DISCUSSION
The recent literature pertaining to insect hormones has been reviewed in several monographs and reviews (Slama et al., 1973; Thomsen, 1975; Highnam and Hill, 1977; Keeley, 1978). Production and release of most of the insect hormones is dependent upon the nervous activity which was initially suggested by Kopec (1922) in the gypsy moth Lymantria dispar. Thus the regulation and synchronisation of insect development is controlled by the neuroendocrine system which represents a functional unit consisting of the nervous system and the endocrine glands. Its basic structure in insects has many features which are common to higher animals including mammals (Scheurer, 1970). The four major components of the system are neurosecretory cells in the brain, the corpora cardiaca, the corpora allata and the thoracic glands. The most important part of the system are the neurosecretory cells in the brain. They are responsible for transmission of neural messages to the other three parts (endocrine glands) as well as other 'target tissues'. The first effect of this function of the neurosecretory cells is to stimulate secretion of hormones from the endocrine glands like corpora allata and thoracic glands (Slama, 1973; Highnam and Hill, 1977). The corpora cardiaca also produce their own intrinsic hormones (Müller and Engelmann, 1968; Steele, 1980) but their major function in most insects is to store and release neurosecretory hormones from the brain i.e. to act as the neurohaemal organs (Müller and Engelmann, 1968; Saini, 1971; Highnam and Hill, 1977).
In keeping with its diverse metabolic functions the fat body is a target tissue for all the major types of insect hormones: neurohormones, corpus allatum or juvenile hormone and thoracic gland hormone or ecdysone or ecdysone. The hormones fall under two categories of action so far as the fat body of insects is concerned. Most of the neurohormones affect homeostatic functions such as the balance between stored and mobile metabolites and the basal metabolic capacities of the tissue. In contrast, some neurohormones, juvenile hormone and ecdysone affect specific metabolic functions related to growth, metamorphosis, development and reproduction (Highnam and Hill, 1977; Keeley, 1978). Fat body cells undergo metamorphosis in response to hormonal stimuli just as do the epidermal cells and imaginal disc cells. Two basic patterns of metamorphosis occur in the fat body. In the simplest pattern as found in mosquitoes (Clements, 1963) and (Banerjee, 1981 a, b) the immature fat body cells remain intact and persist into the adult with little change except possibly a restructuring of the cytoplasm. In the more complicated case of endopterygotes, the larval fat body cells undergo histolysis and an adult fat body forms a new from the precursor cells as in Phormia (Orr, 1964); Drosophila (Butterworth and Bodenstein, 1965); Sarcophaga (Benson and Benson, 1966). Calliphora (de Priester and Vandevoort, 1979) etc. These diverse hormonal regulations as well as a variety of metabolic functions make the fat body a useful research tool not only from the point of its application as a model for
studies on general biological phenomenon but also from the point of insect specific processes and their potential applications for innovative pest control methods.

Keeping the above into consideration and after reviewing the relevant literature as already described, the present investigations were undertaken. Though there have been studies on the neuroendocrine regulation of fat body metabolism in some orthopterans and dipterans, no work has been done so far on the grasshopper of the genus *Poekilocerus pictus* and the local species of the fleshfly *Sarcophaga lineatocollis*. The adult fat body from the former has been studied from the point of view of endocrine regulation with development of both sexes of the adult insect whereas from the latter to elucidate endocrine regulation with reference to larval growth and metamorphosis since this process in a holometabolous species provides unique opportunities for studies of the action of hormones at a cellular level (Slama et al., 1973). The adult fat body of this fly has already been extensively studied in this laboratory (Agarwal, 1976).

Numerous observations during the study for evaluation of effects of various endocrine environment created by experimentation have revealed that the neuroendocrine system attests to its differential regulatory activity on this tissue in both the insects. Moreover, some very interesting results, particularly in the larval fat body of the holometabolous *S. lineatocollis* have been obtained.
The gross anatomy of fat body in adult *P. pictus* as well as larval *S. lineatocollis* is basically similar i.e. fat cells consisting of globular configurations, with central and peripheral globules, around the nucleus as also described by Nair and George (1964) in *Anthrenus* larvae, Odhiambo (1966) in *Schistocerca*; Banerjee (1971, 1981 a, b) in *P. pictus*; Agarwal (1976) in adult *Sarcophaga* etc. The possible origin of peripheral globules from mitochondria in *P. pictus* has been described in detail by Banerjee (1971, 1981 b). These have been variously named as dark bodies (Ishizaki, 1965), acid phosphatase depots (Benson and Benson, 1966), Protein droplets (Locke and Collins, 1965); albuminoid granules (Walker, 1966) etc. In larval fat body of *Sarcophaga* as found in the present study, the fat cells show similar configurations of globules described above at early (feeding/moulting) stages. However, in late larval (wandering) and pupal stages spheroidal bodies appear which increase in size as well as in number with age up to pharate adult stage. This has also been shown by Slama *et al.* (1973); Thomsen, and de Preister and Vandermolen (1979). The larval fat cells show intercellular membranes like the adult fat cells of *Sarcophaga* itself (Agarwal, 1976) and *P. pictus* but in pharate pupal and pupal fat cells these intercellular membranes are obliterated and the fat body appears as a loose mass of cells. The spheroidal bodies which contain RNA, Protein and also Glycogen and Lipids in *S. lineatocollis* larval have been named storage protein granules or microvesicles, proteinaceous spheres, protein droplets etc. by
various authors like Locke and Collins (1965); Price (1973); Keeley (1978); de Priester and Van der Molen (1979) etc. The author assumes that some peripheral globules of the early stages fuse to form these bigger globules. Thomson (1975) has also shown that fusion of microvesicles with each other results in appearance of larger protein spheres which conform to the findings of Locke and Collins (1965). In contrast to the situation in fat cells of S. lineatocollis larvae, the peripheral globules in P. pictus contain only RNA, protein and glycogen and occasionally some phospholipids (Banerjee 1981 a) whereas the central globules alone stain for lipids. In S. lineatocollis larvae central globules are conspicuous in early stages but coalesce with each other forming irregular vacuoles in the later stages of development, showing onset of histolysis. Also, here they are never preserved with any fixative used. Very large vacuoles not preserved by most fixatives have been reported by de Priester and Van der Molen (1979) in Calliphora and Phormia regina. These have been termed water vacuoles by Agarwal (1976). Labour (1974) has described these vacuoles in Leptinotarsa as probably containing protein from coalescent smaller vacuoles. Von Gaudecker (1963) have stressed the role of these vacuoles in the formation of lipid droplets in Drosophila and it is assumed this might be the case in S. lineatocollis larvae too. The increase in size and number of these big spheroidal bodies filled with metabolites after 5-6 days is due to the beginning of larval pupal transformation by diminished synthetic and increased
storage properties, showing loaded cells as also reported by Tojo (1981).

