinococcus granulosus material was obtained by dosing sheep dogs,

from properties near Canberra and Goulburn with $\frac{1}{4}$ to $\frac{1}{2}$ grain of arecoline hydrobromide ('Hydarex'[1]). Adult Hydatigera taeniaeformis was obtained by dosing an adult cat with 1/32 grain of arecoline hydrobromide.

Eggs of fleas (Ctenocephalides felis felis) were collected from naturally infested cats and cultured in the medium and under the environmental conditions recommended by Bruce (1948). The flea larvae were fed small pieces of partially dried gravid proglottids of D. caninum and this procedure resulted in a high incidence of infection. Adult fleas which were infected as larvae were fed for 1 hour daily by holding the gauze-covered end of their glass container in contact with the shaved belly of a kitten (Fig.1.1). However, difficulty was encountered in keeping them alive until the cysticercoids were infective, and only one apparently normal cysticercoid was recovered.

Echinococcus granulosus brood capsules and Taenia hydatigena cysticerci were obtained from infected livers and mesenteries respectively of sheep slaughtered at the Goulburn abattoir. The larval material used for experimental infections of dogs with adult E. granulosus and T. hydatigena originated from the same source.

A Hydatigera taeniaeformis strobilocercus was obtained from a naturally infected wild mouse collected by C.S.I.R.O. Division of Wildlife at Port Essingdon, Coburg Peninsula, Northern Territory of Australia, and supplied through Miss J.L. Hunter, Department of Zoology, A.N.U.

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1 Parke, Davis & Co., Sydney.

Fig. 1.1 Method of restraining cat in order to allow adult fleas to attach through the gauze covering the end of their container.

Taenia pisiformis cysticerci from naturally infected rabbits were fed to dogs in order to obtain adult worms. Oncospheres of T. pisiformis were cultured in vitro by Mr D.D. Heath, Department of Zoology, A.N.U., for 30 days, and small undifferentiated cysticerci were obtained.

Hymenolepis nana adults were obtained from infected mice. The original infected mice were derived from an infected stock maintained at the Department of Parasitology, University of Queensland.

Baerietta criniae minor adults were obtained from frogs, juvenile Pseudophryne corroboree and adult Crinia signifera, collected by Mr R. Pengilley, Department of Zoology, A.N.U., from Coree Flats, Brindabella Ranges, near Canberra.

Details of the techniques used in various aspects of this study are given in the relevant chapters.

TABLE 1.1

Cestode family	Species	Stage	Host	Infected organ	Source of infection	Method of removal
Dilepidae	Dipylidium caninum (Linnaeus, 1758)	adult	dog	small intestine	natural	autopsy dosing
		cysticercoid	Ctenocephalides felis felis (cat flea)	haemocoele	experimental	autopsy
Taeniidae	Echinococcus granulosus (Batsch, 1786)	adult	dog	small intestine	natural experimental	dosing autopsy
		brood capsules	sheep	liver	natural	autopsy
	Hydatigera taeniaeformis (Batsch, 1786)	adult	cat	small intestine	natural	dosing
		strobilocercus	Leggadina delicatula delicatula (wild mouse)	mesenteries	natural	autopsy
	Taenia hydatigena (Pallas, 1766)	adult	dog	small intestine	experimental	autopsy
		cysticercus	sheep	mesenteries	natural	autopsy
	Taenia pisiformis (Bloch, 1780)	adult	dog	small intestine	experimental	autopsy
		cysticercus	rabbit	mesenteries	natural experimental	autopsy autopsy
Hymenolepidae	Hymenolepis nana (Siebold, 1753)	adult	mouse	small intestine	experimental	autopsy
Nematotaeniidae	Baerietta criniae minor Hickman, 1960	adult	Pseudophryne corroboree Crinia signifera	small intestine	natural	autopsy

THE STRUCTURE OF THE CESTODE NERVOUS SYSTEM - A REVIEW

Introduction

What is a nervous system? How do we recognise whether a given animal tissue is, in fact, nervous?

Bullock & Horridge (1965) define the nervous system as 'an organized constellation of cells (neurons) specialized for the repeated conduction of an excited state from receptor sites or from other neurons to effectors or to other neurons'. This definition provides anatomical and physiological criteria to be satisfied by any true nervous system. Thus, presumptive nerve cells must be shown to connect receptor sites with effectors or to connect with each other, and physiological evidence of specialisation for repeated conduction of excitation should support anatomical evidence.

Bullock & Horridge (1965) also point out that there are two practical problems involved in dealing with the nervous system of any one animal: firstly, how to recognise a nerve cell, and secondly, how to prove whether the nervous sytem is, in fact, involved in a particular response to a stimulus. The most basic signs of a nerve cell, even when the axon is reduced in length, are electronmicroscopic or oscillographic evidence of real functional contact with other cells, themselves clearly neurons, and a reasonably brief action potential and refractory period permitting repeated signals at moderate frequencies. These signs may be found even in modified nerve cells, including neuro-secretory cells.

In the Cestoda, the nerve cells and nervous systems have been described on anatomical grounds only - very little physiological evidence on nervous function has been gathered so far.

Interest in the cestode nervous system has been stimulated by the following facts:

(i) The cestodes belong to the phylum Platyhelminthes, the most primitive group of animals at the level of organ-grade construction. The nervous system of these animals is more highly organised than that of the Coelenterata, which, in general, has only an uncentralised network of connected nerve cells. The first centralised nervous systems, including distinct brains, are found in the Platyhelminthes.

(ii) The cestodes are a group of animals extremely adapted to the parasitic habit. They have no gut, no morphologically obvious specialised sense organs, and have no free-living stages apart from embryonic forms which play no active part in entering the host. A knowledge of the nervous system is important in understanding how the parasite interacts with its environment (a) in activation and establishment in the host in which organs sensitive to physico-chemical factors would be important, (b) in co-ordination of movement (probably governed by tactile and rheo-responses of sense organs) which would be important in attachment, and, in apolytic forms, in movement of gravid proglottids in and probably out of the faeces of the host, and (c) in, perhaps, an endocrine control of growth processes and reproduction.

Some of the preceding remarks apply as well to the Trematoda, which is also a highly specialised parasitic group, but which has free-living stages provided with sense organs to deal with their brief sojourn in the outside world.

Although there have been numerous descriptions of the gross morphology of the nervous system of cestodes, very little of the microanatomy and physiology is known. There is considerable difficulty involved in making suitable histological preparations in which the nervous tissue is differentiated sufficiently from the parenchyma for correct identification. This fact has been noted by many authors, and may explain why there are so many discrepancies in detail between descriptions of the nervous system of a particular species by different authors, for example, according to Dollfus (1942), Pintner (1880) described four nerves proceeding anteriorly from the lateral ganglia of Eutetrarhynchus ruficollis; Niemiec (1885), on the other hand, found eight anterior nerves in the same species. It would seem unlikely that this discrepancy is due to intraspecific variation.

Meaningful physiological experimentation on the nervous system of cestodes is difficult, mainly because they remain viable for only a short time on removal from the host. Modern methods of in vitro culture have considerably improved on this state of affairs for several species.

The morphology of the cestode nervous system.

I. Historical.

Until recently, the nervous system of cestodes had been described using morphological criteria only. Because of the difficulty in staining cestode nervous tissue, usually only the main outline of the nervous system was given, with some histological detail.

The first description of the nervous system of a cestode appeared in 1836, when Mueller described a small flat swelling in the scolex of Tetrarhynchus attenuatus (an insufficiently described species of the order Trypanorhyncha). This was followed by the work of Blanchard (1847) who described the nervous system of some taeniids, bothriocephalids and cysticerci. He found in the middle of the scolex a transverse commissure with a ganglionic swelling at both ends. Each ganglion gave rise to a band of nerve fibres which ran posteriorly and anteriorly.

Little was added to this knowledge until the 1880s, when Golgi impregnation techniques, devised for vertebrate nervous tissue, were applied to cestodes. Niemiec (1885) reviewed the literature on the cestode nervous system, and resolved many of the controversies which had arisen since 1836. He gave detailed descriptions of the pattern of the nerve cords and commissures of four cyclophyllidean species, thus providing a basis for further work.

The first microanatomical details of the cestode nervous system were described from silver impregnation and methylene blue preparations. Blochmann (1895)

described a subtegumental nerve plexus, sensory cells and free nerve endings. In 1911 he considered the last mentioned to be parenchymal cells. Zernecke (1895) also described sensory cells and an outer plexus of nerve fibres just beneath the epithelial cells, and, in addition, ganglion cells and an inner plexus at the same level as the nerve cords and ring commissures. Despite the difficulty in distinguishing nervous elements from other tissues e.g. myoblasts, these descriptions have since been found to be remarkably accurate, and very little has been added to this information.

The nervous system of the Platyhelminthes in general, consists of a 'brain' - a complex of ganglia and large commissures near the anterior end - from which a number of longitudinal nerve cords proceed posteriorly. These longitudinal nerve cords lie between the outer, muscular layer, and the internal medullary tissue, and are joined by a number of transverse commissures on the circumference of the medullary tissue. This type of nervous system has been described by Hyman (1951) as the 'barrel-type' longitudinal system. As well as this, they have a fine peripheral network of nerves, the 'diffuse' system of Hyman (1951), which may be homologous with the nerve net of the Coelenterata.

Within this outline, there are variations between and within the Platyhelminth groups. Species of Turbellaria differ considerably from one another in the number of longitudinal nerve cords and commissures, and in the complexity of the brain. The shape of the brain and the number of longitudinal nerve cords (six posterior and six anterior) are remarkably constant in the Trematoda.

The numerically small groups - the Aspidogastrea and the Cestodaria - both have different plans. The Cestoda, although fairly constant in the structure of the 'brain', have a variable number of longitudinal nerve cords (Fig.1.2).

II. The nervous system in cestode groups.

In the following pages, much of our present knowledge of the nervous system of cestodes is summarised. Individual species whose nervous systems have been described are considered together with other members of the order to which they belong. The system of classification used is that given by Yamaguti (1959), unless the species has been described since Yamaguti's treatise was compiled.

1. Order Lecanicephalidea.

So far, knowledge of the nervous system in this order is confined to that of Tylocephalum dierama Shipley & Hornell, 1906 (Lecanicephalidae), whose nervous system was described by Subramaniam (1941 a). The brain is described as a cap-shaped plate, from which approximately 19 pairs of longitudinal nerve cords arise and continue posteriorly. In mature segments this number varies from 32 to 42. These nerve cords are interconnected irregularly by fine fibres, and, at the posterior end of each proglottid, there is a 'plate' commissure, which consists of a meshwork of fibrils. From the main nerves, fibres supply the ovary, testes, vitelline glands, and unicellular sense organs on the inner edge of the tegument.

2. Order Diphyllidea.

Again, knowledge of the nervous system in this order is confined to one species, Ditrachybothrium macrocephalum Rees, 1959. This species resembles closely the diphyllidean genus Echinobothrium, but it does not fit precisely into the order Diphyllidea, since it is similar to the Trypanorhyncha and Pseudophyllidea in the distribution of the yolk glands. The nervous system of this species was described by Rees (1959). Two lateral ganglia are present immediately posterior to the apical organ, and are linked by a transverse commissure. From each lateral ganglion arises one pair of anterior nerves - one dorsal and one ventral, each of which divides into an anterior nerve and a posterior nerve passing backwards to the region of the scolex behind the brain. Two longitudinal nerve cords arise, one in each ganglion, and continue posteriorly. In the scolex, 16 pairs of bothridial nerves originate at intervals in the longitudinal nerve cords.

3. Order Spathebothriidea.

Cooper (1918) briefly described the nervous system of Bothriomonus intermedius Cooper, 1917 (Diplocotylidae). There are two lateral longitudinal nerve cords, each of which gives off a large branch to the lateral wall of the bothria. Anterior to the bothria, the trunks begin to approach each other and each gives off small branches to the other, thus forming an irregular transverse commissure. The tip of the scolex is supplied with a number of small nerves from this commissure.

4. Order Pseudophyllidea.

The nervous system has been described, frequently not in great detail, for a large number of species in the order Pseudophyllidea.

Amphicotylidae.

Cooper (1918) briefly described the nervous system for three species of the Amphicotylidae: Eubothrium crassum (Bloch, 1779), Eubothrium rugosum (Batsch, 1786) and Marsipometra hastata (Linton, 1897). In Eubothrium crassum and E. rugosum, Cooper described two lateral longitudinal nerve cords situated dorsally to the cirrus and the vagina. In E. crassum, the two lateral longitudinal nerve cords enlarge in the scolex, and are united by a small transverse commissure in both E. crassum and E. rugosum. In M. hastata, there are similarly two lateral longitudinal nerve cords which give off a number of branches to the walls of the bothria and enlarge anteriorly to form two ganglia. Each ganglion divides into two large short branches which are joined to the corresponding branches on the opposite side by commissures. An apical branch proceeds anteriorly from each ganglionic branch.

Triaenophoridae.

The nervous system of Fistulicola plicatus (Rudolphi, 1819) was described briefly by Cooper (1918) and that of the plerocercoid of an unidentified species of Triaenophorus, by Lacey (1955). For F. plicatus, Cooper (1918) described only the two lateral longitudinal nerve cords which pass dorsally to the cirrus sac and the vagina. Lacey (1955) described the two longitudinal nerve cords in

plerocercoids of Triaenophorus sp. which, in the scolex, form two lateral ganglionic swellings joined by a median commissure. From each lateral ganglion arises an anterior nerve which probably supplies the hooks.

Diphyllobothriidae.

The nervous systems of a number of species of Diphyllobothriidae have been described: Ligula intestinalis (Linnaeus, 1758) by Cooper (1918), Schistocephalus solidus (Müller, 1776) by Cohn (1898), an unidentified species of Schistocephalus and plerocercoids of an unidentified species of Diphyllobothrium by Lacey (1955). The arrangement of nerves in L. intestinalis is very similar to that in Schistocephalus. Each main longitudinal nerve cord has six accessory longitudinal nerves associated with it, arranged in three groups of two (i.e. two lateral, two dorsal and two ventral). In S. solidus, Cohn (1898) described two ganglia joined by a commissure in the scolex. In the proglottids, the nerve cords provide two series of nerve fibres - one in the transverse plane, supplying the transverse and longitudinal musculature, and the other in a frontal plane, contributing in places to a ring commissure. Lacey (1955) described seven pairs of accessory longitudinal nerve cords in Schistocephalus sp. but no accessory nerves were present in plerocercoids of Diphyllobothrium sp. The bothridia in these plerocercoids were supplied with a pair of nerves from each ganglion.

Bothriocephalidae.

Brief descriptions of the nervous system of Bothriocephalus claviceps (Goeze, 1782), B. occidentalis

(Linton, 1897) and B. manubriformis (Linton, 1889) were given by Cooper (1918), and that of an unidentified species of Bothriocephalus, by Lacey (1955). A more detailed description of the nervous system of B. scorpii (Müller, 1776) was given by Rees (1958) who compared her findings with earlier work by Niemiec (1888) and Lönnberg (1891). Cooper (1918) noted the presence of two lateral longitudinal nerve cords in B. claviceps, B. occidentalis and B. manubriformis. This author showed for B. manubriformis only that these enlarge in the scolex to form lateral ganglia which are connected by a transverse commissure. Lacey (1955) found that, in Bothriocephalus sp., the ganglia are connected by dorsal, ventral and median commissures. In B. scorpii, Rees (1958) described the brain as two bilobed lateral ganglia, connected by a median transverse commissure posteriorly, and dorsal and ventral commissures, anteriorly. A lateral longitudinal nerve cord arises from each ganglion and continues posteriorly into the strobila. Two anterior nerves from each ganglion divide into one or more nerves supplying the tip of the scolex, and a nerve supplying the region of the scolex posterior to the brain. Eighteen pairs of bothridial nerves arising in each longitudinal nerve cord supply the long bothria.

Ptychobothriidae

Cooper (1918) outlined the nervous system in Clesobothrium crassiceps (Rudolphi, 1819). The two lateral longitudinal nerve cords enlarge in the scolex to form lateral ganglia which are connected by a transverse commissure. Anterior nerves arise from the ganglia. Rees (1958) described the nervous system

of C. crassiceps in greater detail. The lateral ganglia are four-lobed structures, connected by dorsal and ventral commissures anteriorly (forming a hexagonal commissure) as well as a median commissure posteriorly. An X-commissure joins diametrically opposite ganglion lobes. Four anterior nerves arise from each ganglion, each dividing into one or more nerves supplying the tip of the scolex, and a nerve supplying that part of the scolex posterior to the brain. From each of the two lateral longitudinal nerve cords, six pairs of nerves supply the bothria.

Haplobothriidae.

Cooper (1918) described the nervous system of the primary scolex (which contains proboscides similar to those of the Trypanorhyncha), and of the primary strobila of Haplobothrium globuliforme Cooper, 1914. Two lateral longitudinal nerve cords are present throughout the strobila. Each is swollen into a very large ganglionic mass at some distance posterior to the proboscis bulbs. The lateral nerve cords are joined further anteriorly, by a large but very irregular transverse commissure. At the point of junction arise large nerves which supply the walls of the proboscis sheaths.

5. Order Tetraphyllidea.

Two families within the order Tetraphyllidea contain species whose nervous systems have been described.

Phyllobothriidae.

The nervous system of Anthobothrium auriculatum (Rudolphi, 1819) has been described by Rees (1943), of Phyllobothrium dohrnii (Oerley, 1885), by Rees (1946),

of the proglottids of Scypophyllideum giganteum (van Beneden, 1858), by Riser (1949) and of Phyllobothrium sinuosiceps Williams, 1959, by Williams (1959). The brain is situated near the anterior end of the scolex and consists of two four-lobed lateral ganglia which are joined by a dorsal and a ventral commissure anteriorly, and a median transverse commissure posteriorly. From each ganglion, a lateral longitudinal nerve cord passes posteriorly and continues throughout the strobila. Four nerves arise from each ganglion and supply the bothridia. In A. auriculatum, each of the four anterior nerves divides into three branches, the two inner branches supplying the myzorhynchus, and the outer nerve extending over the face of the bothridial peduncle. The four ganglia and the commissures of A. auriculatum contain large ganglion cells. P. sinuosiceps has a pair of accessory lateral longitudinal nerve cords supplying each side of the strobila, in addition to the main lateral longitudinal nerve.

Oncobothriidae.

Rees & Williams (1965) and Rees (1966) have described the nervous system of Acanthobothrium coronatum (Rudolphi, 1819). The brain consists of two bilobed lateral ganglia joined by a transverse commissure posteriorly and a dorsal and a ventral commissure anteriorly. Eight anterior nerve cords continue forward from the brain and are joined by an anterior ring commissure. There are five pairs of longitudinal nerve cords throughout the strobila. These are connected by two ring commissures within the scolex, and by approximately 25 ring commissures in each proglottid.

Each bothridium and its pair of hooks is supplied by six pairs of bothridial nerves; two pairs originate from the lateral ganglion, two pairs from the locus of the first ring commissure, and two pairs from the locus of the second ring commissure.

The nervous system is not set off from the parenchyma by a discrete limiting layer of tissue. Ganglion cells are present only in the median transverse commissure, and nerve cells are present in the main longitudinal nerve cords within the scolex. The so-called 'binding cells' of Tower (1900) are present around the longitudinal nerve cords in the strobila. Probable sensory cells, which have long processes extending to muscle fibres, occur in the scolex and may be stretch receptors (Rees, 1966).

6. Order Trypanorhyncha.

The nervous system in species of the Trypanorhyncha is remarkably constant in general form, possibly because of the presence of the proboscides in the scolex. Many of the earlier studies on the nervous sytem of trypanorhynchs have been reviewed by Dollfus (1942), and the information presented by the following authors, Pintner (1880; 1893; 1925; 1930), Lang (1881), Niemiec (1885; 1888), Lönnberg (1891), Poyarkoff (1909), and Nybelin (1918), and given here, has been derived from this source.

Hepatoxylidae

The nervous systems of the adult stage of Hepatoxylon trichiuri and plerocercoid larva of Dibothriorhynchus grossum were described by Lönnberg (1891) and Rees (1941 a)

respectively. According to Yamaguti (1959), these species are synonymous and their correct designation is Hepatoxylon squali (? Martinière, 1797).

The lateral ganglia of H. squali are four-lobed structures, joined by a dorsal and a ventral commissure anteriorly, and by a median commissure posteriorly. Two lateral longitudinal nerve cords arise in the ganglia and continue throughout the strobila. The anterior region of the scolex is supplied by two pairs of nerves from each ganglion, and the bothridia, by nerves from the lateral ganglia and the lateral longitudinal nerve cords. In each ganglion two nerves arise, each supplying a proboscis.

Gilquiniidae.

The nervous system of Gilquinia squali (Fabricius, 1794) has been described by Niemiec (1888), Lönnberg (1891) and Mackenzie (1965), and Aporhynchus norvegicum (Olsson, 1868), by Nybelin (1918) and Rees (1941 b). In G. squali, the ganglia are bilobed structures, joined by three commissures as in H. squali. Two anterior nerves arise in each ganglion, and two pairs of nerves, one pair directed anteriorly and another posteriorly, supply the bothridia. The proboscis nerves, one to each proboscis sheath, arise in the lateral ganglia. Two lateral longitudinal nerve cords are present throughout the strobila. The nervous system of Aporhynchus norvegicum is very similar to that of H. squali. The lateral ganglia are four-lobed structures. From each ganglion two pairs of anterior nerves supply the tip of the scolex. Six pairs of nerves supply each bothridium, three pairs from each ganglion and three pairs from each

lateral longitudinal nerve cord. As proboscides are absent, there are no proboscis nerves.

Lacistorhynchidae.

The nervous system has been described in the following species: Grillotia erinaceus (van Beneden, 1858) by Johnstone (1911), G. scolecina (Rudolphi, 1819) by Rees (1950) and Lacistorhynchus tenue proglottids by Riser (1949). The structure of the nervous system is very similar to that in Hepatoxylon squali. The lateral ganglia are four-lobed in G. erinaceus and bilobed in G. scolecina. In G. scolecina, two pairs of anterior nerves arise from each ganglion, one pair from each ganglionic lobe. In G. erinaceus, two pairs of bothridial nerves arise in each ganglion. In G. scolecina, there are three pairs of bothridial nerves, one pair branching from the anterior nerves, one pair from each lateral ganglion, and one pair from each lateral longitudinal nerve cord. In G. erinaceus a columnar ganglion containing ganglion cells is present as a swelling on each lateral longitudinal nerve cord just posterior to the lateral ganglion. The proboscis nerves originate in the columnar ganglia in G. erinaceus, and the lateral ganglia in G. scolecina. Interproglottidal nerve commissures linking the two lateral longitudinal nerve cords are present in L. tenue.

Tentaculariidae.

Pintner (1925) described the nervous system of Tentacularia coryphaenae Bosc, 1797 (as Stenobothrium macrobothrium) and later (1930), of Nybelinia syngenes Pintner, 1928. The nervous sytem in both these species

is similar to that of Hepatoxylon squali. Four anterior nerves are present, and each main lateral longitudinal nerve cord gives off two accessory longitudinal nerves which lie very close to the main longitudinal nerve cord. The proboscis nerves arise in the lateral ganglia.

Eutetrarhynchidae.

The nervous system of Eutetrarhynchus ruficolle (Eysenhardt, 1829) has been described by Pintner (1880) and Niemiec (1885). Two pairs of anterior nerves arise in each ganglion. A single proboscis nerve from each ganglion divides into two near its origin, each branch supplying a proboscis sheath. Bothridial nerves also arise in each lateral ganglion, just posterior to the junction with the dorsal and ventral commissures.

Gymnorhynchidae.

Lang (1881) described the nervous system in Gymnorhynchus gigas (Cuvier, 1817). The nervous system is very similar to that in Hepatoxylon squali, except that eight anterior nerves are present, four arising from each lateral ganglion.

The nervous system has also been described in a number of trypanorhynch species which are species incertae sedis, according to Yamaguti (1959). These include Tetrarhynchus papillifer (by Poyarkoff, 1909), Tetrarhynchus gracilis (by Lang, 1881), Tetrarhynchus smaridum (by Pintner, 1893) and Tentacularia macropora proglottids (by Subramaniam, 1940). Lacey (1955) has described the nervous system in an unidentified plerocercus larval stage of a trypanorhynch. These descriptions are all very similar to those given above, except for

Tentacularia macropora, in which Subramaniam (1940) claimed that a large number, approximately 60, longitudinal nerve cords are present in the proglottids, Judging from copies of his photographs, it would appear that the structures described as nerve cords are probably longitudinal muscle bundles. He described the innervation of various internal organs including the ovary by fibrils from a nervous plexus situated between the vitelline glands and the testicular vesicles.

For the Trypanorhyncha in general, the brain consists of two lateral ganglia, joined by a dorsal and a ventral commissure anteriorly, and by a median commissure posteriorly. Each ganglion consists of two (a dorsal and a ventral) or four (anterior and posterior dorsal, and anterior and posterior ventral) lobes which are fused posteriorly. There may be two or four anterior nerves arising from each ganglion. Two lateral longitudinal nerve cords are present throughout the strobila each arising from the posterior point of fusion of the dorsal and ventral ganglionic lobes of the corresponding lateral ganglion. The bothridia are supplied with anterior, lateral, and/or posterior nerves from the lateral ganglia, and/or lateral nerves from the longitudinal nerve cords within the scolex. Four proboscis nerves arise, either two from each ganglion, or two from each longitudinal nerve cord, close to the posterior end of the ganglion. These follow the proboscis sheaths to the base of the bulbs.

The median transverse commissure contains bipolar and multipolar nerve cells, and neurocordal cells. There are no nerve cells in the lateral 'ganglia' and the

dorsal and ventral commissures. The lateral 'ganglia' cannot therefore be considered as true ganglia, since they contain no nerve cells. In Grillotia erinaceus, the brain is covered by a delicate capsule containing nuclei (Johnstone, 1911). Neurocords, which have been described only in this group of cestodes, will be considered later.

7. Order Cyclophyllidea.

This is a very large and comparatively diverse order. The main differences in nervous system morphology found within this group are in the number of longitudinal nerve cords, and in the arrangement of nerves in the scolex. The latter is probably associated with the presence or absence of a rostellum.

Taeniidae.

Descriptions of the nervous system include those of the following species: Taenia pisiformis (Bloch, 1780), by Niemiec (1885) and Lacey (1955); Hydatigera taeniaeformis adult by Cohn (1898) and Bartels (1902), and strobilocercus by Rees (1951); Multiceps multiceps (Leske, 1780) by Niemiec (1885), and Taeniarhynchus saginatum (Goeze, 1782) by Niemiec (1885) and by Cohn (1898).

Dilepidae.

The nervous system of Dipylidium caninum has been described by Niemiec (1885).

The pattern in the nervous system is essentially the same in species of Taeniidae and Dilepidae. There are

ten longitundinal nerve cords throughout the strobila, where they are linked by ring commissures. In the scolex, the three lateral nerve cords on each side run together to form the lateral ganglia. The anterior extensions of the lateral ganglia and the median nerve cords continue forward and meet the rostellar nerve ring. The lateral ganglia are joined by a transverse cerebral commissure, and by one or more polygonal commissures which unite them with the median longitudinal nerve cords. The latter are also linked by an X-commissure. Nerves from the rostellar nerve ring supply the rostellum, and nerves from the lateral ganglia and median longitudinal nerve cords supply the suckers.

Anoplocephalidae.

Within this family, the number of longitudinal nerve cords varies from species to species. The nervous system of three species belonging to the sub-family Thysanosomatinae have been described: Thysanosoma actinoides Diesing, 1835 by Lacey (1955); Avitellina lahorea Woodland, 1927 by Subramaniam (1941 b); and A. centripunctata (Rivolta, 1874) by Gough (1911). The nervous systems of four species of the sub-family Anoplocephalinae have also been described: Moniezia expansa (Rudolphi, 1805) by Tower (1896; 1900); M. benedeni (Moniez, 1879) by Tower (1896) as M. planissima; Anoplocephala magna (Abildgaard, 1789) by Becker (1922); and A. perifoliata (Goeze, 1782) by Cohn (1898).

Thysanosoma actinoides, Anoplocephala magna and A. perfoliata all have ten longitudinal nerve cords, and the arrangement of ganglia, nerve cords and commissures in the scolex is similar to that found in the Taeniidae.

Moniezia expansa and M. benedeni have six longitudinal nerve cords, the accessory lateral nerve cords being absent. Within the scolex of M. expansa the nervous system is very similar to that of the Taeniidae, except that the lateral ganglia themselves curve medially and fuse in the centre of the scolex, instead of being joined by a cerebral commissure.

Both Avitellina lahorea and A. centripunctata have only two longitudinal nerve cords. In A. centripunctata, four ganglia, two lateral, one dorsal and one ventral, have been described in the scolex. Each of these is joined to the adjacent and to the diametrically opposite ganglion by commissures. A central nerve plate occurs anterior to this complex, but no connections between the plate and other elements of the nervous system have been established.

Davaineidae.

Siddiqi (1961) described the nervous system of Cotugnia digonopora (Pasquale, 1890). Two longitudinal nerve cords are present, and, in the scolex, the central nervous system comprises an anterior rostellar nerve ring, a cephalic ganglionic mass dorso-ventrally compressed and laterally elongated, and eight sucker nerves, two to each sucker.

Tetrabothriidae.

This family has closer affinities to Pseudophyllidea and Tetraphyllidea (see Wardle & McLeod, 1952) than do most other cyclophyllidean families, and therefore one might expect the nervous system to have a different

pattern. The nervous system of Tetrabothrius affinis (Lönnberg, 1891) has been described by Rees (1956). Six longitudinal nerve cords run throughout the strobila. At the base of the scolex these are linked by a hexagonal nerve ring. The longitudinal nerves continue forwards, the lateral nerves becoming the lateral ganglia. These are connected by the median transverse commissure and a little further forward by a ring commissure. An X-commissure is also present. Two anterior nerves on each side supply the apical organ while bothridial nerves arise from the lateral ganglion and the lateral portion of the hexagonal nerve ring. Spätlich (1909) also described six longitudinal nerve cords in T. laccocephalus Spätlich, 1909.

Tower (1900) described ganglion cells in the lateral ganglia and nerve cords of Moniezia expansa, and also binding cells covering the lateral ganglia, and the nerve trunks within mature proglottids. Nerves occur in the suckers of Avitellina centripunctata and Anoplocephala magna, according to Gough (1911) and Becker (1922) respectively.

III. Basic variation in the morphology of the nervous system.

It is possible to classify the cestode central nervous system into five morphological types based on those described in the foregoing review, provided that nerves supplying structures in the scolex such as suckers and proboscides are omitted from the discussion (see Fig. 1.2).

(i) The basic pattern consists of two lateral longitudinal nerve cords, which become the lateral ganglia in the scolex. The lateral ganglia are joined by a single

median transverse commissure. From the lateral ganglia and lateral nerve cords, nerves arise, supplying the muscular organs and tegument of the scolex and strobila. The Diphyllidea and Spathebothriidea have this type of nervous system. In all other cestodes these features are present, together with additional commissures and/or nerve cords.

(ii) The second type has linking the lateral ganglia two commissures, one dorsal and one ventral, in addition to the median transverse commissure. The dorsal and ventral commissures together form a 'ring' commissure, which joins the ganglia in front of the median transverse commissure. The lateral ganglia may consist of two or four lobes. This condition is found in the Pseudophyllidea apart from Ligula intestinalis and Schistocephalus solidus, in the Tetraphyllidea apart from Phyllobothrium sinuosiceps and Acanthobothrium coronatum, and in the Trypanorhyncha apart from the Tentacularidae.

(iii) Six longitudinal nerve cords are present in the third type of nervous system. There may be two accessory lateral longitudinal nerve cords on each side in addition to the main lateral longitudinal nerve cords (Fig. 1.2), or there may be two dorsal and two ventral median longitudinal nerve cords. The arrangement of the nervous system in the scolex is similar to the second type above, the accessory lateral nerve cords fusing with the main lateral nerve cord on each side to form the lateral ganglia. The tetraphyllidean Phyllobothrium sinuosiceps and the trypanorhynch family Tentacularidae belong to this group.

(iv) In the fourth type, ten longitudinal nerve cords are present in the strobila, three pairs of lateral nerve cords, and two dorsal and two ventral median nerve cords. In the scolex, the arrangement is usually more complex than the patterns described above. There is an additional complete or incomplete dorsal and ventral commissure linking the lateral ganglia with the dorsal and ventral nerve cords, together with an X-commissure linking diametrically opposite median longitudinal nerve cords via the cerebral (median transverse) commissure. The median longitudinal nerve cords and the anterior extensions of the lateral ganglia meet the apical, or rostellar, nerve ring, from which nerves supplying the apex and rostellar structures arise. This is the basic arrangement for the Cyclophyllidea. In this group, some species have a reduction in the number of nerve cords, but the arrangement of nerves in the scolex generally remains the same. Ten longitudinal nerve cords are also present in the tetraphyllid, Acanthobothrium coronatum (Rees, 1966) but this species has no apical nerve ring.

(v) The fifth type is a very simple nervous system, and consists of the basic arrangement, together with additional longitudinal nerve cords. Cohn (1898) claimed that there are 14 longitudinal nerve cords in the pseudophyllid species Ligula intestinalis and Schistocephalus solidus, while Lacey (1955) described 16 in Schistocephalus sp. Subramaniam (1941 a) described a large number (32 to 42) of longitudinal nerve cords in the lecanicephalid, Tylocephalum dierama. Subramaniam (1940) also described a large number of longitudinal nerve cords in an insufficiently described trypanorhynch,

Fig. 1.2. Basic variation in the morphology of the cestode nervous system, drawn from the ventral aspect.

(i) Basic diphyllidean type.

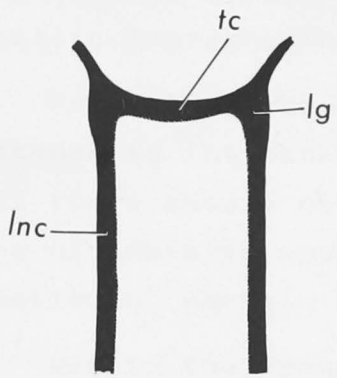
(ii) Pseudophyllidean type.

(iii) Pattern exhibited by the Tentacularidae. The dorsal accessory lateral nerve cords are not shown.

(iv) Basic cyclophyllidean pattern. The dorsal accessory lateral nerve cords are not shown.

(v) Ligula type, the ventral nerves only are shown. (alnc, accessory lateral nerve cord; dc, dorsal commissure; dnc, dorsal nerve cord; lg, lateral ganglion; lnc, lateral nerve cord; mlnc, main lateral nerve cord; pc, polygonal commissure; rnr, rostellar nerve ring; tc, transverse commissure; vc, ventral commissure; vnc, ventral nerve cord; xc, X-commissure).

(i)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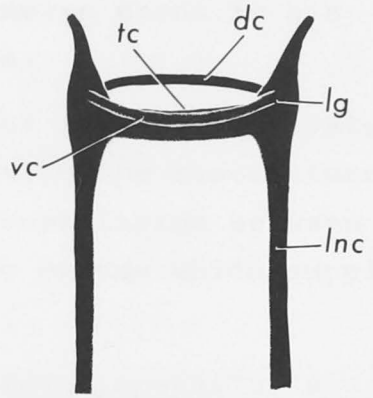
(ii)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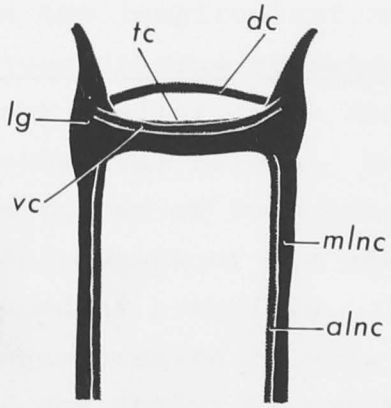
(iii)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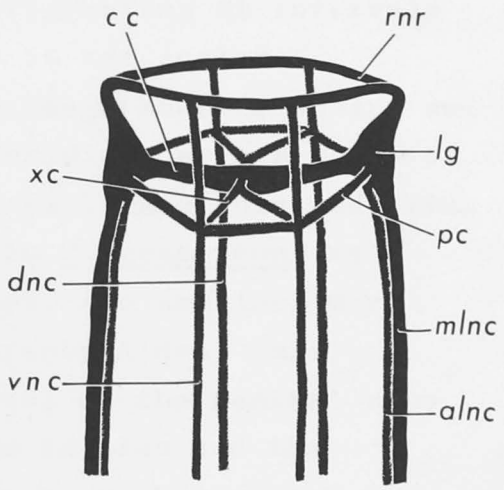
(iv)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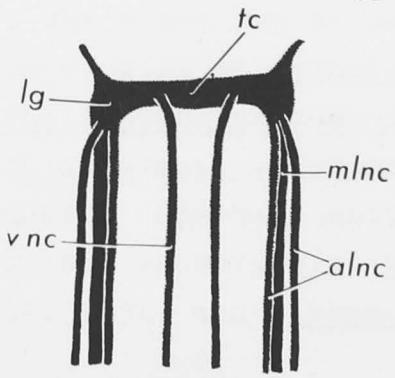
xc
pc
dnc
mlnc
vnc
alnc

(v)


tc
lg
mlnc
vnc
alnc

Tentacularia macropora. As noted earlier, it is possible that in these two species, Subramaniam may have confused longitudinal muscle bundles with nerve cords in his metallic impregnation preparations.

Rees (1958) has suggested that the nervous system in cestodes is intimately connected with the musculature and that there should obviously be a correlation between the type of adhesive apparatus and the nerves which supply its constituent parts.

Within the Pseudophyllidea, Bothriocephalus scorpii, which has two very long, weakly muscular bothridia, has 18 pairs of bothridial nerves originating at intervals in the longitudinal nerve cords in the scolex. Clesobothrium crassiceps, which has a shorter scolex and four smaller, more muscular bothridia, has six pairs of bothridial nerves. Rees (1958) explained this reduction in number of bothridial nerves in C. crassiceps as a consequence of the shorter scolex, and smaller, more muscular bothridia. In the Tetraphyllidea, this concentration is even more marked, as the central zone of the scolex is greatly reduced in size and the bothridial nerves, which are few in number, arise in almost the same place, e.g. in Anthobothrium auriculatum (Rees, 1943) and Phyllobothrium dohrnii (Rees, 1946). Those cestodes with an almost spherical scolex and four highly muscular sucking cups have only one to three separate nerves to each sucker, e.g. Hydatigera taeniaeformis (Rees, 1951) and Tetrabothrius affinis (Rees, 1956).

The principle suggested by Rees (1958) also applies to nerves supplying other types of attachment organs.

In the Trypanorhyncha, as mentioned earlier, each proboscis sheath is supplied with a nerve arising in or near the nearest lateral ganglion. The innervation of the proboscides is very similar in all species, since the gross morphology of the proboscides is essentially uniform.

IV. Sense organs.

Hyman (1951) states that sensory cells are of two types: (i) sensory nerve cells, or neurosensory cells, which are essentially nerve cells with an axon running to the central nervous system, and (ii) non-nervous sensory cells which are modified epithelial cells and must be supplied by fibres from the nervous system.

The sensory cells of cestodes are mostly of the neurosensory type. As mentioned earlier, these were first described by Blochmann (1895) and Zernecke (1895). They consist of a cell body lying deep in the outer, muscular zone of the body, with a process ending below or in the tegument, and an axon to the inner plexus on the periphery of the internal medullary region of the proglottid. Free nerve endings have been frequently described. They have a variety of shapes, and may be a simple process (Morseth, 1966), or a bulb with (Morseth, 1967 b) or without (Blochmann, 1895) a small process protruding beyond the tegument. There may be a small funnel within the tegument with its base near the apex of the bulb (Dollfus, 1942). These are possibly long processes of sensory cells which could not be traced to the cell body.

Other types of sense organs described in various trypanorhynchs have been reviewed by Dollfus (1942).

