5.1 Effect of methoprene on neurosecretory cells of the brain and silk gland:

In the present study, structure, growth and the secretory activity of the neurosecretory neurons of the brain and silk gland cells in *A. assama* have been investigated under the influence of methoprene. Production of silk is the primary objective of the silkworm larvae and formation of cocoon henceforth is obligatory for completion of pupal stage. Biosynthesis of silk protein in the silk gland cells are regulated by a number of biochemical, nutritional and environmental factors. All these factors (extrinsic or intrinsic) extend their respective co-ordinations producing right metabolite at right time fulfilling the ultimate target of the silkworm larvae. In a biological system new materials are synthesized at the expense of the others. Likewise, it is true that silk is synthesized at the cost of the amino acids, pigments, minerals etc. obtained from the food plants by the silkworm larvae through biochemical pathways. From the biochemical analysis of the physiological pathways, it is becoming increasingly clear that synthesis and secretion of silk protein and then formation of cocoon take place under the influence of hormones released from the neuroendocrine centre. Thus, almost precisely, it can be said that the silk used for the formation of cocoon is the resultant product of hormonal co-action and interaction with the metabolic activities of the silkworm larvae taking place through definite biochemical pathways.

5.1.1 NSCs of the brain and effect of methoprene:

The cerebral neurosecretory system is the functional fabric of the neuroendocrine system (Carrow *et al.*, 1984). This system exercises its functions through the production of tropic hormones regulating the target organs which in turn govern the vital processes of the life cycle of the silkworm such as growth.
In this particular species of silkworm (A. assama) three groups of secretory neurons have been recognized on either side of the pars intercerebralis. Of these three groups, medial group of secretory cells which is composed of three different types of cells A-1, A-2 and A-3 is the largest group of cells. It has been noticed that all the three groups of cells during fifth instar larval development and pharate pupa (pre pupal) stage were found to be functional. However, the degree of their functional state is considered to be not the same; some cells contained finer granules (secretory), while some others had coarse secretory product indicating their different involvement in metamorphic changes. It was observed that the PF positive neurosecretion coincides with the active feeding period and with increased rate of spinning associated with gradual decrease in the staining intensity. These observations were in agreement to the work of Loeb and Dodson (1984) for H. virescens, a Lepidopteran insect and Krishnayya and Rao (1995) on H. armigera. As already mentioned, the lateral group of neurosecretory neurons gives rise to the long axon terminals (Fig. 1-6B, E). It may be assumed that the stage-associated production of neurosecretory materials/neurohormones occurs according to the stage-linked genetic instructions of these secretory neurons and the secretory materials are released via these axon terminals. It is very much certain that the various secretory neurons of the brain perform stage-related functions. The same type of cells at a particular stage elaborate a particular neuropeptide/neurohormone. Similar histological profiles of the cerebral neurosecretory system has been studied in a number of Satumiids, such as P. cecropia (Williams 1946, 1969), A. pemyi (Ichikawa and Nishitsuji, 1959) and P. cynthia ricini (Mitsuhashi, 1963). Report on histological aspects of neuroendocrine system of certain species of Aggrotti was reported (Choudhuri, 1990). Similarly, on the neurosecretory cells of brain of third instar larvae of D. obliqua was published (Singh et al., 1975). Mitsuhashi (1963) opined that the presence of three paired groups of NSCs viz. medial, lateral and posterior is a typical Satumiid char-
acter. Immunohistochemically, Mizoguchi et al. (1990) working on B. mori, reported the existence of two pairs of dorso-lateral neurosecretory cells in the brain and demonstrated them as the source of PTTH. The secretory neurons of the brain of the larvae of Lepidoptera are classified into two types, A and B (Kobayashi, 1957; Mc Leod and Back, 1963; Panov and Kind, 1963; Herman and Gilbert, 1965; Mitsuhashi and Koyama, 1969; Takeda, 1972, 1976; Glumac et al., 1979; Lucein et al., 1985; Muszynska-Pyta, 1986), 3 types A, B and C (Nanda and Roy, 1973; Awasti and Singh, 1981a, 1981b, 1982) and 4 types A, B, C, and D (Hinks, 1971; Singh and Arif, 1978, Awad, 1980). J.W. Trumen (1972) working on A. peryi demonstrated the structures essential for the biological clock are located in the dorso-lateral region of each cerebral hemisphere. Davis et al. (1998) working on M. sexta demonstrated that IA-1 neurons are comparable to putative clock cells of A. peryi. In addition to their neurohaemal projections in the CC-CA complex, the IA-1 neurons have processes extending to various areas of brain and also to the suboesophageal ganglion, indicating a capacity for extensive communications.

