CHAPTER 4

SEASONAL CHANGES IN THE IRON CONTENT OF THE LIVER AND MUSCLE OF THE MIGRATORY STARLING, STURNUS ROSEUS

Iron, though a minor constituent in the body plays important roles in many biological processes. In combination with several compounds, iron facilitates oxygen transport and number of oxidative reactions. The oxygen carrying pigment, haemoglobin, incorporates the major amount of iron while the rest is shared by other heme compounds such as myoglobin, mitochondrial cytochromes, several enzymes like SDH, transport protein- transferrin and storage compouds- ferritin and haemosiderin (Bothwell and Finch, 1962). The amount of iron in the body is more or less stable, as there is always a balanced absorption and excretion (Bothwell and Finch, 1962). This constant amount within the body mostly participates in the haemoglobin cycle. It was estimated that 20-25% of the total iron gets stored in the liver or spleen, a part of which is made readily available for the haemoglobin synthesis (Bothwell and Finch, 1962).

The storage of iron takes place mainly in the liver. This iron is reported to be relatively inert. Under normal conditions, the iron released from disintegrating erythrocytes is usually utilized again to produce more haemoglobin, due to which the stored iron is seldom disturbed. Hence, a decrease or increase in the iron content of the liver denotes either a depressed or an elevated erythrocyte production respectively. As an increase in the erythropoiesis was observed in the Rosy Pastor during the premigratory period (Chapter 5) changes in the level of iron may also occur. The present studies were carried out to find the correlation between the amount of stored iron in the liver and the observed increase in erythropoiesis in the migratory bird Sturnus roseus.

Since the oxidative pathway of catabolic processes is the main source of muscular energy during migration, the adaptations in muscles that facilitate increased or efficient uptake of oxygen are of great interest. An increased vascularization and number of circulating erythrocytes could aid in supplying more oxygen to the muscles. The O_2 affinity of the muscles is enhanced by an increase in their respiratory pigments (myoglobin). A study of iron content in the pectoralis muscle was also therefore undertaken.

MATERIALS AND METHODS

The estimation of iron was carried out in dried liver and muscle. The tissues were blotted thoroughly taking care to remove every trace of blood and subsequently dried.

The estimation was carried out by the method of Elvehjem (1930) and Kennedy (1927) as described by Hawk et al. (1954). A suitable amount of the dry tissue was digested in 5 ml of concentrated, iron free sulphuric acid and 2 ml perchloric acid. After digestion the solution was diluted to a convenient volume. Out of this, 10 ml aliquots were processed for photometric measurement by adding 10 ml of amyl alcohol and 5 ml of 20% potassium thiocyanate. The readings were taken on a Bausch and Lomb " Spectronic 20" photoelectric colorimeter at 480 mμ.

For the histochemical demonstration of iron in the liver, Perl's Prussian blue method (Pearse,1960) was employed.

RESULTS

The changes in the levels of iron in the liver of Rosy Pastor during different seasons (post- and pre- migratory periods) are presented in Fig. 1. The average value of iron in the liver was found to be 57.72 mg/100 g fresh tissue by mid August (postmigratory period). But by the last week of August a slight increase (70.83 mg/100 g wet liver) was noticed.

In the premigratory phase, periodical determinations were made from February to the end of April. In February the average value obtained was 81.62 mg/100 g wet liver. Thereafter, a gradual decline in the iron content was recorded. In the last week, the average value was found to be very low (40.73 mg per 100 g wet liver).

Histochemical studies on the liver revealed that the iron, presumably haemosiderin was localized in parenchymal cells in fine granular state. Reticuloendothelial cells (Kupffer cells) were found to contain very little iron (Fig. 3)

Iron estimation in the muscle was carried out in the premigratory period only. A gradual rise though quantitatively negligible was significant (Fig. 2) in view of the fact that

Fig.1. Iron content of the liver Rosy Pastors during post- and pre- migratory periods.

