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Histochemical Analyses of the Nucleic Acids, Lipids and Bound Lipids in the Secretory Dynamics of the Neurosecretory Materials of Earthworm, *Metaphire Peguana* Correspond to the Different Seasons

Trijit Nanda[1]* and Malabika Bhattacharjee[2]
[1]Assistant Professor, [2] Head and Assistant Professor,
[1,2]UG and PG Department of Zoology, Vivekananda College, Thakurpukur, 269, D.H.Road Kolkata-700063, India

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Corresponding Author Email: lt.trijitnanda@gmail.com

Abstract
Impact of seasonal temperature variations on the cytomorphic alterations of the neurosecretory cells of both the ganglia in ***M.peguana*** reveal that the volume of the nuclei declines appreciably and the neurosecretory substance remain stored within the perikarya during the successive seasons. The morphological activation reaches the peak at the early monsoon period. However, during this period the larger neurosecretory cells show enlargement of nuclei and their perikarya. Besides spectacular engorgement of neurosecretory material within the neurosecretory cells are noticeable. So it can be speculated that winter season is the ideal breeding season of the species under study.The intensity of the neurosecretory materials accumulation at the neurohaemal sites in accordance with the seasonal variations both ganglia reveal the following criteria: At the pre- monsson the neuropile do not show arborization of neurosecretory fibres but in some instances accumulation of the neurosecretory materials may be observed at the margin of the neuropile. The early monsoon period also maintains the same trends but at late monsoons rich vascularization are observed in both ganglia and neuropile show finer branches of capillaries and neurosecretory fibers possessing deep stainable substances. Axonal transport may be noticeable in confluence with the capillaries. At post monsoon period neurosecretory material accumulation is noticeable at the accumulation zone where capillaries containing histochemical stainable inclusions are prevalent.

Keywords
Neurosecretory Materials (NSM), Neurohaemal sites (NS), Neurosecretory Fibres (NF), Neurosecretory cells (NSCs)

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INTRODUCTION:

Attention has been made to get an insight pertaining to histochemical natures of the neurosecretoty materials (NSM) in both vertebrates and invertebrates. This has a bearing to spell out the exact relationship between the conventionally stained materials and secretory contents. Series of histochemical tests for the NSM in the divergent groups of invertebrates are already done and the outcome reveals that the neurosecretory cells (NSCs) basically contains three principal constituents – **protein, lipid and glycoprotein** in variable quanta. Chronological studies in the present investigation provide in formations for localization of some important biochemical constituents through acceptable histochemical procedures on the basis of their growth and development. Despite the fact that the procedures are conventional and qualitative the present probe reflects the overall intensity of the reaction in the context of the seasonal fluctuation. In general, **Gallocyanin-Chrome-Alum** reaction shows difference when the nucleus and the cytoplasmic granules are considered. More so, when the profiles of reactive response are taken into account. In general, variable lipid moiety undergo fluctuation in the different temperature regimes. Distribution of the lipid is an important integral part in the neurosecretory elaborations. Understandably, the lipid positive material may undergo fluctuation with reference to the ongoing development of neurosecretory elements as revealed in the various developmental phases in the seasons to come. Heterogeneity in the possession of **Sudan Black –B positive** material becomes imperative in the different seasonal changes.

Bound Lipid (Masked Lipid) in the present investigation despite of graded reaction of Sudan-Black –B in the both intranuclear and extranuclear regions are amenable. Indeed, such discrimination in the localization of masked lipids may be explained in the context of secretory accession of the cells in question based on their types and as well the developmental ages. A close parallelism of the distribution of the masked lipid and histologically demonstrable chrome alum haematoxyline phloxine and Aldehyde Fuchsin positive materials provide evidence for bound lipid moiety for neurosecretory products in *M.peguana.*

MATERIALS AND METHODS

Histochemical studies are being initiated from August through November since secretory physiology of the neurosecretory complements remain conspicuous during this period. Hence, special emphasis has been made to collate localization of important biochemical constituents like nucleic acids, SS- and SH groups, general proteins, carbohydrate containing proteins, general lipids and bound lipids.

