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CHAPTER I

INTRODUCTION

The biological success of the endoparasitic helminths necessitates the existence of efficient and sophisticated sensory mechanisms. That such mechanisms exist is verified by phenomena such as: active positioning in the organs of the host i.e., the vertebrate gut, and larval migrations of many parasitic species. The functional nature of such nervous mechanisms is not well understood and investigations into the functioning of parasitic helminth nervous systems is just beginning. The investigation into the adaptations and functioning of the nervous system in one group of parasitic helminths (Pentastomida) constitutes the subject of this work.

Biochemical and physiological investigations into the functions of parasite nervous systems have gained momentum in recent years. Mellanby (1955) identified and described the acetylcholine contents of three nematodes, Ascaris lumbricoides, Litomosoides carinii, and the microfilariae of Dirofilaria repens. Krotov (1957) demonstrated the sensory and motor nerves of Ascaris sp. to be highly sensitive to acetylcholine. A cholinesterase from Haemonchus contortus which

hydrolyzed both acetyl and butyrylcholine was characterized and tested for its susceptibility to inhibition by organophosphorus substances by Lee and Hodsden (1963). Finally, Sanderson (1969) reported a high specific activity for the acetylcholinesterase isolated from the infective and adult stages of the rat strongyle Nippostrongylus brasiliensis.

In mentioning noteworthy works on the nervous systems of the Trematoda one must consider the isolation and characterization of an acetylcholinesterase from Schistosoma mansoni which is similar to that occurring in the nervous tissue of vertebrates (Bueding, 1952). Bueding (1967) localized, by histochemical methods, the site of cholinesterase activity to the nervous system of this fluke. Pelper (1958) demonstrated acetylcholinesterase activity in the ova of this parasite. Lee (1962) employed cholinesterase histochemistry in a study of the cholinesterase activity of the nervous system and holdfast organ of Diplostomum phoxini. Frady and Knapp (1967) measured the activity of a specific acetylcholinesterase from Fasciola hepatica by employing a radioisotopic assay method for acetyl-1^{14}C-choline iodide. The indoxyl acetate method for esterase activity was employed by Halton and Jennings (1964) as a means of detecting the site of activity in the nervous system of the monogenean Diplozoon paradoxum.

Among parasitic helminths the cholinesterases have been investigated most extensively among the cestodes, as exemplified by the work of Pylkkö (1956) who demonstrated the presence of a specific acetylcholine hydrolyzing enzyme and an enzyme with the ability to split benzoylcholine in the tissues of Diphyllobothrium latum and Taenia

saginata. Lee et al. (1963) made a comprehensive histochemical examination of the cholinesterases in Hymenolepis sp. and Hydatigera sp., demonstrating activity to be localized in the nervous system. A similar investigation showed cholinesterase activity to be localized in the nerve cords and tegument of cyclophyllidean cestodes (Schardein and Waitz, 1965). Wilson (1965) completed a similar work in which he showed acetylcholinesterase activity to be restricted to the nervous system of two hymenolepid cestodes, and Hart (1967) found cholinesterases to be present in the nervous system of tetrathyridian cestodes. Graff and Read (1967) isolated and characterized a specific acetylcholinesterase activity from the tissues of Hymenolepis diminuta. Most recently Shield (1969) employed histochemical methods to identify esterase activity in the nervous systems of other cyclophyllidean cestodes. The works reviewed strongly suggest a role of cholinesters as transmitter substances in the nervous systems of the parasites mentioned.

The role of catecholamines and indolalkylamines in the functioning of parasite nervous systems is now beginning to be investigated. The lone work reported so far is on 5-hydroxytryptamine in Schistosoma mansoni (Bennet and Bueding, 1970).

Although considerable investigation into the functioning of parasite nervous systems appears to be underway, certain helminth groups have been neglected, e.g., the Pentastomida. Heymons (1935) in his monograph on the Pentastomida described in detail the nervous systems of several pentastomes, and Doucet (1965) reinforced the work of Heymons with a histological and histochemical study. This study is the first to be made on the nervous systems of these helminths by biochemical and

histochemical methods. Its purpose is to explain, in part, the functioning of the pentastome nervous system.

CHAPTER II

MATERIALS AND METHODS

Enzyme Assays

Porocephalus crotali and *Porocephalus clavatus* were reared in laboratory white mice and recovered from the lower abdominal tissues four months after infection. They were washed in ice-cold phosphate buffered Ringer (pH 7.0), and all succeeding operations up to the enzyme assays were carried out at -2 to 4 C. Nymphs were homogenized in glass homogenizers containing 3 ml of 0.185 ionic strength saline (0.10 M NaCl, 0.02 M $MgCl_2$, and 0.025 M $NaHCO_3$) for each 300 mg of tissue. Unfractionated homogenates were used for manometric determinations of cholinesterase activity by the methods of Nachmansohn and Rothenberg (1945), and Nachmansohn and Wilson (1955), while fractionated homogenates were obtained by refrigerated centrifugation at 16,000 rpm and 30,000 X gravity for 30 minutes. Dry weights of duplicate aliquots of homogenates or homogenate fractions were determined after heating to a constant weight at 103 C. Acetylcholinesterase activity was measured by manometric methods in a Schoelander respirometer under 95% N_2 - 5% CO_2 at 37 C. After a 15 minute period of gas and temperature equilibration the tissue homogenate was added by means of a calibrated syringe through a vaccine port to achieve a final volume in the reaction vessel of 2.5 ml. Enzyme activity was recorded as microliters (ul) of

CO_2 evolved per mg dry weight during the initial 15 minutes of assay.

