CHAPTER 1

DIURNAL VARIATION IN FAT AND GLYCOGEN CONTENTS OF THE LIVER OF ROSY PASTOR DURING PRE- AND POST-MIGRATORY PERIODS

It is common knowledge that migratory birds store up fat prior to their migratory flight. A large number of reports pertaining to the fat deposition in migratory birds, clearly show that this phenomenon is a prerequisite for such birds to undertake the migratory flight (Odum and Perkinson, 1951; Wolfson, 1954; McGreal and Farner, 1956; Odum and Connell, 1956; King and Farner, 1965). The degree of fat deposition depends upon the size of the birds - higher amounts were deposited in smaller birds - as well as the distance and barriers they cross (Odum, 1960; King and Farner, 1965).

Along with the greater deposition of fat in the body, an increased level was also found in the liver and muscle of the migratory birds (Odum and Perkinson, 1951; King _et_ _al_.,1963). Thus, in the migratory starling, _Sturnus_ _roseus_, the liver (Naik, 1963) and muscle (Vallyathan, 1963) showed increased lipid levels. A corresponding increase in glycogen in the liver (Naik, 1963; John, 1967) and muscle (Vallyathan and George, 1964) was also observed towards the end of April (premigratory phase).

Various physiological factors are known to influence the migratory fattening. King and Farner (1956) and Odum (1960) concluded that an adaptive hyperphagia is the main reason for the increased production of lipids in _Zonotrichia_ _leucophrys_ gambelii and Passerculus sandwichensis respectively, during premigratory periods in spring. Merkel (1958) and Dolnik et al. (1963 a&b) also agreed that hyperphagia was the cause of hyperlipogenesis. A greater energy balance due to decreased activity of birds in the temperate regions where the day length decreases considerably, could influence fat deposition, but this could not be confirmed through experiments on caged birds (Farner et al.,1954; Weise, 1956; King and Farner, 1963).

The role of neuroendocrine mechanisms in bringing about this high deposition is now increasingly realized. Prolactin was one such factor recorded by Meier and Farner (1965) in the migratory white throated sparrows, Z. l. gambelii. King and Farner (1965) suggested that photoperiodic stimulation might primarily effect the 'feeding centers' in the hypothalamus which in turn could furnish the endocrine and enzymic adaptations. The role of thyroid hormone in the fat build up is mentioned else where in this thesis (Chapter 8).

In many respects, this hyperlipogenesis is similar to that of obese animals where the larger amounts or higher turnover of glucose determine the rate of fat production (Tepperman and Tepperman, 1965 a). The availability of glucose depends on the amount of carbohydrates injested. Since the diet is the chief source of blood glucose, the maximum level would be present at the time of absorption. Moreover, as the lipid generating activity of the tissues rests largly on the glucose supply, a diurnal variation of such activities might occur. Thus, a study on the fat and glycogen contents of the liver at different times of the day would provide a basic idea about the production and turnover of these metabolites during resting and active periods. The utilization or synthesis of these two metabolites would alter the RQ of any tissue. Therefore determination of RQ of liver and muscle slices was also carried out.

MATERIALS AND METHODS

In both the post- and pre- migratory periods, the roosting and awakening times of Rosy Pastors (7-7.30 p.m. and 5-5.30 a.m. respectively) were almost constant as the variation in the length of the day is minimal in this tropical region. The birds were shot and brought immediately to the laboratory. The liver pieces were excised, blotted, weighed and digested in 20% KOH for the estimation of glycogen by the Anthrone method of Seifter et al.(1950).

The fat content was determined gravimetrically after extracting the fat from a known quantity of dried tissue using 1:2 methanol- chloroform mixture. The amounts of fat as well as glycogen are expressed as g/100g wet tissue.

The RQ of the muscle and liver slices was determined in a Warburg respirometer using Krebs Ringer-phosphate buffer solution as the medium and atmospheric air as the gas phase (Umbriet et al., 1957).

As the roosting sites of these birds are situated mainly in the University campus, all the estimations were conveniently carried out,on the birds that were shot,within 10 to 15 minutes.

