CHAPTER 6

DIURNAL CHANGES IN GLYCOGEN AND FAT LEVELS IN THE PECTORALIS OF THE MIGRATORY STARLING, STURNUS ROSEUS

That migratory birds deposit large amounts of fat in the body prior to migration and that this "migratory fat" is metabolized for energy during migratory flights, is well known (George and Berger, 1966). Naik (1963) reported in the migratory starling Sturnus roseus, a sharp increase in glycogen as well as fat in the liver towards migration. In the same species towards migration a similar increase in the two metabolites has been recorded in the pectoralis muscle (Vallyathan, 1963; Vallyathan and George, 1964). The aim of the present investigation was to find out if there was any diurnal variation in the glycogen and fat contents of the pectoralis muscle of this starling during the premigratory as well as the postmigratory periods with a view to throw more light on the problem of synthesis and utilization of the two metabolites.

MATERIALS AND METHODS

The birds, (Sturnus roseus) were shot during the months of March and April for the observations during premigratory period and in August for the observations in the postmigratory period after they returned to Baroda from their breeding grounds abroad (probably U.S.S.R.). The observations were made in the morning and evening throughout the periods of investigations, the birds were shot between 4.45 and 5.15 hours and for the evening set

between 19.00 and 19.30 hours. Immediately after the bird was shot, a small piece of muscle about 200 mg was cut from the pectoralis, blotted free of blood and transferred into previously weighed test tubes containing 3 ml of 10% KOH. The test tubes were then closed with glass stoppers and brought to the laboratory within half an hour and reweighed. The muscle was completely digested in KOH by heating in a water bath at 100°C. The carcasses were numbered in the field and brought to the laboratory in plastic bags. The total body weight, sex and the weight of gonads were recorded. For the estimation of total fat in the pectoralis, a piece of the muscle 1 to 2 gm was dried at 85 to 95°C in a hot air oven.

Glycogen was estimated by the anthrone method of Seifter et al. (1950). After digestion of the muscle in KOH and it was allowed to cool, 3 ml 95 % ethanol were added and brought to boiling point to precipitate the glycogen. The samples were then placed in refrigerator and cooled for half an hour. The precipitated glycogen was separated by centrifuging for 15 minutes at 3000 r.p.m. The KOH was decanted off and 1 ml of distilled water was added to redissolve the precipitate. 2 ml of 95% ethanol was then added to reprecipitate the glycogen. These tubes were again centrifuged for 15 minutes at 3000 r.p.m. and the aliquot decanted off. Finally 3 ml of 95% ethanol was again mixed and centrifuged for 15 minutes. The final precipitate of pure glycogen was dissolved in distilled water in such a proportion so as to contain about 30 to 40 gamma per ml. An aliquot in duplicate

was used for the final colour development with the anthrone reagent prepared in 95% sulphuric acid. To every 1 ml of the sample, 4 ml of anthrone reagent was added. A standard glucose solution was also run along with the samples. These tubes were kept in a water bath at 100°C for five minutes for the colour development. The optical density was measured on a Bausch and Lomb "Spectronic 20" colorimeter at 620 mμ. The glycogen content of the muscle per 100 gm wet muscle was calculated.

For the estimation of fat, the muscle pieces were completely dried, weighed again and subjected to Soxhlet extraction with a 1:1 ethanol-ether mixture for 18 hours. The fat extracted was dried, weighed and the percentage was calculated on dry weight basis.

RESULTS AND DISCUSSION

Tables 1 and 2 present the mean values of the percentage of glycogen and fat present in the morning and evening respectively in the pectoralis muscle during the premigratory and postmigratory periods respectively. The total body weight and the weight of the gonads are also presented in the tables. In March the birds form migratory swarms and in April they rapidly put on weight with deposition of fat in preparation for migration which takes place around the last week of April. The data for April were obtained during this last phase. These birds return to Baroda in July/August and the data obtained during August are regarded as those for the postmigratory period.

