CHAPTER 10

HISTOCHEMICAL STUDIES ON THE ALKALINE AND ACID PHOSPHATASES DURING THE PROCESSES OF WOUND HEALING AND REPAIR IN THE PIGEON LIVER

It is increasingly realized, that the hydrolytic enzyme like acid phosphatase (Acid Pase) plays an important role in various processes concerned with wound healing and repair of skin of many vertebrates (Reiner et al., 1957; Moretti and Mescon, 1956; in primates, Carranza and Cabrini, 1962, in rats; Raekallio, 1960, in guinea pigs; Kobayashi et al., 1955, in pigeon; Noback and Paff, 1951, in mammals). The activity of Acid Pase has been investigated in connection with regeneration and development of appendages of animals belonging to different groups of vertebrates. The role of this enzyme was studied during tail regeneration of adult Triturus cristatus (Ghiretti, 1950); limb regeneration of adult newts, Diemictylus viridescens (Schmidt, 1963, Schmidt and Weary, 1963); tail regeneration of adult house lizard, Hemidactylus flaviviridis (Shah and Chakko, 1966). Acid Pase was found to be associated with lysosomes and its increased activity has been demonstrated in macrophages and giant cells participating in the lytic phenomena (Duve et al., 1962;

Novikoff, 1961, 1963). Lymphocytes were also found to contain high Acid Pase activity (see Elves, 1966).

Alkaline phosphatase (Alk Pase) is reported to be associated with carbohydrate metabolism (Cori and Cori, 1952; Cusworth, 1958; Duncan, 1959; Rosenthal et al., 1960). This enzyme has also been found to have certain functions in the formation of fibrous proteins and passage of metabolites across the cell membranes (Verzar and McDougall, 1936; Moog, 1946; Bradfield, 1950; Danielli, 1954).

A very low activity of Alk Pase has been demonstrated in the normal mammalian liver, (Wachstein, 1963). However, its increased activity was noted under many experimental conditions viz., bile duct obstruction (Hard and Hawkins, 1950; Kritzler and Beaubien, 1949; Wachstein and Zak, 1946, 1950; Kaplan and Righetti, 1970), and following partial hepatectomy (Novikoff and Noe, 1955; Yokoyama et al., 1953a).

Despite these facts, the exact role of these two enzymes, viz., acid and alkaline phosphatases, is still not clearly understood. Most of the workers consider that in wound healing there is initially a metabolically inert lag phase of 1 to 4 days. Fell and Danielli (1943) and Firket (1951) demonstrated that during such a lag phase Alk Pase is present only in the invading polymorphonuclear leucocytes. Balazs and Holmgren (1950) found Alk Pase activity at the wound margin, 24 hrs after injury. Needham (1952) considered that Acid Pase plays no part in repair.

However, taking into consideration that Alk Pase is active during the laying down of collagen fibers and the Acid Pase is active in macrophages and also during lytic processes at the wound site, it could be surmised that these two enzymes can assist in the processes of wound healing and repair.

In the present study, changes in the activities of Alk and Acid Pases during wound healing and repair in the pigeon liver following combined surgical and mechanical injury was investigated to find out whether these enzymes participate in the metabolic activities at the wound site (either to provide energy, or to supply or synthesize materials) or in the lytic reactions leading to removal of the injured cells or in both the activities.

MATERIAL AND METHODS

For the experiments, healthy adult pigeons, Columba livia reared under laboratory conditions with balanced diets were used. A small part of the liver was surgically removed and the area adjacent to the wound surface was subjected to high pressure to cause irreversible injury there. The procedure followed for operation was the same as described in Chapter 6. At regular intervals viz., 1, 2, 3, 4, 6, 10, 15, 20, 25 and 30 days after infliction of injury, 3 birds

from a group of 30 operated ones were sacrificed by decapitation under mild anaesthesia. The part of the liver with the injured area was carefully removed and fresh frozen sections of 10 µ thickness were taken using a cryostat microtome. These sections were fixed either in cold 10% neutral formalin or cold acetone for 20 minutes. For the histochemical localization of acid and alkaline phosphatases, Naphthol-AS-BI phosphate and Naphthol-AS-MX phosphate (Sigma) respectively were used as described by Burstone (1962). The diazonium salt used for both the enzymes was Fast Blue B. Suitable controls were also prepared to confirm the authenticity of the localization of the activities of the enzymes.