RESULTS

The glycogen content of the liver in the postmigratory period was found to be 5-6 g/100g wet tissue in the evening and 1-2 g/100g wet tissue in the morning (Fig.1). A depletion of approximately 3g was evident in the morning in these periods. During the premigratory period beginning in March, the glycogen level in the morning and evening hours gradually decreased, followed by a slight increase at the end of the period (last two weeks of April). In March also, the amount of glycogen in the morning was approximately 3 g lesser as compared to that in the evening. But, by the first week of April, it was only 2 g lesser than in the evening. In the last week of April, however, the difference was only 1 g, and the amount of glycogen was higher at both the times of the day than in the first week of April. The glycogen content varied with the sex. But the over all picture of the seasonal and diurnal changes in its level were found to be the same in both the sexes and hence seperate representation of values in the two sexes was not done in Fig.1.

The fat content of the liver was found to be more in the evening than in the early hours of the day (Fig.2). In August (postmigratory period), it was found to be 10 g/100g wet tissue in the evening as against 4 g/100 g in the morning. A gradual increase in the level of fat was observed in the pre-

Fig.1. Diurnal variation of glycogen content in the liver of Rosy Pastor during the post- and pre-migratory periods.

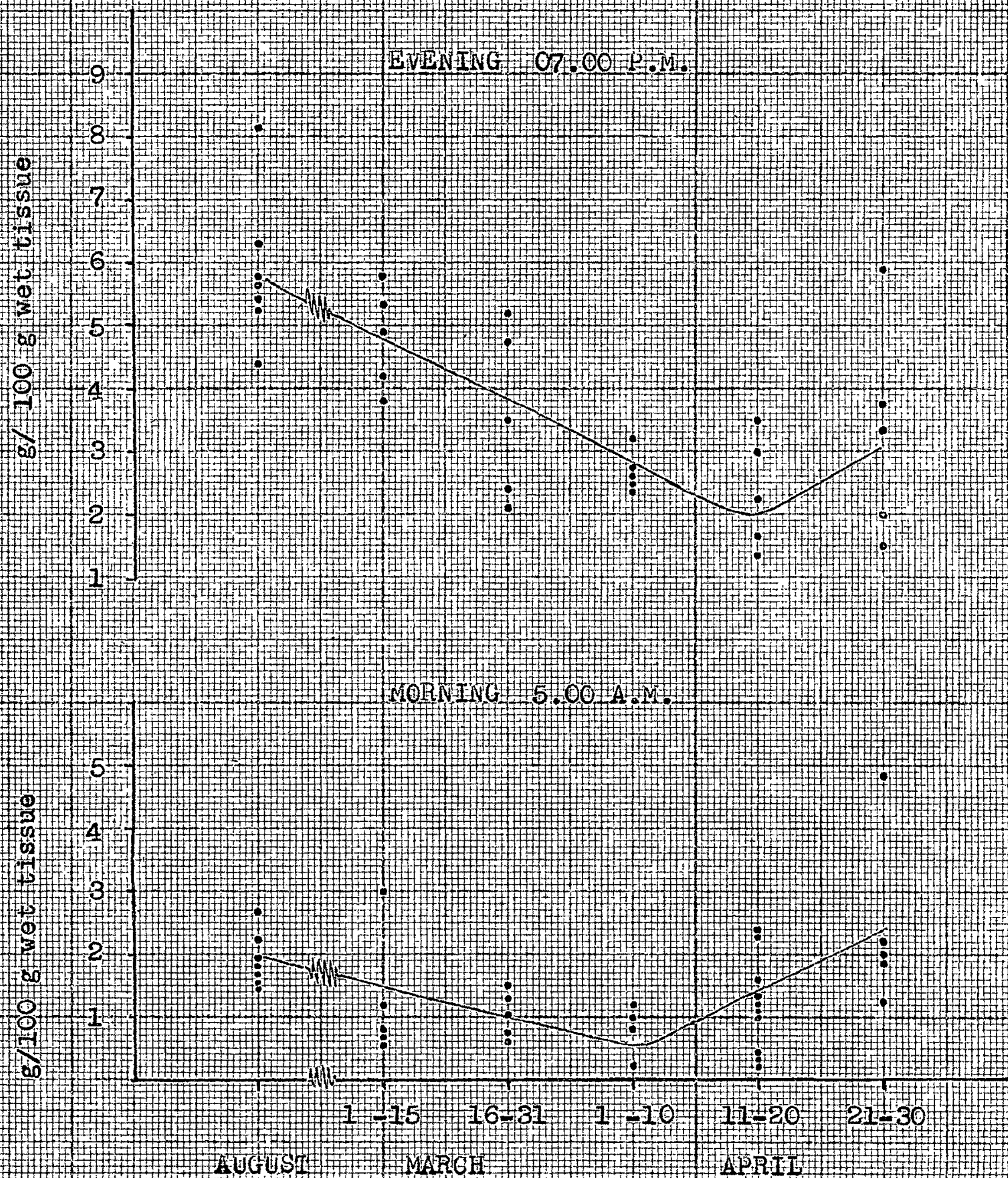
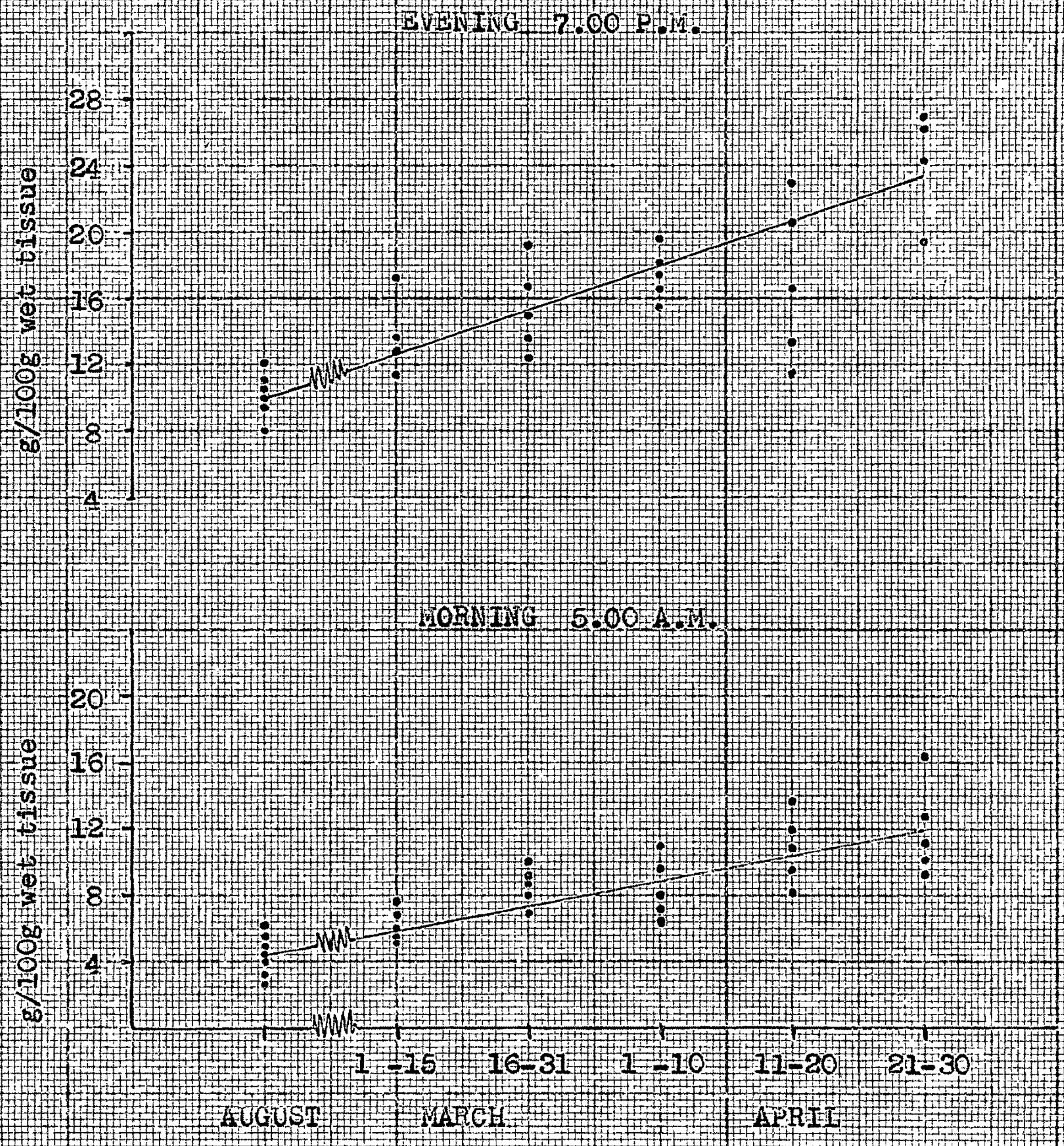


