CHAPTER 4

PHAGOCYTOSIS OF CELLULAR DEBRIS BY THE CELLS OF THE LYMPHOCYTOPOIETIC NODULES IN THE PIGEON LIVER

The presence of lymphocytopoietic nodules in the liver of adult pigeons (Pilo, 1970; Chapter 1) and in that of migratory starling, Sturnus roseus (George and Naik, 1963) is an interesting feature in view of the fact that such lymphocyte producing activity in the liver is seldom noticed in the adults of higher vertebrates. Lymphocytopoietic function of liver in pigeon and starling is all the more interesting when it is known that the bone marrow of active fliers is not lymphocytopoietic (Jordan, 1936). Further, in Chapters 1 & 3 it is suggested that the cells of these nodules in the pigeon liver are in a position to meet the demand of phagocytic activity as well as antibody production. In the present study, the ability of the cells of lymphocytopoietic nodules in the pigeon liver, to phagocytize the cellular debris has been investigated.

MATERIALS AND METHODS

Healthy adult pigeons (domestic variety of Blue Rock Pigeon, Columba livia), reared in laboratory

conditions were selected for the experiments. Two ml of blood, collected from healthy adult pigeons and then haemolysed, was injected intravenously in each of a group of fifteen pigeons. No repeated injections were given.

In another group of fifteen birds, a part of liver of each one was subjected to an irreversible injury by application of high pressure. This was done through an incision made on the lateroventral side of the abdominal wall just posterior to the sturnum. Operations, under sterile conditions, were carried out on ether anaesthetized birds. The part of liver when subjected to high pressure becomes irreversibly injured which provides abundant disintegrating liver and blood cells at the site.

At certain intervals viz., on 1st, 2nd, 3rd, 6th and 9th day after the intravenous injection of haemolysed blood or infliction of high pressure injury on the liver, three pigeons from each of the two groups were sacrificed by decapitation under mild anaesthesia. Their liver and spleen were removed and fixed in formol saline. Frozen sections of 10 to 20 μ thickness were taken on a freezing microtome. Some of these sections were stained with Jenner-Giemsa stain (Gurr, 1956) for the identification of lymphocytopoietic nodules in the liver and spleen, while the adjacent sections of both the tissues were mounted in glycerine jelly without staining, for the observations

of ingested cell debris. The presence of erythrocyte fragments was confirmed from the sections of 10% formaline fixed tissues, stained for iron using the Perl's Prussian Blue method (Pearse, 1960). The nomenclature of different stages of nodules in the pigeon liver suggested in Chapter 1 was followed in this study.

OBSERVATIONS

Phagocytosis of blood cells:

Phagocytized blood cell debris could be detected inside the cells of the lymphocytopoietic nodules of the liver and spleen as brown particles. These particles could be easily identified as the fragments of erythrocytes as they were stained dark blue with Perl's Prussian Blue method, indicating the presence of iron in them. The presence of such ingested particles in the liver and spleen nodules was detectable only by about 3rd day after the injection. This time-lag is perhaps due to delay in the appearence of complement factors or antibodies in the blood which eventually opsonize the injected foreign red blood cell fragments. At this stage (3rd day) a good number of "developing nodules" in the liver showed the ingested cellular debris distributed uniformly inside them (Fig. 1). In the 'mature nodules'

larger concentration of brown particles was found to be localized in the peripheral regions (Fig. 2). However, in these 'mature nodules' the cellular debris, in lesser concentration, was also seen inside the "germinating centres" (Fig. 2). The splenic nodules when examined at this period (3 days after injection) also showed appreciable amount of cellular debris. By about 6th day after the injection of haemolysed blood, the phagocytic reactions in the nodules of liver and spleen reached a maximum level. At this stage almost all the nodules in the liver had taken up large amount of fragments of injected blood cells (Fig. 3). The splenic nodules, where the phagocytic reaction was more profound by 6th day compared to that in the nodules of the liver, showed the ingested particles mainly localized in their peripheral regions (Figs. 4 & 5). These ingested particles inside the nodules of the liver and spleen disappeared by 9th day after the injection (Figs. 6 & 7).