The supernatant from fractionated homogenates was analyzed for nonspecific cholinesterase activity by the spectrophotometric technique of Rappaport et al. (1959). Control tests were performed using mouse serum instead of pentastome supernatant. A standard curve was constructed for pentastome supernatant and mouse serum.

Specific activity for the enzyme assays was read in terms of change in optical density and extrapolated from the above standard curve into millimoles of the substrate broken down per unit time by a definite dry weight amount of serum or supernatant.

Nervous System Histochemistry

Parasites were prepared for histochemical analysis by removal from the hosts and washing in Ringer solution at 23 C. Some were dehydrated in alcohol, embedded in Paraplast (Sherwood Medical Industries Inc., M.P. 56-57 C.) using standard techniques, and sectioned at 6 u. Others were embedded in O.C.T. (Ames Co.), quick frozen with CO_2, sectioned at 15 u on a cryostat and mounted on microscope coverslips.

Catecholamine Histochemistry

Pentastomes were taken from buffered saline and treated using the following histochemical procedures: (1) cryostat cold sections were placed on chloroform cleaned coverslips, treated for catecholamine chromaffin reactions according to the technique of Hillarp and Hokfelt (1955); (2) whole nymphs were fixed in 40% formaldehyde according to the fluorescence method for noradrenaline (Eranko, 1955); and (3) other

pentastome tissues were fixed in Zenker's fixative (12 hrs), paraffin infiltrated, sectioned at 6 u, and examined for adrenochrome (chromaffin) reactions by utilizing the Giemsa method (Sevki, 1934). Mouse adrenals were used as controls in all catecholamine and indolalkylamine histochemical experiments.

Cholinesterase Histochemistry

Histochemical tests for cholinesterase activity in P. crotali and P. clavatus nymphs were performed on cryostat sections by employing the acetylthiocholine iodide method of Koelle (1950 and 1951). Sections were incubated 45 minutes at 37 C in ten ml of Gomori medium as detailed by Pearse (1966). Coverslips were mounted on microscope slides with glycerol gelatin. Mouse brain sections, similarly treated, were used for controls. Heat treated mouse brain sections failed to give positive results.

Physiology Experiments

Solutions of adrenaline, noradrenaline, 5-hydroxytryptamine, and dopamine were prepared by dissolving the appropriate amounts of dry powder in ten ml of 0.9% phosphate buffered (pH 7.0) sodium chloride solution to give a 10^{-3} M concentration of the catecholamine. Eserine sulfate was dissolved in saline to give a 10^{-4} M solution. Portions (10 ml) of these solutions were placed in separate containers; adult and nymph P. crotali were placed in these solutions and their behavior was observed for one hour.

CHAPTER III

RESULTS

Enzyme Assays

In Graph 1 it is evident that the pentastome tissues did not hydrolyze the acetylcholine chloride. Throughout the assay period pentastome tissue homogenates failed to evolve any gas (CO_2) beyond the nonspecific level regardless of the substrate concentration, whereas both the mouse brain and tapeworm tissue (Hymenolepis microstoma) yielded considerable CO_2.

Mouse brain homogenates (Graph 1) incubated in increasing molarity of substrate for fixed time periods revealed an initial low activity, then activation of the enzyme at the intermediate substrate concentrations followed by the characteristic substrate inhibition at the greater substrate concentration levels. The tapeworm tissues showed (Graph 1) similar phenomena but with a reduced magnitude of hydrolysis, while pentastome nymphs failed to hydrolyze any substrate as evidenced by their failure to evolve CO_2. Samples of 8.2 mg/0.1 ml of pentastome tissue evolved 36.8 ul/hr of CO_2,while samples of 16.3 mg/0.1 ml evolved 39.8 ul/hr of CO_2. Samples incubated with medium alone evolved 38.5 ul/hr of CO_2. This indicates that regardless of the concentration of pentastome tissue in the incubation medium the CO_2 evolved over a 15 minute period

was not greatly different from the nonspecific gas evolution of the medium without the tissues. Mouse brain homogenates of 16.3 mg/0.2 ml gave off large volumes of CO_2, 514 ul/hr. Under identical conditions nymph tissue failed to hydrolyze either acetylcholine chloride or butyrylcholine iodide. Mouse brain homogenates demonstrated a preference for the acetyl ester (8.49 ul/mg dry wt/15 min) rather than the butyryl ester (1.32 ul/mg dry wt/15 min); this is characteristic of specific acetylcholinesterase (Nachmansohn and Wilson, 1955).

The clear fractionated supernatant from P. crotali nymphs was assayed for nonspecific cholinesterase activity by the modified spectrophotometric method of Rappaport et al. (1959). Graph 2 indicates that the pentastome nymph supernatant did not hydrolyze the 0.08 M acetylcholine chloride substrate,while mouse serum at 37 C produced rapid hydrolysis of this substrate. The time assay illustrates a rectangular hyperbola for mouse serum hydrolysis of this substrate. Hydrolysis was essentially linear with respect to time for the initial ten minutes of hydrolytic activity.

Nervous System Histochemistry

Catecholamine Histochemistry

When the tissues of P. clavatus adults were treated by the histochemical methods of Hillarp and Hokfelt (1955) (hereafter designated as the "Creaction") to determine the presence of chromaffin the reaction was judged to be poor when compared to that of the mouse adrenal gland control. All of the major areas of interest in the pentastome nervous system gave what was considered to be marginal or negative reactions to

this method of treatment, while the controls showed dark brown coloration in the medulla of the adrenal gland with yellowish-brown cortical responses both of which are characteristic of adrenaline producing cells.

