Chapter I

General Introduction
The phenomenon of neurosecretion was established initially by the critical observations of the conventional staining techniques, (Scharrer, 1928 and Palay, 1945). Bargmann (1949) for the first time succeeded in differentiating the neurosecretory material with Gomori's Chrome-alum-haematoxylin Phloxin (CAHP) method. Latter on Gomori's aldehyde fuchsin (AF) technique was found to stain selectively, (Gabe, 1953; Dawson, 1953). In the subsequent years CAHP, AF, PF and their modifications, Aldehyde thionin, Alcian blue Phloxin, performic acid Victoria blue, (Dogra and Tandan, 1964) and Heidenhain's azan, (Pantin, 1959) techniques have been found fruitful in better understanding the structure of the neurosecretory system in different groups of animals.

Earlier to this Dahlgren (1914) described the neurosecretory cells for the first time. Kopec (1917) suggested that the insect head has some material which controls moulting. However, Wigglesworth, (1934, 1940) experimentally demonstrated the factor to be present in the brain. Since then, the hormone production from the brain cells has been established. Hanström (1938) described large neurosecretory cells in the brain of Rhodnius. Wigglesworth (1940) demonstrated the functions of the neurosecretory cells. Existence of the neurosecretory neurons is well established, (Scharrer and Scharrer, 1945, 1954, 1963 and Scharrer, 1969, 1972).

Bargmann (1949) and Bargmann and Hild (1949) were the first to demonstrate selectively the pre-opticohypophysial tract by using Gomori's CAHP technique. Bargmann and Scharrer
(1951) put forth the transport hypothesis regarding the functioning of the hypothalamo-hypophysial system. Lenhossek (1887) introduced the term supra-optic nucleus (SON) and this term is now generally accepted. In the teleost fishes the nucleus pre-opticus (NPO) was first studied by Herrick (1891). The knowledge of the hypothalamo-hypophysial neurosecretory system has been increased with the works of Palay (1943, 1945, 1953, 1955, 1957, 1960 and 1961); Scharrer, Palay and Milges (1945); Hild (1951); Hanström (1952, 1954); Dispen (1954, 1962); Ghiara (1954, 1966 and 1957); Azzali (1958); Farner (1958, 1961); Dispen et al., (1959); Kobayashi et al., (1959); Legait (1969); Christ (1960); Oksche et al., (1959, 1973); Oksche (1962); Heller and Lederis (1962); Dodd and Kerr (1963); Sathyanesan (1963, 1965, 1968, 1969 and 1970); Saint Girons (1964); Sathyanesan and Gorbmann (1965); Bhargava (1966, 1969); Nishiooka (1967); Rechardt (1969); Haider and Sathyanesan (1971, 1972); Banerjee (1970); Morris (1973) Gopalkrishna and Bhalchandra (1978); Bhatt (1980) and others. Principal vertebrate neurosecretory system serves as a morphological and physiological connection between the hypothalamus and hypophysis.

Invertebrate neurosecretory system has been extensively worked out. In insects, Nayar (1955); Johansson (1958); Ewen (1962); Arvy and Gabe (1962); 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 and Faruqui
Ramamurty (1978); Bhatt and Kashyap (1978); Steel (1978) and others have carried out significant studies. The presence of nervous system in sponges is uncertain, (Jones, 1962; Bullock and Horridge, 1965), although cells with morphological appearance of neurons have been described, (Ledanfeld, 1889a,b, 1891; Tuzet et al., 1962 and Pavans de Ceccatty, 1955, 1957 and 1959). Lents (1966) observed bipolar and multipolar cells in *Spongilla*. Such cells do exist in *Spongilla*.

Hansström (1954) considered the neurosecretory cells of annelids to be primitive. Scharrer (1936, 1937) and Schäfer (1939) described several types of neurosecretory cells in the posterior part of the brain of *Nereis diversicolor* and *N. virens*, which were among the first to be observed in invertebrates. Herlent-Meewis (1955, 1956); Hagadorn (1958, 1962); Nambudiri and Vijayakrishnan (1961); Hagadorn and Nishioka (1961); Dogra (1967); Hanumante and Nagabhushnam (1978); Bhatt (1978) and others have worked on the neurosecretory system of annelids. Burmell et al., (1964); Wood and Burmell (1964) and Lents and Burmell (1965) have identified the neurosecretory cells in cyclostomes. Silk and Spence (1969) and Webb (1976, 1977) have described the neurosecretory system in Helminthes. The neurosecretory system in Mollusca has been worked out by Gabe (1955); Leven (1957); Fahrmann (1961); Bern (1962); Hagadorn (1962); Lane (1964); Nagabhushnam and Muley (1974); Shyamasundari, Banu and Rao (1978) demonstrated the neurosecretory system of various molluscs.
In invertebrates like helminthes, Arthropods and Molluscs, there are cells producing neurosecretion from different areas of the nervous system (Dogra, 1967; Fernandez and Fernandez, 1972; Fahrenbach, 1973; Kulkarni and Nagabhushnam, 1974; Adiyodi, 1974; Grasso et al., 1975; Smith, 1975; Webb, 1976, 1977 and Andrew and Saleuddin, 1976, 1978). The use of electron microscope has been useful in differentiating the various neurosecretory cell types, on the basis of electron dense material contained in the cells.

