## Chapter 6

## A STUDY ON THE LIPID AND LIPASE LEVELS IN THE FAT BODY OF *PERIPLANETA AMERICANA* INDUCED BY STARVATION AND REFEEDING

Depletion of metabolic reserves during inanition has been studied in several insects. Wigglesworth (1953) gives a brief account of the chemical changes associated with starvation in insects. Generally, the available carbohydrates are first utilised and fat forms the chief reserve substance to be utilised. With respect to their ability to metabolize proteins, insects differ greatly. In some insects a high percentage of protein may be oxidized before death while in others there is very little or no utilization of body proteins during complete inanition.

Kreusher (1921) found in adult *Dytiscus* that the fat and protein were considerably reduced during starvation. In the larvae of *Aedes aegypti*, fat, protein and carbohydrate appear to be used up concurrently during starvation (Wigglesworth, 1942) while in *Deilephila* the fat reserves may be reduced as much as 70% when only half of the total energy reserve has been depleted (Heller, 1926). Mellanby (1932) in a study of the effect of atmospheric humidity on the metabolism of the fasting meal worm, observed that in this insect glycogen was used mainly at the beginning of the

starvation period and a loss of approximately 50% fat occurred during the period of a month of fasting,. Ludwig (1950) found in the starved nymphs of grasshoppers that 97% of the glycogen was used during the first week and that fat appeared to be the chief reserve substance to be utilised after the glycogen store was exhausted. The grasshopper nymphs utilized 25% of the fat during the first week and 68.6% during complete inanition. Lafon (1941) observed a loss of 69% of fat during complete starvation of the fly Phormia regina. Newton (1954) found that during starvation, the Japanese beetle larvae had used up 80% of glycogen, 28.6% glucose and 71.7% of fat. In Blattella germanica starvation which may not greatly deplete the fat reserve may lower the iodine number indicating a differential utilization of fat (Mellanby, and Maynard 1937). If the cockroach is given protein alone it exhausts its reserve of fat and then starves to death with the fat body still containing masses of protein crystals (Gier, 1947)

The depletion of the metabolic reserves seems to be a limiting factor in various conditions as in reproduction sustained flight, hibernation and prolonged starvation. Therefore, changes in the different enzyme systems concerned with the metabolism of the food reserves should be expected, Since fat forms the chief reserve to be utilized during starvation, it was thought worthwhile to find out the changes in the fat splitting enzyme, lipase, during prolonged starvation

## MATERIALS AND METHODS

Studies were carried out on the perivisceral fat body of female cockroaches (Periplaneta americana). The animals were maintained on a diet of broken wheat kernels containing 10% brewer's yeast, at room temperature and provided with sufficient water. They were subjected to starvation of varying periods upto a maximum of 30 days. After 10 days of starvation water was made available to the starving animals occasionally. After a period of four weeks the fat body was shrivelled up so that it could not be handled conveniently for biochemical studies.

Insects starved for four weeks were refed on the original food and water and lipase activity of the fat body was measured at intervals of 24 hours after the refeeding was started.

The fat body homogenate was prepared and the lipase activity was determined using the Warburg apparatus as described in earlier chapters.

Simultaneous histochemical studies on lipids of the fat body of starved and fed cockroaches were made. For the study of lipids the tissue was fixed in 10% calcium-formol for 24 hours at room temperature, post-chromated for 18 hours at room temperature and 24 hours at 60°C.

Sections were cut from gelatin blocks on a freezing microtome and stained with Sudan black B or Fettrot 7 B in ethylene glycol. Some tissues were post-osmicated with 1% solution of osmic acid for 24 hours after fixing in 10% calcium- formol for 24 hours, washed in running water to remove osmium tetraoxide and processed for paraffin sectioning using benzene as clearing agent.

## RESULTS

Changes observed in lipase activity of the fat body during starvation and refeeding are shown in fig 1 and Tables 1 and 2.

