CHAPTER 3

ADAPTIVE CHANGES IN THE ACTIVITIES OF ACID AND ALKALINE PHOSPHATASES IN LIVER AND KIDNEY OF MIGRATORY BIRDS, WAGTAIL (*MOTACILLA ALBA*) AND ROSY PASTOR (*STURNUS ROSEUS*)

Hyperphagia that manifest in the migratory birds during the premigratory period causes increased caloric intake. This in turn results in hyperlipogenesis and fat deposition, which is utilized to generate energy required for sustained flight and other purpose during migration. Certain aspects of adaptive changes observed in the alimentary canal of migratory birds, Wagtail and Rosy Pastor have been reported in Chapters 1 & 2. Due to premigratory hyperphagia, an increased amount of digested materials (metabolites) reach the liver via portal blood and are processed for storage, degradation and interconversions. Lipid is synthesized in the liver and transported via blood mainly to the adipose tissue, the liver under such condition is likely to show adaptive changes involving enhanced functional activities of certain enzymes.

Nonspecific acid and alkaline phosphatases are

groups of enzymes which act not only as hydrolases, but are also known to act as transferases. Different physiological functions are ascribed to phosphatases according to their localization in different tissues and cells. These nonspecific phosphatases, according to the site of their localization and characteristics of the cell, are supposed to be involved in variety of cellular activities, viz., absorption, secretion, cellular phagocytosis, synthesis of protein and many phosphorylation reactions. These enzymes not only show difference in pH optima but may also occur in several isoenzymic forms, some of which are sensitive to hormones and other factors.

The distribution patterns of acid and alkaline phosphatases in the liver of birds with different feeding habits have been found to vary considerably (Shah et al., 1972). In the present investigation, the histochemical study of acid and alkaline phosphatases in liver and kidney of two migratory birds (Rosy Pastor and Wagtail) was carried out with the view that the site of localization within the cells or the organ concerned would give an idea about possible physiological functions of the enzymes; while the quantitative study of these enzymes was deemed to provide informations regarding their strength of activity under altered physiological state of these organs which can help

in establishing relationship between the enzymes (acid and alkaline phosphatases) and adaptive physiological activities of the organ concerned during premigratory preparations.

Kidney, the organ for excretion, though not directly involved in premigratory preparations would definitely show changes in its functional activities when metabolism in other organs gets altered. With these facts in view a comparative study on the enzyme activities in kidney of migratory birds was also undertaken.

MATERIALS AND METHODS

Liver and kidney of Rosy Pastor and Wagtail were removed, blotted and were processed for histochemical and quantitative study of acid and alkaline phosphatases. The histochemical demonstration of phosphatases was carried out by the method of Burstone (1962) and for quantitative estimation; the method described in Sigma Technical Bulletine No. 104 was followed. This study was conducted during post and premigratory periods of these birds.

RESULTS

Histochemical observations

EXPLANATIONS FOR FIGURES

Figs. 1-4. Photomicrographs of sections of liver showing alkaline phosphatase activity.

Fig. 1. Liver of Rosy Pastor (POM). 65X.

Fig. 2. Liver of Rosy Pastor (PM). 65X.

Fig. 3. Liver of Wagtail (POM). 125X.

Fig. 4. Liver of Wagtail (PM). 125X.

