

CHAPTER 8

STUDIES ON ASCORBIC ACID IN RELATION WITH COLLAGEN
SYNTHESIS AND CONNECTIVE TISSUE FORMATION AT
THE WOUND SITE IN THE PIGEON LIVER

The role of ascorbic acid in the formation and maintenance of normal collagen in animals is already known (Bourne, 1953; Jeney et al., 1936; Querido ^{and Baillard} et al., 1939). This is evident from the fact that in normal healthy individuals, there is rapid formation of collagen at the wound site, whereas such response is poor in scorbutic mammals (Hunt, 1941; Bourne, 1942; Danielli et al., 1945). Some investigators (Zamanskii and Lopushanskii, 1955; Schauble et al., 1960; Crandon et al., 1961) have reported increase in ascorbic acid during healing of wounds. Gould (1958) distinguished two types of collagen formation, one involved in the body growth (growth collagen) which is independent of ascorbic acid and other (repair collagen) dependent on ascorbic acid (Gould, 1961). Moreover, Chen and Rostlethwait (1961) reported that ascorbic acid is necessary for maintenance of newly formed collagen and the tensile strength of healed wounds.

Relationship of ascorbic acid with collagen

formation is established mostly by studying wound healing in skin (Lanman and Ingalls, 1937; Hartzell and Stone, 1942; Bourne, 1944; Gould and Woessner, 1957). However, very little is known whether the collagen formation at the wound sites in the visceral organs is dependent on ascorbic acid and also whether it helps in maintaining the tensile strength of the connective tissue formed at the wound sites in the visceral organs. The present study was conducted mainly to investigate the participation of ascorbic acid in the collagen formation at the wound site in the pigeon liver.

MATERIAL AND METHODS

Thirty healthy adult pigeons were taken for the experiments. Each one was operated and a small piece of liver was removed and the adjacent area near the wound was subjected to high mechanical pressure. The surgical method, used in the study was same as previously described in Chapter 6. Three experimental birds were sacrificed each time at the intervals of 1, 2, 3, 4, 6, 10, 15, 20, 25 and 30 days after operation. The liver with wound surface was carefully removed and was used for the histochemical studies.

Ascorbic acid (AA) was histochemically demonstrated

by the method of Giroud and Leblond (1936) modified by Chinoy (1969a, b). Control sections were devitaminized in 10% neutral formalin before staining. Some sections of each sample were stained with azan stain for demonstration of collagen (Gurr, 1956). Quantitative estimation of ascorbic acid was carried out by the method of Roe and Kuether (1943) as stated by Roe (1954).

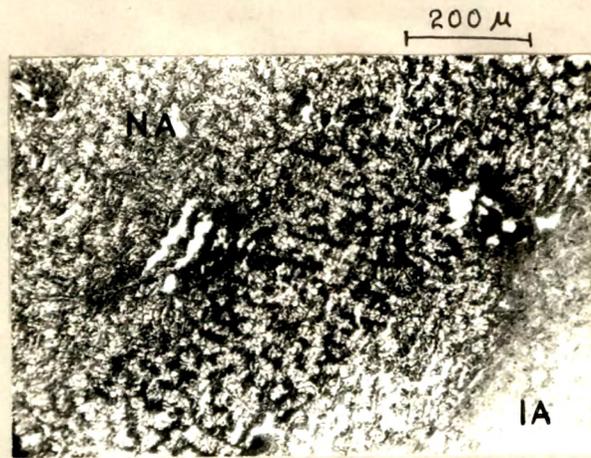
RESULTS

In the early period of wound healing (24 hrs after operation) ~~the~~ ascorbic acid was not noticeable in the healthy tissue subjacent to ^{the} injured area. Its presence became noticeable only by 72 hrs (Fig. 1) when some collagen material was also seen deposited between the intact and injured area (Fig. 2). The maximum concentration of AA was noticed by 96 hrs after operation (Fig. 3). Concomittantly the collagen was laid maximally at this stage of wound healing (Fig. 4). By 6th day the AA seen was mainly localized in the fibroblasts or lymphocytes (Fig. 5) enmeshed in the collagenous fibres (Fig. 6) formed there anew. Tenth day onwards the loosely arranged connective tissue fibres between the injured and subjacent healthy part started getting closely packed and by 15th day the injured part began to separate along with a part of

