

CHAPTER 7

HISTOCHEMICAL STUDIES ON NUCLEIC ACIDS DURING WOUND
HEALING AND REPAIR IN THE PIGEON LIVER

It was observed (Chapter 6) that the wound healing process in the pigeon liver, following surgical and mechanical injury, depended on the participation of cells like lymphocytes, macrophages and fibroblasts. Soon after termination of wound healing which is characterised by completion of the connective tissue over the wound surface, the repair process sets in. Hepatic cell proliferation and collagen synthesis at the wound site could then be reflected in differential concentrations of deoxyribose nucleic acid (DNA) and ribose nucleic acid (RNA). Correlation between peak concentration of DNA and maximum population of fibroblasts in the skin has been shown by Woessner and Robert, (1961). Also they have shown a rise in DNA concentration before collagen accumulation. RNA breakdown products are also believed to have leucotactic property (Tsanev, 1963; Bessis, 1964).

In the present study, histochemical investigation on RNA and DNA during wound healing and repair in pigeon liver was carried out in order to derive possible correlation of nucleic acid concentration with lymphocyte

accumulation, collagen synthesis and hepatic cell proliferation following combined mechanical and surgical injury to a part of the organ.

MATERIAL AND METHOD

Healthy adult pigeons, Columba livia, reared ^{under} in laboratory conditions were selected. From each of 27 birds, a small part of the liver was surgically removed and the area subjacent to the wound surface was subjected to high pressure to cause irreversible injury there. The procedure followed for the operation remained ~~the~~ same as described earlier (Chapter 6). During various post operative periods viz., 1st, 2nd, 3rd, 4th, 6th, 10th, 15th, 20th, and 25th day, each time three birds were killed by decapitation under mild anaesthesia. Liver at the wound along with the subjacent part was quickly removed and pieces were fixed in Carnoy's fluid for 2 hrs. The paraffin sections prepared from such fixed tissues were stained with Methyl Green - Pyronin Y for the demonstration of DNA and RNA following the method of Kurnik (1955) as described by Pearse (1960).

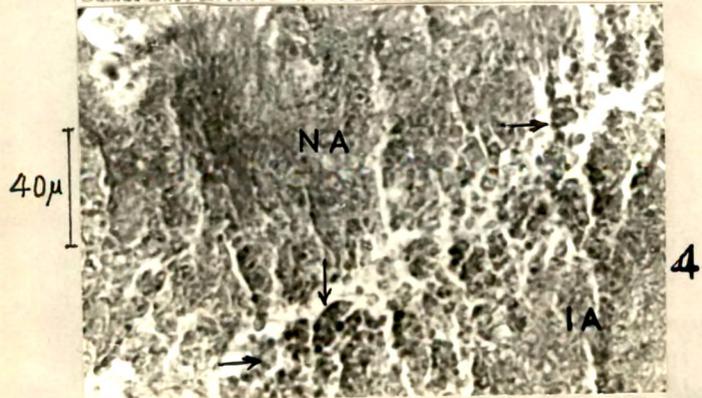
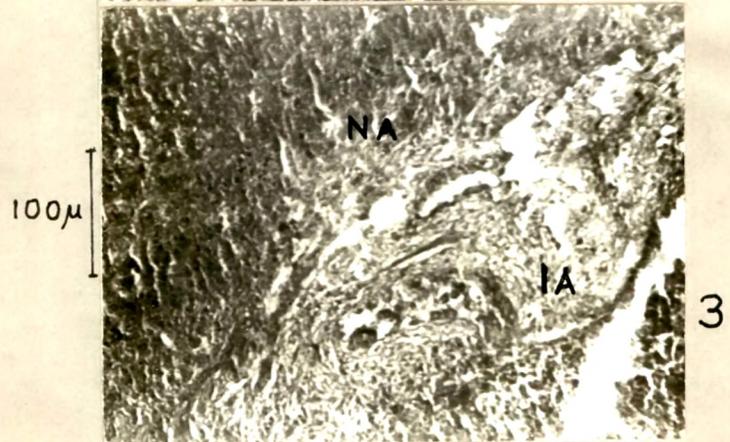
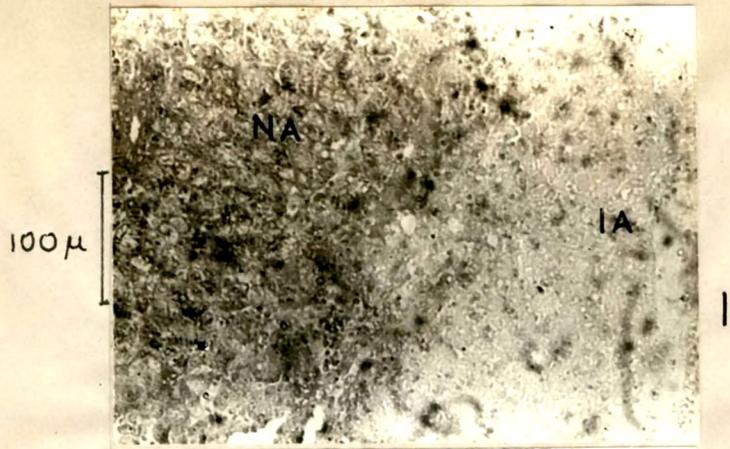
RESULTS