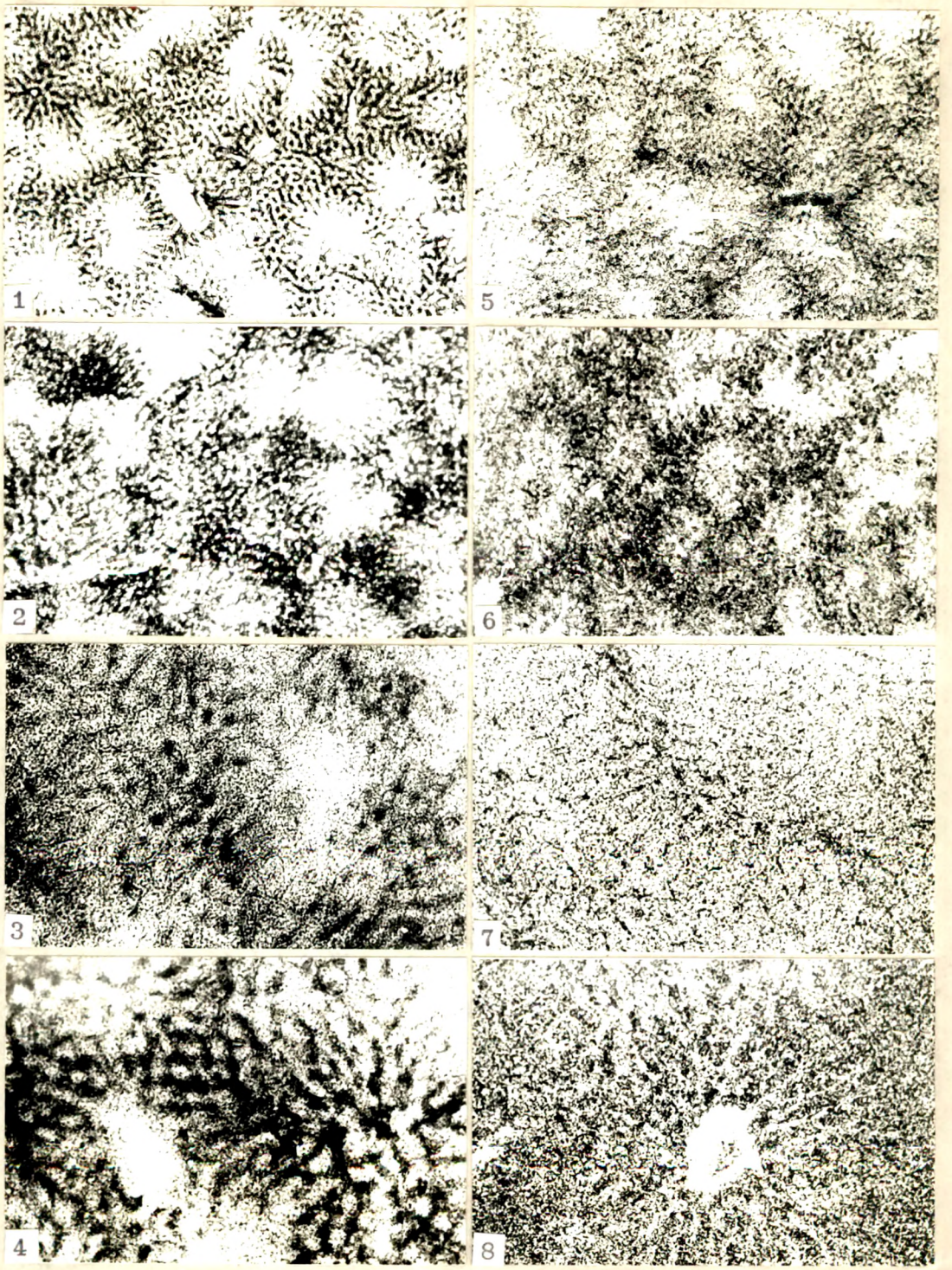
Figs. 5-8. Photomicrographs of sections of liver showing acid phosphatase activity.

Fig. 5. Liver of Rosy Pastor (POM). 65X.

Fig. 6. Liver of Rosy Pastor (PM). 65X.

Fig. 7. Liver of Wagtail (POM). 125X.

Fig. 8. Liver of Wagtail (PM). 125X.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Figs. 9-14. Photomicrographs of sections of kidney showing alkaline phosphatase activity.

Fig. 9. Kidney of Rosy Pastor (POM). 65X.

Fig.10. Cortical region of kidney of Rosy Pastor (PM). 125X.

Fig.11. Medullary region (M) of kidney of Rosy Pastor (PM). 125X.

Fig.12. Kidney of Wagtail (POM). 65X.

Fig.13. Cortical region (C) of kidney of Wagtail (PM). 125X.

Fig.14. Medullary region (M) of kidney of Wagtail (PM). 125X.

Figs. 15-20. Photomicrographs of sections of kidney showing acid phosphatase activity.

