CHAPTER 8

SOME HISTOCHEMICAL OBSERVATIONS ON THE NEUROSECRETORY SYSTEM

The occurrence of the neurosecretory cells i.e. neurons with pronounced glandular activity have been extensively studied in recent years in the central nervous system of several decapod crustaceans (Enami, 1951b; Bliss and Welsh, 1952; Carlisle, 1953b; Bliss et al., 1954; Matsumoto, 1954; Parameswaran, 1955,1956; Nirmal, 1964; Deshmukh and Rangnekar, 1965). Neurosecretory cell groups are found in various parts of the central nervous system including those in the eye-stalk (Hanstrom, 1949). The neurosecretory focal point in the eye-stalk is the sinus gland which consists of swollen endings of many neurosecretory cells, whose cell bodies lie in other parts of the eye-stalk ganglion, the brain and thoracic ganglion (Bliss and Welsh, 1952). The material produced in the neurosecretory cell bodies is transported along axon fibres and these fibres end in the sinus gland in the form of club shaped terminations (Knowles and Carlisle, 1956).

On the basis of certain morphological aspects these neurosecretory cell structures are distinguished into several types and the number of these may vary in different species. In Sesarma, Enami (1951b) had shown the presence of three types of neurosecretory cells in the central nervous system.

Matsumoto (1954a) described three types of secretory cells in the thoracic ganglion of the freshwater crab, Eriocheir. Parameswaran (1956) showed three types of neurosecretory cells in the central nervous system of the freshwater crab Paratelphusa hydrodromous, whereas in Paratelphusa jacquemontii, Nirmal (1964) reported five distinct types of neurosecretory cells. From the histological studies made on the central nervous system of the marine crab, Scylla serrata (Forskal), Deshmukh and Rangnekar (1965) observed five types of neurosecretory cells. Their observation was in agreement with that of Nirmal (1964) in Paratelphusa jacquemontii and on the basis of their secretory activity, structure and staining properties these five types of cells were named as A,B,C,D and E.

Kurup (1963) has reviewed the crustacean hormones in general, which controls the various physiological activities such as moulting, chromatic system, metabolism and reproduction. The eye-stalk endocrine system affects the various aspects of the crustacean metabolism and reproduction. Abramowitz et al. (1944) reported a marked hyperglycemia produced by injecting eye-stalk extracts. However, in Paratelphusa jacquemontii, the presence of a hypoglycemic factor in the eye-stalk has been found out (Rangnekar et al.,1961). A metabolic principle which regulates the rate of O_2 consumption, calcium, phosphorous and water metabolism was also recorded in crustaceans

(Scharrer and Scharrer, 1954). A factor present in the eye-stalk was also shown to influence the gonads, so as to cause alternating periods of reproductive activity and quiesence. Removal of the eye-stalks causes acceleration in the development of the ovaries (Carlisle, 1954) and of testis (Demeusy, 1953). Recently Adiyodi and Adiyodi (1968) reported an ovary inhibiting effect of extract of crab eye-stalk on female American cockroaches.

The present study deals with histological observations on certain parts of the central nervous system of P. jacquemontii with reference to neurosecretory cells in an attempt to correlate certain metabolic and neuroendocrine activities of the animal. Numerous follicles with dense colloid inside, comparable to the thyroid follicles of higher animals were seen in the peripheral connective tissue of the central nervous system (Chapter 1). The storage and release mechanisms of the neurosecretory material in the neurosecretory cells were studied in relation to the formation of these follicles and is correlated with the state of gonadal development.

MATERIAL AND METHODS

The common freshwater crabs Paratelphusa jacquemontii, were collected from the local rivers and were used in the present study. The brain and thoracic ganglion were dissected

out and fixed in Bouin's fluid and 10% neutral formalin. The eye-stalks were carefully removed from the living animals, the outer chitinous exoskeleton was slowly dissected out and the soft parts were fixed in the above fixatives. 5 to 7 μ thick paraffin sections were cut and stained by (1) Gomori's Chrome-alum Haematoxylin Phloxine (CHP) as described by Pearse (1960) and (2) Gomori's (1950) Paraldehyde Fuchsin staining technique as modified by Halmi (1952).