Eversible sensory pits in the frontal margin of the bothridia, occur in the Otobothriidae. Each is a compact organ consisting of a pouch with a slit-like opening. Circular and radial muscle fibres are responsible for the motility of the organ, and it is supplied with sensory hairs similar to those of the rest of the tegument. Peribothridian sensory grooves are similar to the eversible pits, apart from their elongate shape. They are found in the margin of the bothridia of Grillotia scolecina.

A sensory function for these organs is conjectured, purely on morphological grounds and has not been confirmed by physiological experimentation.

V. Neurocords.

Descriptions of these structures in cestodes were reviewed by Dollfus (1942). Neurocords have been reported only by Pintner (1925, 1934), who described them in a number of trypanorhynch species.

The neurocords are giant fibres which closely accompany the lateral longitudinal nerves. They link the various parts of the central nervous system by frequently leaving one nerve and joining another. The cell body of the neurocord which is large and stellate and contains a large nucleus and prominent nucleolus is usually located near the origin of the neurocordal fibre. Some cell bodies are located in the brain, others are found in anastomoses between the proboscis nerves and the longitudinal nerve cords.

Because of their size, structure, and their direct contact with separate parts of the nervous system, they were considered by Pintner (1934) to function in the transmission of motor stimuli.

They are probably analogous to the giant nerve fibres found in many other invertebrate groups (Prosser & Brown, 1961). These giant fibres function in fast conduction of nerve impulses. Thus, in the earthworm, giant fibres mediate quick end-to-end startle contractions, in crustaceans they control quick flipping of the abdomen, and in cephalopod molluscs, stimulation of a giant fibre elicits maximal contraction of the mantle muscle. According to Prosser & Brown (1961), giant fibres have evolved many times and may be absent from animals closely related to those which have them. It is therefore possible that neurocords exist only in a few species of cestodes.

CHAPTER 2

A CONTRIBUTION TO THE MORPHOLOGY OF THE NERVOUS SYSTEM IN SIX CYCLOPHYLLIDEAN CESTODES

INTRODUCTION

When some histochemical tests for esterases are used on platyhelminth material, it has been frequently found that the reaction product of the enzyme hydrolysis is localised in the nervous system (for example, see Lee, Rothman & Senturia, 1963; Halton & Jennings, 1964). Such techniques include the 5-bromoindoxyl acetate technique of Holt & Withers (1952) and a number of different methods involving the use of acetylcholine and butyrylcholine and their corresponding thiol esters and halide salts as substrates. These techniques are therefore very valuable in augmenting the results from routine histological procedures which do not stain the cestode nervous system as selectively; moreover, they reveal fine details which are rarely encountered in routinely prepared material.

In the present study, histochemical techniques for esterases have been applied in the study of the nervous system of six cyclophyllidean cestodes.

MATERIALS AND METHODS

The nervous system in adult Dipylidium caninum, Echinococcus granulosus, Hydatigera taeniaeformis,

Taenia pisiformis, Baerietta criniae minor and cysticercoid of D. caninum were studied in some detail. Additional information on the structure of the cestode nervous system was obtained by observations on E. granulosus brood capsules, H. taeniaeformis strobilocerci, Taenia hydatigena adults and cysticerci, and T. pisiformis cysticerci and cultured oncospheres.

The cestode material and the techniques used for studies described in this chapter are summarised in Table 2.1.

Sections of material fixed in 4 per cent formaldehyde at 4°C were cut in the cryostat, and either the Holt & Withers (1952) technique for non-specific esterases or the Karnovsky & Roots (1964) technique for cholinesterases was employed. Further details are given in chapter 4. Whole mounts of D. caninum and B. criniae minor were incubated in the media of Holt & Withers and Karnovsky & Roots respectively. Cultured oncospheres of T. pisiformis and portions of E. granulosus brood capsule wall and T. hydatigena cyst wall were also prepared as whole mounts. Sections of adult E. granulosus and H. taeniaeformis, after incubation in the medium Karnovsky & Roots, were counterstained in Gower's carmine (see Johri & Smyth, 1956) in order to show the relationship of other organs to the nervous system.

Histological techniques were carried out on paraffin sections. Both Zenker's and Bouin's fixatives contained formaldehyde, but no ethanol.

TABLE 2.1

Species	Stage	Fixative	Staining Technique	Reference
Dipylidium caninum	adult	4% formaldehyde	5-bromoindoxyl acetate	Holt & Withers (1952)
			Acetylthiocholine iodide	Karnovsky & Roots (1964)
		Zenker's fixative	Maximow	see appendix 1
			Heidenhain's azan	Lillie (1954)
		Bouin's fixative	Paraldehyde fuchsin	Cameron & Steele (1959)
			Chromalum-haematoxylin	Gomori (1939)
	cysticercoid	4% formaldehyde	Acetylthiocholine iodide	Karnovsky & Roots (1964)
Echinococcus granulosus	adult	4% formaldehyde	Acetylthiocholine iodide	Karnovsky & Roots (1964)
	daughter cyst	4% formaldehyde	5-bromoindoxyl acetate	Holt & Withers (1952)
Hydatigera taeniaeformis	adult	4% formaldehyde	Acetylthiocholine iodide	Karnovsky & Roots (1964)
	strobilocercus	4% formaldehyde	Acetylthiocholine iodide	Karnovsky & Roots (1964)
Taenia hydatigena	adult	Zenker's fixative	Gabe	see appendix 1
			Mayer's haemalum & eosin	Culling (1963)
	cysticercus	4% formaldehyde	5-bromoindoxyl acetate	Holt & Withers (1952)
			Acetylthiocholine iodide	Karnovsky & Roots (1964)
Taenia pisiformis	adult	4% formaldehyde	Acetylthiocholine iodide	Karnovsky & Roots (1964)
	cysticercus	Zenker's fixative	Gabe	see appendix 1
	cultured oncospheres	4% formaldehyde	Acetylthiocholine iodide	Karnovsky & Roots (1964)
Baerietta criniae minor	adult	4% formaldehyde	Acetylthiocholine iodide	Karnovsky & Roots (1964)

RESULTS

I. The central nervous system:

1. Arrangement.

For the purposes of this description, the central nervous system is considered to comprise the system of nerve cords, ganglia and commissures, and the major nerves supplying structures in the scolex. The arrangement of these elements in adult D. caninum, E. granulosus, H. taeniaeformis, T. pisiformis, B. criniae minor and cysticercoid of D. caninum conforms to the basic cyclophyllidean pattern described in chapter 1. This is illustrated semi-diagrammatically in Figs. 2.1, 2.2, 2.3, 2.4, 2.5.

There are ten longitudinal nerve cords: two prominent 'main' lateral nerve cords, two pairs of accessory lateral nerve cords, and two ventral and two dorsal median nerve cords (Figs. 2.6, 2.8, 2.10, 2.16, 2.23, 2.33, 2.34, 2.40, 2.46). In D. caninum adult, the accessory lateral nerve cords are relatively closer to the lateral margin of the proglottids than in the other species studies. Dorsal and ventral nerve cords were not detected in gravid segments of B. criniae minor (Figs. 2.35, 2.36). The longitudinal nerve cords are linked, in each proglottid, by an interproglottidal nerve ring in D. caninum (Fig. 2.8), H. taeniaeformis and T. pisiformis, but an organised interproglottidal nerve ring was not observed in E. granulosus or B. criniae minor.

In the scolex, the accessory lateral longitudinal nerve cords on each side converge towards the corresponding

main lateral longitudinal nerve cord and fuse to form the lateral ganglia (Figs. 2.7, 2.15, 2.29, 2.39). A large transverse commissure, the cerebral commissure, connects the lateral ganglia (Figs. 2.9, 2.14, 2.22, 2.39). The lateral ganglia are also linked to the dorsal and ventral median commissures by a polygonal commissure (Fig. 2.14). An X-commissure links diametrically opposite median commissures via the cerebral commissure in D. caninum, E. granulosus, H. taeniaeformis (Fig. 2.22) and T. pisiformis, but was not detected in B. criniae minor. In H. taeniaeformis, the X-commissure is connected with the lateral ganglia by small accessory nerves (Fig. 2.30). Large nerves, two from each lateral ganglion and one from each of the median longitudinal nerve cords (Figs. 2.15, 2.29, 2.30, 2.50) supply the suckers.

The anterior extensions of the lateral ganglia and dorsal and ventral median nerve cords meet the rostellar nerve ring (Figs. 2.13, 2.21, 2.38). In B. criniae minor, a similar structure is termed the apical nerve ring (Fig. 2.32) since no rostellum is present. From the rostellar nerve ring, apical nerves arise which supply the rostellar structures - the rostellar pad, the rostellar gland and the tegument (Figs. 2.21, 2.27). The number of these apical nerves was difficult to determine. At least two are present in D. caninum (Fig. 2.6) and there are probably more in E. granulosus (Figs. 2.13). In H. taeniaeformis (Figs. 2.21, 2.27), it was again difficult to count the apical nerves. In these preparations, branches penetrating the rostellar pad number from 18 to 21, while counts of nerves which do not penetrate the rostellar pad varied from 21 to 24. The number counted

depended partly on the angle of the section, but on no occasion was the number of anterior nerves found to equal the number of hooks (30 in the specimens studied). A large number of longitudinal nerve cords is present in the terminal bladder of H. taeniaeformis strobilocercus (Fig. 2.47).

2. Histology.

Histological sections of D. caninum, H. taeniaeformis, T. pisiformis and T. hydatigena revealed the presence of nerve cells in the lateral ganglia, the cerebral commissure, and in the main lateral longitudinal nerve cords.

In D. caninum, nerve cells of the lateral ganglia and cerebral commissure vary in size. There appear to be two main size groups (see Fig. 2.56). The larger cells have large nuclei and prominent nucleoli (Figs. 2.53, 2.54, 2.55), and the cytoplasm stains with both Heidenhain's azan (Fig. 2.56) and chromalum-haematoxylin (Fig. 2.52). Smaller nerve cells are also found in the lateral ganglia and in the main lateral longitudinal nerve cords. Very small cells, which may be binding cells, are present on the outside of the lateral ganglia (Fig. 2.56). Paraldehyde fuchsin did not stain any of the nerve cells.

Elongate neurons which stain with Gower's carmine occur between the two lateral lobes of the lateral ganglion of H. taeniaeformis (Fig. 2.30). Nerve cells also occur in the cerebral commissure.

The lateral ganglia of the cysticercus of T. pisiformis contain large elongate neurons (Fig. 2.48). The cytoplasm of these cells stains lightly with the

haematoxylin of Gabe's stain, and the large nuclei contain prominent nucleoli.

In Gabe-stained sections of adult T. hydatigena, the nerve cells were easily distinguishable. The two lobes of the lateral ganglia contain mostly nerve fibres and are separated by some bipolar neurons (Fig. 2.58). A large number of nerve cells is present in the cerebral commissure (Fig. 2.57), some of which are multipolar (Fig. 2.59). Very small binding cells, whose cytoplasm stains with the light green of Gabe's stain, surround the cerebral commissure (Figs. 2.57, 2.59) and lateral ganglia.

The nerve cords consist of a core of fibres, with nerve cells on the periphery (Figs. 2.60, 2.61). Most of these nerve cells are small and elongate in shape, but there are a few larger cells in the main lateral longitudinal nerve cords of T. hydatigena. Nerve cells are also present in the rostellar nerve ring and polygonal commissure.

II. The peripheral nervous system:

1. The inner plexus.

Zernecke (1895) first used the German equivalent of this term (innerer Gefässplexus) for the series of nerve fibres which link the longitudinal nerve cords in Ligula intestinalis. In the species studied here, the nerves of the inner plexus occur in the same region as the inner circular muscles of the proglottids (Figs. 2.16, 2.23, 2.28, 2.31, 2.33, 2.46), and are oriented mainly in an approximately transverse direction. The ring commissures

found in the proglottids of species mentioned earlier appear to be a concentration of some of these nerve fibres in the interproglottidal region.

2. The peripheral plexus.

This is a network of nerve fibres which is found among the processes of the tegument cells, directly beneath the distal cytoplasm of the tegument (Figs. 2.13, 2.25, 2.26, 2.28). A peripheral network is present in preparations of all species stained with the Karnovsky & Roots (1964) technique, and occur in all parts of the body. In the scolex and proglottids of H. taeniaeformis, the fibres are oriented both transversely and longitudinally. Here, they may supply the peripheral muscle fibres found in the same part of the body.

A network of fibres similar to that observed in the tegument of H. taeniaeformis is present in the bladder wall of the cysticercus of T. hydatigena (Fig. 2.62) and cultured oncospheres of T. pisiformis (Fig. 2.63). In T. hydatigena, these appear to follow the muscle bundles of the bladder wall. In whole mounts of the daughter cyst of E. granulosus, there is a network of 5-bromoindoxyl acetate-positive cells (Fig. 2.65). Near the stalk connecting the daughter cyst wall with a protoscolex, a process of each cell is directed towards, and disappears into, the stalk (Fig. 2.64). The nerve fibre could not be followed further since a localised reaction did not take place within the body of the protoscolex. This was probably due to the inability of the 5-bromoindoxyl acetate medium to penetrate the PAS-positive layer which is known to surround the protoscolex (Morseth, 1967 a). The

germinative cells found in the germinal membrane of the hydatid cyst contain granules which stain positively with 5-bromoindoxyl acetate.

3. Nerve endings.

Nerve endings arise from fibres originating in the longitudinal nerve cords, and from the peripheral nerve plexus. They vary in shape and size, and occur in the tegument of the scolex and strobila. In adult D. caninum, they end in very small bulbs (Figs. 2.11, 2.12). Nerve endings of other shapes may be present as in H. taeniaeformis, see below, but were not observed in this species. The tegument of the cysticercoid also contains bulbous nerve endings which are smaller in the scolex than in the posterior part of the body (compare Figs. 2.39 and 2.40, which are of the same magnification).

In E. granulosus the nerve endings were usually obscured by the intense cholinesterase-positive reaction given by the tegument, but were found in a few thin sections.

There are at least two different types of nerve endings in the tegument of H. taeniaeformis. A bulbous type with a small process (Fig. 2.26) occurs in the tegument of the scolex anterior to the suckers, and a tubular type in the tegument of the scolex, where they are comparatively large (Fig. 2.25) and in the strobila, where they are smaller.

In the scolex of adult T. pisiformis, the nerve endings in the tegument including that of the sucker are almost triangular in shape. In a thin slice preparation

of this species it can be seen that the nerve ending is the terminal of a process from a sensory cell. The cell body occurs close to, and is connected by, an axon to either the nerve plexus in the sucker (Fig. 2.51), or a longitudinal nerve cord, ganglion, or ring commissure, in other parts of the scolex.

Some of the nerve endings in B. criniae minor are tubular (Fig. 2.31), while others resemble a small knob (Fig. 2.34). In one of the nerve endings observed, the base and tip of the structure is strongly cholinesterase-positive, while the middle portion is only slightly positive (Fig. 2.35).

A large number of nerve endings is present in the lip of the sucker and in the tegument immediately surrounding the orifice of the sucker in D. caninum (Fig. 2.12), H. taeniaeformis (Figs. 2.22, 2.30), T. pisiformis and B. criniae minor (Figs. 2.31, 2.32).

4. Innervation of the muscles.

(i) General muscles of scolex and strobila. The main body musculature is supplied with small nerves arising in the longitudinal nerve cords. Innervation of individual muscle bundles was best seen in sections of H. taeniaeformis. In this species, the nerves follow the length of both transversely (Figs. 2.24, 2.45) and longitudinally (Fig. 2.28) oriented muscle bundles. Individual nerve fibres which appear to terminate on individual muscle fibres, have a 'knobbly' appearance.

(ii) Rostellum. Nerves arising in the rostellar nerve ring innervate the rostellum. A number of fine nerves supply the muscles situated immediately posterior to the

rostellar pad (Fig. 2.27). The rostellar pad contains a large number of nerves (Figs. 2.20, 2.27, 2.42, 2.50), some of which terminate in the rostellar gland, and others which presumably supply the muscles of the rostellar pad. These nerves are particularly conspicuous in H. taeniaeformis and T. pisiformis, but are detectable also in D. caninum and E. granulosus.

(iii) Suckers. Large nerves innervate the suckers. As mentioned earlier, some arise in the lateral ganglia, and others in the dorsal and ventral median longitudinal nerve cords. Individual fibres from these nerves enter the sucker in small groups, and reorganize within the sucker into a plexus (Figs. 2.6, 2.15, 2.22, 2.30, 2.32, 2.39, 2.43, 2.51). As mentioned earlier, the nerves supplying the tegument of the sucker in T. pisiformis arise in the sucker plexus (Fig. 2.51). In both the adult and strobilocercus of H. taeniaeformis, nerves arising in the sucker plexus contain small knobs which are in contact with the muscle fibres of the sucker (Figs. 2.30, 2.43). In Gabe-stained sections of the suckers of T. pisiformis cysticercus, large multipolar cells are present among the muscles (Fig. 2.49). These cells are very similar to the nerve cells of the lateral ganglia in staining characteristics. Similar cells are present in the rostellar pad.

5. Innervation of the reproductive system.

In the present study, no nerves were found supplying gonads, uterus, vitellaria, or any of the storage organs of the male or female reproductive systems. However the cirrus and the copulation canal are supplied with nerves

from the adjacent lateral longitudinal nerve cords, and a large number of nerves occur within the cirrus pouch and the wall of the copulation canal of D. caninum (Fig. 2.8), E. granulosus (Fig. 2.17), H. taeniaeformis and B. criniae minor (Fig. 2.35). The tegument surrounding the genital pore is more richly innervated than that of the rest of the strobila (Fig. 2.17). In a whole mount of a B. criniae minor proglottid, small nerves are present just beneath the tegument, and these radiate from the genital pore and closely follow radial muscle fibres immediately beneath the tegument (Fig. 2.37). The genital atrium in B. criniae minor is more richly innervated than the cirrus or the copulation canal (Fig. 2.35).

Infective oncospheres were observed within the uterus of gravid proglottids of D. caninum, E. granulosus, H. taeniaeformis and B. criniae minor. The oncospheres of B. criniae minor did not stain with the Karnovsky & Roots (1964) technique (Fig. 2.36). In D. caninum and H. taeniaeformis, the oncospheres stained, but the results were not sufficiently clear to interpret the deposits as possible nervous structures. However, in the oncospheres of E. granulosus, deposits of stain were found in two general areas. Towards the posterior end there were four deposits, two on each side (Figs. 2.18, 2.68); in the vicinity of the mid-line, there were six deposits, each one near a hook, and an additional deposit between the two middle hooks. An interpretation of these results is given in the discussion.

6. Innervation of the rostellar gland.

The rostellar gland in D. caninum, E. granulosus, H. taeniaeformis and T. pisiformis is supplied with nerves originating in the rostellar nerve ring, some of which pass through the rostellar pad (Fig. 2.27) and others which pass around it (Fig. 2.41). Within the gland, there is an irregular network of nerves, which was most clearly defined in H. taeniaeformis (Fig. 2.19). A few small nerve cell bodies and inconspicuous nerve endings occur in this nerve network.

7. Innervation of the excretory system.

No nerves supplying the excretory vessels were observed in D. caninum, E. granulosus or B. criniae minor. In H. taeniaeformis proglottids, very fine nerves were occasionally found to terminate in the wall of the excretory vessel (Fig. 2.24), while in the scolex, similar nerves were more numerous (Fig. 2.44).

8. Esterase-positive cells in the 'tail' of T. hydatigena cysticercus.

The cysticercus of T. hydatigena has a structure posterior to the scolex, but contained within the bladder, termed the 'tail' or 'proliferative area' by Voge (1962). In a whole mount of the tail stained with 5-bromoindoxyl acetate, large cells with esterase-positive cytoplasm are present (Figs. 2.66, 2.67). These cells give rise to long processes which are oriented longitudinally and approximately parallel to the muscle fibres of the tail (Fig. 2.66). These may be primordial nerve cells, or could be esterase-positive proliferative cells. As

Fig. 2.1. Dipylidium caninum. Central nervous system of the scolex from ventral side. The nervous system in the ventral half only is shown.

Fig. 2.2. Echinococcus granulosus. Central nervous system of the scolex from ventral side. The nervous system in the ventral half only is shown.

Fig. 2.3. Hydatigera taeniaeformis. Central nervous system of the scolex from ventral side. The nervous system in the ventral half only is shown.

Fig. 2.4. H. taeniaeformis. Diagrammatic transverse section through the lateral ganglia showing interconnections of the main longitudinal nervous elements.
(ac, accessory commissure; alnc, accessory lateral nerve cord; an, anterior nerve; cc, cerebral commissure; dnc, dorsal nerve cord; dsn, dorsal sucker nerve; lg, lateral ganglion; lsn, lateral sucker nerve; mlnc, main longitudinal nerve cord; pc, polygonal commissure; rg, rostellar gland; rp, rostellar pad; rnr, rostellar nerve ring; vnc, ventral nerve cord; vsn, ventral sucker nerve; xc, X-commissure.)

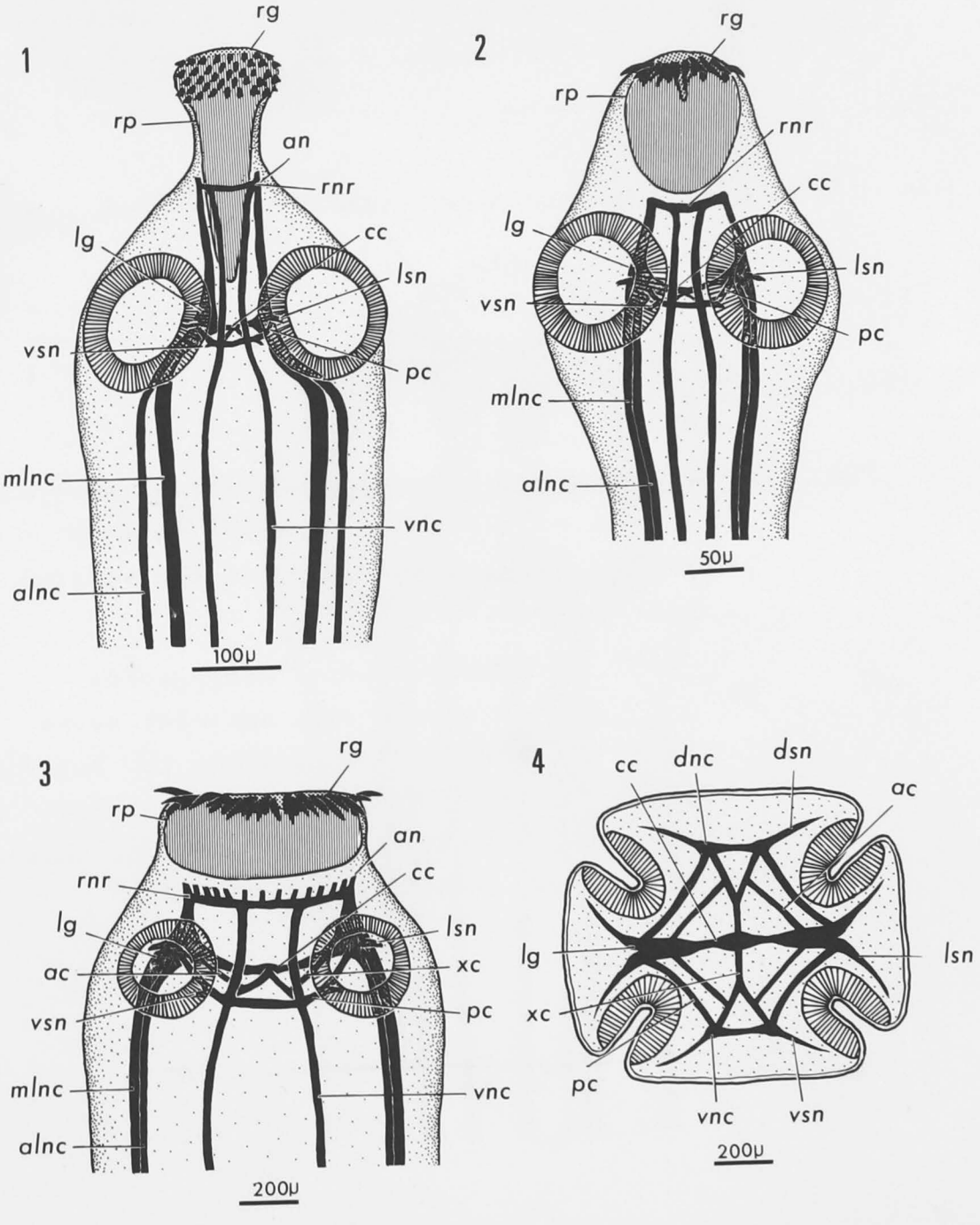

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Fig. 2.5. Baerietta criniae minor. Central nervous system of the scolex from the ventral side. The nervous system in the ventral half only is shown.

(alnc, accessory lateral nerve cord; an, anterior nerve; anr, anterior nerve ring; cc, cerebral commissure; lg, lateral ganglion; mlnc, main longitudinal nerve cord; pc, polygonal commissure; sn, sucker nerve; vnc, ventral nerve cord.)

5

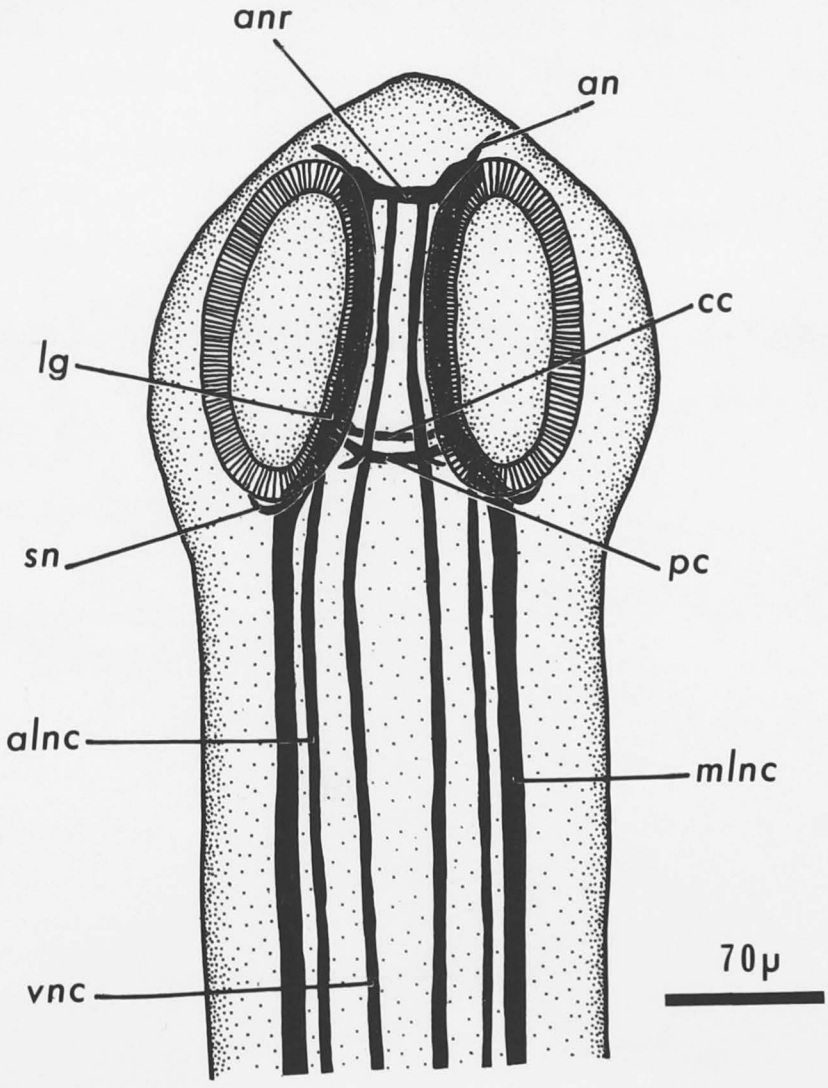


Fig. 2.6 - 2.12. Adult Dipylidium caninum.

Fig. 2.6. Whole mount of scolex showing longitudinal nerve cords (*), anterior nerves (▷) and sucker plexus (→). (5-bromoindoxylacetate).

Fig. 2.7. Cryostat longitudinal section through lateral ganglia (*). Compare with Fig. 2.6. (AThChI, overstained to increase contrast).

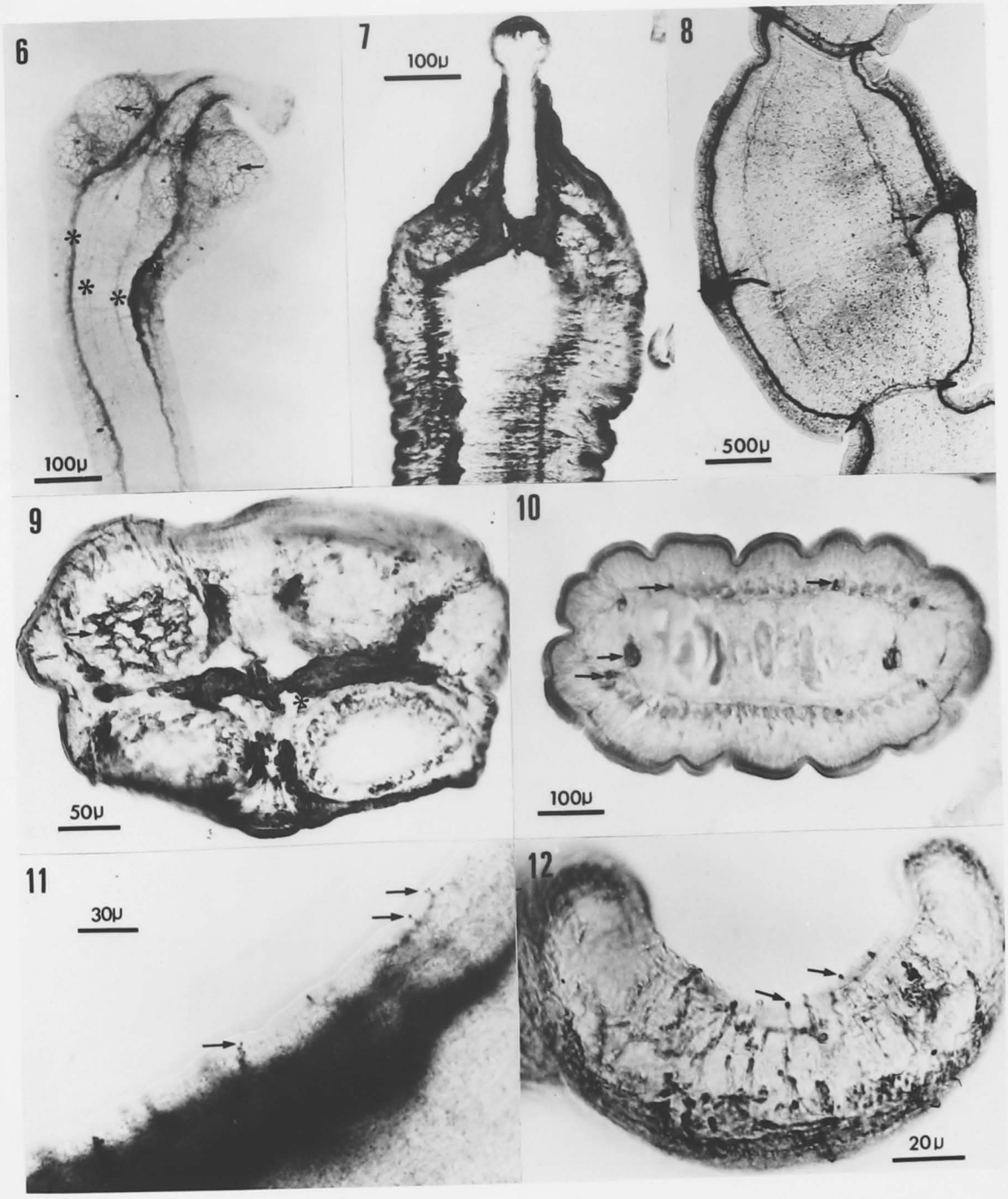
Fig. 2.8. Whole mount of mature proglottid, showing longitudinal nerve cords, inter-proglottidal nerve ring (*), and positively staining cirrus (→), oviduct (↦) and Mehlis' gland (►). (5-bromoindoxylacetate).

Fig. 2.9. Cryostat transverse section of scolex showing cerebral commissure (*) and sucker plexus (→). (AThChI overstained to increase contrast).

Fig. 2.10. Cryostat transverse section of proglottid showing distribution of acetylcholinesterase activity in the nerve cords (→). (AThChI, counterstained light green).

Fig. 2.11. Higher magnification of part of Fig. 2.6 showing nerve endings (→).

Fig. 2.12. Cryostat section through the edge of a sucker, showing nerve endings in the tegument of the sucker (→). (AThChI).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Figs. 2.13 - 2.18. Adult Echinococcus granulosus.

Fig. 2.13. Cryostat transverse section of rostellar nerve ring (→); some anterior nerves (►) and peripheral nerves (▷) can also be seen. (AThChI).

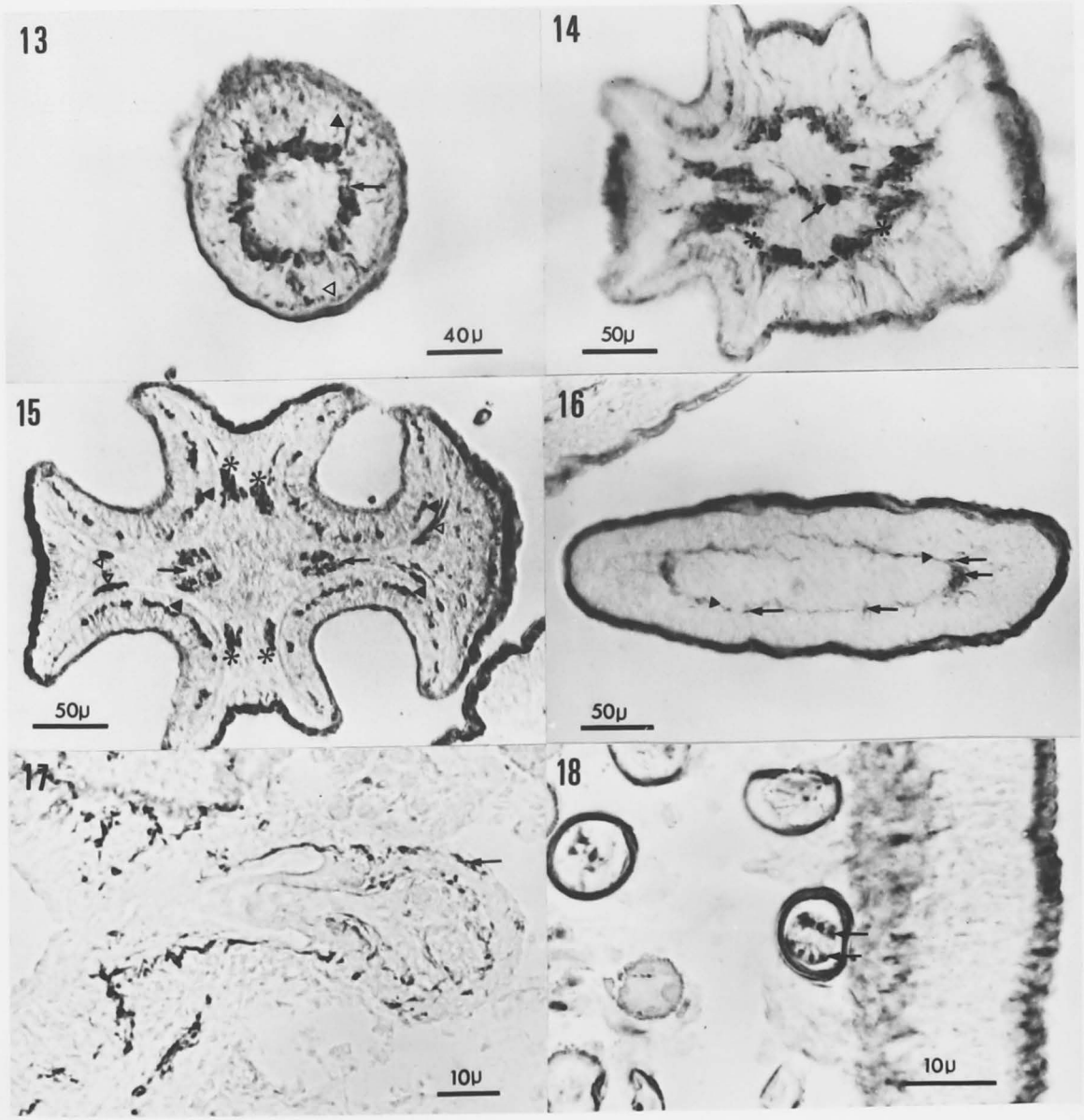
Fig. 2.14. Cryostat transverse section of the scolex showing part of the brain (→) and part of the polygonal commissure (*) joining the lateral ganglia with the dorsal and ventral nerve cords. (AThChI).

Fig. 2.15. Cryostat transverse section of the scolex showing lateral ganglia (→), dorsal and ventral longitudinal nerves (*), sucker nerves (▷) and plexus (►), and cholinesterase-positive tegument. (AThChI).

Fig. 2.16. Cryostat transverse section of neck region showing position of the nerve cords (→) and connecting nerves of the inner plexus (►). (AThChI).

Fig. 2.17. Cryostat longitudinal section showing nerves in the cirrus (→). (AThChI).

Fig. 2.18. Cryostat section of oncospheres showing centres of cholinesterase activity (→). (AThChI).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Figs. 2.19- 2.26. Adult Hydatigera taeniaeformis.

Fig. 2.19. Cryostat transverse section of the rostellar gland showing moderate activity of cholinesterase in the gland, and nerves in the gland (→). (AThChI).

Fig. 2.20. Cryostat transverse section of the rostellar pad showing cholinesterase activity in the nerves (→). Note the large number of nerve endings (►). (AThChI).

Fig. 2.21. Cryostat transverse section through the rostellar nerve ring (→), showing some of the anterior nerves (▷). (AThChI).

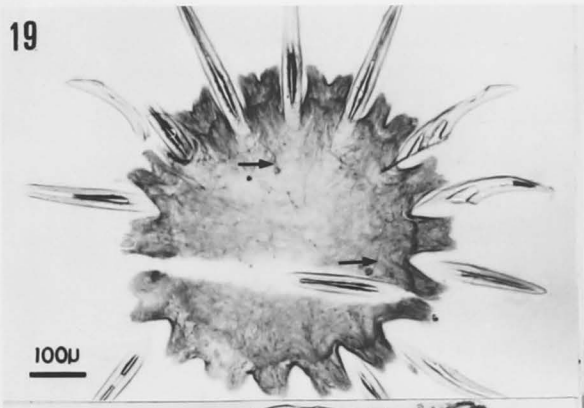
Fig. 2.22. Cryostat transverse section through the cerebral commissure (*) and part of the X-commissure (→). Note the sucker plexus (▷) and large number of nerve endings surrounding the suckers (►). (AThChI).

Fig. 2.23. Cryostat transverse section of the neck region showing distribution of ChE activity in the nerves. Note nerves of the inner plexus which link the longitudinal nerve cords. (AThChI).

Fig. 2.24. Cryostat transverse section of a mature proglottid showing lateral nerve cords (→) and cholinesterase-positive tegument (*). A very fine nerve ending is present in the wall of the excretory vessel (▷). Note that the nerves follow the muscle bundles (►), and that the nerve cord is not entirely composed of AChE-positive nerves.(AThChI).

Fig. 2.25. Cryostat longitudinal section of the scolex showing tubular nerve ending (→), and peripheral nerve plexus (►). (AThChI).

