NERVOUS SYSTEM AND NEUROSECRETION
INTRODUCTION

Nematode endocrinology seems to be in a rudimentary stage of development. The knowledge of neurosecretion in nematodes is scarce. The concept of neurosecretion is that, it is the production of relatively large quantities of materials by nerve cells which can be transported along the axons and released usually by exocytosis at sites called neurohaemal organs (Croll, 1976 eds). Based on the identity of the physiologically active products, two types of secretory neurons are recognised viz. peptidergic and aminergic. In the first type, the hormone has been identified as a peptide or protein. Histochemically they are Gomori positive i.e. stain with paraldehyde fuchsin or chrome haematoxylin stain. The second type is negative to paraldehyde fuchsin stain. The physiologically active products from these cells are various biogenic amines, such as serotonin or various catecholamines. Certain catecholamines are important chemical transmitters in the nervous system of many animals. Zhuchkova and Shishav (1979) have studied catecholamines by a fluorescent
histochemical method in number of nematodes such as Rhabdias bufonis, Ascaris suum, Ascardia galli, Heterakis gallinarum, Gastulerakis spumosa, Cottocomphoronema problematica and so on. They found four catecholaminogenic nerve cells in all species situated in or close to the circumoesophageal ring. Similar neurons have also been found in the lateral chord of middle body part in Heterakis gallinarum, Gastulerakis spumosa and Cottocomphoronema problematica. They state "Stability in number and position of ca-erosic nerve cells especially pronounced in the head region, suggests the conservativeness of both morphological and chemical postteins of the nematode nervous system".

The nervous system of the nematodes like Ascaris, Rhabditis, Thelastoma and Cephalobellus has been studied (Goldschmidt, 1908; Chitwood and Wehr, 1934, Lee, 1959 and Chitwood and Chitwood, 1933). Chitwood and Chitwood (1950) have compiled the most comprehensive review on the nervous system of nematodes. In Ascaris and Rhabditis the nerve ring surrounds the oesophagus and lies close to it. Associated with the nerve ring there are number of ganglionic masses, notably the
ventral ganglion which in these two worms is a symmetrically bilobed structure and lies ventrally on the oesophagus. In Thelostoma and Cephalobellus it is a paired structure. In all cases ventral nerve(s) arises from these ganglia and run posteriorly. Besides these, two important ganglionic masses associated with the nerve ring are the lateral ganglia. The number of cells found in these ganglia differ from species to species e.g. ventral ganglion of Ascaris contains 33 cells and Thelostoma 26 (13 in each).

Nematologists have shown that the nervous system is concentrated in two main areas, oesophageal region and anal region. Both of these two major centres of nervous co-ordination are connected by longitudinal nerves. Ramisz (1965, 1966, 1967) examined the nervous system of Oesophagostomum, Metastrongylus, Protostrongylus and Capillaria.

In the present work the author has attempted to study gross morphology of nervous system of Ganguleterakia spumosa, using Mallory's triple stain. Efforts have also been made to reveal the presence of neurosecretory cells in the nervous system by means of
paraldehyde fuchsin staining technique, chromo- 
haematoxylin method and Mallory's triple staining method. 
Owing to the small quantities of neurosecretion 
occuring in adult specimens of *Ganguleterakis*, 
the functional significance could not be properly 
dealt with. Even in immature specimens, the neuro-
secretion is found to be insignificant.

**RESULTS**

Sections of immature and mature specimens of 
*Ganguleterakis spumosa* were subjected to three 
histological tests viz. Mallory's triple stain test, 
Paraldehyde fuchsin test and Chromo-haematoxylin test. 
The first test enabled the author to study general 
histology and gross morphology of nervous system. 
Paraldehyde fuchsin and chromo-haematoxylin tests 
revealed the putative neurosecretory cells in the 
nervous system. Two halves of each male and female 
worms were treated with Mallory's triple stain. On 
examining these intact pieces as well as the serial 
sections stained by above methods it was found 
that there are two concentrated areas of nervous 
system one near the anterior end and the other at the 
posterior. Besides this the longitudinal nerves are
seen to connect these two areas. The longitudinal nerve cords are thick in the ventral and lateral hypodermal regions than in the dorsal hypodermal. The nerve cords are seen to be composed of a large number of nerve fibres. The fibres are more numerous in the ventral and lateral nerve cords as they have been revealed in the stained sections.

