SUMMARY AND CONCLUSIONS
The present work has been carried out to study the histochemical nature of the NSCs in animals, both invertebrate and vertebrate animals, belonging to different groups, have been taken for study, with the view to make a comparative approach. Various conventional staining techniques, for light microscopic (LM) observation have been applied. Histochemical investigation of the neurosecretory product of the central nervous system, especially the brain of 24 species of animals belonging to different groups of animal kingdom, has yielded the following results:

A. HISTOLOGICAL STUDIES ON THE NEUROSECRETORY CELLS:

On the basis of staining reactions such as paraldehyde fuchsin (PF), chrome-alum-haematoxylin phloxin (CAHP), heiden's azan (Azan) and Squad the NSCs in vertebrates and invertebrates have been recognized and on the basis of their morphology and staining affinities, have been classified into various categories by adopting the patterns of the significant persons in the field.

1. In invertebrates the NSCs represent a vide range of variations and multiplicity. In insects like Dysdercus similis, Poliocerus picta, Periplaneta americana, Polistes hebraeus, Apis indica, 'A', 'B', 'C' and 'D' types of NSCs have been recognized. Similar categories have been recognized in Perionyx sanguinoticus and Hirudo medicinalis. The NSCs almost of the same nature have been studied in the supra-
oesophageal and sub-oesophageal ganglia. In scorpion, *Palamnaeus bengalensis,* 'A', 'B' and 'C' types of the NSCs of the brain have been studied, which exist in three different groups. In *Planaria,* some cells are stained dark and they indicate the existence of 'A' and 'B' types of the NSCs. In *Hydra* and *Spongilla,* the NSCs are of one type only. In *Lymnaea stagnalis,* 'A', 'B' and 'C' types of the NSCs have been recognized.

2. The 'A' type of the NSCs are predominant and are larger in size, which stain heavily in shades of purple colour with PF., dark with CAHP and red with azan staining. In these cells, the cytoplasm is generally stained faint. The nuclei are intensely with red inclusions. The 'B' type of the cells, which are predominant of lateral group of cells in insects, are negative to PF, other categories of the cells including 'B' cells are well demonstrated in CAHP and Azan staining.

3. The NSCs have been found distributed in definite cell groups in the brain of Arthropods, supra and sub-oesophageal ganglion of annelids, cerebral and pleural ganglion of *L. stagnalis* and cerebral ganglia of *Planaria.* In *Hydra* and *Spongilla,* the distribution of these cells is not definite.

4. In fishes, *Clarias batrachus,* *Heteropneustus fossilis,* *Mollinisia sphenops* and in frog, *Rana esculenta,* the hypothalamic NSCs are in the form of a distinct cell group,
the nucleus pre-opticus (NPO), found in the association of the optic chiasma and extends up to third ventricle. The NSCs show long axons passing down to the hypophysis. In *C. batrachus* and *U. fossilis*, apart from the NPO, there exists one more group of cells which has been regarded as nucleus lateralis tuberis, (NLT). The cells of NLT possess similar staining properties.

5. In *Uromastix hardwickii* (Reptilia); doves, *streptopelia tranquebarica* and *S. chinensis*, house sparrow, *Passer domesticus*, (Aves) and bat, *Pipistrellus ceylonicus indicus* and squirrel, *Funambulus pennati* (Mammalia), the hypothalamus shows two distinct cell groups, supra-optic nucleus (SON) and paraventricular nucleus (PVN). The SON is divisible into further sub-groups (rostral, median and caudal neurons). The neurons of the PVN are more scattered. Some cells have been noticed in the infundibular region which forms a third group of cells, the infundibular nucleus. NSCs of these cell groups stain dark purple with PF, dark with CARP and red with azan. The PVN cells in contrast to SON NSCs stain a little less which leads to the probability of the existence of the two types of the neurosecretory neurons. Morphologically these cells are similar and possess long axons.

6. The hypothalamic NSCs are positive to all histological stains. The neuropile is stained light. The neurolemma
sheaths and the non-neurosecretory cells (pituicytes and other brain cells) are lightly stained. In squad staining the neurosecretory material, the cytoplasm and the perineurium are moderately stained. The nuclei are stained dark with red inclusions, with theorcein counterstain. This shows the presence of available nucleic acids (DNA and RNA) in the nucleus.

7. The caudal NSCs in Gymnaster beaehila and Rohtee cotic, show two morphologically distinct cell types. The larger cells are termed as Dahlgren (Dahl.) cells. These cells have been found in the urophysis. The caudal NSCs have been found distributed in definite columns in the caudal region of the spinal cord. These cells show similar staining affinities as the cells of NPO in fishes.

