CHAPTER 9

THE GLYCOGEN CONTENT AND PHOSPHORYLASE ACTIVITY IN THE BREAST MUSCLE OF THE MIGRATORY STARLING, STURNUS ROSEUS

It is well known that several physiological changes take place in a migratory bird prior to migration. One of the better known changes is that migratory birds deposit fat prior to actual migration (Wolfson, 1945; Odum and Perkinson, 1951; MaGreal and Farner, 1956; Farner, 1955; Odum and Connell, 1956; Merkel, 1958). Studies conducted on the fat content (Chapter 8) of the breast muscle and liver (Naik, 1963) have shown that there is an augmented storage of fat in these two organs prior to migration. However, no attempt has hitherto been made to study the changes in the glycogen content and phosphorylase activity in the breast muscle of migratory birds. Recently Farner et al, (1961) reported that fat deposition in a migratory finch, the White-Crowned Sparrow, is accompanied by a reduction in the glycogen content of the pectoral muscle and liver and also showed the existence of a diurnal glycogen cycle in these organs.

The present studies have therefore been carried out in order to assess the changes in the glycogen and phosphorylase levels in the pre-and post-migratory phases.

Material and Methods

All the experiments were conducted in the evenings between (5 to 7 P.M.) of the post-migratory (December) and pre-migratory periods (April). The birds were trapped by means of

mist net or shot with an air rifle and a piece of the breast muscle was immediately excised after decapitation and allowing the blood to drain off. The muscle samples were weighed quickly after blotting off the blood with a piece of filter paper and placed in 30% hot KOH for digestion and estimation of glycogen.

Glycogen was estimated by the anthrone method of Seifter et al, (1950). The digested material was cooled and 2.5 ml. of 95% ethanol was added to the tubes and brought to boiling in order to precipitate the glycogen. The samples were then placed in the refrigerator for 30 minutes. The precipitated glycogen was then separated by centrifuging for 15 minutes at 3000 r.p.m. The KOH was then decanted off and the glycogen residue was washed and reprecipitated twice in the same way. The final precipitated glycogen was dissolved in water and diluted so as to contain 30 to 40 gammas per ml. An aliquot in duplicate was then used for the final colour development with the anthrone reagent prepared in 95% sulphuric acid. To every one ml. of the sample, 4 ml. of the anthrone reagent was added and placed in a boiling water bath for 5 minutes. A standard containing glucose was always run with the samples and the optical density was measured on a Bausch and Lomb " Spectronic 20 " colorimeter at 620 mμ. Glycogen in the muscle is expressed as per centage of glycogen on wet weight of the muscle.

Phosphorylase activity in the muscle was assayed by a modification of the method of Cori, Cori and Green (1943) as adapted by Cahill et al, (1957). A piece of the breast

muscle was immediately excised and blotted well to remove the blood. The muscle piece was then homogenized in a previously chilled mortar with ice cold distilled water. Samples of 0.1 ml. of this fresh homogenate were added to a test tube containing a mixture of the substrate, glucose-1-phosphate (0.05 ml. of 0.2 M); buffer, sodium citrate (0.2 ml. of 0.1 M and pH 5.9) and fluoride (0.3 ml. of 0.154 M). Controls were run with each experiment by adding 1 ml. of 10% trichloracetic acid (TCA) to the incubation mixture before the addition of the homogenate and blanks were prepared by the addition of 1 ml. of TCA to the incubation medium. All samples, controls and blanks were incubated in a water bath for 15 minutes at 30°C and the enzyme activity in the samples were terminated by the addition of 1 ml. of 10% 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 inorganic phosphate. The inorganic phosphate liberated from the glucose-1-phosphate by the enzyme action, was determined by the method of Fiske and Subbarow (1925) and the optical density was read on a Klett-Summerson photoelectric colorimeter using a 660 mμ filter. The controls were subtracted from the samples to determine the phosphate moiety released by the enzyme action. A standard containing inorganic phosphate was always run along with the samples and the enzyme activity was calculated as the amount of μg phosphorous liberated per 10 mg. dry weight of the muscle at 30°C for 15 minutes. Dry

weight of the muscle was obtained by drying 1 ml. duplicate samples of the homogenate for 24 hours in an air oven.

Results

Table 1 presents the glycogen and phosphorylase levels in the pre- and post-migratory periods. It could be clearly seen from the results that there is an increase in both glycogen content and phosphorylase activity of the breast muscle towards the end of the pre-migratory period. The increase in the glycogen content of the muscle is seen to be associated with an increase in the enzyme activity also. Moreover, in some experiments higher figures for glycogen and phosphorylase (2.9% and 60 µg) respectively were also obtained showing a proportional increase in both.