Fig.15. Cortical region (C) of kidney of Rosy Pastor (POM). 125X.

Fig.16. Medullary region (M) of kidney of Rosy Pastor (POM). 125X.

Fig.17. Kidney of Rosy Pastor (PM). 65X.

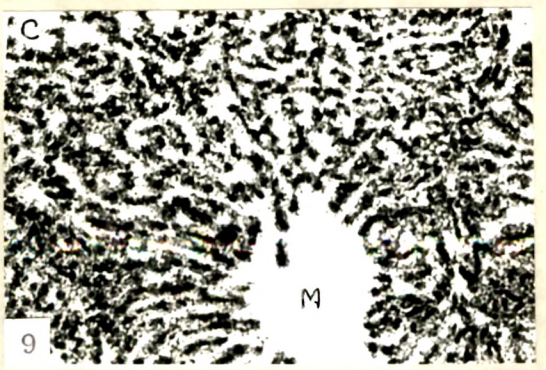
Fig.18. Kidney of Wagtail (POM). 65X.

Fig.19. Cortical region (C) of kidney of Wagtail (PM). 125X.

Fig.20. Medullary region (M) of kidney of Wagtail (PM). 125X.

ABBREVIATIONS

POM = Postmigratory period, PM = Premigratory period



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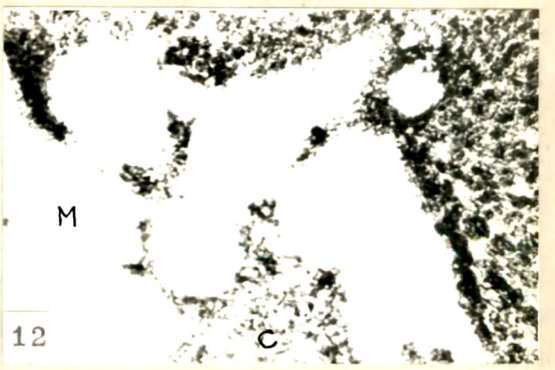

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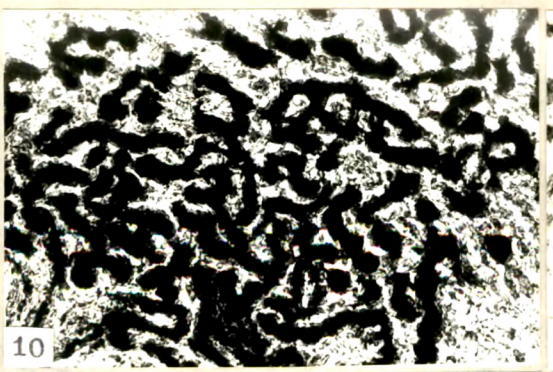

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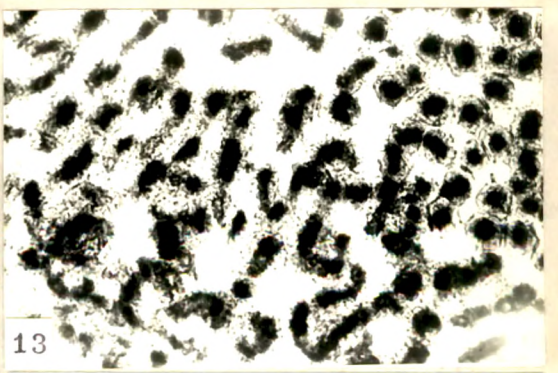

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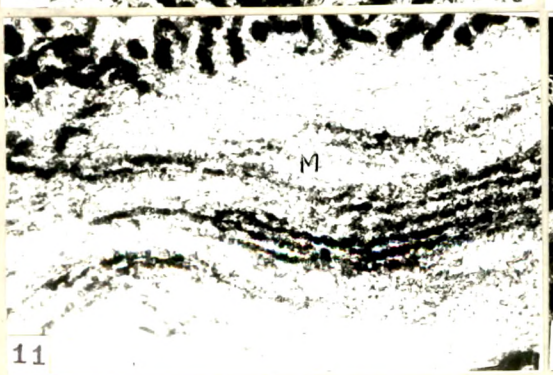

