The adipose tissue or the fat-body of insects is a more definite organ than any other tissues of the body cavity (Wigglesworth, 1965). It is derived from the mesoderm of the walls of the coelomic cavities but in the adult the fat body is arranged as a loose meshwork of anastomosing lobes, invested in delicate connective tissue membranes and joined by connective tissue strands.

Although a phagocytic function is attributed to the cells of the adipose tissue i.e., the trophocytes (Murray and Tiegs, 1935; from Roeder), their primary role appears to be the accumulation, storage, resynthesis and ultimate release of the products of digestion. In addition, materials released from histolyzing larval tissue during metamorphosis may be stored and transformed in the trophocytes.

During recent years, increasing attention has been paid to the possibility that the insect adipose tissue may be a site of intermediary metabolism, as distinct from its more passive role of serving as a depot for the storage of fats, proteins and carbohydrate reserves (Kilby, 1963; Gilmour, 1961; Clements, 1952; Chefurka, 1965; Gilby, 1965). Thus, a
recurrent suggestion regarding insect fat body is that it acts as vertebrate liver. The reserves of the fat body must play a vital role in such crises of insect life as moulting, metamorphosis, maturation of gametes, starvation and hibernation (Buxton, 1935; Snodgrass, 1924; Timon-Devid, 1929, 1930; Scoggins and Tauber, 1950).

Much work has been done on the fat body of insects which have engendered many hypotheses regarding the morphology and arrangement, histology and cytology of fat body as well as its role in storage of reserves and intermediary metabolism.

The arrangement and morphology of the fat body has been described in many insects by Wielowiejsky (1836), Hufnagel (1918), Kreuscher (1922), Bishop (1922), Wigglesworth (1948) and many others. The histology of the fat body has been studied by Kreuscher (1922), Schnelle (1923), Poisson (1924), Poyarkoff (1910), Nair and George (1964), Coupland (1957) and many others.

The cytology of the insect fat body has been little studied (Bishop, 1958; Von Gaudecker, 1963; Wasserman et al., 1963; Locke and Collins, 1965; Walker, 1965, 1966; Ishizaki, 1965 and Wigglesworth, 1967). The only previous cytological investigation of the locust fat body is that of Odhiambo (1967) who made an electron microscope study of the fat body of male desert locust.
Histochemical studies on the fat body of insects have been made by Wiglesworth (1942), MacManus (1946), Wiglesworth (1949), George and Hegdekar (1961), George and Nair (1964), Benson and Benson (1966), Takahashi (1966), Locke and Collins (1968), Collins et al. (1966, 1970). The work of Coupland (1957), Nair and George (1964) and Odhiambo (1967) deserves special mention.

As regards intermediary metabolism, the existence of a system for synthesis of lipids in the fat body has been shown to occur by Zebe and MoShen (1959) in Prodenia and Tietz (1961, 1962) in Locusta. Various enzyme systems concerned with the carbohydrate and protein metabolism were shown to operate in the fat body by Kilby and Neville (1957) and Clements (1959) in locusts, Hearfield and Kilby (1958) in Schistocerca gregaria, Hines and Smith (1963) in S. gregaria, and also by Shigematsu (1958), Levenbook (1961) and Walker (1966). The synthesis of glycogen has also been studied by Wiglesworth (1942). Little is known about the intermediate steps occurring in the process of metabolism, though suggestions have been made by Paillot (1926, 1937), Pardi (1939) and some others. Pfeiffer (1945) and Orr (1964b) have followed changes in lipids and carbohydrates during maturation and have suggested that the corpus allatum hormone regulates their mobilisation from the fat body. Doane (1961, 1962) has made observations on the
hypertrophied fat body of female sterile mutants of Drosophila and suggested that a hormone released by active ovaries controls the mobilization of yolk precursors from the fat body.

On a survey of the entire literature on the subject, it is seen that all these investigations point to the necessity of critical observations on the fat body of the insects. It is also seen that very little work has been done on the fat body of Orthoptera. It was, therefore, thought worthwhile to investigate the problem in greater detail and a locally available grasshopper, Poecillegerus pictus has been chosen for the present work.

