Chapter IX

General Discussion
The presence of neurosecretory cells and the histochemical nature of the neurosecretory product has been studied in 24 species of the animals, belonging to different groups of vertebrates and invertebrates. This study gives useful information in understanding the evolutionary patterns of the neurosecretory system and its products. The morphological, distributional patterns, histochemical and physiological data can be integrated. The phenomenon of neurosecretion has been a matter of much controversy, however, in the light of many publications and day by day increasing researches, the neurosecretory system in animals now has been well established and it has formed a system which can be analysed by histochemical, biochemical and histological methods.

A. Correlation Between the Neurosecretory Cell Types and Their Distributional Patterns:

In invertebrates there exists a diversity of the neurosecretory cell types. Their existence has been studied in the central nervous system, specially the brain. The neurosecretory cells of vertebrates and the 'A' type of the neurosecretory cells of invertebrates seem to possess similar type of the neurosecretory material, as they have a uniformed staining affinity to the paraldehyde fuschin, (PF), chrome-alum haematoxylin phloxin, (CAHP) and Heidenhain’s azan staining techniques. In all the animals, whether invertebrate or vertebrate, there are some neurosecretory cells, which stain deep purple with PF staining. The homologies of such cell types may be derived on this basis, as they possess a
common material positive to basic dyes.

In the brain of arthropods, annelids, molluscs more than one group and type of the neurosecretory cells exist, which have been regarded as 'A', 'B', 'C' and 'D' types and their further sub-types, $A_1$, $A_2$ and $B_1$, $B_2$ etc.. Out of these, the 'A' cells are positive to PF and are similar to neurosecretory cells in lower invertebrates, such as Spongilla, Hydra and Planaria and NPO, SON and PVN neurosecretory cells of vertebrates, in staining properties. The cells, which are negative to PF definitely possess the neurosecretory material of a different nature, which may be responsible for some different physiological functions, such cells are found in invertebrates and a very few in vertebrates.

The existence of the neurosecretory cells in Spongilla, Hydra and Planaria has been described which is similar to the findings of Lentz (1966) in Sycon; Burrnett et al., (1964); Lentz and Burrnett (1965) in Hydra; Wood and Burrnett (1965) in Hydra and Sea anemone; Silk and Spence (1969) in Planaria; Chawda and Karyakarti (1974) and Webb (1976, 1977) in helminthes, of the similar type.

In Perionyx sansibaricus Mich,'A', 'B' and 'C' and in Hirudo medicinalis 'A', 'B', 'C' and 'D' types of the neurosecretory cells have been studied in the brain and sub-oesophageal ganglion, which are similar to the histological findings of a large number of workers, who have described the neurosecretory system in annelids viz., Herlent-

Distribution and classification of the neurosecretory cell types in insects, has been extensively worked out by a large number of authors. Despite minor variations, the insects possess a most generalised type of the neurosecretory system, which consists of the brain, corpus cardiacum-allata complex. In the brain of Poecilocerus picta Fabr, Periplaneta americana L, Apis indica Fabr, Polistes hebraeus Fabr and Dysdercus similis Freeman four types ('A', 'B', 'C' and 'D') of the neurosecretory cells have been demonstrated. The 'A' cells are larger and positive to PF stain. The 'B', 'C' and 'D' cells do not stain with PF, but they are well differentiated with counter stains. The findings agree with the observations of Nayar (1955); Johansson (1958); Ewen (1962); Delphin (1965); Saini (1966, 1971 and 1978); Dogra (1967, 1969 and 1978); Gupta (1970); Hinks (1971, 1975); Awasthi (1973); Pathak (1974); Faruqui (1975, 1976); Rowell (1976); Ghosh Goswami and Faruqui (1977); Ramamurty (1978); Bhatt (1978) and others. 'A', 'B' and 'C' types of the neurosecretory cells have been studied in the brain of scorpion, Palpimanus bengalensis Koch, which resembles with the similar observations of Habibulla (1961, 1962 and 1970) in scorpion, Heterometrus swammerdami.
In *Lymnaea acuminate* 'A', 'B' and 'C' types of the neurosecretory cells have been studied. The finding are in agreement with the similar observations of Gabe (1955); Fahrmann (1961); Nagabhushnam (1962); Nagabhushnam and Muley (1974) and Lomte and Nagabhushnam (1974), but disagree with the findings of Lever (1957) and Shyama Sundri et al., (1978), who have described five types of the neurosecretory cells in pulmonates.