In *P. pictus*, on the other hand, the nymphal fat cells persist in the adult as reported by Banerjee (1981 a) and there is no transformation like histolysis and storage as mentioned above for *S. lineatocollis*. The fat body of larval *Sarcophaga* have very big cells and nuclei in comparison to these of *P. pictus* as well as adult *S. lineatocollis* (Agarwal 1976), the nymphal fat cells of *P. pictus* are also bigger than those of the adult (Banerjee 1971); still they are smaller than cells of *S. lineatocollis* larval fat body which accounts for the majority of the larval body weight as also reported by (Pelt Verkuil 1979) for *Calliphora*. Thomsen (1975) has established that growth in certain tissues by cell enlargement is a general adaptation in holometabolous insects to provide for continuity of cell function without the disruptions necessitated by cytokinesis. According to Thomsen (1975) the significant factor in development of this growth pattern in the fat body is the necessity in this tissue for very large-scale protein synthesis during a short feeding period in larval life.

In order to give a better understanding of the changes induced by various experimentations in the activities of the fat body, the results have been discussed one by one by comparing the changes with the situation found in control insects and correlating the changes wherever possible in the two groups. Since in larval insects, classical gland removal
and reimplantation experiments as performed on *P. pictus*, are not always possible, the equivalent of gland removal has been obtained by ligaturing off the part of the body containing the system as given by Highnam and Hill (1977) and administering hormones topically or by injection.

In *P. pictus* the fat cells along with their nuclei and nucleoli increase in size with age. In the present work growth has been defined as an increase in cell size as in *Drosophila* (Butterworth 1967) and may be due to increase in the background cytoplasm or due to enormous lipid deposition. The cell growth is not always proportional to nuclear and nucleolar growth (Banerjee 1981 b). The cells increase gradually upto 20-22 days in females. Nucleoli enlarge between 7-20 days showing rapid synthesis of protein at this period. Banerjee (1983) has shown formation of nucleolar extrusions at this time of development. Glycogen and Lipids are also elaborated during this period. Before egg laying (30-32 days) the metabolites are depleted and fat cells become smaller. The RNA intensity is cyclic in nature as also mentioned by Hill (1966) in cockroaches and Banerjee (1971, 1981 a) in *P. pictus*. There is a gradual increase in RNA intensity during the time ovary is developing and oocytes are taking maximum amount of protein as has been shown by Banerjee (1981 a) and Sahai and Banerjee (1984). This is the time when nucleolar extrusions migrate to cytoplasm increasing its RNA content as shown by Walker (1965), Butterworth *et al.* (1965), Osborne *et al.* (1968) Pemrick and Butz (1970), Chakko and
Sarojini (1979), Banerjee (1981 a) etc. During egg laying both the metabolites are reduced. Likewise, glycogen and lipid deposition is also cyclic being less in initial stages of development and during egg laying. The male fat cells and nuclei have been found to be bigger than those of females in the newly emerged stage. They increase in size upto 7 days and then shrink. Banerjee (1971, 1981 a) has described this difference in male fat cells upto 7–10 days. RNA and protein are less elaborate than in females most probably due to their slower rate of utilisation. Glycogen is more in male fat cells than females and just the opposite happens with lipid deposition which is more in females fat cells. This result conforms to that given by Banerjee both by quantitative methods (1977) and histochemical methods (1981 a) in P. pictus.

Thus P. pictus fat cells show a sexual dimorphisms. This dimorphism with reference to glycogen and lipid deposition has also been reported in Drosophila by Butterworth and Bodenstein (1968). According to Banerjee (1977, 1981 a) greater amount of glycogen in males may account for testicular development and perhaps for more muscular activity whereas that of lipids in females is due to their utilisation as yolk precursors for oocyte development. The difference between fat body metabolism in males and females may also be due to quantitative differences in their diets in other insects (Orr, 1964; Flint et al., 1971).

Allatostectomy results in distension and hypertrophy of fat cells both in male and female P. pictus. This has also been reported by Pfeiffer (1945) in Melanoplus, Orr (1964) in
Phormia, Odhiambo (1966) and Walker and Bailey (1971) in male
Schistocerca etc. Elliott and Gilliott (1976) have shown
increase in the weight of the fat body of *Melanoplus* following
allatectomy. Nucleoli are also hypertrophied in *P. pictus* upto
15 days in females and 7 days in males. Nuclear chromatin is
clumped showing inactive nature of the cells from which material
is not released since very intensely stained pyroninophilia has
been observed filling up all the space in the cytoplasm in
addition to the peripheral globules. The same is the case with
glycogen and lipids which accumulate in fat cells after
allatectomy, proteins, however, are sparsely distributed.
This may be due to the fact that the synthesis of this
metabolite is dependent on the hormone secreted from the
corpora allata (CA) in the absence of which synthesis is
retarded and whatever amount is present before the operation
is not released either. The effect is most obvious during
15–30 days in females (egg maturation period). Vanderberg
(1963 b) has shown in *Rhodnius* an inhibition of the incorpo-
ration of uridine into RNA after allatectomy. It seems likely
therefore that CA hormone influences the synthesis of RNA and
according to Vanderberg (1963 b) it is the messenger RNA
which obviously helps in protein synthesis. Large patch like
deposition of RNA in fat cells of allatectomised female as well
as males perhaps indicates the non-utilisation of this metabolite
for protein synthesis. The results conform to those of Hill
(1965) for *Schistocerca gregaria*, Engelmann and Penney
(1966) for *Leucophaea maderae*, Pratt and Davey (1972) for
Rhodnius prolкусus, Elliott and Gillott (1977) for Melanoplus
sanguinipes etc., but are different from those of de Loof and
de Wilde (1970) gave shown increased protein in allatectomised
Locusta migratoria, Leptinotarsa decemlineata and Phormia regina
respectively may be that allatectomy has variety of effects
depending upon the species studied. Saini (1971) and Banerjee
and Sahai (1983) have shown that vitellogenesis does not take
place in allatectomised Poekilocerus pictus and there is no
protein synthesis and release in the fat body cells as
interpreted from histochemical studies. Thus in this grass-
hopper CA stimulates Protein uptake in the oocytes and have
no direct effect on fat body protein synthesis as in
Oncopeltus fasciatus (Kelly and Davenport, 1976). Melanoplus
sanguinipes (Elliott and Gillott, 1976) and Calliphora
erythrocephala (Thomsen and Thomsen, 1978) etc. Some protein
synthesis is also stimulated by neurosecretory cells as will
be discussed later.

