CHAPTER 4

A QUANTITATIVE STUDY OF LIPASE AND SUCCINIC DEHYDROGENASE IN THE THORACIC MUSCLES OF THE BEETLE (HELIOCOPRIS BUCEPHALUS.FABR.)

The occurrence of high concentrations of lipase and succinic dehydrogenase in the flight muscles of some insects has been reported and its possible significance in sustained flight has been discussed (Chapter 1, 2 and 3). Edwards and Ruska (1955) studied the coleopteran flight and leg muscles with the electron microscope and observed that the flight muscles are red in colour, intensely tracheated, their fibres possess a large number of sarcosomes and are metabolically very active in contrast to the leg muscles which are white or pale in colour with fibres having a considerably less number of sarcosomes and intracellular tracheoles, and are metabolically less active. This chapter presents the data obtained from a quantitative investigation on the activities of the enzymes lipase and succinic dehydrogenase, in the prothoracic and meso-metathoracic muscles of the beetle (Heliocopris bucephalus).

Materials and Methods

The beetles used were locally collected during flight at night. After decapitation the beetle was fixed on a wax board. The elytra and wings were removed and a median incision on the dorsal side exposed the internal musculature and alimentary canal. After removing the alimentary system and the fat bodies, the muscles from the prothoracic and the meso-metathoracic regions were separately taken out in clean and dry watch

glases and weighed on a "Metlar balance". For lipase, an aqueous extract obtained after homogenizing and centrifuging the tissue at 2500 r.p.m., was used as the enzyme material. Lipase activity was quantitatively determined in the manometric system of the Warburg apparatus employing the method of Martin and Peers (1953), using tributyrin as substrate. The procedure was followed exactly as described in Chapter 1. The lipase value is expressed in terms of μl CO_2/liberated for mg.protein per hour as well as on the basis of the wet weight of the tissue. Protein was estimated according to the micro-Kjeldahl steam distillation method (Hawk, et al, 1954).

The method developed by Kun and Abood (1949) using 2:3:5 triphenyl tetrazolium chloride (TTC) as the electron acceptor was employed to measure the succinic dehydrogenase activity in a colorimeter. The colourless dye (TTC) when reduced through enzymic action gives the coloured formazan which can be extracted in acetone and the intensity of the colour is read on a colorimeter. The amount of formazan formed can be read directly from the standard graph. For experimentation, the muscles separated as above were weighed and crushed in ice cold mortar and diluted with cold distilled water so as to get a 5% homogenate. The incubation mixture contained 0.5 ml 0.1 M phosphate buffer of pH 7.4, 0.5 ml 0.5 M sodium succinate solution, 1 ml of 0.1% freshly prepared TTC solution and 1 ml of the muscle homogenate thus making up the total volume of 3 ml. After shaking, the tubes were incubated for 15 minutes at 37°C. in a water bath. Immediately after the incubation period 7 ml

of acetone were added in each tube and stoppered tightly. After shaking them thoroughly to extract the colour as well as to stop the reaction, the tubes were centrifuged at 3000 r.p.m. for 3 minutes. The clear supernatent was used to read the intensity of the colour on a Klett-Summerson Photo-electric colorimeter at 420 mμ. Duplicate samples were always used during the experiment. The readings were corrected to the blank with zero time incubation. Succinic dehydrogenase activity is expressed in terms of μg of formazan formed per mg. dry weight of muscle per 15 minutes. The dry weight was determined by keeping 1 ml of the enzyme solution in a weighing bottle of known weight, and dried to constant weight at about 100°C.

Results and Discussion

The results obtained show that the meso-metathoracic muscles have a lipase value of 82.76 μl CO_2/mg. protein/hr. and the prothoracic muscles have only 33.51 μl CO_2/mg. protein/hr. When calculated on wet weight basis the values are 632.83 μl CO_2/100 mg. tissue/hr. and 237.49 μl CO_2/100 mg. tissue/hr. respectively. The difference in the lipase activity between the meso-metathoracic and the prothoracic muscles on protein as well as on wet weight basis, is more or less the same. The meso-metathoracic muscles are thrice as active as the prothoracic muscles (Table 1).

