DISCUSSION

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5.4 METHODOLOGY AND TECHNIQUES

A study of tasar silkworm can be carried-out outdoors only. The outdoor rearing exposes tropical tasar silkworms to unfavorable weather conditions and attack of pests and predators. Therefore, selecting of sites, brushing, supervision and maintenance of larvae population play an important role for obtaining reliable information. Several methods for the rearing of tasar silkworm have been suggested. The Central Tasar Research Centre and Training Institute, Ranchi has developed method for its outdoor rearing. Due to very heavy population loss, particularly during 1st instar in outdoor rearing, several workers who made efforts incorporate brushing of twigs and a cyclindrical polythene enclosure with a split bamboo frame. The cut ends of these twigs and the larvae were transferred outdoor after first instar. In another method these sets were placed inside a hot shatuc. Patches of leaf branches (CTR & TI bulletin, booklets and discussion with tasar-culture practitioners and Tasar Suk Centre, Agra, etc.) have suggested complete indoor rearing till the third stage and thereafter the larvae are transferred outdoor. Somanundaram et al. (1986) have suggested rearing of tasar silkworms on maintained chewki plots and later reared on natural leaves of general trees of Terminalia arjuna. The farmers have the tradition of rearing silkworms. Under the traditional rearing system in the first instar alone is found generally 30%. During remaining larval instars the losses from disease and pests are 45-50% with some natural calamities 10%. To protect against these losses by rearing techniques have been advocated. Controlled rearing...
5.1. BIOECOLOGY OF ANOTHERAEA MYLITTA D.

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5.1.1. METHODOLOGY AND TECHNIQUES

A study on tasar silkworm can be carried out outdoor only. The outdoor rearing exposes tropical tasar silkworms to unfavorable weather conditions and attack of pests and predators. Therefore selection of sites, brushing, supervision and maintenance of larval population play an important role for obtaining reliable information. Several methods for the rearing of tasar silkworm have been suggested. The Central Tasar Research Centre and Training Institute, Ranchi has developed method for its outdoor rearing. Due to very heavy population loss, particularly during 1st instar in outdoor rearing, a further improvement was made, which incorporates the indoor rearing of first instar larvae. It consists of water filled bottle holding 3 to 5 twigs with a quality foliage and a cylindrical polythene enclosure with a split bamboo frame. The cut ends of the twigs were inserted well under water. Brushing was conducted on these twigs and the larvae were transferred outdoor after first moult. In another method these sets were placed inside a hut thatched with straw or leaf branches (CTR & TI bulletins, booklets and discussions with tasar culture practitioners and Tasar Silk Centre, Armori experts). BIJAGARI (1988) has suggested complete indoor rearing upto third stage and thereafter the larvae are transferred outdoor. Recently, Somasundaram *et al.*, (1996) have suggested rearing of first three stages on maintained *chawki* plots and later reared on matured leaves of general trees of *Terminalia arjuna*. The farmers have their own way of rearing silkworms. Under the traditional rearing method the loss in the first instar alone is found generally 30%. During the remaining larval instars the losses from disease and pests are 45-50% and from natural calamities 10%. To protect against these losses, various rearing techniques have been advocated. Controlled rearing...
reduces not only the first instar losses to as low as 5% but also mortality during the subsequent stages. The effective yield thereby increases to 50 to 60 cocoon per disease free laying against 15 to 20 with the traditional method.

The presently employed rearing method, combined with the traditional methods of farmers, was found suitable to get a good harvest of cocoon. Accordingly, the host plants on which the brushing was conducted were covered with bamboo mats one day before the date of hatching during first two seasons. This covering was raised with an idea to protect the worms from sun and rain. As there was no rain during brushing in third season the bushes were not covered. Likewise 500 counted eggs were brushed by keeping the leaves over larvae and then tying on plants whereas the remaining eggs kept in leaf cup were tied on plants to allow the worms to crawl over leaves on their own. With leaves tied on plants, some larvae were found perished in the curls of leaf, whereas, in the second method all the larvae crawl over leaves automatically. This mixing of technology coupled with strict vigilance over rearing have resulted in good cocoon harvest in all the three seasons.

Tasar silkworms are very sensitive to various abiotic factors specially during their development. Rainfall washes the worms away. During first two seasons when the rainfall was 278.2 mm to 318.04 mm and 112.71 mm to 142.82 mm respectively, the bushes with worms in early stages were covered with bamboo mats to protect mainly from rains, and this modification was found suitable.

5.1.2. SEASONAL VARIATIONS IN THE POPULATION STRUCTURE

Seasonal variations in the population of A. mylitta were studied on the naturally occurring T. tomentosa which is perennial plant. Terminalia tomentosa plants are full of leaves during all the rearing season which form the diet of tasar silkworm.

In the present investigation observation on population
fluctuation of *A. mylitta* was started from the first crop during July-August to third crop from November to January.

5.1.2a. **Density-Independent Factors**

It was observed that the rearing of *A. mylitta* could be undertaken in distinct seasons, first in July-August, second in September-October and third from November to January. Temperature and humidity seem to intimately influence the fecundity, embryonic duration and hatching of larvae. These parameters were longest, i.e., 118, 7 days and 39.6% respectively during the first season, when the temperature and relative humidity was 22.12 degrees Celsius min to 29.82 degrees Celsius maximum and 73.56% minimum to 90.51% maximum respectively. During third season the minimum temperature and relative humidity range was 12.16 degrees Celsius to 32.56 degrees Celsius maximum, the average fecundity was 186, 10 days and hatching 70.4%, the highest amongst three crops. Therefore, this range of temperature and humidity favours the moths to lay more eggs and more eggs to hatch.

It is worthy to mention that *A. mylitta*, being a semidomesticated species, passes the larval period outdoors. This exposure to diverse meteorological conditions - temperature, humidity, sunlight, rain, storm etc. affects the vigour of the larvae.

The larvae tolerate a very wide range of temperature and humidity because of outdoor rearing. During first season, when the temperature ranged between 22.12 degrees Celsius minimum to 29.91 degrees Celsius maximum, relative humidity 73.56% minimum to 90.51% maximum and rainfall 278.2 mm to 318.04 mm, the larval survival was 42.78% which was lowest amongst three season. There was not much difference in the second season (ERR 43.52%) when the temperature ranged between 22.24 degrees Celsius minimum to 32.2 degrees Celsius maximum, relative humidity 56.66% to 83.23% minimum and maximum respectively and rainfall 112.71 mm to 142.82 mm. The larval survival was highest i.e., 59.56% during third
season when temperature ranged 12.16 degrees Celsius minimum to 32.56 degrees Celsius maximum, relative humidity 33.24% to 78.16% and rainfall was nil in the months of November, December and 94.25 mm in January. It is now evident that the temperature and rainfall play an important role for the success of rearing. During third season when the temperature was 12.16 degrees Celsius minimum to 32.56 degrees Celsius maximum and rainfall nil in first two months and 94.25 mm in the last month (January) the larval survival was maximum.

During low precipitation the larvae continue to feed normally but in heavy rains and gusty winds the feeding is disturbed especially when the early instar larvae are easily washed or blown away.

Temperature and humidity also influence the larval span considerably. In the first season (July-August: Temperature 22.12 degrees Celsius to 29.91 degree Celsius and 73.56% to 90.51% RH) the larval span lasts 30-40 days, in the second season (September-October: 22.24 degrees Celsius to 34.2 degrees Celsius temperature range and 56.66% to 83.23% RH) and third season (November to January: 12.16 degrees Celsius to 32.56 degrees Celsius and 33.24% to 78.16%) it lasts 29-38 and 47-70 days respectively.

Early instar larvae avoid bright sunlight and rest on the underside of the leaves. Late age worms also avoid direct sunlight. During moulting and cocoon formation the larvae prefer shade.

5.1.2b. **Density - Dependent Factors**

The host plant *Terminalia tomentosa* W. & A. (Combretaceae) was available in abundance on both the sites. The larvae during rearing in all the three seasons were transferred to other plants even before the full consumption of leaves on the first plant. Even with the abundance of leaf the larvae tend to aggregate during early
stages and at the time of spinning. The larvae explore a group of leaves for the formation of cocoon when they join the leaves to form a small nest or hammock before the actual spinning of cocoon.