Fig. 2.26. Cryostat longitudinal section of the scolex showing bulbous nerve ending (→) and peripheral nerve plexus (►). (AThChI).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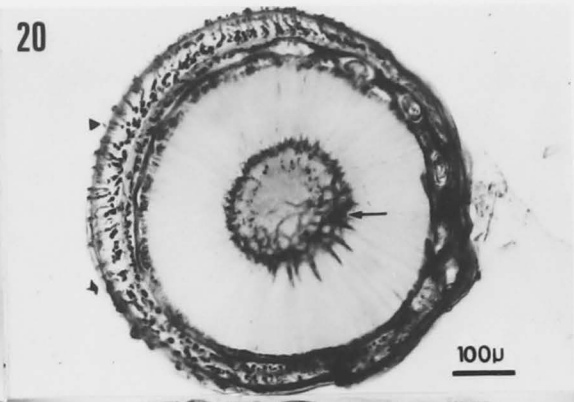
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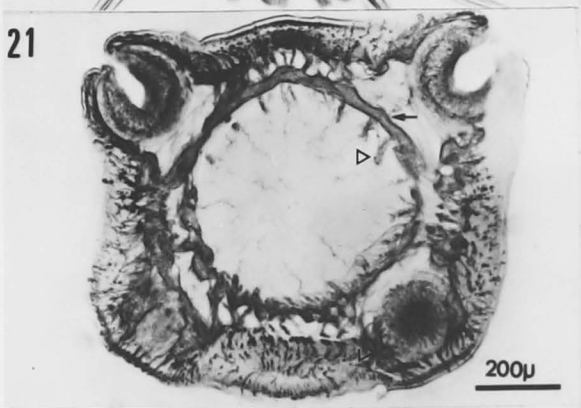
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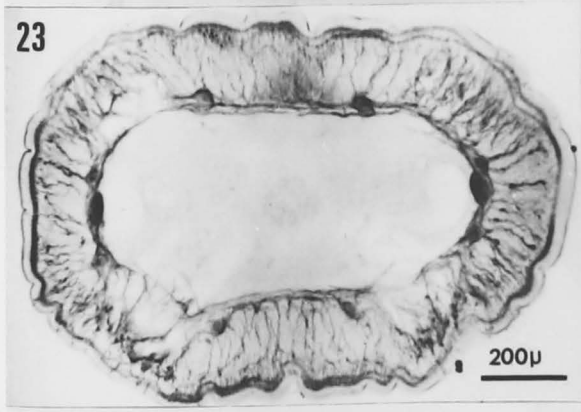
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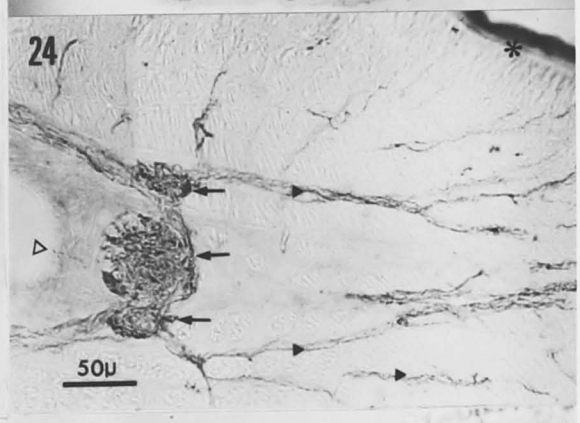
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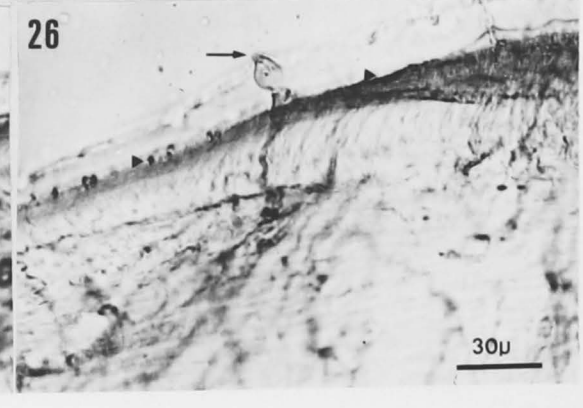
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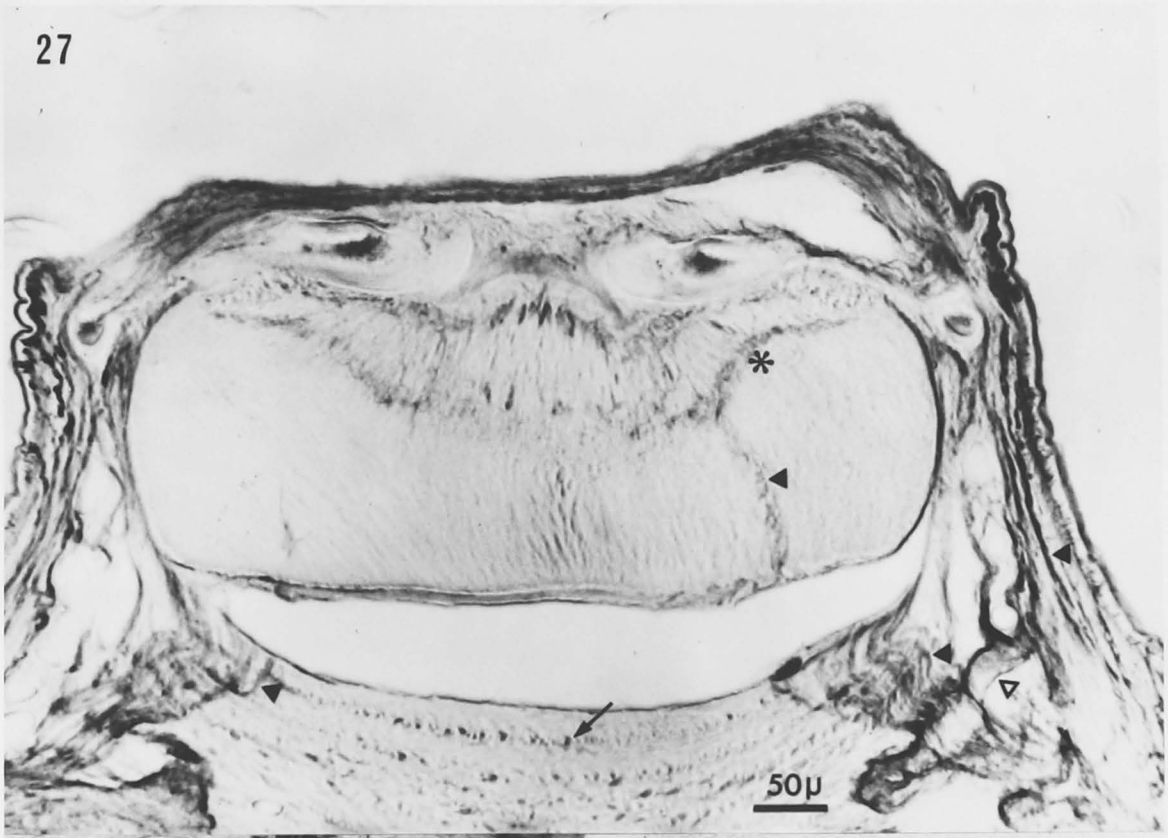
Figs. 2.27 - 2.29. Adult Hydatigera taeniaeformis.

Fig. 2.27. Cryostat longitudinal section through the rostellum. Note the apical nerves (►) arising from the rostellar nerve ring (▻). A diffuse system of nerves is present in the rostellar pad (*), and nerves supplying the muscular cushion posterior to the rostellar pad have been cut transversely (→). (AThChI).

Fig. 2.28. Cryostat longitudinal section through the neck region showing nerves of the inner plexus (*), longitudinal nerves following muscle bundles (→) and the peripheral plexus (►). (AThChI).

Fig. 2.29. Cryostat saggital longitudinal section through a lateral ganglion (*) showing nerves to the suckers (→). (AThChI).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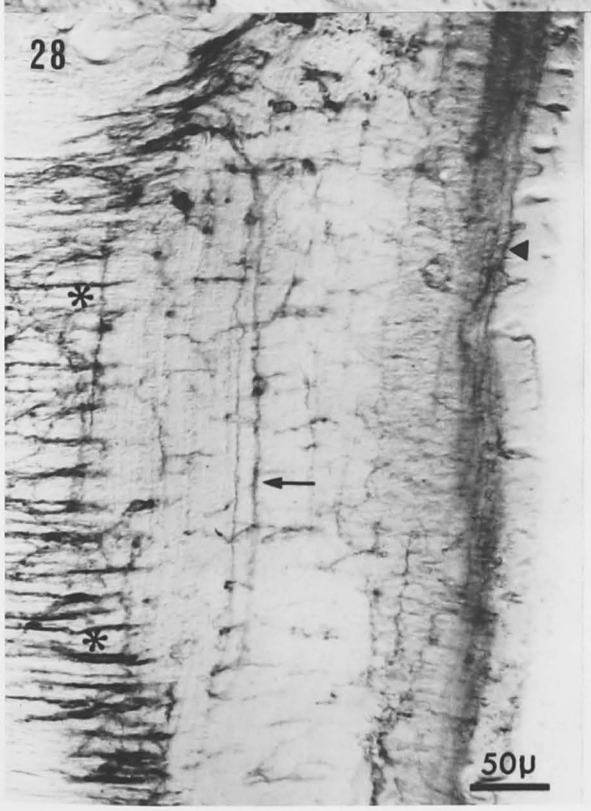
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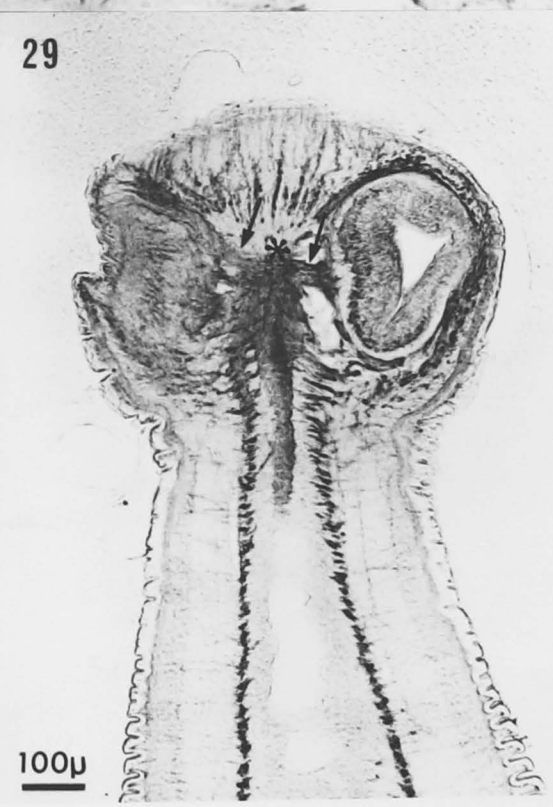
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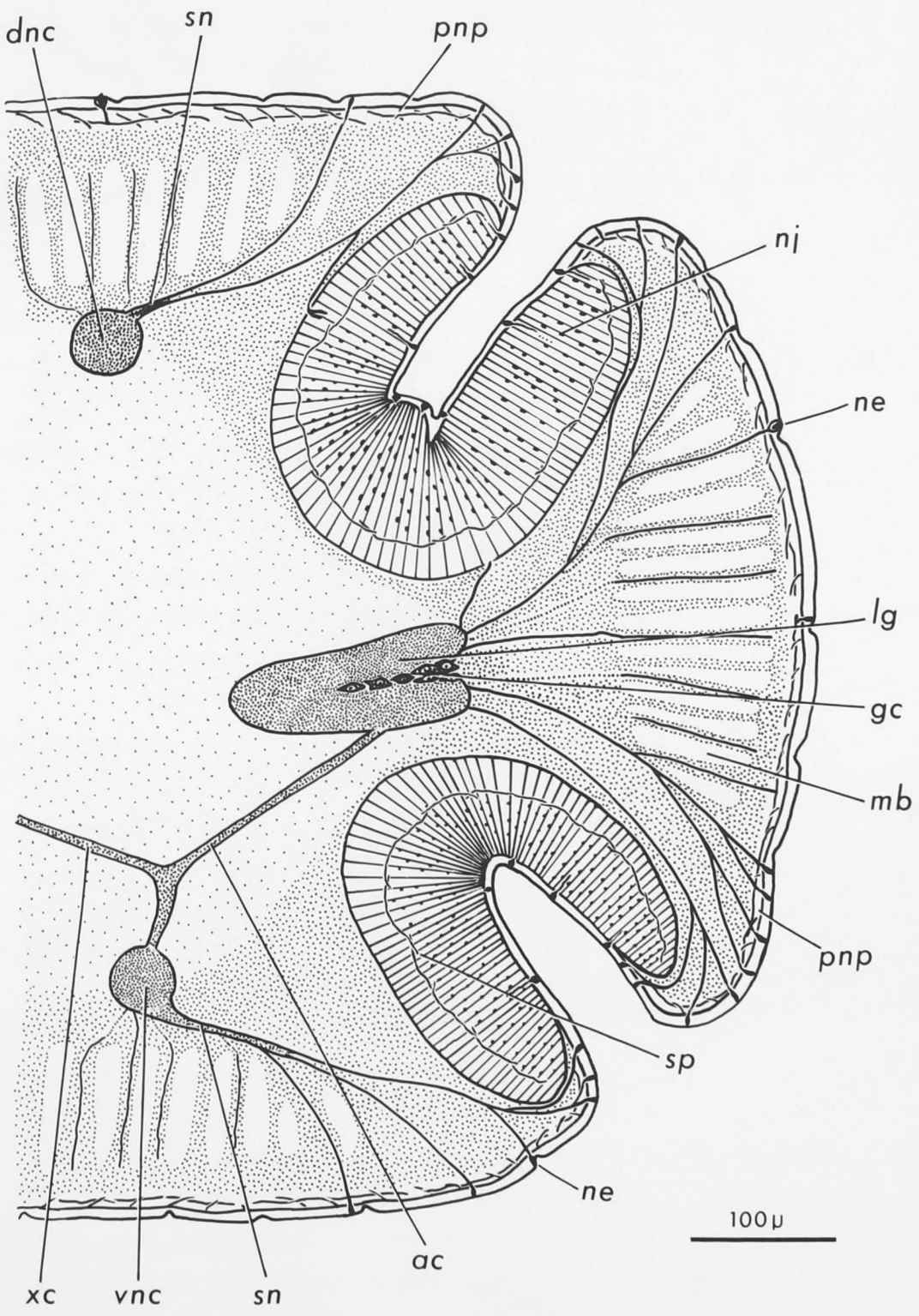
Fig. 2.30. Adult Hydatigera taeniaeformis.

Transverse section through a lateral half of the scolex. Compare with Fig. 2.4. (ac, accessory commissure; dnc, dorsal nerve cord; gc, ganglion cells; lg, lateral ganglion; mb, muscle bundle; ne, nerve ending; nj, possible neuromuscular junction; pnp, peripheral nerve plexus; sn, sucker nerve; sp, sucker plexus; vnc, ventral nerve cord; xc, X-commissure).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Figs. 2.31 - 2.36. Adult Baerietta criniae minor.

Fig. 2.31. Cryostat longitudinal section through scolex showing anterior nerves (→) supplying the anterior tegument, and longitudinal nerve cords (*) with connecting nerves of the inner plexus (►). Note the increased innervation of the lip of the sucker (▷), and a tubular nerve ending in the anterior tegument (↦). (AThChI).

Fig. 2.32. Approximate transverse section through the anterior part of the suckers, showing parts of the apical nerve ring (→). Note the sucker plexus (►) and the increased innervation of the lips of the suckers (▷). (AThChI).

Fig. 2.33. Transverse section through immature region showing the longitudinal nerve cords cut in section (→) and the connecting nerves of the inner plexus (►). (AThChI).

Fig. 2.34. Transverse section through the neck region, showing longitudinal nerve cords (→) and their connectives (►), and a nerve ending in the tegument (▷). (AThChI).

Fig. 2.35. Transverse section through a mature proglottid showing the main longitudinal nerve cords (→) and a nerve ending in the tegument (▷). Note the nerves in the cirrus pouch (►) and the genital atrium (*). (AThChI).

Fig. 2.36. Transverse section through a gravid proglottid, showing the main longitudinal nerve cords (→), longitudinal muscle bundles (►), and oncospheres in the uterus (*). (AThChI).

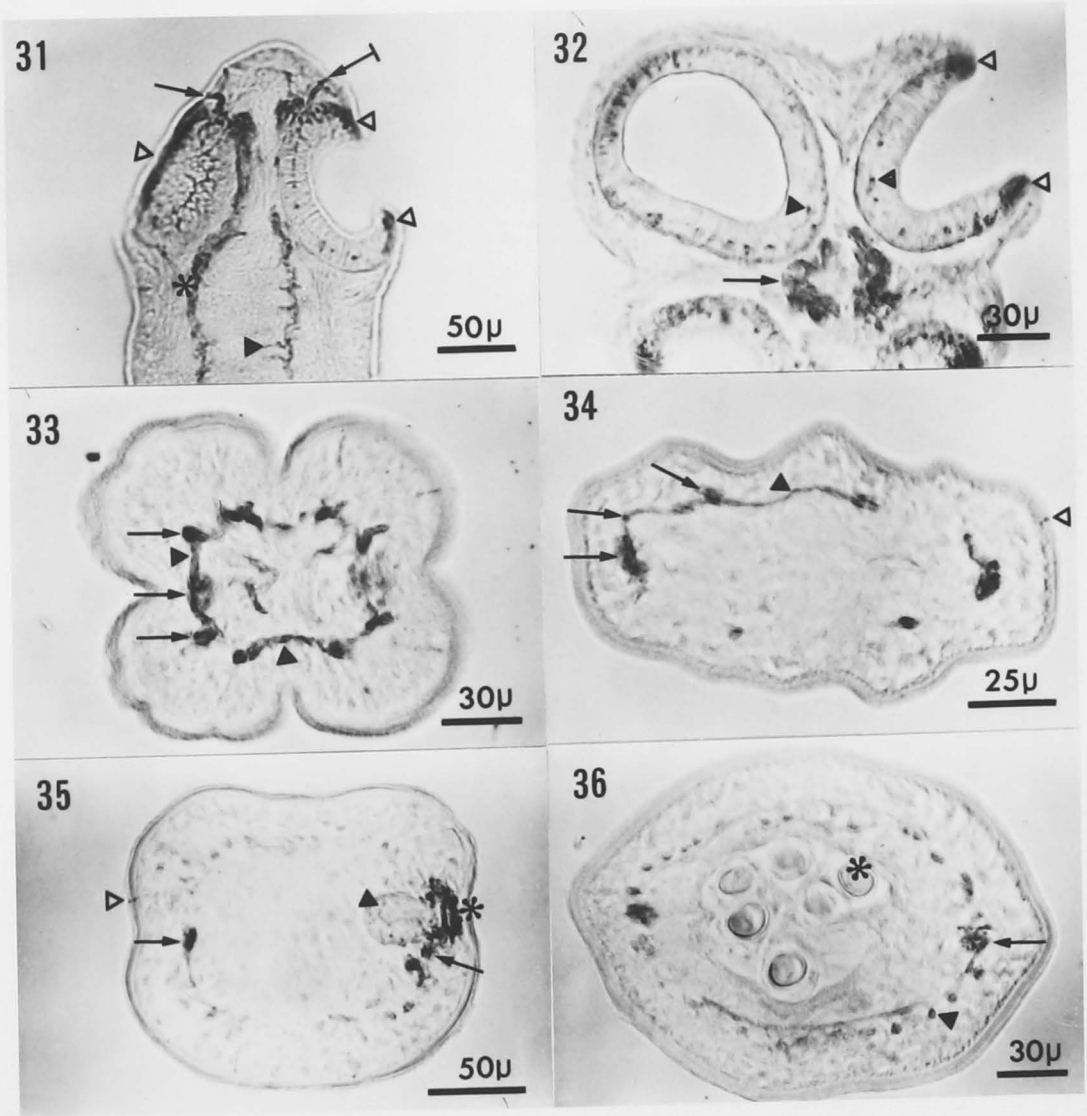
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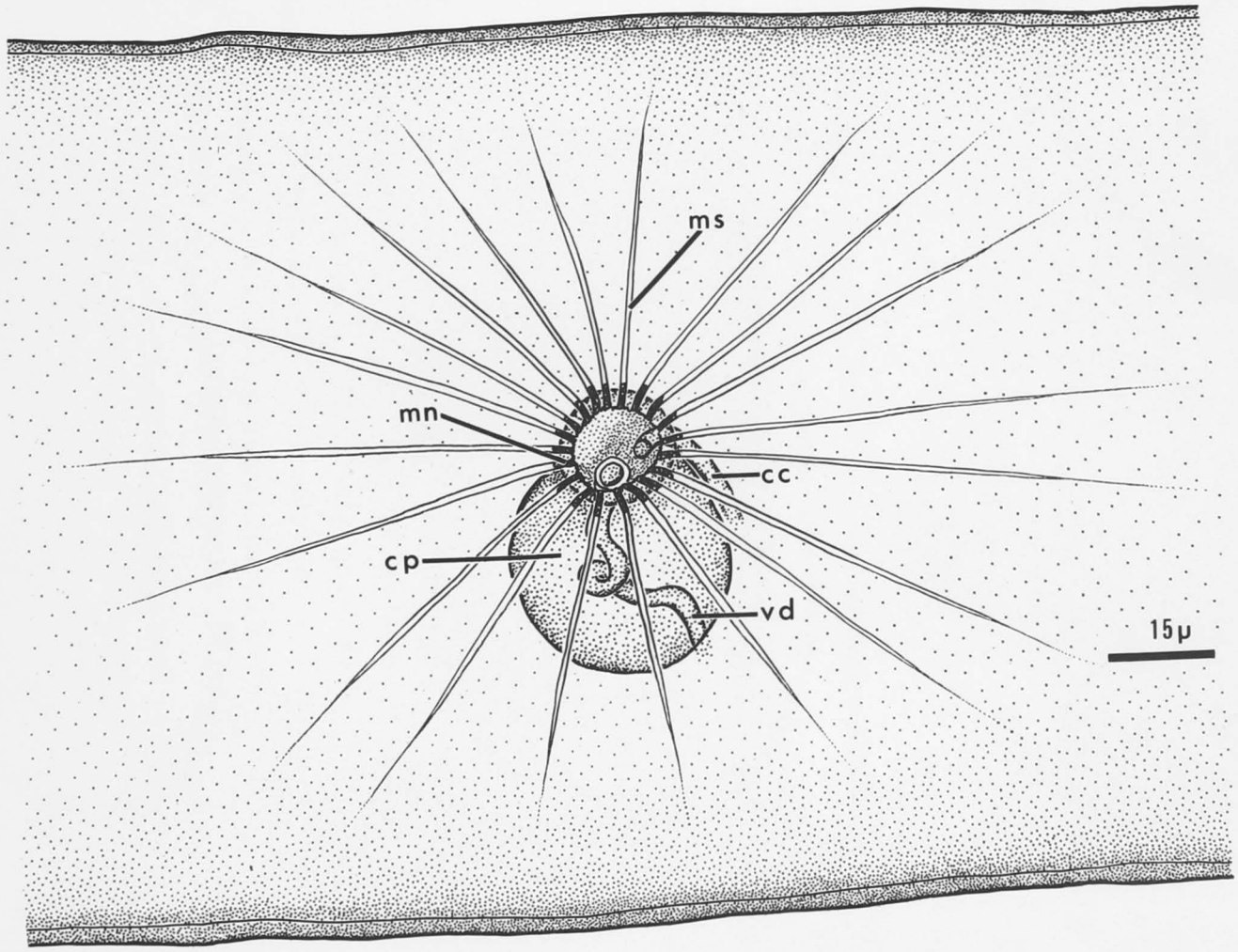
Fig. 2.37. Adult *Baerietta criniae minor*.

Surface view of lateral side of proglottid showing terminal portions of nerve supplying sub-surface muscle strands, presumably dilator muscles of the genital pore.
(cc, copulation canal; cp, cirrus pouch; mn, nerves supplying muscles; ms, muscle strand; vd, vas deferens.)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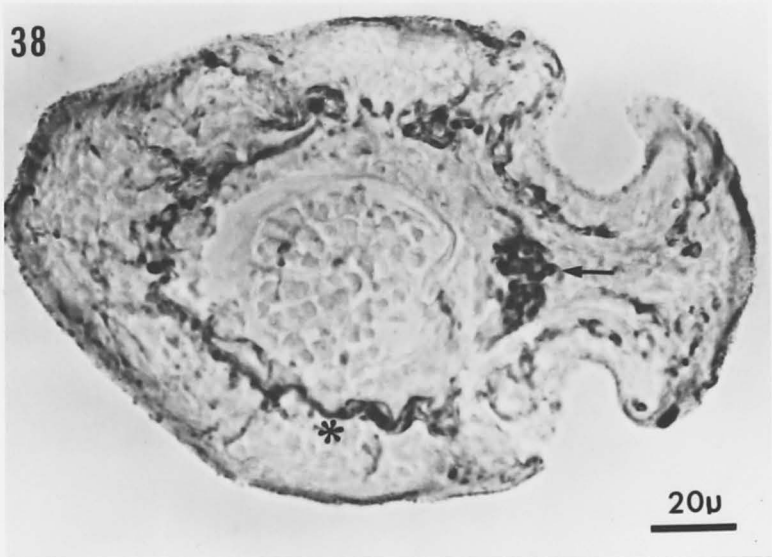
Figs. 2.38 - 2.40. Dipylidium caninum cysticercoid.

Fig. 2.38. Approximate transverse section through the anterior part of the suckers and the rostellar pad. Part of the rostellar nerve ring (*) is shown, and one of the lateral ganglia (→). (AThChI).

Fig. 2.39. Approximate transverse section through the posterior part of the suckers, showing a lateral ganglion (→), part of the cerebral commissure (*) and the sucker plexus (▷). Note the bulbous nerve ending on the lip of a sucker (►). (AThChI).

Fig. 2.40. Approximate transverse section through the posterior part of the cysticercoid, showing the main longitudinal nerve cords (→) and the large nerve endings in the cholinesterase-positive tegument (►). Compare the nerve endings with those in Fig. 2.39, which is of the same magnification.(AThChI).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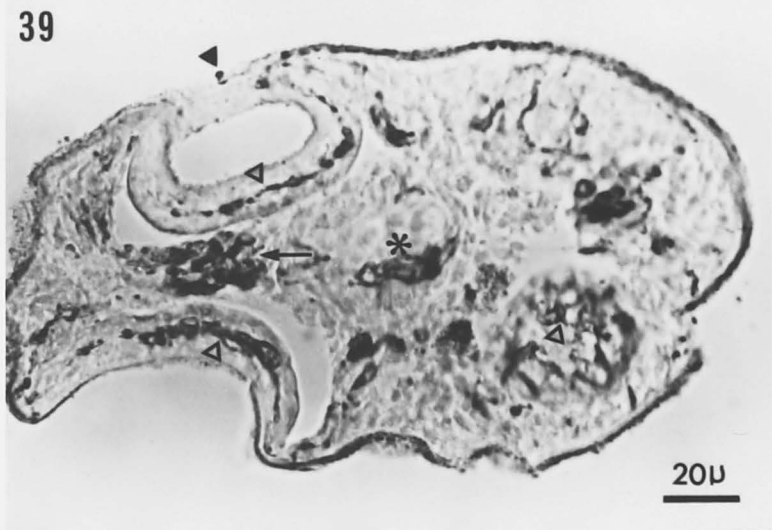
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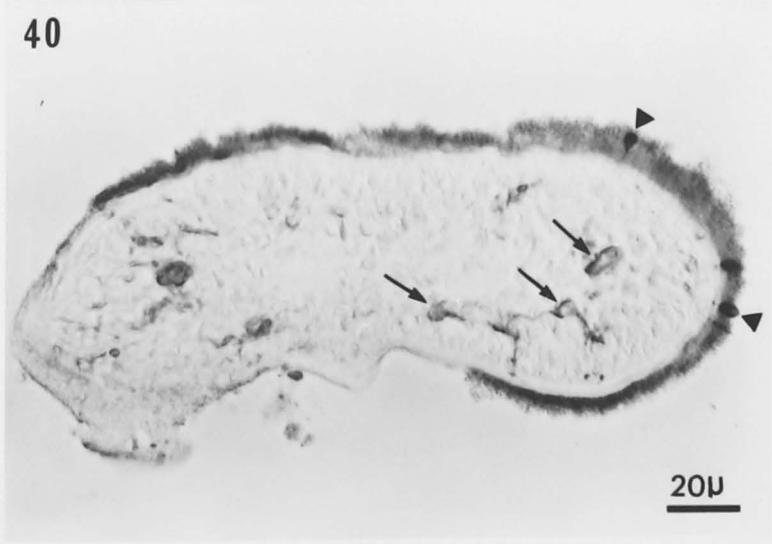
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Figs. 2.41 - 2.47. Hydatigera taeniaeformis strobilocercus.

Fig. 2.41. Approximate transverse section through the anterior tip of the rostellum showing part of the rostellar gland (*), and some hooks cut in section (▷). Note the presence of small peripheral nerves (→) which appear to enter the rostellar gland, anterior to the rostellar pad. (AThChI).

Fig. 2.42. Approximate transverse section of the rostellar pad showing nerves within the pad (→). (AThChI).

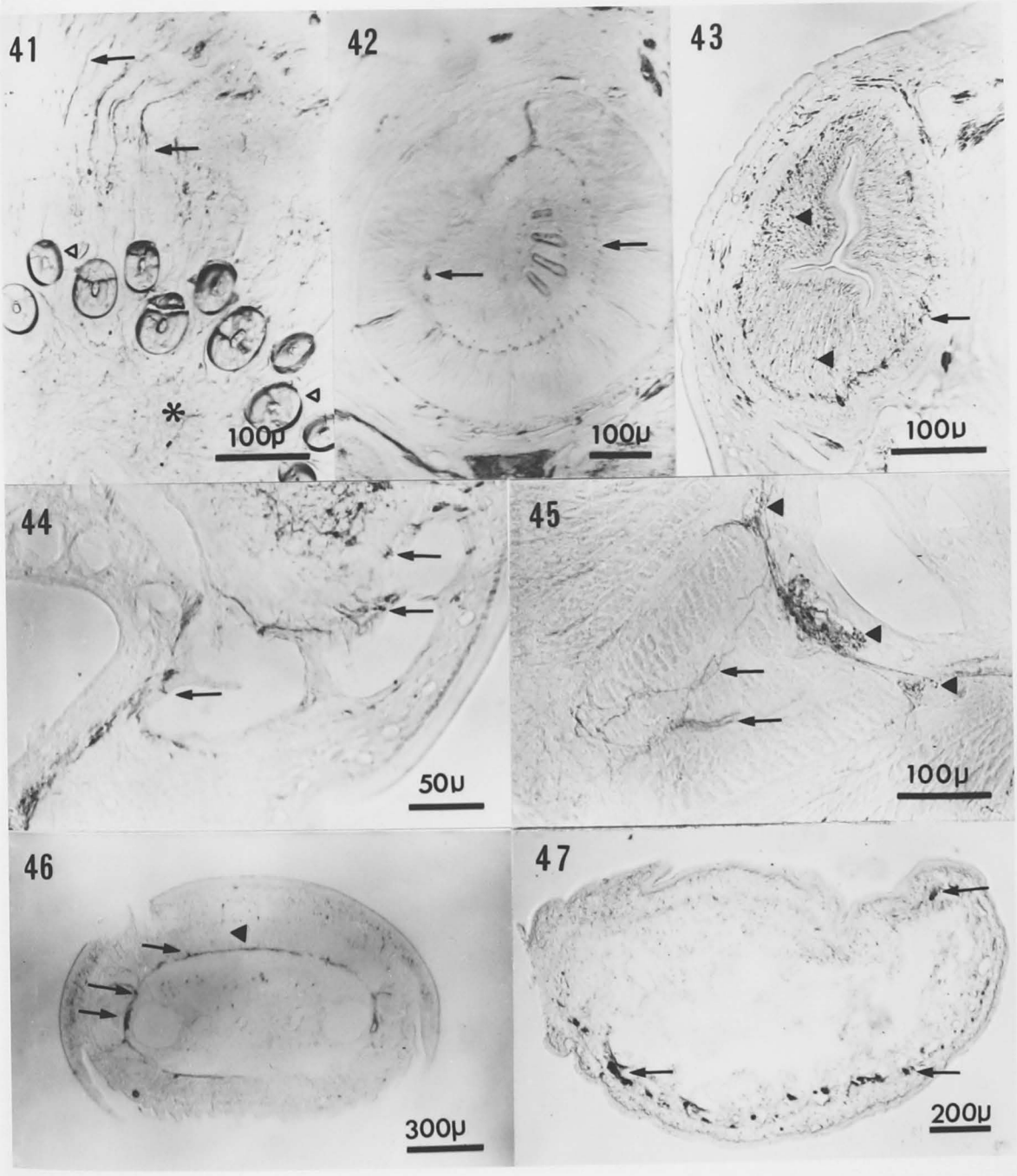
Fig. 2.43. Approximate transverse section through a sucker showing the sucker plexus (→) and small cholinesterase-positive deposits on the sucker muscles, which may be neuromuscular junctions (►). (AThChI).

Fig. 2.44. Approximate transverse section through the posterior part of a sucker, showing parts of the excretory vessels. Note nerves ending in the vessel walls (→). (AThChI).

Fig. 2.45. Transverse section through strobilocercus posterior to the scolex, showing nerve fibres in lateral nerve cords (►), and nerve fibres which follow the muscle bundles (→). (AThChI).

Fig. 2.46. Transverse section of strobilocercus anterior to the terminal bladder, showing longitudinal nerve cords (→) and connecting nerves of the inner plexus (►). (AThChI).

Fig. 2.47. Transverse section through the terminal bladder, showing the many longitudinal nerves (→). (AThChI).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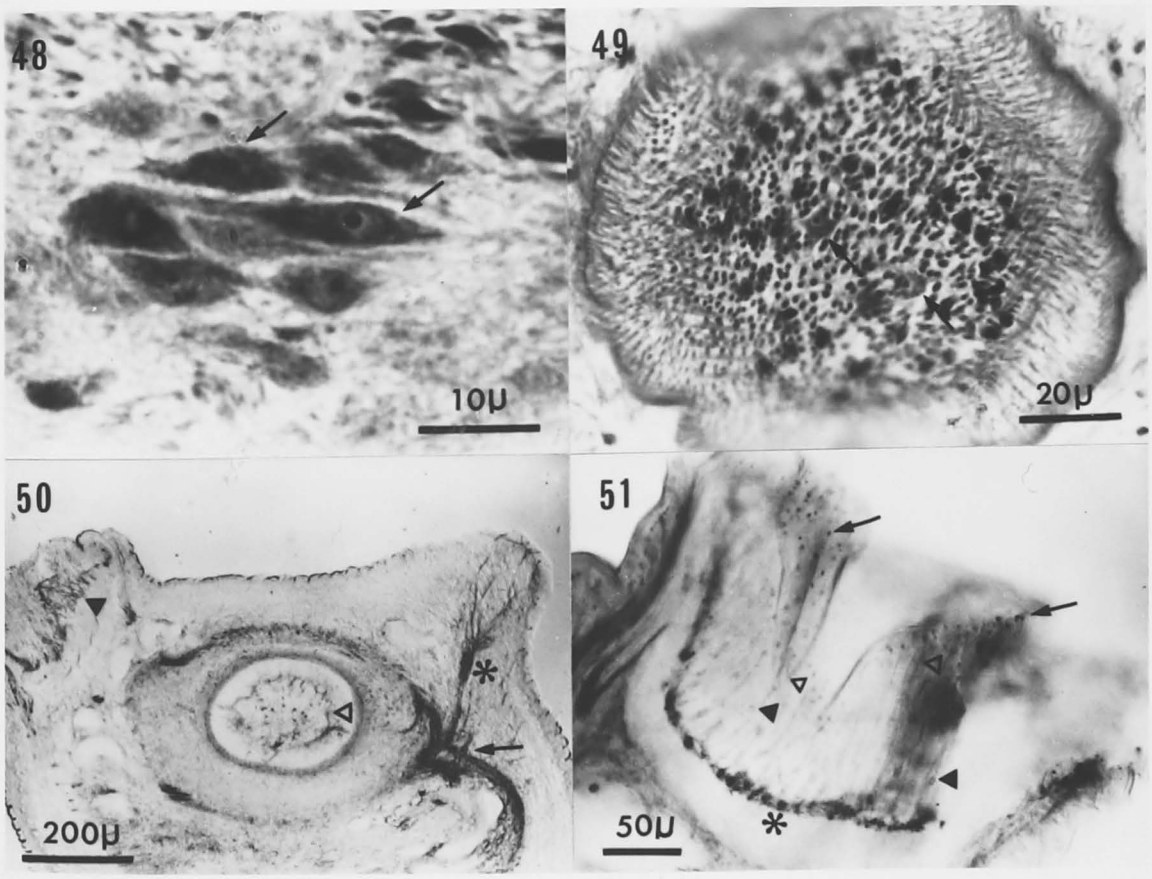
Figs. 2.48 - 2.51. Taenia pisiformis.

Fig. 2.48. Transverse section through a lateral ganglion of the cysticercus, showing ganglion cells (→). (Gabe).

Fig. 2.49. Transverse section through a sucker of a cysticercus showing large, multipolar cells (→). (Gabe).

Fig. 2.50. Oblique cryostat section of the adult, showing a lateral ganglion (→), a sucker nerve (*), nerves within the rostellum (▷) and a sucker plexus (►). (AThChI).

Fig. 2.51. Approximately longitudinal thin slice of a sucker of the adult, showing the sucker plexus (*), nerve cell bodies (►) with processes (▷) terminating as nerve endings (→) in the sucker tegument. (AThChI).


48
10μ
49
20μ
50
200μ
51
50μ

Figs. 2.52 - 2.55. *Dipylidium caninum* adult.

Fig. 2.52. Transverse section through the cerebral commissure showing large cells with cytoplasm staining lightly with haematoxylin (→). (Chromalum-haematoxylin-phloxine).

Fig. 2.53. Longitudinal section through a lateral ganglion (*) showing large ganglion cells (→). (Maximow).

Fig. 2.54. Enlargement of Fig. 2.53 with one of the ganglion cells in focus (→).

Fig. 2.55. Enlargement of Fig. 2.53 with the other ganglion cell in focus (→), and a small ganglion cell almost in focus (►).

