CHAPTER 5

ERYTHROPOIESIS AND HAEMOGLOBIN LEVELS IN THE ROSY PASTOR (STURNUS ROSEUS) DURING POST-AND PRE-MIGRATORY PERIODS

The haematopoietic system is known to be influenced by environmental, nutritional and physiologic changes. A number of factors tend to disturb the circulating blood cell levels. Concurrent with such altered conditions, an increase or decrease in blood cell production may be manifested. The major physiological changes which occur upon migration are concerned in making available a constant supply of energy to the migratory bird. A continuous flight during migration not only calls forth for a high energy supply from stored metabolites, chiefly fat, but also for a higher rate of oxygen supply. Since, the transportation of oxygen is the function of erythrocytes, an elevated red blood cell production should/may occur in such birds. Though several studies on haematopoiesis in birds of different species were carried out (ref. Lucas and Jamroz,1961), much work concerning the different blood cells in a migratory bird have not been reported. The present study was therefore an attempt to elucidate further, the homeostasis of blood cell elements in the Rosy Pastor (*Sturnus roseus*) during different phases of its life cycle.

MATERIALS AND METHODS

Studies were conducted on Rosy Pastor in the post-migratory (August/September) and the premigratory (March/April) periods only.

For the histological investigation, blood and bone marrow smears were stained with haematoxylin-eosin, Jenner, Lieshman's and other suitable dyes. The bone marrow was obtained from various bones such as femur, humerus and tibia.

The haemoglobin estimation was carried out by the method of Wong as described by Hawk et al.(1954). Since, the alterations in the number of circulating red cells would be of interest, only R.B.C. counts were done. This was done using a haemocytometer. All the estimations as well the countings were carried out in birds collected in the early hours of the morning in both the seasons, as diurnal variation occurs in the number of circulating erythrocytes.

RESULTS

The average R.B.C count in the Rosy Pastor in the postmigratory period ranged from 3 to 3.5 million/cmm. Just prior to migration it was found to increase up to 4.5 to 5 million/ cmm.(Fig.1).

The bone marrow showed large number of erythroblasts and reticulocytes while polychromatic (immature) erythrocytes were few in the earlier period (first week of April) of premigratory phase (Fig.3). In the last week of April (just prior to migration) the bone marrow contained enormous numbers of polychromatic erythrocytes as well as nearly mature ones (Fig.4). These cells were seen enmeshed in the reticular net work of marrow surrounded by erythroblasts, reticulocytes and other

Fig.1. R.B.C. counts in the blood of Rosy Pastor during post- and pre- migratory periods