Various predators like bugs, praying mantis, wasp, ant, lizards and birds have been observed to be effective predators of *A. mylitta* during first two seasons. During winter the population of predators is significantly reduced. So the population of predators has effective control over the population of silkworm. Amongst all the predators *Canthecona* and birds were found to be the most obnoxious. At times more than one bugs (*Canthecona*) were found praying on the same larvae. It was due to high incidence of predators only the dfl/cocoon ratio during first season was lowest i.e. 1:20. It rose to 1:41 during second season with a moderate population of predators. However, during third season with almost no population of predators except birds and lizards the dfl/cocoon ratio further rose to 1:78. These predators to the possible extent were mechanically controlled by keeping strict vigil over the rearing. From these observations we may conclude that both density independent and density dependent factors are responsible for regulating the population of silkworms.

5.1.3. BIOECOLOGY AND COMMERCIAL ATTRIBUTES

A systematic study on the bioecology of *A. mylitta* under the climatic conditions of Vidarbha region of Maharashtra has not been made so far.

The Indian tropical tasar silkworm, *A. mylitta*, under present investigation reproduces sexually. The population of *A. mylitta* contains both males and females. Males always outnumber females. The ratio of males to females was found 58:42 in first and third seasons whereas 57:43 during second season.

Moth emergence from the seed cocoons preserved in specially built houses starts late in the evening. Moths are decoupled
after six hours of mating duration. Females are put individually in earthen pots for oviposition. These are allowed to lay eggs for three days. After that moths are microscopically examined for disease freeness and the eggs further processed.

The average number of eggs laid by a single moth during first season is 118 and the same is 151 and 186 for the second and third seasons respectively.

The incubation period for the first season is 7 days whereas 8 and 10 days respectively for the second and third season. The data regarding seasonal variations in respect of average fecundity, incubation period, hatching percentage, larval weight, larval period, single cocoon weight, single pupa weight, single shell weight, shell ratio percentage, DFL/cocoon ratio, effective rate of rearing and sex ratio are given in the Table V. Further the percentage improvement in respect of above characters in successive crops are also analysed and the data are presented in Table V.

It is evident from the tables that the number of eggs laid by an average individual increased by 27.96% in the second season as compared from the first season and by 23.17% in the third season over second crop. As such the temperature and relative humidity ranges of 12.16 degrees Celsius to 32.56 degrees Celsius and 33.24% to 78.16% respectively favour moths to lay maximum number of eggs. Further, decrease in temperature and relative humidity increases the incubation period from 7 days in the first season to 10 days in the third season.

Maximum hatching is recorded, i.e., 70.4% during third season at the temperature and relative humidity range of 12.16 degrees Celsius to 32.56 degrees Celsius and 33.24% to 78.16% respectively indicating that this stage of temperature and relative humidity is most suitable for hatching.
The average weight of a single larva is more or less same in all seasons. It was 30.76 g and 30.62 g for the first and third seasons respectively. However, during second season a slight increase in the larval weight was observed where it was 31.55 g.

Decrease in temperature lengthens the larval period from 30-40 days in the first season to 47-70 days during third season. During second season the larval duration was the shortest i.e. 29-38 days. Climatic fluctuations seem to intimately influence the various physiological activities of the body.

The weight of a single cocoon is lowest i.e. 8.15 g in third season as compared from 8.83 g and 8.87 g during second and first season respectively. Likewise, pupal weight is also lowest i.e. 7.11 g in third crop as compared from 7.52 g and 8.03 gm in second and first season respectively. However, contrary to the lowest cocoon and pupal weight of third season the weight of a single shell is highest i.e. 1.04 g during this season. Shell ratio percentage is highest i.e. 12.76 during third season. It is 9.47 in first and 9.17 during second season.

It can be mentioned here that first and second crops in this area are undertaken as seed crop whereas third crop is taken as commercial crop. Accordingly, most of the seed cocoons harvested during first two seasons are utilized for seed production. Larger cocoon weight during first two seasons are due to larger weight of pupa. A healthy and robust pupa transforms into a healthier moth which lays more eggs as is evident by the increased fecundity of second and third seasons. However, a small part of cocoons harvested in 3rd season go for preservation and seed preparation for the next season and bulk of the cocoons are stifled and go for commercial reeling. The increased shell weight and shell ratio of the third season help the reeler to reel increased quantity of silk filament from a single cocoon required for its commercial use. It is because of high silk content of third crop cocoons the rearing of A.
mylitta during this season is called commercial crop or commercial rearing. The DFL/cocoon ratio of first to third season are 1:20, 1:41, and 1:78 respectively. This again shows the high crop performance during third season when the temperature ranges 12.16 degrees Celsius to 32.56 degrees Celsius, RH 33.24% to 78.16% and rainfall nil in first two months and 94.25 mm in January. The dfl/cocoon ratio is up by 105 percent in the second season and by 90.24 percent in 3rd season over their previous crops. Heavy rain coupled with increased attack of predators during first two seasons are responsible for poor crop performance. Heavy winds followed by heavy rains during first season blown and washed away the larvae particularly during early instars. The loss of larvae during early instars are also not always visible which accounts for the maximum larval loss. However, proper watch and ward and strict vigilance of rearing surely pay and results in good cocoon harvest. The larval survival is highest i.e. 95.56 percent in 3rd season. It is lowest i.e. 42.78 percent in 1st season. There is not remarkable difference in the larval survivality between first and second crop where it is 43.52 percent.

The population of A. mylitta consists of both males and females. The males always outnumber females. The ratio of males and females is more or less the same in all the three seasons. It was found 58:42, in the first and third season whereas, 57:43 in second season.

A comparision in cocoon characters between the Daba ecorace of Vidarbha region with those of Singhbhum, the original ecological niche of the ecorace reveals that the race has deteriorated in respect of all the characters under the ecoclimatic conditions of Vidarbha (Table III). It is evident from the table that the cocoon weight has gone down from 13 to 8.15 g, shell weight from 2.0 g to 1.04 g and SR% from 15.38% to 12.76%. It clearly indicates that the temperature and relative humidity prevailing in Vidarbha region is certainly not suited to Daba ecorace. However, the Indian tasar silkworm, A. mylitta being hardy has adopted the ecoclimate of
Vidarbha and in the process has lost the original characters. It is of utmost importance that Bhandara local ecorace of Vidarbha region be exploited for evolving a suitable ecorace for this region by hybridization.

For a farmer, it is the crop performance that matters. He becomes happy when he gets a good harvest. The harvest being best in the third season (DFL/cocoon ratio, 1:78) the commercial exploitation of A. mylitta be always in this season whereas in the first two seasons the rearing should be restricted to a limited number of selected farmers as per the requirement of seed.

5.2. NEUROENDOCRINE ORGANS AND NEUROSECRETION

The insect possesses an neuroendocrine system which consists of basically the following structures.

1. Neurosecretory Cells: In insects, the presence of neurosecretory cells (the source of neurohormone or brain hormone) in the pars intercerebralis, was first discovered by WEYER (1935) in honeybee, Apis mellifica; later, their presence in the brain and ganglia of the ventral nerve cord was demonstrated by numerous workers (HANSTROM, 1938, 1939, 1940; KLOOT, 1960; WIGGLESWORTH, 1964; DE WILDE, 1964; GILBERT, 1964, 1966; NOVAK, 1966, 1975; ROWELL, 1976; SCHARRER, 1965; SCHARRER and WEITZMAN, 1970; AWASTHI, 1972; RAABE, 1982 etc.). Now it has been well established that the neurosecretory cells are the key endocrine organs of the insects, they not only activate other endocrine glands but also control, directly or indirectly, almost all life processes (NOVAK, 1975; AWASTHI, 1979, 1985). The neurosecretory cells are (i) chiefly lying in the dorsal middle region of the brain (pars intercerebralis) and composed of two or more distinct types of secretory neurons, (ii) lateral neurosecretory cells also lying within the brain, but placed more laterally (pars lateralis), (iii) posterior and tritocerebral
neurosecretory cells of the brain, and (iv) neurosecretory cells of the suboesophaeal ganglion and the ganglia of the ventral nerve cord; and also of anterior sympathetic nervous system.


3. Corpora Allata (CA): CA is a pair (or occasionally single, formed by fusion of two) of glands, lying lateral or ventral to the anterior end of the aorta. The secretory nature of corpora allata was first suggested by NEBART (1913), their endocrine function was indicated by ITO (1918) and the function was elucidated by WIGGLESWORTH (1935). CAZAL (1948) classified them into four types after studying extensively. WIGGLESWORTH (1954) further suggested that the corpora allata are the source of juvenile hormone (JH). It is a well known fact that they are the sites of origin of JH in all insects and their significance in controlling various vital events is well documented (ENGELMANN, 1968, 1970; NOVAK, 1966, 1975; DOANE, 1973; RAABE, 1982, etc).