The most profound effects of allatectomy is accumulation
of enormous amounts of lipid and glycogen in the hypertrophied
fat cells of female as well as male P. pictus. These effects
are less obvious when the individuals are allatectomised at a
late age. The accumulation of glycogen, lipid and protein as
non-utilised yolk precursors in allatectomised female fat cells
has been reported by Slama et al. (1973) but in P. pictus
proteins do not accumulate as already discussed. Banerjee
(1977 b, 1982) has shown that in gonadectomised P. pictus of
both sexes, unlike lipids, glycogen and proteins accumulate showing non-utilisation of these metabolites. The CA of such individuals are hypertrophied and inactive (Banerjee 1977 a) as also shown by Thomsen and Hamburger (1955) and Strangeways-Dixon (1961) in Calliphora and Vogt (1968) in Drosophila. Accumulation of glycogen in fat cells either in the absence of gonads or of the CA, shows that CA alone are not responsible for deposition and depletion of this metabolite and there must be some other factor also.

Accumulation of lipids in the fat body of allatectomised P. pictus is a result obtained by other workers also in different insects like Pfeiffer (1945) in Melanoplus differentialis; Thomsen (1949) in Calliphora, L'Hélias (1953) in Dixippus, Bodenstein (1953) and Vroman and Kalpanis (1965) in Periplaneta; Orr (1964) in Phormia; Minks (1967) in Locusta and Banerjee (1979) in P. pictus. The fat body lipids are normally mobilised and deposited in the developing oocytes as yolk material (Pfeiffer 1945, Sahai and Banerjee 1984) Keeley (1978) has proposed two theories to explain the interaction between the CA, the fat body and the ovaries. First, the CA directly regulate the fat body lipid metabolism and their absence causes an imbalance (Pfeiffer, 1945; Orr 1964 a, b; Vroman and Kaplánis 1965) in the fat body metabolism. The second proposition is that the CA increase the permeability of the ovaries to the metabolites sequestered from the fat body (Highnam, 1964; Highnam et al., 1967; Davey and Huebner, 1974; Kambysselis and Heed, 1974; Keeley and Davenport, 1976).
Banerjee (1977 b, 1982) has shown by quantitative as well as histochemical methods and gonadectomy of female or male \textit{P. pictus} results in depletion of lipids unlike proteins and glycogen in fat body and hypertrophy of its cells. The results are similar to those found in other female or male insects as given by Pfeiffer (1945) in \textit{Melanoplus}; Bodenstein (1947), Doane (1961) and Butterworth \textit{et al.} (1968) in \textit{Drosophila} Thomsen and Hamburger (1955) in \textit{Calliphora}, and Odhiambo (1966) in \textit{Schistocerca} and \textit{Sialiaestava} (1973) in \textit{Syntomus}.

All the above results including those in \textit{P. pictus} itself show that in the absence of gonads CA become inactive and the fat body lipids are reduced, most probably because they are not synthesised. On the other hand, when CA are removed fat body is inactive and hypertrophied accumulating lipids since they are not sequestered by the gonads. This definitely indicates a feed back mechanism between the CA fat body and the gonads, not only in females, but in males as well. The observations by Saini (1971) and Banerjee and Sahai (1983) on \textit{P. pictus} and those of Elliott and Gilliott (1976) on \textit{Melanoplus} that allatotectomised females fail to deposit yolk and wet weight of the fat body increases (Elliott and Gilliott, 1979). Support the postulation made by the authors about an existence of a feedback mechanism in \textit{P. pictus} which is in conformation with the second proposal made by Keeley (1978) as mentioned already. In allatotectomised males accumulation of lipids may be partially due to a decreased locomotor and
muscular activity for which fat is the chief energy reserve (Kilby 1963) as also stated by Odhiambo (1966) for *Schistocerca*. The influence of CA hormone in normal *P. pictus* may be apparent in males up to 6-7 days when spermatogenesis is at its peak (Pandey 1985). After that the effect of this hormone might be limited to stimulation of accessory sexual gland activity. Since these activities are reduced too after allatectomy, the lipids are not utilised. Thus a gonadotropic effect of CA in fat body metabolism of male *P. pictus* can not be ruled out. However this assumption as well as that of Odhiambo (1966) are contradictory to the conclusions drawn by Walker and Bailey (1971) that CA has no effect on utilisation of lipids in male desert locust.

The CA of normal *P. pictus* do not have a suppressing effect on lipid synthesis as in case of *Leucophaea* (Gilbert, 1967) and *Spodoptera littoralis* (El-Ibrashy and Becter, 1970). This is clear from the fact that *Poekilocerus pictus* normally shows a period of intense feeding up to 15-20 days when intense somatic growth also occurs. At this period there is intense lipid deposition, specially in females. May be at this time carbohydrates are converted to lipids.

Allatectomy of 12-15 day old females or 7 day old males does not show much change in distribution of metabolites or the fat cell size. This is obviously due to the fact that the respective individuals mature at this particular period by which time the CA hormone have exerted whatever effects they have on the metabolism. As will be mentioned later, other
operations like cautery and cardiacectomy too, do not affect the fat cells much when performed on old individuals in which gonadal development, oocyte maturation and other processes have passed their peak periods.