The relevant tissues like supraoesophageal and suboesophageal ganglia of *M. peguana,* were dissected out carefully and fixed in appropriate fixatives. Thereafter, these materials are washed, dehydrated and embedded in paraffin (melting point 56°C-58°C). Serial frontal sections were cut at 7µm thickness.

For detection of histochemical importance of the contents, the following methods were adopted.

(a) Chrome-alum-gallocyanin (CAG) method for the detection of nucleic acids Fixative- Carnoy's fluid

(b) Performic acid-alcian blue (PAAB) method for the detection of cystine [1]; Fixative - Carnoy's fluid. [The test was also carried out replacing the performic acid with the permanganic acid (PMAAB)

(c) Sudan black B method (SBB) for the detection of general lipids [2]; Fixative- Aqueous Bouin's fluid.

RESULTS AND OBSERVATIONS: -

It may be mentioned that special attention has been paid to the histochemical characteristics of the large NSCs, since the intracellur profiles of these categories are more discernible. The small type cells, on the other hand, portray poor definition of the biochemically important constituents due to the possession of indistinct intracellular structures.

1. Nucleic acids

(a) Supraoesophageal ganglion

Localization of nucleic acids in general has been demonstrated. Various tinge of bluish coloration has been revealed amongst the neurosecretory elements. The nucleus shows light reaction in contrast with the cytoplasm. The nucleolus gives relatively strong affinity for CAG when compared with other intra-nuclear materials (**Figs.1,2**). The cytoplasm, however, bears fluctuating affinity when quanta and intensity of secretory inclusions are taken into account. In some circumstances there is a rich distribution of stainable granules bearing significant quanta of relevant nucleic acid in contrast with the others having moderate to weak response. I ndications for transparent tinge of gallocyanin positive materials from

axon hillock through the axonal process are also obvious (**Fig.2**).

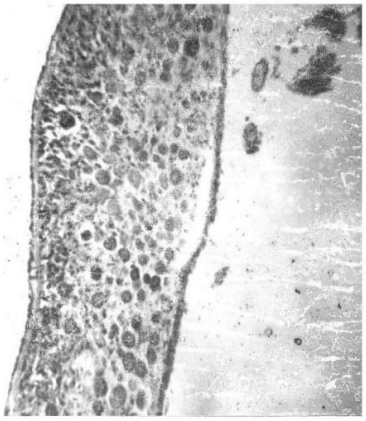


Fig.1: **(X100)** Section of supraoesophageal ganglion of ***M. peguana*** displaying nuctuating concentration of nucleic acids within the secretory perikarya.

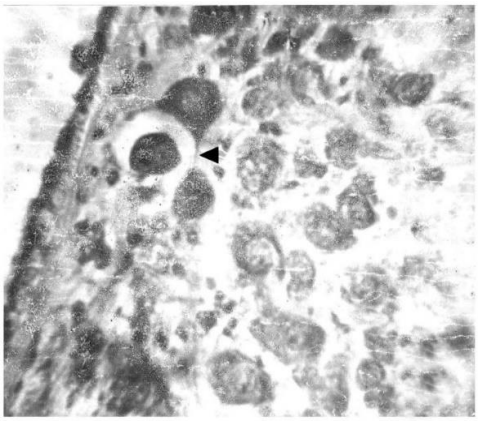


Fig.2: **(X400)** Section of supraoesophageal ganglion of ***M. peguana*** showing rich distribution of nucleic acids and transport of CAG-positive materials from NSCs through the axonal process·,_;.

Fig.3: **(X100)** Section of sub-oesophagcal ganglion of ***M. peguana*** showing intense *C* AG-positive reaction in the neurosecretory cells

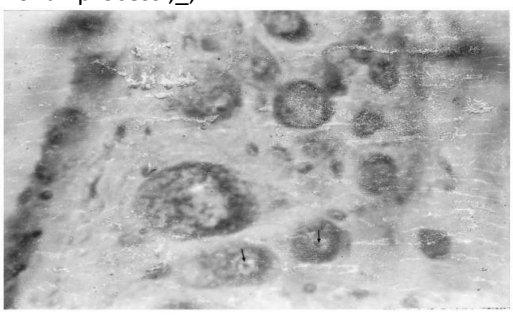


Fig. 4: **(X400)** Section showing transport of nucleic acids po.sitive material from NSCs through the· axonal processes within the suboesophageal ganglion.