During the entire course of present studies on neuroendocrine organs and neurosecretion in A. mylitta and C. furcellata, I have investigated, observed and examined the above mentioned structures on one hand and on the other hand thorough investigations on number, type and distribution of neurosecretory cells in brain, frontal ganglion, suboesophaeal
ganglion and the ganglia of ventral nerve cord; activity of neurosecretory cells during post embryonic development; median and paramedian neurosecretory pathways; neurohormone storage and release sites; and metamorphic histomorphological changes in the corpora cardica - corpora allata complex have also been carried out. The ensuing discussion, for the sake of convenience of study in the subsequent sections, will provide a closer look of these facts, where I am going to compare and contrast my results with the observations made by earlier contributors in this arena of science, the insect neuroendocrinology.

5.2.1. NOMENCLATURE AND CLASSIFICATION OF NEUROSECRETORY CELLS

The classification of neurosecretory cells was initiated by Nayar and followed by subsequent workers. NAYAR (1955) for the first time recognized two types of NSC on the basis of their tinctorial properties in the brain of Iphita limbata Stal. The cytoplasmic inclusions of one type were stained deep blue in chrome haematoxylin-phloxin and dark red in Azan. He designated these cells as “A type”. In the other type, the cytoplasmic contents were stained red in chrome haematoxylin-phloxin and light blue in Azan and these were termed as “B cells”. He could not obtain any selective staining by Gomori’s aldehyde fuchsin. Since then, the NSC of insects have been classified into various types on various grounds such as staining properties, position, size, structure and ultrastructure of their secretory products by a large number of workers. (JOHANSSON, 1958; HIGHNAM, 1961; EWEN, 1962b; HERMAN and GILBERT, 1965; GUPTA, 1970; HINKS, 1971a, b; DOGRA, 1973; PANOV and D. DAVYDOYA, 1976, and BORG and BELL, 1977; SINGH and ARIF, 1978; AWASTHII and SINGH, 1981a,b etc).

JOHANSSON (1958) applying the PF staining technique differentiated Nayar’s “A cells” as PF positive and “B cells” as PF negative cells. He also observed “C” and “D cells” in addition to
these cells. C cells containing large flaky PF positive inclusions, whereas, the D cells were large containing very small PF positive granules. Gersch (1959) classified the brain NSC into three types on the basis of their staining properties. Fraser (1959b) adopted alphabetical system of classification for the NSC of the abdominal ganglia of *Lucilia ceazer*, and divided these into two distinct categories A and B cells. He concluded that A and B types, in fact, represented two aspects of a single type of cell. Highnam (1961) followed Johansson’s (1958) classification and categorized four major groups of cells after staining with PF stain. Raabe (1965a) found two types of fuchsinophilic cells and classified A and B type cells on account of the absence and presence of sulphydryl groups respectively. Chalaye (1975) by the electron microscopic observation supported the diversity in neurosecretory cell types.

Delphin (1963, 1965) classified the neurosecretory cells into four types A, B, C and D in *Schistocerca gregaria*. Dephin’s system of classification included three sub-types of A cells and two subtypes of B cells. Furthermore, by altering the ratio of dyes in the trichrome counterstains, Delphin (1963) was able to impart a green background in the C and D cells which revealed a difference in their cytoplasmic morphology. Herman and Gilbert (1965) studied the multiplicity of cell types and groups in the brain of *Hyalophora cecropia* and classified the neurosecretory cells into two main types - A and B. They further divided the A cells into five and B cells into two sub-categories on the basis of their distribution, size, number and secretory activity.

Hinks (1971a) followed the system of classification advanced by Delphin (1963, 1965). He made a thorough investigation of brain neurosecretory cells in the adults of Lepidoptera belonging to 23 species and reported 8-10 types of neurosecretory cells on the basis of their staining properties with PF.
In 1971b, he further reported four major categories of neurosecretory cells (A, B, C and D) in the brain of adult *Triphaena pronuba* after applying PF and counterstains. These comprised nine subtypes of cells, seven of which were recognizable for analysis of their secretory products.

PRENOTO (1972) after a variety of histochemical tests concluded that the A and B cells of insects are distinct cell types, each elaborating a specific predominantly proteinaceous product. According to him the B type cells and their axons contain high amount of specific material, rich in tyrosine and charged amino acids, while the secretory material of A cells are poor in these amino acids and therefore concluded that it was not fair to consider B cells as a resting stages of A cells, rather they are two entirely different types of cells elaborating different histochemical constituents. Johansson (1958) in *Oncopeltus fasciatus* tried a number of experiments to see whether the A cells change to B cells or vice versa but could not succeed in getting such a change.

NANDA and ROY (1973) divided the neurosecretory cells of *Bombyx mori* into A, B, C types not on the basis of their staining properties but on the basis of their size.

NASKAR and NANDA (1976) found two types of neurosecretory cells (A and B) in regions of proto-, deuto-, and tritocerebrum of the brain of *Chrysomia megacephala*. They found large number of A cells in the region of pars intercerebralis and few in the lateral regions. On the other hand, B cells were present in different regions of the brain including the base of the optic peduncle.

TEMBHARE and THAKARE (1976) in *Orthetrum chrysis* categorized neurosecretory cells into 8 groups and classified these as A1, A2, B and C types on the basis of (i) the presence of stainable granules, (ii) the presence of neurosecretory material in their axons
and terminals and (iii) their cyclic activity on the basis of the quantity of neurosecretory material present during various physiological activities.

The alphabetical system of classification was not universally accepted and there are workers who did not use this system instead, they classified the NSC differently on the basis of their location etc. FRASER (1959a) in the brain of Lucilia caeser, SCHARRER and BROWN (1962); GELDIAY and EDWARDS (1973) in Acheta domesticus, PANOV and D. DAVYDOVA (1976), FLETCHER (1969); in Blaps mucronata GIERYNG (1976) and BANERJEE et al., (1975), are the workers who did not agree with the alphabetical system of classification of neurosecretory cells.

In Dydercus koenigii TIWARI and SRIVASTAVA (1975) failed to obtain any tinctorial difference in the neurosecretory cells and they believed that this insect has only one cells type. Their observations support the findings in Belostoma indica. (DOGRA, 1969); Nezara viridula and Metochus uniguttatus (AWASTHI, 1972a, 1973) SHARMA et al., (1975a) in Dysdercus koenigii doubted whether the neurosecretory cells present in this insect were of distinct type, which in course of their secretory activity showed changes in appearance and stainability. These workers also disagree with the alphabetical classification given by earlier workers, and they believe in grouping of these cells into dorsal and lateral groups. These groups are cytochemically and functionally different and their pattern of secretory activity also differs.

In the brain of Ranatra elongata, Dysdercus koenigi and Rhodnius prolixus DOGRA (1967a,b.;1973) observed green stained B cells which were devoid of any sign of secretory activity. AWASTHI (1972a) observed few small cells in the brain of Nezara viridula which were stained light green with the PF technique like the “so called B type” neurosecretory cells and these were characteristically
lacking granular material and did not exhibit any cyclic activity. Hence, these were not considered as B cells by him. According to these observations it can be inferred that the Heteroptean insects mainly contain A type neurosecretory cells and the B cells, if present at all, do not exhibit a cyclic secretory activity. In contrary to the above notion, NARAIN (1982) demonstrated that the Heteropteran insects definitely have the B cells which are quite different from the A cells. However, in the entire life history of the insects in which the B cells are present he never noted the presence of PF positive NSM.

AWASTHI (1972a) and AWASTHI and SINGH (1981c) made an interesting observation in the certain medial neurosecretory cell of the brain of Nezara viridula and Philosamia ricini respectively where their upper halves were stained purple as A cells, while the remaining lower halves light green, like B cells. These workers reported this to be an indication of the change over of these cells from active phase to passive or vice versa. AWASTHI (1972a) explained his findings in the context of the observations of THOMSEN (1954), KOPF (1957a, b) and GIRARDIE and GIRARDIE (1967). He believed that these two types of cells are, in fact, two different phases of one and the same type of cell, and probably the cell with two types of staining properties might be a transitional stage leading to A or B cells. Further, AWASTHI (1975b, 1976b) in two species of earwig, Labedura riparia and Euborellia annulipes respectively reported in each medial and lateral region of pars intercerebralis a few green stained B cells which do not show secretory activity.