The muscle processes of somatic muscles are seen to be extended convergently to join apparently the dorsal, ventral, and the lateral nerve cords in the respective quadrants. The muscle tails or processes are less stainable but the neuromuscular junctions showed an intense staining with paraldehyde fuchsin (Plate No. 16 a). Apart from dorsal, ventral and lateral regions the interchordal cells are found to be positive to chromehaematoxylin stain.

The entire nerve ring as seen in sections shows that it is composed of numerous nerve cells. The whole structure is positive to Mallory's triple stain, paraldehyde fuchsin stain and chromehaematoxylin stain (Plate 16 b). The axons all over the side of nerve rings are seen to receive muscle tails in the region of oesophagus. The nerve ring is seen to be flattened transversely with two big ganglionic masses situated laterally.
In the lateral hypodermal region a group of nerve fibres has been found to be PF positive. An irregular network of PF positive thick fibres have been found to be distributed on the surface of alimentary canal and gonad (Plate 16 c).

As seen in (Plate No. 16 c) a group of nerve fibres is located in the lateral hypodermal regions. The axons receive the muscle processes in the lateral hypodermal region. The neuromuscular connections are found to be PF positive.

Posteriorly at the level of sucker (Plate No. 16 d) a group of PF positive nerve cells has been observed. PF positive cells have also been located in the spicules and the caudal alae.

Interestingly enough, large spherical cells are found to be distributed in the intestinal epithelium. The number of these cells is found to vary in the immature and mature worms. Two such cells (Plate 16 e,f,) have been observed in the mature worm and more than 15 in the immature form. These cells are stained well using Mallory's triple stain.
PLATE NO. 16

a. A part of the section of G. spumosa showing
a ventral and a lateral nerve cord. PF positive
neuromuscular connections and PF positive thick
axons are seen. Paraldehyde-fuchsin staining
technique X 600.

b. T.S. of G. spumosa adbaci passing through the
nerve ring:

(i) Note two big lateral ganglionic masses,
the peripheral cells of which seem to
be neurosecretory.

(ii) Note a dorsal median group of neuro-
secretory cells;

(iii) Note also ventrolateral group of neurosecretory
cells (a few are multipolar) just below the
lateral ganglion.
Mallory's triple staining method X 600.

c. A magnified portion of lateral hypodermal
region showing a group of PF positive nerve fibres and axons.
PF Staining technique.

LG - lateral ganglion.      VNC - Ventral nerve cord.
NMC - Neuromuscular connections. A - Axons in the lateral hypo-c
DMC - Dorsal median group of neurosecretory cells.
PNC - Peripheral spherical cells (neurosecretory) of the
lateral ganglion.
MC - Multipolar nerve cells (neurosecretory). 
(Arrows indicate nerve fibres in the lateral
hypodermal region).
d. T.S. of G. spumosa through copulatory complex. Note a group of PF-positive cells at the level of sucker. PS staining technique X 60C.

e. T.S. of mature G. spumosa showing two large spherical cells in the wall of the gut stained with Mallory's triple stain X 600.

f. T.S. of immature G. spumosa. Note certain large cells (more than 15) in the wall of the gut stained with Mallory's triple stain X 600.

g. T.S. of female G. spumosa. Note the muscle processes joining the nerve cords. Mallory's triple stain X 600.

NSC - Neurosecretory cells,
S - Sucker, Sp - Spicule
MP - Muscle processes,
DNC - Dorsal nerve cord,
VNC - Ventral nerve cord,
LNC - Lateral nerve cord.
DISCUSSION

