CHAPTER 5

OXIDATIVE METABOLISM IN THE LIVER AND KIDNEY OF MIGRATORY STARLING (*STURNUS ROSEUS*) AND WAGTAIL (*MOTACILLA ALBA*)

During the premigratory period, the amount of reserve fat in Wagtail and Rosy Pastor increased tremendously and for this premigratory obesity, the liver showed adaptive changes facilitating increased lipid synthesis (Chapter 4). Moreover, during the same period, hyperplasia occured in the liver which was realized from the noted increase in mucleic acid (DNA) content in the organ of both the birds (Chapter 7) during this period. It is likely that overall metabolic activities are stepped up in a number of tissues of the migratory birds in preparation for the long journey they are to undertake. The requirement of energy (in the form of ATP) for various metabolic activities in the liver of migratory birds is enhanced during premigratory period and for this the oxidative metabolic machinery has to be further activated. In order to understand the degree of elicitation of Krebs' cycle activity and related aspects of energy metabolism in the liver of the migratory birds (*viz.*, Rosy Pastor and Wagtail), a study on the seasonal changes in the activities of Succinic

dehydrogenase; a key enzyme of Krebs' cycle, and Adenosine Triphosphatase (ATPase) was carried out.

It has been observed that certain metabolic transport mechanisms operate at a higher rate in the kidney of migratory birds during premigratory period (Chapter 3). It is quite logical that the energy demand of this organ would also increase during premigratory period. A study on seasonal changes in the activities of SDH and ATPase in kidney of Rosy Pasotr and Wagtail was also undertaken so as to gather information that would aid in understanding the kidney functions during the premigratory period.

MATERIALS AND METHODS

The birds were shot and brought immediately to the laboratory. Liver and kidney slices were removed quickly and blotted free of blood. Tissue homogenates prepared in chilled distilled water were used for enzyme assays.

Succinic dehydrogenase activity was assayed employing the method of Kun and Abood (1949) using triphenyl tetrazolium chloride (TTC) as a hydrogen acceptor. The readings were taken at 420 μ on Klett-Summerson photoelectric colorimeter. Protein was estimated by the Biuret method (Layne, 1957). The enzyme activity is expressed

as μ gm formazan formed/mg protein/30 minutes.

Quantitative estimation of ATPase (Mg^{++} activated) was carried out employing the method described by Umbreit et al. (1957). Adenosine-5-triphosphate (disodium salt, from equine muscle; Sigma Chemical Co., U.S.A.) was used as a substrate. Inorganic phosphate released was estimated according to the method of Fiske and Subbarao (1925). The readings were taken at 660 μ on Klett-Summerson photoelectric colorimeter. The enzyme activity is expressed as μg of phosphorus released/mg protein/10 minutes.

RESULTS

Succinic dehydrogenase: (Fig. 1 and Table 1)

LIVER: SDH activity in the liver of Wagtail was found to remain at almost a constant level up to February but increased significantly during March (Premigratory period). In the liver of Rosy Pastor also the enzyme activity was found to be higher during premigratory period (April) as compared to that during the postmigratory one (October).

KIDNEY: SDH activity in the kidney of both the birds increased considerably during their premigratory period.

TABLE 1

Succinic dehydrogenase activity in the liver and kidney of Wagtail and Rosy Pastor. Expressed as μg formazan formed/mg protein/30 min. Mean value ± S.D.

Month	Wagtail		Rosy Pastor	
	Liver	Kidney	Liver	Kidney
October	---	---	10.18 ±0.38	7.68 ±0.82
November	4.10 ±0.17	10.25 ±1.00	---	---
December	7.41 ±1.13	10.18 ±1.44	---	---
January	6.25 ±0.49	10.01 ±1.92	---	---
February	7.47 ±1.43	12.20 ±1.00	---	---
March	11.86* ±0.90	19.78* ±0.30	---	---
April	---	---	12.74* ±1.01	10.80* ±0.36
*Significant at the level	P<0.001	P<0.001	P<0.01	P<0.005

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

TABLE 2

Adenosine triphosphatase activity in the liver and kidney of Wagtail and Rosy Pastor. Expressed as μg phosphorus liberated/ mg protein/10 min. Mean value ± S.D.

