Chapter 1

HISTOLOGICAL AND HISTOCHEMICAL OBSERVATIONS ON THE FAT BODY OF POICELOCERA PICTA

Among the tissues occuring in the haemocoel of insects, the fat body forms a more definite organ, usually arranged as loose mesh work of lobes invested in delicate membranes. Though the metabolic importance of insect fat body has been known since long in such events of insect life as growth, metamorphosis, hibernation and starvation little attention was given for its study untill recently.

Extensive studies on the fat body in *Simulium* (Scriban and Dragut, 1935) and the honey bee (Bishop, 1922, 1923, 1960) have been published. Snodgrass (1956) has reviewed the work done on the fat body of Hymenoptera. Studies on insect fat body with special reference to embryogenesis, metamorphosis and growth have been reviewed by Wigglesworth (1953).

Histological and histochemical study on the fat body of Orthoptera is rare (Coupland, 1957). In a general survey of the fat body of some of the common orthopteran insects, it was found that the colour of the fat body varied from creamy white in the cockroach (*Periplaneta americana*) to bright yellow in the banded grasshopper (*Poicelocera picta*). In mammals it is known that there are two types of adipose tissue (Remillard, 1958), yellow and brown, which differ in their histological structure as well as metabolic activity. The present investigation on the histological and histochemical aspects of the yellow fat body of Poicelocera picta, was therefore undertaken.

MATERIALS AND METHODS

Histological and histochemical studies were carried out on the perivisceral fat body of Poicelocera picta . The insects were collected from the field during the months of July and August and reared in laboratories on fresh leaves of Calotropis plants.

After decapitation, the specimens were dissected in insect saline and sheets of perivisceral fat body were removed from the animal and were either spread on clean slides and fixed or fixed directly and processed for paraffin sectioning. Usually sections were cut at 10 μ thick. For histochemical studies either the fat body sheets were spread on clean slides or the tissue fixed and embeded in gelatin (Pearse, 1960) and sections were cut on a freezing microtome at 20 μ thick. For routine histological study Carnoy (6:3:1) and Bouins (alcoholic) fluid were used for fixing and Hematoxylin-Eosine for staining.

Fat was studied by using saturated solutions of Sudan black B and Fettrot 7B in ethylene glycol. Sheets of whole fat body were fixed in calcium-formol for 24 hours at room temperature (Baker, 1946), postchromated for 18 hours at room temperature and 24 hours at 60°C before staining.Phospholipids were studied by using Acid Hematin method of Baker as outlined by Pearse(1960). Baker's pyridine extraction test was applied for controls(Pearse, 1960). Cholesterol and cholesterol esters were studied by adopting Schultz method for formalin fixed material (10% at 4°C) and cholesterol was distinguished from its esters by extracting the esters with alcohol-ether mixture following the proceedure outlined by Pearse (1960).

Alkaline and acid phosphatases and lipase were studied by Gomori's revised methods (Pearse, 1960). Lipase was studied histochemically using Tween 80 as well as Tween 85 as substrates.

Nucleic acids (RNA & DNA) were studied by the Methylgreen-Pyronin method (Pearse, 1060). Treatment with RNase and Trichloracetic acid was used for control material. The details are outlined in chapter 8.

RESULTS

The fat body was found to be of one or two cell layer thickness and consisted of compact cells coloured bright yellow. Two types of cells could be distinguished in the fat body, the fat body cells proper or trophocytes forming the main bulk and oenocytes which are found interspersed among the fat cells. The fat body cells are large with distinct boundary and contained one or two nuclei. The division of the nuclei were also observed. Some of the cells possessed gaint nuclei. The nuclei of the fat body cells were intensely chromatic and the cytoplasm was diffusely eosinophilic(fig.1) The diameter of the normal nuclei varied from 17-30 μ while that of the gaint nuclei varied from 30 to 47 μ. The oenocyte cells were also large (fig.9) with rounded nuclei but their chromatin material was less dense. The oenocyte nuclei varied from 8 to 22 μ in diameter.

