CHAPTER 6

COMPOSITION OF HEPATIC LIPIDS DURING POST AND PREMIGRATORY PERIODS OF THE MIGRATORY STARLING (*STURNUS ROSEUS*) AND WAGTAIL (*MOTACILLA ALBA*)

One of the most widely studied aspects of bird migration has been the premigratory fat deposition. Investigations on flight muscles of migratory birds have clearly shown that deposition of lipid in discrete fat depots form the major source of utilizable energy for prolonged flights during migration (George and Berger, 1966). Along with the greater deposition of fat in the body; an increased lipid level has also been reported in the liver and muscle of the migratory birds (Odum and Perkinson, 1951; King *et al.*, 1963). Increased lipid levels in the liver of Rosy Pastor and Wagtail during premigratory period have been reported by Pilo (1967) and John and George (1966) respectively.

Under various pathological and experimental conditions, accumulation of fat in the liver leading to the development of fatty liver has been noticed. Diet deficient in certain amino acids causes fatty liver

(Niño-Herreratt et al., 1954). Meyer and Hartroft (1960) reported that rats which are polyphagic due to hypothalamic lesions develop fatty liver. Some drug poisoning is also known to cause fatty liver (Leduc and Wilson, 1958). Chickens with hyperphagia due to hypothalamic lesions (Snapir et al., 1969), and force-fed chickens (Lepkovsky and Furuta, 1971) develop fatty liver eventhough they are fed on balanced diet. Similarly chickens force-fed maize diet also developed fatty liver (Leclercq et al., 1974). Dietary doses of carbon tetrachloride in chick alter the liver lipid metabolism, resulting in the accumulation of fat in the organ (Whitehead et al., 1974). Reduced lipoprotein synthesis and altered phospholipid metabolism are also considered to be responsible for development of fatty liver. It is well known that rat fed on choline deficient diet develops fatty liver due to accumulation of triglycerides which is in turn due to hindrance in release of lipids into the systemic circulation. Fatty liver formation in force-fed chicken represents altogether a different situation, wherein lipoprotein synthesis is not impaired but exhibits accumulation of large quantity of triglycerides in the liver which is mainly due to imbalanced lipid synthesis and its removal from the organ (Leclercq et al., 1974).

From the foregoing account one realizes that fatty liver formation is generally a pathological state of the organ. However, in the migratory birds, fatty liver formation is a periodical and a nonpathological phenomenon. No report on details of the types of liver lipids and changes that occur in hepatic lipid composition during premigratory fattening of migratory birds is available.

In the present study analysis of liver lipid is reported and an attempt has been made to explain premigratory lipid accumulation in the liver and adipose tissue of the two migratory birds viz., Rosy Pastor (Sturnus roseus) and Wagtail (Motacilla alba).

MATERIALS AND METHODS

Liver and visceral adipose tissue were obtained from freshly procured Rosy Pastors and Wagtails during their post and premigratory periods. Total liver and a piece of adipose tissue were weighed immediately and a piece of adipose tissue was fixed in Bouin's fluid for histological study.

Liver lipids:

A piece from the previously weighed entire liver was weighed and subjected to lipid extraction employing the method of Folch et al. (1957). Lipid content is expressed as mg/100 mg liver wet weight: and also in terms of mg. lipid/total liver.

Liver lipid analysis:

Liver lipid fractions were separated by thin-layer chromatography. Glass plates (20 x 20 cms size) were layered with Silica gel G (500 μ thick) and activated at 110°C for one hour (Stahl, 1965). The plates were predeveloped overnight in ether, and after drying each plate was marked in six (3 cms) lanes and reactivated. Known quantity of the liver lipid extracts were spotted in four lanes and a mixture of authentic standards in the 5th lane. Only solvent was spotted in the remaining lane and was considered as blank. The plates were then developed unidirectionally using two solvent systems of Freeman and West (1966). The solvent front of the first system was allowed to migrate up to 13 cms from the line of origin. After this, the plate was air dried and developed in the

second solvent system. The second solvent was allowed to migrate up to 17 cms from the line of origin. After development in second solvent system, the plates were dried in an oven for 30 minutes at 60°C. Then the plates were removed from the oven and the spots were visualized by exposing the plates to iodine vapour in a closed glass chamber. The spots of different lipid fractions from sample, standard and corresponding areas of Silica gel from the blank were scrapped and transferred to separate test tubes. These were processed further for the quantitative estimations employing the method described by Marzo et al. (1971).

