# GENERAL CONSIDERATION

Forgoing chapters record the data obtained from certain studies carried out on the pigeon liver. The peculiar phenomenon found in the pigeon liver, namely the lymphocytopoiesis has been given a preponderant attention. Since each chapter is prepared with a view to project certain facts only, an attempt is necessary to record various facts in a sequential manner so as to evolve an overall perspective from the obtained data.

At present the lymphocytopoietic activity in the liver is reported only in isolated groups of birds. In pigeon liver lymphocytopoiesis is not restricted to adults only, but the stem cells are observed even in the embryonic liver itself. This is not strange, since liver is a haematopoietic centre in the embryonic condition. These stem cells multiply in the sinusoidal regions close to the portal spaces forming aggregates of lymphocytes which later get surrounded by connective tissue boundary thus forming a nodule. Such nodules are found even in one day old pigeon. The number of nodules per unit area in the liver increases as young ones grow and mature. Seasonal variation in the number of nodules per unit area may also occur depending upon the demand on lymphocytopoiesis (Chapter 2).

In the liver of one individual itself, the size and shape of the nodules differ greatly due to different stages of their development. A 'developing nodule' does not have connective tissue sheath around it, nor has any definite shape. On acquiring connective tissue covering the nodules become more spherical in shape. Such immature nodule show the presence of a 'germinal centre' where two zones viz., a 'dark' and a 'light' one are seen. The 'dark' zone is the region where active proliferation of lymphoblasts is observed. In a 'mature' round nodule only the 'light zone' containing lymphoblasts and phagocytes, is seen. Such 'mature' nodules usually are present in the liver for only short time and later liberate the lymphocytes formed therein into the blood stream. After liberation of the lymphocytes the mature nodules disappear. This suggested that a nodule is good for production of lymphocytes once only and does not continuously remain in the active phase.

The nodules in the pigeon liver are essentially lymphocytopoietic in function. When a sublethal haemorrhage was caused (under experimental condition), it was found that the number of nodules per unit area increased resulting in an apparent increase in lymphocytopoiesis. Similar increase in lymphocyte production following sublethal haemorrhage was noticed in spleen too. Such concomitant increase in the number of lymphocytopoietic nodules suggest that the activity of liver producing lymphocytes aids the primary function of the spleen where these cells are normally produced. When a total splenectomy was performed on pigeons, the number of nodules per unit area in their liver increased tremendously in contrast to the response of the liver when sublethal haemorrhage was caused or partial splenectomy was performed. Increase in the number of nodules in liver was not only following sublethal haemorrhage and total splenectomy but was also observed when carbon tetrachloride was injected intravenously which caused focal damages to the liver tissue. The increase in the nodular count in this condition (administration of $CCl_4$) could be for providing large number of lymphocytes which could transform into macrophages that would carry out phagocytic activities in removing the damaged liver cells (Chapter 3).

The capacity of lymphocytes for phagocytosis was evident when the cells of the nodules took up large amount of disintegrated pigeon blood cells which were injected intravenously. The nodules were also found to take up damaged cells originated from the body of the same individual (Chapter 4). Many nodules whose cells ingested cellular debris were found to develop a 'germinal centre'. Such 'germinal centre' in a lymphoid follicle is believed to be the site of antibody production. It would be worthwhile if this aspect is studied further with suitable methods using fluorescin etc. This aspect of study is being carried out in our laboratory.

The lymphocytes produced in the nodules are not only capable of phagocytizing the damaged cells or their parts which are brought to the nodules by the circulating blood (Chapter 4) but the lymphocytes from the nodules are also found to migrate to the site of damages or injury. Thus, in the pigeon liver, the area damaged by parasites were found to be surrounded by a large number of lymphocytes. Also many lymphocytopoietic nodules were found to develop in the vicinity of the damaged or infected part. It has been shown that these aggregated lymphocytes not only assist the phagocytic activity but also participate in the formation of connective tissue covering around the damaged part for the obvious reason of protecting the healthy areas of the liver. However, such activity was not noticed in the infected liver of frog where only a few lymphocytes appeared around the site of infection. Presumably,

