CHAPTER 5

EFFECT OF EXERCISE ON THE PHOSPHORYALSE AND SUCCINIC DEHYDROGENASE LEVELS IN THE PIGEON BREAST MUSCLE

Considerable evidence has accumulated in the recent years, to show that the breast muscle of flying birds utilizes fat as the major source of energy for sustained flight (George and Jyoti, 1955; 1957). They also showed that the pigeon breast muscle when exercised by electrical stimulation, utilizes fat as its chief fuel amounting to more than 77% of the total energy expended for the exercise. It has also been shown that the flight muscles of birds are well equipped with high concentrations of the enzymes, lipase (George and Scaria, 1956) and succinic dehydrogenase (George and Scaria, 1958b; George and Talesara, 1960) in contrast to the very low concentrations in the muscles of non-fliers. George and Talesara (1961a) have further demonstrated that the succinic dehydrogenase activity in the breast muscle of a migratory bird e.g. Sturnus roseus increases considerably during the pre-migratory period.

Although evidences are available on the adaptive mechanisms of enzymes depending on the activity of the animal no extensive experimental studies have been done on the effects of exercise on the enzyme systems of the muscle in birds. Most of the published work in this field are on humans and other laboratory animals after the physical training for a certain period of time. Chepinoga (1939a; 1939b) studied α-glycerophosphate dehydrogenase and succinic dehydrogenase activities in the

skeletal muscle of trained rats and reported a 50 to 100% increase in SDH activity. Yampol'skaya (1952) showed an increase in lactic dehydrogenase activity in the skeletal muscles of exercised rats. Some recent studies conducted by Gould and Rawlinson (1959) revealed no significant differences in the levels of lactic and malic dehydrogenases and phosphorylase between the exercised and the control animals. However, Russian workers (Yakovlev, 1950; Yampol'skaya and Yakovlev, 1951; Yampol'skaya, 1952) reported that the analysis of the muscle after training showed higher concentrations of glycogen, phosphagen, ascorbic acid and glutathione and an increase in the phosphorolytic activity and other enzyme levels e.g. hexokinase, succinic dehydrogenase and lactic dehydrogenase. On the other hand Hearn and Wainio (1956) have shown that there is no perceptable change in the SDH activity in the heart and skeletal muscles of rats subjected to more strenuous exercise than in the case of the rats used by the Russian workers.

In view of these conflicting data, it was thought necessary to study the succinic dehydrogenase and phosphorylase activities in the pigeon breast muscle after vigorous muscular exercise. Succinic dehydrogenase and phosphorylase were chosen since they are two important enzymes concerned respectively with the oxidative and glycolytic pathways in muscle metabolism.

Material and Methods

The experiments were carried out on fully grown pigeons weighing between 300 to 320 gm. After having removed few feathers

from the breast, the breast muscles were stimulated directly by an electronic stimulator. In order to prevent the bird from struggling during stimulation, the following device was adopted. A black cloth mask was put over the head blinding the bird completely. By this, the bird remained calm in a state of partial hypnosis. The muscle was stimulated by a current of 20 volts for 30 minutes at a frequency of 5 stimulations per second with a duration of 2 milliseconds. Immediately after the stimulation, the bird was decapitated and a piece of the breast muscle was excised and blotted with a filter paper to remove the blood. Control experiments were conducted on a muscle piece taken out from the muscle of the opposite side before the muscles were stimulated. The region of the wound was plugged with calcium soaked cotton to prevent excessive bleeding. The muscle piece was immediately homogenized in a chilled mortar with ice cold distilled water and a 2% homogenate was prepared after removing all the connective tissues and cell debris.

Phosphorylase activity was determined in the muscle homogenate by a modification of the method of Cori, Cori and Green (1943) as adapted by Cahill et al, (1957). The incubation mixture taken in the test tube contained 0.2 ml. sodium citrate buffer (0.1 M) of pH 5.9 and 0.3 ml. of potassium fluoride (0.154 M) and 0.05 ml. of glucose-1-phosphate (L. Light and Co., England) (0.2 M). Samples of 0.1 ml. of the freshly prepared homogenate were added to the cold incubation medium, incubated for 15 minutes at 30°C and the reaction was terminated by the addition of 1 ml.

of 10% trichlorφacetic acid (TCA). Controls were run with each experiment by adding 1 ml. of 10% TCA to the incubation mixture before the addition of the homogenate and incubated along with the samples. Blanks were prepared by the addition of 1 ml. of 10% TCA in the incubation medium. After incubation and the addition of TCA, the samples and controls were filtered into 10 ml. graduated test tubes by several washings of the filter paper to ensure complete recovery of the phosphate. The filtration procedure was carried out in a cold water bath (10 to 15°C). The inorganic phosphate liberated from the glucose-1-phosphate by the enzyme activity was estimated by the method of Fiske and Subbarow (1925) and the optical density was read on a Klett-Summerson photoelectric colorimeter using 660 mμ filter. The controls were subtracted from the samples to determine the phosphate moiety released by the enzyme action. The enzyme activity was calculated as μg of phosphorus leased per ½ mg. dry weight of the muscle at 30°C for 15 minutes.