Many workers (THOMSEN, 1954; NAYAR, 1955; DELERMA, 1956; HERLANT-MEEWIS and PAQUET, 1956; KOPF, 1957,b) are of the opinion that the B type neurosecretory cells represent a stage in the secretory cycle of A cells. THOMSEN
(1954) found both blue and red inclusions in one and the same cell and considered the “A” and “B” cells to be the same but in different phases of secretory activity. NAYAR (1955) believed that the B type cells are actually the A cells whose stainable colloid has discharged. The phloxinophilic granules are considered by DE LERMA (1956) to represent the first stage of intracellular elaboration and as the granules mature they acquire affinity for chrome-haematoxylin. GAWANADE et al., (1978) in ants also showed that the A and B type cells were active and quiescent phases of a single cell respectively. These findings were further supported by electron microscopic studies of GIRARDIE and GIRARDIE (1967) in Locusta migratoria. They reported intermediate stages between A and B type cells and believed that the two types of cells described by various workers are nothing else but transitional stages of one and the same type of neurosecretory cells. However, BASSURMANOVA and PANOV (1967) confirmed the diversity of these cells on the basis of electron microscopic studies of the brain neurosecretory cells of Bombyx mori. They observed two categories of neurosecretory cells - (i) larger ones, containing both electron luscent vesicles and electron opaque granules, and (ii) smaller ones, filled only with electron opaque secretory granules.

Neurosecretory products, as we have seen in the foregoing pages, are often revealed by standard histological techniques. Chrome haematoxylin phloxin was the first method employed intensively to investigate neurosecretory processes. Other techniques yielding more or less similar results were subsequently developed: paraldehyde fuchsin, alcian blue, paraldehyde thionine-phloxin, paraldehyde thionine-paraldehyde fuchsin, pseudoisocyanine, and for in toto staining, Victoria blue and fuchsin resorcin. These methods successively involved a basic dye, which is fixed on the acidic structures and an acid dye, which is fixed on the basic structures. Neurosecretory products are generally basic and therefore stained by
acidic dyes. After strong oxidation, however, always carried out in these techniques, some of them are modified. Initially basic, they become acidic and retain basic dyes such as chrome haematoxylin, paraaldehyde fuchsins, and alcian blue; these are called type A materials, and the cells which produce such neurosecretory products are termed as A type neurosecretory cells (A NSC). However, other neurosecretory products remain basic and hence acidophilic, and are stained by the acidic dyes used after the basic dyes: phloxin after chrome haematoxylin or alcian blue, chromotrope 2R, orange G, light green, or picric-indigo-carmine after paraaldehyde fuchsin; these are type B products and the cells synthesizing such products are termed as B cells.

Thus it is evident that the terminology employed to designate the type and subtypes of neurosecretory cells lacks uniformity. It is difficult to propose a terminology that satisfies every available case, in view of the rich variety of the neurosecretory system of insects and the diversification of species. The best answer at the present time appears to be to use the designation A, B, (and C) for the major types, and to distinguish numbered subtypes (A1, A2, B1, B2 etc.) within each type as done by certain authors.

During the course of present investigation in A. mylitta the two major types - A and B neurosecretory cells are recorded. The A cells are PF positive and take purple colour of the PF stain whereas the B cells are PF negative and take green colour of counter stain (Orange G, Chromotrope 2R, Light green SF Yellowish). The A and B cells are larger in size than their neighbouring ordinary neurons. Their nucleo-cytoplasmic ratio are also variable. Neurosecretory cells of both these types A and B are distributed in the brain, suboesophageal ganglion and in certain other ganglia of ventral nerve cord and anterior sympathetic nervous system. Depending upon their localization in the brain these are further categorized into their subtypes. The subtypes of A cells are:
A1 (median), A2 (median and tritocerebral), A3 (median) and A4 (lateral); and subtypes of B cells are: B1 (median and tritocerebral) and B2 (lateral). These cells are similar with neurosecretory cells described by the earlier worker (Hinks, 1971; Awasthi and Singh, 1981a,b, 1982c; Singh and Arif, 1978, 1983a,b; Arif and Singh, 1990). The classification and nomenclature of neurosecretory cells followed by me are in conformity with the earlier workers. The comparision of observations made in the Table IX will definitely support my view of the nomenclature and classification of neurosecretory cells in A. mylitta.

The terminology given by Johansson (1958) for the milkweed bug Oncopeltus fasciatus (A, B, C and D) has the drawback of designating cells belonging to type A by three different letters. This terminology, applied to locust by Highnam (1961a), is often used and raises problems because the B cells of Heteroptera are quite different from those of primitive insects which appear to be rare, and because the designation B is generally given to A2 or C cells (Raabe, 1982). In the bulk preparation of C. furcellata, I have observed A principal type of neurosecretory cells in brain. In adults A cells may be further categorized into their subtypes (A1,A2,A3). The presence of A cells in heteropterans has been noted by several workers (Narain, 1982; Raabe, 1982). My observations on the heteropteran species in question here, C. furcellata, are in conformity with the observations made by other workers.

5.2.2. THE BRAIN NEUROSECRETORY CELLS:
THEIR DISTRIBUTION AND MULTIPLICITY

Apropos of the distribution of neurosecretory cells, the most abundant data concern the brain and within it, the pars intercerebralis containing the richest group of neurosecretory cells, which give rise to the pair of nervi corporis cardiaci I (NCCI). Other groups, the lateral protocerebral group and the tritocerebral group, are the source of the other pair(s) of corpora cardiaca nerves NCCII
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In Antheraea mylitta, under present investigation, two main types—A and B neurosecretory cells are present. Furthermore, subtypes of A occupying the pars intercerebralis are A1, A2, and A3; and the subtype of B is B1. These are morphologically similar to the cells of Lepidoptera described earlier (Singh and Arif, 1978; Awasthi and Singh, 1981a, c; Arif and Singh, 1990). The number of neurosecretory cells observed in this region of A. mylitta is also close to the numbers enumerated by Singh et al., 1975; Singh and Arif, 1978, 1983a, b; Singh and Awasthi, 1981; Awasthi and Singh, 1981a, c; Panov, 1983; Khan et al., 1983;
The pars intercerebralis is located mediodorsally at the
junction of the two protocerebral lobes. It contains many types of
neurosecretory cells. In Lepidoptera, Diptera, Coleoptera and
Heteroptera, the neurosecretory cells are larger and few in number;
in Dictyoptera, Phasmoptera, and Orthoptera, they are small but very
numerous. While there are 24 type A cells in Heteroptera and
Lepidoptera (PANOV and KIND, 1963), 1000 and more are found in
Orthoptera (2400 in Schistocerca according to HIGHNAM (1961a),
900 in Acheta according to GELDIAY and EDWARDS (1973). The
pars intercerebralis is not always homologous from one species to
another and neither from one instar to another. In a number of
insects, the neurosecretory cells of the pars intercerebralis, which
form a single group in the adult, are divided into three distinct
groups in the larva, and a migration of neurosecretory cells occurs,
during development. This occurs in Lepidoptera (PANOV and
KIND, 1963; PANOV, 1975, 1976; PANOV and DAVYDOVA, 1976),
Diptera (DOGRA and TANDON, 1965; GIERYNG, 1976; KHAN et
al., 1978b), Odonata and Orthoptera. Furthermore, as shown by
NIJHOUT (1975) the largest lateral group (L1 of Panov) is
paramedian in the larva of Manduca sexta and lateral in the pupa.

In Antheraea mylitta, under present investigation, two main
types – A and B neurosecretory cells are present. Furthermore, subtypes
of A occupying the pars intercerebralis are A1, A2 and A3; and the
subtype of B is B1. These are morphologically similar to the cells
of Lepidoptera described earlier (SINGH and ARIF, 1978;
AWASTHI and SINGH, 1981a,c; ARIF and SINGH, 1990). The
number of neurosecretory cells observed in this region of A. mylitta
is also close to the numbers enumerated by SINGH et al., 1975;
SINGH and ARIF, 1978,1983a,b; SINGH and AWASTHI, 1981;
AWASTHI and SINGH, 1981a,c; PANOV, 1983; KHAN et al., 1983;
ARIF and SINGH, 1990 etc. My observation on the distribution and number of neurosecretory cells occupying in the pars intercerebralis region is in conformity with these workers.