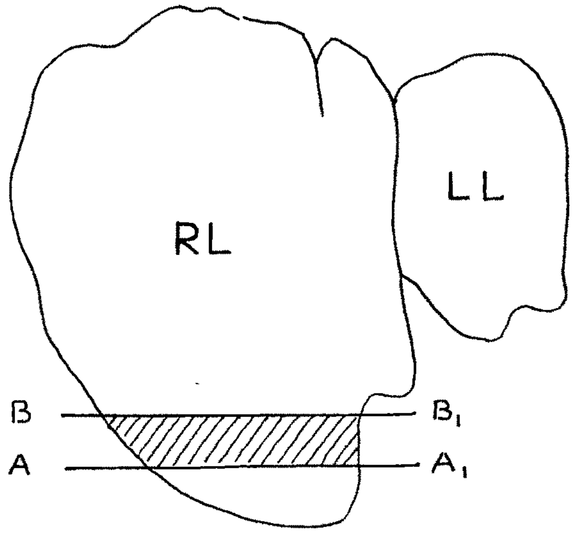


Fig. 1. A schematic representation of the area of pigeon liver where injury is inflicted. The portion below the line $A$-$A_1$ is the portion that is removed surgically. The striped region is the one subjected to a heavy pressure so as to cause an irreversible injury. RL- right lobe; LL- left lobe.

the lymphocytes found in the frog liver (lymphocytopoietic nodules are also found in the frog liver; Chapter 5) do not participate in collagen fibre formation. It would be interesting to survey such reactions around the damaged parts in the liver of ~~the~~ other vertebrates.

When a part of the liver was removed and with proper care during and after operations, it was found that the connective tissue that covers the whole organ (capsule of Glisson) just expands and covers the

Fig. 2. Diagramatic representation of injured area of pigeon liver 12hrs after the injury was inflicted. The injured area is lined by the blood clott (C) on one side and the 'zone' (Z) on the other. There is also a nodule (N) in the normal intact area (NA).

Fig. 3. Diagramatic representation of injured area of pigeon liver 24 hrs after the infliction of the injury. The lymphocytes (L) appeared in the 'zone' (Z) by this time. IA - injured area; NA - normal non-injured area.

