CHAPTER 4

THE FAT BODY OF A FLYING INVERTEBRATE (THE DESERT LOCUST) - LIPASE AND ALKALINE PHOSPHATASE ACTIVITY

The adipose tissue of vertebrates especially mammals, has been the subject of extensive studies in recent years. Evidence is accumulating to show that this tissue in the vertebrates is by no means metabolically less active than other known active tissues. The fat body of insects are often compared with the liver of vertebrates on account of their varied metabolic functions. Using histochemical methods Coupland (1957) demonstrated glycogen, fat, proteins and nucleoproteins in the locust fat body. Certain other aspects of the metabolism of this tissue in the locust were also investigated, by Kilby and Neville (1957), Hearfield and Kilby (1958) and Candy and Kilby (1959). In a recent study Fenwick (1958) distinguished at least two distinct types of particles, in the fat body of the locust, which he obtained by differential centrifugation and according to him they resemble mammalian liver mitochondria and microsomes respectively. Bellamy (1959) studied the oxygen consumption and oxidation of a few metabolites using particulate components of various tissues of the locust, the fat body being one of them. However, no information is available on lipase and alkaline phosphatase activity in this tissue. Results of a quantitative and histochemical study of lipase activity and a histochemical study of alkaline phosphatase activity in the fat body of the desert locust (Schistocerca gregaria) are presented here.

Material and Methods

Fat body was removed from the abdominal region after decapitation of the adult insect. The more readily dissected fat body thus taken consists mainly, if not solely, of true fat cells, the oenocytes being abundant towards the periphery, adjacent to the body wall (Coupland,1957). The enzyme material for lipase was an aqueous extract of ether-defatted fat body which was prepared in the following manner. Fat body was carefully removed free from most of the accompanying tracheae and tracheoles and defatted in two changes of ethyl ether at room temperature for 1 hour. Subsequently it was dried in a vacuum desiccator at room temperature. Almost all the fat was removed within this period. Fat bodies of about 20 locusts were pooled and portions of this used for the assay. Extracts of the material were made in distilled water in cold ($4^{o}C$) for 1 hour by grinding it in a test tube; centrifuged at about 2500 r.p.m for 5 minutes and the supernatant used for the study.

The method used for the assay was a manometric method adopted from Martin and Peers (1953) using the Warburg apparatus, with a bicarbonate carbon dioxide buffer system of pH 7.4 at $37^{o}C$ using tributyrin as substrate. The reaction flask contained 1.5 ml of 0.025 M bicarbonate solution, 1 ml enzyme solution in the main chamber and 0.5 ml 4% (v/v) tributyrin in 0.0148 M bicarbonate (emulsified by shaking with a small drop of "Tween 80") in the side arm, thus making up a total fluid volume of 3 ml. The manometers and flasks were gassed for 3 minutes with a mixture of 95% N_2 and 5% CO_2 from a cylinder. After equilibration for 10 minutes the substrate was tipped in and the readings taken

twice at intervals of 15 minutes. The manometers were shaken at about 100 oscillations per minute allowing an amplitude of 4 - 5 cm/ oscillation.

Lipase activity was calculated on the basis of the protein concentration of the enzyme solution used and is expressed as the number of µl CO_2 produced per mg protein per 30 minutes. The quantity of CO_2 evolved is equivalent to the amount of butyric acid liberated from the substrate by enzymic action. Protein was estimated according to the micro-Kjeldahl steam distillation method for total proteins (Hawk et al,1954).

Fresh frozen sections of the fat body, isolated in the same manner described earlier, was used for the demonstration of lipase and alkaline phosphatase activity. For the demonstration of lipase, Gomori's "Tween" method using "Tween 80" as substrate was employed. Alkaline phosphatase was demonstrated employing the revised method of Gomori making use of sodium glycerophosphate as substrate. The procedures were the same as employed for the histochemical study of the pigeon adipose tissue (Chapter 2).

Results and Discussion

Table 1 presents the lipase value of the fat body of the locust, along with the lipase value of the flight muscles of the same animal for comparison. The lipase activity in the fat body of the locust is more than double the concentration of the same enzyme occurring in the adipose tissue of the pigeon (Chapter 5) and many times more than that in the flight muscles of the locust. The protein concentration of the enzyme solution was on the average 1.8 mg/ ml in locust and 4.7 mg/ ml in pigeon.

