SUMMARY

CHAPTER 1:

The nonspecific (acid and alkaline) phosphatases were histochemically and quantitatively investigated in the alimentary tract of two migratory birds (Sturnus roseus and Motacilla alba) during their post and premigratory periods.

The acid phosphatase was found to be localized in the tubules and glands of proventriculus and gizzard, and in the brush borders, epithelial cells of the villi and glands of the small intestine in both the birds. This phosphatase was found to increase in concentration during the premigratory period in all the parts of the intestine in both the birds.

The alkaline phosphatase was found in the tubules of proventriculus and gizzard, and brush border, lamina propria and absorptive cells of the villi in the small intestine of both the birds. During premigratory period not only there was an increase in the concentration of alkaline phosphatase in all these parts of the intestine in both the migratory birds but even the glands of the proventriculus and gizzard which were negative to this enzyme reactivity during postmigratory period showed appreciable reactivity. The intestinal glands in Wagtail which were negative to enzyme

reaction also revealed intense alkaline phosphatase activity and in Rosy Pastor the activity got enhanced during the same period.

The increase in the acid phosphatase activity in the intestine of both the birds during premigratory period is correlated with the increased protein synthesis and secretory activities, while the increase in alkaline phosphatase activity is associated with the increased absorptive activity. Both these changes are concomitant with the development of hyperphagia in the birds during premigratory period. The difference in the degree of increase in both the phosphatases between the two migratory birds are correlated with the differences in their diets.

CHAPTER 2:

Isoenzymes of alkaline phosphatase in the intestine of a migratory bird, Wagtail (Motacilla alba) were studied histochemically using organ specific inhibitors, L-phenylalanine and Zn^{++}($Zncl_2$). L-phenylalanine inhibits intestinal type of alkaline phosphatase, while Zn^{++} inhibits liver type of alkaline phosphatase. Intestinal type of alkaline phosphatase (L-phenylalanine sensitive) was found to be present in the brush border, epithelial cells of the

intestinal villi and in the intestinal glands. Liver type of alkaline phosphatase (Zn^{++} sensitive) was found to be distributed in lamina propria. Liver type of alkaline phosphatase in the intestine of Wagtail (which feeds on lipid rich diet, insects) seems to be associated with lipid absorption.

CHAPTER 3:

The alkaline and acid phosphatases were histochemically and quantitatively studied in the liver and kidney of migratory starling (Sturnus roseus) and Wagtail (Motacilla alba) during post and premigratory periods. In the liver, alkaline phosphatase was localized more in the sinusoidal linings of the liver, while acid phosphatase was found to be more in the parenchymal cells in both the birds. The alkaline phosphatase activity in the liver of S. roseus did not increase in the premigratory period but that of Wagtail liver showed a significant rise. The activity of acid phosphatase in the liver of both the birds exhibited significant increase in the premigratory period.

The alkaline phosphatase in the kidney of both the birds was found to be localized in the luminal border of the convoluted tubules but medullary zone gave negative results.

During the premigratory period alkaline phosphatase activity showed a significant increase in both the regions of tubules. The acid phosphatase was observed in the glomeruli, cells of the convoluted tubules as well as in the medullary zone in the kidney of both the birds. During their premigratory period, acid phosphatase activity showed a slight increase in Rosy Pastor and significant rise in the kidney of Wagtail.

The significance of the increase in the concentrations of these two nonspecific phosphatases in the liver is correlated to the increased uptake of metabolites and lipid synthesis. In the kidney, these enzymes are assumed to be participating in the reabsorption of metabolites as well as mucin synthesis. The difference in the degree of elicitation of activities of these two enzymes in the liver and kidney of the two migratory birds are perhaps due to variations in the diets of these birds.

CHAPTER 4:

The enzymes, G-6-PDH and "Malic" enzyme were quantitatively determined in the liver and adipose tissue of migratory starlings (Sturnus roseus) and Wagtails (Motacilla alba). These enzymes are concerned with lipogenesis by providing cytoplasmic NADPH, which in turn acts as the

hydrogen donor during lipid synthesis. During premigratory period, both the enzymes were found to show elevated levels in the liver of both the birds. The adipose tissue did not show the presence of measurable quantity of "Malic" enzyme, however, G-6-PDH increased in this tissue. It is concluded that in these migratory birds, the liver is the major site for lipogenesis.