Controversy exists in the mechanism of transport and release of the neurosecretory material, which has not been dealt in the present work.

In lower vertebrates such as *Clarias batrachus* L, *Heteropneustus fossilis* Bl, *Mollisnios sphenops* Cuv and Valen *Rana cyanoplicticus* Schneider, the nucleus pre-opticus (NPO) has been demonstrated within the hypothalamus with PF, CAHP and azan staining techniques. The neurosecretory cells possess a considerable amount of the neurosecretory material in the form of granules. The nuclei and the nucleoli are very distinct. The axons of the cells also show a rich presence of the NSM and are seen clearly passing down to the hypophysis. The position, shape and extention of the nucleus pre-opticus has been found to vary in different species. Several investigations have been made to describe the nucleus pre-opticus in fishes. The present observations agrees with the similar findings of Sathyanesan (1970), who described L-shaped NPO in *C. batrachus* L, divisible into NPO *Pars magnocellularis* and NPO *pars parvocellularis*.
and Chandra Sekhar and Khosa (1972), who described spindle shaped NPO in *H. fossilis* BI.

The nucleus lateralis tuberis (NLT) has been described in *H. fossilis* BI and *C. batrachus* L. The findings are in resemblance with the observations of Stahl and Leray (1962) in *Nugil* sp., Dixit (1967) and Sathyanesan (1970) in *C. batrachus* and Haider and Sathyanesan (1972c) in *H. fossilis* described the nucleus lateralis tuberis. The NPO of *M. sphenops* Valen is a knob like structure and varies from the above mentioned findings, but appears in resemblance with the NPO of *Glassogobius giuris*, Saksena (1974).

The neurosecretory cells in the caudal spinal cord and urophysis of *Oxygaster bacailla* and *Rohtee cotic* have been worked out. There are two types of the neurosecretory cells which are positive to PF and possess distinct axons. The larger cells are the Dahlgren (Dahl) ones which are oval in shape and have distinct large nucleus, the other type of the cells are smaller in size. The present findings are in agreement with a large number of authors who have critically described the caudal neurosecretory system in fishes, viz., Friedberg et al., (1966a); Sano and Hartmann (1969); Gupta and Shrivastava (1971); Jaiswal and Belsare (1974); Lederis et al., (1974); Bern (1976); Bern and Lederis (1978); Bern and Nishioka (1978) and others.

The nucleus pre-opticus in *R. ommoplecticus* Schneider have been studied. The cells are positive to PF and possess clearly visible axons. The observations are in agreement
with the observations of Hild (1951); Dawson (1953); Legait and Legait (1962) and Bhatt (1980) who have described the neurosecretory system in amphibians.

Two neurosecretory centres, supro-optic nucleus (SON) and paraventricularis (PVN) nucleus, have been demonstrated in Uromastix hardwickii, Passer domesticus, Streptopelia chinensis, S. tranquebarica, Pipistrellus ceylonicus indicus and Funambulus pennatil Wroughton, with the help of conventional staining techniques. In PF staining the nuclei stains purple and the NSM stains deep purple. The neurosecretory cells are positive to CAHP and Azan staining. The cells possess long axons. The present findings follow a general vertebrate pattern and are in resemblance with a large number of workers viz., Bargmann et al., (1950); Hild (1951); Scharrer (1951); Chiara (1954, 1956 and 1957); Azzali (1958); Gabe and Saint Girons (1964); Saint Girons (1964); Green (1966); Haider and Sathyanesan (1974); Bhatt (1980) and others in reptiles, Wing Stand (1951); Farner (1958, 1960); Legait (1959); Oksche (1959, 1962); Oksche et al., (1959, 1973); Singh (1973) and others in birds and Scharrer and Scharrer (1954); Diepen (1954, 1967); Diepen et al., (1959); Christ (1960, 1962); Engelhardt and Diepen (1962); Heller and Lederis (1962); Rechardt (1969); A. Mohan (1973); Gopal Krishna and Bhal Chandra (1978) and others in mammals. These authors have described the hypothalamic hypophysial neurosecretory system by using different staining techniques.
The existence of the neurosecretory material or hormone carrier substance in the neurosecretory cells of vertebrates and invertebrates, stainable with common conventional staining techniques indicate a common evolutionary significance of the system.