The observations, here described, deal with cytological and histochemical study of fat cells of 5th and 6th stage nymphs, males and females (virgin and copulated) at different ages in order to understand the developmental changes in both the sexes under different gonadal control. The same observations have been made in the fat body of castrated males, ovarioctomised females, males with ovary implanted and females with testis implanted in order to see the effect of gonadal activities on the metabolism of adipose tissue. The chief interest of the results has been in the deposition and depletion of unsaturated as well as saturated lipids in the adipose tissue with reference to age and sex. Synthesis or
accumulation and depletion of other reserve substances, i.e., carbohydrates and proteins have also been investigated by histochemical methods. Changes in the intensity of distribution of RNA and DNA have been histochemically studied. The search for albuminoid granules, glycogen granules, lipid vacuoles etc. has been made specially when we know that in Aedes (Wigglesworth, 1942), Drosophila (Butterworth, 1965), Philosamia (Ishizaki, 1965) and in Sarcophaga (Benson and Benson, 1966), the protein and carbohydrate granules are formed separately in the cytoplasm, while in Anthrenus (Nair and George, 1964), these depositions are found in the peripheral globules.

Cytologically the changes in cell-size, nuclear and nucleolar size during different growth periods in both the sexes as well as changes in the number and position of mitochondria have been noted. The relationship of mitochondria, peripheral globules and central globules has been studied.

Some biochemical observations on the fat body of P. pictus have also been made at some stages of the normal and castrated insect. This has been done specially keeping in view that Odhiambo (1966) has shown that there is a rapid increase in total lipids during the early development of the adult male S. gregaria and it reaches at its peak at the time
of sexual maturity after which there is no increase but gradual depletion. In the mature fat body, the cells contain enormous lipid and glycogen deposits while the cytoplasmic organelles are little developed. Further, Butterworth and Bodenstein (1968) have shown that the percentage of lipid is either greater or equal in castrated females and the percentage of glycogen decreases in comparison to that in the normal females. So, here, an attempt has been made to collate the picture seen by histochemical methods to the conditions noticed by biochemical methods. Total fats and carbohydrates in the fat body and gonads (ovary or testis) have been estimated. Changes in the weight of fats and carbohydrates in the fat body of castrated males and females have also been investigated.

In addition to all the above experiments, studies on the neurosecretory cells and neuroendocrine complex of some stages, specially of the castrated insects, have been made in order to correlate the hormonal effects of the former, if any, on the adipose tissue. This has been done specially when it is known that the ovary regulates growth in fat cells directly by way of hormones (Vogt, 1942 and Doane, 1961). The opinion of these workers is that the ovary may indirectly regulate growth and proportion of the reserve substances of the fat cells by activating corpus allatum – corpus cardiscum complex (neuro-endocrine complex) which in turn, regulates the adipose
tissue. This has been fully examined in P. pictus.

It may also be mentioned that the adipose tissue of insects behaves differently with reference to the effect of ovaries. It has been noted that gonads have no effect on the adipose tissue in Phormia (Urr, 1964), Lucilia and Sarcophaga (Day, 1943). In order to see this mechanism and extend the studies in other insects, the adipose tissue of P. pictus has been investigated.
Newly emerged nymphs of *Pseudocerus pictus* were collected from the calotropis plants in the fields of Sagar, M.P., India. These were reared in glass fronted cages in the laboratory, being fed every day on fresh calotropis leaves. The nymphs of successive stages of both the sexes, as well as adult females and males, were kept separately and labelled with the exact date of their emergence. The age of each of them was counted from this date of emergence. Some newly emerged females were kept with mature (7 - 8 day old) males. Copulation started at once but the male remained associated with the females until the time of egg-laying. In the present work, such females are termed as copulated females while those which are reared without males are termed virgin females.

Fat tissue has been examined in the 5th and 6th stage nymphs of both sexes at different stages such as newly moulted nymphs, 3 day and 10 day old nymphs and those just before or during moultling to the next stage. Similarly, fat body of females, whether copulated and virgin, have been examined at newly emerged stage and after 3, 7, 15, 20, 25 and 30 days of emergence as well as during and after egg laying. Males have
been investigated at newly emerged stage and after 3, 7 and 15 days of age.