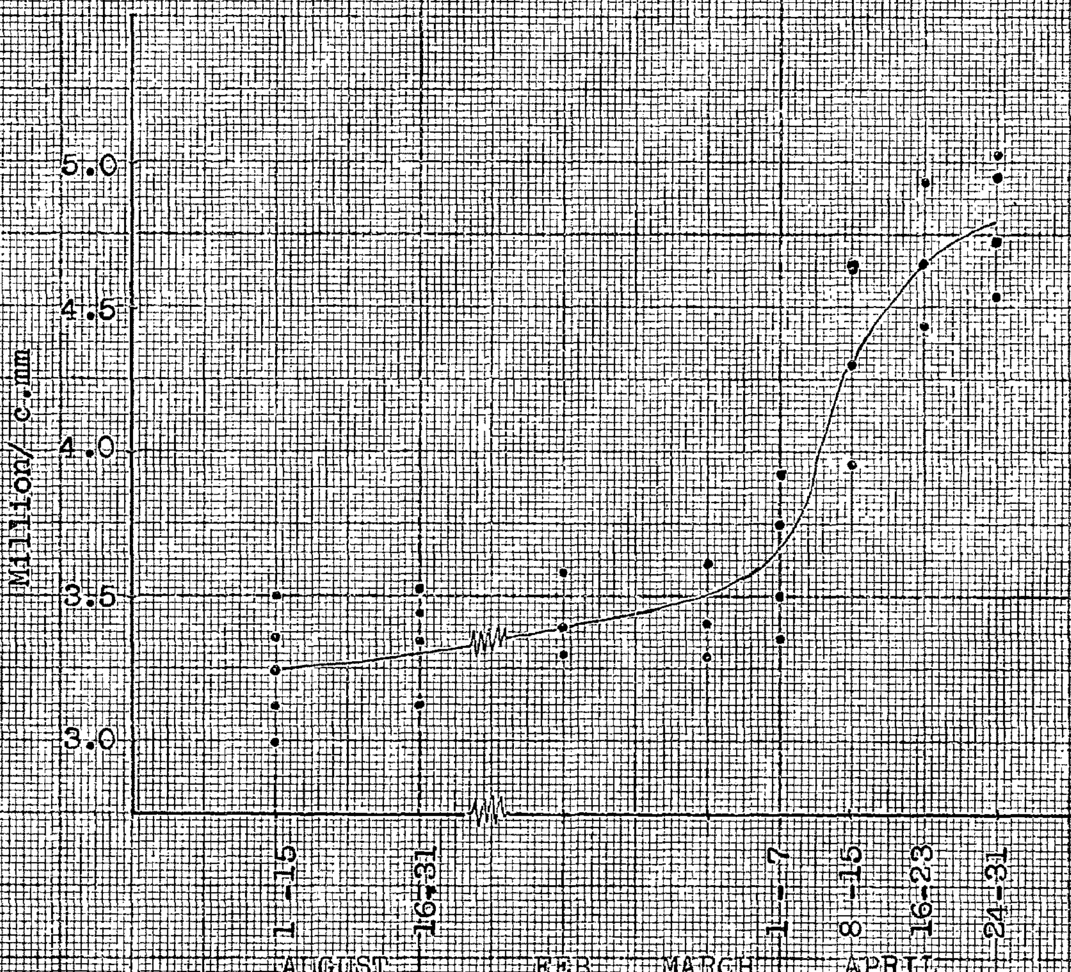
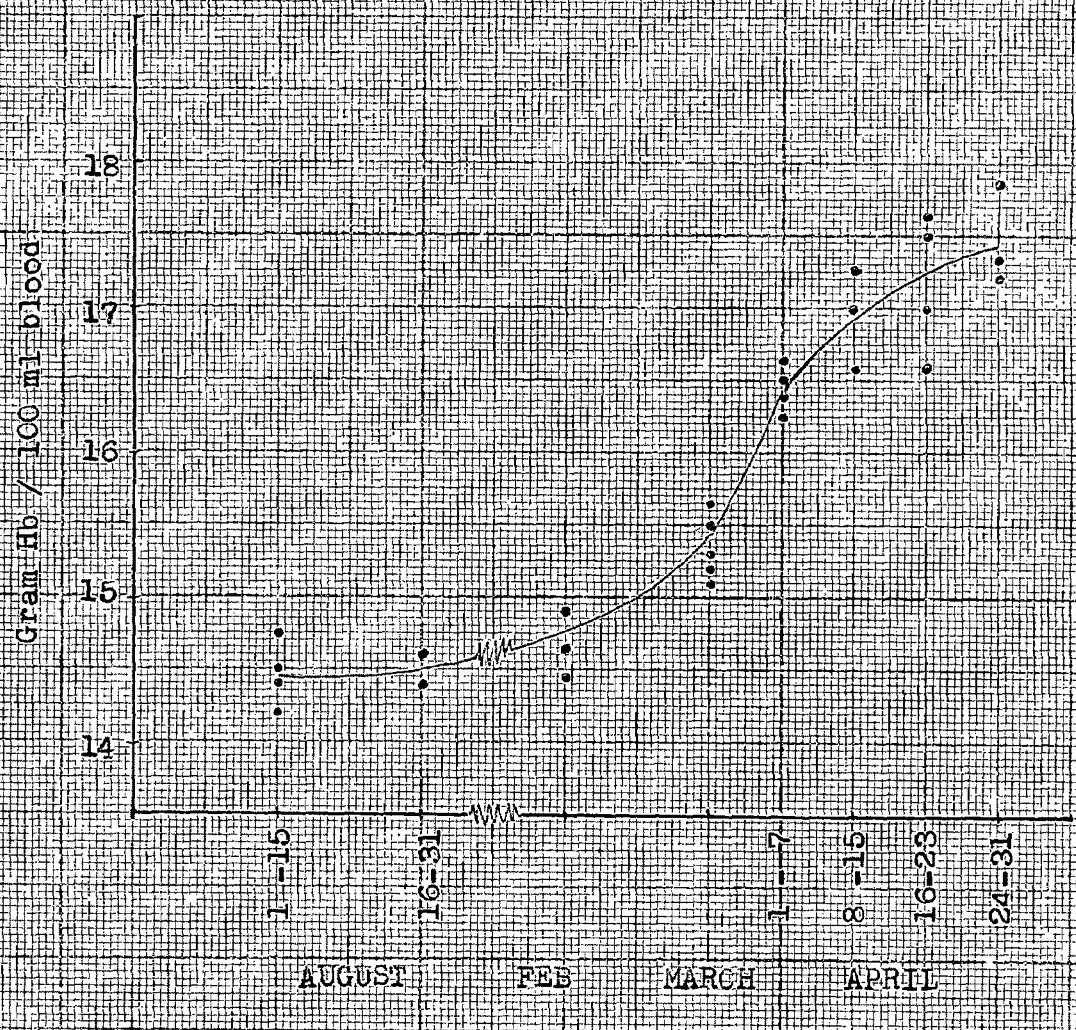