Attempts have also been made to identify the PTTH producing cells using various insects in the brain by different workers at different times. Working on M. sexta, Agui et al. (1979), and Gibbs and Riddiford (1977) demonstrated that the lateral group of secretory neurons are the source of PTTH. Akin observation was made by Mizoguchi et al. (1987) on B. mori and supported the existence of prothoracicotropic activities in the lateral group of neurosecretory neurons. However, much earlier, different conclusions were put forward regarding the source of PTTH by Wigglesworth (1940) in R. prolilxus, Hiruma et al. (1970) in M. brassicae, Steel (1978) in Megoura viciae that the source of PTTH is the medial group of neurosecretory cells. Working on H. cecropia by Van der Kloot (1960) and Williams (1948), on L. migratoria by Girardie and Reggi (1978) and on S. cynthia ricini by Mizoguchi et al. opined the involvement of both medial and lateral NSCs in the PTTH production. Recently, Tembhare and Barsagade (2000) put
forward another different view that the medial A type of cells perform stage-related functions in *A. mylitta*. In the first cycle of A. cells (during 6-9 days of larval life) ATH is synthesized (allatotropic hormone), where-as the second secretory phase (during 15-18 days) of A cells is corelated with the enhance activity of prothoracic glands (Pgs), suggesting the production of prothoracicotropic hormone (PTTH). Chemical analysis of PTTH, on the other hand revealed that there are two different types of PTTH with respect to their molecular mass. This observation is well studied in the three different species of insects namely, Bombyx, Manduca and Samia, and this support the two different sites of production of PTTH. Working on *B. mori*, Kataoka *et al.* (1991) reported that brain of *B. mori* produces PTTH (30 Kd) and another neuropeptide bombyxin (5Kd) that can activate prothoracic glands (Pgs) of Samia but inactive on Bombyx (Ishizaki *et al.*, Nagasawa *et al.*, 1986). In Manduca one big (27Kd) and the other small (7 Kd) peptides were identified (Bollenbacher *et al.*, 1984), and the source of PTTH has been shown to be produced by the pairs of lateral NSCs (O' Brien *et al.*, 1988).

In the present study, it has been observed that the brain, as in other insect in general, is connected to the suboesophageal ganglion (Sg) with a pair of large circumoesophageal connective (Fig.2-2A). This intimate anatomic profile between brain and suboesophageal ganglion may definitely be considered that they maintain neuronal relationship fulfilling the dynamic demands for the synthesis of proteins required for certain specific function(s) at some level of the larval stage. According to Ichikawa *et al.* (1995) in *B. mori*, the neurosecretory cells present in the suboesophageal ganglion are under the control of self neural mechanism and play specific physiological functions. They have also noticed specific morphological profiles of the three groups of cells when injected with Lucifer Yellow (LY).

In case of the methoprene treated-larvae, the nuclei of the lateral group of secretory cells are found to be bigger than that of the normal counterparts of the late stage of fifth instar and also of the spinning period. It may be assumed that the exogenous methoprene imparts the lateral secretory cells to maintain the
larva-like activities through the production of more amount of neuropeptides having prothoracicotropic activities resulting in increase cellular intensity. Furthermore, it may also happen that although there is increase production of PTTH but that increase rate of production is not followed by the same rate of secretion to the CC resulting in slowing down the activities of prothoracic glands. On the other hand, the exogenous methoprene of haemolymph imparts the prothoracic glands to maintain the juvenile nature for another few hours, resulting in delayed moulting. There are supporting literature in this context that the break down of prothoracic glands can be partially prevented by applying juvenile hormone (JH) suggesting that JH is necessary for maintaining the structural integrity of the prothoracic glands (Hazarika and Gupta, 1986). They opined that exogenous application of JH at the critical stage of the last instar nymph, one can prevent the degeneration of the prothoracic glands in B. germanica adultoids and allow them to persist as nymphal-sized prothoracic glands strongly indicate that JH is essential in preserving the integrity of the prothoracic glands.