RESULTS

Acid phosphatases:

Acid Pase was not found to be very active in normal pigeon liver, and usually presented variations in its intensity and localization in different individuals. Nevertheless, in the experimental pigeon, the Acid Pase in the vicinity of the injured area was found to be very active during the early phase of wound healing. The intact hepatic cells adjacent to the irreversibly injured area showed, relatively, a higher concentration of the enzyme as early

as 24 hrs after inflicting the injury (Fig. 1). The cells in the lymphocytopoietic nodules present in the normal pigeon liver (Chapter 1 and Pilo, 1970) were devoid of the enzyme activity (Fig. 2) in the beginning. In the experimental birds the lymphocytes that appeared in the 'zone', a region characterized by the presence of both injured and normal hepatic cells (Chapter 6), were found to contain high concentration of this enzyme (Fig. 3). Such intense Acid Pase activity in the lymphocytes persisted up to 96 hrs. The intensity of the enzyme reaction in the parenchymal cells of healthy liver region adjacent to the injured area remained the same (quite high) as it was after 24 hrs of inflicting injury, till 96 hrs (Fig. 4). But thereafter, the Acid Pase concentration decreased and by 10th day only a poor reaction of the enzyme near the injured area (Fig. 5) was noticeable. As the wound healing progressed, (by 10th day) the cells in the lymphocytopoietic nodules, where the enzyme was not active during the earlier wound healing phase (Fig. 2), became highly reactive (Fig. 6). By 15th day the Acid Pase concentration in the healthy cells near the injured area dropped and reached more or less a preoperative level.

Alkaline phosphatase:

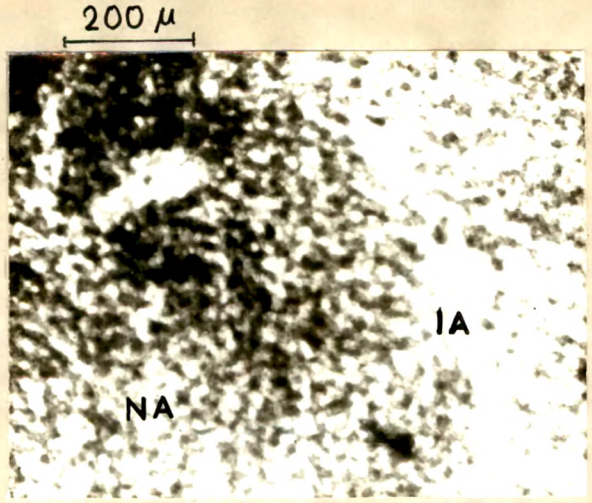
The concentration of Alk Pase like that of Acid Pase in the normal pigeon liver was very low. In the

(Chapter 10: Figs. 1 to 3. Photomicrographs of the pigeon liver showing the histochemical localization of acid phosphatase during the wound healing and repair. Burstone's method)

Fig. 1. 24 hrs after the infliction of the injury. The intact healthy area (NA), adjoining the injured one (IA), shows fairly high intensity of enzyme activity.

Fig. 2. 24 hrs after the infliction of the injury. Note the absence of enzyme activity in the nodule (N).

Fig. 3. 48 hrs after the infliction of the injury. Intact healthy area (NA) and the 'zone' (Z) show high enzyme activity.



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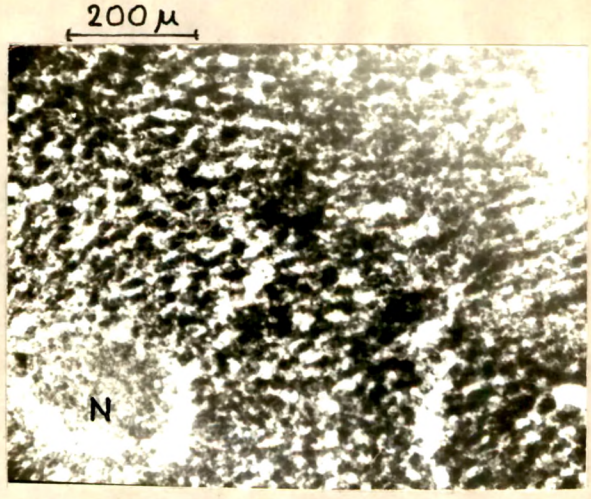