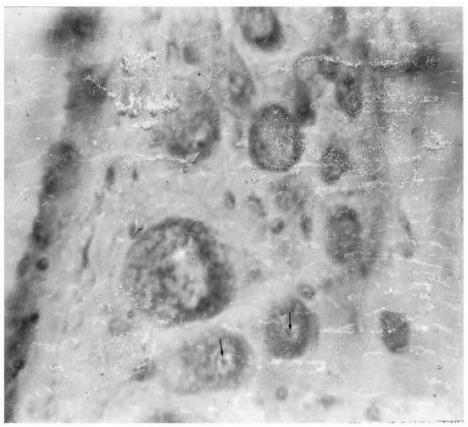


Fig. 5: **(X400)** Section of suboesophagcal ganglion showing that the cytoplasm contains graded distribution of CAG-positive granules within the secretory perikarya. Here, the nucleolus shows deep positive reaction.

Fig.6: **(X400)** Section of supraoesophageal ganglion showing that the cytoplasm of the NS perikarya contain alcianblue positive granules and nucleoli have intense PAAB-positive reaction.

Fig.7: **(X400)** Section of supraoesophageal ganglion showing axoplasmic flow of alcian-blue positive materials from NSCs.

Fig.8: **(X100)** Section of suboesophageal ganglion displaying fluctuating concentration of PAAB positive materials within the secretory perikarya

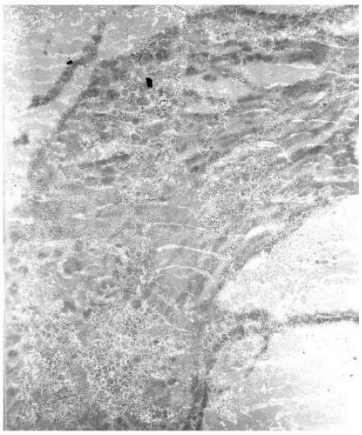


Fig.9: **(X100)** Section of suboesophageal ganglion displaying fluctuating concentration of PAAB positive materials within the secretory perikarya

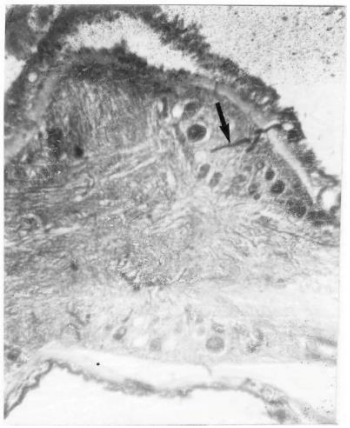


Fig. 10: **(X150)** Section showing the localization of general lipids in the NSCs of supraocsophageal ganglion. Here, the capillaries arc found to contain lipid positive materials

Fig. 11: **(X400)** Section of supraoesophageal ganglion showing transport of SBB positive materials through axonal processes within the neuropile

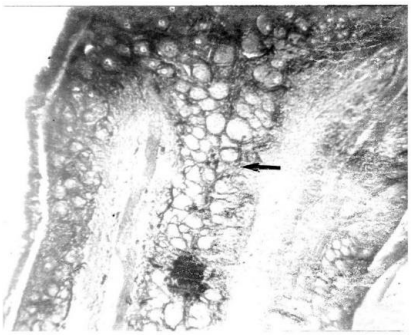


Fig. 12: **(X150)** Section of suboesophageal ganglion showing the localization of general lipids in the medial neurosecretory cell s.

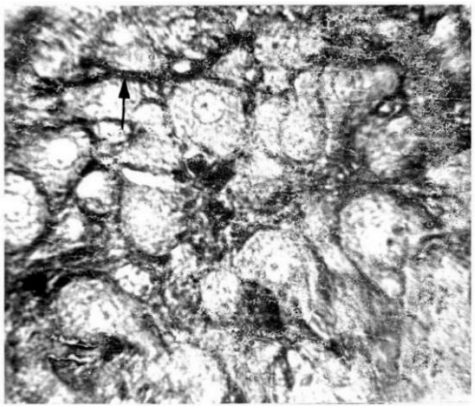


Fig. 13: **(X400)** Section of suboesophageal ganglion showing that the cytoplasm containing SBB-positive granules and cell membrane, nuclear membrane and nucleoli are also conspicuous.