Initial work on the neurosecretory cells of an heteropteran insect, *Iphita limbata*, was done by NAYAR who in a series of papers (1953a,b, 1954a,b, 1955b, 1956, 1957, 1958, 1960, 1962) has elucidated the subject from various aspects and also classified neurosecretory neurons into two (A and B) types on the basis of staining properties. (using PF and CHP techniques). After Nayar's work on *Iphita limbata*, numerous workers have studied neurosecretory cells of heteropteran insects, viz., *Chrysocoris stolii* (GANGULI and DEB, 1960); *Macrocercus grandis* (GANGULI and BANERJEE, 1960); *Iphita limbata* (SESHAN and ITTYCHERIAH, 1966); *Dysdercus koenigi*, *Ranatra elongata*, *Belostoma indica* (DOGRA, 1967a,b, 1969); *Nezara viridula*, *Dysdercus koenigi*, *Metochus uniguttatus*, *Sphaerodema rusticum* and *Lygaeus pandurus* (AWASTHI, 1969b, 1972a,b, 1973b,c, 1980a); *Dysdercus similis* (GUPTA, 1970); *Sphaerodema annulatum* (SRIVASTAVA and TIWARI 1973; SRIVASTAVA et al., 1974); *Leptocoris variocornis* (TIWARI et al., 1975); *Dysdercus koenigi* (TIWARI and SRIVASTAVA, 1975); *Leptocoris acuta*, *Ranatra filiformis* *Sphaerodema rusticum* and *Cyclopelta succifolia* (FARUQUI, 1974a, 1975a, 1977c); *Chrysocoris stolii*, *Bagrada cruciferarum* and *Aspongopus janus* (SINGH and NARAIN, 1980, 1981, 1984) etc. In the above cited works, either one i.e. A or two - A and B main types of neurosecretory cells have been reported. The variable number of A and B cells have been recorded in the medial groups. In the brain of Heteroptera generally 2 medial groups have been recorded. The A cells primarily constitute the medial group; but occasionally their subtypes are also seen. My observation in *C. furcellata*, in which the NSCs are present in medial group is accurately in the line of earlier observations; and also confirms that
the subtypes of A i.e. A1, A2, A3 cells are met in the medial region of heteropteran brain.

Some neurosecretory cells located in the median plane outside the pars intercerebralis have been known for a long time in certain insects orders. They were described under different names in *Melanoplus* (Dogra and Ewen, 1970), *Locusta* (Girardie, 1970), *Acheta* (Geldiay and Edwards, 1973), *Leucophaea* (De Besse, 1978), *Grillus bimaculatus* (Anwar and Ismail, 1979), and where their location was shown to be at the source of the median ocellar nerve. Three groups can be identified in dragonfly larvae, one mediodorsal consisting of four types A cells, and the other two paramedian comprising type A and B cells (Schaller and Meunier, 1968; Charlet and Schaller, 1974). Singh et al., (1975) while working with third instar larvae of *A. mylitta* reported three groups of neurosecretory cells in each half of the protocerebrum - median, lateral and posterior groups. These workers (Singh et al., 1975) further grouped the median cells into two loci namely locus 1 and 2. Those belonging to locus 1 lie below the neural lamella close to the median fissure and those of locus 2 lie a little away from the median fissure (Fig. 1 of their paper). In my observation, the medial cells are located very well in the pars intercerebralis, without slight deviation; and the various subtypes are interpersed in the same region. Furthermore, under present investigations there is no migration of neurosecretory cells during metamorphosis in both of these insects though belonging to different insects orders.

The tritocerebrum, investigated in many species (Raabe, 1963a, 1964; Singh and Arif, 1978, 1983a,b; Arif and Singh, 1990), always contain a small number of neurosecretory cells. The tritocerebral neurosecretory cells are confirmed by the ultrastructural investigation made by Girardie in 1975. Singh and Arif (1978)
reported the presence of A2 cells in tritocerebrum of *P. ricini*. AWASTHI and SINGH (1981a) did not agree with the findings of SINGH and ARIF (1978) and suggested that these cells were of posterior region. However, further SINGH and ARIF in a series of papers (1983a,b; ARIF and SINGH, 1990) confirmed the presence of these cells in the tritocerebrum of lepidoptran brain. SINGH et al., (1975) in *A. mylitta* have already shown the presence of tritocerebral neurosecretory cells. My observation on the presence of A2 and B1 cells in tritocerebrum is in agreement with SINGH and ARIF (1978,83a,b); SRIVASTAVA and PRASAD (1985); ARIF and SINGH (1990); LEE et al., 1990. However, at this point of study, I do not agree with AWASTHI and SINGH, (1981a). However, AWASTHI and SINGH (1981c) have shown the tritocerebral B2 cells in *Philosamia ricini*.

Neurosecretory cells are present in the lateral parts of protocerebrum, pars lateralis, in the vicinity of the calyces of the pedunculate bodies. Few in numbers, they often form distinct group(s). The axons of the lateral protocerebral neurosecretory cells follow a sinuous path and emerge ventrally from the brain, constituting the nervi corporis cardiaci II, which in evolved species fuse with the nervi corporis cardiaci I.

Presence of lateral neurosecretory cells in the protocerebrum has been reported by a large number of workers (WILLIAMS, 1947; CAZAL, 1948, THOMSEN, 1954; NAYAR, 1955; JOHNSON, 1963; DOGRA, 1967a,b,d; SINGH et al., 1975; AWASTHI, 1975b, 1976b; TEMBHARE and THAKARE, 1976 and BORG and BELL, 1977). DOGRA, 1967a,b,d reported light green stained B cells in the lateral regions of the protocerebrum of *Ranatra elongata*, *Dysdercus koenigii* and *Grillotalpa africana* respectively. However, definite selectively stained inclusions were not observed in these cells. SHARMA et al., (1975a) believed that
the neurosecretory cells of three dorsal groups of *D. koenigii* correspond to the A and B cells of the lateral region of other insects. These workers reported the secretory activity of the lateral cells of the unmated females and believed that their secretion induces the urge for mating. They further reported reduced secretory activity in these cells after the beginning of egg laying.

**Awasthi** (1975b, 1976b) reported lateral B type neurosecretory cells in the brain of two species of earwig *Labedura riparia* and *Euborellia annulipes*. **Saini** (1971) in *Poekilocerus pictus* reported the presence of lateral neurosecretory cells but did not clearly indicate their location in the brain. Similarly **Geldiay** (1967) in *Anacridium aegyptium* could not clearly locate the lateral cells and suggested that these might be placed very close to the medial groups. **Krishnanandam** and **Ramamurthy** (1971) in *Pyrilla perpusilla* and **Awasthi** (1972a) in *Nezara viridula* reported the absence of lateral NSC. **Singh** and **Arif** (1978) in *Philosamia ricini* and **Singh** & **Arif** (1983a,b) and **Arif** & **Singh** (1990) reported the presence of PF positive and PF negative lateral neurosecretory cells in all the lepidopteran insects studied by them. **Narain** (1982) did not find these cells in the heteropteran bugs of his study and the mayfly (*A. minor*), but in the earwig *A. annulipes* 2-3 B types neurosecretory cells were noted in pars intercerebralis lateralis of the brain. In the pars lateralis of brain in *C. furcellata*, I did not find even either types A or B cells in nymphs as well as in adult males and females.

**Awasthi** and **Singh** (1981a) in *Amsacta collaris* have shown the presence of A and B cells in lateral region of protocerebrum. In the same year (1981c) these workers again showed their presence in *Philosamia ricini*. In the lepidopteran brain (unlike hemipteran), the presence of A and B neurosecretory cells is well documented. My observation in *A. mylitta* also establishes
their presence, where A and B cells are seen throughout the entire life of the insect, and hence further confirms the findings of earlier investigators (SINGH et al., 1975; KHAN et al., 1983 etc).