As observed in the case of lipase, the activity for succinic dehydrogenase was shown to be more in the meso-meta-

thoracic muscles than that in the prothoracic muscles. The meso-metathoracic muscles have a value of 10.77 µg formazan/ mg. dry tissue/15 minutes, while the prothoracic muscles have only 3.69 µg formazan /mg. dry tissue/15 minutes (Table 2). In the case of succinic dehydrogenase also the meso-metathoracic muscles are nearly three times more active than the prothoracic muscles.

Table 1

Lipase activity in the thoracic muscles of the beetle. The values are the average of 8 experiments.

Tissue	Lipase activity	
	µl CO_2/100 mg. tissue/hr.	µl CO_2/mg. protein/hr.
Prothoracic muscles	237.49	33.51
Meso-metathoracic muscles	632.83	82.76

Table 2

Succinic dehydrogenase activity in the thoracic muscles of the beetle. The values are the average of six experiments.

Tissue	Succinic dehydrogenase activity µg formazan/mg. dry tissue/15 min.
Prothoracic muscles	3.69
Meso-metathoracic muscles	10.77

The results obtained indicate that the meso-metathoracic muscles which operate the functional wings have a greater potential capacity for mobilizing and utilizing fat for energy than the prothoracic muscles, which are mainly responsible for the movement of the pair of legs attached to the prothorax. And it is also known that when a muscle contracts for a long time continuously the metabolic rate of the muscle will also be high and any tissue with a higher metabolic rate should possess a higher concentration of oxidative enzymes which are mainly responsible for the supply of energy through the proper metabolic cycles. Hence the higher concentration of succinic dehydrogenase in the meso-metathoracic muscles can be easily correlated with its higher metabolic activity. Among the skeletal muscles, insect flight muscles are the most metabolically active tissues and this has been established by several workers (Chadwick, 1940, 1953a, 1953b; Barron and Tamhmisian, 1948; Wigglesworth, 1949; Weis-Fogh, 1952; Sacktor, 1955, 1958; Zebe, 1954, 1956, 1957) by measuring the oxygen uptake by insects during rest as well as during flight. They were also able to show that during activity the oxygen uptake rises to 30 to 50 times the normal uptake which is only observed in the case of insects. The higher rate of oxygen uptake is possible only if the necessary substrates and the related enzymes are available for the biochemical reactions. These observations support the view that the meso-metathoracic muscles which operate the wings during flight should naturally be more

active metabolically, than the prothoracic muscles. It is also known that the muscles which utilize fat as chief fuel possess lipase in them which starts the first step in the utilization of fat (George and Scaria, 1956, 1957; George, Susheela and Scaria, 1958a; George, Vallyathan and Scaria, 1958; Chapters 1, 2 and 3) and a higher concentration of lipase should be an asset in a muscle that utilizes more fat. In such a muscle it is also logical to expect the presence of a considerable amount of the oxidizing enzymes which has indeed been shown to be true. It has also been shown that there does exist a relationship between the concentrations of lipase as well as the oxidizing enzymes in the flight muscles of birds (George and Scaria, 1958c) and bat (George, Susheela and Scaria, 1958b), in that the concentrations of these enzymes would be higher in metabolically more active tissues. In the insect muscle too, the occurrence of these enzymes in large quantities indicates that muscles which indulge in long and sustained activity preferably use fat as chief fuel and that the substrate and the related enzyme complex should necessarily be considerably higher in those muscles than the muscles which work only for short duration.

Observations on the histochemical localization of these as well as certain other enzymes in the thoracic muscles of the beetle (Chapter 3) also tend to support the fact that the structural and physiological differences in these muscles have a bearing on function. In the light of

these observations it could be stated that the higher concentration of lipase in the meso-metathoracic muscles of this beetle is for hydrolizing fat at a rapid rate, into its component fatty acids and glycerol which is the first step in the actual utilization of fat for energy. Microscopic observations on the coxal and the prothoracic muscles have shown that the coloration, tracheation and the structural pattern of these muscles are more or less the same unlike that of the meso-metathoracic muscles. The fibrillar muscles of the meso-metathorax contracting at a higher frequency would therefore require a tremendous amount of energy and the available glycogen would not be able to provide it. The energy for such activity has necessarily to be expected from fat. The higher concentration of succinic dehydrogenase in the meso-metathoracic muscles, which is more than double the amount found in the pigeon breast muscle which is one of the most active vertebrate muscles, is also suggestive of a high aerobic metabolism in these muscles.