The region of the liver where the pressure was

applied, showed irreversible injury characterized by the basophilia and disturbed cords of the liver parenchymal cells. There was no appreciable change in the pattern of DNA or RNA distribution in the intact region situated subjacent to the injured area up to 24 hrs after the infliction of injury (Fig. 1). But by 48 hrs the RNA concentration was found to increase in the intact healthy region bordering the injured area (Fig. 2). Maximum concentration of RNA in the hepatic cells in this area was seen by about 72 hrs (Fig. 3). The lymphocytes and fibroblasts present in the "zone" (the region between normal healthy area and the injured part of the liver) showed greater concentration of DNA at this period (72 hrs) (Fig. 4). This period is also characterized by the active formation of collagen fibres (Chapter 8). However, this elevated level of RNA in the healthy parenchymal cells present near the injured area did not last long. It decreased considerably by 4th day (but never lower than the preoperative level) while the nuclei in the cells in the subjacent intact area became prominent with an apparent increase in DNA content (Fig. 5). The DNA concentration continued to increase in the nuclei reaching a maximum level by 10th day. When nuclei were heavily loaded with DNA the proliferative activity at this time was evident (Fig. 6). Thereafter the DNA and RNA concentrations slowly returned to the preoperative level

(Chapter 7: Figs. 1 to 4. Photomicrographs of the pigeon liver showing the localization of DNA and RNA during wound healing and repair. Stained with Methyl Green - Pyronin Y)

- Fig. 1. 24 hrs after the infliction of the injury. The intact healthy area (NA) shows no appreciable change in the distribution of nucleic acids. In the injured area (IA) the DNA is not detectable as the nuclei are almost indistinguishable.
- Fig. 2. 48 hrs after the infliction of the injury, Note the increased concentration of RNA in the intact healthy area (NA) situated near the injured area (IA).
- Fig. 3. 72 hrs after the infliction of the injury. Note the high concentration of RNA in the intact area (NA). The injured area (IA) is almost devoid of both RNA and DNA.
- Fig. 4. 72 hrs after the infliction of the injury. The lymphocytes and fibroblasts seen in the region between normal healthy area (NA) and injured area (IA) show higher content of DNA (arrows).