Scant attention has been paid to the study of neurosecretion in parasitic nematodes. Gersch (1957) and Gersch and Scheffel (1958) were the first workers to draw attention to the possibility of neurosecretion in the nervous system of nematodes. A group of physiologists involved in this type of research includes Davey and his co-workers. Gersch and Scheffel working on *Ascaris* recognised a cell in each lateral ganglion stained by paraldehyde fuchsin and chromo黑客umatoxylin. Commissures connecting the ventral and lateral ganglia have also been shown to contain neurosecretory substance. Ishikawa (1961) found granules stained with Gomori's chromo黑客umatoxylin in the dorsal, ventral and lateral ganglia of *Ascaris*. Davey (1964) reported that the nerve cells in the anterior end of *Ascaris* are neurosecretory on the basis of their fuchsinophilic staining characteristic with paraldehyde fuchsin staining technique. Further he (1966) reported that *Phocanema decipiens* and *Ascaris lumbricoides* possess similar cells in the dorsal and ventral ganglia.
Studying PF staining reactions in *Ascaris*

Davey (1966) observed:

(i) Only a little staining in the cells of the worms fixed immediately after the removal from the host.

(ii) An intense staining in the worms kept in warm saline for a time before fixation, the stain becoming intense first in the cell bodies and after 4-6 hours in the axons as well.

(iii) No longer staining in the worms kept in saline for 24 hours before fixation.

From this he was tempted to hypothesize that this cycle of neurosecretion is a response to stressful situation. According to him the neurosecretory cells exhibit a cycle of secretion correlated with the increased cytological activity which accompanies the deposition of new cuticle. The hypothesis that neurosecretion from the nerve ring controls the deposition of cuticle was not proved in the ligation experiment on *Phocanema*. He argues that if the neurosecretory cells associated with the nerve ring govern the deposition of new cuticle then the
posterior half of the ligated worm should fail to secrete a new cuticle but this was not so and the cuticle was secreted equally well in both the halves of ligated worm. He, therefore, rejected the above hypothesis.

Rogers and Sommerville (1963) suggested that the nematode molting has its root in neurosecretion. Messner and Gunther (1966) using paraaldehyde fuchsin staining technique have reported that the presence of a neurosecretory system in Rhabditis and Oxyerca. Davey (1966) while studying molting specimens of Phocanema observed two groups of neurosecretory cells in the anterior part undergoing a cycle of secretion during molting process. He observed in one group, one or two cells located in the dorsal ganglion just anterior to nerve ring and in other 6 large cells lying in the ventral ganglion posterior to nerve ring. Further Davey and Kan (1967, 1968) have provided both physiological evidence for neurosecretion in nematodes and suggested that these cells undergo a cycle of secretion which is correlated with molting. Rogers (1968) demonstrated neurosecretory vesicles in the axons of Haemonchus contortus close to the excretory pore. These vesicles are found to contain neurosecretory granules. Similar granules have also been shown in the axons of Dipetalonema (McLaren, 1972).
During the exsheathment in *Haemonchus* and *Trichostrongylus* (Rogers, 1965), the release of leucine aminopeptidase (LAP) is essential. Davey (1966) stated that the release of LAP is under neurosecretory control. But regarding growth in *Phocanema*, he says that it is not under the direct neurosecretory control, for cuticle can be formed in the absence of neurosecretory products from anterior end as mentioned earlier.

Pinogenova (1971) found 12 neurosecretory cells on histological examination of the head end of *Ascardia galli*, 4 each in the ventral and 2 in the lateral ganglia. He found all the neurosecretory cells positioned symmetrically relative to longitudinal body axis. Davey (1972) showed the ventral nerve cord of *Phocanema* containing neurosecretory axons.

In the present study two highly concentrated areas of nervous tissue located one in the anterior part and the other in the posterior part of the worm represent the nerve ring or circumoesophageal commissure and the anal ganglion respectively. These two are in turn connected with each other by
longitudinal nerves in the dorsal, ventral and lateral hypodermal regions. As a unique feature of nematode nerves, the nerve fibres receive the muscle processes rather than innervating them. These sites have been identified by PF staining.

In general, the cells of the nervous system of *Gonylepteraakis* which are suspected to be neurosecretory appear in groups. Not only cell bodies but axons are also found to be stainable with PF stain. This indicates that they might transport the neurosecretory material as evidenced by earlier report.