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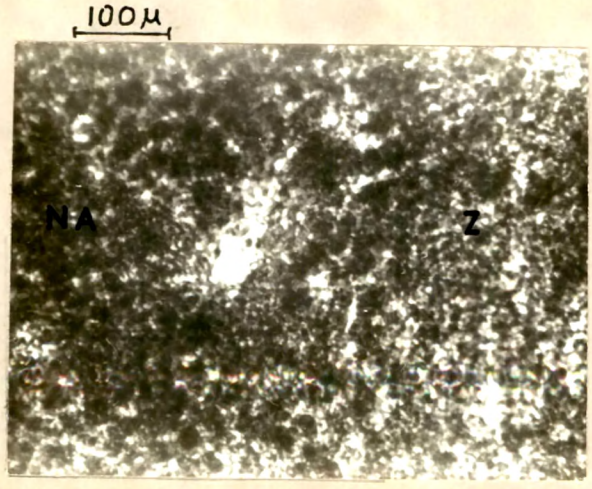

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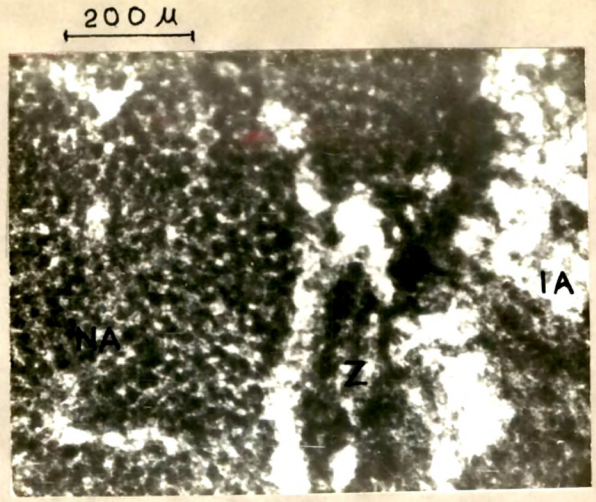
(Chapter 10: Figs. 4 to 6. Photomicrographs of the pigeon liver showing the localizations of acid phosphatase during the wound healing and repair. Burstone's method)

Fig. 4. 96 hrs after the infliction of the injury. The healthy intact area (NA) and the 'zone' (Z) show high enzyme activity.

Fig. 5. 10 days after the infliction of the injury. The enzyme activity decreased in the intact healthy area (NA) of the liver.

Fig. 6. 10 days after the infliction of the injury. Note the high activity of the enzyme in the peripheral part of the nodule (N).



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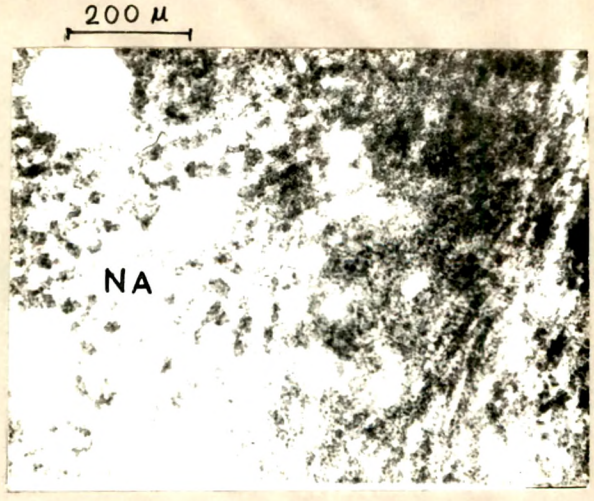

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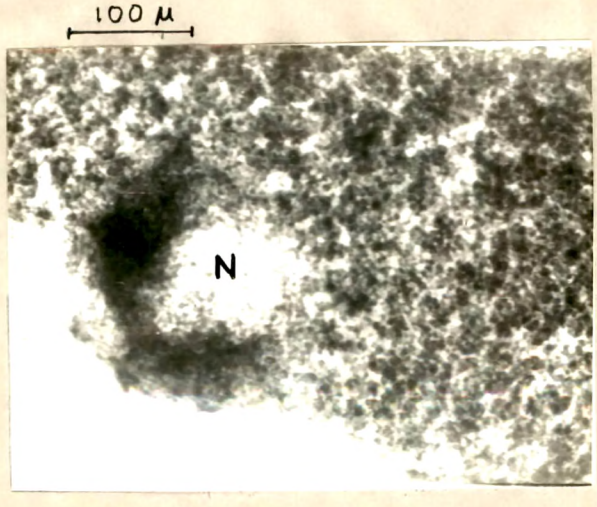