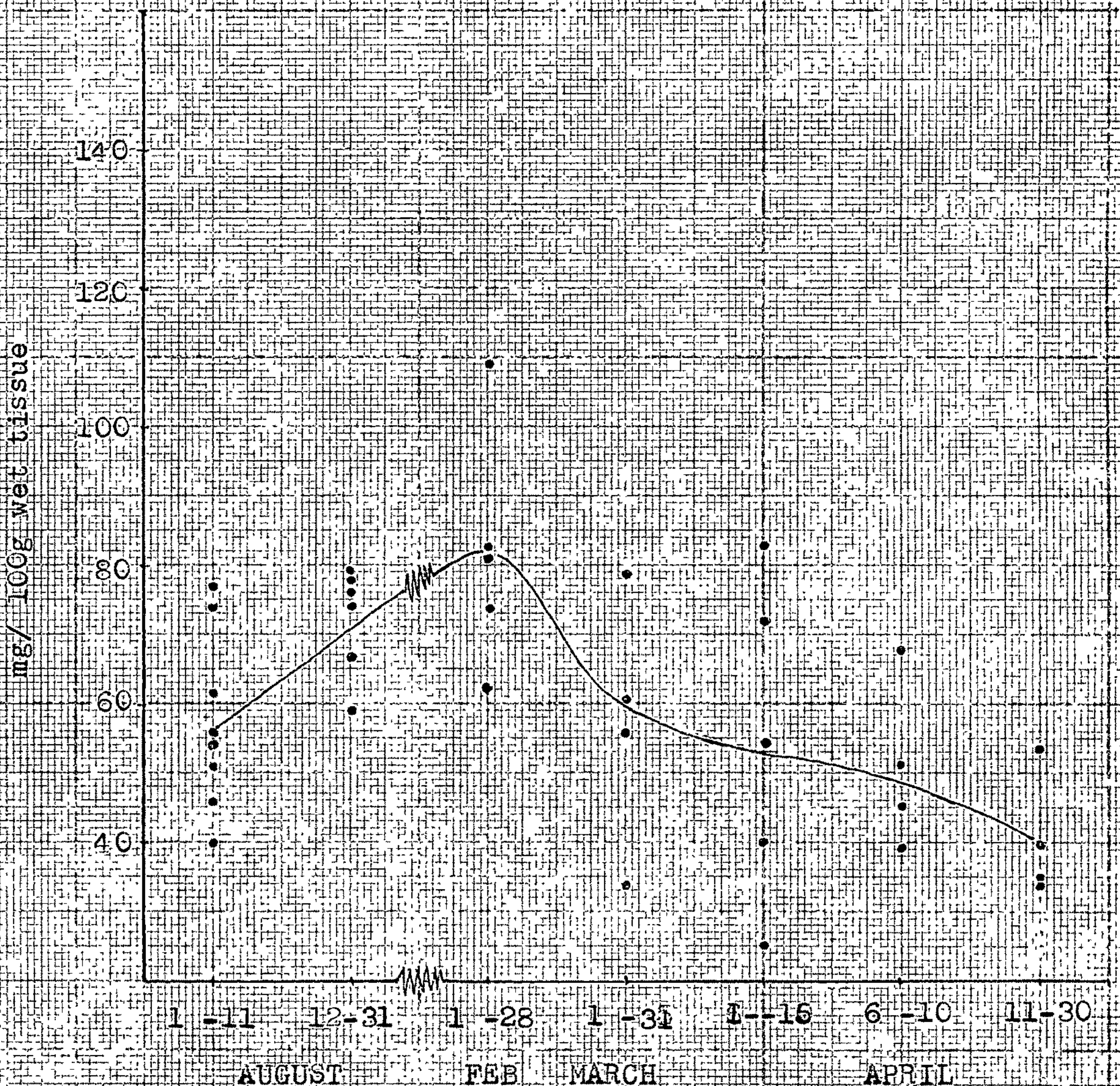
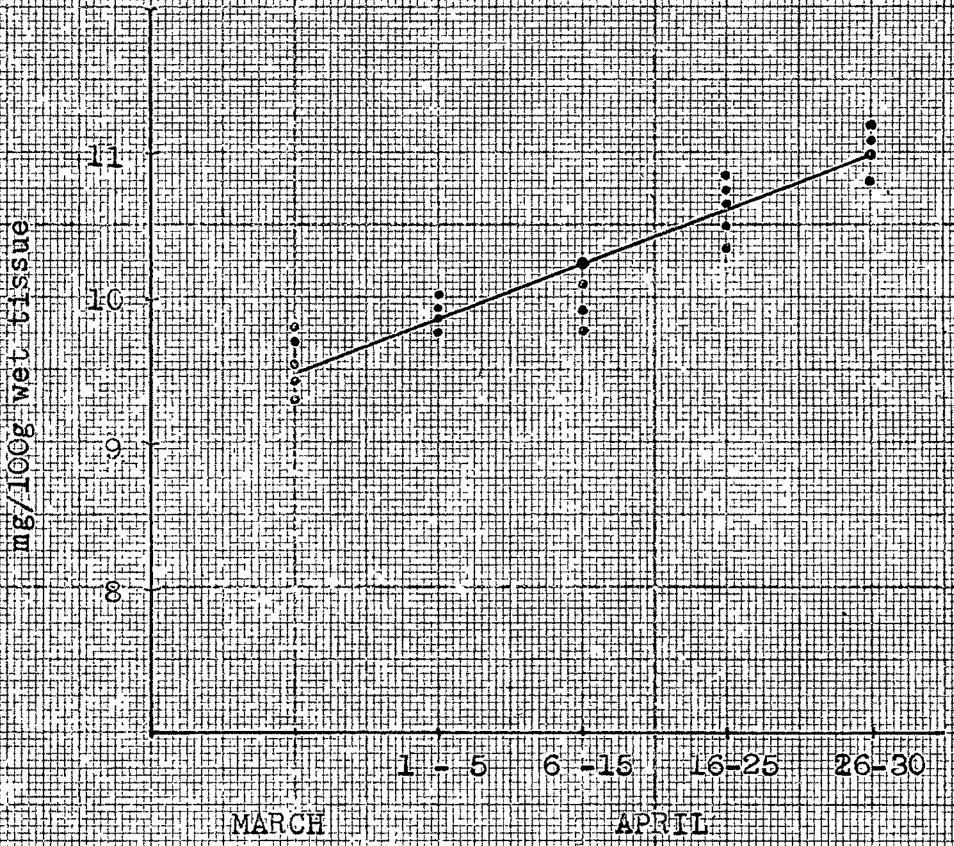