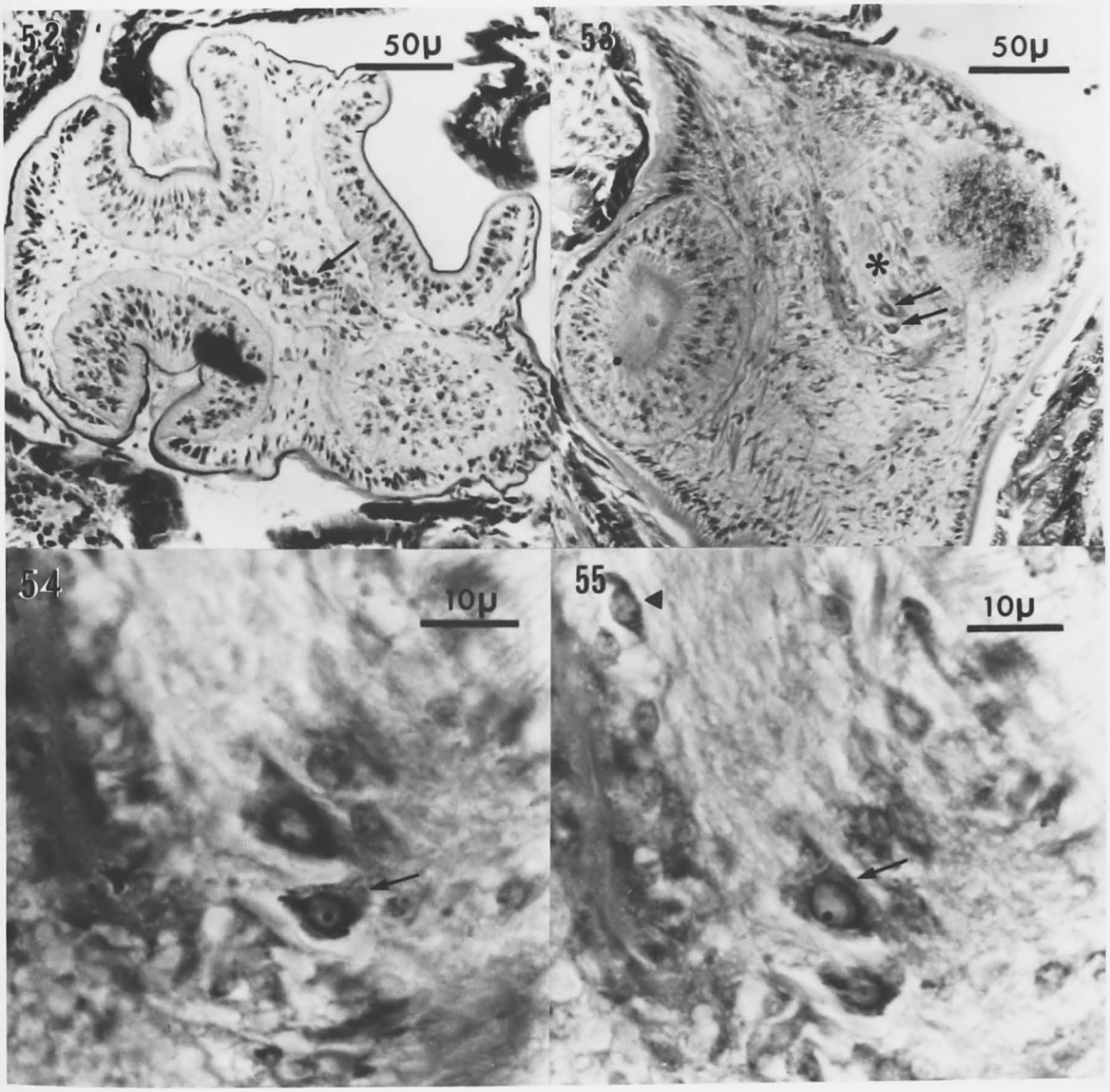

52
50μ
53
50μ
54
10μ
55
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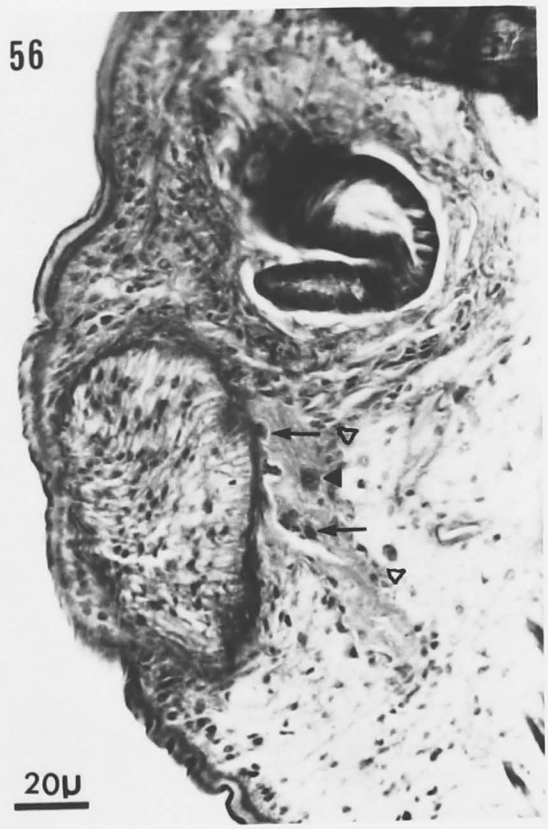
Fig. 2.56. Dipylidium caninum adult.

Longitudinal section through the lateral ganglion showing small nerve cells (→), a large nerve cell (►) and possible binding cells (▷). (Heidenhain's azan).


56
20μ

Figs. 2.57 - 2.61. Taenia hydatigena adult.

Fig. 2.57. Saggital longitudinal section through the cerebral commissure showing large ganglion cells (►) and binding cells (→). (Gabe).

Fig. 2.58. Longitudinal section through a lateral ganglion showing ganglion cells (►) separating the dorsal and ventral lobes of the ganglion. (Gabe).

Fig. 2.59. Section through a nerve in the central nervous system of the scolex showing a multipolar neurone (►) and binding cells (→). (Gabe).

Fig. 2.60. Transverse section through a proglottid showing one set of lateral nerve cords (→) and nerve cells (►). (Mayer's haemalum and eosin).

Fig. 2.61. Longitudinal section through immature proglottids showing the main longitudinal nerve cord (*) and an accessory longitudinal nerve cord (▻). Note the nerve cells on the periphery of the main longitudinal nerve cord (►). (Gabe).

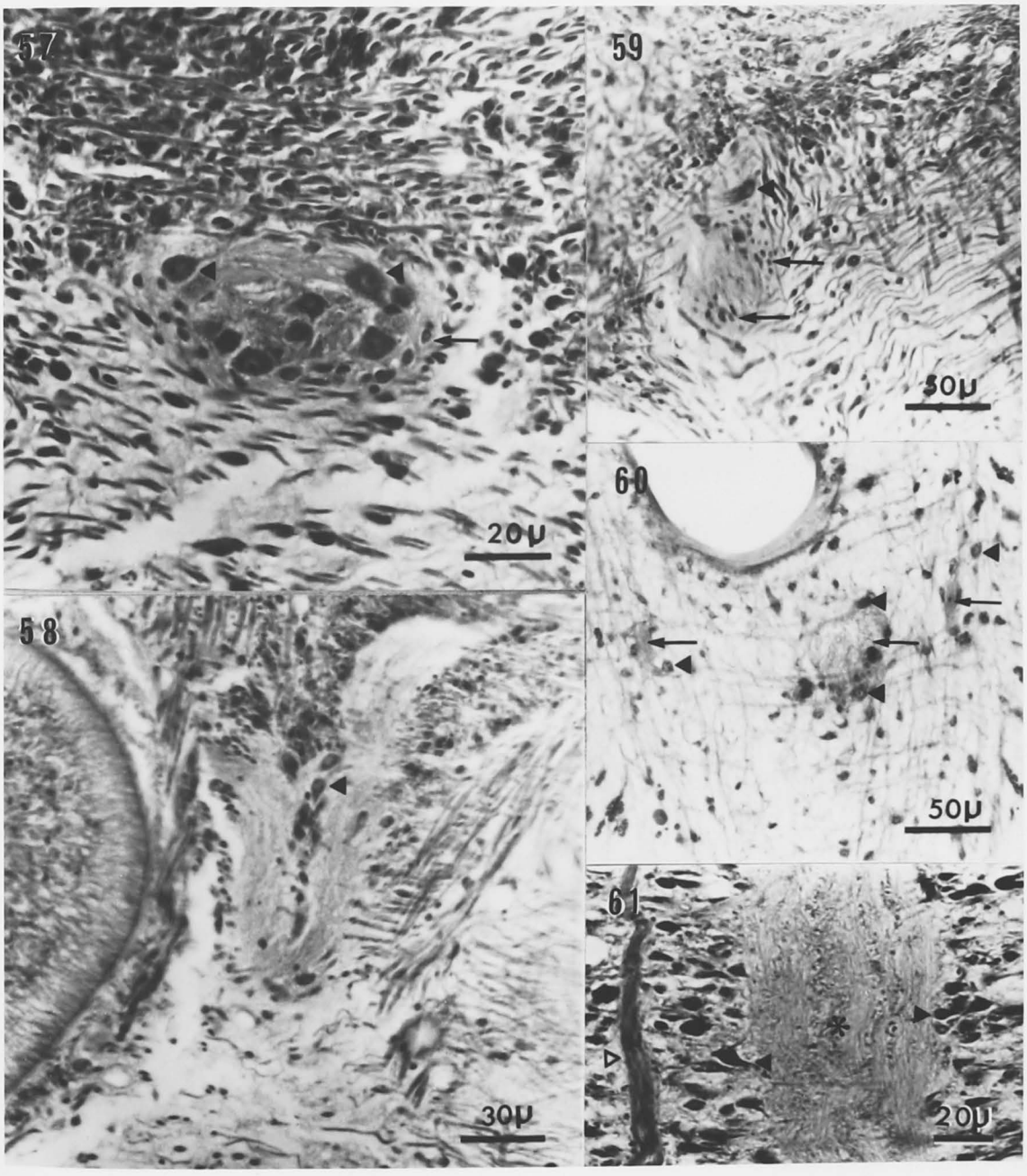
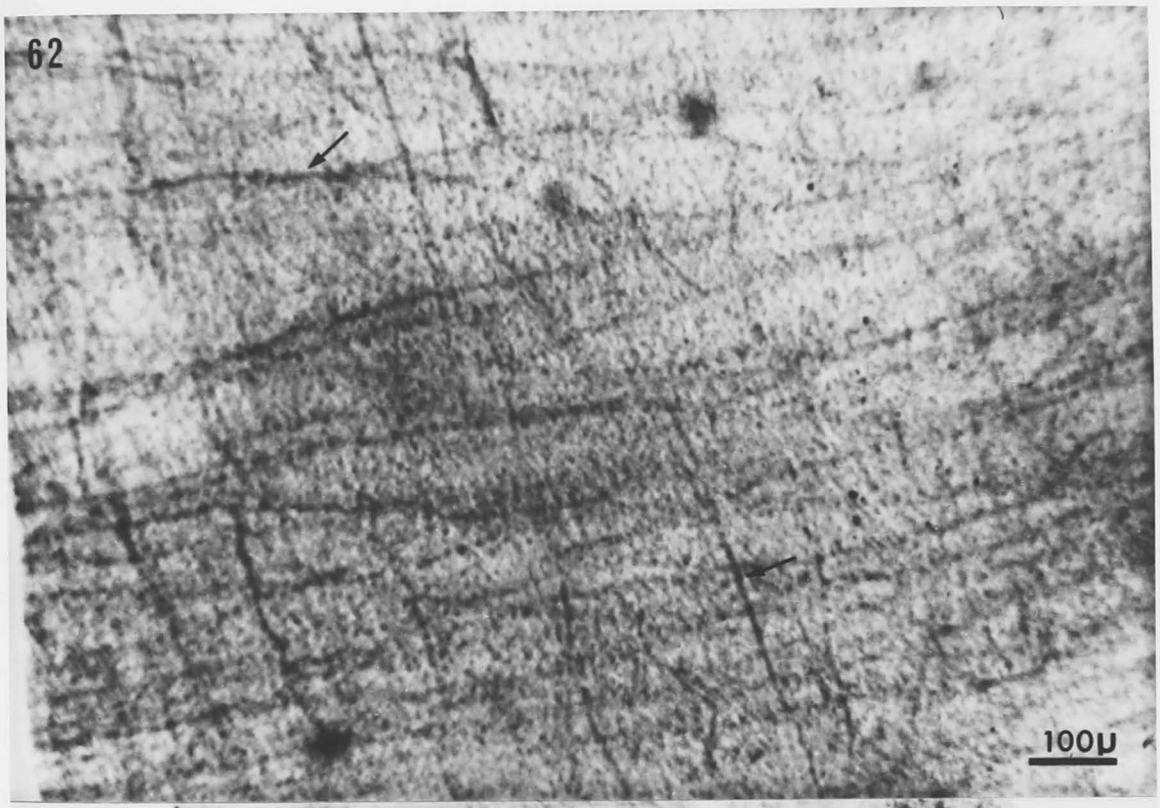

57
20μ
59
50μ
60
50μ
58
30μ
61
20μ

Fig. 2.62. Taenia hydatigena cysticercus.

Whole mount of part of the bladder wall, showing the nerves (→). (AThChI).

Fig. 2.63. Taenia pisiformis oncosphere cultured for 30 days to a small cyst stage.

Part of whole mount showing nerves in the cyst wall (→). (AThChI).


62
100μ

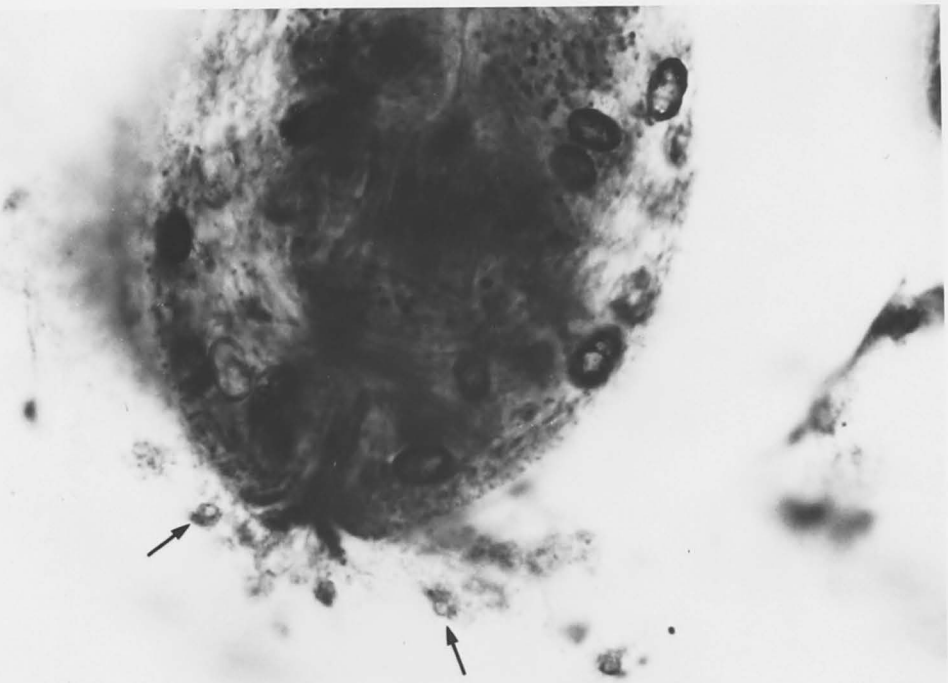

63
50μ

Figs. 2.64 - 2.65. Echinococcus granulosus brood capsule.

Fig. 2.64. Protoscolex with part of brood capsule wall attached to the stalk. Note the esterase-positive cells (→) with processes directed towards the stalk of the protoscolex. (5-bromoindoxyl acetate).

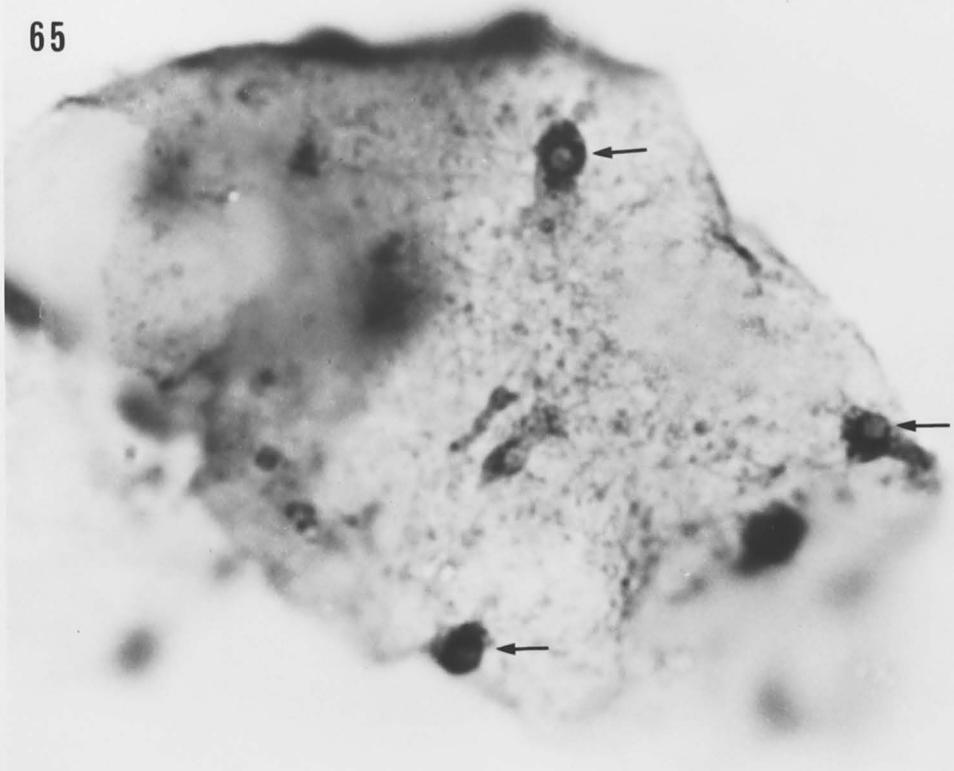
Fig. 2.65. Higher magnification of part of brood capsule wall showing esterase-positive cells (→). (5-bromoindoxyl acetate).

64



20μ

65



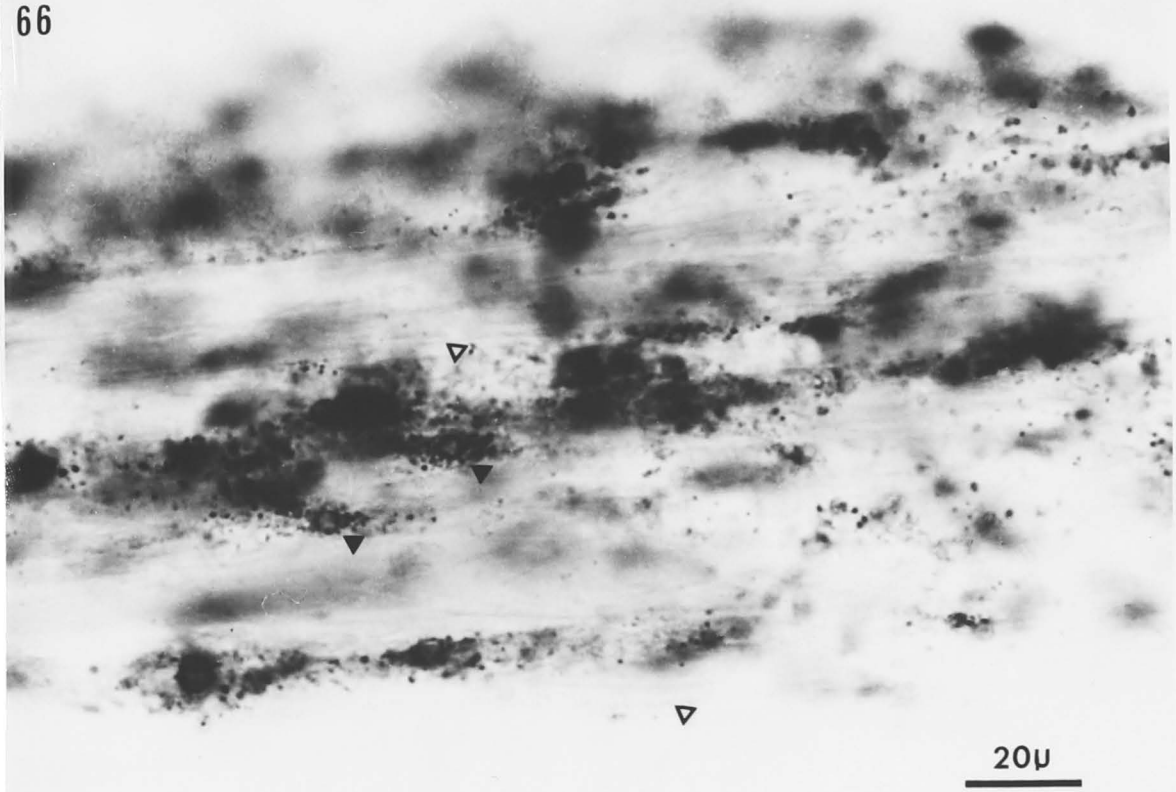
10μ

Figs. 2.66 - 2.67. *Taenia hydatigena* cysticercus.

Fig. 2.66 Whole mount of part of the tail region, showing esterase-positive cells (►) and muscle bundles (▷). (5-bromoindoxyl acetate).

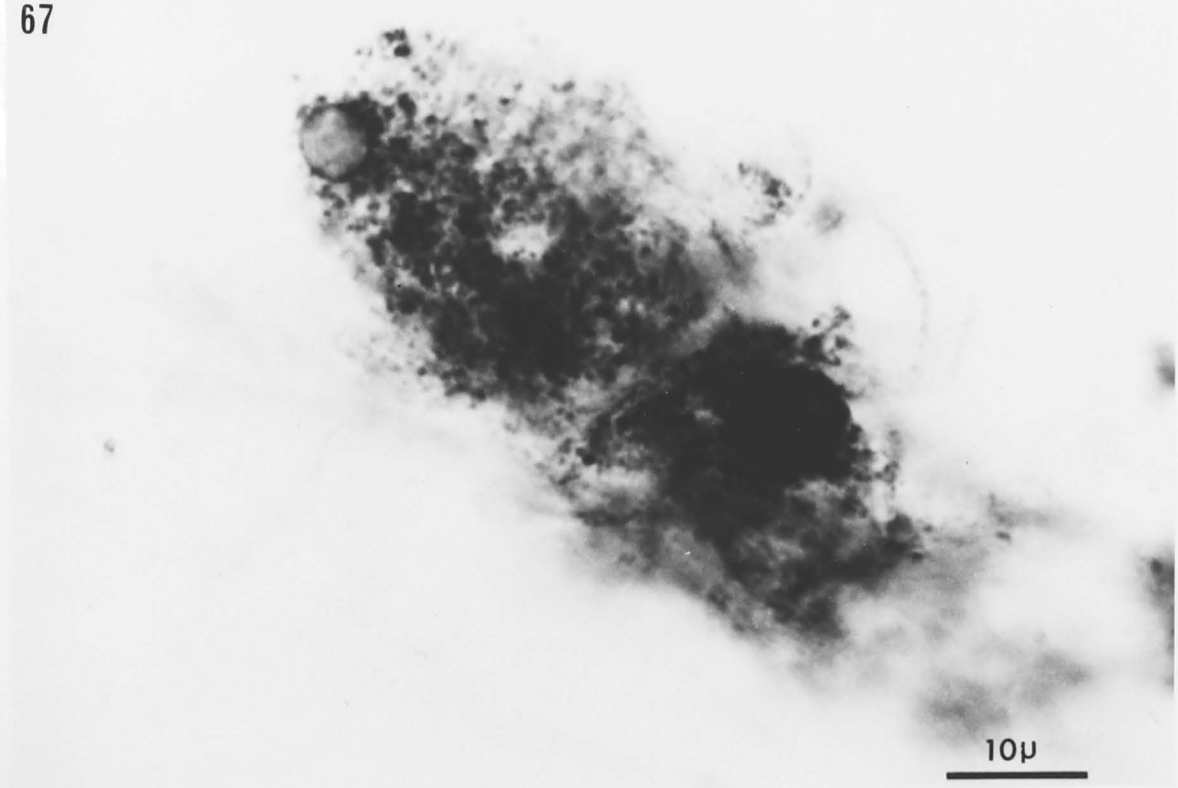
Fig. 2.67. Two of the esterase-positive cells at a higher magnification. Note the granular appearance of the indigo reaction product. (5-bromoindoxyl acetate).

66



20μ

67



10μ

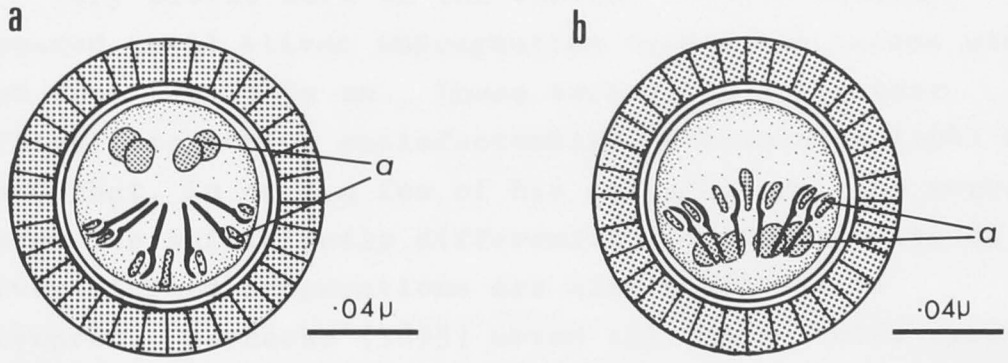
Fig. 2.68. Echinococcus granulosus oncosphere.

Position of cholinesterase deposits.

(a) Interpretation of Fig. 2.18, posterior end facing upwards.

(b) Position of reaction product deposits as they would appear in dorsal view.

(a, sites of cholinesterase activity.)


a
a
·04μ
b
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mentioned earlier, the germinative cells in E. granulosus cyst contain esterase-positive granules.

DISCUSSION

I. Techniques.

As noted in chapter 1, difficulty in preparing cestode material so that the nervous system is selectively stained is frequently encountered.

Very little work on the cestode nervous system appeared until silver impregnation techniques became widely used from the 1880s on. These techniques are rather difficult to employ satisfactorily. Subramaniam (1941 b) found that, in only a few of his preparations, the nervous system was sufficiently differentiated. The results of silver-stained preparations are also difficult to interpret. Zernecke (1895) noted that nerve cells were easily confused with myoblasts. However, a large contribution to the knowledge of the morphology of the cestode nervous system has been derived from these techniques.

Other metallic impregnation techniques have been used with some success. Staining of cestode tissue in von Rath's fluid, which contains osmium (see Tower, 1900), has been employed by a number of workers including Johnstone (1911), Subramaniam (1941 a) and Siddiqi (1961).

Some information on the cestode nervous system has been accumulated by routine histological methods. Rees has made a considerable contribution in this way using stains such as Ehrlich's haematoxylin and eosin or orange

G, and Heidenhain's azan (see Rees & Williams, 1965). The arrangement of nerves and the morphology of neurones has been resolved for a number of species by the use of these methods. However, only occasional descriptions of peripheral nerves and sense organs, for example, the probable stretch receptors of Acanthobothrium coronatum (Rees, 1966) have appeared.

Histological techniques are of limited value in that, while nervous elements in some cestode species are stained satisfactorily, in others, these elements are very difficult to distinguish from other tissues. Niemiec (1885) was able to describe the nervous system of Multiceps multiceps in some detail, whereas his description of the nervous system of Dipylidium caninum was much shorter because he could only distinguish the larger nerve elements in his sections.

Recently, it has been found that histochemical techniques for non-specific esterases and cholinesterases will stain nervous tissue in platyhelminths. Lee, Rothman & Senturia (1963) noted in sections of the cestodes Hymenolepis diminuta, H. citelli, H. microstoma and Hydatigera taeniaeformis stained with the 5-bromoindoxyl acetate method for non-specific esterases, that a positive result occurred throughout the nervous system, including nerve endings in the tegument and nervous tissue within the suckers and rostellar pad. Halton & Jennings (1964) showed that this technique stained the nervous system in whole mounts of the monogenetic trematode, Diplozoon paradoxum. A number of papers have since reported similar results with other species (see chapter 4). However, it has yet to be determined whether or not esterases are

present throughout the entire nervous system in platyhelminths, and thus whether the entire nervous system is in fact revealed by esterase techniques.

In the present study, it was found that the 5-bromoindoxyl acetate technique has some disadvantages. Firstly, it gave inconsistent results when used on whole mounts, the amount of indigo deposited in the nervous system varying within and between specimens of D. caninum. Secondly, granules of the reaction product could be seen with the microscope, giving a diffuse localisation (see Fig. 2.67). Thirdly, some slight non-specific staining occurred throughout the parenchyma.

The Karnovsky & Roots (1964) cholinesterase method as applied in the present study did not have any of the above objections. It gave consistent and very precise localisation of cholinesterase activity throughout the nervous system, and no background staining occurred unless the sections had been incubated for considerably longer than the time required for good development of colour. The incubation period was short, 10 to 20 minutes being required for the species studied here, as opposed to two to three hours for the 5-bromoindoxyl acetate technique in D. caninum. This short incubation period is convenient, and probably contributes to the high specificity of localisation of the reaction product. The Karnovsky & Roots (1964) technique has also been applied successfully to vertebrate tissues at the electron microscope level (Karnovsky, 1964; Robinson & Bell, 1967) and therefore it is potentially a very useful tool for the study of the cestode nervous system. However, the technique does have one disadvantage when used for

light microscopy - the reaction product is yellowish and highly refractile, and is not readily recorded by photomicrography.

Other cholinesterase techniques have been successfully applied to studies on the cestode nervous system. Hart (1967; 1968) used a modification of the Koelle-Friedenwald technique in studies on Mesocestoides tetrathyridia. In the present study, preliminary work using Gomori's modification of the Koelle method (Pearse, 1960) was carried out. It was found that the reaction product was crystalline, and not localised as finely or as specifically as in the Karnovsky & Roots technique.

The electron miscroscope is also a very useful tool in the study of the nervous system. More morphological criteria are available at this level of magnification for the satisfactory identification of nervous structures, than at the light microscope level. Esterase techniques which are suitable for electron microscopy are now available, and such work should be able to establish whether all cestode nervous elements are cholinesterase-positive. However, apart from the work of Morseth (1966; 1967 b), the electron microscope has so far not been exploited in the study of the cestode nervous system.

II. Discussion of nervous structures:

1. Arrangement.

The nervous system of D. caninum has already been described briefly by Niemiec (1885) using a carmine borax staining technique. Because of the small size of the

scolex, he could identify elements of the nervous system in longitudinal sections only, and not in transverse sections. He described the lateral ganglia joined by the cerebral commissure, as well as a lower polygonal commissure and an upper polygonal commissure which was probably complete, joining the median longitudinal nerve cords with the lateral ganglia. He stated that he could not positively identify the rostellar nerve ring, the X-commissure or the accessory lateral nerve cords, but he did observe nerve fibres leaving the longitudinal nerve cords in the strobila.

In the present study, owing to the use of the more specific esterase techniques, the rostellar nerve ring, the X-commissure and the accessory lateral nerve cords have been positively identified, confirming the similarity of D. caninum with taeniid species in the arrangement of the main nervous elements. However, only one polygonal commissure was found, as opposed to two described by Niemiec (1885).

The nervous system of E. granulosus adult has not been described previously. This is perhaps not surprising since the scolex is extremely small, and the nervous system particularly refractory to selective histological staining.

Cohn (1898) was the first to describe the nervous system in adult Hydatigera taeniaeformis, and he commented on the similarity of the nervous system in this species to other taeniid species described by Niemiec (1885). Bartels (1902) described the nervous system in immature adults and strobilocerci, and Rees (1951), in the strobilocercus.

It is interesting to note that all these descriptions agree basically with the present description. However, there are some differences in detail, especially in the arrangement of nerves in the scolex:

(i) Cohn described three polygonal commissures. Bartels agreed that these are present, but considered that, since they are small, they do not require detailed description. In contrast, the results of the present study agree with those of Rees, who described only one polygonal commissure.

(ii) Rees also described two additional commissures, one linking the two dorsal median longitudinal nerve cords, and the other the two ventral cords. Neither Cohn nor Bartels made any mention of these, and they were not observed in this study.

(iii) In the specimens studied here, four accessory nerves are present, linking the arms of the X-commissure with the lateral ganglia. Both Cohn and Bartels described similar nerves, but they were not present in specimens examined by Rees.

(iv) The suckers are innervated by nerves arising from the lateral ganglia and from the intersection of the dorsal and ventral median nerve cords with the polygonal commissure of specimens examined in the present study. Cohn and Bartels described a similar arrangement, while Rees described sucker nerves arising from the dorsal and ventral loci only.

(v) An unpaired lateral nerve and an outer anterior nerve arising from the lateral ganglia were described by Rees, but these were not mentioned by Cohn or Bartels, and were not observed here.

(vi) Cohn and Rees found eight nerves proceeding anteriorly from the level of the lateral ganglia to the rostellar nerve ring. The findings of the present study agree with those of Bartels who described six anterior nerves in all but one of his specimens (see below).

(vii) An apical nerve ring, anterior to the rostellar nerve ring, was described by Bartels. This was not mentioned by Cohn, and was not found by either Rees or in the present study. However, Bartels found that in one strobilocercus, the accessory nerves, the rostellar nerve ring and the apical nerve ring were absent, and eight anterior nerves were present.

(viii) Rees found 36 apical nerves proceeding anteriorly from the rostellar nerve ring. Bartels found 20, and in the present specimens 18 to 21 apical nerves were identified.

Rees (1951), in comparing her work with that of Bartels (1902), suggested that the nervous system in H. taeniaeformis can vary. The additional information given above supports this view. These variations may be both real and apparent.

A real variation is probable with regard to details of the smaller nerves, for example, the accessory nerves linking the arms of the X-commissure with the lateral ganglia. In the present study, it was found that there are many small nerves present, linking the longitudinal elements and supplying the tegument and musculature (for example, see Fig. 2.22), and these nerves are probably subject to intraspecific variation.

Apparent variation may be due to the state of contraction of the scolex, small nerves linking the longitudinal nerve elements being more compact in contracted specimens and perhaps resembling commissures. An apparent variation may also be due to the use of different staining techniques by various authors, so that different interpretations of the results were given. The variation in the number of nerves proceeding from the lateral ganglia to the rostellar nerve ring could be explained by differing interpretations of a similar structure. In the present study, each lateral ganglion consists of two lobes, dorsal and ventral, separated by ganglion cells (see Figs. 2.30, 2.58). At the anterior end, this structure joins the rostellar nerve ring, the lateral parts of which may bend posteriorly towards the lateral ganglia. One author may interpret the lateral ganglion and its anterior nerve as one structure, while others might interpret it as two structures lying close together.

The number of apical nerves arising in the rostellar nerve ring is of interest. Rees (1951) found 36, corresponding exactly to the number of hooks, and suggested that they possibly supply muscles which control movements of the hooks. In the present study, only approximately 20 apical nerves were found, although 30 hooks were present. The difference in number of apical nerve cords is probably due to intraspecific variation, as the number of hooks in H. taeniaeformis varies (see Wardle & McLeod, 1952). However, the results of the present study do not support the view that the main function of the apical nerves is to supply hook muscles.

These nerves appear to divide and supply all parts of the rostellum.

Rees (1951) found that the ten longitudinal nerve cords continue from the pseudostrobila into the bladder of the strobilocercus. The median and accessory lateral nerves could be traced for about half the length of the bladder, and the main lateral nerves, a little further. In the present study, by comparison, a large number of small longitudinal nerves were found throughout the bladder wall. It is possible that none of these represents the original longitudinal nerve cords, but that they are branches supplying the muscles of the bladder.

Niemiec (1885) first described the nervous system of T. pisiformis. Most of the details he gave agree with those given in the present study. However, he described two polygonal commissures, and four ganglionic swellings in the rostellar nerve ring, whereas only one polygonal commissure has been detected here, and no rostellar ganglia were observed.

A brief description of the nervous system of B. criniae minor was given by Hickman (1960). In histological preparations, he found a 'thick nerve ring', probably the polygonal commissure, which was enlarged on each side to form the lateral ganglia. From each ganglion, a lateral longitudinal nerve was given off to the strobila. More detail has been given here, owing to the selectivity of the Karnovsky & Roots (1964) technique.

2. Histology.

In 1885, Niemiec described the nervous system of the taeniid cestode, Multiceps multiceps in considerable

detail, and included information on the histological structure of elements of the central nervous system. In the 'central ganglion' (the central portion of the cerebral commissure) and in the lateral ganglia, he described ganglion cells similar to those seen in the present study. In the central ganglion, most cells were located in the posterior portion, while in the lateral ganglia, they were dispersed throughout the central granular mass. Nerve cells also occurred in the polygonal commissures, the intersections of the median longitudinal nerve cords with the polygonal commissures, and the rostellar nerve ring. Niemiec also found that the cells of the parenchyma built up around the central ganglion, forming a limiting layer. In all these respects, the histological structure of M. multiceps is similar to that of T. hydatigena, as described here.

Ganglion cells have been described in the lateral ganglia and the cerebral commissure in most cestode species studied histologically. Since chromalum-haematoxylin-phloxine and Heidenhain's azan frequently stain neurosecretory substance (see Gabe, 1966), it is possible that the larger ganglion cells of D. caninum have a neurosecretory function. However, elucidation of this problem would require detailed studies to establish whether a secretory cycle in these cells could be correlated with any physiological function.

The lateral ganglia and cerebral commissure appear to comprise a primitive brain or co-ordinating centre in cestodes. In trypanorhynchs, it has been found that nerve cells are present in the transverse commissure, but absent in the lateral ganglia (see Dollfus, 1942).

Rees (1966) observed a similar condition in the tetraphyllid, Acanthobothrium coronatum. The term lateral 'ganglion', although convenient, is a misnomer for the lateral concentration of nervous tissue in these species, since the term 'ganglion' imples a concentration of nervous tissue containing neurons. The concentration of nerve cells in the transverse commissure, and their absence in the lateral 'ganglia' is probably indicative of a more highly organised brain than that found in the Cyclophyllidea.

Binding cells, noted here in sections of T. hydatigena where they surround the central nervous system in the scolex, have been described in other cestode species. As mentioned earlier, Niemiec (1885) considered that these were a concentration of parenchymal cells built up around the cerebral ganglion of Multiceps multiceps. Elliptical cells scattered over the surface of the ganglia and main longitudinal nerve cords were found in Moniezia expansa by Tower (1900). Johnstone (1911) described in the trypanorhynch Grillotia erinaceus 'a very delicate capsule on which are situated some scattered nuclei' surrounding the central nervous system in the scolex, with the exception of the posterior transverse commissure. Each main lateral longitudinal nerve cord of the tetraphyllid, Acanthobothrium coronatum has binding cells on the outer side (Rees, 1966).

These binding cells are probably either modified parenchymal cells, as suggested by Niemiec (1885), or modified nerve cells. Those described in M. multiceps, G. erinaceus, and T. hydatigena are probably of parenchymal origin, as they are extremely small, and in

T. hydatingena, completely different in appearance and in staining characteristics from the nerve cells. However, the binding cells in M. expansa and A. coronatum may be nervous in origin, as they are similar in appearance to cholinesterase-positive cells on the periphery of longitudinal nerve cords, observed in the present study in T. pisiformis. The binding cells in cestodes may be a functional counterpart of the connective tissue limiting structure found surrounding the nerve bundles in higher animals (see Bloom & Fawcett, 1962, p.230). When the longitudinal muscles of the strobila are extended, the nerve cords are straight, but when the longitudinal muscles are contracted, the nerve cords take a sinuous course. Rees (1966) suggested that the binding cells may protect the nerves, preventing them from tearing when contraction of the longitudinal muscles occurs.

3. The inner plexus.

This system of nerve fibres probably serves to provide nervous pathways between the longitudinal nerve cords, thus providing local co-ordination in the proglottid. Some of the nerves supply the inner circular muscles of the proglottid, and others diverge from the plexus to the longitudinal muscle bundles of the frontal portions of the proglottid. As mentioned in chapter 1, Zernecke (1895) first described this structure in Ligula intestinalis. Cohn (1898) described a similar network between the longitudinal nerve cords of Bothridium pithonus and Hydatigera taeniaeformis. In the strobila of Tylocephalum dierama, the younger proglottids have an inner plexus, while more mature proglottids have an inner plexus anteriorly and a series of ring commissures

posteriorly (Subramaniam, 1941 a). More recently, the inner plexus has been observed on application of histochemical esterase techniques to cestode tissue. Lee, Rothman & Senturia (1963) figured the cut ends of 'transverse nerves' in longitudinal sections of H. taeniaeformis proglottids. Schardein & Waitz (1965) presented photographs showing sections of similar nerves in H. taeniaeformis and D. caninum, but did not comment on these nerves. Hart (1967) described numerous 'circular nerves' interconnecting the longitudinal nerve trunks in Mesocestoides tetrathyridia.