The nervous system of P. clavatus nymphs, treated by the histochemical methods of Sevki (hereafter referred to as the "GA reaction") for the presence of chromaffin gave a general positive response when compared to mouse adrenal gland controls (Figure 1). This reaction varied in intensity depending upon the location in the pentastome nervous system. The most intense reaction was in the axon portion of neurons forming synaptic junctions of what was considered to be sensory nerves terminals. These sensory nerve terminals included many of the neurons of the anterior and posterior nerve tracts emanating from the supra and subesophageal ganglia which gave chromaffin positive responses. The synaptic termini of the third and fourth ganglionic pair innervate the muscles of the hooks, buccal cavity, and cephalic hypodermal sensory cells. The termini of the fifth, sixth, and seventh ganglionic pairs supply neurons to the lateral hypodermis and muscles, gut, genital organs, and diffuse posterior nerve net. All of the above mentioned neurons gave a weak chromaffin positive response to the "GA reaction". The terminal sensory knobs (hereafter referred to as "SK cells") as figured by Heymons (1935) surrounding the anus and vaginal orifice were not visible. In the area of these orifices were numerous nerve endings which gave positive results with the "C and GA reactions".

P. crotali adult tissues were treated with the "C reaction" and giemsa stained. The terminal nerve endings in the muscles at the base of

the hooks gave a chromaffin positive reaction as did the nerve endings to the buccal cavity. Figure 2-A demonstrates chromaffin positive granules in the axons of nerves ending in muscles (Figure 2-B) at the base of a hook. The supraesophageal ganglia gave weaker chromaffin reactions than did the hook region or the buccal cavity neurons. The neuropile of the supra and subesophageal ganglia was only slightly positive when treated with the "C reaction" and stained with Giemsa. In the hypodermis (Figure 3) numerous small delicate sensory cone cells (hereafter designated as "SDS cells") and sensory cones (hereafter designated as "SC cells") were chromaffin positive. This is also true for the synaptic junctions (Figure 4) of multipolar neurons in the subhypodermal region of the forebody.

Neurons were frequently seen to end in close proximity to the hypodermal "SDS cells" (Figure 5-A) in a fashion similar to that described by Barrington (1967) for the echinoderms. Adult and nymphal Kiricephalus pattoni were observed to give faint chromaffin positive reactions at the basal portion of these "SDS cells" near the "secretory cells" of the nymphal hypodermis (Figure 5-B) when treated by the Sevki method. Chromaffin granules were not observed in the "SK cells" of the anterior end of the nymph (Figure 6). The nerve endings in the buccal cavity and hooks were minimally chromaffin positive suggesting the presence of catecholamine in the terminal neurons of the anterior supra and subesophageal ganglionic masses. The nerve endings in the neuropile of the supraesophageal ganglionic mass were chromaffin positive (Figure 8-A) with granules present in the nerve ends (synaptic junctions?) and cell bodies of the area immediately under the perineurium (Figures 8-B

and 9-B). Figure 9-A indicates the origin of the posterior nerve tracts from the brain. The posterior sixth or seventh nerve tract may be seen directed toward the genital organs and posterior body, while the anterior fourth and fifth nerve tract is seen to innervate the muscles of the posterior hooks and anterior "SK cells" (Figure 8-C). These are probably mixed nerve tracts. At least some of the cell bodies in the ganglionic masses are motor ganglion cells and others are sensory ganglion cells.

The "SDS cells" of the hypodermis, mentioned earlier in the reference to Barrington (1967), were observed to form a close association with the subhypodermal neurons (Figures 5-A and 5-B). Granules appearing to be chromaffin in nature were aggregated along the basal portion of the hypodermal cell membrane surface (Figure 5-B). The granules were visibly paler than others and had absorbed some of the azure blue component of the Giemsa stain. No nerve connection to the reproductive system was observed in either the adults or the nymphs and only small neurons which were chromaffin negative were associated with the genitalia.

Raillietiella orientalis adults were examined for catecholamine by the "GA reaction". The nervous system of R. orientalis, as tested here, was chromaffin negative. In Sambonia sp. the nervous system of the nymphs resembled that of the porocephalids with chromaffin positive material being deposited in only a few neurons innervating hook muscles of specimens treated with the "GA reaction".

P. crotali nymphs were treated by the fluorescence method of Erankö (1955) (hereafter designated the "F reaction") and the only area of strong fluorescence was associated with the gut. Mouse adrenals fluoresced intensely in the medulla.

Cholinesterase Histochemistry

The acetyl and butyrylthiocholine method for cholinesterase activity applied to adults and nymphs of P. crotali and P. clavatus yielded consistently negative results. Control tests applied to sections of mouse brain gave positive results (Figure 7).

Physiology Experiments

P. crotali nymphs and adults were emersed in 10^{-3} M solutions of suspected transmitter substances: adrenaline, noradrenaline, 5-hyroxytryptamine, and dopamine and their behavior observed visually for one hour. Their behavior in adrenaline and dopamine was similar to that of pentastomes in buffered saline at room temperature. Activity consisted of mild contractions and undulations at 10-15 second intervals. Adult and nymphal worms in noradrenaline and 5-hydroxytryptamine began rhythmic contractions and rapid undulations (one every 2-3 seconds) about ten minutes after being emersed in these solutions. This activity persisted throughout the one hour study. Removal of these pentastomes to buffered saline resulted in normal activity after 20-30 minutes.