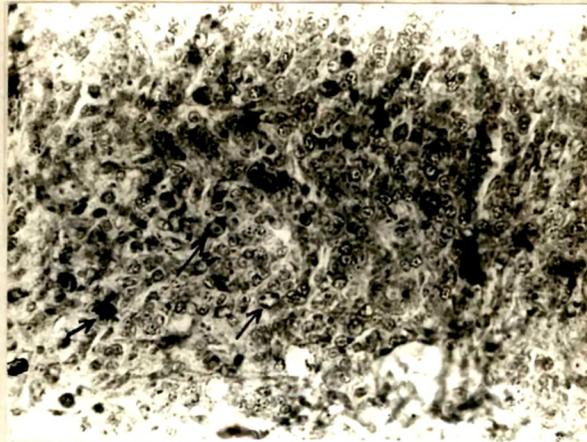
(Chapter 7: Figs. 5 to 7. Photomicrographs of the pigeon liver showing the localizations of DNA and RNA during wound healing and repair. Stained with Methyl Green - Pyronin Y)

Fig. 5. 96 hrs after inflicting the injury. Note the prominence of the nuclei due to increased DNA content. Many dividing cells could be seen (arrows).

Fig. 6. 10 days after inflicting the injury. A large number of nuclei are still loaded with DNA. The completed connective tissue (CT) is seen on the right.

Fig. 7. 20 days after inflicting the injury. The parenchymal cells of the liver have regained the normal pattern of nucleic acid distribution.

