CHAPTER 7

HYPERPLASIA AND HYPERTROPHY OF HEPATIC CELLS OF MIGRATORY STARLING (STURNUS ROSEUS) AND WAGTAIL (MOTACILLA ALBA) DURING ADAPTIVE HYPERLIPOGENESIS IN THE PREMIGRATORY PERIOD

Hyperlipogenesis becomes an important function of liver of the migratory birds during their premigratory period. The adaptive hyperlipogenesis is associated with changes in the metabolic machinery of the hepatic cells and these changes probably involve the synthesis of number of enzyme proteins. During premigratory period, the liver cells of the migratory birds could be presumed to undergo hypertrophy and/or hyperplasia to cope up with the enhanced functional activities of the liver.

It was therefore, thought necessary to ascertain whether hypertrophy and/or hyperplasia of the liver cells of Rosy Pastor and Wagtail occurs or not during their premigratory period. For this purpose protein and nucleic acids content of the liver of these two migratory birds were estimated during their post and premigratory periods.

MATERIALS AND METHODS

Rosy Pastors and Wagtails during their post and premigratory periods were shot and immediately brought to the laboratory. Liver from each bird was excised and total liver weight was recorded. A piece of liver was removed, weighed and homogenized in cold distilled water. The homogenate was used for the estimations of nucleic acids and protein. A piece of the liver was fixed in Bouin's fluid and processed for routine histological study. 6 μ thick sections were cut and stained with Haematoxylin-eosin for histological observations.

Nucleic acids:

Method described by Schneider (1957) was employed for extraction and estimation of nucleic acids. Nucleic acids were extracted from an aliquot of liver homogenate, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) contents were estimated using diphenylamine reaction for DNA (Dische, 1930) and orcinol reaction for RNA (Mejbaum, 1939). Results are expressed as mg nucleic acid (DNA) or (RNA)/g liver and also as mg nucleic acid (DNA) or (RNA)/liver (total weight).

Protein:

Protein was precipitated by adding cold 10% trichloroacetic acid to an aliquot of liver homogenate. After centrifugation the precipitates were collected and dissolved in 1N sodium hydroxide and protein content was estimated according to the method of Lowry et al. (1951) using Spectronic 20 colorimeter. Protein values are expressed as mg protein/100 mg liver weight as well as mg protein/total liver.

RESULTS AND DISCUSSION

Histological observations of the liver of Rosy Pastor (Figs. 1 and 2) and Wagtail (Figs. 3 and 4) clearly indicated that during the premigratory period the volume of hepatic cells increased greatly indicating the occurrence of hepatic cell hypertrophy.

In the histological preparations of the liver of Rosy Pastor and Wagtail, during their premigratory period, the places where fat was accumulated appeared as vacuoles. Such vacuoles in the case of Rosy Pastor were larger compared to those in Wagtail. From these observations it could be surmised that liver steatosis was more pronounced

in Rosy Pastor compared to Wagtail. This fact finds support from the observation reported earlier that triglyceride content (%) in Rosy Pastor liver is higher than that in Wagtail (Chapter 6).

In both the birds during their premigratory period, some of the hepatic cells were found to be binucleated and some appeared to have been divided. Such features gave an indication of hyperplasia occuring in the liver of both the migratory birds during their premigratory period. Thus the increased liver weight recorded during the premigratory period could be due to both hypertrophy as well as hyperplasia of the hepatic cells.

Total DNA content of the liver is considered as a measure of the number of cells in the case of rats (Thomson et al., 1953). When the present observations were analyzed applying this criterion, it was found that the considerable increase in DNA content of the liver of Rosy Pastor and Wagtail during the premigratory period (Table 1a & b, Fig. 5) was due to the hyperplasia of hepatic cells.

Paradoxically, during the premigratory period, concentration of protein (%) in the liver of Rosy Pastor decreased while the same was found to be elevated in Wagtail.

TABLE 1 a

Nucleic acids in the liver of Wagtail. Mean value ± S.D.