The presence of ring commissures in cestode proglottids has been noted more frequently. They are present for example, in Moniezia expansa (Tower, 1900), Tetrabothrius laccocephalus (Spätlich, 1909), Anoplocephala magna (Becker, 1922), Anthobothrium auriculatum (Rees, 1943), Scyphophyllidium giganteum and Lacistorhynchus tenue (Riser, 1949), Thysanosoma actinoides and Taenia pisiformis (Lacey, 1955), Cotugnia digonopora (Siddiqi, 1961) and Acanthobothrium coronatum (Rees & Williams, 1965). In the present study, the interproglottidal nerve ring in D. caninum, H. taeniaeformis and T. pisiformis appears to be a localised concentration of nerves of the inner plexus. Such ring commissures may be important in co-ordination of the movement of the proglottid when it becomes detached from the rest of the strobila.

4. The peripheral plexus.

Blochmann (1895) and Zernecke (1895) both described an outer plexus in Ligula intestinalis, Triaenophorus lucii

and Taenia pisiformis, but this network of nerves occurred beneath the tegument cells. In the present study, the peripheral plexus is located between the cell bodies and the distal cytoplasm of the tegument, in the same general area as the peripheral muscle bundles first described in cestodes by Blochmann and Zernecke, and confirmed more recently by electron microscopy (Threadgold, 1965; Béguin, 1966; Lumsden, 1966; Morseth, 1966; 1967 a; Bräten, 1968). The nerves of the peripheral plexus probably control the function of the peripheral muscles.

The nerve fibres of the peripheral plexus in the bladder wall of T. hydatigena and T. pisiformis probably serve as a diffuse network, co-ordinating the movements of the bladder, as well as supplying the muscle fibres of the bladder wall. In the cyst wall of E. granulosus brood capsules, the nerve fibres may form pathways of communication between the protoscoleces of the brood capsule.

The nervous system within the brood capsule wall of E. granulosus may have an additional function. The protoscolex is capable of developing into either a cyst if injected into the peritoneal cavity of a sheep, or an adult if eaten by a dog (Smyth, Howkins, & Barton, 1966). Since the protoscolex does not undergo differentiation in either direction while attached to the wall of the brood capsule within the hydatid cyst, the diffuse nerve net within the capsule wall may contain a suppressor substance which inhibits such differentiation.

5. Nerve endings.

As mentioned in chapter 1, nerve cells which are probably sensory were first observed in cestodes by

Blochmann (1895) and Zernecke (1895). Since that time, 'free' nerve endings have been described in a number of trematodes and cestodes. Rohde (1966; 1968 a;b), using a urea-silver nitrate stain, demonstrated nerve endings in the tegument of the trematodes, Multicotyle purvisi and Diaschistorchis multitesticularis. Esterase techniques have revealed nerve endings in a number of cestodes. Schardein & Waitz (1965) found that nerve endings in the tegument of D. caninum, H. taeniaeformis, Hymenolepis diminuta and H. nana were intensely reactive; Hart (1967) described 'heavy' fibres which penetrated into and through the tegument of Mesocestoides tetrathyrida; and Öhman-James (1968) described a nerve ending in the tegument of Diphyllobothrium dendriticum.

The nervous nature of these structures has been confirmed for a number of cestodes and trematodes by electron microscopy. Morseth (1966) described unmyelinated nerves penetrating the distal cytoplasm of the tegument of T. pisiformis, and (1967 b) bulb-like nerve endings in the tegument of E. granulosus, each with a distal process projecting beyond the tegument. These two types of nerve endings may correspond to the tubular and bulbous nerve endings in the tegument of H. taeniaeformis. Bulbous nerve endings each with a distal process projecting beyond tegument have also been described in several trematode species (Dixon & Mercer, 1965; Erasmus, 1967; Morris & Threadgold, 1967). It is therefore likely that nerve endings occur throughout the Platyhelminthes.

Each nerve ending may be part of a sensory cell. In T. pisiformis, a nerve fibre could be traced from the nerve ending to a cell body which had a second process

leading to a co-ordinating nervous structure. Similar arrangements have been found in the cestodes Ligula intestinalis, Triaenophorus lucii and Taenia pisiformis by Blochmann (1895) and Zernecke (1895), in trematodes by Blochmann & Bettendorf (1895), Bettendorf (1897) and Havet (1900), and in turbellaria (see Bullock & Horridge, 1965), and the cells described were considered by these authors to be sensory neurones because of their morphology. It is probable that nerve endings in the Platyhelminthes are terminals of processes from sensory neurones, and could be demonstrated as such in suitable preparations. The variation in morphology of the nerve endings may indicate a specialisation in function. However, their sensory nature has yet to be proved.

6. Innervation of the muscles.

(i) The neuromuscular junction. The neuromuscular junction in H. taeniaeformis appears to be very similar to the end-plates of mammalian unmyelinated nerves on smooth muscle (see Bloom & Fawcett, 1962, p.232), but their similarity has yet to be confirmed by electron microscope studies. Muscle end-plates have also been described by Lee & Tatchell (1964) in the cestode Anoplocephala perfoliata, and by Wilson & Schiller (1969) in Hymenolepis diminuta.

Probable neuromuscular junctions have been found during electron microscope studies in the rostellar pad of the cestode Echinococcus granulosus (Morseth, 1967 b) and in the pharynx of the trematode Fasciola hepatica (Dixon & Mercer, 1965). The description of these structures by Dixon & Mercer is similar to that given by Caesar, Edwards & Ruska (1957) of axon-muscle contact regions in mammalian smooth muscle. At these mammalian neuromuscular junctions,

the nerve and muscle membranes were very close together, the basement membrane which invests the muscle fibre being absent at this point; the axon contained a significantly greater number of mitochondria and vesicles than the rest of the axon; and the contact region of the muscle cells showed subcytolemmal aggregation of mitochondria and augmented endoplasmic reticulum. These features were considered by Caesar, Edwards & Ruska to be sufficient to satisfy the criteria for a synapse. Although a basement membrane surrounds the muscle fibres in mammals (Caesar, Edwards & Ruska, 1957) and trematodes (Dixon & Mercer, 1965), no such membrane was found investing the muscle fibres in the cestodes Calliobothrium verticillatum, Phyllobothrium foliatum, Lacistorhynchus tenue and Hymenolepis diminuta by Lumsden & Bryam (1967). The absence of a muscle basement membrane would make neuromuscular synapses more difficult to recognise in cestodes.

(ii) The rostellar pad. Lee, Rothman & Senturia (1963) and Eränkö, Kouvalainen, Mattila & Takki (1968) found esterase-positive nerves in the rostellar pad of H. taeniaeformis. In the present study, this observation has been confirmed for this species, and similar nerves are also present in D. caninum, E. granulosus and T. pisiformis.

(iii) The suckers. Gough (1911) found large multipolar ganglion cells in the suckers of the cestode Avitellina centripunctata which were usually closer to the delimiting membrane of the sucker than to the tegument. He had earlier observed similar cells in Stilesia centripunctata and Anoplocephala magna. Becker (1922) also described nerves in the suckers of A. magna. Lee, Rothman & Senturia

(1963) and Eränkö, Kouvalainen, Mattila & Takki (1968) found esterase-positive nerves in the suckers of Hydatigera taeniaeformis. The latter observation has been confirmed in the present study, and it has been found that the suckers of D. caninum, E. granulosus, T. pisiformis and B. criniae minor are also richly supplied with nerves.

The concentration of nerve endings on and surrounding the lip of the sucker may indicate that this area of the sucker is very sensitive to the environment, and may be capable of recognition of host tissue suitable for attachment.

The sucker plexus in the cestodes studied here is derived from nerves which enter the sucker as single or small groups of fibres. This structure may serve to co-ordinate the activity of the sucker. The multipolar cells present in the suckers of T. pisiformis cysticerci are probably nerve cells. Similar large multipolar ganglion cells were observed by Gough (1911), and such cells may be part of the sucker plexus.

Sensory receptors which are probably stretch receptors have been described by Rees (1966) in the scolex of Acanthobothrium coronatum. These cells are triangular in shape, the apex of the triangle being continuous with an axon which passes into the nerve cord. Processes extend from the base of the triangle to muscle fibres of the bothridia. A nerve arising from the nerve cord near the axon and running parallel to it towards the muscle could be seen in places.

Stretch receptor organs in decapod crustacea have been studied in some detail (for references, see Pilgrim, 1960)

for two main reasons: firstly because they possess most of the essential elements which are found in vertebrate muscle spindles, and secondly, because they are readily accessible, they can be studied under direct observation, and the properties of their individual cell components can be investigated in isolation or within the body of the animal (Kuffler, 1954). According to Pilgrim (1964), each muscle receptor organ comprises typically a specialised receptor muscle and direct inhibitory innervation of the stretch receptor cell.

Rees (1966) suggested that stretch receptors would seem to be essential in a cestode scolex which adheres by muscular action to the intestine of the host. According to Kuffler (1954), an appreciable portion of the nervous system in vertebrates is exclusively devoted to the indirect control of posture and movement by muscle spindles. A similar situation might be expected in cestodes in the maintenance of attachment to host tissue.

In cyclophyllidean cestodes, the scolex possesses suckers, attachment organs whose musculature is more compact than that of the bothridia of Acanthobothrium coronatum. Accordingly, it might be expected that stretch receptors would be present in the suckers, and also in the rostellar pad in the species where this structure is present. Although no nerve cells resembling typical crustacean stretch receptors were observed in the species studied here, it is possible that other nervous structures have a stretch receptor function. Two nerve elements are worth considering in this context:

(i) The large multipolar nerve cells in the sucker and rostellum of T. pisiformis. Processes of these cells may

be attached to muscle fibres. It was not possible to determine whether this was so, in the present study. However, in such a situation, the multipolar cells could be sensitive to change in shape of the sucker as a whole, measured in terms of increase or decrease in distance between the radial muscle fibres. In T. pisiformis, no accessory nerves to the multipolar cells were observed. Rees (1966) suggested that in A. coronatum, the motor nerve, instead of accompanying the stretch receptor, may take some other route to the muscle. The nerve inhibitory to the stretch receptor may also be absent. Pilgrim (1964) found in the second thoracic segment of the crustacean Squilla mantis a cell resembling a stretch receptor in electrical characteristics, but for which no accessory nerve was detected. The stretch receptor would then consist simply of a sensory nerve cell whose processes are sensitive to deformation either by stretch or compression.

(ii) Some fibres of the sucker plexus. Although the main function of the sucker plexus is likely to be control of muscular activity within the sucker, some of the fibres could have a stretch receptor function. In the crustacean Palinurus vulgaris, the nerve cell body of the stretch receptor in the coxo-basipodite is located in the central nervous system (Alexandrowicz, 1967). A similar situation could be present in cestode suckers, all histological evidence of the stretch receptor being confined to dendritic nerve endings on muscle fibres.

It is not known how the sucker operates in cyclophyllidean cestodes. In view of the importance of the suckers in attachment, hypotheses on their function may be valuable in the stimulation of research on this subject.

Three possibilities will be considered here, the first two suggested by Smyth (1969 a) as alternative mechanisms by which E. granulosus may remain attached to host tissue, and the third suggested by M.J. Howell (pers. comm.) for trematode suckers which are morphologically very similar to those of cyclophyllidean cestodes.

(i) The 'catch' mechanism. This term is applied where persistent muscle tonus is maintained in the absence of continued stimulation. According to Prosser (1967) it is best studied in the anterior byssus retractor muscle of the mollusc Mytilus. However, a similar phenomenon is also seen in many other smooth muscles, notably in vertebrate vascular muscle. The precise mechanism involved in the 'catch' is not understood. Prosser (1967) suggested that there is some truth in both the two main hypotheses explaining the 'catch' mechanism: (a) that such muscle differs from other muscles only in the rate of relaxation and in requiring fewer impulses to maintain tension and (b) that the participation in contraction of paramyosin, which occurs in relatively large quantities in such muscles (about 40 per cent of the anterior byssus retractor muscle of Mytilus) in conjunction with actomyosin is responsible for the phenomenon.

Smyth (1969 a) pointed out that a problem comparable to that of the anterior byssus retractor muscle of Mytilus appears to exist in many trematodes and cestodes. In order to maintain their position in the gut, the muscles of the attachment organs must be maintained in a state of continual contraction. He posed the question that a comparable 'catch' mechanism might operate here.

(ii) Smyth (1969 a) also suggested that if the sucker muscles do not operate on the 'catch' principle, then presumably they require large quantities of energy in order to maintain contraction. Since, in an intestinal site, unlimited supplies of food would be available, and since the presence of microtriches on the tegumental surface of the suckers (Morseth, 1967 a) suggests that the suckers themselves are capable of continuously absorbing nutrient, it is possible that the rate of absorption may be sufficient to supply continuously enough energy for sustained contraction.

If such large quantities of energy were required for the attachment of the cestode scolex, the cestode would consume large quantities of carbohydrate, thereby depriving the host of a portion of its necessary energy supply. This would have a detrimental effect on the host unless it consumed considerably more food than necessary for its own requirements. However, Rees (1967), in a review on cestode pathogenicity, concluded that in general adult cestodes have little effect on their hosts and are rarely pathogenic. Moreover, there are a number of cestodes whose scoleces are capable of surviving in the intestine when the host is undergoing starvation, notably Raillietina cesticillus of the fowl (Reid, 1942). In this case, presumably enough carbohydrate is available between the villi and in the crypts of Lieberkühn for maintenance of scolex attachment. This suggests that the energy requirements of the suckers is not great.

(iii) Howell (pers. comm.) has suggested that it is possible to erect a hypothesis on the mode of operation of the suckers which does not assume that the muscles remain in a state of

continued contraction. When the sucker is attached to the host, the musculature may be in a relaxed state. On initial contact of the sucker with host tissue, the orifice may be enlarged by contraction of peripheral muscles of the scolex surrounding the sucker and radial muscles of the sucker, in a manner similar to the dilation of the pupil of the vertebrate eye. Contraction of the muscles surrounding the basement membrane of the sucker would then bring the sucker concavity in contact with the host tissue. Relaxation of all muscles involved in this process would enable the sucker to regain its normal shape, drawing a piece of host tissue into the cavity of the sucker as it does so.

It is of interest, in considering the above ideas, to note that the shape of the suckers in fixed D. caninum is the same in both attached and free scoleces. In living D. caninum, although the suckers may be protruded at different angles, the shape of the sucker does not appear to change. This suggests (a) that the sucker shape changes only in the act of attachment and (b) that the maintenance of attachment requires little effort on the part of the parasite. There can be little doubt that the muscles in the sucker are functional since they contain large quantities of glycogen (Lumsden & Bryam, 1967). However, whether they operate only in the act of attachment, or whether they remain in a state of tonic contraction involving a 'catch' mechanism, remains to be seen.

Byram/

7. Innervation of the reproductive system.

No nerves supplying the medullary organs of the reproductive system were observed in any of the species studied here. In contrast, Becker (1922) found that the

reproductive organs in Anoplocephala magna were supplied with nerves arising in the inner plexus, and Subramaniam (1941 a) described nerves supplying the ovary, testes and vitellaria in Tylocephalum dierama. Lee, Rothman & Senturia (1963) found that cholinesterase activity was present in the vicinity of the sperm ductules, sperm duct and oviduct of H. taeniaeformis, and suggested that this was associated with the nervous supply to these organs. A similar situation was found in Anoplocephala perfoliata by Lee & Tatchell (1964). In the present study (see chapter 4) the parenchyma in H. taeniaeformis was cholinesterase positive, but the reaction did not appear to represent nerve fibres. Possibly there was poor fixation of the medullary region, as the strobila was fixed whole, and formaldehyde has poor penetration properties. However, this criticism could not be applied to the preparations of D. caninum, E. granulosus or B. criniae minor, as the greatest distance which has to be penetrated by the fixative in these species is less than the depth of the muscular cortex of H. taeniaeformis, in which there was good preservation of the nerve fibres.

In many papers describing the nervous system in the proglottids of cestode species, there was no mention of innervation of the medullary reproductive organs (see, for example, Niemiec, 1885; Tower, 1900; Gough, 1911; Riser, 1949; Siddiqi, 1961; Rees & Williams, 1965). There are two possible explanations for this: either these reproductive organs have no nervous supply in the species studied, or the nerves supplying the reproductive organs were not selectively stained by the techniques used.

It might be expected that there is some mechanism for controlling and co-ordinating the function of the medullary reproductive organs. In vertebrates, there is a sympathetic nervous supply to ovaries and testes, and, in addition, a complex hormonal system controlled ultimately by neurosecretory cells in the hypophysis. It is possible that in cestodes, the reproductive organs are functionally maintained by hormones produced in the nervous system, and transported to their target site by diffusion through the parenchyma. If this is so, then a nervous supply may be unnecessary. However, this hypothesis has yet to be substantiated.

In contrast to the lack of nervous supply to the ovary, testes and vitellaria, the cirrus and the wall of the copulation canal are richly innervated, and there is a concentration of nerves in the tegument surrounding the genital pore. Genital nerves supplying the cirrus pouch and the copulation canal are also present in Cotugnia digonopora (Siddiqi, 1961). Lee, Rothman & Senturia (1963) found that cholinesterase was present in the cirrus sac of Hymenolepis diminuta, H. citelli and Hydatigera taeniaeformis, but was absent in the walls of the copulation canal. Eränkö, Kouvalainen, Mattila & Takki (1968) described cholinesterase-positive nerves innervating the genital apparatus in H. taeniaeformis.

The nerves supplying the genital apparatus may control copulation. Those in the tegument are probably sensory and responsible for initiating copulation, while those in the cirrus pouch and the wall of the copulation canal probably control the muscles which would operate in copulation. In B. criniae minor, the presence of a large

number of nerves supplying the genital atrium may indicate that this structure is more important in copulation in B. criniae minor than in D. caninum, E. granulosus and H. taeniaeformis. The subsurface muscle strands which radiate from the genital pore of B. criniae minor may be capable of dilating the genital pore.

The presence of cholinesterase-positive structures in the oncospheres of E. granulosus is of particular interest. Rybicka (1967) stated that it seems reasonable to expect the existence of some centres stimulating the activity of hook muscles in hatched embryos of Hymenolepis diminuta. The structures near the hooks of E. granulosus oncospheres are possibly nerve centres supplying hook muscles. Sub-surface muscles attached to the pivot of the hook occur in oncospheres of H. citelli (Collin, 1968), and similar muscles are probably present in E. granulosus oncospheres. The four larger structures may be associated with the penetration glands of the oncosphere.

8. Innervation of the rostellar gland.

It has been established that the rostellar gland of E. granulosus is secretory (Smyth, 1964). The rich innervation of this organ may indicate that its secretory cycle is under nervous control.

CHAPTER 3

RESPONSES OF *DIPYLIDIUM CANINUM* PROGLOTTIDS TO CHOLINERGIC DRUGS

INTRODUCTION

The fact that nerves in *Dipylidium caninum* are cholinesterase-positive (chapters 2 and 4) suggests that these nerves may be cholinergic. However, although cholinergic nerves contain acetylcholinesterase, this enzyme is also associated with processes occuring outside the nervous system. Thus the presence of cholinesterase in the nervous system does not necessarily indicate that the nerves are cholinergic.

In this chapter, the effect of a number of cholinergic drugs on movements of *D. caninum* proglottids was studied in order to determine whether the neuromuscular junction in this species is cholinergic. Cholinergic drugs either mimic or block the action of acetylcholine (ACh) at cholinergic synapses. The drugs whose effects were studied here were the cholinergic drugs acetylcholine chloride (AChCl), pilocarpine, nicotine, atropine and tubocurarine chloride. In vertebrates, pilocarpine has a weak ACh-like activity, atropine is an antagonist at postganglionic cholinergic synapses, while nicotine is an agonist and tubocurarine an antagonist at voluntary muscle synapses (Barlow, 1964).

MATERIALS AND METHODS

Dipylidium caninum was obtained from naturally infected dogs, either by purging with arecoline hydrobromide, or, on autopsy, after carbon monoxide poisoning. The worms were washed in several changes of Hanks' balanced salt solution (Hanks' BSS) (Hanks, 1948) before use, and proglottids from about half way along the length of a fully gravid worm were selected for the experiments. A proglottid was suspended in a muscle bath containing Hanks' BSS, by means of cotton thread tied to each end. One end was attached to a glass rod in the bottom of the bath, and the other to a light drawn-plastic lever. Essential details of the apparatus used are given in Fig. 3.4. The muscle bath was maintained at 37°C in a water bath. The writing lever was almost balanced by attaching a piece of plasticine to the end opposite the writing point so that the tension on the proglottid was small enough for the proglottid to contract against, but not so small that the writing point could not move downwards when the proglottid relaxed. The fulcrum was arranged so that magnification of the proglottid movements was approximately 5 to 1. To reduce friction to a minimum, the kymograph paper was smoked very lightly.

The cholinergic agonists, acetylcholine chloride[1] (AChCl), pilocarpine nitrate,[2] and nicotine,[1] and the antagonists, atropine sulphate[2] and D-tubocurarine chloride[3]

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1 Koch-Light Laboratories, Colnbrook, England.

2 Macfarlan Smith Ltd., Edinburgh, Scotland.

3 Pierce Chemical Company, Rochford, Illinois, U.S.A.

were made up to 10^{-3}M aqueous solutions and diluted with Hanks' BSS for use at concentrations of 10^{-4} or 10^{-5}M.

The Hanks' BSS was drained from the muscle bath and a drug solution which had previously been brought to 37°C was added to the muscle bath until the proglottid was completely immersed. After 1 to 2 minutes, the drug solution was drained, and replaced by Hanks' BSS from the reservoir in the water bath. A further two rinses with Hanks' BSS followed in order to remove all traces of drug solution.

RESULTS

When an entire D. caninum is placed in a physiological salt solution, successive waves of contraction pass from the anterior to the posterior end. These are caused by localised contraction of longitudinal muscles accompanied by relaxation of circular muscles. When proglottids are isolated, contraction waves originating at the anterior end of the proglottid and passing posteriorly, still occur. The successive changes in shape that take place in a proglottid as a wave of contraction passes along its length are shown in Fig. 3.5. When all the longitudinal muscles are relaxed, the proglottid is longest, when the longitudinal contraction wave is in the middle of the proglottid, it is shortest. Since in the experimental procedure it is the change in length which is registered on the kymograph paper, the movements of the proglottid will be described in terms of longitudinal muscle contractions only. If a chain of proglottids is set up in the muscle bath there may be no recordings of contraction

on the kymograph, since frequently there is a constant number of contraction waves proceeding from the anterior to the posterior end and no overall lengthening and shortening of the proglottid chain. Hence observations were restricted to single proglottid preparations.

The number of contractions per minute in a proglottid of D. caninum in Hanks' balanced salt solution at 37°C varies, the number of contractions per minute becoming gradually less with time. There is also some variation between proglottids, the number of contractions per minute ranging from 6 to 15. When the Hanks' BSS bathing a proglottid of D. caninum was changed, there was no variation in the contraction pattern of the proglottid.

Responses of two proglottids of D. caninum to some cholingergic drugs are shown in Figs. 3.2 and 3.3 respectively.

Proglottids of D. caninum responded to a 10^{-4}M solution of AChCl by a general increase in the tone of the longitudinal muscles, consequent decrease in the degree of relaxation following each contraction wave, and a decrease in the frequency of contraction waves. When the drug was removed, there was a slackening of the muscle tone over the next 2 to 3 minutes, followed by recovery of the proglottid as indicated by a trace similar to that obtained before the application of the drug.

Similar responses occurred when nicotine 10^{-4}M, tubocurarine chloride 10^{-5}M, pilocarpine 10^{-4}M and atropine 10^{-4}M respectively were applied to the proglottid. When 10^{-5}M tubocurarine chloride and 10^{-4}M nicotine were applied together, the proglottid again responded in a similar

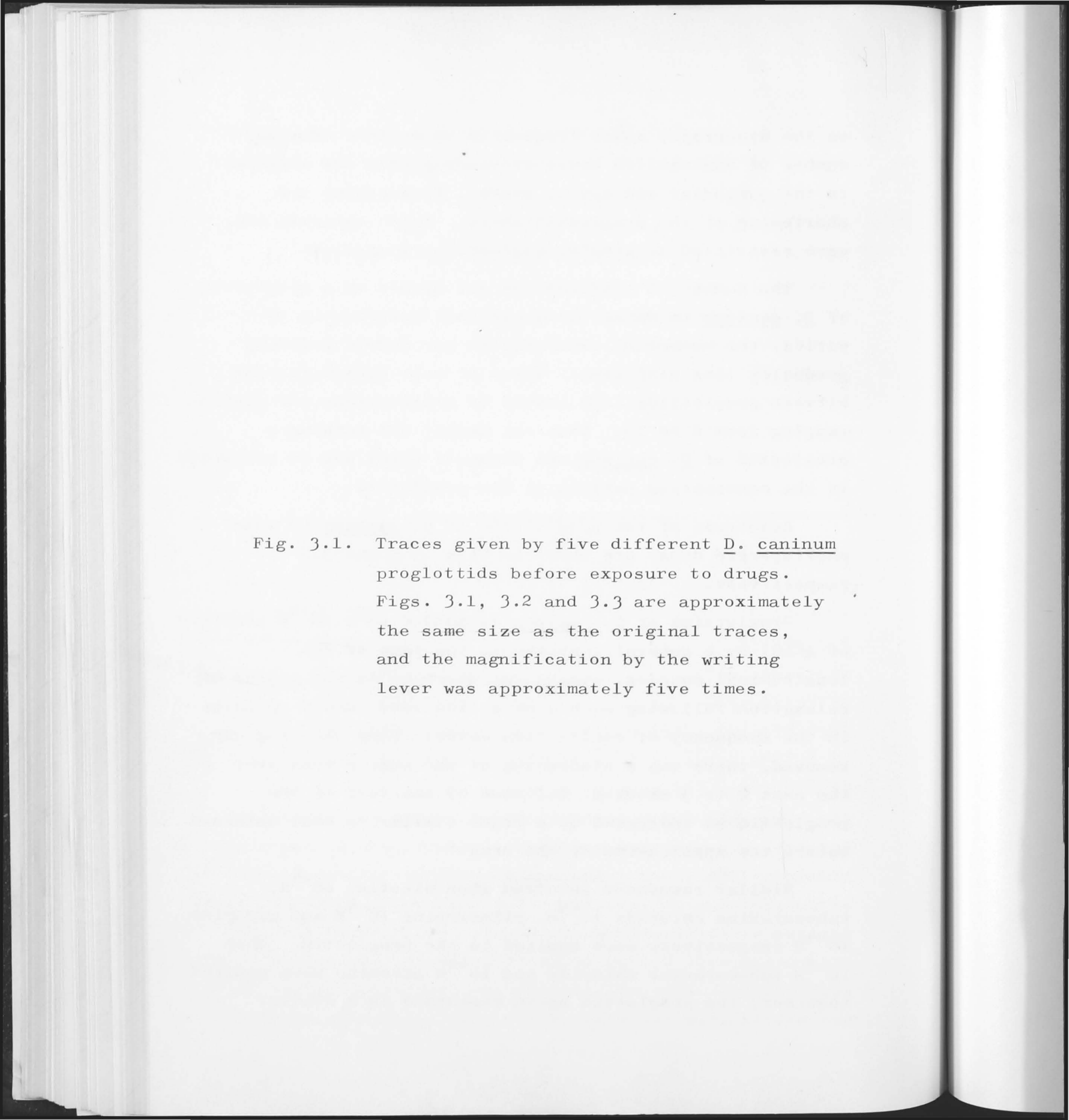
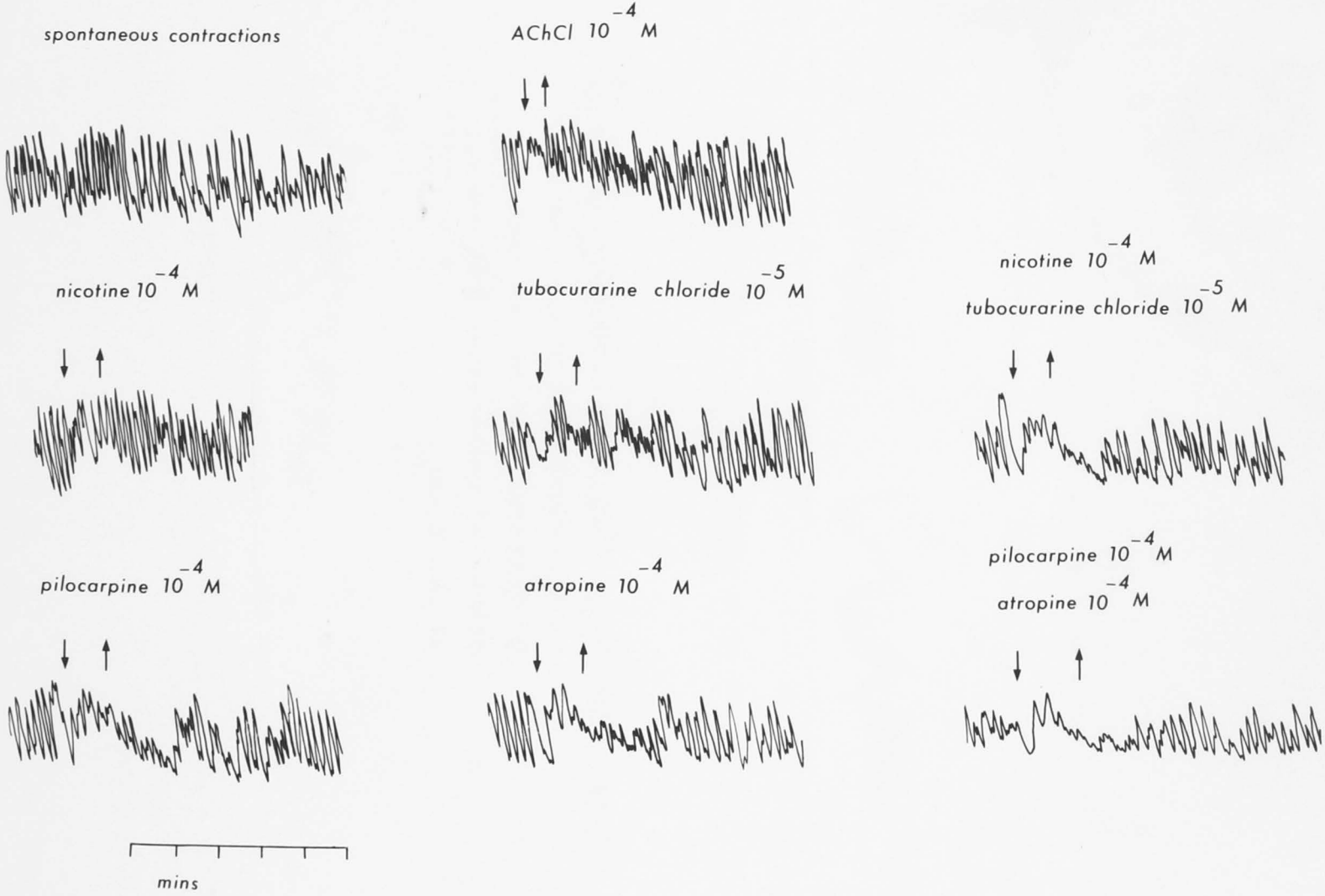


Fig. 3.1. Traces given by five different D. caninum proglottids before exposure to drugs. Figs. 3.1, 3.2 and 3.3 are approximately the same size as the original traces, and the magnification by the writing lever was approximately five times.

1

Fig. 3.2. Effects of five cholinergic drugs on contractions of a D. caninum proglottid. The arrows indicate the points of application and removal of the drug.

2


spontaneous contractions
AChCl 10^{-4} M
nicotine 10^{-4} M
tubocurarine chloride 10^{-5} M
nicotine 10^{-4} M
tubocurarine chloride 10^{-5} M
pilocarpine 10^{-4} M
atropine 10^{-4} M
pilocarpine 10^{-4} M
atropine 10^{-4} M
mins

3

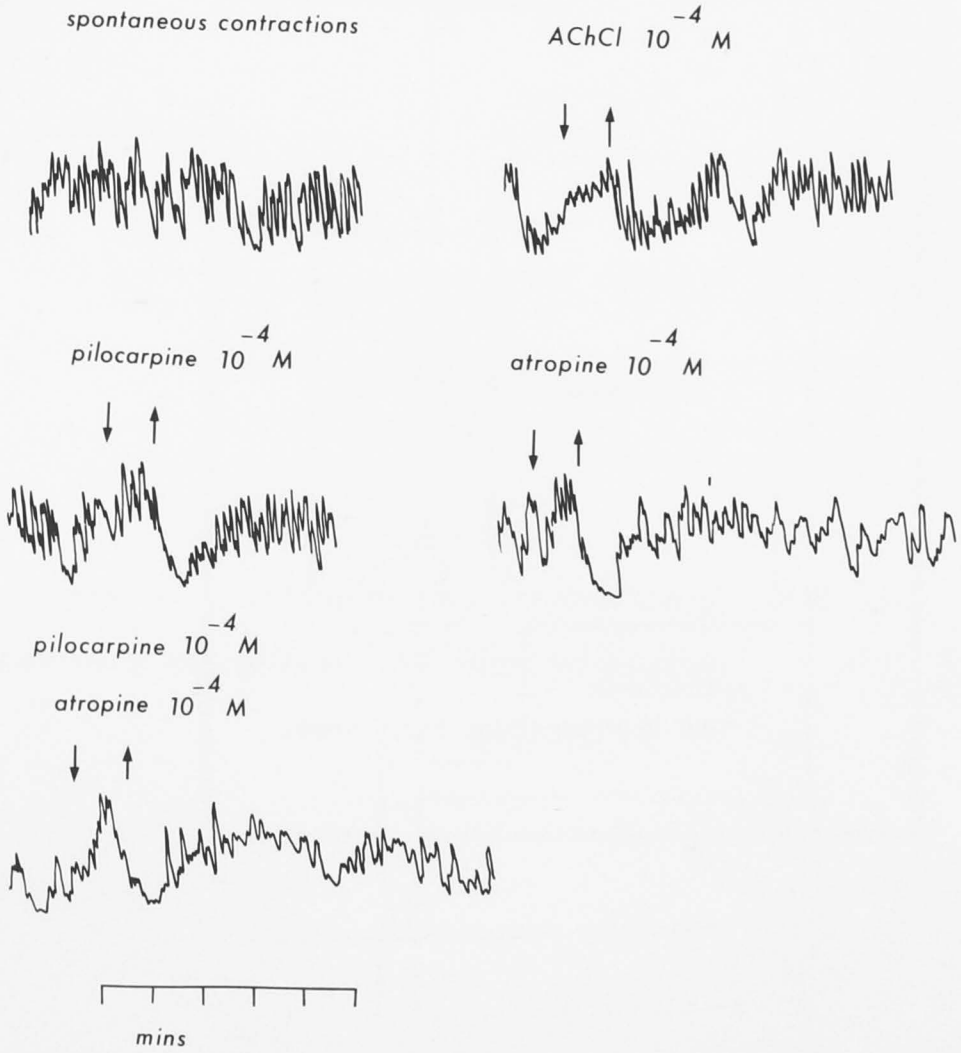

spontaneous contractions
AChCl 10^{-4} M
pilocarpine 10^{-4} M
atropine 10^{-4} M
pilocarpine 10^{-4} M
atropine 10^{-4} M
mins

Fig. 3.4. Diagrammatic representation of the apparatus used in testing the responses of *D. caninum* to drugs.

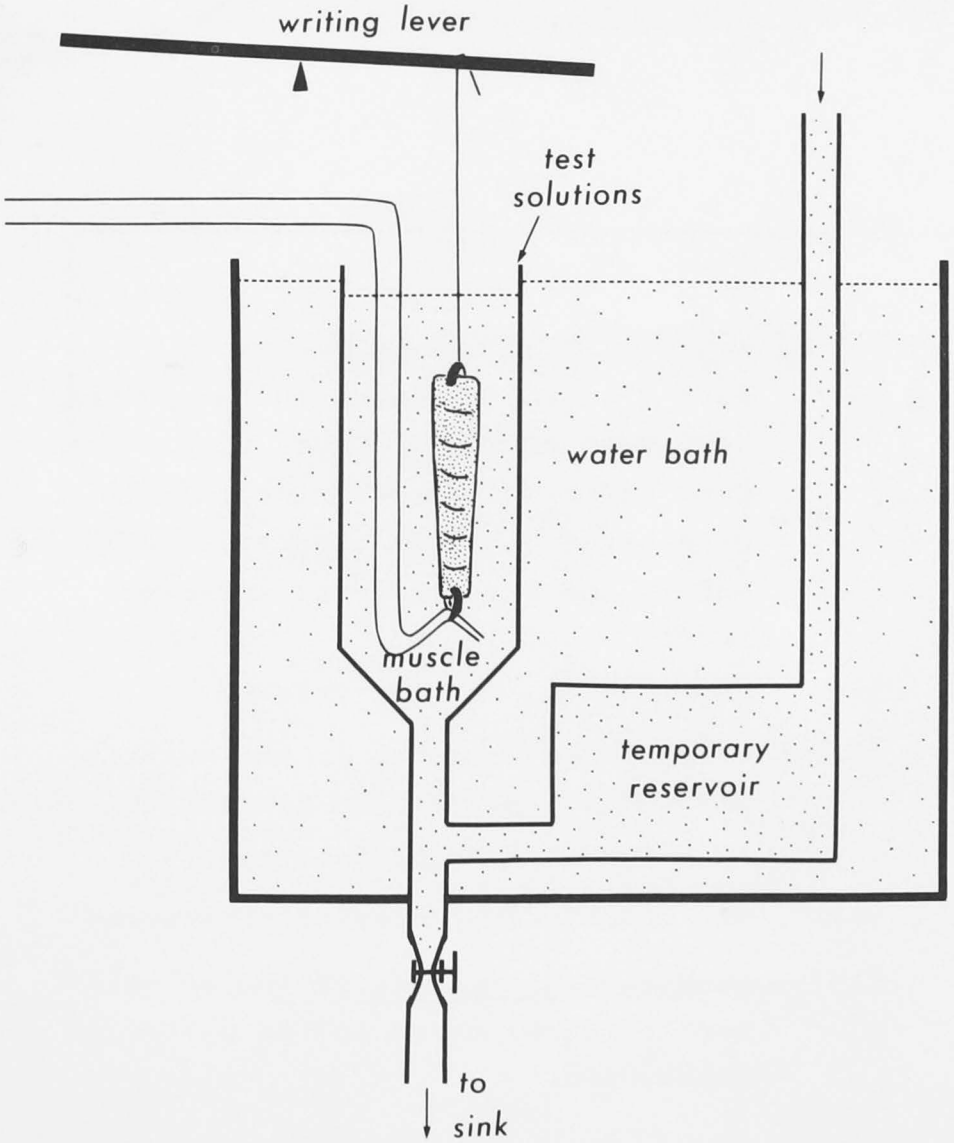

writing lever
test
solutions
water bath
muscle
bath
temporary
reservoir
to
sink

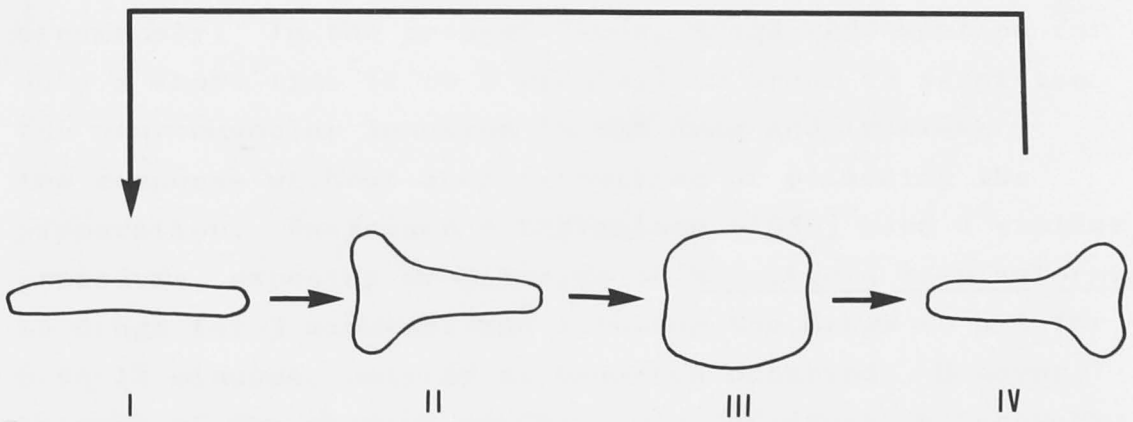
Fig. 3.5. Four successive stages in the passage of a wave of longitudinal muscle contraction from anterior to posterior end of a proglottid. In the diagram, the left hand end of the proglottid is anterior.

