CHAPTER 4

GLUCOSE-6-PHOSPHATE DEHYDROGENASE AND MALIC ENZYME DURING ADAPTIVE HYPERLIPOGENESIS IN MIGRATORY STARLING (*STURNUS ROSEUS*) AND WAGTAIL (*MOTACILLA ALBA*)

Migratory birds have a remarkable ability to accumulate reserve energy in the form of fat prior to migration. Only scattered information is available regarding the mechanism of accelerated lipid synthesis and possible enzymatic adaptations of the organs involved in the adaptive hyperlipogenesis during the premigratory period in migratory birds. Biosynthesis of lipid requires hydrogen ion for which the cytoplasmic NADPH is considered to be an important donor and hyperlipogenesis in cells depends on the availability of cytoplasmic reducing equivalents (e.g. NADPH). It has been postulated that as an efficient NADPH generating system, participation of Glucose-6-phosphate dehydrogenase (G-6-PDH) and "Malic" (MDH-NADP dependent) enzyme is essential for the hyperlipogenesis. Several reports regarding the effects of dietary and hormonal stimuli on the activities of G-6-PDH and "Malic" enzyme are available (Glock *et al.*, 1956; Hill *et al.*, 1958; Fitch and Chaikoff, 1960; Lee *et al.*, 1962; Tepperman and Tepperman, 1964; Butterworth *et al.*, 1966;

Cohen and Teitelbaum, 1966; Goodridge and Ball, 1966, 1967; Freedland et al., 1968; Tepperman et al., 1968; Chandrabose and Bensadoun, 1971a, 1971b). It is known that the adipose tissue is the important site for lipogenesis in the rat (Leveille, 1967; Romsos and Leveille, 1974), while Goodridge and Ball (1967) have reported that the liver is the major organ for lipogenic activities in birds. De Graw (1975) has reported adaptive changes in the activity of "Malic" enzyme in the liver of migratory White-crowned Sparrows and has shown that the enzyme activity increases during premigratory period.

In the present investigation changes in the activities of G-6-PDH and "Malic" enzyme in the liver and adipose tissue of Rosy Pastor and Wagtail were studied to evaluate the role of these enzymes in hyperlipogenesis in the two birds and to probe into the relative importance of the adipose tissue and liver in the hyperlipogenesis during premigratory period. During premigratory period Rosy Pastor shows a shift to a carbohydrate rich diet cosisting of fruits, from a mixed diet (fruits, seeds and insects) on which it subsists during the postmigratory period. Wagtail on the other hand continues to feed on protein and fat rich diet cosisting mainly of insects during post as well as premigratory periods. The present study on G-6-PDH and

"Malic" enzyme is also deemed to throw light on the mechanism by which these two birds bring about hyperlipogenesis with their different dietary preferences.

MATERIALS AND METHODS

Birds:

Rosy Pastors and Wagtails during their post and premigratory periods were shot and immediately brought to the laboratory for assaying G-6-PDH and "Malic" enzyme in their liver and adipose tissue.

Enzyme studies:

Liver and adipose tissue were homogenized in cold 0.15M KCl and centrifuged at 20,000 g for 20 minutes. The intermediate layer of the adipose tissue homogenate (the lower most layer is of sediment, the topmost layer consists of lipids and the intermediate layer consisting microsomes etc.) and the supernatant layer from the liver homogenates were collected and used for assaying enzymes. Glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) was assayed employing the method of Kornberg and Horecker (1955) with

modification as described by Marks (1966). NADP-malate dehydrogenase (E.C. 1.1.1.40) was assayed as described by Hsu and Lardy (1969). The protein content of the portion of the homogenates selected for the study was estimated by the method of Lowry et al. (1951). Enzyme activities are expressed as μ mole NADPH formed/mg protein/min. at 30°C.

RESULTS

Glucose-6-phosphate dehydrogenase: (Fig. 1 and Table 1).

The level of Glucose-6-phosphate dehydrogenase in the adipose tissue of both the birds was slightly higher than that in the liver of respective birds. During the premigratory period higher level of the enzyme activity was evident in both liver and adipose tissue of Rosy Pastor as well as Wagtail.

"Malic" enzyme: (Fig. 2 and Table 2).

In the liver of both the birds "Malic" enzyme level was found to be significantly higher than that of Glucose-6-phosphate dehydrogenase irrespective of their migratory phase. Failure to detect any measurable amount of

TABLE 1

Glucose-6-phosphate dehydrogenase activity in the liver and adipose tissue of Wagtail and Rosy Pastor. Expressed as mμ Mole NADPH formed/mg protein/min. Mean value ± S.D.

Month	Wagtail		Rosy Pastor	
	Liver	Adipose tissue	Liver	Adipose tissue
October	---	---	4.78 ±0.45	5.88 ±0.30
November	4.06 ±0.30	5.00 ±0.45	---	---
December	3.79 ±0.37	4.95 ±0.23	---	---
January	4.77 ±0.40	6.41 ±0.33	---	---
February	6.63 ±0.55	7.92 ±0.29	---	---
March	7.83* ±0.40	11.70* ±0.46	---	---
April	---	---	10.83* ±1.23	12.73* ±0.77
*Significant at the level	$P<0.001$	$P<0.001$	$P<0.002$	$P<0.001$

*P values refer to differences between post and premigratory periods. The Student's 't' test was used to analyze differences in means.