The above has been confirmed in the present study by implantation of CA from maturation insects of different age groups into allatectomised young insects. The lipids were still abundant in the fat body of both females and males.

Implantation of CA as above shows slight decrease in fat cell as well as nuclear size than in allatectomised individuals. Although no regular pattern of this change has been found and the decreased size does not actually reach normal fat cell size but it is clear from the repeated observations that the CA hormone might regulate the growth of the fat cells in *P. pictus* - a function which is considered the primary one by Walker and Bailey (1971). That CA stimulate protein synthesis in *P. pictus* is confirmed by implantation experiments as mentioned above, which results in an increase in amounts of RNA and proteins in comparison to those in allatectomised individuals. The nuclei of these individuals have dispersed chromatin. This shows presence of active cells in the fat body and restoration of proteins synthesis after introducing CA. From histochemical observations on protein deposition in fat cells of both females and males implanted with CA, it can be said that CA from 7-15 day old individuals are more effective, probably because they are more active according to Saini (1971) in this insect.
Effect of CA implantation on lipid deposition has already been described above. So far as accumulation of glycogen is concerned, implantation of CA in allatectomised individuals do not show any change by histochemical methods applied in the present study.

Cautery of median neurosecretory cells results in decreased fat cell and nuclear size up to 15 days after which cells continue to decrease in size whereas nuclei hypertrophy i.e. they are not active. These results agree with those of Thomsen (1952), Slama (1964), Highnam and Hill (1977) etc. who have shown arrest of the general growth by cauterity of neurosecretory cells. This change is more noticeable in female than in males which has also been shown with reference to general body growth by Slama (1964). Most probably in P. pictus the neurosecretory material from the brain affects growth of the fat body and in the absence of this material they shrink with depleted reserves.

Slama et al. (1973) have also shown that cauterised insects do not accumulate reserves like glycogen, fat and protein.

In P. pictus, nucleic acids accumulate after cauterity except in early stages after the operation. The nuclear chromatin is clumped and densely stained showing inactive nature of the fat body. These conditions, except the cell size are comparable to those found in allatectomised P. pictus which indicates that the CA are perhaps controlled by the
brain by some allatotropic hormone so far as the synthesis of RNA is concerned. According to Saini (1971) CA of cauterised P. pictus do not develop. This might be the reason of inactivity of fat cells and accumulation of nucleic acids seen in the present study. It is also possible that mNSC cautery induced reduced enzyme activity as also reported by Coles (1964) and Wigglesworth (1970) which affects the nucleic acid synthesis.

Proteins reveal themselves as faintly HgBPB positive material in fat cells of female and male P. pictus cauterised at a newly emerged stage except at the most active stages that is, 15 day old females and 7 day old males. On the other hand, this metabolite shows no change when the operation is performed at a later age. Removal of cerebral neurosecretory cell (NSC) in desert Locust as well as Egyptian locust causes low concentration of total blood proteins and reduced protein synthetic activity of the fat body according to Highnam (1962) who has postulated that these effects must be partly due to the absence of JH, since removal of the NSC prevents normal increase in the activity of the CA as also shown by Saini (1971) in P. pictus. Hill (1962) has also shown decreased haemolymph protein level in adult Schistocerca gregaria after cautery of brain neurosecretory cells. Since the haemolymph proteins are products of the fat body, neurohormonally dependent changes in the protein should reflect changes in the synthetic capacity of the fat body. This was confirmed by
Hill (1965) by measuring the in vivo incorporation of $^{14}C$ glycine into the fat body protein. Allactectomy in Hill's experiments (1965) caused only a partial decrease in the protein synthesis and gave results that were intermediate between the controls and the cauterised animals. This conforms to the results obtained for P. pictus in the present study. Scheurer (1969) has shown a depressing effect of NSC cauterity on protein synthesis of Leucophaea maderae. Banerjee and Sahai (1983) have shown that cauterity of mNSC in P. pictus delays privitellogenesis growth and stops vitellogenesis and mature oocyte production. Vitellogenesis is restored by CA implantation according to them. It is suggested that although CA in P. pictus have a gonadotropic effect through which it controls fat body protein metabolism, presence of the neurosecretory material from the brain must also be there for its proper activities.

The effect of cauterity on lipid and glycogen deposition in the fat body of P. pictus seems to be quite insignificant. PAS and Sudan Black B staining of the tissue show no change in the deposition of glycogen or lipids in both sexes. These results are different from those found in Calliphora (Thomsen 1952) in which cauterity of mNSC reduced fat, as well as those in Rhodnius (Coles 1966) which showed accumulation of fat following decapitation. It seems that the response of the fat body lipid metabolism to neurohormones depends on the insect species studied as also suggested by Keeley (1978). Since corpora cardiaca are closely associated with the
metabolism of lipid and glycogen in the fat body of insects (Steele 1980), as discussed later, and they also act as storage glands for the neurosecretory material in *P. pictus* (Saini 1971) like in other insects (Highnam and Hill 1977), the results obtained in cauterised *P. pictus* in the present work so far as distribution of glycogen and lipid is concerned may be due to the fact that even in the absence of the median neurosecretory cells, corpora cardiaca which are intact, control distribution of lipids and glycogen as usual. However, the results in male *P. pictus*, whether cauterised at a newly emerged or a late stage, are different. In the fat cells of these males lipids accumulate to different extents. It is assumed that this is simply due to the fact that in males there is no need of sequestration of lipid yolk precursors from the fat body which consequently accumulate. The usual extra ovarian utilisation found in males is also not there since the cauterised male *P. pictus* are seemingly inactive i.e. have less need for energy.

Implantation of Corpora Allata into cauterised individuals results in an increase in fat cell nuclear and nucleolar size in *P. pictus*, so much so that the fat cells almost regain their normal size. The most effective result is obtained by implantation of CA from 7-15 day old females and males. This again confirms the fact that corpora allata regulate the fat cell size as mentioned already and supported by the work of Walker and Bailey (1971) but in the presence also of same factor from the neurosecretory cells of brain.
However, it is not clear from the present work, why implantation of these glands show very clear and regular effect on fat cell size than in case of the same experiment in alltectomised females.