A large number of workers have attracted towards the multiplicity found in the brain neurosecretory cells in certain insects. PANOV and KIND (1963); HERMAN and GILBERT (1965); VIJVERBERG (1970); HINKS (1971a); SHARMA et al., (1975a); SINGH et al., (1975); PANOV and E.D. DAVYDOVA (1976); PANOV and D. DAVYDOVA (1976); SINGH and ARIF (1978) etc have described multiplicity of the neurosecretory cells. VIJVERBERG (1970) in Calliphora erythrocephala reported an increase in the number of neurosecretory cells in the brain and suboesophageal ganglion from first to third larval instars while HINKS (1971a) supported the view of PANOV and KIND (1963) and HERMAN and GILBERT (1965), and reported a remarkable uniformity in the number of medially placed A1, A2, A3 and C and D cells, and lateral A4 and B2 cells in all the insects studied by him. PANOV and D. DAVYDOVA (1976) in the brain of Mecoptera and Neuropteroiodes reported 3 paired groups (M1, M2 and M3). These workers did not find any change in the composition of M2 and M3 groups during metamorphosis but the number of NSC of the M1 group, including A1, A2 and B cells varied considerably. On the other hand, PANOV and E.D. DAVYDOVA (1977) in the scorpion flies Panorpa communis and Panorpa cognata found equal number of medial A cells in the larval and adult stages. These workers proposed a possible mode of increase in the number of B cells of the pars intercerebralis during pupal period and thought it probably due to mitotic activity of neuroblasts located in these clusters. SINGH and ARIF (1978, 1983a,b) and ARIF and SINGH (1990) in Philosamia ricini, Pericalla ricini, Hypsa alciforun and Diacrisia obliqua reported a continuous increase in the number of anteromedian lateral and tritocerebral A, B, C and D cells.
Variations in the number of neurosecretory cells in a particular centre of ganglion in different individuals of the same stage have been reported by several workers. Fuller (1960) recorded variations in the number of neurosecretory individuals of the suboesophageal ganglion in various individuals of Periplaneta americana and reported the presence of A cells in 30%, B cells in 12% and C cells in extremely low percentage of the sacrificed specimens. Similarly Mitsuhashi (1963) reported considerable variations in the types of cells and their number in 8 species of Lepidoptera studied by him. Delphin (1965) reported variations in the number of B1 and D cells of 1st and 2nd thoracic and 5th abdominal ganglia of different individuals of Schistocerca gregaria. On the other hand, a remarkable uniformity has been reported in both, types and numbers of cells in twenty species of insects studied by Panov and Kind (1963).

Al Sharook et al., (1970) proposed a possible mode of replacement of median neurosecretory cells from globuli cells in the brain of cockroach Periplaneta americana. They observed PF positive granules in some globuli cells and believed that some small sized median neurosecretory cells possibly recruited from time to time from these globuli cells which helped not only in increasing number of median neurosecretory cells but also to replace the old and dead median neurosecretory cells in the brain.

Singh et al., (1975) have thoroughly investigated the 'summer crop' third instar larvae of A. mylitta. These workers observed three groups of neurosecretory cells in each half of the protocerebrum. They are medial (MNSC), lateral (LNSC) and posterior (PNSC) groups. Two types of NSC namely A and B types are recognized. In one day (1d) old third instar larvae of A. mylitta there are not less than ten large and medium sized MNSC (A type), two LNSC (B type) and two PNSC (A type) in each half of the protocerebrum. These workers further reported the number of A
cells in median region 7(2d), 8(3d), 8(4d), 7(6d), 8(7d) and 4(8d); and in posterior region 2(1d) and 2(4d). The median B cells are 5(2d), 4(3d), 2(6d) and 3(7 and 8d). The LNSC are of B types only and are 2(1 and 2d), 6(7d) and 1(8d). There is an increase and decrease in all types of cells in each region according to the age. Thus according to these workers (SINGH et al., 1975) there exists a remarkable multiplicity in protocerebral neurosecretory cells in A. mylitta. And further, the appearances and disappearances of neurosecretory cells are related with secretory activity and molting by these investigators.

KHAN et al., (1983) in winter crop 2nd instar larvae of A. mylitta have reported the presence of three groups-medial, lateral and posterior group of neurosecretory cells in brain. These workers observed A-, B- and C- neurosecretory cell types. The number and the secretory activity of neurosecretory cells have also been correlated with the molting cycle. I have investigated each larval instar (from 1st to 5th instar), pupae (of the varying ages viz; early, late) and adult moths (both males and females), under pre- and post-mated stages) of A. mylitta. In each brain hemisphere there are three groups and two principal types of neurosecretory cells during the entire life of this insect. The three groups observed are - medial (pars intercerebralis), lateral (pars lateralis) and tritocerebral; and the two principal types of neurosecretory cells are A and B. The A cells are further categorized in four subtypes (A1, A2, A3 and A4) and the B cells into two (B1 and B2) types, SINGH et al., (1975) and KHAN et al., (1983) have also observed the presence of A and B cells in the larval instars of A. mylitta. These workers have emphasized on the presence of three groups of neurosecretory cells in the protocerebral lobe of the brain only, whereas, in my opinion the third group lies in the tritocerebral region of the brain. This group is comprised of A2 and B1 cells. My above finding is also in accordance with the observations made by SINGH and ARIF (1978,
83a,b) and ARIF and SINGH (1990) in lepidopteran insects. Furthermore, SINGH et al., (1975) have reported a day-to-day variation in the number of neurosecretory cells and correlated these variations in number with the activity of neurosecretory cells which again are related with the moulting. KHAN et al., (1983) have also proposed that the number and secretory activity of neurosecretory cells have a correlation with the moulting cycle. AWASTHI and SINGH (1981c) in Philosamia ricini have reported four groups (medial, lateral, posterior and tritocerebral) and their principal types (A, B and C) of neurosecretory cells in each brain hemisphere of larvae, pupae and adults. These workers further classified A, Band C cells in their subtypes viz., A1, A2, B1, B2, C1 and C2. Further, AWASTHI and SINGH (1981c) have made a very noteworthy observation on the variation (increase and/or decrease) of neurosecretory cells. In eri silkworm, the number of few cells (medial - A1, A2, C1, C2 and posterior A3) are observed unaltered whereas, other cells (median - B1, B2, lateral - A2, B2, posterior - B2 and tritocerebral - B2) increased gradually in their number throughout the entire life - from 1st larval instars to adults. Moreover, this gradual increase in number of some cells is found to be related with age. These workers have not mentioned, even in exceptional cases, the sudden and swift alteration in the number of neurosecretory cells. In A. mylitta under present investigations, there are some cells (A1, A4) which remain unaltered, few others (A2, A3, gradually increase in number, while few more others (B1, B2 and tritocerebral A2) virtually increase in number during some parts of the life and in later stages, especially in adulthood, they disappear at all. Thus, there is no fixed pattern of neurosecretory cell multiplication attributed to A. mylitta. However, in the other insect studied by me, C furcellata, there is a continuous increase in the number of PF-positive neurosecretory cells. In the 5th instar nymph total number of A cells (A1, A2 and A3) are 6 in each brain hemisphere, whereas, in males, it rises to 7, and in females it
further increases to 13. This pattern of neurosecretory cell multiplicity in heteropteran brain has already been established by the earlier workers (NARAIN, 1982 etc).

5.2.3. NEUROSECRETORY CELLS OF THE ANTERIOR SYMPATHETIC NERVOUS SYSTEM

The presence of neurosecretory cells has sometimes been demonstrated in the frontal and hypocerebral ganglia of cockroaches, locusts, stick insects and lepidopteran larvae (SMITH, 1968; CAZAL et al., 1971; CHANUSSOT, 1972; DORN, 1978; KHAN, 1976a; SINGH and AWASTHI, 1981b) and in the frontal ganglion of moth larvae (BORG et al., 1973; BELL et al., 1974; YIN and CHIPPENDALE, 1975; AWASTHI, 1980; SINGH and AWASTHI 1981b). Dense granules of 105 nm have been observed in Corethra (TOMBES and MALONE, 1977), and dense granules from 100 to 300 nm and 90 to 120 nm in the hypocerebral ganglion of the bug Oncopeltus (UNNITHAN et al., 1971). Based on the size and density of the granules, one may conclude that two types of secretion exists. It is not known at present exactly where the neurosecretory products of the sympathetic ganglia are delivered (RAABE, 1982). Those of the frontal ganglion pass either towards the brain (TOMBES and MALONE, 1977) or towards the recurrent nerve, from where they probably reach oesophageal effectors. In fact, neurosecretory granules have been reported in the oesophageal nerves of Melanoplus (DOGRA and EWEN, 1970) and Calliphora (THOMSEN, 1969). A detailed study of the neuronal connections of the frontal ganglion has been carried out in Periplaneta (GUNDEL and PENZLIN, 1978).

SINGH and AWASTHI (1981b) have described neurosecretory cells of the frontal and hypocerebral ganglia of Amsacta collateralis. These workers have shown A and B cells in the frontal and A cells in the hypocerebral ganglion. The axons of the neurosecretory cells of frontal ganglion were also shown directed
towards the recurrent nerve. In *A. mylitta*, under present investigations, both types- A and B cells are present in the frontal ganglion, whereas, the hypocerebral ganglion was not noticed. The A and B cells are similar as described by AWASTHI (1980) and SINGH and AWASTHI (1981b). Though the cumulative number of these cells (A-15 and B-12) in *A. mylitta* is more than the number noted by SINGH and AWASTHI (1981b). Furthermore, the frontal ganglionic neurosecretory cell axons are also found to be directed towards the recurrent nerve, suggesting the release of neurosecretory material into it.