Nymphs and adults of P. crotali emersed in 10^{-4} M eserine sulfate solution, a specific cholinesterase inhibitor, became increasingly active after approximately ten minutes emersion. After 5-10 minutes of increased contractile activity the parasites exhibited complete tetanus, contracting to one-half their original length. This condition was irreversible indicating that the inhibitor had completely and irreversibly blocked the vital functioning of the parasite.

PLATE I

Figure 1. A cross section through the mouse adrenal gland (400X). Tissue treated by the Sevki method for catecholamines. The arrow indicates chromaffin granules.

Figure 2. A longitudinal section through the hook region of a Porocephalus crotali adult (1,000X). The tissue was treated by the Hillarp and Hokfelt method for catecholamines.

A. Chromaffin positive granules in the axons of nerves innervating the muscles of the hooks.

B. Striated muscle of the hooks.

Figure 3. A cross section through the midbody of a Porocephalus clavatus adult (400X). The tissue was treated by the Hillarp and Hokfelt method for catecholamines. The arrow marks a small delicate sensory cell.

Figure 4. A cross section through the midbody of a Porocephalus clavatus adult (1,000X). The tissue was treated by the Hillarp and Hokfelt method for catecholamines.

A. A multipolar subhypodermal cell body with numerous chromaffin positive granules.

B. An axonal junction with a chromaffin positive granule.

C. The hypodermis just beneath an invisible cuticle.

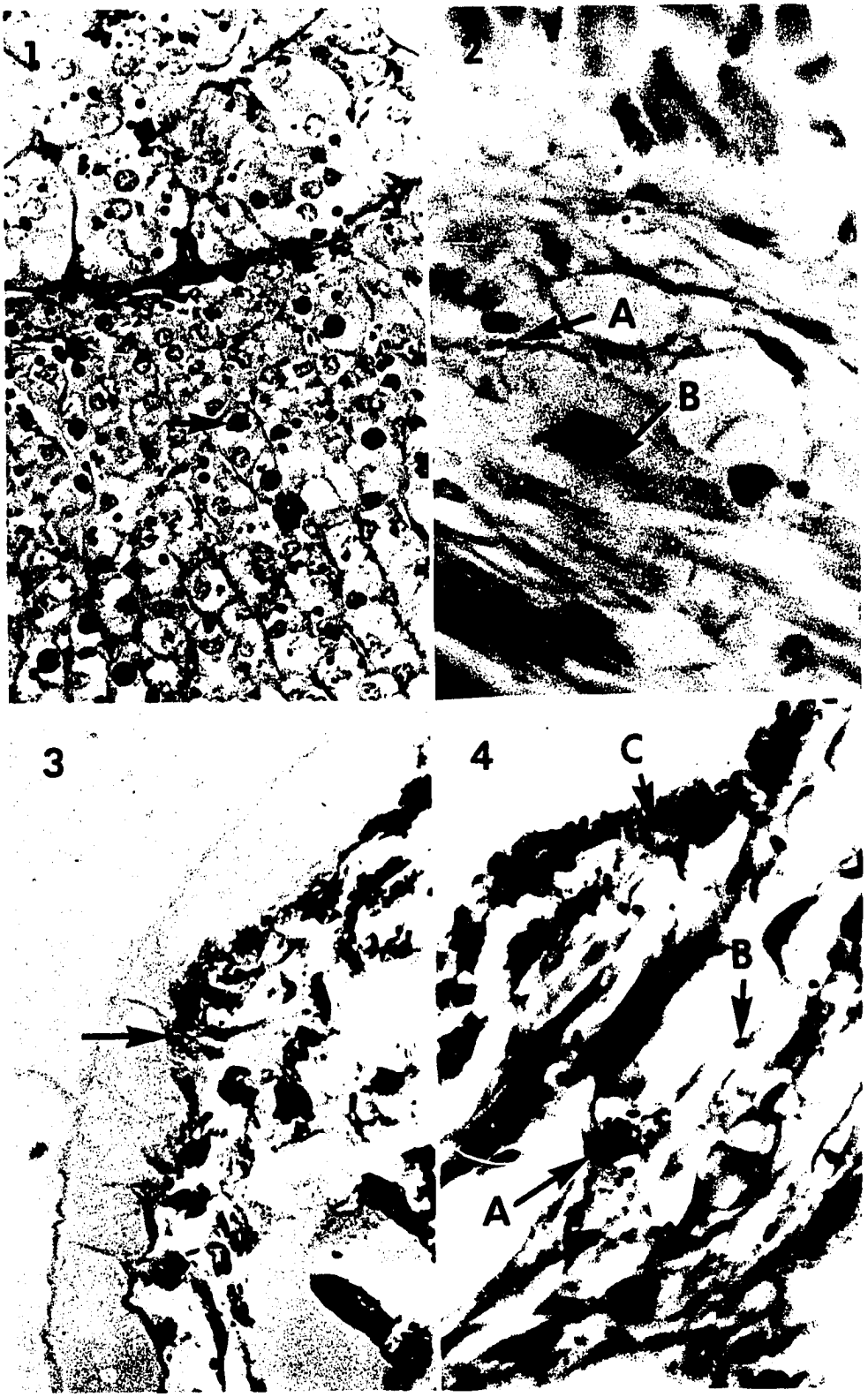

1
2
A
B
3
4
C
B
A

PLATE II

Figure 5. A longitudinal section through a Kiricephalus pattoni nymph (400X), treated by the Sevki method for catecholamines.