The fat cells after CA implantation following cautery also regain their activity in the sense that they show dispersed chromatin and intense distribution of nucleic acids. Proteins increase slightly by implantation of active CA, that is, from 7-15 day old females and 7 day old males. However, inspite of this increase, the protein deposition can not be compared histochemically to that found in normal individuals. It is therefore assumed that although the corpora allata control protein synthesis mainly by their gonadotrophic action as discussed above, they also need a stimulating factor from the median neurosecretory cells of the brain. This conforms to the statements given about brain and CA hormone functions by Slama et al. (1973). Banerjee and Sahai (1983) have shown that implantation of CA into cauterised female *P. pictus* accelerates previtellogenic growth and vitellogenesis, although the rate is slower than in normal females. It is clear from these experiments, however, that both CA and mNSC are necessary for protein synthesis by the fat body and a successful oocyte development in *P. pictus* as also in *S. gregaria* (Highnam et al., 1962, 1965); and *L. maderae* (Wyss-Huber and Lüscher, 1967); Lüscher et al. (1969) etc. According to Lüscher (1968) CA alone maintains oocyte growth and protein synthesis in the fat body in *Nauphaeta*. However
he has shown that some protein synthesis takes place without corpora allata, and it is possible that some stimulation is also due to the neurosecretory material since brains have a slight stimulatory action on respiration of this cockroach. Since the protein synthetic activity of the fat body in cauterised *P. pictus* is lower as seen histochemically, than in allatectomised individuals, the neurosecretory hormone must be exerting a direct effect. A similar situation exists in *Locusta migratoria* (Highnam and Hill 1977) and the most likely explanation is that the neurosecretory hormone controls the overall fat body protein synthesis and that the CA hormone switches at least a part of this activity into production of vitellogenic proteins as also suggested by Minks (1967) and Highnam and Hill (1977). The interpretation of similar results in males after cautery, allatectomy or CA implantation as in female *P. pictus*, so far as protein synthesis is concerned, is difficult, may be it is under the control of mNSC and CA and the proteins are diverted for some other metabolic activities than reproduction. Very little is yet known of the metabolic demands of reproduction in males according to Highnam and Hill (1977).

Implantation of CA into cauterised individuals, since their own glands are inactive due to cautery as shown by Saini (1971), results in an increase in deposition of lipids in both sexes. Glycogen increases in comparison to that in fat cells of cauterised females. In males however it can not be definitely said by histochemical methods alone that
glycogen increases since this metabolite is already present in large amounts in cauterised male fat cells. Since previtellogenic growth and vitellogenesis is accelerated by implantation of CA in cauterised female P. pictus (Banerjee and Sahai, 1983), increase of metabolites in the fat body is feasible. In males it may be due to restoration of fat cell activities.

Cardiacectomy causes slight distension of fat cells alongwith their nuclei in P. pictus. They are inactive with clumped chromatin at most post-treatment periods. Hypertrophy of a high degree in the fat bodies of Calliphora erythrocephala after removal of corpora cardiaca has also been shown by Thomsen (1952). This operation in P. pictus induces accumulation of the metabolites, although to a lesser degree of proteins than lipids and glycogen. The effect must be due to slowing down of rate of mobilisation of the reserves for egg development in females and other processes in males. As the corpora allata were left intact; the results of cardiacectomy so far as the distension of cells is concerned is difficult to interpret. Corpora cardiaca have been removed in the present work by severing NCC I and II and according to Saini (1971) such severance inhibits the growth of CA in P. pictus. The effects of cardiacectomy specially on cell size may be through this indirect pathway. The same reason may apply so far as accumulation of protein in cardiacectomised individuals is concerned i.e. absence of some factors from the CC which might activate the CA for synthesis and release of
proteins causes their accumulation in fat cells. According to Scheurer (1969) protein synthesis in general is stimulated by a neuroendocrine factor from the parsintercerebralis – corpus cardiacum complex in *Leucophaea maderae*. In the same insect Müller and Engelmann (1968) who have shown that severance of CC from brain results in increased basal metabolism of the fat body irrespective of CA. So the results in *Leucophaea* (Müller and Engelmann 1968, Scheurer 1969) do not support those of the present author in the sense that they point to independent activities of CC and CA, irrespective of one another. The male fat cells after cardiacectomy do not show much change. The reason for this is not known and needs further investigations.

More recently, corpora cardiaca have been established as the endocrine organs responsible for controlling carbohydrate and lipid metabolism in insects (Steele, 1980). In experiments with *P. pictus* removal of these glands shows accumulation of lipids in fat body as mentioned above. In *Locusta migratoria* (Mayer and Candy, 1969) and *Schistocerca gregaria* (Goldsworthy and Mordue, 1972) a factor from the corpora cardiaca facilitates the release of diglycerides from the fat body. This factor serves the dual purpose of stimulating the release of the fat body lipids and the preferential uptake and use of these lipids by the flight muscles (Robinson and Goldsworthy 1974). In *Poekilocerus pictus* most probably the removal of CC causes inhibition of release of lipids which thus accumulate in both sexes since
they are not utilised in oocyte development and other activities in females and males as already mentioned. The operation performed at a late age does not have any effect.

