CHAPTER V:

THE TRANSPORT AND THE STORAGE OF THE NEUROSECRETORY MATERIAL.
INTRODUCTION:

The neurosecretory pathways were observed with the ordinary histological methods long before the neurosecretory cells were recognised in insects (Baldus, 1924). The hypothesis that the neurosecretory material is transported from the cells to the neurohaemal organs, along the axons was first put forward by Scharrer and Scharrer (1944). Later on it has been supported by several investigators. Substantial evidences in this direction have been provided by Scharrer (1952 a,b) and Thomsen (1954, a,b), who applied obstruction and ligature experiments respectively, to the nerves carrying this material to the neurohaemal organs.

The nerves that come out of the brain have been variously named. Cazal (1948) named these two nerves as nervi paracardiacus externus and nervi paracardiacus internus. Ewen (1962) followed the same nomenclature. Johansson (1958) and many other later workers (Dogra, 1967 a,b; 1969; Gupta, 1970; Awasthi, 1972, 1973) have used the term nervi corporis cardiaci I and II for these structures.
Many workers (Cazal, 1948; Junqua, 1956; Johansson, 1958 and Ewen, 1962) have reported that the cardiac nerves (NPC I and II or NCC I and II) after their independent emergence fuse to form a common cardiac or paracardiac nerve. But Nayar (1956), Dogra (1967 a,b; 1969), Gupta (1970), Awasthi (1972, 1973) and Faruqui (1974 a,b,c; 1975 a and b) have reported that both of these nerves - NCC I and NCC II and in some cases, where it occurs, a third nerve NCC III (Dogra, 1967, 1969) keep their independent identity throughout their course.

The question as to which organ serves as the neurohaemal organ in Heteropteran bugs is again the most controversial point of the insect endocrinology. In most of the insects so far studied, the corpus cardiacum serves as the neuro-haemal organ for the cerebral neurosecretory material i.e., the median and lateral cell secretions. In hemipterans, however, the position is some what different. In cryptoceratatan species the neurosecretory material is reported to be stored in the 'lame fibrogliale' (Cazal, 1948) or 'cardioglia' tissue (Janqua, 1956). Benwitz (1956) has reported in Corixa punctata that most of the axons of nervous paracardiacus enter the aorta after running on the mid dorsal line of the corpora cardiaaca, where as only a few enters this gland. Some authors have reported the presence of the median cell neurosecretory material in the corpus cardiacum of the bugs (Nayar, 1956, in Iphita limbata; Ewen, 1962 in Adelphocoris lineolatus; Wigglesworth, 1964 in Rhodnius prolixus; Gupta, 1970 in Dysdercus similis; Pathak, 1974 in Dysdercus similis and Sphaerodema rusticum). Johansson (1958) in Oncopeltus fasciatus
has reported the absence of any amount of NSM from the corpora cardiaca while others (Seshan and Ittycheriah, 1966; Dogra, 1967 a, b, 1969; Srivastava, 1970; Awasthi, 1972, 1973 and Faruqui, 1974 a, b, c, 1975 a, b) have not only reported the absence of this material in the corpus cardiacum but have also demonstrated its presence in the aorta wall in large quantities and have suggested the aorta to be the neurohaemal organ for the median cell material. The corpus cardiacum however, serves as a neurohaemal organs for the lateral neurosecretory cell material in all the insects so far studied.

With the view to uncover the factors for such variations and to know as to which organ actually serves as the neurohaemal organ in this group of insects, a large number of bugs have been taken for this study. Some of these insects have been studies earlier on these lines but the findings are controversial. The closely related species of such insects have also been included in the present project. These insects have been studied with the help of both techniques used by earlier workers as well as the recent workers. Many Reduvid bugs have also been taken because, perhaps, except Rhodnius, no other reduvid insect has been studied on these lines. An attempt has also been made to study the neurohaemal organs of Rhodnius prolixus, the blood sucking bug, to see whether it is like all other heteropterans or differs from them. Keeping all these points into view, the twenty heteropteran species have been studied in the present project with the help of both histological preparations and the bulk staining methods.
OBSERVATIONS:

The neurosecretory cell groups i.e., median and lateral, have their independent transport and storage system in heteropteran insects. For the cells of tritocerebrum it is not very clear. The same is with the cells of the ventral nerve cord except for the A1 cells of the sub-oesophageal ganglion. In the following lines these systems have been described.