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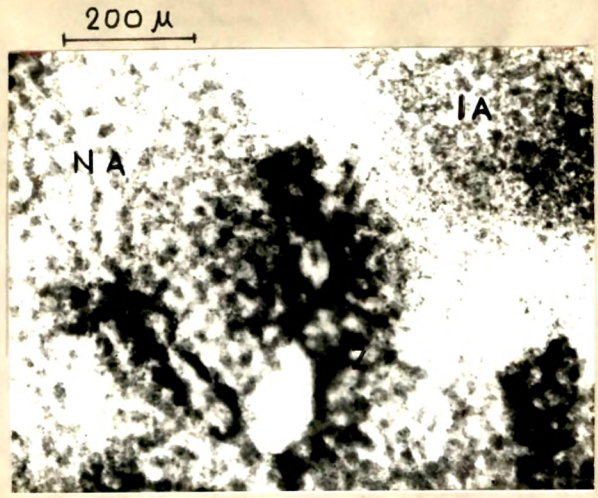
(Chapter 10: Figs. 7 to 9. Photomicrographs of the pigeon liver showing the histochemical localization of Alkaline phosphatase during the wound healing and repair. Burstone's method)

Fig. 7. 24 hrs after the infliction of the injury. The 'zone' situated between the normal intact area (NA) and the injured area (IA) shows high enzyme activity.

Fig. 8. 48 hrs after the infliction of the injury. The 'zone' (Z) shows high enzyme activity in contrast to the normal (NA) and injured (IA) areas.

Fig. 9. 96 hrs after the infliction of the injury. Only the 'zone' (Z) shows increased enzyme activity.



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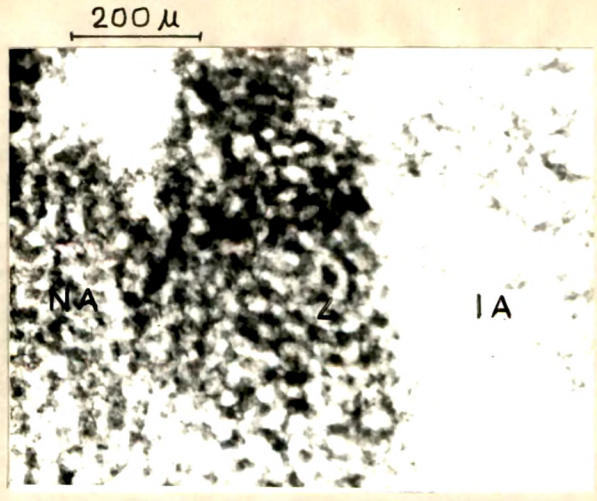

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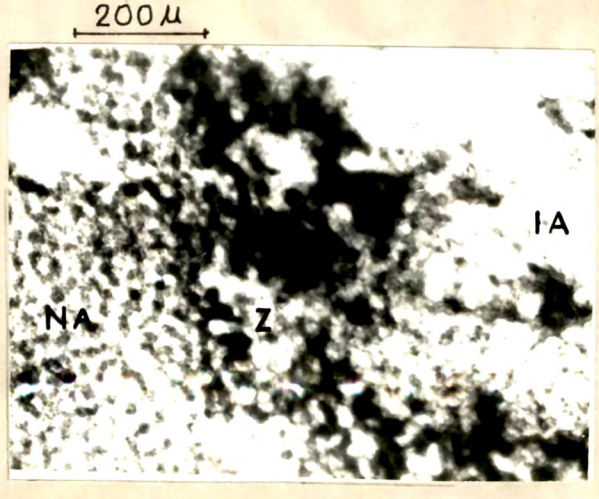