Accumulation of glycogen in fat cells after cardiectomy in *P. pictus* could be possibly due to decreased phosphorylase activity as described by Keeley (1978) and Steele (1980). A well-defined aspect of carbohydrate metabolism concerns the action of the corpora cardiaca on trehalase synthesis. Since the present study involves only histochemical observations, interpretations about actual alternations in biochemical pathways are practically impossible. However, from established facts given by Keeley (1978) and Steele (1980), it is known that the CC activate fat body phosphorylase to degrade glycogen providing new intermediates for trehalase synthesis. It is therefore assumed that absence of these glands will cause reduced haemolymph trehalase, as also shown in *Locusta migratoria* by Cazal (1971). This will be due to decreased phosphorylase activity. Consequently the glycogen is not utilised and accumulate in the fat body. According to Wiens and Gilbert (1965) corpora cardiaca hormone acts on the fat body by decreasing the amount of carbohydrate available for glycolysis while increasing the quantity available for other tissues in the form of newly synthesised trehalase. It is quite possible therefore that in the absence of corpora cardiaca, the fat body carbohydrate i.e. glycogen will accumulate which can be histochemically detected as in the present work.
Isolation of corpora cardiaca into the abdomen of the same individual shows sparse distribution of protein in fat cells. The other metabolites do not show any change. This may be again due to an effect on the corpora allata rather than a direct effect on the fat body. It is more similar to the consequences of mNSC cautery than cardiaectomy. The cells are slightly distended than normal and inactive. This may be simply a pathological effect of the operation. The experiment has almost no effect on the distribution of lipids and glycogen. These metabolites definitely show release according to the age as in normal females and males in contrast to accumulation by complete removal of the glands as described. In this way, perhaps the position of CC does not apparently affect deposition and release of lipids and glycogen. This shows that CC, on one hand, might influence protein metabolism of the fat body via the corpora allata and on the other, they can act independently for release or accumulation of lipids and glycogen. This is supported by the findings of Müller and Engelmann (1968) for Leucophaea moderae.

Thus, in P. pictus it has been found in the present study that the growth of the fat body is mainly controlled by the NSC of the brain and the CA, both of which also regulate the protein synthesis and release. These neuroendocrine centres do not have much effect on the metabolism of lipids and glycogen which is regulated mainly by the corpora cardiaca. The effect of the CA on storage/release of these metabolites as well as the proteins is an indirect one through
their gonadotropic action. The CC also might regulate the CA activity since they store neurosecretory material and are connected to the CA.

All the above experiments are not quite feasible in the small and delicate larvae of the holometabolous Sarcophaga lineatocollis. The effects of the neuroendocrine system on fat body activities of this insect with special reference to metamorphosis have been studied by alternate methods of ligation and hormone application as suggested by Highnam and Hill (1977). Thoracic ligation isolates almost the whole neuroendocrine system including a blockage in the secretion of thoracic glands, a component of the neuroendocrine system which is absent in adult insects. Ligation can be thus compared to a combined operation of neurosecretory cell destruction, allatostomy, and cardiacectomy as also mentioned by Highnam and Hill (1977).

The general structure of fat cells of larval S. lineatocollis has already been discussed in light of that of adult insects including Poekilocerus pictus in the beginning of this chapter. The larval life in Sarcophaga lineatocollis extends up to 8–9 days showing 3 distinct phases of activity. Successive growth synthesis for storage and export and finally uptake and storage in preparation for pupariation. These results are in conformation to those of Thomson (1975) for Calpodes and Pelt-VerkHil (1979) for Calliphora. The larval fat cells of S. lineatocollis grow up to 6–7 days and then decrease abruptly in the pharate pupa. The pupal fat cells
are smaller than the pharate pupal cells at 2 days and then they are deformed. de Priester and van der Molen (1979) have also shown in *Calliphora erythrocephala* larvae, an increase in the cytoplasmic area of fat cells up to 7 days and severe shrinkage at the pharate pupa stage. The results of the present study are also supported by (Thomson, 1975) who has shown shrinkage of individual cells during imaginal development. The variations between individual cells in this case is great as also observed in different developmental stages. As is quite usual in Diptera, fat cells are often seen with many nucleoli per nucleus. This may be due to the large sized cells being involved in massive protein synthesis in the larval fat body. RNA and protein increase gradually in the larvae. Very active nature of cells is depicted by dispersed chromatin of the nuclei in which DNA is intensely deposited. In pupal fat cells RNA increases and decreases cyclically i.e. it is very little in early pupae, increase at 4-6 days and decrease again in pharate adult fat cells. This is most probably related to the release and storage of proteins in preparation for imaginal development. The protein in the early larvae are deposited into small peripheral globules as usual. Gradually as the larva grows bigger globules appear which increase in size and number through the pupal stages. The most interesting result is found in 6 day old pupae in which the big spheroidal globules are peculiar in the sense that they stain darkly at the margins whereas faintly in the inside. These are termed in the present work as 'coated vesicles' after de Priester and van der Molen (1979) who have
described these vesicles as being derived from isolation bodies of the late larva and contain areas of 'engulfed cytoplasm'. Proteins for storage are apparently derived from the haemolymph as described for Calliphora and other insects (Martin et al., 1971; Thomassen and Mitchell 1972; Price 1973; Collins 1975 and Pelt Verkuil 1979). This process is helped by ecdysterone (Thomassen and Mitchell, 1972; Butterworth et al., 1979, 1984).

Glycogen deposition after repeated observations was found to be different than in Drosophila larvae (Butterworth and Forest, 1984) who have shown accumulation with age. The observations on S. lineatocollis are like those on Calliphora (de Priester and van der Molen, 1979) i.e. very little glycogen around the nuclei in early larvae, which decreases up to 6 days and then accumulates in late larval and pupal fat cells. No explanation has been given by de Priester and van der Molen (1979) for these variations in glycogen deposition. It is assumed in the present study that this metabolite before pupariation is utilised for cuticle formation as in Rhodnius (Wigglesworth 1947) and Drosophila (Butterworth et al., 1965). In nymphs of P. pictus also (Banerjee 1971) glycogen deposition varies cyclically during moult and intermoult periods. Its depletion in the pharate adult S. lineatocollis is due to the utilisation in some imaginal tissues like flight muscles besides cuticle formation. Lipids are present in moderate quantities up to 4 days in larvae, then they increase and accumulate through pupal development.
Thoracic ligation of feeding stage larvae shows interesting results during 2–8 days of the operation. The fat body of normal S. lineatocollis larvae is divided into anterior and posterior lobes as already described, against that in the adult fly (Agarwal 1976), in which the fat body is in the form of flat sheets uniformly distributed throughout the body like the fat body of nymphal or adult Poekilocerus pictus. In ligation experiments with Calliphora (Pelt-Verkuil 1979) the anterior fat body has been carefully pushed back so that the whole tissue is considered treated. However, in the present work the author thought it worthwhile to leave the fat body as such as and to see the effect of ligation on both parts of the fat body i.e. in front (untreated) part and behind (treated part) the isolation of the neuroendocrine system. The anterior part of the fat body after ligation has shown remarkable changes instead of being like that of a normal (control) larva. Also externally the larva shows tanning of cuticle in this part, by 24–36 hours of ligation and during the later days it becomes darker and tougher like a pupa. This is in confirmation to the studies of Karlson and Sekeris (1962) who have shown anterior pupated and posterior unpupated portions of larvae after ligation. This may be due to accumulation of neurosecretion and other hormones on the "brain side" of the ligature, which change this portion of the body accordingly as also explained by Highnam and Hill (1977). However, this result is different from that found in Calpodes (Collins 1974) in which thoracic ligation does not induce pupation.
The fat cells of the anterior part of the body (pupated part) after ligation are initially hypertrophied as found in the present study along with their nuclei. However, between 4–8 days they shrink gradually in size. The intercellular membranes get distorted and after 6–8 days of ligation the fat cells exactly look like pupal fat cells. At later stages RNA and protein are stored into big globules whereas glycogen and lipids are sparsely distributed as in pupal fat cell. This confirms that all the hormones after ligation accumulate in the anterior part and affect the metabolism of the fat body in the usual way. So far as pupation of this part is concerned, it is precocious after this experiment since the process occurs at 8–9 days in normal larvae. Price (1973) has shown a precocious induction of lysosomal activity after ligation and sequestration of blood proteins normally related to wandering larvae.