(i) Longitudinal muscles relaxed.

(ii) Longitudinal muscles in the anterior part of the proglottid contracted, those in the posterior part, relaxed.

(iii) All longitudinal muscles contracted.

(iv) Longitudinal muscles in the anterior part relaxed, those in the posterior part contracted.


I
II
III
IV

manner, and the simultaneous application of 10^{-4}M pilocarpine and 10^{-4}M atropine also had a similar effect on the proglottid.

DISCUSSION

Several studies on the effects of drugs on the activity of cestodes and trematodes have been carried out previously. In the present study, drugs were applied for only a short time (1 to 2 minutes) in order to sensitise the neuromuscular junction to the drug and initiate the response without over-activiting or poisoning the preparation. Paarsonen & Vartiainen (1958) used a similar procedure, exposing proglottids of Hydatigera taeniaeformis to drugs for 3 minutes, and allowing the drugs to act for 6 to 12 minutes, only if no reaction occurred. However, in most of the studies of the action of drugs on tapeworm movements, the aim was to test whether the drugs would cause paralysis, in view of their possible use as anthelmintics, and the tapeworms were therefore exposed to drugs for longer periods (Duguid & Heathcote, 1950 a; b; Rusak, 1964). The results of these studies are considered below.

Duguid & Heathcote (1950 a) found that arecoline, which is a drug pharmacologically similar to pilocarpine (Barlow, 1964), caused relaxation and cessation of activity in proglottids of the cestodes Taenia saginata and Moniezia expansa. However, these authors (1950 b) found that pilocarpine had little or no effect on M. expansa proglottids. ACh had a similar but higher activity, which was slightly potentiated by the cholinesterase inhibitor,

eserine, and was not blocked by atropine. Nicotine had a more moderate but similar effect.

In Hydatigera taeniaeformis, intact proglottids and proglottids whose lateral margins had been removed were sensitive to ACh, but only after application of eserine (Paasonen & Vartiainen, 1958). ACh then caused relaxation and cessation of activity, but neither stropine nor D-tubocurarine blocked this effect. Nicotine caused a slow decrease in tone. Wilson & Schiller (1969) found that arecoline caused relaxation and cessation of activity in proglottids of Hymenolepis diminuta. Eserine had a similar effect, while atropine caused stimulation of activity in the scolex and neck, followed by cessation of activity or depressed activity.

Rusak (1964) studied the effects of several cholinergic drugs on movements of the cestodes Hymenolepis nana and Taeniarhynchus saginatum. When he applied 10^{-5} g/ml (approximately 6.8×10^{-5}M) AChCl to T. saginatum proglottids and to H. nana, he obtained results similar to those obtained with D. caninum proglottids in the present study following application of 10^{-4}M AChCl. However, arecoline caused a gradual decrease in tone of the longitudinal muscles of T. saginatum accompanied by gradual cessation of the contractions, while in H. nana, it caused increased lateral and spiral movements with gradual slackening of the strobila. Atropine caused an increase in tone and an increased amplitude of contraction in T. saginatum proglottids, but in H. nana, it caused an increase in the rate of contraction, followed by a sudden reduction in length of the strobila and cessation of movement. Atropine was able to block the effects of AChCl,

arecoline and pilocarpine in these cestodes. Nicotine caused a gradual increase in tone together with an increased rate and a decreased amplitude of contraction in T. saginatum proglottids, whereas in H. nana, this drug caused convulsive movements of the strobila and increased movements of the rostellum. This was followed by a general contraction of the whole worm, retraction of the rostellum and suckers, and cessation of movement.

From the foregoing account, it can be seen that ACh and arecoline in general had similar effects on the proglottids of all the cestodes which have been investigated. However, the results with other drugs varied according to the species under consideration. Atropine did not block the effect of agonist drugs in some cestodes, but did in others, whereas nicotine caused a slackening in tone in some and an increase in others. Thus the type of response given by cestodes to these drugs appears to depend on the species which is being investigated.

Similar results have also been obtained with trematodes. Chance & Mansour (1953) found that AChCl caused contraction followed by gradual relaxation and cessation of movement in deganglionated Fasciola hepatica preparations. However, carbamoylcholine chloride, which has a similar pharmacological effect, but which is not readily destroyed by cholinesterases, caused relaxation and complete cessation of movement. Nicotine caused rapid contraction followed by a gradual relaxation to half-tone, while D-tubocurarine chloride and atropine sulphate partially blocked the effect of AChCl and carbamoylcholine chloride. Low concentrations of eserine sensitised the preparation to AChCl. Barker, Bueding & Timms(1966) found that ACh,

arecoline, carbamoylcholine chloride and several ChE inhibitors caused a flaccid paralysis of Schistosoma mansoni, while pilocarpine had nicotine lacked any effect on motor activity. Atropine reversed carbamoylcholine chloride-induced paralysis, but D-tubocurarine failed to block this paralysis.

In the present study, the failure of the antagonist drugs to block the effect of the agonists presents a problem in interpreting the results. It is probable, of course, that the drugs were not applied in equipotent molar concentrations. The equipotent molar ratios of cholinergic drugs referred to ACh vary according to the species of animal and the site being studied (see Barlow, 1964) and have not yet been determined for the muscle receptors of any cestode species. Paarsonen & Vartiainen (1958) concluded that neither ganglionic nor neuromuscular mechanisms of the classical type were included in the action of ACh on Hydatigera taeniaeformis proglottids. However, it is possible that these authors did not apply the drugs in equipotent molar ratios. Cholinergic synapses are probably similar throughout the animal kingdom, since their action is the depolarisation of nerve membranes by ACh or a closely related substance. Thus it might be expected that the cholinergic neuromuscular junction in cestodes would have some properties in common with vertebrate cholinergic synapses.

The addition of drugs to the incubation medium of a whole proglottid preparation is an empirical method of obtaining pharmacological information, since all the organs of the proglottid are present and therefore other cholinoceptive systems may be involved in the responses

to drugs, e.g. the drugs may trigger some sensory receptors involving a neuromuscular response. The result seen is the combined effect on all sensitive systems in the proglottid on muscular contractions. Factors such as the ability of the drug to diffuse through the tissue and to penetrate membranes may also greatly affect the results of pharmacological experiments (Barlow, 1964). Further pharmacological studies using micropipettes, which can deliver a precise quantity of drug close to a sensitive receptor site, may give information which can clarify the anomalous effects of some drugs observed here. However, the fact that cestode movements are affected by cholinergic drugs supports the hypothesis that cestodes contain cholinergic neuromuscular junctions.

CHAPTER 4

A HISTOCHEMICAL STUDY OF CHOLINESTERASES IN FOUR CYCLOPHYLLIDEAN CESTODES

INTRODUCTION

The role of acetylcholinesterase in the removal of free acetylcholine (ACh) diffusing from cholinergic synapses is well known.

It was first suggested by Dale (1914) that an esterase was responsible for the rapid removal of free ACh. Since that time, it has been established that two types of cholinesterases (ChEs) exist in mammals. 'True' or 'specific' ChE which hydrolyses ACh at a high rate and other cholinesters much more slowly is exemplified by ChE of mammalian nerve and muscle tissue and erythrocytes (Nachmansohn & Rothernberg, 1945). 'Pseudo' or 'non-specific' ChE which hydrolyses cholinesters other than ACh more rapidly than ACh is present in mammalian blood serum (Stedman, Stedman & Easson, 1932). In this thesis, 'true' ChE is referred to as acetylcholinesterase (AChE) and 'pseudo' ChE as butyrylcholinesterase (BuChE), since salts of acetylcholine and butyrylcholine respectively were used for their detection.

Bacq & Oury (1937) demonstrated the presence of ChEs in a number of invertebrates, including the trematode Fasciola hepatica. In 1941, Artemov & Lurje demonstrated ChE activity in homogenates of the cestodes Hydatigera taeniaeformis and Dipylidium caninum. Pennoit-de Cooman & Van Grembergen (1942) found significant ChE activity

in a planarian, in F. hepatica and in the cestode Taenia pisiformis, and Bullock & Nachmansohn (1942) found that the turbellarians Procotyla fluviatilis and Planaria maculata contained very high concentrations of ChE. Subsequent work has confirmed that ChEs are present in many other platyhelminth species (see discussion).

In the present study, histochemical techniques were used to study the localisation and identification of ChEs in adults of the cestode species Dipylidium caninum, Echinococcus granulosus, Hydatigera taeniaeformis and Baerietta criniae minor.

MATERIALS AND METHODS

Adult Dipylidium caninum from naturally infected dogs and Echinococcus granulosus from the small intestine of naturally and experimentally infected dogs were obtained on autopsy, or following dosing with arecoline hydrobromide. Hydatigera taeniaeformis adults were obtained on dosing a naturally infected cat and Baerietta criniae minor adults were obtained on autopsy from the small intestine of adult Crinia signifera and juvenile Pseudophryne corroboree. All specimens were immediately washed, D. caninum, E. granulosus and H. taeniaeformis, in mammalian Ringer solution, B. criniae minor in amphibian Ringer solution, and fixed in cold (5°C) 10 per cent phosphate buffered formalin (4 per cent formaldehyde) pH 7.0, for 24 hours to 4 weeks. There was no evidence that enzyme activity became reduced during this period. Specimens of D. caninum and E. granulosus intended for whole mounts were fixed and flattened simultaneously.

Flattened worms were washed briefly in distilled water and placed in the medium of Holt & Withers (1952) as given by Pearse (1960), using 5-bromoindoxyl acetate[1] (5-BIA) as the substrate for 2 to 4 hours at 37°C. Metacercariae of Philophthalmus burrili Howell & Bearup, 1967, which have a well-defined nervous system, were used for comparison.

Portions of unflattened worms were washed and supported in blocks of 10 per cent gelatine, which were frozen on to the chuck of an International model CTD cryostat. Frozen sections 10 to 30μ thick were cut at -18°C, mounted on slides and air dried. Some sections of D. caninum were incubated at 37°C in the 5-BIA medium for 4 hours, and sections of all four species were incubated for 10 to 15 minutes at room temperature (approximately 22°C) in the medium of Karnovsky & Roots (1964), using acetylthiocholine iodide[1] (AThChI) and butyrylthiocholine iodide[1] (BuThChI) as substrates.

Characterisation of the enzyme was attempted, using the following inhibitors (Pearse, 1960): For AChE - BW284C51[2] (1, 5-bis(4-allyldimethylammoniumphenyl)pentan-3-one dibromide); for BuChE - Mipafox[3] (N,N-diisopropyl-phosphorodiamidic fluoride) and iso-OMPA[1] (tetramonoisopropylpyrophosphortetramide); for both ChEs - eserine[4] (physostigmine), DFP[1] (diisopropylphosphoro-fluoridate) and E600[3] 10^{-7}M (diethyl-p-nitrophenyl phosphate);

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1 Koch-light Laboratories Ltd., Colnbrook, England.

2 Courtesy Burroughs Wellcome & Co., Sydney, Australia.

3 K & K Laboratories Inc., Hollywood, California, U.S.A.

4 British Drug Houses, Ltd., Poole, England.

for A-esterase - PCMB[1] (para-chloromercuribenzoate); for B-esterase - E600 10^{-5}M; and for C-esterase - β-phenyl-propionic acid.[1] (For concentrations used, see Tables 1 and 2). Whole worms and sections were pre-incubated in the appropriate medium containing inhibitor, but not substrate, for 30 minutes before being transferred to medium containing both substrate and inhibitor. No colour developed during the pre-incubation period. Material used for comparison of the effect of inhibitors was always run simultaneously.

RESULTS

I. Whole mounts; 5-bromoindoxyl acetate technique.

In contrast to the trematode controls, difficulty was encountered in obtaining consistent results with the cestode material. This was probably due to poor and uneven penetration of the 5-BIA medium. In Dipylidium caninum, the substrate penetrated the body of the worm and stained the nervous system, Mehlis' gland, the tegument anterior to the suckers, and some cells of the rostellum (Figs. 2.6, 2.7). However, the deposition of indigo tended to be uneven, and some non-specific precipitate occurred on the tegument and in the tissues. In Echinococcus granulosus, which is smaller than D. caninum and therefore would be expected to fix more satisfactorily and be more readily penetrated by components of the substrate medium, very little reaction took place except in the tegument. On

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1 Koch-Light Laboratories Ltd., Colnbrook, England.

occasions when incubation was carried out for longer periods, some precipitate aggregated apparently non-specifically in the tissues; experimentation with other fixatives such as alcohol and acetone, and different fixation times, failed to give an improvement on these results.

The effect of various esterase inhibitors on the reaction in the nervous system in D. caninum whole mounts, compared with that in Philophthalmus burrili metacercariae, is shown in Table 4.1. The reaction in both D. caninum and P. burrili was insensitive to DFP 10^{-5}M, while that in D. caninum was also insensitive to the specific AChE inhibitor BW284C51 10^{-5}M. Because of the variability in intensity of the reaction product formed under control conditions, it was impossible to judge whether any partial inhibition had occurred.

II. Sections.

In sections of D. caninum, E. granulosus, H. taeniaeformis and B. criniae minor, the nervous system was positive for esterases (Figs. 2.8 to 2.26). Note, however, that in Fig. 2.24, ChE-positive nerve fibres do not occupy the entire nerve cord. The results given by incubating the sections in various combinations of substrates and inhibitors are given in Table 4.2. The intensity of the reaction was judged visually. For convenience, the intensity of the reaction in D. caninum with 5-BIA is regarded as the same as that with AThChI with no inhibitors present. In all four species, only a slight inhibitory effect on the hydrolysis of AThChI was given by BW284C51 and this substance inhibited the hydrolysis of BuThChI partially in D. caninum and H. taeniaeformis and completely in E. granulosus and

TABLE 4.1

Reaction in the nervous system of whole mounts incubated in 5-bromoindoxyl acetate.

Inhibitor		Esterase inhibited	*Dipylidium caninum* adult	*Philophthalmus burrili* metacercaria
None		none	+	+
BW284C51	10^{-5}M	AChE	+	0
Eserine	10^{-5}M	AChE ChE	0	0
Mipafox	10^{-3}M	ChE B-esterase	+	+
DFP	10^{-5}M	AChE ChE	+	+
E600	10^{-7}M	AChE ChE	0	0
E600	10^{-5}M	AChE ChE B-esterase	0	0
E600	10^{-2}M	AChE ChE B-esterase A-esterase	0	0
PCMB	10^{-4}M	A-esterase	+	+
β-phenyl propionic acid	10^{-2}M	C-esterase	+	+

+ = indigo precipitated in nervous system

0 = no indigo precipitated in nervous system

B. criniae minor. BW284C51 also partly inhibited hydrolysis of 5-BIA in D. caninum.

Mipafox caused slight inhibition of the hydrolysis of BuThChI in H. taeniaeformis and of AThChI and BuThChI in B. criniae minor, but had no effect on enzyme activity in D. caninum and E. granulosus or on the hydrolysis of AThChE in H. taeniaeformis. Iso-OMPA had no visible effect on enzyme activity on the substrates used. When Miapfox and iso-OMPA were used in conjunction with BW284C51, no additional inhibitory effect on enzyme activity was observed except in B. criniae minor where the additional Mipafox resulted in complete inhibition, and the additional iso-OMPA in further inhibition of the hydrolysis of AThChI. However, eserine completely inhibited the hydrolysis of both AThChI and BuThChI in all species, and 5-BIA in D. caninum.

The enzymic hydrolysis in D. caninum, E. granulosus and H. taeniaeformis was only slightly inhibited by E600 10^{-7}M, and concentrations of 10^{-5}M or 10^{-4}M were required for complete inhibition. In B. criniae minor, ChE activity was not affected by 10^{-5}M E600. DFP caused some reduction in the activity of the enzyme in D. caninum and E. granulosus, 10^{-4}M preventing hydrolysis of 5-BIA and BuThChI completely, but this inhibitor had no effect on enzyme activity in B. criniae minor. The effect of DFP on H. taeniaeformis ChE was not investigated.

There was a slight ChE-positive reaction around the excretory ducts and in the parenchyma of gravid segments of H. taeniaeformis (Fig. 2.24). The excretory ducts and parenchyma in D. caninum, E. granulosus and B. criniae minor were negative.

TABLE 4.2

Histochemical reactions of the nervous system to combinations of cholinesterase substrates and inhibitors.

Inhibitor		Cholinesterase inhibited	Indoxyl acetate	Dipylidium caninum		Echinococcus granulosus		Hydatigera taeniaeformis		Baerietta criniae	
				AThChI	BuThChI	AThChI	BuThChI	AThChI	BuThChI	AThChI	BuThChI
None	0	none	++++	++++	++	++++	++	++++	++	++++	+++
BW284C51	10^{-5}M	AChE	-	-	-	-	-	+++	+	-	-
BW284C51	10^{-4}M	AChE	++	+++	+	++++	0	-	-	++	0
Eserine	10^{-5}M	AChE, BuChE	-	-	-	-	-	0	0	-	-
Eserine	10^{-4}M	AChE, BuChE	0	0	0	0	0	-	-	0	0
Mipafox	10^{-3}M	BuChE	++++	++++	++	++++	+	++++	+	+++	++
iso-OMPA	$3x10^{-5}$M	BuChE	++++	++++	++	++++	+	++++	++	++++	+++
DFP	10^{-6}	AChE, BuChE	+++	++++	++	++++	++	-	-	++++	+++
DFP	10^{-5}M	AChE, BuChE	+	++++	+	++++	+	-	-	++++	+++
DFP	10^{-4}M	AChE, BuChE	0	++	0	++	0	-	-	++++	+++
E600	10^{-7}M	AChE, BuChE	+	++++	+	++++	+	-	-	++++	+++
E600	10^{-5}M	AChE, BuChE	0	0	0	0	0	-	-	++++	+++
E600	10^{-4}M	AChE, BuChE	-	-	-	-	-	0	0	-	-
BW284C51 +Mipafox	10^{-5}M 10^{-3}M	AChE, BuChE	-	-	-	-	-	++	+	-	-
BW284C51 +Mipafox	10^{-4}M 10^{-3}M	AChE, BuChE	+	++++	+	+++	0	-	-	0	0
BW284C51 +iso-OMPA	10^{-5}M $3x10^{-5}$M	AChE, BuChE	-	-	-	-	-	++	+	-	-
BW284C51 +iso-OMPA	10^{-4}M $3x10^{-5}$M	AChE, BuChE	+	+++	+	+++	0	-	-	+	0

++++ = intense reaction
+++ and ++ = progressively less intense reactions
\+ = slight reaction
0 = no reaction
\- = not tested

The tegument of D. caninum posterior to the suckers was negative with both the indoxyl acetate and the thiocholine iodide methods. In E. granulosus the tegument reacted strongly with AThChI, and was also positive with BuThChI. The tegument surrounding the genital pore showed less activity, whereas the lining of the pore was negative. In H. taeniaeformis, the tegument was positive in gravid and mature proglottids, but deposition of reaction product became progressively less anteriorly, with the immature segments and scolex being negative. The tegument surrounding the genital pore did not show less activity than that of the rest of the proglottid, but the lining of the genital pore was negative. ChE activity observed in the subtegument area was not localised within the proximal cytoplasm of the tegument cells but in the intercellular region of the cytoplasmic extensions of these cells. Counterstaining with Gower's carmine ensured that the tegument cells were correctly identified. The tegument of B. criniae minor was negative. The action of ChE inhibitors on the reaction in the tegument of D. caninum, E. granulosus and H. taeniaeformis followed the same pattern as that in the nervous system (see Table 4.2).

The rostellar gland of E. granulosus and H. taeniaeformis was moderately ChE-positive, and the effect of the various inhibitors on this reaction was similar to that on the reaction in the nervous system. The rostellar gland of D. caninum was negative, but some anterior cells of the rostellar pad were positive.

Oncospheres enclosed in gravid proglottids of D. caninum, E. granulosus and H. taeniaeformis were also ChE-positive. The reaction was not a general one, but localised

in discrete deposits, which were probably associated with structures of the oncosphere (see chapter 2). The ChE activity of E. granulosus oncospheres was more resistant to DFP than the ChE of the nervous system, as it was still present, though with very reduced activity, following treatment with 10^{-4}M DFP. In D. caninum and H. taeniaeformis, the oncospheral membrane was ChE-positive. The effect of ChE inhibitors on the reaction in these oncospheres was not investigated. No activity was detected in oncospheres of B. criniae minor.

In contrast with the 5-BIA technique, the Karnovsky & Roots technique was found to give a very precise localisation of the reaction product within the nervous system, with no general precipitate which might be judged as non-specific. It has an advantage also in being specific for ChEs, although non-specific esterases will also catalyse some hydrolysis of the substrate.

DISCUSSION

ChEs have previously been demonstrated histochemically in the nervous system of the following cestodes: Hymenolepis diminuta (Lee, Rothman & Senturia, 1963; Schardein & Waitz, 1965; Wilson, 1965; Douglas, 1966; Bogitsh, 1967; Wilson & Schiller, 1969), H. citelli (Lee, Rothman & Senturia, 1963), H. microstoma adult (Lee, Rothman & Senturia, 1963; Bogitsh, 1967) and cysticercoid (Bogitsh, 1967), H. nana (Schardein & Waitz, 1965; Wilson, 1965; Wilson & Schiller, 1969), Hydatigera taeniaeformis (Lee, Rothman & Senturia, 1963; Schardein & Waitz, 1965; Eränkö, Kouvalainen, Mattila & Takki, 1968),

Anoplocephala perfoliata (Lee & Tatchell, 1964), Dipylidium caninum (Schardein & Waitz, 1965), Ligula intestinalis (Arme, 1966), Mesocestoides tetrathyridia (Hart, 1967; 1968), Diphyllobothrium dendriticum adult and plerocercoid Öhman-James, 1968), Choanotaenia unicoronata (Ramisz, 1967) and Dilepis undula (Ramisz, 1967).

The results of inhibitor studies in histochemical identification of cestode ChEs have frequently been difficult to interpret, since they do not exactly fit results obtained with mammalian ChEs.

Eserine completely inhibited the esterase reaction in D. caninum, E. granulosus, H. taeniaeformis and B. criniae minor. Since eserine inhibits ChEs but not non-specific esterases, the major component of the histochemical reaction in these cestodes is due to one or more ChEs. If there were some non-specific esterases present, they were not detectable by the methods used.

Since AChE will sometimes hydrolyse BuThChI, although not as rapidly as AThChI, it is possible that at least some of the BuThChI hydrolysed was due to AChE. Because BW284C51 is a specific inhibitor of AChE but not BuChE, it is likely that all the hydrolysis of BuThChI in E. granulosus and B. criniae minor was due to AChE, and that at least some of the hydrolysis of BuThChI in D. caninum and H. taeniaeformis was due to AChE. Similarly, AChE will hydrolyse 5-BIA, so at least some of the reaction observed with this substrate is probably due to AChE. Thus it may be concluded that a specific AChE, which is less sensitive to BW284C51 than mammalian AChE, is present in these four species. BuChE may be present in

D. caninum and H. taeniaeformis, but is absent in E. granulosus and B. criniae minor.

Iso-OMPA and Mipafox, both inhibitors of BuChE activity, had little inhibitory effect on enzyme activity, and caused only slight additional inhibition when used in combination with BW284C51. These results may mean either that BuChE is absent in D. caninum and H. taeniaeformis and that therefore BW284C51 is not as effective an inhibitor of cestode AChE as of mammalian AChE, and/or that BuChE is present and was only slightly inhibited by Mipafox and iso-OMPA. More definite conclusions require biochemical characterisation of the enzyme and quantitative evaluation of various reaction conditions and inhibitors on its activity.

The organophosphate inhibitors, DFP and E600, had little effect on the ChE activity of D. caninum, E. granulosus, H. taeniaeformis and B. criniae minor (see Table 4.2) except in high concentrations. However, E600 did cause complete inhibition of 5-BIA hydrolysis in whole mounts. It is possible that, in the whole worm, this inhibitor penetrated to the enzyme site more effectively than the substrate. The results obtained from the sections are therefore more likely to be reliable. No additional information on the nature of the ChE in the nervous system of these cestodes was given by these results, even though mammalian ChEs are very sensitive to these compounds.

The effects of the ChE inhibitors, eserine, BW284C51, and iso-OMPA on cestode ChEs found in the present study are in general agreement with earlier work on platyhelminth ChEs.

Eserine has been found to inhibit esterase activity in the nervous system of all the cestode species in which its inhibitory activity has been investigated (Schwabe, Koussa & Acra, 1961; Lee, Rothman & Senturia, 1963; Lee & Tatchell, 1964; Schardein & Waitz, 1965; Arme, 1966; Bogitsh, 1967; Hart, 1967; Öhman-James, 1968).

Some sensitivity of platyhelminth ChEs to BW284C51 and a similar inhibitor 62C47 has been noted previously. Schardein & Waitz (1965) found that 62C47 slightly reduced the esterase activity in the cestodes D. caninum, H. taeniaeformis, Hymenolepis diminuta and Hymenolepis nana. Within the Trematoda, the esterase activity of Philophthalmus burrili metacercariae was completely inhibited by BW284C51 (see Table 4.1) while 62C47 has been reported to completely inhibit the esterase in some trematodes (Pepler, 1958; Panitz & Knapp, 1967) but only slightly reduce the activity of the enzyme in others (Fripp, 1967; Halton, 1967).

Iso-OMPA had little or no effect on the ChE activity in a number of cestodes (Schardein & Waitz, 1965) but prevented the slight hydrolysis of BuThChI in Hymenolepis diminuta embryos (Rybicka, 1967). Iso-OMPA also had little effect in some trematodes (Pepler, 1958; Halton, 1967), but prevented the hydrolysis of BuThChI in some schistosome adults (Fripp, 1967).

Eränkö, Kouvalainen, Mattila & Takki (1968) showed that in Hydatigera taeniaeformis the distribution of ChE after incubation with AThCh together with iso-OMPA was identical with that demonstrated after incubation with BuThCh together with BW284C51. In mammalian tissues, the former combination selectively demonstrates AChE, while the latter combination,

BuChE. When both substrates, together with both inhibitors were used in the incubation medium, the distribution of ChE was again similar, whereas the ChE in the duodenum of the host cat, was effectively inhibited.

The effect of Mipafox on ChEs in the nervous system of platyhelminths has not been investigated previously.

The effect of DFP and E600 on ChE activity of platyhelminths in general is not clear. Among the cestodes, DFP has been found by Hart (1967) to inhibit completely BuThChI hydrolysis, and sharply reduce that of AThChI, in Mesocestoides tetrathyridia, while Graff & Read (1967) found that AChE activity in H. diminuta homogenates was unaffected by 10^{-4}M DFP. Krvavica, Lui & Bečejac (1967) and Halton (1967) noted some inhibitory effect of DFP on the esterase activity in the nervous system of the trematode, Fasciola hepatica, whereas Frady & Knapp (1967) obtained no inhibition by 10^{-4}M DFP in homogenates. E600 10^{-5}M gave complete or almost complete inhibition of esterase activity in the cestode species studied by Lee & Tatchell (1964), Schardein & Waitz (1965) and Arme (1966). Halton (1967) found only a slight reduction of activity in Fasciola hepatica.

The effectiveness of organophosphates as anthelmintics against certain species of helminths is probably partly due to their efficiency as inhibitors of ChEs. Hart & Lee (1966) found a good correlation between the efficiency of the organophosphate Haloxon as an anti-ChE and as an anthelmintic in a number of nematodes. The ineffectiveness of this preparation as an anthelmintic for certain other nematode species was thought by Hart & Lee (1966) to be due to a rapid recovery of the ChEs from the effect of the

(1966)/ inhibitor. Campbell & Cuckler (1964) found that the organophosphate Phthalphos was effective against the trematode Schistosoma mansoni in mice. However, Werbel & Thompson (1967) found that none of the organophosphates they tested showed a high antischistosomal activity in monkeys at dose levels which are tolerated by the host. Their results discourage the expectation of a direct relationship between ChE inhibition by organophosphates and antischistosomal activity. However, the effectiveness of organophosphates as ChE inhibitors appears to be a factor in their possible anthelmintic activity. The efficiency of organophosphate compounds as ChE inhibitors of both host and parasite ChEs can be measured in vitro, and such measurements should be useful as preliminary screening for anthelmintic compounds. Helminth species whose ChEs are inhibited by concentrations of organophosphates too low to inhibit host ChEs are most likely to be susceptible to organophosphates when used as anthelmintics. However, as with other chemical control measures, care should be taken in management to prevent possible selection of organophosphate resistant strains as, for example, has happened in the control of cattle ticks (Boophilus microplus). Organophosphate resistance in strains of this species is associated with low levels of ChE activity in the brain (Stone, 1968 a; b).

Several criteria may be used when attempting to identify ChEs: (i) the relationsip of enzyme activity to substrate concentration, (ii) the relative activity of the enzyme towards various substrates, and (iii) the effect of specific inhibitors on enzyme activity. In histochemical work, two of the above criteria are available, the effect of specific

inhibitors, and, to some extent, the relative activity of the enzyme towards various substrates. However, from the foregoing discussion it can be seen that the behaviour of platyhelminth ChEs towards standard ChE inhibitors is different from that of mammalian ChEs. These critera are discussed below in conjunction with what is known about the ChEs of cestodes.

(i) When the enzyme activity of AChE on certain cholinesters including ACh, is plotted against log concentration, a bell-shaped curve results, with the optimum at a low concentration of the substrate (e.g. 10^{-2} to 10^{-3}M). When non-choline esters, e.g. triacetin, are used, there is an increase of enzyme activity with increase in substrate concentration. A similar curve plotted for BuChE has the more usual S-shape. Pylkkö (1956 b), using homogenates of the cestode Diphyllobothrium latum, obtained a bell-shaped curve when AChCl and propionylcholine chloride were used as substrates, and with a purified enzyme preparation, obtained a bell-shaped curve for benzoylcholine chloride also. When triacetin and ethyl acetate were used as substrates, there was a low activity which increased with increasing substrate concentration. Graff & Read (1967) demonstrated that the ChE activity of homogenates of Hymenolepis diminuta with the substrates ACh and triacetin was similar to that in D. latum. Eränkö, Kouvalainen, Mattila & Takki (1968) also found that in Hydatigera taeniaeformis, the inhibition of ChE activity occurred at high concentrations of the substrates AChCl, acetyl-β-methylcholine chloride and butyrylcholine iodide. Bueding (1952) observed that homogenates of the trematode Schistosoma mansoni were capable of hydrolysing ACh very

rapidly and BuCh slowly. The enzyme in S. mansoni showed an activity-substrate concentration relationship similar to mamalian AChE.

(ii) The relative activity of the enzyme towards different substrates is also useful in defining whether AChE is the enzyme present. Augustinsson (1963) reviews much of the work done on this topic. AChEs isolated from mammalian brain (grey substance), erythrocytes, or electric tissue from certain fish show much the same substrate specificity. Other types of AChE, present in high concentrations in cobra venoms, Helix blood and insect brain show specificity and other properties which differ slightly from those of mammalian AChE. These enzymes split cholinesters or thiocholine esters at decreasing velocity in general, in the following order: ACh > propionylcholine > BuCh, the latter ester being hydrolysed at a very low rate or not at all. Acetyl-β-methylcholine is hydrolysed, but not benzoylcholine. AChEs can also hydrolyse many non-cholinesters, the optimim acyl group being acetate for aliphatic esters. ChEs which hydrolyse BuCh or propionylcholine at a higher rate than ACh also show a divergence in specificity towards various choline and non-cholinesters. It is therefore not possible to apply results obtained with enzyme preparations of one species to those of any other species.

Pylkkö (1956 a) found that in homogenates of Diphyllobothrium latum and Taenia saginata, enzyme activity was highest towards AChCl, benzoylcholine chloride was hydrolysed at a lower rate than AChCl and at a higher rate than dl-acetyl-β-methylcholine chloride, and BuChCl was not hydrolysed at all, or at a very low rate. These results

were confirmed for D. latum by Pylkkö (1956 b) who noted also that propionylcholine chloride was hydrolysed at a rate between that of AChCl and benzoylcholine chloride. Activity of the prepartion towards the non-cholinesters triacetin, ethyl acetate and tributyrin was low. When AChCl and tributyrin were used together, the rate of hydrolysis of the mixture of substrates was approximately equal to the sum of the individual rates. From this, and from the fact that eserine did not inhibit hydrolysis of tributyrin, Pylkkö concluded that there was a lipase-like enzyme present, in addition to the ChE. Graff & Read (1967) using homogenates of Hymenolepis diminuta found that at concentration at which ACh was hydrolysed at a high rate, BuCh was not hydrolysed as rapidly. Eränkö, Kouvalainen, Mattila & Takki (1968) found that in Hydatigera taeniaeformis homogenates, hydrolysis of acetyl-β-methylcholine chloride occurred at the highest rate, followed by AChCl, whereas butyrylcholine iodide (BuChI) was hydrolysed at a somewhat lower rate.

(iii) The effects of the ChE inhibitors used in the present study on the cestodes studied has been discussed above and compared to the respective effects found histochemically by other workers on cestode and trematode ChEs. Eränkö, Kouvalainen, Mattila & Takki (1968) have studied ChE inhibition in Hydatigera taeniaeformis homogenates manometrically. They compared inhibition by eserine salicylate and iso-OMPA on H. taeniaeformis ChE with that of rate brain ChE (AChE) using 0.03M acetyl-β-methylcholine. The tapeworm ChE was more sensitive to eserine salicylate than human serum ChE. High concentrations (10^{-3}M) of iso-OMPA were quite ineffective (25 per cent inhibition) in

inhibiting tapeworm ChE, though human serum ChE was readily inhibited at only 10^{-6}M concentration. Complete inhibition of rat brain ChE was obtained by BW284C51 10^{-6}M, while complete inhibition of H. taeniaeformis ChE was obtained at 10^{-3}M. They suggest that the activity of the H. taeniaeformis ChE resembles that of mammalian AChE more than that of mammalian BuChE.

The above evidence suggests that the ChEs in cestodes and other platyhelminths are predominantly AChEs. AChE is found in those parts of the mammalian nervous system where the transmitter is ACh. Since ACh has been demonstrated in the tissues of some cestodes, it is probable that the presence of AChE in cestode nervous systems is also associated with cholinergic transmission.

The significance of AChE present in the tegument or part of the tegument in many cestode species has not been established. Schwabe, Koussa & Acra (1961) found that an AChE was present in homogenates of E. granulosus cysts, and that inhibitors of this enzyme accelerated the passage of glucose from inside the cyst wall into the isotonic solution surrounding them. From this, they suggested that the AChE present was involved in permeability control and osmoregulation. AChE in the tegument and in the walls of the excretory ducts of cestodes may also be involved in osmoregulation.

Rybicka (1967) has demonstrated that AChE is present in embryos and oncospheres of Hymenolepis diminuta. Lee & Tatchell (1964) demonstrated non-specific esterase activity in early embryos, and in the pyriform apparatus in oncospheres of Anoplocephala perfoliata. They could not

demonstrate any activity when AThChI was used as substrate. Lee, Rothman & Sentura (1963) showed that the embryo of Hydatigera taeniaeformis contains small amounts of non-specific esterase. Wilson & Schiller (1969) could not demonstrate AChE in Hymenolepis diminuta and Hymenolepis nana embryos. AChE activity in oncospheres may be associated with nervous elements and histolytic glands, and AChE activity in the oncospheral membrane of D. caninum, with permeability regulation.

CHAPTER 5

MONOAMINES IN THE NERVOUS SYSTEM OF *DIPYLIDIUM CANINUM*, *HYMENOLEPIS NANA* AND *TAENIA HYDATIGENA*

INTRODUCTION

The concept of neurohumoral transmission in the nervous system, and the existence of two different types of nerves, some (cholinergic) liberating acetylcholine (ACh) and some an adrenaline-like substance, was finally confirmed experimentally in the period between 1920 and 1950 (Norberg, 1965). The adrenergic transmitter in mammals was identified as noradrenaline by Von Euler (1946; 1948).

In parallel with early studies on transmitter substances in the mammalian nervous system, similar work on the invertebrate nervous system was also carried out. It was shown cytologically by Gaskell (1914; 1919), that some cells in the leech ventral nerve cord contained a substance which gave a positive chromaffin reaction. He considered that this substance was adrenaline.

In recent years, monoamines including 5-hydroxytryptamine (5-HT) and catecholamines such as adrenaline, noradrenaline and dopamine have been identified in the nervous system in a large number of vertebrates and invertebrates following the development of both histochemical (Falck, 1962; Falck, Hillarp, Thieme & Torp, 1962) and biochemical (Welsh & Moorhead, 1960; Anton & Sayre, 1964) techniques. These have also made possible

the elucidation of mechanisms involved in the synthesis, storage and release of nervous system transmitters.

It has also been found that, whereas noradrenaline is the main adrenergic transmitter in mammals, in a number of groups of animals, the adrenergic nervous elements may contain predominantly other catecholamines or 5-HT, while noradrenaline is either present in small quantities only, or frequently absent. In nerves innervating the lung of the amphibian, Bufo marinus, for example, adrenaline is predominant (McLean & Burnstock, 1967), and among the invertebrates, 5-HT is predominant in the leech Hirudo medicinalis (Kerkut, Sedden & Walker, 1967) and dopamine, in the earthworm Lumbricus terrestris (Rude, 1969). The term 'adrenergic' is used in this thesis to describe nerves containing any biogenic monoamines.

In the present study, the histochemical fluorescence technique has been used to study the localisation of monoamines in the nervous system of the cestodes Dipylidium caninum, Hymenolepis nana and Taenia hydatigena. Although these substances have been reported from turbellarians (Dahl, Falck, Von Mecklenburg & Myhrberg, 1963 a; b), no previous attempt has been made to localise them histochemically in cestodes.

MATERIALS AND METHODS

Adult D. caninum and T. hydatigena were obtained on autopsy of naturally infected dogs, and H. nana, on autopsy of experimentally infected mice and washed in

Hanks' balanced salt solution. Subsequent processing essentially followed that given by McLean & Burnstock (1967). Thus, the tapeworms were immediately frozen in liquid propane cooled with liquid nitrogen, maintained at -50°C in a Dynavac Freeze-Dryer, and dried at 5×10^{-7} torr for 2 to 3 days. The tissue was then allowed to warm to room temperature overnight. Immediately on releasing the vacuum, the tissue was transferred to a desiccator containing paraformaldehyde (which had been stored at 70 per cent relative humidity for at least 4 days), sealed with clips, and incubated at 80°C for 1 or 3 hours. The tissue was transferred to 63°C melting point paraffin wax, infiltrated under vacuum, and embedded. Controls were incubated at 60°C for 1 hour in a formaldehyde-free oven, and embedded in formaldehyde-free wax. Some H. nana were incubated in 2×10^{-5} g/ml α-methyl noradrenaline in Hanks' balanced salt solution continuously gassed with carbogen for 1 hour at 37°C, blotted on filter paper and freeze-dried and exposed to formaldehyde vapour.

Sections 10μ in thickness were cut, placed on warmed glass microscope slides, mounted in liquid paraffin, and covered with a coverslip. The material was observed on the heated stage of a Leitz fluorescence microscope using a Schott BG12 excitation filter which transmits U.V. light in the range of 330 to 500 mμ. Microphotographs were taken on Kodak Tri-X film.

