CHAPTER 1

DEVELOPMENT OF LYMPHOCYTOPOIETIC NODULES IN THE LIVER OF ADULT PIGEON

Liver is a haematopoietic centre atleast in the embryonic period in many vertebrates, but lymphocytopoiesis in this organ is seldom noticed. It is a well known fact that the adult liver has no haematopoietic function which means that when the other sites become competent to meet the demand, the liver suspends this activity (Barker, 1968). However, it should be realized that adult liver never loses the potentiality of producing blood cells. There are reports of haematopoiesis in the adult liver when additional demands of blood cells are to be met with or when the usual site like bone marrow gets damaged (Popper and Schaffner, 1957). Though this is essentially a pathological condition, cases of normally occurring haematopoiesis in the adult liver are not altogether lacking. Recently, George and Naik (1963) reported that nodules containing developing blood cells (predominantly lymphocytes) were found in the liver of adult migratory starlings (Sturnus roseus). In an attempt to find whether such nodules are present in other species of birds, Pilo (1967) noticed that the pigeon liver

too has such haematopoietic nodules which according to him were exclusively lymphocytopoietic in nature.

The presence of haematopoietic nodules in the liver of these two birds viz., Sturnus roseus and Columba livia, reported by George and Naik (1963) and Pilo (1967; 1970) respectively, was considered by them to be a commonly occurring normal phenomenon and not a pathological one. However, no mention was made by them about the development of such nodules in the liver of any of these birds. Hence, the present study was undertaken to further the knowledge about these nodules especially their development in the pigeon liver.

MATERIAL AND METHODS

Liver pieces, from healthy adult pigeons (domesticated variety of blue Rock Pigeon, Columba livia), reared in aseptic conditions and fed standard balanced diet, were removed; fixed in suitable solutions and frozen as well as paraffin sections were cut. For the frozen sections, 10 % neutral formaline and calcium formol were used as fixatives and sections of 10 to 15 μ thickness were cut on a freezing microtome. These sections were stained with haematoxylin and mounted in glycerine jelly. This way the general characteristics of

the nodules was well preserved as distortion due to shrinkage was very much minimized. For histological studies, pieces of liver were fixed in Bouins fluid and embedded in wax. Sections of 5 to 8 μ thickness were cut and stained with haematoxylin and eosin. Identification of lymphocytes was done by staining the sections, cut from formol-saline fixed and paraffin embedded tissue, with Jenner-Giemsa stain as described by Gurr (1956).

OBSERVATIONS AND DISCUSSION

Large number of nodules of varying shape and size were observed in the liver of all the individuals studied. The variation noticed in the size and shape of nodules was due to the different stages of their development. The nodules were seen always in the vicinity of the portal areas of the liver lobules and invariably developed in close association with the blood vessels (Fig. 2). Young nodules in the beginning were usually irregular in shape (Fig. 1) but later they become rounded as their cells aggregated together (Fig. 2). Further during the development of these nodules, simple enlargement of these may take place by the increase in number of the cells. Once the nodules become rounded they get surrounded by connective tissue fibres. Such

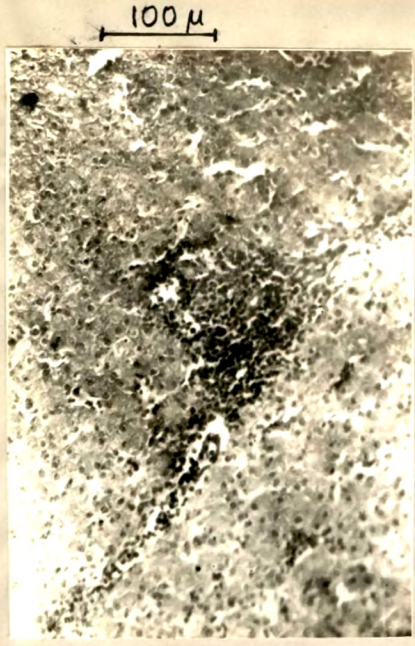
(Chapter 1: Figs. 1 to 4. Photomicrographs of the liver of pigeon showing various stages in the development of lymphocytopoietic nodules)

Fig. 1. A developing nodule. Note the irregular shape of the nodule without a connective tissue boundary.

