CHAPTER 2.

HAEMATOPOIETIC NODULES AS CENTRES OF FAT SYNTHESIS IN THE LIVER OF THE MIGRATORY STARLING, STURNUS ROSEUS

In the higher vertebrates the haematopoietic part of the reticulo-endothelial system is normally found in the adult in the bone-marrow, spleen, and lymph glands (Bessis, 1956). The liver is known to be a site of haematopoietic activity during embryonic life and, under abnormal circumstances such as depressed bone-marrow function, in postnatal life also (Popper and Schaffner, 1957). In certain conditions of damage to the liver, spleen and bone-marrow, or under cicumstances of heavy loss of blood, the liver has been reported to take on haematopoietic activity (Bessis, 1956; Popper and Schaffner, 1957). In certain pathological conditions of the liver such as myeloid metaplasia (Block and Jacobson, 1950) or panmyelosis (Black-Schaffer and Stoddard, 1953), haematopoiesis is also known to occur in the liver. In these cases the changes in the bone-marrow are considered a part of a generalized mesenchymal reaction (Peace, 1953) in which the liver is believed to participate by becoming enlarged and by developing haematopoietic foci (Black-Schaffer and Stoddard, 1953; Peace, 1953).

In the chick embryo the erythrocytes and thrombocytes are the only cell types normally present in the circulating blood. The other cell types that develop within the haematopoietic organs are retained there until after hatching (Lucas and Jamroz, 1961). Dantschakoff (1908) observed that the liver of the normal chick embryo was not haematopoietic. This was in sharp contrast to the

claim of Haff (1914) that the liver does function as a site for the production of erythrocytes and granulocytes and that the endothelial cells of the liver sinusoids are the sites of origin of the different cell types. Sandreuter (1951) observed continued erythropoiesis even after hatching in the liver of the starling, Sturnus vulgaris, whereas in the chick, Gallus domesticus, it is confined to the bone-marrow as soon as blood formation in the yolk sac ceases.

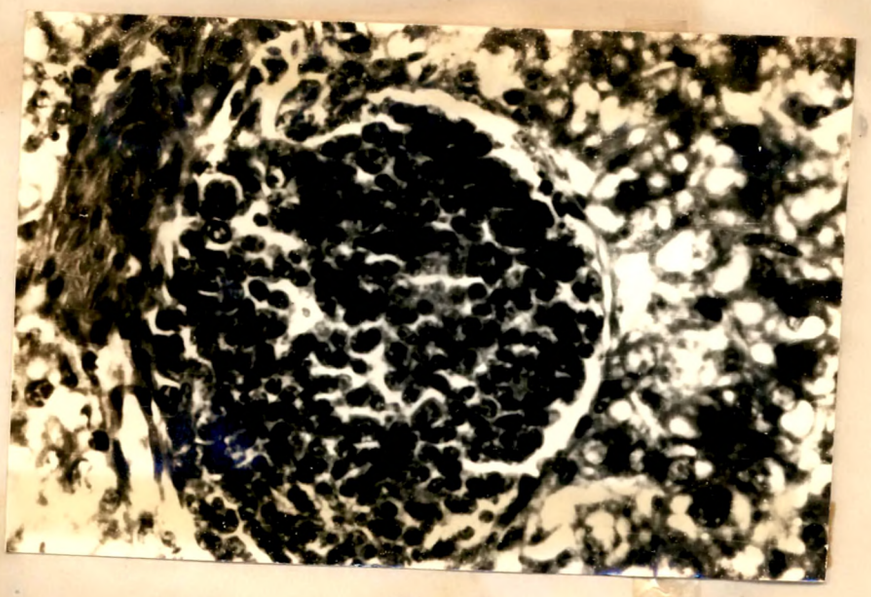
Material and Methods

Sturnus roseus comes to India about September and leaves for its breeding grounds in southern Europe in April. The birds were collected from October to April; they were either shot with an air-rifle or trapped alive in nets. In each month 4 or 5 birds were collected and histological and histochemical observations on the liver were carried out. For the histological study the liver was cut into small pieces and fixed in Helly, Bouin, Carnoy and 10% formalin. Paraffin blocks were prepared and 6 µ sections cut. The sections were stained with Delafield's haematoxylin/eosin Y/ azure II as described by Gray (1954) with the following modification. Five millilitres of 1% aqueous eosin Y and five ml of 1% aqueous azure II were added to 90 ml of distilled water to make the staining medium. After staining with Delafield's haematoxylin the slides were transferred to this mixture and left in it for 24 hours. The sections were differentiated in 96% alcohol until the cytoplasm of erythrocytes was pink, that of the lymphocytes, lymphoblasts, monoblasts, and monocytes blue to pale blue and of granular leucocytes different shades of pink. The sections were then dehydrated and