Evidence exists for the control of corpus allatum functions by nerve stimulation and humoral factors. It is presumed that the former is the result of transmitter substances released from the neurons with cell bodies that lie in the brain and processes that synapse on corpus allatum cells. Doane (1973) demonstrated that action potential might release either stimulatory or inhibitory substances from the presynaptic membrane and these would interact with receptors on the post synaptic membrane. The signal would then have to be transduced through one or more steps to stimulate or inhibit the processes of hormone biosynthesis. In the case of humoral factors, various organs, including possibly the brain or corpus cardiacum, might release chemicals from axons of neurosecretory cells into the haemolymph; these would be transported to the corpus allatum cells, where they would either enter the cells or interact with membrane receptors to stimulate (allatotropin) or inhibit (allatinhibin) hormone synthesis (Doane, 1973). Tobe et al. (1977) demonstrated in S. gregaria, rapid declination occurs in the ability of the
glands to synthesize JH, if the nerve is cut that lead to the corpus allatum during the first gonadotropic cycle, as monitored by incorporation of metheonine methyl group during a short incubation in vitro period.

The biogenic amines are important neurosecretory products of the central nervous system in which the dopamine the most abundant while epinephrine and norepinephrine (Welsh, 1972) and octopamine (Dymond and Evans, 1979) occur in smaller amount. Certain biogenic amines, dopamine (DA), L-DOPA, norepinephrine (NE), epinephrine (EP), deoxyepinephrine (DE), metanephrine (ME), normetanephrine (NM), tyramine (TA), octopamine (OA), serotonine (5-HT) and melatonin (MT) have been tested on the apolysis of decapitated larvae (M. Idriss et al.). The apparent effect of NE, EP, 5-HT and MT on apolysis of decapitated *Ph. ricini* larvae has initiated a clearly defined concept for the function of the catecholamines and tryptophane derivatives on the induction of insect development.

Schooneveld (1974) described synaptic contacts between neurosecretory axons and axons containing dense-core vesicles of a size to be expected in aminergic cells in *L. decemlineata*. Thus there is ultrastructural evidence that median neurosecretory cells may be controled synaptically by aminergic neurons.

As has been reported in vertebrates (Weiner and Ganong, 1978) there is also a close association between aminergic and peptidergic neurosecretory cells in insects. NSCs and aminergic cells are grouped together within the central nervous system and neurohaemal organs contain axons belonging to both classes of cells. Octopamine, dopamine and norepinephrine have been identified within neurohaemal organs using radioenzymatic assays and fluroscent histochemistry.

Presumably the nerve endings are supplying stimuli to the cells to promote hormone synthesis. Working on *L. decemlineata* and *D. punctata* respectively by Schoonedveld et al. (1979) and Tobe and Stay (1979) suggested that administration of JH or its analogues results in feedback control of synthesis. Whether this is a direct effect on the corpus allatum, or operated through an effects of the
hormone on the brain or some other endocrine organs, is not yet known. Kramer and Law (1980) working on adult female *M. sexta* demonstrated that there appears to be no feedback control by hormone. Stay *et al.* (1980) and Friedel *et al.* (1980) suggested that the ecdysone, the moulting hormone can also inhibit hormone production under certain circumstances. Sehnal and Granger (1978) reported indirect evidence for a neurohormonal allatotropic factor from the brain of the wax moth, *G. mellonella*. They postulated that the JH synthesis in the corpus allatum is inhibited by neurotransmitter action and stimulated by allatotropin. Brain stimulation by means of an electrode, which presumably causes the neurosecretory cells to release their hormones, caused ovarian development into two insect species, and this was taken as evidence for an allatotropic neurosecretory hormone (Moulins *et al.* 1974). With the help of Glands implantation techniques on *M. sexta* last instar larva Bhaskaran *et al.* (1980) explored the factors responsible for the decrease in hormone production at this time. They oppined that an inhibitory neurohormone from the brain initiates the inactivation of the corpus allatum and this is followed by a neurally mediated inhibitory process later in the instar.