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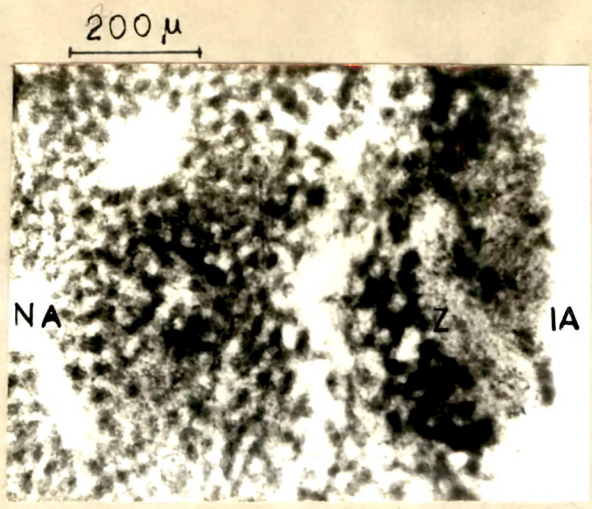
(Chapter 10: Figs. 10 to 12. Photomicrographs of the pigeon liver showing the histochemical localization of alkaline phosphatase during wound healing and repair. Burstone's method)

Fig. 10. 6 days after the infliction of the injury. While the 'zone' (Z) still shows high enzyme activity, the beginning of increased activity in the nearby intact area (NA) is evident at this stage.

Fig. 11. 10 days after the infliction of the injury. Note the elevated activity in the intact healthy area (NA) adjoining the injured area (IA). The activity in the 'zone' (Z) decreased considerably.

Fig. 12. 15 days after the infliction of the injury. The enzyme concentration in the healthy area has returned to a normal level. The injured area (IA) is getting separated at this time.



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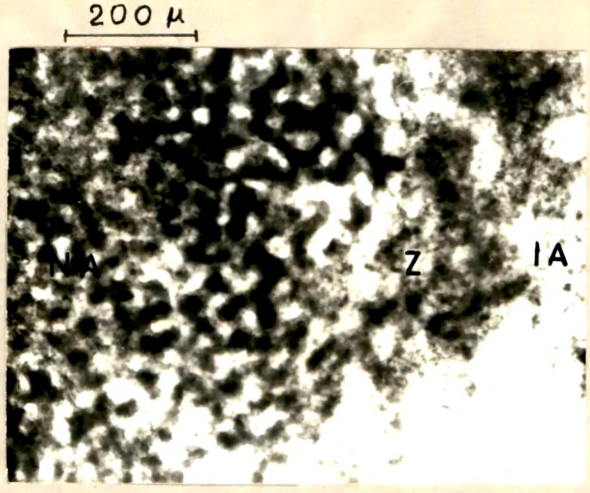

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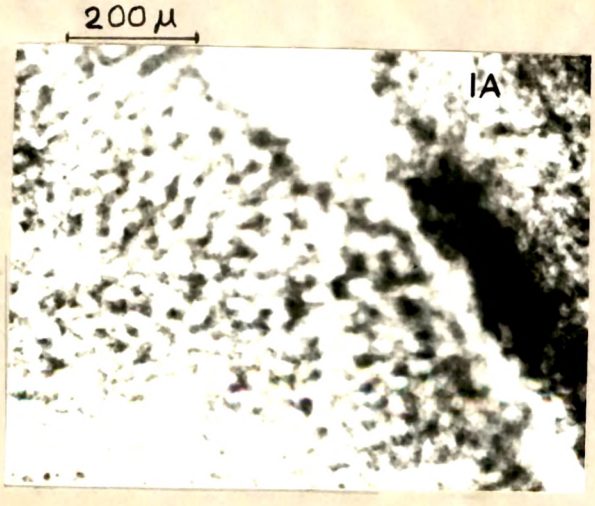