It is usually known that these two enzymes show fluctuating levels of activities according to the diet consumed by the birds. A carbohydrate rich food normally elevates the enzymes activities, while fat and/or protein rich diet depresses the activities. Since the migratory starling (S. roseus) shifts the dietary preference to a carbohydrate rich fruits during premigratory period, instead of usual diet consisting of insects and seeds, the hyperlipogenesis is easily manifested with the help of the enzymes investigated. However, the Wagtails shows no such dietary changes and continues to eat insects and grubs all through out. It is suggested that elevated levels of these two enzymes in the liver of this bird might be maintained with the help of hormones, viz., thyroxine and insulin wich are known to have stimulatory effect on the activities of these enzymes.

CHAPTER 5:

In order to understand the oxidative metabolism in the tissues of migratory birds during premigratory period, the key enzymes such as SDH and ATPase were quantitatively estimated in the liver and kidney of migratory starling (Sturnus roseus) and Wagtail (Motacilla alba).

It was observed that SDH and ATPase in both the liver and kidney showed considerable increase in the levels of their activities during premigratory period in both these birds.

It is concluded that oxidative metabolism was maintained at a higher rate during premigratory period to cope up with tremendous increase in the functions of both the liver and kidney.

CHAPTER 6:

The composition of liver and adipose tissue lipids were determined during post and premigratory periods of the two migratory birds, Sturnus roseus and Motacilla alba.

It was seen that, inspite of a tremendous increase in the total lipid content, the monoglycerides, cholesterol

ester and free fatty acids per 100 mg lipid did not vary from post to premigratory periods. However, their concentrations increased per total liver. The phospholipid fraction (as percentage of lipids) in the liver of both the birds showed a reduction during premigratory period. The percentage of free cholesterol, diglycerides and triglycerides increased during premigratory period and the total concentration of these per total liver increased tremendously.

It is concluded that the rate of fatty acids synthesis and their esterification in the liver gets stepped up during premigratory period. But perhaps the rate of transportation of so synthesized lipids from liver to adipose tissue may be lagging behind resulting in an increased lipid contents of the liver during premigratory period.

CHAPTER 7:

It is believed that the adaptive hyperlipogenesis in the migratory birds is concomitant with increased hepatic cell number and size and elevation of their functional potentialities. Hyperplasia and hypertrophy of liver cells in the two migratory birds, Rosy Pastors and

Wagtails, were determined from the data on nucleic acids and protein levels in the liver as well as its histological observations.

The increase in the nucleic acids content and total protein in the liver along with its histological observations clearly indicate that the hyperplasia and hypertrophy take place in the liver of these two migratory birds during their premigratory period. These adaptive features enable the liver to meet the increased demand to deal with greater influx of metabolites as well as the synthesis of lipids during premigratory period.

CHAPTER 8:

Arginase was quantitatively determined in the liver and kidney of the two migratory birds, *viz.*, *Sturnus roseus* and *Motacilla alba* during their post and premigratory periods. Eventhough, all the enzymes of the ornithine cycle are not present in the avian tissues, arginase was found to be active in the liver and kidney of both the birds. During premigratory period the liver and kidney of Rosy Pastor (*S. roseus*) were found to have low arginase activity, while during the same period its activity increased

considerably in the kidney and slightly in the liver of Wagtails (M. alba). Significance of these changes is correlated with the dietary habits of the birds studied. Rosy Pastor consumes mainly carbohydrate rich food during premigratory period while Wagtail consumes protein and fat rich insect food. It is suggested that arginase converts the dietary arginine into products that ultimately can be converted into Krebs cycle intermediates which in turn may be utilized for gluconeogenesis and/or lipogenesis.

CHAPTER 9:

The adaptive hyperlipogenesis in the migratory birds is as a result of adjustments in the intermediary metabolism with the help of hormones. Other physiological changes in the migratory birds such as hyperphagia, hyperplasia and/or hypertrophy of tissues and the dietary preferences of the birds also lead to the enhanced lipid synthesis and its deposition. These changes are also under the influence of hormones.