(a) The Transport and Storage System for the NSC of the Median Group:

The axons, that are given out by each neurosecretory cell converge to form the median neurosecretory pathways. The axons of two groups of neurosecretory cells form separate pathways. These pathways immediately after their formation travel a little towards the dorsal side, bend ventrally and then travel downwards. At the point of their turning towards the ventral side the axons of the two sides cross over each other so that the axons of the left side come to the right and those of right side come to travel on the left side (Figs. 66, 69). Thereafter they are quite apart from each other and travel all the distance of protocerebrum and deutocerebrum. This position is found in all the insects except Cyclopelta sicciifolia. In this insect, the axons after crossing over to each other travel towards the lateral side and run up to three fourth part of the total width of the brain and then they turn back to come to travel more or less in the same way they had gone (Fig. 6). After reaching the point of crossing over, they travel backwards in the form of a common bundle of axons which
Fig. 66 : Whole mount of brain and associated neurosecretory structures of *Ranatra filiformis*.
(PAVB X 100). HCC I is seen entering the aorta.

Fig. 67 : Whole mount of brain of *Gerris nepalensis*,
showing the emergence of HCC I. (PAVB X 400).

Fig. 68 : Section of the brain of *Sphaerodema rusticum*,
showing the median neurosecretory pathways.
(PAVB, Stained cut. X 600).

Fig. 69 : Section of the brain of *Spilostethus pandurus*,
showing median neurosecretory cells and their pathways. (PF X 450).
Fig. 70 : Section of the brain of _Cyclopelta siccifolia_, showing the median neurosecretory pathways. (PF X 450).

Fig. 71 : Section of the brain of _Laccotrephes griseus_, showing the median neurosecretory pathways. (PF X 450)

Fig. 72 a : Longitudinal section of the brain of _Gerris nepalensis_, showing the median neurosecretory pathways. (PF X 450)

Fig. 72 b : Longitudinal section of the brain of _Codophila macculicollis_, showing the median neurosecretory pathways. (PF X 450)
PLATE XX
Fig. 73: Whole mount of the aorta of Sphaerodema rusticum, showing the arrangement of axons, loaded with NSM. (PAVB X 450).

Fig. 74: Whole mount of the aorta and CA of Dysdercus similis, showing the arrangement of axons, loaded with NSM and also the axonal network on the CA. (PAVB X 450)

Fig. 75: Longitudinal section of the aorta of Ranatra filiformis, showing the arrangement of axons loaded with NSM. (PF X 450).

Fig. 76: Longitudinal section of the aorta of Gerris nepalensis, showing the arrangement of axons loaded with NSM. (PF X 450)
however gets separated after reaching the point of their emergence (Fig. 70). The axons in this insect show a peculiar tendency of retaining the major part of NSM, within them (Fig. 6). The amount of the material is always noted to be greater in these axons than those in the aorta.

The median neurosecretory pathways after travelling the proto and deuto-cerebrum (Figs. 68, 69, 70) come out of the brain from the tritocerebral region of the brain as nervi corporis cardiaci I (NCC I) (Figs. 24, 66, 71, 72). The transportation of the neurosecretory colloid by the axons in the brain and the NCC I is very clearly visible. The entire course of the median neurosecretory pathways is distinct in the protocerebrum, deuto-cerebrum and the tritocerebrum.

Up to their emergence from the brain, the course of these pathways is similar in all the cases except for a few minor points of variations, as has been described earlier, but the distal fate of NCC I show some very remarkable differences. Here after these insects can be grouped under two heads - (i) the heteropterans other than Reduvids and (ii) the Reduvids.