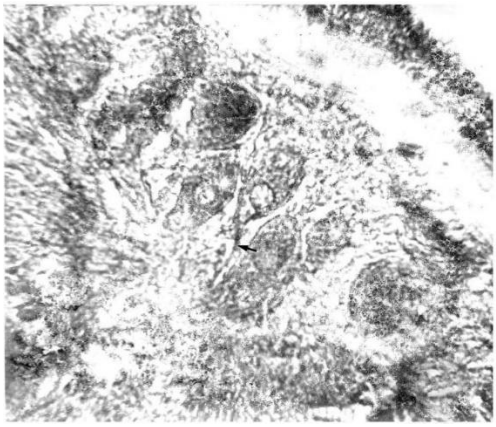
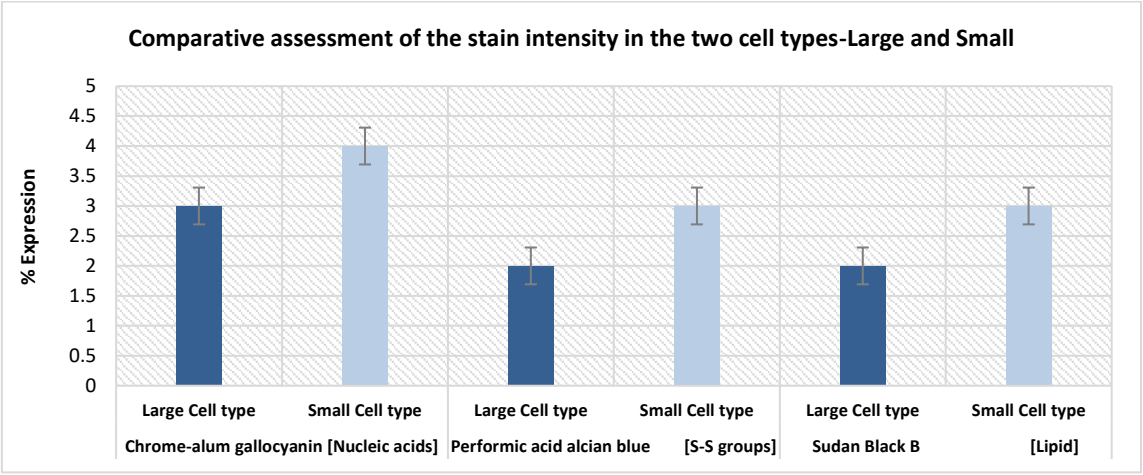


Fig. 14: **(X400)** Section of suboesophageal ganglion showing the presence of SBB-positive granules within the secretory perikarya and their transport through axonal processes.



Moderately high quanta of nucleic acids are detected in the Small Types of Cells. But the polypeptide rich cysteine commonly reacting with Neurosecretory materials exhibits the higher trends in the small types of cells. The same trend even reflects in case of bound lipids and such discrimination may be explained in the context of secretory cycle**.**

(b) Suboesophageal ganglion:

There is not much variation m the distribution pattern of gallocyanin positive substances amongst the NS components. In general, the nucleolus shows deep positive reaction than the rest of the intra-nuclear materials (Figs.3,4 and 5). Cytoplasm, however, assumes graded distribution of granules that are responsive to this reaction (Figs. 3 and 5). In general, most of the cellular elements remain moderately positive. The orientation of nucleic acids positive granules is clearly explicit, especially when the axonal transport is referred to (Fig.4). The neuropile however, may have distribution of CAG positive substances but their intensity does not exceed appreciably (Fig.4).

2. Dithio-groups (S-S linked protein)

(a) Supraoesophageal ganglion

Both types of NSCs in the brain possess of S-S positive amino acids in a fluctuating manner (**Figs.6** and **7)**. The nucleoli have intense alcian blue positive reaction and are readily visible (**Fig.6**). Overall profiles of the NS components are demonstrable, when cytoplasm containing alcian blue positive granules are noted (**Figs. 6** and **7**). Instances for the discrepancy in the staining intensity are vivid when the anterior and the posterior portion of some of the NS elements are examined. Evidence for axopl asmic flow are remarkable, when neuropile

interspersed by NS-axon fibers are verified. Indeed, individualization of the NS axonal tract may be possible on the basis of the efficacy of the reaction as well as the extent of axoplasmic flow of the NSCs in quest ion (**Fi g. 7**).