mounted in Canada balsam. Some sections were stained with Heidenhain's azan as described by Gurr (1956) for study of the connective tissue of the haematopoietic nodules. DNA was studied by the Feulgen method as described by Pearse (1960). For the histochemical study of lipids, pieces of liver were fixed in formaldehyde-calcium and embedded in gelatin (Baker, 1946); the sections were stained with Sudan Black B.

Observations

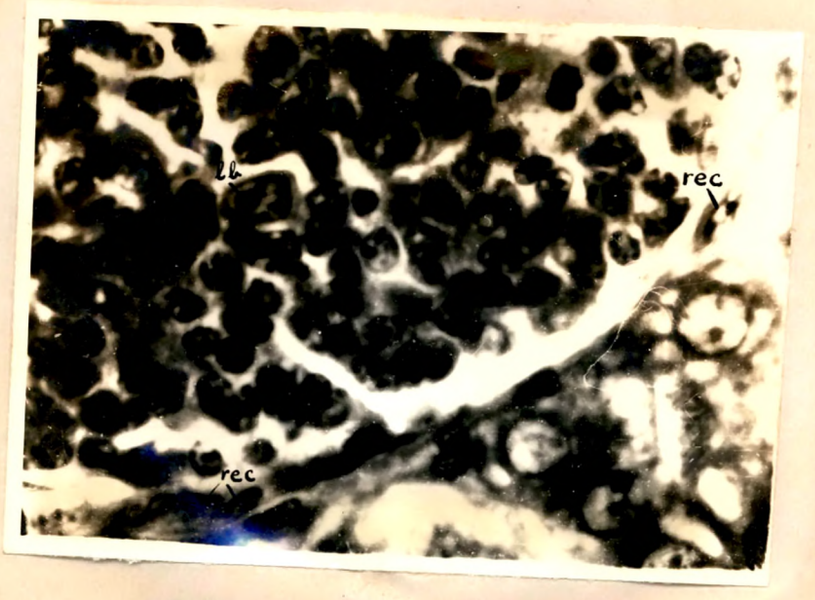
Circular or oval haematopoietic nodules were clearly seen by staining sections of the liver with eosin Y/azure II or Azan. With Feulgen, the nodules appear bright pink and stand out distinctly from the typical liver cells owing to the greater mitotic activity in them. One or more such nodules were seen in each 9 or 10 sq. mm of the section. A fully formed nodule is circular or oval, whereas the incomplete ones have an irregular shape. They are always located near or attached to a small blood vessel (Fig. 1). By means of serial sections it was found that a complete nodule had a diameter of 120 μ in two directions at right angles to each other. Azan stained blue the connective tissue sheath and the reticular net work of the haematopoietic nodule (Figs. 1, 2). The cells are long with tapering ends and possess cytoplasmic processes and a dense, oblong nucleus. The connective tissue sheath is continuous except where it is in contact with the blood vessel. In this region the sheath appears extremely thin if not altogether absent. In the vicinity lymphocytes, monocytes, heterocytes, macrophages and very rarely other granular leucocytes are



25μ

Fig. 1

Fig. 1. Section of the liver of Rosy Pa[illegible]or, passing through a haematopoietic nodule. The atta[illegible]ed blood vessel is seen to the left of the nodule. (Haematoxylin/eosin Y/azure II)