Table 1

Glycogen content and phosphorylase activity in the muscle of the Rosy pastor in the post-migratory and pre-migratory periods

Migratory phase and month	Glycogen gm. % in wet muscle	Phosphorylase activity µg phosphorous / 10 mg. dry muscle /15 minutes	No. of Expts.
Post-migratory (December)	0.9281 ± 0.0910	21.86 ± 3.50	6
Pre-migratory (March & April)	1.8240 ± 0.6920	43.86 ± 11.87	14

Discussion

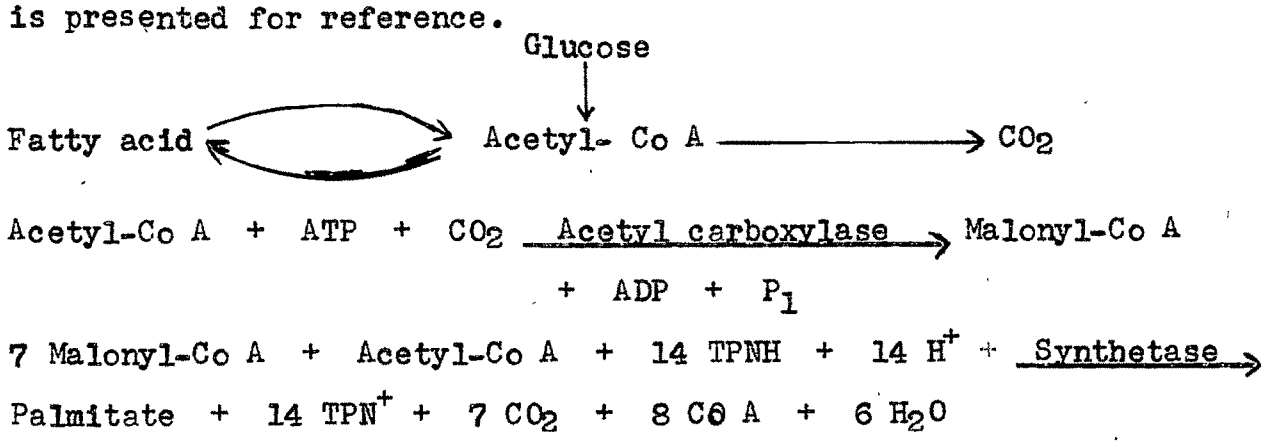
The changes in the muscle glycogen content and phosphorylase activity observed during the post- and pre-migratory phases suggest that there is an increased glycogen metabolism during the pre-migratory phase. Although it is generally known that there is a gradual increase in the body fat and particularly that of the liver (Naik, 1963) and muscle (Chapter 8) no data are available on the glycogen and phosphorylase levels of the muscle other than the recent studies on the changes in the glycogen content conducted by Oksche et al, (1959) and Farner et al, (1961).

From the studies reported in Chapters 10 and 8, it is logical to suggest that in the Rosy pastor muscle there is less utilization of fat during the pre-migratory phase and the muscle is adapted for an increased synthesis of fat. In this context it is also reasonable to expect a greater utilization of a metabolite other than fat by the muscle during this period. Studies conducted on the capacity for fatty acid oxidation by the muscle homogenate in the post-migratory and pre-migratory periods have shown that there is lesser oxidation of fatty acids (butyrate) in the pre-migratory period (Chapter 10). It should be mentioned here that these birds during the pre-migratory period feed on fruits which are rich in carbohydrates and poor in fat content. It could be assumed from the marked increase in body fat and the intake of carbohydrates during this period, that there is a high rate of metabolic conversion

of glucose to fatty acids which would then be esterified to form lipids for storage. However, Farner et al, (1961) noted a low concentration of glycogen in the pectoral muscles and liver of the migratory finches subjected to long day photo-periodic treatment (20 hr.) and suggested that in these birds there is an increased rate of glycolysis which in turn has a significant acetate-sparing effect thereby making more acetate available for fat synthesis.

Evidence presented by Cahill et al, (1957) in accordance with the observation that increased phosphorylase activity is associated with increased glycogenolysis, and only in the presence of low phosphorylase levels has net glycogen synthesis been obtained. Thus the increased phosphorylase activity during the pre-migratory phase could be considered as an index for the increased rate of glycogenolysis and hence more production of glucose which in turn should promote greater fatty acid synthesis through increased production of acetyl-Co A.

The following abbreviated metabolic scheme which is considered to be the major pathway of fatty acid biosynthesis is presented for reference.



It is well known that carbohydrate metabolism promotes fatty acid synthesis. Studies presented in this chapter are suggestive of the fact that there is a greater metabolism of carbohydrates during the pre-migratory period indicated by the greater glycogen concentration and phosphorylase activity. The greater glycogen content and phosphorylase activity in the muscle during this period could also be due to a change in the metabolite used for muscular energy in the sense that more glycogen is expended for muscular energy in short flights during this period.