B. CORRELATION BETWEEN HISTOCHEMICAL REACTIONS AND THE HISTOCHEMISTRY OF THE NEUROSECRETORY PRODUCT:

In the foregoing account, the neurosecretory cells and their staining affinities have been mentioned. To understand the histochemical nature of the different types of the neurosecretory cells and their secretory product, different histochemical tests have been applied. Most of the findings are in agreement with the earlier authors, but there are many observations, which are quite different from them and disagree with their findings.

Presence of proteinaceous compounds have been reported in the neurosecretory material of the various species of the animals undertaken for study. The proteins exist in the form of smaller and larger bodies as revealed by mercury bromophenol blue staining technique, (Bonhag, 1955). The neurosecretory cells in invertebrate and vertebrate animals have been histochemically found rich in sulphydryl (-SH), disulphide (-SS) and bound NH₂ proteins. Tyrosin has been reported to occur universally in poor concentrations. Cystine or cysteine and tryptophan rich proteins have been demonstrated. Arginine has been
found universally absent as tested by Sakaguchi oxine reaction, (Baker, 1947).

The caudal neurosecretory cells of the two teleost fishes, O. bacinula and R. coting show a doubtful presence of sulphhydryl rich proteins. Although the neurosecretory material in all the animal species studied, has been reported positive to both sulphhydryl and disulphide proteins, but the disulphide proteins have been observed to occur in small concentrations. More over, the NSCs of insects, A. indica and P. hebraeus the disulphide proteins show a doubtful presence.

The observations on the distribution of protein bound sulphhydryl and disulphide groups are uniform and are in agreement with the findings of Barman and Saligman (1952); Gomori (1956), Sloper (1955, 1957, 1958) and Adams (1956), who have demonstrated the presence of these proteins by DDD reagent neotetrazolium and ferric ferricyanide reactions and Sloper (1955, 1957) showed the existence of these proteins uniformly in vertebrate and invertebrate neurosecretory material. The present observations are based on ferric ferricyanide reaction, (Chevremont and Frederic, 1943) for -SH groups and performic acid oxidation and Schiff reaction, (Pearse, 1951) for -SS groups. Sloper employed the ferric ferricyanide reaction after reduction with sodium thioglycolate for the demonstration of -SH groups in Leuconoea maderae. Dogra and Tandan (1964)
claimed that performic acid Victoria blue staining also
gave better results for sulphhydril rich proteins, superior
than alcian blue. The present observations are in
complete disagreement with Rehm (1965), who claimed that
the protein bound disulphide and sulphhydril proteins do
not exist in the neurosecretory product of insects.

The protein bound cystine or cysteine have been
demonstrated in the NSCs of all the species studied. The
present findings support the observations of Sloper (1958);
Dogra and Tandan (1966); Dogra (1967b); Faruqui (1974);
Bhatt (1979, 1980) and Bhatt et al., (1980, unpublished
data). Hinks (1971) denied the presence of these amino-
acids in the brain neurosecretory cells of Triphaena pronuba.
According to him, these amino-acids exist in other insects,
in appreciable quantities. In the staining reactions, the
neurosecretory material in invertebrates and vertebrates
has been found positive to chrome-alum-haematoxylin, PF
and alcian blue, which has been interpreted by Sloper (1950);
Delphin (1965); Sien (1965) and Naisse (1966), as
the oxidised sulphhydril groups of the cystine or cysteine
amino-acids, which are responsible for this affinity. Sloper
(1955) suggested that chrome-alum-haematoxyphil vertebr-
ate neurosecretory material or material in its exact dis-
tribution is essentially a protein. Further, Sloper (1967)
has drawn the significant conclusions that the neurosec-
tory proteins are rich in protein bound cystine or cysteine
and are common to vertebrate and invertebrate materials.
alike. The present observations do agree with this view.

The tyrosin and tryptophan amino-acids have been demonstrated in the neurosecretory material, in almost all the species studied, but the arginine contents have been found totally absent.