Table 1

Tissue	Lipase activity: $\mu l\ CO_2$/ mg protein/ 30 min
Fat body	312.3
Flight muscles*	9.5

* George, Vallyathan and Scaria,1958.

Both lipase and alkaline phosphatase are present in appreciable concentrations (Figures 1 and 2).

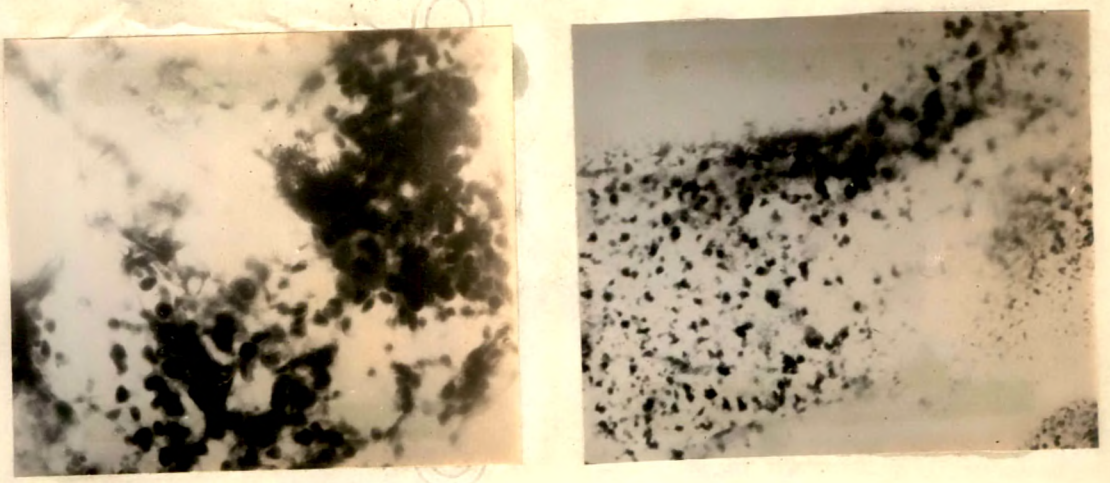

Fig. 1 Fig. 2

Fig. 1 and 2 - The photomicrographs of sections of the fat body of the locust treated respectively for lipase and alkaline phosphatase activity.

In the case of the sections treated for alkaline phosphatase, there were present numerous round or ovoid bodies which stained intensely for the enzyme. These appeared to be the nuclei. The quantitative study of lipase (Table 1) not only confirms the results obtained by the histochemical study of this enzyme in the

fat body, but has also shown the presence of very large quantities of the enzyme. Lipase has been recently demonstrated in the flight muscles of the desert locust (George, Vallyathan and Scaria,1958). The same authors (1958) obtained a value of 50 µl CO_2/ mg protein/ 30 minutes for the flight muscles of the dragon fly (Pentala flavescens) and suggested that the comparatively lower lipase content in the locust flight muscles may be due to the fact that the locusts used were laboratory bred and kept in captivity throughout their lives. In my present study also, locusts which were reared and maintained in the laboratory were used.

The lipase present in the muscle should split the muscle fat into their component fatty acids and glycerol which can be further oxidized for energy purposes. But the fat occurring in the muscle itself may not be adequate for replenishing the fat used up during sustained muscular exercise. Then fat has to be made available to the muscle from extraneous sources like fat body. The fat body therefore could provide the fat required by the muscle, since it contains large quantities of fat. The high concentration of lipase is useful for the synthesis of the large quantities of fat present in the fat body and also for the breakdown of the fat for utilization for energy purposes. This is significant due to the fact that the desert locust has been shown to utilize chiefly fat - about two-thirds of the total energy - for muscular energy during sustained flight (Weis-Fogh, 1952). The fat body could thus synthesize, store and supply the fat needed to meet the various demands for energy, particularly for sustained muscular activity such as during migratory flights.

However, for the confirmation of this, further experimental evidences are necessary.