STUDIES ON THE NERVOUS SYSTEM AND NEUROENDOCRINE COMPONENTS IN SOME TREMATODE PARASITES

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I certify that the thesis entitled **"Studies on the Nervous System and Neuroendocrine Components In Some Trematode Parasites"** submitted by Mrs Neerja Mishra for the degree of Doctor of Philosophy in Zoology of the North-Eastern Hill University, Shillong embodies the record of original investigation carried out by her under my supervision. She has been duly registered and the thesis presented is worthy of being considered for the award of the Ph. D. degree. This work has not been submitted for any degree of any other university.

V Tandon

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CONTENTS

PREFACE

An organism has an environment in which it lives. The environment may be friendly, i.e., there may be plenty to eat, mate, reproduce and enough protection from enemies. The environment may be hostile also, i.e., it may not suit. There is an internal environment also in every organism. For a successful living there must be co-ordination between the organism and the environment or, to be more appropriate, between the external and internal environment of the organism. One of the basic facts of the various vital activities of the body is that the optimal function of cells and tissues is obtained through the maintenance of an optimal internal environment. The regulation and maintenance of the internal environment thus brought about is of two types: (1) the endocrine regulation that is based on hormones, a chemical substance formed by a specialized tissue or gland and carried in the blood stream to act as an excitant to some other tissue or organ; and (ii) the neurosensory regulation brought about by the nervous system made up of some specialized cells or neurons which send and receive the instructions through some special pathways. Thus both these systems (endocrine and nervous) constitute a chief regulatory mechanism that coordinates and integrates operation of other systems of the body and bring about adjustment to meet outside environmental changes. Because their functions are similar, it is not surprising that they are related. But the interdependence, closeness and intricacy of this relation have only come to be appreciated in recent years.

In the vertebrate and invertebrate groups, the best known neuroendocrine mechanisms are those controlling the pituitary secretion (Bargmann and Scharrer, 1951). Following Scharrer and Scharrer's (1963) hypothesis of the neurosecretory cells (NSC) as the functional endocrine system and their role in metabolism, growth and maturation in invertebrates (Gabe, 1966; Wigglesworth, 1967, 1970), attempts have been made to ascertain the application of these concepts in helminth parasitic group also. From within this group, nematodes have received the best attention, so far, in this direction (Davey and Kan, 1967; Finogenova, 1975; McLaren, 1972a,b) and the phenomenon of ecdysis in them is suggested to be correlated with the neurosecretory activity. NSC have also been detected in some cestode species (Davey and Breckenridge, 1967; Webb, 1976; Webb and Davey, 1976) and a regulatory role in muscle modulation has been suggested as a possible function of the NSC in this group (Specian **et al.**, 1979).

Since their demonstration in the cerebral ganglion of **Dicrocoelium lanceatum*** by Ude (1962), NSC have been detected in some other digenetic trematode species also (see Mehrotra and Bhutia, 1979). However, in most of these studies the NSC have not been mapped in relation to the various components of the nervous system and their functional roles are not correlated. With this objective in view, it is proposed to undertake studies in this direction.

The present work aims to study the nervous system in toto in some trematodes, commonly parasitizing the edible mammalian hosts like sheep, goats, cattle and pigs. Detailed localization of the putative NSC has been investigated using specific histochemical staining techniques and these observation have been supplemented by electron microscopy to confirm the secretory nature of these cells. To know the nature of the neurosecretory material in these parasites cytochemical studies have been performed for different metabolites. In order to know the possible role they play in the physiology of the worm an attempt has been made to study the NSC in different developmental phases of the adult worm, i.e., juvenile, immature and mature, in its final host to compare their activity.

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*The scientific names of the species typed herein in bold script are to be taken as in italics.

ABBREVIATIONS USED IN FIGURES

Ace	Acetabular nerve
ACE	Acetabulum
AD	Antero-dorsal
AIV	Antero-inner ventral
AM	Antero-marginal
AOV	Antero-outer ventral
AV	Antero-ventral
Br	Brain
Cg	Cerebral ganglia
CS	Cirrus sac
GenP	Genital papilla
GP	Genital pore
INT	Intestine
LC	Longitudinal connective
LYM	Lymph vessel
MG	Mehlis' gland
N	Nucleus
NC	Nerve cord
Nu	Nucleolus
OES	Oesophagus
ONR	Oral nerve ring
OV	Ovary
OVD	Oviduct
OVP	Opening of the ventral pouch
PD	Postero-dorsal
PH	Pharynx
Phn	Pharyngeal nerve
PIV	Postero-inner ventral
PL	Postero-lateral
PM	Postero-marginal
PMUS	Pars musculosa
PMV	Postero-Median ventral
POV	Postero-outer ventral
PV	Postero-ventral
ST	Subtegument
SV	Seminal vesicle
TC	Transverse connective
TES	Testes
UT	Uterine coil
VIT	Vitellaria
Vitd	Vitelline duct
VS	Ventral sucker

CHAPTER I

NERVOUS SYSTEM AND NATURE OF NERVOUS TISSUE

INTRODUCTION

Since early days of classical gross morphology of trematodes, their nervous system has received little attention. The work on the nervous system was initiated by some classical zoologists among whom the most notable are Wright and MacCallum (1887), Looss (1892; 1894; 1896), Bettendorf (1897) and Abraham (1929). Later, the system was further explored in detail with histological observations by Ulmer (1953) in **Postharmostomum helicis**; by Dixon and Mercer (1965) in **Fasciola hepatica**; by Jeong **et al.** (1978) in **Clonorchis sinensis**; and by Dei-cas **et al.** (1980) in **Schistosoma mansoni**. Among paramphistomes the few early accounts pertain to **Fischoederius elongatus** by Brandes (1898) and Lee (1971), **Gastrothylax crumenifer** by Otto (1896) and Brandes (1898), some unidentified amphistomes by Fukui (1929), **Fischoederius cobboldi** by Gupta and Dutta (1967); and **Paramphistomum calicophorum** and **Parorientodiscus magnus** by Lee (1971).

In recent years the association of esterases with the nervous system has been successfully exploited by many workers for the demonstration of nerve arrangement and other nervous components in several trematode parasites. Halton and Jennings (1964) were the pioneers in demonstrating the complete nerve arrangement in toto in a monogenetic trematode, **Diplozoon paradoxum**, based on esterase localization. Following their work there have appeared many reports pertaining to the complete nervous disposition in many trematode species. Venkatanarsaiah and Kulkarni (1980) studied the nervous system in some other monogenetic species, viz. **Vallisiopsis contorta, Alidiscocotyla chorinemi, Protomicrocotyla** spp., **Cyclobothrium insistii, Vallista chorinemi** and **Heteromicrocotyla carangis**. Among the larval monogenea the oncomiracidium of **Pricea multae** was investigated by Venkatanarsaiah (1981). Ramulu **et al.** (1981) studied the nerve arrangement in an aspidobothrian, **Lissemysia indica.** Notable work on this aspect in digenea pertains to **Singhiatrema longifurca** and **Paradistomoides orientalis** by Simha and Rao (1977); **Prosthodendrium** spp. by Ramulu and Rao (1979); **Gangesia** sp. by Krishna and Simha (1980); **Ceylonocotyle scoliocoelium** by Roy (1980); **Schistosoma spindalis** by Rao **et al.** (1982); **Echinostoma revolutum** by Krishna (1981); **Isoparorchis hypselobagri** by Simha and Fernandez (1982); **Singhiatrema najai** by Fernandez **et al.** (1982); **Euparadistomum herpestesi** by Kishore **et al.** (1982); **Ganeo tigrinum** by Anitha **et al.** (1983); **Gastrothylax crumenifer** by Choubisa **et al.** (1982) and **Echinochasmus mordax** by Simha and Bhandarkar (1984).

Rohde (1968a) described the nervous system in **Diaschistorchis multitesticularis** and also in the aspidobothrian, **Multicotyle purvisi** by using the silver nitrate technique of Gurr (1956). Shipokova (1982, 1984) demonstrated the nervous system in **F. hepatica** and **Dicrocoelium lanceatum** with the help of methylene blue technique. Kotikova **et al.** (1984) studied the nervous system of **Prosorhynchus squamatus.**

Besides the above-mentioned, the nervous system in toto has also been traced in the various larval forms of several trematode species : embryonated eggs of **S. mansoni** by Pepler (1958); sporocysts of **F. hepatica** by Wilson (1970); larvae of **S. mansoni** by Lewert and Hopkins (1965) and Bruckner and Voge (1974); **Catatropis indica** and **Diaschistorchis multitesticularis** by Rohde (1968c); **Plagiorchis elegans** by LeFlore (1979); **Cloacetrema michiganensis** by LeFlore **et al.** (1980); **Haplometra cylindracea** by Grabda-Kazubska and Moczon (1981); and **Diplostomum pseudospathaceum** by Niewiadomska and Moczon (1982); and the metacercariae of **Tetracotyle lymnaei** by Choubisa and Sharma (1983), and **Diplostomum pseudospathaceum** by Niewiadomska and Moczon (1984). The neuroanatomy of the redia of **Cercaria buckleyi** and also of several cercarial species was also described by Choubisa (1986).

Very little is known about the histocytology of nervous system in trematodes. Relatively few studies deal with monogenea. In the latter group the absence of nerve sheath around the nerves has been reported in several species (Halton and Jennings, 1964; Halton and Morris, 1969; Rohde, 1968b, 1972a). However, a nerve sheath has been demonstrated, though only around portions of the posterior ventral nerve cord, in the aspidobothrian, **Multicotyle purvisi** (Rohde, 1970, 1971, 1972b). The presence of a myelin sheath around the main nerves in the digenetic fluke, **Clonorchis sinensis**, has been reported by Jeong **et al**. (1978), whereas in several other digenetic species the nerves are unmyelinated (Dixon and Mercer, 1965; Lee, 1971; Mandawat and Sharma, 1978; Dei-cas **et al**., 1980). The neuroplasm of nerve fibres comprises dense-core granules (Dei-cas **et al**., 1980).

Reports on histochemical nature of the nervous tissue deal with the detection of specific esterase types. Non specific esterase (NSE) and acetylcholinesterase (AChE) have been reported by Probert and Durrani (1977) in **Fasciola gigantica** and **F. hepatica** and in many other digenetic species (Ramulu and Rao, 1979; LeFlore, 1979; LeFlore **et al**., 1980; Krishna and Simha, 1980; Krishna, 1981; Fernandez **et al**., 1982). Both AChE and butylcholinesterase (BChE) have been reported in the nervous system of **F. hepatica** by Krvavica, Lui and Becejac (1967). Sood (1977) demonstrated a strong BChE activity in the synapses of nerve fibres and nerve cells of **Glossimetra orientalis**. No NSE activity was reported in the nervous system of **C. scoliocoelium** and **Pharyngostomoides adenocephala** by Roy (1980) and Allen and Harkema (1972), respectively. These authors evidenced AChE activity in the nervous system.

MATERIAL AND METHODS

The material pertaining to the investigation reported in the present work was collected from the freshly killed and autopsied host animals (Table-1.1) from the local abattoirs

TABLE 1.1 Trematode species investigated for the nervous system

S.No.	Name of the parasite	Family : Subfamily	Host	Location
1	**Fischoederius elongatus** (Poirier, 1883) stiles et Goldberger, 1910	Paramphistomidae:Gastrothylacinae	**Bos indicus** L. **Ovis aries** L. **Capra hircus** L.	Rumen " "
2	**F. cobboldi** (Poirier, 1883) Stiles et Goldberger, 1910	"	**B.indicus**	"
3	**F. brevisaccus** Eduardo,1981	"	**B.indicus**	"
4	**Gastrothylax crumenifer** (Creplin, 1847) Poirier, 1883	"	**B.indicus** **O.aries** **C.hircus**	"
5	**Olveria indica** Thaper et Sinha, 1945	Paramphistomidae:Cladorchiinae	**B.indicus**	"
6	**Cotylophoron cotylophorum** (Fischoeder, 1901) Stiles et Goldberger, 1910	Paramphistomidae:Paramphistominae	**B.indicus**	"
7	**Paramphistomum epiclitum** Fischoeder 1904	"	**B.indicus** **O.aries,** **C.hircus**	"
8	**P. gracile** Fischoeder, 1901	"	**B.indicus** **O.aries** **C.hircus**	"
9	**Orthocoelium orthocoelium** (Fischoeder, 1901) Price et McIntosh, 1953	"	**B.indicus**	"
10	**O. streptocoelium** (Fischoeder, 1901) Yamaguti 1971	"	**B.indicus**	"
11	**O. narayanai** (Gupta et Gupta, 1972) Eduardo,1980	"	**B.indicus**	"
12	**Explanatum explanatum** (Creplin, 1847) Fukui,1929	"	**B.indicus**	Bile duct
13	**Homalogaster paloniae** Poirier, 1883	Paramphistomidae:Gastrodiscinae	**B.indicus**	Intestine
14	**Eurytrema pancreaticum** (Janson, 1907)Looss, 1907	Dicrocoeliidae:Dicrocoeliinae	**B.indicus**	Bile duct
15	**Fasciola gigantica** Cobbold, 1855	Fasciolidae:Fasciolinae	**B.indicus** **O.aries** **C.hircus**	Liver

of Shillong, these animals mostly come from the plains of the neighbouring Assam state. The forms studied include thirteen paramphistomes representing four subfamilies of Paramphistomidae, one dicrocoeliid and one fasciolid, the common Indian liver fluke.

Nervous system in toto preparation

Live specimens were collected in 0.9% physiological saline and were immediately flattened and fixed in 10% neutral formalin buffered at pH 7 for 6 to 8 hrs. The technique employed for the demonstration of nervous system was basically that of Holt and Withers (1952) with slight modification. The specimens were washed in several changes of distilled water at 10°C and incubated at room temperature (10-18°C) for 12-14 hr in the standard bromoindoxyl acetate incubation medium (O-acetyl-5-bromoindoxyl - 1.3mg, dissolved in 0.1ml ethanol and mixed with 2.0ml of 0.1M Tris, 1.0ml of 0.05M potassium ferricyanide, 1.0ml of 0.05M potassium ferrocyanide, 1.0ml of 0.1M calcium chloride, and final volume made to 10ml with distilled water) at a pH between 5.8 to 7.4. Following incubation the specimens were washed in distilled water and dehydrated in ascending grades of ethyl alcohol, cleared in methyl benzoate and mounted in Canada balsam. The nervous system stains deep indigo blue and its components are clearly visible in intact worm.

Camera lucida drawings of the nervous system were made using Wild M5APO stereoscopic and Leitz Ortholux-2 research microscopes. For clarity sake, in some cases the thickness of nerves has not been drawn to scale. To give a complete picture of the nerve disposition in the worms, diagrams have been drawn with slight reconstruction using more than one specimen. The photomicrographs were taken using Wild M5APO and Leitz Ortholux-2 research microscopes.

Histocytological and histochemical studies

The histocytology of the system was based on series of sagittal sections of the specimens. Cajal's pyridine silver method (modified by Davenport **et al.**, 1934) was used to reveal the nervous nature of these tissues. The material was fixed in absolute ethyl alcohol and concentrated ammonia in a ratio of 98:2 for 1-4 days. The fixed material was treated with 5% aqueous pyridine for 24 hr followed by washing in distilled water for 5-6 hr, then impregnated in 1.5-2.0% aqueous silver nitrate solution at 37°C for 2-4 days, washed again with distilled water for 1 hr, giving a fresh change at every 10 min, and finally reduced in 4% pyrogallol solution for 4 hr. After dehydration through ascending grades of alcohol this material was processed for paraffin sectioning. 8-10u thick sections, after deparaffinization and clearing in xylene and mounting in Canada balsam, revealed the nerve cells to be yellow to brown and non-myelinated nerve fibres, black.

To demonstrate the presence of myelin sheath around the longitudinal nerves and brain, Ora's method (1958) was used after fixation of the material in 10% neutral formalin. The slides containing 12-15 u thick paraffin sections were treated with absolute alcohol for 2 min and then celloidin coated by dipping them in celloidin solution (2.0 ml of gum mastic saturated in absolute alcohol, 7 ml of 18% celloidin, 45.0 ml of anhydrous ether, 45.0 ml of absolute alcohol and 10ml of acetone). These were treated at 60°C in a mordant solution (25.0 gm potassium dichromate and 1.0 gm sodium sulphate dissolved in distilled water to make 100 ml, to this solution was added 1.0 ml of 5% aqueous ferric alum) for 30 min, and then stained for 30 min in haematoxylin solution (1.0 gm haematoxylin dissolved in 10.0 ml absolute ethyl alcohol, and added to 90.0 ml distilled water and 4.0 ml aqueous, saturated lithium carbonate) pre-heated at 60°C, washed in running water till the sections turned blue, differentiated in 0.5% aqueous potassium permanganate solution for 30 sec, washed in running water, dehydrated, cleared and mounted. Myelin if present is revealed as black coloured in a contrast yellow background.

To study the histochemical nature of nervous tissue, the live worms were processed for Gerebtzoff's method* following Bancroft (1975) for demonstration of acetyl and butylcholinesterase enzymes, using acetyl and butylthiocholine iodide, respectively. The controls consisted of incubating the worms in media which contained 10^{-3}M eserine (Jennings and LeFlore, 1972).

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*Procedural details given in Appendix.

OBSERVATIONS

A. Nervous system

Following the intense localization of the NSE, it was possible to trace the details of the nervous system in the parasites.

A pair of small thickenings, the cerebral ganglia, connected to each other by a transverse commissure, constitute the brain mass in all the species investigated, but their position varies in some of the species.

The two cerebral ganglia lie immediately posterior to the muscular pharynx and dorsal to the oesophagus in the paramphistomes. From each cerebral ganglion anteriorly and posteriorly directed longitudinal nerves are given out and they are named according to their position.

Fischoederius cobboldi

From each cerebral ganglion three anteriorly and three posteriorly directed longitudinal nerves are given out (Pl. 1.1, Figs. 1,2; Pl. 1.2; Fig.1).

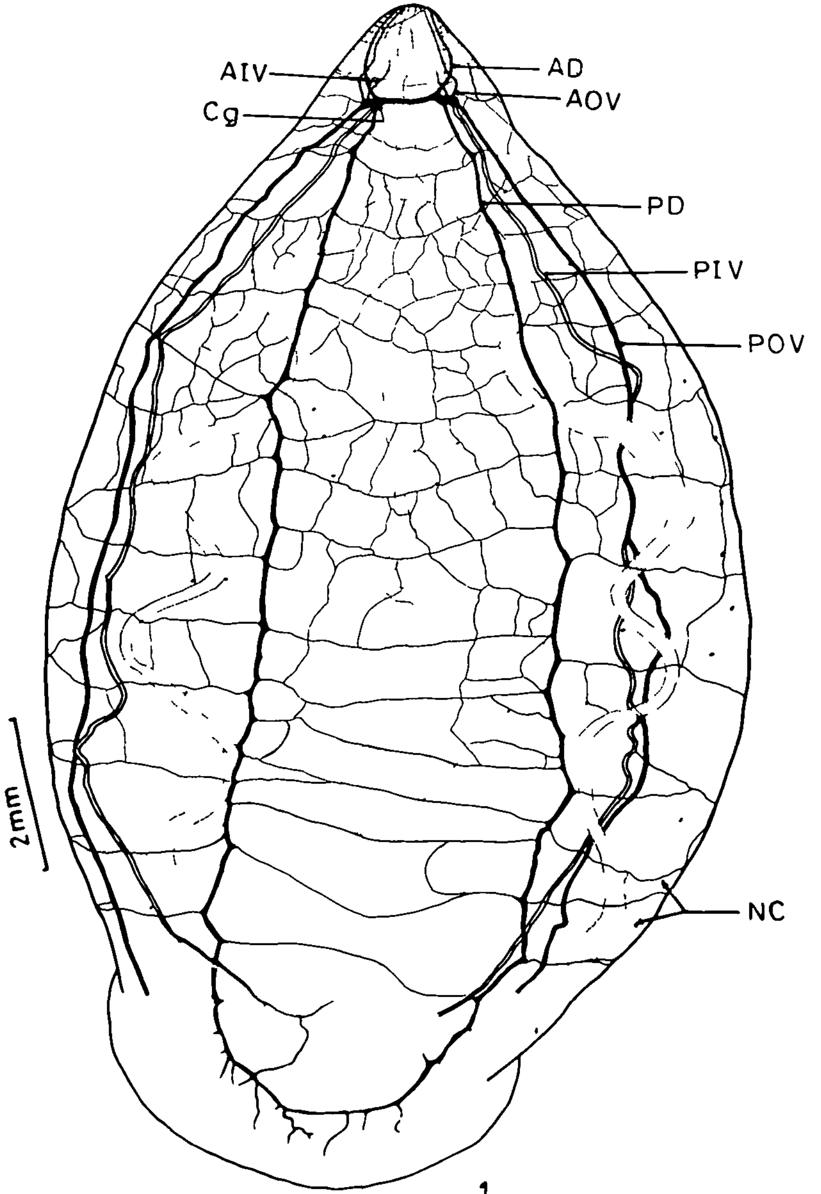
Of the nerves traversing cephalad, one pair is of the antero-dorsal nerves, each of which arises from the inner border of the cerebral ganglion but soon comes to lie close to the lateral margins of the body, innervating the oral rim. The other two pairs of nerves are ventral in position. Each antero-outer ventral nerve arises from the outer border of the cerebral ganglion; it proceeds inner

PLATE 1.1: Fischoederius cobboldi

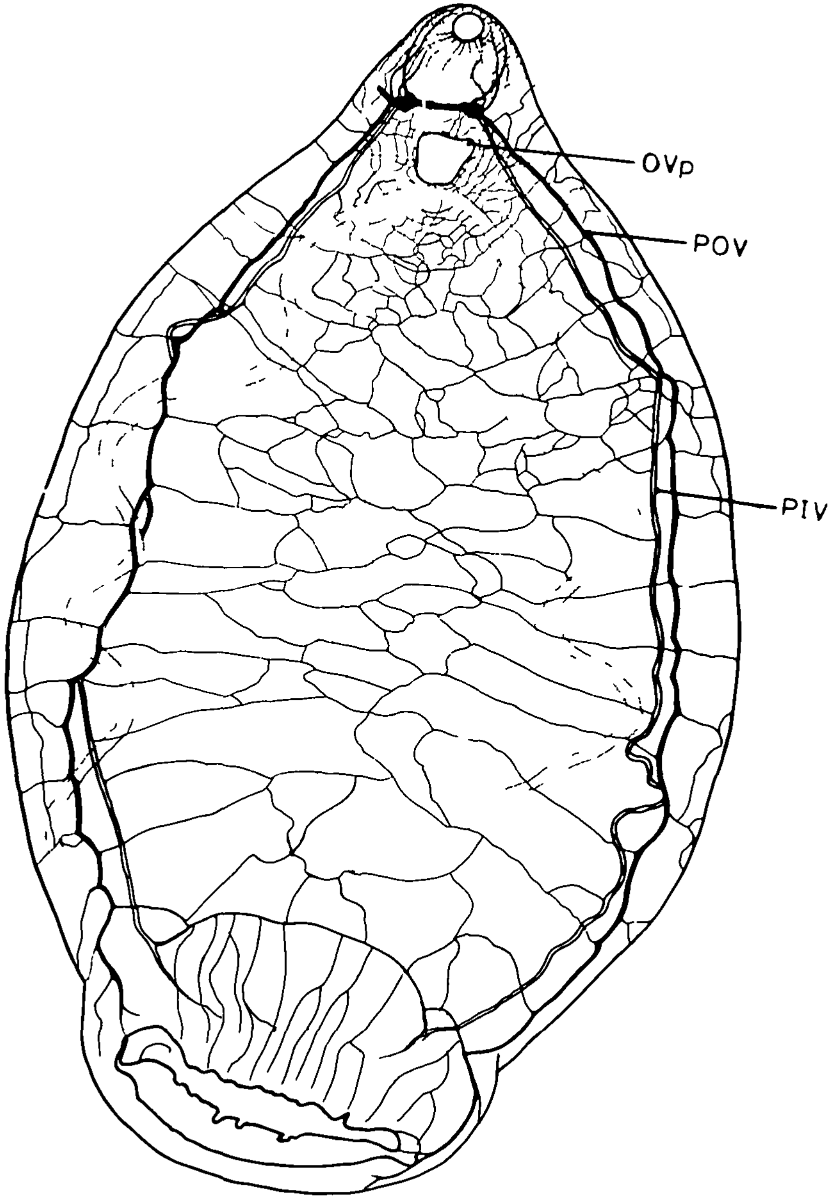
Fig. 1,2 Diagrammatic representation of the nervous system in the whole mount of the worm (Camera lucida).

1. Dorsal view, showing the main nerves and the nerve net of postero-dorsals in the post cerebral region.
2. Ventral view, showing the postero-ventral nerves and their nerve net.

PLATE 1·1



1



2

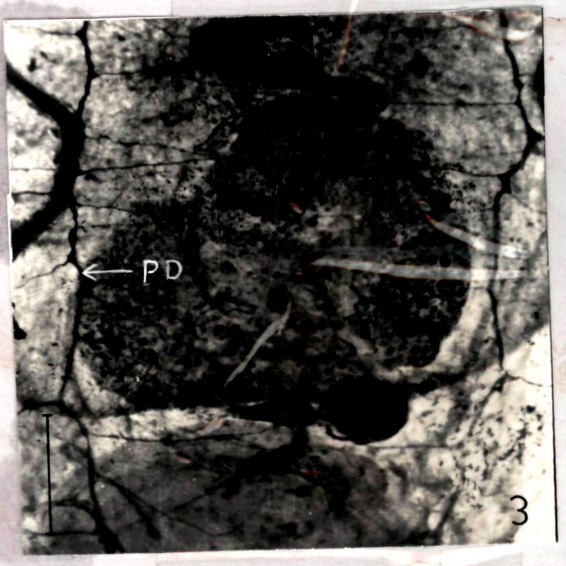
to the antero-dorsal nerve and immediately close to the pharynx, innervating the side walls and rim of the latter. The antero-inner ventral nerves originate between the antero-dorsal and antero-outer ventral nerves. They extend only upto the middle length of the pharynx, giving out many branches to supply its walls and lower half. All these are connected to one another by transverse connectives encircling the pharynx. These rings get closer and denser towards the oral opening and together with numerous longitudinal connectives and their branches constitute a fine basket-like nerve net in the oral region (Pl.1.2, Figs. 2a-b). Fine branches from the transverse connectives also innervate the tegument in this area.

From amongst the three pairs of the posterior longitudinal nerves, the innermost (nearer the median axis of the body) comprises the postero-dorsal nerves (Pl.1.1, Fig.1; Pl. 1.2, Figs. 1,3). These, on their way towards the acetabulum, innervate the gut, the reproductive organs and the excretory bladder, also giving off branches to the tegument. In the region of the acetabulum the two postero-dorsal nerves of either side join with each other and innervate the middle portion and posterior edges of the sucker (Pl.1.3; Fig.1). The postero-outer ventral nerves (Pl. 1.1, Fig.2; Pl. 1.2, Fig.1), running somewhat parallel to the lateral body margins, give off branches to the reproductive organs and paranchyma. Just a little ahead of the acetabulum each postero-outer ventral nerve bifurcates into two (Pl.1.3, Fig.2), of which

PLATE 1.2: Fischoederius cobboldi (Photomicrographs)

Fig.1	Brain mass and the main anterior and posterior nerves (Scale bar = 1.0 mm).
Fig.2a	Nerve net in the oral region, in top view (Scale bar = 0.4 mm).
Fig.2b	A portion of same under high resolution. The ramification of the nerves between the transverse connectives is evident (Scale bar = 0.15 mm).
Fig.3	Postero-dorsal nerve and its network in the posterior half of the body (Scale bar = 1.0 mm).

PLATE 1.2



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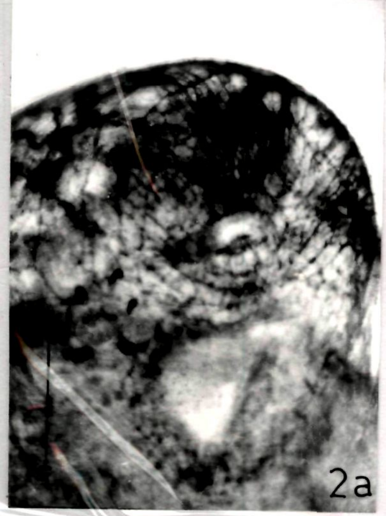

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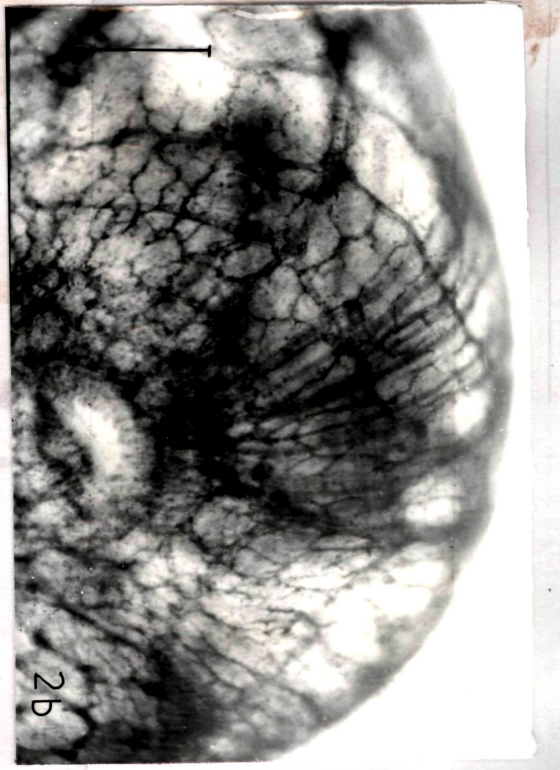


2b

the inner branch meets its fellow of the other side making a prominent transverse connective. From the latter, numerous fine branches proceed downward, ramify and supply the rim of the sucker (Pl.1.3, Fig.3). The outer branch of the postero-outer ventral nerves runs lateral to the acetabulum and terminates, giving off branches to its lateral walls. The postero-inner ventral nerves, running between the dorsal and outer ventral nerves, in each longitudinal half of the body, supply the tegument, parenchyma, reproductive organs and also the excretory bladder. Before terminating in the floor of the acetabulum, this nerve joins the transverse connective of the postero-outer ventral nerves through a side branch. A dense nerve net that is contributed by the branches of the postero-outer and inner ventral nerves encircles the opening of the ventral pouch within which also terminate the genital ducts (Pl.1.3, Fig.4). The ventral pouch is innervated by the branches from all the posterior nerves. Each postero-dorsal nerve has transverse connections with the postero-outer ventral nerve of its side. Minute branches from these connectives are observed terminating in the tegument. The postero-outer ventral nerve is, likewise, connected to the postero-inner ventral nerve. All the three posterior longitudinal nerves are joined to their counterparts by means of numerous, thin ring-like transverse connectives which in turn are connected to one another by still thinner longitudinal connectives. Thus a fine nerve net is formed on both the dorsal and the ventral sides of the worm (Pl.1.3, Fig.5,6).

PLATE 1.3: Fischoederius cobboldi (Photomicrographs)

Fig.1 Postero-dorsal nerves joining in the acetabular region.

Fig.2 Postero- outer ventral nerves bifurcating in the acetabular region.

Fig.3 Transverse connective (arrow) formed by the postero-ventral nerves just before the acetabulum. Fine branches from it are seen supplying the latter.

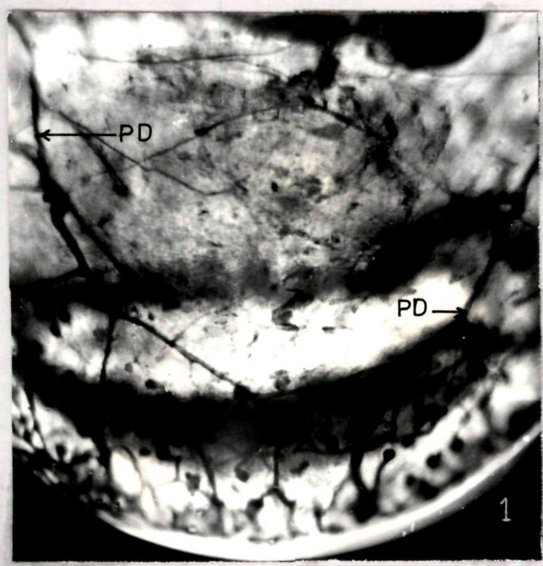
Fig.4 The dense nerve net around the opening of the ventral pouch.

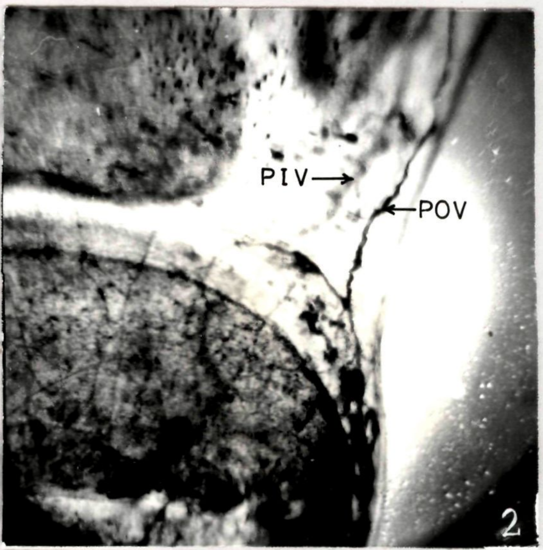
Fig.5 Nerve net of the postero-dorsal nerve in the mid body region. Note the associated nerve cells (arrows).

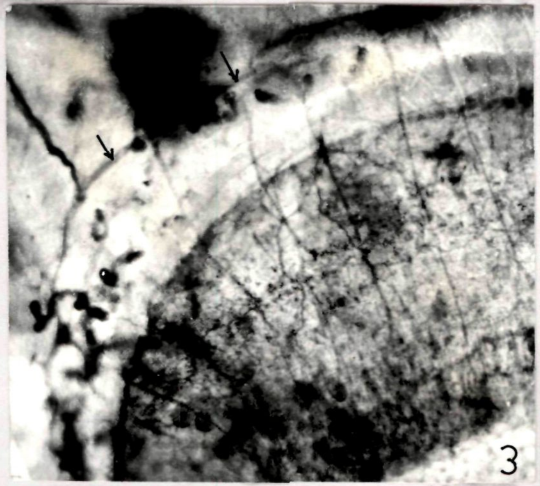
Fig.6 Postero-ventral nerves and their nerve net in the anterior half of the body.

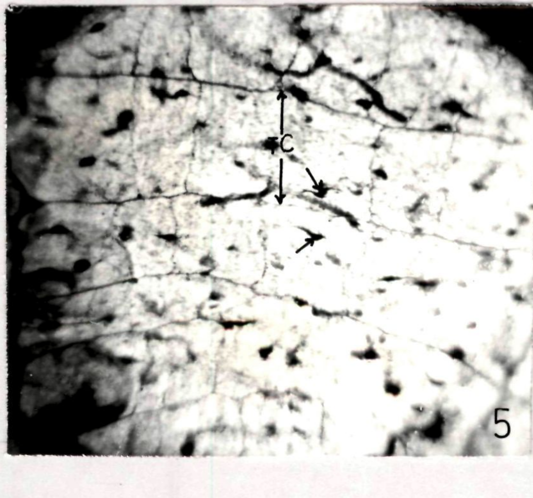
(Figs.1-6, Scale bar = 0.05 mm).

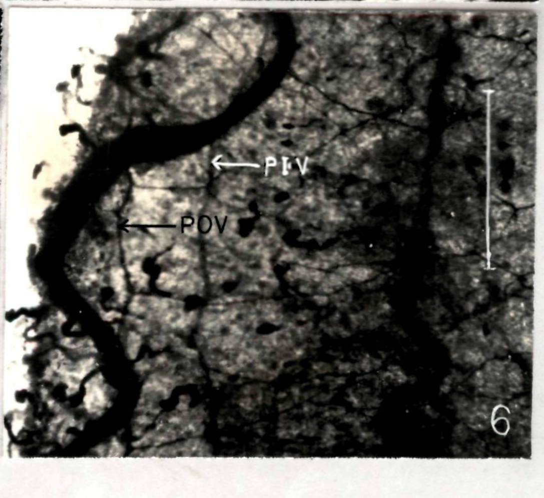
PLATE 1.3











Fischoederius elongatus

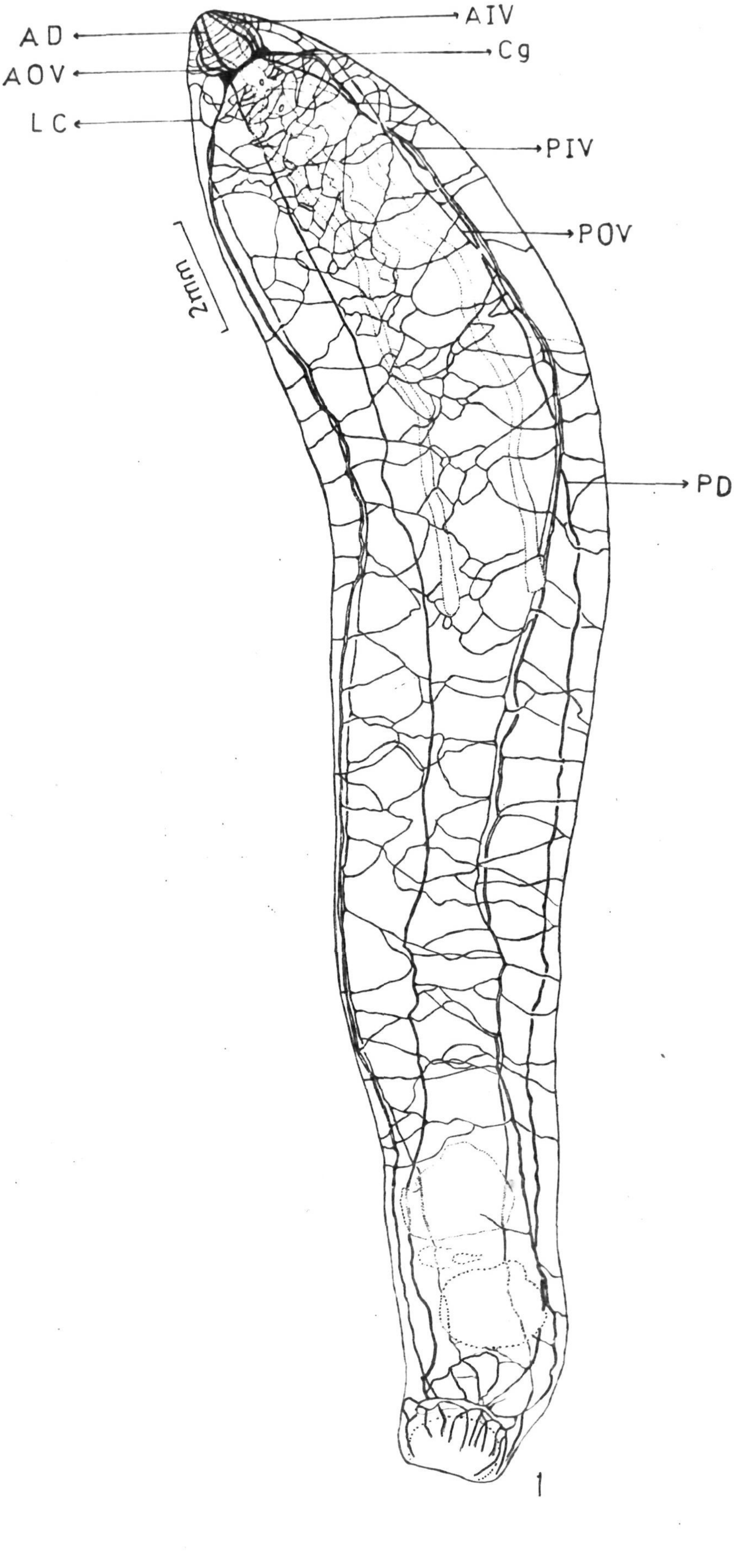
Three pairs of nerves run cephalad and three pairs of nerves proceed caudad from the cerebral ganglia (Pl.1.4, Fig.1; Pl.1.5, Fig.1). The antero-dorsal nerves which are the innermost, i.e., nearer to the median axis, arise from the inner border of the cerebral ganglia and travel dorsally upto the tip of the pharynx, supplying the oral tip and the dorsal surface of the pharynx and tegument. The lateral walls of the pharynx are supplied by the antero-inner ventral nerves that arise from the antero-median facet of the ganglia. The antero-outer ventral nerves, arising from the outer lateral side of the cerebral ganglia, innervate the whole ventral surface of the pharynx. Thin transverse connectives, completely encircling the pharynx, join the anterior nerves with one another (Pl.1.5, Figs. 1,2). Very fine branches from these are observed petering out into the circumpharyngeal tegument.

The postcephalic longitudinal nerves comprise a pair of postero-dorsals and two pairs of postero-ventrals (Pl.1.4, Fig.1; Pl. 1.5, Figs.1,2). The postero-dorsals originate from the inner facet of the cerebral ganglia and on their way to the acetabulum fine branches of these nerves innervate the gut, reproductive system and excretory bladder. The postero-dorsals of the two sides are joined with each other by thin transverse connectives throughout their course; the connectives, in turn, are connected to one another by

PLATE 1.4: Fischoederius elongatus

Fig.1 Camera lucida sketch of the nervous system in the whole mount of the worm (ventral view).

PLATE 1·4



still thinner, two to three longitudinal connectives, thus forming a complete nerve net under the dorsal surface of the body (Pl.1.4, Fig.1; Pl.1.5, Figs. 1,4). The postero-dorsals are superficially placed nerves.

Of the postero-ventral nerves, the postero-inner ventral nerves arise from the postero-ventral border of the cephalic ganglia (Pl.1.4, Fig.1; Pl.1.5, Fig.1). They travel posteriad up to the region of the acetabulum where they terminate. The postero-outer ventral nerves arise from the lateral aspects of the brain. Initially these nerves run lateral to the inner ventrals for some distance but thereafter are seen changing their course and running inner to the latter nerves at places. A thin, longitudinally-running lateral connective is observed joining the antero and the postero-outer ventral nerves of the same side. Along most of their length the two postero-ventral nerves (i.e., outer and inner) of either side are joined with each other by many rather conspicuous but thin, laterally placed loop-like connectives which appear as 'C' - or titled 'V' - shaped with the apex of 'V' directed towards the median line. Fine branches from these loops converge towards the mid-ventral field of the body and, joining with their counterparts of the other side, constitute a dense nerve net on the ventral side (Pl.1.5, Figs.1,3). Minute branches from this nerve net innervate the tegument and wall of the ventral pouch. The tributaries of postero-ventrals also innervate the reproductive organs, vitellaria and the opening of the ventral

PLATE 1.5: Fischoederius elongatus (Photomicrographs)

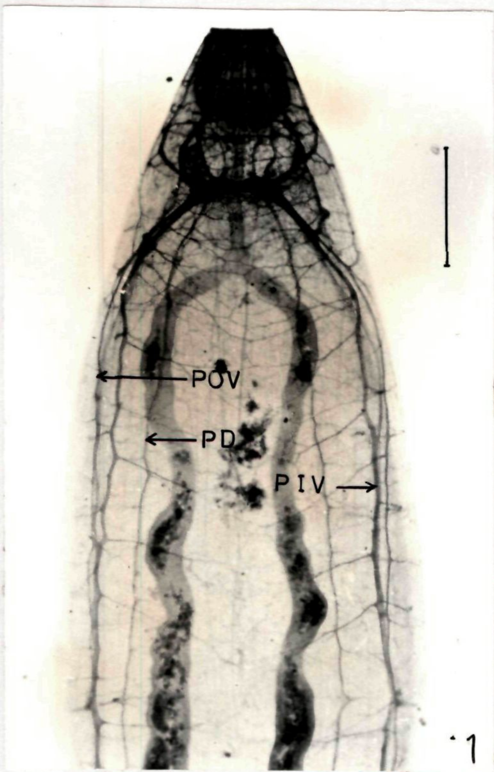
Fig.1 Brain mass and the main anterior and posterior nerves (Scale bar = 1.0 mm).

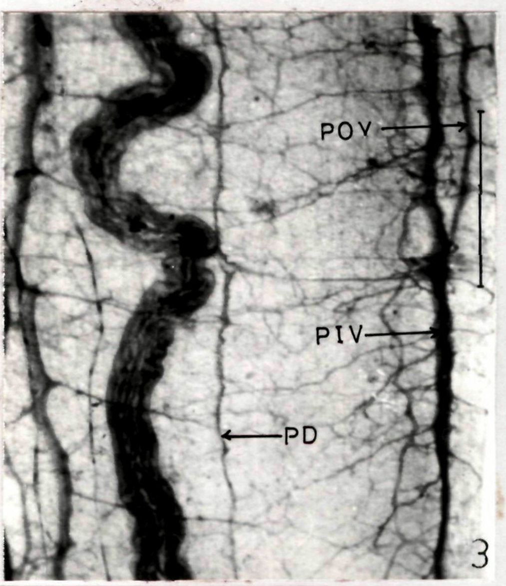
Fig.2 Anterior region magnified to show ring-like transverse connectives of anterior nerves, encircling the pharynx. (Scale bar = 0.4mm).

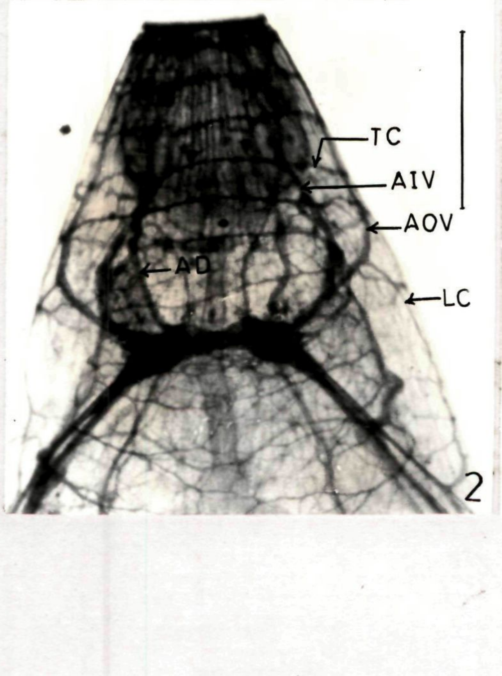
Fig.3 Posterior nerves and their nerve net in the mid body region (Ventral view) (Scale bar = 0.4 mm).

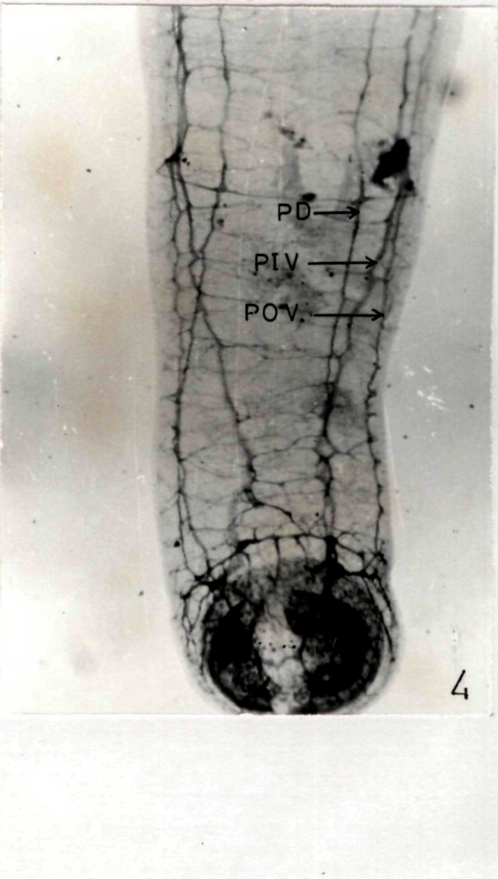
Fig.4 Terminal course of the posterior nerves and innervation of the acetbulum (Scale bar = 1.0 mm).

PLATE 1.5









pouch. The postero-dorsals are connected only to the postero-outer ventrals and not to the inner ventrals by means of lateral connectives. While the postero-inner ventrals are deep seated in the parenchyma, the postero-outer ventrals like the postero-dorsals, are superficially lodged.

All the posterior longitudinal nerves join with one another and form a conspicuous ring-like nerve just in front of the anterior border of the acetabulum. Gradually tapering branches given out from this nerve and further secondary fine branches of these innervate the sucker (Pl.1.4, Fig.1; Pl.1.5, Fig.4).

Fischoederius brevisaccus

Three pairs of nerves run cephalad and three pairs, posteriad from the cerebral ganglia (Pl.1.6, Figs.1,2).

Of the anterior nerves, the antero-dorsals arise at the dorso-inner facet of the cerebral ganglia and, proceeding dorsal to the pharynx, terminate in the oral nerve ring. The antero-inner ventrals originate from the latero-ventral facet of the cerebral ganglia and run anteriorly taking a course parallel to the lateral wall of the pharynx and finally terminate at the oral tip. The antero-outer ventral nerves originate from the lateral facet of the ganglia and run along the lateral body wall. A nerve basket encircling the pharynx and contributed by circumpharyngeal transverse connectives associating all the anterior nerves is not conspicuous. Very few and randomly distributed longitudinal

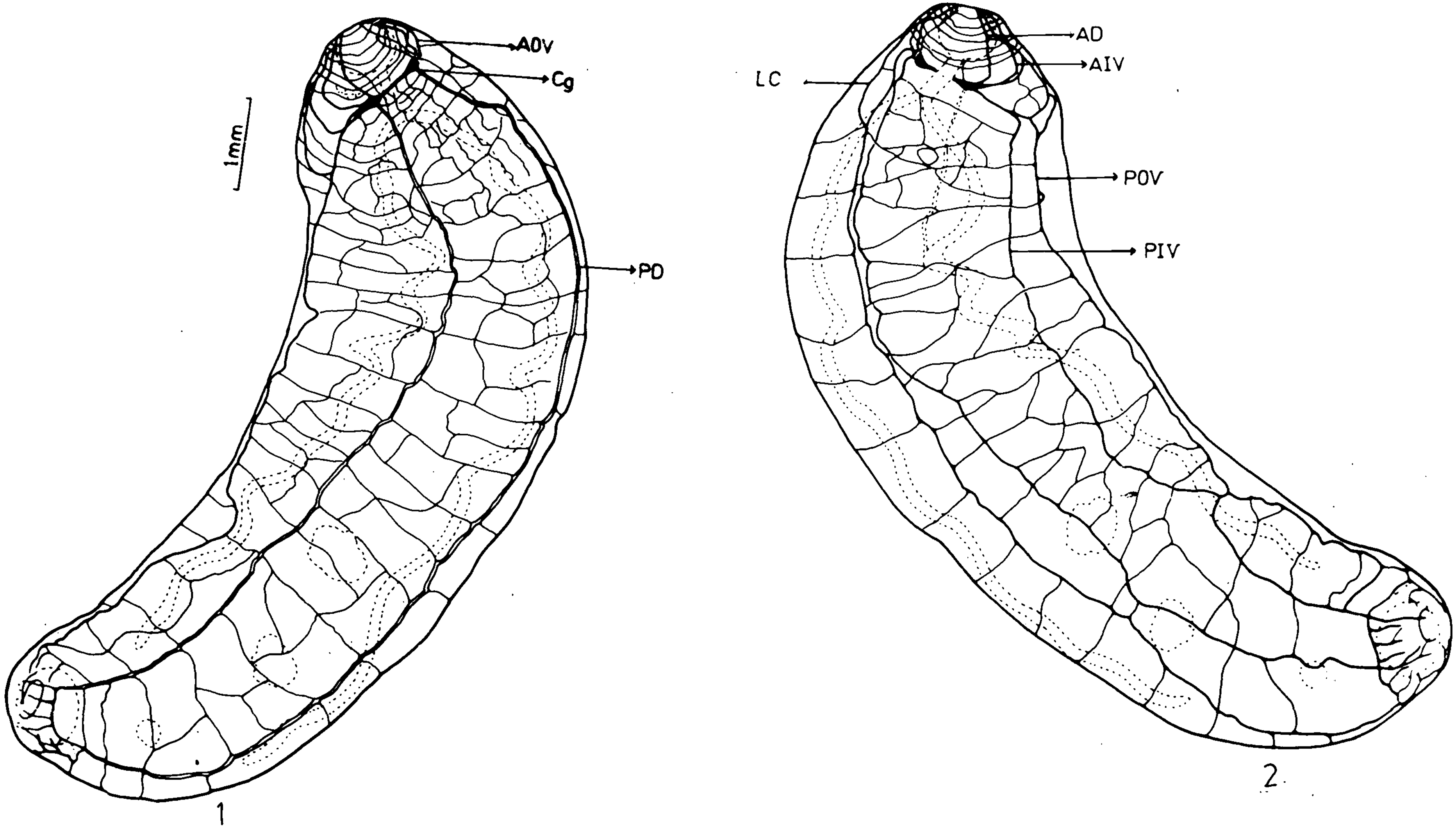
PLATE 1.6: Fischoederius brevisaccus

Figs1,2 Diagramatic representation of the nervous system in the whole mount of the worm (Camera lucida).