(b) Suboesophageal ganglion:

The reactive response amongst the NS elements maintains a low gear as observed in cerebral ganglia. In any case, the intra-nuclear contents including nucleoli are weakly positive (**Figs.8** and **9**). The cytoplasmic contents leave differential response in accordance with quanta of the secretory material s. Alcian blue positive NS axon fibres are hardly amenable to the respective NS territories. Characterization of NSCs on the basis of the quanta of the S-S positive materials is not hard to assess (**Fig. 8 and 9**).

3. Lipid in general

(a) Supraoesophageal ganglion

Distribution of lipid positive materials amongst the NSCs is clearly revealing. Intensity of lipid positive reaction may undergo fluctuation and all that depends upon the functional state of the NS neurons (**Figs.10** and **11**). Axonal processes are found to bear Sudan black B positive material s, which sometimes demonstrate relatively less tinctorial depth in contrast with the perikaryon (**Fig.11**). Incidentally, the capillaries are al so found to contain the distribution of lipid positive materials and content resemblance with the stainable substances that are detected within the cells or along the axonal processes (**Fig.10**)

(b) Suboesophageal ganglion:

Lipid positive reaction of NS elements is spectacular (Figs. **12, 13 and 14**). But the intensity of the reaction turns to be in lower pitch in contrast with the cerebral ganglion. Nevertheless, the clarity of the secretory materials becomes non-clear so as to denote their morphological characteristics. The cell membrane, nuclear membrane and nucleoli are quite· conspicuous owing to graded lipid reaction (**Fig. 13** and **14**). Nucleoli remain conspicuous under the background of less reactive intra-nuclear materials (**Fig.13**) The capillaries are also found to have the distribution of lipid positive materials (**Fig.13**). In this context, it is interesting to note that the axial extension of medial cells along with their lipid positive reaction provide clear indication for the unilateral fusion of two ganglia (**Fig.12**). This is in other way accounts the histomorphic nature of the development in this ganglion to form the ganglionic ventral nerve cord.

DISCUSSION

Since inception considerable le attention has been paid to get an insight pertaining to the biochemical nature of the NSM in both vertebrates and invertebrates. This has a bearing to. spell out the exact relationship between the conventionally stained materials and the secretory contents. Series of histochemical tests for the NSM in divergent groups of invertebrates like *Cracothemis servilia* (Odonata), *Marpissa tigrina* Tikadar (Araneida), *Zoofecus* sp. (Pulmonata:l, *Thais bufo* (Gastropoda), *Squilla hofoschisw* (Crustacea) *Eutyphoeus gammiei* (Oligochaeta) etc. have revealed that the NSCs contain three principal constituents (protein, lipid and glycoprotein) in variable proportion. And accordingly, in some cases the NSM may be considered as glycoprotein, lipoprotein or glyco-lipoprotein as the case may be **[3-8] .**Earlier **[9]**, reported that NSM may contain catecholamine and dopamine as major components in case of a polychaete, *Ophryotrocha puerillis* .

Moderately high content of nucleic acids in the NSCs of some invertebrates has been demonstrated **[6, -8,10,11]**. In the brain of *M. peguana, feeble* reaction of the nucleus with gallocyanin component indicates traces of DNA and may be associated with the functional state of the nucleus. Moderate quanta of RNA are, however, detected in the perikarya or the species under stud y. As the nucleolus gives relatively strong affinity for CAG, it is indicative of the presence of moderate amount of RNA. In suboesophageal ganglion, there is not much variation in the distribution pattern of gallocyanin positive substances amongst the NS components. The variability in the nucleic acid components are histologically demonstrable stainable inclusions which indicate a close relationship within neurosecretory substances. Indeed, distribution 'of nucleic acids has relevance for the active synthesis of protein. Indirect evidence further substantiates that the periods of RNA synthesis 'accumulation phase' of secretion within the cytoplasm of NSCs are concurrent events.

