CHAPTER 10

CAPACITY FOR FATTY ACID OXIDATION OF THE BREAST MUSCLE HOMOGENATE OF THE MIGRATORY STARLING (STURNUS ROSEUS) IN THE PRE-MIGRATORY AND POST-MIGRATORY PERIODS

It is now well established that fat forms the chief source of energy in the flight muscles of birds for sustained flight. George and Jyoti (1955) found a considerable amount of reduction in the fat store of the pigeon breast muscle when the muscle was stimulated electrically. They (1957) showed that in the pigeon, nearly 77% of the energy expended during sustained muscular activity was derived from fat. It was also shown by several authors (Wachs, 1926; Zedlitz, 1926; Wolfson, 1945; Farner, 1955; McGreal and Farner, 1956; Odum, 1951; Odum and Connell, 1956; Merkel, 1958) that migratory birds store large amounts of fat in the body prior to migration and that fat is reduced during migration (Williamson, 1952; 1955). Weis-Fogh (1952) showed that in the locust at least 2/3 of the energy expended during flight was derived from fat. Recent studies reported in Chapter 8 on the seasonal changes in the fat store of the breast muscle of the migratory starling Sturnus roseus have shown that there is a great reduction in the muscle fat store when the birds arrive in India and that the fat content gradually increases reaching the peak level just prior to their return.

If fat is used as chief fuel for muscular energy, it is logical to expect that fat is first brokendown enzymically

into the component fatty acids for β-oxidation. George and Scaria (1958a) demonstrated the presence of a hydrolytic enzyme, lipase in the breast muscle of birds, which could split fat into fatty acids and glycerol. They also showed that lipase is highest in the red fat-loaded fibres of the pigeon breast muscle.

Weinhouse et al, (1950) showed that the pigeon breast muscle is capable of oxidizing fatty acids. George and Talesara (1962a) have shown that the pigeon breast muscle mitochondria and homogenate are not capable of oxidizing triglycerides directly under in vitro conditions thereby indicating the important role of a lipase in splitting fat into fatty acids prior to β-oxidation. It has also been shown that the heart, diaphragm, kidney, liver and skeletal muscles are capable of oxidizing exogenous octanoate, butyrate, palmitate and acetate (Fritz, 1958; Fritz and Kaplan, 1960; Fritz, 1960; Fritz and Kaplan, 1961; Schwartzman and Brown, 1960; Neptune et al, 1960a; Neptune et al, 1960b; George and Iype, 1963).

Though several studies have been conducted on fatty acid oxidation by several tissues specially skeletal muscle, no such attempt has ever been made with the muscle of a migratory bird. The flight muscles of a migratory bird should be expected to possess efficient Krebs cycle enzyme systems for a higher fatty acid oxidizing capacity. It has already been reported by George and Talesara (1961a) that the concentration of succinic dehydrogenase which is one of the major enzymes in the Krebs cycle increases to a high level in the breast muscle

of Sturnus roseus just before migration. The present investigation was undertaken to elucidate the changes occurring in the breast muscle of this bird in the pre-migratory and post-migratory periods.

Material and Method

The Rosy pastors (Sturnus roseus) arrive in Baroda in large numbers during September and October and leave by the end of April. The birds were shot with an air rifle and brought to the laboratory immediately . A piece of the breast muscle was quickly cut out and placed on a filter paper to remove the blood as completely as possible. It was then homogenized in a chilled mortar kept on cracked ice and a 5% homogenate was prepared in cold distilled water. After being freed of the cell debris and connective tissue this homogenate was used as the material to study the oxygen uptake with sodium butyrate as the substrate.

The in vitro oxidation of sodium butyrate in the presence of a sparker was determined by the oxygen uptake in a standard manometric system. Malate was used as the 'sparker' since it is known that the presence of a citric acid cycle intermediate is essential for the fatty acid oxidation system (Knox et al, 1948; Green, 1951; 1955; Weinhouse et al, 1949). The experiments were conducted at 37°C and the gas phase in the flask was atmospheric air. Each reaction flask contained 0.6 ml. 0.1 M phosphate buffer of pH 7.4; 0.15 ml. of 0.15 M $MgCl_2$; 0.2 ml. of 0.0225 M ATP; 0.15 ml. of 0.00225 M cytochrome C ; 0.2 ml. of 0.015 M DPN; 0.2 ml. of 0.0075 M coenzyme A ; 0.2 ml. of 0.15 M sodium