Fig. 2. Haemoglobin content of the blood of Rosy Pastor during post- and pre- migratory periods.



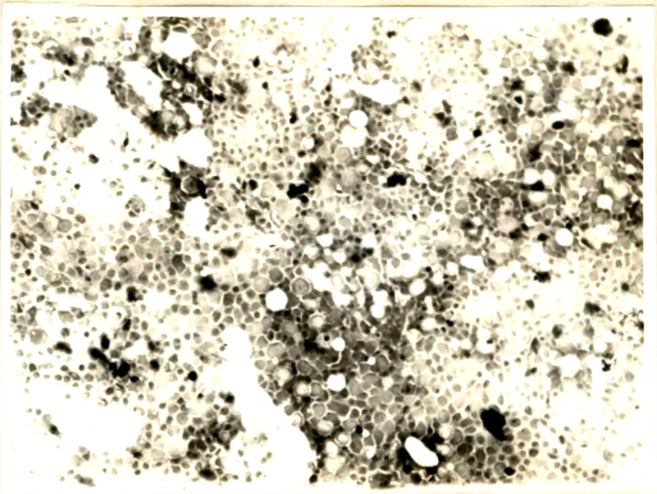


Fig.3 A. Microphotograph of bone marrow of Rosy Pastor during the first week of April (premigratory period). Note the large number of reticulocytes and other precursor cells. The mature erythrocytes are few in number. Stained with haematoxylin-eosin. 128x.

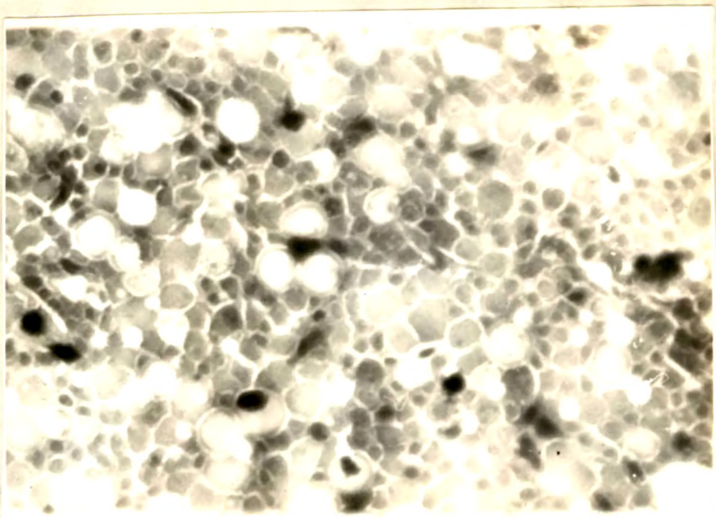


Fig.3 B. Higher magnification of Fig.3 A. Note the erythroblasts and reticulocytes which are in great numbers. 200x.

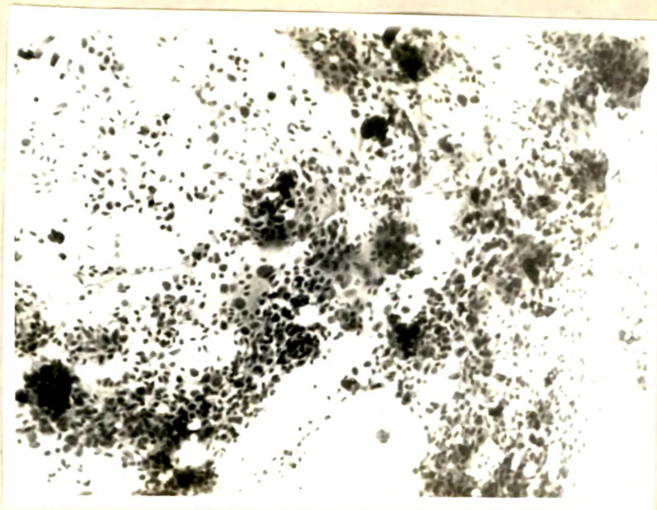


Fig.4 A. Microphotograph of bone marrow of Rosy Pastor during the last week of April (just before migration). Note the large number of polychromatic erythrocytes (immature erythrocytes). Stained with haematoxylin-eosin. 128x.