The existence of the neurosecretory cells has been widely studied, both in vertebrates and invertebrates. Different authors have described different types of neurosecretory cells viz., 'A', 'B', 'C' and 'D' types and their sub-types, on the basis of their reactions to the specific neurosecretory stains and their morphology. Invertebrates show a multiplicity of the neurosecretory cells; but in vertebrates the cells are found arranged in definite cell groups. Nucleus pre-opticus (NPO) in fishes and amphibians and nucleus supra-opticus (SON) and paraventricularis nucleus (PVN) in amniotes. Eneströn (1967) and Rechardt (1969) described 'A' and 'B' cells in mammals, on the basis of their staining qualities and electron microscopic appearance. In the hypothalamus of fishes, a nuclear centre, other than the NPO has been recognised as nucleus lateralis tuberis (NLT). Dixit (1967) and Sathyanesan (1970) in Clarias batrachus and Haider and Sathyanesan (1972c) in Heteropneustus fossilis described the NLT. It has also been reported in other fishes; but it not necessarily occurs in all fishes. Its role in
seasonal sexual cycle has been established by Leray (1962) in *Mugil* sp.

The classification of the insect neurosecretory cells, based on staining affinities have been endlessly proposed, sub-divided, criticised, modified and acclaimed by many authors (Rowell, 1976). Rowell, used some new electrophysiological techniques for the demonstration of neurosecretory cells in insects. With the development of the markers suitable for intercellular labelling of cells in both light microscopy (LM) and electron microscopy (EM), such as the filling of neuron with dye from several nerve stumps (Mulloney, 1973) and filling of neurons with dye through an intracellular micropipette electrode (Kater and Nicholson, 1973). Intersalification of cobalt staining by silver precipitation (Tyrer and Bell, 1974) has been tried.

The cells have been discovered which have the appearance of neurosecretory cells by EM, but which do not stain with any of the usual LM procedures (Sandifer and Tombes, 1972; Chalaye, 1974a), it is not known how common such cells are. Many attempts have been made to correlate LM and EM pictures (Ramade, 1966; Geldiary and Edwards, 1973; Chalaye, 1974), but no study has been made with truly identified cells. For this there is some histochemical evidence that the proteins show real differences in their concentration in different cell types, (Raabe and Mónjó, 1970 and Prentō, 1972).

Centres located outside the insect brain have been established by Wigglesworth (1960); Maddrell (1962, 1963).
and Finlayson and Osborn (1968). Caudal neurosecretory system of teleost fishes has been worked out by Bern et al., (1973); Lederis et al., (1973); Jaiswal and Balsara (1974); Swanson et al., (1975); Bern and Lederis (1978); Bern and Nishioka (1978); Pryor et al., (1978) and others. These authors have demonstrated the urotensin I and II in the caudal neurosecretory cells.

The brain hormone, the neurosecretion is a mucopolysaccharide, although researchers, earlier had thought it to be lipoidal in nature related to cholesterol etc., (Williams, 1967). The workers like Ichikawa and Ishizaki (1963); Kobayashi and Yamizaki (1966); Williams (1967) are of the opinion that the neurosecretion is a water soluble, heat stable, non-dialyzable resistant to peptidases, but is destroyed by microbial peptidase. It is a protein or a complex sensitive to pronase and Nagase.

Neurosecretory material has been studied histochemically by a large number of authors and the presence of individual substance or a number of substances have been reported in it. It seems probable that it is a mixture of a large number of organic, hormone carrier substances. Pantin (1956) has pointed out that pharmacologically active substances may be present in cells as accidental features prior to the evolution of nervous system. With the aid of performic acid alcian blue method, it has been shown that the neurosecretory material is rich in cystine or cysteine, (Sloper, 1955; Adams and Sloper, 1955 and 1956). Ortmann
(1951) and Bargmann (1954) have suggested that the stainable neurosecretory material is firmly tied with the posterior pituitary hormones. Acher (1958) and Hartmann (1968) have found the stainable NSM to act as a carrier substance for the posterior pituitary hormones. Certain mammalian posterior pituitary principles have already been shown to be exceptionally rich in cystine, (Dyke et al., 1953). Sloper (1957) has shown that in general the cystine/cysteine are present in the neurosecretory cells of vertebrates and invertebrates. Adjacent cystine/cysteine rich neurosecretory cells, show a positive dark blue staining with chrome-alum-haematoxylin phloxin. Sloper (1955) and Howe and Pearse (1956) did not find any lipoidal material in the neurosecretory substance of vertebrates. Castel (1977) and Minnen et al., (1977) applied alcian blue-alcian yellow staining techniques for the identification of different types of NSM in paraffin sections. Dogra and Tandan (1964) adapted performic acid Victoria blue (FAVb) technique (modification of the original Rumberstone's method) for in situ demonstration of the neurosecretory substance (cystine/cysteine) in invertebrates and vertebrates. The method has been applied in insects, (Dogra and Tandan, 1966; Dogra, 1969 and Faruqui, 1974).