As far the presence of arginine is concerned, the present observations are in disagreement with Hinks (1971), who has reported the presence of arginine in the neurosecretory cells of *Triphaena pronuba*. Bargmann (1958) and Howe (1959 and 1962) demonstrated the arginine positive material throughout the pars nervosa and interpreted that the distribution corresponds in general to that of the neurosecretory material stained by GAFP method. Present observations agree with them only in one point as they have observed no arginine contents in the hypothalamic NSCs and also are in agreement with Shyama Sundri et al., (1978), who reported the absence of arginine in the NSCs of *Discodoris* sp..

The tyrosin and tryptophan have been demonstrate to occur in poor quantities in the NSCs. The observations are in support to the findings of Dyke (1953); Arvy and Gabe (1962) and Hinks (1971), who reported the existence of tryptophan and tyrosin in the NSCs, but are in complete disagreement with Shyamasundri et al., (1978), who denied the presence of these amino-acids in the NSCs in the brain of *Discodoris* sp..
The bound NH₂ proteins have been observed in a uniform distribution in the neurosecretory material of a large number of species, but its concentration varies from species to species. In the NSCs of Planaria, leech, H. medicinalis and Earthworm, P. sansibaricus Mich a negative reaction has been observed. The entire observations are based on the chloramin-T. Schiff method, (Chu, 1953; Burstone, 1955).

The proteins as a whole have been demonstrated by mercury bromophenol blue method, (Bonhag, 1955). The proteins exist in the NSCs in the form of larger and some smaller bodies, deeply stained and distributed in the weakly stained cytoplasm. The findings are in resemblance with a large number of authors, who have demonstrated proteins in the neurosecretory material in different animals, Rehm (1955); Defretin (1955); Sloper (1957); Arvy and Gabe, (1962); Ramade (1966); Wendelaar and Benga (1970); Banhawy and Anwar (1971); Beattide (1971); Hinks (1971); Gupta (1971); Pathak (1974); Saksera (1974); Shyamasundri et al., (1978); Bhatt and Kashyap (1978); Bhatt (1980) and others. The proteins have been regarded by Schlieber (1952) to exist in the form of a glycoprotein complex or bearer substance soluble in lipid solvents, in the hypothalamus and hypophysis of vertebrates. Banhawy and Anwar (1961) did not support this view. The present observations are in favour of the previous authors. Dyke (1953) and Arvy and Gabe (1962) demonstrated proteins by a positive alloxan-Schiff reaction. Dyke has observed
that the posterior pituitary principles in mammals are exceptionally rich in proteins.

The cytoplasmic RNA has been demonstrated in the NSCs of all the species studied. The 'A' cells of invertebrates and the hypothalamic NSCs of vertebrates show rich presence of RNA in the form of fine granules. The other varieties of cells also show a positive reaction. Present observations are in resemblance with the findings of Banhawy and Anwar (1971); Gupta (1971); Hinks (1971); Pathak (1974) and Bhatt (1980). Pipa (1961) noticed a parallel relationship between RNA inclusions, number and size of the secretory granules in the NSCs. Brachet (1957) suggested that there is a close relationship between RNA and protein synthesis in the NSCs of insects. The present observations are in complete disagreement with Rehm (1955), who claimed that the RNA do not exist in the neurosecretory products.

The lipids have been demonstrated in the neurosecretory material by a positive Sudan black 'B' reaction. It gives the most intense colouration of lipids. Lipids stain black and are present in the form of fine granules uniformly distributed in the cytoplasm. The concentration in 'A' cells of invertebrates is greater and can be correlated with the hypothalamic NSCs of vertebrates. The other type of the cells in invertebrates also show a positive reaction. The bound lipids exist only in poor quantities as studied
by Sudan black 'B' saturated in acetone (Pearse, 1960). As far the presence of lipids is concerned, the present observations agree with Hinks (1971); Gupta (1971); Nayar (1954); Pipa (1961); Pathak (1974); Shyamare sundri et al., (1978) and Bhatt (1980), but/in complete disagreement with Sloper (1955); Howe and Pearse (1956); Arvy and Gabe (1962) and Banhawy and Anwar (1971), who did not find any lipoidal material in the neurosecretory substance of vertebrates.

