

VITAMIN C DEFICIENCY AND WOUND HEALING

AN EXPERIMENTAL AND CLINICAL STUDY

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SPONTANEOUS breakdown of a surgical wound in the absence of infection occurs with relative frequency in patients with the cachexia of cancer, in debilitated individuals, and in young patients; notably those who have some congenital anomaly of the gastro-intestinal tract. There exists in these patients a likelihood of ascorbic acid deficiency. Since the relationship of ascorbic acid to collagen formation has been clearly demonstrated, we have endeavored to reëxamine the problem of noninfectious wound breakdown from the point of view of a possible specific relationship to vitamin C deficiency.

Cases of frank scurvy are not of frequent occurrence even in patients of the age group treated at the Children's and Infants' Hospital in Boston. There is being disclosed at this hospital by laboratory tests, however, a far greater incidence of asymptomatic scurvy than hitherto could be recognized by the usual methods of clinical examination. The term "asymptomatic scurvy" may be defined as scurvy existing in a patient without characteristic symptoms, physical signs, or roentgenologic evidence during life, but in whom the histologic changes of early scurvy are demonstrable at autopsy, as is illustrated in the appended case report.

Case Report.—No. A204,665, L. F., male, age four weeks, was admitted to the hospital because of a congenital atresia of the bowel. He had had an ileostomy performed at another hospital at the age of two days. On admission he was markedly emaciated and dehydrated. For ten days an attempt was made to improve his nutritional state. In addition to breast milk, he was given several transfusions, and daily administrations of parenteral fluids—glucose and normal saline. On the eleventh day a laparotomy was performed, and a side-to-side anastomosis around the site of the obstruction effected. The ileostomy was closed. For the next three days the patient received a continuous intravenous drip of glucose, as well as two transfusions. The abdominal wound opened on the third postoperative day. It was resutured but the patient died the following day.

At autopsy microscopic sections of the costochondral junction were described as follows: "There is a definite lattice formation and many of the cartilage spicules are fractured. Osteoblasts are present along the cartilage spicules but there is no osteoid formation." This is evidence of early scurvy. It is significant that roentgenologic examination of the wrist, taken during life, showed no evidence of scurvy. It is also suggestive, in view of our experimental findings, that sections of the skin at the operative site showed numerous fibroblasts without formation of collagen. Sections of the

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WOUND HEALING

intestinal wall adjacent to the anastomosis showed proliferation of connective tissue without definite formation of collagen.

This infant had asymptomatic scurvy at six weeks of age, but during life did not show any of the clinical or roentgenologic evidences of the disease. Although this patient received some breast milk during his six weeks of life, it seems clear that he did not have enough ascorbic acid to meet metabolic requirements. It is our opinion that this was at least one factor in the failure of the wound to heal.

Minot¹ (1936) has recently stated: "The major problems of nutrition do not concern clean cut deficiency diseases, but the prevention of partial deficiency. Border line states of nutritional instability are much more common than is usually appreciated. There is a wide zone between optimal nutrition, and the level at which classic symptoms of recognized dietary deficient states develop." It is becoming increasingly apparent that vitamin C nutrition is a relative term embracing several zones of ascorbic acid depot; between saturation on the one hand and absolute depletion on the other. A large body of evidence is being assembled to show that the conception of vitamin C deficiency should include not only absolute depletion of the ascorbic acid stores, but also such low levels of supply as place the functioning of connective tissue cells at a physiologic disadvantage.

This conception of vitamin C deficiency, as well as the wide range of plasma ascorbic acid levels in human beings, is borne out by a recent study by one of us² at the Infants' Hospital. The ascorbic acid in the plasma was determined in 54 patients. These patients were divided into four groups on the basis of their ascorbic acid nutrition as suggested by the feeding history. The results are shown in Table I.

TABLE I

Group*	Plasma Ascorbic Acid (Mg. %—Average for Group)
I Well Baby Clinic.....	1.00
II Ward patients with history of good vitamin C content in diet.....	0.73
III Ward patients with history of poor vitamin C content in diet.....	0.22
IV Ward patients with frank scurvy.....	0.08

*It is noteworthy that only in Group IV was there clinical evidence of scurvy. Groups II and III were admitted for a great variety of causes. Group I showed no disease.