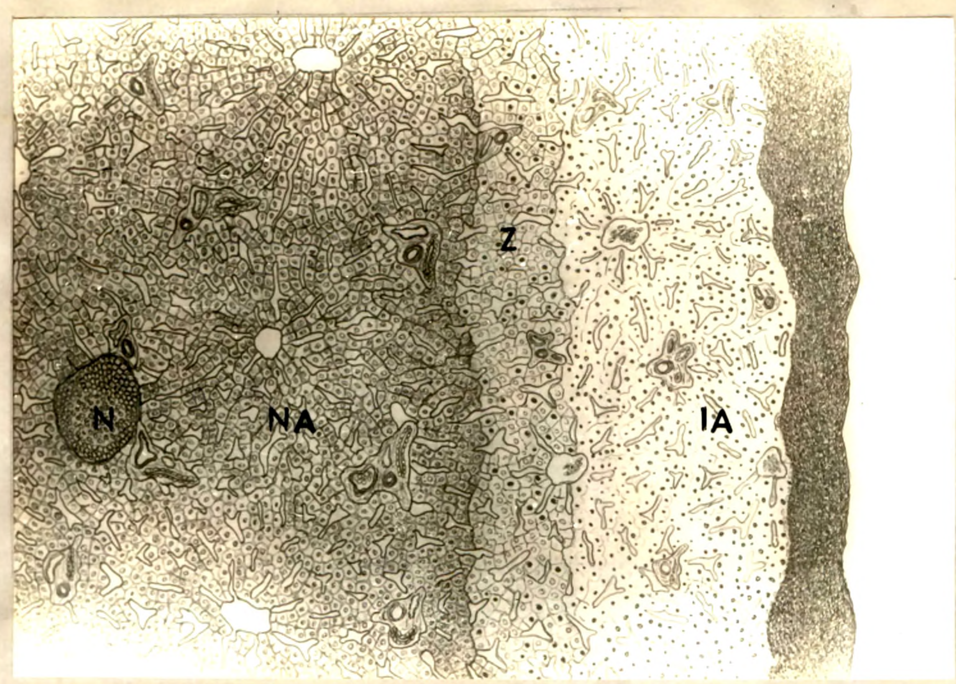


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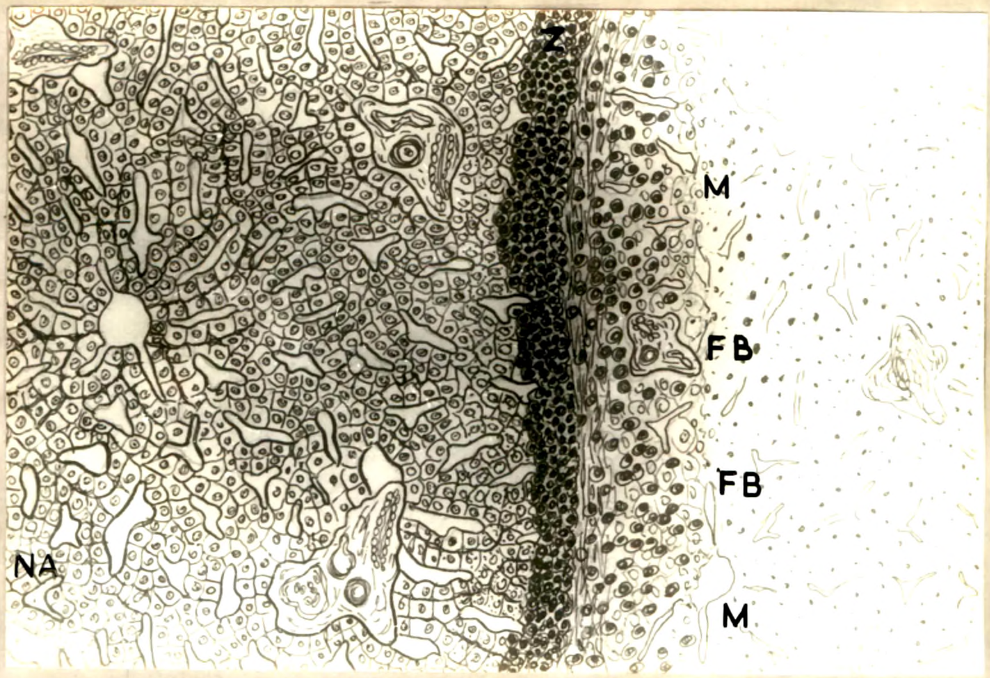
Fig. 4. Diagramatic representation of the injured area of pigeon liver 72 hrs after the infliction of the injury. The 'zone' shows many lymphocytes (L), fibroblasts (FB) and macrophages (M). IA - injured area; NA - normal non-injured area.

Fig. 5. Diagramatic representation of the injured area of pigeon liver 6 days after the infliction of the injury. Proliferation of hepatic cells (HC) is seen near the 'zone' (Z). The connective tissue band (CB) between the normal (NA) and injured (IA) areas, is almost complete.