16μ

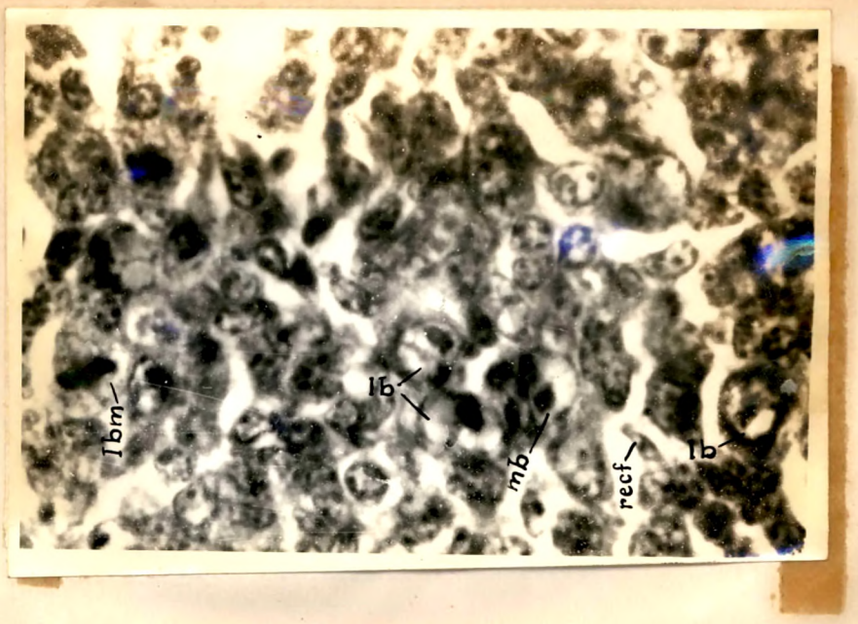
Fig. 2

Fig. 2. Magnified portion of fig. 1 showing the sheath of reticulo-endothelial cells(rec) of the nodule and the tightly packed lymphocytes. A lymphoblast(lb) is seen in the centre.

seen. Most of the erythrocytes appear to contain less haemoglobin than ordinary erythrocytes. The cytological details of the cells of the haematopoietic nodule were studied under oil immersion. The majority of the cells in the nodule were found to be lymphocytes and monocytes. Among the lymphocytes different sizes can be noticed, small, medium and large. Besides these cells there are also the large lymphocytes, monocytes and erythroblasts. There are about 50 erythroblasts, each about 10 to 16 μ in diameter, in each section that passes through the centre of a nodule. The cytoplasm of these cells is stained in different shades of blue with eosin Y/azure II. Their nuclei are very large (Figs. 2, 3). The nucleoli are very distinct in all the formative cells. The lymphoblasts contain a single nucleolus and the monoblasts two.

In some of the cells the nuclei were very lightly stained; these can be recognized as young lymphocytes. These young lymphocytes are always found in pairs and are obviously newly divided cells. The lymphocytes can be distinguished from monocytes without much difficulty because the nuclei of the latter are kidney-shaped and slightly larger than those of lymphocytes. The cytoplasm is also more abundant than that of the lymphocytes. The young monocytes were found to have more cytoplasm than their adult forms.

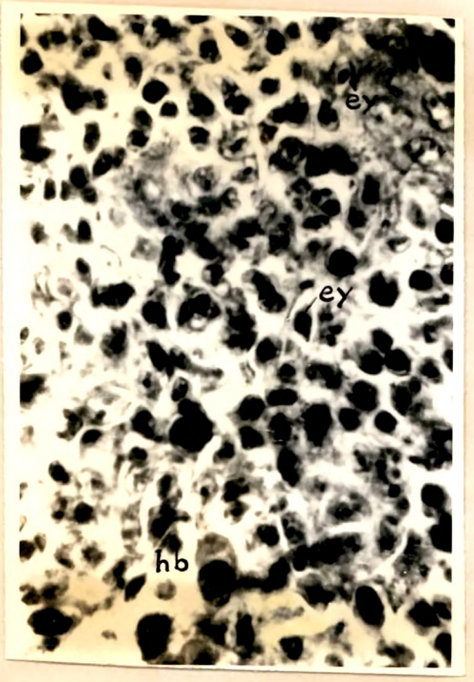
A few haemocytoblasts were found within the nodules(Fig. 4). They measure 12 to 18 μ in diameter. The nucleus is often located on one side of the cell and the cytoplasmic mass shows its amoeboid nature. The nucleus is round or oval with either two or three nucleoli. The chromatin can be distinctly seen as a beaded structure.



