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.

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CONTENTS

Preface i
Abbreviations used in figures iii

Chapter I **NERVOUS SYSTEM AND NATURE OF NERVOUS TISSUE** **1 - 56**

INTRODUCTION 1
MATERIAL AND METHODS 4
OBSERVATIONS 8

A. Nervous System
- **Fischoederius cobboldi** 8
- **F. elongatus** 11
- **F. brevisaccus** 13
- **Gastrothylax crumenifer** 15
- **Olveria indica** 17
- **Cotylophoron cotylophorum** 20
- **Paramphistomum epiclitum** 23
- **P. gracile** 25
- **Orthocoelium orthocoelium** 27
- **O. streptocoelium** 30
- **O. narayanai** 31
- **Explanatum explanatum** 33
- **Homalogaster paloniae** 36
- **Eurytrema pancreaticum** 38
- **Fasciola gigantica** 40

B. Histocytology of the nervous tissue 42
DISCUSSION 45

Chapter II **NEUROENDOCRINE COMPONENTS - presumptive neurosecretory cells (pNSC) and neurosecretory material (NSM)** **57 - 91**

INTRODUCTION 57
MATERIAL AND METHODS 61
OBSERVATIONS 63

A. Localization of pNSC
- **Fischoederius cobboldi** 66
- **F. elongatus** 68
- **Olveria indica** 69
- **Cotylophoron cotylophorum** 70
- **Paramphistomum gracile** 71
- **Orthocoelium streptocoelium** 71
- **Explanatum explanatum** 72
- **Eurytrema pancreaticum** 73
- **Fasciola gigantica** 74

B. Histochemical Nature of the NSM 75

DISCUSSION 76

SUMMARY **92 - 96**

REFERENCES **97 - 111**

APPENDIX **112 - 120**

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

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.