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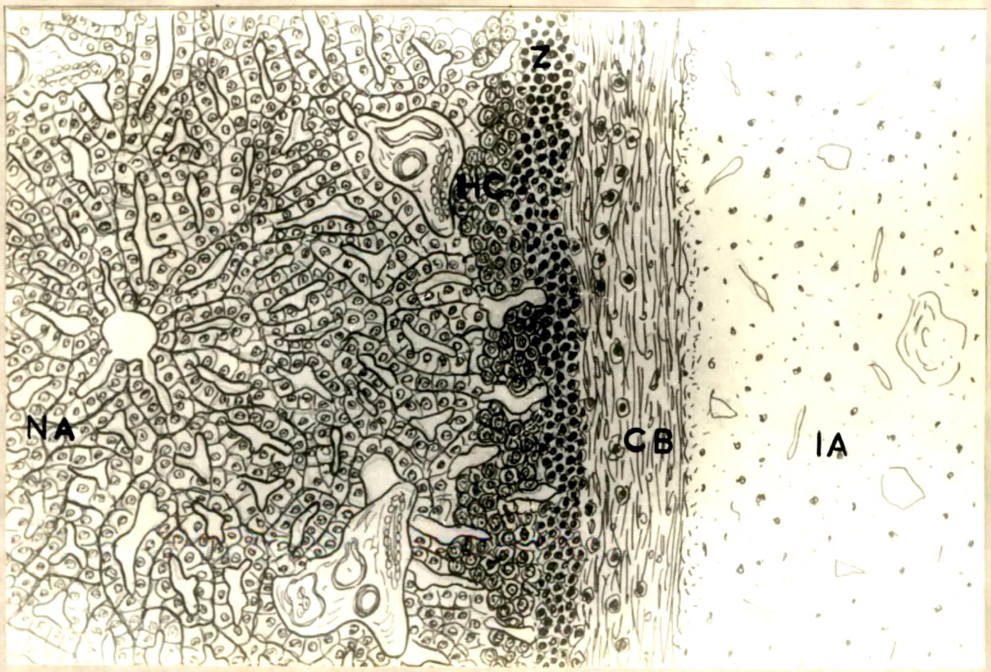


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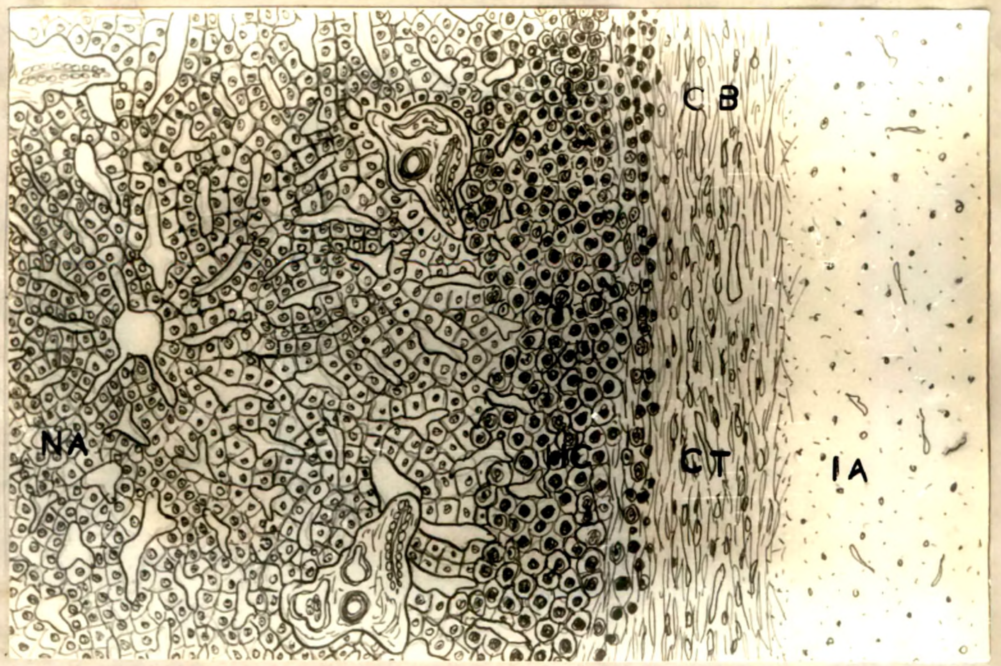
Fig. 6. Diagramatic representation of the injured area of the pigeon liver 10 days after the infliction of the injury. The connective tissue (CT) has established a band (CB) separating the normal (NA) and injured (IA) areas. The proliferating hepatic cells (HC) are seen near the newly formed connective tissue band (CB).

Fig. 7. Diagramatic representation of the injured area of the pigeon liver 15 days after the infliction of the injury. The connective tissue (CT) begins to split (arrow). The newly formed hepatic cells (HC) have rearranged to give the normal liver cord appearance.

