CHAPTER 6

ON THE LIPASE ACTIVITY IN THE MUSCLES OF THE DIFFERENT THORACIC SEGMENTS OF THE COCKROACH, PERIPLANETA AMERICANA

It has been shown in the earlier chapters (1, 2 and 4) that insects which indluge in prolonged muscular activity possess a considerably high concentration of the enzyme lipase which indirectly indicates the extent of fat being used up in their flight muscles for energy. The extensive studies of Krogh and Weis-Fogh (1951) and Weis-Fogh (1952) on the metabolism of the desert locust and subsequently by George, Vallyathan and Scaria (1958) who detected a fat-splitting enzyme lipase in the same insect provided further support to the fact that in locusts fat is chiefly utilized for energy. The report of Fulton and Romney (1940) on the fat content of the beet-leafhopper also is suggestive of fat being metabolized in their muscles during their migratory flights. The cockroach which is also an orthopteran and is a good runner but not a good flier, has been the subject of several biochemical and physiological investigations. It has been reported that the cockroach muscle is as metabolically active as any highly metabolically active vertebrate muscle. Barron and Tahmisian (1948) reported that all the enzymes and the related substrates tested for are found to be concentrated comparatively more in the cockroach muscle than in the vertebrate muscle. They also pointed out a difference in the metabolic activity in the sexes, the activity being more in the male than in the female. Since no information is available on the muscle lipase in this insect which is a poor flier in comparison with other orthopterans like locusts, the present investigation was undertaken.

Material and Methods

The cockroaches were collected locally and maintained in the laboratory under appropriate conditions and only adult forms were used. After severing the head and abdomen the remaining part of the insect was pinned on a wax board. The legs were removed and the thorax cut opened on the ventral side. Any part of the alimentary canal remaining was pulled out and the fat bodies were also removed as far as possible. The three thoracic segments were separated out at their joints and blotted on a filter paper. After removing the exoskeletal cuticle as far as possible the muscles of the different thoracic segments were homogenized separately in ice cold mortars, in distilled water, centrifuged for 3 minutes at 2500 r.p.m. and the supernatent was used as the enzyme solution in all the cases. The lipase activity of these muscles were manometrically determined quantitatively according to the method followed by Martin and Peers (1953). The enzyme solution was incubated in a Warburg apparatus with the appropriate substrate - tributyrin (4% v/v emulsified with a drop of "Tween 80" in 0.0148 M sodium bicarbonate) in a gas phase of 95% nitrogen and 5% carbon dioxide at 37°C. The procedure was as described in the earlier chapters.

The lipase activity is expressed as μl CO_2 liberated per hour per mg. protein of the enzyme solution. Protein was estimated using the micro-Kjeldahl steam distillation method (Hawk, et al, 1954). All experiments were performed in duplicate and each experiment was repeated 5 times.

Results and Discussion

The results obtained show that the metathoracic muscles in either sex possess the highest activity for the enzyme lipase. It is also evident from the results (Table 1) that the male thoracic muscles in comparison with the same muscles of the female show a much greater concentration of the enzyme. In the earlier chapters it has been suggested that the activity of lipase could be considered as an index of the extent of fat utilization for energy during activity.

Table 1. Lipase activity in the different thoracic segments of the cockroach, *Periplaneta americana*.

S e x	Lipase activity: ul CO_2/mg.protein/hr.		
	Prothorax	Mesothorax	Metathorax
Male	104.91	111.78	133.96
Female	82.48	90.12	96.69

Cockroach muscle has been the material used in several biochemical investigations and much of our present knowledge of the intermediary metabolism in insect muscle is obtained from the investigations of Barron and Tanhmisian (1948) Humphrey (1953) Sacktor and Boadenstein (1952), Sacktor and Thomas (1954), Kubista (1954) and Richards (1956). These investigators have studied the different aspects of the energy balance sheet in the cockroach muscle. They were able to show a marked difference between the male and female sexes, the male being more active than the female. The striking difference seen in the concentration of lipase in the male and female cockroaches indicates that the male cockroach muscle has a greater capacity to burn fat for energy during activity. The data obtained by Barron and Tanhmisian (1948) indicate an equal distribution of the oxidative enzymes and the related substrates, being more in the male cockroach. They also suggested that sex hormones may be playing a part in the regulation of the muscle metabolism. Again the cytochrome system has been found to be present in high concentrations in the male, which might partly be responsible for the higher metabolic activity of cockroach muscle. The observations of Sacktor and Bordenstein (1952) on the cytochrome oxidase activity of the different tissues of the cockroach have shown that the muscle tissue contains more of this enzyme than any other tissue. They also found a marked difference in the concentration of this enzyme in the same muscles in the two sexes, the male having three times more than in the female. Allen and Richards (1954) reported a two fold difference in the oxygen up-take of the thoracic muscles of the cockroach in the presence of added substrates. The quantitative study in the blood proteins on the male and female cockroaches (Stephin, 1957) also suggest that there exist a quantitative difference in the different proteins in the blood. All these observations tend to confirm the fact that sex in cockroach has a marked influence on the overall metabolism of the insect.

Recent studies on lipogenesis in the cockroach using labelled C^{14} acetate have shown that fatty acid synthesis from C^{14} acetate was 2.5 to 4.7 times greater in the male than in the female (Louloudes, 1961). This suggests that lipase should play an important role in the hydrolysis as well as in the sythesis of fat. The higher concentration of lipase in the male therefore could be expected for its greater capacity to sythezise or to break down fat. In attempting to correlate the colouration and the activity of the oxidative enzymes in the red and white muscles of the male and female cockroaches respectively, Nakatsugawa (1961) isolated the mitochondria from both the sexes and spectrophotometrically estimated the cytochrome content. No great difference in the concentration of the cytochromes in the mitochondria of the male and the female was obtained. Nevertheless, the difference of 3 to 4 times in the content of cytochromes in the muscles of the male and famale is largely due to the greater number of mitochondria present in the male muscle. The muscle of the male yielded more than 3 times as much mitochondria as the muscle of the female. The studies on the beetle muscle (Chapter 5) have shown that the mitochondria contain a considerable amount of lipase and it is an established fact that mitochondria can burn fat (Green, 1954, 1956). The higher lipase activity can therefore be correlated structurally with the greater amount of mitochondria in the muscle.