Month	DNA mg/ gm liver	DNA mg/ liver	RNA mg/ gm liver	RNA mg/ liver	RNA/DNA
November	2.901 ±0.252	1.887 ±0.012	11.615 ± 0.671	7.607 ±1.147	4.028 ±0.582
December	2.743 ±0.487	1.831 ±0.210	10.267 ± 0.855	6.874 ±0.135	4.632 ±1.571
January	2.240 ±0.170	1.666 ±0.285	10.112 ± 1.595	7.347 ±0.588	4.506 ±1.124
February	3.086 ±0.415	2.520 ±0.344	16.182 ± 1.849	13.208 ±1.476	5.331 ±1.315
March	3.226* ±0.193	3.054* ±0.286	17.318* ± 1.195	16.401* ±1.157	5.367* ±0.198
*Significant at the level	NS	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

*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 1b

Nucleic acids in the liver of Rosy Pastor. Mean value ± S.D.

Month	DNA mg/ gm liver	DNA mg/ liver	RNA mg/ gm liver	RNA mg/ liver	RNA/DNA
October	1.554 ±0.062	3.828 ±0.167	14.048 ± 0.172	33.951 ± 0.569	8.691 ±0.157
April	1.315* ±0.004	4.985* ±0.077	13.610* ± 0.747	51.585* ± 3.457	10.344* ±0.532
*Significant at the level	NS	$P<0.005$	NS	$P<0.02$	$P<0.05$

*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

Protein content of the liver of Wagtail and Rosy Pastor. Mean value ± S.D.

Month	Wagtail		Rosy Pastor	
	mg protein/ 100mg wet liver	mg protein/ liver	mg protein/ 100mg wet liver	mg protein/ liver
October	---	---	22.03 ±0.18	565.14 ±43.42
November	22.20 ±1.13	120.93 ±14.82	---	---
December	23.30 ±0.71	134.95 ±14.94	---	---
January	22.27 ±1.64	163.80 ± 8.06	---	---
February	22.77 ±0.52	177.15 ±11.90	---	---
March	24.19* ±0.74	218.31* ±17.50	---	---
April	---	---	20.59* ±0.78	774.03* ±54.11
*Significant at the level	P<0.05	P<0.002	P<0.05	P<0.01

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

EXPLANATIONS FOR FIGURES

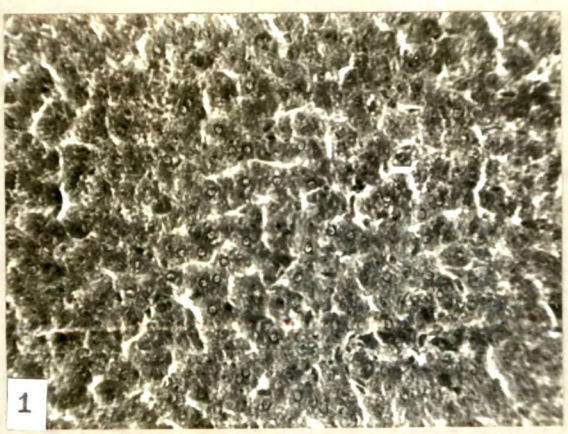
Figs. 1-4. Photomicrographs of liver sections stained with haematoxylin-eosin during postmigratory (POM) and premigratory (PM) periods of Rosy Pastor and Wagtail. Each one is of 200 X magnification.

Fig. 1. Section of Rosy Pastor liver (POM).

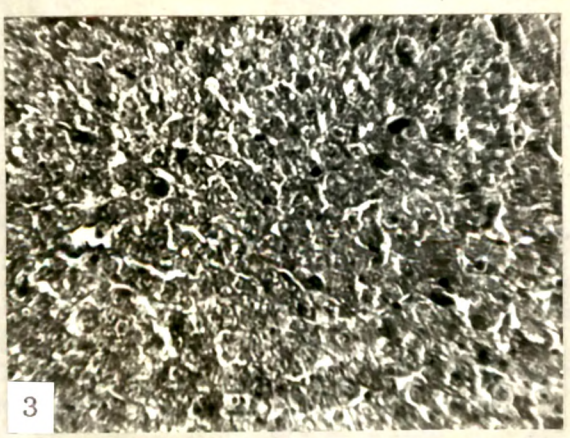
Fig. 2. Section of Rosy Pastor liver (PM).