1. Dorsal view; only the postero-dorsals with their nerve net are depicted in the post-cerebral region.

2. Ventral view; showing in the post-cerebral region [illegible] the postero-ventral nerves and their nerve net.

PLATE 1·6



connectives interconnect all these transverse connectives. Fine branches from the latter terminate in the tegument of the anterior region and the pharynx (Pl. 1.6, Figs.1,2; Pl.1.7, Fig.1).

The postero-dorsal nerves emerge from the postero-inner facet of the cerebral ganglia and extend up to the acetabulum (Pl. 1.6, Fig.1; Pl.1.7, Figs.1-3). They innervate the reproductive system, alimentary canal and excretory bladder. Conspicuous transverse connectives and a fine net work of nerves in between them constitute a dense dorsal nerve net (Pl.1.6, Fig.1; Pl.1.7, Fig.2). The transverse connectives are more dense in the anterior region of the body and they become lesser towards the posterior end. The two postero-dorsals innervate the acetabulum, dorsally joining with each other through a transverse connective in the mid-acetabular region (Pl.1.7, Fig.3). The branches from the latter supply the middle, lateral and lower edges of the suckers. The postero-inner ventrals emerge from the postero-ventral facet of the cerebral ganglia and turn caudad. They also supply the alimentary canal, reproductive organs and also the vitellaria. The postero-outer ventrals originate from the latero-ventral facet of the cerebral ganglia and extend up to the acetabulum, supplying the excretory system, alimentary canal and vitellaria (Pl. 1.6, Fig.2; Pl.1.7, Fig.3). The two ventrals of each longitudinal half of the body closely approximate each other near the testicular region of the body but soon separate out and run further

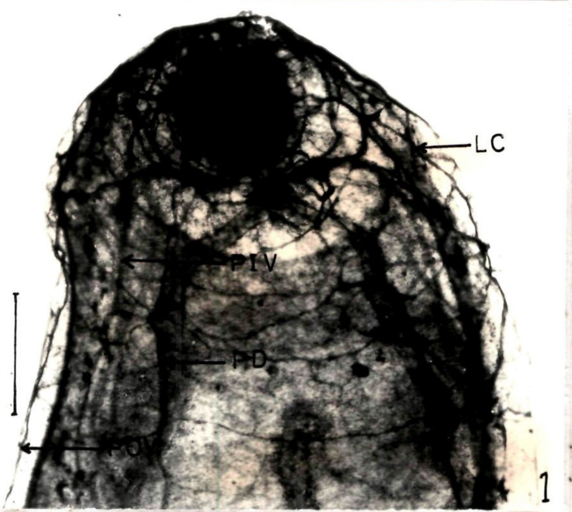
PLATE 1.7: Fischoederius brevisaccus (Photomicrographs)

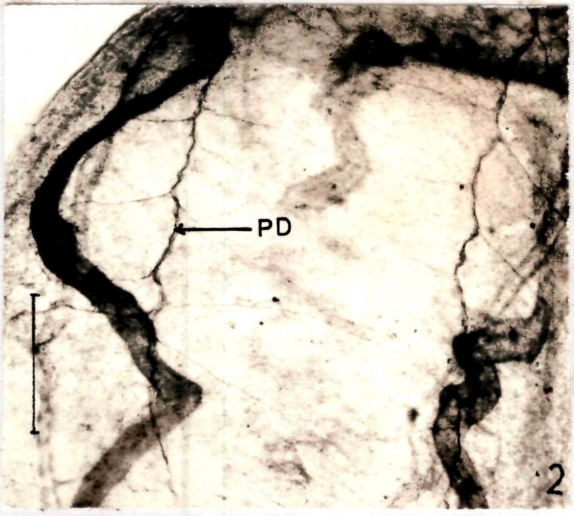
Fig.1 Brain mass and the main anterior and posterior nerves (Scale bar = 0.5 mm).

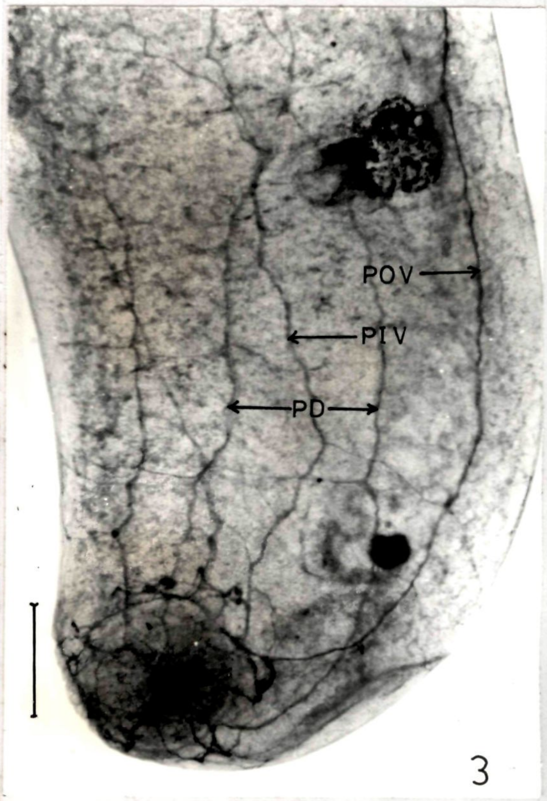
Fig.2 The postero-dorsals and their nerve net in the mid body region (Scale bar = 0.05 mm).

Fig.3 Terminal course of the posterior nerves and innervation in the acetabulum (Scale bar = 1.0 mm).

PLATE 1.7







caudad. During their course towards the hind end both the postero-ventrals send branches towards the median axis from their inner facets, which ramify and anastomose with one another and with their counterparts of the other side, thus constituting a nerve net on the ventral surface as well (Pl.1.6, Fig.2; Pl.1.7, Fig.1). Minute branches emerge from the body nerve net and innervate the whole of the tegument.

The genital pore region is supplied by the tributaries of the nerve net of the ventral surface (Pl.1.7, Fig.1).

A longitudinal connective interconnects the postero- and the antero-outer ventrals of each longitudinal half (Pl. 1.6, Figs.1,2; Pl.1.7, Fig.1).

The postero-inner ventrals are deep seated in the parenchyma, while the postero-outer ventrals and dorsals are superficially lodged.

Gastrothylax crumenifer

The neuroanatomy of **G. crumenifer** follows the same pattern as that of **F. elongatus**. The number, origin and position of the anterior and posterior nerves are the same as described for the latter species (Pl.1.8, Figs.1,2). All the anterior nerves of both the sides are joined with one another by means of few thin connectives encircling the pharynx. However, a conspicuous circumpharyngeal basket is not formed (Pl.1.8, Figs.1,2; Pl.1.9, Fig.1; Pl.1.10,Fig.1).

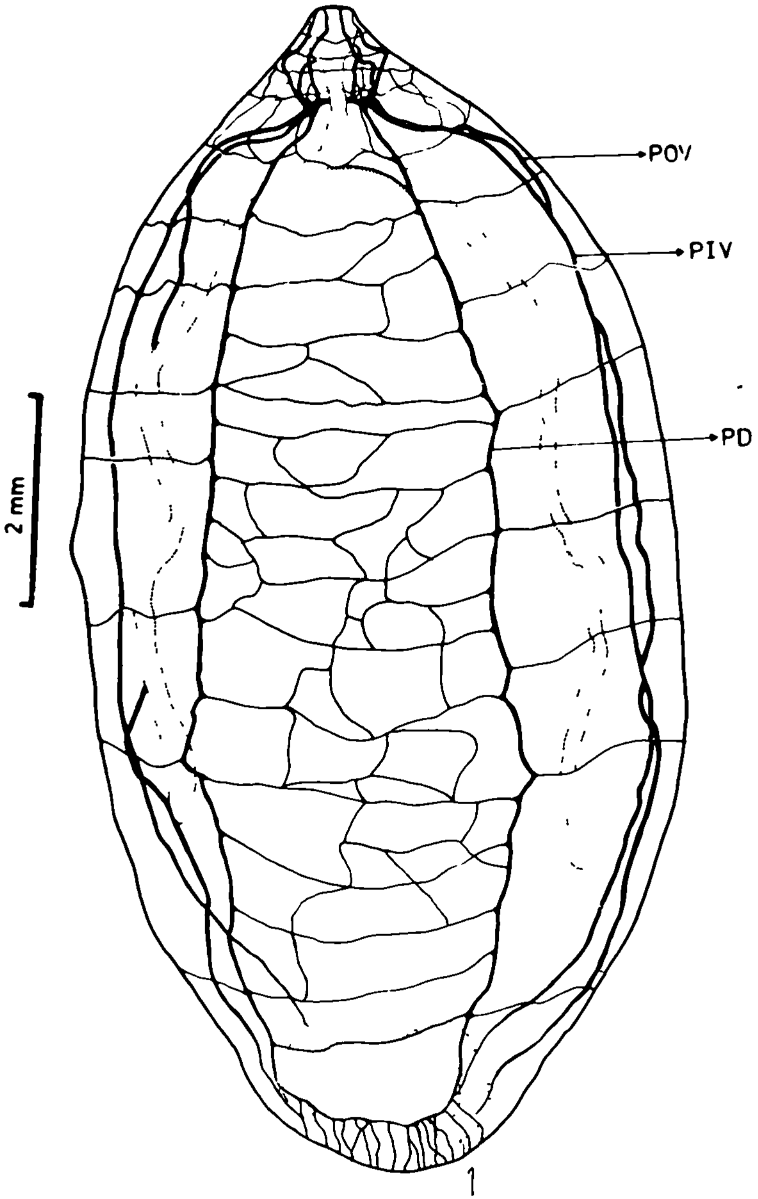
PLATE 1.8: Gastrothylax crumenifer

Figs1,2 Demonstration of nervous system in the whole mount of the worm (Camera lucida).

1. Dorsal view, showing the anterior nerves and postero-dorsals and their nerve net in the post-cerebral region.

2. Ventral view, showing the anterior nerves and nerve net of the postero ventrals.

PLATE 1·8



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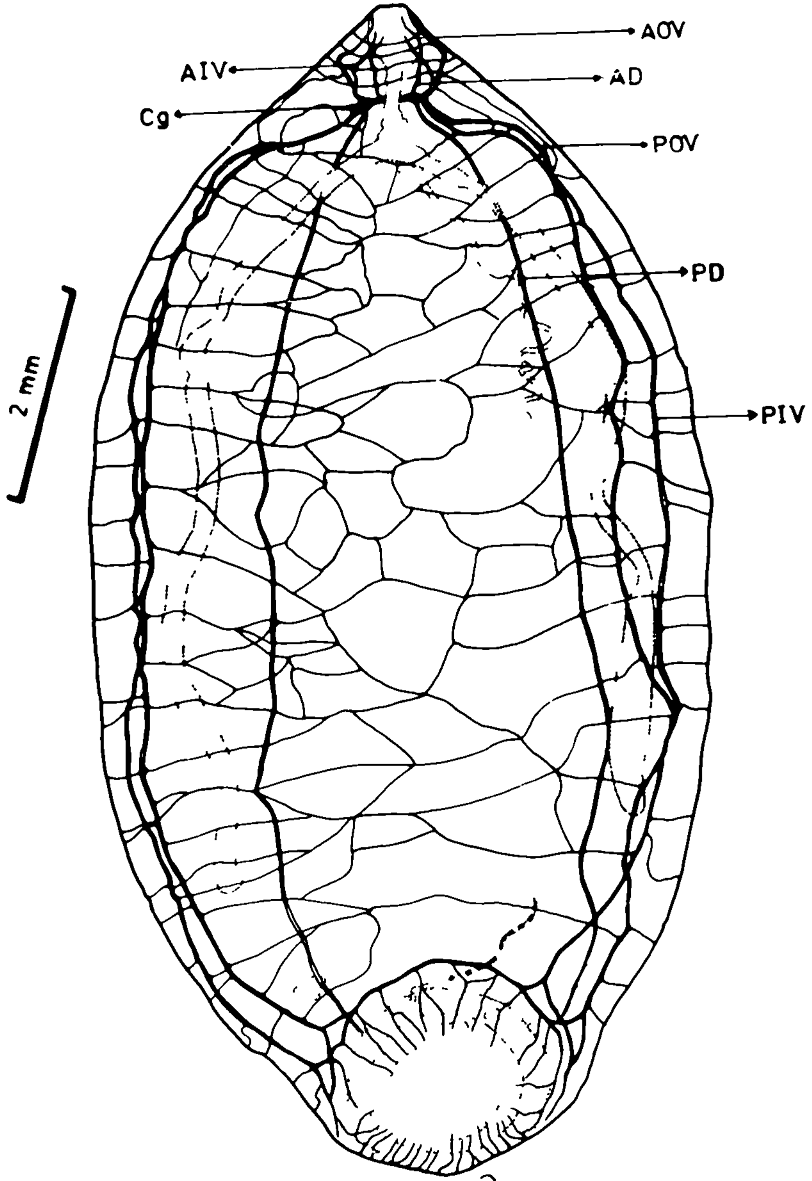

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A complete nerve net is constituted on the dorsal surface by the transverse and longitudinal nerve connectives between the postero-dorsals of the two sides (Pl.1.8, Fig.1; Pl.1.9, Figs.1,2). The postero-dorsals of either side join with each other and innervate the middle and lower edges of the sucker (Pl.1.9, Fig.3).

The postero-outer and inner ventral nerves send branches towards the median axis which ramify and anastomose with one another, thus constituting a nerve net on the ventral surface (Pl.1.8, Fig.2, Pl.1.10, Fig.2). Both these nerves also send branches towards the lateral body wall.

Each postero-outer ventral nerve bifurcates just a little ahead of the acetabulum into two, of which the inner branch meets its fellow of the other side, making a thin transverse connective (Pl.1.10, Fig.3). From the latter many fine branches proceed downwards, branch further and supply the rim of the sucker. The other branch of the postero-outer ventral runs lateral to the acetabulum and terminates supplying its lateral and lower walls. The postero-inner ventral nerve before terminating in the acetabulum also bifurcates into two; the inner branch joins the transverse connective of the postero-outer ventral, while the outer branch proceeds downwards and joins the postero-outer ventral to supply the lateral walls of the sucker.

The innervation to various regions and organs of the body is same as observed in **F. elongatus.**

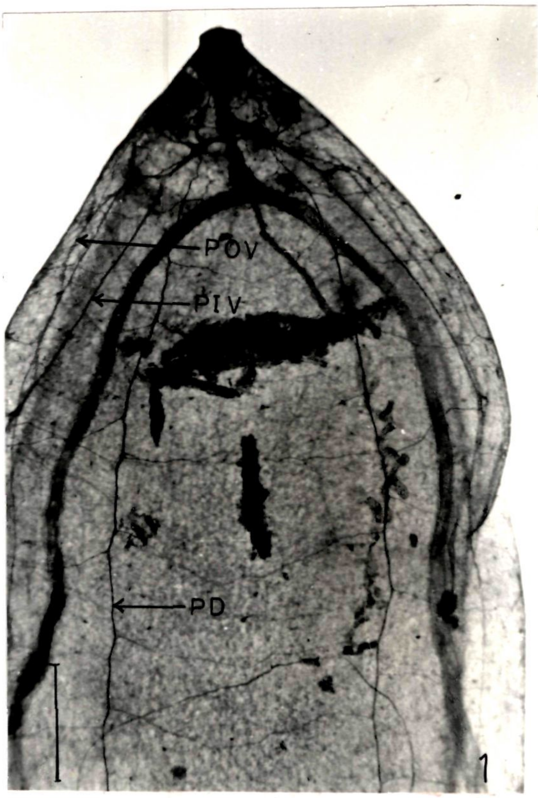
PLATE 1.9: Gastrothylax crumenifer (Photomicrographs)

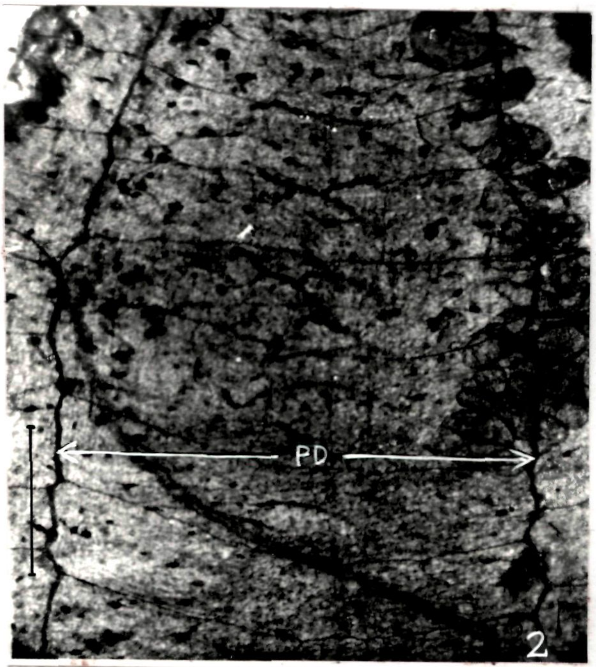
Fig.1 Dorsal view, showing the brain mass and main anterior and posterior nerves in the anterior half of the body. (Scale bar = 1.0 mm).

Fig.2 The postero-dorsals and their nerve net in the mid body region (Scale bar = 0.5 mm).

Fig.3 The postero-dorsals terminating in the acetabular region (Scale bar = 0.5 mm).

PLATE 1.9





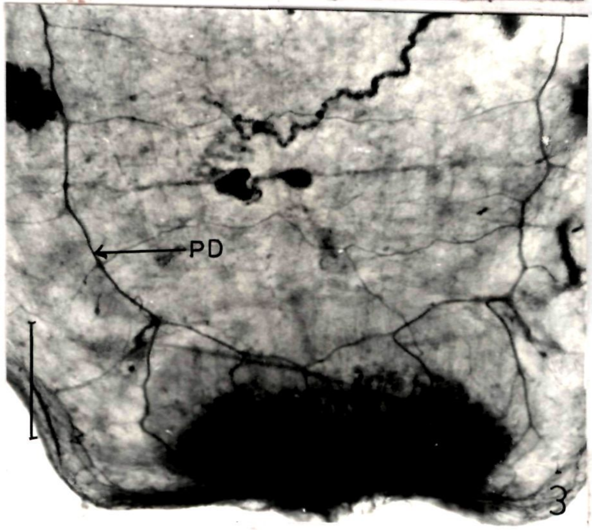


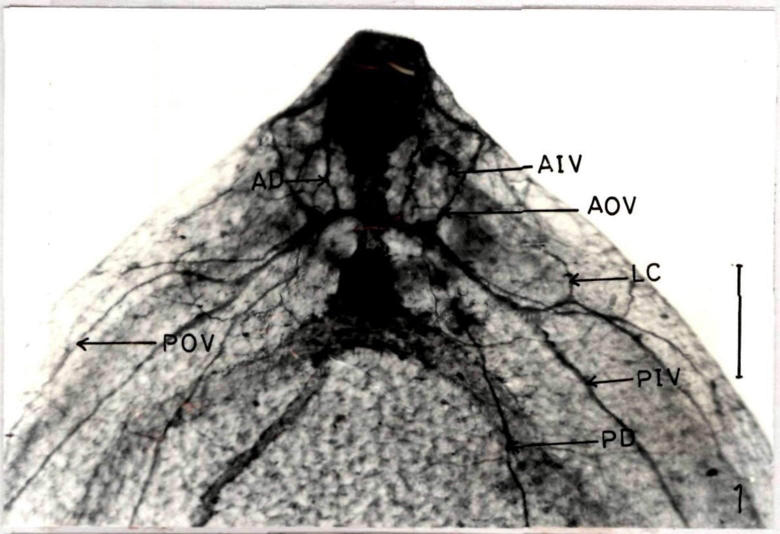
PLATE 1.10: **Gastrothylax crumenifer** (Photomicrographs)

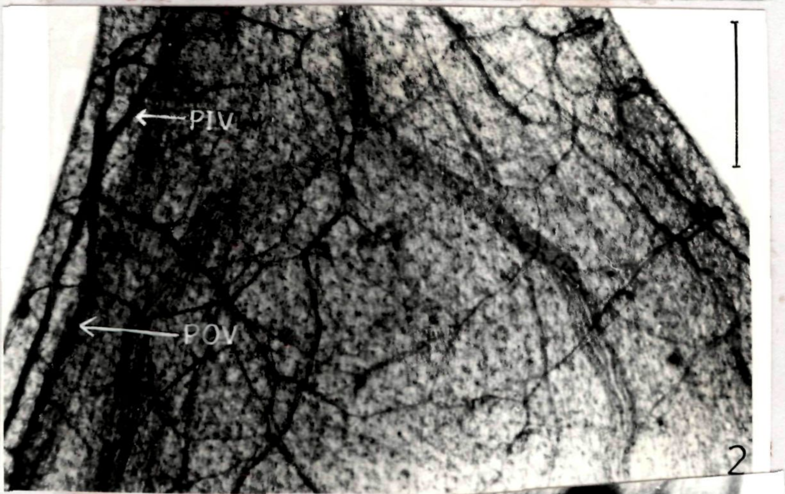
Fig.1 Ventral view, showing the main anterior and posterior nerves (Scale bar = 0.05 mm).

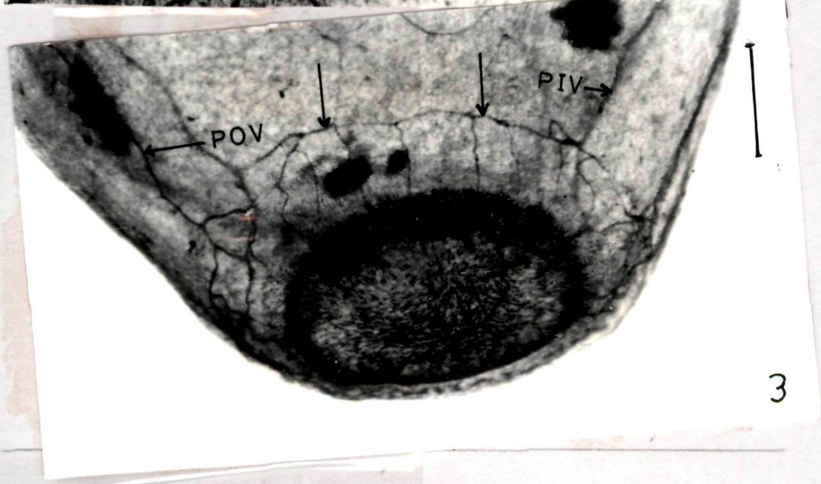
Fig.2 The nerve of the postero-ventral in the mid body region (Scale bar = 0.5 mm).

Fig.3 The postero-ventrals bifurcating and forming a transverse connective (arrows) just anterior to the acetabulum (Scale bar = 0.5 mm).

PLATE 1.10







Olveria indica

In this non-pouched paramphistome four pairs of nerves run anteriad from the cerebral ganglia. However, the number of posterior longitudinal nerves is the same as in the earlier described forms (Pl.1.11, Figs. 1,2; Pl.1.12, Fig.1).

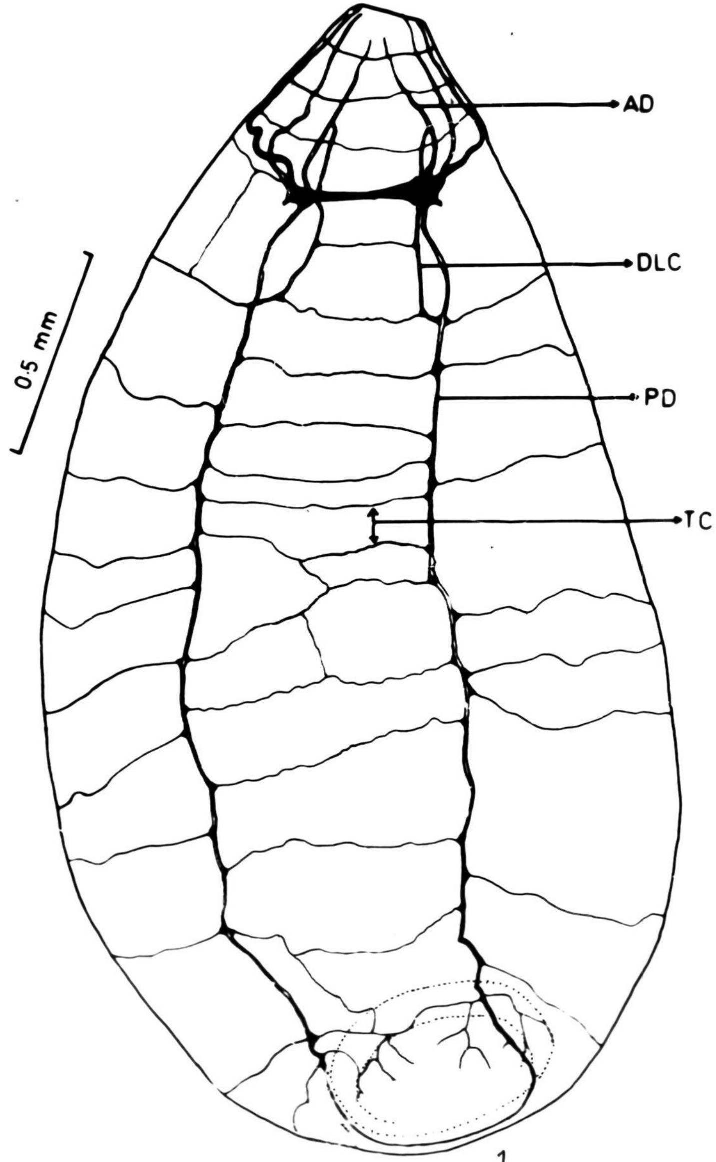
Of the anterior nerves, the innermost pair are the small and stout pharyngeal nerves which shortly beyond their origin, penetrate the pharynx at its postero-lateral margins (Pl.1.11, Figs.1,2; Pl.1.12, Figs.2,3). The antero-dorsal nerves of either side arise from the dorsal side of the cerebral ganglia and proceed to the oral tip, where they become gradually thinner and finally peter out in the tegumental parenchyma (Pl.1.11, Figs.1,2; Pl.1.12, Figs.2,3). The two pairs of anterior ventral nerves are particularly well developed and arise from the ventro-anterior part of the cerebral ganglia (Pl.1.11, Figs.1,2; Pl.1.12, Figs.2,3). Of these, the median ventral nerve, shortly beyond its origin separates from the pharynx and, running parallel to the lateral body wall, reaches to the oral extremity, while the outer ventral nerve runs laterally to the pharynx. All the anterior longitudinal nerves, but the pharyngeal nerves, are interconnected with one another by means of two or three thin transverse connectives (Pl.1.11, Figs.1,2). Of the anterior nerves, only the pharyngeal nerves are deep-seated in the parenchyma, the others being superficially located.

PLATE 1.11: Olveria indica

Figs1,2 Demonstration of nervous system in the whole mount of the worm (Camera lucida).

1. Dorsal view, showing the main anterior nerves and nerve net of the postero-dorsals in the post-cerebral region.
2. Ventral view, showing the antero- and postero-ventrals and the nerve net of the latter.

PLATE 1·11



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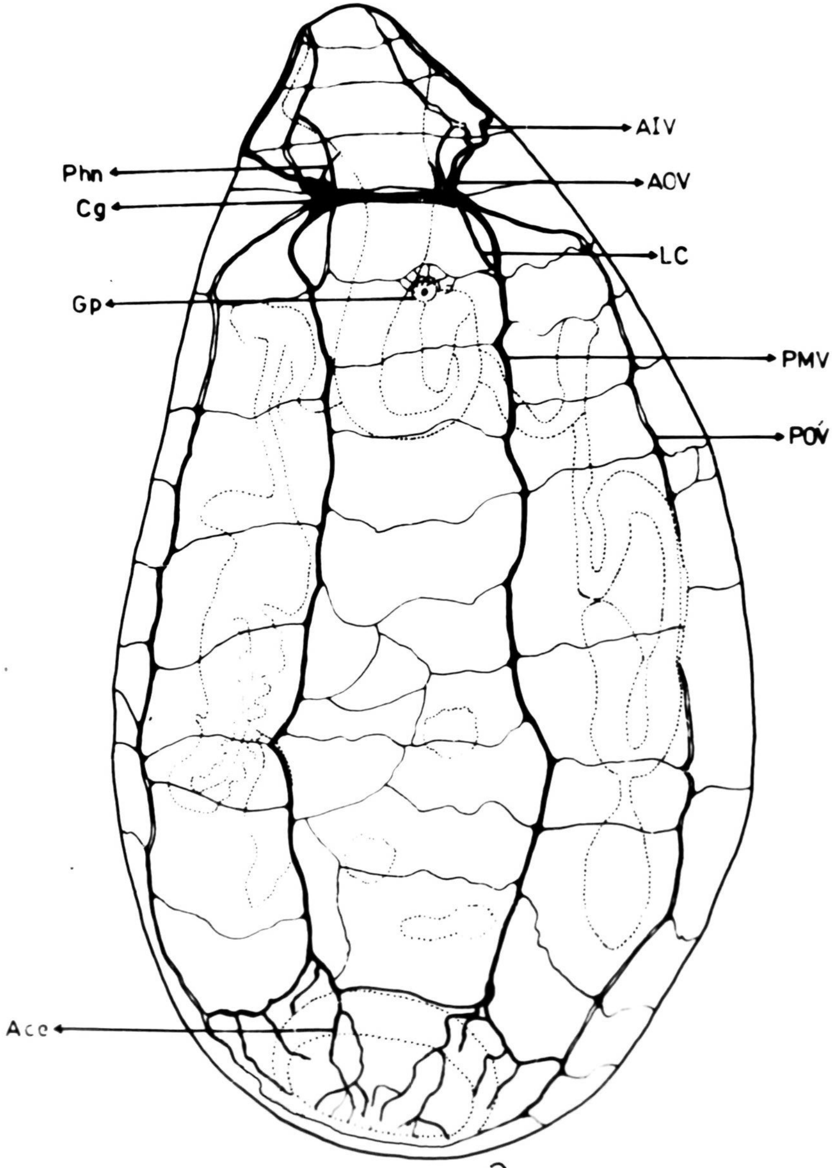

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The post-cephalic longitudinal nerves consist of a pair of postero-dorsals and two pairs of postero-ventrals. The postero-dorsals originate from the middle facet of the cerebral ganglia and proceed posteriad (Pl.1.11, Fig.1; Pl.1.12, Fig.2); on their way to the acetabulum fine branches from these nerves innervate the gut, the reproductive system and the excretory bladder. The postero-dorsals of the two sides are joined with each other by thin transverse connectives throughout their course; the connectives in turn are further connected to one another by two or three still thinner longitudinal connectives in some places (Pl.1.11, Fig.1; Pl.1.13, Fig.1). Each postero-dorsal is also joined with the postero-outer ventral on the same side by means of another set of transverse connectives. A prominent longitudinal connective joins the postero-dorsal nerve with the antero-dorsal on the same side a short distance behind the cerebral ganglia. In the acetabular zone, the branches of the postero-dorsals innervate the floor and lateral walls of the acetabulum. The two nerves extend to the posterior extremity of the body where they join medially (Pl.1.11, Fig.1; Pl.1.13, Fig.3).

Of the posterior ventral nerves, the two postero-median ventral nerves arise from the inner facet of the cerebral ganglia, proceed downwards close and parallel to the median axis of the body, whereas the postero-outer ventral nerves shortly after their origin from the lateral edge of the cerebral ganglion turn obliquely towards the lateral body wall and then run posteriad (Pl.1.11, Fig.2; Pl.1.12, Figs.2,3). Two

PLATE 1.12: **Olveria indica** (Photomicrographs)

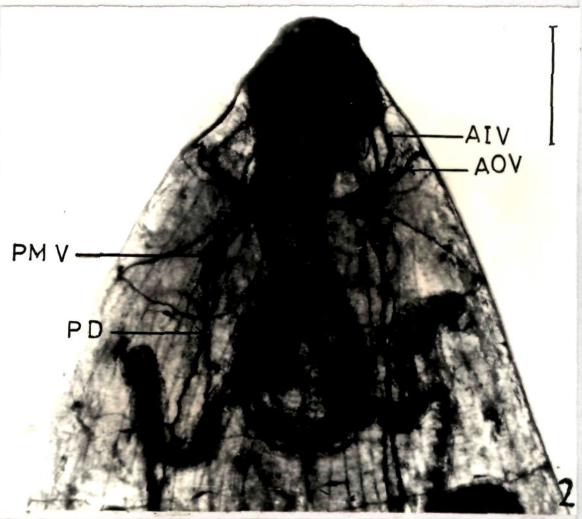
Fig.1 Complete nervous system in the whole worm (Scale bar = 1.0mm).

Figs.2,3 Brain mass and the anterior and posterior longitudinal nerves (Scale bar = 0.5 mm).

2. As seen in a dorsal view, and
3. as seen in a ventral view.

PLATE 1.12


1



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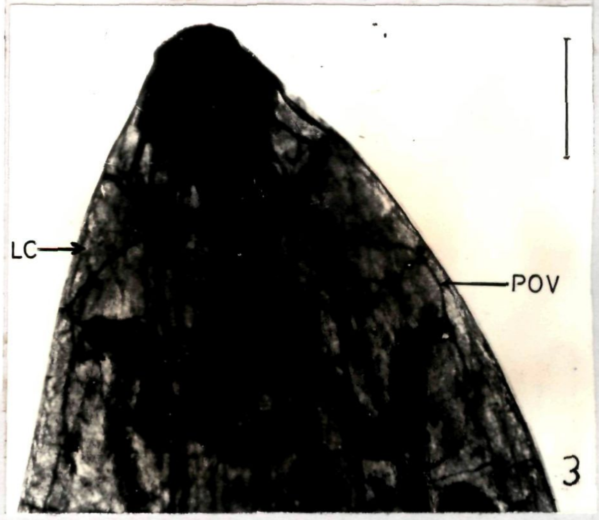

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sets of transverse connectives join the postero-ventrals, one joining the postero-median ventrals of either side and the other interconnecting the postero-median ventral with the postero-outer ventral on each side. At few places these transverse connectives are also interconnected by one or two still thinner longitudinal connectives. Thus, on the ventral surface of the body a nerve net is also formed (Pl.1.11, Fig.2; Pl.1.13, Fig.2). The branches from the postero-ventrals supply the ventral tegument, gut, the reproductive system and the excretory bladder. However, the genital pore is solely supplied by a pair of transverse genital nerves derived from the postero-median ventrals. The genital nerves of both the sides join just anterior to the genital pore forming an arch. Minute branches from the latter approach the genital pore.

In the region immediately anterior to the acetabulum all the postero-ventrals join one another. The postero-median ventrals proceed further as acetabular nerves, branches from which supply the different regions of the sucker (Pl.1.11, Figs.1,2; Pl.1.13, Fig.3).

A longitudinal connective, similar to the one occurring between the antero- and postero-dorsals, joins the postero-median ventral nerve with the antero-inner ventral of the same side, at a level a little away from the point of their origin (Pl.1.11, Fig.2; Pl.1.12, Fig.3). With a slight increase in the concentration of the substrate (5-bromoindoxyl acetate) in the incubation medium a superficial net work of longitudinal

PLATE 1.13: **Olveria indica** (Photomicrographs)

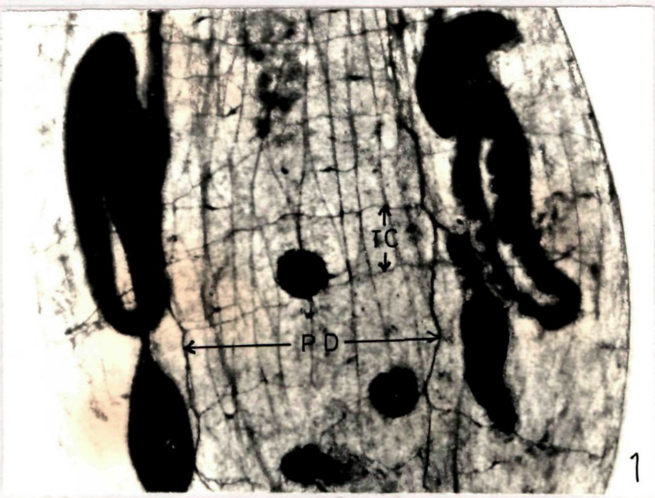
Fig.1 Postero-dorsals and their nerve net in the middle third and testicular region of the body.

Fig.2 Postero-outer and median ventrals in the same region.

Fig.3 Termination of the posterior longitudinal nerves in the acetabular region.

(Figs. 1-3, Scale bare = 0.5 mm).

PLATE 1.13



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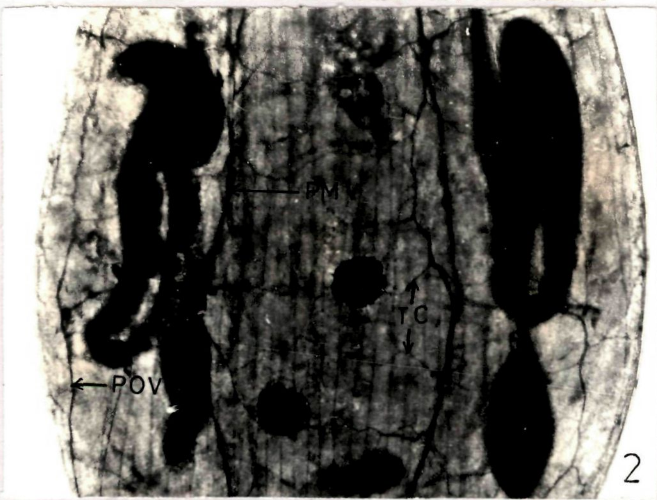

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and circular fibres in the tegument is also revealed (Pl.1.12, Fig.1, Pl.1.13, Figs.1-3).

Cotylophoron cotylophorum

The longitudinal nerve strands given out from the cerebral ganglia comprise three anterior and three posterior pairs (Pl.1.14, Figs.1,2).

Of the anterior nerves, the innermost pair is of dorsal nerves, emerging from the dorso-middle facet of the cerebral ganglia (Pl.1.14, Figs.1,2; Pl.1.15, Fig.1). These nerves take a course dorsal to the pharynx and finally terminate near the oral tip where a circumoral ring is formed by the merging together of all the anterior nerves. The remaining two pairs of anterior nerves comprise inner and outer ventrals. The anterior inner ventrals originate from the ventro-lateral facet of the cerebral ganglia and proceed towards the anterior extremity, running laterally to the pharynx. The outer ventrals emerge from the lateral facet of the cerebral ganglia and run somewhat parallel to the lateral body wall. All the anterior nerves are also interconnected with thin, ring-like transverse connectives. The latter are further joined with one another by still thinner longitudinal connectives at some places, thus constituting a nerve basket encircling the pharynx (Pl.1.14, Figs.1,2; Pl.1.15, Fig.1). Fine branches from the transverse connectives also innervate the tegument. All the anterior nerves are lodged superficially. They supply the dorsal,

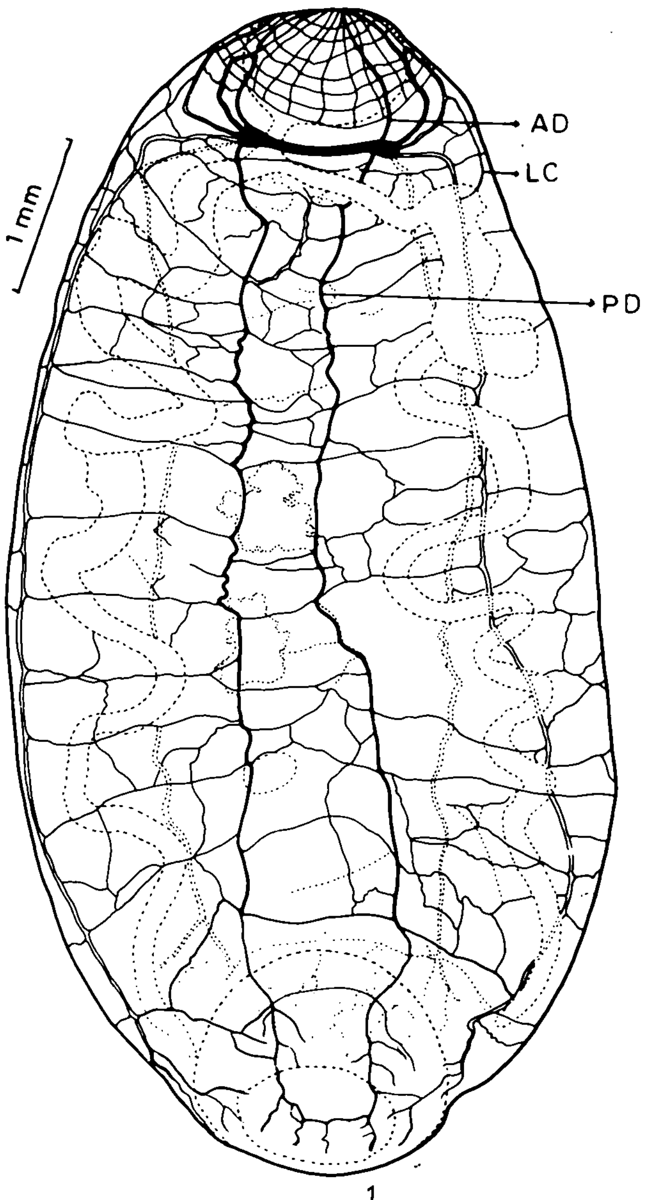
PLATE 1.14: **Cotylophoron cotylophorum**

Figs.1,2 Demonstration of the nervous system in the whole mount of the worm (Camera lucida).

1. Dorsal view, showing the anterior nerves and the nerve net of the postero-dorsals in the post-cerebral region.

2. Ventral view, showing the postero-ventral and lateral nerves and their nerve net.

PLATE 1·14



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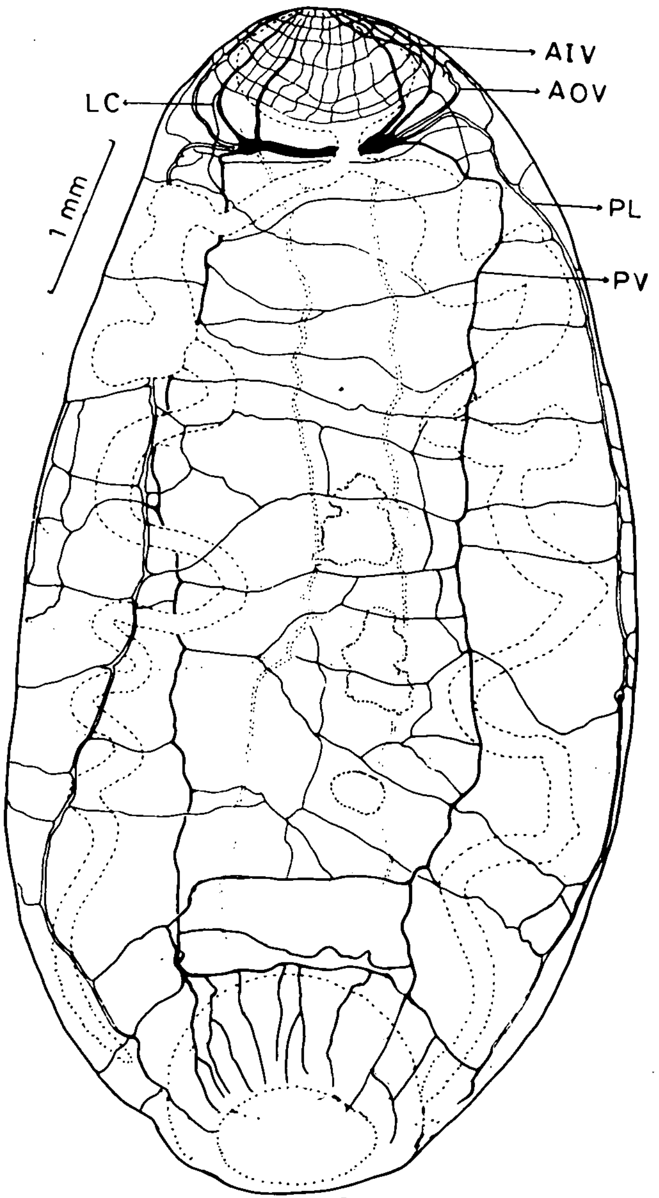

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lateral and ventral parts of the anterior region of the body including the pharynx.

The innermost pair of posterior nerves is of the postero-dorsals, which arise from the postero-dorsal surface of the cerebral ganglia and proceed medially (towards the median axis) up to the acetabular region. On their way to the acetabulum, these nerves innervate the alimentary canal, vitellaria, excretory bladder and the reproductive system (Pl.1.14, Fig.1; Pl.1.15, Figs.2,3). Just anterior to the acetabulum both the postero-dorsals branch and rebranch, reaching to the floor of the sucker and then join medially with each other. The branches from the postero-dorsals supply the middle, lateral and lower portions of the sucker (Pl.1.14, Fig.1; Pl.1.15, Fig.4).

The postero-ventral nerves arising from the postero-ventral border of the cerebral ganglia run between the other two posterior nerves and supply the reproductive system, excretory duct and bladder (Pl.1.14, Fig.2; Pl.1.15, Fig.3).

The postero-laterals arise from the postero-lateral facet of the cerebral ganglia and proceed first obliquely towards the lateral body wall and then run posteriorly, being parallel to the latter (Pl.1.14, Fig.2; Pl.1.15, Fig.3; Pl.1.16, Fig.1). They innervate the vitellaria and alimentary canal (Pl.1.16, Figs.1,2). Just anterior to the acetabulum both postero-ventrals are joined with each other and also with the postero-laterals of their side by a thin transverse connec-

PLATE 1.15: **Cotylophoron cotylophorum** (Photomicrographs)

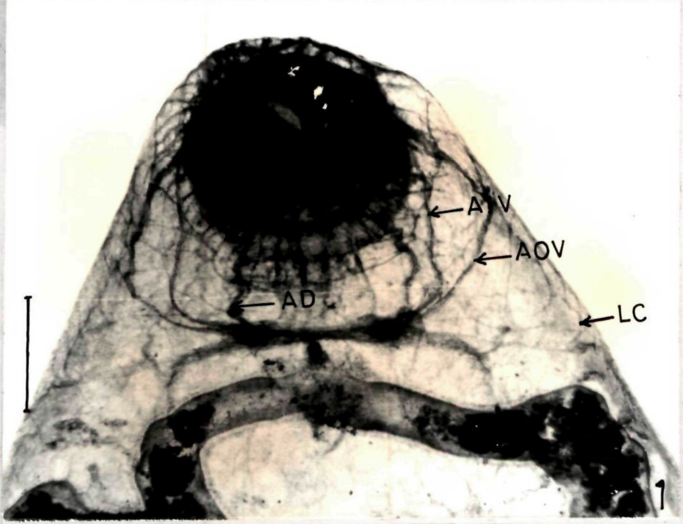
Fig.1 Cerebral mass and the main anterior longitudinal nerves in the pre-cerebral region (Scale bar = 0.5 mm).

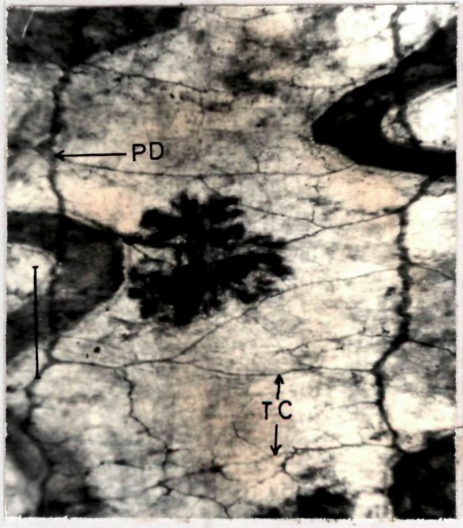
Fig.2 Postero-dorsals and their nerve net in the mid-body region (Scale Bar = 0.5 mm).

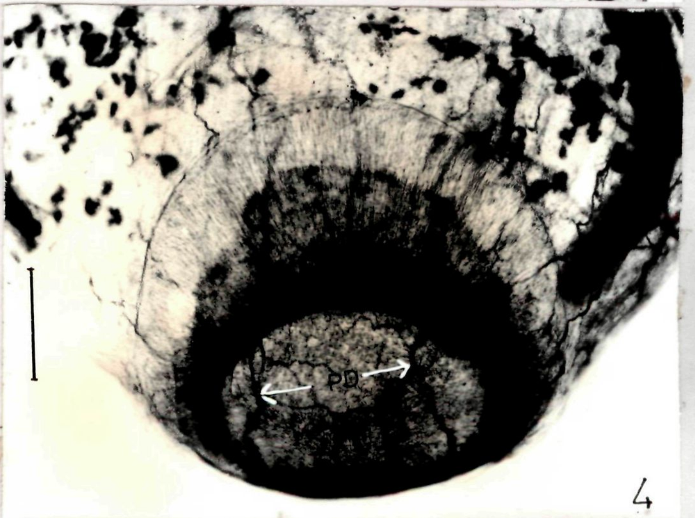
Fig.3 The main posterior longitudinal nerves (Scale bar = 1.0 mm).

Fig.4 Termination of the postero-dorsals in the acetabulum (Scale bar = 0.5 mm).

PLATE 1.15







tive. Many thin slender branches emerging from this connective reach up to the floor of the sucker and innervate the whole ventral surface of the latter (Pl.1.14, Fig.2; Pl.1.16, Fig.2). The postero-laterals, running laterally to the acetabulum innervates its lateral and posterior edges (Pl.1.14, Fig.2; Pl.1.16, Fig.2).

Several thin transverse connectives interconnect all the posterior nerves to one another. Still thinner and oblique connectives, joining all the transverse connectives, constitute a dense and fine nerve net on the dorsal and ventral surfaces of the worm (Pl.1.14, Figs.1 and 2; Pl.1.15, Fig.2; Pl.1.16, Fig.1).

The genital pore region is solely supplied by a pair of thin transverse genital nerves given off from the postero-ventrals. The latter gives few minute branches in the vicinity of the genital pore region to innervate it (Pl.1.15, Fig.3).

The postero-ventrals are embedded in the parenchyma, whereas the others are superficially located. All the nerves are well developed, the postero-dorsals being the thickest of all.

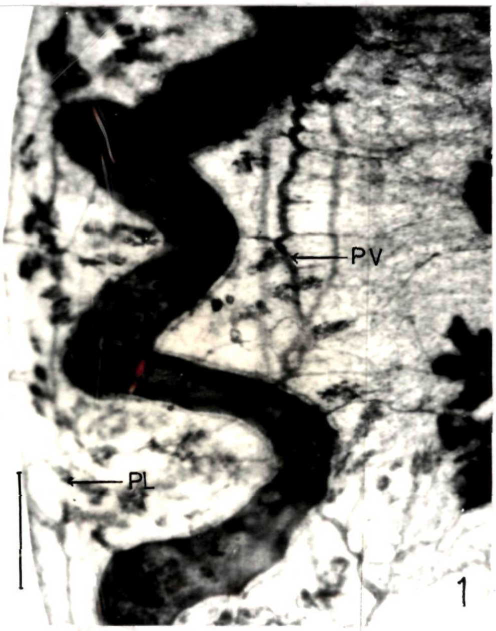
Both postero-ventrals and postero-laterals are connected with the antero-inner ventral and antero-outer ventral, respectively, of their side by way of long and thin longitudinal connectives. Branches from the latter innervate the lateral

PLATE 1.16: **Cotylophoron cotylophorum** (Photomicrographs)

Fig.1 The postero-ventral and lateral nerves and their nerve net in one longitudinal half of the body (Scale bar = 0.5 mm).