A. A subhypodermal nerve.

B. A sensory cone cell.

C. A "secretory cell".

Figure 6. A longitudinal section through a Kiricephalus pattoni adult (1,000X), treated by the Sevki method for catecholamines. The arrow marks a sensory knob.

Figure 7. A cross section through mouse brain (400X), treated by the Koelle method for cholinesterases. The sites of activity are the dark deposits.

Figure 8. A longitudinal section through a Kiricephalus pattoni nymph (400X), treated by the Sevki method for catecholamines.

A. The posterior nerve tracts of the ganglionic mass leading to the genital organs, lateral nerves, and posterior body.

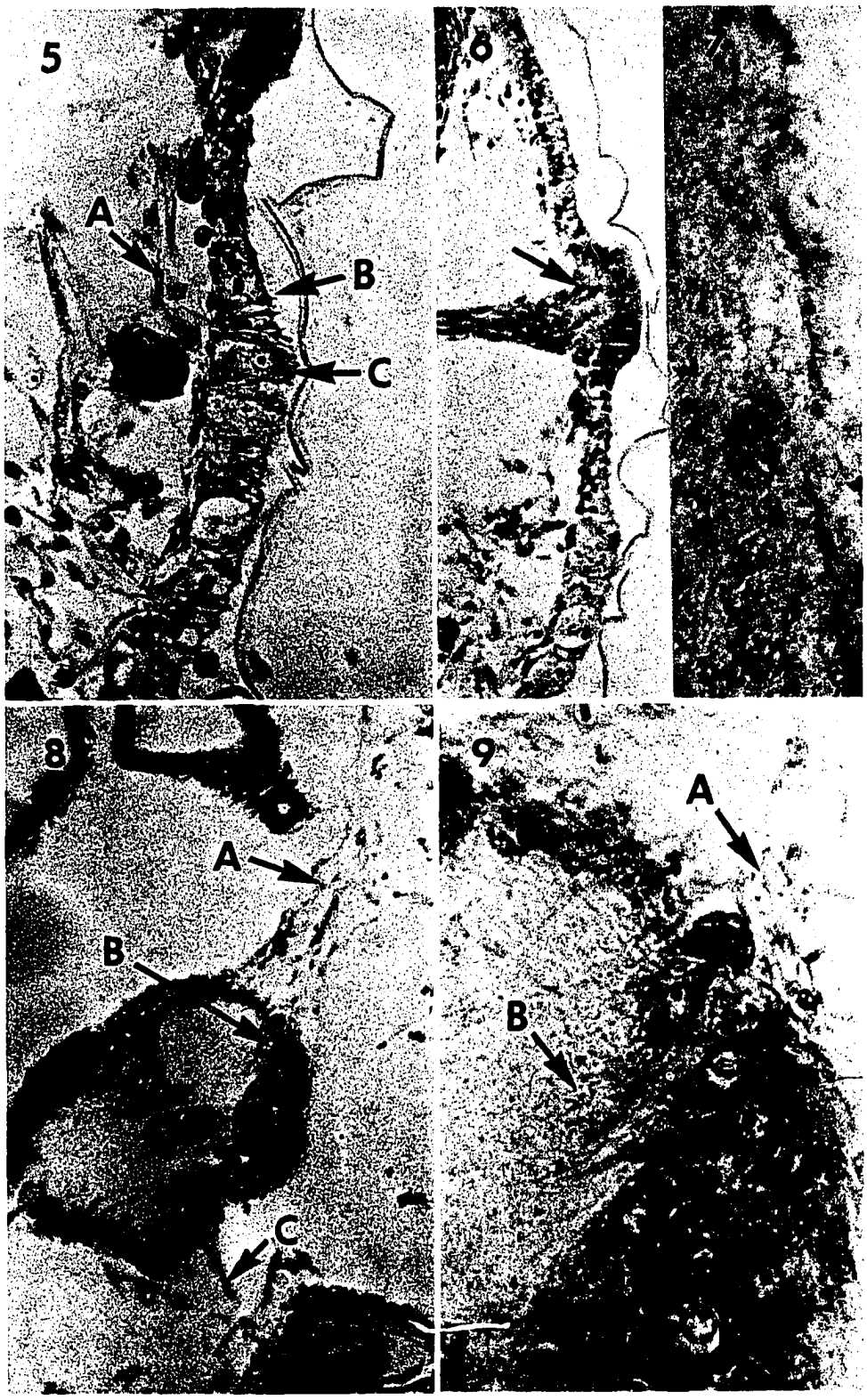
B. Synaptic junctions where the posterior nerves enter the ganglionic mass.

C. The anterior nerve tracts to the hooks and anterior sensory knobs.

Figure 9. A longitudinal section through area B. of Figure 8 (1,000X).