Phagocytosis of injured liver cells:

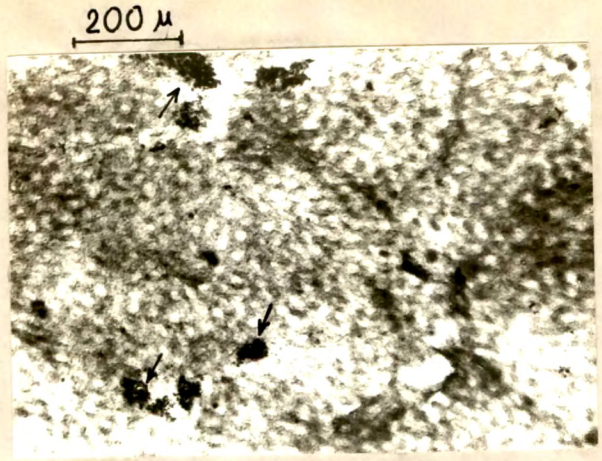
Since, application of high pressure causes irreversible injury (see Tsanev, 1963) to the hepatic cells, these injured cells must be removed from the site. It was observed that a large number of lymphocytes appeared near the injured site in the liver and got

(Chapter 4: Figs. 1 to 3. Photomicrographs of the liver of pigeon showing the ingested cellular debirs in the lymphocytopoietic nodules)

Fig. 1. 3 days after the injection of the haemolyzed blood. Many nodules show cellular debris in the form of brown particles (arrows).

Fig. 2. 3 days after the injection of the haemolyzed blood. A big round 'mature nodule' with 'germinal centre' (G). The ingested particles (arrows) are seen inside the 'germinal centre' as well as in the peripheral regions of the nodule.

Fig. 3. 6 days after the injection of haemolyzed blood. Almost all the nodules show ingested cellular fragments (arrows).



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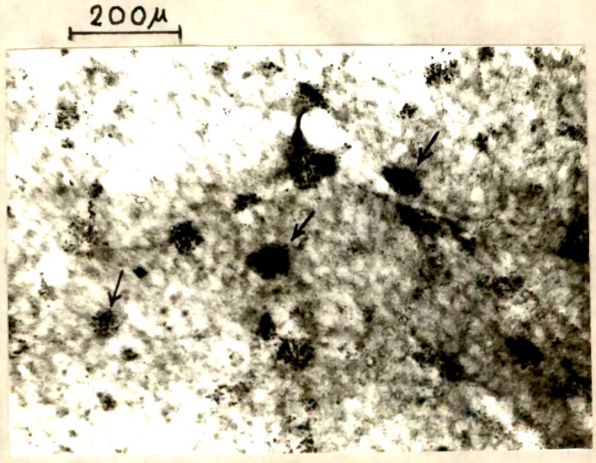

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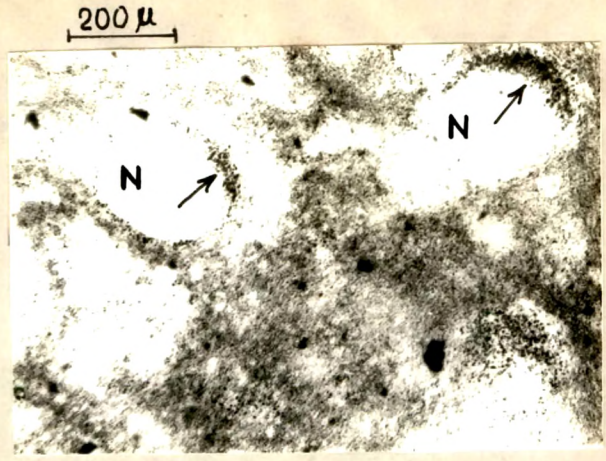
(Chapter 4: Figs. 4 to 6. Photomicrographs of spleen (Figs. 4 & 5) and liver (Fig. 6) showing the ingested cellular particles in the lymphocytopoietic nodules)

Fig. 4. 6 days after the injection of the haemolyzed blood. Spleen. The nodules (N) show greater concentration of ingested cellular debris (arrows) in the peripheral regions.