Fig. 2. A nodule attached to the portal space in the liver. Note the proximity of an artery (A), vein (V) and the bile duct (B). The nodule is surrounded by a thin layer of connective tissue (arrow).

Fig. 3. A immature nodule with 'germinal centre' where two zones, a 'dark zone' (D) and a centrally situated 'light zone' (L) are seen.

Fig. 4. A mature nodule with the 'germinal centre' (G). Note the large blast cells and macrophages inside the centre (G).



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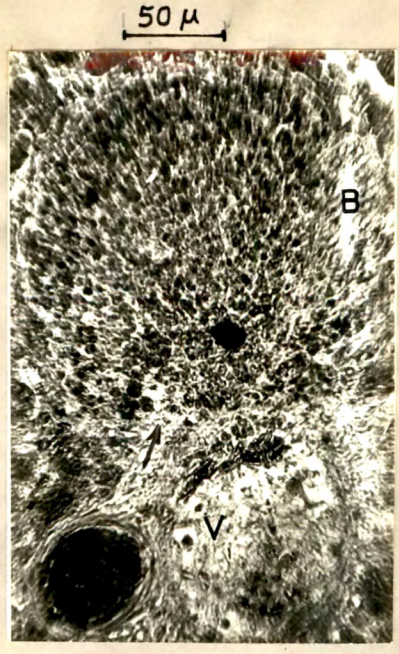

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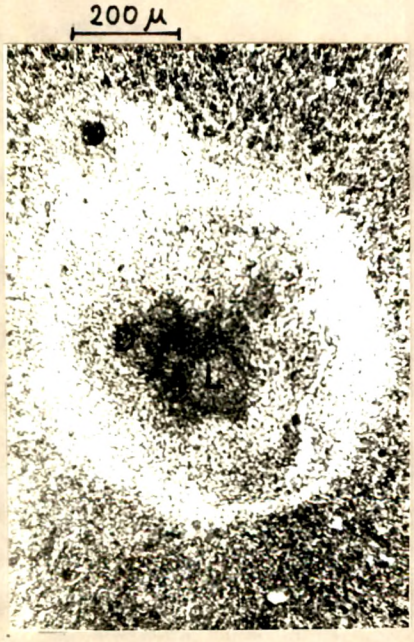

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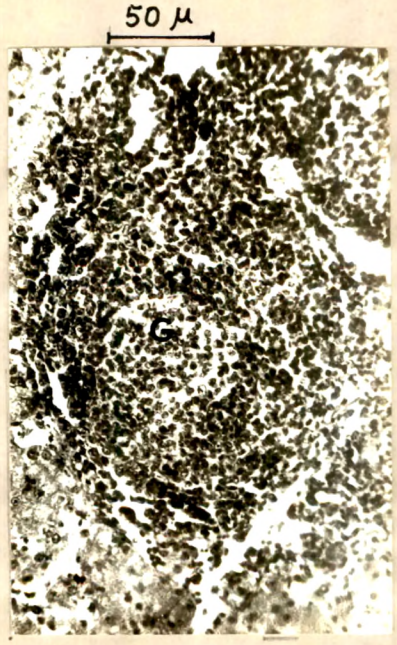

