HISTOCHEMISTRY
MATERIAL AND METHODS

Supraoesophageal (cerebral) ganglia of *T. lumbricinus* were chosen for histochemical probe as arthropodan brains are found to contain well arranged neurosecretory territories enriched with large quanta of neurosecretory cells and bear evidence to act as an important centre for neuroendocrine control over multiple physiological events to promulgate metabolic maintenance. Therefore, the intact fresh brain tissues from the acclimated adults were fixed in suitable fixatives and were dehydrated, cleared and embedded in paraffin. Serial frontal sections were made at 5 to 7 micra (μm) thickness and were subjected to the following histochemical tests.

1] Calcium-cobalt method for alkaline phosphatase (Gomori, 1946).
   Fixative - 80% chilled alcohol. Optimum incubation period: 16 hours.

   Fixative - Cold acetone. Optimum incubation period: 20 hours.

3] Periodic acid - Schiff (PAS) technique of Mc Manus (1946a,b) for glycogen and carbohydrate containing proteins.
   Fixative - Carnoy's and Bouin's fluid.
4] Sudan black B method for the detection of general lipids (Mc Manus, 1946a). Fixative - 1 gm cobalt nitrate in 80 ml of distilled water, 10 ml of 10% calcium chloride and 10 ml of commercial (40%) formalin.


7] Alkaline tetrazolium reaction (Pearse, 1968) and performic acid-alcian blue method (Adams and Sloper, 1955; 1956) for dithio groups (Cystine - SS and/or Cysteine - SH). Fixative - Formalin/ and Carnoy's fluid respectively.

OBSERVATIONS

1] Phosphatases

a) Alkaline phosphatase

Large cells have their nuclei conspicuous owing to the intense reaction of the nucleoli, intranuclear material and nuclear membrane (Fig. 19). Cytoplasm may be endowed with varied concentrations of deep brown inclusions which are traceable along the axonal processes. Besides these, accumulation of alkaline phosphatase positive material at the axonhillock region is not scarce. From the nature of reaction, it is very likely that difference in the staining intensity among neurosecretory compliments has close correlation with their content present within the perikarya. Medium sized cells, too, exhibit moderate reaction. Concentration of deep brown particles either in the vicinity of the nuclei or at the axonhillock region is a common feature in these categories of cells apart from the possession of conspicuously positive nuclei. Distribution of alkaline phosphatase positive material is rather less spectacular along the axonal processes. In contrast, cells of smaller dimension showing rich positive reaction do exhibit migration of alkaline phosphatase positive along their tiny axonal processes.
b) Acid phosphatase

Moderate to weak positive reaction for acid phosphatase test in the neurosecretory cells has been encountered. The nuclei, however, remain distinct due to the intense black colouration of the nucleoli and brown to deep black hue of the nuclear membrane. Cytoplasmic inclusions, too, exhibit moderate to weak reaction. Cells of larger dimension show frequent accumulation of acid phosphatase positive material at the apex as well as at the distal axonal end of the perikarya (Fig. 20a). Cells of smaller dimension remain conspicuous for rich distribution of this enzyme both around the nucleus and along the tiny axonal processes (Fig. 20b).

2) PAS reactive substances

Small to medium types of cell show response to PAS reaction. Nuclei remain weakly positive although the nucleoli become purplish red (Fig. 21). In some cells, presence of PAS reactive substances is not difficult to locate at the perinuclear and axonhillock regions. Migration of purplish red granules along the axonal processes is not always discernible. In contrast, large cells hardly demonstrate response to such reaction.
3] General lipid

Majority of the NSCs are lipid-positive but the extent of reaction does differ with respect to the type of neurosecretory cells and their content which again is dependent upon the secretory status of the cell concerned. In general, the nucleolus and the nuclear membrane remain Sudan black B positive but the intranuclear material do not exhibit much response. The cytoplasm show light to moderate reaction and in such cases deep brown to black particles are usually detectable in the vicinity of the nucleus and at the axonhillock area as well. Sometimes streaming of lipid positive material along the axonal tract is visible (Fig. 22). Besides these, accumulation of such lipid positive substances at the intracerebral storage site is not ruled out.