Most of the cells of the nerve ring are small spherical in appearance but peripheral cells are slightly bigger with centrally aggregated PF positive material. In a few cells the material seems to be discrete, granulated in the form of a ring. Each lateral ganglion shows a group of distinct, spherical cells containing PF positive material. A dorsally placed group of cells in the nerve ring shows dense material in the centre of each cell. Few cells occupying ventrolateral position in the nerve ring are bipolar and multipolar in nature which can be assumed as neurosecretory cells.
A group of cells in the lateral hypodermal region (Plate 16 c) seems to be composed of multipolar nerve cells since ultimately thick axons have been formed which in turn are seen to receive the muscle processes. These cells being PF positive can be called neurosecretory, even then, the author feels that these cells need confirmation to assign them to neurosecretory system.

Another observation made by the author is the presence of an intense PF staining at the neuromuscular junctions and almost no staining in the parts of muscle processes traversing through the pseudocoelom (Plate 16 a). This indicates that the so-called neuromuscular junctions contain higher concentration of neurosecretory material probably because at these sites the neurosecretory substance (NSC) might be released.

The ventral and the lateral nerve cords (Plate 16 a) seem to be composed of numerous axons positive to PF stain. These axons after receiving the muscle processes spread over the gut wall and the gonad in the form of a network. The author feels, these thick axons to be neurosecretory type.
Two groups of PF positive cells which have been observed at the level of sucker may be of neurosecretory type (Plate 16 d). Spicule cores and spicule margins are also shown to be PF positive. Whether these parts are neurosecretory needs further investigation. At present it can be said that the neuron processes do exist in the spicules of Genouleteraakis. This observation is in agreement with the recent findings of Wright (1978) in the spicule of Capillaria hepatica and Trichuris muris, who observed sensory dendrites in the spicules of these two species and proposed a chemoreceptory function.

As stated already, an interesting observation made by the author is the presence of certain cells in the intestinal epithelium. These cells are distributed randomly among the intestinal epithelial cells which are arranged in a single layer. The author, therefore, feels that these typical cells are in no way concerned with digestive functions and they may constitute the part of peripheral nervous system. At this stage the author does not venture to call them neurosecretory and hence a matter of investigation to the workers in future.
To sum up, overall the PF positive material appeared to be in little quantity. These findings are in agreement with those of Davey (1966). The author feels that a very small amount of neurosecretion occurs in the adult worm. In this connection, Davey (1966) stated that the nematode neurosecretory cells never contain very large quantities of stainable material unlike that of pars intercerebralis of insects. The granules are usually small, discrete and blue in colour. The possible explanation for small amounts of neurosecretion in parasitic nematodes given by Davey is "The cells may not accumulate secretion before they release it. In this case production and release of the secretion occur simultaneously. There is no obvious centre for release of the products".
**SUMMARY**

Various chemical constituents have been revealed in the different parts of the two worms using histochemical techniques. Histochemical changes in the carbohydrates, proteins and lipids are noticed during the development of gonads. Similarly, sexual differences in these constituents have been observed. Depletion in glycogen content has been found during the formation of fertilized eggs and consequent eggshell formation.

In general, the concentration of the three constituents is found to be more in females than in males. This might be due to the greater metabolic demands in the females.

As regards the distribution of glycogen, the lateral hypodermal cords are found to be the richest parts in glycogen content. Mucopolysaccharides are found to be distributed in collagenous parts of the body. Their occurrence in the lateral alae is noteworthy.

Among proteins, tyrosine is the best revealed. Its presence in the egg shell indicates its role in the egg shell formation. It occurs in the hard part like spicule where it may contribute to the
collagen. -SS and -SH groups of protein are detected in the hard parts like cuticle, spicules, eggs etc.

Lipids are found to be abundant in females than in males. Female reproductive organs are the main sites of lipids.

Among enzymes, lipase has been localized for the first time in the gut cells of *Trichuris muris*. Esterase has been detected in the cuticle, body muscles and gut. Localization of polyphenol oxidase is indicative in the tanning of egg shell.

Nervous system of *Ganqueleterakis spumosa* follows the same pattern as in other nematodes. Neurosecretory material in this worm is very less. Neurosecretory cells are spherical in the nerve ring. A few bipolar and multipolar cells are found to be PF positive. The axons also contain PF positive material.