Diurnal variations in muscle glycogen:

From the results obtained it is found that in the evening there was a higher percentage of glycogen in the muscle than in the morning in all months (March: (morning) 1.037 ± 0.199, (evening) 1.729 ± 0.694; April: (morning) 1.227 ± 0.543, (evening) 2.714 ± 0.921; August: (morning) 0.561 ± 0.197, (evening) 0.810 ± 0.215). On the statistical analysis of the data employing the "t" test the difference was found to be highly significant for March and April and significant for August.

The reduction of glycogen in the morning suggests that during night glycogen is utilized in the muscle and that its increase during the day, is due to it being built up. In the postmigratory period (August) the reduction was 0.25 gm (30.9%), and in the premigratory period it was 0.69 gm (40.0%) in March and 1.48 gm (54.6%). This shows that the greatest increase of glycogen was in April and least in the postmigratory period (August). The increase in glycogen in the muscle during the day may be attributed to two possible reasons. 1. The birds feed during the day and the food intake might register a rise in glycogen in the tissues. 2. The bird is active during the day and the muscle while metabolizing fat for energy, might spare glycogen or even stimulate glycogen synthesis in muscle. That bird muscles use fat for energy is well established (George and Berger, 1966). Shipp et al. (1961) have shown that when glucose and fatty acids are present as substrates, the heart muscle preferentially oxidizes the latter and that the total

myocardial glycogen is increased by diverting more glucose to form glycogen thus sparing cardiac glycogen. Recently it has been demonstrated histochemically (George and Nene, 1965) that when the pigeon pectoralis muscle is electrically stimulated the white fibres which normally contain more glycogen than the red ones get quickly depleted of its glycogen store whereas the fat utilizing red fibres increase in their glycogen reserves. This increase in glycogen in the red fibres may well be due to increase in the activity of glycogen synthetase in these fibres. Support for this suggestion is available from the observations of Stubbs and Blanchear (1965). They showed from quantitative estimations that glycogen synthetase activity is more in the red muscles than in the white of the guinea pig and that it further increased when the muscle was electrically stimulated.

The reduction in the muscle glycogen during night, may be attributed to the fact that during that time the bird does not feed. Another possible explanation is that the bird being inactive, a lower body temperature is to be expected and during that time glycogen may be preferentially utilized since it is known that in rats glycogen stores of the cardiac and skeletal muscles get depleted under conditions of hypothermia (Itzhaki and Wertheimer, 1957). Such a possibility is unlikely in the case of birds because feathers serve as good insulators against heat loss. In the next chapter it will be seen that in pigeons subjected to cold stress considerable reduction in muscle glycogen occurs only when the feathers in the region of the

breast were removed and not otherwise. So the high reduction of glycogen (54.6%) during the night in this starling in the late premigratory period (April) is due to the conversion of this metabolite into fat. This also suggests that fat synthesis in the migratory period takes place in the night. Evidence in support of this possibility is available from the work of George and Vallyathan (1964). These authors estimated the lipase and succinic dehydrogenase activity in the particulate fractions of the pectoralis muscle of Sturnus roseus during the pre- and postmigratory periods and observed that the oxidative capacity of the mitochondrial fractions was low during the night but lipase activity in the microsomal fraction was high. Since microsomes are known to be the sites of fat synthesis they attributed the high lipase activity there during the night, to increased esterification of fatty acids by the reversible action of lipase. The mitochondria being the sites of fatty acid oxidation, the low oxidative capacity of the mitochondrial fractions was suggestive of low oxidative metabolism.

