Chapter 3

A QUANTITATIVE STUDY OF LIPASE ACTIVITY IN THE FAT BODY OF A FEW ORTHOPTERAN INSECTS

Fat metabolism has been studied in considerable detail mostly in laboratory mammals and it has now become possible to follow several of the intermediate steps of fat synthesis and degradation. Comparatively little is known about the metabolism of fats and the enzyme systems involved in insects. The occurrence of lipases capable of splitting the ester linkages of fatty acids with glycerol, are known in some insects. George and Eapen (1959 a, b)who studied lipase and alkaline phosphatase activity in the fat body of the desert locust Scistocerca gregaria, reported high lipase activity (more than double the lipase activity in the pigeon adipose tissue and many times that in the flight muscles of the locust) in the fat body.

Since fat forms the chief reserve material in the orthopteran fat body, a study on the activity of lipase in a few representative orthopteran insects was undertaken. In the orthopteran insects studied, the colour of the fat body tissue varied from opaque white to various shades of yellow and bright yellow. The lipase activity in the fat body of each species was estimated quantitatively. It was found that the lipase activity of the tissue was related in some way to the degree of pigmentation of the fat body cells.

MATERIALS AND METHODS

The fat body tissue in the following insects was studied.

Periplaneta americana
Grillus sp.
Gryllotalpa africanus
Mecapoda elongata
Acrida exaltata
Aeolopus affinis
Epacromia dorsalis
Poicelocera picta
Schistocerca gregaria

Cockroaches were obtained from stock culture maintained in the laboratory. The grasshoppers were secured locally and kept in laboratory till used. Locusts were supplied by the locust entomologist, Government of India, and were kept for some time in cages under laboratory conditions.

The animals were anaesthetized by chilling for 15-30 minutes and dissected. Thin sheets of the fat body mostly from the perivisceral region were quickly removed, seperated from air sacs and tracheal tissue, blotted on filter paper, weighed on a precision balance and homogenized in ice cold distilled water in a chilled mortar so as to obtain a 2.5% homogenate. The enzyme was extracted for 15 minutes. The homogenate was centrifuged at 2500 r.p.m. for 3 minutes. The sediment and the floating lipid scum were discarded and the infranatant solution was pippetted out and mixed well.

Female insects of more or less the same age as judged on the basis of the development of their ovaries were used throughout the study. The colour of the fat body in the various insects studied ranged from opaque white in Periplaneta americana to various shades of yellow in the grasshoppers and the locust. One of the grashoppers viz. Mecapoda elongata contained two types of fat body, a lightly pigmented (mostly ventrolateral and peripheral) and a highly pigmented (mostly perivisceral and dorsal). The lipase activity of the two types of fat body was estimated separately. The assessment of the intensity of the yellow pigmentation of the fat body cells was done through visual approximation.

The method adopted for the estimation of lipase activity was that of Martin and Peers (1953), using the conventional Warburg apparatus. The reaction was carried out in a bicarbonate-carbondioxide buffer system at pH 7.0 using a $N_2:CO_2$ (95:5) mixture as gas phase. Tributyrin was used as substrate. Protein in the enzyme solution was estimated by the micro-Kjeldahl method (Hawk et al, 1954).

Each Warburg flask contained 1.5 ml of 0.01 M sodium bicarbonate buffer, 0.5 ml of enzyme solution and 0.5 ml of distilled water in the main chamber and 0.5 ml of 4% (W/V) of tributyrin in 0.0058 M sodium bicarbonate emulsified with a drop of tween 80, in the side arm. After 10 minutes of equilibration of the Warburg flasks in a constant temperature water bath at 37°C the substrate was tipped in and initial readings were taken after 3 minutes of tipping in of the substrate. Throughout the experiment the manometers were oscillated horizontally 120 times per minute with an amplitude of 2.5 cms. Readings were taken at intervals of 30minutes each for one hour and the results are expressed as μl CO_2 /mg protein/ initial 30 minutes.