The thyroid hormone is believed to play an important role in the hyperlipogenesis and as such the thyroid is found to become active in the premigratory period of Sturnus roseus. Thyroidectomy performed in Rosy Pastor during the

premigratory period resulted in a decreased food intake as well as decreased fat deposition in these birds. Concomitantly, activity of alkaline phosphatase in the small intestine, G-6-PDH and "Malic" enzyme in the liver and that of SDH in the liver and muscle were also found to be significantly lowered in the thyroidectomized birds. However, total lipid content of the liver was found to be same in thyroidectomized and control birds. This anomaly is explained in the text. In contrast, pectoralis major muscle of thyroidectomized birds had very low fat content.

From the data obtained, it is concluded that thyroid hormone assists this migratory bird in the development of hyperphagia; activation of alkaline phosphatase in the intestine for the increased absorption of the digested food; the activation of NADPH generating enzymes in the liver; in the maintenance of a high rate of oxidative metabolism in the tissues and an increased mobilization of lipids from the liver to other tissues.

ADAPTIVE PHYSIOLOGICAL AND BIOCHEMICAL CHANGES UNDERLYING THE HYPERLIPOGENESIS IN MIGRATORY BIRDS: A GENERAL CONSIDERATION

Physiology of bird migration is still a baffling topic to the initiated and uninitiated ornithologists alike, although curious intelligentia have been mounting multifarious assaults on the problem from the time immemorial. The early trickle of informations regarding several aspects of physiology of migration has grown steadily into a swollen torrent, to include a review of all these informations will be beyond the scope of the general consideration of presently investigated aspects of physiological and biochemical adaptations leading to premigratory hyperlipogenesis. However, a constant recourse of the information regarding the activities of hypothalamo-hypophysial system and other endocrine glands is necessary because they are integral components of the regulatory mechanism responsible for the manifestation of premigratory preparations. There is a general agreement that adaptive hyperphagia is the principal factor for hyperlipogenesis during the premigratory period. Hypothalamus gets stimulated in response to the changes in environmental factors. The neurosecretory centre in the hypothalamus induces changes in the activities of other endocrine glands, while the

"feeding centre" which is situated in the lateral hypothalamic area stimulates hyperphagia (hunger feeling and associated increased food intake). Increased food intake in rats has been noticed when its lateral hypothalamus was electrically stimulated (Delgado and Anand, 1953; Anand and Dua, 1955). In the migratory birds also the hypothalamus (ventromedial area) is implicated in regulation or control of premigratory hyperphagia (Kuenzel and Helms, 1967). The state of hyperphagia combined with the increased daylight period results in a tremendous influx of metabolites. Meanwhile it has been already established that the hormones from hypophysis and other endocrine glands influence number of adaptive biochemical and physiological changes in the tissues to deal with extra amount of so gathered metabolites. The studies on hypothalamo-hypophysial system of Rosy Pastor (George and Naik, 1965; Naik and George, 1966) and Wagtail (John and George, 1967b) revealed that this system, in both the birds, is highly active during premigratory period. The pituitary gland in these birds exhibited higher capacity to produce prolactin, ACTH, TSH and FSH etc., as evident from the general increase in number and activity of the cells concerned (Naik and George, 1965, John, 1967). During the premigratory period, an increased thyroidal (John and George, 1967a; Pilo and George, 1970) and adrenal (Naik and George,

1963; John, 1966) activities were also noticed. The effect of higher release of metabolic hormones such as thyroxine, corticosteroids, STH and prolactin induces changes in the machinery of organs and tissues to bring about increased uptake of metabolites (mostly carbohydrates and proteins) and their conversion into storable products (esterified fat).

Presently, it has been demonstrated that thyroid gland activity is well involved in the expression of certain changes in the intestinal functions (Chapter 9). From the observations on the changes in the activities of acid and alkaline phosphatases in the intestine it is possible to believe that the efficiency of digestion and absorption increase during premigratory period. It is also evident that alimentary canal of these two birds with different dietary habits during premigratory periods have different basic physiological adaptations to deal with the specific type of food ingested by them (Chapters 1 and 2). On the basis of these findings it could be suggested that along with hyperphagia certain events occur in gastrointestinal tract, ultimately presenting a challenge to the internal organs like liver with an overload of metabolites.