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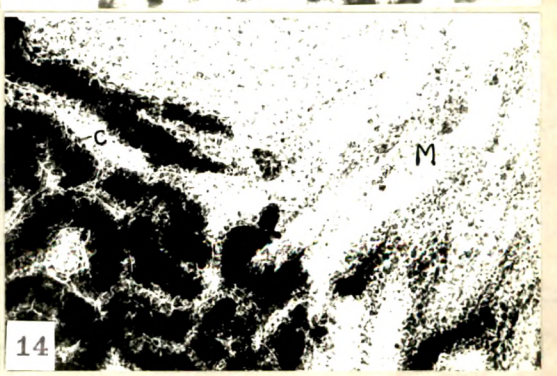

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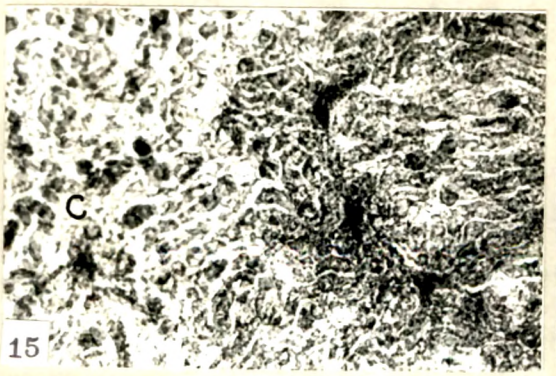

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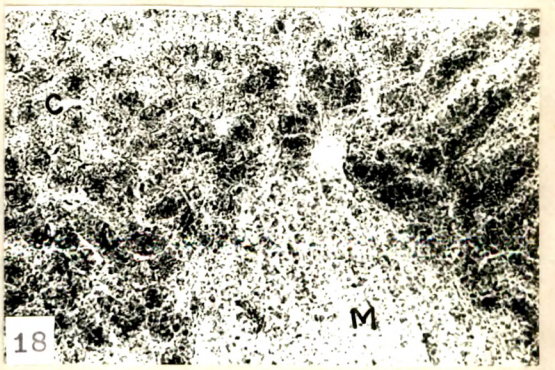

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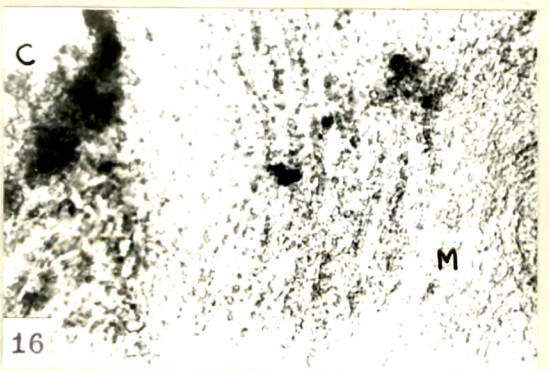

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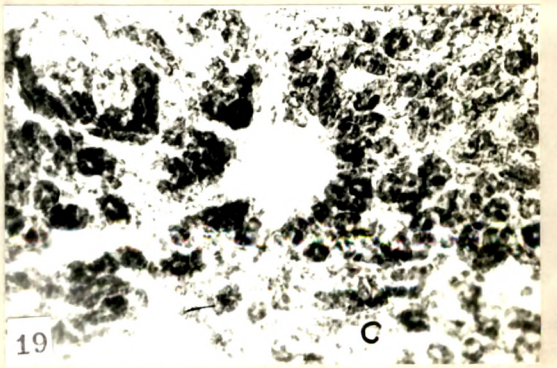

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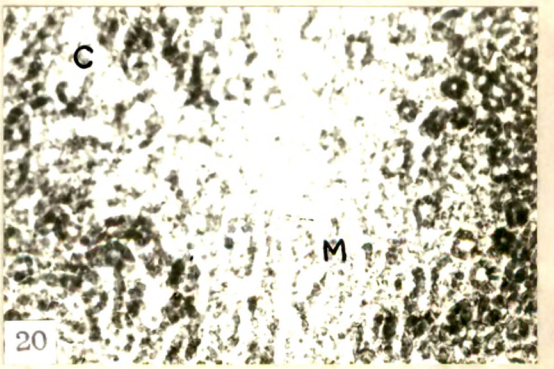

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Alkaline phosphatase in the liver of Rosy Pastor and Wagtail:

During post as well as premigratory periods the alkaline phosphatase reactivity in the liver of Rosy Pastor and Wagtail was seen in the sinusoidal linings and predominantly in the periportal areas (Figs. 1, 3 and 2, 4). In Rosy Pastor the enzymes concentration did not show any appreciable change during premigratory period (Fig. 2) but in the Wagtail during the same period the enzyme reactivity had increased (Fig. 4).