20 μ



5

100 μ



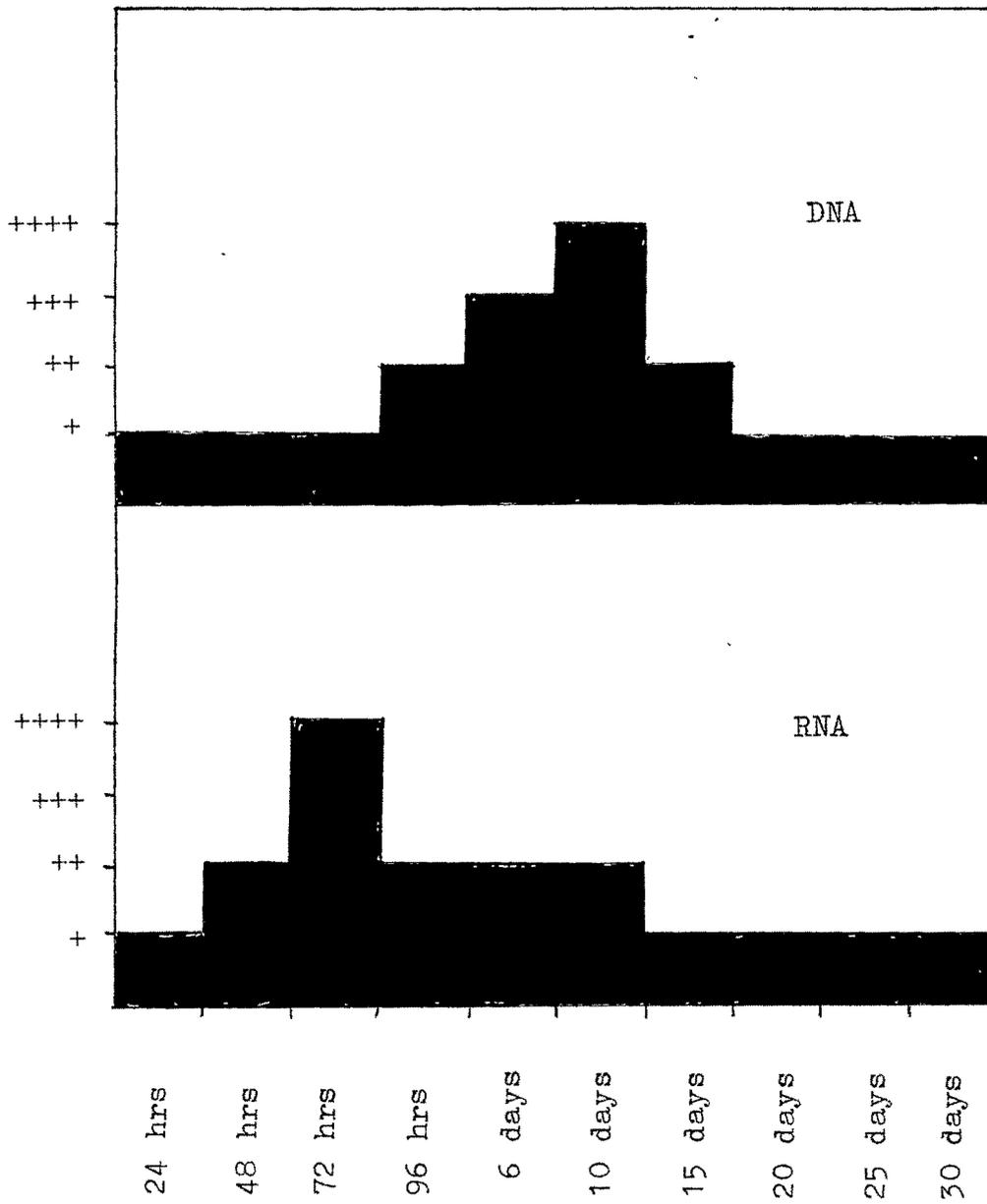
6

100 μ



7

Fig. 8. Graphic representation of the changes in the concentration of DNA and RNA near the injured region in the pigeon liver.



while the newly formed hepatic cells near the injured region began to arrange themselves into hepatic cords acquiring normal characteristics of the organ (Fig. 7). During this phase the sloughing off of the injured part had already been more or less completed.

DISCUSSION

From the histochemical observations it was evident that the RNA concentration increased in the early part of wound healing and repair in the pigeon liver following the infliction of surgical and mechanical injury.

The RNA increase then coincides with the connective tissue formation especially the collagen synthesis^(Fig. 8). It has already been reported that RNA content and collagen synthesis decrease~~s~~ in animal (mammals) tissues having vitamin C deficiency, without affecting the DNA content (Tsanev, 1958). However, in the case of pigeon liver the hepatic cells do not participate in the collagen synthesis since it has been already stated (Chapter 6) that lymphocytes and fibroblasts were responsible for the collagen formation. Taking into consideration the report of Woessner and Robert (1961), the increased DNA content observed in the lymphocytes and fibroblasts that were present in the "zone" (Fig. 4) where collagen fibres were

being laid down, could be correlated to the collagen synthesis.

In this case, then the increase in RNA content could be correlated to the synthesis of enzymes that was found to increase during the wound healing processes. Tsanev's (1963) observation, that RNA degradation products act as leucotactic substance, is highly significant here, since, in the pigeon liver lymphocytes were found to migrate towards the injured area. He further stated that immediately after injury, there was within the neighbouring cells a rise in free RNA due to the breakdown of RNA protein complex. This free RNA according to him is rapidly degraded followed by a rapid synthesis of new RNA thereby increasing the RNA content to the maximum. He also stated that this response of nucleoproteins to mechanical injury is the starting point of regeneration following injury. The fact that high mitotic stimulation of cells takes place in the presence of damaged cells of the same tissue was already reported in the epidermis (Bullough and Laurence, 1960) and in the present study on the pigeon liver (Chapter 6).

The increase in the DNA content was noticed at a period when the wound healing process was almost complete. The formation of connective tissue band demarcating the intact and injured areas of the liver

by 4th day, arbitrarily marks the completion of wound healing process. The increase in DNA thus coincided with the beginning of proliferation of hepatic cells.

Thus it could be said that the increase in RNA content immediately after the infliction of injury greatly augments the repair process and also stimulates the proliferation of intact cells neighbouring the injured area in the pigeon liver. The appearance of lymphocytes at the wound site could also be correlated to the release of nucleic acid breakdown products which exerts a leucotax^{ic} influence over these mononuclear leucocytes. Active hepatic cell proliferation during the repair phase is preceded by the^a rise in DNA concentration.