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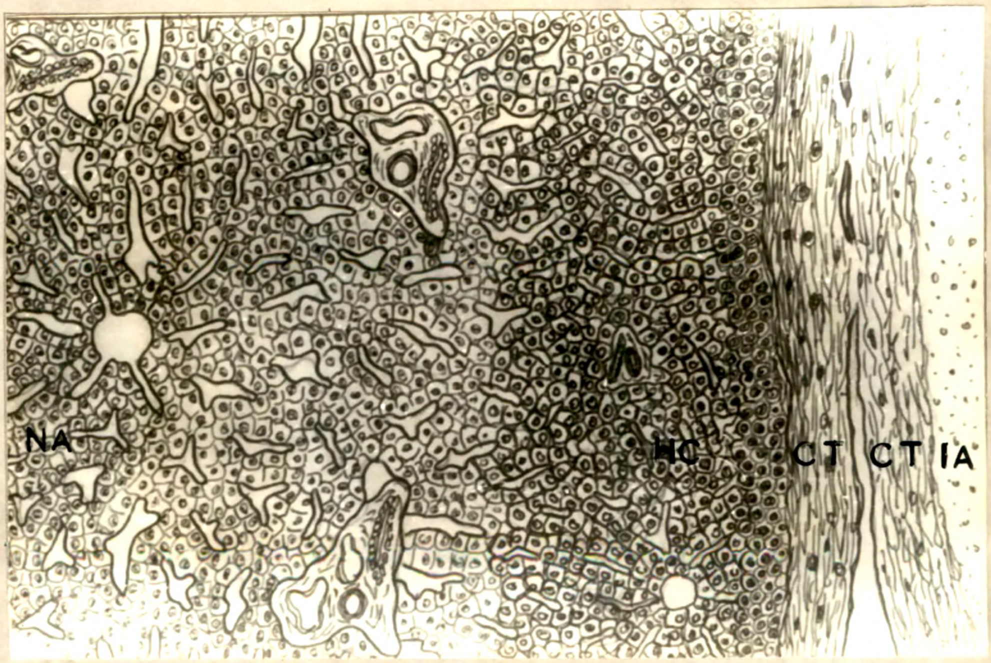


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wound surface. But if the region adjacent to cut surface (striped region marked in Fig. 1) was subjected to a high mechanical pressure, such expansion of capsule of Glisson did not take place. Since the region where the pressure was applied contained only damaged part, there was a necessity for forming a new connective tissue boundary to separate the injured region from the healthy part of the organ. Immediately after the formation of the wound covering the hepatic cells adjacent to it started multiplying. Since the mechanism of wound healing and repair is not so simple, the various activities and changes taking place at the wound site are given under appropriate time based phases.

12 to 48 hrs:

The region that separate the injured and intact region, for convenience termed 'zone' is composed of both injured and intact cells (Fig. 2). Most of the wound healing reactions occurred in the 'zone' region. This region became clearly distinguishable only by 24 hrs after inflicting the injury (by applying high mechanical pressure), when lymphocyte infiltration was observed to have been initiated (Fig. 3) and a maximum concentration of lymphocytes was noticed by 36 hrs.

This was the period when large number of phagocytes appeared in the 'zone'; presumably they were transformed ones from the lymphocytes. The phagocytic reaction, thus, was more profound between 1st and 2nd day. Many of the lymphocytes and macrophages were found to have a high acid phosphatase activity. The intact area near the 'zone' also showed high acid phosphatase activity. The Acid Pase being a lysosomal enzyme evidently figured in the autophagocytosis which was prerequisite in the region near the 'zone' where the cells have to undergo dedifferentiation prior to division. From the healthy parenchymal cells near the 'zone', fat as well as glycogen got depleted from the very beginning itself (after inflicting injury). While, fat was removed from the cells, the glycogen might be getting utilized for energy purposes. This was evident from the fact that lactate dehydrogenase was very active between 1st and 6th day after the infliction of the injury, while β-hydroxy butyrate dehydrogenase (BDH) activity was not at all found to increase significantly during this period. However, the aerobic metabolism suffered no reduction from the region in the beginning as evident from the SDH activity which dipped low only by 4th day.