Acid phosphatase in the liver of Rosy Pastor and Wagtail:

The liver of Rosy Pastor and Wagtail showed acid phosphatase in all the parenchymal cells (Figs. 5 and 7). However, the intensity of the enzyme in the liver of Rosy Pastor was slightly more in the cells around the portal areas. The level of the enzyme activity in both the birds increased during their premigratory period (Figs. 6 and 8).

Alkaline phosphatase in the Kidney of Rosy Pastor and Wagtail:

During post and premigratory periods the alkaline phosphatase reactivity in glomeruli of the birds was sparse while that in luminal border of the proximal and distal

convoluted tubules was intense (Figs. 9 and 10; 12 and 13). The 'medullary zone', the region of the collecting tubules, in both the birds was enzyme negative during postmigratory period (Figs. 9 and 12) however, became fairly enzyme positive during premigratory phase (Figs. 11 and 14).

Acid phosphatase in the kidney of Rosy Pastor (Figs. 15, 16 and 17) and Wagtail (Figs. 18 and 19, 20):

During post and premigratory periods the acid phosphatase reactivity was found to be localized in the glomeruli, in the cytoplasm of the cells of the proximal and distal convoluted tubules and their brush border, in the cells of medullary loop and collecting ducts. The intensity of the enzyme was highest in the brush borders of the proximal and distal convoluted tubules, least in the cells of medullary loops and collecting ducts and intermediate in the cytoplasm of the cells of glomeruli and those of the proximal and distal convoluted tubules. During the premigratory period, the enzyme reactivity was found to be higher than that observed during the postmigratory one.

Quantitative study

Liver:

Data on quantitative estimations of acid and alkaline

TABLE 1

Acid and alkaline phosphatases activities in liver of Wagtail and Rosy Pastor. Expressed as μ Mole p-Nitrophenol released/mg protein/30 minutes. Mean value ± S.D.

Month	Wagtail		Rosy Pastor	
	Acid phosphatase	Álkaline phosphatase	Acid phosphatase	Alkaline phosphatase
October	---	---	0.7108 ±0.0346	1.1056 ±0.1346
November	0.5802 ±0.0523	0.7788 ±0.0206	---	---
December	0.4068 ±0.0008	0.7305 ±0.0890	---	---
January	0.4594 ±0.0276	0.7959 ±0.0276	---	---
February	0.6379 ±0.0471	0.9364 ±0.0304	---	---
March	0.7940* ±0.0610	1.4901* ±0.0622	---	---
April	---	---	1.4338* ±0.1288	1.0996* ±0.2238
*Significant at the level	P<0.01	P<0.005	P<0.01	NS

*P values refer to differences between post and premigratory periods. The Student's 't' test was used to analyze differences in means. NS = Change observed is not significant.

TABLE 2

Acid and alkaline phosphatases activities in kidney of Wagtail and Rosy Pastor. Expressed as μ Mole p-Nitrophenol released/mg protein/30 minutes. Mean value ± S.D.