TABLE 2

"Malic" enzyme activity in the liver of Wagtail and Rosy Pastor. Expressed as mμ Mole NADPH formed/mg protein/min. Mean value ± S.D.

Month	Wagtail	Rosy Pastor
October	---	23.02 ±2.71
November	23.53 ±2.30	---
December	21.43 ±2.79	---
January	28.50 ±2.16	---
February	44.68 ±3.38	---
March	134.11* ±27.9	---
April	---	115.07* ±12.28
*Significant at the level	P<0.002	P<0.001

*P values refer to differences between post and premigratory periods. The Student's 't' test was used to analyze differences in means.

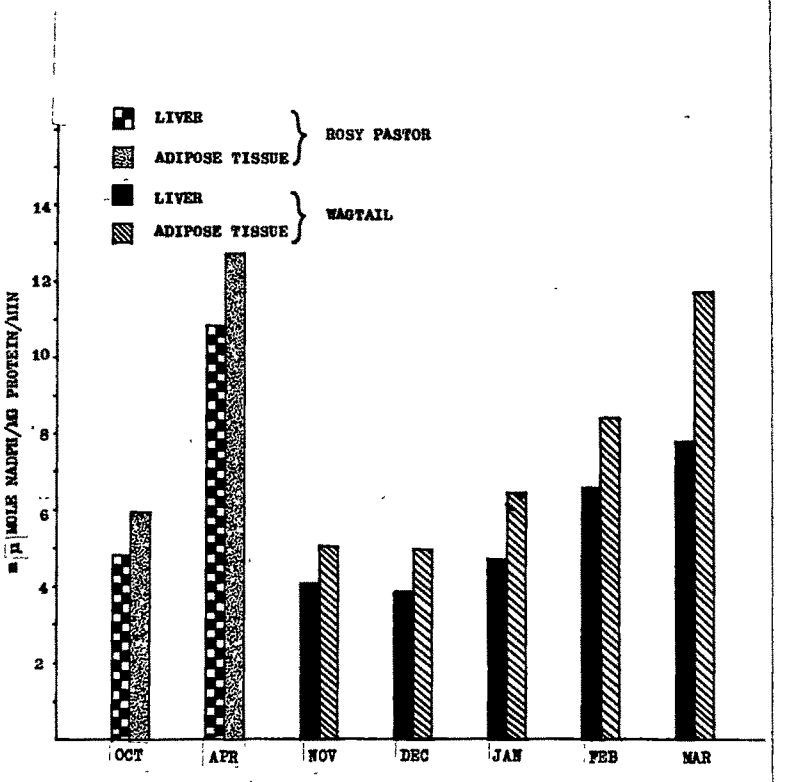

FIG. 1 LEVELS OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY IN THE LIVER AND ADIPOSE TISSUE OF ROSY PASTOR AND WAGTAIL. ENZYME ACTIVITY IS EXPRESSED AS m μ MOLE NADPH FORMED/MG PROTEIN/MINUTE.

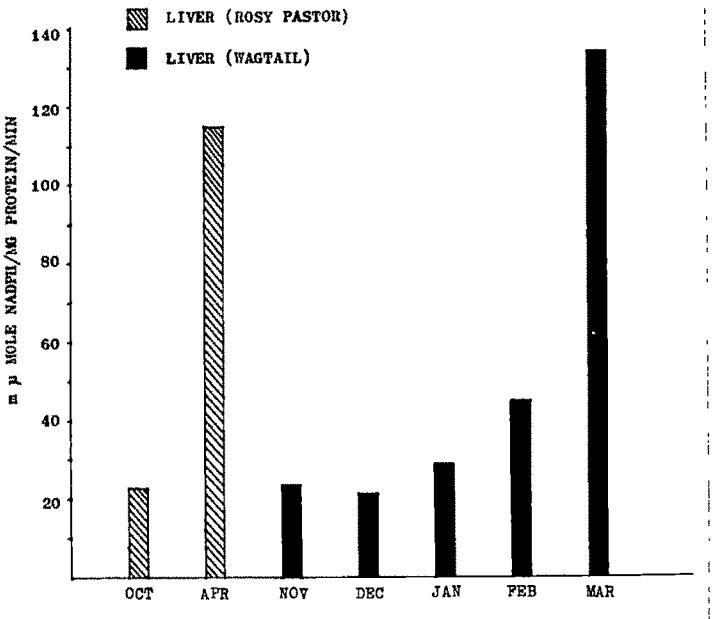

FIG. 2 LEVELS OF "MALIC" ENZYME ACTIVITY IN THE LIVER OF ROSY PASTOR AND WAGTAIL. ENZYME ACTIVITY IS EXPRESSED AS m μ MOLE NADPH FORMED/MG PROTEIN/MINUTE.