Fig.2. Diurnal variation of fat content in the liver of Rosy Pastor during the post- and pre- migratory periods.



migratory period which reached the maximum in the last week of April wherein the values for the morning and evening were 12% and 23% respectively (Fig.2). No differences were notived in the amount of liver lipids with regard to sex.

A gradual rise of RQ above 1 was noticed in the liver and muscle slices by the end of March and biginning of April (Table I). But, by the end of April it came down to 1 in both the tissues.

TABLE I

The RQ of liver and muscle slices of Rosy Pastor during the pre-migratory period

Date	Body weight	RQ of liver slices	RQ of muscle slices
MARCH			
8-15	65	0.92	1.2
16-23	68	1,1	1.2
24-31	68	1.4	1.4
APRIL			
1-10	69	1.5	1.4
11-20	78	1.3	1.2
21-31	84	1.1	1.0

DISCUSSION

The increased hepatic fat content in the evening as well as in the morning towards the time of migration indicates a higher rate of fat synthesis during this period. The fat content in the evening was roughly twice as more than in the morning. This discrepancy between the values for evening and morning was maintained constant in post- and pre- migratory periods. This obviously denoted that the rate of removal of fat from the liver was equally high under an increased rate of fat synthesis. Since, the determinations of fat and glycogen were done first in the evening when the birds roost and then the following morning just before they fly away from their roosting place, any observed changes in the contents of these metabolites occurred during the resting period. The expenditure of energy in this resting condition should be minimal. Naturally one would expect the *de novo* synthesis of fat during the night using the carbohydrates (glycogen) that were stored in the day. In that case, the fat content should be highest in the morning, but the data obtained tends to disagree with the above suggestion. The fat present in the liver was not drawn from the diet, since the dietary source of fat was negligible. The fruits of *Pithaglobium dulce* which the birds eat in the premigratory period contains only 0.2 to 0.4 per cent fat, while the carbohydrate amounts to 16.0 %. Then the high fat content in the evening denotes two things: (1) a *de novo* synthesis from carbohydrates and (2) the synthesis takes place simultaneously with the absorption of carbohydrate during the day time.

An increased food intake and hyperphagia might have greatly influenced the hyperlipogenesis as reported by King and Farner (1965) in Z.l.gambelii. But the changeover to a rich carbohydrate food in the premigratory period by the Rosy Pastors might be more influential in this connection. The change in the pattern of feeding and in the preference of food might have been induced by the hypothalamus, as observed in adaptive hyperlipogenesis in obese, overfed or refed (after starvation) rats (Tepperman and Tepperman, 1964 a). This changeover of diet was not due to the scarcity of food, since the related resident species in the same locality were found to prefer insect food. The carbohydrate rich diet thus provided an increased amount of glucose that was turned over to the liver for fat and glycogen synthesis. This resulted in higher fat and glycogen levels in the liver during the evening hours.

The decreased amount of glycogen in the morning suggests the occurrence of glycogenolysis during the night time. But, glycogen did not seem to be utilized for fat synthesis as a correspondingly increased amount of the latter was not observed in the early morning hours. This glycogen then, might have been transported to the muscle where it gets converted to fat, since the fat content of the muscle of Rosy Pastor was found to be more in the morning (Chandra-Bose, 1967). During the last 10 to 12 days of April, only a slight difference was evident between the glycogen values of moring and evening while the amount itself increased at both the times. This could mean that, firstly, less glycogen was sent from liver to other tissues like muscle and secondly, more glucose was made available for glycogen formation. The gradual decrease in glycogen from the liver in the beginning of the premigratory period agrees with the report of King et al.(1963) for Z.l.gambelii. But, they did not determine the glycogen content periodically upto the last week before the white throated sparrows migrate, and hence might have failed to observe any increase in glycogen deposition as seen in the Rosy Pastor.