Month	Wagtail		Rosy Pastor	
	Acid phosphatase	Alkaline phosphatase	Acid phosphatase	Alkaline phosphatase
October	---	---	0.7978 ±0.0053	5.9150 ±0.2502
November	0.6667 ±0.1166	9.4350 ±0.3606	---	---
December	0.6535 ±0.0406	9.9340 ±2.5116	---	---
January	0.6832 ±0.0203	6.4270 ±0.2991	---	---
February	1.0423 ±0.1336	9.2985 ±1.4743	---	---
March	1.5401* ±0.2720	19.0350* ±2.1283	---	---
April	---	---	1.0046* ±0.1050	7.4580* ±1.0663
*Significant at the level	P<0.02	P<0.05	P<0.05	P<0.05

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

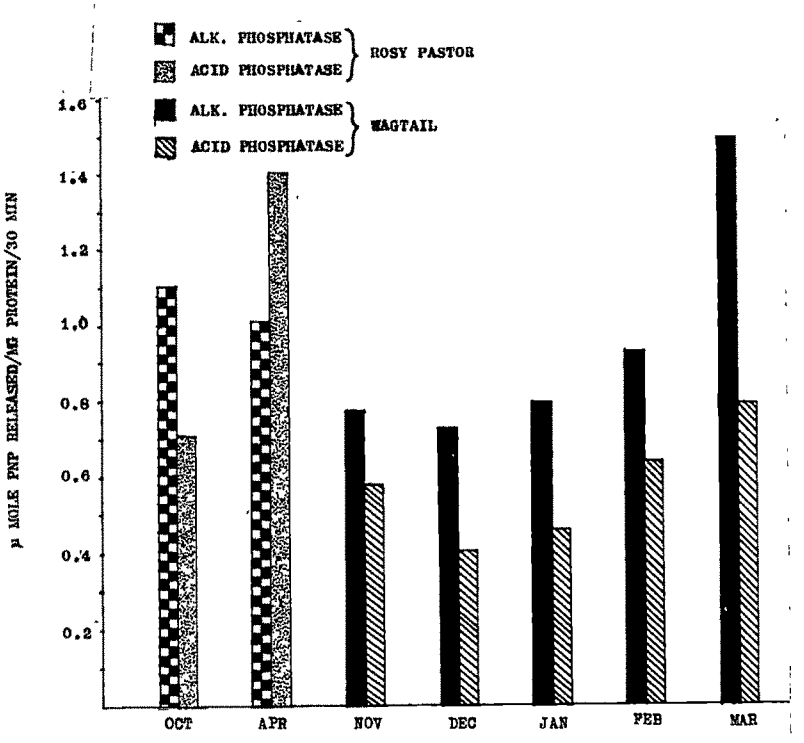

FIG. 21 LEVELS OF ALKALINE AND ACID PHOSPHATASE ACTIVITY IN THE LIVER OF ROSY PASTOR AND WAGTAIL. ENZYME ACTIVITY IS EXPRESSED AS μ MOLE p-NITROPHENOL (PNP) RELEASED/MG PROTEIN/30 MINUTES.