RESULTS

I. Dipylidium caninum.

In sections of the proglottids of D. caninum prepared after exposure to formaldehyde vapour for 1 hour, a number

of nerve fibres show a moderately bright, yellowish fluorescence against a low green background fluorescence. This fluorescence faded quickly, but did not change in colour, on exposure to U.V. light and was absent from the control sections which had been incubated in the absence of formaldehyde vapour. Exposure of tissue to formaldehyde vapour for 3 hours before embedding and sectioning did not result in any noticeable intensification of fluorescence in the nerves or the background. Nerve fibres which fluoresce after formaldehyde treatment occur in all the longitudinal nerve cords (Fig. 5.1) and in the nerves of the inner plexus connecting the longitudinal nerve cords (Fig. 5.2). Fine fluorescent varicose nerves are also found in the musculature of the proglottids, where the fibres run both longitudinally and transversely (Fig. 5.2), in the sub-tegument region (Fig. 5.3), in the cirrus pouch and in the walls of the copulation canal (Fig. 5.4). No fluorescent fibres were observed in the distal cytoplasm of the tegument, or in the central medullary region of the proglottid. Bright green fluorescence in the embryophoric blocks of the oncospheres and in the calcareous corpuscles may be classed as auto-fluorescence, since it also occurs in tissue which has been incubated in the absence of formaldehyde vapour.

In the scolex of D. caninum, fluorescent varicose nerves were observed in the rostellar pad, the rostellar gland and the suckers. No fluorescent nerve fibres or nerve cell bodies were found in the lateral ganglia or cerebral commissure. However, considerable difficulty was encountered in obtaining satisfactory preparations of this region of the scolex. Thus the results obtained were not unequivocal.

Fig. 5.1 - 5.4. Dipylidium caninum proglottids. Sections treated with formaldehyde gas for 1 hour.

Fig. 5.1. Longitudinal section. Yellow fluorescent fibres in the main longitudinal nerve cord (→). The calcareous corpuscles show a bright green autofluorescence.

Fig. 5.2. Longitudinal section showing yellow fluorescent fibres of the inner plexus (→) and fluorescent fibres of the cortex, some of which are oriented longitudinally, some transversely (*).

Fig. 5.3. Longitudinal section showing yellow fluorescent varicose fibres close to the periphery of the proglottid (→). The calcareous corpuscles are autofluorescent (*).

Fig. 5.4. Longitudinal section showing yellow fluorescent nerves in the cirrus pouch (→). The embryophoric blocks of the oncospheres have a green autofluorescence. Towards the left of the photograph is a reflection of light.

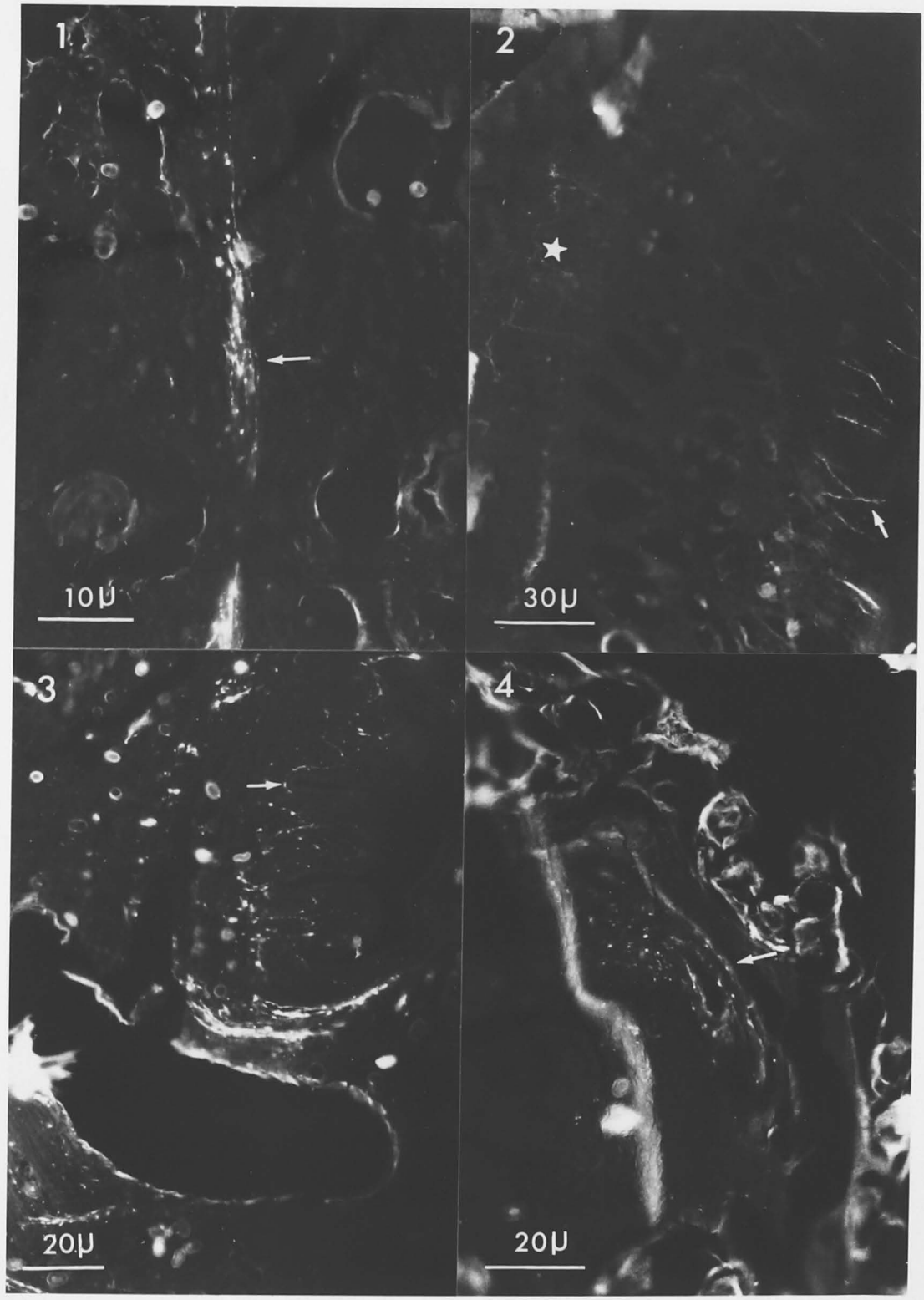
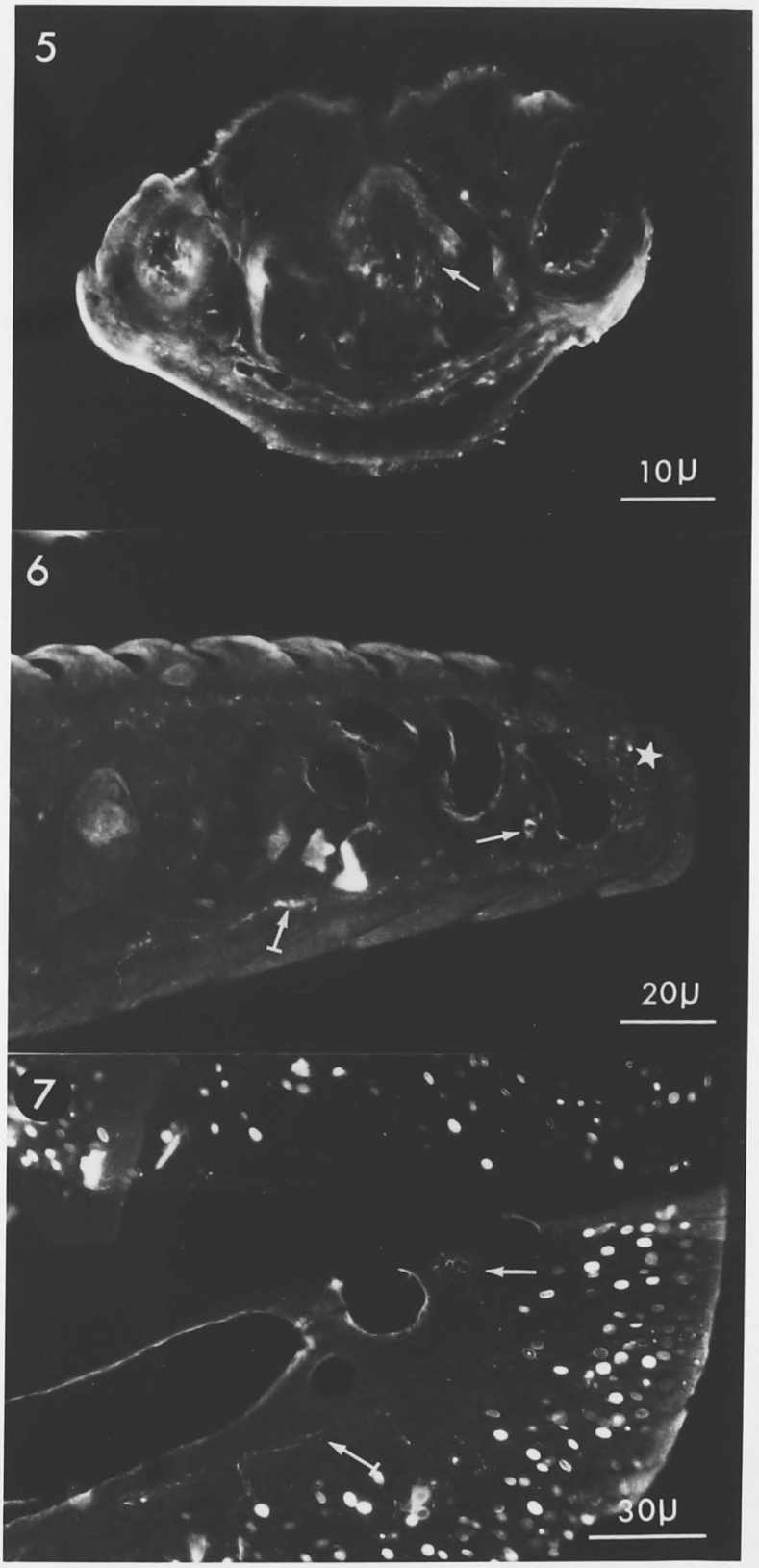

1
10μ
2
30μ
3
20μ
4
20μ

Fig. 5.5. Hymenolepis nana scolex. Approximate longitudinal section of scolex which had been incubated in α-methyl noradrenaline. Yellow fluorescent fibres (→) are present in the rostellum and in the cerebral commissure. There is a high background fluorescence, owing to incomplete removal of background α-methyl noradrenaline.

Fig. 5.6. Hymenolepis nana. Transverse section of gravid proglottid showing yellow fluorescent fibres in the main longitudinal nerve cord (→), connecting nerves of the inner plexus (⊢) and cirrus pouch (*).

Fig. 5.7. Taenia hydatigena. Transverse section through interproglottidal region showing yellow fluorescent fibres in the main longitudinal nerve cord (→) and in the nerves of the inner plexus (⊢). The calcareous corpuscles are highly fluorescent.


5
10μ
6
20μ
7
30μ

II. Hymenolepis nana.

In H. nana, a yellowish fluorescence which was weaker than in D. caninum, occurred in nerve fibres (Fig. 5.6) occupying similar positions to the fluorescent fibres described in D. caninum. Thus sections of H. nana were more difficult to photograph satisfactorily than sections of D. caninum. However, incubation in α-methyl noradrenaline resulted in an increase in the level of fluorescence in these fibres (Fig. 5.5), indicating that these are capable of accumulating this catecholamine. Difficulty was again encountered in obtaining satisfactory preparations of the scolex. Although fluorescent nerve fibres could be seen in the cerebral commissure, ganglion cells could not be identified with certainty (Fig. 5.5). No increase in fluorescence was noted after 3 hours exposure to formaldehyde vapour.

III. Taenia hydatigena.

Preparations of T. hydatigena contained nerves which had a weaker yellowish fluorescence than those of D. caninum, and there was also a high background fluorescence. Fluorescent fibres occurred in the longitudinal nerve cords, nerves of the inner plexus, and nerves supplying the musculature (Fig. 5.7). No fluorescent nerves were found in the sub-tegument region, though the high level of background fluorescence might have obscured these. There were no fluorescent nerve fibres in the central medullary region of the proglottids, or in the tegument. No sections through the cirrus or the copulation canal were examined.

DISCUSSION

In the fluorescence technique for monoamines, a yellow fluorescence obtained after exposure of freeze-dried tissue to formaldehyde vapour at 80°C for 1 hour can indicate the presence of 5-HT (Falck, 1962) or high concentrations of primary catecholamines (e.g. dopamine or noradrenaline) (Corrodi & Jonsson, 1967), while a green fluorescence obtained under the same conditions indicates the presence of lower levels of primary catecholamine (Falck, 1962). The appearance of a green or yellow fluorescence only following more severe reaction conditions, namely 3 hours exposure at 80°C, indicates the presence of a secondary catecholamine (e.g. adrenaline) (Falck, 1962). This treatment does not destroy primary catecholamines or 5-HT. It has been found also that the velocity of photodecomposition for the formaldehyde condensation product of 5-HT is much greater than that for the corresponding condensation products of catecholamines (Corrodi & Jonsson, 1967).

The yellow fluorescence which developed in nerve fibres of D. caninum, H. nana and T. hydatigena after exposure to formaldehyde vapour for 1 hour appears therefore to be due to either a primary catecholamine, or 5-HT. Since there was no detectable increase of fluorescence in nerve fibres when the incubation time in formaldehyde vapour was increased to 3 hours, it is probable that little or no adrenaline is present. It is likely that most, if not all, the fluorescence obtained is due to 5-HT, since the fluorescence was yellow, and faded rapidly on exposure to U.V. light.

The monoamine-containing nerve fibres in cestodes together with the cholinesterase-positive nerves are probably responsible for dual innervation of most cestode tissues. The significance of this will be discussed in chapter 7. However, the monoamine-containing nerves may be analogous to the adrenergic nerves of mammals, whereas the ChE-positive nerves may correspond to mammalian cholinergic nerves.

No fluorescent fibres were found in the ovary, testes, uterus, vitellaria, auxillary organs of the reproductive system, or in any other part of the medullary region of the proglottids in D. caninum, H. nana or T. hydatigena. A similar absence of ChE-positive medullary nerves in D. caninum and some other cestodes was noted and discussed in chapter 2. Either adrenergic nerves in the medulla contain monoamines in concentrations too low to be detected by the fluorescence technique, or adrenergic nerves do not exist in this region of the cestode.

The absence of adrenergic nerve endings in the tegument of D. caninum and H. nana , and the presence of ChE-positive nerve endings in the tegument of D. caninum and other cestodes (see chapter 4) may indicate that the sensory nerves in cestodes are cholinergic.

It might be expected that some nerve cell bodies containing monoamines exist in cestodes, since in mammals, monoamine storage granules have been found to be synthesised in the adrenergic cell body and transported via the axon to the adrenergic nerve terminals (Dahlström & Häggendal, 1966). There are two likely sites for the localisation of such nerve cell bodies in cestodes - the

periphery of the nerve cords, and the lateral ganglia-cerebral commissure complex in the scolex, since there are a large number of neurons in these regions (see chapter 2). However, no fluorescent nerve cells were found in any of the sections examined. It is unlikely that adrenergic nerve cell bodies are absent in the proglottids, since the proglottids are able to function independently when they are separated from the rest of the strobila. It is possible that the nerve cell bodies were too small to be clearly recognised in the 10μ sections at the magnifications used, or that they contained monoamines in concentrations too low to be detected by the formaldehyde fluorescence technique. Adrenergic nerve cell bodies may also exist in the scolex, but the elucidation of this problem in D. caninum and H. nana is dependent on the development of more suitable techniques for handling very small pieces of tissue in the freeze-drying technique. An example of the difficulties encountered in the present study is the problem of removing surface moisture completely from the scoleces before freezing. The presence of this excess moisture was found to interfere with preservation of monoamines in the freeze-drying process. In T. hydatigena, the main problem in establishing whether adrenergic nerve cell bodies are present in the scolex is the high background fluorescence of the tissues. Further experimentation may result in finding other tapeworm species which are more suitable for this kind of work than those used in the present study.

In a study of the distribution of 5-HT in a number of invertebrate species, Welsh & Moorhead (1960) found

that this substance was present in extracts of three species of the turbellarian genus Dugesia, but was not detected in extracts of the trematode Pneumonoecis similiplexus. However, Mansour, Lago & Hawkins (1957) found that extracts of the trematode Fasciola hepatica contained a substance which had a 5-HT-like effect on a rat uterus preparation, and Mansour (1957) demonstrated that small doses of 5-HT stimulated muscular activity in F. hepatica. Varicose nerve terminals containing unidentified monoamines have been demonstrated in turbellarians (Dahl, Falck, Von Mecklenburg & Myhrberg, 1963 a) and adrenergic nerve cells, presumed by the authors to be sensory, occur in the penis papilla of turbellarians (Dahl, Falck, Von Mecklenburg & Myhrberg, 1963 b). It is probable, therefore, that adrenergic nerves are of wide occurrence among members of the Platyhelminthes.

The vitellaria of many platyhelminths, including a number of trematodes and pseudophyllidean cestodes, contain the enzyme polyphenol oxidase which functions in the oxidation of phenolic compounds to quinones which then take part in the tanning process of the egg shell (Smyth, 1954; Smyth & Clegg, 1959). This enzyme has been also termed phenol oxidase (Fruton & Simmonds, 1958). Mansour (1957) found that phenolamines such as adrenaline were oxidised by phenol oxidase from the trematode Fasciola hepatica, and (1958) that 5-HT was not oxidised by this enzyme. This evidence suggests that in animals where phenol oxidase occurs in relatively large quantities, it is unlikely that catecholamines are important in adrenergic innervation, and 5-HT is the most likely adrenergic transmitter.

Nimmo-Smith & Raison (1968) have demonstrated the presence of monoamine oxidase activity in extracts of the trematode Schistosoma mansoni. They found that the enzyme activity consisted of two components. Both oxidised catecholamines, whereas only one oxidised 5-HT. It is possible that one of the components may have been phenol oxidase which has been demonstrated in S. mansoni vitellaria (Clegg, 1965) and which does not oxidise 5-HT (Mansour, 1958). The existence of a distinct monoamine oxidase in this platyhelminth species may be associated with the presence of physiologically important monoamines such as 5-HT. However, it has been found that there is no relation between chemically estimated monoamine oxidase and 5-HT in the mammalian duodenum, and the histochemical distributions of these two substances in the mammalian duodenum differ (Pentillä, 1968). Therefore no definite conclusions about the presence of adrenergic neurones can be drawn at this stage from the presence of monoamine oxidase activity in platyhelminths.

Apart from its possible function as a nervous system transmitter, 5-HT may be important in the control of platyhelminth metabolism. Mansour, Le Rouge & Mansour (1962) found that in the trematode Fasciola hepatica, 5-HT stimulated carbohydrate metabolism by activating phosphofructokinase. Glucose uptake was also stimulated by 5-HT (Mansour, 1959). Mansour, Sutherland, Rall & Bueding (1960) found that 5-HT caused a rapid and specific increase in the formation of adenosine 3', 5' phosphate by a particulate fraction from the liver fluke. Thus the nervous system may be responsible for releasing substances which act as metabolic regulators.

Duguid & Heathcote (1950 b), in an investigation on the effects of a number of drugs on movements of Moniezia expansa proglottids, found that adrenaline caused a slight decrease in tone, and that its effect was potentiated by ephedrine. Rusak (1964) found that these drugs had a similar effect on movements of Taeniarhynchus saginatum proglottids. However, Paasonen & Vartiainen (1958) found that adrenaline was ineffective on intact and denervated proglottids of Hydatigera taeniaeformis, but that 5-HT induced activity in a quiescent denervated proglottid. These results indicate that the nervous system and/or the musculature in these tapeworms possess some sensitivity to monoamines.

It is evident that the results of the present study, combined with those of others discussed above, support the hypothesis that monoamines, in particular 5-HT, are involved in nervous transmission in cestodes. The possible significance of this will be discussed in chapter 7.

CHAPTER 6

HISTOCHEMISTRY AND ATTACHMENT OF THE SCOLEX

INTRODUCTION

The cestodes studied in chapter 2 were found to contain a considerable amount of nervous tissue in the scolex. The attachment organs (suckers and rostellum) were particularly richly supplied with nerves, indicating that their activity is under nervous control. Although the secretory activity of the rostellar gland has been established only for Echinococcus granulosus (Smyth, 1964), it is likely that homologous structures in other cestodes are also secretory, and it was suggested in chapter 2 that the process of rostellar secretion is under nervous control.

A histochemical study of the localisation of a number of protoplasmic constituents in the scolex of Dipylidium caninum was carried out in order to obtain some information on the nature of nervous tissue in the scolex, the organs under its control, and thus the relation of the nervous system to attachment. The attachment of Taenia hydatigena to the small intestine of the dog was also studied.

MATERIALS AND METHODS

Adult Dipylidium caninum, obtained after dosing naturally infected dogs with arecoline hydrobromide, were

washed in mammalian Ringer solution at 37°C and immediately placed into an appropriate fixative. Pieces of dog intestinal wall with scoleces of D. caninum attached were obtained on autopsy of a naturally infected dog and fixed immediately. Attached Taenia hydatigena from an experimentally infected dog was fixed in Zenker. The histochemical techniques and fixation employed are listed in Table 6.1. The methods used were carried out as described by Pearse (1960), except for the aqueous bromophenol blue technique, which was discussed by Davenport (1960). Zenker-fixed sections of T. hydatigena attached to dog gut were stained with Gabe's stain and with Maximow (see appendix 1), whereas Bouin-fixed sections of D. caninum attached to dog gut were stained with paraldehyde fuchsin and Halmi's stain (Cameron & Steele, 1962).

1959/

RESULTS

I. Anatomy of the scolex of Dipylidium caninum.

The main features of the scolex of D. caninum are illustrated in Fig. 6.22, and are briefly described below:

1. Tegument.

The tegument anterior to the suckers (anterior tegument) has some different staining properties from the tegument posterior to the suckers (posterior tegument). The perinuclear cytoplasm of the anterior tegument is almost round in shape and larger than that of the posterior tegument which is elongate.

TABLE 6.1

Method	Constituent	Fixatives used	Control	Positive control
PAS	glycogen	Zenker	saliva (22°C)	none
Alcian blue	acid mucopoly-saccharide	Zenker	none	none
Toluidine blue	acid mucopoly-saccharide	Zenker	none	none
Sudan black B	lipids	4% buffered formaldehyde	methanol/chloroform extraction at 52°C	none
Hg bromophenol blue	general protein	Zenker, 4% formaldehyde	none	Fasciola hepatica
Aqueous bromophenol blue	basic protein	Zenker, 4% formaldehyde	none	Fasciola hepatica
DDD	-SH	Zenker, Bouin	iodoacetate	none
	-SS-	Zenker, Bouin	none	rat skin
Millon	tyrosine	4% formaldehyde	iodination	Fasciola hepatica
Feulgen	DNA	Zenker	none	none
Methyl green pyronin	RNA	Carnoy	RNAase, heated saliva	rat pancreas
5-bromoindoxyl acetate	esterases	4% buffered formaldehyde	specific inhibitors	none

2. Musculature.

There are three main groups of muscles in D. caninum.

(i) The main body musculature which lies between the tegument and the central medulla consists of large longitudinal and circular muscle bundles posterior to the suckers, which are responsible for the movement of the segments.

(ii) The musculature of the scolex is responsible for (a) movement of the suckers and (b) protrusion and withdrawal of the rostellum.

(iii) The tegument musculature consists of very small muscle bundles situated immediately beneath the distal cytoplasm of the tegument.

In addition, the suckers are attachment cups composed mainly of muscle fibres, and the rostellar pad is also a specialised muscular organ.

3. Rostellum.

The rostellum is the anterior hook-bearing structure which presumably acts as an accessory attachment organ. When D. caninum is attached to the intestinal wall of the host, the rostellum is usually extended. However, individuals freed from the intestinal wall are capable of retracting the rostellum into the scolex (Fig. 6.3). The greatest part of the rostellum is occupied by a rostellar pad which extends from the middle of the scolex to the rostellar gland. The rostellar pad is bounded by a muscular sheath-like structure. When the rostellum is protruded, the anterior part of the rostellar pad, which contains a number of longitudinal muscle bundles, is about

three times the diameter of the posterior portion (Figs. 6.11, 6.22), and when the rostellum is retracted, the rostellar pad coils slightly, and the anterior part is only marginally wider than the posterior part (Figs. 6.2, 6.4). Internally, there are a number of large, elongate cells, and those in the anterior part, the apical cells have some different staining properties from the basal cells in the posterior part.

At the anterior end of the rostellum is a group of small cells which is similar to those forming the rostellar gland in Echinococcus granulosus, as described by Smyth (1964). Centrally, between these cells and the rostellar pad is a space which opens anteriorly through the centre of the rostellar gland. This space is bounded by tegument similar in appearance to that covering the rostellar gland and in some specimens is filled with material. It is therefore termed the 'secretion reservoir'.

The anterior part of the rostellum is surrounded by five rows of small hooks.

4. Nervous system.

The nervous system has been described in chapter 2.

II. Histochemistry of the scolex of Dipylidium caninum.

The results of the histochemical tests applied are summaried in Table 6.2. All nuclei were positive with the Feulgen test for DNA. In sections stained with the methyl green pyronin technique, all nuclei were stained pink with pyronin, although nuclei in the positive controls

stained green with methyl green. However, in RNAase and heated saliva controls, all the pyronin-staining components were removed, and the nuclei were stained with methyl green. This indicates that the nuclei contain substantial amounts of RNA as well as DNA. The results given by a number of specific inhibitors of esterase activity in the 5-bromoindoxyl acetate technique were essentially the same in all esterase-positive tissues in the scolex including the nervous system. The inhibition of nervous system esterase is given in Table 4.1, and is discussed in chapter 4. Thus all the esterase-positive tissues in D. caninum contain cholinesterases.

1. Tegument.

In general, the perinuclear cytoplasm and distal cytoplasm of the anterior tegument had similar staining properties. Thus, in contrast with the posterior tegument, they were positive with alcian blue (Fig. 6.3), and the perinuclear cytoplasm and microtriches exhibited ethanol-stable β-metachromasia with toluidine blue (Fig. 6.4) indicating the presence of acid mucopolysaccharide. Both regions of the anterior tegument were also weakly positive for esterases which had properties of cholinesterases (see Table 4.1). However, both the anterior and posterior tegument stained weakly with Sudan black B and Millon's reagent, and the anterior tegument cells contained no PAS-positive material, whereas the anterior tegument and posterior tegument were weakly PAS-positive. No part of the tegument stained with aqueous bromophenol blue, DDD or methyl green-pyronin.

2. Musculature.

The muscles were positive with the protein stains, aqueous bromophenol blue and mercuric bromophenol blue, and the membranes of the muscle fibres were stained by Sudan black B, indicating that they contained lipids as well. However, the muscles were negative for all other constituents considered.

3. Rostellum.

(i) Rostellar pad.

The apical cells of the rostellar pad were weakly positive with alcian blue, and exhibited a weak β-metachromasia, indicating the presence of acid mucopolysaccahride. However, the basal cells were negative for these substances. The apical cells, in contrast with the basal cells, were esterase-positive (Figs. 6.8, 6.9, 6.10), and the esterase-positive cells had processes which appeared to lead into the secretion reservoir. Esterase-positive cells were also present in the rostellum of the cysticercoid (Fig. 6.10). All the cells of the rostellar pad contained PAS-positive diastase-labile substances (Fig. 6.2), and were weakly positive with methyl green-pyronin. Thus, large quantities of glycogen and small amounts of RNA were present. The cytoplasm of these cells did not stain with bromophenol blue in the presence or absence of mercuric ions, nor with DDD or Millon's reagent, indicating that it contained low levels of protein.

(ii) Rostellar gland.

The cells of the rostellar gland were positive for lipid and RNA, and gave a weakly positive reaction with alcian blue (Fig. 6.3) and a weak toluidine blue metachromasia, indicating that some acid mucopolysaccaride was present. They were also weakly positive for proteins with the bromophenol blue techniques (Figs. 6.6, 6.7), and showed very little reactivity for DDD after thioglycollate reduction, and for Millons reagent, indicating very low concentrations of disulphide bonds and tyrosine respectively. Globules staining positively with PAS were present in the rostellar gland. Saliva controls were not carried out. The tegument of the rostellar gland appeared to be different from the anterior tegument of the scolex in sections stained with alcian blue (Fig. 6.3) and mercuric bromophenol blue (Fig. 6.5). The rostellar gland tegument appeared thinner and stained more lightly with these reagents. Sections stained with alcian blue also showed that globules of material were present in the tegument of the rostellar gland, and these appeared to be escaping to the exterior. The periphery of these globules stained with alcian blue (Fig. 6.3) indicating that they probably have an acid mucopolysaccharide rind, but the core of the globules did not stain with histochemical reagents in any of the sections examined.

(iii) Secretion reservoir.

The tegument lining the secretion reservoir appeared to be morphologically similar to that of the rostellar gland, and was slightly PAS-positive. However, in contrast

to the rostellar gland tegument, the secretion reservoir tegument was positive with alcian blue (Fig. 6.3) and showed β-metachromasia with toluidine blue (Fig. 6.4) indicating the presence of acid mucopolysaccharide. The cavity of the secretion reservoir appeared to be empty in sections of most of the specimens examined. However, in a number of specimens, the cavity contained globules similar to those seen in the tegument of the rostellar gland, surrounded by material which was positive with alcian blue (Fig. 6.3) and weakly positive with mercuric bromophenol blue (Fig. 6.1). Thus this material contained acid mucopolysaccharide and low levels of protein. The contents of the secretion reservoir were also esterase-positive.

4. Nervous system.

In chapter 2, two types of neurones, large and small, were described on the basis of size. However, no histochemical differences between them could be detected, and therefore they are discussed together in this chapter. In contrast to the nerve fibres, the nerve cells were weakly positive with the PAS and alcian blue techniques, exhibited β-metachromasia with toluidine blue which was not ethanol stable, and were positive for RNA (Figs. 6.20, 6.21). The neurones and nerve fibres were positive with the bromophenol blue techniques indicating the presence of some protein, but negative with Sudan black B, DDD and Millon's reagent.

II. Attachment of Dipylidium caninum and Taenia hydatigena.

In the small intestine of the host dog, adults of T. hydatigena occur in the anterior two-thirds, and adults of D. caninum, in the posterior two-thirds. However, both these species were attached at about the same depth in the intestinal wall, the suckers being attached to intestinal villi, and the rostellum extending into the crypts of Lieberkühn (Figs. 6.11, 6.13). In the suckers, there was usually a 'plug' of host tissue, which was little modified apart from flattening and distortion of the epithelial layer. Similarly, the rostellum of D. caninum caused little histologically evident damage to the crypts of Lieberkühn (Fig. 6.11) apart from flattening the epithelium adjacent to the hook-bearing regions. However, T. hydatigena, which is a much larger species, caused considerably more damage to the host tissue. The rostellum was immediately surrounded by mucus contained in a zone of compressed lamina propria (Figs. 6.14, 6.15, 6.16). Beyond this, the tissue appeared to be normal. The tegument of the rostellar gland was very irregular in configuration (Figs. 6.15, 6.16) and extensions appeared to be projecting anteriorly into the mucus (Figs. 6.16, 6.17). Anterior to the scolex was an area of mucus (Figs. 6.17, 6.18) in which a few plasma cells could be seen, and deeper still was an area where haemorrhage was evident (Figs. 6.18, 6.19).

TABLE 6.2

Histochemistry of the scolex of Dipylidium caninum.

Test	Nervous system		Rostellar gland			Rostellar pad		anterior tegument proximal cytoplasm	anterior tegument distal cytoplasm	posterior tegument	Musculature
	neurons	nerve fibres	gland	reservoir	tegument	apical cells	basal cells				
PAS	++	0	+	0	+	+++	+++	0	+	++	0
Saliva control	0	0	0	0	0	0	0	0	0	0	0
Alcian blue	+	0	++	++	0	++	0	+++	+++	0	0
Toluidine blue	+++(β)	0	+(β)	+++(β)	0	0	++	+++(β)	+++(β)	0	0
Sudan black B	0	0	+++	empty	+++	0	0	+++	+++	+++	0
Methanol/chloroform control	0	0	0	0	0	0	0	0	0	0	0
Hg bromophenol blue	+++	++	++	++	++	0	0	0	0	0	+++
Aqueous bromophenol blue	++	++	++	empty	++	0	0	0	0	0	+++
DDD (-SH)	0	0	0	0	0	0	0	0	0	0	0
(-SS-)	0	0	+	empty	0	0	0	0	0	0	0
Millon reaction	0	0	+	0	+	0	0	+	+	+	0
Iodination control	0	0	0	0	0	0	0	0	0	0	0
Feulgen	+++	0	+++	0	0	+++	+++	+++	0	0	0
Methyl green pyronin	+++	0	+++	0	0	+++	+++	+++	0	0	0
RNAase control	0	0	0	0	0	0	0	0	0	0	0
Heated saliva control	0	0	0	0	0	0	0	0	0	0	0
5-bromoindoxyl acetate	+++	+++	++	+++	0	+++	0	++	++	0	0

+++ = strong reaction
++ = weak reaction
+ = very weak reaction
0 = no reaction

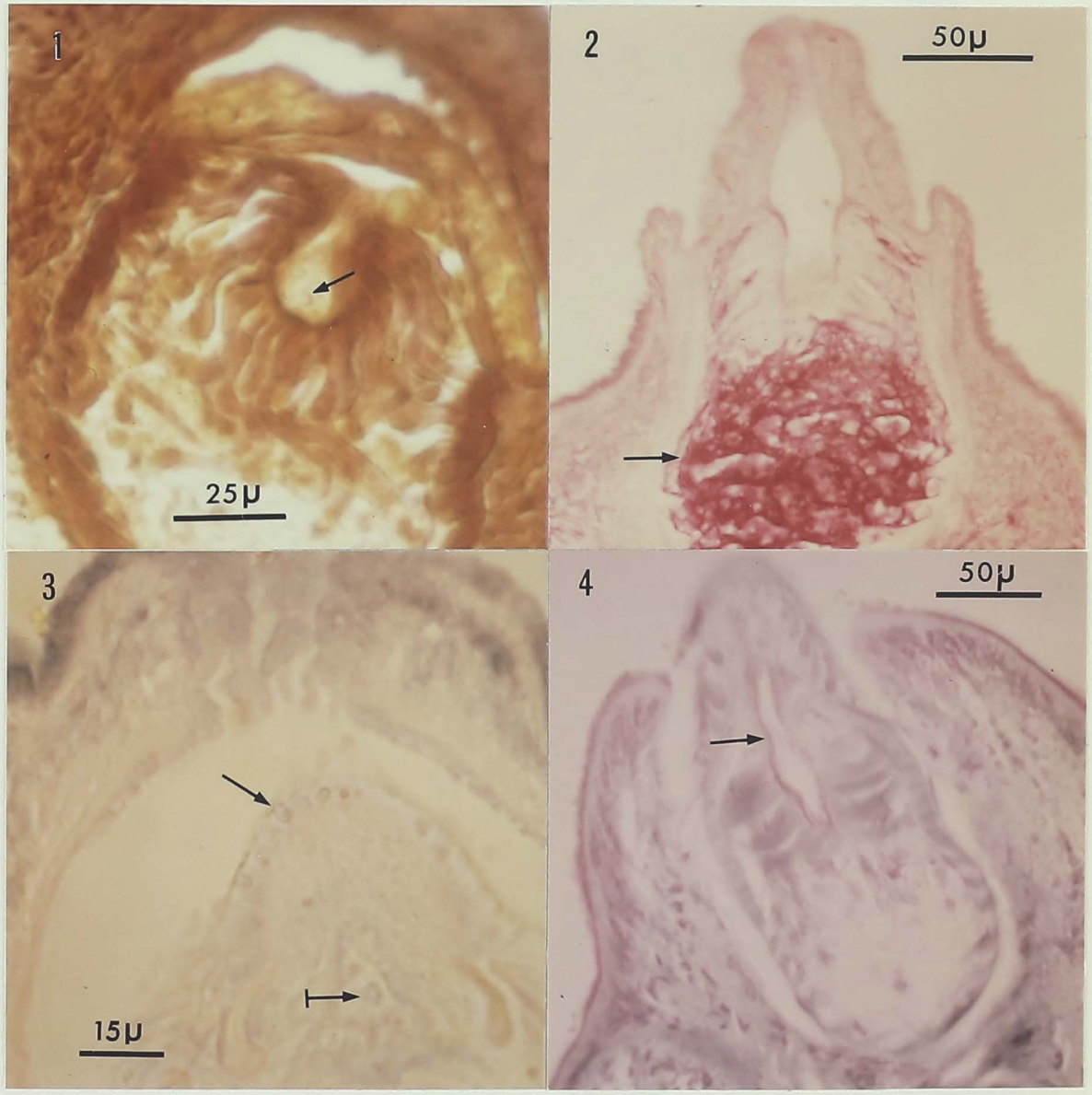
Figs. 6.1 - 6.4. Histochemical tests on the rostellum of Dipylidium caninum.

Fig. 6.1. Slightly oblique frontal section, rostellum retracted, stained with mercuric bromophenol blue. Note the globules (→) in the secretion reservoir.

Fig. 6.2. Median frontal longitudinal section, rostellum partly retracted, stained with PAS, and counterstained Mayer's haemalum. Note the abundance of PAS-positive material in the rostellar pad (→).

Fig. 6.3. Almost median frontal longitudinal section, rostellum retracted, stained with alcian blue, counterstained with eosin. Note the alcian blue-stained globules in the tegument of the rostellar gland (→) and alcian blue-positive material lining the secretion reservoir (↦) The anterior tegument and anterior tegument cells are also positive.

Fig. 6.4. Median frontal longitudinal section, rostellum partly retracted, showing alcohol-stable toluidine blue metachromasia. Note β-metachromasia of lining of the secretion reservoir (→), apical cells of the rostellar pad, anterior tegument cells and microtriches of the anterior tegument.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Figs. 6.5 - 6.7. Dipylidium caninum, rostellum protruded. Three approximately transverse serial sections through the rostellar gland stained with mercuric bromophenol blue.

Fig. 6.5. Section through the tegument of the rostellar gland, showing the opening of the secretion reservoir (→). Note the spongy texture of the tegument. A few hooks are also present in the section (▷).

Fig. 6.6. Section through the sub-tegument region showing rostellar gland cells (→) and hooks (▷).

Fig. 6.7. Section through the rostellar gland anterior to the secretion reservoir, showing cells staining heavily with mercuric bromophenol blue (→) surrounded by loose tissue.

5

30μ

6

30μ

7

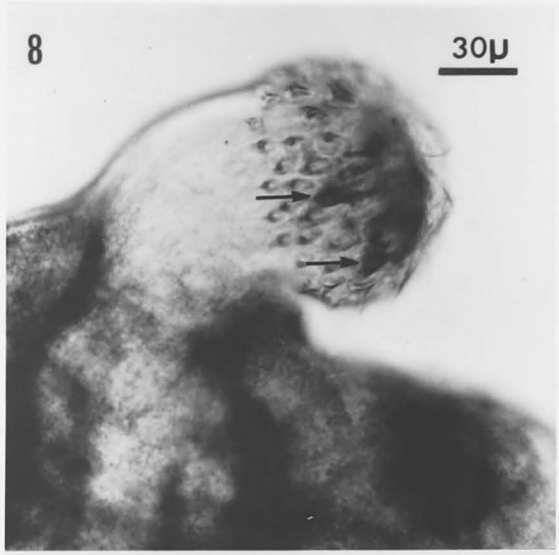
30μ

Figs. 6.8 - 6.10. Dipylidium caninum. Esterase activity in the rostellum.