4] Bound lipid

Majority of the NSCs are intensely bound lipid positive. Cells of larger dimension have their perikarya deep black and accordingly the distribution of secretory inclusions becomes inconceivable (Fig. 23). The nuclei are conspicuous for their relatively light reaction although the nuclear membrane, the chromatin material and nucleoli remain positive. Cells of smaller dimension, however, possess cytoplasm beset with a distribution of deep brown to black
secretory inclusions which can be traced both at the axonhillock region and along the axonal processes.

5] **Protein in general**

A good positive reaction for all the types of neurosecretory cells is well conceivable following application of MBB method for the localization of protein. In general, the nuclei, specially the nucleoli and nuclear membrane are deep blue but the nucleoplasm remain sluggish red. Cytoplasmic inclusions have various shades of blue and are sometimes detectable along the axonal processes (Fig. 24). Those cells which possess rich content of MBB positive material become more prominent in contrast with the other as the latter contain relatively less protein positive substances in their perikarya.

6] **SS and SH linked protein**

a) **Alkaline tetrazolium reaction**

Not all the cells do equally respond to the alkaline tetrazolium reaction and seems to depend upon the quanta of cystine and cysteine contained by the cells in question (Figs. 25a, b). Accordingly, spectacular variation is noticed
in the concentration of sulphur containing amino acids in the brain NSCs. Of the nucleus, the nucleolus and the nuclear membrane show dull blue colouration. Evidence for the presence of alkaline tetrazolium positive material within the cytoplasm and their eventual migration along the axonal processes are not scarce (Fig. 25b). In some cases, presence of SH- and/or SS-positive substances is detectable at the zone of accumulation.

b) **Performic acid-alcian blue reaction**

Fluctuation in the distribution of alcian blue positive material within the neurosecretory perikarya, irrespective of their types, is obvious. The nucleolus and the nuclear membrane remain pale blue in colouration. But the cytoplasm exhibit relatively stronger reaction and presence of dark steel-blue secretory granules especially at the apical portions of some cells is conceivable. Axonal migration of cystine positive secretory inclusions becomes apparent amongst the cells that are located in the vicinity of the outer peripheral margin of the neuropile. Accumulation of cystine (SS) positive material within the neuropile is very much conspicuous as they form bright-blue streak in the zone of accumulation (Fig. 26).
7] **Nucleic acids**

There is not much variation with regard to the reactive response of the neurosecretory cells when Kurnick's method is followed upon for simultaneous localisation of RNA and DNA. Majority of the neurosecretory cells exhibit strong positive reaction of their nucleoli for being brilliantly red but the chromatin materials remain light green and thus demonstrate the sites for the localisation of RNA and DNA respectively (Fig. 27). The cytoplasm, however, show fluctuation in the distribution of RNA positive secretory inclusions. Indeed, their presence is more defined when they form aggregates or clusters either at the outer periphery of the nucleus or at the axon hillock. Such characteristics are more visible in cells of larger dimension. Axonal transport of red particles (RNA-positive) and their subsequent concentration in the intracerebral zone of accumulation is not seldom.
### Table Showing the Histochemical Reactions of the NSCs in the Cerebral Ganglia of *T. lumbricinus*

