CHAPTER 9

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

Since long, it is known that nerves play some role in regeneration, especially in amphibians (Singer, 1960; Thornton, 1960; Steen and Thornton, 1963; Tweedle, 1969a, b). Thornton (1970), however, has tressed that, only the early stages of regeneration i.e., wound healing is under nervous control, whereas aggregation of mesenchymal cells, differentiation and blastema formation can proceed in the absence of nerves. Wound healing, is a complex process which calls for different events including active proliferation of cells at the wound site. Hey (1960) has shown that epidermal hyperplasia could be stimulated through nerves. Thus one of the functions of nerves in wound healing could be to stimulate the division of cells. Such tropic effects by nerves may be necessary only for local stimulation of cell division, especially at the wound site. During the regenerative processes on large scale such as morphallaxis and epimorphosis, the nervous influence may get superimposed by humoral factors.

In the epidermal region where injuries occur quite often, the speedy wound healing and repair, according to

available informations, may largely depend on nervous participation. However, very little is known about the influence of nerves in the wound healing and repair in visceral organs.

It was shown that wound healing and repair in the pigeon liver following the infliction of injury differs in many respects to that which occurs in epidermal regions (Chapter 6). When sufficient amounts of injured cells are present at the wound site proliferation of hepatic cells at that site was observed (Chapter 6). Since the liver has a fine network of nerve fibers (Youssef and Saleh, 1961; Sutherland, 1964), nerves could influence wound healing. An increased or decreased of nervous activity at any site could be inferred through studies on the activity of cholinesterases, the intensity of which fluctuates according to the amount of acetylcholine secreted by the nerve endings. The normal pigeon liver has been shown to have a good amount of specific as well as non-specific cholinesterases (Pilo, 1969).

In the present investigation, histochemical observations on cholinesterases activity were made with a view to obtain information regarding possible influence of nervous activity on the wound healing and repair processes in the pigeon liver.

MATERIAL AND METHODS

Wound healing and repair were studied in the liver of pigeons (Columba livia) reared in the laboratory. Healthy adult pigeons were selected and from each, a small piece of liver was surgically removed and the adjacent region to the cut surface was subjected to high pressure to inflict surgical and mechanical injury as described earlier in Chapter 6. At regular intervals three operated pigeons were sacrificed and the part of the liver with wound surface was removed and fixed in formol saline for 12 to 14 hrs at 4°C. After fixation, the tissue was washed thoroughly with distilled water and 12 to 18 μ thick sections were cut on a freezing microtome. The histochemical demonstration of specific and nonspecific cholinesterases was carried out using the method of Koelle and Friedenwald (1949) as modified by Coupland and Holmes (1957). The substrates used for specific ChE (AChE) was Acetylthiocholine iodide and for nonspecific ChE (BuChE) was butyryl thiocholine iodide.

RESULTS

In the normal healthy liver both AChE and BuChE were localized in the cells of the liver cords. Both these

enzymes showed similar variation in intensity and localization in the affected parts of the liver tissue during wound healing and repair.

Twenty four hrs after injury neither the normal intact part nor the area near the wound showed any change in the activity of the enzymes from the preoperative (normal) level. Even the irreversibly injured area (where the cords presented a disarranged picture due to pressure applied) showed same concentration as in the intact normal region (Fig. 1).

By about 48 hrs after the infliction of injury a gradual disappearance of enzyme activity was seen in the injured area. Simultaneously a general reduction in their activities all over the liver was also evident (Fig. 2). This overall decrease of the enzymes activity remained so even by about 72 hrs. By this time, however, there was a slight increase in the enzyme activity at the "zone", the junction of the injured and intact areas.

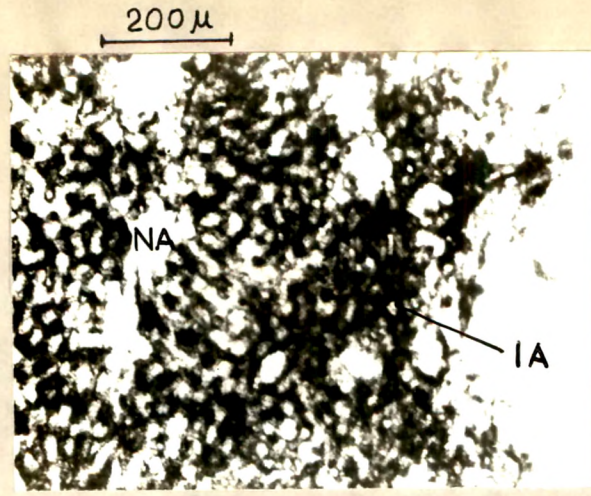
Thereafter there was a gradual increase in the intensity of the activity of these enzymes in the "zone" area and by about 96 hrs, a high concentration of AChE and BuChE in the same site was observed (Fig. 3). However, their activity in the subjacent healthy region was relatively very much less than that in the normal liver. By 6th day the region near the injury ("zone") showed a

(Chapter 9: Figs. 1 to 3: Photomicrographs of the liver of pigeon showing AChE localization near the injured area)

Fig. 1. 24 hrs after inflicting the injury. Note the more or less comparable AChE activity in both intact normal area (NA) and injured area (IA).