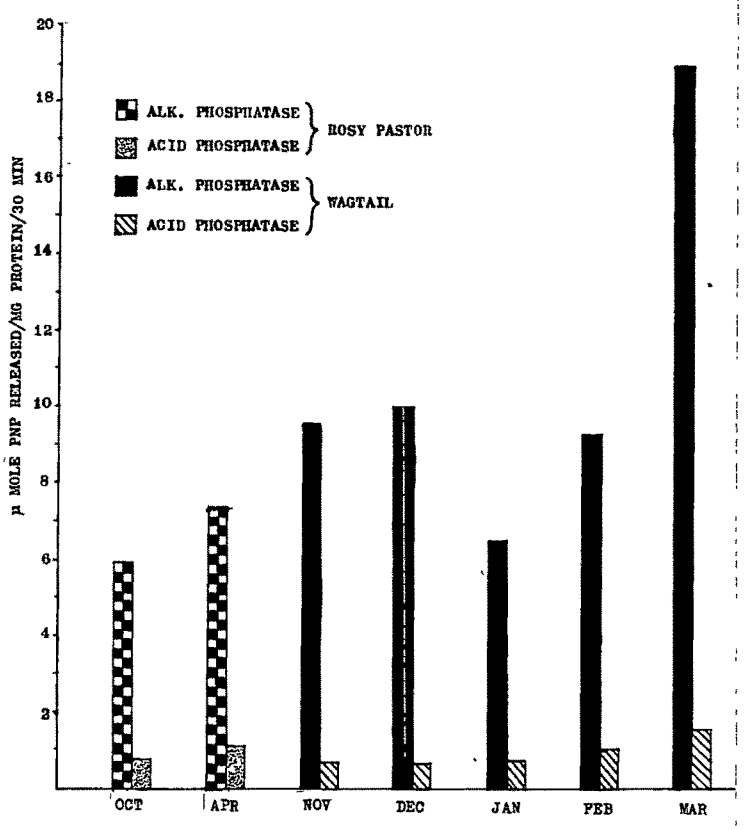

FIG. 22 LEVELS OF ALKALINE AND ACID PHOSPHATASE ACTIVITY IN THE KIDNEY OF ROSY PASTOR AND WAGTAIL. ENZYME ACTIVITY IS EXPRESSED AS µ MOLE p-NITROPHENOL (PNP) RELEASED/MG PROTEIN/30 MINUTES.

phosphatases in the liver of both the birds are presented in Table 1 and Fig. 21. From the data it becomes obvious that both the acid and alkaline phosphatases increased in the liver of Wagtail during premigratory period, while in Rosy Pastor during the same period, only the acid phosphatase showed similar trend whereas the alkaline phosphatase activity did not change. Unlike in Wagtail, it was also noticed that the activity of acid phosphatase was more than that of alkaline phosphatase in the liver of Rosy Pastor during the premigratory period.

Kidney:

Both the acid and alkaline phosphatse activities in the kidney of Rosy Pastor and Wagtail were at a higher level during premigratory period as compared to that during postmigratory one (Table 2 and Fig. 22). Acid phosphatase activity in the kidney of Wagtail and Rosy Pastor was almost at the same level during the postmigratory period, but during the premigratory period the enzyme activity in the kidney of Wagtail was significantly higher than what was noticed in the kidney of Rosy Pastor.

DISCUSSION

In the liver of both the migratory birds, alkaline

phosphatase activity was noticed in the periportal areas where the uptake of metabolites (viz., amino acids, carbohydrates and lipids) by hepatocytes normally occurs. Since alkaline phosphatase is associated with transport mechanism across the cell membrane, in this case also it must be involved in transporting the metabolites across the cell membranes of the hepatocytes. Shah et al. (1972) reported a similar localization of alkaline phosphatase in the liver of some birds and suggested similar function to this enzyme. During the premigratory period, activity of alkaline phosphatase was found to be higher in the liver of Wagtail (which consumes lipid and protein rich diet) than that in Rosy Pastor (whose diet is not so rich in lipids). Such a difference in the activities of the enzyme in the liver of these two migratory birds points to the adaptive feature of the organ to the respective diets. Nimni (1957) has reported that feeding of a high fat diet increases the alkaline phosphatase activity of rat liver during absorptive period. Thus in Wagtail liver, the high activity of alkaline phosphatase could be explained in terms of its involvement in greater absorption of digested materials, especially fat.

The hepatic parenchymal cells of both the birds showed granular localization of acid phosphatase activity.

Nonspecific acid phosphatases are known to act as phosphotransferases also. Glucose-6-phosphatase (G-6-Pase) is active at acidic pH and hydrolyses Glucose-6-phosphate so as to liberate glucose. Stetten (1964) has reported Glucose-6-phosphatase as a multifunctional enzyme capable not only of degrading Glucose-6-phosphate but also of synthesizing Glucose-6-phosphate via the reaction catalyzed by PPi (inorganic pyrophosphate) glucose phosphotransferase activity. These phosphatases (which are functional optimally at acidic pH) may be active in the hepatocytes of both the birds and Glucose-6-phosphate generated as a result of their activities can be utilized through Hexose monophosphate shunt as well as Embden-Meyerhof, Parnas pathways for the production of NADPH and energy respectively. Need for more NADPH and energy during premigratory period for a higher rate of lipogenesis would demand higher rate of operation of HMP shunt and EMP pathways; which in turn would need greater amount of Glucose-6-phosphate for their adequate functioning. Such a high need for Glucose-6-phosphate can be met with when acid phosphatases are in action at a higher rate. Since the acid phosphatase, in the liver of both the migratory birds, is highly reactive during their premigratory period and also it is known that higher rate of lipogenesis exists in their liver during this period, one