5.2.4. NEUROSECRETORY CELLS OF THE VENTRAL NERVE CORD

The presence of neurosecretory cells in the different ventral nerve cord ganglia has been noted in all insects orders. The most thorough investigations have been conducted on dragonflies (CHARLET, 1969; TEMBHARE and THAKARE, 1977), stick insects (RAABE, 1965a,b,1967), cockroaches (DE BESS'E, 1965), locusts (FREON, 1964; DELPHIN, 1965; CHALAYE, 1965, 1974a, 1975), the beetle *Blaps mucronata* (FLETCHER, 1969), the moth *Galleria mellonella* (DELEPINE, 1965), and the blood-sucking bug *Rhodnius prolixus* (BAUDRY, 1968). The neurosecretory cells of the ventral nerve cord are less numerous and more widely dispersed than the brain cells, but they belong to many types and form rich and diversified systems. DAY (1940) was the first to describe the presence of neurosecretory cells in the ventral ganglia of certain Lepidoptera. The other lepidopteran species examined are *Bombyx mori* (BOUNHIOL et al., 1953; FUKUDA and TAKEUCHI, 1967), *Galleria mellonella* (DELEPINE,1965) etc and the heteropterans are *Oncopeltus fasciatus* (JOHANSSON, 1958), *Adelphocoris lineolatus*, (EWEN, 1962), *Lethocerus indicum* (BHARGAVA, 1967), *Iphita limbata* ( NAYAR, 1955; SESHAN, 1968), *Rhodnius prolixus* (BAEHR, 1968; BAUDRY, 1968). *Nezara viridula* (AWASTHI, 1972)
Chrysocoris stollii (SINGH et al., 1978) etc.

A comparative examination of the most thoroughly investigated species reveals that four major categories of neurosecretory cells are found in almost all of the insect groups (RAABE, 1982; page 26-29, Table-VI). The first category consists of a single pair of cells located medioventrally in the suboesophageal ganglion, and is present in almost all of the groups. The axons of both cells run into the circumoesophageal connectives and terminate within the brain (Calliphora, VJIVERBERG, 1970; Acheta, STRAMBI et al., 1979). In Acheta, they terminate in a definitive neurohaemal area (GELDIAY and KARACALI, 1980). Immunocytological studies indicate that in Locusta these cells also send processes back towards the thoracic and abdominal ganglia (REMY and GIRARDIE, 1980) and terminate in the somatic nerves (GIRARDIE and REMY, 1980). A second category is represented by the 'thoracic' cells that form two or three lateral pairs. These are large pyriform neurons whose secretion assumes the appearance of granules. A third category is exclusively abdominal. It includes two to five pairs of posterior lateral smaller cells. A fourth category of Cr type cells are also present.

The neurosecretory cells of the ventral cord are generally of type A (NARAIN, 1972; GUNDEVIA and RAMAMURTHY, 1972b; AWASTHI, 1972a; GAUDE, 1975; NARAIN, 1982 etc). But neurosecretory cells with type B affinities are also noted in the ventral nerve cord and more frequently in the abdominal ganglia (HIGHNAM, 1965; NARAIN, 1972 etc). I have observed PF positive A type neurosecretory cells in the suboesophageal, thoracic and abdominal ganglia of A. mylitta and in the suboesophageal ganglion of Canthecona furcellata. In certain specimens of A. mylitta PF negative B cells have also been noted in the suboesophageal ganglion. Morphologically, the PF positive cells of the ventral nerve
cord resemble with the A1 cells of the pars intercerebralis of brain. The distribution and number of ventral nerve cord neurosecretory cells under present investigations are in harmony with the earlier observations (RAABE, 1982), but Cr cells remain unnoticed by me.

5.2.5. ACTIVITY OF NEUROSECRETORY CELLS

A large number of workers have studied the cyclic changes in the neurosecretory cells in different insects during their metamorphosis. An uniform criterion is not yet established by the insect endocrinologists (ARIF and SINGH, 1991). Hence, different interpretations have been given for a particular phase by different workers. HIGNAM (1962) is of the opinion that the active cells have a small amount of neurosecretory material and inactive cells are full of neurosecretory material. According to GILBERT (1964) when the neurosecretory cells contain a small amount of stainable material, indicates that it is synthesizing and releasing the material and when packed with neurosecretory material indicates inactive stage. According to HIGNAM (1962, 1965) the neurosecretory activity is regulated by three factors: (i) the rate of synthesis of neurosecretory material in the cells (ii) the rate of transport of material along the axons and (iii) the rate of release of the transported material into the blood. Thus the amount of neurosecretory material in the cells represents a balance between synthesis and release. If both the processes run on parallel lines i.e., the rate of synthesis and the rate of release is equal, the cells will appear empty. According to HIGNAM (1962) the neurosecretory cells with a small amount of material is actively synthesizing and releasing developmental factors, whereas the system with large amount of material is synthesizing but not releasing its factor.

AWASTHI and SINGH (1981a) investigated dynamics of the neurosecretory cells of the brain of Amsacta collaris during post-embryonic development. Their studies have shown a distinct cyclic change in the amount of neurosecretory material in the
neurosecretory cells. The authors are of the view that (i) the empty cells indicate inactive stage, (ii) when the cells contain a small amount of neurosecretory material and granules are sparsely distributed in the cytoplasm represent the initial secretory phase, (iii) when the cells contain a moderate amount of neurosecretory material (not fully packed), the granules are distinctly seen with vacuoles and the material under transport along the axons is visible, indicates the most active stage, and (iv) when the cells are heavily loaded with neurosecretory material granules fused together and lose their identity nucleus gets obliterated and the material is not seen under transport shows a resting or storage phase (second type of inactivity). In the latter case material may be synthesized but not transported and released in the circulatory system. AWASTHI and SINGH (1981a) agreed in principle to the views of HIGHNAM (1962) with certain modifications. These workers presumed that the synthesized material necessarily may not be discharged or transported with the same rate i.e. the rate of synthesis must not be equal to the rate of transport of synthesized material. It is well known that the secretory granules reach the axon terminals by proximodistal-axonal transport (SCHARRER, 1967). The latter process depends on the capacity of cells generating and conducting impulses. The action potential with characteristics slow conduction velocity have been demonstrated in the neurosecretory cells of several invertebrates (SCHARRER and WEITZMAN, 1970). These authors (AWASTHI and SINGH, 1981a) further presumed that the rate of synthesis of neurosecretory material and its transport along the axoplasm via impulse conduction may not run with the same speed. AWASTHI and SINGH (1981a) thus concluded that the transport of material gradually or suddenly, depends on the rate and speed of impulse-conduction. Thus a cell showing enhanced activity must contain a moderate amount of neurosecretory material and it should not be empty, and at the same time it must indicate the axonal transport of neurosecretory material. These workers
appropriately realized and mentioned that the light microscopy has its own limitations and only the ultrastructural study can throw a definite light on this aspect.

**ARIF and SINGH (1991)** described secretory activities of the PF-positive neurosecretory cells of the brain during life histories of four lepidopteran insects. The four species investigated are *Philosamia ricini*, *Pericalla ricini*, *Diacrisia obliqua* and *Hypsa alciforom*. These workers mentioned four phases of secretory activity viz., Synthesis phase, Coalescence phase, Release phase and Resting phase of neurosecretory cells during the life of all the species examined by them.

In the synthesis phase of cell activity the synthesis of neurosecretory material takes place, the neurosecretory granules are seen scattered in the entire parikaryon of the cells. The synthesis phase is followed by a coalescence phase in which the distribution of neurosecretory material is uniform in the cell body. The synthesized neurosecretory granules are accumulated at certain places and vacuoles appear in the parikaryon. Coalescence phase is followed by the release phase. The neurosecretory cells have large to less quantity of neurosecretory material according to their degree of release. The neurosecretory material is mostly accumulated towards the axonal ends. The axons also have neurosecretory material. After the release of neurosecretory material the cells enter a resting phase. Neurosecretory cells show larger nuclei with less cytoplasmic area. Cells stain faintly with PF stain and no trace of neurosecretory granules is found in the cytoplasm. In my view, an active stage is one which shows the sign of synthesis of neurosecretory material in a previously empty cell. In the coalescence phase the cell shows the presence of vacuoles; and in release phase the neurosecretory materials are seen in the axons or toward the axonal end. The resting phase is attributed by the absence of neurosecretory material. The larger cells with smaller nuclei are also in the phase
of having greater quantity of neurosecretory material and therefore, may be regarded in the stage of intense activity. I have observed all four phases of secretory activity in brain neurosecretory cells of *A. mylitta* during its life span. The cells repeat these phases in a cyclic fashion. In pupal stage this cycle is repeated more than once. Thus, my observation is in harmony with the earlier workers especially with SINGH and ARIF (1978, 1983a,b) and ARIF and SINGH (1990, 1991). I did not investigate this aspect in the brain of *C. furcellata* because rearing of this insect was not done due to its predatory habit.