Thus the neurosecretory cells and the neuronal responses conveyed by them either in the form of action potential or through the elaboration of certain neuropeptides/hormones controls the biosynthetic pathway of juvenile hormone (JH). In addition to it, various humoral factors and hormones including JH itself control the biosynthetic pathway of juvenile hormone. Exogenous agents can also inhibit JH synthesis. Some of these are competitors, whereas others may be essentially prodrugs that operates only after metabolic alteration at the site of their action.

5.1.2 : Cells of the silk gland and effect of methoprene :

The light microscopic investigations of structure, growth and secretory activity of silk gland of untreated and treated larvae are made in detail during the fifth larval stadium and during spinning period. It has been noticed that in both the cases, during the early part of the fifth instar larval development (0 day - 96 hrs), rapid nuclear activity of the gland cells has been noticed compared to the later
part of the fifth instar (96-192 hrs). This may explain why the nuclear to cytoplasmic ratio is more at early part than that of the later part of the fifth instar (Fig.1-18). Most probably, during the early part of the fifth instar, rapid synthesis of DNA and RNA takes place and as a result, increased accumulation of nuclear materials, staining intensity of the nuclei increased. The observations on ultrastructural basis of these structural changes and the secretory activities of the gland cells of untreated larvae will be discussed in detail in the later section of this chapter (5.2.2). The increased nuclear activities of the gland cells during the early part of the fifth instar (0-96 hrs) can be attributed to the increased production and secretion of sericotropic hormone from the neurosecretory cells of the brain. It can be assumed that due to the increased release of the sericotropic hormone in the early part of the fifth instar the nuclear activities of the silk gland increases resulting in increased synthesis and secretion of silk protein in the gland.

In methoprene-treated larvae the size of the nuclei of the gland cells are larger than that of the normal with increased staining intensity (Fig. 1-10C,1-17B). Larval weight and shell dry weight of the treated larvae are also recorded to be more than that of the untreated counterparts (Fig.1-19). Supportive observations were made by Sehnal and Akai (1990) and they reported that when water-emulsion-containing methoprene was sprayed on silkworms on 2nd and 3rd day of the last instar larvae, the length of the instar was extended by 2-3 days and enhanced the silk yield by 5-10%, some times by upto 30%. In the later part of the fifth instar (96-192 hrs) in both the treated and untreated glands gradual increase in the accumulation of silk in the gland's lumen was observed. This observation is consistent with the work of Biswas (1997). The nuclei of the gland's cells from this stage gradually became smaller and narrower, and towards the end of spinning (72 hrs of spinning period) were not visible. But the rate of degeneration of the nuclei of treated larvae seemed to slower. Similar was the case even in the late spinning period (48-72 hrs.) (Fig.1-16, 1-17). This increased stainability and retention of conspicuous nuclei at the later part of spinning of the treated larvae
may be attributed to the effect of methoprene. Cellular atrophy and degeneration of the nuclei at the later part of the spinning period are observed in the untreated earlier than the treated (Fig. 1-17). This delay in the degenerative activities of the gland must be due to the juvenile effect of exogenous methoprene.

Juvenile hormone controls the type of development pattern and is realized in the silk gland. The growth and function of the silk gland depend upon the availability of nutrients to the silkworm from the food plants during larval stage, and after the cessation of feeding from the body reserves. Development of silk gland in the early part of the last larval instar (0-96 hrs) is characterized by the regular DNA replication that can account for the increase nuclear cytoplasmic ratio (Tashiro et al. 1968). But during this time there were moderate level of RNA transcription and protein synthesis. Low level of JH during ecdysing last larval instar of both B. mori and G. mellonella (Rembold and Sehnal, 1984) briefly restrains the function of silk gland to the level of previous instar but during the following period of JH absence, the silk gland develops according to a new metamorphic pattern. This includes higher growth rate, increase silk production and certain changes in silk quality. This pattern can be reverted to the larval type with exogenous JH until about 24 hrs (partial reversion until about 96 hrs) of the last instar in the B. mori and until 60-144 hrs. of the last instar G. mellonella (Sehnal and Akai, 1989). The complete loss of silk gland sensitivity to JH coincides in both species with the termination of DNA synthesis.