On the basis of staining reactions it may be concluded that in the evolutionary sequence of the animals the number of the cell types has been exhausted. Only 'A' type of the cells which are positive to PF in invertebrates are perhaps prevailing in the hypothalamus of the vertebrates. This may be perhaps due to the development of advanced endocrine system in vertebrates which is lacking or poorly developed in invertebrates, where the various cell types are responsible for various secretions.

'B. HISTOCHEMICAL STUDIES ON THE NEUROSECRETORY CELLS:

1. Metachromasia:
Staining with toluidine blue gives a strongly positive reaction to the NSCs of vertebrates and invertebrates. The rich metachromasy is represented by the appearance of dark violet colour neurosecretory elements in the weakly stained cytoplasm. The nuclei are stained dark and show a strong metachromatic reaction. The perineurium, neurolemma sheath and the non-neurosecretory cells are fairly stained. In some species like *P. hebraeus*, *P. similis*, *L. acuminate* and *F. pennati*, the reaction is moderate.

2. Sulphydryl and disulphide (-SH and -SS) rich proteins:

Protein bound sulphydryl groups often exist in considerable quantity in all the species examined. In the NSCs of *Hydra*, *U. hardwickii* and both the species of doves, the reaction is fairly strong. The poor reaction has been noticed in the NSCs of *H. fossilis* and *C. batrachus*. The caudal NSCs show a doubtful presence of -SH groups. The disulphide rich proteins occur in small concentrations. The reaction in *A. indicus* and *P. hebraeus* is doubtful.

3. Sulphated mucopolysaccharides:

The sulphated elements are rich in the NSCs of vertebrates and invertebrates. These elements are found in the form of granules in fair quantities in the perikarya of the NSCs. The axons are fairly rich in these elements. There are only a few sulphated elements in the non-neurosecretory cells. Dense accumulation is seen in the neurolemma sheath.
The caudal NSCs of *R. kotio* and *O. bacaila* show a poor reaction.

4. Bound lipids:

The presence of bound lipids varies from species to species. In *R. cyanoploticus*, *P. domesticus*, doves and in squirrel, *E. pennati* the reaction is moderate, in *A. indicus* and *D. similis* the reaction is doubtful, but in rest of the species the reaction is very poor. A small amount of lipid granules is visible in the cytoplasm of the NSCs and some granules are seen in the axons, descending to the respective neurohaemal organ.

5. Glycogen:

Glycogen particles exist in the NSCs of all the species of animals studied. These particles are clearly visible in the weakly stained cytoplasm. The perineurium and the non-neurosecretory cells also possess a considerable amount of these particles. In the NSCs of *C. betrachus*, *H. fossilis*, *U. hardwickii*, *P. domesticus*, *E. pennati*, *P. ceylonicus indicus* and the caudal NSCs of *O. bacaila* and *R. kotio*, the reaction is strongly positive. In other species the reaction is moderate.

6. Nucleic acids:

RNA is demonstrated in the NSCs, in the form of small bodies distributed in the cytoplasm. The perineurium and the non-neurosecretory cells also show the presence of RNA
in a small quantity. But in the NSCs, RNA inclusions, show marked variations in the different cells and form granular bodies in different species. In *P. ansibaricus*, *H. medicinalis*, *P. picta*, *P. americana*, *P. bengalensis*, *C. batrachus*, *H. fossilis*, *M. sphenops* and all the species of amniota taken for study, RNA inclusions are fairly rich. In caudal NSCs of *R. otio* and *O. bacilla* a rich presence of animals RNA is present in moderate quantities. The axons of the NSCs show a moderate presence of RNA.

7. Carbohydrates:

The NSCs of all the species of animals studied, give a positive PAS reaction. Carbohydrates are moderately present in the form of fine granules, distributed in the cytoplasm. The nucleoli and the nuclear membrane are intensely stained. Non-neurosecretory cells and the perineurium give a poor reaction. Axons possess a fair amount of these particles. PAS reaction is perhaps due to the presence of available amount of neutral polysaccharides in the form of glycogen or glycogenproteins and acid mucopolysaccharides.

8. Proteins:

Mercury bromophenol blue method (Bonhag, 1955) has been applied. The NSCs in all the species of the animals studied give an intense reaction. The smaller and larger bodies of neurosecretory substance are readily visible in the
cytoplasm of the NSCs. Axons are positive to this reaction. The perineurium and the non-neurosecretory cells also possess a considerable amount of proteins. The available proteins are perhaps the proteinic fraction of the mitochondria. Proteinic inclusions show marked variations in the NSCs. This variation is due to the active and non-active stages in cells. In active cells the amount is greater, proteins in the NSCs include -SS, -SH groups, bound NH₂ proteins, lipo and glycoproteins.