<table>
<thead>
<tr>
<th>Reactions</th>
<th>Staining intensity</th>
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<tr>
<td></td>
<td>Nucleus</td>
<td>Cytoplasm</td>
<td>Nucleus</td>
<td>Cytoplasm</td>
<td>Nucleus</td>
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<tr>
<td></td>
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<td>Small cell</td>
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<tr>
<td>1) Alkaline phosphatase (Calcium Cobalt Method)</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+++</td>
<td></td>
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<tr>
<td>2) Acid phosphatase (Kobayashi and Kambara Method)</td>
<td>+++</td>
<td>+</td>
<td>++/+</td>
<td>+++/+++</td>
<td></td>
</tr>
<tr>
<td>3) PAS-reactive substances (Periodic Acid Schiff Method)</td>
<td>+</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td></td>
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<tr>
<td>4) General Lipid (Sudan Black B Method)</td>
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<td>+</td>
<td>++/++</td>
<td>+/+</td>
<td></td>
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<tr>
<td>5) Bound Lipid (Acetone Sudan Black Method)</td>
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<td>+</td>
<td>+++</td>
<td>++/+</td>
<td></td>
</tr>
<tr>
<td>6) Protein in general (Mercury Bromophenol Blue Method)</td>
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<td>++/+</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td>7) SS- and SH-Groups (Alkaline Tetrazolium Method)</td>
<td>++</td>
<td>.</td>
<td>+</td>
<td>++</td>
<td>++/+</td>
</tr>
<tr>
<td>8) SS-Group (Performic Acid-Alcian Blue Method)</td>
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<td>+/±</td>
<td>++/+</td>
<td>++/+</td>
<td></td>
</tr>
<tr>
<td>9) Nucleic acids (Methyl Green Pyronine-Y Method)</td>
<td>+</td>
<td>+</td>
<td>++/+</td>
<td>++/+</td>
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+, ++, +++ reactions of increasing intensity.

± doubtful reaction.
EXPLANATION OF FIGURES

Fig. 19. Showing the distribution of alkaline phosphatase positive substances in the NSCs of the cerebral ganglia of *T. lumbricinus*; (80% chilled alcohol fixation; calcium-cobalt staining). X 568

Fig. 20. Showing the distribution of acid phosphatase positive substances in the cerebral neurosecretory cells of *T. lumbricinus*; (cold acetone fixation; Kobayashi and Kambara method).

Note intense to moderate reaction of the nuclei and difference in the distribution of the enzyme in two types of cells, (a) cells of larger dimension, (b) cells of smaller dimension. X 125

Fig. 21. Showing the distribution of PAS-reactive substances in the NSCs of cerebral ganglia (Carnoy fixation; Mc. Manus method).

Note not too intense reaction of the nuclei and faintly positive cytoplasmic inclusions. X 532
EXPLANATION OF FIGURES

Fig. 22. Showing the localization of general lipid in the NSCs of cerebral ganglia; (Sudan Black B method).

Note both nucleolus and nuclear membrane remain intense positive in contrast with the intranuclear material and streaming of lipid positive material along the axonal tract. X 568

Fig. 23. Showing the distribution of bound lipid in the NSCs of cerebral ganglia; (Carnoy fixation; Acetone Sudan Black method).

Note rich bound lipid positive reaction of the cytoplasm without ascribing the identity of discrete inclusions within the perikaryon. X 568

Fig. 24. Showing the distribution of protein in general in the neurosecretory perikarya of cerebral ganglia; (Carnoy fixation; MBB method).

Note the occurrence of mercury bromophenol blue positive material in variable proportion within the cell body and their eventual migration through the axonal process. X 532
EXPLANATION OF FIGURES

Fig. 25. Showing the distribution of alkaline tetrazolium blue reactive substances (SS- and/or SH-groups) in the NSCs of cerebral ganglia (formalin fixation; Alkaline tetrazolium reaction).

Note feeble reaction of the nuclei and fluctuation of SS- and SH-linked protein content in the cytoplasm of the neurosecretory cells (a). Direct dispatch of such substances along the axonal processes for their intracerebral accumulation is also visible (b).

X 278

Fig. 26. Showing the concentration of performic acid alcian blue reactive substances in the neurosecretory cells of the cerebral ganglia; (Carnoy fixation; Performic acid-alcian blue method).

Note concentration of cystine (SS-) positive material in the form of streak within the neurosecretory zone of accumulation.

X 125

Fig. 27. Showing the distribution of nucleic acids in the cerebral neurosecretory cells; (Carnoy fixative; Methyl green-pyronin Y method).

Note RNA positive secretory inclusions within the cytoplasm and strong positive reaction of the nucleolus.