In the first type of insects, the NCC I after emergence travel a short distance independently and then gets attached to the inner margin of the corpus cardiacum of their sides. At about the point of attachment of the CC with the CA, it (NCC I) gives off a very thin branch which goes to the CA. These axons subdivide and make a superficial network on it. A few of these secondary axons penetrate the gland and release the neurosecretory
material in its intercellular spaces. The major part of the NCC I axons penetrate the aorta wall from its lateral sides. The axons now divide into the secondary axons which fan out in all the directions and also backwards upto one fourth of the total length of the aorta. The aorta in this region has a rich supply of tracheae and tracheoles. It has thicker lateral wall which is made up of loose connective tissue. This loose connective tissue lodges the axons which carry the neurosecretory material elaborated by the cells of median group. The axons are seen loaded with a large amount of neurosecretory colloid (Plate XXI). Probably it is because of this fact, that the aorta appears silvery white in the living condition. The aorta seems to serve as the storage and release organ for the median cell material in all the heteropteran bugs except Reduvids. *Cyclopelta siccifolia* shows a little difference from the pattern described above. In this insect though the aorta shows the presence of very fine axons in it, the amount of neurosecretory material in these axons is very little as compared to those in the brain. It is possible that the axons which form an extra loop, and thus run greater distance than those of the other bugs retain the major part of neurosecretory material with them and release it directly in the leumen of the aorta. The axons of the aorta in this insect in no stage show the presence of equal or higher amounts of NSM then those in the brain.

In Reduvids the axons in the distal part of the NCC I enter the body of corpus cardiacum from the latero-anterior side, travel whole the length of the gland and ultimately reaches the
Fig. 77 : Section through the CC and the aorta of *Acanthaspis quinquespinosa*, showing the NSM stored in the "Cardioglial tissue". (PF X 450).

Fig. 78 : Section through the CC and the aorta of *Oncocephalus* sp., showing the NSM stored in the "Cardioglial tissue". (PF X 450).

Fig. 79 : Section through the CC and the aorta of *Ectomogoris quadriguttatus*, showing the NCC I axons reaching the cardioglial tissue after bypassing the CC. Stored NSM in this area is also seen. (PF X 450).

Fig. 80 : Section through the CC and the aorta of *Oncocephalus* sp., showing the NCC I axons reaching the cardioglial tissue after bypassing the CC. (PF X 450).
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Fig. 81 a : Whole mount of aorta of *Ectomocoris quadriguttatus* showing the NSM carrying axons. (PAVB 400).

Fig. 81 b : Section of CC, CA and aorta of *Rhodnius prolixus*. Note the absence of NSM in the CC. cardioglial tissue and aorta are seen with NSM. (PF X 400).

Fig. 82 a : Whole mount of retrocerebral endocrine glands and aorta of *Acanthaspis quinquescinosa*, showing the NSM in the cardioglial tissue. (PAVB X 200).

Fig. 82 b : Section of the CC and CA of *Rhodnius prolixus*, showing the NSM in the cardioglial tissue and also the innervation of axons in the CA. (PF X 450).
posterior part at which the glands of the two sides fuse with each other. At this point the aorta is also in very close contact with the gland. Here a large amount of the median cell neurosecretory material is seen to be stored (Figs. 78-81). Some axons are also seen to enter in the aorta (Figs. 82, 83). This area, the 'Cardiofugal tissue' (Janqua, 1956) or 'lame fibrogliale tissue' as termed by Cazal (1948), serves as the storage and release organ for the median cell neurosecretory material in all the reduvids studied. A few axons of the cerebral origin do not terminate in the aorta or the cardiogliale tissue but penetrates the NCA region to enter into the corpus allatum. These axons release their neurosecretory material in the intercellular spaces of this gland. But this cerebral neurosecretory material is not noticed in the corpus allatum of all the individuals studied. A few axons of the same origin fan out on this gland and form a network.

The arrangement of axons in the aorta wall is different in Cryptocerata and Gymnocerata. In the first group of insects i.e. the cryptoceratans, the axons run downwards, more or less parallel to each other but in gymnoceratans these primary and secondary axons form a compact network.

(b) Transport and Storage System for the Lateral Cell NSM:

The lateral neurosecretory cells, like the median cells, give off an axon each(Figs. 34, 37, 42). These axons converge to form the lateral neurosecretory pathways (LNP). These axons travel downwards and after running all the distance of
protocerebrum and deutocerebrum come out of the brain from the tritocerebrum at a point just outer to that of the emergence of the median cell axons (Fig. 48).

The entire course of the lateral cell axons could not be followed, because they are not very clear and not clearly differentiated from the neighbouring tissue. They are clearly seen at the point of their formation and in the tritocerebral region, at the point of their emergence.