OBSERVATIONS

The histological features of the five different types of neurosecretory cells in the central nervous system of Paratelphusa jacquemontii, were the same as that described by Nirmal (1964).

A - cells: were the largest of all the cell types and measured from 50 to 160 μ in diameter. The nucleus was round with a prominent nucleolus and was 15 to 30 μ in diameter. The cytoplasm showed deep blue granules with Chrome alum-haematoxylin phloxin and deep purple with Paraldehyde fuchsin stain (Fig. 1). Small characteristic vacuoles were seen in the cytoplasm of certain cells which increased during the secretory phase.

B - cells: were smaller than A types, measuring 30 to 60 μ in diameter, with homogenous cytoplasm and densely packed

granules in it. The nucleus was mostly spherical. While discharging the neurosecretory material it showed peripheral vacuoles and in the resting stage the vacuoles were not seen (Fig. 2).

C - cells: varied from 20 to 50 μ in diameter and showed characteristic clumpy, irregular cytoplasm. The nucleus was round with conspicuous nucleolus (Fig. 3).

D - cells: ranged from 8 to 25 μ in diameter with large round nucleus and distinct nucleolus (Fig. 4).

E - cells: were the smallest of all the cell types and were 8 to 15 μ in diameter. The cytoplasm appeared as a thin layer around the nucleus (Fig. 5).

All the five types of cells were seen in the thoracic ganglion whereas the C type cells were not found in the brain and eye-stalk ganglion. The detailed description of these cells and their distribution were presented by Nirmal (1964).

The cytoplasm of all these secretory cell types in the various parts of the central nervous system were filled with neurosecretory granules in July/August, i.e. before the gonads start functioning. During the climax of the storage phase the nucleus was considerably large with conspicuous

nucleolus. The bulbous endings of the sinus gland contained only very little amount of the secretory material (Fig. 6). Most of the cells showed thickly packed secretory granules in the cytoplasm, and only few A-type cells had certain small characteristic vacuoles in the cytoplasm. The initial releasing vacuoles were found in September/October (Fig. 7) and the vacuoles gradually increased in number and size towards the exhaust phase. During this initial releasing period a remarkable increase in the thyroid-like follicles (Chapter 1) was noted in the peripheral connective tissue of these nervous tissues as well as in the intertubular connective tissue of the hepatopancreas and testis. Groups of numerous small follicles were abundant in the peripheral connective tissue of the brain, thoracic ganglion and eye-stalk ganglion.

Regarding the state of the gonad, the testis was found to start functioning in August and the ovary in late August or early September. The formation of the initial releasing vacuoles in the neurosecretory cells were found to follow these changes in the gonads. These vacuoles were first seen in the male neurosecretory cells and then in the females, hence the development of the testis was found to take place first. Large vacuoles at the periphery and in the cytoplasm of the cells were noted towards the later stage of spermatogenesis in December/January (Figs. 8 to 10). In the same

way relative abundance of the secretory material and the presence of large number of peripheral vacuoles were observed in the gravid and ovigerous females. These large peripheral vacuoles indicated the height of the secretory activity and the cytoplasmic granules decreased in number. More and more vacuoles appeared gradually in the cytoplasm of these cells and they appeared to be shrunken after the secretory phase. Towards the completion of the gamete formation in both the sexes the peripheral vacuoles increased in number and size and the cell entered the exhaust phase. Almost all the neurosecretory material was discharged from the cells during this exhaust phase in February/March. Only very few granules were scattered here and there and the cytoplasm in general appeared to be greenish in colour when stained with Paraldehyde Fuchsin stain (Fig. 11). In addition to these changes in the cytoplasm the nucleus also exhibited remarkable changes. The nucleus of the large A-type cells reduced in size to 12-30 μ in diameter and most of them assumed an elongated shape(Fig.12).