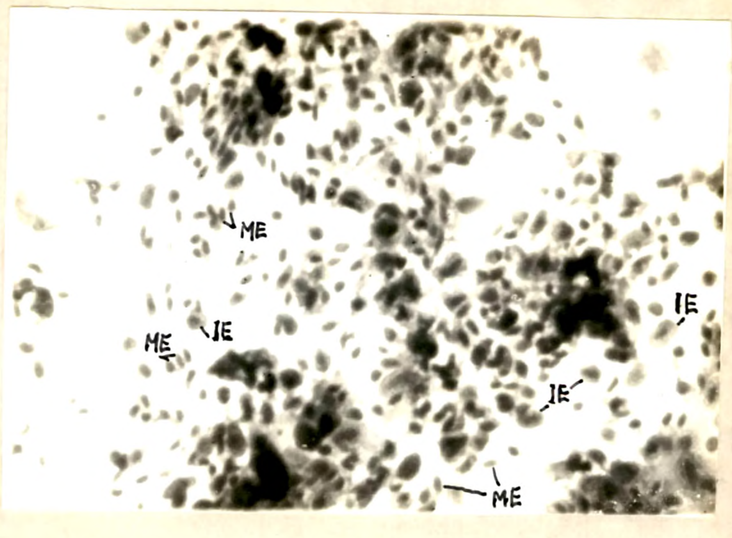


Fig.4 B. Higher magnification of Fig.4 A. ME- mature erythrocytes., IE- immature erythrocytes. 200x.

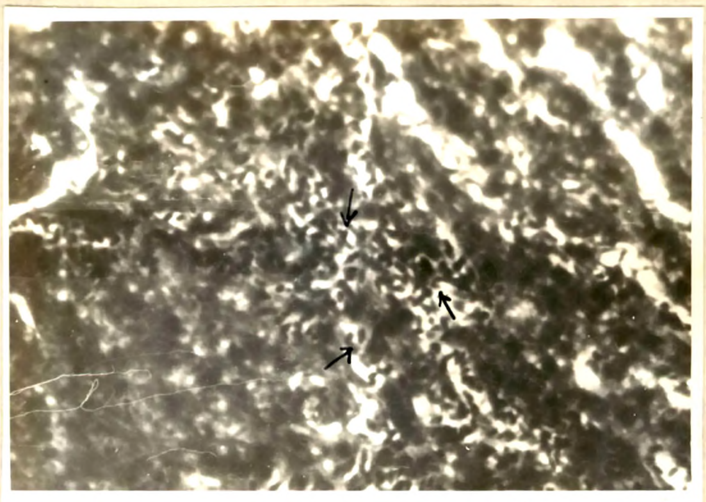


Fig.5. Microphotograph showing the erythrocytes in the sinusoids of the liver of Rosy Pastor during the post migratory period. Haematoxylin-eosine stained. Arrow shows the erythrocytes. 320x.

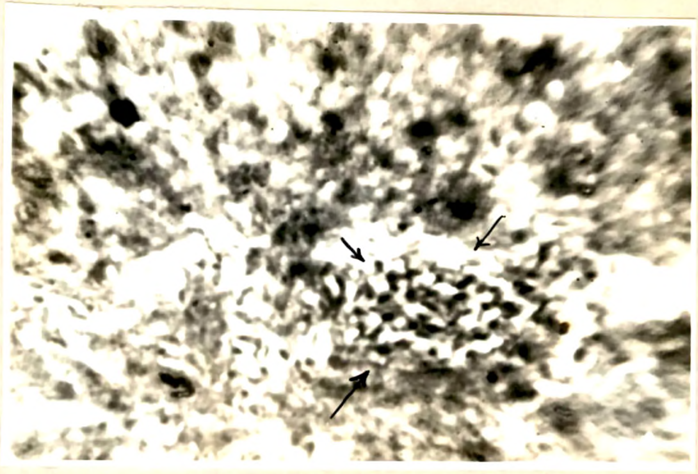


Fig.6. Microphotograph showing the pycnotic nuclei (arrow) of erythrocytes all clustered together in the sinusoids of the liver of Rosy Pastor during post migratory period. 500x.

stem cells. In the postmigratory period, however, the bone marrow showed a normal pattern with numerous fat cells, occupying the spaces where originally the active erythropoietic loci were present.