Fig.2. Iron content of the muscle of Rosy Pastor during the premigratory period.



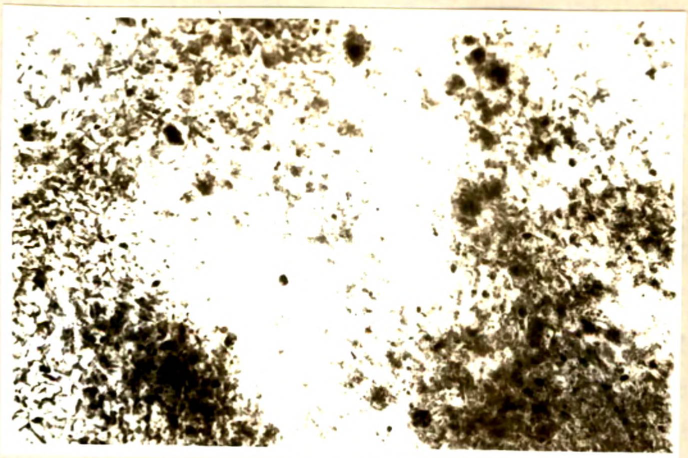


Fig.3A. Microphotograph of section of liver of Rosy Pastor during the postmigratory period showing the granular distribution of iron in the parenchymal cells. Perl's Prussian Blue method. 160x.