The fat cells from the posterior part which remains larva like till the experiment is terminated (8–9 days) show shrinkage of fat cells. This effect is more like that of NSC cautery found in adult *P. pictus* fat body in the present study and also described by Thomsen (1952), Slama (1964) and Highnam and Hill (1977) rather than an effect of allatectomy may be neurosecretory hormone is important for growth of fat cells, either by itself as in both the cases studied here or through its action on the CA as in *P. pictus*. There is no report on shrinkage of fat cells by ligation in dipteran larvae. It is assumed that this may also be due to reduced synthesis and
storage in the fat body as discussed in the following paragraphs.

All the metabolites are present up to 4 days after ligation in moderate quantities but they gradually decrease later to very little quantities. Presumably, absence of a normal hormonal milieu prevents synthesis and storage which should otherwise occur by 6-8 days. Whatever amount is seen is perhaps that synthesised already before the operation which is then utilised or released. de Priester and Van der Molen (1979) have shown a cessation of protein accumulation after ligature in Calliphora which supports the findings of the present study. The author does not agree to the views of Arking and Shaaya (1969) who have stated that ligation does not apparently adversely affect the synthetic capabilities of the fat body in Calliphora. Collins (1974) demonstrated that pinocytosis of blood proteins occurs in the larval fat body of Callipodos ligated to prevent pupation.

Thus the present study has revealed a depressing effect of ligation on synthesis of protein similar to the effect of cautery as well as allatectomy in adult P. pictus (present study), Calliphora (Thomsen, 1952) and Leucophaea maderae (Scheurer 1969). Lipids and Glycogen are also sparsely distributed in the fat cells of ligated S. lineatocollis larvae as in cauterised adult Calliphora (Thomsen 1952).

Application of a Juvenile hormone analogue – Z 515 – to the ligated posterior parts to elucidate its effects on fat
body, has shown a peculiar effect morphologically. This part after 2 days shows tanning of the cuticle and sclerotisation to some extent. This effect is not so pronounced with the lowest concentration used (0.25%) but with 0.5% and 1% it can be seen within two days, so far as development in insects is concerned, good experimental foundations are there to establish that the function of juvenile hormone is to maintain larval characters and delay in metamorphosis until the larva has accumulated enough reserve material for future use (Slama et al., 1973; Highnam and Hill 1977). According to Slama et al. (1973) the usual effect of an extraneous source of JH is an inhibition of metamorphosis in some endopterygotes as indicated by absence of pupation causing formation of extra larval or pupal intermediates as also in some exopterygotes. In the present study, the results of high concentrations of JH analogue inducing darkening of skin which is an indication of beginning of metamorphosis may indicate some uncommon phenomenon, caused by this juvenoid.

Application of this hormone induces deposition of RNA and protein in comparison to the controls, that is, in the fat cells of ligated larvae. A low concentration induces accumulation between 2-4 days like the normal larval fat cells. By 6 days a considerable amount of protein in big globules characteristic of pharate pupal cells is found. May be that juvenile hormone plays some part in sequestration and storage of blood proteins as in Rhodnius prolixus and P. pictus as discussed already. According to Highnam and Hill (1977), the
major hormone affecting differentiation in insects is JH (and not ecdysone) which could exert its effects either at the level of DNA or at some other point of the protein synthetic pathway. Vanderberg (1963 b) and Karlson and Sekeris (1965) have shown that Juvenoids give rise to new mRNA molecules by activating specific gene sets. In S. lineatocollis larvae, increased protein synthesis caused Juvenoid application might be obviously due to synthesis of more RNA molecules. Increased concentration induces the effect earlier than the low concentrations.

Glycogen and Lipids also show accumulation by Z-515 application. It has been shown in P. pictus in the present study by experiments of allatectomy and implantation of CA experiments that the CA hormone regulates synthesis of proteins as well as accumulation of glycogen and lipids although in that case the action is also gonadotropic (Saini 1971, Banerjee 1977, Banerjee and Sahai 1983) as in many other insects including diptera (Thomsen, 1949; Bodenstein, 1953; Orr 1964; Engelmann and Penney 1966; Minks 1967; Luscher 1968; Agami and Morrison 1973; Elliott and Gillott 1977 etc.). In ligated S. lineatocollis larvae, however, where all the neuroendocrine hormones are purposely isolated from the posterior part (abdomen) of the body, possibility of any gonadal development is not feasible. Therefore transformation of larval fat body induced by the juvenoid through its gonadotropic effect is not at all possible in this case. Moreover, Engelmann (1971) and Stoppie et al. (1981) have recently postulated that in diptera
JH is not a gonadotropic hormone as in some other insects. The present findings in JH treated ligated *S. lineatocollis* larvae so far as protein deposition in fat cells is concerned are also different from those of Tojo et al. (1981, 1985) who have reported an inhibitory action of JH application on protein synthesis.