Month	Wagtail		Rosy Pastor	
	Liver	Kidney	Liver	Kidney
October	---	---	78.64 ±6.65	76.30 ±3.52
November	60.30 ±8.88	139.60 ±13.53	---	---
December	65.90 ±2.74	135.76 ± 6.73	---	---
January	60.85 ±2.05	149.23 ±13.41	---	---
February	60.51 ±8.60	117.60 ± 9.25	---	---
March	79.61* ±8.51	250.43* ±30.25	---	---
April	---	---	129.90* ±20.08	200.68* ±28.39
*Significant at the level	P<0.05	P<0.005	P<0.05	P<0.005

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

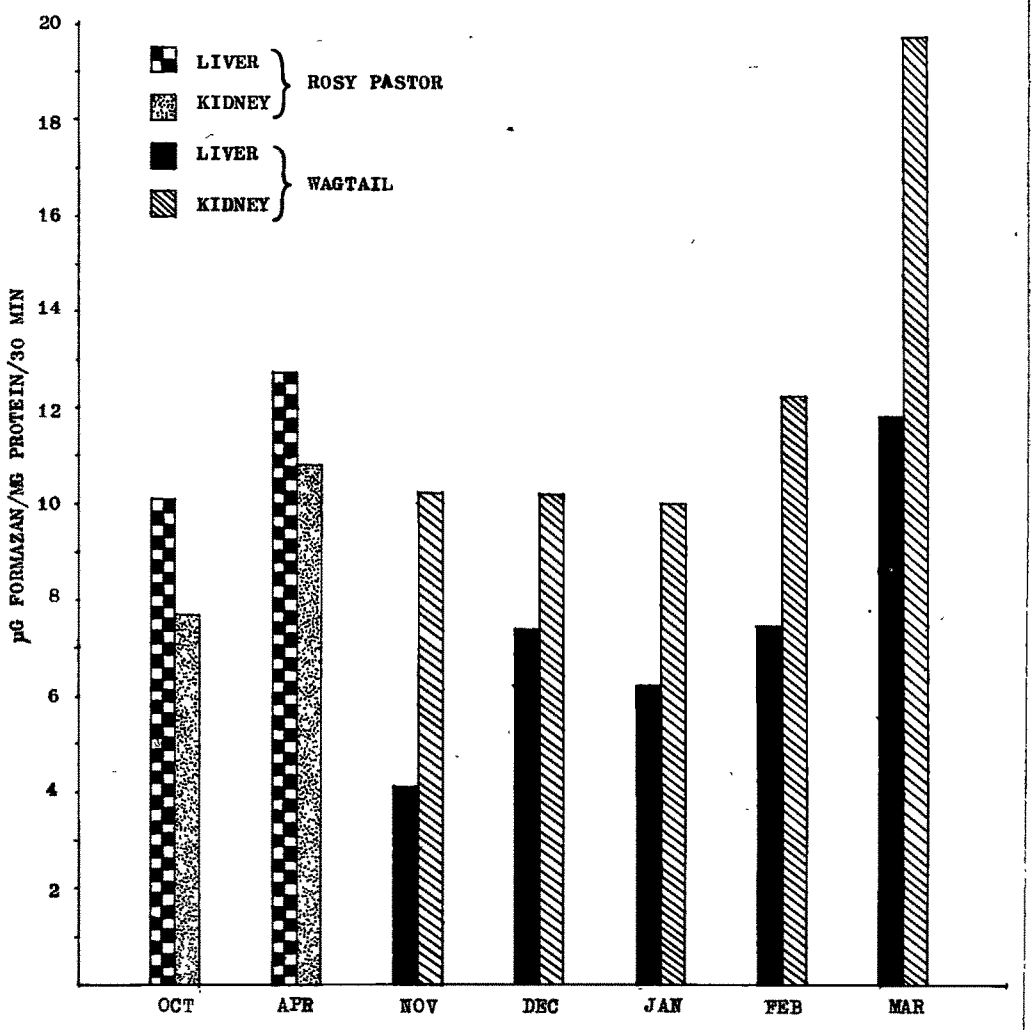

FIG. 1 LEVELS OF SUCCINIC DEHYDROGENAGE ACTIVITY IN THE LIVER AND KIDNEY OF ROSY PASTOR AND WAGTAIL. ENZYME ACTIVITY IS EXPRESSED AS µGM FOMAZAN FORMED/MG PROTEIN/30 MINUTES.