X 278
DISCUSSION

Histochemical probe of the neurosecretory product in invertebrates is of paramount importance since such study will provide clue to work out the exact relationship existing between granules, particles or more extensive deposits, staining with conventional stains [Peraldehyde-fuchsin (PAF), Chromo-alum haematoxylin-phloxin, Azan etc.] and neurohormone. Nevertheless, experimental studies of ganglionic neurosecretion coupled with histochemical investigations have immense possibility to reveal precisely the biochemical nature of the neurosecretory product that are stained by selective staining methods.

Cytochemical tests for the localisation of alkaline phosphatase in neurosecretory cells of both vertebrates and invertebrates portray debatable ground. The perikaryons of the hypothalamus show absence of this enzyme in large number of vertebrates (Gabe, 1966). However, restricted alkaline phosphatase positive responses could be recorded in this area and is considered to be due to the intense reaction in the vascular endothelium particularly in the venules (Arvy, 1962). Lack of these enzymes has also been detected in the brain neurosecretory perikarya of Iphita limbata (Nayar, 1959). This may well be interpreted in the context of some technical pitfalls like insufficient reaction time, temperature of liquid paraffin and
selection of chemical reagents like ethanol and xylene used during paraffin embedding of the tissue which otherwise may hinder localisation of enzyme in question. In contrast to these findings, positive sites for the distribution of alkaline phosphatase in the neurosecretory systems of both vertebrates and invertebrates have been detected by a number of investigators (Kobayashi and Kambara, 1959; Ganguly and Basu, 1962; Ganguly and Biswas, 1966; Banerjee and Pahari, 1975). Besides these, they have also reported the parallelism between the distribution of the alkaline phosphomonoo-esterases and NSM and have demonstrated a correlation between the activity of these enzyme and ready transportation of the secretory material. In fact, Banerjee and Pahari (1975) also opined that a distinct interrelationship exists between the elaboration of alkaline phosphatase positive material and secretory cycle of the neurosecretory cells of *Calotes versicolor* subjected to environmental stimuli. As observed by Tewari and Awasthi (1968), the present findings reveal almost similar microanatomical distribution of NSM and alkaline phosphatase in the neurosecretory perikarya particularly in the cells of larger dimension distributed in the cerebral ganglia of *I. lumbricinus*. Indeed, involvement of alkaline phosphatase for the synthetic ability of NSM ('membrane bound') appears to be a working principle for the secretory property (Kimura and Ichihara; 1980) of the cells in question which necessarily triggers the elaboration of the proteinaceous secretion (Danielli, 1954).
Nevertheless, the role of golgi bodies for the 'fashioning' of NSM (B. Scharrer, 1963; Smith and Smith, 1966) should be considered particularly with respect to the synthesis programme. Thus mediation of alkaline phosphatase for the elaboration of neurosecretory product is rather imperative. Variation in the reactive intensities amongst the cell types as well as within the same type of cell tends to indicate involvement of this enzyme for the regulation of metabolic processes that are implicated in the secretory dynamics apart from the process of permeability (Tewari and Dabholkar, 1968) in the brain neurosecretory cells of T. lumbricinus.