Akai and Kobayashi (1971) demonstrated that the prolongation of the feeding period in the JH-treated last instar silkworms is associated with additional body growth. The growth of silk gland is curbed with JH, but over the extended developmental time, the silk gland reach the considerable size. According to Kurata (1978), the content of DNA in the silk glands of the treated larvae may grow twice as high as in the control. There are reports that exceptionally large silk glands develop in the dauerlarvae that are exposed continuously to JH. In the silk gland of normal last instar larvae, the DNA synthesis ceases in about 4 days
(96 hrs), whereas, it continues at a low level with some fluctuations for nearly as long as dauerlarvae survive (Akai et al. 1973).

The silk gland of JH-treated larvae are observed to be larger enough compared to the untreated counterparts and are induced to a high rate of silk production when JH disappears and the larvae prepares for pupation. This shift can be considered as the most important effect of JH treatment in practical sericulture. Other than the size, these JH-induced giant larvae are like the spinning controls in terms of ratio of silk gland weight to body weight (Daillie, 1979), synthetic rate of DNA and RNA in the silk gland (Kurata, 1978; Daillie, 1979), in the translation efficiency of available mRNA (Kurata and Daillie, 1978) as well as the ultrastructure of the silkworm (Akai, 1982).

Application of methoprene results in increase in total body weight and wet weight of silk gland of the treated compared to the control. Likewise, it also results in increased size and weight of the cocoon than their control counterparts. Light microscopical observation of the posterior silk gland shows that the nuclei of the treated-gland cells remain conspicuous even at the late stage of spinning. The process of degeneration of the cells of the treated larvae is noticed to be slower and lower compared to the control. This tendency of restoration of cellular activity even at the late stage of the fifth instar may be attributed to exogenous JH (Akai, 1990). It was also observed by Biswas (1997) in muga silkworm.

Since the last instar differs from the previous instar primarily by the lack of juvenile hormone (JH), it is reasonable therefore to assume that JH control the pattern of silk gland growth (Akai, 1965). Lack of sufficient dose of JH converts larval moult to pupal moult and the silk glands are stimulated accordingly (Nishimura, 1957; Akai, 1965). By the beginning of the last larval instar, when the insect larvae attains a certain size and weight, its brain is reprogrammed from the larval pattern to neurohemal regulation to the metamorphic pattern, which includes absence of JH (Sehnal, 1985). The growth and functional properties of the silk gland and other tissues of silkworm are totally dependent on the supply of nutrient from the diet.
Thus application of exogenous JH or analogue (methoprene) causes reversion of the pattern to the larval type resulting in increased silk production and prolongation of larval duration.

5.2 Study of the ultrastructure of different types of neurosecretory cells of the brain and cells of the silk gland under scanning and transmission electron microscope:

The discovery of the brain's competency for multiple modes of chemical signaling, ranging from the use of locally acting neuroregulators to the use of neurohormones has removed the sharp border line formerly separating the two systems, the nervous system and the endocrine system. It becomes increasingly clear that the two system work in coordination with one another (Scharrer, 1987). The ultrastructural studies of the subcellular organization of the neurosecretory cells of the brain can explain the distribution and its versatile roles in neurochemical signaling. In this context, the membrane bound electron dense secretory materials (Fig. 2-10B) passing through the axon terminal of the different types of neurosecretory cells located at various regions in the protocerebrum can be used as tools for elucidation of activity pattern of those cells at that stage. The changing cellular ultrastructures such as ER from lamellar type to vesico-tubular type, Golgi bodies from cisternae type to vesicular type, increased mitochondrial size with developed crista mitochondialis and count, increased appearance of rER compared to the previous stage of the insect life cycle can definitely be considered for the determination of activity range and pattern of that cell or neurosecretory neuron. These stage-dependent changes of the cellular ultrastructures in the neurosecretory neurons of the brain of this particular silkworm (A. assama) are discussed in relation to the concomitantly changing cellular ultrastructures of the silk gland in relation to the silk protein biosynthesis in the present study.