Fig. 2. 48 hrs after inflicting the injury. Note the absence of AChE activity in the injured area (IA). There is a general reduction in the activity in the normal intact area (NA) also.

Fig. 3. 96 hrs after inflicting the injury. Note the high activity of AChE in the 'zone' (Z) demarcating the injured area (IA) and healthy intact area (NA).



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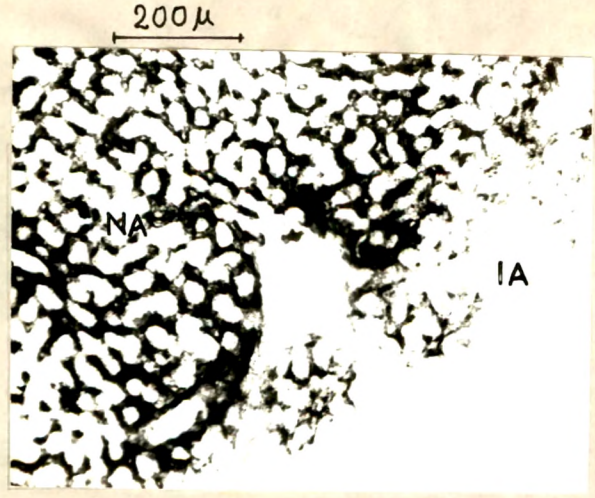

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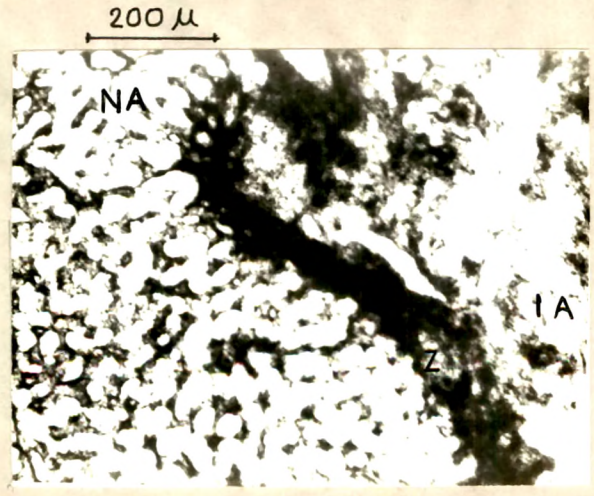

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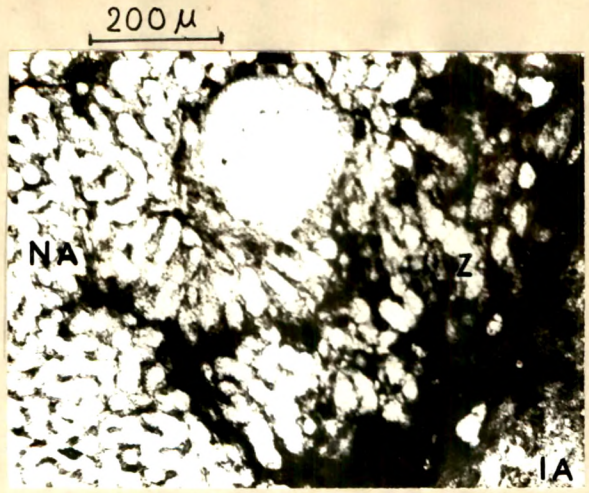
(Chapter 9: Figs. 4 to 6: Photomicrographs of the liver of pigeon showing AChE localization near the injured area)

Fig. 4. 6 days after inflicting the injury. Note the very high concentration of AChE in the 'zone' (Z) as well as in the healthy intact area (NA) situated adjacently to the 'zone'. Injured area (IA) is seen in the lower right side.

Fig. 5. 10 days after inflicting the injury. Note the decreased AChE activity at the junction of injured area (IA) and healthy intact area (NA).

Fig. 6. 15 days after inflicting the injury. The injured area has already been cast off. The AChE activity in the hepatic cells have returned to a more or less preoperative level.