The importance of acid phosphatase in relation to ribonucleoprotein reserves for general metabolism and cell maintenance is well documented. Infact, the NSCs of both vertebrates and invertebrates are reported to be acid phosphatase positive. The quanta of this enzyme may differ and is dependent upon the cytophysiological criteria (Lomte and Nagabhusanam, 1974) of the cell types, but their occurrence is more or less ubiquitous (Shiebler, 1951; Kobayashi and Kambara, 1959; Ganguly and Basu, 1962; Nanda and Goswami, 1978). Cytochemical test for the detection of this enzyme has shown strong positive reaction to nucleus especially to the nuclear membrane and nucleolus of all the neurosecretory cells under study. Intense reaction of the nucleus may be corroborated with the findings of Mazia and Presscott (1955) who attributed it as the centre of
protein synthesis. Strong acid phosphatase reaction of the nucleolus is very likely associated with the synthesis of neurosecretory products which are, according to Holmgren (1959), protein in nature and can be considered as octapeptides (Berlind, 1977). Moreover, it is further substantiated that physiology of secretion is always associated with active protein synthesis as could be encountered in the cells of the anterior lobe of the pituitary (Kobayashi and Kambara, 1959). Cytoplasmic localisation of acid phosphatase positive substances has been considered as one of the confirmatory indices for the recognition of NSCs by Bargmann (1949) and Holmes (1962), although relative concentration of the enzyme in both the neurosecretory and non-neurosecretory regions is to be assessed before arriving at such contention. Variability in the enzymatic activity of the NSCs in *T. lumbricinus* may be interpreted by the fact that acid phosphatase activity is probably working at different levels depending upon the functional status of the cells in question. Indeed, similar interpretation has been attributed by Farner et al. (1962) who also reported the parallelism between the distribution of AF-positive cells and "acid phosphatase positive cell" in the neurosecretory centres of white-crowned sparrow. Furthermore, association of this enzyme for the formation of dithio-group containing proteins may have possible relevance for the elaboration of neurohormone and could be interpreted in the line as suggested by Pearse (1968) for the production of insulin.
Detection of PAS-positive substances in some of the neurosecretory perikarya and near absence of these substances in the neurosecretory pathway of the supraoesophageal neurosecretory territories of *T. lumbricinus* provide ground of scepticism with regard to the glucidic moiety of NSM. Difference in the PAS-positive response may be due to the "state of secretion" and/or discrimination in synthetic ability of glycocidic molecules amongst the neurosecretory cells (Herlant-Meewis, 1956b; Arvy and Gabe, 1962; Tasso and Rua, 1975; Parent *et al.*, 1976). Fraser (1959) and Takeuchi (1965b) have denied the occurrence of polysaccharide component in the neurosecretory substances. But host of other investigators (Scharrer, B., 1937; Defretin, 1955, 1956; Gabe, 1966; Gupta, 1971), on the other hand, reported the polysidic nature of the neurosecretion in majority of the invertebrates. In this context, it is mentionable that results concerning the PAS reaction of the neurosecretory product are not always in strict agreement with their affinity for selective stains like chrome alum haematoxylin phloxin and paraaldehyde fuchsin (Schiebler, 1951; Gabe, 1966; Gupta, 1971). In view of the above contention and admitting the importance of glucidic component (Takeuchi, 1965b) of the secretory product in cerebral neurosecretion of *T. lumbricinus*, it is reasonable to assume that these differences are possibly due to specific variations in the occurrence of "carrier substances" of the neurohormone being represented as the neurosecretory inclusions detectable with selective stains.
The present investigation has confirmed the view that the lipid is one of the important moieties of the neurosecretory product of *T. lumbricinus*. In their report, Joly and Devauchilli (1970) also stated that lipid can be considered necessary chemical constituent of the neurosecretory granules encountered in the cerebral gland of Lithobias forficatus. Furthermore, occurrence of cerebrocides in the golgi complex is worth noting (Casselman and Baker, 1955; Pipa, 1962) and the fact that the latter does provide positive contribution for the fabrication of NSM (Scharrer and Brown, 1962) has cogent reasons for such moiety in the process of elaboration. In contrast with the observations of Divry (1934) and Howe and Pearse (1956) who used various lipid positive stains to test the cytochemical nature of NSM in man, rat and dog, the present investigation clearly demonstrates a close parallelism between the distribution of the neurosecretory products and sudanophil structures not only within the perikarya but also at the distal part of the axonal pathways that ultimately release their product (sudanophilic) at the intracerebral accumulation zone of the species under study. Kobayashi et al. (1962) and Gupta (1971) supported the view that the Sudan black B staining for lipids in paraffin sections is due to the presence of cholesterol, an active principle of the brain hormone. Such contention may have significance when association of principal constituents like protein as well as carbohydrates is considered. Variable positive reaction of the neurosecretory neurons in the brain of *T. lumbricinus* has possible bearing with the secretory cycle and...
thus confirm the views of Schiebler (1952a,b) and Clark (1959). The fact that a close correspondence between the distribution of both CAHP- and AF-positive material and lipid positive substances within the neurosecretory perikarya of the species under study has relevance with the observations of Boer (1965), Hagadorn (1966b) and Lalitha Gabardhan et al. (1978) in Lymnaea stagnalis, Hirudo medicinalis and Hydrous triangularis respectively, although one of the investigators (Boer, 1965) demonstrated the presence of lipid moiety in "Gomori negative" cells.