The subject of wound healing is covered by a large literature that has recently been surveyed by Arey³ (1936). He lists 12 general factors influencing wound healing, each one of which has been the subject of numerous researches. Among other phases of nutrition, the influence of fats, carbohydrates, and proteins, in the diet, has been investigated. Likewise considerable attention has been given to the rôle of vitamins in regenerative

processes. Avitaminosis A, B, C, and D has each in its turn been reported to inhibit normal healing, with resumption of healing following ingestion, or local application, of the specific vitamin involved in the deficiency.

The attention given to local application of vitamins, hormones, peptones, chick embryo juice, and sulphydril compounds rests largely on the emphasis given by Carrel⁴ (1921) to "external irritation," as the initiating mechanism of wound repair. He was able to delay cicatrization and epidermization for as long as 20 days by protecting the wound with an overlying sheet of fascia sutured to its edges. The multiplicity of specific remedies advocated for the promotion of wound healing, however, is at variance with the simple clinical fact that most wounds, not directly contaminated by gross infection, heal satisfactorily in healthy people, while those in debilitated patients may be difficult to improve under any regimen. As Höjer⁷ (1924) has aptly put it: "Even if, as Carrel has pointed out, the cicatrization of wounds is apparently initiated by an external factor, the answer of the organism to the irritation is decided by internal factors." He concludes that a full supply of anti-scorbutic "is necessary to an answer of normal intensity."

Normal repair of connective tissue is primarily a process of fibroblastic proliferation and the formation of collagen. Wolbach⁵ (1933) has concluded that this formation of collagen is "a product of secretory activity of fibroblasts."

Wolbach and Howe⁶ (1926) have characterized scurvy as an "inability of the supporting tissues to produce and maintain intercellular substance. Direct proof of this conclusion has been obtained in the study of repair of soft tissue in regard to the collagen of connective tissue." Höjer has observed that: "Before the formation of collagen is completely arrested, there is a stage where it is very unevenly distributed and irregularly arranged."

These facts rest securely upon proved pathologic studies and form the basis for an experimental study of wound healing in guinea-pigs, which were partially depleted of their vitamin C depot and subsequently maintained at a low level of ascorbic acid supply (0.25 mg. daily). The nutritional state of these animals resulting from this diet may be held analogous to asymptomatic scurvy.

Experiments.—During this study two groups, comprising normal and scorbutic guinea-pigs, were operated upon and incisions made through the abdominal and stomach wall. Subsequently at periods of ten, 20, and 30 days, representatives of each group were sacrificed for histologic study, and for the measurement of the pressure required to rupture the scars in the abdominal and gastric walls.

Diet.—Twenty-four growing guinea-pigs weighing about 200 Gm. were separated in two groups; one group was fed solely on a scorbutogenic diet, and the other on this same basic formula but with an ample supplement of green vegetable tops.

WOUND HEALING

Rolled oats.....	35.0%	35.0%
Wheaten bran.....	34.0%	34.0%
Skimmed milk powder (heated and stirred for 1 hr.).....	22.5%	
Butter fat.....	7.5%	
Sodium chloride.....	1.0%	1.0%

In addition 0.5 cc. of oleum percomorphum (for vitamins A and D) was given twice a week.

The group selected for the production of scurvy was given the above diet exclusively for nine days in order to accomplish, to a large degree, depletion of the vitamin C depot. On the tenth day 2.0 mg. of crystalline ascorbic acid dissolved in distilled water were administered with a pipet to each of these 12 guinea-pigs. Thereafter 0.5 mg. was given every other day for the duration of the experiment.

The scorbutogenic properties of this diet and the adequacy of the control feeding were attested by roentgenologic studies of the wrists at ten day intervals on representatives of both groups, and by the histology of the ribs at autopsy.

Operative Technic.—On the fifteenth day after starting the above diets, the 12 scorbutic and the 12 normal animals were operated upon as follows: Under ether and using aseptic technic, a one inch incision was made slightly below the xiphoid in the midline, the abdominal musculature divided by blunt dissection, and the peritoneum incised. The stomach was delivered into the wound and part of the anterior stomach wall was placed in an intestinal clamp. An incision one-half inch long was then made through the anterior stomach wall. This incision was closed with a running suture of silk, no attempt being made to invert the edges. The abdominal wound was closed in two layers, interrupted silk sutures to muscles and peritoneum, and the skin by a running suture of silk. No dressing was applied to the skin, which was cleaned with alcohol and painted with collodion.