Concentrations of different lipid fractions are expressed as percentage of lipid analyzed (mg/100 mg lipid), as percentage of liver fresh weight (mg/100 mg liver) and also as mg/total liver.

Adipose tissue:

Previously weighed adipose tissue was dried in an air oven (maintained at 80°C) to a constant weight and total lipids were extracted with chloroform:methanol (2:1 v/v). Lipid content is expressed as percentage of adipose tissue dry weight.

Bouin's fixed adipose tissue was dehydrated, embedded in paraffin wax and 6 μ thick sections were cut; Sections were stained with Haematoxylin-eosin. Considering the adipocytes to be almost spherical, diameter was measured with the help of occular eye piece fitted on a microscope. Ten different fields were selected in each section for the measurement of diameter. Mean values were used to calculate the volume of the adipocytes.

RESULTS

During premigratory period, as compared to postmigratory one, weight of the liver and its total lipid content increased significantly in the case of both the migratory birds studied (Table 1a & b). In both the birds, lipid content when expressed as a percentage of the liver fresh weight, was also significantly increased during the premigratory period (Table 1a & b).

Analyses of the liver lipids in both the birds indicated that concentrations of monoglycerides, cholesterol ester and free fatty acid fractions, expressed as percentage of lipids, did not change significantly from postmigratory period to premigratory one (Table 1a & b). However, the concentration of monoglycerides, cholesterol

TABLE 1a

Changes in liver weight, liver lipid content and liver lipid composition in Wagtail. Lipid fractions expressed as % of lipid. Mean value ± S.D.

Month	Total liver weight gm	Lipid mg/100mg wet liver	Lipid mg/liver	PH	MG	FFA	CH	DG	TG	CHE
October	0.5493 ±0.060	9.15 ±0.34	50.22 ±5.07	52.70 ±2.38	1.49 ±0.46	2.92 ±0.40	4.49 ±0.52	1.57 ±0.17	30.03 ±2.09	4.42 ±0.65
November	0.5240 ±0.100	9.56 ±0.45	50.12 ±10.5	50.90 ± 1.94	1.31 ±0.35	2.48 ±0.48	5.14 ±0.39	1.12 ±0.29	33.20 ±1.33	4.38 ±0.29
December	0.6350 ±0.055	10.73 ±0.38	68.08 ± 8.51	49.02 ±1.32	1.67 ±0.35	1.99 ±0.12	5.46 ±0.56	2.32 ±0.32	35.40 ±0.96	4.10 ±0.30
January	0.6875 ±0.105	11.23 ±0.39	77.31 ±12.71	51.36 ±1.40	1.48 ±0.23	2.34 ±0.28	5.35 ±0.41	2.38 ±0.34	36.04 ±1.26	3.12 ±0.19
February	0.7900 ±0.032	13.58 ±0.73	107.59 ± 8.24	50.00 ±1.61	1.59 ±0.26	2.03 ±0.31	6.85 ±0.62	1.80 ±0.46	36.57 ±0.85	4.61 ±0.13
March	0.9010* ±0.084	15.78* ±0.94	141.92* ± 4.27	46.33* ±0.76	1.38* ±0.19	1.08* ±0.24	6.82* ±0.28	4.15* ±0.36	54.16* ±1.42	4.53* ±0.29
*Significant at the level P	<0.005	<0.001	<0.001	<0.001	NS	NS	<0.005	<0.001	<0.001	NS

*P value 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.

CH = cholesterol, CHE = cholesterol esters, DG = diglycerides, FFA = free fatty acids, MG = monoglycerides, PH = phospholipids, TG = triglycerides.

TABLE 1b

Changes in liver weight, liver lipid content and liver lipid composition in Rosy Pastor. Lipid fractions expressed as mg/100mg lipid. Mean value ± S.D.