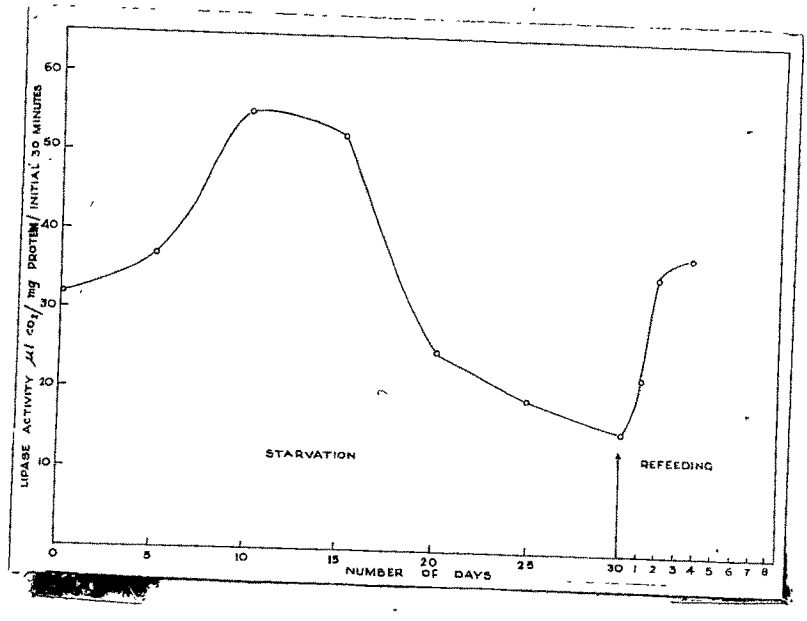



FIG. 1

Graph showing the effect of starvation and refeeding on the activity of the fat body lipase.

TABLE 1

Table showing the effect of starvation on the lipase activity and the rate of fat depletion in the fat body of Periplaneta americana.

| No. of days starved | Lipase activity µl $CO_2$/mg protein/ 30 minutes | Activation (%) | Inhibition (%) | Amount of lipid in the fat body |
|---|---|---|---|---|
| - | 32.5 ± 1.2 | - | - | 8+ |
| 5 | 37.0 ± 1.8 | 13.9 | - | 7+ |
| 10 | 55.5 ± 3.1 | 72.0 | - | 5+ |
| 15 | 52.0 ± 2.5 | 61.2 | - | 4+ |
| 20 | 24.5 ± 3.0 | - | 24.0 | 3+ |
| 25 | 19.5 ± 7.0 | - | 40.1 | 2+ |
| 30 | 15.5 ± 3.0 | - | 52.3 | 1+ |

TABLE 2

Table showing the effect of refeeding on the lipase activity of the fat body of Periplaneta americana, starved 30 days.

| No of days refed | Lipase activity µl $CO_2$/mg protein/ 30 minutes | Activity recovered (%) | Amount of fat in the fat body |
|---|---|---|---|
| 1 | 23.5 ± 2.5 | 72.3 | 1+ |
| 2 | 30.5 ± 2.0 | 98.7 | 1+ |
| 3 | 39.0 ± 2.5 | 120.0 | 3+ |

There was a gradual increase in the enzyme activity from the fifth day of starvation. The maxium activity, an increase of about 72%, was observed on the tenth day. Though after this the activity declined, it was maintained at a considerably high level till the fifteenth day of starvation. In the subsequent period, the level of lipase activity fell and by the end of the thirtieth day 52% inhibition was noted.

Refeeding the cockroaches starved for 30 days brought about gradual increase in the lipase activity of the fat body and normal conditions were restored by the end of the third day of refeeding.

Simultaneous histochemical observation of lipid depletion revealed a gradual and uniform decrease of fat from the fat body cells though during the period between the fifth and the tenth day the lipid depletion was more (fig. 2,3,4,5). It was noticed in the late stage of starvation that fat in the fat cells was more labile and easily diffusible.

At the end of 30 days starvation, it was observed that there was some amount of fat still remaining in the fat body cells.

## DISCUSSION

In insect life the metabolic store of the fat body is important during metamorphosis, hibernation, migration,