11 μ

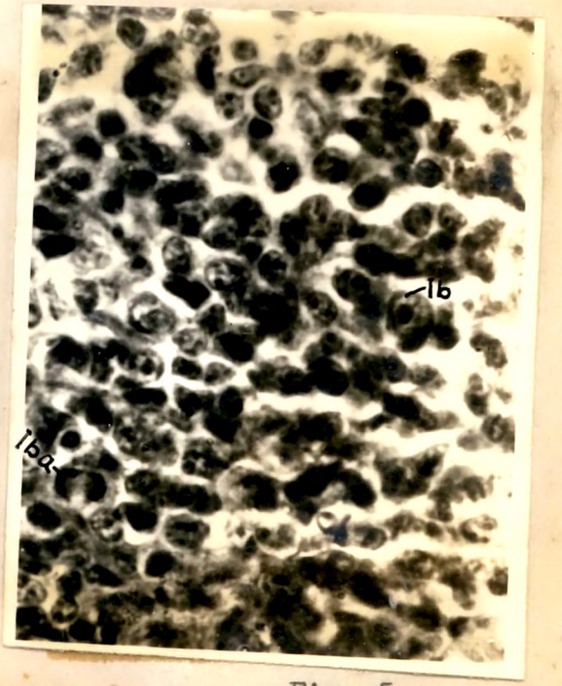
Fig. 3

Fig. 3. Section of the liver showing the developed nodule with lymphoblasts(lb), monoblast(mb), and a free moving reticulo-endothelial cell(recf). Note the metaphase of a lymphoblast (lbm), ans also the cytoplasm and nucleoli of the resting lymphoblasts and monoblasts.



20 μ

Fig. 4



16 μ

Fig. 5

Fig. 4. Section of the liver with a developed nodule showing the young erythrocytes (ey), the haemocytoblasts(hb), and lymphocytes and monocytes.(Haematoxylin/eosin Y/azure II)

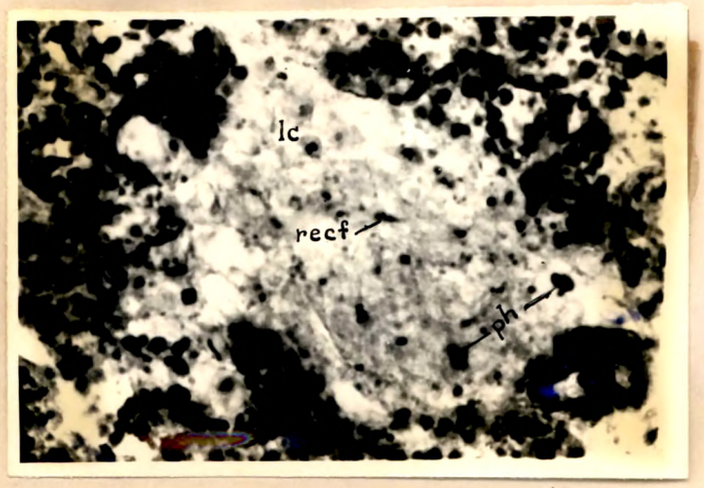
Fig. 5. Section of the developed nodule showing the different types of cells and mitosis. Note lymphoblasts (lb); anaphase of lymphoblasts(lba). (Azan)

The cell boundary stains deeply and is basophil. The haemocytoblast that would subsequently become an erythroblast shows a characteristic intense violet basophilia in its cytoplasm.

There are a few immature erythrocytes in the nodules(Fig.4). They contain little haemoglobin and taper at the two ends; this suggests an amoeboid nature. The nuclei of the immature erythrocytes are circular or oval instead of oblong. According to Blount (1939) a thrombocyte is an erythrocyte in which the process of haemoglobinization of the cell has not proceeded to maturity. Young erythrocytes of this kind are also found outside the connective tissue lining of the haematopoietic nodule, as well as in blood smears. A fully developed erythrocyte in a blood smear measures about 12.5 μ in length and 6.2 μ in breadth.

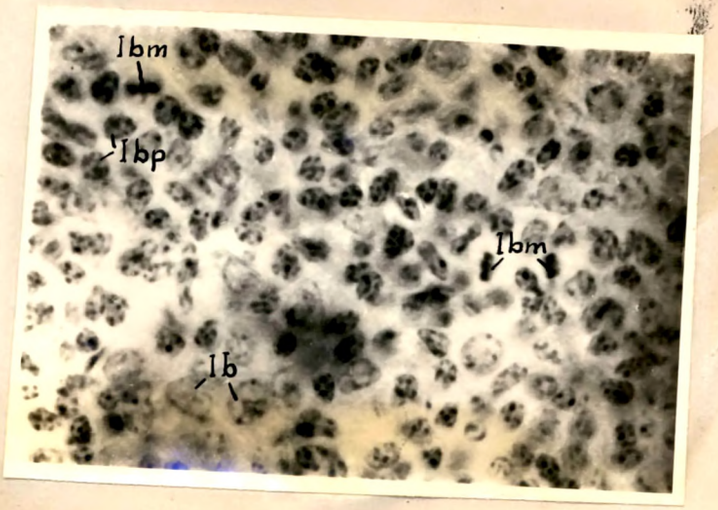
Some free-moving reticulo-endothelial cells, much smaller than the Kupffer cells, are also seen in the central portion of the nodule.