5.2.1 Ultrastructure of brain:

Brain being the vital part of the central nervous system does play important role in the neuroendocrine regulation of biological activities controlling growth,
development and reproduction. The paired group of secretory neurons in the protocerebrum of insects are analogous to the hypothalamic neurosecretory centres of vertebrates (Scharrer, B., 1987). Their axon bundles (NCC) enter the corpus cardiacum which, in its neurohemal capacity, corresponds to the posterior lobe of the pituitary gland. In addition to the neurohormones of the cerebral origin, the corpus cardiacum delivers products of its intrinsic neuroglandular cells into the hemolymph. Some axonal projections from corpus cardiacum enters the adjacent corpus allatum. According to Scharrer (1987), this important non-neural endocrine insects gland, the third component of the brain-cardiacum-allatum system can be considered the analog of adenohypophysis of vertebrates.

Knowles (1969) demonstrated two types of neurosecretions, viz. A fibres with spherical electron dense vesicles of over 1000 Å in diameter producing peptide hormones, and B. fibres showing smaller irregular vesicles smaller than 1000 Å in diameter containing mono-amines. Electrophysiological studies to elucidate the neural nature (conduction property) of the cells have shown that there is a long duration of action potential from neurosecretory fibres, nearly 10 times that of adjacent neurons (Bern and Yagi). They also show low conduction velocity. Possibly these two help to maintain prolonged and continuous release of neurohormone from the terminals.

In the present study, it has been observed that the activities of the cellular ultrastructures immediately after last larval moulting of the NSCs were very less compared to the later part of the instar. The marked increase in the number and size of rER, mitochondria, towards the later part can be assumed that, immediately after last larval moulting the various type of neurosecretory cells get actively engaged with the synthesis of neuropeptides/neurohormones essential for rapid synthesis of silk protein and for the process of pupation. Increased appearance of large sized secretory materials in the secretory neurons (Fig.2-7A) indicates the importance of that materials in neurochemical signalling. These released secretory material may be directly released in to the haemolymph for the stimulation of target tissues or may be collected into the neurohaemal organ, corpora cardiaca.
In the corpus cardiacum, further processing of these neurosecretions perhaps takes place and periodically released into the haemolymph following the genetic instruction of the cells of the store house or after getting feed back response from the target tissues. Change in the type and number of rER, mitochondria and Golgi complex could be considered as the index for the determination of the neurosecretory activities. It has been observed that the secretory neurons in the very early part were characterized by the presence of lamellar ER. Golgi complex were of cisternae type, and the mitochondria were not many in number and were spherical in shape. But in the later part of fifth instar the lamillated cisternae transform into vesicullar or tubular type. Golgi cisternae transformed into vesicular type filled with secretory materials, mitochondria became elongated with increased number of cristae mitochondriales. These changing ultrastructures observed in the later part of the fifth instar can be attributed to the increased neurosecretory activity of the cells. The appearance of secretory materials in the ER cisternae (Fig. 2-8A) is indicative of synthesis of neurohormones. Thus, it may be suggested that the NSCs of the brain maintain coordinations through neurochemical signaling with the vanous endocrine and exocrine tissues.

One of the interesting ultrastructural observations is that the appearance of increased number of NSCs, (Fig.2-6A, 2-7B) is probably related to the requirement of increasing demand for hormonal secretion for growth, including silk protein biosynthesis, development and ecdysis. Simmilar type of observations were made in *S. gragaria* (Highnam, 1961) and in *A. lineolatus* (Ewen, 1962b). They strongly suggested the existence of cyclic activity among the secretory cells. Singh and Gangrade (1988) in *D. obliqua*, demonstrated the cycle of appearance of cells, their filling and release of neurosecretory materials and ultimate invisibility is repeated in each larval instar untill transformation to pupa. Moreover, they reported that the axons of the MNCs do not cross over from one hemisphere to another to form the chiasma in the larval instar which is in contrast to the observation made by Tembhare and Barsagade (2000). In the present study, no such cross over forming chiasma by the axon terminals coming from two hemisphere have been observed in this particular silkworm (*A. assama*).
5.2.2 Ultrastructure of silk gland:

Electron microscopic observations of the subcellular organization and their changing pattern in the posterior silk gland as the last larval stage progresses; it stands in good agreement with the light microscopic observations. The increased nuclear activity of the gland cells during the early part of the fifth instar was very much visible under electron microscopic investigation. The increased amount of condensed chromatin materials (C) were reported in the early part of the fifth instar (0-96 hrs) than the later part (96-192 hrs). These chromatin blocks detected in the nucleus may explained that there may be rapid synthesis of DNA and RNAs (Tanaka et al., 1968). This increased rate of synthesis of nuclear materials may be assumed that immediately after fourth moultion, rapid adjustment of subcellular constituents in the gland cells necessary for the early part of the cells occurs. Simillar observations were made by Tashiro et al. (1968) in B. mori. They reported an increased amount of DNA and RNA during the early part (0-96 hrs) of the fifth larval instar. Since the number of cells remain constant through out the post embryonic development (Tanaka, 1928 and Ono, 1951), these observations strongly suggest that the various subcellular constituents such as nucleus, ribosomes, ER, GB and other structures of some individual cells proliferated rapidly while that of others they were not so. This is further supported by the ultrastructural observations of the silk gland, in which the cytoplasm of the gland cells was dominated with free ribosomes large sized nuclei and electron dense chromatin materials immediately after fourth moultion.

That the transformation of ER from lamellar to vesico-tubular form apparently proceeded in parallel with the increase in the rate of silk protein biosynthesis. Therefore, it may be suggested that transformation is intimately correlated to the biosynthesis of fibroin on the one hand and increased accumulation of fibroin granules in the cell cytoplasm in the later stage of the fifth instar on the other hand. It is very much probable that the fibroin thus synthesized in the ribosomes attached to the ER is transported to the intracisternal space of the ER which results in the distension of the intracisternal space henceforth, results in the transformation of ER into vesico-tubular type. The fibroin granule accumulated in
the intracisternal space of the ER is supposed to be transported to the Golgi vacuoles via Golgi vesicles or 'transition elements' (Jamieson and Palade, 1966).

At early stage of the fifth instar, the Golgi apparatus was poorly developed and vesicular forms were not found. With the transformation of ER from lamellar to vesicular type, Golgi complex also starts to develop into vesicular forms. Golgi vacuoles, when become fully packed leave the Golgi region as fibroin globules (Tashiro et al., 1968) move towards the glandular lumen and then secrete their content into the luminal space. Radiographic observation also suggest that when glycine $^3$H is injected into fifth instar larvae it is accumulated into fibroin globules (Akai and Kobayashi, 1965). Fibroin globules were transported to the apical cell pole by radial system of microtubules (Fig. 2-24A) that become confluent with system of circular microtubules (Fig. 2-22B) (Sehnal and Akai, 1990).

Mitochondria having circular and oval profiles predominate in the cytoplasm throughout the early part of the fifth instar and increase in number and size towards the later stage suggesting the increased metabolic activities of the gland cells. Microtubules can be detected in the specimens which are fixed in glutaraldehyde (Ledbetter and Porter, 1963). The secretion of fibroin culminates in the increase of the number of structures of the later period of fifth instar (Sasaki and Tashiro, 1976; Sasaki, 1977) and become more complicated by the formation of cytoplasmic processes (Sasaki and Nakagaki, 1980).

From the ultrastructural studies of NSCs of brain and cells of the silk gland, it is suggested that there exist a direct functional relation between these two tissues which is realized in the biosynthesis of silk protein. The different types of secretory neurons synthesize their stage specific neuropeptides or neurohormones and exercise their respective roles over the body including the synthesis of silk protein. The increased activity of the silk gland cells as observed in the present study during the later half of the last larval instar (96-192 hrs) might be due to the trophic effect exerted by the sericotropic hormones (STA) released from some specific neurosecretory neurons. Although the precise cell type for the production of the sericotropic hormone is not known, it is assumed that the secretory neurons responsible for the production of the hormone become most active during the
fifth instar larval development and enhances the production and secretion of silk protein fulfilling the ultimate target of the larvae.