The fat body stained intensely with Sudan black B (fig.2) and Fettrot 7B. The fat was found to be distributed as small globules of uniform size throughout the cytoplasm. The nuclei of some of the cells were stained with Sudan black. The fat globules in the cells of the fat body of Periplaneta americana stained moderately with Sudan and faintly with Fettrot. Moreover, the fat globules were of varying sizes and usually a bigger fat globule was surrounded by smaller ones.

The acidhematin test gave a positive reaction for phospholipids. The nuclear membrane and the chromatin material were stained in the nucleus. In the cytoplasm the fat globules seemed to have a phospholipid envelope. The fat body cells were rich in cholesterol and cholesterol esters. Cholesterol was

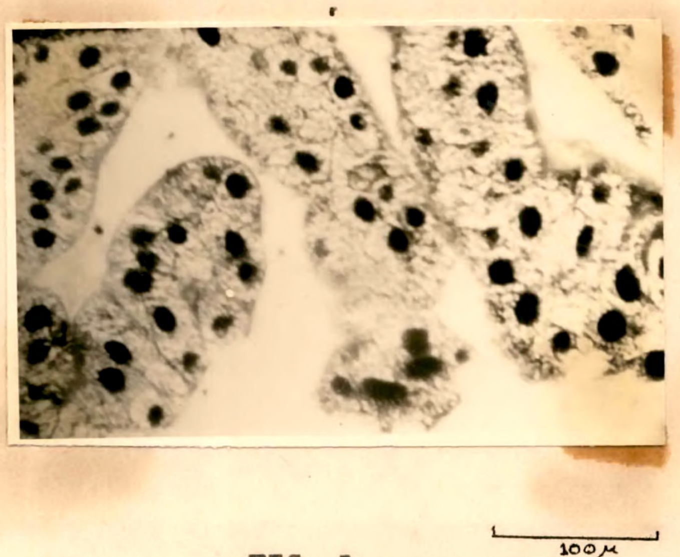

FIG. 1

Photomicrograph of the fat body of <u>Poicelocera picta</u> showing two cell thick fat body. Nuclei large, densely chromatic and centrally situated. Cytoplasm eosinophilic and granular.
(Hematoxylin-Eosine)

NUCLEI

TRACHEA

FIG. 2

Photomicrograph of the fat body of <u>Poicelocera picta</u> stained for fat. (Sudan black B)

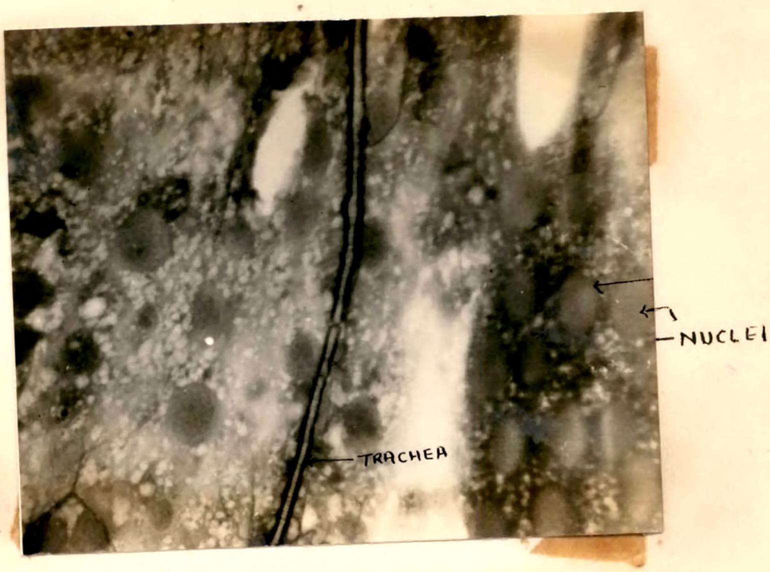

FIG. 3

Photomicrograph of the fat body of <u>Poicelocera picta</u> stained for phospholipids. (Acid-hematin)

NUCLEUS ←

FIG. 4

Photomicrograph of the fat body of <u>Poicelocera picta</u> stained for cholesterol. (Schlutz)