**Surgical Methods**

Ovaries from newly emerged females were removed under ether anaesthesia through a slit made on the left terga in segments 5 and 6, which were surface sterilised with streptomycin. These were sealed with wax after the operation. The insect was then kept in a dry sterilised bottle taking care that it was always dry. The fat body of such ovarioctomised females was studied after 3, 7, 15, 25 and 30 days. The newly emerged males were similarly castrated and studied after 3, 7, 15 and 19 days.

Ovaries of 3 day, 7 day and 15 day old copulated females were implanted in adult males (8 - 15 day old) by the method given below after removing their testes. The adipose tissue of such males with ovary implanted was investigated after 10 days, 7 days 10 days respectively. The ovary of a 15 day old female was also implanted in the abdomen of a 2 day old male after removing its testis and the adipose tissue of this male was studied after 5 days. Similarly, testes of 2 - 3 day old males were implanted in newly hatched females, 7 day and 10 day old females after removing their ovaries. The adipose tissue of such testis containing females were investigated after 5 days,
7 days and 10 days respectively.

The tissue to be implanted (testis or ovary) was dissected in ice cold Ringer's solution. This solution contained antibiotic streptomycin and was always prepared just before the operation. One gram of streptomycin was dissolved in 10 ml of distilled water and made to 100 ml with Ringer's solution (Butterworth, 1965). The testis or the ovary was implanted in the abdomen of female or male, as the case may be, under ether anaesthesia, through a slit made on the left terga in segments 5 and 6 which were surface sterilised with streptomycin. Prior to this, the ovary or testis of the insect in which implantation was to be made, was removed. After implantation, the wound was sealed with molten paraffin wax and the insect kept in a separate sterilised jar taking care that it was always dry.

The adipose tissue was dissected out in Ringer's solution at varying stages of growth and at different periods after ovariectomy, castration or implantation of gonads as stated earlier. Some observations have been made of fresh tissue under light or phase contrast microscope.

Histochemical Methods

For histochemical studies, the tissue has been fixed mostly in Carnoy's solution and the paraffin blocks cut at 6 μ
thickness. For the study of lipids, the tissue was fixed in 2% Osmium tetroxide and stained with ethyl gallate as stated below. Tissue was also fixed in Formol-calcium for studying the lipids in paraffin sections.

The procedures for histochemical staining were in general those of Pearse (1960) and Davenport (1960), which are as follows:

1. **Nucleic Acids**: For DNA, Feulgen method of Feulgen and Rosenthal (1924) was followed (Davenport, 1960).

For studying both the DNA and RNA, Pyronin/Methyl green (P/MG) method of Baker et al. (1955) was used. Extraction of RNA by ribonuclease was done in the usual way (Brachet from Pearse, 1960) in order to confirm that the P/MG positive material in the tissue was RNA.

2. **Proteins**: Mercury/Bromophenol blue (Hg BPB) method of Bonhag (1955) was used (Pearse, 1960). Carnoy fixed material was stained in 1% alcoholic bromophenol blue solution saturated with mercuric chloride.

3. **Carbohydrates**: Periodic acid/Schiff (PAS) technique of McManus (1948b; 1954) was used (Davenport, 1960). Schiff's reagent was prepared by the method of McManus (Stain Technology, 1948). Confirmation of the fact that positive PAS reaction
in the tissue sections was due to the presence of carbohydrates was obtained by acetylation of the sections with 40% acetic anhydride in anhydrous pyridine as described by Lillie (1954) and consequent blocking of their staining with PAS. To reverse the acetylation reaction, the acetylated sections were treated with 20% ammonia in 70% alcohol as recommended by Lillie (1954). Thus the granules in the tissue giving colour with PAS, obliterated by acetylation and restored by subsequent treatment with alkali show that they possess 1 : 2 glycol groups and are likely to be carbohydrates. Since glycogen is the only naturally occurring carbohydrate remaining in all animal tissues after fixation and paraffin embedding (Pearse, 1960), these granules are assumed to be those of glycogen. Some sections were digested with saliva for 30 minutes before applying the PAS test and consequent blocking of the staining confirmed that the PAS positive granules are those of glycogen.