During this period the bulbous axon terminations in the sinus gland were found to contain considerably large amount of the neurosecretory material (Fig. 13). The long axon fibres leading from the various neurosecretory cells might have carried the secretory material to the bulbous axon endings of the sinus gland, from where it might be released into the neighbouring

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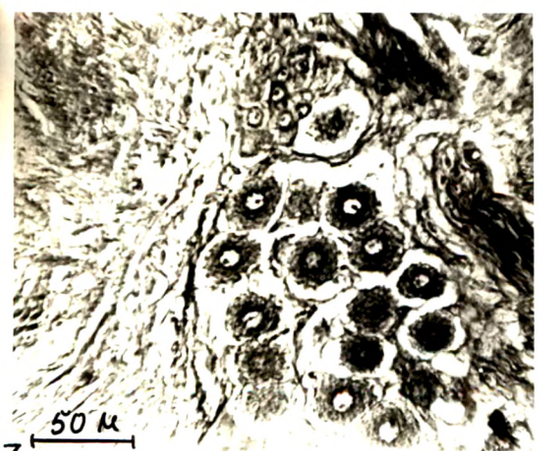

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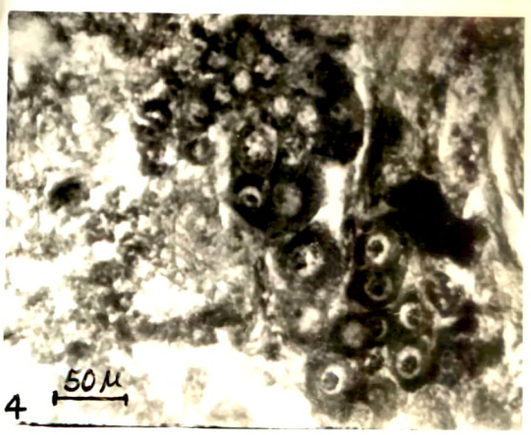

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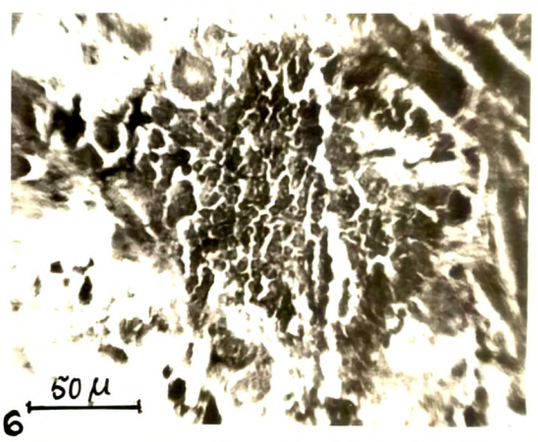