Barrnett and Seligman (1952); Rehm (1955); Gomori (1956) and Arvy and Gabe (1962) demonstrated both sulphydryl and disulphide (-SH and -SS groups) groups to occur in various proteins. Sulphydryl rich lipoprotein
(Brousse et al., 1968; Ramade, 1969); sulphydryl rich glycoproteins (Arvy and Gabe, 1962) and sulphydryl rich proteins, (Schreiner, 1966 and Naissa, 1966) have been demonstrated in the neurosecretory material of different animals. Tryptophan and tyrosin showed significant concentrations only in adult cell secretions, cysteine (sulphydryl group) and arginine show uniform distribution in the brain of *Triphaena pronuba*, (Hinks, 1971); but no other worker, including the present observations reported arginine contents in the brain or hypothalamic neurosecretory cells. Howe (1962) reported the presence of arginine in the pars nervosa of some mammalian species. Schlieber (1952) reported proteins in the hypothalamus and hypophysis of vertebrates in the form of glycoprotein complex. Sulkin (1960) reported the distribution of mucopolysaccharides in the cytoplasm of the vertebrate nerve cells. Spicer (1960) has presented a comparative account of acid mucopolysaccharides in rodents. Banhawy and Anwar (1971) reported rich acid mucopolysaccharides in *Gryllotalpa gryllotalpa*. Acid mucopolysaccharides were also demonstrated by Ashrast (1961) in the extra cellular spaces of the cockroach ganglia.

Significant histochemical observations on the neurosecretory cells for studying carbohydrates, proteins, nucleic acids (RNA and DNA), glycogen contents, mucopolysaccharides, amino-acids (tyrosin, tryptophan, cystine/cysteine), sulphydryl and disulfide rich proteins and lipids have been made by a large number of workers in this field. (Rehm,
1955; Gomori, 1956; Brachet, 1957; Sloper, 1957; Pipa, 1961; Ewen, 1962; Arvy, 1962; Arvy and Sabe, 1962; Delphin, 1965; Wandelaar Benga, 1970; Bakhawy and Anwar, 1971; Hinks, 1971; Gupta, 1971; Franto, 1972; Maruqui, 1974; Pathak, 1974; Bhatt and Kashyap, 1978; Bhatt and Gupta, 1980 and others). A large number of hormones are now known to be secreted by nerve cells or their derivatives. Neurosecretory cells have been recognized as source of hormones (Jenkin, 1962). In annelids the hormone produced is regarded as morphogenetic hormone, but in molluscs, arthropods and vertebrates the same is known as vascular hormone with kinetic and metabolic actions.

The definition of the neurosecretory cell has been quite clear since the Napples symposium (First International Symposium on Neurosecretion, 1953); it is not solely based on the presence of neurosecretory granules, but also on the fact that neurosecretory cells do not form synapses with other neurons or effector organs. Their axons end at blood spaces into which neurosecretory material is released in the neurohaemal organs. It was already concluded at Lund Symposium, (Second International Symposium on Neurosecretion, Lund, 1957) that the main function of the neurosecretory cell is the release of a substance or substances into the blood to act either on distant effectors (organs) or to stimulate the cells of another gland. Cross and Green has studied the electrical activity of single neurons in the supra-opticus and paraventricular nuclei by means of extracellular micro-electrodes and had recorded action potentials indistinguishable from those obtained
from non-neurosecretory sites, but Berta Scharrer (1973, at the VI International Symposium on Neurosecretion, London, 1973) concluded that the neurosecretory cells operates in transmitting the neurally derived information to various receptors. The neurons behave much like conventional neurons. Further Saini (1978, at the Symposium on Modern Trends on Neurosecretion, at Saugar University, Sagar) added that the neurosecretory cells are the true nerve cells, which are secretory in nature and their secretions reach to a target organ which may be quite far from the place of secretion.