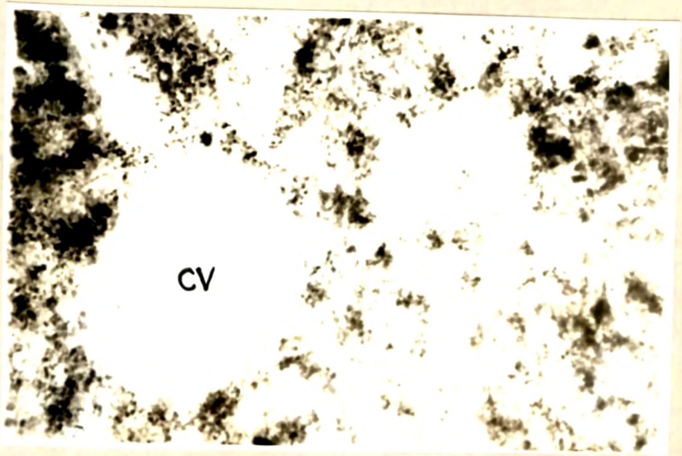


Fig.3B. Higher magnification of fig.3A. Note the granular deposition of iron (haemosiderin) in the parenchymal cells around the central vein (CV). 250x.

even slight changes in the level of myoglobin and other iron containing compounds such as cytochromes could be of adaptive value in the oxidative capacity of the muscle.

DISCUSSION

The histochemical preparations of the liver usually indicate the presence of iron in reticuloendothelial cells or in the parenchymal cells. The source of iron in these two locations were reported to be different. Thus, the deposite in the reticuloendothelial cells were derived largely from the disintegrating red cells after their phagocytosis while that in parenchymal cells was from transferrin, an iron binding protein in the plasma (Finch and Finch, 1955; Bothwell and Bradlow, 1960). Usually iron is stored in the liver as ferritin but in excess, it is laid down as haemosiderin (Shoden et al., 1953). The latter is largely composed of ferritin (Richter, 1957). In Rosy Pastor liver sections, stained for iron, the parenchymal cells were found to contain large amounts of iron as granular inclusions (Fig. 3). Since only the large haemosiderin molecules are detectable under the light microscope, these granules in the parenchymal cells could be therefore haemosiderin. The hepatic deposition of iron was more profound in the postmigratory period. It could be seen (Fig. 1) that the liver contained less iron in the middle of August than at the end of the month. The two factors that might affect such rise towards the end of August are: (a) an increased absorption or (b) a high rate of red cell destruction. The presence of large number of disintegrating cells in the liver and a drop in the R.B.C. count (Chapter 5) in the Rosy Pastor during this period, indicates a probable elevated cell destruction. Large build up of iron in the liver was found to occur when haemolysis was induced (Hahn <u>et</u> <u>al</u>.,1943). By the end of August, haemolysis might have completed resulting in the rise of iron in the liver.

The parenchymal stores of iron will also increase when more iron gets absorbed through the intestinal tract. However, its absorption is a regulated process. An increased absorption takes place only when a considerable amount of iron is depleted from tissues. The limiting factor being the capacity to take up iron or saturation of ferritin and tissue receptors (Bothwell and Finch, 1962). An enhanced erythropoiesis could also induce such large scale uptake by the intestine (Bothwell <u>et</u> <u>al</u>.,1957). Since the capacity of the liver to remove iron from transferrin is limited as compared to that of bone marrow (Elmlinger <u>et</u> <u>al</u>.,1952; 1953; Huff <u>et</u> <u>al</u>.,1952), significant hepatic uptake occurs only when the transferrin attains maximum saturation with iron (Jandl <u>et</u> <u>al</u>.,1959). Such high saturation of transferrin was reported to occur under conditions of ineffective erythropoiesis and/ or when a block in the incorporation of iron into haemoglobin took place (Whipple and Bradford, 1936; Howell and Wyatt, 1952; Ellis <u>et</u> <u>al</u>., 1954; Lukl <u>et</u> <u>al</u>., 1958). Since, in postmigratory period, an active blood cell production was not found to occur nor was there an increased absorption from the diet, the sudden increase of iron content in the liver by the end of August could be due to an increased red blood cell destruction.