Fig.2 Thin transverse connective (arrow) formed by the postero-ventral and lateral nerves just before the acetabulum. Fine branches from it are seen supplying the latter (Scale bar = 1.0 mm).

PLATE 1.16



body wall and join the ventral nerve net of the body (Pl.1.14, Figs.1,2; Pl.1.15, Figs.1,3).

Paramphistomum epiclitum

Three pairs each of anterior and posterior nerves originate from the cerebral ganglia (Pl.1.17, Fig.1; Pl.1.18, Fig.1).

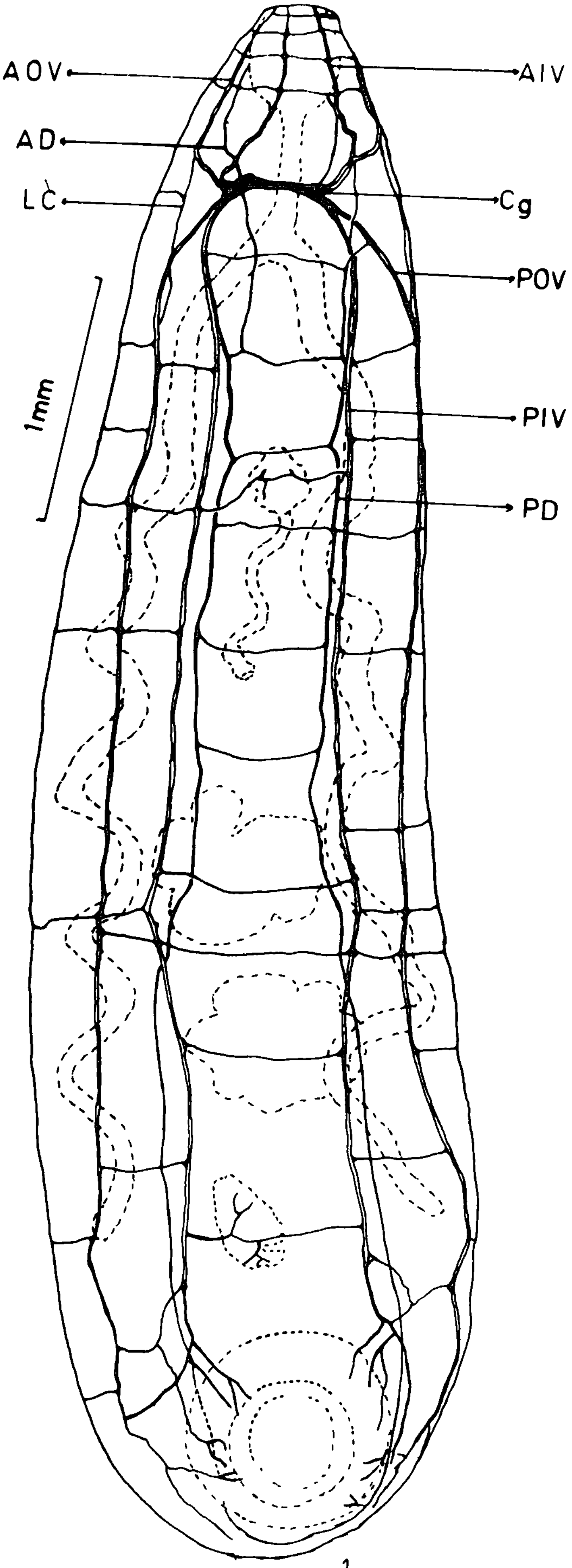
Of the anterior nerves that run cephalad, the inner most pair is of the antero-dorsals, which arise from the antero-dorsal facet of the ganglia and pass anteriad to the oral tip (Pl.1.17, Fig.1; Pl.1.18, Figs.1,2). The antero-inner ventrals arise from the latero-ventral facet of the ganglia and extend along the lateral walls of the pharynx, thinning gradually and finally merging into the nerve ring of the oral aperture. The antero-outer ventral nerves originate from the antero-lateral facet of the ganglia and travel up to the oral rim along the lateral body wall (Pl.1.18, Figs.1,2). The anterior transverse connectives are few (four to six) in number. Minute branches from these connectives innervate the circumpharyngeal tegument and pharyngeal region. All the anterior nerves are superficially lodged.

The post-cephalic longitudinal nerves comprise a pair of postero-dorsals and two pairs of ventral nerves. The postero-dorsals arise from the dorso-posterior facet of the cerebral ganglia and pass posteriad (Pl.1.17, Fig.1; Pl.1.18, Figs.1,2). They innervate the alimentary canal,

PLATE 1.17: **Paramphistomum epiclitum**

Fig.1 Demonstration of nervous system in the whole mount of the worm (Camera lucida sketch).

PLATE 1·17



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reproductive system and excretory bladder. Both the postero-dorsals are extended up to the posterior extremity of body, joining each other just below the acetabulum (Pl.1.17, Fig.1; Pl.1.18,Fig.3). Throughout their length 8-9 thin transverse connectives connect the postero-dorsals to each other and with outer ventral of their side. At two or three places they are further interconnected with very thin longitudinal connectives (Pl.1.17, Fig.1; Pl.1.18, Fig.4). Minute thin branches from the dorsal nerve net innervate the tegument.

The postero-inner ventrals arise from the ventro-posterior part of the cerebral ganglia (Pl.1.17, Fig.1; Pl.1.18, Fig.1). They innervate the reproductive system and alimentary canal. The postero-outer ventrals emerge from the latero-ventral border of the ganglia and are the outer most in disposition (parallel to the lateral body wall) throughout their course in each longitudinal half (Pl.1.17, Fig.1; Pl.1.18, Figs.1,4). They innervate the vitellaria and the alimentary canal region.

Two sets of thin transverse and longitudinal connectives interconnect the posterior-ventrals with one another throughout their length (Pl.1.18, Fig.4). The two postero-inner ventrals innervate the anterior border of the acetabulum by giving away two prominent branches whereas the two postero-outer ventrals extend lateral to the acetabulum to supply the lateral aspects of the latter (Pl.1.17, Fig.1; Pl.1.18, Fig.3).

PLATE 1.18: Paramphistomum epiclitum (Photomicrographs)

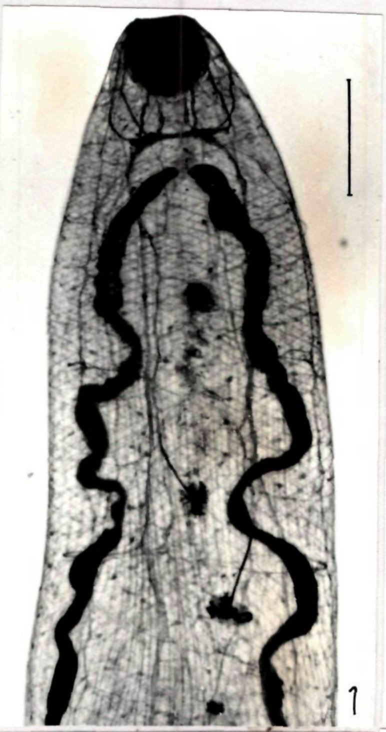
Fig.1 Cerebral mass and the anterior and posterior longitudinal nerves (Scale bar = 1.10 mm).

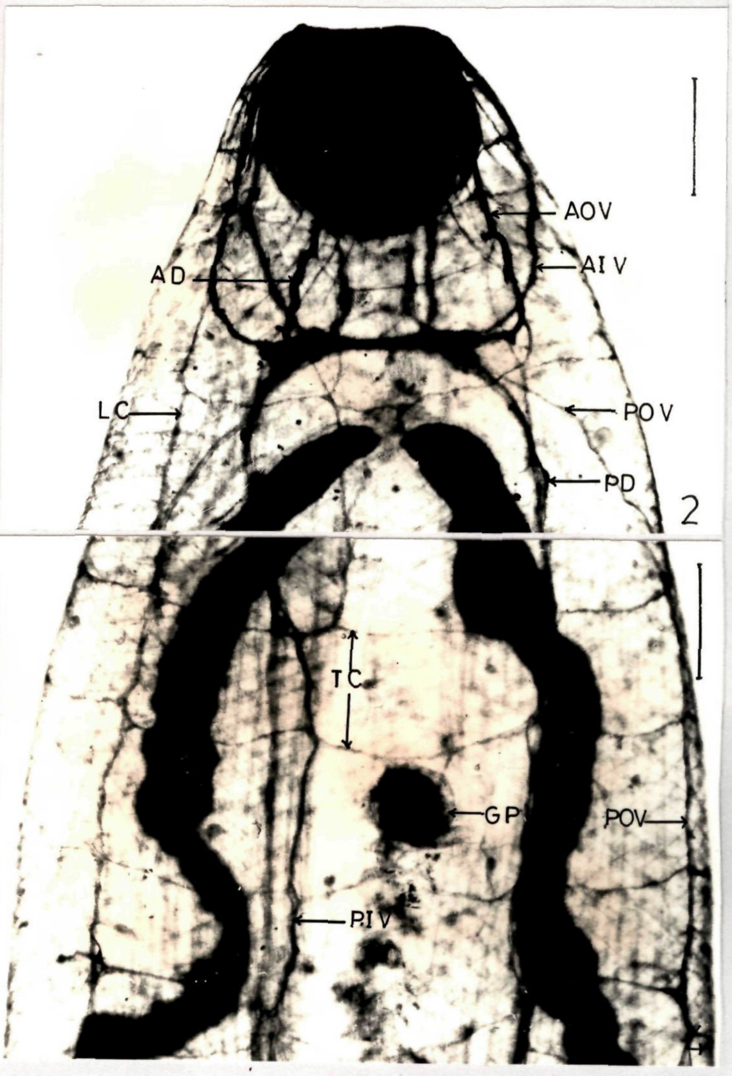
Fig.2 Anterior region of the body under higher resolution. The transverse connectives of anterior nerves and lateral longitudinal connectives are evident (Scale bar = 0.5 mm).

Fig.3 Terminal course of the posterior longitudinal nerves and innervation in the acetabulum (Scale bar = 1.0 mm).

Fig.4 The posterior longitudinal nerves and their nerve net in the mid body region. A pair of transverse genital nerves forming a connective anterior to the genital pore is evident (Scale bar = 0.5 mm).

PLATE 1.18





Thin and long longitudinal connectives interconnect the antero and postero-inner ventrals and also the antero and postero-outer ventrals with one another (Pl.1.18, Fig.2). These longitudinal connectives join the ventral nerve net of the body by thin transverse connections.

The genital pore is innervated by a pair of transverse genital nerves from the postero-inner ventrals (Pl.1.18, Figs.1,4).

Of the posterior nerves, only the postero-inner ventrals are deep-seated in the parenchyma, whereas the outer ventrals and dorsals are superficially located.

Paramphistomum gracile

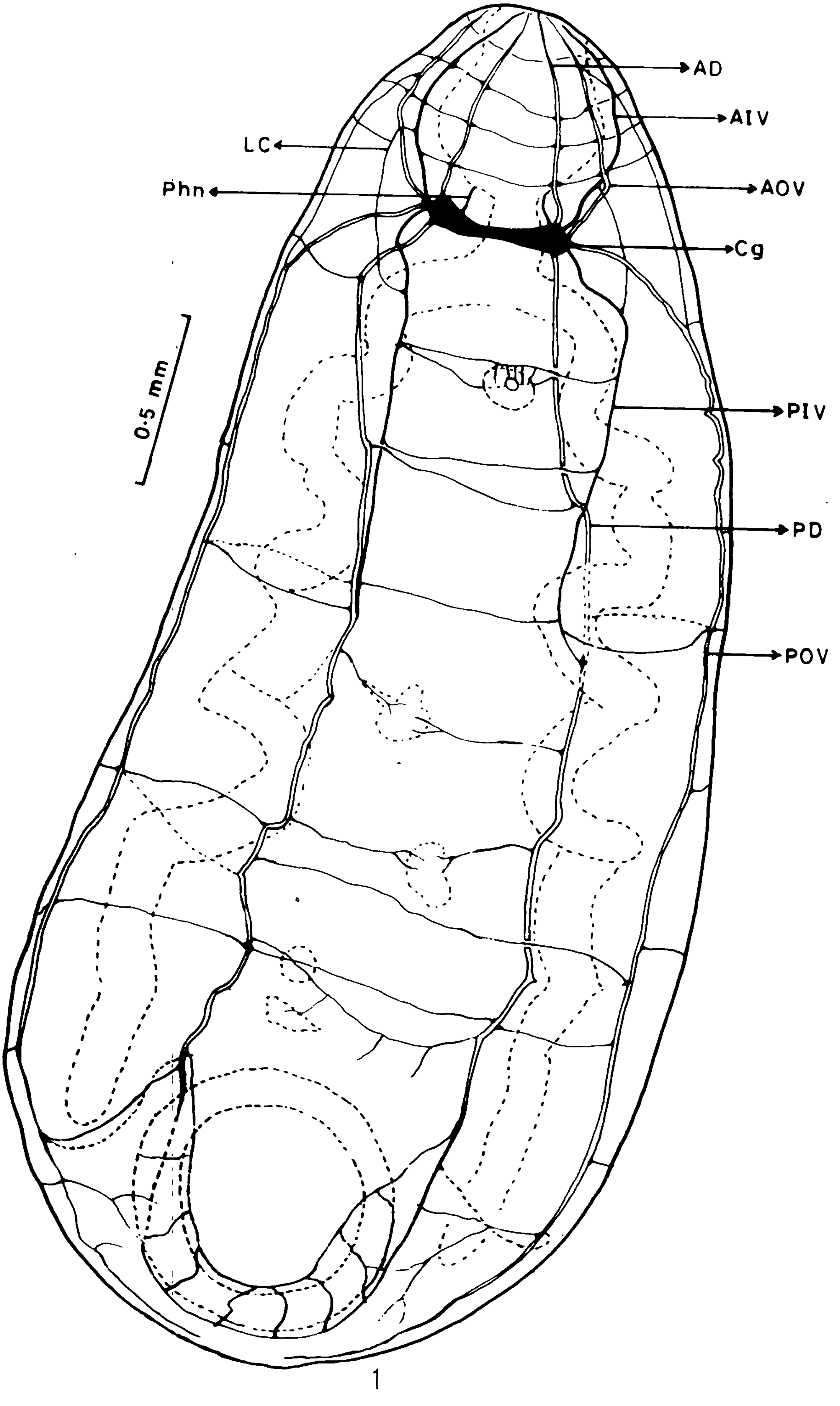
Four pairs of nerves run cephalad and three pairs, caudad from the cerebral ganglia (Pl.1.19, Fig.1; Pl.1.20, Fig.1).

Among the anterior pairs of nerves, all of which are superficially lodged, the inner most pair comprises the small and slender pharyngeal nerves. Shortly after their origin, these nerves penetrate the pharynx at its postero-lateral margins (Pl.1.19, Fig.1; Pl.1.20, Fig.1). The remaining nerves include a pair of antero-dorsals, antero-inner ventrals and antero-outer ventrals (Pl.1.19, Fig.1; Pl.1.20, Fig.1). All of these proceed anteriad to the oral rim, thinning gradually. *The antero-dorsals originate from the dorso-inner facet of the cerebral ganglia in between the pharyngeal and*

PLATE 1.19: Paramphistomum gracile

Fig.1 Demonstration of the nervous system in the whole mount of the worm (Camera lucida).

PLATE 1·19



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antero-inner ventral nerves. The two pairs of antero-ventral nerves are relatively well developed and arise from the antero-ventral aspect of the cerebral ganglia. The inner ventrals take a course along the lateral walls of the pharynx, whereas the outer ventrals run parallel to the lateral body wall. Ring-like four to five thin transverse connectives interconnect all the anterior nerves, but the pharyngeal nerves, encircling the pharynx (Pl.1.19, Fig.1; Pl.1.20, Fig.1). Fine tributaries from these nerve connectives innervate the tegument of the anterior region and also the pharynx. The pharyngeal nerves solely supply the pharynx.

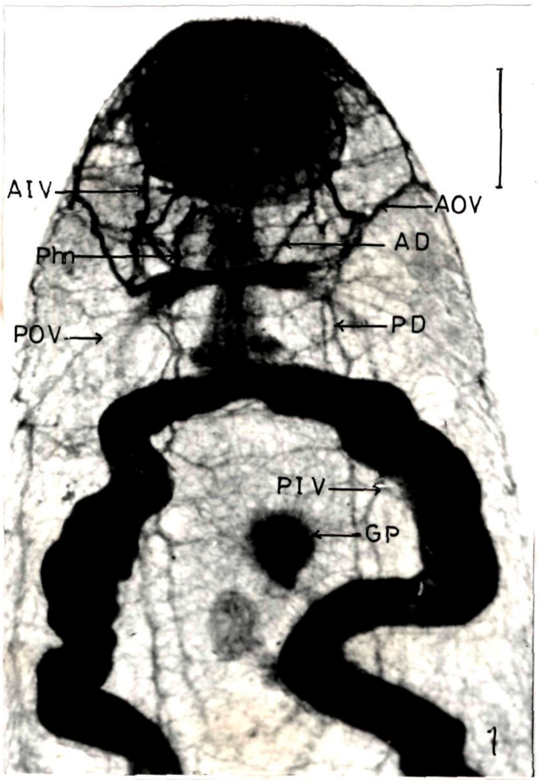
In the post-cerebral region, one pair of postero-dorsals and two pairs of postero-ventrals (inner and outer) are present (Pl.1.19, Fig.1; Pl.1.20, Fig.1). The two postero-dorsals originate from the dorsal facet of the cerebral ganglia and innervate the alimentary canal region and reproductive system. The postero-ventrals arise from the postero-ventral aspect of the brain. Of these, the outer ventrals run along the lateral body wall, whereas the postero-inner ventrals travel along the postero-dorsals. The former nerves, which fall short of the acetabulum, fuse with the postero-dorsals in the pre-testicular region to form a single nerve. Just anterior to the acetabulum the two postero-dorsals bifurcate; the inner branch extends posteriorly in the acetabulum to join the fellow of the other side through a transverse connective. From the latter many thin branches emerge and supply

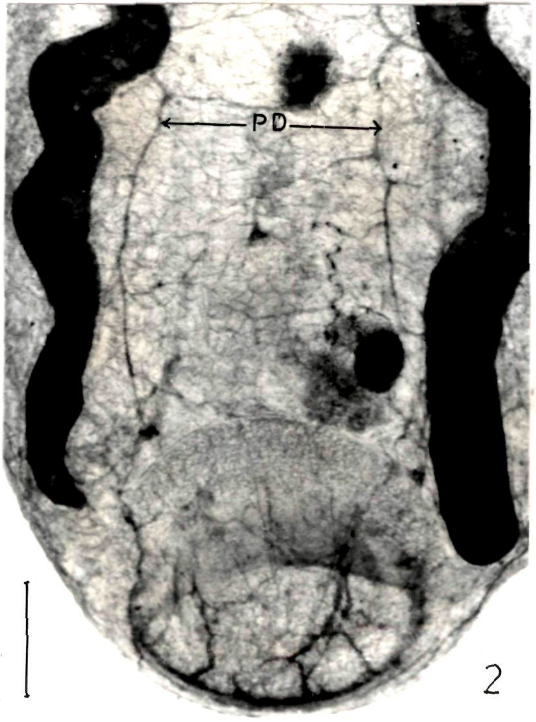
PLATE 1.20: **Paramphistomum gracile** (Photomicrographs)

Fig.1 Brain mass and the main anterior and posterior nerves (Scale bar = 0.5 mm).

Fig.2 Terminal course of the posterior nerves and innervation of the acetabulum (Scale bar = 0.5 mm).

PLATE 1.20





the lateral, middle and posterior aspects of the sucker (Pl.1.19, Fig.1; Pl.1.20, Fig.2). The outer branch gets connected with the postero-outer ventral on its side and proceeds laterally in the acetabulum, merging near the lower edges of the sucker (Pl.1.19, Fig.1; Pl.1.20, Fig.2). The postero-outer ventrals further run lateral to the acetabulum and innervate its lateral walls and finally terminate near the posterior extremity of the body. Both the postero-ventral nerves supply the alimentary canal, vitellaria and excretory systems.

The transverse connectives interconnecting the posterior longitudinal nerves are thin (Pl.1.19, Fig.1; Pl.1.20, Figs.1,2). Minute branches from these connectives penetrate the tegument of the dorsal and ventral surfaces. All the posterior nerves are deep seated in the parenchyma.

The pre- and post-cerebral nerves are interconnected by way of two pairs of longitudinal connectives : one pair interconnects the antero- and postero-inner ventrals and the second, the antero- and postero-outer ventrals (Pl.1.19, Fig.1; Pl.1.20, Fig.1). The connective between the latter two nerves looks like a continuation of the postero-outer ventrals in the pre-cerebral region. Both these longitudinal connectives are interconnected with each other and with the nerve net of the body.

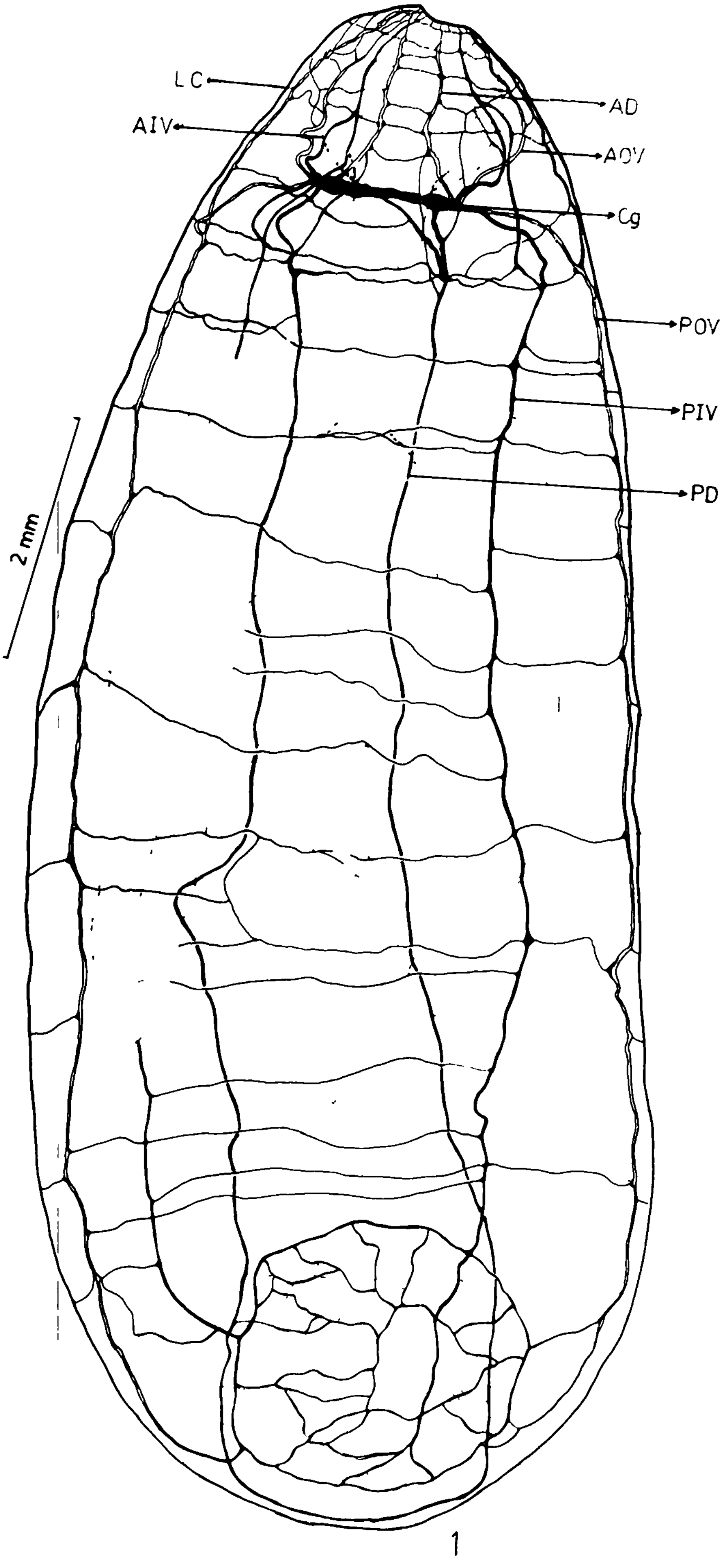
Orthocoelium orthocoelium

Three pairs each of anterior and posterior nerves

PLATE 1.21: **Orthocoelium orthocoelium**

Fig.1 Demonstration of nervous system in the whole mount of the worm (Camera lucida).

PLATE 1·21



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emanate from the cerebral ganglia (Pl.1.21, Fig.1; Pl.1.22, Figs.1,2).

The anterior nerve pairs include one pair each of antero-dorsals, antero-inner ventrals and antero-outer ventrals (Pl.1.21, Fig.1; Pl.1.22, Figs.2,3). The antero-dorsals being the innermost pair (nearer the median axis) emanate from the inner border of the cerebral ganglia and proceed anteriad dorsal to the pharynx. Outer to this pair are the antero-inner ventrals which emerge from the antero-lateral aspects of the cerebral ganglia. The outermost nerve pair is of the antero-outer ventrals. They emanate from the lateral extremities of the cerebral ganglia and proceed anteriorly lateral to the pharynx. Thin transverse connectives interconnect all the anterior longitudinal nerves with one another (Pl.1.22, Figs.2,3). A little behind the oral rim, all the anterior nerves terminate in a thick oral nerve ring. From the latter many minute, thin nerves emerge and further extend to innervate the outer and inner aspects of the oral rim constituting a fine nerve plexus (Pl.1.22, Fig.3).

Of the nerves traversing posteriad, one pair is of the postero-dorsal nerves, each of which emerges from the inner facet of the cerebral ganglia and proceeds parallel to the median axis of the body (Pl.1.21, Fig.1; Pl.1.22, Fig.2). These on their way to the acetabulum innervate the gut, reproductive organs and excretory bladder. The postero-inner ventrals arise from the postero-ventral border of the

PLATE 1.22: **Orthocoelium orthocoelium** (Photomicrographs)

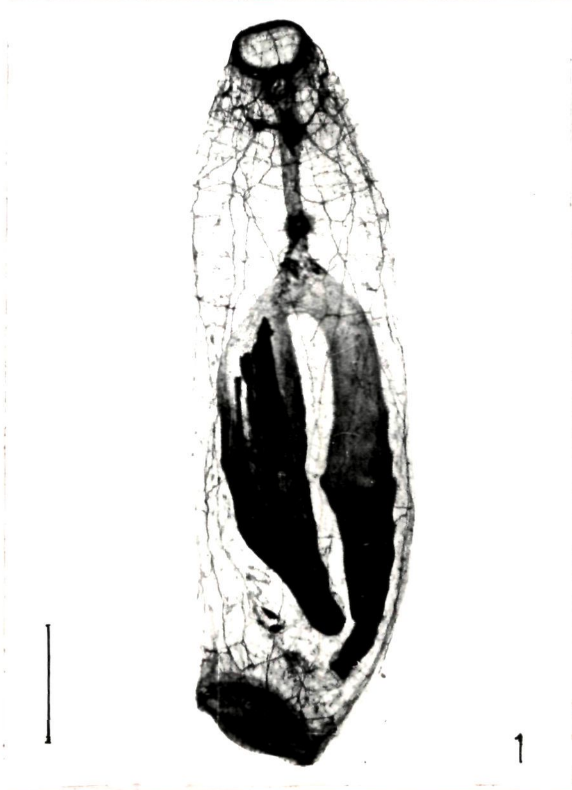
Fig.1 Complete nervous system in the whole worm (Scale bar = 1.0mm).

Fig.2 Brain mass and the main anterior and posterior nerves (Scale bar = 0.5 mm).

Fig.3 The pre-cerebral region under higher resolution. The oral nerve ring a little behind the oral rim all contributed by the anterior nerves is evident. Tributaries from the former are seen innervating the inner aspects of the latter (Scale bar = 0.5 mm).

Fig.4 Termination of the posterior longitudinal nerves in the acetabulum (Scale bar = 0.5 mm).

PLATE 1.22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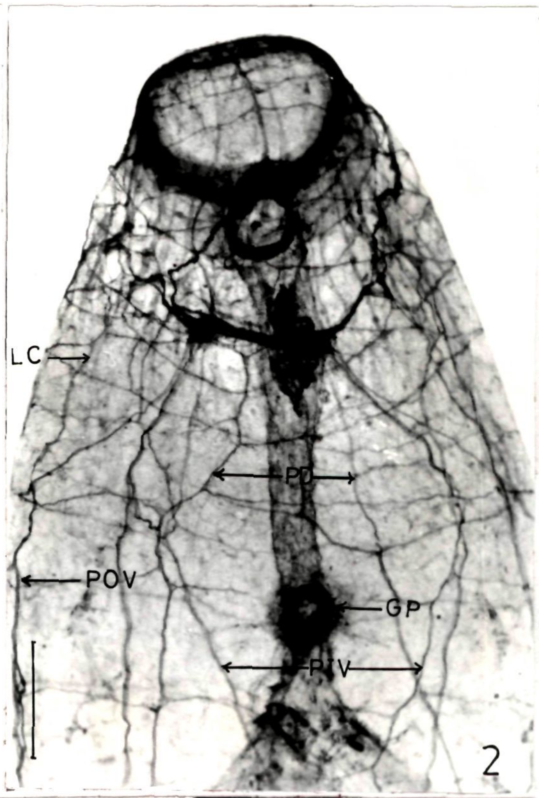

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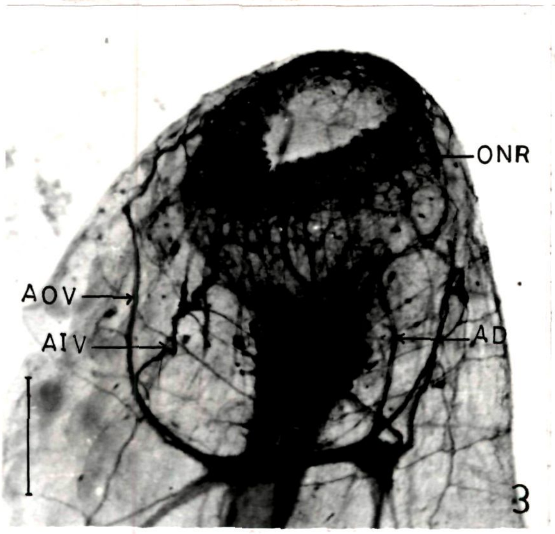

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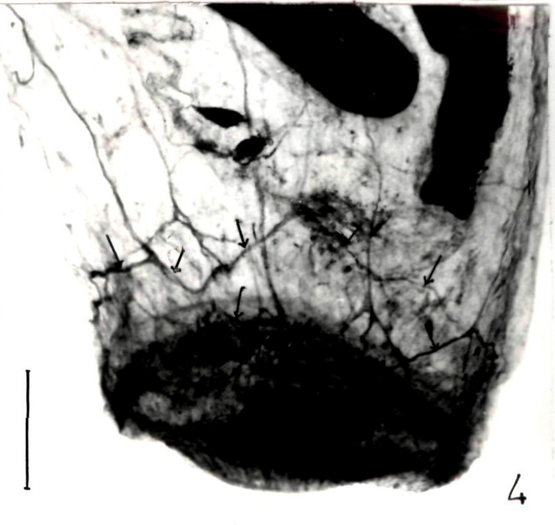
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cephalic ganglia and proceed caudad innervating the genital pore region (Pl.1.21, Fig.1; Pl.1.22, Fig.2), reproductive organs and excretory bladder. Each postero-outer ventral nerve emerges from the postero-lateral extremity of the cerebral ganglia and runs posteriad close and parallel to the lateral body wall and gives off branches to the tegument, vitellaria, parenchymatous tissue and alimentary canal (Pl.1.21, Fig.1; Pl.1.22, Figs.1,2). Both the postero-ventrals, i.e., inner and outer, and postero-dorsals of each lateral longitudinal half of the body join with each other and with the fellow of the other side to constitute a circular connective just anterior to the acetabulum; from this connective many thin, slender branches emerge and innervate various aspects of the acetabulum, finally terminating in a nerve plexus (Pl.1.22, Fig.4).

Several transverse and longitudinal connectives join these posterior nerves with one another to constitute a conspicuous nerve net on both dorsal and ventral surfaces of the body (Pl.1.21, Figs.1; Pl.1.22, Figs.1,2).

All these posterior nerves are connected with their counterparts in the pre-cerebral region by way of long and thin longitudinal connectives. Branches from the latter join the nerve net of the body (Pl.1.21, Fig.1; Pl.1.22, Fig.2).

Both anterior and posterior longitudinal nerves are superficially located.

Orthocoelium streptocoelium

Of the three pairs of anteriorly directed nerves, one pair is of dorsal nerves, the second, of antero-inner ventrals and the third and the outermost pair, of antero-outer ventral nerves (Pl.1.23, Fig.1; Pl.1.24, Figs.1,2). The antero-dorsals emerge from the dorso-middle facet of the cerebral ganglia and run anteriad, dorsal to the pharynx. The antero-inner ventrals emerge from the antero-ventral facet of the brain and take a course along the lateral walls of the pharynx. The antero-outer ventrals emerge from the lateral aspects of the ganglia, run cephalad in between the antero-inner ventral nerve and the lateral body wall. All the anterior nerves terminate at the oral tip. A few thin transverse connectives interconnect all the anterior nerves and encircle the pharynx (Pl.1.23, Fig.1; Pl.1.24, Figs.1,2). Minute branches from these transverse connectives innervate the tegument of the lateral, ventral and dorsal sides of the worm. All the anterior nerves are superficially located below the tegument.

Amongst the three pairs of the main posterior nerves, the thin postero-dorsals constitute the innermost pair (Pl.1.23, Fig.1; Pl.1.24, Fig.1). They emerge from the postero-middle facet of the cerebral ganglia and run caudad, parallel to the median axis. They innervate the alimentary canal and excretory bladder. The postero-inner ventrals arise from the ventro-middle facet of the cerebral ganglia. These nerves overlap the postero-dorsals at places but they generally run parallel

PLATE 1.23: **Orthocoelium streptocoelium**

Fig.1 Demonstration of the nervous system in the whole mount of the worm (Camera lucida sketch).

PLATE 1·23

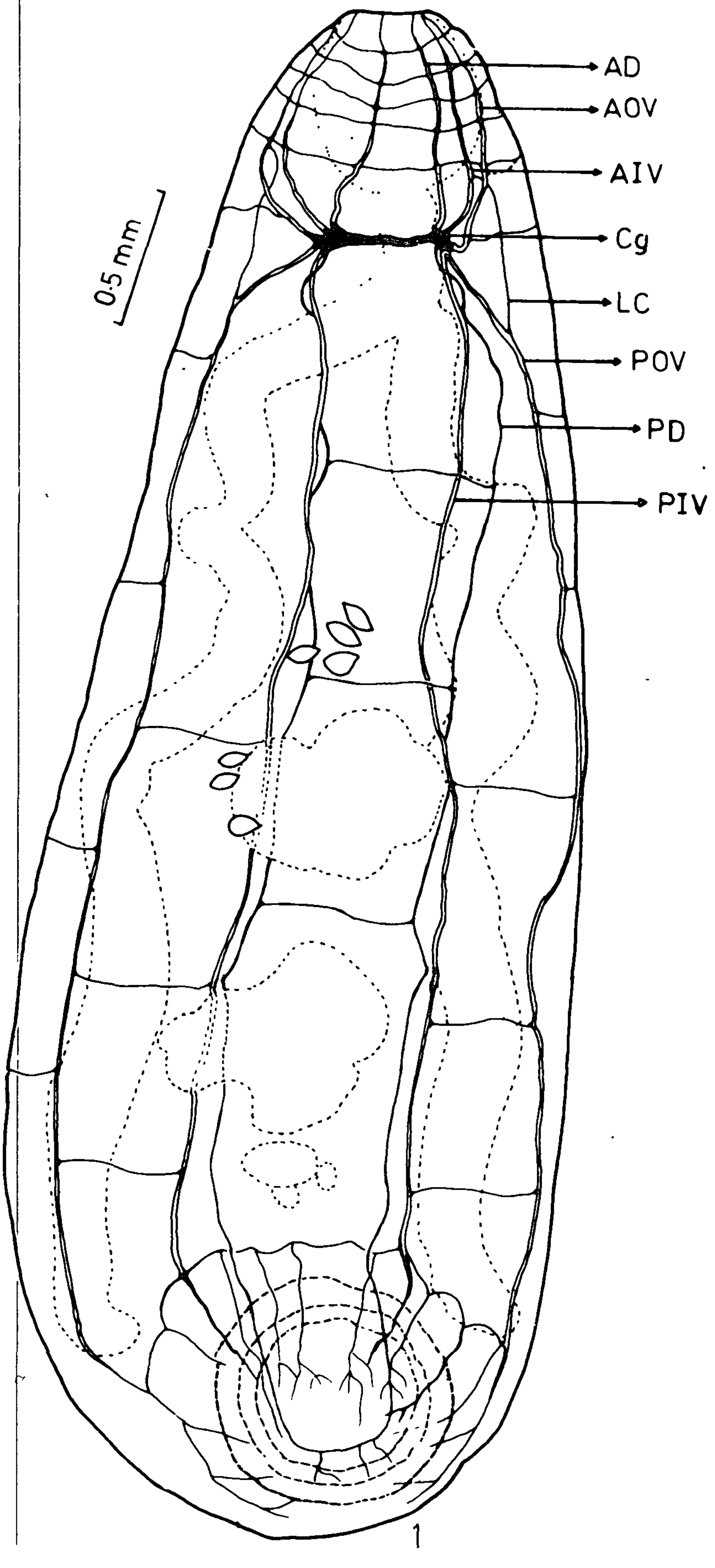

PLATE 1.24: **Orthocoelium streptocoelium** (Photomicrographs)

Fig.1 Brain mass and the anterior and posterior longitudinal nerves (Scale bar = 1.0 mm).

Fig.2 Anterior region of the worm under higher resolution. Transverse connectives of the anterior nerves are conspicuous (Scale bar = 0.5 mm).

Fig.3 Termination of the posterior longitudinal nerves in the acetabulum (Scale bar = 0.4 mm).

PLATE 1.24

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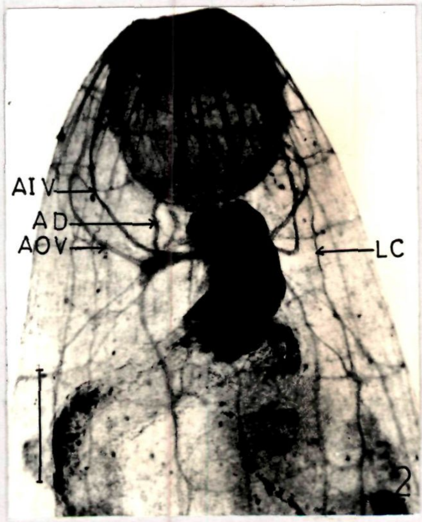

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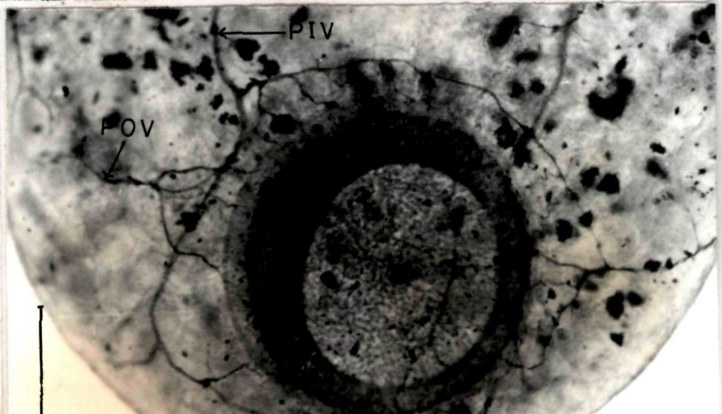

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to the latter up to the acetabular zone. The postero-outer ventrals originate from the lateral aspects of the cerebral ganglia and proceed parallel to the lateral body wall. Both the posterior ventral nerves are interconnected just anterior to the acetabulum by a transverse connective, covering lateral and ventral sides of the acetabulum (Pl.1.24, Fig.3). The postero-outer ventral nerves run posteriad beyond this connective lateral to the acetabulum to branch and supply its lateral aspects. Both the postero-dorsals extend and join medially in the acetabulum through a transverse connective. Many thin minute branches, given off from this transverse connective, also supply the dorsal aspects of the acetabulum (Pl.1.23, Fig.1; Pl.1.24, Fig.3).

Only a few transverse connectives interconnect the various posterior nerves at some places. One pair of longitudinal connectives, connecting the antero- and postero-outer ventrals, are present (Pl.1.23, Fig.1; Pl.1.24, Figs.1,2). These connectives in turn are interconnected with each other and with nerve net of the body.

The postero-inner ventrals are embedded in the parenchyma and the other two, i.e., postero-dorsals and outer ventrals, are superficially lodged.

Orthocoelium narayanai

The anterior pairs of nerves include one pair of dorsals and two pairs of ventrals. (Pl.1.25, Fig.1; Pl.1.26,

PLATE 1.25: **Orthocoelium narayanai**

Fig.1 Demonstration of the complete nervous system in the whole worm (Camera lucida).

PLATE 1·25

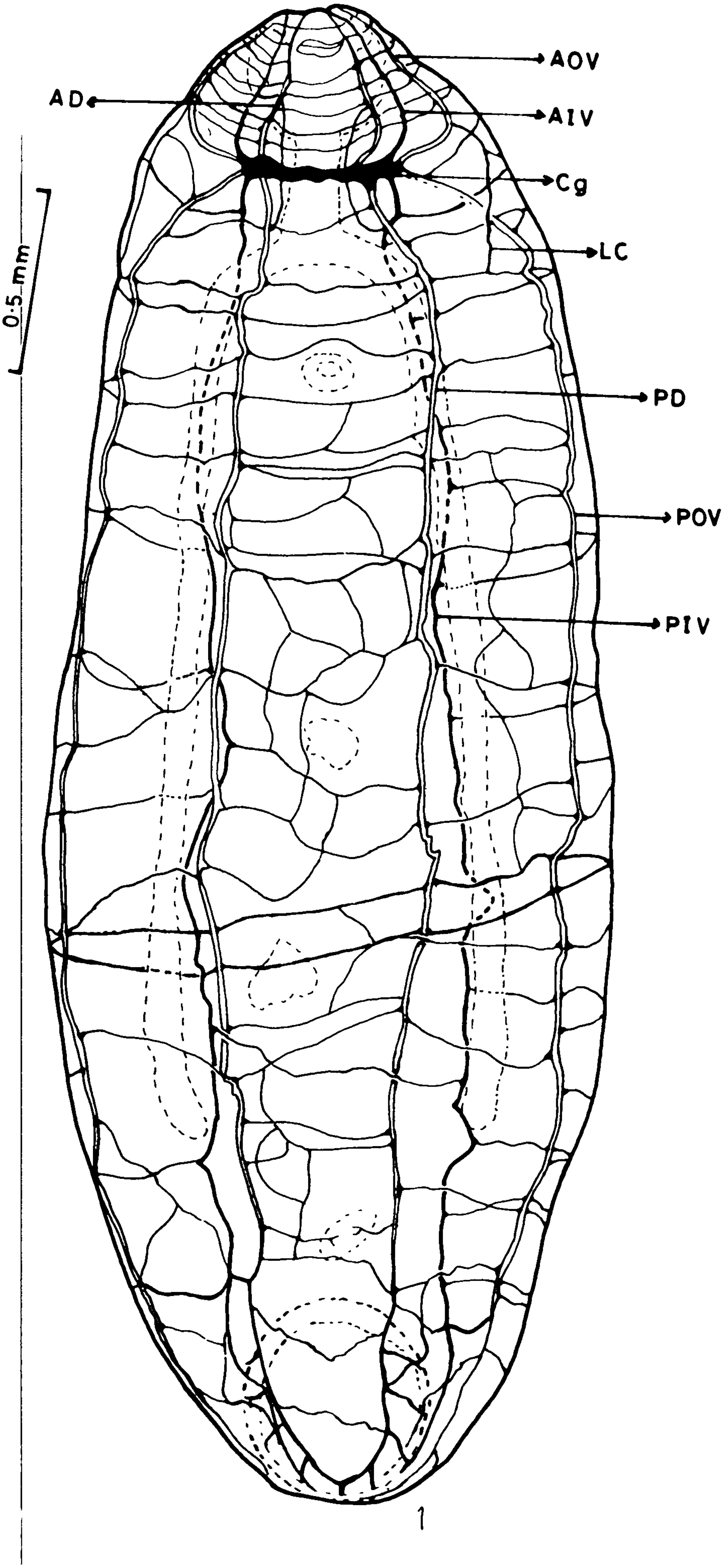


Fig.2). Thin ring-like conspicuous transverse connectives, encircling the pharynx, interconnect all of them with one another (Pl.1.26, Fig.2). The uppermost connective, lodged in the rim of the mouth, constitutes the oral nerve ring. Minute branches from the anterior transverse connectives innervate the pharynx and tegument of the region.

The posterior pairs of longitudinal nerves consist of one pair of dorsal nerves which are embedded deep in the parenchyma and two pairs of ventrals which are superficially lodged (Pl.1.25, Fig.1, Pl.1.26, Figs.1,3). The postero-dorsals emerge from the postero-dorsal aspects of the brain and extend up to the acetabulum. Tributaries from the postero-dorsals innervate the alimentary canal, excretory bladder and reproductive system. Posteriorly each of these nerves bifurcates just anterior to the acetabulum; the inner branch (i.e., towards the median axis) passes posteriorly and joins its fellow of the other side in the posterior region of the acetabulum (Pl.1.25, Fig.1; Pl.1.26, Fig.4).

The two pairs of ventrals include the postero-inner and outer ventrals (Pl.1.25, Fig.1; Pl.1.26, Figs.1,3). The inner ventrals arise from the ventro-middle facet of the cerebral ganglia and the outer ventrals, from their ventro-lateral aspect. All these nerves innervate the reproductive system, alimentary canal, excretory system and vitellaria. The two inner ventrals join each other and the postero-outer ventral of their side to form a prominent connective anterior to the

PLATE 1.26: **Orthocoelium narayanai** (Photomicrographs)

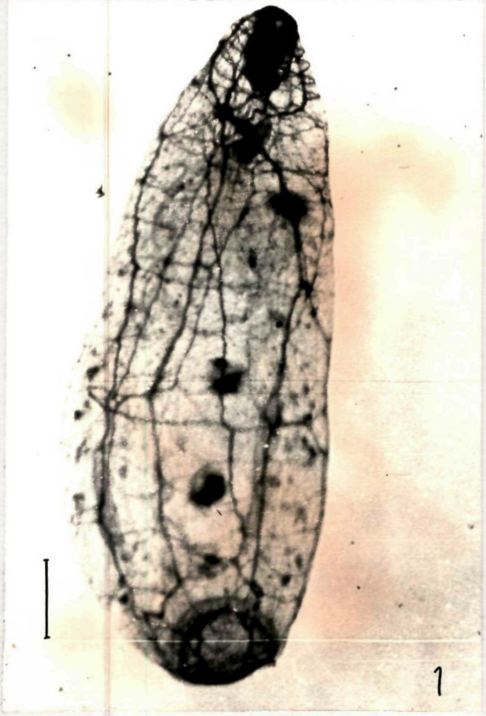
Fig.1 Complete nervous system in the whole mount of the worm (Scale bar = 1.0 mm).

Fig.2 Anterior region of the worm under higher resolution. Brain mass, anterior nerves and their tansverse connectives are evident (Scale bar = 0.5 mm).

Fig.3 The posterior longitudinal nerves and their nerve net in the post-cerebral region (Scale bar = 1.0 mm).

Fig.4 Terminal course of the posterior longitudinal nerves and innervation in the acetabulum (Scale bar = 1.0 mm).

PLATE 1.26



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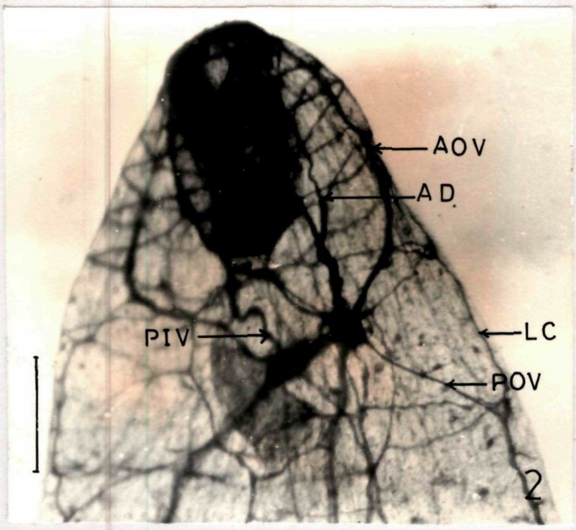

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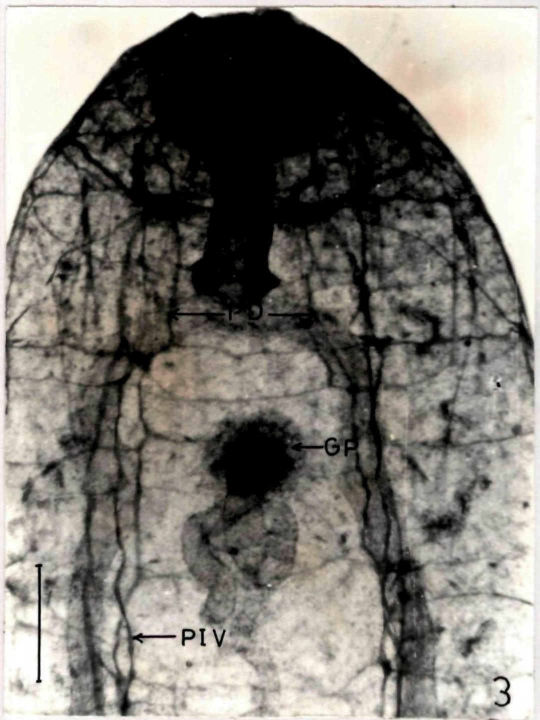

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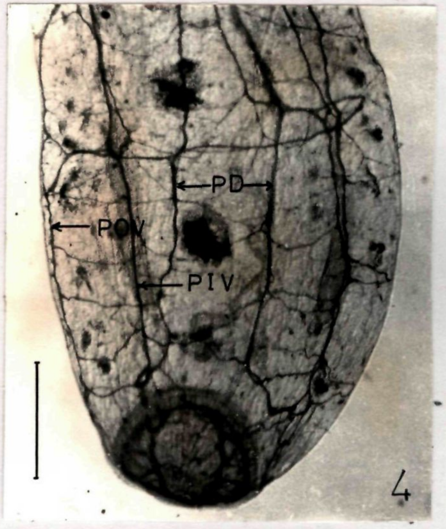

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acetabulum and terminate near the ventro-lateral aspects of the sucker. Each postero-outer ventral continues to proceed lateral to the acetabulum and terminates near the posterior edges of the acetabulum and innervates the lateral walls of the latter, giving off a few minute branches (Pl.1.25, Fig.1; Pl.1.26, Fig.4). The dorsal and ventral body nerve nets are constituted by thin transverse and longitudinal connectives interconnecting all the posterior nerves (Pl.1.25, Fig.1; Pl.1.26, Figs.1,3,4). A relatively thick and conspicuous ring-like connective joins all the posterior nerves in the testicular region (Pl.1.25, Fig.1; Pl.1.26, Fig.4).

One pair of genital nerves, derived from the inner ventrals, form an arch above the genital pore region and branches from this nerve arch innervate the genital pore (Pl.1.26, Fig.3).

A dorsal longitudinal connective connects the antero- and postero-dorsals of the same side. Likewise, an outer ventral longitudinal connective, present along the lateral body wall, interconnects the antero- and postero-outer ventrals of the same side. A third longitudinal pair joins the antero- and postero-inner ventrals (Pl.1.25, Fig.1; Pl.1.26, Figs.1,3). All the three longitudinal connectives are also connected with one another and with the nerve net of the body.