Month	Total liver weight gm	Lipid mg/100mg wet liver	Lipid mg/liver	PH	MG	FFA	CH	DG	TG	CHE
October	2.565 ±0.210	7.61 ±0.49	195.75 ±27.21	51.63 ±1.87	2.19 ±0.32	1.92 ±0.17	5.35 ±0.39	1.35 ±0.26	32.70 ±1.67	3.35 ±0.35
April	3.756* ±0.146	12.70* ±0.50	476.61* ±10.51	22.85* ±1.17	1.96* ±0.22	2.06* ±0.30	6.70* ±0.35	3.91* ±0.46	61.65* ±3.09	3.46* ±0.27
*Significant at the level P	<0.002	<0.001	<0.001	<0.001	NS	NS	<0.02	<0.005	<0.001	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. Abbreviations - same as in Table 1a.

TABLE 2a

Changes in liver lipid composition of Wagtail, Lipid fractions expressed as mg/100 mg liver wet weight. Mean value ± S.D.

Month	PH	MG	FFA	CH	DG	TG	CHE
October	4.82 ±0.24	0.139 ±0.045	0.273 ±0.033	0.410 ±0.035	0.144 ±0.012	2.75 ±0.28	0.413 ±0.054
November	4.86 ±0.07	0.131 ±0.042	0.243 ±0.037	0.492 ±0.034	0.107 ±0.027	3.17 ±0.13	0.429 ±0.013
December	5.25 ±0.07	0.183 ±0.043	0.211 ±0.002	0.587 ±0.079	0.248 ±0.030	3.80 ±0.23	0.447 ±0.020
January	5.86 ±0.38	0.169 ±0.021	0.268 ±0.047	0.610 ±0.022	0.267 ±0.032	4.05 ±0.26	0.353 ±0.092
February	6.78 ±0.15	0.212 ±0.045	0.270 ±0.018	0.943 ±0.082	0.248 ±0.019	4.97 ±0.31	0.612 ±0.047
March	4.16* ±0.37	0.222* ±0.019	0.323* ±0.055	1.078* ±0.110	0.675* ±0.094	8.57* ±0.46	0.733* ±0.007
*Significant at the level	$P<0.05$	NS	NS	$P<0.001$	$P<0.001$	$P<0.001$	$P<0.002$

*P values refer to differences between post and premigratory periods. The Student's 't' test was used to analyze differences in means. Abbreviations - same as in Table 1a.

TABLE 2b

Liver lipid composition in Rosy Pastor. Lipid fractions expressed as mg/100 mg wet liver. Mean value ± S.D.

Month	PH	MG	FFA	CH	DG	TG	CHE
October	3.93 ±0.40	0.167 ±0.029	0.146 ±0.021	0.407 ±0.046	0.103 ±0.026	2.49 ±0.29	0.256 ±0.038
November	2.89* ±0.03	0.248* ±0.018	0.261* ±0.028	0.850* ±0.012	0.497* ±0.085	7.82* ±0.094	0.441* ±0.045
*Significant at the level	$P<0.02$	$P<0.02$	$P<0.02$	$P<0.001$	$P<0.002$	$P<0.001$	$P<0.01$

*P values refer to differences between post and premigratory periods. The Student's 't' test was used to analyze differences in means. Abbreviations - same as in Table 1a.

TABLE 3a

Changes in liver lipid composition of Wagtail. Lipid fractions expressed as mg/liver. Mean value ± S.D.

Month	PH	MG	FFA	CH	DG	TG	CHE
October	26.55 ±3.88	0.738 ±0.130	1.496 ±0.411	2.224 ±0.397	0.772 ±0.054	14.791 ±1.572	2.261 ±0.463
November	25.53 ±5.22	0.748 ±0.185	1.413 ±0.324	2.599 ±0.165	0.5454 ±0.115	16.718 ±3.815	2.484 ±0.268
December	33.41 ±3.22	1.216 ±0.284	1.234 ±0.097	3.758 ±0.378	1.572 ±0.138	24.228 ±3.577	2.940 ±0.106
January	40.55 ±8.28	1.331 ±0.172	2.112 ±0.606	4.369 ±0.587	1.926 ±0.430	29.195 ±5.759	2.786 ±0.471
February	53.69 ±2.36	1.644 ±0.336	2.102 ±0.599	7.472 ±0.824	1.969 ±0.574	39.373 ±3.568	4.760 ±0.309
March	37.35* ±0.78	1.941* ±0.341	2.780* ±0.255	9.671* ±0.113	5.823* ±0.313	77.122* ±3.784	6.371* ±0.617
*Significant at the level	P<0.01	P<0.01	P<0.01	P<0.001	P<0.001	P<0.001	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. Abbreviations - same as in Table 1a.