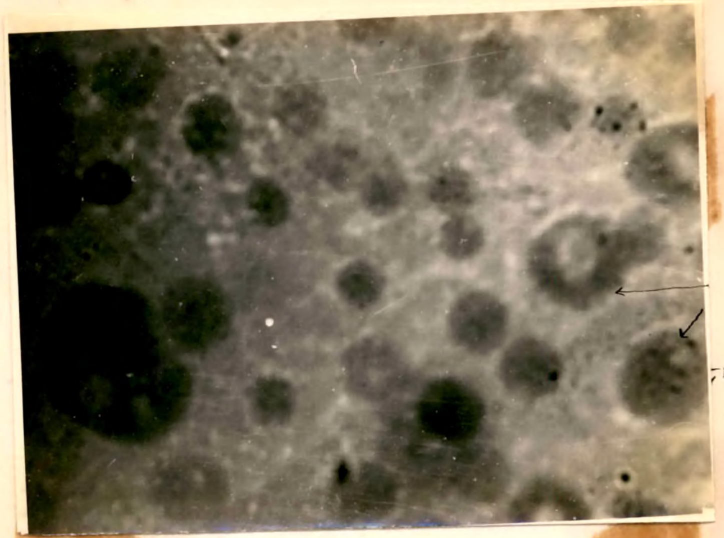

FIG. 5

Photomicrograph of the fat body of Poicelocera picta stained for cholesterol plus cholesterol esters.

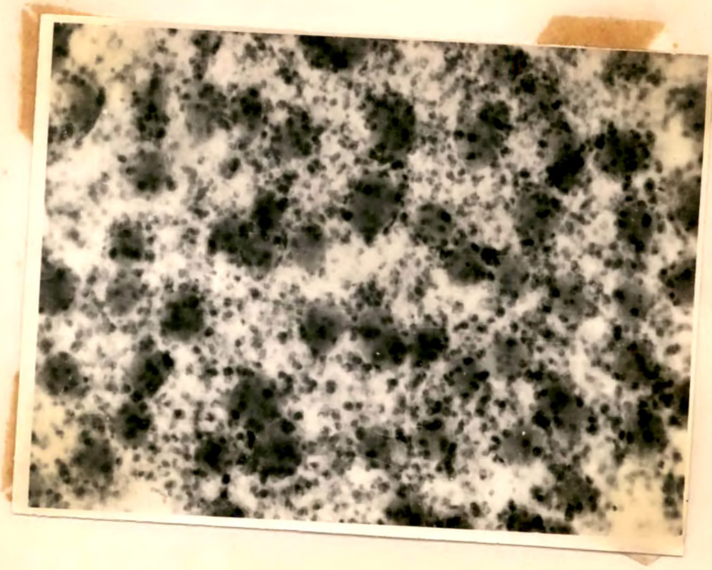

FIG. 6

Photomicrograph of the fat body of Poicelocera picta treated for demonstrating lipase (Tween 80)

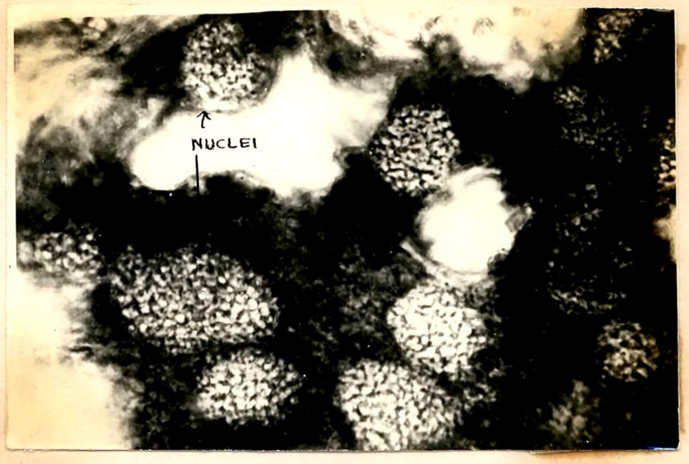

FIG. 7

Photomicrograph of the fat body of Poicelocera picta showing the distribution of alkaline phosphatase activity. (Gomori)