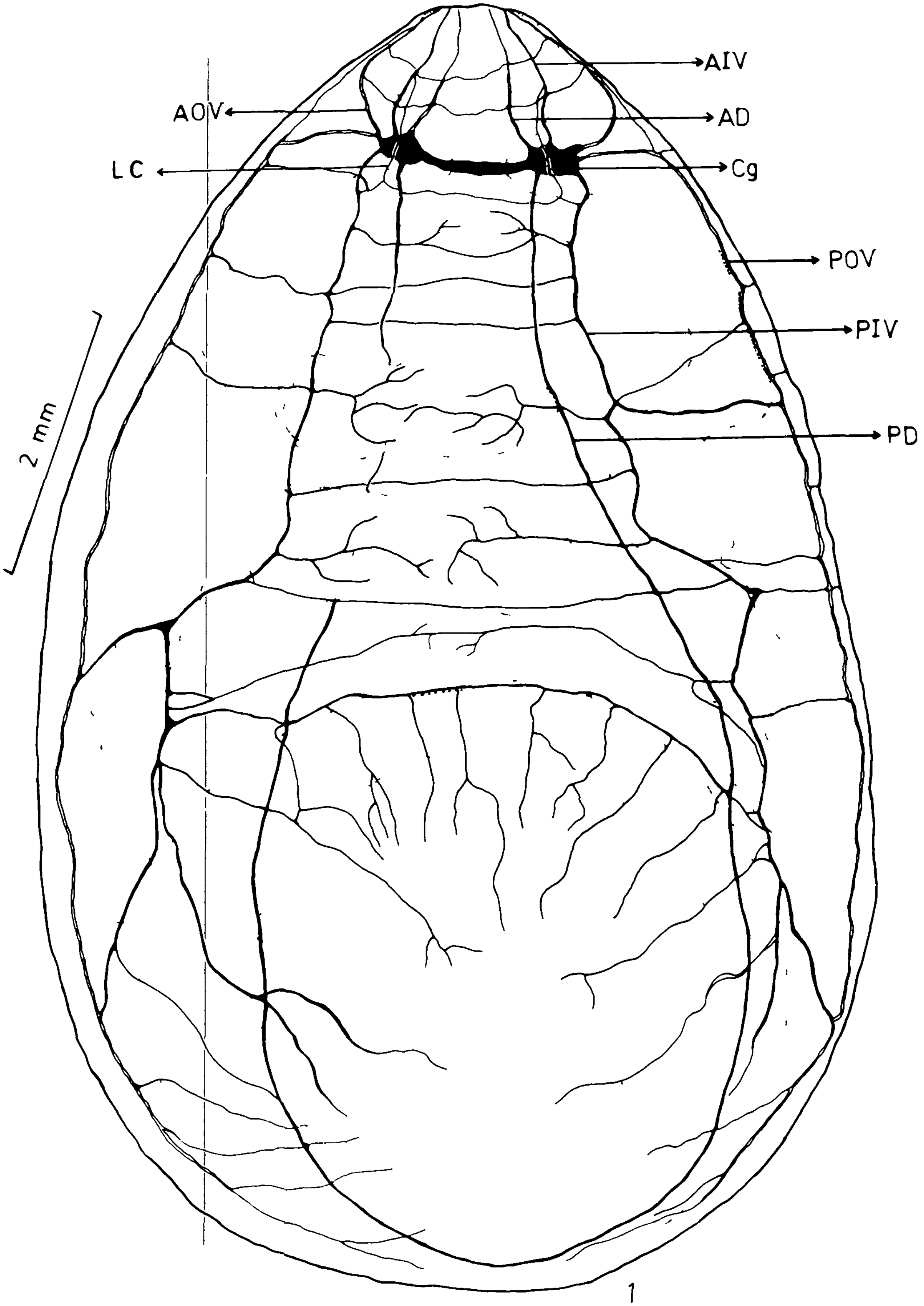
Explanatum explanatum

Three pairs each of anterior and posterior nerves

PLATE 1.27: **Explanatum explanatum**

Fig.1 Diagrammatic representation of nervous system in the whole worm (Camera lucida).

PLATE 1·27



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given out from the cerebral ganglia (Pl.1.27, Fig.1). As all of these nerves are deep seated in the parenchyma, the intervening thick tissue impairs their resolution in one focus, hence the problem in photomicrographic presentation of the nerve pattern in this worm.

Of the anterior nerves, the innermost pair comprises the antero-dorsals, which arise at the junction of the cerebral ganglia with the cerebral commissure and proceed upwards, supplying the oral tip and dorsal surface of the pharynx. Outer to this pair are the antero-inner ventrals, emerging from the antero-lateral aspect of the cerebral ganglia and running anteriad they innervate the pharynx at its posterior edges. The outermost nerve pair is of the antero-outer ventrals. They emerge from the antero-outer margin of the cerebral ganglia and proceed upwards parallel to the lateral body wall, supplying the ventro-lateral aspects of the pharynx. The anterior longitudinal trunks terminate near the oral tip. Only two or three ring-like transverse connectives were seen interconnecting all the anterior nerves (Pl.1.27,Fig.1; Pl.1.28, Fig.1).

The postcephalic longitudinal nerves comprise a pair of postero-dorsals and two pairs of postero-ventrals (Pl.1.27, Fig.1). The postero-dorsals originate from the postero-inner facet of the cerebral ganglia and run caudad. Fine branches from these nerves innervate the gut, reproductive system, excretory bladder and excretory duct. The postero-dorsals of the two sides are joined with each other near the posterior

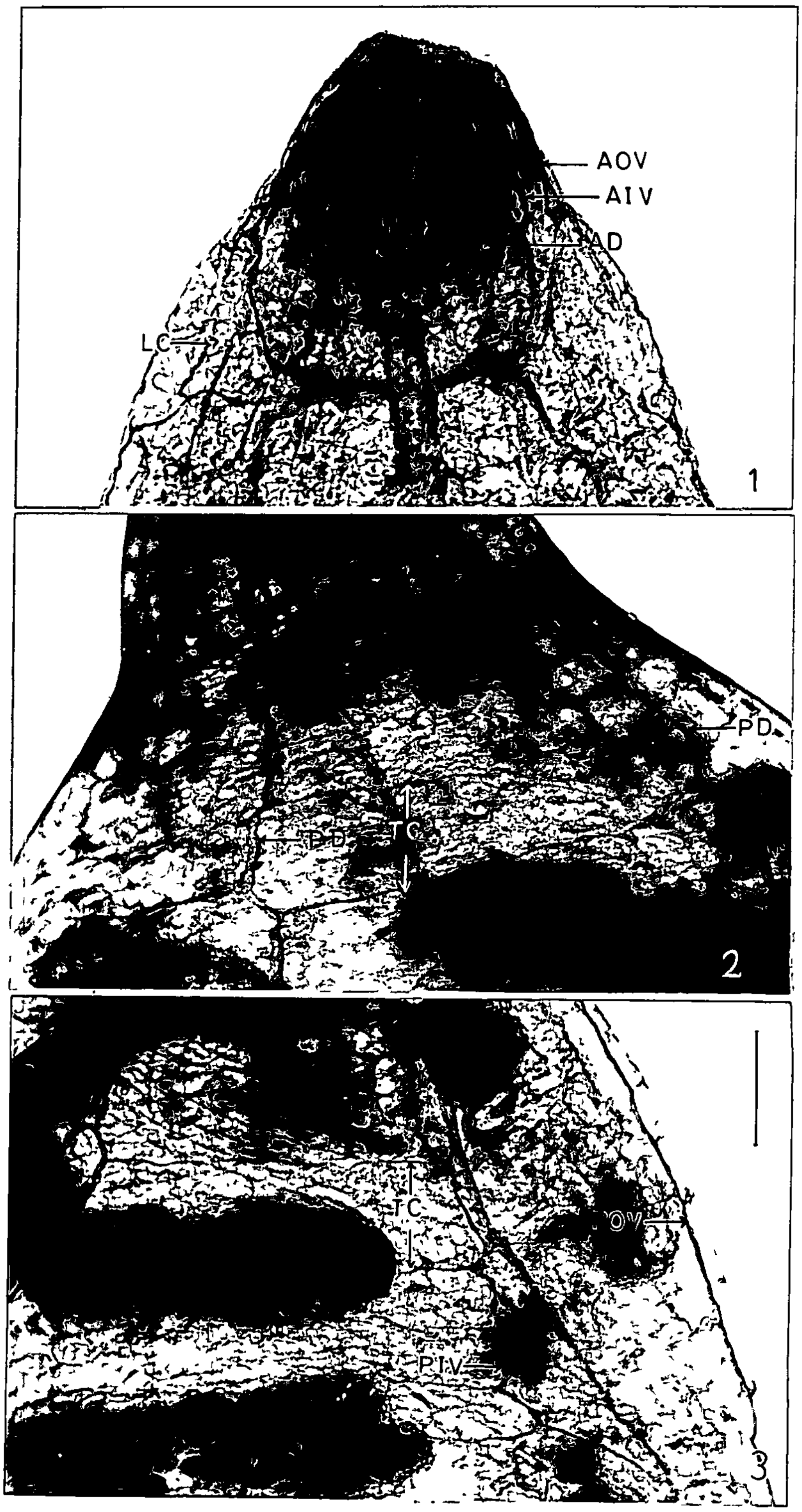
PLATE 1.28: **Explanatum explanatum** (Photomicrographs)

Fig.1 Brain mass and the main anterior longitudinal nerves in the pre-cerebral region (Scale bar = 1.0 mm).

Fig.2 Postero-dorsals and their nerve net in the anterior half of the body (Scale bar = 0.5 mm).

Fig.3 Postero-ventrals and their transverse connectives in the longitudinal half of the body (Scale bar = 0.5 mm).

PLATE 1.28



extremity of the acetabulum (Pl.1.27, Fig.1). Thin transverse connectives interconnect these two nerves with each other and with the postero-outer ventrals of their side throughout their course (Pl.1.28, Fig.2), thus forming a fine nerve net under the dorsal surface of the body. The postero-inner ventrals arise from the postero-ventral border of the cephalic ganglia and terminate in the mid-ventral region of the acetabulum where they split out into a few branches to supply the anterior and mid ventral aspects of the latter. These nerves also innervate the genital pore, reproductive components and excretory bladder. The postero-outer ventrals originate from the postero-lateral facet of the cerebral ganglia and run posteriad to terminate near the hind extremity of the body, innervating on the way the vitellaria, excretory duct, lateral body wall and lateral edges of the acetabulum (Pl.1.27, Fig.1).

Along their whole length the two postero-inner ventrals of either side are joined with each other and with the postero-outer ventrals of their side by many thin but conspicuous transverse connectives (Pl.1.28, Fig.3), constituting a ventral surface nerve net. The postero-inner and outer ventrals are also connected with their corresponding anterior trunks of their side by means of thin, longitudinal connectives (Pl.1.27, Fig.1; Pl.1.28, Fig.1).

A slight increase in the substrate concentration in the incubation medium also reveals the subtegumental muscle fibres along with the nerves (Pl.1.28, Fig.3).

Homalogaster paloniae

In this intestinal fluke also, like the earlier described paramphistomes the central nervous system comprises a pair of large cerebral ganglia that are connected by a stout cerebral commissure, situated dorsal to the oesophagus near its mid length, and three pairs each of the anterior and posterior longitudinal nerves (Pl. 1.29, Fig.1).

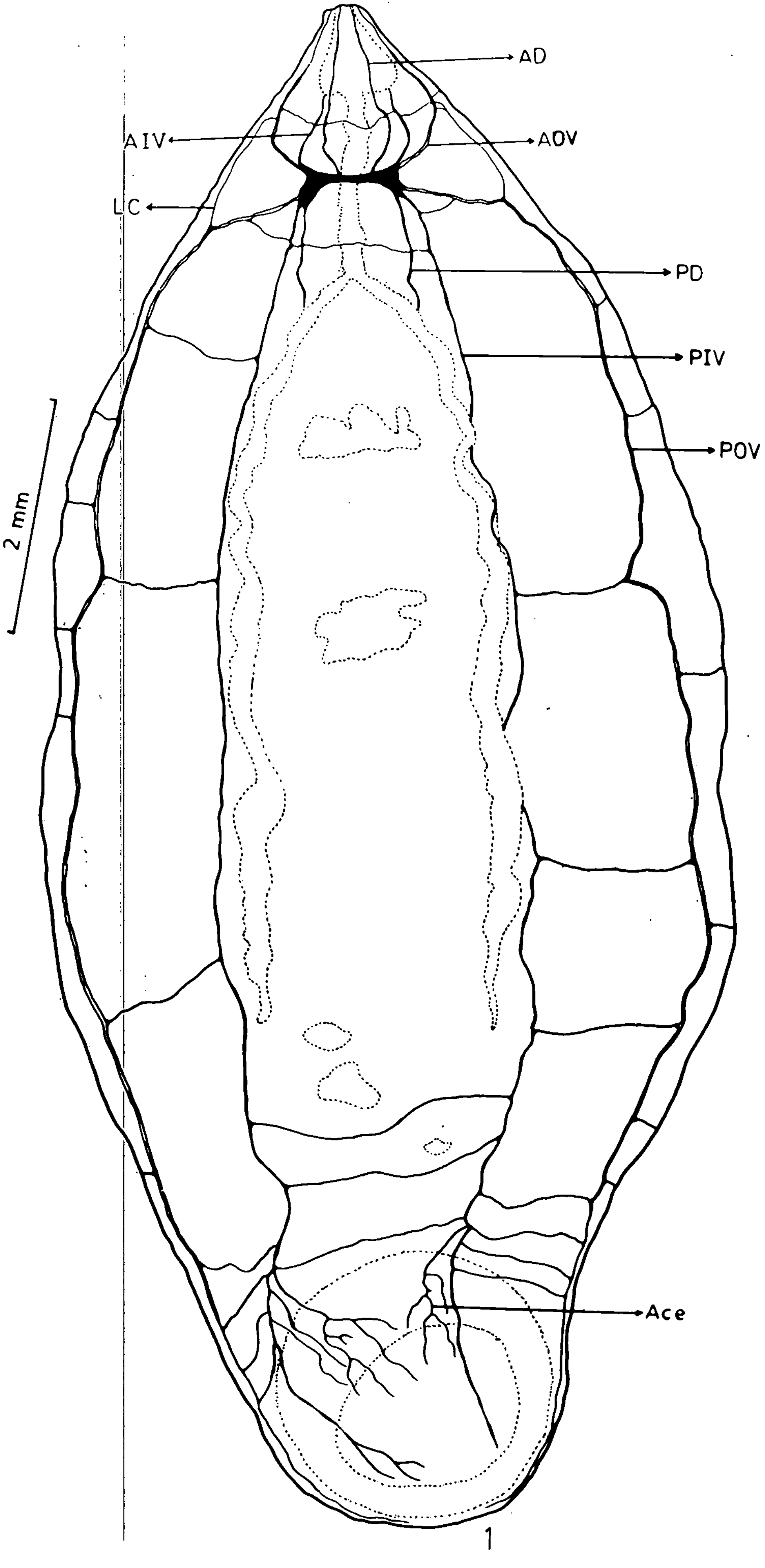
In the pre-cerebral region the anterior longitudinal nerves include one pair each of antero-dorsals, antero-inner and outer ventrals (Pl. 1.29, Fig.1; Pl.1.30, Fig.1). The anterior trunks terminate in a ring surrounding the oral opening. The antero-dorsals emerge from the cerebral commissure, one on either side of the oesophagus and run anteriad parallel to the latter and dorsal to the pharynx. Outer to these are the antero-inner ventrals which, emerging from the ventro-lateral aspect of the cerebral ganglia and running anteriad, innervate the pharynx at its posterior edges. The antero-outer ventrals form the outermost nerve pair. These emerge from the lateral aspects of the cerebral ganglia and also proceed parallel to the pharynx. A single transverse connective interconnects all the anterior nerves, a little distance away from the pharynx (Pl.1.29, Fig.1).

The posterior nerve trunks are unequal in their extension. Among the more conspicuous nerves are the postero-inner ventrals and postero-dorsals (Pl.1.29, Fig.1; Pl.1.30, Fig.1). The postero-dorsals are the innermost pair, thin and least

PLATE 1.29: **Homalogaster paloniae**

Fig.1 Diagrammatic representation of the nervous system in the whole worm (Camera lucida).

PLATE 1·29



developed, emerging from the postero-inner facet of the cerebral ganglia and extend caudad up to the region of intestinal bifurcation where they peter out in the parenchymatous tissue (Pl.1.29, Fig.1). Fine branches from these nerves innervate the oesophagus and anterior portions of the intestinal diverticula. The postero-inner ventrals emerge from the postero-middle facet of the cerebral ganglia and run posteriorly, parallel to the intestinal caeca up to the posterior extremity of the acetabulum. Just anterior to the latter these nerves bifurcate to give two branches — the inner one proceeds as acetabular nerve to supply the anterior and median aspects of the acetabulum, whereas the outer branch joins the postero-outer ventral of its side by thick transverse connective (Pl.1.29, Fig.1; Pl.1.30, Fig.2). On their way to the acetabulum the postero-inner ventrals innervate the gut, components of the reproductive system, excretory bladder and duct and ventral aspects of the acetabulum. Just anterior to the latter also these nerves of either side are joined with each other by means of two to three transverse connectives (Pl.1.30, Fig.3). The postero-outer ventrals emerge from the lateral facets of the cerebral ganglia in an arch and proceed caudad, parallel and close to the lateral body wall, terminating just below the acetabulum near the hind end of the body (Pl.1.29, Fig.1; Pl.1.30, Fig.2). Throughout their course they are connected with the postero-inner ventrals by way of 4-5 distantly placed transverse connectives. These nerves also innervate the lateral body wall through transverse branches. The postero-

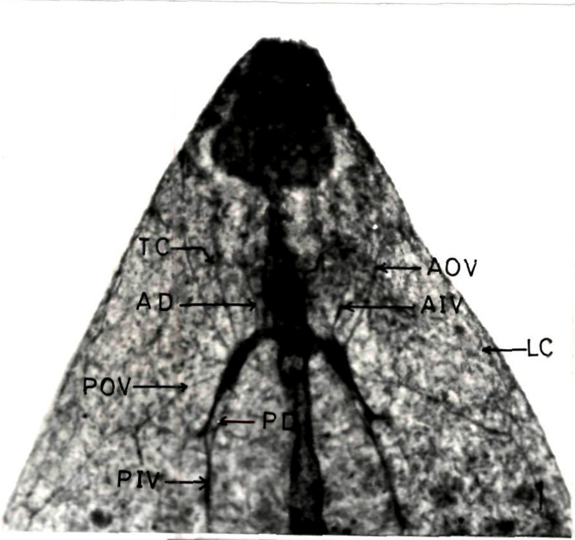
PLATE 1.30: **Homalogaster paloniae** (Photomicrographs)

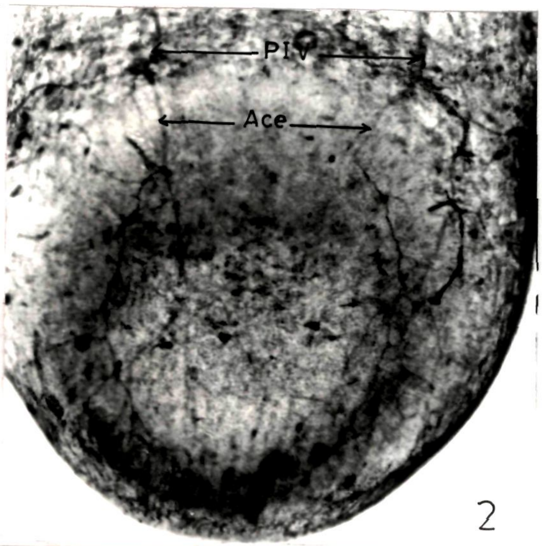
Fig.1 Brain mass and the anterior and posterior nerves (Scale bar = 0.5 mm).

Fig.2 Postero- inner ventrals birfurcating just anterior to the acetabulum to give rise to the acetabular nerves (Scale bar = 0.5 mm).

Fig.3 2-3 thin transverse connectives between the postero-inner and outer ventrals, present anterior to the acetabulum (Scale bar = 0.5 mm).

PLATE 1.30

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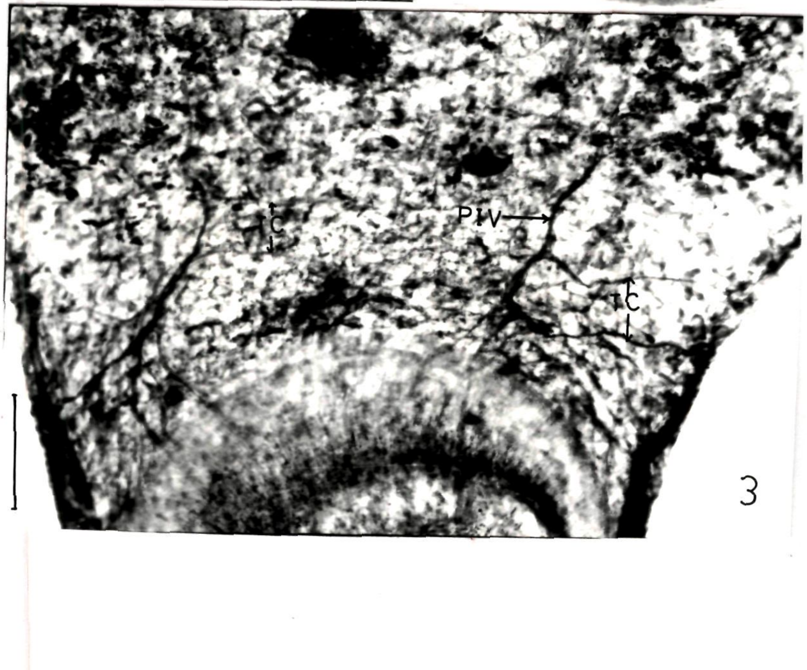
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outer ventrals are connected with their corresponding anterior trunks by longitudinal connectives (Pl.1.29, Fig.1; Pl.1.30, Fig.1). Both these nerves of either side innervate the vitellaria, excretory duct and lateral aspects of the acetabulum.

Eurytrema pancreaticum

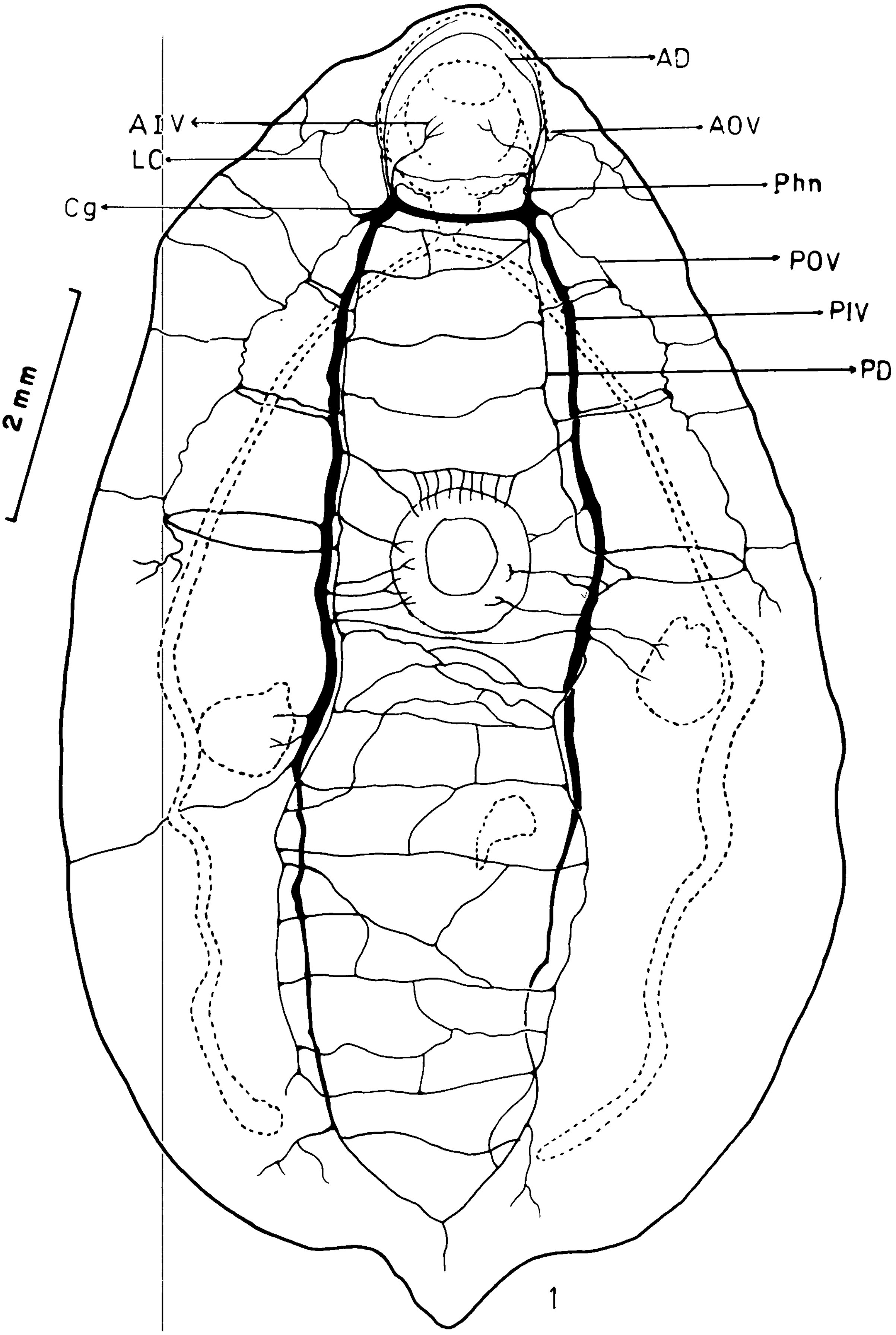
Like paramphistomes, in this trematode also the nervous system is essentially rectilineal. The two cerebral ganglia are situated postero-lateral to the oral sucker and dorsal to the pharynx, connected by a dorsal cerebral commissure (Pl.1.31, Fig.1; Pl.1.32, Fig.1).

From the cerebral ganglia four pairs of anterior nerves are given out three of which namely, the antero-dorsal, antero-inner ventral and antero-outer ventral proceed cephalad, while the fourth pair comprises the thin pharyngeal nerves that innervate the lateral aspects of the pharynx (Pl.1.31, Fig.1; Pl.1.32, Fig.2). Each antero-dorsal arises at the junction of the cerebral ganglion with the cerebral commissure. From here it proceeds anteriorly, dorsal to the oral sucker and just below the oral tip the two nerves of either side join with each other. The antero-inner ventrals, just after their emergence from the mid-ventral facet of the cerebral ganglia, form a roughly 'S'-shaped curve and innervate the pharynx at its posterior aspects. The antero-outer ventrals emerge from the ventro-lateral aspects of the cerebral ganglia and proceed anteriad, terminating near the oral extremity of the body. A thin single transverse connective joins the

PLATE 1.31: **Eurytrema pancreaticum**

Fig.1 Diagrammatic representation of the nervous system in the whole worm (Camera lucida sketch).

PLATE 1·31



anterior nerves of either side near the lower portion of the oral sucker.

Of the three pairs of longitudinal nerves extending in the post-cerebral region, one pair is of the postero-dorsal and the rest are ventral nerves that include a pair each of postero-inner and outer ventrals (Pl.1.31, Fig.1; Pl.1.32, Fig.2). The postero-dorsals arise from the dorso-posterior portion of the cerebral ganglia and run posteriorly up to the region of caecal termination. Thin transverse connectives join the two postero-dorsals throughout their length to constitute a dorsal nerve net (Pl.1.31, Fig.1; Pl.1.32, Fig.4). The postero-inner ventrals arise from the ventro-lateral margins of the cerebral ganglia and assume a similar course like that of the dorsals. However, in the region of the ventral sucker thin branches are given out from the inner facet of these nerves. The anterior most of these branches unite medially to form a transverse connective just in front of the ventral sucker (Pl.1.31, Fig.1; Pl.1.32, Figs.2,3). Many thin, slender branches emerge from this connective and supply the anterior edges of the sucker, while the second and third pair of transverse branches ramify immensely and supply its middle and posterior edges. The thin transverse connectives and their branches joining the two inner ventrals also contribute a ventral nerve net in the post-acetabular region. The outermost nerve pair is of the postero-outer ventrals that originate from the postero-lateral facets of the cerebral ganglia and continue running posteriad only up to the pre-testicular

PLATE 1.32: **Eurytrema pancreaticum** (Photomicrographs)

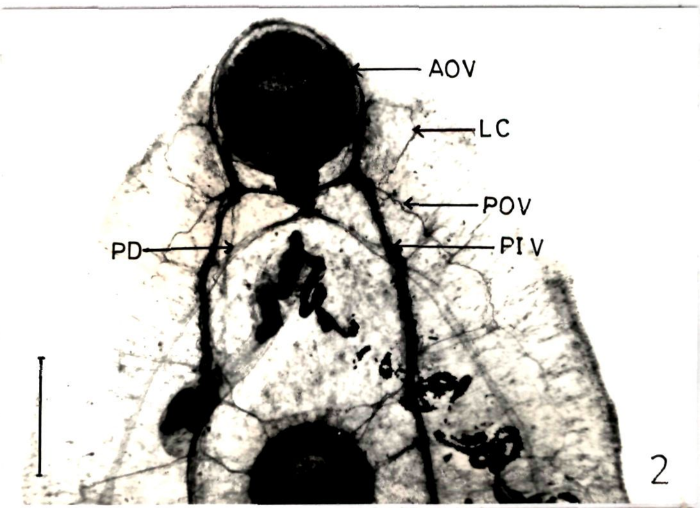
Fig.1 Complete nervous system in the whole mount of the worm. (Scale bar = 1.0 mm).

Fig.2 Brain mass and the main anterior and posterior nerves (Scale bar = 1.0 mm).

Fig.3 Transverse connective (arrows) formed by the postero-inner ventral nerves just before the ventral sucker. Fine branches from it are seen supplying the latter (Scale bar = 0.4 mm).

Fig.4 Nerve net of the postero-dorsals in the mid body region (Scale bar = 0.4 mm).

PLATE 1.32



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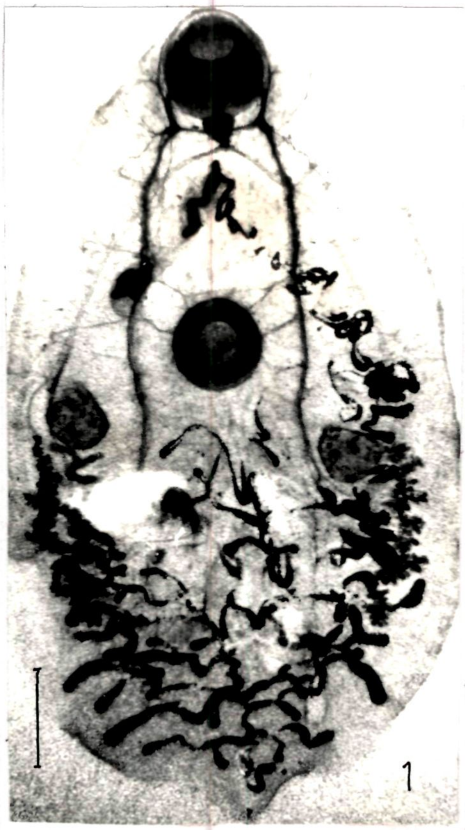

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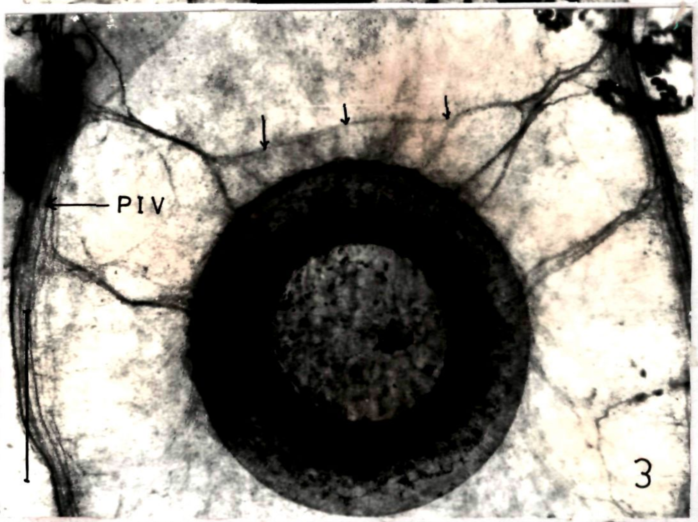

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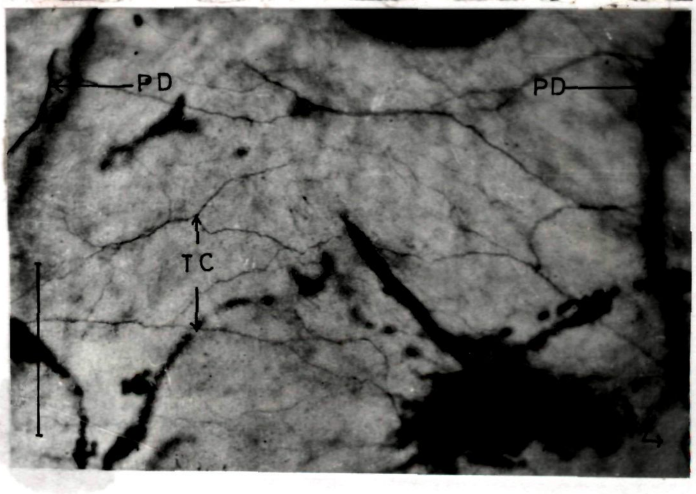

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region, where they peter out in the parenchymatous tissue (Pl.1.31, Fig.1; Pl.1.32, Figs.1,2). These nerves are connected with the other two pairs of nerves at three places by means of ring-like connectives. The postero-outer ventrals are also connected with their corresponding anterior trunk by a semi-circular connective towards the lateral body wall (Pl.1.32, Fig.2).

Fasciola gigantica

A pair of cerebral ganglia connected by a cerebral commissure lie near the anterior margin of and dorsal to the pharynx. Like other digeneans in **F. gigantica** also the main longitudinal nerves include three pairs each of anterior and posterior nerves (Pl.1.33, Fig.1; Pl.1.34, Fig.1).

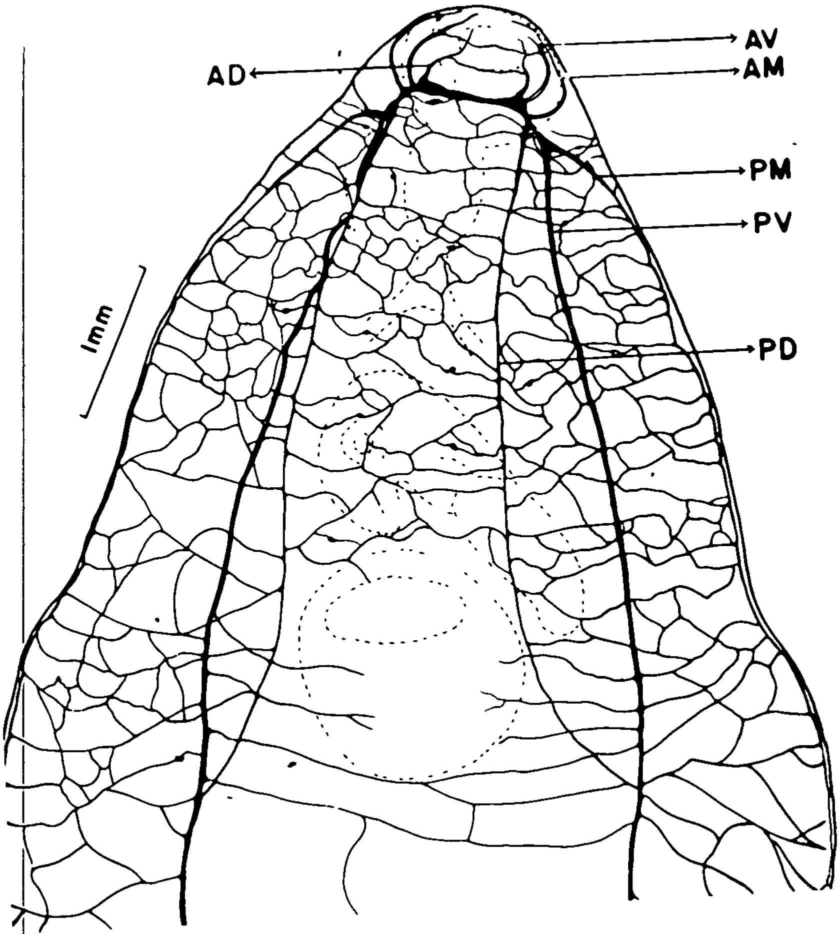
Among the nerves running anterior to the cerebral ganglia, the innermost pair is of antero-dorsals, emerging from the dorso-middle facet of the cerebral ganglia, running dorsal to the oral sucker and terminating at the oral rim (Pl.1.33, Fig.1). These nerves of the two sides are joined with a single transverse connective. Outer to this pair are present the antero-ventrals, originating from the lateral aspects of the cerebral ganglia (Pl.1.33, Fig.1). These travel anteriad along the lateral margins of the oral sucker, becoming thinner towards the oral-rim where they peter out in the tegument of the anterior region. These two nerves are also inter-joined by a thin transverse connective. The nerves

PLATE 1.33: *Fasciola gigantica*

Fig.1,2 Diagramatic representation of the nervous system in the whole worm.

1. Anterior region of the worm, showing the brain mass, anterior and posterior longitudinal nerves and their nerve net.
2. Posterior portion of the body, showing the postero-ventral andmarginal nerves and their nerve net.

PLATE 1·33



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of the marginal pair, which is the outer-most pair, emerge from the lateral sides of the cerebral ganglia, run anteriad lateral to the oral sucker and terminate near the anterior extremity of the body, innervating on their way the lateral and anterior edges of the oral sucker (Pl.1.33, Fig.1). All the anterior nerves innervate the tegument through fine minute branches.

Among the posterior pairs of nerves, the best developed is the ventral pair, which is the main nerve to give off branches to supply the various organs of the body (Pl.1.33, Figs.1,2; Pl.1.34, Figs.3,5). The postero-dorsals are the innermost pair consisting of thin, slender nerves, originating from the inner facet of the cerebral ganglia (Pl.1.33, Fig.1; Pl.1.34, Fig.1) and running posteriad up to the immediate post-acetabular region where they join the main ventral trunks on their side, a little distance away from the ventral sucker. The two postero-dorsals are interlinked in the pre-acetabular region with each other and also to the postero-marginal nerve of their side by means of numerous thin transverse connectives which further branch and anastomose to constitute a fine nerve net. A few presumptive neurosecretory cells appear strewn all along the nerve net in the pre-acetabular region (Pl.1.34, Fig.2). The outermost pair is of extremely thin marginal nerves which emerge from the lateral aspects of the cerebral ganglia and run caudad (Pl.1.33, Fig.1; Pl.1.34, Fig.1), parallel and close to the lateral body wall up to the hind end of the body to terminate near the excretory

PLATE 1.34: **Fasciola gigantica** (Photomicrographs)

Fig.1 Brain mass and the main anterior and posterior nerves (Scale bar = 1.0 mm).

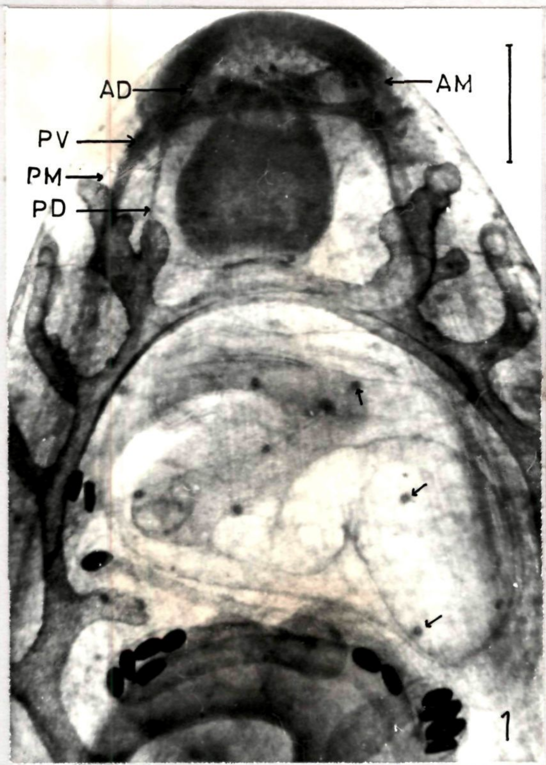
Fig.2 Postero-dorsals and their nerve net in the pre-acetabular region. Note the associated nerve cells (arrows) (Scale bar = 1.0 mm).

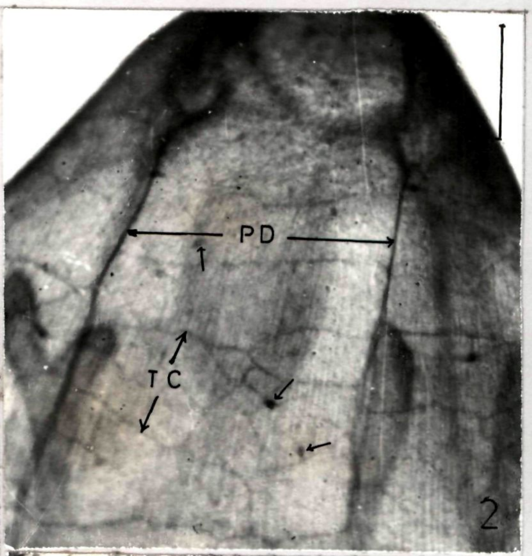
Fig.3 Postero-ventrals in the anterior half of the body, innervating the ventral sucker through their transverse nerves (Scale bar = 1.0 mm).

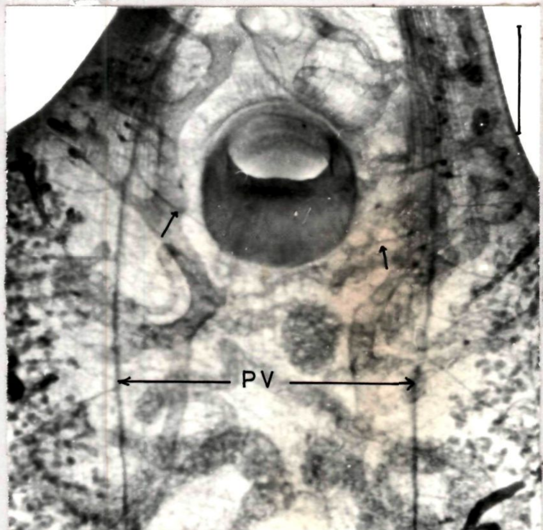
Fig.4 A fine nerve net between the postero-ventral and marginal nerves approximating the lateral body wall of the worm (Scale bar = 0.4 mm).

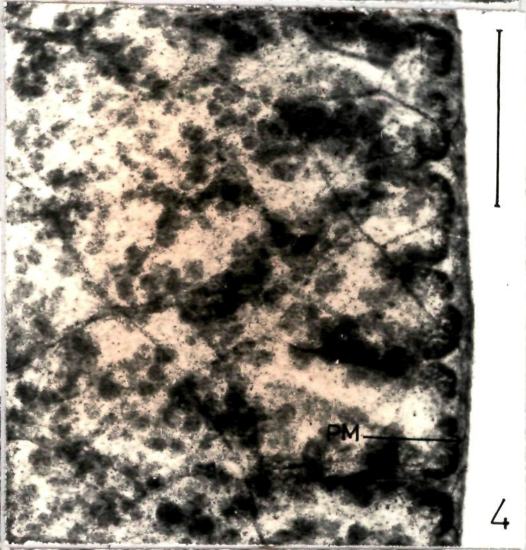
Fig.5 Postero-ventrals joining near the posterior extremity of the body (arrow) (Scale bar = 1.0 mm).

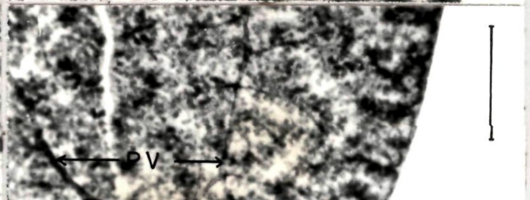
PLATE 1.34











pore. Minute fine branches from these nerves are seen terminating in the body tegument. The postero-ventral pair consists of two thick nerves originating from the ventro-lateral aspects of the cerebral ganglia. They run caudad parallel to the longitudinal vitelline duct. Just a short distance away from the hind extremity of the body, the postero-ventrals of either side join each other and further split out to give a few slender, downwardly directed branches, which further branch to join the marginal nerves (Pl.1.34, Fig.5). Both ventrals are connected with each other and also with the postero-marginals of their side by means of long, diagonal and transverse connectives (Pl.1.33, Fig.2; Pl.1.34,Fig.3,5). Numerous fine branches may be seen stemming from these connectives towards the lateral body wall and branching further to join finally the marginal nerves. In this way a conspicuous nerve plexus approximating the lateral body margins is formed (Pl.1.33, Fig.2; Pl.1.34, Fig.4). Throughout their course, the postero-ventrals give off numerous branches to supply the ventral sucker, intestinal diverticula, components of the reproductive system, excretory system and the tegument.

B. Histocytology of the nervous tissue

Brain:

The so-called "brain", consisting of a pair of cerebral ganglia that are connected by a band-like cerebral commissure, is composed of a fibrous ground substance that makes up the central zone or neuropile; cellular components restricted

PLATE 1.35: **Fischoederius cobboldi** - Histocytology and histochemistry of the nervous tissue (Photomicrographs)

Fig.1 Cerebral ganglion in a sagittal section revealing the presence of fibrous ground substance, neuroglial cells (small arrow), ganglion cells (nerve cell/large arrow) and putative neurosecretory cell(pNSC) CHPh.

Fig.2 A portion of longitudinal nerve cord in a sagittal section revealing the presence of ganglion cells, neuroglial cells and fibrous ground substance.

Fig.3 A large multipolar nerve cell (arrow) (putative neurosecretory cell) approximating a nerve cord in the general parenchyma. Silver impregnation method.

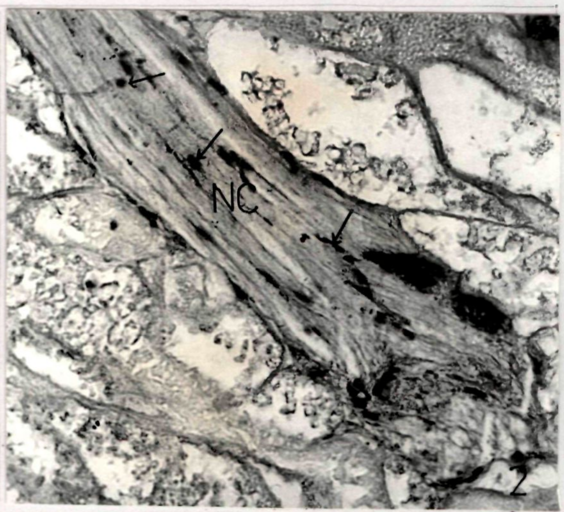
Fig.4 A unipolar nerve cell (arrow) in the subtegumental region. Silver impregnation method.

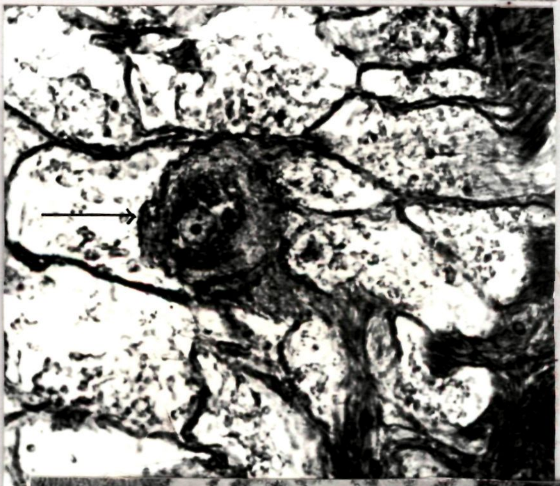
Fig.5 Cerebral ganglia in a sagittal section, showing a weakly positive reaction for Butylcholinesterase. Thiocholine iodide method.

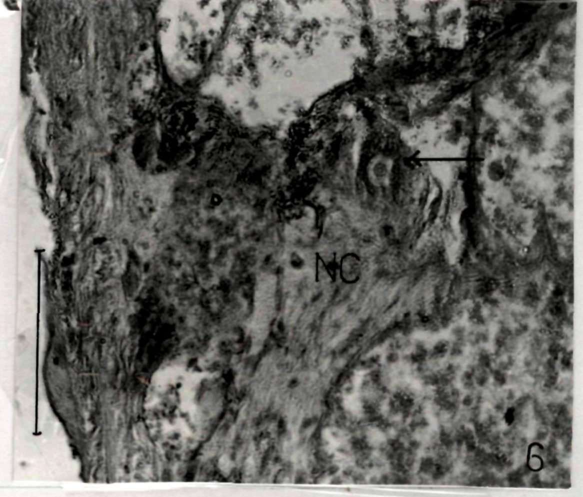
Fig.6 A portion of the nerve cord in a sagittal section showing a moderately positive reaction for Acetylcholinesterase. Thiocholine iodide method.

(Figs 1-6, Scale bar = 0.05 mm).

PLATE 1.35







towards the periphery constitute the outer zone or cortical layer of the brain (Pl.1.35, Fig.1).

The differentiation of neuropile was conspicuous in the anterior and posterior lateral aspects of the brain where the nerve fibres are arranged in parallel tracts just before forming longitudinal cords. The cellular components included two types of cells : (i) the nerve cells or ganglion cells, characterized by a distinct cell body, moderate to dense cytoplasm, a conspicuous nucleus and a well-marked nucleolus, and probably having a nervous function (Pl.1.35, Fig.1), and (ii) neuroglial cells, comparatively small in size and characterized by having a little cytoplasm which is often not markedly exhibited, and a prominent nucleus; their position between the nerve fibres (Pl.1.35, Fig.1) indicates a possible supporting function. The ganglion cells constitute the outer zone of the brain. They are mostly uni-or bipolar. Some ganglion cells were relatively large in size, with conspicuous granular secretion in their cytoplasm, exhibiting a presumptive neurosecretory nature (Pl.1.35, Fig.1) (see chapter II - presumptive neurosecretory cells - pNSC). The neuroglial cells are scattered in between the fibrous tissue and ganglion cells. These cells stain bright red with Heidenhain's azan method and pinkish red with chrome haematoxylin phloxin method.

Longitudinal nerves

All the longitudinal nerves are also composed of closely packed slender 'neurofibres', running parallel to one another and are found to be unmyelinated. Both the types of cellular components, i.e., ganglion cells and neuroglial cells, were found scattered in the fibrous tissue of nerves and their transverse connectives. The neuroglial cells were numerous, mostly oval in shape and compressed in between the nerve fibres (Pl.1.35, Fig.2). The ganglion cells were relatively very few in number (Pl.1.35, Fig.2). A few uni- and bipolar pNSC identical with those observed in the brain were also visible in the nerve cords.

The silver impregnation technique revealed the nervous tissue that constitutes different parts of the nervous system and some nerve cells in, and in association with, the nervous system and also in the body of the worm, approximating various organs like oesophagus, intestine, reproductive components, lymph vessels, vitellaria, and oral and ventral sucker regions (Pl.1.35, Figs.3,4).

Histochemically, the NSE activity was demonstrated intensely throughout the nervous system and was further completely inhibited when eserine was included in the incubation medium, thereby indicating that AChE was the principal enzyme present. For the specific cholinesterase type, both AChE and BChE were evidenced in the nervous tissue, the former being much more intense than the latter (Pl.1.35,Figs.5,6).

DISCUSSION

Nonspecific esterases (NSE) of carboxylesterase group hydrolyse the indoxyl acetate (Pearse, 1972). The components of nervous system were clearly visible using this substrate for localizing the nonspecific esterases. The principle involved in the method is that hydrolysis of a soluble ester of indoxyl (indoxyl acetate) by the enzyme NSE brings about the liberation of free indoxyl and this free indoxyl is further oxidized by atmospheric oxygen to bright indigo, an insoluble product. The intensity of the indigo (crystal) is favoured by an acidic pH of the solution (Pearse, 1972).