RESULTS

Results are tabulated in table 1 and illustrated in figure 1. It is seen that the fat body of Poicelocera picta has the maximum pigmentation and highest lipase activity, though in enzyme activity slightly less than in Schistocerca gregaria. The fat body of Periplaneta americana lacks any yellow pigmentation in the fat body cells and hence is called white fat body. The fat body of Gryllus sp. and Gryllotalpa africanus shows slight pigmentation. The lipase activity differed considerably between the white and the yellow types of fat body. In Mecapoda elongata both the types of fat body are present in the same animal and showed a difference in lipase activity between them. (that of the yellow variety having 1½ times that of the white type).

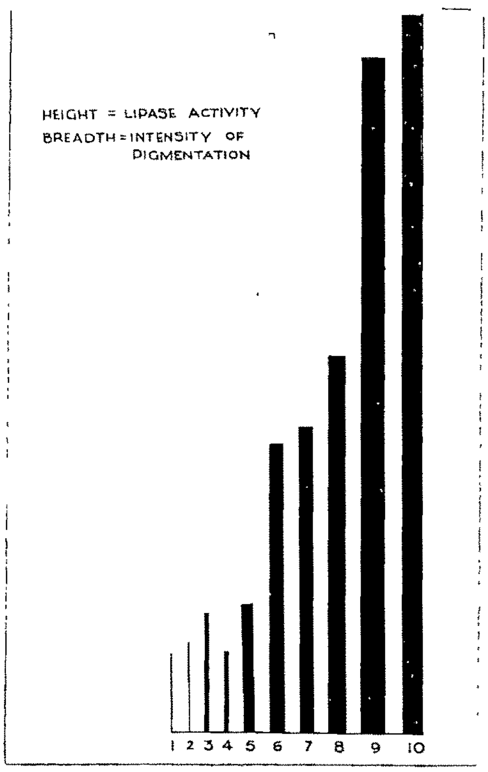

FIG.1.

Histogram illustrating the relation between lipase activity and the degree of pigmentation of the fat body tissue. 1. Periplaneta americana, 2. Gryllus sp., 3. Gryllotalpa africanus, 4. Mecapoda elongata, (white type), 5. Mecapoda elongata (yellow type), 6. Acrida exaltata, 7. Aeolopus affinis, 8. Epacromia dorsalis 9. Poicelocera picta , 10. Schistocerca gregaria.

TABLE 1

Table showing the relation between the lipase activity and the degree of pigmentation of the fat body tissue.

Name of insect	Lipase activity µl CO_2/mg protein/30 min. White type	Lipase activity µl CO_2/mg protein/30 min. Yellow type	*Intensity of pigmentation
<u>Periplaneta americana</u>	32.5 ± 1.5	-	-
<u>Gryllus sp</u>	55.0 ± 3.0	-	1+
<u>Gryllotalpa africanus</u>	73.5 ± 5.5	-	1+
<u>Mecapoda elongata</u>	48.0 ± 1.5	-	1+
<u>Mecapoda elongata</u>	-	78.0 ± 3.5	3+
<u>Acrida exaltata</u>	-	175.5 ± 8.0	4+
<u>Aeolopus affinis</u>	-	186.0 ± 11.2	4+
<u>Epadromia dorsalis</u>	-	230.0 ± 10.5	5+
<u>Poicelocera picta</u>	-	411.0 ± 11.5	8+
<u>Schistocerca gregaria</u>	-	433.0 ± 7.0	7+

* Intensity of pimentation of the fat body tissue was assessed visually and has been denoted as +

DISCUSSION

From the results obtained, it is seen that the enzyme solution obtained from the fat body of different insects studied, hydrolysed tributyrin at different rates which denotes different lavels of lipase actvity in the fat body of the different insects studied. This also may be regarded as an indirect measure of the extent to which the animal is able to esterify fatty acids and hydrolyse fats in its fat body

Lipids serve as an important source of fuel for the various phases of physiological activity in all organisms. The fat body of insects has been shown in recent years to synthesize lipids (Nelson, 1958; Clements, 1959; Zebe and McShan, 1959; Tietz, 1961) but information regarding the site and mechanism of fat utilization is scanty. However, it has been suggested that one of the sites of fatty acid oxidation may be the fat body (Gilmour, 1961). Moreover, the occurrence of the highly active alpha glycerophosphate dehydrogenase in insect fat body (Zebe and McShan, 1957) suggests the existence of an efficient pathway for the oxidation of the glycerol moiety of neutral fats. The role of lipase in the fat body should, therefore, be in the esterification as well as hydrolysis of fats.