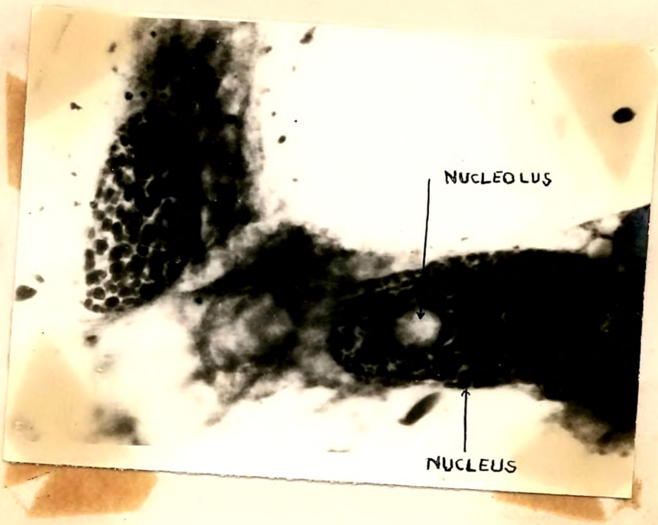

FIG. 8

Photomicrograph of the fat body of Poicelocera showing the distribution of acid phosphatase activity. (Gomori)

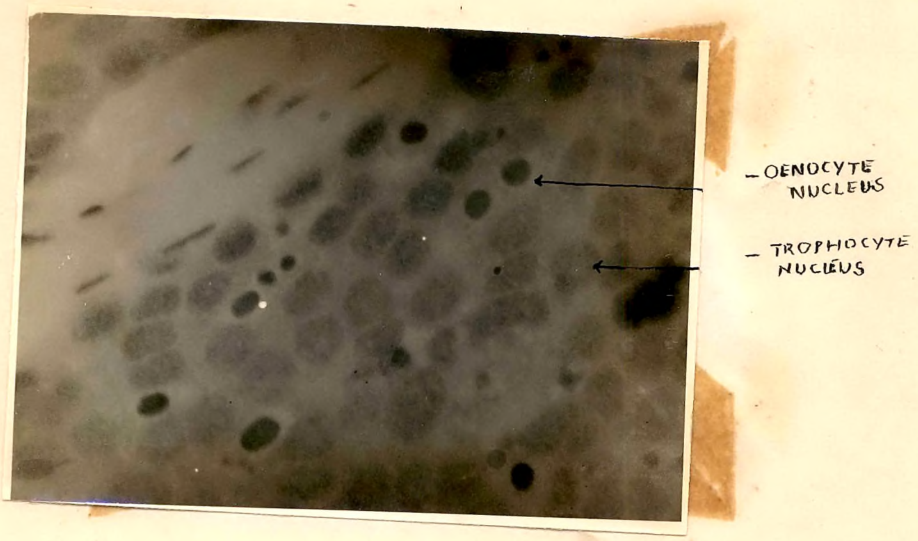

FIG. 9

Photomicrograph of the fat body of <u>Poicelocera picta</u> stained for RNA. (Methylgreen-pyronin)

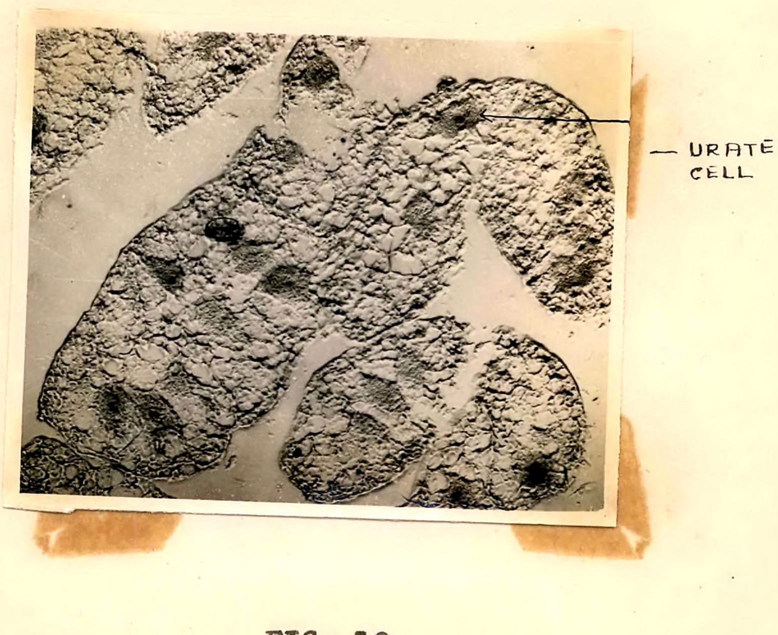

FIG. 10.