In the present investigation the neuroanatomy of thirteen paramphistome species and two parenteric flukes has been revealed by indigogenic technique for localization of NSE, the association of which with the nervous system has been established. However, Roy (1980) observed no NSE activity in the nervous system of **Ceylonocotyle scoliocoelium,** a bovine paramphistome, using napthyl acetate as the substrate. Similarly, in **Pharyngostomoides adenocephala** the nervous system lacks NSE activity (Allen and Harkema, 1972). Instead, in both these worms AChE activity is evidenced in the nervous tissue.

A comparison of the nerve pattern in all the paramphistomes studied herein with other paramphistomes studied earlier, i.e., **F. elongatus, Parorientodiscus magnus** and **Paramphistomum calicophorum** (see Lee, 1971); **F. elongatus** and **G. crumenifer** (see Brandes, 1898; Otto, 1896) and **F. cobboldi** (see Gupta and Dutta, 1967) brings out several differences, although revealing some general resemblances. In all these forms in the pre-cerebral region there are three pairs of anterior longitudinal nerves except in **O. indica** and **P. gracile** where an extra pair of pharyngeal nerves also exist. Otto (1896) and Gupta and Dutta (1967) have also reported only 3 pairs of anterior longitudinal nerves. Fukui (1929) in some unspecified Japanese amphistomes and Lee (1971) and Brandes (1898) have also reported the fourth pair of pharyngeal nerves in their studies. An additional pair of anterior nerves, called the lateral nerves, referred to by Lee and Brandes in **P. calicophorum** could not be traced in the present investigation. Brandes reported two accessory (dorsal and ventral) anterior nerves in **F. elongatus** and three accessory anterior nerves in **G. crumenifer**; these nerves arise independently and are distinct from the basic pairs of anterior nerves. However, no such nerves were observed in the present study of paramphistomes. Lee stated the splitting of one of the anterior ventral nerves into two, thus mentioning the occurrence of three ventral nerves on both sides. In the present investigation all the anterior nerves run singly up to the oral tip. Ring-like thin transverse connectives, joining the anterior pairs of nerves of the two sides and completely encircling the pharynx, are conspicuous in all the paramphistome species under the present investigation. In **O. orthocoelium** an oral nerve ring, a little behind the oral rim is very conspicuous.

From the former many thin and minute nerves emanate and extend to innervate the inner aspects of the oral rim. Lee (1971) mentioned only few poorly developed ventral commissures in the three paramphistome species studied. Fukui (1929) also mentioned some transverse commissures but did not state which nerves they connect. Anterior commissures are figured in **F. elongatus** and **G. crumenifer** by Brandes (1898). The paired oesophageal nerves or a single unpaired oesophageal nerve, as described by Lee (1971), Otto (1896) and Brandes (1898), were not found to be present in the forms under the present study.

The occurrence of three pairs of posterior longitudinal nerves is the most constant feature of the nervous system of the paramphistomes studied till now. In the present investigation also there is one pair of postero-dorsals and two pairs of postero-ventrals; the latter include the outer ventral or lateral nerves and the inner ventral or median nerves, termed so in accordance with their course of run. Lee (1971) also reported the presence of one pair of postero-dorsals along with one pair each of postero-laterals and postero-ventrals in **F. elongatus** and similar is the observation of Gupta and Dutta (1967) in **F. cobboldi**. In all the paramphistome species studied herein except **P. gracile** and **Homalogaster paloniae** all the posterior longitudinal nerves terminate in or near the acetabulum, an observation which agrees with the findings of Lee, Brandes and Otto; in **P. gracile the** postero-inner ventrals terminate in the pre-testicular region by joining the postero-dorsals of their side and in **H. paloniae** the postero-dorsals extend only up to the level of intestinal bifurcation. However, according to Gupta and Dutta (1967) it is only the postero-dorsals (= postero-inner ventrals herein) that reach the acetabulum. The occurrence of an additional pair of collateral nerves resulting from the splitting of the postero-inner ventrals, as reported by Lee (1971), Brandes (1898) and Otto (1896), could not be observed in the forms under the present study. Fukui (1929) did not mention the existence of collateral nerves. Lee (1971) reported the fusion of lateral and ventral nerves in the pre-acetabular region and also of the dorsal and ventral nerves in the acetabular region. The union of the posterior longitudinal nerves at places was also reported by Brandes (1898) in **G. crumenifer** and **F. elongatus**. In the present study fusion between the postero-inner ventrals and postero-dorsals was observed in **P. gracile**. According to Lee's (1971) description, the nerves separate again to pursue their respective course but in **P. gracile** the fused nerves continue to run posteriad as a single postero-dorsal nerve which terminates in the acetabulum. In **F. elongatus** and **O. orthocoelium** all the posterior longitudinal nerves join in the immediate pre-acetabular region to form a conspicuous circular connective from which many branches emerge in the posterior direction, supplying the whole wall and floor of the sucker. In other paramphistome species the postero-ventral nerves are interconnected to form a transverse connective just anterior to the acetabulum and they further continue to run still posteriad to supply the whole sucker, whereas the postero-dorsals terminate individually in the acetabulum, joining each other. The presence of a posterior transverse connection between the nerves of the two sides was also reported by Brandes (1898) and Lee (1971) in **F. elongatus**. The latter author also reported the occurrence of an acetabular nerve in this pouched paramphistome, but in the present study no such nerve was traced in **F. elongatus**. However, in **O. indica** and **H. paloniae** an acetabular nerve, solely derived from the postero-median and inner ventral nerve respectively, supplies the acetabulum. In the post-cerebral region conspicuous transverse connectives connect the three pairs of posterior nerves with one another, establishing a direct communication between the system of two sides. In the present study all the pouched paramphistome species have a larger number of transverse and longitudinal connectives than the non-pouched ones; however, from amongst the latter it is only in **C. cotylophorum** that a relatively dense nerve net is formed on both dorsal and ventral surfaces of the body. Further, in **H. paloniae** the number of transverse connectives is reduced to two to three, occurring only in the region, immediately anterior to the acetabulum. Among the three paramphistome species studied by Lee (1971), **P. magnus** has the maximum number of transverse connections and **P. calicophorum**, the least. In **P. magnus**, Lee further reported a direct dorsoventral connection traversing the parenchyma in between the postero-dorsal and postero-ventral nerves (the latter corresponding to the postero-inner ventrals in the present description). No such dorso-ventral connection is present in the forms under the present investigation.

A comparison of the nerve pattern among the pouched paramphistomes, **F. elongatus, F. brevisaccus, F. cobboldi** and **G. crumenifer** brings out some notable variations. The nerve distribution in the pre-cerebral region of **F. elongatus F. brevisaccus** and **G. crumenifer** is simple in not possessing a conspicuous circumpharyngeal nerve basket which is a prominent feature in **F. cobboldi**. The 'C' or titled 'V' (>)-shaped connectives of the postero-outer and postero-inner ventrals in **F. elongatus** are conspicuous by their absence in the other three forms. In **F. elongatus** all the posterior nerves join to form a circular connective anterior to the acetabulum and thus do not terminate individually in the acetabulum as they do in the other three species.

Amongst the non-pouched paramphistomes there occur some minor differences regarding the position of nerves, the number of anterior nerves and transverse and longitudinal connectives, and innervation pattern in the acetabulum. Apart from the commonly observed pattern of three pairs of anterior nerves, an extra fourth pair, i.e., pharyngeal nerves, is present in **O. indica** and **P. gracile**. In **C. cotylophorum** and **O. narayanai** the anterior transverse connectives are relatively more in number and are further connected at places with the help of thin longitudinal connectives. In contrast, only two to three transverse connectives are present in **O. indica** and **H. paloniae**. In **E. explanatum** the ventral nerve net supplies the genital pore but in the other species a pair of genital nerves are present. A dense nerve net, formed by numerous transverse connectives, occurs in **C. cotylophorum**, in contrast to this in **H. paloniae** only a few connectives constitute the nerve net. In all these forms, all the posterior nerves extend up to the acetabulum except in **P. gracile** and **H. paloniae** where the postero-inner ventrals and dorsals, respectively, are relatively short nerves.

In comparison to the other paramphistomes, the pouched forms were found to have more complicated nervous organization, which can be attributed to the presence of a ventral pouch in the latter category. The neuroanatomy of **C. cotylophorum** resembles greatly with that of the pouched species in respect of having a dense body nerve net and pharyngeal nerve basket around the pharynx. In **E. explanatum** also the innervation pattern of the genital pore is the same as in pouched forms.

The genital organization of nerves in the two distome trematodes namely, **E. pancreaticum**, the dicrocoeliid fluke and **F. gigantica**, the common fasciolid, conforms to the typical pattern observed in the paramphistome species, although several important deviations have also been observed. Like **O. indica** and **P. gracile**, an extra fourth pair of anterior nerves in the form of pharyngeal nerves is present in **E. pancreaticum** The genital pore in **E. pancreaticum** and **F. gigantica** is innervated by several branches from the postero-inner ventrals. In resemblance with **H. paloniae** in these species also the anterior longitudinal nerves are interconnected with one or two transverse connectives. The lateral longitudinal connectives between the anterior and posterior nerves also occur in these two species like many paramphistomes studied herein, but in general the former have a comparatively less dense and simple nerve net than the latter. However, the peculiar fine nerve plexus approximating the lateral body margins remains a feature unique to **F. gigantica**, which is not shared by any other species.

A comparison of the nerve pattern in **E. pancreaticum** with that in the other dicrocoeliid forms, namely, **Paradistomoides orientalis** (see Simha and Rao, 1977) and **Euparadistomum herpestesi** (see Kishore **et al**., 1982) reveals some noticeable differences. While in **E. pancreaticum** the anterior nerves comprise four pairs, in **P. orientalis** there are three pairs of them and in **E. herpestesi**, five pairs. In the last-mentioned two species there occur several transverse connectives in the anterior region. As in **P. orientalis**, **E. pancreaticum** has three pairs of posterior nerves, the innermost pair being of thin postero-dorsals (towards the median axis) that terminate near the hind end of the body. However, in **E. herpestesi**, wherein only two pairs of posterior nerves are reported, these nerves terminate in the testicular region (Kishore **et al**., 1982). The postero-inner ventrals are the most developed nerve pair innervating the various organs of the body in all the dicrocoeliids studied so far. The innervation pattern of the ventral sucker and other organs in **F. pancreaticum** is similar to that in **P. orientalis**. In **E. pancreaticum** both the postero-dorsals and postero-inner ventrals constitute a fine nerve net in the mid body region on both dorsal and ventral surfaces of the body. No such nerve net is described for **P. orientalis**. A nerve net is reported in **E. herpestesi** by Kishore **et al.**(1982).

F. gigantica lacks the pharyngeal pair of anterior nerves and the thin postero-dorsals are very short nerves as they terminate in the region of much anteriorly situated ventral sucker. In contrast, in **E. pancreaticum** these nerves extend beyond the sucker up to the posterior extremity of the body. The postero-inner ventrals are the main nerves to innervate the various organs of the body in both the species. A fine nerve net is present in the whole body of **F. gigantica** which is specially characterized by a dense nerve plexus approximating the lateral body walls, whereas in **E. pancreaticum** the nerve net is restricted only to the mid body region.

An interesting aspect of the present investigation is the presence of a dorsal and a ventral nerve nets which are constituted by transverse and longitudinal connectives and provide a direct communication system between all the anterior and posterior nerves. Transverse connectives connecting the main tracts along the body are reported in cercariae of **Cloacetrema michiganensis** by LeFlore **et al.** (1980), **Plagiorchis elegans** by LeFlore (1979) and **Diplostomum pseudospathaceum** by Niewiadomska and Moczon (1982), and also in adults of **Prosthodendrium** spp. by Ramulu and Rao (1979) and **Singhiatrema** spp. by Simha and Rao (1977) and Fernandez **et al.** (1982). A dense nerve net is also reported in metacercariae of **D. pseudospathaceum** (Niewiadomska and Moczon, 1984).

Ramulu and Rao (1979) in **Prosthodendrium** spp. and Simha and Rao (1977) in **S. longifurca** and **P. orientalis** have reported the ganglionated nature of nerves. No ganglionated thickenings were observed in the parasites under the present investigation. The innervation of the pharynx, genital pore, ventral sucker, vitellaria, and excretory and reproductive systems suggests a well developed nervous control over these organs (Simha and Rao, 1977). The concentration of fine tributaries of nerves, seen terminating towards the tegument in all these regions, suggests their association with the tegumental papillae as reported in many paramphistome species (Tandon and Maitra, 1981; 1982).

It is also of interest to note that increasing the substrate concentration, a superficial network of longitudinal and circular muscle fibres is revealed in the tegument of the worm. This system seems to operate at a low functional level of NSE activity and thus requires a higher concentration of the substrate. This network resembles the one described by LeFlore (1979) for the cercariae of **Plagiorchis elegans.**

The cerebral ganglia and cerebral commissure constituting the "brain" are composed of both fibrous and cellular components. Among the few earlier studies, Brandes (1898) reported a distinct sheath around the brain, which is lacking in the species under the present investigation. Looss (1896) reported the presence of uni-, bi- and multipolar ganglion cells and figured a few nuclei similar to those considered as neuroglia, in the nervous system of **Gastrodiscus aegypticus.** The ganglion and neuroglia cells were also demonstrated in the nervous system of **Gastrothylax** species by Brandes (1898) and of **F. elongatus, P. calicophorum** and **P. magnus** by Lee (1971). The presence of some unipolar and bipolar nerve cells has been described in some digenetic and monogenetic flukes (Rohde, 1968a, b, c; Halton and Morris, 1969). Jeong **et al.** (1978) reported a distinct myelin sheath around the main nerves in the digenetic fluke, **Clonorchis sinensis.** However, the nerves were found to be unmyelinated in the present study. In several other digenetic species the nerves are unmyelinated (Dixon and Mercer, 1965; Lee, 1971; Mandawat and Sharma, 1978; Dei-cas **et al.,** 1980). In monogenea also, the nerves were found to be unmyelinated (Halton and Jennings, 1964; Rohde, 1968b, 1972b; Halton and Morris, 1969). However, the presence of nerve sheath around portions of the posterior ventral nerve cord in the aspidobothrean **Multicotyle purvisi** has been demonstrated by Rohde (1970, 1971, 1972b). Among cestodes, in **E. granulosus** the nerve fibres are reported to be unmyelinated (Morseth, 1967).

Histochemically the nervous tissue revealed the presence of NSE, AChE and BChE enzymes, the first mentioned showing more intensity than the latter two. However, Roy (1980) and Allen and Harkema (1972) evidenced only the AChE activity in the nervous tissue of **Ceylonocotyle scoliocoelium** and **Pharyngostomoides adenocephala**, respectively. Both AChE and BChE activity in the nervous tissue were reported by Krvavica **et al.** (1967) in **F. hepatica.** Sood (1977) demonstrated a strong BChE activity in the nerve synapses of **Glossimetra orientalis.** The association of esterases with neurotransmission has been demonstrated in many trematodes (Pepler, 1958; Becejac **et al.**, 1964; Lewert and Hopkins, 1965; Bueding **et al.**, 1967; Bueding and Bennet, 1972).

CHAPTER II

NEUROENDOCRINE COMPONENTS - presumptive neurosecretory cells (pNSC) and neurosecretory material (NSM)

INTRODUCTION

Ever since the development of chrome haematoxylin-phloxin staining technique of Bargmann (1949) and aldehyde fuchsin method of Gomori (1950), these techniques were used extensively to discover neurosecretory cells (NSC) in many invertebrates including the helminth parasites.

Among trematodes, the early observations were made on the adults of **Dicrocoelium lanceatum** by Ude (1962) who first succeeded in bringing out Gomori-positive granules in the nerve cells of both cerebral ganglia and ventral nerve cords. Following Ude's work, the NSC have been investigated in many digenetic trematode species, namely, **Fasciola** species (Gresson and Threadgold, 1964; Grasso, 1967a, b; Shyamasundari and Rao, 1975; Radlowski, 1975/'76), **Opisthodiscus diplodiscoides** by Matskasi (1970); **Acanthoparyphium spinulosum** by Steele (1971); **Leucochloridiomorpha constantiae** by Harris and Cheng (1972); **Paramphistomum epiclitum** and **Gastrothylax** sp. by Mehrotra and Bhutia (1977, 1979); **Paragonimus compactus** by Shyamasundari and Rao (1980); **Proalarioides tropidonotis** by Kalyankar and Kankal (1981); **Encyclometra colubrimurorum** by Kishore and Shyamasundari (1981); **Fischoederius elongatus** by Krishna and Rao(1981); and **Ceylonocotyle scoliocoelium** by Bhatnagar **et al.**(1980) and Sharma and Sharma (1981). All these studies revealed the presence of NSC in the brain, nerve cord, suckers and also in the various other regions of the body. The NSC were also demonstrated in larval trematodes like xiphidiocercaria by Karyakarte and Yadav (1977) and in isoparorchid metacercariae by Kalyankar and Mane (1977).

The ultrastructural evidence for secretory nature of neurons was provided by Gresson and Threadgold (1964) and Grasso and Quaglia (1974) for adult **Fasciola hepatica.** Similar studies were also made on the putative NSC in **Schistosoma mansoni** (see Silk and Spence, 1969; Reissig, 1970) and in cercariae and metacercariae of **F. hepatica** (see Dixon and Mercer, 1965 and Wilson, 1970, respectively).

On the basis of their electron microscopic observations Gresson and Threadgold (1964) distinguished two types of neurons; the cells typified as β cells contained intense secretion, granules and vesicles and were in diverse forms; the other type of cells, referred to as ∝ cells, were of relatively small size and with less vacuolation. These authors concluded that β cells were the neurosecretory cells while ∝ cells could get transformed into β type. However, Matskasi (1970) maintained that both be regarded as neurosecretory since the difference between ∝ and β cells pertained chiefly to the polarity (β neurons usually being unipolar and ∝ , bi- or multipolar) and the amount of secretory content in the cell perikarya. Shyamasundari and Rao (1975) used the terms 'A' and 'B' corresponding to β and ∝ cells, respectively, of earlier authors to designate the two types of NSC. Their terminology has been followed in the later studies as well.

Histochemically, the nature of the neurosecretory material (NSM) is found to be varying in the various trematode species studied. While in **Fasciola** spp., **Paragonimus compactus** and **Euparadistomum** spp. the NSM contains proteins, RNA and phospholipids (Shyamasundari and Rao, 1975, 1980); in **Tremiorchis ranarum** the nature of the NSM is found to be mainly glycoproteinaceous (Karyakarte and Baheti, 1977). Likewise, in **Fischoederius elongatus** also the NSM was found positive for glycogen, proteins, and acid mucopolysaccharide (Krishna and Rao, 1981).

The presence of acetyl and butyl cholinesterases, acid and alkaline phosphatases, succinic dehydrogenase and nonspecific esterases in the NSM of the bovine paramphistomid fluke, **Ceylonocotyle scoliocoelium** has also been reported (Bhatnagar **et al**., 1980; Sharma and Sharma, 1981).

There is relatively little information on the possible functions of neurosecretion in trematodes. Matskasi (1970) reported a daily rhythm of fluctuation in neurosecretory activity in the adult **Opisthodiscus diplodiscoides** from frogs. Steele (1971) suggested the neurosecretory material to be responsible for gametogenesis and possible differentiation of the entire reproductive system in **Acanthoparyphium spinulosum**. Harris and Cheng (1972) postulated an involvement of NSC in the development and maturation of gonads in **Leucochloridiomorpha constantiae**, as the neurosecretory activity apparently decreased from metacercariae to adult. A similar role of these cells was also suggested in other digenea by Karyakarte and Baheti (1977) and Kalyankar and Kankal (1981). A graded reduction in the intensity of stain of neurosecretory substance from juvenile to the sexually mature adults of **Paramphistomum epiclitum** indicated towards an association of the NSC with maturation of the fluke (Mehrotra and Bhutia, 1977).

While studying the nervous system in the various digenean species under the present investigation, numerous dark staining bodies were invariably observed associated with the main nerves or nerve net of the worm. With the use of specific histochemical techniques these bodies were revealed to have staining properties characteristic of NSC. The present work aims at studying these cells and their secretory material (NSM) in some more digenetic species and adding to the existing information on the NSC in trematodes. By studying the putative neurosecretory cells (pNSC) and their activity in the various maturation phases, i.e., from the juvenile to the reproducing adult stage in its final host of two worm species, it is also aimed to reveal the involvement, or otherwise, of the NSC in growth and development of the flukes.

MATERIAL AND METHODS

pNSC Demonstration

For this aspect of study adult worms of nine species viz. **F. cobboldi, F. elongatus, O. indica, P. gracile, O. streptocoelium, E. explanatum, C. cotylophorum, E. pancreaticum** and **F. gigantica** were fixed in Bouin's and Hally's fluid for 18-48 hr. and the fixed material was then processed for paraffin embedding. For morphological identification of the NSC serially cut sections of 6-7 u thickness were stained with Bargmann's (1949) chrome haematoxylin phloxin (CHPh), Gabe's (1953) paraldehyde fuchsin (PAF). Herlant's (1958) Alcian blue phloxin (AbPh) and Raabe's (1963) modified Heidenhain's Azan method all following Panov (1980)*. The measurements of NSC (n=5 per cell type and per location) were taken using occular and stage micrometers. All the measurements are in microns. The photomicrographs were taken under the Leitz Ortholux-2 research microscope.

Ultrastructural demonstration of NSC and NSM

In order to establish the secretory nature of the cells under investigation, those in **F. cobboldi** were also studied electron microscopically. Live specimens of **F. cobboldi** were obtained from the rumen of freshly slaughtered cow and fixed for overnight in 4% Glutaraldehyde solution in 0.1 M phosphate buffer at pH 7.4. After required fixation the material was washed 4-5 times in the same buffer solution and then post-fixed for 1-2 hrs in 1% osmium tetroxide in 0.2 M phosphate

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* Procedures of all histochemical techniques, followed herein, are given in Appendix.

buffer (pH 7.4). After post-fixation the material was dehydrated in acetone and embedded in araldite. Serial sections of the various zones of the anterior, i.e., pre-intestinal, region of the worm containing the cerebral ganglia and anterior nerves were cut with glass knife on an LKB ultramicrotome and picked up on formvar-coated single slot copper grids. In this manner, grids holding several sections were stained with uranyl acetate and viewed in a Hitachi HUIIE TEM.

Histochemistry of NSM

For this aspect of study five species, namely **F. elongatus, F. cobboldi** (both representing the pouched paramphistome types), **E. explanatum** and **C. cotylophorum** (representing the non-pouched paramphistome species) and **E. pancreaticum** (a typical distome digenean) were chosen. The technique employed in respect of the various metabolites are following Pearse (1968, 1972) and Bancroft (1975) and are presented along with the results in table-2.15. The serial sections of paraffin-embedded material were cut at a thickness of 8-9 µm. The frozen sections were cut at 12-15 µm, using HR SLEE cryostat.

NSC activity

With a view to revealing the involvement, if any, of NSC in growth and maturation of worms, the same were studied in different growth phases. For this purpose, **F. cobboldi** (the rumen-dwelling fluke) and **E. pancreaticum**, a parenteric fluke were chosen. Of both these, NSC in three growth phases of the worm, i.e., juvenile (without or only with traces of genital primordia), immature (with gonads but the latter still not in a functional state), and mature (with functional gonads, i.e., with evidence of gametes) in its final host were studied. The serial sections of worms representing these growth phases were stained only with chrome haematoxylin-phloxin as this technique gave more specific and better results than the others.

OBSERVATIONS

By application of standard staining methods for neurosecretion, the putative neurosecretory cells (pNSC) are distinguishable from non-neurosecretory cells in having a larger size and phloxinophilic or fuchsinophilic material in their cytoplasm and axonal extensions. The neurosecretory material was stainable with CHPh, AbPh, modified Heidenhain's Azan and at times with PAF techniques. The secretory product usually exhibited a fine granular appearance, some times even showing a tendency towards aggregation.

A. **Localization of pNSC**

In all the species of trematodes studied herein, two types of pNSC were distinguished light microscopically on the basis of the size of the cell body and the nature of the cytoplasm : (i) larger, polygonal or somewhat spherical in contour, mostly unipolar, sometimes bipolar and multipolar, having granular or fibrous and more or less vacuolated cytoplasm with single, mostly spherical or oval nucleus and mostly one or some times more nucleoli; as the number and size of vacuoles increase the cytoplasm appears almost empty of the stainable secretory material (these cells are similar to type 'A' cells of Shyamasundari and Rao (1975) and β - cells of Gresson and Threadgold (1964)), (ii) smaller, spherical, oval or pyriform in shape, with homogeneous cytoplasm, uni-, bi- and multipolar, with spherical or oval nucleus having one or more nucleoli (these cells correspond to the type 'B' cells of the former authors and α -cells of the latter).

The terminology of Shyamasundari and Rao (1975) has been adopted to define and designate the cell types in the present investigation.

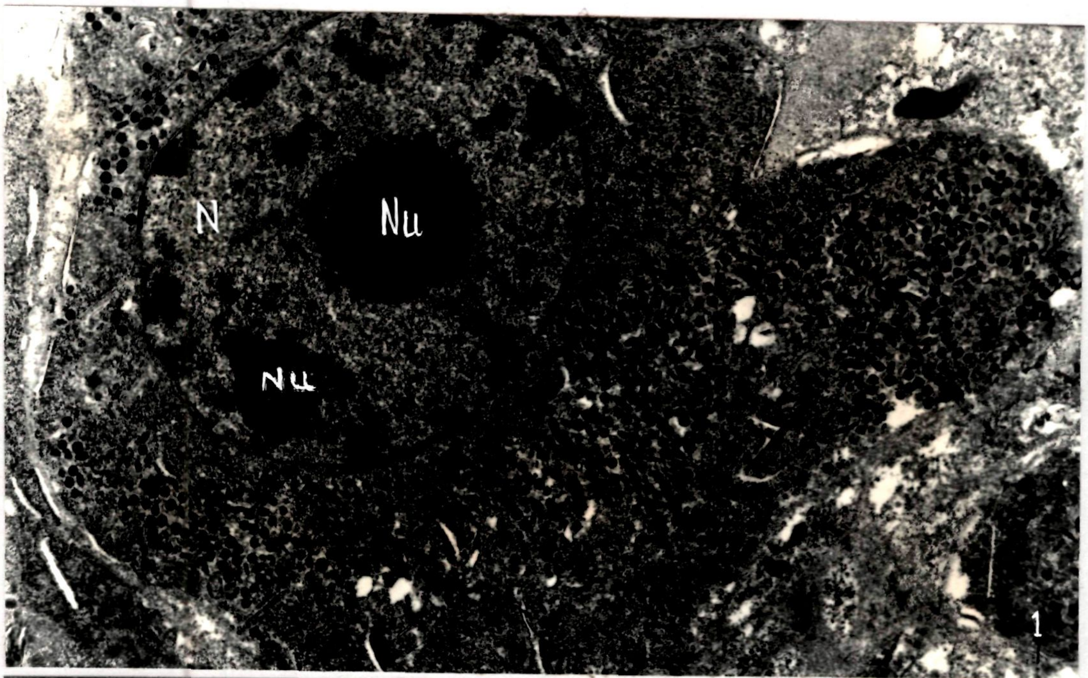
To confirm the presence of secretory granules in the perikarya of pNSC, the morphological evidences based on light microscopy were further supplemented ultrastructurally with the help of electron microscopic studies performed on the cerebral plexus of one of the species, **F. cobboldi**. The brain, consisting of two cerebral ganglia and a commissure localized anteriorly after the pharynx, was easily located in sections of the anterior region of **F. cobboldi**. Examination of the pNSC revealed the shape to be more ovoid rather than spherical, with a sharp and conspicuous cell membrane and having a prominent nucleus with chromatin-like material and two conspicuous nucleoli (Pl.2.1, Fig.1). In the perikaryon of the cell are present two types of vesicles. The larger

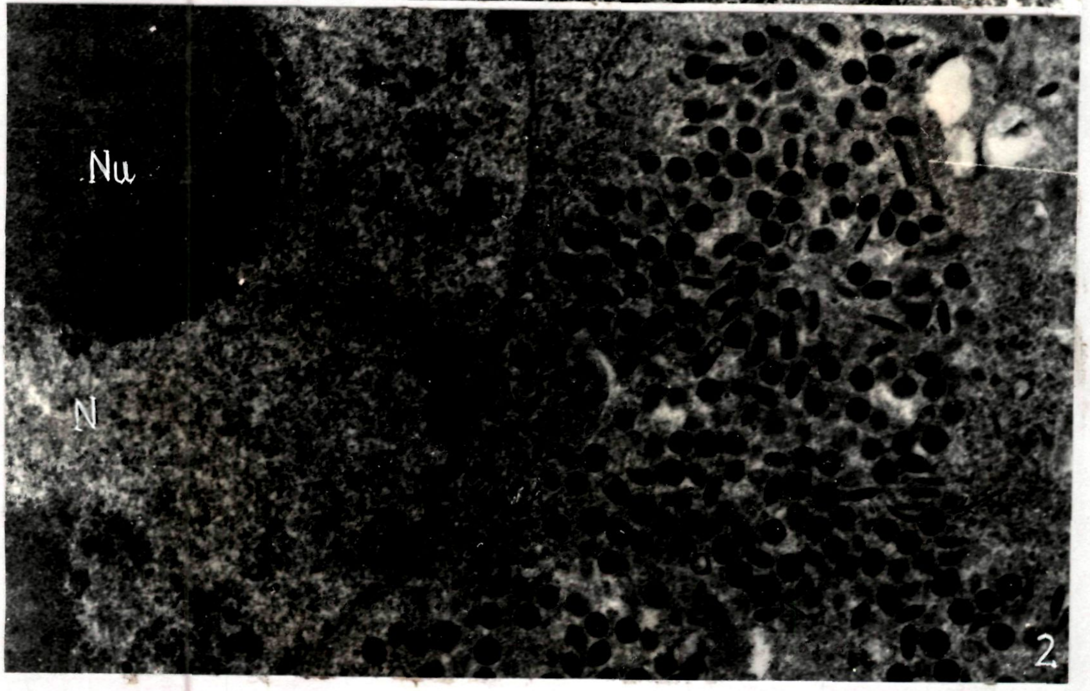
PLATE 2.1: Fischoederius cobboldi (Electronmicrographs)

Fig.1 A bipolar pNSC revealing the presence of electron-dense granules in its cytoplasm and axonal extensions. Note the presence of two nucleoli in the nucleus (5400x2).

Fig.2 A portion of the same cell in a magnified view (13,000 x 2).

PLATE 2.1





vesicles, 700 to 900 A° in diameter, have electron dense contents enclosed by a membrane. The other vesicles are rounded and smaller in size, 200 to 250 A°, with less dense contents (Pl.2.1, Fig.2). The other structures visible in the perikaryon were golgi complex, rough endoplasmic reticulum, free ribosomes and mitochondria. Electron dense granules were also observed in the process (axon) of the cell.

Some important and uniform features encountered in all the forms under the present study are: (i) multipolar 'A' cells occurred always approximating either the musculature or the subtegument and at times the lymphatic system, (ii) the 'A' cells observed in the oral sucker or pharynx were always round or oval in shape and without traceable axonal extensions, (iii) the cytological changes in the 'A' type cells, exhibiting cyclicity in their activity, were quite apparent, whereas the 'B' cells never showed any cyclic activity in their cytoplasm, and (iv) the multipolar 'B' type pNSC occurred frequently in the ventral sucker and at times in the oral sucker and pharynx.

The localization and measurements of the pNSC in various parts of the body and in association with various organ systems are depicted in Tables (2.1-2.13) in respect of all the species taken up for study; the account of pNSC for each species that follows incorporates only the special features pertaining to their distribution and morphology.

Fischoederius cobboldi

i) Juvenile form (Table 2.1) - Mostly 'B' cells were found present in the various locations of the body; the majority of them were mostly unipolar with a centric nucleus and centric or eccentric nucleolus (Pl.2.2, Fig.1), a few cells in the acetabulum were bipolar too (Pl.2.2, Fig.2). Unipolar 'B' cells were observed in the nerve cord (Pl.2.2, Fig.3).

The 'A' type cells were very few and localized only in the parenchyma and subtegumental region. They were unipolar, spherical or rounded, with only one or two small vacuoles in their dense cytoplasm (Pl.2.2, Fig.4).

In general the intensity of phloxinophilia was observed to be more in the juvenile stage as compared to the later stages of the worm.

ii) Immature form (Table 2.2) - This form of the fluke showed the presence of both 'A' and 'B' types of pNSC, the former being more in number.

Both cell types were observed in the brain region, 'B' being more in number (Pl.2.2, Fig.5). Mostly 'A' cells, with spherical or oval contour, were observed in the nerve cords (Pl.2.2, Fig.6). In both these regions, the cells were restricted towards the periphery.

The 'A' cell present at various locations of the body were found exhibiting two forms, one completely filled and

PLATE 2.2: Fischoederius cobboldi (Photomicrographs)

Figs.1-4, Juvenile form -

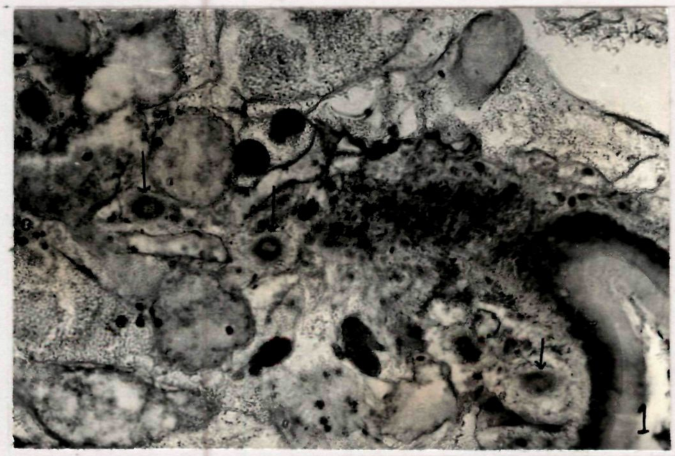
1. 'B' cells (arrows) in general parenchyma in a row. CHPh.
2. Bipolar 'B' cells (arrow) in the acetabulum. CHPh.
3. Unipolar 'B' cells (arrow) in the nerve cord. CHPh.
4. 'A' cells (arrows) in the genral parenchyma. CHPh.

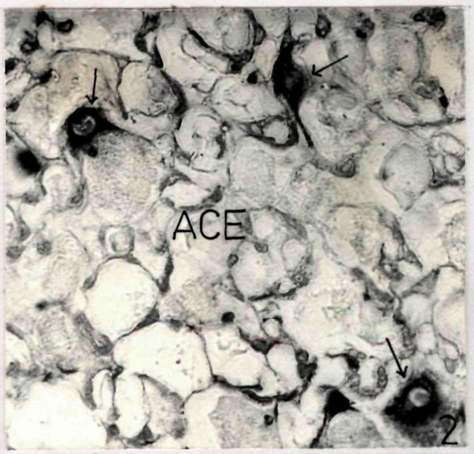
Figs.5-6, Immature form -

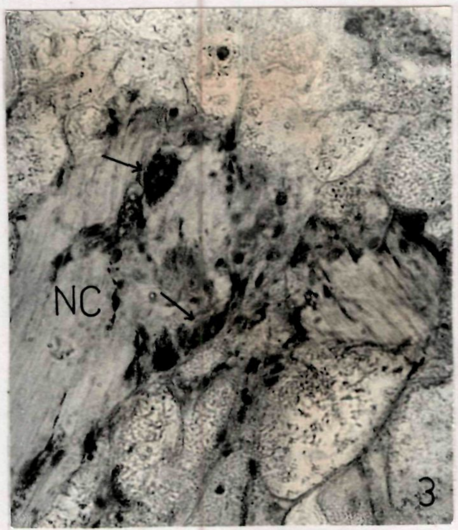
5. 'B' cell in the brain (large arrow) and 'A' cells (small arrow) approximating the latter. CHPh.
6. 'A' cells (arrow) in the nerve cord. CHPh.

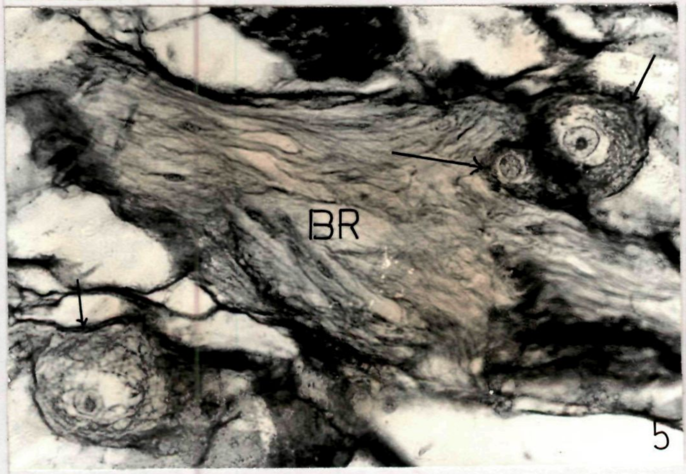
(**Figs.1-6** Scale bar = 0.05 mm.)

PLATE 2.2









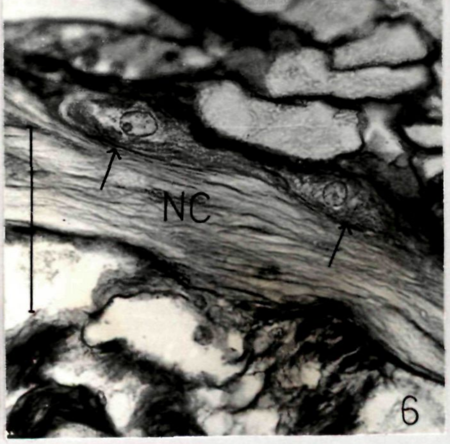


TABLE 2.1: pNSC in **Fischoederius cobboldi:** Juvenile stage

Location (in or in association with the organ)	Type	Cell body	Nucleus	Neucleolus (Diameter)
Brain	B	10.0 - 16.0 x 7.2 - 12.8	4.0 - 6.2 x 3.0 - 4.8	0.6 - 0.8
Nerve cord	B	9.2 - 15.0 x 8.0 - 12.0	4.0 - 7.2 x 3.2 - 6.0	0.4 - 0.6
Pharynx	B	7.2 - 14.6 x 6.4 - 14.0	5.2 - 8.2 x 4.8 - 7.8	0.6 - 0.8
Intestine	B	16.0 - 20.8 x 12.8 - 16.8	8.4 - 10.2 x 8.0 - 9.8	0.6 - 0.8
General parenchyma	A	18.0 - 24.2 x 15.2 - 20.4	6.2 - 8.2 x 4.8 - 7.0	0.8 - 1.0
	B	8.4 - 18.2 x 5.2 - 16.8	5.8 - 8.2 x 4.2 - 6.4	0.6 - 0.8
Subtegument	A	18.2 - 22.2 x 14.0 - 20.8	4.8 - 8.0 x 4.2 - 6.4	1.0 - 1.2
	B	8.2 - 14.8 x 6.4 - 12.8	5.2 - 10.4 x 4.2 - 9.4	0.6 - 0.8
Mehlis' gland (primordia)	B	8.2 - 14.8 x 4.8 - 10.0	4.0 - 8.4 x 4.0 - 6.0	0.6 - 0.8
Lymph vessel	B	10.2 - 14.2 x 8.4 - 12.0	7.2 - 8.2 x 6.0 - 6.4	0.8 - 1.0
Acetabulum	B	12.4 - 20.2 x 10.0 - 18.8	8.4 - 9.2 x 8.0 - 8.4	1.2 - 1.4

PLATE 2.3: **Fischoederius cobboldi:** Immature form (Photomicrographs)

Fig.1 Completely filled 'A' cell in the parenchyma near a vitelline follicle. CHPh.

Fig.2 Half filled 'A' cell in the general parenchyma near a nerve cord. The peripheral concentration of the contents is clear. CHPh.

Fig.3 Incompletely filled 'A' (large arrow) and 'B' (small arrow) in the general parenchyma near pars prostatica. CHPh.

Fig.4 'A' cell in the general parenchyma showing the irregular contour of the nucleus (arrow). CHPh.

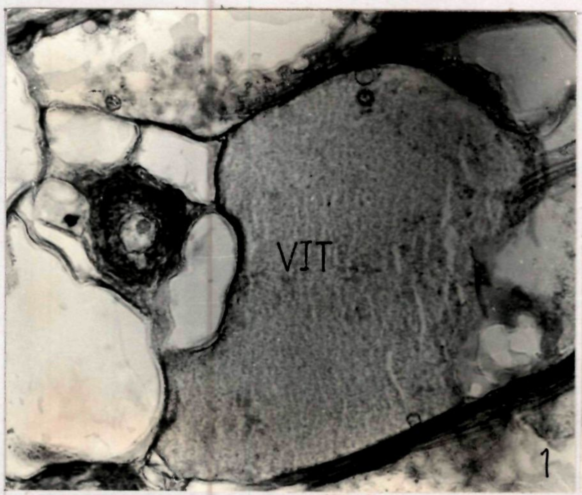
Fig.5 'A' cell in the general parenchyma showing a pyriform contour. CHPh.

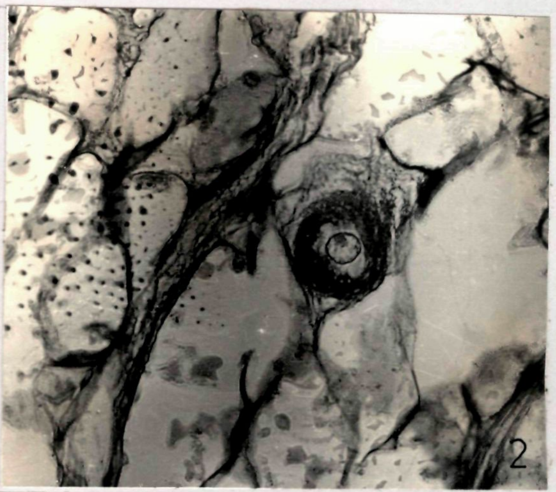
(Figs. 1-5, Scale bar = 0.05 mm).

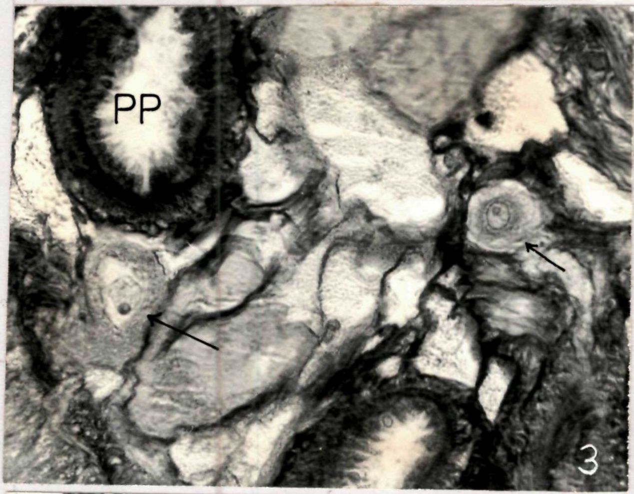
Fig.6 Uni- (small arrow) and multipolar (large arrow) 'B' cells in the acetabulum. CHPh.

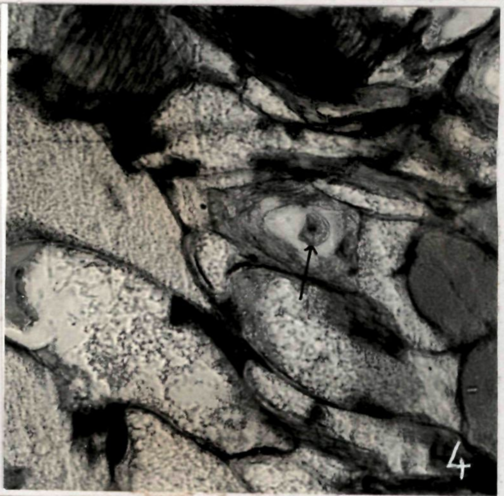
(Scale bar = 0.1 mm).

PLATE 2.3









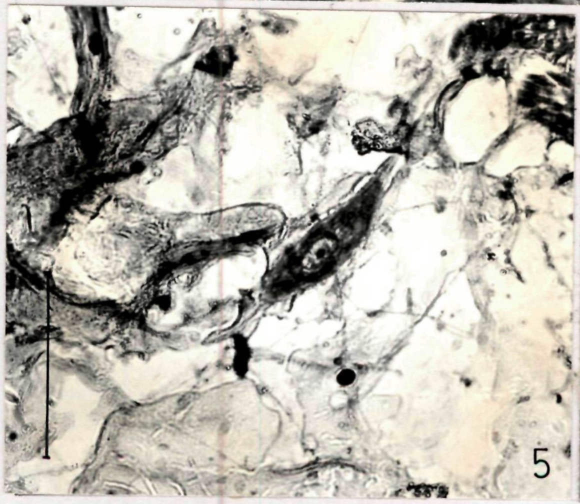


TABLE 2.2: pNSC in **Fischoederius cobboldi:** Immature stage

Location (in or in association with the organ)	Type	Cell body	Nucleus	Neucleolus (Diameter)
Brain	A	22.4-32.4 x 18.2-28.8	8.4-11.2 x 7.4-10.0	0.8-1.0
	B	16.2-22.8 x 12.8-18.2	8.0-10.0 x 6.8-8.2	0.6-0.8
Nerve cord	A	28.4-34.4 x 20.2-30.2	8.2-10.8 x 7.4-10.0	0.8-1.0
	B	18.2-24.2 x 14.8-18.4	8.0-10.2 x 7.2- 9.4	0.6-0.8
Pharynx	A	30.2-40.8 x 24.4-36.8	6.4-10.2 x 5.2- 9.4	0.8-1.0
Intestine	B	16.2-24.4 x 12.8-18.8	6.4-9.8 x 4.8 - 8.8	0.6-0.8
General parenchyma	A	34.2-42.2 x 18.2-36.4	9.4-12.4 x 8.8-10.8	1.4-1.8
	B	22.4-36.4 x 16.0-32.0	8.8-11.4 x 8.0-10.8	0.8-1.2
Subtegument	A	32.4-44.0 x 24.4-40.2	9.4-12.2 x 8.8-11.2	0.8-1.2
	B	20.0-34.2 x 18.2-30.2	8.0-11.2 x 7.2-10.4	0.8-1.2
Testes	B	18.8-32.4 x 12.0-28.0	8.8-10.4 x 7.2 - 9.2	1.2-1.8
Pars prostatica	B	14.6-24.8 x 12.0-22.4	8.0-10.2 x 6.4- 9.4	1.2-1.4
Pars musculosa	B	14.8-24.4 x 11.2-20.2	7.4-10.4 x 6.2-9.2	1.2-1.4
Ovary	A	20.2-28.2 x 14.2-24.6	7.2-10.2 x 5.8-9.0	1.2-1.4
	B	16.8-22.8 x 14.2-20.8	7.2-11.2 x 6.4-10.4	1.2-1.4
Uterine wall	A	22.4-30.4 x 18.0-28.2	7.2-10.4 x 6.0-9.8	1.2-1.4
	B	18.2-22.8 x 14.2-18.4	8.2-11.2 x 7.2-10.0	1.2-1.4
Vitellaria	A	28.2-36.4 x 20.2-32.8	8.8-11.4 x 8.0-10.2	0.8-1.0
Lymph vessel	A	30.0-38.2 x 22.8-32.2	8.8-11.2 x 7.2-10.4	0.8-1.2
	B	20.0-28.2 x 16.8-24.4	8.2-10.4 x 7.4-9.8	0.8-1.0
Acetabulum	A	22.8-30.8 x 16.4-28.8	8.2-9.4 x 7.4- 8.8	1.2-1.4
	B	16.2-28.4 x 14.2-22.4	8.0-10.0 x 7.2 -9.4	0.2-1.2

PLATE 2.4: **Fischoederius cobboldi** (Photomicrographs)

Fig.1-4, Immature form -

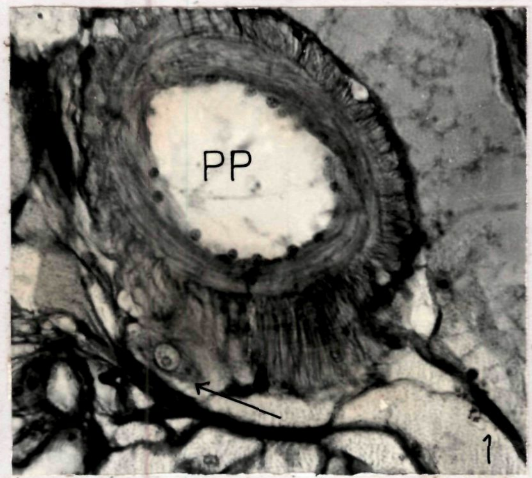
1. A unipolar 'B' cell (arrow) in the wall of pars prostatica. CHPh.
2. A unipolar 'B' cell with a long axon (arrow) in the general parenchyma near testes. CHPh.
3. Unipolar 'B' cell in the general parenchyma near a nerve cord. CHPh.
4. Bipolar 'B' (arrow) near a muscular strand. CHPh.

Figs.5-6, Mature form -

5. 'A' cells (small arrow) confined to the periphery of the brain region. One 'A' cell (large arrow) in the parenchyma approximating the latter is also conspicuous. CHPh.
6. 'A' cell in the nerve cord (arrow) with a large, spherical nucleus. CHPh.

(Figs. 1-6, Scale bar = 0.05 mm).

PLATE 2.4



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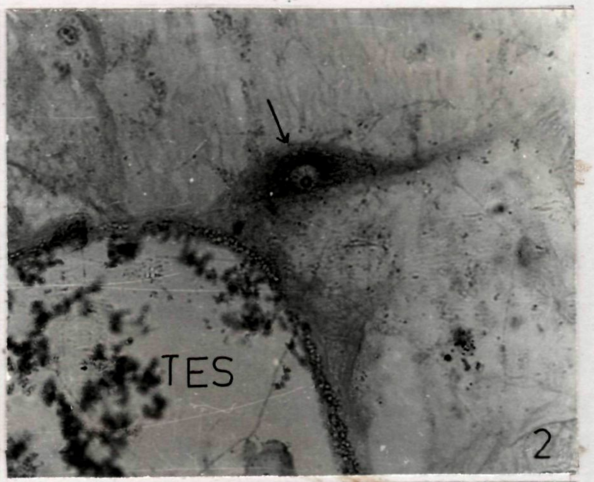

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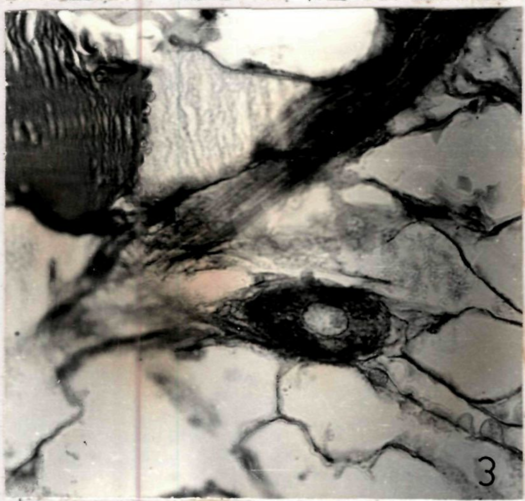

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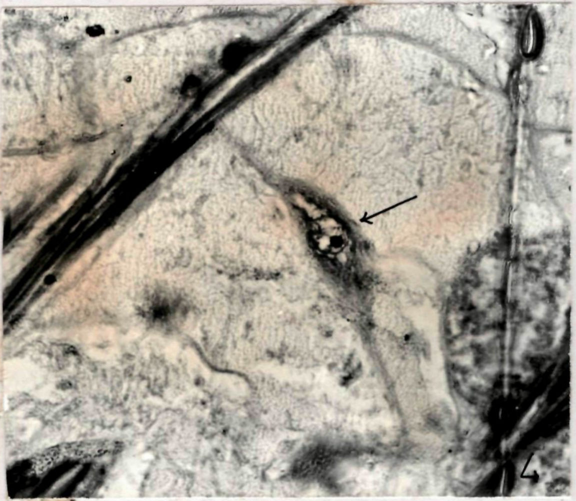

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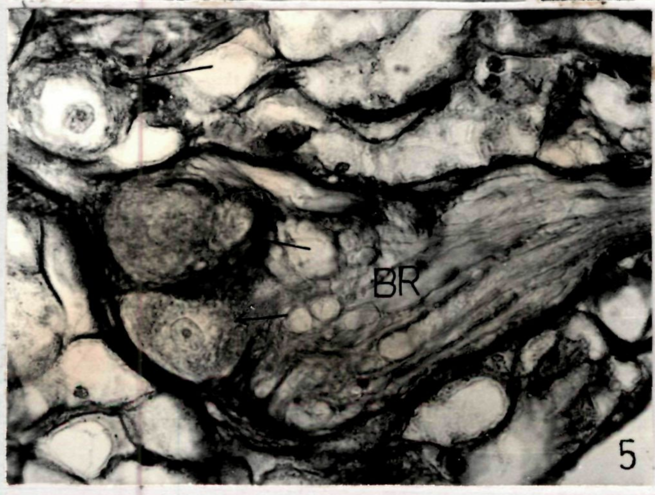


5

6

the other incompletely or half filled, probably in accordance with their state of activity (Pl.2.3, Figs.1-3). The cytoplasm of these cells was slightly vacuolated and granular, showing a tendency towards aggregation of granules. The nucleus of these cells was mostly spherical or oval in shape except in a few cells in which it had an irregular outline (Pl.2.3, Fig.4). In half-filled cells the cytoplasm was confined to the peripheral region. A few unipolar 'A' cells having a pyriform or elongated contour, at times with long axonal extension, were observed in general parenchyma. (Pl.2.3, Fig.5).