Fig. 6.8. Whole mount of an adult, rostellum protruded, showing esterase-positive cells (→) in the rostellum. (5-bromoindoxyl acetate).

Fig. 6.9. Whole mount of an adult, rostellum partly retracted, showing esterase-positive cells (→) in the rostellum. These have stained more heavily than those in Fig. 6.8. (5-bromo-indoxyl acetate).

Fig. 6.10. Whole mount of a cysticercoid, rostellum retracted, showing esterase activity (→) in the rostellum. (AThChI).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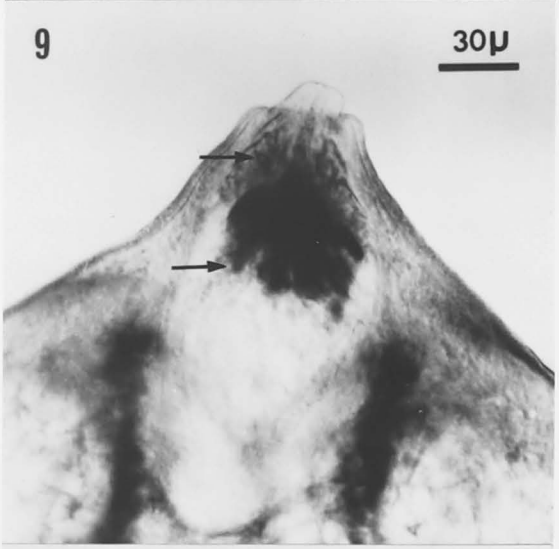
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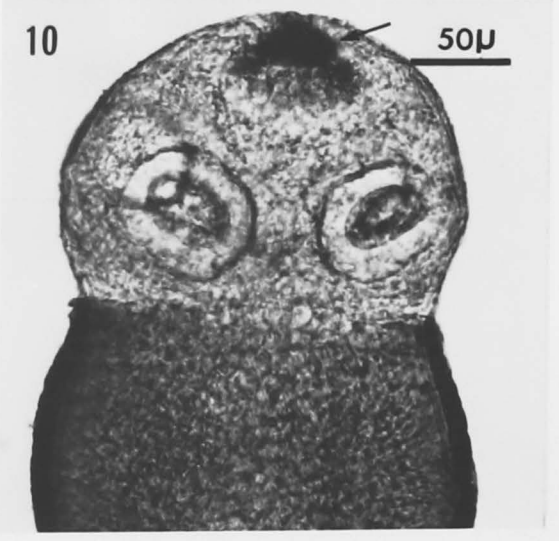
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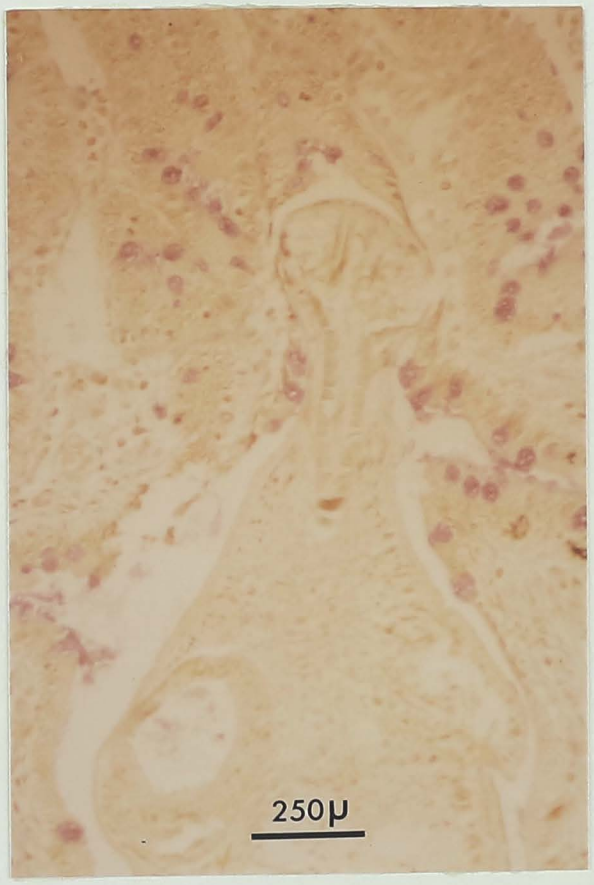
Fig. 6.11. Dipylidium caninum. Longitudinal section of scolex attached to the small intestine of the host dog. (Paraldehyde fuchsin counterstained with Halmi's stain).


250µ

Figs. 6.12 - 6.19. Taenia hydatigena. Effect of the rostellum on the small intestine of the host dog. Figs. 6.13 - 6.19 are all sections of the same series.

Fig. 6.12. Longitudinal section showing hooks embedded in the host tissue. (Gabe).

Fig. 6.13. Approximate transverse section through the rostellar pad and the base of the rostellar gland, showing the position of the scolex in the intestinal wall. Note the crypts of Lieberkühn towards the left of the photograph and the villi towards the right. (Gabe).

Fig. 6.14. Transverse section through the anterior tip of the rostellar gland (*) showing parts of parasite tissue (→) in a mucus filled cavity surrounded by normal crypts. (Gabe).

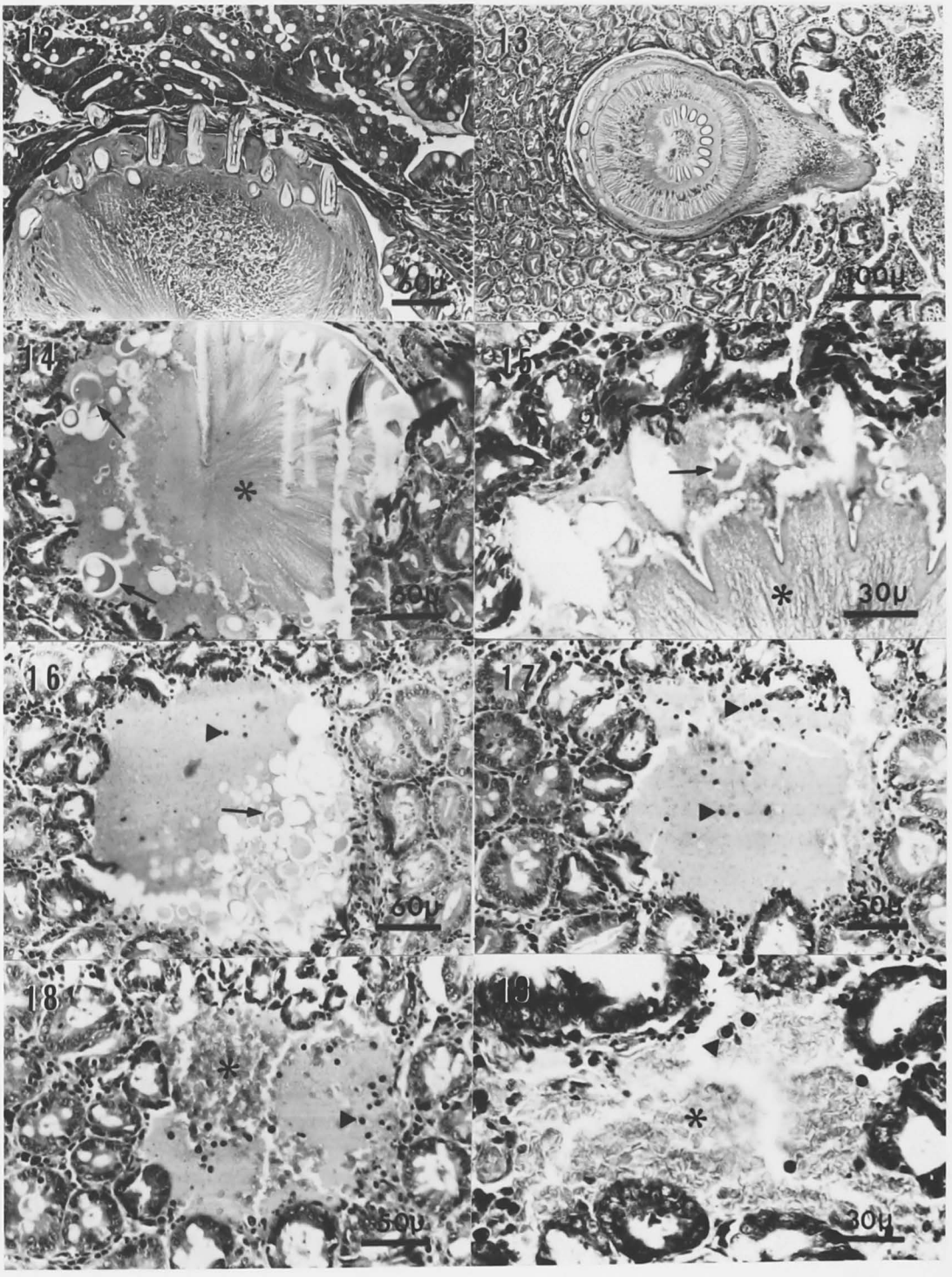
Fig. 6.15. Similar section to that shown in fig. 6.14 at a higher magnification. (Gabe).

Fig. 6.16. Section through the anterior tip of the rostellum, showing parasite tissue (→) surrounded by mucous (►). (Gabe).

Fig. 6.17. Section through the crypts of Lieberkühn approximately 40μ anterior to the parasite, showing host mucous containing a few plasma cells (►). (Gabe).

Fig. 6.18. Section approximately 70μ anterior to the parasite. Some bleeding is apparent (*). (Gabe).

Fig. 6.19. Section approximately 100μ anterior to the parasite, at the deepest point of host tissue erosion. The space is filled mainly with erythrocytes (*) but a few plasma cells are also present (►). (Maximow).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Figs. 6.20 - 6.21. *Dipylidium caninum* stained with methyl green pyronin.

Fig. 6.20. Transverse section showing pryonin-staining cell (→) in a lateral ganglion.

Fig. 6.21. Transverse section posterior to that in Fig. 6.20 showing pryonin-staining cells (→) in the lateral ganglion.

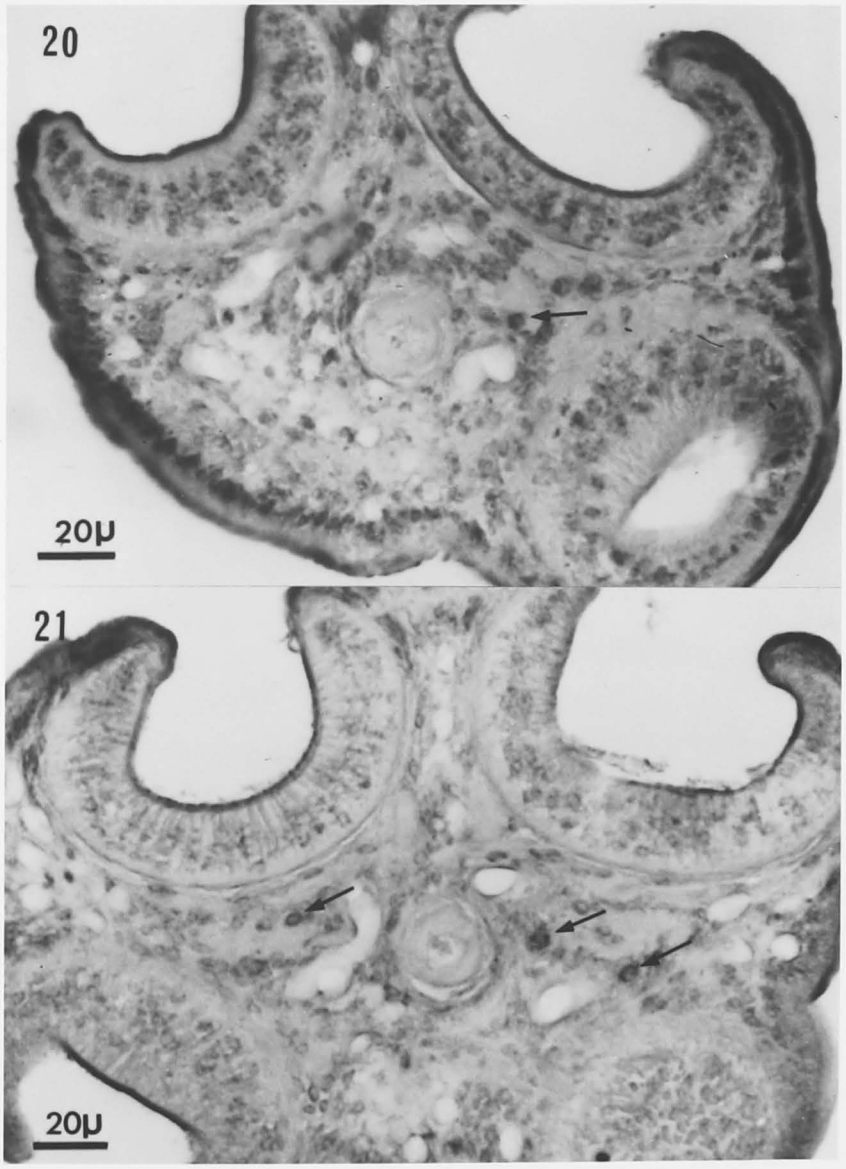
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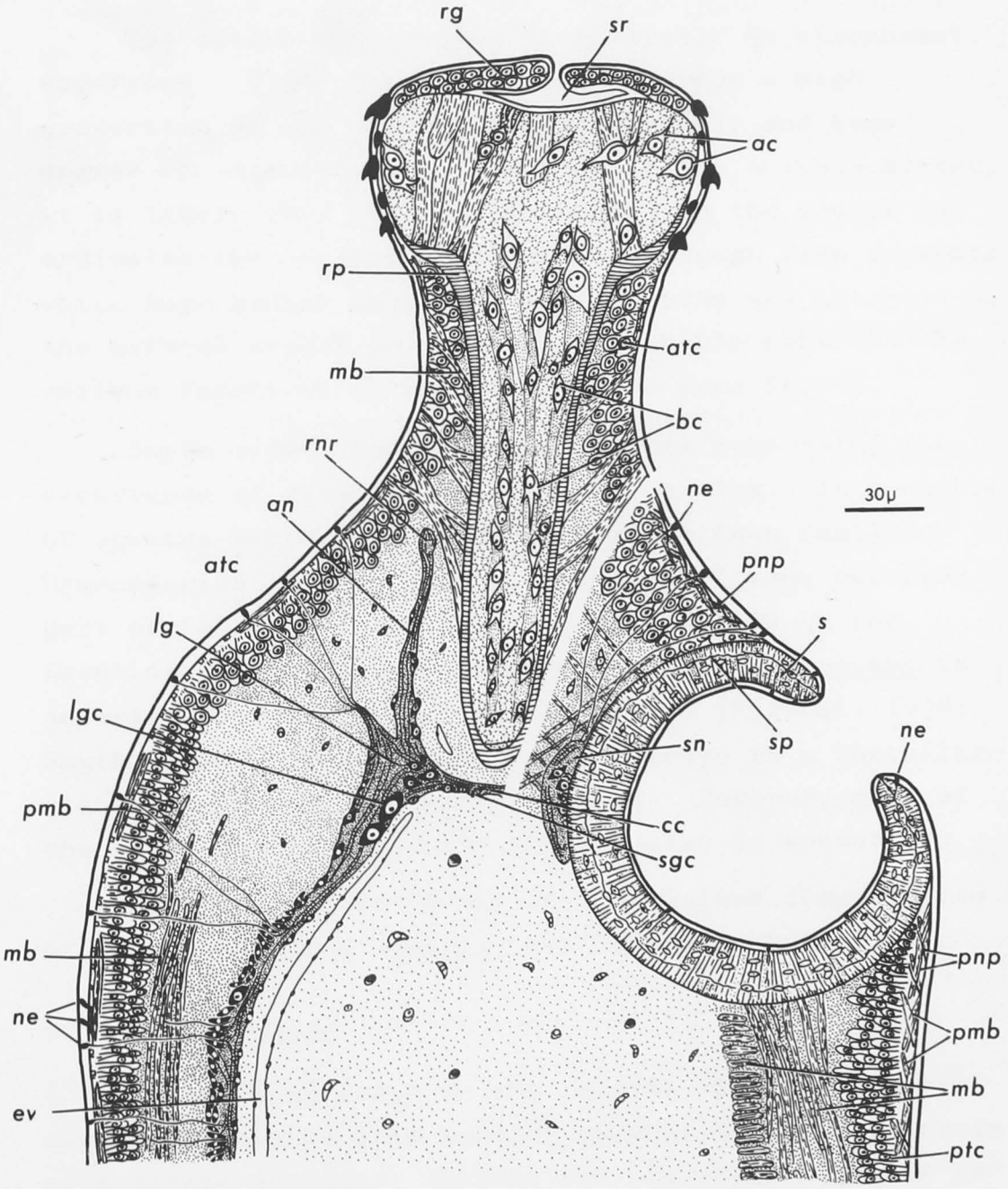
Fig. 6.22. Dipylidium caninum. Semi-diagrammatic drawing of the scolex with the rostellum protruded. Left of the gap is portion of a median frontal section, to the right, a frontal section through a sucker. (ac, apical cell; an, anterior nerve; atc, anterior tegument cell; bc, basal cell; cc, cerebral commissure; ev, excretory vessel; lg, lateral ganglion; lgc, large ganglion cell; mb, muscle bundle; ne, nerve ending; pmb, peripheral muscle bundle; pnp, peripheral nerve plexus; ptc, posterior tegument cell; rg, rostellar gland; rnr, rostellar nerve ring, rp, rostellar pad; s, sucker; sgc, small ganglion cell; sn, sucker nerve; sp, sucker plexus; sr, secretion reservoir).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DISCUSSION

The scolex of cestodes is primarily an attachment apparatus. Since nervous tissue occupies a high proportion of the scolex (see chapter 2), and some degree of organisation is evident in the nervous system, it is likely that the nervous system in the scolex co-ordinates the attachment organs. Although free segments which have broken away from the strobila are autononous, the nervous system in the scolex probably controls the various functions of the strobila to some degree.

Smyth (1964) has reviewed reports concerning the occurrence of glands in the cestode scolex. In a number of species belonging to the cyclophyllidean families Hymenolepidae and Taeniidae, glandular tissue occupies part of the rostellum. The rostellar gland in the taeniids Taenia solium and Echinococcus granulosus is anterior to the muscular rostellar pad (Farooqi, 1958; Smyth, 1964). In D. caninum also, there is a rostellar gland anterior to the rostellar pad. However, some of the cells in the rostellar pad may also be secretory.

The possible functions of the various constituents of the scolex in D. caninum are discussed below.

1. Tegument.

It has been noted in the results that the anterior tegument contains acid mucopolysaccharide and low levels of esterase activity, whereas the posterior tegument is negative for these constituents. This probably indicates a difference in function between these two areas of tegument. It is possible that the anterior tegument is

important in attachment, since it is situated between the suckers and the rostellum, and is in close contact with the tissue of the host. It is also possible that, since the scolex is likely to require different nutrients from the strobila, the anterior tegument is specialised for absorbing different nutrients from the posterior tegument and thus these two areas of tegument are morphologically different.

The presence of esterase in the anterior tegument is also of interest. Öhman (1966) has reviewed suggestions which have been put forward on the possible functions of esterases in platyhelminth tissues. These fall into five categories: (i) cholinesterase associated with the nervous system (ii) cholinesterase involved in permeability regulation in the hydatid cyst wall (Schwabe, Koussa & Acra, 1961) (iii) esterases which may be involved in transport across membranes in Hymenolepis diminuta (Lee, Rothman & Senturia, 1963) (iv) esterases which may be involved in the metabolism of absorbed nutrients in Polymorphus minutus (Crompton, 1963) (v) esterases in strigeid trematodes which may be involved in histolytic action (Lee, 1962; Erasmus & Öhman, 1963). The fact that there is no marked damage to host tissue in the region of parasite attachment suggests that no histolytic enzymes are secreted by the tegument of D. caninum. However, it is possible that the esterase in the anterior tegument is involved in permeability regulation, active transport, or metabolism of nutrients required by the scolex. Smyth (1969 b) has speculated that a process of membrane (= contact) digestion is occurring in Echinococcus granulosus, and the same process may be occurring in the anterior tegument of D. caninum.

2. Rostellum.

(i) Rostellar pad.

The rostellar pad in D. caninum differs from the rostellar pad in taeniids, where its histological structure is very similar to that of the suckers. The large number of cells in the rostellar pad could have other functions apart from their probable role of assistance, possibly hydrostatic, in the protrusion and retraction of the rostellum.

The apical cells of the rostellar pad contained low levels of acid mucopolysaccharide and they also contained RNA and esterase. The presence of RNA suggests that these cells are involved in the synthesis of the esterase which is present. Since the esterase-positive cells have processes which appear to open into the secretion reservoir, and since esterase is present in the secretion reservoir, it is probable that the anterior cells of the rostellar pad are secretory and that their secretion products are stored in the secretion reservoir. The high concentration of glycogen in the cells of the rostellar pad may be present as energy stores for the rostellar pad. Since in sections stained with both PAS and alcian blue the material in the secretion reservoir stained with alcian blue only, it is unlikely that the PAS-positive material in the apical cells is secreted, unless its nature is changed in the process of secretion.

The basal cells, in contrast to the apical cells, did not contain acid mucopolysaccharide or esterases in detectable quanities. The high levels of RNA in the cytoplasm of the basal cells may indicate that active

protein synthesis is taking place. However, no protein was demonstrated in the basal cells, but they contained high levels of glycogen.

Davey & Breckenridge (1967) described paraldehyde fuchsin-positive cells in the rostellum of Hymenolepis diminuta which they considered to be neurosecretory cells. These authors were able to demonstrate a cycle of secretion of paraldehyde fuchsin-positive material in these cells which was associated with the development of the adult worm. Morseth (1967) figured an electron micrograph of a nerve containing possible neurosecretory vesicles in the rostellar pad of Echinococcus granulosus. However, in the present study, fuchsinophilic material was not demonstrated in any of the cells of the rostellar pad of D. caninum, and although nerve fibres could be seen in the rostellar pad, it was not established that any of the cells of the rostellar pad were part of the nervous system in this species.

(ii) Rostellar gland.

The histochemical tests have revealed that (a) PAS-positive globules were present in the rostellar gland and (b) globules, the periphery of which stained with alcian blue, were present in the tegument of the gland. The PAS-positive globules may be precursors of the globules in the tegument of the gland. The polysaccharide nature of the secretion in D. caninum is in contrast to the situation found in E. granulosus where the secretion is a lipoprotein (Smyth, 1964). However, the rostellar gland secretion in D. caninum is similar to that in Davainea proglottina and Aploparaxis furcigera, where it consists

of acid mucopolysaccharide (Slais, 1958; 1961). The presence of RNA in the gland cells suggests that protein synthesis takes place. Thus the esterase and other protein present in the gland are probably synthesised by the gland cells. It may be speculated that the esterase is important in the elaboration of the secretion product in the rostellar gland.

It was noted earlier that the tegument of the rostellar gland differs in appearance from the tegument of the rest of the scolex. This may reflect adaptation of the tegument of the rostellar gland for secretion, whereas the tegument of the rest of the body is absorptive. The cells of the rostellar gland are probably modified tegument cells.

Smyth (1964) suggested that the secretion of the rostellar gland in E. granulosus could be hormonal in nature, and its release may be related to the regulation of growth and maturation of the strobila. It can be speculated that the secretion in D. caninum has a similar function to that in E. granulosus, and its secretion may be controlled by neurosecretory processes in the nervous system.

(iii) Secretion reservoir.

The secretion reservoir is probably a storage area for products of secretion from the rostellum. There are two possible sources of the material contained in the secretion reservoir: the apical cells of the rostellar pad, and the rostellar gland. The fact that both the apical cells of the rostellar pad and the secretion

reservoir are strongly esterase-positive suggests that the esterase in the reservoir originates in these cells. Similarly, the material staining with mercuric bromophenol blue probably originates in the rostellar gland. However, the acid mucopolysaccharide material could originate from either or both structures. Discharge of the material from the secretion reservoir could be either a mechanical process, occurring when it has been completely filled, or it may be under nervous control, so that it occurs at an appropriate time, such as on attachment to the intestinal wall of the host.

It is difficult to speculate on the function of the esterase present in the secretion reservoir, since it is unlikely that the esterase is secreted in large quantities in D. caninum as there is little damage to the intestinal wall of the host. It is possible that the esterase may participate in a condensation reaction in the final stage of the preparation of the polysaccharide material for secretion to the exterior.

It is likely that secretion from the rostellar gland can take place either directly to the exterior, or into the secretion reservoir. The advantage of the latter is that the secretion could be accumulated so that a larger effect than that usually required from the rostellar gland is available to the animal. It is possible that a large amount of rostellar secretion is required for attachment, while only small continuous amounts of secretion are required for maintenance of attachment.

3. Nervous system.

The presence of large quanties of RNA in the nerve cells of D. caninum is probably associated with protein synthesis in these cells. It is possible that, in addition to the cholinesterase present in the nervous system, neurosecretory substances may be produced by the nerve cells. However, although neurosecretory substances often contain high concentrations of disulphide bonds, (Gabe, 1966), the nerve cells were negative for these with the DDD test, and all other attempts to stain neurosecretory substance in D. caninum were unsuccessful.

GENERAL DISCUSSION

1. Evidence for neurohumoral transmission in cestodes.

From the data presented in previous chapters, it can be seen that cestodes have at least two chemically distinct types of nerves, one type (cholinergic) containing AChE, and the other (adrenergic), probably containing 5-HT.

The presence of AChE in the cestode nervous system is highly suggestive that a pharmacologically active choline such as ACh is present in these nerves and functions as a chemical transmitter. According to Bullock & Horridge (1965), the presence in the nervous system of synaptic clefts of over 200A^{o} wide and of synaptic vesicles are associated with chemical transmission. The chemical transmitter is released from the terminal of the first nerve and diffuses across the synaptic cleft causing depolarisation of the receptor membrane of the next nerve, and so initiating the electrical impulse in the next nerve. Morseth (1967 b) has shown that the synaptic clefts in Echinococcus granulosus are 300A^{o}, and that many vesicles are present at these junctions. This suggests that there is a chemical mode of transmission across the synapse in E. granulosus, possibly involving ACh or 5-HT.

Evidence for the presence of ACh in the cestode nervous system is summarised below:

(i) AChE has been localised in the cestode nervous system (chapter 4).

(ii) It has been found by biochemical experiments that most of the esterase present in cestodes hydrolyses ACh and very closely related cholinesters much more rapidly than other cholinesters, and this enzyme has similar properties to AChE of the vertebrate nervous system. This has been discussed in chapter 4.

(iii) ACh or a substance which has very similar pharmacological properties to ACh is present in cestode homogenates. Artemov & Lurje (1941) carried out biological assays for ACh in homogenates of Dipylidium caninum and Hydatigera taeniaeformis, using leech dorsal muscle, and calculated the concentration of ACh in the tissue of these cestodes. D. caninum was found to contain between 1 and 1.5 µg ACh per g wet weight, and H. taeniaeformis contained between 2 and 3 µg per g wet weight. Pylkkö (1956 b), using a similar method of assay, calculated the amount of ACh present in preparation of Diphyllobothrium latum tissue, and found that this varied between 4.0 and 12.0 µg per g wet weight of tissue. In the present study, an assay of the ACh content of Dipylidium caninum was carried out using the body wall of the Australian land leech, Chtonobdella limbata Grube, 1966 in order to confirm the results of Artemov & Lurje (1941). Although only uncontrolled experiments have been carried out at this stage, it has been calculated that a sample of D. caninum contained approximately 0.5 µg per g wet weight of tissue. This is approximately half that found by Artemov & Lurje (1941). The difference could be due either to the different methods used in preparing the homogenate, or to biological variation between samples, similar to that

encountered by Pylkkö (1956 b). It has been established by biological assay techniques that ACh is also present in trematodes (Chance & Mansour, 1953; Barker, Bueding & Timms, 1966). Unfortunately, limited accuracy in both identification and quantitative estimation of the pharmacologically active substance is obtainable when biological methods are employed, and future studies should involve chemical methods as these are developed.

(iv) Muscular activity of cestodes is affected by cholinergic drugs. This has been discussed in chapter 3.

Histochemical evidence for an 'adrenergic' nervous system in cestodes, in which 5-HT is the probable transmitter, has been presented in chapter 5.

Although it has not been definitely established whether cholinergic and adrenergic transmitters are present together in the same nerve in cestodes, the evidence tends to suggest that separate cholinergic and adrenergic nerves exist. The fact that sensory nerve endings were found to contain AChE but not monoamines in D. caninum indicates that the sensory nerves are most probably cholinergic and not adrenergic. It appears also that separate cholinergic and adrenergic nerves occur in the longitudinal nerve cords, since only a few nerve fibres in the longitudinal nerve cords of D. caninum contained monoamines (chapter 5), and in transverse sections of Hydatigera taeniaeformis stained for AChE, it could be seen that AChE was absent from part of the main longitudinal nerve cord (chapter 2). Histochemical staining of the same sections with both the fluorescence technique and for AChE may solve this problem.

2. Possible functions of the two types of nerves.

At present, it seems reasonable to postulate that there are two kinds of innervation of organs which contain both AChE-positive and monoamine-positive nerve fibres, the cholinergic nerves probably having different functions from the adrenergic nerves. It is of interest to note that in the nematode Ascaris lumbricoides, a neurohumour, probably ACh, causes depolarisation of muscle membranes and stimulation of muscular activity, while an inhibitory neurohumour, probably γ-aminobutyric acid or a closely related compound, has a paralysing effect (Debell, 1965). It is possible that, of the cholinergic and adrenergic nerves of cestodes, one type may stimulate muscular activity while the other inhibits it. However, further pharmacological studies using cholinergic and adrenergic drugs as well as amino acids such as γ-aminobutyric acid, and a neuromuscular preparation which is devoid of other tissues will be required before a possible stimulatory, inhibitory action of the nerves is investigated.

3. Evidence for neurosecretion in cestodes.

Some evidence has been presented which supports the view that neurosecretory cells are present in cestodes. Davey & Breckenridge (1967) described paraldehyde fuchsin-positive nerve cells in the rostellum of Hymenolepis diminuta, and Morseth (1967 b) described nerve cells in a ganglion of Echinococcus granulosus which contained electron dense bodies similar to those present in neurosecretory cells of vertebrates. In invertebrate groups,

neurosecretion may control one or more of a number of physiological processes: reproduction, regeneration (in annelids), regulation of somatic pigmentation, moulting (in crustaceans), growth, differentiation, moulting (in insects), (Bullock & Horridge, 1965), and moulting (in nematodes) (Davey, 1966; Davey & Kan, 1967).

Within the Platyhelminthes, nerve cells with staining properties or with ultramorphology similar to vertebrate neurosecretory cells have been demonstrated in a number of turbellarians (Oosaka & Ishii, 1965; Morita & Best, 1966; Lentz, 1967) and trematodes (Ude, 1962; Gresson & Threadgold, 1964). A cycle of neurosecretion has been demonstrated in a number of other turbellarians, and has been correlated with reproduction (Grasso, 1966), regeneration (Török, 1958; Lender & Klein, 1961; Ude, 1964; Grasso, 1966) and osmoregulation (Ude, 1964). It is therefore possible that neurosecretion in cestodes could control reproduction, osmoregulation in intestinal forms, and regeneration in some forms, e.g. Mesocestoides tetrathyridia (Hart, 1968). Neurosecretion in cestodes may be associated with development, since these two processes in Hymenolepis diminuta have been correlated (Davey & Breckenridge, 1967). It is also possible that a neurosecretory process could control secretion of the rostellar gland, thus participating in attachment.

4. Role of the nervous system in movement.

In chapter 3, it was found that movements of Dipylidium caninum proglottids were sensitive to

cholinergic drugs, and that the neuromuscular junction in this and other cestodes is probably cholinergic. Rietschel (1935) investigated the propagation of slow waves of longitudinal muscle contraction in the cestode Catenotaenia pusilla which spread from the anterior end and proceeded posteriorly. A series of overlapping partial transections of a proglottid did not interrupt the passage of the contraction wave. He concluded that the transmission of the contraction wave does not depend on the nervous system but that the muscles contracted in response to mechanical stimulation. Thus the organised contraction of cestode muscle is probably independent of the nervous system. In nematodes, the 'finger tissue' of the muscle cells acts as a co-ordinating centre, but the nerve cord modifies the action of the muscles by the secretion of neurohumours (Debell, 1965). It is possible that in cestodes there is some mechanism in the muscle cells for co-ordinating activity, and that the nervous system modifies this activity.

5. The nervous system in oncospheres.

The metacestode stage in cestodes is generally the same as the adult stage apart from the absence of proglottids, and the nervous system is correspondingly similar except for nerve fibres in the cyst wall. However, the oncosphere is the larval stage, and is quite different from the adult in organisation. Rybicka (1967) suggested that some nervous centres stimulating the activity of hatched oncospheres probably exist. However, in this study, it could not be demonstrated unequivocally whether AChE-positive structures in oncospheres of Echinococcus

granulosus were elements of the nervous system. Collin (1969), using the electron microscope was also unable to demonstrate that nerve fibres were present in hatched oncospheres of Hymenolepis citelli. Further electron microscope studies may be successful in elucidating this problem.

6. Comparison of the cestode nervous system with that of other animals.

The cestode nervous system appears to have all the same elements as the mammalian nervous system. Neurons are linked functionally by synapses, and neuromuscular junctions with muscles are present (Morseth, 1967 b) and, in addition, two chemically distinct types of nerves are present, providing dual innervation to muscular organs. However, the nervous system is clearly not as centralised as that in vertebrates and arthropods. Florey (1967) stated that:

> All animal life functions according to the same principles and the same physiological laws. There are no higher and no lower kinds of physiology, just as there are no higher or lower forms of life.

This appears to hold true for the nervous system of cestodes. A relatively decentralised nervous system is more able to deal with local situations in a body which is very long compared with its width, and with serially repeated reproductive units. However, some centralisation is present in the form of the nerve cords and the system of ganglia and commissures in the scolex. The concentration of nervous tissue in the scolex is probably related to the importance of the scolex in attachment (chapter 6).

SUMMARY

1. Much of the nervous system of the cestodes Dipylidium caninum, Echinococcus granulosus, Hydatigera taeniaeformis, Baerietta criniae minor, and Taenia hydatigena has been shown to contain cholinesterase, and the nerves were stained selectively by a number of histochemical techniques for non-specific esterases and cholinesterases. These techniques revealed ganglia, commissures, longitudinal nerve cords, nerves of the inner plexus and peripheral plexus, possible neuromuscular junctions, sensory nerve endings, and nerves supplying organs and tissues except those occurring in the internal medullary region.

2. The effects of specific inhibitors of acetylcholinesterase and butyrylcholinesterase on the histochemical reaction in D. caninum, E. granulosus, H. taeniaeformis and B. criniae minor were investigated. The cestode cholinesterases were not as sensitive to the inhibitors as mammalian cholinesterases, and the results were therefore not very clear cut. However, the cholinesterase is probably one or more acetylcholinesterases which are not as sensitive to the specific inhibitor BW284C51 as mammalian acetylcholinesterases.

3. Movements of D. caninum proglottids were affected by cholinergic agonist and antagonist drugs, and this suggests that the neuromuscular junction in D. caninum is cholinergic.

4. Nerve fibres containing biogenic monoamines demonstrable by the histochemical formaldehyde fluorescence technique were identified in the longitudinal nerve cords, inner plexus, peripheral plexus, and nerves supplying the cirrus and copulation canal of D. caninum, Hymenolepis nana and Taenia hydatigena. Characteristics of the fluorescence and the reaction conditions which produced it strongly suggest that it is due to 5-hydroxytryptamine.

was/

5. The rostellum of D. caninum were found to contain two groups of cells which are probably secretory: (i) the rostellar gland, which is anterior to the rostellar pad, and (ii) the apical cells of the rostellar pad. Globules present in the cytoplasm of the rostellar gland cells and in the tegument of the rostellar gland contained polysaccharide. The apical cells of the rostellar pad and the secretion reservoir contained acid mucopolysaccharide and cholinesterase. It is speculated that the tegument anterior to the suckers and the glands in the rostellum may participate in attachment to the intestine of the host.

APPENDIX I

Maximow Technique

as modified by the Department of Parasitology, University of Queensland.

Used after fixation in Zenker-formaldehyde.

1. Fix tissue 12 hours at room temperature. Use thin pieces of tissue - 1 mm. for dense tissue e.g. lymph nodes; 2 mm. for gut.

2. Remove fixative by washing in running water (5 mins.).

3. Place in a solution of equal parts Lugol's iodine and 70 per cent alcohol for about 24 hours.

4. Dehydrate and embed as usual.

5. Cut sections at 4μ.

6. Deparaffinise and bring sections to 70 per cent alcohol.

7. Remove mercuric chloride deposits by immersing in Lugol's iodine (5 mins.) followed by sodiumthiosulphate (5 mins.).

8. Bring to water and wash in water to remove sodium-thiosulphate.

9. Stain Delafield's haematoxylin solution.

5 mls. stock solution of haematoxylin.

100 mls. distilled water.

Leave ½ hour or until sections appear dove-grey in colour. Use a fresh solution each time.

10. Wash distilled water (two changes) for 3 hours.

11. Counterstain overnight in the following solution:

5 mls. 1/1000 eosin in distilled water

40 mls. Sorensen's buffer pH 6.8

5 mls. 1/1000 Azure II.

added in the above order. The correct Azure II is Grubler's stain sold under the name of 'Chromo' or 'Revector' stain (Hopkins & Williams). Note: the stain tends to precipitate.

12. Rinse in distilled water. If slides are too blue, differentiate in 1 drop glacial acetic acid to 50 mls. water ($\frac{1}{2}$ min.).

13. Differentiate in 96 per cent alcohol (2 changes) until nuclei are sufficiently differentiated to show the chromatin formation; the cytoplasm is clear and the collagen fibres, pale pink.

14. Dehydrate rapidly in 2 changes of absolute alcohol. (This continues to remove the stain).

15. Clear in 2 changes analar xylene.

16. Mount in neutral mountant.

Do not expose stained slides to sunlight, as they fade very rapidly.

Results: nuclei, deep blue; smooth muscle, pale blue or pink; connective tissue fibres, pink; granulocytes, (acidophil), pink; basophil granules, blue; red blood cells, pink or pale green.

Gabe's Stain

After Rybicka.

Solutions:

Gabe's trichrome A

ferric ammonium sulphate	1 gm.
distilled water	50 ml.
sulphuric acid	0.8 ml.
95% ethyl alcohol	50 ml.
haematoxylin	0.5 gm.

Gabe's trichome B

yellow eosin	1 gm.
light green	0.2 gm.
phosphotungstic acid	0.5 gm.
glacial acetic acid	0.5 ml.
distilled water	100 ml.

1. Stain Gabe's A $1\frac{1}{4}$ mins.
2. Rinse in tap water until the colour changes to 'bluish'.
3. Stain Gabe's B 3 mins.
4. Blot dry with filter paper.
5. Pass quickly through two changes of absolute alcohol.
6. Clear in xylol.
7. Mount in neutral mountant.

APPENDIX 2

List of abbreviations

ACh	acetylcholine
AChCl	acetylcholine chloride
AChE	acetylcholinesterase
AThChI	acetylthiocholine iodide
BuCh	butyrylcholine
BuChE	butyrylcholinesterase
BuChI	butyrylcholine iodide
BuThCh	butyrylthiocholine
BuThChI	butyrylthiocholine iodide
BW284C51	1,5-bis(4-allyldimethylammoniumphenyl) pentan-3-one dibromide
ChE	cholinesterase
DFP	diisopropylphosphorofluoridate
E600	diethyl-p-nitrophenyl phosphate
5-HT	5-hydroxytryptamine
Mipafox	N,N-dissopropylphosphorodiamidic fluoride
iso-OMPA	tetramonoisopropylpyrophosphortetramide
PCMB	para-chloromecuribenzoate
βPPA	β-phenylpropionic acid

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