The origin of the neurosecretory granules is still a matter of controversy. A group of workers believe, the secretory granules bud off the Golgi apparatus, some believe it the transformation of mitochondria into neurosecretory granules. Earlier observations with light microscopic techniques demonstrate the origin of secretory granules with in the nuclei of certain groups of neurosecretory neurons in some teleost fishes. Ernst Scharrer (1961) remarked, whether the granules are fully formed in the perikaryon and are merely transported to the axon terminals, or whether they undergo a process of maturation on the way. It is still an unsolved problem.

The role of the axonal spike potentials in the release of neurosecretory mediators has been demonstrated in Molluscs (Kandal and Kupfermann, 1970) and insects (Normann, 1973). The neurosecretory neuron with its distinctive biosynthetic
and bioelectric properties has found its place within an impressively rich specturm of possibilities available for neural communication. The place of release, the type of release and the mechanism of release is a complicated process, (De Robertis, 1964). The release organs have been described in different ways in the blood, or corpus cardiacum, (Nayar, 1956; Highnam, 1961; Gupta, 1970; Awasthi, 1973) or in the cardioiglial tissue, as a result of fusion of CC and aorta (Faruqui, 1977) or in the aorta (Dogra, 1967). In many cases, the neurosecretory axons are seen to run right up to the target organs, (Johansson, 1963).

Heller (1961) in his introductory lecture (at the third International Symposium on Neurosecretion, at the University of Bristol, 1961) felt the necessity for a comparative approach to the characterization to the hormone carrier to understand more about the nature of the carrier substance specificity and to know whether each neuron synthesize only one hormone or several hormones.

As the neurosecretory complexes of vertebrates and invertebrates can very well be studied by using common staining and histochemical techniques. Prof. W. Bargmann (1973) in his concluding remarks as a chairman of VI Int. Nat. Symp. of Neurosecretion, London, felt it necessary that "The invertebrates and vertebrates should not be discussed separately in separate Symposia. The neurosecretory system and their products are admittedly different in different"
groups of animals, but the basic phenomenon of neurosecretion are processes at the cellular level that investigators try to understand the mechanism of elaboration, transport and release of neurohormones and neurohumors". The neurosecretory material, specially the reducing group of pars-intercerebralis of insects corresponds with the similar observations in vertebrate material, ( Gabe, 1956 ).

Experimental and pharmacological studies on the neurosecretory system have shown that the neurosecretory substance plays an important role in gonad maturation, caste determination, metamorphosis, differentiation, colour change mechanism, oocyte development, Osmoregulation and other growth and metabolic processes.

In the present study, the neurosecretory system of 24 animals, including both vertebrates and invertebrates, has been studied by conventional staining and histochemical techniques. The review of the literature on neurosecretion clearly suggests to make a comparative approach to study the histochemical nature of the neurosecretory material in different groups of animals. Present observations show the following results :-

1. PF positive neurosecretory cells have been demonstrated in all the animals undertaken for study. The 'A' cells of invertebrates stain deep purple and homologise with the hypothalamic neurosecretory cells, ( neurosecretory cells of NPO, SON and PVN ) of vertebrates. The other varieties of cells, viz., 'B', 'C' and 'D' cells and their sub-
types are quite different from those of the 'A' cells. These cells have been recognized with the help of counter stains. Some lower invertebrates which do not possess variety of cell types, they show a positive reaction to PF. Suppression of the cell types in vertebrates may be probably due to the elaborate development of endocrine system.

2. The neurosecretory cells are positive to chrome-alum haematoxylin phloxin and the adjacent cells give a positive response to cystine/ cysteine.

3. Sulphydryl and disulphide ( -SH and -SS ) groups have been observed in the neurosecretory cells, in the form of proteins.

4. Proteins, sulphated and acid mucopolysaccharides, cytoplasmic RNA and tyrosin ( in small quantities ) have been demonstrated in the neurosecretory cells.

5. Lipids and glycogen contents have been demonstrated in the neurosecretory cells, but their concentrations varies in different individuals.

6. Bound lipids and bound NH₂ proteins have been reported in small quantities. In the neurosecretory cells of some individuals they show a doubtful presence or they are totally absent.

7. Tryptophan show variations in its concentration in the neurosecretory cells in different species.
8. No arginine contents have been found in the neurosecretory cells of vertebrates and invertebrates.

9. In squad staining the nuclei are intensely stained with orcein counterstain, which shows the presence of nucleic acids.

10. Metachromatic nature of the neurosecretory material has been demonstrated by positive responses to the toluidine blue staining, in which the nuclei are intensely stained.

The variability of the histochemical characteristics is an argument in favour of the view that the neurosecretory material stained by conventional staining techniques in both vertebrates and invertebrates, consists of the carrier substance, not the hormones themselves, (Arvy and Gabe, 1962). Further, it seems that the neurosecretory cells produce a variety of substances which carry more than one type of the hormones.