9. Cysteine and cystine:

Alcian blue and Victoria blue stains after performic acid oxidation have been applied. Cystine/cysteine are present in the NSCs of all species of the animals studied. They are present in moderate quantity, but the reaction is more intense in annelids. Black mollie, M. sphenops, house sparrow, P. domesticus and the caudal NSCs of O. biceida and R. cotio also show a strong reaction. Axons are intensely stained. The perineurium and the non-neurosecretory cells also give a positive reaction, indicating the poor amount of cystine or cysteine.

10. Tyrosin:

The neurosecretory cells in all the species of the animals give a positive reaction, showing the poor presence of tyrosin. The axons are not very clear. The reaction in block mollie, M. sphenops remained doubtful. In the
caudal NSCs of *O. bcailla* and *R. cotio* the reaction is moderate.

11. Bound NH$_2$ proteins:

The presence of bound amino proteins varies from species to species. In most of the species the reaction is doubtful and negative. In *P. picta*, *P. americana*, fish species and the bat, *P. ceylonicus indicus* the reaction is positive. The bound NH$_2$ proteins are present in the NSCs of these species of the animals studied, in considerable quantities. The non-neurosecretory cells and the perineurium give a poor reaction.

12. Tryptophan:

Tryptophan, as tested with DMAB nitrate test, (Adams, 1957) is present in the neurosecretory material of most of the animals undertaken for study, in considerable amount. The reaction remained doubtful in *Planaria*, *A. indicus*, *P. hebraeus* and house sparrow, *P. domesticus*. The non-neurosecretory cells and the perineurium give a poor reaction. Axons of the NSCs give poor reaction. The neurosecretory material rich in tryptophan containing proteins, is granular and stains purplish red.

13. Arginine:

The arginine, has been tested by Sakaguchi oxine,
(Baker, 1947) reaction. The reaction remained negative in all the species of the animals studied. This indicates the absence of arginine in the NSCs.

14. Lipids:

Lipids have been demonstrated in the NSCs of all the species of the animals in the form of fine sudanophilic granules. Fair amount of these granules is also present in the axons. The non-neurosecretory cells and the perineurium also give a positive reaction, showing the presence of a little lipid granules, fairly distributed in the cytoplasm.

15. Acid mucopolysaccharides:

Acid mucopolysaccharides show a considerable presence, in the form of fine green colour bodies, in the NSCs of all the species of animals studied. The reaction is moderate in some animals as is revealed by Alcian blue staining, such as planaria, D. simillae, species of birds and squirrel, but in rest of the animals the reaction is poor. The NSCs in fishes showed a doubtful reaction, or the alcian blue stained bodies are present in negligible quantities. The non-neurosecretory cells and the perineurium show a positive reaction, indicating the little presence of acid mucopolysaccharides. The axons of the NSCs are poorly stained.

In invertebrate, variety of NSCs exist and it is probable that they secrete different type of hormones, as there
are only a few endocrine centres located outside the brain. But in vertebrates the multiplicity of the NSCs is less, as there are many endocrine centres outside the brain, located in different parts of the body and the secrete their own hormones of some specific function. The vertebrate NSCs homologise with the 'A' type of the NSCs of invertebrates and secrete only 'Topic material' which is either a precursor or a labile material used by other endocrine centres for secreting a new hormone.

From the foregoing account, it is clear that the neurosecretory material shows uniformed staining reactions. The variations in the histochemical nature of the neurosecretory product in different NSCs and the species of the animals undertaken for study, are minor ones. The neurosecretory material is a carrier substance which is proteinaceous in nature and is rich in sulphhydril, disulphide, NH₂ bound proteins, tyrosin, tryptophan, cystine/cysteine and mucopolysaccharides. The lipids, cytoplasmic RNA, glycogen contents and the strong metachromasy is demonstrated in the neurosecretory material. In all the animals, the NSCs showed a negative reaction for arginine. The neurosecretory material is responsible for the production of hormones, as it acts as a carrier substance, but it is not a hormone in itself.

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Table: Staining Reactions and Histochemistry of the neurosecretory product in the Neurosecretory Cells in some vertebrate and invertebrate animals.

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0 = negative reaction; 1 = doubtful reaction; 2 = less positive reaction; 3 = positive reaction and 4 = strongly positive reaction.