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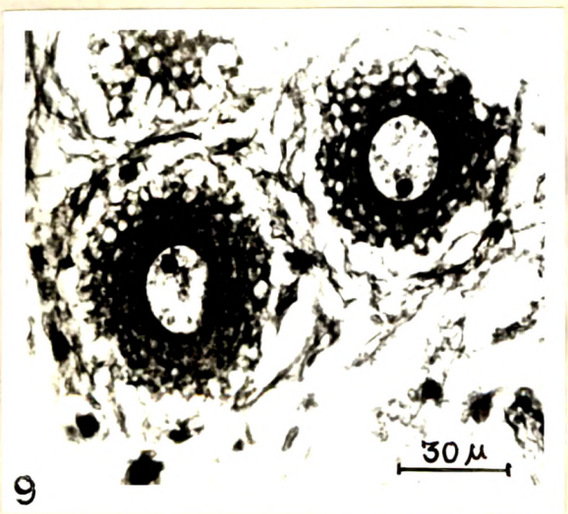
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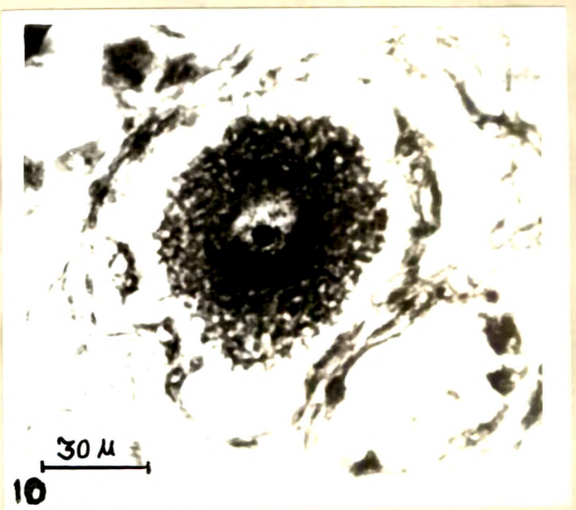
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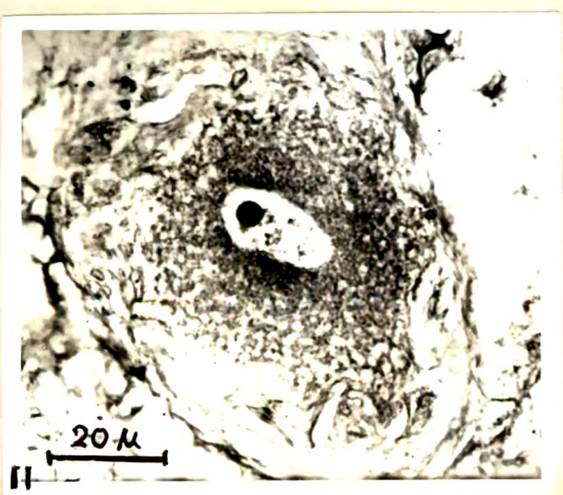
20 μ
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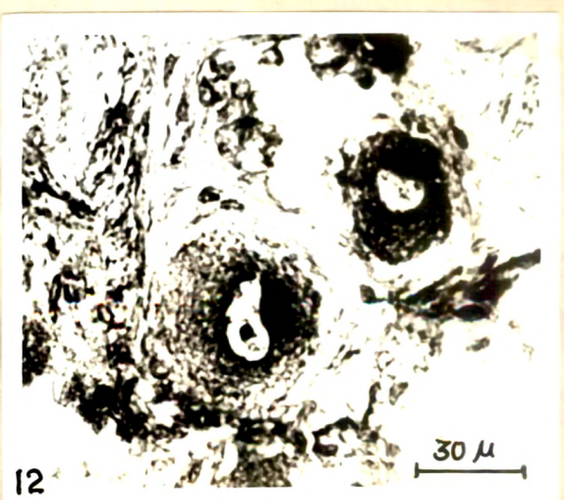
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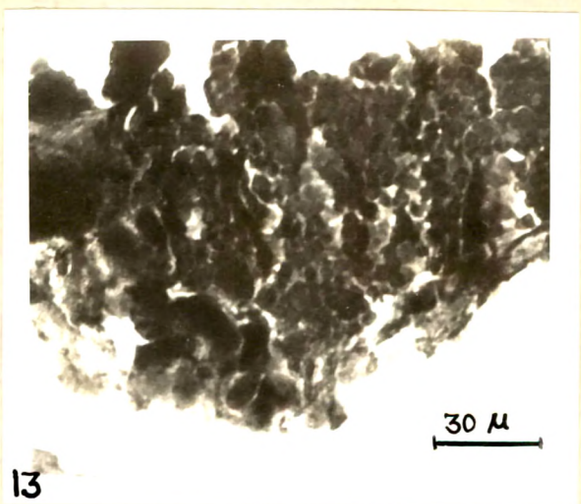