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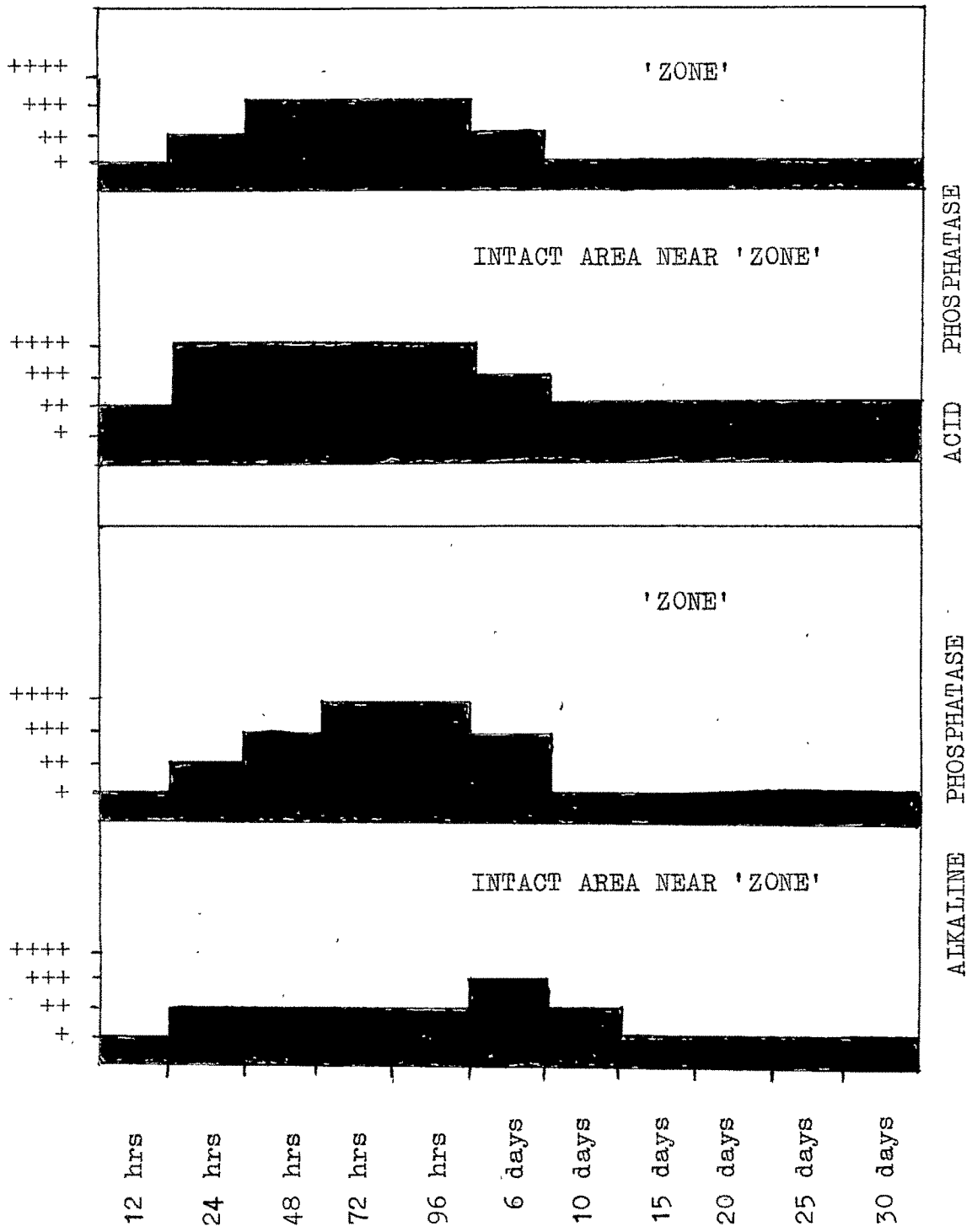
Fig. 13. Graphic representation of changes in the activities of acid and alkaline phosphatases near the injured region in the pigeon liver.



experimental pigeons by about 24 hrs after inflicting the injury, the periportal areas in the "zone" region, adjacent to the injured area, an intense activity of Alk Pase, was noticed (Fig. 7). In the 'zone' area its activity was maximum between 48 hrs and 96 hrs (Figs. 8 & 9). And by 6th day, the healthy cells adjacent to 'zone' also showed similarly high concentration of the enzyme (Fig. 10). Thereafter, i.e., from 10th day onwards the normal parenchymal cells near the 'zone' revealed a gradual decline in the enzyme concentration, while, that in the 'zone' completely disappeared (Fig. 11). 15th day onwards the activity of Alk Pase in the parenchymal cells near the wound area dropped and reached the level comparable to that of the normal liver (Fig. 12).

The concentrations of both Acid and Alkaline phosphatases during the processes of wound healing and repair in the pigeon liver are presented diagramatically in Fig. 13.

DISCUSSION

Acid Pase, being a hydrolytic enzyme, is usually associated with phagocytic processes as well as with cellular autolysis (Duve, 1959; Weber and Nichas, 1961). Thus the presence of this enzyme in macrophages and

lymphocytes as well as in histiocytes like Kupffer cells is quite understandable (Dannenberg et al., 1960; Duve et al., 1962; Novikoff, 1963; Thorbecke et al., 1961; Carranza and Cabrini, 1962; Elves, 1966).