Thus the action of the juvenoid Z-513 on *Sarcophaga lineatocollis* larvae, so far as induction of darkening of cuticle like that of a pupa even in the absence of ecdysone along with transformation of fat cells to storage cell-like structures indicates some physiological mechanisms not common to JH or CA hormones. According to Slama et al. (1973) some physiological anti-juvenile mechanisms may be effective at the end of larval development which has been shown by them in *Dermestes vulpinus* being caused by low titres of ecdysone. However the same authors have not obtained anti juvenile activity of juvenoids in experiments with *Pyrhocoris*. The effect of the juvenoids is therefore complicated and difficult to interpret. According to Thomsen (1975) hormonal control of the synthetic processes in the premetamorphic larval fat body has not yet been successfully demonstrated. The situation described in *S. lineatocollis* larvae so far as the effect of the particular juvenoid used, on metamorphosis is concerned, indicates that the juvenoid helps in sequestration of proteins needed for tanning but does not play an active role in differentiation, obviously because a normal condition of the factors from the neurosecretory cells of the brain are not
present after ligation. The high concentration of Z-515 could also have an anti-juvenile effect as suggested by Slama et al. (1973).

When ecdysone is injected into the ligated abdomens of S. lineatocollis larvae it promptly changes into a pupal form with a tanned and sclerotised cuticle. It is of interest to note that by 10-12 hours of injection about 55% of the larvae exhibited darkening and tanning of the cuticle which is the first visible sign of the sclerotisation process. The phenomenon is absent in uninjected ligated posterior parts and is rather a slow process in juvenoid treated ligated larvae.

RNA and protein increase immensely in the fat body, much more in comparison to JH treated fat cells. These metabolites accumulate in big globules gradually with increased dose as well as post-treatment periods. It is assumed that ecdysone accelerates RNA and protein synthesis in Sarcophaga larvae more than the JH analogue, Z-515 does. Accumulation of synthetic proteins in ecdysone treated ligated larvae has also been shown in Calliphora by Arking and Shaaya (1969) and Thomsen et al. (1971) and in Calpodes by Collins (1974). The author's findings are different from those of Neufeld et al. (1968) who showed that ecdysone treatment caused inhibition of both protein and RNA synthesis within the fat body. The differences in results might only be a reflection of the different distribution patterns of the hormone as also agreed by Neufeld et al. (1968).
Accumulation of protein by ecdysone injection could be due to its utilisation in cuticular tanning (of puparium) which is brought about by the cross bonding protein molecules to form sclerotin (Highnam and Hill 1977). Stimulatory action of ecdysone on RNA synthesis has also been reported by Karlson and Sekeris (1965) in Calliphora, Kobayashi and Akai (1967) in Musca and Raikow and Fristrom (1971) in Drosophila, in different tissues including fat body. Karlson and Sekeris (1965) working with isolated fat body nuclei of Calliphora concluded that ecdysoids as well as juvenoids gave rise to new mRNA molecules whereas Wyatt (1972) and Keeley (1978) recorded stimulation of all kinds of RNA by ecdysone. Increased protein synthesis is a consequence of increased RNA synthesis which may be induced by juvenoids and ecdysoids as well as found in the present study.

Glycogen also accumulate in big spheroidal globules with α-ecdysone treatment. Lipids do not show any change, depletion or accumulation whatsoever, in fat cells treated with 0.25% and 0.5% concentrations. However the highest concentration (1.0%) used in this study after the longest post treatment period i.e. 6 days, has showed slight accumulation of this metabolite. Accumulation of glycogen may be due to its utilisation in cuticle formation since it is the precursor for structural polysaccharide of cuticle - chitin, as also stated by Wyatt (1974) and Steele (1980). Wyatt (1980) has shown that after ecdysone injection the rate of respiration increases which results in increased basal metabolism. There is no
report an effects of ecdysoids on lipid metabolism as such so far as the author is aware. It is assumed that neurosecretory cells and the ring gland (CA-CC complex) regulates this aspect of the fat body metabolism. Ligature prevents flow of secretions from all the above centres and application of the JH-analogue stimulates lipid deposition to same extent in *S. lineatocollis* larvae but α-ecdysone does not seem to have any apparent effect.

A combined treatment of the ligated larvae with similar concentrations of Z-515 and α-ecdysone has given interesting but varied types of results. A low concentration of both the hormones show accumulation of RNA at two days but gradually this metabolite decreases and disappears either at the same period with increased concentrations or by 6 days with the same concentrations. Most probably with increased dose and post treatment periods, the JH analogue reverses the RNA synthesising capacity of α-ecdysone. This is in conformation to Buckner's results (1982) on *Manduca sexta* with reference to uric acid storage. The highest concentration (1%) of both hormones, when applied together shows accumulation of RNA indicating a more dominating role of ecdysone and inhibitory role of JH.

The ligated larvae of *S. lineatocollis* show sclerotisation of cuticle of the posterior part as the α-ecdysone treated larvae. However the larva does not show differentiation most probably because no factor from the neurosecretory
cells of the brain is present.

Proteins accumulate with all concentrations and post-treatment periods after a combined treatment as above. Most probably, in this case also the action is mainly due to ecdysone. Glycogen accumulation depends upon the concentration as well as treatment time. Depletion is seen with a low concentration of 6 days and with the high concentration at 2 days, otherwise an usual accumulation is there. Most probably the two hormones act synergistically so far as glycogen storage/synthesis and release is concerned. Lipids usually accumulate as a consequence of the function of Z-515, rapidly with low concentrations and vice versa. There is almost no report on above types of experiment in other insects so far as the author is aware. The experiments indicate two possibilities of action of the JH analogue in relation to \( \Delta \)-ecdysone, that is, it either (i) reverses or inhibits (e.g. RNA and protein deposition, lipid deposition) or (ii) acts synergistically to (e.g. darkening of cuticle and glycogen deposition) to the ecdysone induced metabolic functions of the larval fat cells in *S. lineatocollis*. Thus findings in *S. lineatocollis* larvae in present work conform to those of Highnam (1977) that JH can not be merely a status quo inhibitory hormone.