72 to 96 hrs:

This is the most active period as far as the wound healing is concerned. Maximum number of fibroblasts and equally elevated collagen synthesis manifested during this period (Fig. 4). The RNA concentration increased during 72 hrs. This increase in RNA concentration could be due to the enhanced synthesis of this nucleic acid which would be required for the formation of many new proteins and enzymes during this stage.

The concentration of ascorbic acid in the 'zone' as well as in the nearby intact area showed a maximum increase by 96 hrs. Concomitantly the collagen synthesis was at its highest level at this juncture. Since it is known that ascorbic acid aids in the differentiation of lymphocytes into fibroblasts and in the synthesis of collagen, the increased ascorbic acid content during this time could be reasonably accounted. By 96 hrs not only the collagen fibres were laid down maximally but also the onset of proliferative activity of the hepatic cells was evident. Since acetylcholinesterase became quite active in this region by 96 hrs one could imagine that nervous interplay might be responsible for the initiation of mitotic activity at the wound site.

6 to 10 days:

By 6th day the connective tissue formation was almost complete (Fig. 5) and the division of hepatic cells near the 'zone' gathered momentum by about this time. The increase in the concentration of DNA which reached a maximum level between 6th and 10th day could be thus easily correlated to the mitotic activities. Due to proliferative activity, there occurred a temporary reduction in the aerobic metabolism which could be inferred from the decrease in the SDH activity during this period. Since the glycogen level in the cells near the 'zone' also decreased maximally at this time it could be stated that the dividing cells were utilizing glycogen predominantly through anaerobic reactions. As the hepatic cell proliferation reached the climax, the connective tissue formed anew between the healthy and injured area completely separated the injured area from the intact region. The lymphocytes as well as macrophages disappeared from the 'zone' as the region became completely obliterated by the connective tissue (Fig. 6).

15 to 30 days:

By 15th day the separation of the injured area began (Fig. 7). Which occurred by splitting the connective tissue band formed between the injured and intact areas. The hepatic cells near the newly formed 'wound covering' ceased to proliferate by 15th day. It was from 15th day onwards that the metabolic activities began to come to a more or less preoperative level. The fat content that was decreased considerably by 10th day rose and so also did the SDH activity. Thus from this period onwards, the newly formed hepatic cells progressively switched over to aerobic metabolism. Morphologically also it was observed that the newly formed hepatic cells began to rearrange themselves in a typical liver lobular pattern.

In conclusion it could be said that lymphocytes formed in certain specific regions may have different capacities and perhaps different functions too. For example it is reported that in mammals the lymphocytes from lymph nodes and spleen have lesser ability to repopulate the bone marrow than the lymphocytes produced in the bone marrow itself (Yoffey, 1966). Thus, there are reasons to believe that such differences in capacities in their activities are due to differential origin of lymphocytes. Ackerman and Knouff (1964) reported that lymphocytes of bursa of Fabricius are probably formed from endodermal epithelial cells, while in many other sites, as originally believed, mesenchymal cells may give rise to lymphocytes. It will be worthwhile to establish such developmental details regarding the nodules in the pigeon liver so that the true nature of such lymphocytes could be brought to the light. Another interesting feature about the lymphocytopoietic nodules in the pigeon liver is that they could easily function like lymph nodes of mammals as already suggested in Chapter 1. It is shown that lymph nodes found in the mammals are absent in pigeon and many other birds (Romanoff, 1960).

Besides, ~~repair~~ the wound healing phenomenon in the visceral organ like liver in pigeon is highly interesting. It is observed that lymphocytes take part significantly in the wound healing processes. The metabolic activities at the wound site centre around glucose or its storage form. It is also found that ascorbic acid is highly essential for the formation of wound covering in the internal organs too. Hence in tissues where wound healing reactions were to take place, certain prerequisite conditions as well as the requirements of certain chemicals have to be satisfied. Such studies should be extended to all other internal organs of other vertebrates so as to evaluate the mechanism by which each organ responds to injury and wounds. These studies should also take into consideration of various metabolic and hormonal factors that may tend to change the wound healing patterns of tissues. These studies will also show the requirement of each organ and tissues for an effective wound healing and thus, many of the disorders arising due to belated or defective wound healing could be corrected. Some of these aspects are at present being investigated in our laboratory.