Appreciable bound lipid positive reaction of the brain neurosecretory perikarya particularly in the cytoplasmic contents of T. lambricinus unequivocally demonstrate the lipoprotein moiety of NSM. Absence of such masked lipids in the neurosecretory systems of some animals (Sloper, 1955; Howe and Pearse, 1956) may be due to "Strong binding" of lipoproteins in laminated form (Sjöstrand, 1953) so as to prevent the affinity for fat soluble colouring agents by simple steric hinderences (Berenbaum, 1958) or depends on the acidophilic nature of NSM endowed with protein rich in tyrosine. Thus, it may be concluded that the bound lipid is an important moiety of the neurosecretory substances and lends support to the views advocated by Schiebler (1952a,b), Ganguly (1962), Nanda and Goswami (1978). It seems very probable that masked lipids serve as the carrier cement (Scharrer and Scharrer, 1954b) to act as a labile barrier (Berenbaum, 1958) in the event of neurohormone synthesis. Such screening is necessary to
ward off "interference" prone to develop from different inappropriate cell constituents as suggested by both Berenbaum (1958) and Ganguly (1962).

Peptide nature of NSM has been strongly admitted by various investigators. This is confirmed by the fact that when the hypothalamic neurosecretory territories of various mammals were pretreated with trypsin and pepsin yield completely negative results after adopting chrome alum haematoxylin and paraldehyde fuchsin staining techniques (Schiebler, 1952a,b; Howe and Pearse, 1956). In this context, it may be stated that the results of some histochemical reactions for tyrosine, tryptophan, histidine as well as arginine of the neurosecretory product do not prove decisive. Hence special emphasis has been paid to detect protein as well as sulphhydryl rich protein in the neurosecretory material of *T. lumbricinus*.

In so far the efficacy of the mercury bromphenol blue staining method is concerned particularly after its introduction by Durrum (1950) and its subsequent application as a general stain for protein by Mazia *et al.* (1953), it has been established that the staining intensity is proportional to the amount of protein present over a wide range of tissue preparations. Since then, this method after some modification, was employed by Bonhag (1955) for the investigation of the composition of ovary in milk weed bug, *Oncopeltus fasciatus*. Despite its limitation for using this method as histochemical test
for general protein (Baker, 1958; Pearse, 1968), many investigators (Hagadorn, 1958; Ganguly and Biswas, 1966; Beattie, 1971; Gupta, 1971; Habibulla, 1971; Banerjee and Pahari, 1975) have widely applied this method to determine the proteinaceous nature of the neurosecretory substances in arthropods and non-arthropods. Positive reaction of the neurosecretory substances to this stain provides evidence for the peptide composition of the neurosecretory cell elaboration of *T. lumbricinus* in variable proportion. And such reaction is presumably not associated with the acidophilic nature of the neurosecretory material as reported by Beattie (1971) in *P. americana*. Furthermore, Mc Alpine (1951) has shown that there occurs a distinct relationship between the phosphatase and proteins and is in agreement with the present study. Exposition of variable shades of colouration (ranging from reddish purple to blue) in the neurosecretory material of *T. lumbricinus* has possible implication with the "secretory nature" (Takeuchi, 1965a,b) of the cells concerned especially when remain "masked by some other material" (Gupta, 1971). Indeed, successful application of this method certainly helps to locate "unique identifiable neurons" in this millipede despite its histochemical significance as reiterated by Ganguly and Biswas (1966) and Nanda and Goswami (1978) in *P. americana*.