The 'B' type pNSC in the acetabulum were uni-, bi- and multipolar (Pl.2.3, Fig.6), whereas those near the reproductive organs and parenchyma were mostly unipolar (Pl.2.4, Figs.1-3); a few bipolar cells, and even provided with long axonal extensions, were also observed, at times terminating in muscular or lymphatic tissues (Pl.2.4, Fig.4). All these cells had a spherical or oval nucleus with centric or eccentric nucleolus.

iii) Mature form (Table 2.3) - Both 'A' and 'B' types were present, but comparatively less in number than in the earlier described forms.

Both these cell types in the brain and nerve cord were mostly unipolar, the 'A' cells being comparatively more in number. However, those in the brain were found restricted towards the periphery of the cerebral mass (Pl.2.4, Fig.5).

PLATE 2.5: **Fischoederius cobboldi:** Mature form (Photomicrographs)

Fig.1 Completely filled 'A' cell (arrow) in the general parenchyma. Heidenhain's Azan (Scale bar = 0.05 mm).

Fig.2 Incompletely filled 'A' cells (arrows) in the general parenchyma. CHPh. (Scale bar = 0.1 mm).

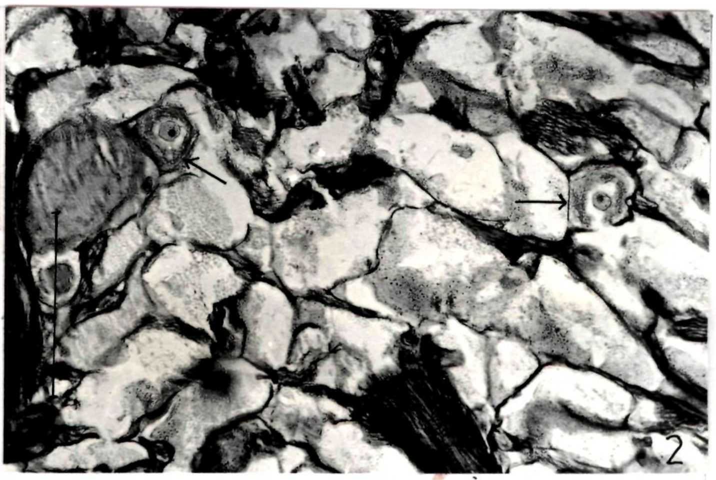
Fig.3 Incompletely filled 'A' cells (arrow) in the acetabulum. CHPh. (Scale bar = 0.05 mm).

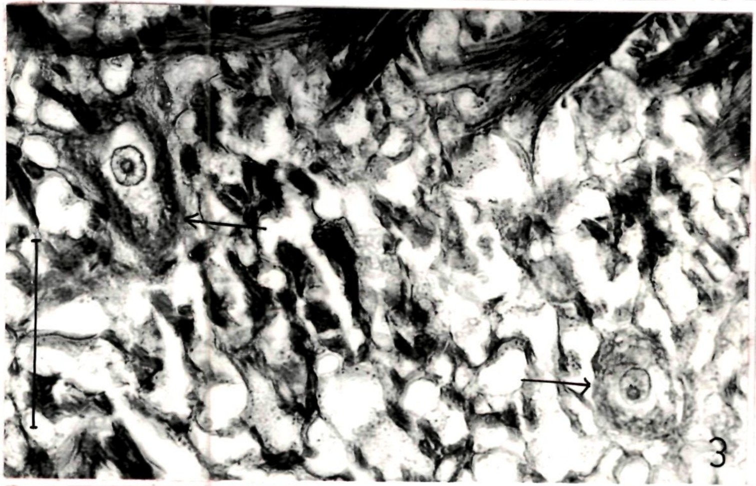
Fig.4 Empty 'A' cells (arrow) devoid of secretory material in the pharynx. CHPh. (Scale bar = 0.05 mm).

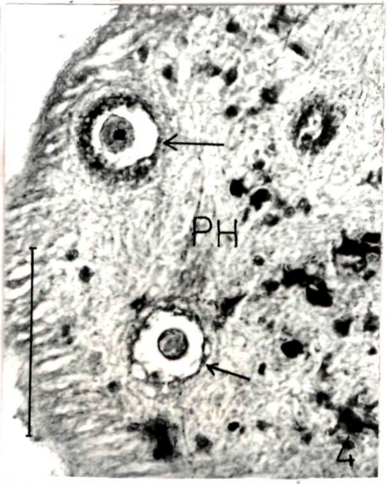
Fig.5 'B' cell (arrow) near uterine coils. CHPh. (Scale bar = 0.05 mm).

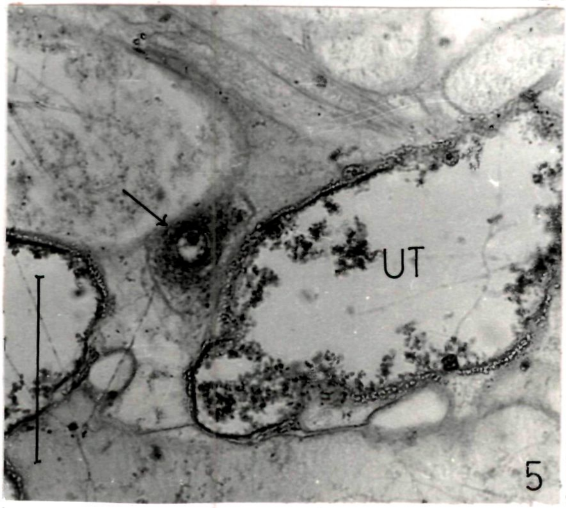
Fig.6 'B' cell (arrow) near pars musculosa. CHPh. (Scale bar = 0.1mm).

PLATE 2.5









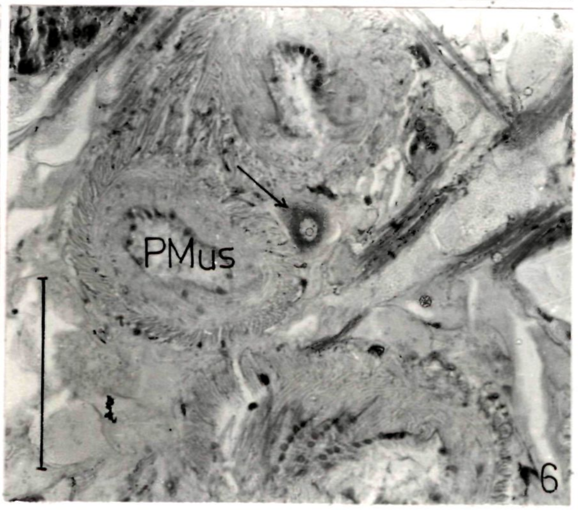


TABLE 2.3: pNSC in **Fischoederius cobboldi:** Mature stage

Location (in or in association with the organ)	Type	Cell body	Nucleus	Neucleolus (Diameter)
Brain	A	28.0-38.8 x 22.0-32.0	8.0-10.0 x 6.0-8.2	1.2-1.4
	B	18.0-20.4 x 16.8-18.6	6.0-7.5 x 4.5-5.0	1.2-1.4
Nerve cord	A	36.0-42.0 x 32.5-40.4	10.0-15.0 x 9.0-12.8	1.4-1.8
	B	18.0-20.0 x 10.6-16.5	8.0-8.5 x 6.8-7.2	1.2-1.4
Pharynx	A	28.0-40.0 x 34.5-38.0	10.0-12.2 x 9.0-9.8	1.2-1.4
Intestine	A	24.5-28.0 x 22.0-24.5	5.5-6.0 x 4.0-4.6	1.2-1.4
	B	20.0-24.0 x 14.0-16.0	6.5-8.0 x 6.0-6.5	1.5-1.6
General parenchyma	A	34.2-44.0 x 28.0-40.0	8.0-12.0 x 6.0-10.0	1.2-1.4
	B	24.0-34.0 x 18.0-30.0	6.0-10.0 x 6.0-8.4	1.2-1.4
Subtegument	A	36.0-44.0 x 24.0-32.0	10.4-12.0 x 8.2-10.0	1.2-1.4
Pars musculosa	B	18.2-20.4 x 12.0-16.0	7.8-10.0 x 6.0-8.2	1.2-1.8
Uterine wall	B	20.0-24.0 x 16.0-24.0	7.0-8.0 x 6.0-7.2	1.2-1.4
Vitellaria	A	34.0-40.0 x 16.0-32.0	9.2-10.0 x 8.0-8.4	1.2-1.4
Lymph vessel	A	34.0-44.0 x 28.0-36.4	10.0-12.0 x 8.4-9.2	1.2-1.4
Acetabulum	A	32.4-42.0 x 28.0-38.0	10.0-12.4 x 8.4.-9.4	1.2-1.4
	B	20.0-28.4 x 16.0-22.0	8.0-11.4 x 7.2 - 9.2	1.2-1.4

A few incompletely filled cells were also observed in this region. A few 'A' cells present in the nerve cord had a large conspicuous, spherical and centric nucleus with centric or eccentric nucleolus (Pl.2.4, Fig.6).

The 'A' cells present in the pharynx, general parenchyma and acetabulum were spherical or oval in shape, mostly unipolar, having a rounded or oval nucleus and a centric or eccentric nucleolus. Those in the parenchyma were comparatively large in size, mostly spherical with a few vacuoles in their cytoplasm (Pl.2.5, Fig.1). Some of these cells seemed to be containing a little secretory material or none at all (Pl.2.5, Figs.2-4). 'A' cells in the pharynx were restricted towards the periphery. All these cells had few big vacuoles in their cytoplasm with less dense secretory material. The 'B' cells near the reproductive organs were mostly unipolar (Pl.2.5, Figs.5,6), whereas those in the acetabulum were also multipolar.

Fischoederius elongatus (Table 2.4)

The 'A' cells present in the various locations of the body were mostly unipolar. These cells had mostly oval or at times spherical cell body with a few big vacuoles in their dense cytoplasm (Pl.2.6, Fig.1). A few multipolar cells were also observed in the subtegumental region (Pl.2.6, Fig.2). At times these cells had more than one nucleolus.

The 'B' cells present in or in association with the various organs of the body such as the intestine, gonads and

PLATE 2.6: **Fischoederius elongatus** (Photomicrograph)

Fig.1 'A' cells in the general parenchyma. Heidenhain's Azan.

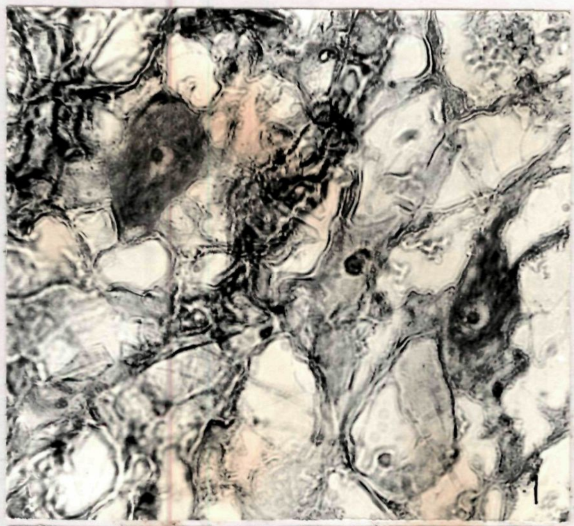
Fig.2 Multipolar 'A' cell (arrow) in the subtegumental region. AbPh.

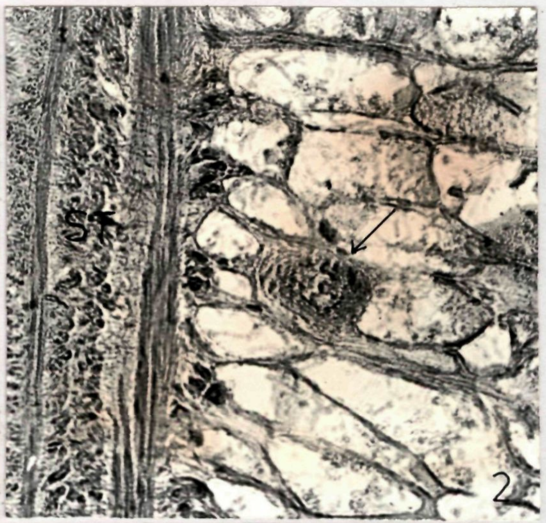
Fig.3 'B' cell (arrow) in the acetabulum. Heidenhain's Azan.

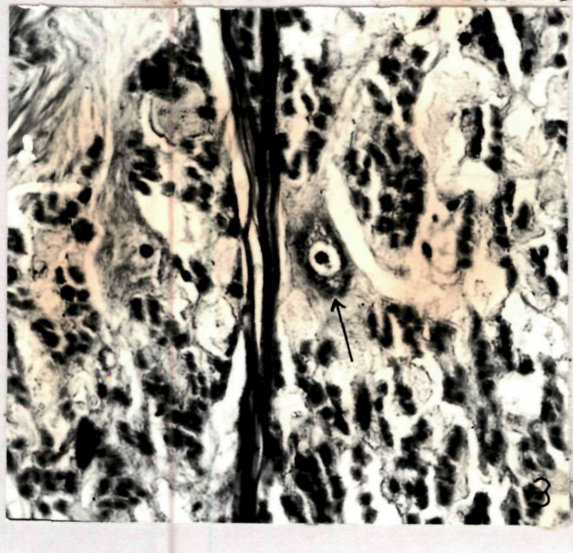
Fig.4 'B' cell (arrow) with a long axon near a uterine coil. CHPh.

(**Figs. 1-4,** Scale bar = 0.05 mm).

PLATE 2.6







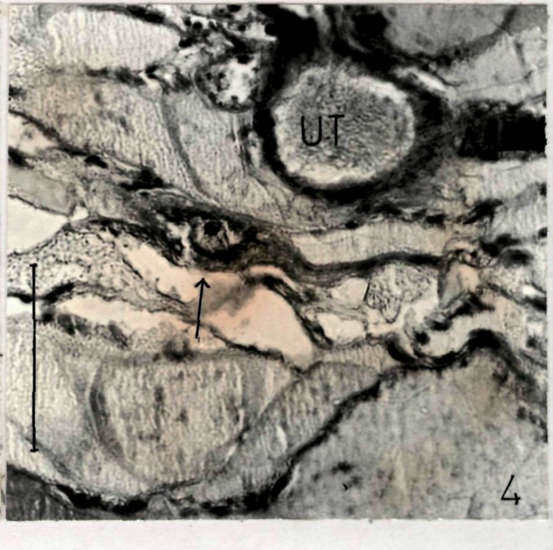


TABLE 2.4: pNSC in **Fischoederius elongatus**

Location (in or in association with the organ)	Type	Cell body	Nucleus	Nucleolus (Diameter)
Brain	A	28.6-40.0 x 23.0-34.8	9.0-10.2 x 8.2-8.6	1.8-2.0
	B	20.4-28.4 x 14.0-24.0	7.2-8.4 x 6.0-7.0	1.6-1.8
Nerve cord	A	24.0-36.4 x 23.2-28.4	6.0-8.2 x 5.2-7.2	1.6-1.8
	B	20.0-28.2 x 16.0-18.2	7.2-8.0 x 5.2-6.0	1.6-1.8
Pharynx	A	34.8-42.0 x 30.0-38.0	10.0-10.2 x 9.2-10.4	1.6-1.8
Intestine	A	28.2-32.4 x 24.0-28.8	7.6-10.0 x 6.4-8.4	1.2-1.4
General parenchyma	A	32.4-44.0 x 28.0-40.0	6.4-10.0 x 6.0-8.8	2.0-2.4
	B	24.2-38.2 x 18.0-34.4	9.2-10.4 x 8.0-10.2	2.8-3.0
Subtegument	A	32.0-44.4 x 26.4-40.2	8.4-10.0 x 7.2-9.6	1.4-1.8
	B	24.0-32.0 x 21.6-24.6	7.2-8.6 x 6.2-8.4	1.4-1.8
Uterine coils	B	20.0-28.4 x 18.6-24.4	7.2-8.4 x 6.4-8.2	2.4-2.8
Vitellaria	A	34.0-46.2 x 28.0-36.8	8.0-10.4 x 7.2-9.6	1.8-2.0
Lymph vessel	A	34.4-42.0 x 28.0-36.2	10.0-11.2 x 7.2-10.0	1.8-2.0
Acetabulum	A	32.0-40.0 x 28.4-34.0	8.0-12.2 x 7.2-11.2	1.8-2.0
	B	24.2-32.6 x 18.0-20.0	8.2-12.0 x 6.2-9.4	2.4-2.8

acetabulum had similar morphological characters; they were small rounded, oval or pyriform in shape, mostly unipolar, had centric, oval or spherical nucleus and centric or eccentric nucleolus (Pl.2.6, Fig.3). These cells were more in number in the acetabulum than in other locations of the body. In addition to uni- and bipolar cells multipolar cells were also present in the sucker. Unipolar 'B' cells often with long axonal extensions were also observed in the general parenchyma, at times approximating reproductive components (Pl.2.6, Fig.4).

Olveria indica (Table 2.5)

The 'B' cells were very few in number, mostly uni- or bipolar, often with long axons when present in, or in association with, the nerve cords and reproductive components (Pl.2.7, Fig.1). These cells in the pharynx were mostly oval in shape with spherical or oval centric nucleus and very little secretory material (Pl.2.7, Fig.2).

The 'A' cells were observed mostly in association with the wall of oesophagus and intestine, having a large size, oval or spherical contour and moderate to dense secretion in their perikarya with large vacuoles in the cytoplasm (Pl.2.7, Figs.3,4). All these cells had a centric or eccentric nucleus with centric or eccentric nucleolus.

An assemblage of 2-3, 'A' and 'B' cells were also observed in the region of genital papilla (Pl.2.7, Fig.5).

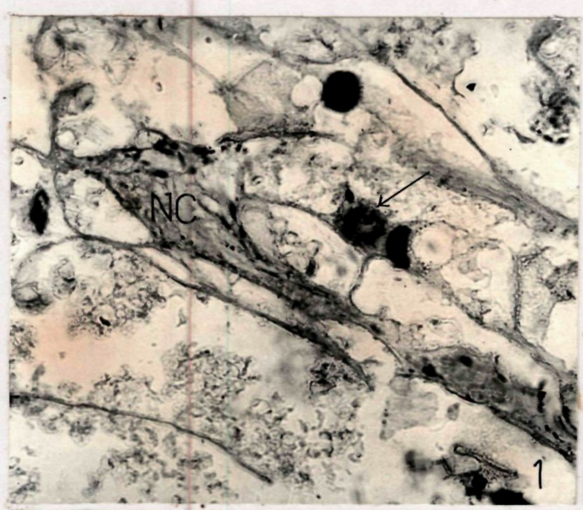
In the acetabulum, along with the commonly occurring

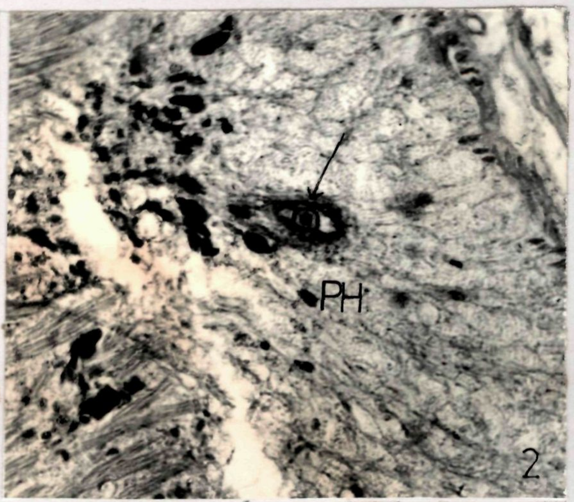
PLATE 2.7: **Olveria indica** (Photomicrographs)

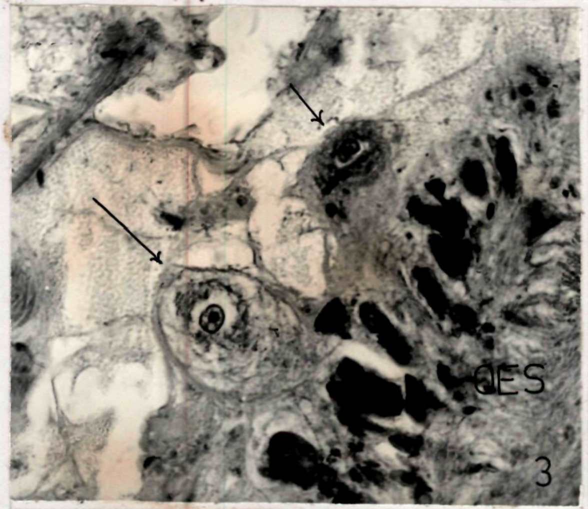
Fig.1	'B' cell (arrow) approximating a nerve cord in the general parenchyma. CHPh.
Fig.2	'B' cell (arrow) in the pharynx. CHPh.
Fig.3	'A' cell (large arrow) and 'B' cell (small arrow) approximating the oesophagus. CHPh.
Fig.4	'A' cell (arrow) with a long axon approximating the intestinal coil. CHPh.
Fig.5	Assemblage of 'A' (large arrow) and 'B' cells (small arrow) in the genital papilla. CHPh.
Fig.6	Uni- and bipolar 'A' cells (arrow) in the acetabulum. CHPh.

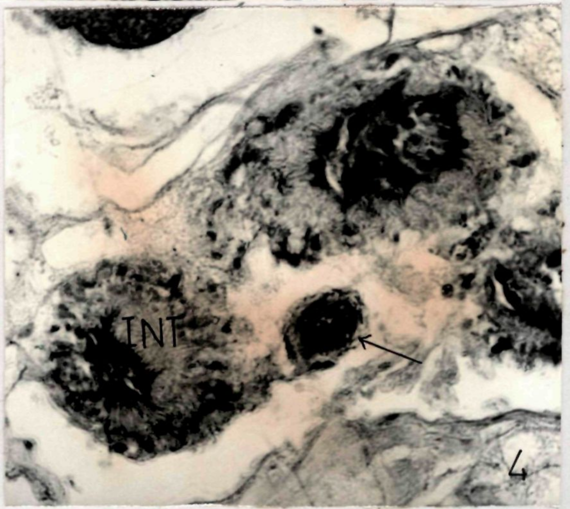
(**Figs. 1-6**, Scale bar = 0.05 mm).

PLATE 2.7









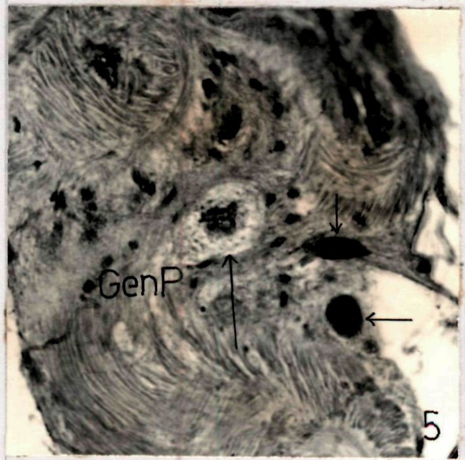


TABLE 2.5: pNSC in **Olveria indica**

Location (in or in association with the organs)	Type	Cell body	Nucleus	Nucleolus (Diameter)
Brain	A	18.8-24.4 x 16.0-20.2	8.0-8.0 x 4.8-8.0	1.0-1.2
	B	16.0-24.0 x 14.4-18.4	6.0-8.0 x 8.0-8.0	0.6-0.8
Nerve cord	A	28.4-30.0 x 24.0-26.4	8.8-10.0 x 6.2-8.0	0.6-0.8
	B	20.4-24.0 x 12.0-16.0	7.2-8.0 x 4.8-6.0	0.4-0.6
Pharynx	A	32.0-36.0 x 26.4-32.2	8.0-10.0 x 6.0-8.0	1.4-1.8
	B	18.0-24.0x 12.2-20.0	7.2-9.8 x 5.4-7.8	1.2-1.4
Oesophagus	A	30.0-42.0 x 26.8-28.0	7.4-8.8 x 6.0-8.0	1.2-1.4
	B	24.0-34.2 x 20.2-32.0	7.0-8.0 x 6.0-7.0	1.2-1.4
Intestine	A	32.2-42.0 x 28.4-32.4	7.2-8.8 x 6.0-8.0	0.8-1.2
General parenchyma	A	32.4-40.0 x 28.2-34.2	8.0-9.0 x 6.2-8.0	0.8-1.2
Subtegument	A	32.4-42.2 x 28.4-36.4	8.4-9.0 x 5.8-8.2	0.8-1.2
Genital papilla	A	24.4-32.2 x 18.8-26.0	7.0-8.4 x 5.2-7.0	0.6-0.8
	B	12.4-18.4 x 10.2-16.4	4.8-8.0 x 4.0-6.2	0.4-0.6
Acetabulum	A	32.0-40.0 x 28.0-34.0	8.0-10.0 x 6.0-8.2	2.8-3.0
	B	18.0-20.0 x 12.5-16.0	6.0-8.8 x 5.0-7.0	1.2-1.4

unipolar 'A' cells, a few bipolar cells were also observed (Pl.2.7, Fig.6).

Cotylophoron cotylophorum (Table 2.6)

Both cell types in this species exhibited a special feature in having a darker zone around the nucleus that encircled the latter in a band-like manner and was sharply demarcated, from the rest of the cytoplasm by virtue of its strong basophilia (Pl.2.8, Figs.1-6).

The 'A' cells present in the parenchyma were mostly spherical or at times oval in contour, mostly uni-, at times bipolar and some times even with long axonal extensions, with centric nucleus and centric or eccentric nucleolus (Pl.2.8, Figs.1,2). The 'B' cells, present in this region were few, mostly uni- or some times bipolar, oval or pyriform and often with long axonal extensions, with oval or spherical centric nucleus and centric or eccentric one or two nucleoli (Pl.2.8, Figs.3-5). Some times a few darkly staining basophilic masses were also observed in the cytoplasm of these cells (Pl.2.8, Fig.3).

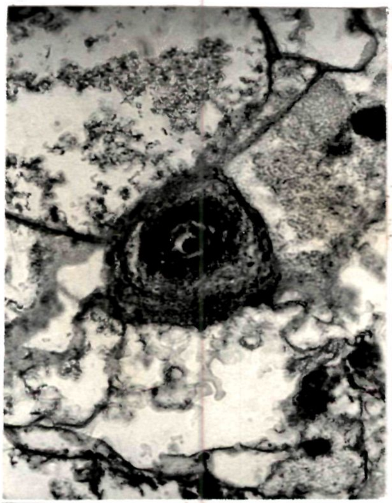
The 'B' type pNSC located in, or in association with, the intestinal coils were small in size, mostly ovoidal or spherical, unipolar, with centric nucleus and centric or eccentric nucleolus. Some times in the intestinal wall, five or six small 'B' cells were observed in a row (Pl.2.8, Fig.6). The 'B' cells associated with the lymph vessels exhibited

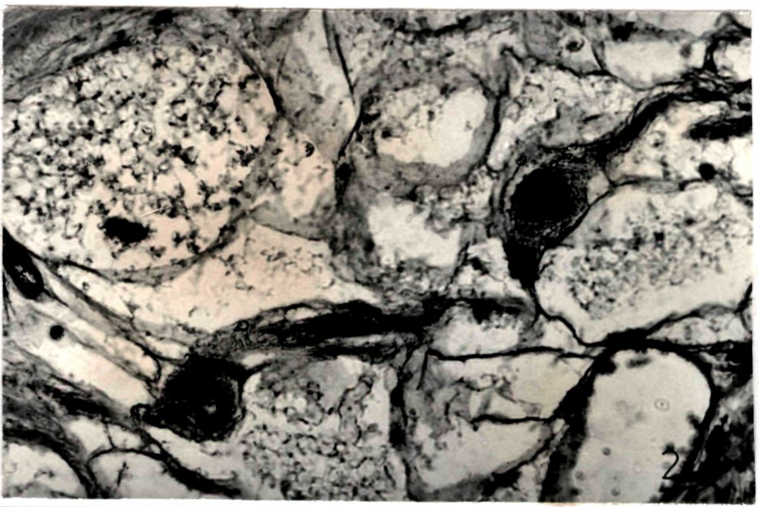
PLATE 2.8: **Cotylophoron cotylophorum** (Photomicrographs)

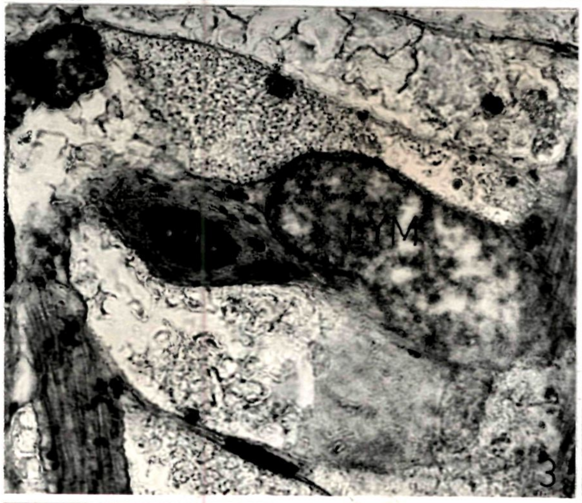
Fig.1	'A' cell in the general parenchyma. CHPh.
Fig.2	'A' cell with long axonal extensions in the general parenchyma. CHPh.
Fig.3	'B' cell near a lymph vessel. Many dense aggregations of darkly staining material abound in the cytoplasm. CHPh.
Fig.4	'B' cell (arrow) with a long axon near the testes. CHPh.
Fig.5	'B' cell with a long axon near the seminal vesicle. CHPh.
Fig.6	Small 'B' cells (arrow) nearing the intestinal wall. AbPh.

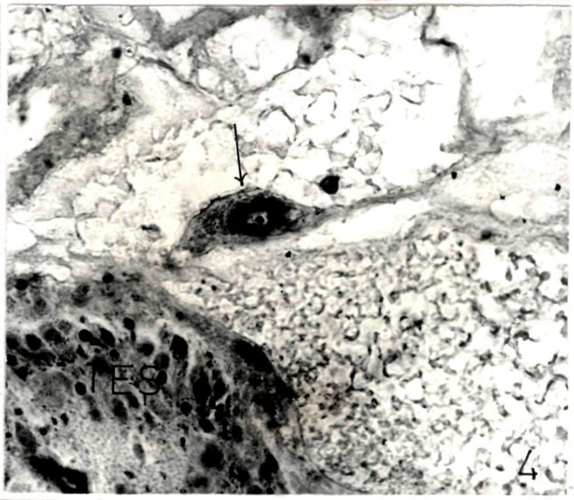
(Figs. 1-6, Scale bar = 0.05 mm).

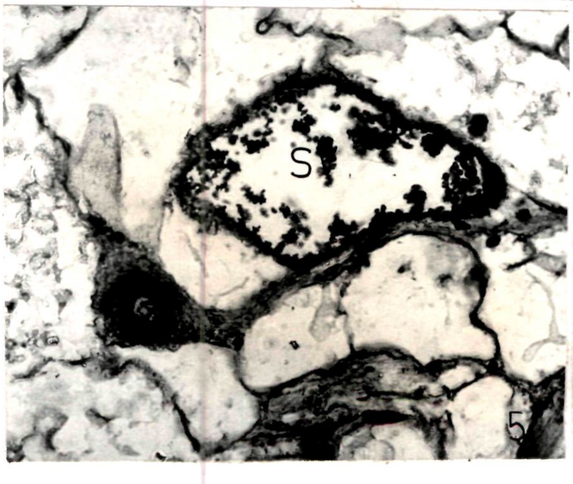
PLATE 2.8











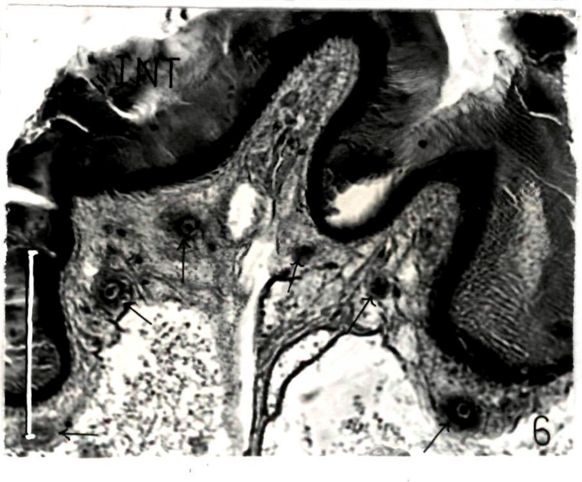


TABLE 2.6: pNSC in **Cotylophoron cotylophorum**

Location (in or in association with the organs)	Type	Cell body	Nucleus	*Nucleolus* (Diameter)
Brain	A	20.4-28.0 x 14.0-24.4	6.0-8.4 x 5.2-6.0	0.8-1.2
	B	16.2-24.0 x 12.4-18.8	6.0-8.2 x 5.2-6.4	0.8-1.2
Nerve cord	B	20.0-28.0 x 12.0-16.0	6.0-7.2 x 5.2-6.4	0.8-1.0
Pharynx	A	22.2-30.0 x 18.8-24.4	7.0-10.2 x 5.4-8.0	0.8-1.2
Intestine	B	12.0-20.0 x 8.4-16.0	6.0-8.0 x 4.8-6.2	0.8-1.2
General parenchyma	A	32.0-50.0 x 28.2-44.0	9.2-12.0 x 7.0-10.2	2.4-2.8
	B	24.0-40.0 x 18.0-32.0	8.2-12.0 x 7.0-10.2	1.4-1.8
Subtegument	A	32.0-40.0 x 28.0-32.0	8.0-10.8 x 6.4-8.8	1.4-1.8
Testes	B	18.2-32.8 x 14.2-25.0	8.2-9.2 x 7.0-8.0	0.6-0.8
Seminal vesicle	B	20.4-36.4 x 16.2-32.2	8.0-9.2 x 7.2-8.2	0.8-1.2
Vitellaria	A	24.0-34.0 x 18.0-30.0	8.0-9.2 x 6.0-8.2	1.2-1.4
Lymph vessel	A	32.2-44.0 x 26.2-38.0	10.0-12.2 x 9.2-10.8	0.8-1.2
	B	30.0-42.0 x 22.0-38.0	10.0-12.0 x 8.8-10.2	0.8-1.2
Acetabulum	A	24.0-32.0 x 18.0-26.4	9.2-10.2 x 8.0-8.4	0.8-1.2
	B	16.0-24.0 x 12.0-18.0	7.2-10.0 x 5.0-8.4	0.8-1.2

large dimensions.

Paramphistomum gracile (Table 2.7)

The 'A' type cells were mostly ovoid or spherical in shape, with few small vacuoles in the cytoplasm. These cells at various locations were mostly unipolar (Pl.2.9, Fig.1) but few multipolar cells were also observed in the subtegumental region (Pl.2.9, Fig.2).

The 'B' type pNSC approximating various organs were spherical, pyriform or oval in shape. These cells approximating various reproductive components often showed the presence of two nucleoli in their nucleus (Pl.2.9, Figs.3-5). A few 'B' cells present in the general parenchyma and acetabulum were comparatively large in size and at times even had two nucleoli (Pl.2.9, Fig.6).

Both cell types had distinctly granular secretion in their perikarya.

Orthocoelium streptocoelium (Table 2.8)

The 'B' cells approximating the reproductive components were mostly of relatively small size. All these cells had oval or pyriform contour with spherical or slightly oval centric nucleus and centric or eccentric nucleolus (Pl.2.10, Fig.1). A few bipolar 'B' cells with long axonal extensions were observed in the parenchyma (Pl.2.10, Fig.2). Unipolar 'B' cells with long axonal extensions were often observed in the acetabulum

PLATE 2.9: Paramphistomum gracile (Photomicrographs)

Fig.1 Unipolar 'A' cell (arrow) in the general parenchyma. AbPh.

Fig.2 Multipolar 'A' cells (arrow) in the subtegumental region. AbPh.

Fig.3 'A' cell (large arrow) in the general parenchyma and 'B' cell (small arrow) near testes with two nucleoli in the nucleus. Heidenhain's Azan.

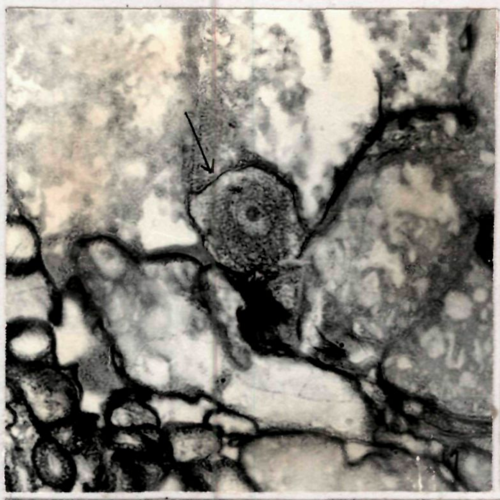
Fig.4 'B' cell (arrow) with two nucleoli near oviduct. CHPh.

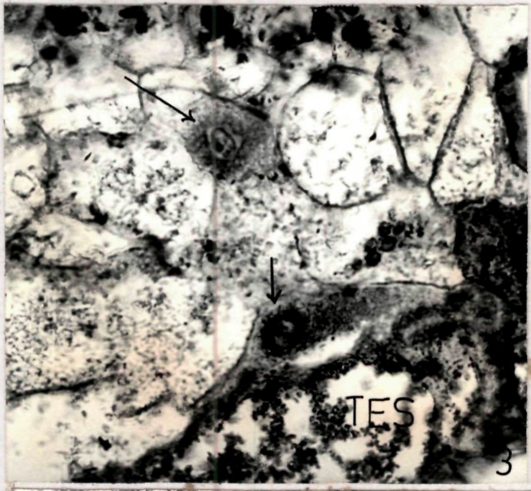
Fig.5 'B' cell (arrow) near uterine coil with two nucleoli in the nucleus. CHPh.

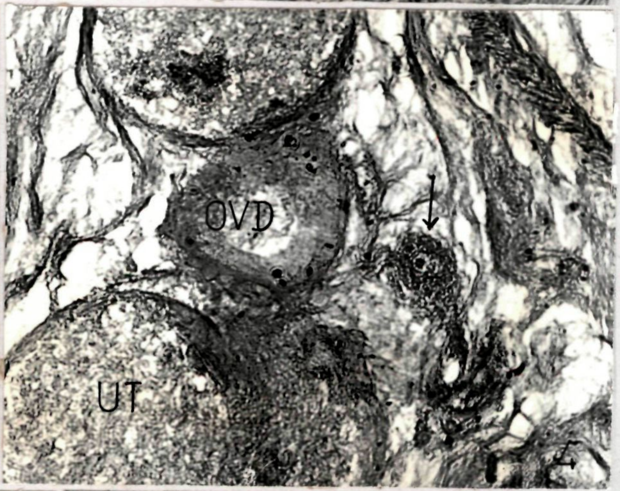
Fig.6 'B' cell (arrow) in the general parenchyma. Two nucleoli are evident in the nucleus. CHPh.

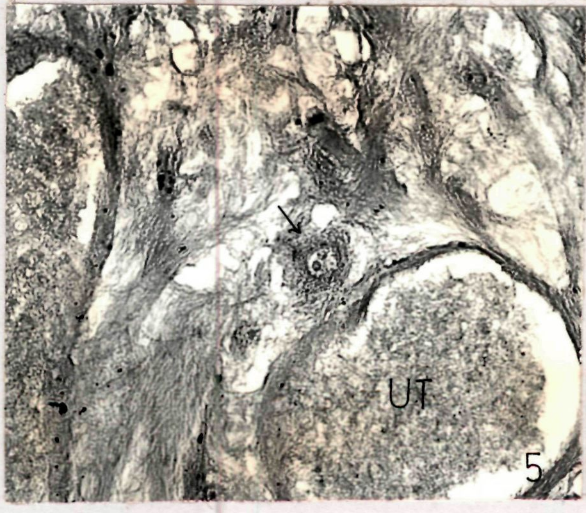
(Figs. 1-6, Scale bar = 0.05 mm).

PLATE 2.9









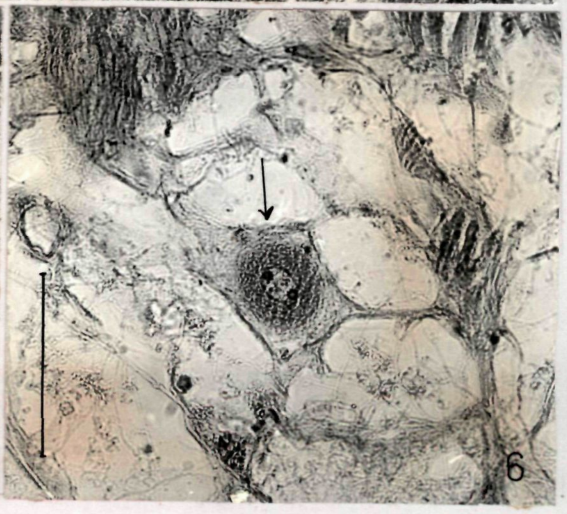


TABLE 2.7: pNSC in **Paramphistomum gracile**

Location (in or in association with the organs)	Type	Cell body	Nucleus	Nucleolus (Diameter)
Brain	A	20.0-28.0 x 16.0-24.6	8.0-10.0 x 6.8-7.2	1.2-1.8
	B	14.8-22.0 x 12.8-18.0	6.0-8.0 x 6.0-7.2	1.2-1.4
Nerve cord	B	21.6-28.0 x 16.0-20.8	7.2-8.0 x 6.0-7.2	1.2-1.4
Pharynx	A	28.4-32.0 x 24.0-28.4	10.0-10.2 x 8.4-9.2	1.2-1.4
Oesophagus	A	27.2-32.4 x 20.8-28.0	7.2-10.4 x 5.2-8.4	1.2-1.4
Intestine	B	20.2-28.0 x 18.0-21.6	8.0-10.0 x 7.2-8.8	1.2-1.4
General parenchyma	A	32.0-42.0 x 24.4-38.0	10.0-12.2 x 8.2-10.4	2.4-2.8
	B	24.4-34.0 x 20.0-30.0	8.2-12.2 x 6.8-8.8	1.8-2.0
Subtegument	A	32.0-44.0 x 28.4-36.0	9.0-11.2 x 8.0-9.4	1.4-1.8
	B	21.6-28.0 x 14.8-24.0	7.2-8.0 x 6.0-7.2	1.2-1.4
Uterine wall	B	16.0-28.0 x 14.8-22.6	8.0-10.4 x 7.2-8.8	1.8-1.0
Lymph vessel	B	22.4-32.0 x 20.6-28.0	8.4-10.4 x 6.0-8.0	1.2-1.8
Acetabulum	A	30.0-36.0 x 24.0-34.2	10.0-10.4 x 8.2-9.6	1.2-1.8
	B	24.0-28.0 x 21.6-22.0	8.0-10.0 x 6.0-8.6	1.2-1.8

PLATE 2.10: **Orthocoelium streptocoelium** (Photomicrographs)

Fig.1 'B' cell (arrow) near mehlis' gland. CHPh.

Fig.2 Bipolar 'B' cell (arrow) with long axons in the general parenchyma approximating a vitelline follicle. CHPh.

Fig.3 'B' cell (arrow) with a long axon in the acetabulum. AbPh.

(Figs. 1-3, Scale bar = 0.05 mm).

Fig.4 'A' cells (large arrow) and 'B' cell (small arrow) approximating a nerve cord. CHPh.

(Fig. 4, Scale bar = 0.1 mm).

PLATE 2.10

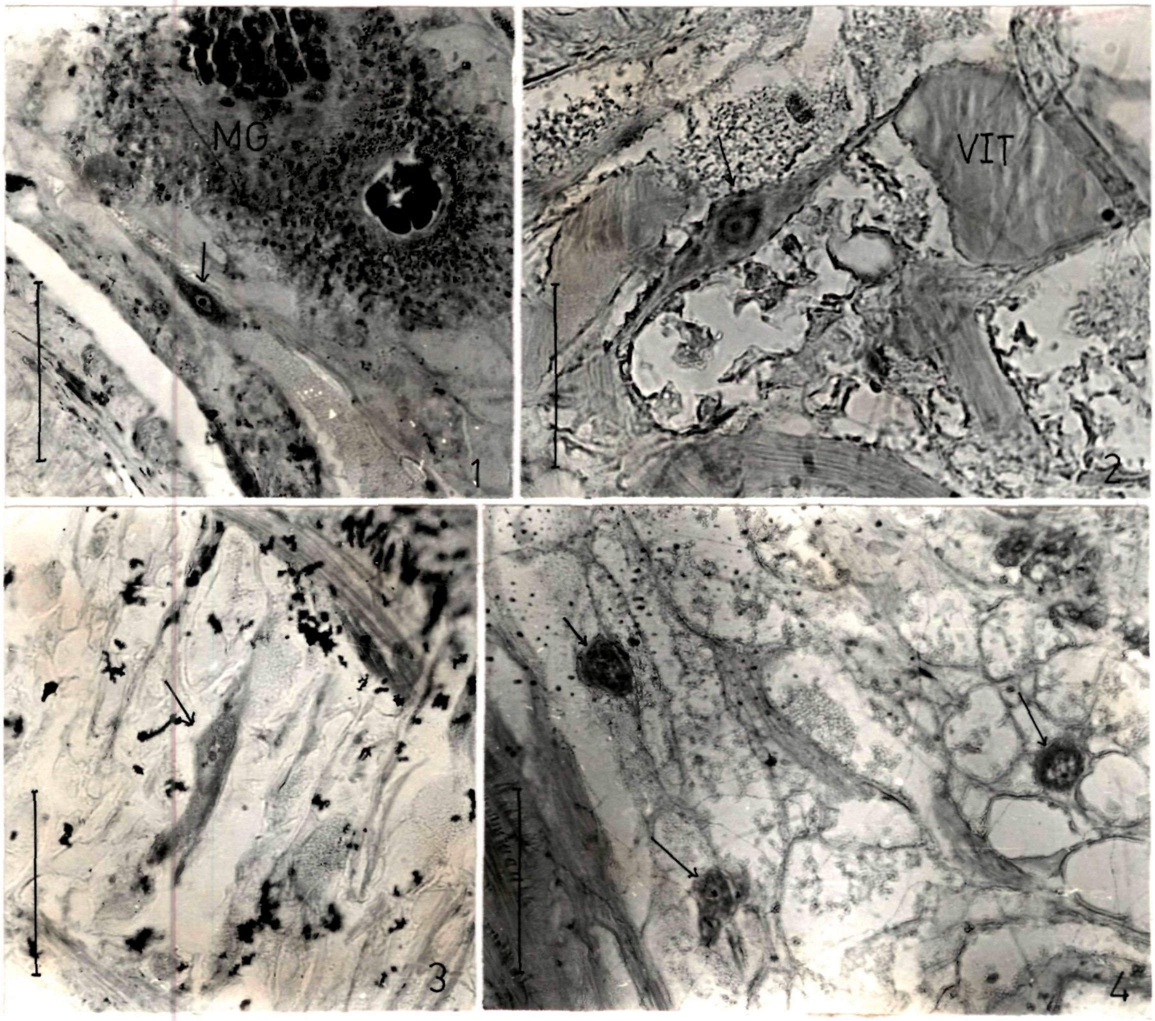


TABLE 2.8: pNSC in **Orthocoelium streptocoelium**

Location (in or in association with the organs)	Type	Cell body	Nucleus	Nucleolus (Diameter)
Brain	A	16.2-24.8 x 12.6-20.2	5.2-6.8 x 4.0-4.0	0.4-0.6
	B	14.0-20.0 x 10.0-16.0	6.0-6.8 x 4.2-5.4	0.4-0.6
Nerve cord	A	20.0-24.0 x 16.0-20.0	6.0-7.2 x 4.2-5.2	0.4-0.6
Pharynx	A	18.0-24.8 x 14.0-22.0	6.8-8.0 x 6.0-6.4	0.6-0.8
	B	12.0-18.0 x 10.0-14.0	6.0-7.0 x 4.2-6.2	0.6-0.8
General parenchyma	A	20.0-32.0 x 16.0-30.0	7.2-8.0 x 4.2-6.4	0.6-0.8
	B	26.0-28.0 x 14.0-24.0	6.4-7.2 x 5.0-6.0	0.6-0.8
Subtegument	A	22.8-32.0 x 18.4-26.0	7.2-8.0 x 5.4-8.0	0.6-0.8
Testes	B	14.0-16.0 x 8.0-12.0	7.2-8.0 x 5.4-7.2	0.4-0.6
Seminal vesicle	B	8.8-16.2 x 6.0-12.2	4.8-7.0 x 3.2-6.2	0.4-0.6
Mehlis' gland	b	8.8 -16.8 x 6.4-14.0	5.2-7.2 x 4.0-6.2	0.6-0.8
Uterine wall	B	8.8 -17.6 x 6.0-12.4	4.0-6.8 x 3.2-5.2	0.6-0.8
Lymph vessel	B	12.0-20.0 x 8.8-14.8	8.0-8.4 x 7.2-8.0	0.4-0.6
Acetabulum	A	18.0-24.0 x 12.2-18.0	7.0-8.2 x 5.6-6.4	0.6-0.8
	B	16.0-24.0 x 12.8-20.0	7.0-8.0 x 6.0-6.2	0.6-0.8

(Pl.2.10, Fig.3).

At times both cell types were also observed approximating nerve cords in general parenchyma (Pl.2.10, Fig.4).

Explanatum explanatum (Table 2.9)

The 'A' type pNSC present at various locations of the body exhibited similar characters, i.e., mostly spherical or oval with dense cytoplasm and very few small vacuoles, unipolar with centric nucleus and nucleolus (Pl.2.11, Figs.1,2). Spherical, unipolar 'A' cells were often observed in the nerves approximating various reproductive components (Pl.2.11, Figs.3,4). Those present in the parenchyma exhibited comparatively large dimensions. One or two bipolar 'A' cells were also observed near reproductive components (Pl.2.11, Fig.5).

Both cell types were found scattered throughout the tissue in the pharynx. They were oval in contour with large oval and centric nucleus and centric or eccentric nucleolus or at time two nucleoli (Pl.2.11, Fig.6).

The 'B' cells in the parenchyma were oval or pyriform, at times spherical with a conspicuous oval or spherical nucleus and centric or eccentric nucleolus (Pl.2.12, Figs.1,2). The 'B' cells approximating various reproductive components were comparatively small in size, oval or pyriform, unipolar with centric nucleus and centric or eccentric nucleolus (Pl.2.12, Figs.4,5).

PLATE 2.11: **Explanatum explanatum** (Photomicrographs)

Fig.1 'A' cells (large arrow) and 'B' cell (small arrow) in the general parenchyma. Heidenhain's Azan.

Fig.2 'A' Cell (arrow) near lymph vessel in the general parenchyma. CHPh.