A. The posterior nerve tracts.

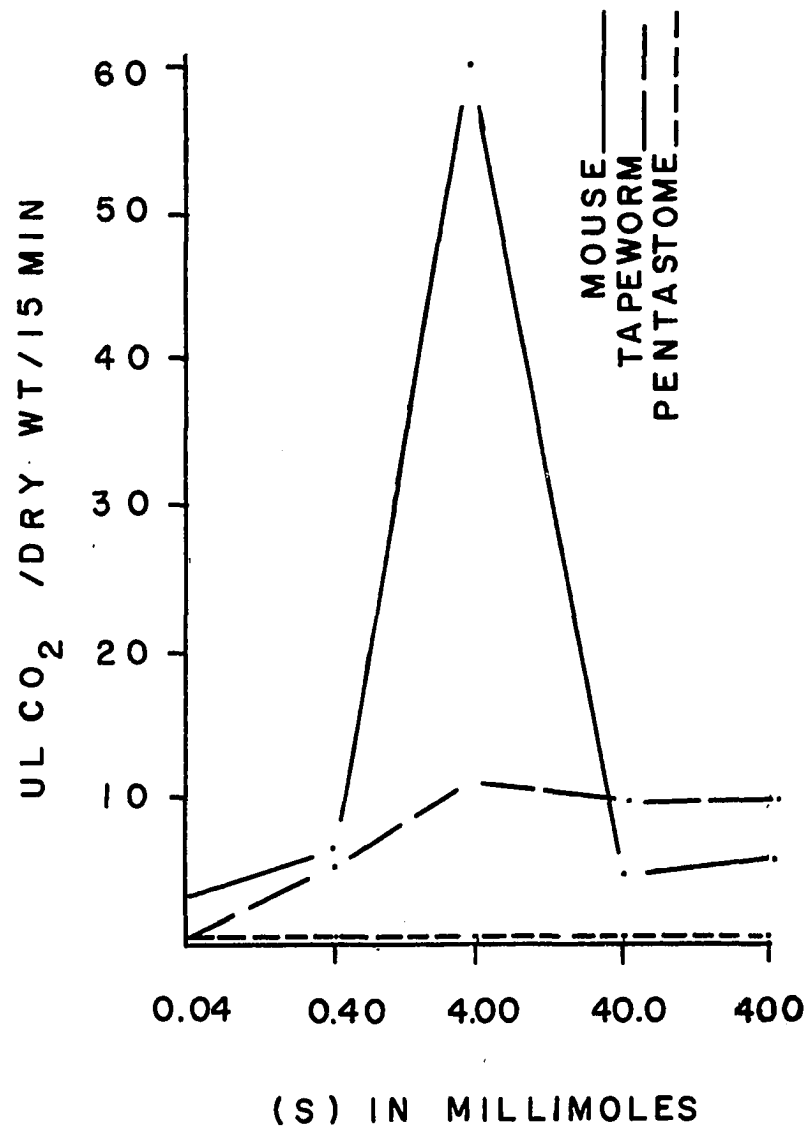
B. Chromaffin positive granules in synaptic junctions.


5
A
B
C
6
7
8
A
B
C
9
A
B

GRAPH 1

The Hydrolysis of Acetylcholine Chloride Demonstrated by a Manometric Method

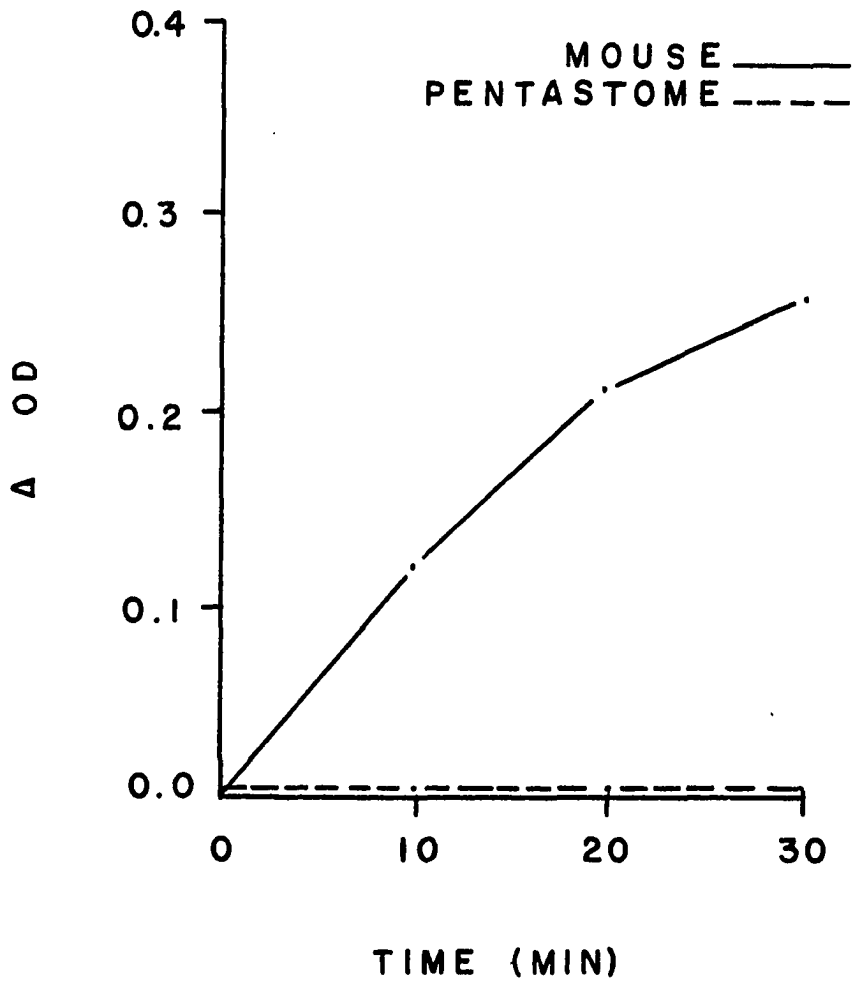
Effect of substrate concentration on the cholinesterase activity of mouse brain, tapeworm, and pentastome tissue using unfractionated homogenates. Each point represents a mean of five values.