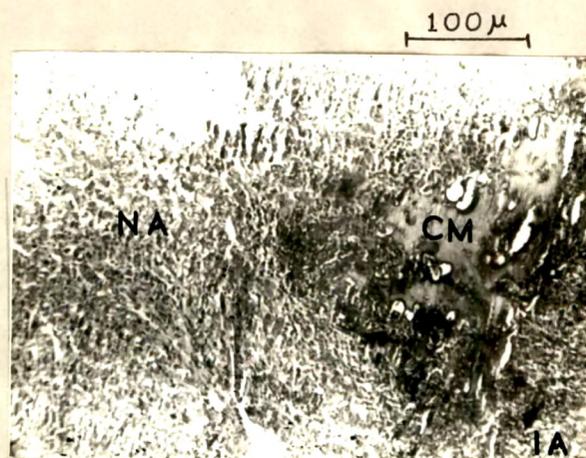
(Chapter 8: Figs. 1 and 2. Photomicrographs of the liver showing ascorbic acid localization and collagenous materials at the wound site)

Fig. 1. 72 hrs after inflicting the injury. The normal healthy area (NA) shows fairly high concentration of ascorbic acid (dark spots) and in the injured area (IA) the vitamin is absent.

Fig. 2. 72 hrs after inflicting the injury. Note the presence of collagen materials (CM) between the normal healthy area (NA) and the injured area (IA).



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(Chapter 8: Figs. 3 to 6. Photomicrographs of the liver showing the ascorbic acid localization and connective tissue formation at the wound site)

Fig. 3. 96 hrs after inflicting the injury. Note the presence of ascorbic acid as fine granular deposition (arrows). Many cells (darkly stained) are also loaded with the vitamin.

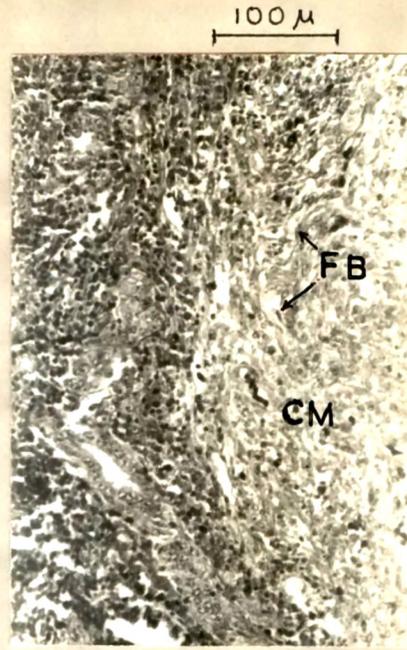
Fig. 4. 96 hrs after inflicting the injury. Note the increased number of fibroblasts (FB) and collagen materials (CM).

Fig. 5. 6 days after inflicting the injury. Note the large number of lymphocytes and fibroblasts and lymphocytes loaded with ascorbic acid (arrows).

Fig. 6. 6 days after inflicting the injury. Note the band of connective tissue demarcating the normal intact area (NA) from the injured area (IA).