Fig.3 'A' cell (arrow) in the nerve cord approximating a uterine coil. AbPh.

Fig.4 'A' cell (large arrow) and 'B' (small arrow) in a nerve cord approximating the testes. CHPh.

Fig.5 Bipolar 'A' cell with long axons approximating the seminal vesicle. CHPh.

Fig.6 'B' cells in the pharynx. Two nucleoli are evident in the nucleus of one of the cells (arrow). AbPh.

(**Figs. 1-6,** Scale bar = 0.05 mm).

PLATE 2.11

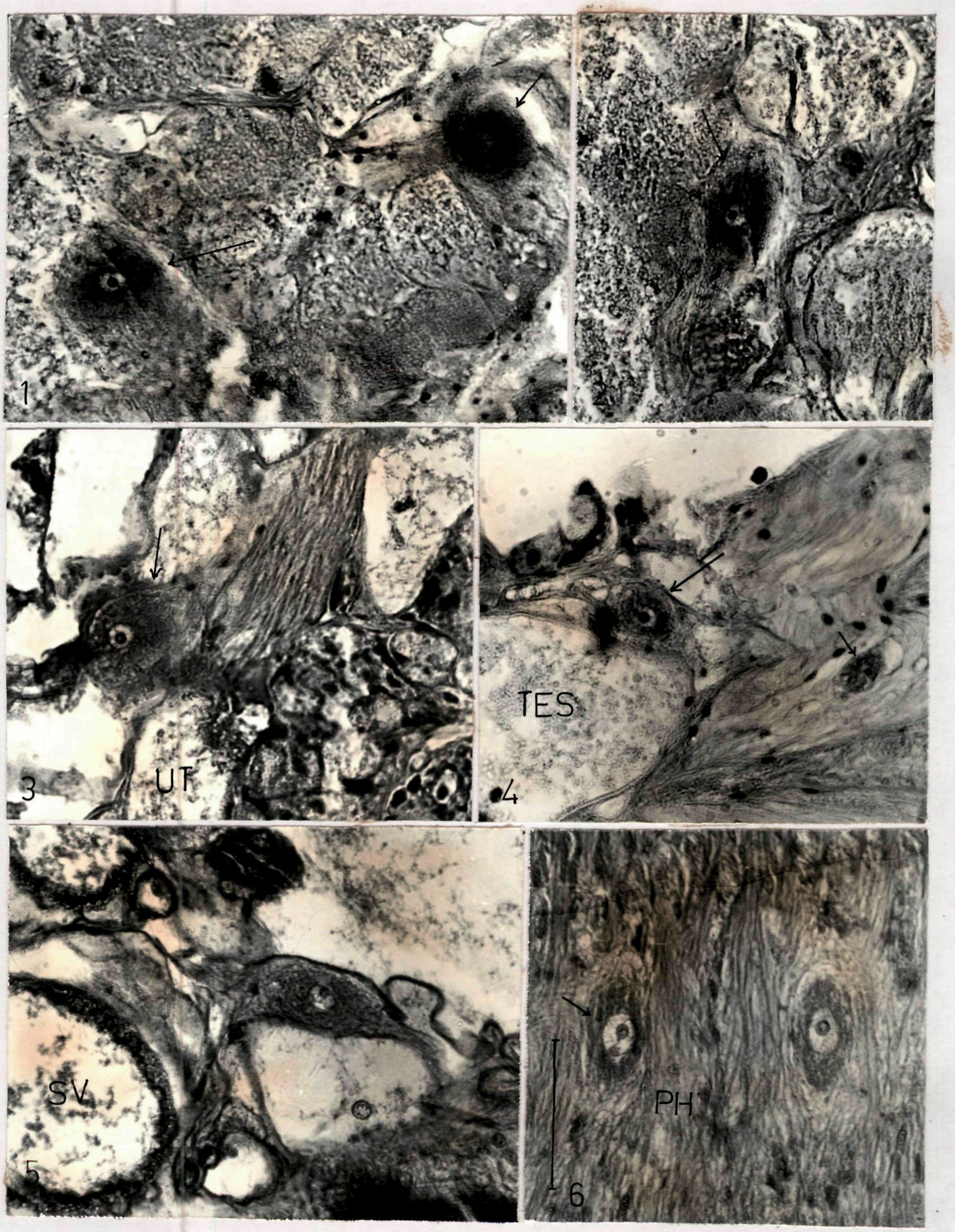


PLATE 2.12: **Explanatum explanatum** (Photomicrographs)

Fig.1 'B' cell (arrow) approximating a nerve cord. Heidenhain's Azan.

Fig.2 'B' cell (arrow) in the wall of lymph vessel. CHPh.

Fig.3 'B' cell (arrow) in between the uterine coils. CHPh.

Fig.4 'B' cell (arrow) in between the seminal vesicle and oviduct. CHPh.

(**Fig.1-4**, Scale bar = 0.05mm)

PLATE 2.12

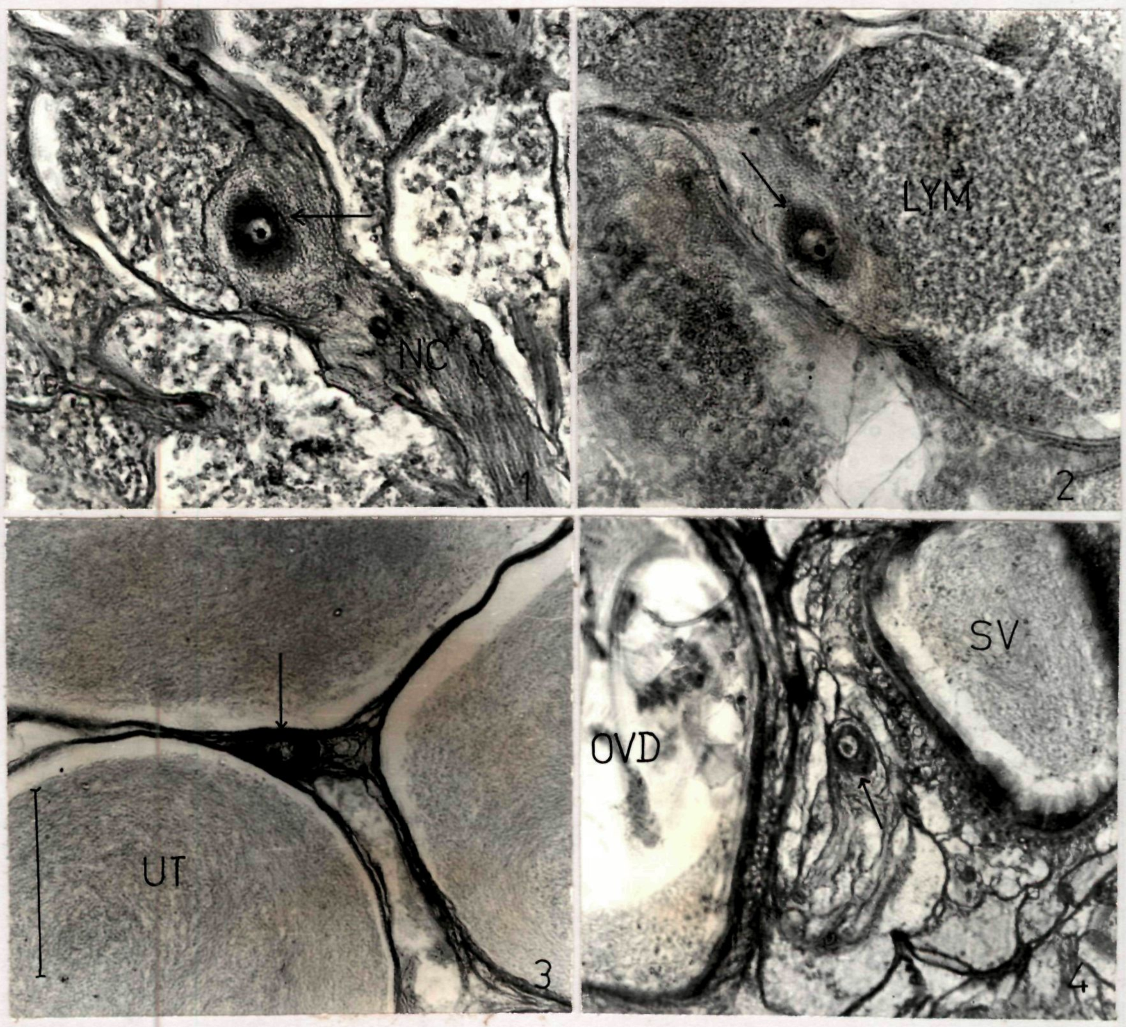


TABLE 2.9: pNSC in **Explanatum explanatum**

Location (in or in association with the organs)	Type	Cell body	Nucleus	Nucleolus (Diameter)
Brain	A	20.0-36.0 x 16.4-30.2	8.2-12.0 x 6.8-10.0	2.4-2.8
	B	16.4-24.0 x 14.2-22.8	8.0-10.0 x 7.2-9.0	2.4-2.8
Nerve cord	A	20.0-42.6 x 16.2-38.0	8.0-10.4 x 6.2-8.0	2.4-2.8
	B	20.0-28.8 x 16.2-24.4	8.0-10.4 x 7.2-9.2	3.0-3.2
Pharynx	A	28.0-36.0 x 20.0-30.0	8.0-12.0 x 6.8-10.0	3.0-3.2
	B	24.0-36.0 x 18.0-28.0	10.2-14.0 x 8.0-10.4	3.0-3.2
Intestine	A	24.2-34.4 x 18.2-30.2	6.2-9.2 x 6.0-8.2	2.8-3.0
	B	16.2-40.0 x 14.4-32.4	6.0-8.4 x 6.0-7.4	2.8-3.2
General parenchyma	A	30.4-54.0 x 24.2-48.0	8.0-10.2 x 6.8-8.6	2.4-2.8
	B	24.2-38.4 x 18.4-34.0	9.0-12.2 x 7.2-10.0	2.4-2.8
Subtegument	A	32.0-44.0 x 26.4-34.0	9.2-12.0 x 8.2-10.0	2.4-2.8
Testes	A	28.4-36.8 x 24.0-32.4	7.2-9.8 x 6.2-8.0	2.2-2.4
	B	16.6-22.4 x 14.2-18.6	6.0-9.2 x 6.0-8.4	2.2-2.4
Seminal vesicle	A	30.2-38.4 x 24.2-34.0	6.2-8.4 x 5.0-7.2	2.2-2.4
	B	16.4-24.4 x 14.2-20.2	6.0-8.8 x 6.0-8.4	2.2-2.4
Uterine coils	B	14.6-20.8 x 12.4-16.4	7.2-8.0 x 5.4-6.4	1.8-2.2
Vitellaria	A	28.2-36.4 x 22.4-32.2	8.4-10.0 x 8.0-9.4	3.0-3.2
Lymph vessel	A	18.8-34.2 x 16.0-28.8	7.2-9.2 x 6.0-8.4	2.6-2.8
Acetabulum	A	20.2-34.0 x 16.2-30.8	7.2-9.4 x 5.2-8.2	2.6-2.8
	B	18.2-28.8 x 16.0-24.2	7.6-10.2 x 7.2-9.4	2.4-2.8

Eurytrema pancreaticum

Juvenile form (Table 2.10) - Mostly 'B' type pNSC were observed in this form of the worm.

In the brain and nerves small unipolar 'B' cells with little secretory material were observed (Pl.2.13, Fig.1).

The 'A' type pNSC were located only in the general parenchyma and subtegumental region. These cells were oval or spherical with a large nucleus and a centric or eccentric nucleolus. They had little secretory material and their axonal extensions could not be traced out (Pl.2.13, Fig.2).

The 'B' cells in the oral and ventral suckers were uni- and biopolar (Pl.2.13, Fig.3).

Immature form (Table 2.11) - Both cell types were located in the immature stage of the worm.

The most peculiar feature was the presence of a darkly staining basophilic material in the nucleoplasm surrounding the nucleolus (Pl.2.13, Figs.4-6).

The 'A' cells were lesser in number, having one or two small vacuoles in the cytoplasm and with a granular secretory material.

Mature form (Table 2.12) - Both cell types were present, the 'B' type being relatively more in number.

PLATE 2.13: **Eurytrema pancreaticum** (Photomicrographs)

Figs.1-3, Juvenile form -

1. 'B' cells (arrow) in the nerve cord. CHPh.
2. 'A' cells (arrow) in the general parenchyma. CHPh.
3. 'B' cells (arrow) in the ventral sucker. CHPh.

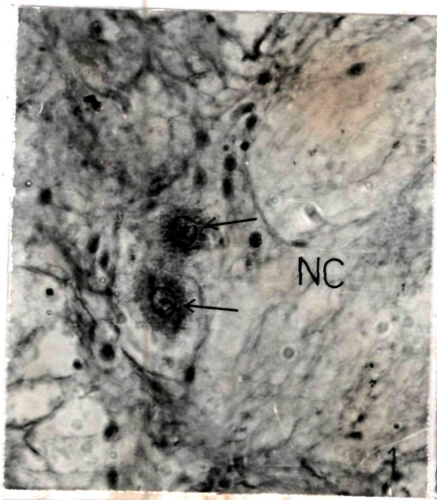
Figs.4-6, Immature form -

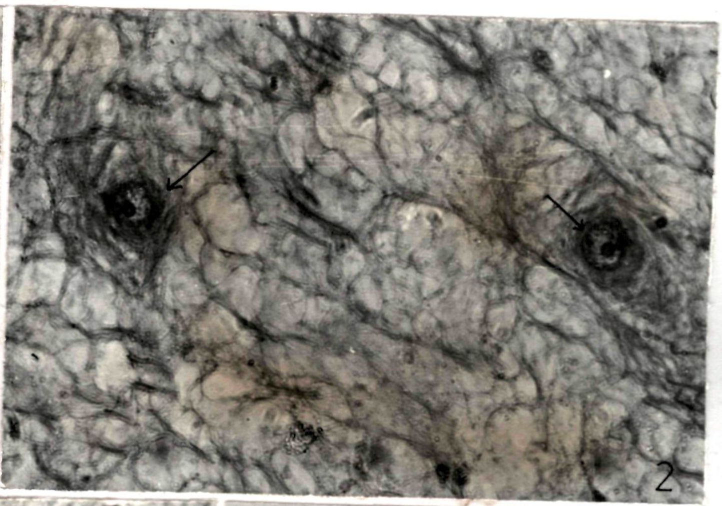
4. 'B' cell (arrow) in the subtegumental region showing the appearence of a basophilic material in the nucleoplasm. CHPh.

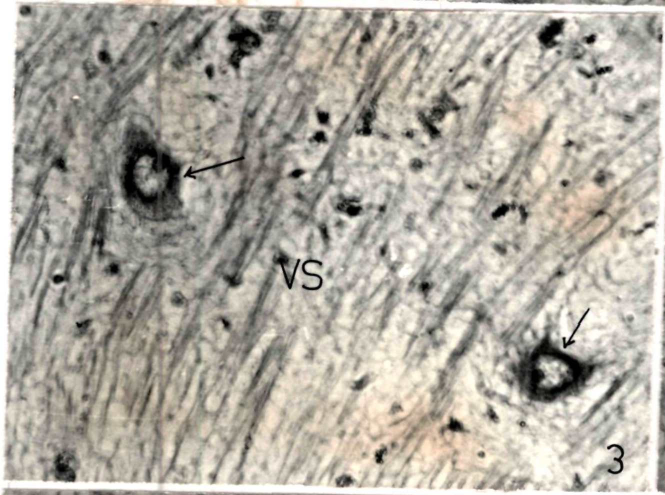
5,6. 'A' cells in the general parenchyma. The basophilic material (arrow) in the nucleoplasm is conspicuous. CHPh.

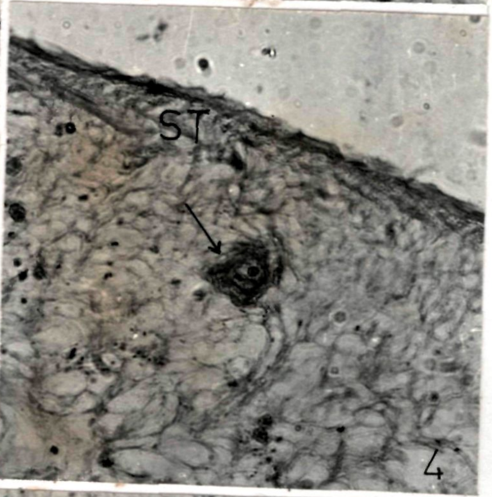
(Figs. 1-6, Scale bar = 0.05 mm).

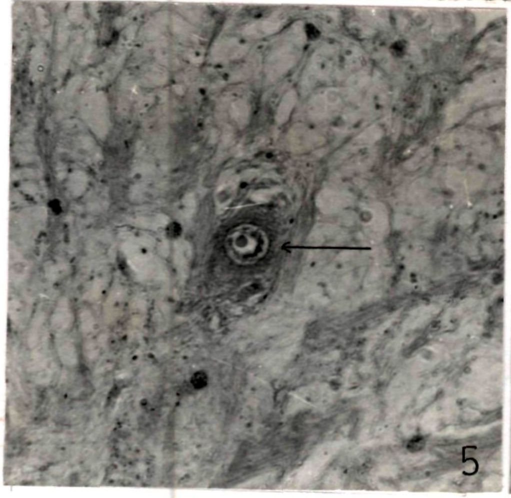
PLATE 2.13











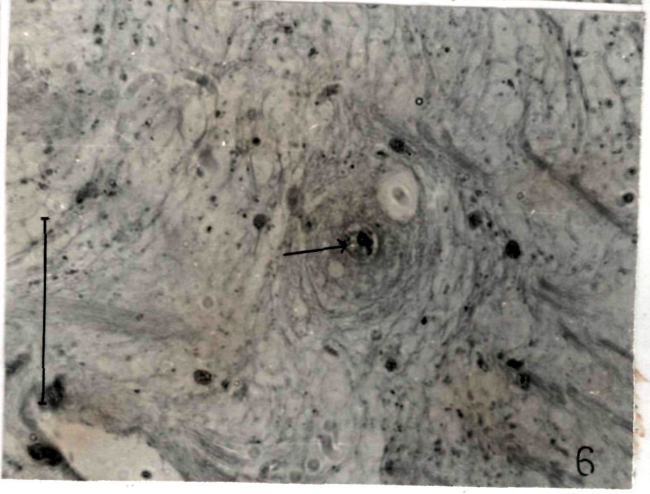


TABLE 2.10: pNSC in **Eurytrema pancreaticum:** Juvenile stage

Location (in or in association with the organs)	Type	Cell body	Nucleus	Nucleolus (Diameter)
Brain	B	12.8-18.0 x 10.0-16.2	8.0-10.2 x 7.0-8.2	2.8-3.2
Nerve cord	B	12.8-22.0 x 10.0-18.8	8.0-10.4 x 8.0-7.0	1.8-2.0
Oral sucker	B	12.2-20.8 x 8.8-16.8	8.0-9.4 x 6.2-7.8	1.8-2.0
Phorynx	B	12.0-20.2 x 8.0-16.8	8.0-10.4 x 6.4-8.0	1.8-2.2
Intestine	B	12.0-24.2 x 8.0-16.8	8.8-9.8 x 6.2-8.2	1.8-2.0
General parenchyma	A	20.4-30.8 x 14.2-24.6	8.4-12.8 x 6.2-10.2	1.8-2.0
	B	20.2-26.4 x 16.4-24.0	10.2-12.0 x 8.0-10.8	2.8-7.2
Subtegument	A	21.2-28.4 x 16.2-24.8	8.2-11.8 x 6.0-9.2	2.8-3.2
	B	14.0-26.2 x 8.2-22.0	8.8-12.2 x 7.2-9.2	2.8-3.2
Testes (primordia)	B	8.8-20.0 x 6.8-14.0	8.0-10.2 x 6.2-8.4	2.8-3.2
Ventral sucker	B	12.0-24.8 x 8.8-18.0	9.2-15.0 x 7.5-12.0	2.8-3.2

TABLE 2.11: pNSC in **Eurytrema pancreaticum:** Immature stage

Location (in or in association with the organs)	Type	Cell body	Nucleus	Nucleolus (Diameter)
Brain	A	18.4-24.8 x 12.4-20.2	12.2-15.2 x 10.0-12.8	2.6-2.8
	B	15.4-20.2 x 10.8-18.2	10.4-14.2 x 8.8-12.2	2.6-2.8
Nerve cord	B	15.2-20.4 x 10.8-16.4	10.4-12.8 x 8.0-12.4	2.4-2.6
Oral sucker	B	16.4-22.2 x 10.4-16.2	10.8-14.4 x 8.0-12.2	2.6-2.8
Pharynx	B	10.8-20.4 x 8.8-16.8	9.2-14.2 x 8.0-12.8	2.4-2.6
Intestine	B	12.2-20.8 x 10.2-16.2	10.2-12.8 x 8.4-12.0	3.2-3.4
General parenchyma	A	30.8-48.0 x 22.4-42.2	8.8-13.8 x 8.0-12.8	3.2-3.6
	B	16.8-24.2 x 12.0-20.8	8.2-10.8 x 8.0-11.4	3.4-3.8
Subtegument	A	24.0-32.8 x 20.2-28.0	8.0-10.0 x 7.8-9.4	3.2-3.4
	B	28.4-40.0 x 12.0-34.2	10.2-12.2 x 8.8-12.8	3.2-3.4
Testes	B	14.2-18.0 x 10.2-14.0	10.2-12.4 x 8.8-11.2	3.2-3.4
Ovary	B	10.0-18.0 x 8.4-12.8	8.8-12.0 x 8.0-10.8	3.2-3.4
Uterine wall	B	12.8-16.8 x 8.0-12.8	6.2-12.4 x 4.8-11.4	3.2-3.4
Ventral sucker	A	20.4-30.4 x 16.0-22.8	10.2-12.8 x 8.4-10.8	3.2-3.4
	B	12.4-18.2 x 8.0-15.2	8.0-14.2 x 6.2-12.8	2.0-2.4

PLATE 2.14: **Eurytrema pancreaticum :** Mature form (Photomicrographs)

Fig.1 'A' cell with a long axon in the subtegumental region. CHPh.

Fig.2 'B' cells in the subtegumental region. CHPh.

Fig.3 'A' cell near the cirrus sac. CHPh.

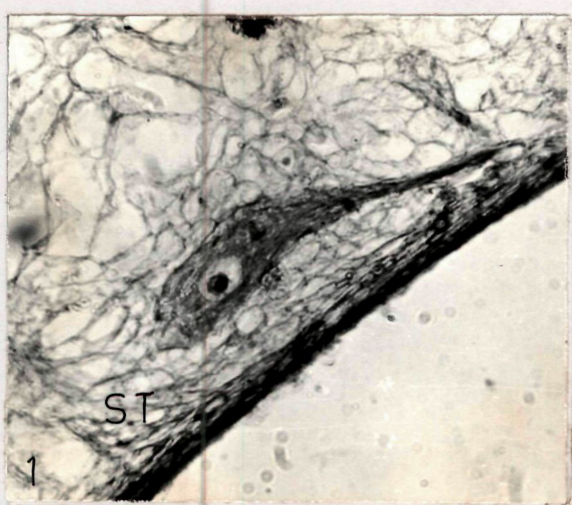
Fig.4 'A' cell (arrow) near the uterine coils, showing the nucleoli in its nucleus. CHPh.

Fig.5 'A' cell (arrow) with dense secretion and long axon in the general parenchyma. CHPh.

Fig.6 'B' cell (arrow) in the ventral sucker. AbPh.

(Figs. 1-6, Scale bare = 0.05 mm).

PLATE 2.14



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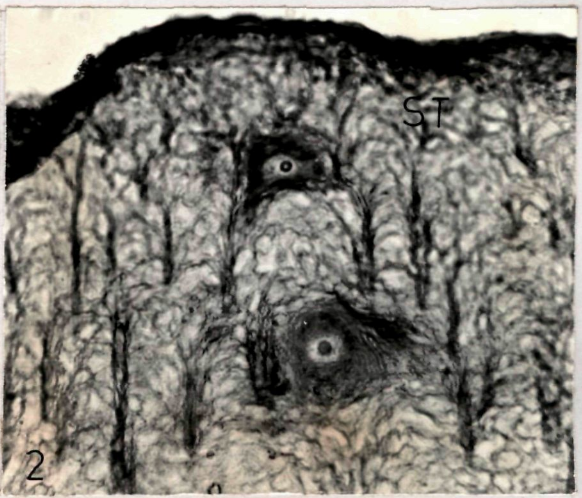

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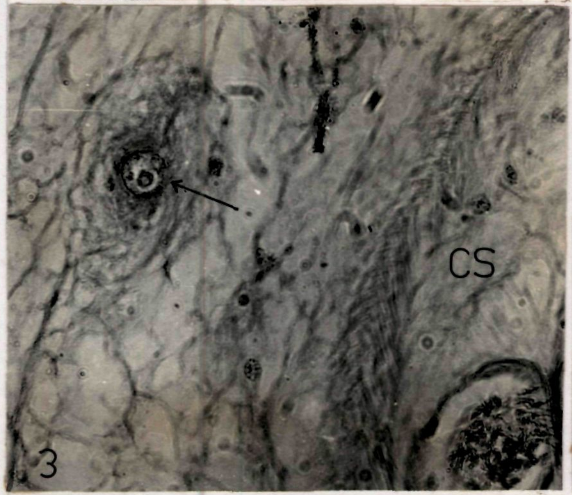

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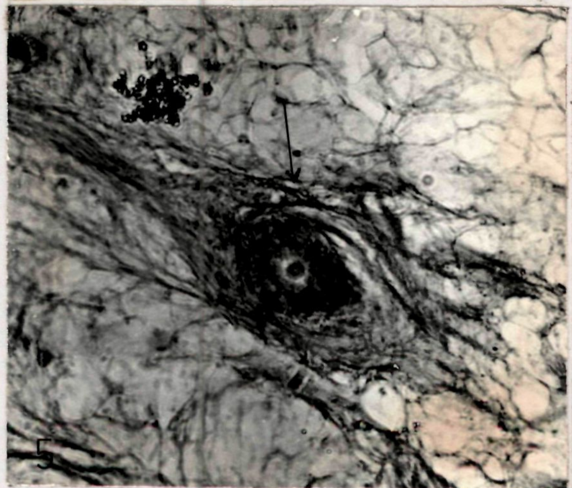

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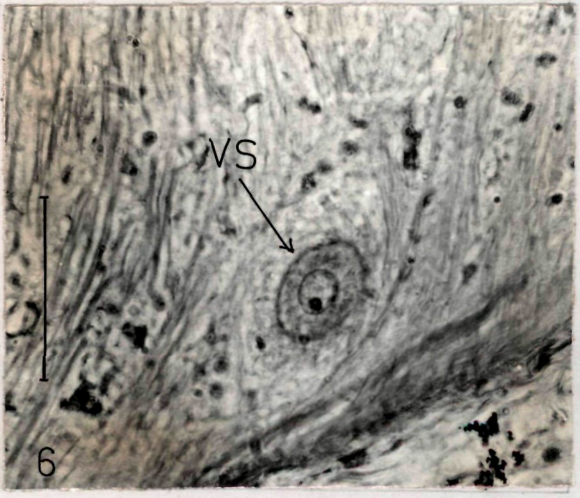

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TABLE 2.12: pNSC in **Eurytrema pancreaticum:** Mature stage

Location (in or in association with the organs)	Type	Cell body	Nucleus	Nucleolus (Diameter)
Brain	A	40.0-44.4 x 32.4-36.2	9.2-10.4 x 8.4-9.6	2.6-2.8
	B	18.0-20.0 x 14.0-16.0	8.4-10.0 x 8.0-9.2	2.6-2.8
Nerve cord	A	28.0-40.0 x 18.0-24.4	10.0-10.4 x 9.2-10.0	2.6-2.8
	B	18.4-24.6 x 16.0-18.0	8.0-9.2 x 7.2-8.8	2.6-2.8
Oral sucker	A	24.6-34.4 x 20.0-28.2	10.0-10.2 x 9.2-10.4	2.2-2.4
	B	14.2-24.4 x 14.0-18.6	7.4-8.0 x 6.2-7.4	2.2-2.4
Pharynx	B	14.4-24.6 x 14.0-18.6	8.4-9.2 x 7.2-8.8	2.2-2.4
Intestine	A	32.2-40.0 x 22.6-28.4	10.0-10.4 x 8.0-8.4	3.0-3.8
General parenchyma	A	32.4-48.0 x 30.2-42.4	8.0-12.4 x 7.2-10.4	3.0-3.6
	B	28.0-44.0 x 22.0-36.8	8.2-12.4 x 6.2-8.0	2.4-2.6
Subtegument	A	40.6-60.2 x 32.0-54.4	8.4-13.2 x 8.0-12.2	3.8-4.0
	B	24.2-38.0 x 22.4-32.6	8.4-10.4 x 8.0-10.2	2.8-3.2
Testes	B	24.2-32.6 x 20.0-24.0	7.2-8.0 x 7.2-7.4	2.8-3.0
Uterine coils	A	24.6-34.2 x 18.0-28.4	7.2-9.2 x 3.4-8.2	2.4-2.6
Vitellaria	A	34.6-48.4 x 18.2-40.6	9.2-12.4 x 8.4-10.6	3.6-3.8
Ventral sucker	A	30.6-44.4 x 28.0-36.4	11.2-12.8 x 9.2-10.4	2.4-3.0
	B	20.2-34.2 x 18.2-28.2	10.0-15.2 x 8.4-12.0	2.4-2.8

In both these pNSC, the chromatin-like basophalic nuclear material was absent (Pl.2.14, Figs.1,2) or reduced in quantity, some times occurring in the form of only 2-3 dots (Pl.2.14, Figs.3,4). The 'A' cells were more frequently observed in the anterior region of the worm than in the posterior. These cells were spherical or pyriform, unipolar with moderate to dense secretion in their perikarya and a few big vacuoles. Often long axonal processes from these cells, filled with the secretory material and terminating in the parenchymatous tissues, were also observed (Pl.2.14, Fig.5).

The 'B' cells in the ventral sucker and pharynx were oval or spherical with large rounded centric nucleus and one or more centric or eccentric nucleolus (Pl.2.14, Fig.6).

Fasciola gigantica (Table 2.13)

Mostly 'A' type pNSC were located in the body approximating various organs.

A few bipolar 'A' cells with dense cytoplasm and 2-3 big vacuoles were observed near the uterine coils (Pl.2.15, Fig.1). The 'A' cells present in the general parenchyma had *immensely vacuolated cytoplasm and centric, spherical or oval* nucleus with one or two nucleoli (Pl.2.15, Figs.2,3).

Two or three spherical, unipolar 'B' cells were observed in a group in the nerve cord region. All these cells had large oval or spherical nucleus with conspicuous centric or eccentric nucleolus (Pl.2.15, Fig.4).

PLATE 2.15: **Fasciola gigantica** (Photomicrographs)

Fig.1 Bipolar 'A' cell near a uterine coil. CHPh.

Fig.2 'A' cell (arrow) with two nucleoli in the general parenchyma. CHPh.

Fig.3 'A' cell (arrow) with two nucleoli near cirrus sac. Heidenhain's Azan.

Fig.4 'B' cells (arrow) in the nerve cord region. CHPh.

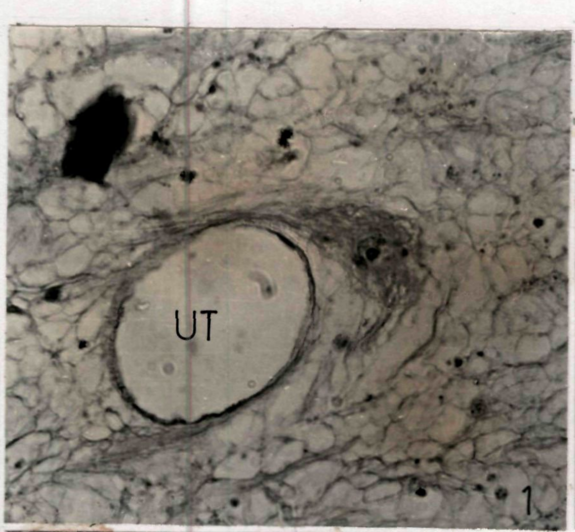
Fig.5 'B' cell (arrow) near the uterine coil. CHPh.

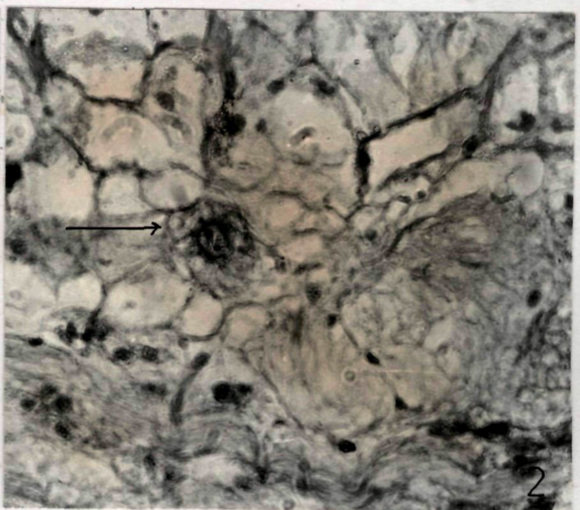
(**Figs. 1-5,** Scale bar = 0.05 mm)

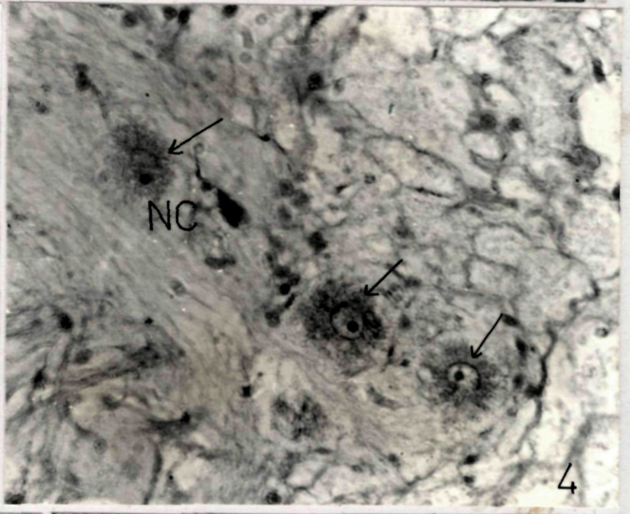
Fig.6 'A' cell (large arrow) and 'B' cell (small arrow) in the ventral sucker. CHPh.

(**Fig.6,** Scale bar = 0.1 mm).

PLATE 2.15







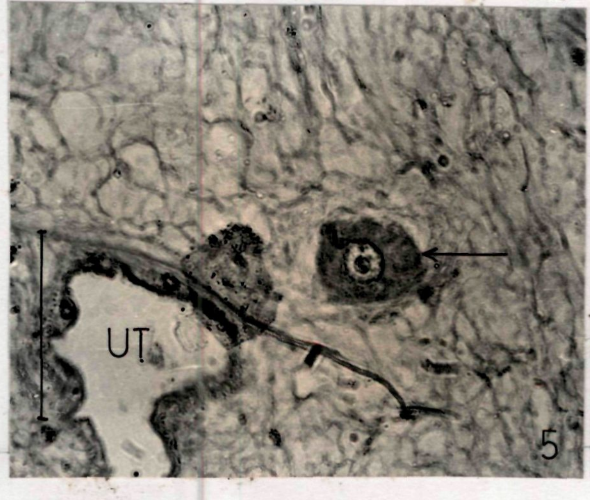


TABLE 2.13: pNSC in **Fasciola gigantica**

Location (in or in association with the organs)	*Type*	*Cell body*	*Nucleus*	Nucleolus (Diameter)
Brain	A	24.4-32.4 x 22.0-26.8	10.0-11.2 x 6.2-10.4	1.2-1.4
	B	18.4-26.4 x 12.4-18.8	8.0-10.0 x 8.0-9.0	1.2-1.4
Nerve cord	A	20.4-32.2 x 16.0-26.2	10.0-12.0 x 8.2-9.0	2.4-2.8
	B	16.4-36.0 x 12.0-24.2	9.0-11.2 x 8.0-10.0	1.2-1.4
Pharynx	A	28.4-40.0 x 24.4-32.0	9.0-12.0 x 6.0-10.0	2.4-2.6
Intestine	A	24.0-40.2 x 20.8-38.2	12.0-12.4 x 10.0-11.2	1.8-2.0
General parenchyma	A	32.4-44.0 x 28.8-38.2	10.2-13.8 x 8.8-12.0	2.4-2.8
	B	20.4-28.4 x 18.2-24.4	8.0-12.0 x 7.0-10.0	2.2-2.4
Subtegument	A	32.8-56.0 x 28.8-48.8	10.0-12.0 x 10.0-11.2	2.4-2.8
Testes	B	16.0-20.0 x 18.2-14.2	10.0-11.2 x 6.0-8.0	3.0-3.2
Uterine wall	A	34.2-44.0 x 28.2-40.8	10.0-14.2 x 8.0-12.4	2.2-2.4
	B	12.2-24.6 x 10.0-20.8	6.2-10.2 x 4.8-8.0	2.8-3.2
Cirrus sac	A	30.8-40.0 x 24.4-40.2	11.2-12.0 x 10.0-12.0	2.8-3.0
Ventral sucker	A	32.2-48.2 x 30.0-44.0	11.2-15.4 x 10.0-12.0	2.4-2.8
	B	20.4-28.2 x 16.8-24.8	11.2-15.0 x 6.0-8.0	2.8-3.2

A few comparatively large 'B' cells, mostly unipolar with dense secretory material, were present near the reproductive components (Pl.2.15, Fig.5).

Both the cell types were present in the oral and ventral suckers. The 'A' cells were mostly unipolar, whereas bipolar 'B' cells occurred frequently in the suckers (Pl.2.15, Fig.6).

The comparative distribution of pNSC, localized in respect of various organs of the body in all the species studied herein is depicted in Table 2.14.

B. **Histochemical nature of the NSM**

The results of the histochemical tests employed to ascertain the nature of the NSM are depicted in Table 2.15. While all the species gave a very strongly positive reaction for proteins, different endgroups of proteins was from moderate to strongly positive (Pl.2.16, Figs.3-6; Pl.2.17, Figs.1-4). Only mild reaction was observed for RNA (Pl.2.17, Figs.5,6). A moderately positive reaction was also exhibited for PAS, indicating the presence of glycol group (Pl.2.18, Figs.1,2). No reaction or a weakly positive reaction was also observed for lipids (Pl.2.18, Figs.5,6). A moderately positive reaction for AChE and weakly positive reaction for BChE was also observed. (Pl.2.19, Figs.1-4). Moderately positive to strongly positive reaction was also exhibited for NSE in different species (Pl.2.19, Figs.5,6).

TABLE 2.14: pNSC types and their localization in the trematode species studied

Organ	F. cobboldi			F.elongatus	O.indica	C.cotylo-phorum	P.gracile	O.strepto-coelium	E.expla-natum	E. pancreaticum			F.gigantic
	Juvenile	Immature	Mature							Juvenile	Immature	Mature	
Brain (Cerebral ganglia +commissure)	B	A,B	A,B	A,B	A,B	A,B	A,B	A,B	A,B	B	A,B	A,B	A,B
Nerve cord	B	A,B	A,B	A,B	A.B	B	B	A	A,B	B	B	A,B	A,B
Oral sucker	-	-	-	-	-	-	-	-	-	B	B	A,B	A,B
Pharynx	B	A	A,	A	A,B	A	A	A	A,B	B	B	B	A
Oesophagus	-	-	-	-	A,B	-	A	-	-	-	-	-	-
Intestine	B	B	A,B	A	A	B	B	B	A,B	B	B	A	A
General parenchyma	A,B	A,B	A,B	A,B	A	A,B	A,B	A,B	A,B	A,B	A,B	A,B	A,B
Subtegument	A,B	A,B	A,B	A,B	A	A	A,B	A,B	A	A,B	A,B	A,B	A,B
Testes	-	B	-	-	-	B	-	B	A,B	B	B	B	B
Seminal vesicle	-	-	-	-	-	B	-	-	A,B	-	-	-	-
Pars musculosa	-	B	B	-	-	-	-	-	-	-	-	-	-
Pars prostatica	-	B	-	-	-	-	-	-	-	-	-	-	-
Ovary	-	A,B	-	-	-	-	-	-	-	-	B	-	-
Mehlis' gland	B	-	-	-	-	-	-	B	-	-	-	-	-
Uterine coils	-	A,B	B	B	A,B	-	B	B	B	-	B	A	A,B
Vitelleria	-	A	A	A	-	A	-	-	A	-	-	A	A
Cirrus sac	-	-	-	-	-	-	-	-	-	-	-	A	A
Lymph vessel	B	A,B	A	A	-	A,B	B	B	A	-	-	-	-
Acetabulum (=Ventral sucker)	B	A,B	A,B	A,B	A,B	A,B	A,B	A,B	A,B	B	A,B	A,B	A,B

TABLE 2.15: Histochemical tests performed for NSM

Metabolites	Reaction	Author	Fixative	Sections	**F.cobboldi**	**F.elongatus**	**C.cotylophorum**	**E.explanatum**	**E.pancreaticum**
Proteins	Mercuric Bromophenol Blue method	Mazia **et al.** (1953)	10% neutral buffered formalin	Paraffin sections	++++	++++	++++	++++	++++
Disulphides	Performic acid-Alcian Blue method	Adams & S'oper (1955-'56)	"	"	++	++	++	++	++
Arginine	Sakaguchi method (1925)	Baker (1947)	"	"	-	-	-	-	-
Tyrosine	Millon's (1849)	Baker (1956)	"	"	+	+	+	+	+
Histons	Alkaline Fast Green	Alfert & Geschwind (1953)	"	"	+++	+++	+++	+++	+++
Proteins with amino group(NH_2^-)	Ninhydrin -Schiff method	Yasuma and Itchikawa(1953)	Bouin's	"	+++	+++	+++	+++	+++
RNA	Methyl green /pyronin	Modified by Kurnick (1955)	Carnoy's	"	+	+	+	+	+
DNA	Feulgen nuclear	Feulgen & Rossenback (1924)	"	"	-	-	-	-	-
Carbohydrates	PAS	McManus (1946)	Formol-alcohol	"	++	++	++	++	+
Glycogen	Best's Carmine method	Best(1905)	"	"	-	-	-	-	-

TABLE 2.15: Histochemical tests performed for NSM (contd..)

Metabolites	Reaction	Author	Fixative	Sections	**F.cobboldi**	**F.elongatus**	**C.cotylophorum**	**E.explanatum**	**E.pancre-aticum**
Acid muco substances	Alcian Blue method	Scott & Dorling (1965)	Bouin's	Paraffin	-	-	-	-	-
"	Toluidine Blue	Kramer & Windrum (1955)	"	"	-	-	-	-	-
Neutral muco-substances	Alcian Blue PAS method	Mowry (1956)	10% neutral buffered formalin	"	-	-	-	-	-
Lipids (general)	Sudan Black B	Lison & Dagnelie (1935)	Formol-Calcium	"	+	+	+	-	+
Unsaturated lipids	Performic Acid-Schiffs	Lillie (1951)	"	"	-	-	-	-	-
Phospho-lipids	Acid Haematein method	Baker (1946)	10% cold neutral formalin	"	-	-	-	-	-
AChE	Thiocholine method	Gerebtzoff (1959)	10% cold neutral formalin	Fresh-frozen	++	++	++	++	++
BChE	"	"	"	+	+	+	+	+	+
NSE	Indoxyl Acetate method	Holt (1958)	"	"	+++	+++	+++	+++	++

- = Negative; + Weakly positive reaction: ++ = Moderately positive; +++ = Strongly positive; ++++ = Very strongly positive.

PLATE 2.16: **Histochemical tests** for NSM (Photomicrographs)

Fig.1 'A' cell in the acetabulum showing a very strongly positive reaction for proteins in **F. cobboldi.** Bromophenol blue.

Fig.2 'B' cell (arrow) in the uterine wall showing a very strongly positive reaction for proteins in **E. explanatum.** Bromophenol blue.

Fig.3 'A' cell (arrow) in the subtegumental region showing a strongly positive reaction for amino group (- NH_2^-) in **E. pancreaticum.** Ninhydrin Schiff's.

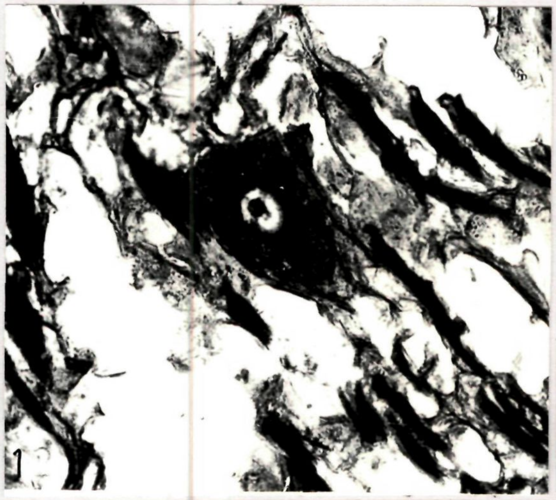
Fig.4 'A' cell (arrow) in the general parenchyma approximating a nerve cord, showing a strongly positive reaction for amino group (- - NH_2^-) in **E. explanatum.** Ninhydrin Schiff's.

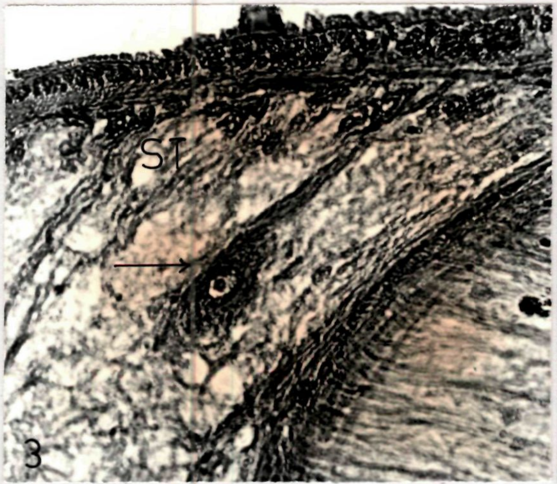
Fig.5 'A' cell (arrow) in the parenchyma approximating pars prostatica, showing a moderately positive reaction for Cysteine in **F. cobboldi.** Performic acid/Alcian blue.

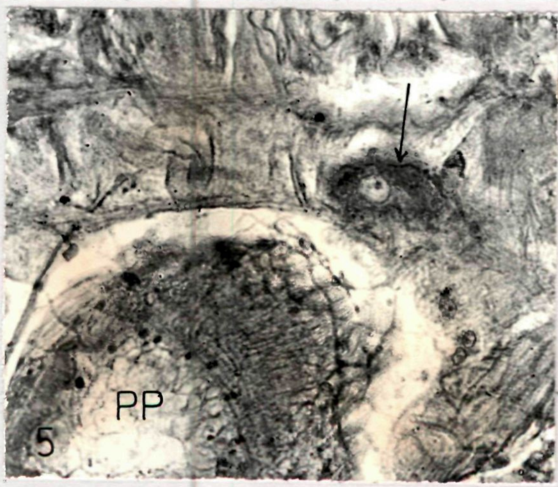
Fig.6 A multipolar 'A' (arrow) cell in the subtegumental region associated with the wall of lymph vessel, showing a moderately positive reaction for Cysteine in **C. cotylophorum.** Performic acid/Alcian blue.

(**Figs. 1-6**, Scale bar = 0.05 mm).

PLATE 2.16







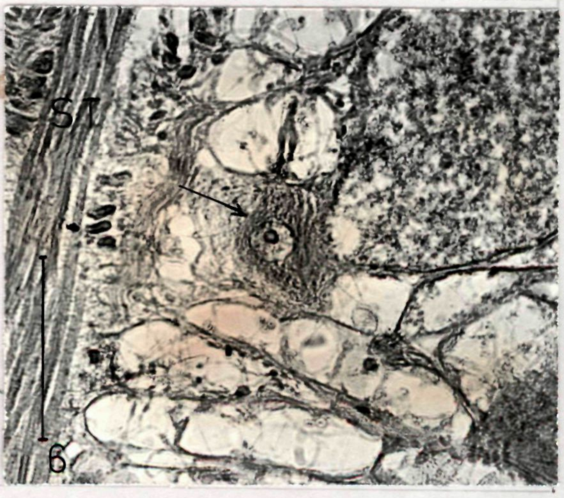


PLATE 2.17: **Histochemical Tests** for NSM (Photomicrographs)

Fig.1 'B' cell (arrow) in the subtegumental region showing a strongly positive reaction for histones in **F. elongatus.** Alkaline Fast green.

Fig.2 Bipolar 'B' cell (arrow) with two nucleoli in the general parenchyma near ventral sucker showing strongly positive reaction for histones in **E. pancreaticum.** Alkaline Fast green.

Fig.3 'A' cell (arrow) in the general parenchyma showing a moderately positive reaction for tyrosine in **F. cobboldi.** Millon's test.

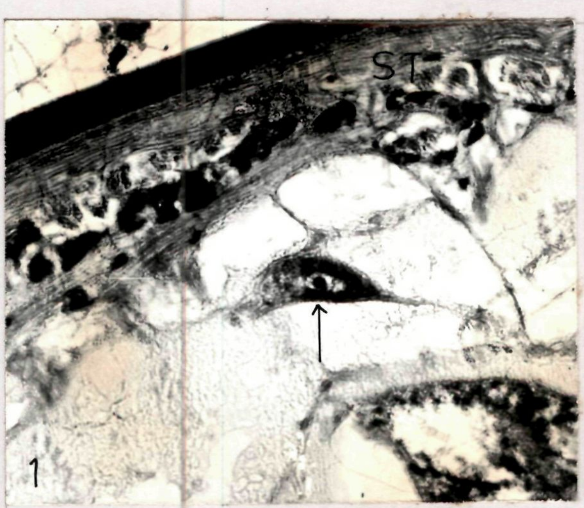
Fig.4 'B' cell (arrow) in the general parenchyma showing a moderately positive reaction for tyrosine in **E. explanatum.** Millon's test.

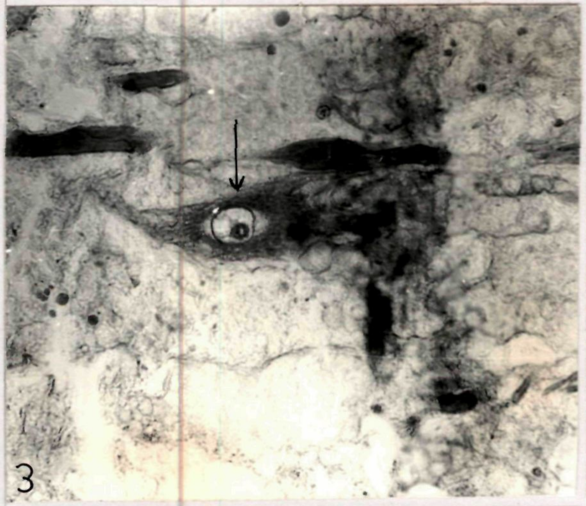
Fig.5 'A' cell (arrow) in the subtegumental region showing a weakly positive reaction for RNA in **F. cobboldi.** Methyl green-Pyronin.

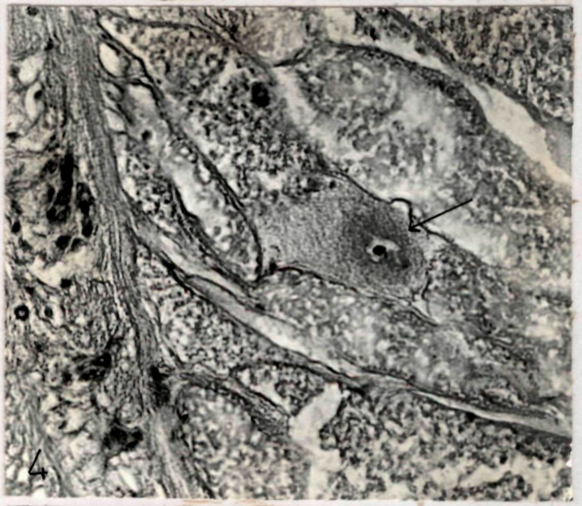
Fig.6 'A' cell (arrow) in the general parenchyma showing a weakly positive reaction for RNA in **E. pancreaticum.** Methyl green-pyronin.