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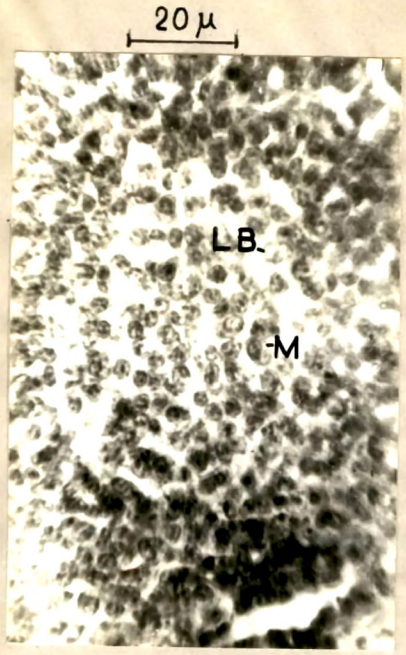
(Chapter 1: Figs. 5 to 7. Photomicrographs of the pigeon liver showing various stages in the development of lymphocytopoietic nodules)

Fig. 5. Magnified portion of Fig. 4, showing the macrophages (M) and lymphoblasts (LB) in the 'germinal centre'.

Fig. 6. A mature nodule, with a connective tissue covering in the process of liberating the lymphocytes. Note the proximity of the blood vessel (BV) and the connective tissue extension between blood vessel and nodule (CE).

Fig. 7. Mature nodules in the same connective tissue boundary. Note the number of nodules (shown by the arrows) inside the same connective tissue capsule. The lymphocytes have already started disappearing, leaving loose cells behind (see inset).



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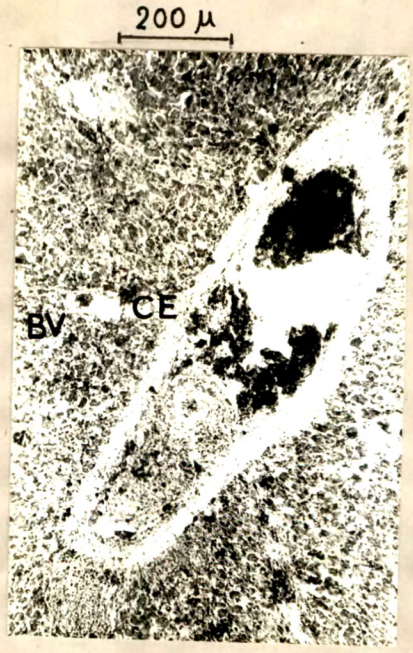

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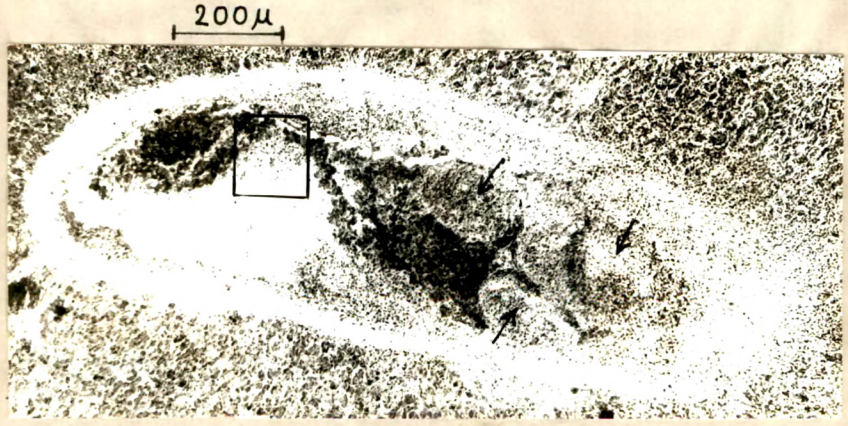