Fig. 5. 6 days after the injection of the haemolyzed blood. Spleen. Higher magnification of part of Fig. 4, showing the peripherally concentrated cellular debris (arrows).

Fig. 6. 9 days after the injection of the haemolyzed blood. Liver showing a nodule (N) devoid of the cellular debris. The nodular boundary is shown by arrows.



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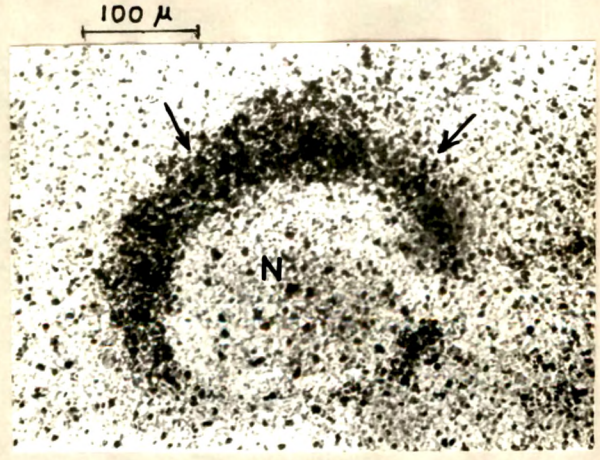

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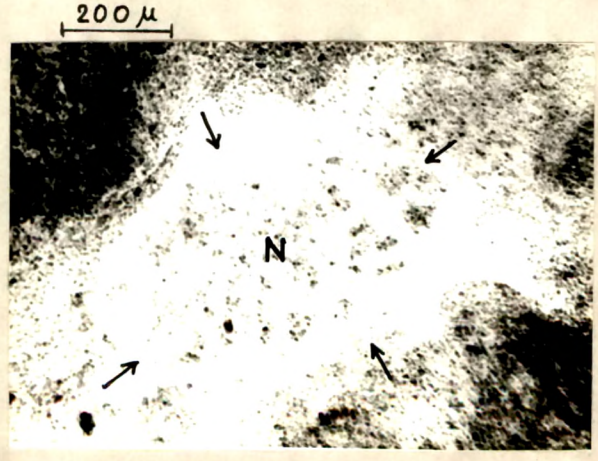

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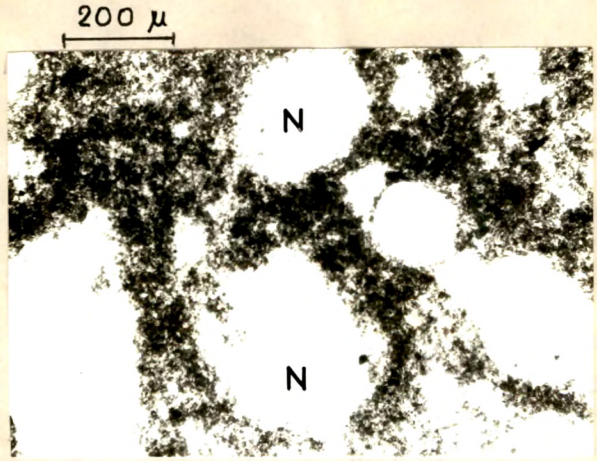
(Chapter 4: Figs. 7 and 8. Photomicrographs of spleen (Fig. 7) and liver (Fig. 8) showing the presence of ingested cellular fragments in the lymphocytopoietic nodules)

Fig. 7. 9 days after the injection of haemolyzed blood. Spleen. Note the absence of cellular debris inside the nodules (N).

Fig. 8. Liver, 6 days after the infliction of irreversible injury to a part. The cellular particles (arrows) are seen inside the nodule (N) which is situated near the injured area of the liver (IA).