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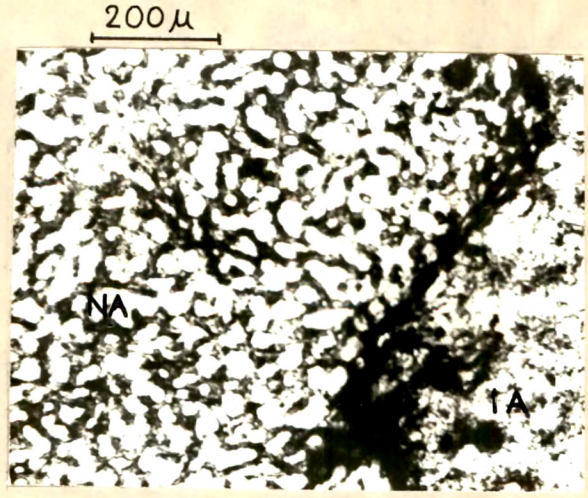

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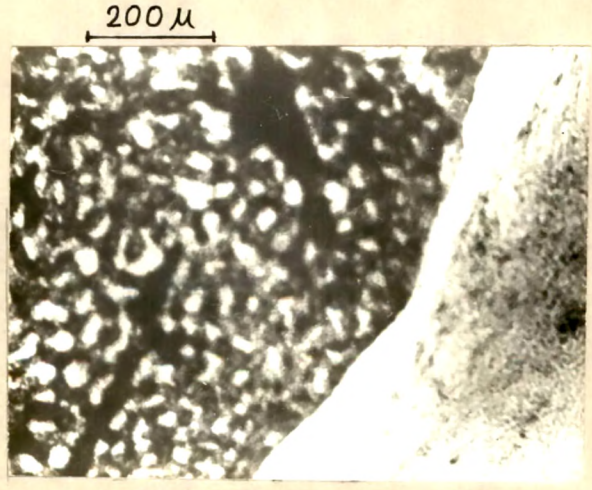

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very high concentration of cholinesterase activity. Later the enzyme activity at the wound site began to fall gradually while in the healthy normal liver cords it began to increase. The last evidence of increased cholinesterase activity at the injured area was noticed by about 10th day (Fig. 5). The injured area was separated ~~off~~ from the normal area by 15th day and at this time the enzyme activity in the cords almost returned to the normal level (Fig. 6).

DISCUSSION

From the experiments on pigeon liver it was clear that during certain stages of wound healing the cholinesterases were more active at the wound site, especially between 4th and 6th day after the infliction of injury. This period was also characterized by the active formation of connective tissue as well as the initiation of proliferation of the hepatic cells near the injured area (Chapter 6). The connective tissue formation was preceded by a massive accumulation of lymphocytes which later became transformed into macrophages and fibroblasts. Since it was shown that such cellular transformations could be induced by addition of choline to the culture medium (Cheveremont, 1949) it is possible to believe that

acetylcholine secreted by the nerves at the injured area (which of course, also induces the AChE to become more active) may be playing an active role or influencing the transformation of lymphocytes into macrophages and fibroblasts. This contention find support from the work of Van Stone (1964) who suggested that cell differentiation during wound healing may be depending upon nervous stimulation.

Increased blood supply to the injured area during the wound healing in the pigeon liver has been reported earlier (Chapter 6). Perhaps the augmented blood supply might be bringing more cholinesterases at the wound site. Besides, nerves are also known to regulate the flow of the blood through organs (Julia et al., 1967). Thus the decreased activity of the enzymes in other parts (intact healthy) of the liver could be due to selective inhibition of nervous activity or due to increased secretion of cholinesterase into the blood due to wound shock as suggested by Yntema (1962).

Most important influence of the nerves during wound healing and repair may be the initiation of local hepatic cell proliferation which was found to occur by about 6th day after infliction of injury. Obviously the ChE activity was also maximum at the wound site at that period. D'Agostini and Rossatti (1958) observed a variable

intensity of acetyl cholinesterase in the 'germinal centers' of lymphatic follicles in the lymphatic tissues of mammals and said that "the proliferation and differentiation of cells in the germinal centre of lymphatic follicles might be related to the presence and metabolism of acetylcholine in the capillary vessels of this structure". By extrapolating, it could be stated that hepatic cell proliferation could have been influenced by the acetylcholine secretion by the nerves at the wound site.

Though, the increased activity of acetylcholinesterase could be correlated to the increased nervous function, the increase in the activity of nonspecific cholinesterase (BuChE) is difficult to explain. Since specific inhibitors are not used in the control, it is difficult to infer which of the enzymes is more active. However, the increase in BuChE activity could be correlated to the enhanced lipolytic activity at the wound site. Szendzikowski et al., (1961/62) observed that about 50% of the lipolytic activity of rat aorta depends on nonspecific cholinesterase. Ballantyne and Burwell (1965) studied the histochemical distribution of cholinesterase in the lymph nodes and suggested that the enzyme may be associated with macrophages. According to them this enzyme probably functions to remove some lipid component from the tissue fragments so as to make them presentable to 'receptor cells' of the lymphocytic series.

Since, choline assists in lipid clearance, BuChE could also provide more choline at the wound site thereby clearing most of the fat present there. Such clearance of fat has been observed from the histochemical studies on fat during wound healing process in the liver (Chapter 11).

The present study, thus clearly suggests that in pigeon nervous participation does occur in the processes of wound healing and repair in the visceral organs such as liver.