The parenchymatus cells of the liver are uniformly granular and loaded with fat. There is less fat in the haematopoietic nodule (Fig.6). Some of the cells of the nodules, distinct from the others, are, however, loaded with fat. The free-moving reticulo-endothelial cells as well as the phagocytes show quite a different type of localization of fat. In these cells fat is present as fine, dense globules, packed together in large masses. In lymphocytes and monocytes it is evenly distributed as fine globules. The rest of the cells in the nodule show fewer globules.



21 μ Fig. 6

Fig. 6. Section of the liver passing through a nodule, showing the distribution of lipids. Note the greater deposition of lipid in the phagocytes (ph), lymphocytes(lc), and free-moving reticulo-endothelial cell(recf). (Sudan Black B)



20 μ Fig. 7

Fig. 7. Section of the liver passing through a nodule. Note the lymphoblasts (lb), with prophase (lbp) and metaphase of lymphoblasts (lbm). (Feulgen)

The reticulo-endothelial cells of the sheath did not contain fat globules.

Preparations made by the Feulgen and by other staining techniques revealed a considerable amount of mitotic activity in the nodules (Figs. 3, 5, 7).

Discussion

Haematopoietic nodules are well developed throughout the period from October to April. Sandreuter (1951) observed that in Sturnus vulgaris haematopoiesis continued in the liver for some days after hatching and that the formation of erythrocytes was most intense during the first five days after hatching, but gradually ceased between the 10th and the 20th days. Granulocytopoiesis was found to be reduced after the fifth day and the lymphoid elements, including the monocytes, increased after the 10th day. In Sturnus roseus it is possible that the liver continues its haematopoietic activity from the embryonic stage. However, there is no opportunity to verify this, since this bird does not breed in India.

From the present observations it appears that some of the lymphocytes and monocytes captured in the sinusoids of the liver undergo further development there and then the sinusoids develop into nodules. Bloom (1937) claimed that the small lymphocytes become transformed into eosinophils, myelocytes, and granulocytes. Downey (1948), from histological studies, concluded that granulocytes develop from small lymphocytes in the thymus of the rabbit and man. Jordan (1936, 1939) suggested that blood lympho-

cytes enter the bone marrow where they turn into erythroblasts and eventually into erythrocytes. Yoffey (1956) stimulated erythropoiesis and found in the bone-marrow an increased number of lymphocytes and also many cells in all the stages of the transition from small lymphocytes to erythroblasts. There is good evidence that lymphocytes can become transformed into monocytes and macrophages under certain conditions, particularly in inflammation (Bourne and Danielli, 1958). Harris (1956) studied the bone-marrow of guinea-pigs recovering from X-irradiation and found a marked accumulation of lymphocytes together with many cells which appeared to be transitional between lymphocytes and typical precursors of myeloid and erythroid cells. If, as is now believed, the large lymphocytes develop from reticulo-endothelial cells, this transformation of small lymphocytes to macrophages represents a sort of lymphopoiesis. This suggests that reticulo-endothelial cells become transformed into lymphocytes and conversely (Trowell, 1955, 1957). In the present study it has been shown that some of the sinusoids of the liver have many lymphocytes, some monocytes and few granular leucocytes. Some of the sinusoids are packed with newly formed cells of different types, whereas in others the cells are fewer and not closely packed. The sinusoids that are fully packed with cells have a regular shape, either oval or circular in section, while the others have a rather irregular outline (Fig.1). After entering the empty sinusoids from the blood stream the lymphocytes and monocytes develop into reticulo-endothelial cells and then differentiate into haemocytoblasts and histocytoblasts and finally into lymphocytes, monocytes, granular

leucocytes and erythrocytes.

In the liver of the Rosy Pastor, small developing haematopoietic nodules are perhaps being continuously formed according to demand. Ultimately these small nodules would develop into full-sized nodules, with different mother cells giving rise to their respective cell types. As a result of the rapid formation and growth of the blood cells in the haematopoietic nodules and of the pressure exerted on the sheath of the nodule, the nodule becomes distended, and the adjacent parenchymatus cells of the liver are moved away, thereby allowing the nodule to attain an ovoid or spherical shape. When the nodule has attained full growth, the various cells enter the blood stream by way of the adjacent blood vessels. Most of the cells produced from these haematopoietic nodules are lymphocytes and monocytes. The reticulo-endothelial cells of the haematopoietic nodular sheath might have given rise to various types of blood cells by mitosis at the beginning, but it is unlikely that in the fully formed nodule the sheath cells were the main source of the blood cells.