The liver of Rosy Pastors showed some peculiar characteristics at the time of their (birds') arrival(July-August) in India. The sinusoidal spaces were found to be enlarged lodging large number of erythrocytes (Fig.5). In some such aggregation of erythrocytes, majority of the red cells were observed to be in a disintegrating stage. In others the nuclei were seen detached from the cytoplasm, clumped together and were pycnotic (Fig. 6).

In the premigratory stage, in accordance with an elevated erythropoiesis, a corresponding increase in haemoglobin (Hb) content of the blood was also recorded (Fig.2). By the first week of April it was found to be 16.5 gm/100 ml of blood, which increased to 17.5 g/100 ml by the end of the month. In the postmigratory period the amount of Hb was 14 to 15 g/100 ml of blood.

DISCUSSION

Massive collection of erythrocytes observed in the liver sinusoids of birds collected in August (postmigratory period), suggests that the liver may act as a temporary erythrocyte storing organ. This storing of cells is usually done by the spleen. But this happens only in the cases of mammals, wherein the spleen has smooth muscles and is contractile

(Barcroft,1925). Klemperer (1938) reported that the spleen of birds consisted of thin capsule with few muscle fibres and has only a slight power to contract. But, Harman et al.(1932) ascribed an erythrocyte storage function to the spleen of chicken. Sturkie (1943), however, could not obtain the same results. In the spleen of Rosy Pastors also, such red cell storage was not seen as on no occasion a large collection of erythrocytes either in the morning or in the evening were found in it. It also lacked smooth muscles. Since, in human beings, the spleen does not work as a storing organ.(Lewis,1930; Bierman et al.,1953), most of the erythrocytes, are in circulation (Ross and Chapin, 1942; Hahn et al.,1942; 1943). In this bird also the same could be the case. As the spleen is relieved of a storing function, in conditions wherein such a need arises, the liver with its vast vascular channels could function as a temporary storing site. The enlargement of hepatic sinusoids may then be an adaptation to accomodate a large number of erythrocytes which are not necessary for the normal functioning of oxygen transport in the postmigratory period. This could be the reason for the sudden decrease of erythrocytes in the Rosy Pastors, just after their arrival in India from their breeding grounds abroad.

The stored cells were also found to undergo disintegration during this period. The function of blood cell destruction is also usually attributed to the spleen but it too, here, occurs in the liver. In case of mammals this is true as large amounts of bilirubin could be detected in the spleen. Sturkie (1954) reported that the avian spleen may not function as an erythrocyte destructing organ since he could not observe appreciable amounts of bilirubin therein. More over, Krumbhar (1928) opined that in birds the liver is the main site for such a function. This could occur in one or more ways. The red cells break up into smaller and smaller fragments and then finally get absorbed by the cells of reticuloendothelial system. Disintegration could also set in after they are phagocytosed by the histiocytes of the liver or phagocytes of the spleen (Sturkie, 1954). In the former case, the iron would be liberated in the blood which would be taken up by the transferrin (the iron binding protein in the blood). In the latter case, the reticuloendothelial cells would show the presence of iron (Bothwell and Bradlow,1960; Finch and Finch,1955). As described in the previous chapter, iron deposits were not found in histiocytes (Kupffer cells) of the liver. This denotes that phagocytosis of intact red cells as such is not taking place, but only the particles get absorbed releasing the iron to the blood. As mentioned earlier (Chapter 4) the increase in iron content towards the end of August could be due to an increased haemolysis which could take place in the liver.

