CHAPTER 7

ON THE LIPASE AND SUCCINIC DEHYDROGENASE ACTIVITY IN THE SLOW AND FAST CONTRACTING LEG MUSCLES OF THE COCKROACH, *PERIPLANETA AMERICANA*

The recent studies of Becht and Dresdon (1956) and Smit (1958) have brought to light certain physiological and histological features of the slow and fast contracting mesothoracic leg muscles of the cockroach, *Periplaneta americana*. Smit (1958) has shown that the slow contracting muscle fibres contain more lipids and possess a stronger reducing capacity for methylene blue, than the fast contracting ones. An histochemical investigation on these two types of muscles (Chapter 8) has shown that the activity of lipase and certain other enzymes is more in the slow contracting red muscle. To assess the exact relative difference in the concentration of the enzymes, a quantitative study of the hydrolyzing enzyme, lipase and the oxidative enzyme, succinic dehydrogenase, was carried out and the results are presented in this chapter.

Material and Methods

The male cockroach being known to be metabolically more active than the female, the leg muscles of male cockroaches were used in the present investigation. The cockroaches were locally collected and maintained in the laboratory under uniform conditions. According to Becht and Dresdon (1956) the muscles 136, 137 and 135a and c (Carbonell's numbering) are fast contracting and 135b, d and e are slow contracting. The slow muscle in this case is the *tergo-trochanteral* muscle,

which

which is an exceptionally large muscle, passing down the coxa to be attached to the trochanter. The fast muscles which are the extensors and act antagonistically to the above muscle arise from the coxal rim and are attached on the coxa-trochanteral joint. These two types of muscles were carefully dissected out and removed separately from the coxa of the leg into separate ice cold watch glasses and weighed, and used for the following observations.

Lipase:

Separate aqueous extracts of the above muscles, were prepared at $4^{o}C$. The extracts were centrifuged at 2500 r.p.m. for 3 minutes and the supernatent was used as the enzyme material in each case. The method used for the assay was adopted from Martin and Peers (1953) using the Warburg apparatus and tributryrin as substrate with a bicarbonate-carbon dioxide buffer system of pH 7.4 at $37^{o}C$. The procedure was exactly as described in the earlier chapters. The reaction flask contained 1.5 ml 0.025 M sodium bicarbonate solution and 1 ml enzyme solution in the main chamber and 0.5 ml substrate (4% v/v tributyrin in 0.0148 M sodium bicarbonate solution emulsified by shaking with a drop of "Tween 80") in the side arm. The flasks and the manometers were gassed for 3 minutes with a mixture of 95% nitrogen and 5% carbon dioxide. After equilibrating the flasks in the constant temperature water bath of the apparatus for 10 minutes, the substrate was tipped in and initial readings were taken after 3 minutes. The subsequent readings were taken 15 minutes interval for 1 hour.

Succinic dehydrogenase:

The colorimetric method of Kun and Abood (1949) was employed to estimate the succinic dehydrogenase activity. 2:3:5 Triphenyl tetrazolium chloride (TTC) when reduced through enzymic reaction, formed the coloured formazan which was extracted in acetone and the intensity of the colour read on a colorimeter. The amount of formazan formed was then read directly from the standard graph.

The muscles separated as mentioned above, were homogenized separately in ice cold mortars and diluted with cold distilled water to obtain a 5% homogenate. The incubation mixture contained 0.5 ml of 0.5 M sodium succinate, 0.5 ml 0.1 M phosphate buffer of pH 7.4, 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 in each test tube. 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 to each tube, stoppered tightly and centrifuged at 3000 r.p.m. for 3 minutes. The clear supernatent was used for measuring the intensity of the colour on a Klett-Summerson photoelectric colorimeter, at 420 mμ. The readings were corrected for the blank with zero time incubation. SDH activity is expressed in terms of μg formazan formed per mg. dry tissue per 15 minutes. Dry weight of the same volume of homogenate used was determined by keeping 1 ml of the homogenate in a hot air oven and dried to constant weight.

Results and Discussion

From the results obtained it is evident that the slow contracting muscles have a higher lipase as well as SDH activity than the fast contracting ones. This confirms the histochemical observations (Chapter 8).

Table 1. Lipase and SDH activity in the slow and fast contracting leg muscles of the cockroach. The data presented is the average of 12 experiments.

Tissue	Lipase activity $\mu l\ CO_2$/mg.protein/hr.	SDH activity μg formazan/mg.dry tissue/15 min.
Slow muscle	129.31	8.79
Fast muscle	75.65	2.03

The higher concentration of lipase and SDH in the slow muscles can be correlated with their higher lipid content and greater capacity to reduce methylene blue (Smit, 1958). In a similar study on the beetle thoracic muscles the red fibrillar muscles showed more activity for these enzymes than the white leg muscles of the same. Hence it may be suggested that the concentration of the different enzymes could be considered as an index of the level of metabolic activity of the muscle in general. It follows therefore that the higher concentrations of lipase in the slow contracting red muscle of the cockroach leg, could be correlated with the greater capacity to hydrolize fat for oxidation for energy during activity. The higher concentration of SDH in this muscle also supports this contention.

It is known (Becht and Dresdon, 1956) that the slow contracting muscles contract at a lower frequency of contraction and continue for a long period while the fast contracting muscles contract rapidly at a higher frequency but for a short duration. For muscles indulging in sustained contractions, fat is to be considered as the chief fuel source. Higher fat content and the higher lipase and SDH activity of the slow muscle also indicate a higher level of aerobic metabolism in which fat forms the chief fuel.

In the earlier chapters (1, 2 and 3) it was possible to show a relationship between the lipase concentration and the diameter of the fibres of a muscle. This holds good for the slow and fast contracting leg muscles of cockroach also where it has been shown (Smit, 1958) that the slow muscles fibres are smaller in size than the fast muscle fibres.

The higher metabolic activity of the slow contracting leg muscles in comparision with the fast contracting muscles could also be attributed to the greater number of mitochondria in the former (Chapter 6).