The carbohydrates have been demonstrated in the neurosecretory material by periodic acid/Schiff (PAS) method of Mc Manus (1948). Intensely stained PAS positive material has been noticed in the NSCs of vertebrates and invertebrates. The 'A' cells of invertebrates are exceptionally rich in carbohydrates. The observations are in agreement with Arvy and Gabe (1945, 1953, 1955); Rehm (1955); Gabe (1960); Gupta (1971); Banhawy and Anwar (1971); Hinks (1971); Pathak (1974); Bhatt and Kashyap (1978); Bhatt (1980) and others, but are in disagreement with the view of Rehm, who interpreted that in Galleria larvae, the positive reaction for carbohydrates is due to the presence of residual lipids in the tissues.

The positive PAS reaction indicates the presence of mucopolysaccharides. The glycogen and the carbohydrates show a uniform distribution in the neurosecretory cells. The glycogen particles may be correlated with the lipids,
which perhaps exist in the form of glycoproteins and the
PAS reaction is due to these proteins only. Sulkin (1960)
and Spicer (1960) were of the opinion that the positive
reaction to PAS test indicates the presence of neutral
mucopolysaccharides or of glycoproteins, in quantities that
vary from species to species. Present observations agree
with this view, as supported in the species studied.

The neurosecretory material has been demonstrated
positive to glycogen contents, metachromasia, acid and
sulphated mucopolysaccharides. The findings are in support
to the observations of Kobayashi et al., (1958, 1962);
Sulkin (1960); Spicer (1960); Lane (1964); Banhawy
and Anwar (1971); Bhatt and Kashyap (1978); Shyamasundri
et al., (1978) and Bhatt (1980), but disagree with Arvy
and Gabe (1962), who denied the existence of glycogen,
metachromasia, mucopolysaccharides and lipids in the
neurosecretory material of insects.

Ewen (1962); Thomson (1965) and Banhawy and
Anwar (1971) noticed that there was a correlation between
the secretory activity and the size of the nuclei and
nucleoli. Delphin (1965) denied this, but the present
findings are in confirmation to the views of former authors.
The existence of the neurosecretory substance in the axons,
shows that the neurosecretory material passes through the
axons to the respective neurohaemal organs. In appearance,
after permagnate oxidation of the reducing groups, in the neurosecretory material of invertebrates, corresponds with a similar observation in vertebrate material, (Gabe, 1955).

The neurosecretory substance is clearly noticed by staining methods in 'A' type of cells. The 'A' type of cells, which take characteristic colour, staining alike with staining techniques like PF, CAMP and alcian blue. The 'A' cells are noticed to take similar stain in invertebrates and vertebrates, (sponges to mammals). These show common staining properties, although these are of different sizes and shapes. The caudal NSCs of fishes are also homologous to these cells in staining affinities. The 'B', 'C' and 'D' type of cells are differentiated by these staining methods, but are not deeply and characteristically stained by PF, CAMP and alcian blue. These cells definitely have secretions of some type. Such multiplicity of the cell type, which are noticed in invertebrates like annelids, arthropods and molluscs is not present in vertebrates. It is probable that in invertebrates different types of the hormone are secreted from all cells present in the brain and they have various functions as there are only a few endocrine centres located out side the brain, which are quite numerous in vertebrates. In vertebrates the brain cells, which show not much of multiplicity and the cells are nearly of one or two types only. They only secrete a 'topic material', which is either a precursson or topic
material or labile material, which is used by other hormones and endocrine centres for the secretion of a new hormone, as other endocrine centres are located outside the brain in vertebrates. Other new referred techniques would show new type of hormone secreted by one type of the stained cells of vertebrates and invertebrates.

As regards to the histochemistry, the nature of the secretion and the NSCs are similar in vertebrates and 'A' cells in invertebrates. The neurosecretory material is a proteinaceous and metachromatic substance rich in mucopolysaccharides, sulphhydryl and disulphide groups and cystine or cysteine. Tyrosin, bound NH₂ proteins and tryptophan show their presence in varying concentrations in different animals studied. Arginine is totally lacking in the neurosecretory material. RNA has been demonstrated in the form of fine granules and show uniform distribution in the NSCs of all the species studied. The NSCs show the cytoplasmic origin of the neurosecretory substance and its axonal transport to the respective neurohaemal organs.

From the foregoing account it is evident that the histochemical characteristic of the neurosecretory substance, is an argument in favour of the view, (Arvy and Gabe, 1962) that 'the neurosecretory material stained by conventional staining techniques, in both invertebrates and vertebrates consists of carrier substance and not the hormones themselves. This carrier substance is mostly common to all the animals'.