In the scorbutic group there was one immediate operative fatality, and a second guinea-pig died during the night following operation. Two of the controls were found unsatisfactory for the purpose of this experiment, one because of gross infection of the wound and healing by second intention, and another because of faulty technic during measurement of wound strength. There were left ten guinea-pigs of each group (20 in all), for the purpose of the study.

Subsequent Procedure.—The skin sutures in both groups were removed one week after operation. At periods of ten, 20, and 30 days, three members of each group were sacrificed; two for the purpose of wound measurement, and the third for histologic study.

There were no complicating factors in the experiment as far as could be ascertained. The wounds healed by first intention and at about the same gross rate. The smooth, even abdominal scars of the normally fed group, however, at ten, 20, and 30 days postoperatively, contrasted with the rough

appearance of the scars of the scorbutic group at the same time interval (Fig. 1).

Technic of Measurement.—As in the experiments of Harvey and Howes⁸ (1930), the strength of the wound was estimated in terms of its breaking point when distended by air. Our apparatus differed from theirs, however, and was constructed from a mercury sphygmomanometer with its cuff wrapped around a small bottle. Its closed circuit was connected with a free length of tubing to which a lumbar puncture needle was attached (Fig. 2). The needle was inserted into the peritoneal cavity, care being taken to avoid subcutaneous emphysema, and the intra-abdominal pressure slowly increased. The highest point reached by the mercury column, before gaping and rupture of the wound occurred, was recorded. The same general procedure was used



FIG. 1.—The normal animal is on the left. Note difference in gross appearance of operative wounds. (Ten days postoperative.)

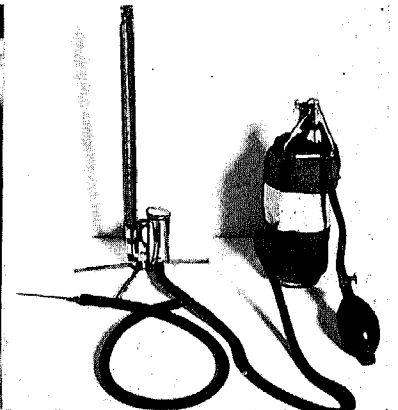


FIG. 2.—Apparatus used for distending wounds in the experimental animals.

for testing the stomach wound. The organs were left in situ after opening the abdominal wall. The esophagus was clamped close to the cardia, the small intestine tied about one inch below the pylorus, and the needle inserted just proximal to this tie. We may quote Harvey and Howes in regard to the accuracy of this method of measuring wound strength: "It is conceded at once that this gives no value of the (absolute) strength of the wound, but inasmuch as the data desired, are those of relative changes in strength, the method seems adequate."

Results.—During the process of abdominal distention and rupture, there was a characteristic gross difference in tensile strength, behavior, and appearance of the two types of wounds. The abdominal wounds of the normal animals uniformly withstood far greater distending pressure than did those of the scorbutic animals. The wounds of the normal animals burst with great suddenness when they reached the breaking point; the wounds of the scorbutic animals first gaped widely at the site of the incision, and then ruptured at a slightly higher pressure. The scar tissue of the wounds of the normal animals after rupture was pink and firm, while that of the wounds

WOUND HEALING

of the scorbutic animals was livid and of soft consistency. It resembled closely the appearance of the broken down wound in the debilitated human patient (Figs. 3 and 4).

It was found that the wound in the abdominal wall served better to demonstrate the difference in wound strength of the two groups, as the wound in the stomach was not always suitable for direct observation. In addition, the gastric incisions with their subsequent adhesions to liver and omentum were not precisely similar in each guinea-pig. It is notable, however, that in all the scorbutic animals rupture occurred at the wound, while in the normal animals the stomach ruptured in all cases elsewhere than at the wound, and at higher levels of pressure (Tables II and III).

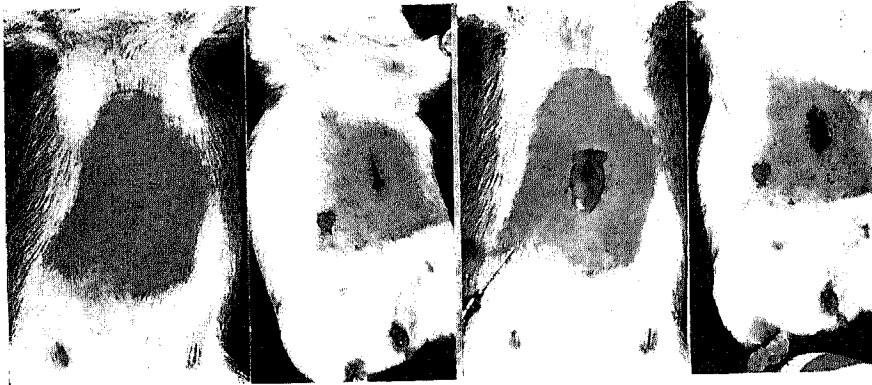


FIG. 3.—Same animals as in Fig. 1, 30 days postoperative. The peritoneal cavity of the normal animal on the left has been distended to 70 Mm. of mercury air pressure. The scorbutic animal on the right is distended to only 38 Mm. of mercury air pressure, and the wound has begun to gape.