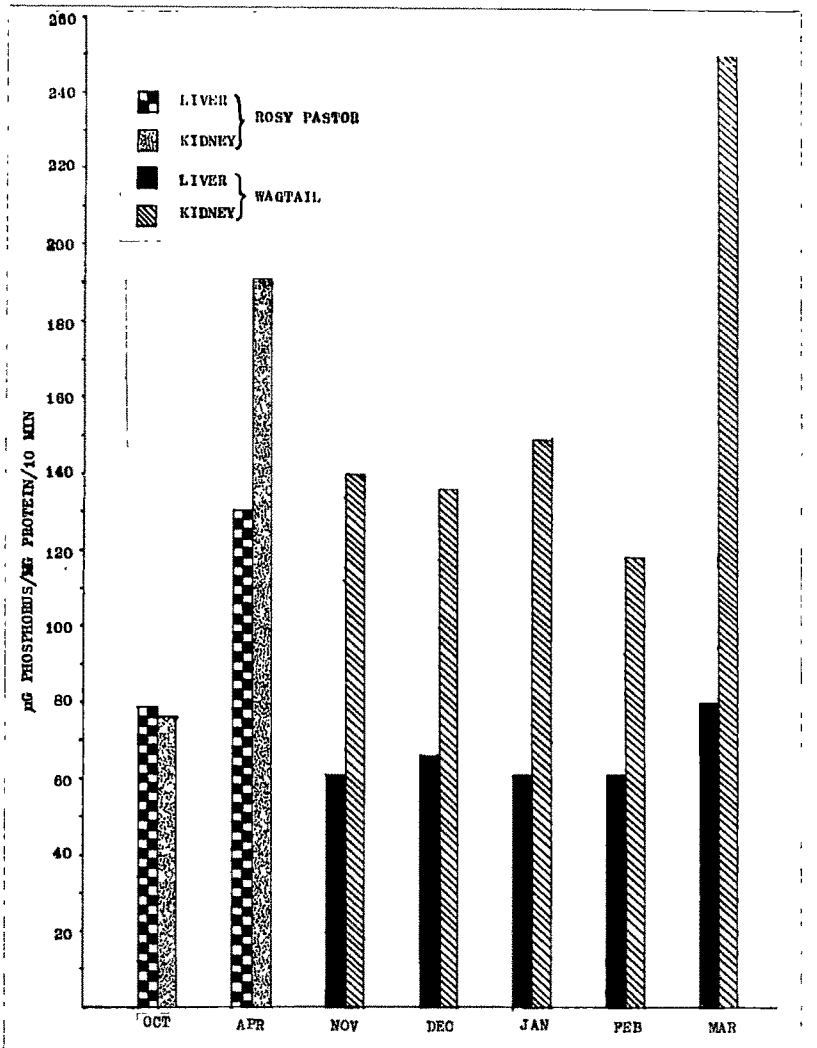

FIG. 2 LEVELS OF ADENOSINE TRIPHOSPHATASE ACTIVITY IN THE LIVER AND KIDNEY OF ROSY PASTOR AND WAGTAIL. ENZYME ACTIVITY IS EXPRESSED AS µG PHOSPHORUS/MG PROTEIN/10 MINUTES.

Adenosine Triphosphatase: (Fig. 2 and Table 2)

LIVER: ATPase activity in the liver of Wagtail exhibited a little fluctuation from November to February but in March (premigratory period) it reached to a level which was higher than any observed during other months. Similarly, ATPase activity in the liver of Rosy Pastor during the premigratory period was found to be higher than that observed during its postmigratory period.

KIDNEY: During premigratory period, the activity of the enzyme in the kidney of both the birds was significantly higher than that noted during the postmigratory period. Like in the liver, the enzyme activity in the kidney of Wagtail showed fluctuation during different months, highest being during the premigratory period (March). Besides, ATPase activity in the kidney of Wagtail was more than that noted in the kidney of Rosy Pastor.