Release or deposition of iron is known to match the needs of the bone marrow (Bothwell and Finch, 1962). Thus, when erythropoiesis was activated by bleeding, a sufficient mobilization of iron from depots took place which permitted the rate of cell production to rise 1.5 to 2 times that of normal (Finch et al.,1950; Haskins et al., 1952; Bothwell et al., 1957). As a considerable reduction in the iron content from the liver was observed in the premigratory period, greater uptake by bone marrow might have occurred. A marked fall was observed in the last week of April which also coincides with the recorded high R.B.C. count (Chapter 5) during the same period. Though a decrease in iron level was evident from March onwards, a correspoding increase in the erythrocyte number was not observed. However, a large number of erythrocyte precursor cell were found in the bone marrow in the first week of April. According to Baldini et al.(1959), the precursor cells actually determine the amount of iron utilized. This is because only reticulocytes and not mature erythrocytes could absorb iron from the plasma (Walsh et al., 1949; London et al., 1950).

Iron is not only utilized for haemoglobin synthesis but also for many other compounds and enzymes wherein it forms an integral part. Thus, increase of other heme compound such as myoglobin cytochromes, catalases, peroxidases and non heme substances like SDH, DPNH-cytochrome reductase, xanthine oxidase etc., also reduces the store of iron.

The gradual increase in the iron content of the pectoralis muscle of Rosy Pastor in premigratory period might point to higher myoglobin level. Myobglobin is essential for the O_2 utilization as well as for a certain amount of intracellular O_2 retention.(Hurtado et al., 1937; Lawrie, 1953; Vannotti, 1964). Vaughan and Pace (1956) were of the opinion that this pigment may keep a constant optimal oxygen tension between cellular membrane and the mitochondria. The myoglobin concentration in muscle is usually associated with the degree of glycolytic processes. More the myoglobin less its capacity for glycolysis but greater its ability to use oxidative metabolism and synthesize ATP (Lawrie, 1953a; 1953b). It could be mentioned here that muscle fibres containing high amount of myoglobin (red fibres) could withstand prolonged activity whereas the white ones, using glycolytic pathway for energy requirements, contract more rapidly and get exhausted quickly (Gordan and Phillips, 1953; George and Berger, 1966). Since it is emphatically knowm that the fat forms the chief fuel during migratory flight (George and Berger, 1966) the muscle requires more myoglobin for the oxidative processes. It was also shown that excercice elevates myoglobin level (Lawrie, 1953). Thus the increase of iron in the muscle may be indicative of a corresponding rise in myoglobin. But this need not be necessarily so, since cytochromes, SDH etc., which are also associated with oxidative metabolism too, contain iron. Moreover, myoglobin is more stable than haemoglobin and subjected to less variation even under conditions of anaemia and malnutrition (Hurtado _et_ _al_.,1937). An increased myoglobin content is not essential in the muscles of birds for a higher O_2 utilization. The Pigeon breast muscle, although has high oxidative activity, nevertheless contain low levels of myoglobin. This is due to the efficient blood supply which actually reduces the need for O_2 storage (Lawrie, 1952). The Rosy Pastor, with an effective blood supply combined with an increased red blood cell number could cppe up with the oxygen demand of the muscles. This means that increase in myoglobin is only an obligatory process to increase the O_2 affinity of the muscles in active fliers. What may be needed is an efficient oxygen transfer system and enzymatic adjustments for the increased oxidative processes. Repeated electrical stimulation of rabbit muscle caused a rise in the quantities of cytochromes, cytochrome oxidase,riboflavines and some iron containing coenzymes (Vannotti, 1946). Hearn and Wainio (1956) observed an increase in the SDH activity in the muscles of exercised rats. Since, iron is an essential part of all these compounds and enzymes, the increase in the amount of iron in the pectoralis muscle of Rosy Pastor could mean an increase of all these too.