60
50
40
30
20
10
UL CO_2 /DRY WT/15 MIN
MOUSE
TAPEWORM
PENTASTOME
0.04
0.40
4.00
40.0
400
(S) IN MILLIMOLES

GRAPH 2

The Hydrolysis of Acetylcholine Chloride Demonstrated by a Spectrophotometric Method

The hydrolysis of 0.08 M acetylcholine chloride by mouse serum and pentastome supernatant as a function of time. Each point represents a mean of three values. Reading were made at 440 mu.


0.4
0.3
0.2
0.1
0.0
Δ OD
MOUSE
PENTASTOME
0
10
20
30
TIME (MIN)

CHAPTER IV

DISCUSSION

The occurrence of cholinesterases in the nervous tissue of parasitic helminths appears to be ubiquitous as demonstrated by the works of Bueding (1952), Mellanby (1955), Pylkkö (1956), Krotov (1957), Pelper (1958), Lee (1962), Lee et al. (1963), Lee and Hodsden (1963), Halton and Jennings (1964), Schardein and Waitz (1965), Wilson (1965), Bueding et al. (1967), Frady and Knapp (1967), Graff and Read (1967), Hart (1967), Sanderson (1969), and Shield (1969). According to Florey (1966), cholinergic nervous systems are common to the animal kingdom; the sensory neurons of invertebrates (arthropods) are cholinergic, but not those of the vertebrates. According to Krotov (1957), acetylcholine and cholinesterase have been found in the tissues of all free-living invertebrates which have a nervous system and in numerous animals which lead a parasitic existence.

As shown in Graph 1 mouse brain homogenates and homogenates of Hymenolepis microstoma demonstrated maximal hydrolysis of acetylcholine chloride at 60 and 12 ul of CO_2/mg dry wt/15 min, respectively. The latter figure does not differ significantly from the figure of 7 ul of CO_2/mg dry wt/15 min for H. diminuta which was obtained by Graff and Read (1967). Pentastome tissues so treated failed to evolve CO_2 showing

they did not hydrolyze significant quantities of the substrate. A twofold increase in the concentration of the pentastome tissue from 8.2 mg/0.1 ml to 16.3 mg/0.1 ml did not change significantly the CO_2 emitted from that which was evolved by tubes incubated in buffer and substrate alone. When near equal quantities of mouse brain homogenate were utilized (16.3 mg/0.2 ml) the rate of substrate hydrolysis was high (514.0 ul/hr) in comparison to the tapeworm tissues. Increased carbon chain length of the choline ester did not alter the hydrolysis situation with respect to pentastome homogenates.

The results of spectrophotometric assays of the fractionated supernatant from pentastome homogenates (6.1 mg dry wt/0.2 ml) shown in Graph 2 indicates no hydrolysis of the 0.08 M acetylcholine chloride substrate, while mouse serum (16.6 mg dry wt/0.2 ml) effectuated significant hydrolysis.

Pentastome tissues analyzed using standard manometric and spectrophotometric enzyme assay techniques failed to give any indication of cholinesterase activity. When whole adult and nymphal P. crotali were emersed in 10^{-4} M eserine sulfate solutions (a cholinesterase inhibitor) a marked increase in activity was noticed after ten minutes emersion and was followed by complete tetanus after 15 minutes of emersion. Although cholinesterase activity was not detected by biochemical and histochemical assay techniques the behavior of intact living pentastomes in the inhibitor solution suggested the presence and functioning of cholinesterase in these parasites. It must be concluded that the specific activity of cholinesterases in P. crotali and P. clavatus nymphs though quite low may occur as indicated by the response to the cholinesterase inhibitor.

The esterase activity appears to be low and could not be detected by standard biochemical assay techniques or conventional histochemical methods.

The evaluation given below for the (nor-) adrenergic nature of pentastome nervous systems is not conclusive. It is apparent from the evidence gathered that chromaffin positive granules are most frequently visible in the synaptic junctions of the neuropile area of the supraesophageal ganglionic mass of K. pattoni adults and nymphs (Figures 8 and 9); in the axons of possible sensory nerves near the hypodermis; and in the motor end-plates and axons of nerves ending in striated muscles of P. crotali (Figure 2). Nerve cells in the subhypodermal area of adult P. clavatus, which resembled multipolar ganglion cells of the echinoderms (Barrington, 1967), contained granules in their axonal processes (Figure 4). The interpretation of this evidence is that catecholamine and indolalkylamine substances (=chromaffin granules) are present and probably act as transmitter substances. These results were not observed in the tissues of Raillietiella orientalis adults and Sambonia sp. nymphs.

The sensory knobs (van Hafner and Heymons, 1935) in the area of the anus and head of porocephalid pentastomes were not seen in this work after a diligent search through hundreds of stained sections. There is some question as to whether such structures exist in the porocephalids.

One note of interest is the obvious close association between nerve termini and the basal portion of some hypodermal cells. This anatomical relationship suggests a possible sensory nature for this layer of cells. The sensitivity of this layer to touch is easily demonstrated

when pentastomes respond to probing with undulations and by contraction, indicating the cuticle of pentastomes is thigmotactic.

The fluorescence of catecholamines and indolalkylamines when treated with concentrated formalin or paraformaldehyde fumes was first demonstrated by Erankö (1955); the techniques of fluorescence microscopy have been revised by Falck et al. (1962). Fluorescence was observed throughout the midgut of P. clavatus and P. crotali whole mounted nymphs, but significant fluorescence of the nervous tissue was not observed. The results obtained are explained by the fact that pentastomes feed on the host's blood and the red blood cells are known to be fluorescent. Fluorescent particles of less than 1 u diameter were observed on sections of P. crotali nymphs which were treated by the "F reaction" and viewed under oil emersion on a fluorescent microscope. Although fluorescent particles were observed they could not be associated with certainty to any part of the pentastome nervous system.