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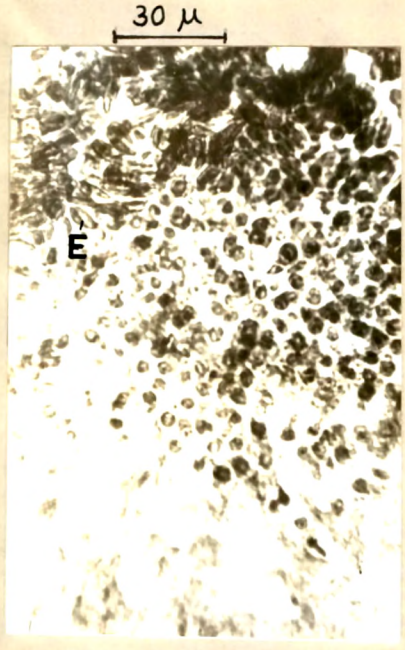
(Chapter 1: Figs. 8 and 9. Photomicrographs of the pigeon liver showing the various stages in the development of the lymphocytopoietic nodules)

Fig. 8. A portion of the Fig. 7 (inset) magnified to show the loose lymphocytes and the erythrocytes (E).

Fig. 9. A mature nodule, where only a few lymphocytes adhering to the connective tissue and mature erythrocytes are only seen. The central blank area denotes the space left after the liberation of lymphocytes.



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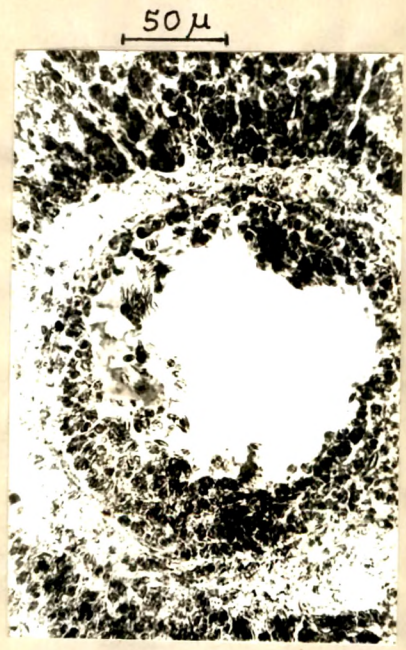

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encapsulation is a characteristic of fully developed (mature) nodules (Fig. 2). At times a few nodules were seen encased in a single connective tissue covering (Fig. 7).

In a fully developed (mature) nodule, the central area was usually found different from the peripheral one in having fewer and larger cells (lymphoblasts) (Figs. 3, 4 & 5). This region was comparable to the 'germinal centre' observed in lymphoid organs such as spleen and lymph nodes. The presence of dividing cells and also the lymphoblasts could easily vouche for the contention that this central region in a nodule is the site of lymphocyte proliferation as originally suggested by Flemming (1885) with regard to the 'germinal centre' in lymph nodes. Yoffey et al., (1958) also noted that the presence of 'germinal centres' in the mesentric lymph nodes of guinea pigs coincided with the increased output of lymphocytes through the thoracic duct. But it is generally accepted that 'germinal centres' are concerned with the production of antibodies rather than that of lymphocytes (Elves, 1966). The experimental studies of Ehrich (1929a & b), Habel et al., (1949), Harris and Harris (1949), and Marshall and White (1950), showed that the appearence of the 'germinal centres' in the lymph nodes was corresponding to the appearence of antigen in the blood.

Ehrich (1929) who did not regard the 'germinal centre' as the site of lymphocyte production, suggested that the lymphocytes are formed from the walls of the blood vessels where the 'pseudosecondary nodules' are formed. Autoradiographic studies also showed that such 'centres' are not concerned with the production of new lymphocytes (Murray and Murray, 1964; Yoffey et al., 1959). However, the active proliferation of cells in the 'centres' was also proved by the increased uptake of ^{32}P (Gyllensten, et al., 1956).

It is also known that, whatever be the stimulation (which may be antigenic, non-specific or regenerative) the 'germinal centres' may show active proliferation of cells (Hill and Pospisil, 1960). This may be true in the case of nodules in the pigeon liver, where it has been observed that injection of foreign particles as well as toxic substances caused the appearance of large number of nodules in the liver (Chapters, 3 & 4). Total and subtotal splenectomy also showed increased hepatic lymphocytopoiesis (Chapter 3). Thus, an increased demand of lymphocytes as well as the presence of toxic substances and antigens in the body appear to influence an increase in the number of nodules in the liver. Hence, it could be stated that the nodules which develop in the liver of pigeon are in a

position to meet both the demands *viz*., of more lymphocytes and antibodies.