is tempted to surmise that the acid phosphatase which is in higher concentration is well implicated, though indirectly, in the premigratory hyperlipogenic activity of the liver in these migratory birds. Phosphatidic acid phosphatase is another enzyme acting at acidic pH and is essential for triglycerides and phospholipid synthesis. This enzyme can play an important role during premigratory hyperlipogenesis. Thus, acid phosphatase activity detected in the hepatic cells may be due to the active participation of several phosphatases mentioned above. During premigratory period, there was an increase in the activities of acid and alkaline phosphatases in the liver of Wagtail while only acid phosphatase showed similar trend in Rosy Pastor but alkaline phosphatase remained relatively unchanged. At this stage it is significant to note that during the premigratory period Rosy Pastor is mainly a frugivore i.e. its diet is not rich in lipids. While studying alkaline and acid phosphatase activities in the liver of birds with different dietary preferences, Shah et al. (1972) observed that alkaline phosphatase was more active in the liver of carnivorous, insectivorous and omnivorous birds than in that of frugivorous and graminivorous ones. In the latter two groups, acid phosphatase has been found to be more active.

These facts support the present findings wherein it is noticed that acid phosphatase activity in the liver of Rosy Pastor is higher than that in the liver of Wagtail during their premigratory period. Thus premigratory changes in acid and alkaline phosphatase activities in the liver of both the birds (each having different dietary preferences) could be associated with the specific metabolic activities; indicating their indirect involvement in the process leading to hyperlipogenesis.

Intense alkaline phosphatase activity was observed in the brush border of the proximal convoluted tubules of the kidney of both the birds studied where it may be involved in reabsorption of glucose by transphosphorylation. As the glomerular filtration is a passive process, no alkaline phosphatase was found in the glomerular cells. During the premigratory period, alkaline phosphatase was detected to be fairly active in the medullary loops as well as collecting tubules of nephrons which is suggestive of the increased rate of reabsorption. It must be noted at this point that Pilo (1967) has reported an increase in Na^+ concentration in the kidney of Rosy Pastor; which suggests an increased reabsorption of this cation during premigratory period.

Wachstein and Bradshaw (1965) demonstrated acid phosphatase activity in the glomeruli, proximal convoluted tubules and in the luminal border of the collecting ducts in the kidney of three mammalian species. Lin and Fishman (1972) reported microsomal and lysosomal acid phosphatase in mouse kidney. Straus (1964) showed the presence of acid phosphatase activity in the cells of the proximal convoluted tubules in the kidney of rat and suggested its involvement in the protein absorption. Since the histochemical localization of acid phosphatase activity observed in the kidney of both the migratory birds is similar to that reported for mammalian kidney, one would like to suggest that in these birds also the enzyme is involved in functions similar to those suggested for mammalian kidney. During postmigratory period poor activity of acid phosphatase was observed in the cells of medullary loops and collecting ducts which became a little more intense during their premigratory period. It is known that the collecting tubules secrete mucus into the lumen which facilitates the passage of uric acid (Poulson, 1965). A relationship between acid phosphatase and mucin production in the intestine has been suggested by Ogawa et al. (1962). It is tempting to suggest a similar role for the acid phosphatase in the collecting

ducts in birds presently studied. The Rosy Pastor shows a change in its dietary preference from a mixed diet during postmigratory period to a diet of fruits and seeds during premigratory period. However, Wagtails subsist on more or less same diet (insects and other animal matters-rich in lipids and proteins) all through out. The study on the activity of acid phosphatase in the kidney of these birds shows that the enzyme activity rises in both the cases during premigratory period, but its rise in the Wagtail is noticeably higher than what is seen in Rosy Pastor. Such a difference can be correlated with dietary differences and consequent removal of nitrogenous wastes. Thus, quantitatively observed increase in the activities of both the phosphatases in the kidney of Rosy Pastor and Wagtail can be considered to be adaptive changes in the renal tissues during pre and postmigratory periods. Changes observed in the activities of both the phosphatases, on the basis of quatitative analysis, during pre and postmigratory periods, in the liver and kidney of Wagtail and Rosy Pastor are in agreement with the histochemical observations made herein.