Photomicrograph of the fat body of <u>Periplaneta americana</u> stained for RNA. (Methylgreen-pyronin) was found mostly restricted to the cytoplasm while cholesterol esters to the nucleus (fig. 4,5)

A high lipase activity was found in the fat body cells and each fat globule seemed to possess the enzyme (fig.6) adsorbed on its surface at one or more places. Lipase activity was found comparatively very little in the cockroach fat body.

The fat body was found to possess a very high concentration of phosphatases. Alkaline phosphatase was restricted mostly to the cytoplasm of the cells and gave intense reaction for the enzyme. The chromatin material of the nuclei also showed some activity (fig. 7) . The nuclei were very rich in acid phosphatase. The sites of the enzyme activity were seen as coarse granules distributed along the chromatin material of the nuclei (Fig. 8). Comparatively very little activity of acid phosphatase was noticed in the cell cytoplasm.

The cytoplasm of the fat body cells showed intense ribonucleic acid basophilia with methylgreen-pyronin stain. A detailed study of this has been reported in chapter 8. However, in contradistinction to this finding, the fat body cells pf the cockroach showed negative reaction for RNA basophilia. The urate cells ~~xxxx~~ in the fat body of Periplaneta were rich in RNA while the cytoplasm of the oenocytes of the fat body of Poicelocera was free from any RNA basophilia.

DISCUSSION

The arrangement, distribution and compactness of the fat body is a characteristic of the species and shows some consistency within the insect order (Buys, 1923). The arrangement of the fat body described for Poicelocera did not seem to differ from that in other orthopteran insects. The lipid droplets seem to be mainly neutral fats as they are stained with Fettrot 7B which is by and large specific for glyceride fats. The fat globules of the fat body of the cockroach however, seem to differ in arrangement as well as its chemical nature from that in the fat body of Poicelocera. In the cockroach many small fat globules were seen around a central big one. Fettrot staining was poor. The lipid globules, however, in the fat body of Poicelocera were of uniform size.

The phospholipid coat around the globules of neutral lipids is of interest. The demonstration of a high content of cholesterol and cholesterol esters suggests an important role in the synthesis of these substances to the fat body.

The high concentration of lipase activity seen in the fat body is consistent with the importance of this tissue in fat metabolism. Wigglesworth (1958) found esterase caps for each individual fat globules in the fat body of Rhodnius prolixus and contended that exchange of fatty acids might take place at these points. The present observations regarding lipase localization, also agrees with his conclusions.

The fat body of Poicelocera is rich in phosphatases. Phosphatases in the fat body have been studied during post-embryonic development in Drosophila melanogaster and Phormia regina by Yao (1950) and Stay (1959) respectively. Yao (1950) found only nuclear activity, by the Gomori Technique, of the alkaline phosphatase. He further found increase in cytoplasmic activity of the dissociated fat body cells after pupation. Stay (1959) could not find any alkaline phosphatase activity in the fat body of Phormia by the azo-dye method. The acid phosphatase activity was however, present and increased at puparium formation. In the fat body of Poicelocera the acid phosphatase was mainly restricted to the nuclei and alkaline phosphatase to cell cytoplasm.

Intense phosphatase activity has been correlated with protein synthesis by the cell (Wolf, et al, 1943; Kivalo et al, 1958). The fat body of insects is also known to synthesize protein (Shigematsu, 1958; Chapter 8). Alkaline phosphatase activity has been variously related to such functions as tranport of material (Moog, 1946), activities of pinocytotic apparatus and secretion vacuoles (Barka, 1962) and lipid metabolism (Eapen, 1959). It is also possible that in the fat body of insects, a role for alkaline phosphatase in relation to lipid metabolism is more probable.