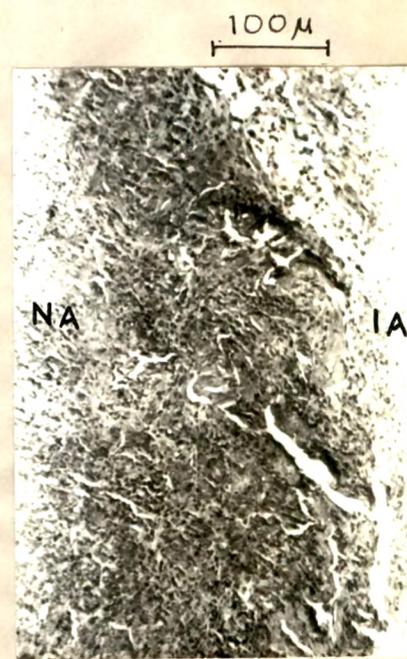
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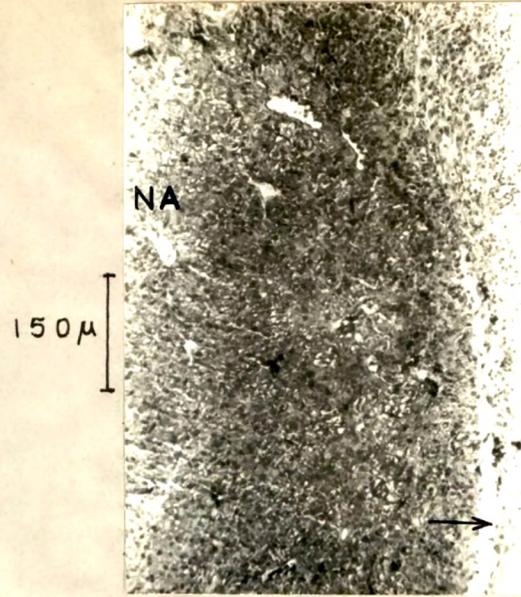
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(Chapter 8: Figs. 7 to 9. Photomicrographs of the liver of the pigeon showing the connective tissue formed at the wound site and the ascorbic acid in the liver cells and connective tissue)

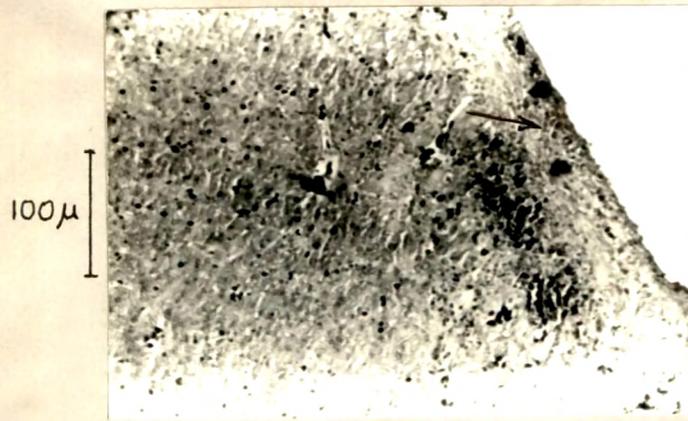
Fig. 7. 15 days after inflicting the injury. Stained for collagen. Note the beginning of the separation (arrow) of the injured part from the intact area (NA).

Fig. 8. 20 days after inflicting the injury. Stained for collagen. The connective tissue (arrow) has thinned out very much.

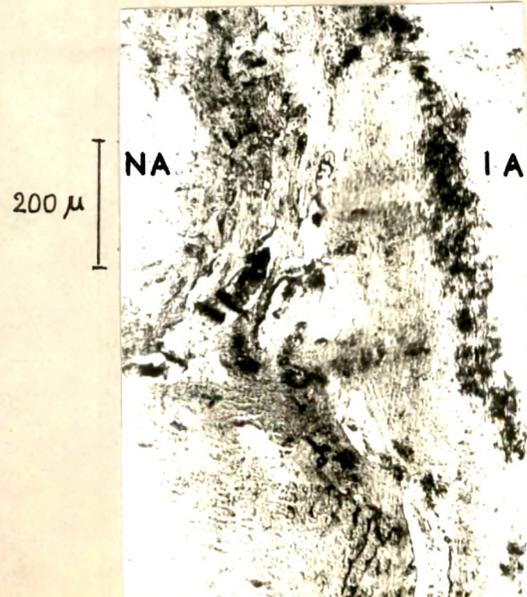
Fig. 9. 10 days after inflicting the injury. The ascorbic acid is seen in the connective tissue that separate the injured part (IA) from the normal area (NA). The splitting up of the connective tissue is also seen clearly here.