TABLE 3b

Liver lipid composition in Rosy Pastor. Lipid fractions expressed as mg/liver. Mean value ± S.D.

Month	PH	MG	FFA	CH	DG	TG	CHE
October	98.21 ±12.27	4.33 ±1.08	3.76 ±0.87	10.52 ±2.06	2.69 ±0.49	64.29 ±12.14	6.62 ±1.54
April	108.95* ± 5.53	9.35* ±1.05	9.84* ±1.44	31.94* ±1.65	18.71* ±3.48	293.84* ±14.51	16.56* ±1.30
*Significant at the level	NS	P<0.005	P<0.005	P<0.001	P<0.002	P<0.001	P<0.002

*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. Abbreviations - same as in Table 1a.

TABLE 4

Adipose tissue lipid content and volume of adipocyte in Wagtail and Rosy Pastor. Mean value ± S.D.

	Wagtail		Rosy Pastor	
	Lipid mg/100 mg	Adipocyte volume X $10^6\mu$	Lipid mg/100 mg	Adipocyte volume X $10^6\mu$
Postmigratory period	94.03 ±0.32	0.012 ±0.004	93.70 ±0.61	0.011 ±0.003
Premigratory period	98.48* ±0.19	1.142* ±0.019	98.41* ±0.56	1.055* ±0.020
*Significant at the level	P<0.001	P<0.001	P<0.001	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.

EXPLANATIONS FOR FIGURES

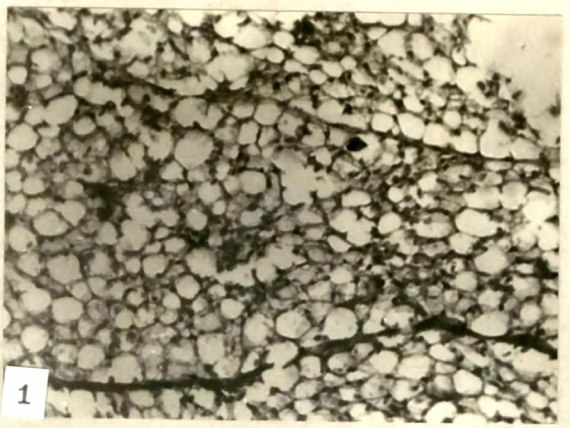
Figs. 1-4. Photomicrographs of adipose tissue sections stained with haematoxylin-eosin during postmigratory (POM) and premigratory (PM) periods of Rosy Pastor and Wagtail. Each one is of 150 X magnification.

Fig. 1. Section of Rosy Pastor adipose tissue (POM).

Fig. 2. Section of Rosy Pastor adipose tissue (PM).

Fig. 3. Section of Wagtail adipose tissue (POM).