These lateral cell axons come out of the brain from tritocerebrum as nervi corporis cardiacl II (NCC II), one on each side. In all the cases i.e. the bug studied the NCC II is seen to enter the corpus cardiaclum of its side from the apical portion. The majority of these axons terminate in this gland. Varying amounts of lateral cell neurosecretory material is seen in the median region of this gland (Figs. 85, 89). The NCC II axons are seen terminating in this gland and a large amount of the lateral cell NSM is also seen the leuman of the gland.

Two or three secondary axons of the NCC II origin are seen to reach the corpus allatum via the nervi corporis allatai, but these axons could not be traced in the gland.

(C) Transport and Storage System for Tritocerebral Cell NSM:

The scattered cells of the tritocerebrum gives off an axon each, that are seen coming downwards. Their course could not be followed up after a very short distance. These axons also do not show the formation of any separate nerve after coming out
of the brain. It is very much possible that these axons join with the lateral cells axons and come out of the brain with NCC II. Such a thing is very clearly seen in case of *Rhodnius prolixus* (Fig. 48).

(d) **Transport and Storage of the Neurosecretory Material Elaborated by the Ganglia of the Ventral Nerve Cord**:

The pathways and storage organ for the neurosecretory material elaborated by the cells of the ventral nerve ganglia could not be ascertained except for the $A_1$ type cells found in the sub-oesophageal ganglion in a few insects.

The two $A_1$ cells which occur on the ventral surface of the sub-oesophageal ganglion are seen to give off two axons which first travel towards the dorsal side and then towards the anterior side (Figs. 49, 50) till they reach the tritocerebrum of the brain. After this, these axons could not be distinguished because of the presence of a large number of axons of the median neurosecretory cells (Figs. 73-76).

The axons of the other type of cells are directed inwards towards the neuropilar region (Figs. 53, 59). After this, their course is not traceable.

**DISCUSSION**:

The transportation of the neurosecretory material along the axons and the intracerebral routes of these axons in the heteropteran bugs studied are similar to all other insects. The median and the lateral group cell axons converge to form the
median and the lateral neurosecretory pathways like all other insects (see review by Van der Kloot, 1960; Gabe, 1966; Tombes 1970 and Nayar, 1973). The transportation of neurosecretory material along the axons have been demonstrated experimentally by Scharrer (1952 a,b) and Thomsen (1954 a,b).

The intracerebral course of these axons except those of *Cyclopelta siccifolia*, is similar to all the other heteropterans, as described by Cazal (1948), Nayar (1956), Ewen (1962), Dogra (1967 a,b; 1969), Gupta (1970), Awasthi (1972 a,b; 1973) and Pathak (1974). In the bug *Cyclopelta siccifolia* the axons of the two sides after crossing over to each other form a loop towards the lateral side and then travel in the usual manner. Such axonal routes have been described by Siew (1965) and others.

The median and the lateral neurosecretory pathways in these heteropteran bugs come out of the brain from the tritocerebrum. In other groups of insects these axons come out of the brain from the protocerebrum (Scharrer, 1952; Highnam, 1961; Siew, 1965; Saini, 1971). The emergence of NCC I and II from the tritocerebrum have been reported in some other heteropterans (Ewen, 1962; Dogra, 1967 a,b, 1969; Srivastava, 1970; Pathak, 1974). Both these nerves i.e. NCC I and II emerge from the brain as two independent nerves and keep independent identity throughout their course. These findings are similar to those of Gupta(1970) and Pathak (1974). But Cazal (1948) in five Cryptoceratan species viz. *Naucoris cimicoides, Ranatra linearis, Nepa cinerea* and *Notonecta glauca*, has reported that paracardiacus internus and
externus after their emergence fuse to from a common paracardiac nerve. Junqua (1956), Benwitz (1956), Johansson (1958), Ewen (1962) have also reported the same findings i.e. the occurrence of a common paracardiac or cardiac nerve.

As regards the occurrence of a third nerve i.e. the NCC III, which has been noticed in many bugs viz. Oncopeltus fasciatus (Johansson, 1958), Ranatra linearis and Belostoma indica (Dogra, 1967 a, 1969) in the insects studied here, no such nerve has been noticed, not even in the very closely related species to the insects mentioned above, like Spilostethus pandurus, Ranatra filiformis and Sphaerodema rusticum.