The sections after staining with any of the above methods were mounted in D.P.X., a proprietary neutral synthetic resin (Gurr, 1962).

4. For the study of lipids, the Osmium/Ethyl gallate method of Wigglesworth (1959) was used. The tissue was fixed in 2% osmium tetroxide, stained with ethyl gallate and embedded in agar. The agar block was dehydrated and re-embedded in ester-wax. These blocks were cut at 1 μ thickness and the coverglasses
containing sections were mounted in Farrant's medium.

The paraffin blocks of formol-calcium fixed tissue were cut at 6 μ thickness, stained with a saturated solution of Sudan Black B in 70% alcohol (McManus, 1946) and mounted in glycerine jelly. These sections showed lipids in paraffin sections. Some of the sections were stained with 1% Nile blue sulphate solution at 60°C, by the method of Cain (Davenport, 1960) and mounted in glycerine jelly to study the phospholipids and fatty acids in the fat body.

**Cytological Methods**

The Osmium/Ethyl gallate method of Wigglesworth (1959) was also used for the study of mitochondria in the fat cells.

Some tissue was fixed in Regaud's fluid and the paraffin sections were stained with iron haematoxylin (Davenport, 1960) in order to observe Golgi elements, if present, in the fat body.

The general histological studies of the fat body were made by staining sections of aqueous Bouin fixed tissue with eosin/haematoxylin. The size of the cells, nucleoli and nuclei, was measured from sections stained with Feulgen, P/MG and eosin/haematoxylin methods. Since cells nuclei and nucleoli of a particular stage of the insect have areas which vary very slightly, a single average value has been used in all the tables.
This average value for each stage was obtained from the mean of the areas of 15 - 20 nuclei, 10 - 15 nucleoli and about 10 - 15 cells from that very stage of the insect. Usually all observations for a particular stage were made out from the study of at least 4 - 5 insects.

**Biochemical Methods**

1. **Extraction and estimation of lipids**: Lipids were extracted by the method of Bieber et al. (1961) as modified by Hodgson (1970) and estimated chemically. The entire insect, its fat body or gonad (ovary or testis), in which lipid was to be estimated was first of all weighed. Then it was homogenised and extracted three times with chloroform : methanol (2 : 1) allowing the residue to extract for 2 hours after each of the first two homogenisations. The final volume of chloroform : methanol (2 : 1) was made 20 times the volume of insect material i.e., the homogenate from one gram of tissue was diluted to a volume of 20 ml. The lipid extract was evaporated to dryness and weighed. From this weight, the percentage of total lipids in the entire insect body, fat body or gonad was calculated.

2. **Extraction and estimation of glycogen**: Glycogen was extracted and estimated by the method of Lovern (1963) and BeMiller (1965). The tissue to be used (fat body or gonad) was quickly dissected out from anaesthetised insects and
immediately frozen. When glycogen was to be estimated from the entire body of the insect, it was weighed, anaesthetised and then extracted. The frozen tissue was weighed and ground with a threefold volume of 10% Trichloroacetic acid (TCA) at 0°C. Grinding was performed in a precooled mortar with sand. The mixture was then quickly centrifuged and glycogen was immediately precipitated by pouring the centrifugate into 3 volumes of ethanol. The residue was quickly re-extracted with 3 volumes of 5% TCA at 0°C. Re-extraction, centrifugation and precipitation were repeated 3 times more (a total of 4 extractions). The crude glycogen precipitate was isolated by decantation. It was then purified by repeated precipitations with ethanol from clarified aqueous solutions. It was finally washed with diethyl ether. The precipitate thus purified was air dried, weighed and its percentage in the entire body, fat body or ovary, as the case may be, was calculated.

Method for Neuroendocrine complex

The sections of aqueous Bouin fixed brains and corpora allata of castrated insects were stained by Harker’s modified Paraldehyde/Fuchsin method and Bwen’s method (1962).

Some adipose tissue was fixed in alcohol and stained by Gomori’s Hexamine Silver method in order to see uric acid, if present, in it.