Fig. 4. Section of Wagtail adipose tissue (PM).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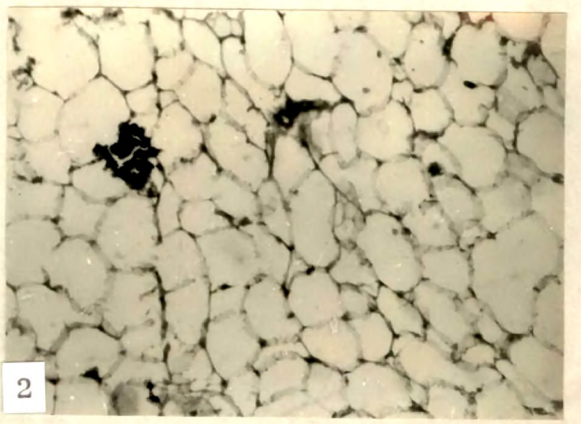
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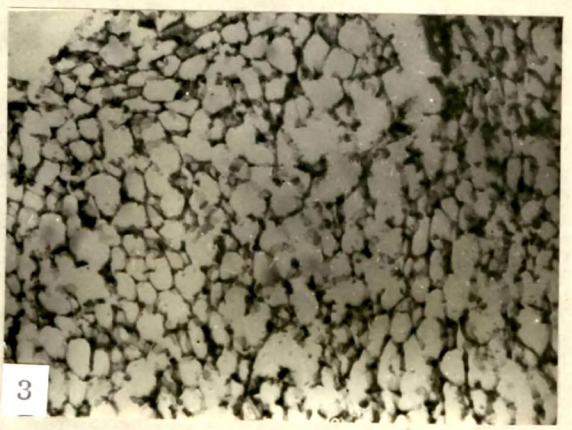
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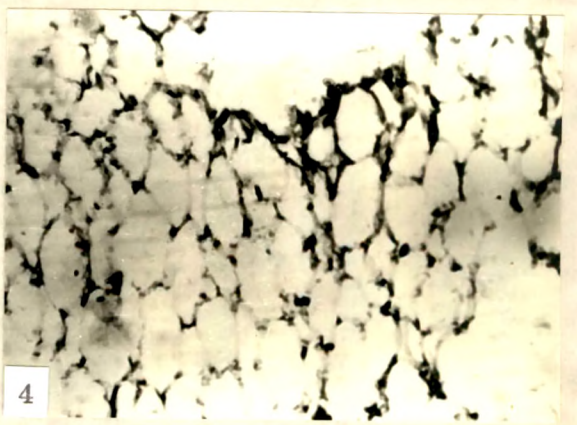
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ester and free fatty acids when expressed for the total liver content significantly increased during the premigratory period (Table 3a & b).

The amount of phospholipid fraction of the extracted lipid (mg phospholipid/100 mg lipid) in the liver of both the birds during their premigratory period was significantly lower as compared to that during their postmigratory period (Table 1a & b). Whereas the quantity of the phospholipid expressed per total liver in the Wagtail was significantly higher during its premigratory period. Such a significant difference was not noticed in the case of Rosy Pastor.

In Rosy Pastor as well as Wagtail, the quantity of free cholesterol, diglycerides and triglycerides (when expressed as mg/100 mg liver wet weight or per total liver) increased significantly (Table 1a & b, 2a & b and 3a & b) during the premigratory period. It was noticed that, during the premigratory period, triglycerides per total liver in Rosy Pastor were more than that in the liver of Wagtail. Total cholesterol content (free cholesterol + cholesterol ester, expressed as a percentage of liver wet weight) of liver of Wagtail was observed to be more than that in Rosy Pastor liver.

Adipose tissue: (Table 4, Figs. 1, 2, 3, 4)

Lipid content of the adipose tissue of Rosy Pastor as well as Wagtail was significantly higher during the premigratory period. Size of the adipocytes in the adipose tissue of both the birds increased significantly during this period.

DISCUSSION

Changes observed in the concentration of total liver lipids are in good agreement with those reported by Pilo (1967) for Rosy Pastor, and John and George (1966) for Wagtail. Liver lipid analyses reported herein suggest that phospholipid constitutes major part of hepatic lipids in Rosy Pastor and Wagtail during the postmigratory period. Triglyceride concentration was found to be highest among the neutral lipids during the same period. The fractions of liver lipid in Rosy Pastor and Wagtail did not differ much (Qualitatively) from those reported for domestic chicken (Christie and Moore, 1972).

Changes in the liver lipid concentration during premigratory period in Rosy Pastor and Wagtail are accompanied by only quantitative changes in the levels of

different lipid classes. Inspite of a higher rate of fatty acid synthesis in the liver, during their premigratory period; free fatty acid fraction in the liver lipid of these migratory birds did not show any significant increase (Table 1a & b). This fact suggests that the rate of esterification and transport of free fatty acid must be keeping pace with high rate of synthesis during the premigratory period. Highly increased level of triglycerides in the liver (Table 1a & b) during the premigratory period in both the birds obviously points out that newly synthesized free fatty acids are immediately esterified to form triglycerides and other esterified lipid components. In support of this contention it could be stated that if the hepatic cells were incapable of incorporating newly synthesized fatty acids into triglycerides, phospholipid and cholesterol esters at a faster rate, the whole process of fatty acid synthesis would grind to a halt because of the feed back inhibition of fatty acid synthesizing enzymes. Such inhibition is not noticed which is evident from the enzymological study reported in Chapter 4. However, during the premigratory period, the total free fatty acids in the liver of both the birds was found to be higher due to the increase in the total liver lipid (Table 3a & b).