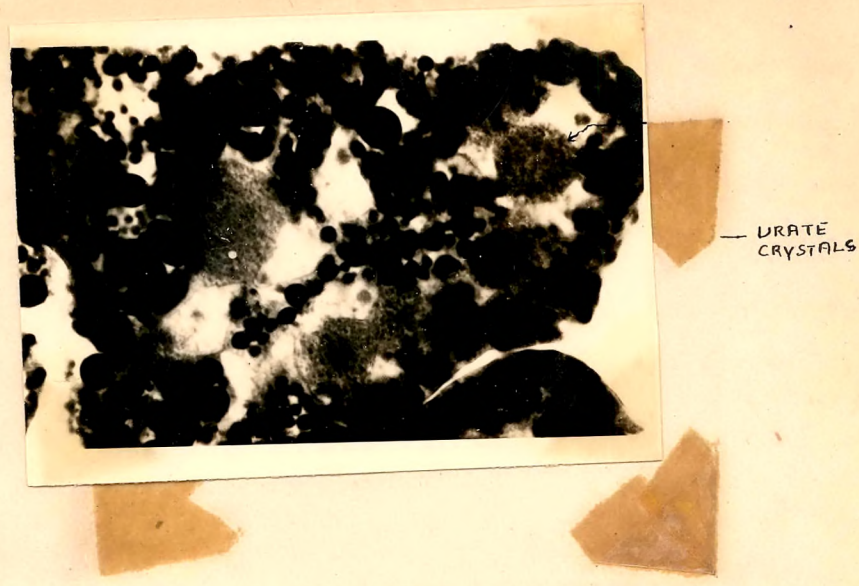
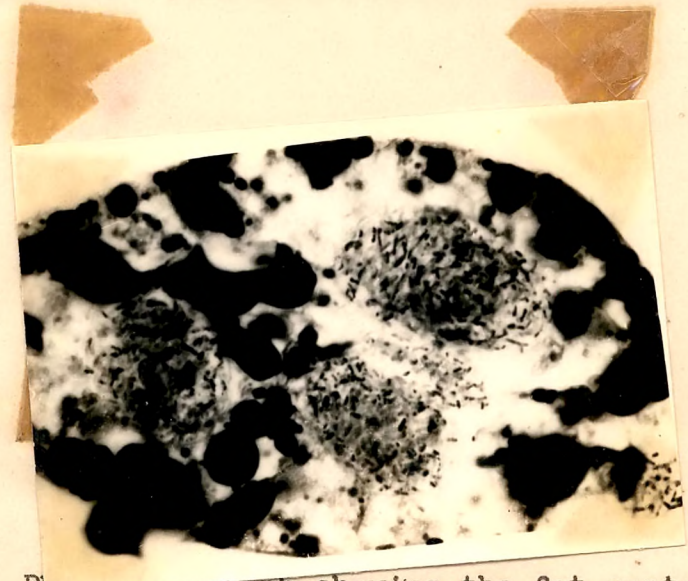



FIG. 2

Photomicrograph showing the fat content of the fat body of normal Periplaneta americana. (post-osmicated)



Photomicrograph showing the fat content of the fat body of Periplaneta americana starved for 5 days. (post-osmicated)

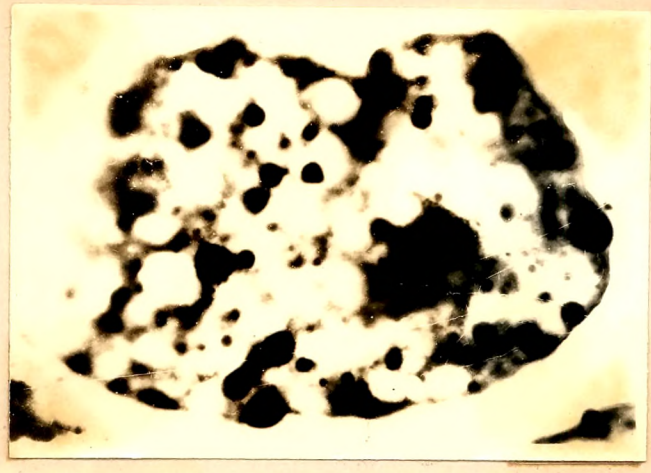


FIG. 4

Photomicrograph showing the fat content of the fat body of Periplaneta americana starved for 10 days. (post-osmicated)

FIG. 5

Photomicrograph showing the fat content of the fat body of Periplaneta americana starved for 20 days. (post-osmicated)

starvation etc. In these circumstances, the depletion of metabolic reserves such as carbohydrate, protein and fat from the storage depots takes the role of a limiting factor in the activity of the insect.