It is possible that due to the elevated levels of metabolites in the blood during the postabsorptive period; glucose and amino acids may get filtered through glomeruli

into the kidney tubules. These, if not reabsorbed effectively would get excreted out and be wasted. Therefore, logically; to prevent such a loss, tubular reabsorption has to be stepped up. In fact increased alkaline phosphatase activity in the kidney of both the migratory birds, which was histochemically found to be localized mainly in the proximal and distal convoluted tubules (Chapter 3), is indicative of the enhancement of renal reabsorption to conserve the useful constituents. Besides, increased metabolic activities found in the kidney of both the birds; judged from the enzyme study made thereon; is surmised to provide necessary energy for the required enhanced renal functions (Chapter 5).

In addition to this increased reabsorption, at least in the insectivore bird, like Wagtail; gluconeogenesis in its kidney is likely to be activated. This presumption, which ofcourse needs to be substantiated, has its roots in the findings that during premigratory period when Wagtail is confronted with large amount of dietary proteins, arginase activity is noticed to be increased in its kidney (Chapter 8). In the absence of the complete ornithine cycle in birds the activity of this enzyme (which catalyses the Ist step of the ornithine cycle) leads to the formation of glutamate which could be utilized for gluconeogenesis.

The hyperphagia, and the resultant increase in the digestion and absorption of carbohydrates and amino acids would be expected to trigger increased insulin release from the β-cells. The result would be a rise in the concentration of insulin in blood which has generally anabolic consequences. The study of George and Naik (1964b) suggested a possibility of increased insulin production in pancreatic islets of Rosy Pastor during their premigratory period. Such an increased level of insulin when the level of metabolites (glucose and amino acids) is known to be high, would help to present increased amount of substrate to the interior of lipogenic cells (hepatic cells). The adaptive changes in the levels of nonspecific phosphatases (acid and alkaline) in liver also aid in increasing the influx of the substrates (Chapter 3). Such increased availability of the intracellular substrates as well as certain hormonal factors would activate specific intermediary metabolic pathways to intensify lipogenesis. The association between high rate of lipogenesis and the increase in activities of G-6-PDH and "Malic" enzyme is known. The significant increase in the activities of G-6-PDH and "Malic" enzyme observed in the liver of both the birds during their premigratory period (Chapter 4) would provide more NADPH required for fatty acid synthesis. The higher degree of "Malic" enzyme activity as compared to that of G-6-PDH suggests that

"Malic" enzyme catalyzed reaction may be a more important component of NADPH generating system or the enzyme is more sensitive to certain hormones. Besides, as suggested by Lardy et al. (1964) "Malic" enzyme can perform a very important function of reformation of pyruvate and hence can continuously generate acetyl CoA precursors. Equally interesting aspect of the present findings is that the liver is a major site of fatty acid synthesis and the activities of NADP dependent enzymes increased concomitantly with the hyperlipogenesis in both the migratory birds, eventhough each have a different dietary habit. Increased functioning of NADP dependent enzymes in liver of Rosy Pastor could be explained on the basis of the fact that its diet during the premigratory period consists of mainly fruits which are rich in carbohydrates; and carbohydrate rich diet is known to stimulate lipid synthesis as well as increase in activities of these enzymes (Fitch and Chaikoff, 1960). Wagtail on the other hand feeds on a diet rich in protein and lipids during both the pre and postmigratory periods, but protein rich diet at least in this case does not inhibit lipogenesis which otherwise is noticed in case of chick (Yeh and Leveille, 1969). An explanation given for the inhibition of lipogenesis by protein rich diet is based on the fact that conversion of amino acids to pyruvate via transamination reactions results

in the oxidation of redox potential in cytoplasm i.e. NADH/NAD ratio decreases. This in turn interferes with the formation of NADPH required for fatty acid synthesis (Yeh and Leveille, 1969). They have further demonstrated a decreased "Malic " enzyme activity in the liver of chick fed on protein rich diet. Contrary to this, we observed increased activities of NADP dependent enzymes ("Malic" enzyme and G-6-PDH) and lipogenesis in the liver of Wagtail during its premigratory period. Thus in the liver of Wagtail wherein the major source of substrate for lipogenesis is mainly amino acids, the intermediary pathways leading to premigratory hyperlipogenesis would differ from those operating in the liver of Rosy Pastor. With these facts at hand a question naturally arises as to how metabolic pathways in the liver of Wagtail are oriented to bring about hyperlipogenesis in spite of high protein intake. Unlike the mechanism observed in chick fed on protein rich diet (wherein pyruvate concentration increased due to protein rich diet), the situation in Wagtail could be leading to metabolic desposition of pyruvate in such a way that it could serve a double purpose viz., (1) remove the possibility of condition leading to oxidation of redox potential in cytoplasm by increasing lactate/pyruvate ratio, (2) to provide precursor i.e. acetyl CoA, for fatty acid synthesis. A flow of pyruvate metabolism to fatty acid synthesis and TCA cycle can be