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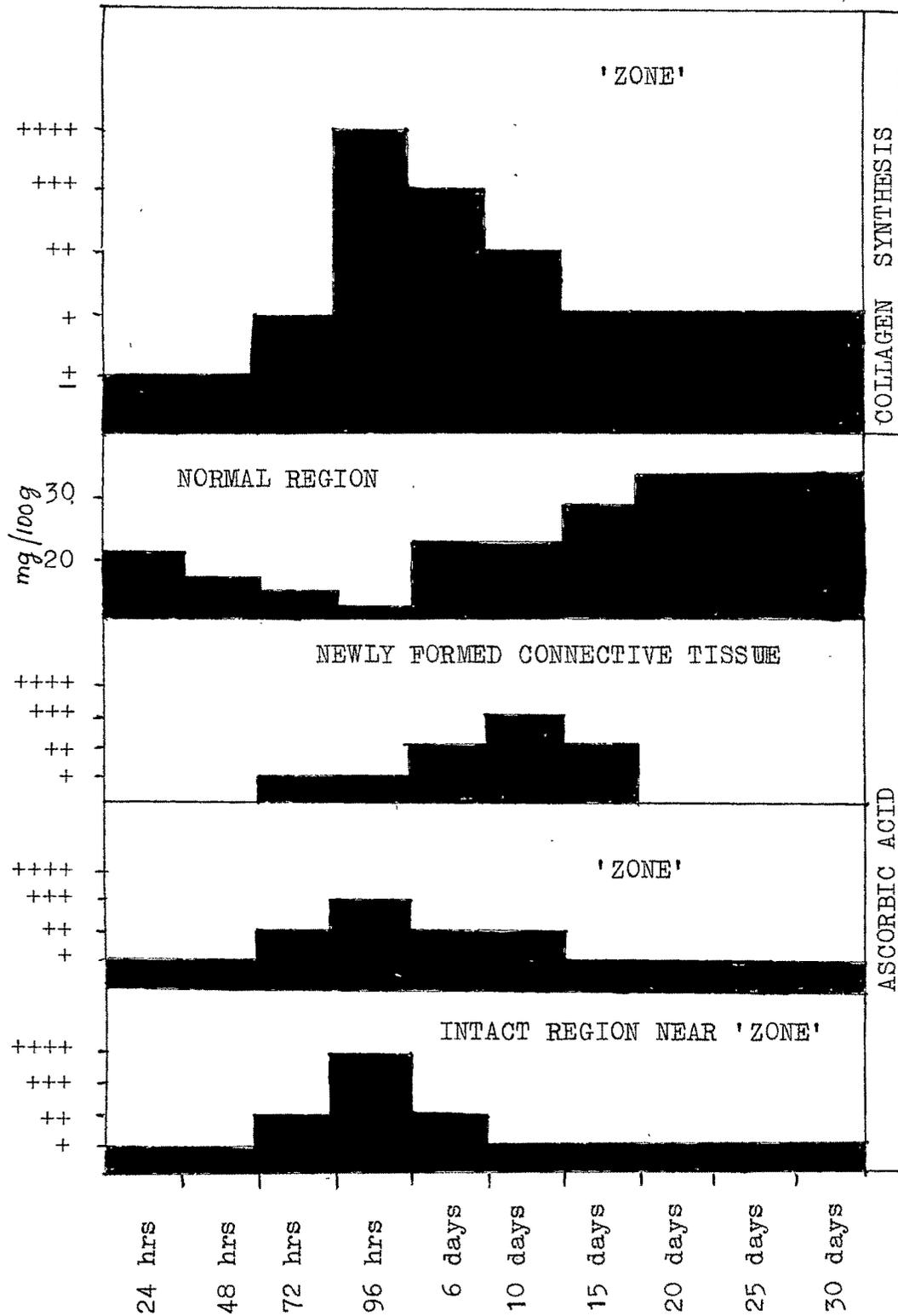
TABLE I

Ascorbic acid content of the normal liver of pigeon and
that undergoing wound healing and repair