DISCUSSION

The enzymatic hydrolysis of ATP by ATPase is an ubiquitous property of the cells which is possibly of importance for intracellular transfer of energy. It is

suggested that, there are several kinds of ATPases, localized in various cell organelles and functioning at different pH optima. Potter et al. (1953) reported that the ATPase activity of the various cell fractions may have different optimal conditions as well as functions. Histochemically ATPase was demonstrated in the liver mitochondria (Wachstein et al., 1960, 1962), in bile canaliculi (Wachstein and Meisel, 1957; Novikoff et al., 1958; and Shah et al., 1975). Na^+-K^+ dependent ATPase has been reported to be present on the cell membrane. Novikoff et al. (1953) reported the presence of ATPase in microsomal fraction of liver. Mitchell (1966) proposed a chemiosmotic system operating in mitochondria and postulated that ATPase is a part of such system. He explained that such a system plays an important role in ATP synthesis by mitochondria during oxidative phosphorylation. It is reported that there is a membrane bound actomyosin like ATPase in mitochondria which is implicated in contraction of their membrane. Ohnishi et al. (1964) isolated two proteins from rabbit liver mitochondria, one resembling actin and other resembling myosin. Thus the available evidences quoted above corroborate the view that mitochondrial ATPase is well involved in the

functional activity of mitochondria. Potter et al. (1953) suggested that total ATPase activity of liver homogenate is dominated by the mitochondrial activity therein.

Present study on seasonal changes in SDH and ATPase activities in the liver of Rosy Pastor and Wagtail showed that activities of these enzymes in the organ were at higher level during premigratory period, as compared to that during their postmigratory period. As SDH activity reflects the metabolic state of mitochondria, the increased activity of the enzyme is suggestive of increased oxidative metabolism in the liver during the premigratory period of these birds. Increased ATPase activity could also be due to its possible involvement in increased mitochondrial functions.

From these facts, it can be inferred that there is a high degree of enhancement of the rate of energy yeilding reactions in order to build up required concentration of ATP needed for various synthetic activities in the liver of migratory birds during their premigratory period. Besides, increased ATPase activity in the liver during the same period may also be due to an increase in the activity of the specific ATPase which participates in active transport of metabolites, synthesized products and bile.

It is well known that oxygen consumption in the liver rises when the animals are treated with thyroxine. Activity of thyroid gland of Wagtail (John and George, 1967a) and Rosy Pastor (Pilo and George, 1970) increases during the premigratory period. Pilo (1967) also showed increased RQ of liver and muscle of Rosy Pastor during premigratory period.

Thus, the observed changes in SDH activity which alter the rate of oxidative metabolism could be considered as an adaptive change which may partly be under the control of hormone like thyroxine.

Higher SDH activity in the kidney of Rosy Pastor and Wagtail during premigratory period obviously suggests that energy metabolism in kidney is also stepped up, thereby facilitating enhanced renal functions. Na^+-K^+ ATPase is associated with transport mechanism in the nephrons of kidney (Kinsolving et al., 1963). In the present study; Na^+-K^+ ATPase was not estimated separately but the changes in total ATPase activity can be considered as an index of Na^+-K^+ ATPase because the latter is the major part of the total ATPase activity. Bonting et al. (1961) studied total ATPase and Na^+- K^+ activated ATPase in several tissues of cat and reported that all the tissues examined with significant total ATPase activity were found to contain

Na^+- K^+ activated ATPase activity. During the premigratory period, increased ATPase activity was noted in the kidney of Rosy Pastor and Wagtail, which is suggestive of increased active transport and active secretion of excretory products. Pilo (1967) reported that Na^+ concentration in the kidney of Rosy Pastor increases during the premigratory period. Accumulation of Na^+ against concentration gradient would then require energy. Thus the increased ATPase activity in kidney during premigratory period can provide the required energy for such purpose.

Dietary preference of these two birds seems to have an effect on physiological adaptations of their renal tissues. Due to the habit of feeding on protein rich diet, uric acid excretion in Wagtail would be higher than that in Rosy Pastor which is mainly frugivorous during premigratory period. It is tempting to consider that the higher value of ATPase activity observed in the kidney of Wagtail as compared to that in Rosy Pastor could be associated with relatively higher rate of uric acid excretion in the former.