There is a certain degree of similarities in the production of antibodies and lymphocytes in the nodules as the production of both could be stimulated by the same factor. When the cells that have ingested the antigen (macrophage or transformed lymphocytes) liberate their cellular content at the 'germinal centre' in a nodule, the formation of antibody in the nodule is stimulated. It is also known that the presence of cell debris activates the cell proliferation in the 'germinal centres' of the splenic nodules (Hill and Pospisil, 1960). Hence, for both antibody and lymphocyte production certain amount of cellular debris, especially that of lymphocytes and that too of their nuclear components, could become a stimulating factor.

As stated earlier, the presence of large cells (lymphoblasts) in the 'germinal centre' indicates the proliferative activity in this area. Apart from this, the presence of a 'dark zone' surrounding a 'lighter one' in the 'germinal centre' in the developing nodules in the pigeon liver (Fig. 2) supports the observations of Rohlich (1933) who had also distinguished similar two different zones in the 'germinal centres' of the lymph nodes, *viz*., a dark mitotic zone and a light phagocytic one.

He regarded the dark zone as the "germinative portion of the centre" while the 'light one' as the site of "reaction to noxious substances". In the pigeon liver nodule; such demarcation of 'germinal centre' into the zones, was seen only in the young developing nodules (Fig. 3), whereas in the comparatively mature ones (Fig. 4) only the 'light zone' (which usually contained lymphoblasts and phagocytes) was present. From these observations it could be reasoned out that, the mature nodules in pigeon liver may have an immunological or phagocytic function, as only the 'light zone' is present in their 'germinal centre' while in the immature nodules, active proliferation of lymphocytes may also take place as these nodules also contain 'dark zones'.

The sequence of events during the development of nodules could be thus surmised as follows: Some blast or stem cells, either produced by the liver itself or migrated from some other regions, with the potentiality to divide, get attached to the portal areas in the liver lobules and proliferate to form a lymphocytopoietic nodule by pushing aside the liver parenchymal cords. (Nodule of this stage is referred to as a developing one). Then, gradually connective tissue covering is formed around the aggregated mass of cells of the nodules (referred to as developed or mature nodule). In immature nodule a 'germinal centre' is seen, where two

zones; a dark zone and a light one, are present. In the mature nodule only the light zone is present. Such nodules remain in the liver for some period (not determined), assisting in the phagocytic and immunological reactions of the body. Afterwards, the nodules liberate the lymphocytes into the blood stream and finally they themselves disappear.

The liberation of lymphocytes from these nodules was difficult to understand. The close proximity of blood vessels to the nodules (Fig. 6) may suggest an easy passage for the cells ~~from~~ to enter the blood stream. Many nodules were found to be in the process of lymphocyte liberation (Figs. 6 & 7). Since the liberation of cells was from the central region (Fig. 9) some connection should exist between the nodules and blood vessels. Such a suggestion could be envisaged in view of the fact that the connective tissue covering of the nodule was seen extending towards the blood vessel (Fig.6), as well as by the presence of mature erythrocytes inside the nodules at this stage (Fig. 8). It is also likely that the lymphocytes may gain direct entry into the circulating lymph itself. There are some other possibilities of lymphocytes gaining entry into the liver sinusoids which may be through the loose connective tissue, by diapedesis. Like the lymphoid nodules in the regular sites such as

spleen and lymph nodes, continuous liberation of cells could not be envisaged in the case of hepatic nodules in the pigeon. Here, once the cessation of cell proliferation takes place, and all the lymphocytes become mature, they are liberated from the nodule and finally the nodule itself disappears.