Synthesis of triglycerides and phospholipids is

biosynthetically related through a common intermediate 1,2 diglyceride (Kennedy, 1961). A marked increase in the concentration of diglycerides in the liver of Rosy Pastor and Wagtail during the premigratory period is indicative of its increased synthesis from the free fatty acids. It could be suggested that the increased availability of such a precursor would significantly facilitate an enhanced triglyceride synthesis.

Liver is one of the major sites of cholesterol synthesis (Lorenz, 1954). Cholesterol level in the liver of Wagtail and Rosy Pastor was found to rise during their premigratory period. These results are in accordance with the report of John and George (1967c). The increased quantity of total cholesterol during the premigratory period was mainly due to the increase in the free cholesterol. Sperry and Stoyanoff (1935) reported that free cholesterol constitutes the major portion of the total cholesterol in the liver of fowl. Higher level of free cholesterol as compared to that of esterified cholesterol plays a dynamic role in view of the fact that free cholesterol is exchanged readily between tissues and lipoprotein (Harper, 1975). Free cholesterol from the liver can then be easily transported with lipoprotein to the organs like adrenal and

gonads where it can be utilized for steroidogenesis. This contention finds support in the work of Naik (1963) and John (1967) who have reported that steroid synthesis is higher during premigratory period of Rosy Pastor and Wagtail.

One of the important functions of cholesterol in the liver is its utilization for bile formation. During premigratory hyperphagia, increased demand for bile acid may be anticipated to facilitate lipid digestion and absorption. Increased cholesterol synthesis as is evident from its higher concentration in the liver during this period would help in meeting the demand. Higher cholesterol content in liver of Wagtail as compared to that in Rosy Pastor can be explained as an adaptation to lipid rich diet of the former.

The reduction in percentage of phospholipid (Table 1a & b) in the liver, a few weeks prior to migration could be due to its rapid removal from the organ and/or perhaps due to a decreased hepatic phospholipid synthesis. Pilo (1967) presumed that reduced level of phospholipid in the liver of Rosy Pastor may be due to its decreased synthesis under the influence of increased thyroxine level. However, total phospholipid content per total liver in

Rosy Pastor did not show any significant change during its premigratory period; while the phospholipid content per total liver increased in the liver of Wagtail till February but slightly decreased in the month of March (Table 2a & b and 3a & b) indicating its rapid removal from the liver.

During premigratory period the amount of triglycerides accumulated in the liver of Rosy Pastor was more than that found in the liver of Wagtail (Table 3a & b). The study on hyperlipogenesis in migratory birds (Chapter 4) proved that intense fatty acid synthesis occurs a few weeks prior to migration and the liver is the major site for hyperlipogenesis. Results of liver lipid analyses (Table 1a & b, 2a & b and 3a & b) show that there is a rapid increase in the concentration of triglycerides in the liver of both the migratory birds. Major portion of triglycerides is immediately transported to the adipose tissue where the lipid content increases rapidly. During this phase the size of the adipocytes increases (Figs. 2 & 4) to accomodate large amount of fat. Since the lipogenesis in adipose tissue of migratory birds is practically insignificant (Chapter 4), the fat present in the adipose tissue is derived from the liver. Though the phospholipid fraction of the lipid in the liver of the migratory birds studied was at a significantly

low level during the period of intensive fat synthesis, it did not have adverse effect on the removal and transport of triglycerides from the liver to adipose tissue. Thus, liver steatosis in the migratory birds differs from the conditions leading to pathological state of fatty liver in the rat fed on choline deficient diet, where alteration in phospholipid metabolism is considered to be the main factor. It appears that liver steatosis occuring in the Rosy Pastor and Wagtail during their premigratory period is due to accumulation of triglycerides and is not as the result of weak "packaging and export" mechanisms of their liver. Nevertheless, the increase in the rate of synthesis of hepatic triglycerides beyond the maximal rate of their removal from the site of synthesis naturally results in their accumulation in the liver itself.