CHAPTER 11

THE IN VITRO LIPASE ACTIVITY AND OXIDATION OF BUTYRATE BY THE HONEY BEE (APIS MELLIFERA) FLIGHT MUSCLE HOMOGENATE

George, Vallyathan and Scaria (1958) found that the bumble bee flight muscles contain a lipase. They suggested that the occurrence of this enzyme is an indirect indication of the extent of fat being utilized in the flight muscles. This suggestion was confirmed by detecting a lipase in the flight muscles of the desert locust, Schitocerca gregarea which is known to utilize chiefly fat for energy during flight (Krogh and Weis-Fogh, 1951, Weis-Fogh 1952). The occurrence of this enzyme in sites where fat is utilized and the significance of the same in relation to fat utilization has been discussed in the previous chapters (Chapter 1, 2, 4, 5, 6, 8). In the flight muscle homogenates of Periplaneta americana (Barron and Tahmisian 1948) and Musca domestica (Sacktor, 1955) it has been shown that acetate is oxidized in considerable amounts Sacktor (1955) suggested that fat utilized for energy during muscular activity, must have been broken-down to the level of acetate and transported to the muscles for further oxidation. Recently an acetate activating enzyme in the flight muscle mitochondria of the honey has also been discovered (Hoskins et al. 1956). Supporting the suggestion that acetate is a

substrate that could be oxidized in the honey bees flight muscles. It has been recorded that honey bees flight muscles contract at a very high frequency upto 1000/sc Sotavalta, 1947). The energy for such intense contractions for short durations have been shown to be glucose. The Lepidopteran insects which also feed on nectar, are found to utilize fat instead of glucose in their flight muscles (Zebe, 1954). Apis mellifera is a comparatively bigger Hymenopteran and the workers indulge in continuous flights. In the present chapter is given the resutls of an investigation carried out to assess the lipase activity in the flight muscles and the capacity to oxidize fat.

Materials and methods

The bees are collected during flight as well as from their hives during night and used separately. It was observed during experiments that considerable variations in the oxidation of butyrate occurred and was found to be related to the state of the insects depending on whether they were in flight or in rest. Hence the oxidation of butyrate was done during the day time at the peak period of activity (found to be after 2 P.M.) and during rest at night after 9 P.M.

Lipase activity of the flight muscles was determined adopting the method of Martin and Peers (1953) and the procedure was exactly as described in the previous chapters.

For the oxidation of butyrate, the neutral sodium salt of butyric acid prepared was used as the substrate, in the Warburg apparatus. Since it is known (Knox, et al, 1948; Green, 1951) that the presence of a citric acid cycle intermediate is necessary as 'sparker' in the fatty acid oxidation system, malate was used as the sparker. The gas phase for the reaction was air and the experiments were conducted at 37°C. Each reaction flask contained 0.6 ml 0.1 M phosphate buffer of pH 7.4, 0.15 ml 0.15 M $MgCl_2$, 0.2 ml 0.0225 M ATP, 0.15 ml 0.00225 M cytochrome c (Sigma Chemical Company), 0.2 ml 0.015 M DPN, 0.2 ml 0.0075 M Coenzyme A (Sigma Chemical Company), 0.2 ml 0.15 M sodium malate, 0.5 ml of 0.03 M sodium butyrate and 0.8 ml of the homogenate making a total volume of 3 ml. This gave a final concentration of 20 µM phosphate buffer, 7.5 µM $MgCl_2$, 1.5 µM ATP, 0.1125 µM cytochrome c, 1 µM DPN, 0.5 µM Coenzyme A and 10 µM sodium malate per Warburg flask. The final concentration of butyrate was 5 µM. 0.2 ml of 20% KOH was pipetted in the central well of the reaction flask and placed a roll of filter paper. The homogenate was added last. The homogenate prepared was centrifuged for 2 minutes at about 600 r.p.m. so as to remove the particles of the cuticle if any remained in the homogenate. The flasks were placed on an ice slab till they were mounted on the manometers. The manometers were shaken at 100 oscillations per minute with an amplitude of 4.5 cms. After 10 minutes of temperature equilibration, readings

were taken at every 10 minutes for 1 hour. The value is expressed as µl O_2 per mg. dry tissue per hour. The dry weight of the tissue was determined as described in Chapter 10.

Results and Discussion

The results obtained for lipase activity and butyrate oxidation are given in table 1. The oxygen uptake due to butyrate oxidation was more during the day time thanthat in the night as is evident from the results.

Table I

Honey bee flight muscle homogemate	µl O_2 uptake for malate	µl O_2 uptake for malate + butyrate	Lipase activity µl CO_2/mg.protein/hour
Day	7.70	9.58	13.25
Night	6.88	7.18	

In the presence of malate, butyrate was oxidized nearly 25% more in the day time during the peak period of activity (between 2 to 5 P.M.) and during night the oxidation of butyrate was comparatively little. Malate oxidation also was less at night. This shows that during the resting period the metabolic rate of the flight muscles is low. The utilization of butyrate during intense activity suggests that though honey bees feed on nectar and are known to

utilize chiefly glucose as fuel they do possess the ability to utilize fat also as a fuel. This is further supported by the lipase activity of the flight muscles. Lipases are known to break down fat into fatty acids and glycerol. Therefore the lipase should play its role in bringing down the fat to the level of fatty acids for the subsequent step in the oxidation of fat via TCA cycle in the mitochondria. Butyrate is next higher to acetate in the length of the carbon chain and the enzyme for acetate oxidation was already detected in the flight muscle mitochondria.

During experimentation it was observed that oxidation of butyrate is not so much in the morning hours as was obtained in the afternoon. This suggests that the intensity of the activity has a bearing on the utilization of the fuel concerned. Though the honey bee is not equipped for the complete oxidation of most fatty acids as has been observed in the case of the desert locust, its capacity to utilize shortchained fatty acids, (Acetic acid and butyric acid) is quite evident. This also shows that the relationship between the type of the muscle fibres and the type of the fuel used as pointed out by some earlier workers (Wiggles-worth, 1949) is not unflexible.