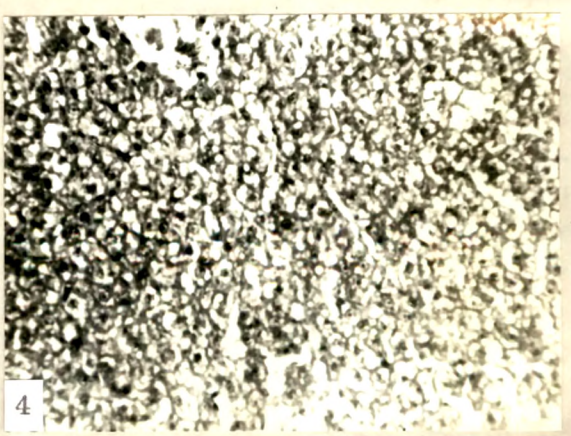
Fig. 3. Section of Wagtail liver (POM).

Fig. 4. Section of Wagtail liver (PM).


1


2


3


4

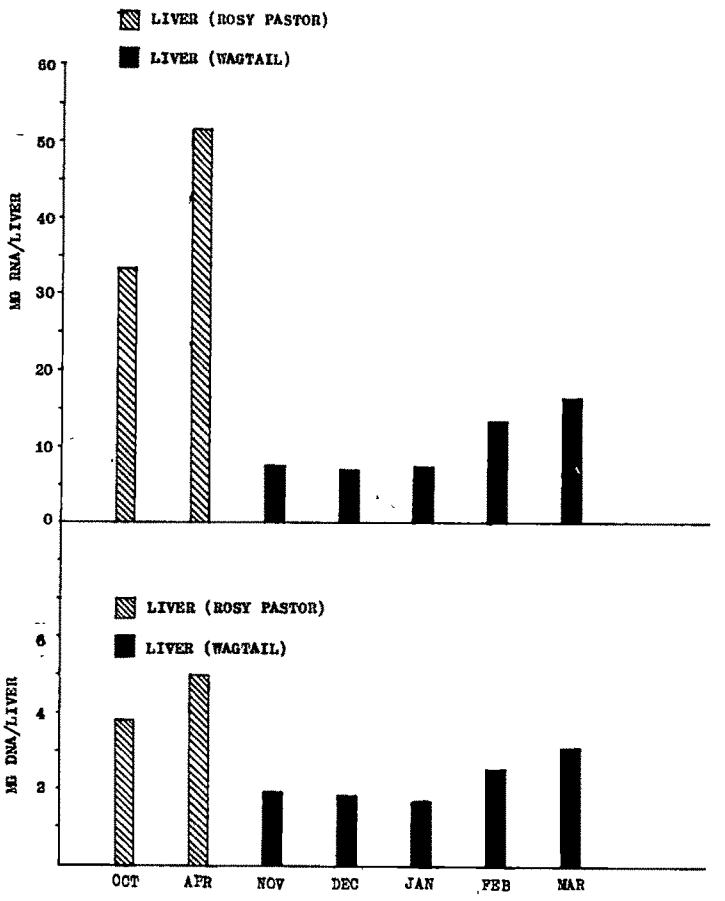


FIG. 5. NUCLEIC ACIDS IN THE LIVER OF ROSY PASTOR AND WAGTAIL. (RNA:UPPER; DNA:LOWER).