During the hyperlipogenesis, the Rosy Pastor depends on carbohydrates as the energy source, sparing fats. In this connection it is interesting to note that the cpacity of the muscle of Rosy Pastor for fatty acid oxidation was reported to be low in the premigratory period than in the post migratory period (George and Vallyathan,1964). That the fat was sparingly used during the premigratory period was also evident as the RQ of liver and muscle slices was either 1 or more. An RQ more than 1 usually denotes fat synthesis from glucose (glucose RQ). Dickerson et al. (1943) reported a high glucose RQ in the liver slices obtained from those rats which were trained to feed the 24 hour quota of food within a very short time. Tepperman et al. (1943) also observed an elevated RQ in rats with hypothalamic obesity. The high RQ due to over feeding was found to be accompanied by the appearance of a high amount of saturated fatty acids in the liver (Dickerson et al.,1943).

Tepperman and Tepperman (1964a) thus concluded that the high glucose RQ was due to increased lipogenesis. Then the high RQ of the tissues of Rosy Pastor could also be considered as of a very high rate of lipogenesis. The above authors also noted that an enhanced lipogenesis was not accompanied by a reduction in hepatic glycogen. On the contrary, an increased amount was observed by them. The glycogen deposition then could not bear any relation to fat synthesis. It also showed that the fat synthesis was not at the expence of glycogen as suggested by King et al.(1963), nor did it reduce the formation of glycogen. Since, both fat and glycogen, in this case have glucose as the precursor, it could be suggested that in the premigratory period only a small amount of glucose was diverted to form glycogen which naturally results in a reduced content of glycogen in the liver. It was only during the last week of April that more glucose was made available for such synthesis. Perhaps a feedback inhibition of fatty acid synthesis by free fatty acids (FFA) as postulated by Weber et al. (1966), could ~~have~~ have influenced this diversion. Since, only the FFA and not esterified fatty acids were found to have such a feedback inhibition of fatty acid synthesis as well as glycolysis, it is logical to believe that the rate of fatty acid production exceeded the rate of its esterification in the liver by the end of April. Such a feedback inhibition might play an important role in effecting the switch over to fat utilization by the birds during migratory flight. Thus, one could state that the amount of glycogen deposited in the liver solely depends upon the amount of glucose spared for its formation and the reduction in its level in the premigratoryy period need not necessarily mean that the glycogen is brocken down for the synthesis of fat.

Tepperman and Tepperman (1965a) reported that adaptive hyperlipogenesis occurred when the tissues like liver and adipose tissue were faced with an overload of glucose. If such is the case, the glucose turnover being highest in the day time (when large amount of carbohydrate food was injested by these birds), the lipogenesis would also be highest during this time. The glucose turnover to these tissues is under the influence of insulin. A reduction of blood glucose level was observed in the Rosy Pastor by the last week of April (George and Naik,1964). Correspondingly, an increased activity of pancreatic islet cells was aslo observed (George and Naik,1964).

The glucose, not only provides acetyl CoA by undergoing lysis but, through the direct ~~ing~~ oxidative (HMP-shunt) pathway, also supplies the essential $NADPH_2$. In order to ensure sufficient supply of the latter, active HMP-shunt dehydrogenases as well as malic enzyme should be present in the tissues. Several factors were found to activate these enzymes, such as, high carbohydrate diet (Willmer and Foster, 1962; Niemeyer et al.,1962), increased utilization (oxidation) of $NADPH_2$ due to active lipogenesis (Tepperman and Tepperman, 1965b) and thyroxine (Tepperman and Tepperman,1964b). Since, increased release of thyroid hormone was observed in the Rosy Pastor (Chapter 8), it could be reasoned out that these enzymes must be active in the liver.

In conclusion it could be mentioned that the high carbohydrate diet provided the basie stimulus for hyperlipogenesis aided by hormonal and other physiological factors that paved the way for the necessary enzymic adaptations. The shift to fat utilization was effected by hormones like thyroxine (in high amount), adrenal hormones etc., which influenced the mobilization of non-esterified fatty acids from adipose tissue. The free fatty acids then exerted their feed-back inhibition on glycolysis as well as fatty acid synthesis.