"Malic" enzyme in the adipose tissue was perhaps due to its very low concentration. During the premigratory period, highly significant increase in the activity of "Malic" enzyme was noticed in the liver of both the birds, which was almost six fold in the case of Wagtail and five fold in the case of Rosy Pastor. Even during the premigratory period the "Malic" enzyme activity in the adipose tissue of both the birds was not detected.

DISCUSSION

It has been reported that the alimentary canal of Rosy Pastor and Wagtail show certain adaptive changes during their premigratory period which help them in maintaining an increased flux of metabolites to the liver (Chapter 1). The liver too showed adaptive changes to bring about hyperlipogenesis which is evident from the fact that increased activities of phosphatases have been noticed in the liver of both Rosy Pastor and Wagtail during premigratory period (Chapter 3). Similarly, an increased cholinesterase activity and changes in ionic concentration (Pilo, 1967, 1969) have been reported in the liver of Rosy Pastor during its premigratory period. It is evident that during premigatory period certain physiological adjustments are

induced in the liver of the migratory birds most of which are geared to ensure increased lipid synthesis.

In the present study it was noticed that during premigratory period, the activities of G-6-PDH and "Malic" enzyme in the liver of Rosy Pastor and Wagtail increased. Such an increase in the activities of these two enzymes in the liver of both the birds would ensure increased supply of NADPH and support the adaptive hyperlipogenesis. Hyperphagia could be one of the factors that induces increase in the activities of these enzymes. This contention is supported by findings of Cohn and Joseph (1959,1962) who have reported increased HMP shunt enzyme activity and increase lipid synthesis in rat liver following forced feeding. Increased lipogenesis has been observed in force-fed chick also (Lepkovksy and Furuta, 1971). A few weeks prior to migration is known to be the period when premigratory fat synthesis and its deposition are at the maximum. A sudden increase in the activities of G-6-PDH and especially "Malic" enzyme during March and April could be considered as adaptive changes occurring in the liver at this phase to facilitate high lipogenic activities.

Further, since the level of "Malic" enzyme as compared to that of G-6-PDH is higher in the liver of both the birds, it is logical to believe that the former

contributes to a greater extent in the production of NADPH. O'Hea and Leveille (1968) have reported that "Malic" enzyme plays a predominant role in providing NADPH for lipogenesis in chick amply supports the belief. Besides, higher level of G-6-PDH observed in both the migratory birds during their premigratory period can also be accounted for enhanced turn over of pentose sugar through HMP pathway, which would support the increased nucleic acids synthesis and help in hyperplasia which is observed in the liver of both the birds during their premigratory period (Chapter 7).

In pigeon (Goodridge and Ball, 1966) and chick (O'Hea and Leveille, 1969), the liver is the major site for de novo synthesis of fatty acids, besides, it is said that avian adipose tissue is practically inert lipogenically (O'Hea and Leveille, 1969). Presently demonstrated very high "Malic" enzyme activity in the liver on one hand and failure to demonstrate measurable amount of the enzyme in the adipose tissue of the Rosy Pastor and Wagtail amply suggest that in these birds also the liver is the major site of lipid synthesis.

Weiss et al. (1967) have reported that inclusion of fat in the diet results in a reduction in hepatic lipogenesis, while increased level of dietary protein has been reported to depress fatty acid synthesis in the liver

of chick (Yeh and Leveille, 1969). On the other hand a carbohydrate rich diet is known to stimulate lipogenesis (Romsos and Leveille, 1974). The increase in the activities of G-6-PDH and "Malic" enzyme in the liver of Rosy Pastor which indirectly helps in hyperlipogenesis may be due to its habit of feeding mainly on carbohydrate rich diet during its premigratory period. In the case of Wagtail, though it feeds on diet rich in fat and protein during the premigratory period, the activities of G-6-PDH and "Malic" enzyme are not inhibited but are enhanced. Perhaps, the inhibitory effect of diet in this case is not manifested or is modified due to some other factors prevailing in the body of this migratory bird during its premigratory period. Hence the enhanced enzyme activities observed and concomitant hyperlipogenesis may be under the control of both diet (hyperphagia) and hormones. Goodridge et al. (1974) have demonstrated that thyroxine stimulates fatty acid synthesis in the isolated chick hepatocytes. Freedland et al. (1968) have reported that excess of thyroxine given to a rat fed on high protein diet caused a marked increase in "Malic" enzyme activity of its liver. Lardy et al. (1965) and Goodridge (1973) suggested that "Malic" enzyme is also responsive to the administration of insulin. Thus the

available data are suggestive of the existence of modifying effects on the activities of NADP-linked enzymes by several hormones in animals with diverse dietary habits. It has been reported that thyroidal activity increases in Rosy Pastor (Pilo and George, 1970) and Wagtail (John and George, 1967a) during their premigratory period. This leads to suggest that the increase in the activities of NADP-linked enzymes observed in the liver of both Rosy Pastor and Wagtail was due to the induction of G-6-PDH and "Malic" enzyme under the influence of increased dietary intake as well as hormonal activity.