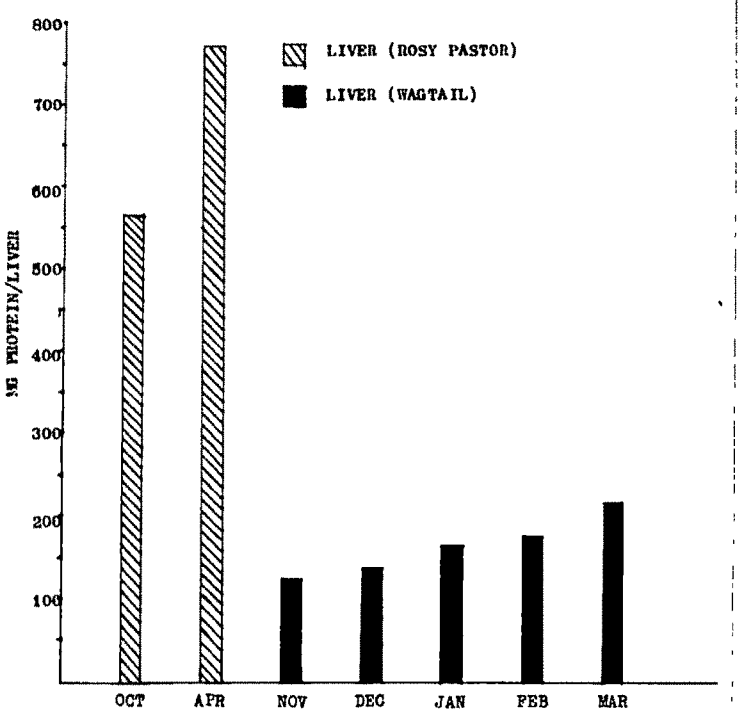


FIG. 6 LIVER PROTEIN IN ROSY PASTOR AND WAGTAIL EXPRESSED AS MG PROTEIN/LIVER

However, total liver protein content in both the birds, increased significantly during the premigratory period (Table 2 and Fig. 6). Taking this fact into consideration, it could be suggested that decrease in the concentration of liver protein noticed in Rosy Pastor was not due to actual decrease in the level of protein but was as a result of increase in the liver weight due to accumulation of lipids and hence the protein percentage appeared to be low. The increase in protein per total liver in both the birds, during the premigratory period suggests that accelerated protein synthesis in the liver of both the birds does occur. It was observed that the total amount of RNA (mg RNA/total liver) significantly increased during premigratory period in the liver of both the birds (Table 1a & b, Fig. 5). Elevations in RNA/DNA ratios, in the liver of both the migratory birds during their premigratory period, would support higher rate of protein synthesis during the same period. The protein synthesised may be the enzyme proteins (as concentrations of several enzymes have been observed to increase during this period); besides, it could be the structural proteins (as hypertrophy and hyperplasia occur during this period) and also it could be the protein moiety of the lipoproteins.

Thus the changes in nucleic acids and protein content are positively correlated with hypertrophy and hyperplasia of the liver of both the birds during their premigratory period. Obviously the physiological changes leading to hyperlipogenesis in the migratory birds seem to be associated with hypertrophy and hyperplasia of their liver. Similarly, flight muscles of migratory birds are also known to show morphophysiological changes such as hypertrophy during their premigratory period (Fry et al., 1972). Some supports to the present observation could also be had from the reports of Blum et al. (1968) and Leclercq et al. (1968) who have shown that the hepatic hyperlipogenesis that manifests in the liver of force-fed goose is associated with an activation in enzyme activity (G-6-PDH) and increase in its RNA content.

The increased protein and nucleic acids levels in the liver of both Rosy Pastor and Wagtail could be due to their increased synthesis under the influence of several hormones. Feigelson et al. (1962) reported that a single, low dose of cortisone acetate exerts anabolic influence on nucleic acids and protein of both normal and regenerating liver. Wyatt and Tata (1968) using hybridization studies in many species have suggested that

thyroid hormone plays a role in stimulating the synthesis of ribosomal RNA. It is also reported that thyroxine stimulates protein synthesis (Tata, 1974). Previous studies carried out in this laboratory on both these migratory birds, have pointed out that adrenocortical and thyroid activities in Rosy Pastor (Naik and George, 1963; George and Naik, 1964a) and Wagtail (John, 1966; John and George, 1967a) increase during the premigratory period. On the basis of these facts and the present study, it is tempting to suggest that the hormones like adrenal corticoids and thyroid hormone influence the increased synthesis of the nucleic acids and protein in the liver of Rosy Pastor and Wagtail during their premigratory period.