The free-moving reticulo-endothelial cells showed fine, dense globules of lipid in the cytoplasm (Fig. 6). Some of the lymphocytes and monocytes also showed a considerable amount of lipid, while the other cells of the nodules contained very little. Perhaps the fat which is synthesized by the lymphocytes, monocytes and other granular leucocytes (Elsbach, 1959; Buchanan, 1960; Marks, Gellhorn and Kidson, 1960; Rowe, Allison and Lovelock, 1960) is carried out by the free-moving reticulo-endothelial cells into

the blood stream. These free-moving reticulo-endothelial cells may also synthesize fat (Day and French, 1959; Day, 1960; French and Morris, 1960), but I did not detect fat in the reticulo-endothelial cells of the connective tissue sheath of the nodule. It therefore seems that the free-moving reticulo-endothelial cells and the phagocytes are responsible for the transport of fat rather than for its synthesis. Petrikis, Davis and Lucia (1961) found that <u>in vitro</u> the nongranular mononuclear leucocytes (lymphocytes and /or monocytes) appear to hypertrophy into cells having the form of macrophages and histocytes. These cells appeared to have a variety of forms and were designated as 'polyblast macrophages'. Ultimately these would form the fibroblasts, but in several instances they were found to become transformed into fat-cells. In histological preparations most of these phagocytes were found towards peripheral regions of the nodule, towards the blood vessel. Probably such phagocytes, after accumulation of fat, may enter the circulation and after reaching the fat-depots become converted into fat-cells. Chang (1940) has reported the transformation of the histocytic macrophages of the peritoneal cavity into fat-cells. From histochemical and quantitative studies on the fat in the liver (Chapter 1), it is known that fat is uniformly distributed in the parenchymatous cells in the form of fine droplets: these are not fat-cysts. According to Hartkoft (1961) fat-cysts are only seen in definitely fatty livers. Even though a certain increase in the fat content of the liver is seen, it should not be considered as a pathological change, as there is an overall increase in the fat content of the various

tissues of the body of the Rosy Pastor during the period of its residence in India. The fat which is synthesized by the haematopoietic nodule is continuously removed by the phagocytes and the free-moving reticulo-endothelial cells; the nodule can thus continue to synthesize more fat.

From the above histological and histochemical observations it is clear that the liver of this bird is not comparable to one in the condition of fatty liver disease (Popper and Schaffner, 1957) since there are no fat-cysts and no excess fat deposition and the lipid granules are uniform in shape and size. The Azan stain does not reveal any distortion of the nodular architecture nor any increase in connective tissue. There is no fatty infiltration or fatty degeneration such as has been described in man (Popper and Schaffner, 1957; Virchow, 1961). No scars of bacterial infection could be seen and there is no possibility of this being a case of liver sarcoma. The histological study of the liver did not show any sign of dead hepatic cells with negative or pycnotic nuclear staining. It must, therefore, be concluded that the liver of this bird shows normal haematopoiesis.

Summary

1. An histological and histochemical study of the haematopoietic nodule in the liver of the Rosy Pastor was made from October to April. The formation and development of the haematopoietic nodule by aggregation of lymphocytes and monocytes was found to be a continuous process, in which new nodules are formed regularly.

2. The older nodules after a certain period of high activity show a considerable decline in the activity of blood cell formation. The fully formed haematopoietic nodules give rise to different types of blood cells - erythrocytes, granular leucocytes, lymphocytes, and monocytes.

3. During the premigratory period the lymphocytes and monocytes were found to be more numerous in the nodules. It is likely that the number of erythrocytes increases during migration and that the formation of different types of blood cells depends upon demand.

4. It is concluded that fat is synthesized in the lymphocytes, monocytes and free-moving reticulo-endothelial cells, but that this synthesis is mainly carried by the reticulo-endothelial cells. The phagocytes may also be the carriers of fat and it is suggested that they are later modified into fat-cells in the fat-depot.

5. Haematopoiesis in this bird is a normal phenomenon occuring in the liver in all seasons and is not a pathological condition.