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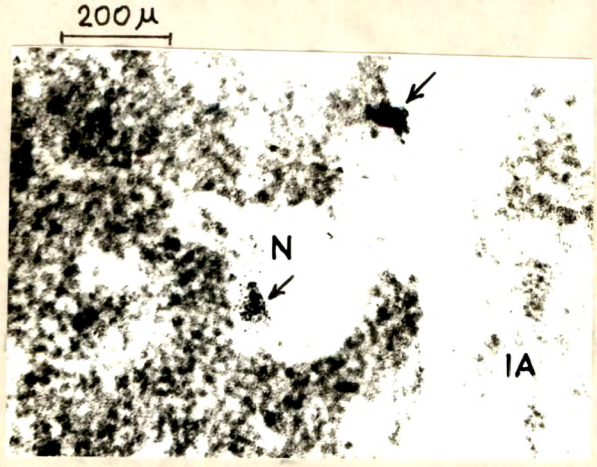

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transformed into macrophages which ingested most of the disintegrating cells present in this area. The lymphocytes which appeared near the injured area could have been migrated from the nodules in the liver itself. It was observed that at this stage the number of lymphocytopoietic nodules per unit area in the liver increased greatly. This increase suggests that the presence of injured cells in the liver may have initiated the development of more nodules in this organ for the supply of lymphocytes in greater number. Moreover, the phagocytic reaction was also clearly observed in the nodules situated either in the region adjacent to the injured area or elsewhere in the intact part of the liver. The phagocytized particles appeared in the cells of the nodules of the liver between the 3rd and 6th day after the infliction of the injury (Fig. 8). Most of these ingested particles were of erythrocytic in origin as evidenced by the selective staining for iron. Nevertheless, some of the cells in the nodules did show the presence of cellular debris of other than erythrocytic origin. It is quite likely that the blood borne cellular particles were trapped by the cells of these nodules. Such ingested particles persisted inside the nodules until 7th or 8th day after the infliction of injury to the liver cells and by 9th day they totally disappeared. Similar phagocytic activity also

occurred simultaneously in the splenic nodules.

DISCUSSION

The phagocytosis is a characteristic function of reticuloendothelial system which includes the lymphoid tissues also. The presence of blood cell fragments in the cells of the lymphocytopoietic nodules in the pigeon liver definitely suggests to their possible phagocytic function. Since nodules in the liver and spleen of pigeon have similar ability to phagocytize autochthonous or foreign cellular debris, it could be inferred that the nodules in both these organs are of same category and are concerned with not only the production of lymphocytes but also with phagocytic activity. Thus, it is surmised that in this bird, some of the splenic functions are supplemented by the nodules in the liver. The "germinal centres" in lymphoid nodules are generally considered to be associated with the antibody production (see Elves, 1966). The fact that "germinal centres" were seen only in the nodules of the liver and not in those of spleen after the injection of blood cells, may suggest that the production of antibodies in pigeon is mainly taking place in the nodules of the liver. The presence of cellular debris in the lymphoid nodules has been reported to be one

of the factors that influences the proliferation of cells in the central regions forming "germinal centres" in the nodules (Hill and Pospisil, 1960). In the light of relationships between the presence of cellular debris and the appearence of "germinal centres" in the nodules in liver as well as the 'germinal centres' and the antibody production, it could be reasonably suggested that in the nodules of the pigeon liver, where 'germinal centres' as well as cellular debris inside them were observed a few days after the injection of blood cells, the production of antibodies may be taking place. However, the absence of 'germinal centres' in the splenic nodules at this stage need not necessarily mean that the splenic nodules have no immunological functions. Perhaps they are not activated to produce antibodies with a single dose of antigens and/or the immediate immunological reactions are carried out only by the nodules in the liver. In this respect, since the pigeons lack a well developed lymphoid system comprising of lymph nodes (Romanoff, 1960), the liver nodules in this bird may be acting as lymph nodes as in the case of mammals wherein most of the immunological reactions occur.