In the present investigation when the irreversible injury to the hepatic cells was inflicted by applying pressure, the cells in that region became necrotized and were partially removed by macrophages formed from lymphocytes that appeared in the region between intact and necrotic parts of the liver (Chapter 6). Increased concentration of Acid Pase in the lymphocytes and macrophages present in this region definitely indicated the lytic reactions occuring there. Thorbecke et al. (1961) had shown an increased activity of Acid Pase in the reticuloendothelial cells of the liver when colloidal particles were injected into the blood stream. A high concentration of Acid Pase is reported in the isolated cells (probably histiocytes or macrophages) in the connective tissue of the healing wounds (Carranza and Cabrini, 1962).

In this context it is interesting to note that the lymphocytopoietic nodules present in the pigeon liver did not show any Acid Pase activity during earlier phases of wound healing, but 6 to 10 days after infliction of the injury, cells of the nodules showed an appreciable

concentration of the enzyme. Presence of lymphocytopoietic nodules in the pigeon liver has been reported (Chapter 1 and Pilo, 1970) and their participation in the phagocytic processes has been observed (Chapter 4). When haemolyzed blood was injected, cells in the nodules were found to take up the cellular debris, and that remained in them for about 9 days (Chapter 4). Incidentally the Acid Pase activity in the cells of nodules noted in the present investigation of injury indicated that the cells in these nodules received the injured cells or their fragments concurrently the lytic action of the cells became more intense.

The Acid Pase activity found to be more profound near the injured area during early wound healing phase could also be correlated to the fact that the injured and neighbouring areas were found to have acidic environment in the beginning (Chapter 6). Perhaps the demolition process is enhanced in an acidic medium. A change of pH to acidic level could take place due to the liberation of nucleic acids (Tsanev, 1963) or due to increased ionic concentration (Needham, 1952). However, the function of increased Acid Pase activity need not be only for the demolition process in the injured area. Acid Pase activity could also be associated with autophagocytosis, a part of the mechanism by which the hepatic cells become

partially dedifferentiated prior to division (Becker and Lane, 1965).

The next important process that takes place after the initial phagocytic reaction near the injured area in the liver was the demarcation of intact region from the irreversible injured parts by the formation of connective tissue (Chapter 6). The laying down of collagen fibres which began by about 48 hrs after infliction of injury, coincided with the increased activity of Alk Pase (Fig. 8). Fell and Danielli (1943) have suggested that this enzyme has been found to participate in the collagen formation. Its role in synthesis in fibrous proteim formation and differentiation of collagen has been stressed by many other authors (Marchant, 1949; Junquiera, 1950). The increase in the concentration of this enzyme in the 'zone' by about 4 to 6 days thus corresponded with the active collagen synthesis. However, maximum intensity of Alk Pase activity in the parenchymal cells near the wound area was by about 10 days after the infliction of injury which was the period of active proliferation of hepatic cells (Chapter 6). It was also observed that during the wound healing in the liver, there was a significant depletion of glycogen from the parenchymal cells situated subjacent to the wound area in the initial stages (4 to 6 days). This denotes an increased requirement of glucose for the wound healing process and also for the dividing hepatic cells at the

wound site which apparently caused the depletion of glycogen from the nearby cells. Availability of glucose is of utmost importance for dividing cells. Bullough (1949) pointed out that the mitotic rate in the mouse skin showing physiological regeneration is related to blood sugar level. There was no further division of hepatic cells after 10th day (Chapter 6) and simultaneously an increase in glycogen level was also noticed. These facts show that the glucose requirement was reduced after 10th day, and hence the excess of glucose might have been converted into glycogen thereby increasing the glycogen content in the hepatic cells. There was also a parallel decrease in the Alk Pase activity in the parenchymal cells by 10th day, which suggests that Alk Pase may be aiding the breakdown of glycogen or helping the transport of it or its intermediary product across the cell membrane. Perhaps this enzyme may also be playing a supplementary role to other phosphatases in releasing the energy from the phosphate bonds of ATP and other phosphate-esters. Hence the increase in the Alk Pase activity (between 6th and 10th days) could be correlated to the metabolic activities in the hepatic cells during the processes of wound healing and repair.