The activity of neurosecretory cells includes the synthesis of neurohormones, their transport along the axons, their release at the endings, and sometimes autophagous mechanisms. This regulation of these steps is complex, depends on different factors, and follows various procedures. Neurosecretory processes are influenced by environmental conditions such as temperature and photoperiod, and by internal factors such as the repletion of the digestive tract, the metabolic composition of the haemolymph, and the level of circulating hormones. The existence of connections between the sensory organs and the brain neurosecretory centres have already been established (BROUSSE-GAURY, 1968, 1971a,b). The anterior sympathetic nerves system also exerts control over neurosecretory activity. Furthermore, independent of the regulatory pathways described above, the neurosecretory cells also exhibit cyclic activity governed by circadian rhythms (CYMBOROWSKI and DUTKOWSKI, 1969, 1970a; HEINZELLER, 1976 etc). Though, I have not worked on these aspects, but in my bioecological observations, it is found that the temperature and photoperiod have greatly influenced the biology of the silk worms. The duration of larval span varies in respective seasons. This indicates a correlation between the neurohormone system with the environment. The tasar silkworms chosen for neuroendocrinological investigations belong to the second crop and the blueprint of their neuroendocrine organs and neurosecretion are prepared and discussed. The species is open for
future study, if carried out in all the three seasons of the year, will
definitely throw more light on these aspects and will also help us to
understand the latent life activities in a more precise way.

5.2.6. RETROCEREBRAL ENDOCRINE COMPLEX AND
NEUROHORMONE STORAGE AND RELEASE SITES

The retrocerebral endocrine complex (=retrocerebral
neuroendocrine complex = retrocerebral neuroendocrine-aortal
complex) of A. mylitta consists of corpora cardiaca, corpora allata,
nervi corporis cardiacci I and II, nervi corporis allati I and II, inner
cardiac nerves I and II; and in Caneheonu furcellata it constitutes
corpora cardiaca, fused corpus allatum, nervi corporis cardiacci I and
II and the aorta, which acts as a neurohaemal organ in this insect.
The most significant and a noteworthy observation of the present
work is the occurrence of multilobed corpora allata in A. mylitta;
which act as neurohaemal organ in this insect. Both features of A.
mylitta i.e. presence of multilobed corpora allata and their function
of neurohaemal organ are significant and are found deviated from
the general pattern of Insecta. However, in heteropterans, the
neurohaemal nature of dorsal aorta has already been reported

Several reviews dealing with neurosecretion (VAN DER
KLOOT, 1960; GABE, 1966; SCHARRER and WEITZMAN, 1970),
the endocrine control of development, growth and reproduction in
insects (GILBERT, 1964; WILDE, 1964; WIGGLESWORTH, 1964b,
ENGELMANN, 1968; DOANE, 1973), neurohaemal organs in insects
(AWASTHI, 1974) and the neurosecretory - neurohormonal system of
insects; anatomical, structural and physiological data (RAABE, 1983
e tc.) have been published. Although there is no definite criteria for
the classification of the neurohaemal organs in insects, it has been
observed that in each insect bulk of neurosecretory material is
stored and released from one main organ, and besides this, there
may be numerous other small either discrete organs or parts of the
main organ which store and release neurosecretory material. For the sake of convenience, therefore, neurohaemal organs have been classified into two types, viz., (i) principal neurohaemal organs and (ii) associated neurohaemal organ. The neurohaemal organ may be defined as portion of the surface of nervous system providing passage for neurohormones from the central nervous system to the target areas. But this definition cannot hold good because other non-nervous tissues like part of the aorta are also associated with it. Thus the neurohaemal organ, therefore more appropriately can be defined as any organ or part of an organ which stores neurohormones and releases them into the blood. From the latter, the neurohormones reach the target organs.

The retrocerebral endocrine complex of *A. mylitta* is in principle similar to those of other lepidopterans (CAZAL, 1948; YEN and CHIPPENDALE, 1973; RAINA and BELL, 1978; SINGH and AWASTHI, 1980, 1982) but certain specialities are also evidenced. In most individuals of *A. mylitta*, each corpus cardiacum is innervated by NCC I and NCCII as shown by RAINA and BELL (1978), SINGH and AWASTHI (1981), SINGH and ARIF (1981) but in certain cases NCC III is also seen (GABE, 1966), but under no condition the fusion of NCC I and NCC II is observed as reported by SINGH and AWASTHI (1982) in *Philosamia ricini*.

The corpora cardiaca of *A. mylitta* are non-neurohaemal structures and act merely as 'cross-roads' for the neurosecretory material which ultimately reach the corpora allata. SINGH and AWASTHI (1982) have already mentioned that in *P. ricini* the corpora cardiaca act as a cross-road through which neurosecretory axons pass to the corpora allata via a thick allatic nerve. My this aspect of observations is also in accordance with NIJHOUT (1975). Some of the target organs receive neurosecretory material directly via axons which emerge from the inner and outer part of the
corpora cardiaca (SINGH and AWASTHI, 1982).

In *A. mylitta* the corpora allata are very peculiar structures. These are multilobed bodies in which the neurosecretory materials are brought through nervi corporis allati I. The corpora allata also show metamorphic changes during the course of development of the insect. In majority of the lepidopterans, the larval corpora allata are either oval or elongate bodies (MITSUHASHI, 1963; SRIVASTAVA et al., 1975; SINGH and SRIVASTAVA, 1976; RAINA and BELL, 1978; SINGH and AWASTHI, 1981b etc). In *P. ricini* each corpus allatum is composed of four unequal lobes. The NCA I is quite distinct in the larva, less in pre-pupa and mid-pupa and completely reduced in the late pupa and adults (SINGH and AWASTHI, 1982). My observation on this aspect is in accordance with *P. ricini* described by SINGH and AWASTHI (1982) except that the each corpus allatum is composed of six unequal lobes. This lobular nature is well manifest throughout the entire life span of *A. mylitta*. Histologically, the corpora allata are similar to the other lepidopterans described earlier (GABE, 1966; NOVAK, 1966; SINGH and AWASTHI, 1981b, 1982).

**KOBAYASHI and YAMASHITA** (1959) as well as **ICHIKAWA** and **NISHIITSUTSUJI-UWO** (1959) and **NISHIITSUTSUJI-UWO** (1961) are of the view that the corpora allata of *Bombyx mori* and *P. cynthia ricini* act as neurohaemal organ for neurosecretory material of neurosecretory cells of the brain. **BOUNHIOIL** (1963) has also reported the storage of neurosecretory material in the corpora allata. Finally, **AWASTHI** (1976), have further confirmed that the lepidopteran corpora allata act as neurohaemal organ for the cerebral neurosecretion. He (with SINGH, 1981b, 1982) verified this fact in *Amsacta collaris* and *Philosamia ricini*. My observation on this aspect also falls in the same line and further confirms the earlier observations on the
neurohaemal nature of lepidopteran corpora allata. In *A. mylitta* the corpora allata are enclosed by a connective tissue sheath consisting primarily of a limiting acellular basement membrane. Most of the axons of neurosecretory cells of brain, except some that innervate the adjacent organs, terminate on to the corpora allata. These axons bend around the lobes of the corpora allata inside the acellular basement membrane. Here they make a network and store neurosecretory material. No neurosecretory material is observed inside the body of the corpora allata at any developmental stage. However, in some cases of adult individuals a small amount of neurosecretory material is observed inside the central lumen which is in fact the outer space formed as a result of the fusion of unequal lobes. Thus the peripheral part of the corpora allata acts as a neurohaemal organ. In *Canthecona furcellata* the corpus allatum is a fused mass. In this insect the neurosecretory material is brought to the dorsal aorta by the nervi corporis cardiaci I and the aorta acts as a neurohaemal organ. The dorsal aorta of heteropterans is well attributed as a neurohaemal organ by the earlier workers (DOGRA, 1967a,b,c, 1969; AWASTHI, 1972; SRIVASTAVA et al., 1974; TIWARI et al., 1975; SINGH and NARAIN, 1980, 1981 etc).