Inspite of the fact that common reacting components of the NSM is a polypeptide rich in cystine both positive and near negative responses of the NSCs are being reported in several invertebrates following performic acid alcian blue (PAAB) test. Indeed, sulphur association with NSM has been repeatedly

stressed upon in several members of invertebrates like annelids **[7]**; molluscs **[4]** and in vertebrates. On the contrary, this could not detect disulphide (S-S) groups in the NSCs of earthworm, *Pheretima communisima* and *P. vitetta.* Hemimetabolous insects too, demonstrate: doubtful presence of dithio groups in NSM. In case of the NSCs of holometabolous insects, especially in Diptera and Lepidoptera, indication for the presence of cysteine or cystine cast doubt on the plea that they may not be the integral part of NSM in these insects **[11]**. In case of the flesh fly, *Sarcophaga ruflcornis,* out of a total of twenty-six PAF positive NSCs, only eight are positive to PAAB specific for cystine and cysteine . Among different cell categories, only type A neurones of the cerebral ganglion in earthworm, *Lumbricus herculeus* and *Eisenia foetida* and type a-cells in the CNS of *P. posthuma* possess cystine and cysteine. Likewise, in an oligochaete earthworm *Eutyphoeus gammiei,* cystine is detected only in a few divergent secretory cells (homologous to type 'a' or 'A' cells of other investigators) while others show doubtful reaction**[8]** . In the present probe on *M. peguana,* in supraoesophageal ganglion, both types of NSCs possess S-S positive amino acids to a graded fashion. The nuclei have strong alcian blue positive reactions and the cytoplasm contain distribution of alcian blue positive granules. Sometimes, evidence for axoplasmic flow are remarkable, when neuropile possessing NS axon fibers are verified. In suboesophageal ganglion, the reactive response amongst the NS elements maintains the same trend. In any case the intra-nuclear contents including nucleoli are weakly positive and cytoplasm show fluctuating response in accordance with the amount of the secretory materials contained by them. The weak sulphur contents of NSCs may be due to their low gear synthetic activity. All these facts seem to indicate that the basic constituent; of the NS products are proteinaceous though predominant amino acid residue is different amongst several types of cells or species.

Available literature reveals that in polychaeta, the NSM contain only a lipid moiety, in hirudinea, the secretion of the NSCs show weak to moderate staining affinity for SBB and in oligochaeta NSM possess variable lipid moiety **[7,8]**. Indeed, in Euchytraeidae the NSM is composed largely, if not solely, of lipids .The present study on Megascolicid worm, *M. peguana* also corroborates with these findings to elucidate the fact that lipid as an important integral part of the NS elaborations. In cerebral ganglion of *M. peguana,* intensity of lipid positive reaction may undergo fluctuation and all that depend upon the functional state of the NS neurones. Incidentally, the capillaries also possess SBB positive ma1erials otherwise corroborate the presence of NSM in the blood vessels. In suboesophageal ganglion, although the staining intensity is comparatively less than the brain, the nucleoli are mostly conspicuous under the background of Jess reactive intra- nuclear materials.

Bound lipids positive reaction of NSM in invertebrates has been claimed by several investigators **[6,7]**.In *M. peguana* intense bound lipid positive reaction in supra and suboesophageal ganglionic NSCs corroborates with the previous observations made by**[7]** in the ventral ganglionic NSCs of *M. peguana.* In cerebral ganglion, majority of the NSCs though possess bound lipid positive materials but their intensity of reaction is not so high. But this reaction, however, turns to be more in case of suboesophageal ganglionic NSCs. Indeed, such discrimination in the localization of masked lipids may be explained in the context of the state of secretory cycle existing in the NSCs in question. Moreover, a close parallelism between the distribution of masked lipid and histologically demonstrable CAHP 'and AF positive materials provide evidence for bound lipid moiety of the NS products in the species under study. These materials probably serve as 'labile barrier' for guarding the neurohormone **[6,7,8]** from inappropriate reaction supposed to occur within the cells.

However, the histogram reflects the array of histomorphological changes in concurrence with the secretory dynamics of the neurosecretory cells in the different seasonal fluctuations in both supra& suboesophageal ganglia of the Earth worm (*Metaphire peguana*)

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