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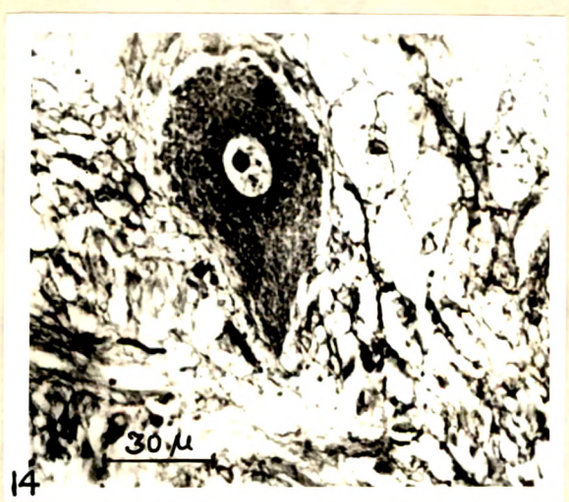

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EXPLANATION TO THE FIGURES

Fig. 1. A cell from the thoracic ganglion showing long axon and the cytoplasm filled with neurosecretory material.

Fig. 2. B cells from the thoracic ganglion with long axon.

Fig. 3. Group of C cells from the thoracic ganglion showing clumpy irregular cytoplasm and round nucleus.

Fig. 4. Group of D cells from the brain with large nucleus and conspicuous nucleolus.

Fig. 5. Group of E cells from the thoracic ganglion. Few B and D cells are also seen.

Fig. 6. Section of sinus gland region showing less neurosecretory material inside.

Fig. 7. A cells with initial releasing vacuoles at the periphery of the cytoplasm, during the early stage of gonadal development.

Figs.8,9 & 10. Stages in the release of the neurosecretory material, showing an increase in the number of vacuoles at the periphery of the cytoplasm as well as large peripheral cavity.

Fig. 11. A cell in the exhaust phase. Note the less neurosecretory material in the cytoplasm and the elongated shape of the nucleus.

Fig. 12. A cell with elongated nucleus during the release of the neurosecretory material.

Fig. 13. Section of the sinus gland region with an increase in the storage of the neurosecretory material.

Fig. 14. A cell towards summer. Note the resynthesis of the neurosecretory material in the cell cytoplasm and the large nucleus with prominent nucleolus.

blood sinus. Both acidophilic and basophilic material were seen in the sinus gland whereas the axon fibres carried only acidophilic material.

However, again the initial regaining or the resynthesis of the neurosecretory material in the various neurosecretory cells was observed prior to aestivation in April/May (Fig.14). The brain, and thoracic ganglion when examined soon after the aestivation period were seen to be filled with the neurosecretory material and the cells also showed enlarged nucleus with conspicuous nucleolφus. When the resynthesis of the neurosecretory material started in the neurosecretory cells prior to aestivation, an increase in the thyroid-like follicles also was noted in the gonads and hepatopancreas but not in the nervous tissues.

DISCUSSION

The histological observations reported in the present investigation were similar to those of the other workers, made on the central nervous system of several Indian freshwater and marine crabs (Parameswaran, 1956; Nirmal, 1964; Deshmukh and Rangnekar, 1965). It is well established that the axon fibres leading from the MGTX terminate in bulbous endings in the sinus gland (Bliss, 1951; Bliss and Welsh, 1952; Enami, 1951b; Passano, 1951a). The neurosecretory material is

transported through these axon fibres to the sinus gland where it is stored. In Sesarma, Enami (1951b) had shown the movement of the neurosecretory products along the axon fibres of the sinus gland. Deshmukh and Rangnekar (1965), reported that in Scylla serrata, the neurosecretory material in the axon fibres leading to the sinus gland is acidophilic in nature and they observed both acidophilic and basophilic inclusions in the nerve endings of the sinus gland.

In the present investigation also, both these types of secretory materials were seen in the bulbous axon fibre terminations of the sinus gland in P. jacquemontii, The relative proportion of these two materials depends upon the physiological state of the animal (Deshmukh and Rangnekar, 1965).