The fluorescence histochemical tests preclude any chromatographic separation of catecholamines or indolalkylamines from the parasites, i.e., fluorescent host material found in the gut and pseudocoelomic fluid of the parasite would have masked any fluorescent material of the parasite nervous system.

According to Florey (1966), catecholamines and indolalkylamines are suspected transmitter materials in the nervous systems of many animals. These compounds occur throughout the neuron and it is generally assumed that they accumulate in the nerve endings; they are presumed to be released from the nerve endings upon stimulation and apparently diffuse to the subsynaptic membranes ". . . producing specific, rapid, and

transient permeability changes in subsynaptic membranes." With respect to vertebrate nervous systems the (nor-) adrenergic nerve cell manufactures, stores, and releases the (nor-) adrenaline or 5-hydroxytryptamine to the subsynaptic membrane and it then causes the (nor-) adrenoceptive or "tryptaminoceptice" cells to respond to this released material. The same is true for (nor-) adrenaline or 5-hydroxytryptamine applied to the subsynaptic membrane.

These compounds have an effect on the behavior of pentastomes as indicated by their differential response to 10^{-3} M solutions of adrenaline, noradrenaline, 5-hydroxytryptamine, and dopamine. The first three chemicals have been suspected as transmitters in vertebrate and invertebrate nervous systems (Florey, 1966).

When adult and nymphal P. crotali were emersed in these solutions no change in activity of the parasites was observed in adrenaline and dopamine solutions. This indicates these substances have no such effect on the symaptic membranes as described above. When noradrenaline and 5-hydroxytryptamine were used, a definite increase in motor activity was observed which subsided shortly after the parasites were removed from these solutions to the saline. This indicates these materials have the ability to act as excitors of the pentastome nervous tissue and possibly function in the manner described by Florey (1966).

On the basis of the histochemical evidence and the pentastome's reaction to the catecholamine (noradrenaline) and indolalkylamine (5-hydroxytryptamine) one must conclude that such compounds play a significant role in the permeability of cell membranes and possibly function in the transmission of stimuli from the environment to the

ganglionic neuropile and from the ganglionic synaptic centers of the neuropile to the effector organs (e.g., striated muscles of the hooks).

Cholinesterase activity in the porocephalids could not be measured by the standard manometric and spectrophotometric methods employed, but it is apparently functioning as indicated by the inhibitor study. The nature of the mechanism of operation remains obscure, but the inhibitor study suggests that the cholinergic mechanism is operable, as demonstrated by the complete tetanus of the poisoned worms.

Pentastomes, like most parasitic helminths, are capable of only feeble movements, and motor neuron activity is limited. Pentastomes, like all other helminths, must have well developed sensory mechanisms which enable them to sample substrates and actively position themselves in the proper biotope. The paucity of cholinergic activity, and motor capacity of the porocephalids is in stark contrast with that of their suggested ancestors, the arthropods and annelids. The arthropods and annelids have active cholinesterases and high concentrations of acetylcholine in their nervous systems (Prosser and Brown, 1962), whereas the pentastomes, as shown by this work, have low cholinesterase activity. This condition indicates a supression of cholinesterase biosynthesis in the porocephalids suggesting a loss of genetic capacity as described by Fairbairn (1970).

The histochemical evidence presented here indicates that the pentastome nervous system has adapted to the parasitic mode of existence by relying upon the catecholamines and indolalkylamines as transmitter substances. The diminution of the "cholinergic" mechanism in favor of an "adrenergic" mechanism of nerve transmission in the porocephalids

shows a selective advantage of the latter over the former possibly suggesting an epigenetic adaptive process as described by Fairbairn (1970).

CHAPTER V

SUMMARY

The biological success of the pentastomida necessitates the existence of a sophisticated nervous system; the subject of inquiry in this work is the mechanistic operation of such a nervous system in pentastomes

Manometric and spectrophotometric enzyme analyses for acetylcholinesterase activity in Porocephalus crotali and Porocephalus clavatus nymphs yielded negative results, while emersion of adult and nymphal P. crotali in 10^{-4} M solutions of eserine sulfate (a cholinesterase inhibitor) resulted in complete tetanus of the worms. Thus porocephalids possibly have cholinesterase activity at a level not detectable by standard assay techniques. Cholinesterase histochemistry of porocephalids gave negative results.

Catecholamine and indolalkylamine histochemistry revealed positive granular deposits (chromaffin granules) in the nervous systems of P. crotali, P. clavatus, and Kiricephalus pattoni, but not in Raillietiella orientalis or Sambonia sp. Adults and nymphs of P. crotali were stimulated to greater activity by 10^{-3} M noradrenaline and 5-hydroxytryptamine but not by 10^{-3} M adrenaline or dopamine. Therefore, the role of noradrenaline and 5-hydroxytryptamine as transmitter substances in the porocephalid and kiricephalid nervous systems is

strongly suggested.

The Pentastomida would be expected to have mixed nervous systems with highly active cholinergic and adrenergic mechanisms of impulse transmission because of their theorized common ancestry with the Arthropoda and Annelida. Cholinesterases appear to be nonfunctional in the porocephalids studied, while the adrenergic nature of the porocephalid and kiricephalid nervous systems was demonstrated. The reduced cholinesterase activity is viewed as a loss of genetic capacity, possibly through suppression of genetic information, while the apparent dependency on catecholamines is viewed as an epigenetic adaptive phenomenon.

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