Diurnal variation in muscle fat:

In the month of March during the premigratory period, the fat content of the muscle in the mornings was higher (Morning: 12.685 ± 1.46; Evening: 10.322 ± 1.438 per 100 gm of dry muscle) whereas in April during the last week prior to migration the values were more or less the same (Morning: 13.451 ± 1.62; Evening: 13.44 ± 1.931). Employing the "t" test the differences were found to be statistically highly significant. It should be mentioned here

that the values obtained for April were highest for the periods under investigation. In the August birds, the fat content of the muscle was higher in the mornings (Monning: 13.080 ± 5.129; Evening: 7.567 ± 6.103 per 100 gm of dry muscle). The higher fat content of the muscle in the morning and its fall towards evening during the months of March and August clearly indicate that fat is built up in the night and utilized as fuel during the day. The high fat content in the muscle of the August birds in the morning indicate that these birds must have actually arrived earlier and ~~are~~ were actively feeding to build up their normal energy reserve. However, April birds during the last week prior to migration, there was practically no difference between the morning and evening fat contents of the muscle but at the same time the fat content was the highest recorded. It was also observed that in these birds the glycogen levels showed the highest increase during the day. The maintenance of the fat level indicates that towards the end of April, as migration time approaches the birds utilize hardly any fat during the day. It was pointed out earlier that when the pigeon breast muscle or a red muscle in the rat is put into continuous activity by electrical stimulation, there is increased glycogen synthesis in the muscle as a result of increased activity of glycogen synthetase. In the present investigation it was found that the highest level of glycogen was reached in April when there was hardly any utilization of fat at all. Normally the active bird muscle utilizes fat as the chief fuel as is indicated in the postmigratory (August) or early premigratory

(March) periods. If fat is not the fuel for the muscular activity in the April birds which are near-ready for their migratory flight the supply of energy, particularly in a system such as this adapted for aerobic metabolism, must come from the complete oxidation of pyruvate. Some evidences to support the above inferences and suggestions are available from the studies of earlier investigators. It has been shown that the levels of succinate dehydrogenase activity in the pectoralis muscle of this bird was more than doubled in the month of April from what it was in November (George and Talesara, 1961). This would suggest that the muscle should be metabolizing considerably more fat in April. But on the contrary it was found from in vitro experiments that the homogenate of the pectoralis muscle of this bird during its postmigratory period oxidized considerably more fatty acid than by the muscle homogenate of the bird in its premigratory period whereas malate oxidation in the manometric system was higher in the latter case (George and Vallyathan, 1964^{a}). It has also been shown that the muscle homogenate of a nonmigratory starling, Acridotheres tristis could oxidize more fatty acid than by that of Sturnus roseus in the premigratory phase unlike that in the postmigratory phase (George and Iype, 1964). It is also known that when fat is to be metabolized for muscular energy it has to be first broken down to fatty acids by the enzyme lipase (George and Talesara, 1962^{a}). So a higher lipase activity in the muscle should be expected in the postmigratory phase. That this is indeed the case was shown by Vallyathan (1963).

TABLE 1

DIURNAL CHANGES IN THE GLYCOGEN AND FAT CONTENTS IN THE PECTORALIS OF *STURNUS ROSEUS* DURING THE PREMIGRATORY PERIOD

Months	Total body weight	Weight of testis	Weight of ovary		Glycogen content per 100 gm wet muscle	Fat content per 100 gm dry muscle	No. of Expts.
March	72.610 ± 6.800	0.037 ± 0.014	0.031 ± 0.002	Morning:	1.037 ± 0.199	12.685 ± 1.460	16
				Evening:	1.729 ± 0.694 **	10.322 ± 1.438 **	14
April	80.660 ± 10.260	0.196 ± 0.031	0.058 ± 0.018	Morning:	1.227 ± 0.543	13.451 ± 1.521	20
				Evening:	2.714 ± 0.921 **	13.442 ± 1.931	20

** Denotes that the differences are highly significant

TABLE 2

DIURNAL CHANGES IN THE GLYCOGEN AND FAT CONTENTS IN THE PECTORALIS OF *STURNUS ROSEUS* DURING THE POSTMIGRATORY PERIOD

Months	Total body weight	Weight of testis	Weight of ovary		Glycogen content per 100 gm wet muscle	Fat content per 100 gm dry muscle	No. of Expts.
August	69.410 ± 5.499	0.020 ± 0.007	0.019 ± 0.006	Morning:	0.561 ± 0.197	13.080 ± 5.129	12
				Evening:	0.810 ± 0.215 *	7.567 ± 6.104 **	10

* Denotes that the differences are significant

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