5.3 Study of the effect of secondary host plant extract (*Mejankari, L. cubeba, Pers*) along with normal diet on the peptide/protein profiles in the brain and in the silk gland of muga silkworm *A. assama*

Factors like plant growth hormones, phytohormones, dietary supplements at specific levels play a vital role in establishing pattern of growth and reproduction in phytophagous insects. There are reports that the dietary supplementations of abscisic acid (ABA), gibberlic acid (GA 3) on kinetin affect the reproduction, egg viability and longevity in grass hopper, *A. elliotti* (Visscher, 1982) and carnivorous Dipterans, *S. bullata* (De man *et al*., 1981), Kamada and Ito (1984) demonstrated the influence of indole acetic acid (IAA) and GA 3 to silkworm and reported increased larval and cocoon weights. Common phytohormones like indole butyric acid (IBA), indole propionic acid (IPA), IAA and GA 3 were suplemented to silkworm and reported improved precocooning and post cocooning parameters (Magadum and Hooli, 1989, 1990, 1991a & b). Santakumari *et al*. (1989) reported increased larval weight, cocoon weight and fecundity in *B. mori* fed with GA 3 sprayed mulberry leaves.

In the present study, experiments were planed to demonstrate the effect of mejankari (*L. cubeba*) leaf extract on the protein profiles of the brain and silk gland along with the normal diets. It was observed that there were some additional peptide/protein bands (Fig.3-1, 3-2) in the treated group compared to their counterparts of the control. The appearance of these additional peptides/proteins bands in both the tissues (brain and silk gland) of the treated group may be attributed to the direct or indirect effect of this secondary host plant extract. Unni *et al*. (1997) demonstrated higher soluble carbohydrates in the leaves of mejankari than the primary host plants such as som (*P. bombycina*). It is reported by Visschar (1982) that the application of phytohormones alter the rate of DNA synthesis. The appearance of additional bands on both the tissues in comparison to the control found in the present investigations, it may be due to the involvement of the plant extracts directly or indirectly in altering the rate of DNA synthesis and
as a result protein synthesis. Nair (1997), Saikia and Goswami (1997) working on *A. assama* demonstrated that the mejankari-fed cocoons were much smaller than som fed cocoons. But the S/R (silk ratio) of the mejankari fed cocoons were much higher than that of som-fed cocoons. They opined this is due to the fact that the pupa of mejankari fed cocoons are smaller than the som fed cocoons.

Nakanishi *et al.* (1968) during the course of a chemical study on the leaves of *P. nakaii*, a traditional medicine for cancer in Formosa isolated three constituents and named the compounds ponasterone A, B and C having strong moulting hormone activity. From the roots of *A. fauriei* Takemoto *et al.* (1968) obtained two constituents and named one as ecdysterone 2 and the other was assumed as a positional isomer designated as inokosterone and possessed potent moulting hormone activity. These were the first ecdysones recognized in the plant kingdom. These discovery raised the possibility that the other plant sources might contain similar substances. After extensive screening it was found that substances having the hormone activity were widely distributed in plant sources. Since ecdysones are polar substances for the extraction of ecdysones polar solvant like ethanol, aqueous ethanol, methanol, aqueous methanol were commonly utilized. In the present study, the ethanol extract of the said plant were applied to the silkworm to see the effect on the electrophoretic protein profiles of the brain and silk gland.

Since insects lack the capacity for the de novo sterol biosynthesis, they in general require a dietary or exogenous source of sterol for normal growth, development and reproduction. In phytophagous insects, exogenous phytosterol such as sitosterol (Kaplanis *et al.*, 1975), sigmatosterol and campesterol under go side chain dealkylation at the C-24 position to afford cholesterol (Galbraith *et al.*, 1971) which serves as a precursor for ecdysone and is an important composition of the cell membrane.

Horie, 1962), nutritional requirement of silkworm larvae (Ito, 1967, 1972), artificial
diets for silkworms (Yoshida et al. 1960; Fukuda et al. 1960; Yokoyama, 1963-64)
and phytosteroids with strong moulting hormone activity (Takemoto et al., 1967)
contributed much to the development of sericulture.

On the basis of the above results and discussion it is clear that the incor-
poration of nutritional supplements either in the form of diet or any other exogenous
incorporation play significant effect on the protein moiety of the brain and silk
gland. Takemoto et al. (1967) isolated insect moulting hormone from mulberry
leaves and many other workers as already discussed in above suggested that the
phytohormones present in the host plants of the phytophagous insects play a
significant role in the biology of the insect. In the present study it may be assumed
that the changes in the protein profiles of this two tissues is either due to the
additional nutrient supplements present in the plant extract or due to hormonal
influence present in the said extract.