In these bugs, as in other insects there are evidences of the transport of NSM from the cells in the pars-intercerebralis to the neurohaemal organ. But the available literature posed a problem to determine as to which organ actually serves as the neurohaemal organ in the heteropteran bugs. In Iphita limbata, Nayar (1956 a,b,c), has traced the NSM from pars-intercerebralis to both, the CC and the CA. According to Benwitz (1956), a considerable part of nervi corporis cardiaci passes to the wall of the aorta in Corixia punctata. She, however, does not give any information about the NSM. In Hydrocyirus columbianus, Junqua (1956), has reported the formation of tissue named as 'tissu-cardio-glial' as a result of the union of aorta and the corpora cardiaca. Cazal (1948) has also reported the formation of a similar structure in five heteropteran species viz. Naucoris cimicoides, Ranatra linearis, Nepa cineria and Notonecta glauca.
which was termed as 'lame fibrogliale'. Johansson (1958) has noticed the presence of a large amount of neurosecretory material in the aorta wall but has reported the absence of NSM from the CC and CA. Ewen (1962), Gupta (1970) and Pathak (1974) have reported the presence of cerebral neurosecretory material in the corpora cardiaca of Adelphocoris lineolatus, Dvysercus similis and Sphaeroedema rusticum respectively. But some other workers like Seshan and Ittycheriah (1966), Dogra (1967 a,b; 1969), Srivastava and Dogra (1969), Srivastava (1970) and Awasthi (1972 a,b; 1973) have reported the presence of neurosecretory material elaborated by the MNC in the aorta wall, which they considered to be the main neurohaemal organ for this material in heteropteran bugs.

In the insects studied in this project, the corpora cardiaca serves as the neurohaemal organ for the lateral cell neurosecretory material, as reported by all the previous workers. But the MNC material is seen to be present in the aorta. The NCC I is seen to penetrate the aorta directly, independent of the corpora cardiaca in Leptocorisa acuta, Petalocrinis sp., Dvysercus similis, Antilochus coqueberti, Bagrada hiliaris, Dorpius indicus, Codophila maculicollis, Cyclopelta siccifolia, Erthesina fullo, Gerris nepalesis, Laccotrephes griseus, Ranatra filiformis, Sphaeroedema rusticum and Enithares indica. In these insects the aorta wall serve as the storage and release organ for the median cell neurosecretory material.

In Reduvids, the NCC I axons are seen to penetrate the CC of their side and travel superficially to reach the area termed
as 'Cardioglia tissue' (Junqua, 1956). A fairly large amount of NSM is seen in this area, but some axons travel onwards to reach the aorta wall where they are seen with the NSM. Thus in Reduvids, parts of both the CC and the aorta serves as storage and release organ. As such the neuro-endocrine system of Reduvids represent a transitional stage in between the Orthopteran endocrine system, where the CC serves as the principle neurohaemal organ and the Heteropteran endocrine system, where the aorta serves as the neurohaemal organ. These findings are similar to some extent, to those of Cazal (1948) for cryptoceratan species. But in some very closely related species to those studied by Cazal (1948), no such structure (lame fibro-gliale) is seen.

The axonal routes of the neurosecretory cells of the ganglia is not very clearly known. The axons of the A1 type cells of Pelatocnemis sp., Bagrada hiliaris, Dorpius indicus, Erthesina fullo, Codophila meculicollis, are seen to reach the aorta wall which seems to serve as the storage and release organ for these cells material. These findings are similar to those for Nezara viridula and Metochus uniguttatus (Awasthi, 1972 a, 1973). As far as the other NSC of the ganglia of the ventral nerve cord are concerned, it seems very likely that the NSM passes both backwards and forwards along the ventral nerve cord and along the nerves to the periphery as has been demonstrated by Delphin (1965) in Schistocerca gregaria.

It can be concluded that in heteropterans there are two independent neurohaemal organs for the cerebral neurosecretory
material - (i) the CC for the lateral cell material and (ii) the aorta for the median cell material. In Reduvids, however, both the parts of CC and the aorta serve to store the MNC material.