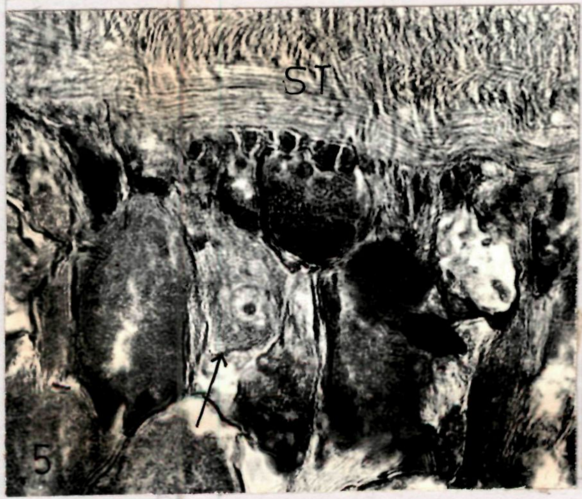
(Figs. 1-6, Scale bar = 0.05 mm).

PLATE 2.17









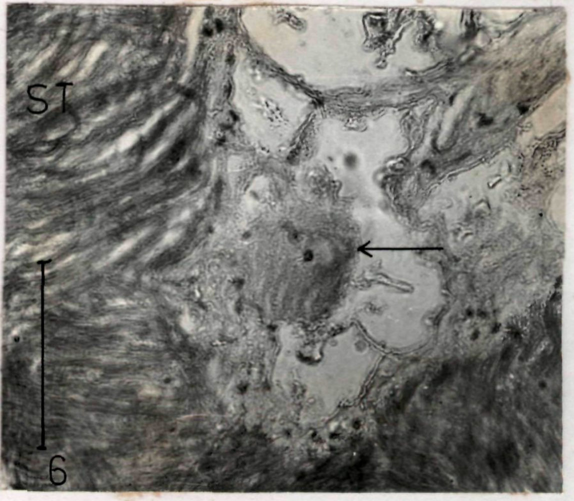


PLATE 2.18: **Histochemical Tests** for NSM (Photomicrographs)

Fig.1 'B' cell in the general parenchyma showing a moderately positive reaction for glycol group in **E. explanatum.** PAS.

Figs.2 Two unipolar 'A' cells in the general parenchyma showing a moderately positive reaction for glycol group in **C. cotylophorum.** PAS.

Fig.3 'A' cell (arrow) approximating a nerve cord in the general parenchyma showing a negative reaction for glycogen in **F. cobboldi.** Best's carmine.

Fig.4 A spherical unipolar 'A' (arrow) in the pharynx showing a negative reaction for glycogen in **F. elongatus.** Best's carmine.

Fig.5 'A' cell (arrow) in the subtegumental region showing a weakly positive reaction for lipids in **F. cobboldi.** Sudan Black B.

Fig.6 Bipolar 'B' cell (arrow) near vitelline follicle showing a weakly positive reaction for lipids in **C. cotylophorum.** Sudan Black B.

(**Figs. 1-6,** Scale bar = 0.05 mm).

PLATE 2.18

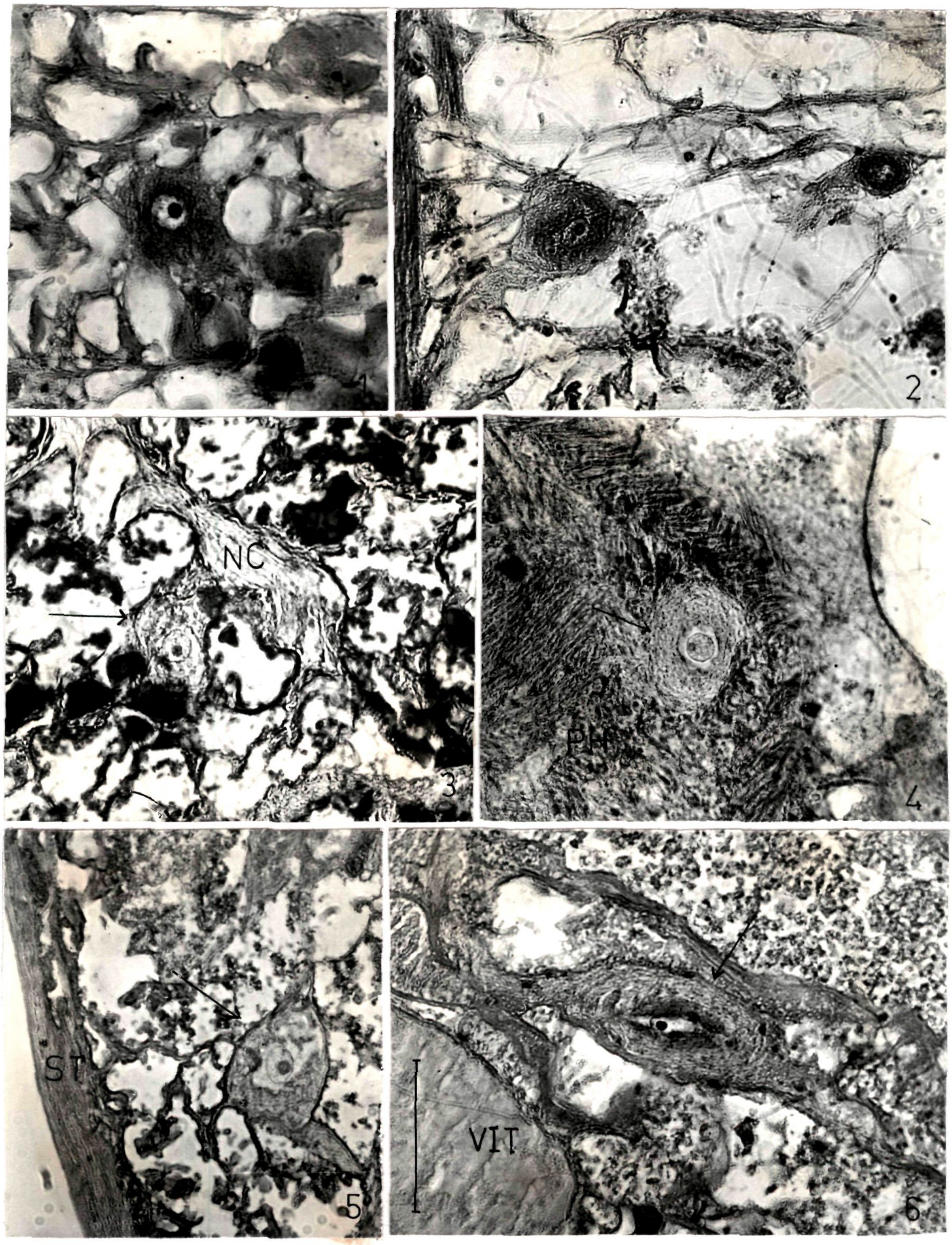


PLATE 2.19: **Histochemical tests** for NSM (Photomicrographs)

Fig.1 'B' cell (arrow) approximating a nerve cord, showing a moderately positive reaction for acetyl cholinesterase in **F. cobboldi.** Thiocholine iodide method.

Fig.2 'A' cell (arrow) approximating the testes showing a moderately positive reaction for acetyl cholinesterase in **F. elongatus.** Thiocholine iodide method.

Fig.3 'A' cell (arrow) in the subtegumental region showing a weakly positive reaction for butyl cholinesterase in **E. pancreaticum.** Thiocholine iodide method.

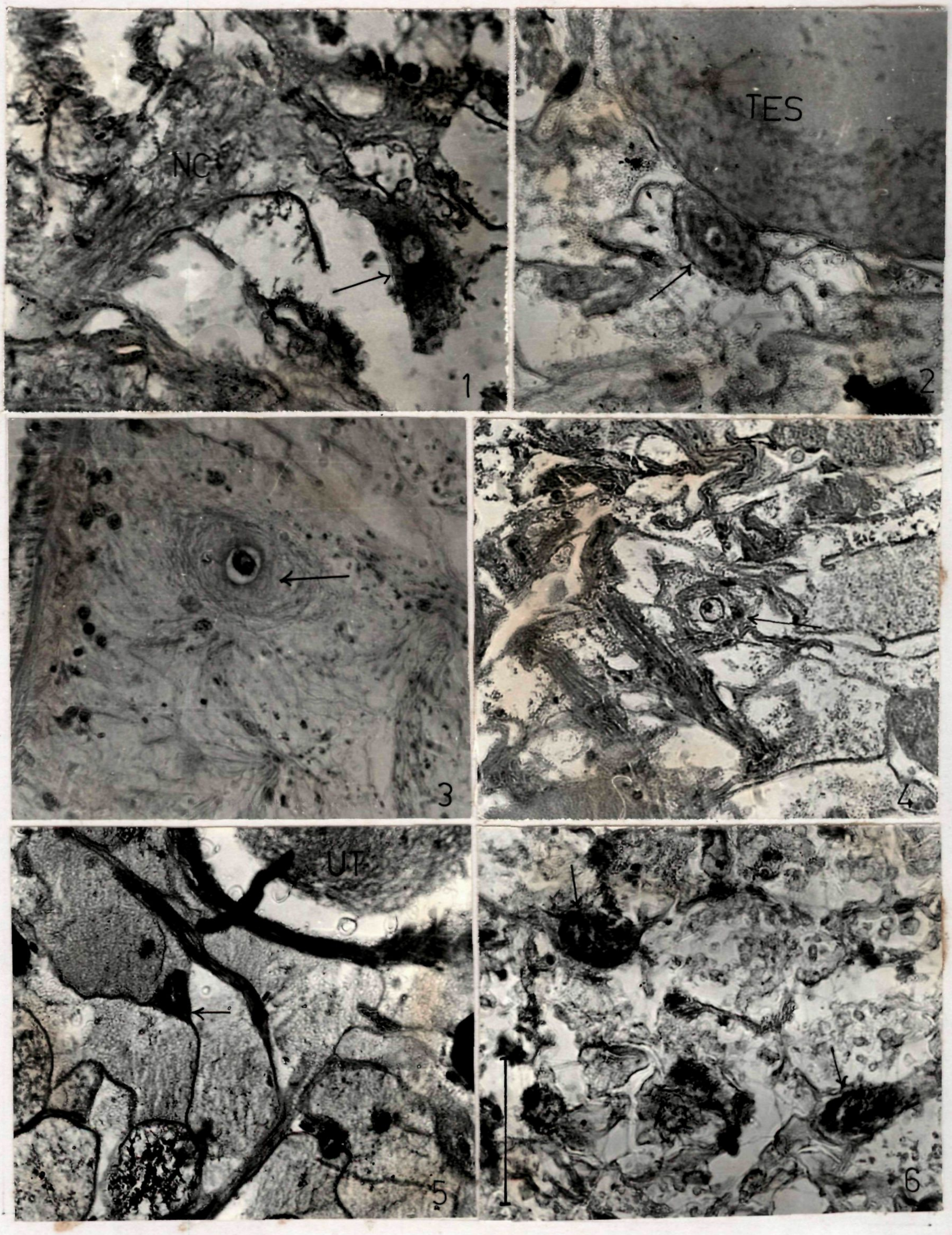
Fig.4 'A' cell (arrow) in the general parenchyma showing a weakly positive reaction for butyl cholinesterase in **C. cotylophorum.** Thiocholine iodide method.

Fig.5 Bipolar 'B' cell (arrow) nearing a uterine coil showing a strongly positive reaction for nonspecific esterases in **E. explanatum.** Indoxyl acetate method.

Fig.6 'A' cell (arrow) in the general parenchyma showing a strongly positive reaction for non specific esterases in **F. cobboldi.** Indoxyl acetate method.

(Figs. 1-6, Scale bar = 0.05 mm).

PLATE 2.19



DISCUSSION

In the course of evolution, the primitive nervous system had also evolved by giving way to more elaborate and advanced structures in both vertebrate and invertebrate groups. One of these ways is associated with the property of some of the "conventional" nerve cells to display cytological signs of neurosecretory activity. These neurosecretory cells, therefore, may play an important and coordinative role in forms like platyhelminths which belong to a group of primitive invertebrates that lack both a proper circulatory system and regular glands for internal secretion.

To determine a nerve cell as neurosecretory, several criteria are generally agreed upon; these are (I) the presence of a morphologically demonstrable secretory product, typically stainable by CHPh or PAF or by visualization of electron-dense granules, called elementary neurosecretory granules (ENG) (Bern, 1962), (II) the presence of α-glycogen in the neurites (Webb, 1977) (III) morphological changes in the neurosecretory cells correlating with the physiological changes in the organism, and (IV) an identifiable physiological hormonal effect of the product from the NSC (Charniaux-Cotton and Kleinholz, 1964; Simpson **et al.**, 1966). In the present study, the cells described on the basis of the light and electron microscopy satisfy the first criterion, i.e., the morphological criterion, to be considered as neurosecretory. To fulfil the second criterion an effort has been made to correlate the possible alteration in a developing worm with the changes in the activity of the cells. Since only one of the criteria is established authentically the cells in the present study are designated as presumptive neurosecretory cells (pNSC).

The pNSC described herein revealed a fuchsinophilic or phloxinophilic nature of their cytoplasm. The product of neurosecretion exhibited similar variability in its staining affinities in respect of all the species studied herein. The cells were found to have affinity for CHPh but intensity of PAF was too low to be photomicrographed. The granules in both the cell types assumed an intense red colour, whereas some of the 'B' cells present in the acetabulum took up both the stains, azocarmine and aniline blue and varied from bluish to violet when stained with Heidenhain's Azan method. With AbPh the NSM stained in different shades of blue. The intensity of different stains was more or less the same in all the species studied herein. However, the minute differences observed in the tint can be attributed to the differences in the quantity of the NSM present and not to the variation in its affinities for the various stains used. Recently, a few cells present in the oral and ventral suckers of **Orthocoelium scoliocoelium** were considered non-neurosecretory in the absence of any cyclicity of activity by Bhatnagar **et al.** (1980), although they responded positively for CHPh and PAF stains. In cestodes also Specian and Lumsden (1980) confirmed the PAF positive nature of the rostellar glands but demonstrated them to be non-neurosecretory; these cells were earlier thought to be neurosecretory by Davey and Breckenridge (1967) based on the

PAF-differential staining. The PAF technique is known to reveal substances other than NSM also (Pearse, 1968).

The pNSC belonging to two morphological categories, i.e., 'A' and 'B', were located close to the central as well as peripheral nervous system in conformity with their location in other trematodes species (Gresson and Threadgold, 1964; Shyamasundari and Rao, 1975; Matskasi, 1970; Rao and Shyamasundari, 1977; Mehrotra and Bhutia, 1977, 1979; Kalyankar and Kankal, 1981; Sharma and Sharma, 1981). However, Grasso (1967, a,b) did not describe two morphological types of cells in **Fasciola hepatica** but mentioned the presence of uni-, bi- and tripolar neurons. Prasad and Shyamasundari (1977) localized three basic types of NSC in **Cymatocarpus undulatus**, designating them as A, B and C. NSC of three types (1,2 and 3) have also been reported in **Paragonimus westermani** and **Euparadistomum** sp. by Shyamasundari and Rao (1980). However, Bhatnagar **et al.** (1980) could detect only one morphologically distinct NSC type (i.e., 'A') in the paramphistome, **Ceylonocotyle (=Orthocoelium) scoliocoelium.**

While the 'A' cells have been described as occurring only in the brain, nerve cord and commissure (Shyamasundari and Rao, 1975; Rao and Shyamasundari, 1977), in general parenchyma, subtegumental region, near uterine coils (Mehrotra and Bhutia, 1977, 1979) or only in the pharynx and oral sucker (Gresson and Threadgold, 1964; Matskasi, 1970), the 'B' type NSC have been reported in the various locations like suckers, pharynx and parenchyma (Gresson and Threadgold, 1964; Matskasi, 1970), near cirrus sac and Mehlis' gland region (Shyamasundari and Rao, 1975) and near oesophagus, intestine, vitelline follicles, acetabulum and various reproductive components (Mehrotra and Bhutia, 1977, 1979). In the present investigation, both the cell types were detected in the brain, nerve cords, subtegumental region, parenchyma, approximating lymph vessels (in paramphistomid species), pharynx, oesophagus, intestine, oral and ventral suckers and various components of the reproductive system.

The ultrastructure of cerebral plexus of **F. cobboldi** was revealed with the help of electron microscopic studies demonstrating the presence of uniform calibre nerve fibres and cells that exhibited characteristics regarded as representative of neurosecretion, i.e., large electron dense granules (ENG) side by side with small clear or less dense vesicles. Electron-opaque granules are also reported in other platyhelminths such as planarians (Morita and Best, 1965; Oosaki and Ishii, 1965; Grasso and Quaglia 1970a,b), trematodes (Gresson and Threadgold, 1964; Dixon and Mercer, 1965; Reissig, 1970) and cestodes (Morseth, 1967; Davey and Breckenridge, 1967, Webb, 1977). Regarding the origin of such granules, it is most probable that they are synthesized in the perikarya of the nerve cells and migrate from there along the axons. The approximation of these granules with Golgi complex is evidenced in trematodes and cestodes (Grasso and Quaglia, 1974; Webb, 1976, 1977).

The size and shape of the pNSC varied from species to species and also among the individuals of the same species. These cannot be considered authentic criteria for the identification of the different NSC types. The change in dimension can also be attributed to the cyclic activity of cells (Thompson and Lea, 1968; Srivastava and Srivastava, 1976).

In the present study the pNSC designated as 'A' type exhibited variation in respect of the degree of vacuolization in their cytoplasm; the cells ranged from those having small vacuoles to those with a single or several large vacuoles. Besides empty cells (i.e., completely devoid of the NSM), half filled ones with their cytoplasm confined to the peripheral region of the cell were also observed with their nucleus often showing a deformity in the nuclear wall. These variabilities may indicate towards either the existence of several cell types or the changes in the product elaborated by a single cell type in the course of its secretory cycle. Matskasi (1970) also reported a change in the cytoplasmic nature depending on the rhythmic process of secretion and attributed the increase in vacuolization to the decreased activity of the cell. While the resorption of the product of secretion is exhibited by vacuolization of the cytoplasm, the presence of a large amount of NSM in the cytoplasm is suggestive of more or less complete synthesis to be followed by discharge (Gabe, 1966). The disappearance of the NSM probably indicated the discharge of the active substances finally leading to a completely empty cell, devoid of the NSM. The deformity in the shape of the nuclear wall, as observed in the present study in **F. cobboldi** and **C. cotylophorum**, has also been reported in polychaetes (Clark, 1955a,b; 1959) where it is regarded as a stage of secretory cycle associated with the decreased activity of the cell. In **C. cotylophorum** a dark band like zone around the nucleus was very conspicuous probably exhibiting the concentration of secretory material in this region only. A similar phenomenon has been observed by Ishikawa (1961) in a nematode that the secretory granules first appear around the nucleus and then migrate to the periphery of the nerve cells and are secreted to the tissues surrounding the cells.

Variations were also observed with regard to the appearance of the NSM with respect to different species studied herein: distinctly granular as in **P. gracile**; granular with a tendency towards aggregation of granules as in **F. elongatus, F. cobboldi, O. indica, E. pancreaticum, O. streptocoelium** and **E. explanatum** and at times also with some darkly staining basophilic masses scattered in the cytoplasm as evident in **C. cotylophorum**; or apparently fibrous as in **F. gigantica**. A size variation in electron dense granules ranging from 400-1200 A° has been reported in many trematode and cestode species (Morseth, 1967; Grasso and Quaglia, 1972, 1974; Webb, 1976, 1977). The aggregation of neurosecretory granules in the cell perikarya forming big droplets and visible at light microscopic level is also reported in teleost and elasmobranch fishes (Fredberg, 1962; Saenko, 1970; Brady, 1984). The occurrence of neurosecretory granules in various forms like globules, droplets and colloids is observed in many invertebrate species (see Gabe, 1966).

The cytoplasmic characteristics of 'B' cells were found to be uniform in the species of present study. No changes were observed indicating the synthesis or discharge of NSM leading to an empty stage of the cell. The differences were chiefly in the size and shape of these cells present at various locations in the body. The regular occurrence of uni- and bipolar cells with long axonal processes is in conformity with the earlier reports of Mehrotra and Bhutia (1977, 1979) on **P. epiclitum** and **Gastrothylax** sp.

An important disputable feature of the NSC is related with their multipolar nature. In the present investigation multipolar 'A' cells with long processes were frequently observed in the subtegumental region, whereas 'B' cells with minute processes were observed in the acetabulum and at times in the oral sucker. These processes were observed associated with the cell perikarya and are frequently revealed by CHPh, PAF and AbPh techniques. On the basis of the observed similarity between the secretory product present in these processes and that in the cell perikarya in **Ceylonocotyle scoliocoelium**, Bhatnagar **et al.** (1980) regarded them as dendritic processes. The presence of dendritic processes in insects has frequently been reported in the ordinary neuron (Chapman, 1972) and also in the neurosecretory neurons (Adiyodi and Bern, 1968; Srivastava, 1969). In the absence of a conventional circulatory system, the occurrence of long processes leading from the pNSC seems an advantageous proposition in that the cells can reach the distantly located target (Gustaffsson and Wikgren, 1981). Further extensive branching of the cells, providing an increased surface area for the exchange of material, is considered to be yet another characteristic of NSC (Berlind, 1977). Despite the so assumed characteristic morphological feature exhibited by some cells in **Hymenolepis** species, Specian **et al.** (1979) regarded them as endocrine and not as neurosecretory, since these cells were observed far away from the central nervous system (CNS). However, in the present study the pNSC located at a distance from the CNS were always observed to be associated with some nerve or its tributaries. Frequent abundance of multipolar pNSC with their terminals in the subtegumental region of the body appears to be significant in view of the absorptive role of the tegument in platyhelminths. The involvement of neurosecretion in the various metabolic processes indicating the control on the membrane permeability has been well emphasized in invertebrates (Scharrer and Weitzman, 1970).

In order to reveal the nature of NSM, various histochemical tests were performed. The main histochemical feature that emerged in the present investigation is the presence of a PAS - positive, diastase labile compound differing from glycogen in the product of neurosecretion. The PAS reaction stained the NSM very intensely in some species like **F. cobboldi,**

F. elongatus, C. cotylophorum and **E. explanatum**. In **E. pancreaticum** the colouration of the cytoplasm was paler and thus the reaction, very mild. The NSM did not stain with Alcian blue at pH 1.0 or 2.5, so the possibility of it being acid muco-polysaccharide is eliminated. Furthermore, the NSM was found to be negative for toulidine blue test, confirming the absence of acid muco-substances. In addition, the occurrence of neutral muco-polysaccharide may also be eliminated, since the substrate was not stainable with PAS/Alcian blue method of Mowry (1955). None of the species showed a positive reaction for glycogen when stained with Best's carmine method. The present observations on the absence of different classes of carbohydrates are uniform and in agreement with those on the other trematode species studied so far (Harris and Cheng, 1972; Shyamasundari and Rao, 1975; Kalyankar and Kankal, 1981; Sharma and Sharma, 1981) and in disagreement with Krishna and Rao (1981) who reported the presence of appreciable quantity of acid mucopolysaccharide and metachromatic substances in **F. elongatus**. The extensive studies in many invertebrate and vertebrate species have also confirmed the presence of a PAS positive, glucide component other than glycogen in the NSM (Howe and Pearse, 1956; Arvy and Gabe, 1962).

The staining with Sudan Black B gave a mild reaction in all the species studied herein except **E. explanatum** where no reaction was observed. It is of course possible that lipids exist in much more quantity in the NSM but these are dissolved during impregnation by a solvent of the paraffin or during dehydration. Shyamasundari and Hanumantha Rao (1975) mentioned an intense sudanophilia in the NSM of **Fasciola** species. The other studies, done so far in helminths have not reported the occurrence of lipids in the NSM. In arthropods also, the NSM is devoid of lipid components (Arvy and Gabe, 1962).

Since the contents of the pNSC exhibited a mild affinity for methyl green - pyronin in general, it is concluded that the NSM contained minute quantities of RNA. However, Shyamasundari and Rao (1975) could detect appreciable quantities of RNA in the NSM of **F. gigantica** due to the pyroninophilia exhibited. In other helminth groups and in higher invertebrates and vertebrates, no mention is made about their occurrence (Rehm, 1955; Howe and Pearse, 1956).

The presence of proteinaceous compounds in the NSM appears indisputable in all the species investigated. The contents of the pNSC stained intensely with mercury bromophenol blue, indicating the presence of proteins. Further, of the specific end groups tyrosine, disulphide and amino groups were also revealed in the NSM. Even so, considerable differences were observed in the intensity of staining reactions for the three end groups, it being maximum for the amino group and minimum for tyrosine in all the species studied herein. The presence of tyrosine and disulphide groups has also been reported in **F. elongatus** and **Fasciola** species by Krishna and Rao (1981) and Shyamasundari and Rao (1975), respectively. Kalyankar and Kankal (1981) reported the amino group proteins in the NSM of **Proalariodes tropidonotis**. The occurrence of protein-bound disulphide group, i.e., cystine and or/cysteine "neurophysine" has been reported only in 'A' type NSC in insects by Dogra and Tandon (1964) and in the paramphistomid fluke, **C. scoliocoelium** by Bhatnagar **et al**. (1980). "Neurophysins", the special carrier proteins present in the NSM, are supposed to be responsible for the selective staining of the NSM throughout the animal kingdom (see Scharrer, 1977). The present observations regarding the occurrence of basic proteins in all the species investigated tally with the findings of Harris and Cheng (1972) who also reported the presence of tyrosine in the NSM of **Leucochloridiomorpha constantiae**. The protein bound disulphide groups, i.e., cysteine/cystine and tyrosine are a significant constituent of the NSM of pars intercerebralis of many insects (Arvy and Gabe, 1962). The NSM has been reported as having glycoproteinaceous nature in turbellarians (Vendrix, 1963) and trematodes (Harris and Cheng, 1972; Sharma and Sharma, 1981). The present observations also suggest that the neurosecretory product is predominantly a glycoprotein, sometimes even with small amount of lipid (as observed in **Fischoederius** species, **C. cotylophorum** and **E. pancreaticum**).

The higher intensity of staining reaction (in turn reflecting higher enzymatic activity), observed when acetyl thiocholine was used as the substrate than that occurring with the ester butyl thiocholine iodide, indicates that apart from the presence of NSE, AChE is the active enzyme in the NSM of the parasites under present investigations. The association of AChE with the nervous system has been demonstrated histochemically in many trematode species, namely **Schistosoma mansoni** (see Pepler, 1958; Lewert and Hopkins, 1965; Bueding 1952; Bueding **et al.**, 1967); **Dicrocoelium lanceatum** (Becejac **et al.**, 1964) and **F. hepatica** (Krvavica **et al.**, 1967). In all these studies a possible function of this enzyme as a neurotransmitter has been suggested. Pharmacological evidences indicate that AChE is an inhibitory neuromuscular transmitter in **S. mansoni** and **F. hepatica** (Bueding and Bennett, 1972). A similar function was also attributed to this enzyme in **Hymenolepis diminuta** by Wilson and Schiller (1969). The presence of AChE activity is also suggestive of the cholinergic nature of neurons and hypothetically the cholinergic stimulation is responsible for the liberation of the neurosecretory product (Gabe, 1966).

In the absence of a vascular tissue or extracellular transport mechanism in most platyhelminths, the release of NSM to the effector site may take place in one of the several possible ways. A cell to cell transfer of the NSM from the pNSC to the target organs (Dixon and Mercer, 1965) or its diffusion through the intercellular fluid to reach the site of action (Webb, 1977; Gustaffson and Wikgren, 1981) would eliminate the need of any intervention of a circulatory system (Scharrer and Weitzman, 1970; Scharrer, 1976). The non-sheathed nature of the nerves allows the release of the NSM into the intercellular space. The presence of NSM in the axonal extensions, as reported by many authors, is suggestive of its migration along the axonal process. The ubiquitous distribution of the pNSC at locations away from the cerebral ganglia, as observed in all the species in the present study, also indicates a cell to cell transfer mechanism. However, in respect of the paramphistomid species, which possess a lymphatic system that is regarded as the equivalent of a circulatory system (Tandon, 1960), the involvement of a vascular avenue also seems probable (Rao and Shyamasundari, 1977; Mehrotra and Bhutia, 1979). In all the paramphistomid species under the present investigation, the pNSC were observed to be frequently occurring in association with the walls of the lymphatic vessels. A parallel can be drawn to a similar situation occurring in crustacea, where Carlisle and Knowles (1959) noted the frequency of the grouping of neurosecretory terminals in close contact with blood vessels or their physiological equivalents and suggested the concern of these organs in the discharge of the active substances elaborated in the perikarya and carried along the axonal paths.

The comparative studies made on the three different growth phases, i.e., juvenile, immature and mature adult of the rumen dwelling paramphistome, **F. cobboldi** and parenteric worm, **E. pancreaticum** revealed that the juvenile form had only 'B' type pNSC in brain, nerve cords, pharynx and acetabular region and the pNSC localized in the parenchyma included mostly 'B' and only a few 'A' cells. However, both 'A' and 'B' pNSC occurred in the above-mentioned locations, in the immature and mature adult stages of the worm, the number of 'A' cells being relatively less in the former stage as compared to the latter. These observations are in conformity with those of Mehrotra and Bhutia (1977) on **P. epiclitum**. The presence of mostly 'B' type cells in the juvenile form of the worms studied herein lends support to the suggestions made by Gresson and Threadgold (1964) and Matskasi (1970) that ubiquitously occurring smaller NSC get transformed into the larger cell type which occur to a lesser extent in the immature, and to a greater in the adult form of the parasite. Besides, 'A' cells, multipolar 'B' cells were also missing in the juvenile forms which showed the occurrence mainly of the uni- and bipolar 'B' cells. This seconds the assumption that transformation of cells from one morphological phase to another occurs, made by Morita and Best (1965), on the basis of their studies on the planarian, **Dugesia dorotocephala**.

In immature form of **E. pancreaticum** a dense basophilic, chromatin-like material surrounding the nucleolus was observed. Finogenova (1975) also reported the presence of chromatin in the NSC of several nematode species and suggested that their occurrence is associated with the synthesis of neurosecretory material.

The reduction in the intensity of occurrence of NSC is also suggested in the present study because of the observed decrease in the number of cells per section of **F. cobboldi** and **E. pancreaticum** indicates towards an apparent association of the NSM with the maturation of the fluke. In addition, the 'A' and 'B' cells in approximation with gonads were also found to have dense secretion and no or less vacuoles. The involvement of the NSM in controlling directly or indirectly the maturation and development of the gonads has been suggested in several earlier studies made on digenetic species (Clegg and Smyth, 1968; Grasso and Quaglia, 1972; Harris and Cheng, 1972; Mehrotra and Bhutia, 1977; Kalyankar and Kankal, 1981). A similar phenomenon occurs in nereid annelids (Durchon, 1960) where the gonadal maturation is believed to be controlled by an inhibitory neurosecretion and only proceeds when the level of secretion decreases. In contrast, there is some evidence for an increase in the neurosecretory activity during sexual maturation in turbellarians (Lender, 1964; Grasso and Quaglia, 1970a,b). Notwithstanding the increase or decrease in the level of secretion, the participation of the neurosecretory material in growth and differentiation has been well documented in lower vertebrates like coelenterates (Lentz, 1968) and a regulatory role in certain reproductive events is ascribed to the NSC in planarians (Grasso and Benazzi, 1973).

In the context of the present investigation it emerges as a fact that many of the neuronal cells exhibit cytological signs of their being neurosecretory. However, precise determination of the release and effector sites as well as the physiological demonstration of specific functions involving the neurosecretion seem essential before the substance of the pNSC could be categorized as neurohormonal in trematode group of flat worms.

The present work incorporates a study on the detailed nerve arrangement and localization, distribution and structure of putative neurosecretory cells (pNSC) in some digenetic trematode parasites. The species of flukes chosen for the study are parasites of mammalian hosts namely sheep, goat, cattle and pig. An attempt has also been made to establish the nature of neurosecretory material (NSM) from the pNSC and the possible role of the latter in the physiology of the worm.

I. (i) With the localization of non specific esterases nervous system in toto is studied and described in fifteen trematode species. Of these, thirteen are paramphistomes representing four subfamilies of the family Paramphistomidae: **Fischoederius cobboldi, F. brevisaccus, F. elongatus** and **Gastrothylax crumenifer** (Gastrothylacinae); **Olveria indica** (Cladorchiinae); **Cotylophoron cotylophorum, Paramphistomum epiclitum, P. gracile, Orthocoelium orthocoelium, O. streptocoelium, O. narayanai** and **Explanatum explanatum** (Paramphistominae), and **Homalogaster paloniae** (Gastrodiscinae). The remaining two species are **Eurytrema pancreaticum** (Dicrocoeliidae: Dicrocoeliinae) and **Fasciola gigantica** (Fasciolidae: Fasciolinae).

The general organization of nerves conform to the typical pattern found in other digenea, i.e., a pair of cerebral ganglia connected by a cerebral commissure situated in the anterior region of the body and giving off three pairs each of anterior and posterior longitudinal nerves running anteriad and caudad, respectively. In **O. indica, P. gracile** and **E. pancreaticum**, an extra fourth pair of anterior nerves, i.e., pharyngeal nerves, is observed. A fine nerve net on both ventral and dorsal surfaces of the body, contributed by transeverse and longitudinal connectives of anterior and posterior nerves and providing a direct communication system between all the longitudinal nerves, is a special feature encountered in the present investigation.

(ii) The various nervous components constituting the central nervous system (i.e., cerebral ganglia, cerebral commissure, longitudinal nerves and their connectives) consist of a fibrous ground substance and cellular components that include both ganglion and neuroglial cells. The nerves are found to be unmyelinated in the present study. Histochemically the nervous tissue revealed the presence of non specific esterase, acetyl cholinesterase and butylcholinesterase.

II. (i) On the basis of the phloxinophilic and/or fuchsinophilic nature of their cytoplasm, putative neurosecretory cells are detected with the application of conventional staining methods of neurosecretion, namely, Bargmann's chrome haematoxylin phloxin, Gabe's paraldehyde fuchsin and Alcian blue phloxin methods; besides, Heidenhain's Azan method following fixation in Hally's fluid has also been used for demonstration of the NSM.

Two morphologically distinct pNSC types were identified (a) 'A'- large cells with vacuolated cytoplasm and mostly single nucleolus and (b) 'B'- small cells with homogenous, non-vacuolated cytoplasm and one or more nucleoli. Both the cell types were found ubiquitous in distribution and localized in both nervous and non-nervous tissues approximating various organs of the body.

To confirm the secretory nature of pNSC the light microscopic studies are supplemented with electron microscopic observations on the cerebral plexus of one species, **F. cobboldi**. The occurrence of electron dense vesicles of a size of 700-900A° (elementary neurosecretory granules) along with some small less dense vesicles in the cell perikarya and also in their cytoplasmic extensions is an evidence of secretory nature of the cells in question.

(ii) For determining the nature of NSM the histochemical studies have been performed on five species namely **F. cobboldi, F. elongatus, C. cotylophorum, E. explanatum** and **E. pancreaticum,** the last mentioned representing a non-paramphistome fluke. Histochemically, the contents of both pNSC types (A+B) are found to be identical. The NSM is primarily proteinaceous in nature, being specifically positive for disulphide group, amino group, basic proteins and tyrosine. The presence of a carbohydrate-protein complex such as muco- or glyco-protein is also suggested based on the PAS positive reaction of the NSM even after diastase digestion, together with a strong reaction for proteins. The presence of mucopolysaccharide in the NSM is eliminated. RNA in small amounts is also evidenced in the NSM and so is the occurrence of some lipid substance in **Fischoederius** spp., **C. cotylophorum** and **E. pancreaticum.**

The NSM is intensely positive for nonspecific esterases and moderately so for acetylcholine esterase. A mild reaction for BChE is also visualized in the NSM.

(iii) In view of the localization of pNSC in places away from the cerebral ganglia and in association with the various organs/organ systems of the body, a cell to cell transfer of the NSM from the pNSC to the target organ is suggested. A probable role of lymphatic system in extracellular transport is also suggested in paramphistome flukes in which this primitive circulatory system exists.

(iv) A comparative study has been performed on three developmental phases of **F. cobboldi** and **E. pancreaticum** in their final host. It is revealed that the juvenile form has mostly 'B' type pNSC, whereas the immature and mature forms have both 'A' and 'B' types. On the basis of these observations, morphological transformation from one cell type to another is suggested. A considerable variation is noticed with regard to the number of phloxinophilic cells in the developmental phases, these being maximum in juvenile and least in mature adult. This is indicative of a possible correlation between the neurosecretory activity and gonadal maturation.

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APPENDIX

PROCEDURES FOR HISTOCHEMICAL METHODS

1. Bargmann's Chromehaematoxylin-Phloxine (CHPh) Method

Fixation in Bouin's, paraffin sections.

Sections were deparaffinized and hydrated to water; oxidized with permanganate solution (1 part each of 2.5% aqueous potassium permanganate and 5% H_2SO_4 with 6-8 parts distilled water) — 20 sec; washed in distilled water — 1 min; bleached with 1% aqueous oxalic acid — 1 min; washed in running water — 5 min; stained in chromehaematoxylin (equal volumes of 1% aqueous haematoxylin and 3% aqueous chrome alum mixed together and to 100 ml of this mixture added 2.0 ml of 0.5 N H_2SO_4, and used after 4-8 weeks' ripening) — 10 min; differentiated in 0.5% acid alcohol — 30 sec; washed in running water — 3 min; stained with 0.5% aqueous phloxine — 3 min; treated with 5% phosphotungstic acid — 2 min; wahsed in running water — 5 min; dehydrated, cleared in xylene and mounted in Canada balsam.

Neurosecretory material — light to deep purple, nucleoli, pink and nuclei, lighter purple.

2. Paraldehyde-Fuchsin (PAF) Method

Fixation in Bouin's, paraffin sections.

Sections were deparaffinized and hydrated to water; oxidized with freshly prepared mixture of permanganate solution (0.3 gm potassium permanganate, 0.3 ml H_2SO_4 and 100 ml distilled water) — 1 min; rinsed rapidly in tap water, immersed in 3.0% aqueous potassium metabisulphite — 20 sec; rinsed briefly in distilled water; stained in Gabe's paraldehyde fuchsin (1 gm basic fuchsin added to 200 ml of boiling water, again boiled for 1 min, cooled and filtered; the filtrate was further mixed with 2 ml each of concentrated HCl and paraldehyde; the solution filtered after 4 days when it loses its red colour and the precipitate dried at 50°C; final staining solution prepared by dissolving 0.12 gm of

~~final staining~~ crystals in 50 ml of 70% alcohol and 1.0 ml of glacial acetic acid) — 3 min; differentiated in 0.5% HCl in absolute alcohol — 20 sec; dehydrated, cleared and mounted in Canada balsam.

Neurosecretory material - different shades of purple.

3. Alcian Blue-Phloxine (AbPh) Method

Fixation in Bouin's, paraffin sections.

The deparaffinized and hydrated sections were oxidized in freshly prepared mixture of equal volumes of 0.6% potassium permanganate and 0.6% H_2SO_4 — 1 min; bleached in 2.5% potassium metabisulphite — 20 sec; washed in running tap water — 5 min; stained in 0.5% solution of alcian blue in 3% acetic acid (pH 2.5) — 30 min; rinsed in distilled water; stained in 0.5% aqueous solution of phloxine — 5 min; immersed in 5% phosphotungstic acid — 1 min; washed in running water — 30 sec to 5 min; differentiated in 95% ethyl alcohol; dehydrated, cleared in two changes of xylene and mounted in Canada balsam.

Neurosecretory material - basophilic material stains in different shades of blue, nucleoli bright pink.

4. Heidenhain's Azan Method (Raabe's modification)

Fixation in Helly's fluid for 24 hr, washed in running water — 24 hr, dehydrated with iodinized ethyl alcohol, cleared in chloroform; paraffin sections.

The deparaffinized and hydrated sections were treated with 0.25% sodium thiosulphate — 5 min, to remove traces of iodine; washed in running water — 5 min; rinsed in distilled water; stained with 0.1% azocarmine G at 50°C — 15 min; cooled to room temperature and rinsed with distilled water; differentiated in 0.1% aniline in 90% alcohol — 3 min; treated with 5% phosphotungstic acid — 1 hr; stained in a solution containing 0.5 gm aniline blue, 2.0 gm Orange G, 8.0 ml glacial acetic acid and finally made to 100 ml with distilled water — 2 hr; differentiated in rectified alcohol; dehydrated, cleared and mounted in Canada balsam.

Neurosecretory material - bright orange to red colouration.

5. Mercuric Bromophenol Blue Method

Fixation in 10% neutral formalin, paraffin sections.

Sections were deparaffinized and hydrated to water; immersed in absolute ethyl alcohol — 2 min; kept in the staining solution (10 gm mercuric chloride and 100 mg bromophenol blue dissolved in 95% ethyl alcohol to make up final volume of 100 ml) — 15 min; wahsed in 0.5% acetic acid to remove excess dye — 20 min; immersed in tap water to convert dye to blue alkaline form — 3 min; dipped in tertiary butyl alcohol for dehydration with two changes, cleared and mounted in Canada balsam.

General proteins - blue.

6. Performic acid - Alcian blue method

Fixation in 10% neutral formalin, paraffin sections.

Following deparaffinization and hydration, sections were immersed in the performic acid reagent (4 ml of 30% fresh hydrogen peroxide) added to 40 ml of 98% formic acid and 0.5 ml concentrated H_2SO_4; this mixture allowed to stand for an hr before use) — 5 min, with vigorous stirring to remove dissolved gas; washed in tap water — 10 min; rinsed in 70% alcohol, blotted with filter paper and again rinsed with tap water; warmed at 50-60°C until the section is just dry; rinsed again in absolute alcohol and finally in tap water — 1 min; stained in acid alcian blue mixture (3% (w/v) alcian blue dissolved in 2N-H_2SO_4 at 70°C, pH 0.2 to 0.3) — 1 hr at room temperature; washed in tap water — 5 min; dehydrated, cleared and mounted in Canada balsam.

Structures containing cysteine - pale blue to dark steely blue.

7. Sakaguchi method (1925) modified by Baker

Fixation in 10% neutral formalin, paraffin sections.

Sections were deparaffinized and hydrated; rinsed in 70% alcohol; stained in the incubating mixture containing 2.0 ml of solution A

(1% sodium hydroxide), 2 drops of solution B (1 gm - Naphthol dissolved in 100 ml of 70% alcohol) and 4 drops of solution C (1 ml of stable sodium hypochlorite dissolved in 99 ml of distilled water) — 15 min; the slides were drained and blotted dry; dipped in pyridine-chloroform solution (30 ml pyridine and 10 ml chloroform) — 2 min; mounted in pyridine-chloroform mixture.

Arginine - orange-red.

8. Millon's Reaction

Fixation in 10% neutral formalin, paraffin sections.

Deparaffinized sections were hydrated; stained in a beaker containing 10 ml of solution A (10 gm mercuric sulphate dissolved by heating in a mixture of 10 ml H_2SO_4 and 90 ml distilled water; after cooling, 100 ml distilled water was further added to the solution) and 1.0 ml of solution B (250 mg sodium nitrite dissolved in 10 ml of distilled water) and boiled gently — 2 min; cooled to room temperature and washed in distilled water — 2 min, thrice; dehydrated, cleared and mounted in DPX.

Tyrosine - pink to yellowish red.

9. Alkaline fast green method

Fixation in 10% neutral formalin, paraffin sections.

Dewaxed sections were hydrated to water; treated with 5% aqueous trichloroacetic acid at 100°C — 15 min; washed with 3 changes of 70% alcohol — 10 min for each change; washed in distilled water; stained at 22°C in 0.1% aqueous fast green, pH 8.0 to 8.1 — 30 min; washed in distilled water — 5 min; dehydrated in 95% alcohol, cleared, and mounted in Canada balsam.

Basic proteins - bright green.

10. Ninhydrin - Schiff method

Fixation in Bouin's fluid, paraffin sections.

Sections were deparaffinized and hydrated to 70% alcohol; stained with 0.5% Ninhydrin solution in absolute ethyl alcohol at 37°C — over night; washed in running tap water; immersed in Schiff's reagent (1 gm basic fuchsin dissolved in 200 ml of distilled water and boiled; solution cooled to 50°C, filtered and to the filtrate added 20 ml of N-HCl, further cooled the solution to 25°C and to it added 1 gm of sodium metabisulphite and stored in the dark, overnight; to this solution added 2 gm of activated charcoal, and filtered; solution stored in dark container) — 45 min; washed in running water — 5 min; dehydrated, cleared, and mounted in Canada balsam.

-amino groups - pink to purplish red.

11. Methyl green - Pyronin method

Fixation in Carnoy's, paraffin sections.

Deparaffinized and hydrated sections were stained in methyl green-pyronin solution (7.5 ml of 2% aqueous, methyl green purified with chloroform and 12.5 ml of 2% aqueous pyronin mixed with 30 ml of 0.1 m acetate buffer, pH 4.8) — 10 min; blotted dry; rinsed rapidly in absolute acetone, 10% acetone in xylene, 50% acetone in xylene and finally in pure xylene, twice; and mounted in DPX.

RNA - red, DNA-green.

12. Feulgen nucleal reaction

Fixation in Carnoy's, paraffin sections.

After deparaffinizing, the sections were hydrated to water; rinsed in N- HCl at room temperature — 1 min; immersed in N- HCl at 60°C — 5 min; rinsed in N- HCl at room temperature — 1 min; stained in Schiff's reagent — 45 min; rinsed in bisulphite solution (5 ml of 10% potassium metabisulphite and 5 ml N- HCl in 90 ml distilled water) — 3 min, repeating this rinsing twice more; rinsed in distilled water; counter-stained with 1% aqueous light green — 2 min; washed in water; dehydrated and cleared; mounted in Canada balsam.

DNA - red to purple.

13. PAS method

Fixation in Formol-alcohol, paraffin sections.

Sections were deparaffinized and hydrated to water; immersed in 0.5% periodic acid — 5 min; rinsed in distilled water; stained in Schiff's reagent (as in method 10) — 15 min; dipped in sulphurous acid solution (0.9 gm potassium metabisulphite mixed with 7.5 ml N- HCl and 150 ml distilled water) — 5 min; washed in running tap water — 5 min; dehydrated and cleared in xylene; mounted in DPX.

Substances containing 1,2-glycol group - magenta.

14. Best's Carmine method

Fixation in Formol-alcohol, paraffin sections.

Sections were deparaffinized and hydrated to 70% alcohol; stained in alum haematoxylin — 10 min; washed in tap water; stained in Best's carmine (2 gm Carmine, 1 gm potassium carbonate and 5 gm potassium chloride dissolved in 60 ml distilled water, boiled gently, cooled and filtered; the staining solution comprised 12 ml of stock solution and 18 ml each of ammonia (.880) (and methyl alcohol) — 30 min; rinsed in two changes of best's differentiator solution (8 ml absolute ethyl alcohol, 4 ml methyl alcohol and 10 ml distilled water) — 20 sec; washed briefly in 90% alcohol; dipped in absolute alcohol — 2 min; cleared in xylene and mounted in Canada balsam.

Glycogen - red, nuclei - blue.

15. Alcian Blue method

Fixation in Bouin's, paraffin sections.

Sections were deparaffinized and hydrated to water; stained in Alcian Blue solution (50 gm Alcian Blue in 100 ml acetate buffer pH 5.8, made 0.06 M by adding 1.2 gm magnesium chloride) — overnight; rinsed in distilled water; dehydrated, cleared and mounted in Canada balsam.

Carboxyl and sulphated mucosubstances - blue.

16. Toluidine Blue method

Fixation in Bouin's, paraffin sections.

Deparaffinized sections were hydrated to water; stained in Toluidine Blue solution (100 mg Toluidine Blue dissolved in the mixture of 30 ml absolute alcohol and 70 ml distilled water) — 15 min; rinsed in 95% and absolute alcohols; cleared, and mounted in DPX.

Acid mucosubstances - pink (metachromatic)

17. Alcian Blue - PAS method

Fixation in 10% neutral formalin, paraffin sections.

Deparaffinized sections were hydrated to water; stained in alcian blue solution (1 g Alcian Blue in 100 ml of 3% acetic acid, pH 2.5) — 5 min; washed in distilled water; oxidized in 1% periodic acid — 5 min; washed in distilled water; oxidized in 1% periodic acid — 5 min; washed in distilled water; immersed in Schiff's reagent — 8 min; washed in running tap water — 10 min; dehydrated, cleared and mounted in DPX.

Acid mucosubstances - blue, neutral mucosubstances - red, mixtures - purple.

18. Sudan Black B method

Fixation in Formol-calcium, paraffin sections.

Sections, after dewaxing and hydration up to water, were dipped in 60% triethyl phosphate; stained in Sudan Black B solution at 20°C (1 gm Sudan Black B dissolved in 60 ml triethyl phosphate and 40 ml distilled water by heating at 100°C for 5 min with constant stirring, filtered while hot and again, just before use) — 10 min; immersed in 60% triethyl phosphate — 30 sec; washed in distilled water; mounted in glycerine jelly.

Lipid material - black.

19. Performic acid - Schiff method

Fixation in Formol-calcium, paraffin sections.

Sections were deparaffinized and hydrated to water; rinsed with tap water; treated with performic acid solution (40 ml, 90% Formic acid, 4 ml of 20% hydrogen peroxide) with 0.5 ml concentrated sulphuric acid; this mixture was allowed to stand 1 hr before use) — 30 min; washed in tap water — 15 min; placed in Schiff's reagent — 40 min; washed in running tap water — 1 min; dehydrated, cleared and mounted in DPX.

Unsaturated lipids - red.

20. Acid Haematein method

Fixation in Formol-calcium - 6 to 18 hr, transferred to post-chroming solution (5 gm potassium dichromate and 1 gm calcium chloride dissolved in 100 ml distilled water) at 22°C - 18 hr; further post-chromed at 60°C - 24 hr; washed well in distilled water, and processed for paraffin sectioning.

Sections were deparaffinized and hydrated to water; immersed in post-chroming solution at 37°C - 1 hr; washed in distilled water - 5 min; stained in acid haematein solution (50 mg haematein with 1.0 ml of 1% sodium iodate and 49 ml of distilled water, heated to boiling point, cooled and to the solution added 1 ml glacial acetic acid) at 60°C - 5 hr; rinsed in distilled water; kept in differentiating solution (250 mg potassium ferricyanide and 250 mg sodium tetraborate dissolved in 100 ml of distilled water) at 37°C - 18 hr; washed in tap water - 10 min; mounted in glycerine jelly.

Phospholipids - dark blue.

21. Gerebtzoff's Thiocholine method

Fixation in 10% cold neutral formalin, frozen sections.

The frozen sections were incubated at 37°C in the incubating medium (5.0 ml of solution A (0.1 M acetate buffer, pH 5-6.2), 0.8 ml of solution B (15 mg acetyl or butyl thiocholine iodide and 7 mg cupric sulphate, dissolved in 1.4 ml distilled water; this solution centrifuged at 4,000 rpm for 15 min and the supernatant used), 0.2 ml of solution C (375 mg glycine in 10 ml distilled water), and 0.2 ml of solution D (250 mg cupric sulphate in 10 ml distilled water), the final volume made to 10 ml with distilled water) — 15 to 30 min; rinsed in distilled water; immersed in 2% aqueous ammonium sulphide — 2 min; washed in distilled water and mounted in glycerine jelly.

Esterases - brown.

22. Indoxyl acetate method

Fixation in 10% cold neutral formalin, frozen sections.

The frozen sections (12-15u thick) were incubated at 37°C in incubating solution (1.3 mg O-acetyl 5-bromo-indoxyl acetate dissolved in 0.1 ml of ethanol and mixed with 2 ml of 0.1 M Tris buffer pH 7.2; 1.0 ml of 0.05 M potassium ferricyanide, 1.0 ml of 0.05 M potassium ferrocyanide and 1.0 ml of 0.1 M calcium chloride dissolved in 1.0 M NaCl and the solution, mixed with indoxyl acetate solution; final volume made to 10 ml) — 10-12 hr; rinsed briefly in tap water; mounted in glycerine jelly.

Esterases - indigo blue.