Time after the infliction of injury	Ascorbic acid content mg/100g wet liver
0 hrs (normal)	38.45 (35.6-42.8)*
24 hrs	21.26 (20.6-24.3)
48 hrs	16.85 (15.5-18.4)
72 hrs	14.75 (15.6-16.7)
96 hrs	11.96 (10.8-14.9)
6 days	23.77 (22.9-24.8)
8 days	23.77 (22.7-24.9)
10 days	23.50 (22.2-25.2)
15 days	28.86 (27.3-30.5)
20 days	34.21 (33.9-35.5)

* Figures in parenthesis denote range of
readings from 3 experimental birds

Fig. 10. Graphic representation of changes in the collagen synthesis and ascorbic acid content near the injured region in the pigeon liver.



the newly connective tissue (Fig. 7). When the sloughing off of the injured part became complete by 20th day the repaired healthy part was covered by only a thin membrane formed of connective tissue (closely packed collagen fibres) (Fig. 8). During the earlier period (i.e., about 10th day) an appreciable amount of AA was present in the loosely packed connective tissue (Fig. 9) but later by 20th day as connective tissue became very thin due to close and compact arrangement of fibres, AA totally disappeared from the connective tissue. Quantitative estimations of AA in the liver during different phases of wound healing and repair were made and the results obtained are presented in the Table I^{& Fig. 10}. From the Table it could be seen that a significant depletion of ascorbic acid occurred from the liver during the period between 48 and 96 hrs after inflicting injury. Thereafter, the vitamin content increased to reach the normal level (35 to 40 mg/100g fresh liver).

DISCUSSION

Since the concentration of ascorbic acid in the part of liver subjacent to the wound site was maximum by about 4th day after the infliction of injury- a period characterized by the massive laying down of collagen fibres at the "zone" area (Fig. 3)- it could be logically

argued that during wound healing and repair in the liver, the collagen formation is dependent on ascorbic acid. The most interesting feature of wound healing in the liver following infliction of injury is the participation of lymphocytes. As stated in Chapter 6, these lymphocytes transform into fibroblasts at the wound site. The evidence now strongly suggests that for such differentiation of the mononuclear leucocytes (lymphocytes) into the fibroblasts, the ascorbic acid plays a direct role (Gould, 1963). Prior to the formation of collagen fibres for the purpose of demarcating the injured part from the healthy part of the liver, a large number of fibroblasts and lymphocytes was found in the "zone" area. It is known that accumulation of AA at the wound site is due to the aggregation of fibroblasts and leucocytes loaded with the vitamin (Bartlett et al., 1940 & 1942). Thus in pigeon also it seems that at the wound site in the liver the vitamin is brought in from other parts of the organ and is taken up by the lymphocytes which aggregate in large number at the site. There is no report that AA is actively synthesized at the wound site. The observations of Schilling et al., (1953) show that AA content is higher in the tissue fluids of healing area than in the blood serum which points to the fact that AA is brought from other parts of the body to the wound site. Similar observations were reported by Lauber and Rosenfeld (1938) and Candlish ^{and Chandra} ~~et al.~~, (1967). From the

Table it is evident that AA gets depleted from the healthy parts of the liver during the period between 48 to 96 hrs. From 6th day onwards the vitamin concentration in the liver was found to increase to reach a preoperative level by 20th day. Concomittantly, AA disappeared from the intact region adjacent to injured area where connective tissue was being formed. This may indicate the end of connective tissue formation and hence a high AA concentration is no more necessary. Though AA disappeared from the parenchymal cells of the liver, from the histochemical preparations it was found that AA was present (though in less concentration) in the connective tissue covering the injured area. The presence of the vitamin in the newly formed connective tissue in the liver could be explained on the basis of the report of Chen and Rostlethwait (1961), that AA is required for the maintenance of collagen formed anew as well as to maintain its tensile strength.

X Thus the present study ^{strongly supports} proves the requirement of AA for the wound healing and repair process in the pigeon liver like that in the epithelial tissues.