One of the physiological consequences of starvation is the depletion of the food reserves from the depots. As a result of two opposite processes, esterification and lipolysis, a dynamic equilibrium appears to exist in the fat body tissue between the stored triglycerides and the metabolic pool of tissue fatty acids. Therefore, changes either in the total glycerides or tissue fatty acids could potentially alter the rate of breakdown or the rate of triglyceride storage. Since most insects during the initial period of starvation draw upon the carbohydrate reserves (Wigglesworth, 1953), soon they are depleted of their carbohydrate store resulting in a greater demand for tissue fatty acids.

It is known from studies on vertebrate adipose tissue that a relationship exists between lipogenesis and the nutritional state of the animal. Fasting markedly diminishes carbohydrate metabolism and lipogenesis (Hausberger et al, 1955; Perry, 1958; Jeanrenaud, 1961) and during impaired glucose metabolism (like starvation and diabetes), the rate of lipolysis exceeds the rate of esterification. This stimulates the release of fatty acids from the adipose tissue.

It is also reported in vertebrate adipose tissue that extracts prepared from starved animals contained significantly high level of lipolytic activity than tissues from fed animals(Rizak, 1961). The increased lipase activity observed in insect fat body after a period of 5 days' starvation may be correlated with an increased demand for lipid mobilization from the fat store as a consequence to an impaired carbohydrate metabolism which evidently is due to the depletion of carbohydrates during the early period of starvation. The dynamic equilibrium existing between the triglycerides and the tissue fatty acids, thus disturbed, it could be expected, that the rate of lipolysis would exceed the rate of esterification.

Apart from the reduced lipogenesis during fasting, a secondary activation of the insect fat body lipase is also possible since the activation of certain tissue lipases by the product of their hydrolysis has been reported (Wertheimer, et al, 1960)

The involvement of a neuroendocrine mechanism in the activation of lipase activity cannot be ruled out (see chapter 9 & 10) as it is known that in many insects allatectomy causes hypertrophy and increase in the fat content of the imaginal fat body (Thomsen, 1942; Pfeiffer, 1945; Bodenstein, 1953). The metabolic effect induced by the starvation on the activity of lipase may possibly be

dependent on the release of an endocrine secretion which activated the fat body lipase. Some evidence on this contention is available from the work on vertebrate adipose tissue, where the existence of a lipase which is sensitive to both epinephrine (Rizak, 1961) and ACTH (Hollenberg, 1960) has been shown.

Histochemical observations on fat depletion, however, presesented slightly different picture. The rate of lipid depletion was not always proportional to the level of lipase activity. The decrease in fat was uniform throughout the period of starvation once lipolysis sets in, though during the initial period it was more.

Tietz (1962) in a study of fat transport in the locust, found that when fat body tissue was incubated with palmitate-1-$C^{14}$ in phosphate saline, the acid was readily taken up by the tissue. Of that 80-90% was esterified and recovered in the glyceride fraction but when prelabelled fat body tissue was incubated in haemolymph, glycerides were released from the tissue into the medium but were not released from the tissue in phosphate saline, bovine serum, buffered solution of bovine serum albumin or egg albumin.

Since the fat body lies in the haemocoel and is immersed in heamolymph, which also circulates through the interspaces of the tissue, a free exchange of metabolites is possible between the cells of the fat body and the haemolymph. It is possible that the high lipase activity

observed during the initial period of starvation can bring about partial hydrolysis of the stored triglycerides and the product of this partial hydrolysis namely fatty acids and lower glycerides are released into the surrounding haemolymph. Histochemical observation on the more labile nature of the fat in the fat body cells during the later stages of starvation, lends support to this assumption. In this context it should be noted that the blood of Peri planeta americana has a very high lipase activity (unpublished observations)

The subsequent decrease observed in the lipase activity if the fat body during the later period of starvation may quite possibly be due to the substrate becoming a limiting factor in the cell system. Therfore, it seems that though fatty acids are stored exclusively as triglycerides in the fat body, the release of lipid reserve during starvation from the fat body may not involve the complete hydrolysis of the triglycerides. But it is likely that one of the factors which regulate lipid depletion from the fat body during starvation would be lipase.

The restoration of enzyme activity was very quick within 3 days the lipase activity reached the normal value.

It may be noted that the insect fat body lipase is quite distinct from the lipoprotein lipase of vertebrate

adipose tissue (Hollenberg, 1956; Cherkes and Gordon, 1959) and lipase in the rat diaphragm (George and Susheela, 1962) because the activity of these enzymes decreased during starvation while the insect fat body lipase increased during starvation.