Though it is obvious that increased cell destruction takes place in the postmigratory period, the cause remains obscure. It was seen that environmental factors such as oxygen tension and temperature have profound influence on the packed cell volume or on the erythrocyte number. In man and domestic animals, a decrease of packed cell volume was observed when the environmental temperature was raised by 15-20° C and a lowering of the temperature, similarly increased the cell number (Spector, 1956). Thus an inverse relationship exists between temperature and erythrocyte number. Since Rosy Pastors migrate from a temperate zone (breeding grounds) to a tropical one (wintering area), they face an increase in the ambient temperature at least by 15 to 20°C . This change in the environmental temperature might be the reason for the observed decrease in R.B.C counts. The accelerated cell destruction must be then a compensatory step to bring down the number of circulating erythrocytes from the persisting high level during migration.

A similar explanation would not be void in interpreting the gradual increase of erythrocytes during the premigratory period. Actually, a rise in ambient temperature was observed in this period (March/April). This naturally would have caused a further reduction in the cell number. Since, the reverse was the case, the factors bringing about such a rise in R.B.C count would then be other than environmental. As physiological changes may reflect upon haematopoietic system, it could be assumed that these changes occurring in Rosy Pastor prior to migration might have influenced the blood cell production. The necessity of an increased rate of oxygen supply required during migration, also could activate the production.

Hormonal factors too, should be considered in this connection, since, thyroxine, testosterone and prolactin were found to activate bone marrow function (Rall et al.,1964). Hypophysectomy in rats caused marrow hypoplasia with decreased erythropoiesis and haemoglobin levels (Meyer et al.,1940; Vollmer et al.,1942). A combined thyroidectomy and adrenalectomy resulted in an anaemia stimulating hypophysectomy in rats (Crafts,1953). These observations indicate that the thyroid gland has a definite erythropoiesis stimulating function. In Rosy Pastors, the thyroid gland was found to hypertrophy and release its colloidal secretion just prior to migration (George and Naik,1964a ; in the present investigation, Chapter 8). From this it could be infered that the elevated erythropoiesis might be a thyroxine mediated manifestation. The haematopoietic activity in the bone marrow is also influenced by the anterior hypophysis, adrenal cortex and gonads, since their increased activities in the premigratory period have been confirmed in the Rosy Pastor (Naik and George, 1963; 1964; 1965a; 1965b; George and Naik,1965).

The elevated erythropoiesis was also evidenced by the changing picture of bone marrow. Relatively few immature erythrocytes (polychromatic erythrocytes) and the presence of large numbers of erythroblasts in the first week of April, gave the impression that the marrow at this time was recipient of some stimulating factor (erythropoietin?), whereby a large number of precursor cells were formed therein. By the end of April, developmental processes of erythroid cells were nearly completed as large numbers of immature erythrocytes were seen in the marrow at this time. These immature erythrocytes were in a position to develop into mature ones within 36 hours as they contained their full complements of haemoglobin (Wright and VanAlstyne, 1931). These cells then could be either in the process of entering the vascular channels or temporarily stored for release when the birds start the migratory flight.

In the postmigratory period the erythrocyte number was found to be 3 -3.5 million/cubic mm. This was increased to 4.5 - 5 million in the premigratory period. Nice et al. (1935) reported that the slate coloured junco (*Junco hyemalis*) which is also a migratory bird has the highest R.B.C count, 7.65 million/cmm. Although in Rosy Pastor, red cell values of 4.5 to 5 million were observed in the period just prior to migration, in all probability, this number might rise further during the migratory flight. The number of erythrocytes were found to increase with activity and more active a bird the higher was its R.B.C. count. Thus Hummingbird (*Chrysolampis elatis*) has a very high value 6.59 million/cmm (Nice et al .,1935) as against those of the non-fliers like the fowl (3.6 million) and Ostrich (2.19 million)(Nice et al.,1935). Therefore, an increase in R.B.C count in the premigratory period could have also been caused by the increased activity of the bird at this stage.

It could be concluded that the increased erythropoiesis in the premigratory period might be the result of one or more interplaying physiological and hormonal factors and this might be an adaptation to increase the oxygen carrying capacity of the blood. But an increase in the red blood cells alone cannot elevate the oxygen carrying capacity of the blood. A corresponding rise in the haemoglobin level should also occur. Increased mobilization of iron from the liver (Chapter 4) points out to an increased production of Hb. The determination of Hb concentration in the blood during both the post- and pre-migratory periods (Fig.2) confirmed this. This rise in the Hb level in the premigratory period (from 14.5g/100 ml of blood to 17.5g per 100 ml) is to be taken as a characteristic confined to this migratory bird alone since the Hb value of a related, non-migratory species (Common Myna) had only 14.5 g/100ml of blood in the corresponding periods.