accelerated by insulin (Jungas, 1970). Further, Freedland et al. (1968) reported that on injection of thyroxine into rat fed on protein rich diet, "Malic" enzyme activity in its liver increases. In light of these observations it is obvious that dietary effect could be modified by hormones. Since thyroid activity in both the birds has been known to increase during premigratory period (John and George, 1967a; Pilo and George, 1970) and also increased insulin production is expected, it could be suggested that these hormones may be modifying the inhibitory effect of protein rich diet on lipogenesis in Wagtail. Further research is in progress to elucidate the regulatory processes. However, present studies on the thyroidectomized birds proved beyond doubt that by influencing the activities of G-6-PDH and "Malic" enzyme and energy yielding processes; the thyroid is well involved in inducing the premigratory preparations (hyperlipogenesis) in the migratory birds (Chapter 9).

Premigratory adaptive hyperlipogenesis, in the migratory birds would not be accomplished without optimum provision for energy (ATP). In the liver of migratory birds studied; mitochondrial activity was found to be enhanced (Chapter 5) which would result in production of increased amount of energy to meet the demands for fatty acid synthesis and their esterification.

The newly synthesized fatty acids in the liver undergo esterification quickly as denoted by the increased concentration of diglycerides and triglycerides in the liver of both the migratory birds during their premigratory period (Chapter 6). Such speedy esterification of free fatty acids would prevent feed back inhibition by free fatty acids on lipid synthesis. The major part of the fat produced in the liver, is transported to adipose tissue resulting in the increased deposition of fat in the adipocytes. To confirm this, the size of adipocytes were observed which increased during the premigratory period. However, some amount of lipid in the form of triglycerides accumulated in the liver too, resulting in the increased liver lipid content. Some other physiological changes such as reduction in liver water content and changes in ionic balance (Na^+, K^+) occuring during premigratory period as reported by Pilo (1967) make adjustments for the increase lipid deposition in the hepatocytes.

Lipids in blood are usually in the forms of phospholipids, cholesterol and neutral fat, which are in complex state of lipoproteins. The reduction in the level of phospholipid in liver (Chapter 6) during the hyperlipogenesis (premigratory period) is suggestive of higher rate of removal

of this lipid together with neutral lipid in the form of lipoproteins complexes and are carried through blood to the site of deposition. For such lipoprotein synthesis choline is essential, which can be made available by releasing it from choline-esters by the activities of the specific esterases. Pilo (1969) reported increased activities of cholinesterases in the liver of migratory birds which easily substantiates the suggestion made herein. Thus mechanism responsible for the transport of lipid in the form of lipoproteins from the site of synthesis (liver) to place of deposition (adipose tissue) is also active during premigratory period of these birds.

Evidently, the liver of migratory birds which presents physiological adaptive changes also shows hypertrophy and hyperplasia of the hepatic cells during premigration period (Chapter 7). Such anatomical changes would further aid in enhanced lipogenic and lipid storing capacities of the organ.

From the present study on the liver, alimentary canal, kidney and adipose tissue of the two migratory birds during their pre and postmigratory periods, the major physiological adaptive feature that emerges is the hyperlipogenesis during premigratory period. For this, the factors involved themselves are also adaptive changes occuring in the birds for the specific purpose. The factors are:

(1) Increased source of precursors and energy for hyperlipogenesis made available by hyperphagia and increased efficiency of digestion and absorption. (2) Adaptive changes in the intermediary metabolism in the liver in accordance with dietary differences leading to hyperlipogenesis. (3) Hypertrophy and hyperplasia of hepatic cells which help in hyperlipogenesis. (4) Effective packing and removal of lipids synthesized in the liver to the depot sites and (5) The involvement of the hormones in such regulatory mechanism. By emphasizing this, one should not minimize the possible importance of other adaptive changes which have not been elucidated in the present study on the migratory birds.