The significance of the occurrence of lipase in tissues like the flight muscles of birds indulging in sustained flight (George and Scaria, 1956), heart muscles vertebrates (George and Scaria, 1957; George and Iype, 1963), flight muscles of insects (George et al, 1958; George and Bhaktan, 1960 a, b, c), the adipose tissue of pigeon (George and Eapen, 1958) and the fat body of the locust(George and Eapen, 1959 a, b) has been established. It is also established that fat forms the chief fuel in the long and sustained flights of migratory birds and insects. Weis-Fogh (1952) showed that in the desert locust Schistocerca gregaria at least $\frac{2}{3}$ of the energy liberated during flight was derived from fat. The monarch butterfly, Danais plexippus which is a continental migrant lays down a store of fat as do migratory birds and apparently utilizes it during flight (Beall,1948; Zebe, 1954).

The free fatty acids present in the blood or the fat in the flight muscles however, are not sufficient to provide a continuous supply of the energy fuel so as to meet/the energy demands of sustained flight. Here again the mechanisms whereby lipids can be converted to yield energy at rates adequate to support prolonged flight in insects are not well understood. Sactor (1955) suggested that fats may be converted to acetates in the fat body which would then be transported to the flight muscles and oxidised there through the tricarboxylic acid cycle. The presence of an acetate activating enzyme has been detected in the flight muscle mitochondria of the honey bee (Hoskins et al, 1956)

Recent studies have shown that utilization of fat takes place through β-oxidation in insect muscle (Meyer, 1960). Zebe (1960) has shown the presence of a condensing enzymes which aids the entry of acetic acid into the citric acid cycle and a β-keto-acyl-thilase which leads β-keto acids into the fatty acid cycle, in the mitochondria of the locust flight muscle. The activity of these enzymes was many times more when compared with that in the pigeon and rat tissues. It should, however, be stated that fatty acids could be directly oxidised through β-oxidation. Honey bee flight muscle has been shown to possess high lipase activity and also to oxidise butyrate *in vitro* (George and Bhaktan, 1963). These authors have also shown that lipase activity in the honey bee flight muscle is higher in that part of the day when the insects are more active. The role of lipase in breaking down fat to fatty acids is thus evident. It has now been shown that in birds as the muscle fat gets depleted, fatty acids are transported to the muscle from the adipose tissue (George and Vallyathan, unpublished).

The high activity of lipase observed in the yellow fat body of the various grass-hoppers may be correlated with their ability for sustained flight. *Poicelocera picta*, however, is an exception because it does not indulge in sustained flight. But on the other hand its voracious feeding habit and reproductive prodigality perhaps accounts for the presence of an actively fat synthesising and degrading system.

The apparent relation between lipase activity and the yellow pigmentation is however, difficult to explain and calls for further investigation. The occurrence of two types of adipose tissue, brown and yellow, in certain mammals may be mentioned here(Fawcett, 1952; Remillard, 1958) Fawcett (1952) using histochemical methods reported a much higher concentration of lipase in the brown than the yellow adipose tissue of the rat. But no detectable difference between the brown and yellow adipose tissue in the bat was noted in a recent histochemical study with respect to lipase (George and Eapen, 1959 c). However, a quantitative study by the same authors (1959 d) revealed that in the bat the yellow adipose tissue has a higher lipase activity than the brown. The lipase activity in the pigeon adipose tissue, which is of the yellow type was found to be about six times greater than that of the yellow adipose tissue of the bat (George and Eapen, 1958). This higher concentration of lipase activity in the yellow adipose tissue has been attributed to the ability of the yellow adipose tissue to mobilize fat to a greater measure and at a faster rate (George and Eapen, 1958).