Succinic dehydrogenase activity of the same homogenate prepared for the phosphorylase assay was determined according to the method of Kun and Abood (1949) using triphenyl tetrazolium chloride (TTC) as the electron acceptor as mentioned in Chapter 2. The incubation mixture contained 0.5 ml. of 0.1 M phosphate buffer of pH 7.4; 0.5 ml. of 0.5 M sodium succinate and 1 ml. of freshly prepared 0.1% TTC in distilled water. 1 ml. of the freshly prepared homogenate was added to this incubation medium and incubated for 30 minutes at 37°C. After 30 minutes

incubation the enzyme activity was terminated by the addition of 7 ml. of acetone. The tubes were shaken well and the formazan extracted in the acetone was centrifuged for 5 minutes at 2500 r.p.m. and the clear supernatant was drawn off and the intensity of the colour read at 420 mμ on a Klett-Summerson photoelectric colorimeter. The readings were corrected for a blank with zero time incubation and the amount of formazan formed was calculated from a standard graph. The enzyme activity is expressed as μg formazan formed per mg. dry weight of the muscle per 30 minutes at 37°C under aerobic conditions.

Results

Table 1 presents the results obtained. Significant differences between exercised and non-exercised animals with regard to enzyme levels are seen. The increase in the enzyme activity was also observed when compared with the values obtained from the control muscle piece taken from a different pigeon. In these cases,however, the increase in the enzyme activity was not very sharp as was seen in comparisons made with the control samples taken from the same pigeon before the muscle was stimulated. Nevertheless, when these data were put to statistical test by the method of analysis of variance, it was found that a sharp difference between the stimulated and the resting muscle was indeed noted. When the t.test was performed on the variations observed between the resting and the exercised animals it was found that the increase in the enzyme activity observed was highly significant in both the cases.

Table 1

Effect of exercise on the phosphorylase and succinic dehydrogenase activities of the pigeon breast muscle.

	Phosphorylase activity µg phosphorøus/ mg. dry muscle/ 15 minutes			SDH activity µg formazan / mg. dry muscle/ hr.			No.of Expts.
Resting	29.478	±	3.364	7.870	±	0.669	7
Exercised	44.091	±	6.235	9.039	±	0.654	7
t.	= 6.258 (Highly significant)			6.326 (Significant)			

Discussion

The results of the present study are in agreement with those of Chepinoga (1939a; 1939b) and of the Russian workers Ykovlev (1950), Yampol'skaya and Yakovlev (1951), and Yampol'skaya (1952). The increased activation of phosphorylase after the stimulation of the muscle is also confirmed. It is known that phosphorylase activity is enhanced after the stimulation of the muscle (Cori, 1956; Rulon et al, 1961). It has been also shown by Leonard and Wimsatt (1959) that glycogen and phosphorylase levels are increased in the bat after the arousal of the animal from hibernation. However, the results obtained by Gould and Rawlinson (1959) do not agree with these results reported here and that appears to be due to the difference in the enzyme assay

and the technique of the exercise employed by them. It is possible that if there was a delay in the assay of the enzyme activity after exercising, a different result might be obtained due to some interconversion reactions.

The present studies alsc indicate that during muscular exercise the enzyme systems in vivo are probably operating at a considerably high rate, so as to cope with the greater metabolic activity of the muscle. It has been observed that during electrical stimulation of the pigeon breast muscle the respiratory quotient of the muscle was nearly 0.7 denoting fat metabolism (Pishawikar, 1961). George and Jyoti (1957) had shown that the pigeon breast muscle utilized fat as the major fuel during electrical stimulation of the muscle. Studies on free fatty acids of the pigeon blood during rest and electrical stimulation of the breast muscle have indicated that free fatty acids are being extracted from the blood stream during muscular activity (Chapter 4). The enhanced activity of SDH observed in the present study indicates that it is to meet the greater demands of the activity of the enzyme for fat utilization since SDH is one of the prime movers in the fatty acid oxidation cycle.