In arthropods, several workers have reported the presence of NSM rich in disulphide groups. Rehm (1955) recorded the occurrence of axon
endings containing NSM rich in disulphide groups in the sinus gland of the crab *Carinus maenas*. Otsu and Sonobe (1965) found that extracted chrome activating substances from the thoracic ganglion of crab, *E. japonicus* contain cystine and they made the suggestion that these substances are "Polypeptides with S-S connection". In insects, presence of NSM rich in disulphide groups has been well documented (Sloper, 1957; Pipa, 1961; Schreiner, 1966; Girardie and Girardie, 1972). Arvy and Gabe (1962) and Busselet (1967, 1968) have detected the presence of NSM rich in both sulphhydryl and disulphide groups in variable proportion to a variety of insects. In other invertebrate groups the distribution of NSM rich in cystine (SS) has been reported in leeches by Hagadorn (1966) and in basommatophoran molluscs by Boer (1965). Gabe (1955), Adams and Sloper (1956) and Imoto (1958) have advocated a cystine-rich component in the NSM of vertebrates. On the basis of biochemical investigations, they opined that the selective affinity of the hypothalamic neurosecretory product for both chrome alum haematoxylin in the Gomori's method and paraldehyde fuchsin (Gabe, 1955) is due to sulphur rich proteins.

However, the reasons for the presence of sulphur in many protein secreting systems are not yet clear (Berlind, 1977). The present investigation reveals that the NSCs in the brain of *I. lumbricinus* are endowed with a distribution of dithio-groups similar to the secretory inclusions that stained with conventional staining.
methods (with either AF- or CAHP-) adopted for cytomorphic studies. Variation in the intensity of reaction may be due to the fluctuation in the amount of cystine and/or cysteine containing protein in the neurosecretory cells apart from their types. Such criteria may also be indicative of the level of synthetic activity of the cells in question (Steel and Moris, 1975). In any case, it is worthwhile to presume that indispensability of sulphur rich "cytoplasmic precursor" of NSM in NSCs of T. lumbricinus may bear physiological implications in the synthetic competence of the cell.

It seems very reasonable to state that basophilia of the Nissl bodies possess RNA (Casperson and Schultz, 1939; Gersch and Bodian, 1943) and necessarily the ability for protein synthesis is likely to be controlled by the amount of RNA present in the cytoplasm (Prescott and Mazia, 1954). Indeed, the peptide nature of the neurosecretory products has an implication with these basophilic milieu available in the perikaryon. In this context it is worthwhile to mention that varied concentration of one of the nucleic acids (RNA) has often been observed in majority of NSCs of the species under study when methylene-green-pyronin\textsubscript{\textgamma} method (Kürnik) was followed upon. Furthermore, presence of high content of nucleic acids in NSCs was reported by Miyawaki (1960), Ganguly and Basu (1962), Boer (1965); Shyamasundari (1977) and Nanda and Goswami (1978) in various mandibulate arthropods. Furthermore, in his studies on the histochemical nature of neurosecretion in the silkworm B. mori, Kobayashi (1957)
demonstrated moderate distribution of RNA-positive substances in the neurosecretory cells of both brain and suboesophageal ganglia but Gupta (1971) has detected meagre amount of such material in the brain neurosecretory cells of *Dysdercus*. In contrast to these findings, absence of nucleic acid component has been reported by Arvy and Gabe (1962) and Lake (1970) in a number of pterygote insects and *Chirocephalus diaphanus* (Crustacea). Gabe (1966) however, contended that only energetic oxidation produce an affinity for basic stains in the product and none of the staining affinity of the neurosecretory product is modified by the action of ribonuclease. In view of the above facts, it is suggested that fluctuation in the content of RNA within the neurosecretory perikarya of *T. lumbricinus* may have implication in the fabrication programme (synthesis) of neurosecretory material (Schreiner, 1966) especially when other secreting cells are considered where role of RNA becomes imperative for the synthesis of adequate amount of protein.