malate (neutralized with KOH) and 0.8 ml. of freshly prepared muscle homogenate. The samples contained 0.5 ml. of 0.03 M sodium butyrate and the blanks contained o.5 ml. of distilled water making a total volume of 3 ml. in the reaction flask. 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, 10 µM sodium malate in each flask. The final concentration of sodium butyrate was 5 µM in the samples. In the central well of the reaction flask 0.2 ml. of 20% KOH and a roll of filter paper were placed. The muscle homogenate was added last and the flasks were placed on ice slabs until they were mounted on the manometers. The manometers were shaken at 100 oscillations per minute and an amplitude of 4.5 cm. The readings were taken at every 10 minutes for 1 hour. Protein was determined by the micro-Kjeldhal steam distillation method (Hawk et al, 1954).

Results

The results obtained for the oxidation of sodium butyrate by the muscle homogenate during the post- and pre-migratory periods are given in Table 1. As can be seen from the table, oxygen uptake due to the added butyrate in the system during the pre-migratory period was considerably lower than that in the post-migratory period. However, there was a slight change in the total oxygen uptake between the two periods and also a slight decrease in the complete system was observed in the pre-migratory period. During the pre-migratory period it was also observed that the addition of added butyrate in the system under the same experimental conditions inhibited to a certain extent the oxidation

of malate. It could be seen from the data presented in Table 1, that there is a decrease in the total oxygen consumption of the muscle during the pre-migratory period.

Table 1

Fatty acid (Sodium butyrate) oxidation by the breast muscle homogenate of the migratory starling Sturnus roseus during the post-migratory and pre-migratory periods.

Time	µl O_2 uptake due to the oxidation of malate (Incomplete system)		µl O_2 uptake due the oxidation of malate and butyrate (Complete system)		Difference due to the oxidation of butyrate
	Mean	S. D.	Mean	S. D.	
Post-migratory (December)	5.1602	± 0.6243	5.7242	± 0.6198	0.5640
Pre-migratory (April)	4.4135	± 0.8016	4.2545	± 0.8866	0.1590*

The figures indicate the average values of 5 sets of experiments in each case.

* This difference is due to the malate oxidation which is higher than in the complete system with the butyrate.

Discussion

It has been shown that the fat content of the breast muscle (Chapter 8) and liver (Naik, 1963) in these birds steadily increase reaching the peak toward the time of migration. It

therefore seems possible that the pectoral muscles like the liver and adipose tissue, are also adapted for the biosynthesis of fat during the pre-migratory period. The results obtained in the present study have shown that the oxygen uptake due to the oxidation of added butyrate was more in the case of the muscle of the pre-migratory bird which has a lower fat content. The lowering of the capacity for fatty acid oxidation during the pre-migratory phase could be considered as indicative of a shift in the regulative mechanism for the conversion of fat. The results obtained in a study reported in Chapter II on the lipase and succinic dehydrogenase levels in the different sub-cellular fractions of the muscle homogenate suggest that fat is being conserved and actively synthesized in the muscle during the pre-migratory period. The mitochondrial lipase and SDH levels have been shown to be strikingly low in the pre-migratory phase which is suggestive of lowered mitochondrial oxidation, and hence less fat utilization. The low mitochondrial enzyme levels is definitely of significance with regard to lowered fat utilization since it is known that fatty acids are believed to be oxidized exclusively within the mitochondrion (Schneider and Potter, 1949; Lehninger and Kennedy, 1949). Moreover, it is also known that the skeletal muscles have the enzymatic machinery to oxidize long and short-chain fatty acids to CO_2 (Fritz et al. 1958). It is also of interest to note that the diaphragm and other skeletal muscles are also able to transport, esterify and oxidize palmitic acid under in vitro conditions

(Bodel et al, 1962). At present, however, it is not clearly understood as to how the muscle during the pre-migratory period does not oxidize fatty acids even with its higher succinic dehydrogenase levels (George and Talesara, 1961a). But it could be said, on the other hand that the higher lipase and succinic dehydrogenase activity in the microsomes and the higher lipase activity in the supernatant fraction of the breast muscle homogenate of the pre-migratory bird is indicative of a higher degree of active esterification of fat from increased fat synthesis in the microsomes.