The fact that the reproductive activity of crustaceans depends upon the seasonal and other extrinsic factors as well as hormonal action is well known. The removal of the eye-stalk causes an acceleration in the development of the ovaries and testis (Carlisle, 1954; Demeusy and Veillet, 1952;Demeusy,1953). Eye-stalk and brain extract inhibit the ovarian growth and development (Carlisle, 1954; Matsumoto, 1951). Again the relationship between the gonad and the central nervous system is also evident from the fact that in parasitized crabs with

degenerated gonads the sinus glands are enlarged (Iwakura, 1951).

Regarding the other classes of the arthropod group Englemann (1957) had shown that the cyclic endocrine activity which causes the insect ovaries to remain quiescent during "pregnancy" depends upon the inhibiting nerves connecting the corpora allata with the brain. Further, the presence of an ootheca in the uterus, which prevents the onset of a new reproductive cycle is signaled to the corpora allata by a hormonal stimulus which maintain the action of the inhibitory nerves (Engelmann, 1957). Comparable neuroendocrine mechanisms exist in vertebrates also. In the control of the gonadotropic function in duck, the state of gonad depends on the hypothalamic control of the adenohypophysis. There is additional evidence in other vertebrates that the feed back from the gonad reaches the anterior lobe by way of the hypothalamus (Donovan and Van der Werfften Bosch, 1956a; Donovan and Harris, 1956b).

In the present investigation also a relationship was noticed between the gonadal development and the level of storage and release of the neurosecretory material in the neurosecretory cells. The release of the neurosecretory material which was evident from the formation of more and more neurosecretory releasing vacuoles, was found to start

with the onset of gametogenesis in both the sexes. An increase in the number and size of these vacuoles denoting the height of the neurosecretory release was observed gradually and the neurosecretory cells entered an exhaust phase towards the maturation of the gonads. Parameswaran (1955) in P. hydrodromous also reported a relative abundance of the neurosecretory material in the gravid and ovigerous females and the presence of a large number of peripheral vacuoles.

George and Naik (1965) showed that in the migratory starling, Sturnus roseus the release of the neurosecretory material from the median eminence and neurohypophysis was quickly followed by the release of the thyroidal colloid, just prior to migration. Similarly, in the present investigation an increase in the thyroid-like follicles in the connective tissue of the various tissues studied, was observed towards the height of the neurosecretory storage and initial releasing periods as well as at the time of aestivation. In the second case the increase was seen only in the gonads and in the hepatopancreas but not in the nervous tissues. Moreover, during that period the neurosecretory material already released might have through the circulating blood, initiated the increased production of these follicles with colloid in the testis and hepatopancreas.

Thus if we consider the basic mechanisms of hormone action, invertebrates and vertebrates show a remarkable parallelism. The structural and functional resemblance between the neurosecretory centres in invertebrates and vertebrates manifest itself particularly well, in the existence, in both the groups, of neurosecretory organ systems. The similarity between the hypothalamic-hypophyseal system of vertebrates and the corresponding systems in crustaceans and insects is striking. Their essential features are groups of secretory nerve cells that send their glandular products via their axons to the nerve terminals located in separate organs viz: neurohypophysis in vertebrates, sinus gland in crustaceans, and the corpus cardiacum in insects. However, convincing evidence exists for the axoplasmic transport of the neurosecretory materials in the vertebrate hypothalamic-posterior pituitary system and in the analogous system of insects and crustaceans. Hild (1951) showed that cutting the pituitary stalk in frog resulted in an accumulation of neurosecretory substance in the fibres proximal to the cut. Similarly, when the axons of neurosecretory cells of the insect Leucophaea were cut, there was a similar piling up of neurosecretory substance proximal to the cut (Scharrer,1952). Similar observations have been made on a variety of crustaceans when their neurosecretory axons have been interrupted

or when the sinus gland was removed (Bliss and Welsh, 1952; Passano, 1953). Thus the neurosecretory system of invertebrates resemble in almost every detail, the corresponding hypothalamic-hypophyseal neurosecretory system of vertebrates.