FIG. 4.—Same animals as Figs. 1 and 2: The normal animal on the left is now distended to 200 Mm. of mercury air pressure. The wound has ruptured. The animal on the right is distended to 47 Mm. of mercury air pressure and complete rupture of the wound has occurred. Note difference in gross appearance of both wound edges and the granulation tissue in the two animals.

Histology.—Sections were made through three representative areas of each wound, and stained with hematoxylin and eosin, and with Mallory's aniline blue.

The operative incision through the corium in each of the partially scorbutic animals showed connective tissue repair with but very little collagen production. A defect in the corium resulted that was easily apparent under low magnification (Fig. 5). The subcutaneous fibroblastic tissue was abnormally thick and irregular in pattern in the partially scorbutic animals, and there was very little collagen deposition ten, 20, and 30 days postoperatively (Fig. 6). Numerous extravasations of red blood cells were seen, in many of which were strands of fibrin. Hemosiderin was conspicuous in the two animals sacrificed 20 and 30 days after operation. In all control animals there was a thick collagenous layer at the site of the incision, and the original point of incision through the corium could be ascertained only by its relation to the subcutaneous scar. Healing in the controls proceeded with a plentiful

TABLE II

AIR PRESSURE REQUIRED (IN MILLIMETERS OF MERCURY) TO RUPTURE ABDOMINAL WOUNDS

Normal Animals				Scorbutic Animals			
Number	Date	Mm. of Mercury		Number	Date	Mm. of Mercury	
		Gaped	Ruptured			Gaped	Ruptured
1	10 days P.O.	..	128	1	10 days P.O.	67	85
2	10 days P.O.	..	145	2	10 days P.O.	37	60
3	20 days P.O.	..	165	3	20 days P.O.	40	50
4	20 days P.O.	..	135*	4	20 days P.O.	45	65
5	30 days P.O.	..	210†	5	30 days P.O.	62	85
6	30 days P.O.	..	200	6	30 days P.O.	38	47
	Average	..	160		Average	48	65

* Postoperative hernia.

† Abdominal wall ruptured elsewhere before wound gave way.

TABLE III

AIR PRESSURE REQUIRED (IN MILLIMETERS OF MERCURY) TO RUPTURE GASTRIC WOUNDS

Normal Animals			Scorbutic Animals		
Number	Date	Mm. of Hg.	Number	Date	Mm. of Hg.
1	10 Days P.O.	95	1	10 Days P.O.	25
2	10 Days P.O.	90	2	10 Days P.O.	34
3	20 Days P.O.	60	3	20 Days P.O.	20
4	20 Days P.O.	45	4	20 Days P.O.	40
5	30 Days P.O.	45	5	30 Days P.O.	25
6	30 Days P.O.	43	6	30 Days P.O.	34
	Average	70		Average	30

In all cases stomach ruptured elsewhere than at wound.

In all cases wound ruptured before stomach.

deposition of collagen by the tenth postoperative day, and in the wounds of these animals hemorrhage and hemosiderosis were minimal. Both groups showed foreign body giant cell reaction about the sutures.

DISCUSSION.—Other workers have established experimentally the inability of tissues to produce intercellular substance in absolute scurvy. That the data so obtained have a valid application to human beings is the conclusion of Wolbach⁹ (1937) as recently expressed in the following statement: "The gross and microscopic pathologic changes of human scurvy as seen in the infant, and experimental scurvy as seen in the guinea-pig, are so nearly identical that no reasonable doubt can be entertained with regard to applying to the human being the facts ascertained from the experimental studies." One can therefore state with a fair degree of certainty that in a patient with abso-

lute scurvy, the effective healing of such lesions as require fibroblastic repair comes to a standstill. Absolute scurvy, however, is of relatively rare occurrence. The most significant clinical aspect of this study is its possible bearing on states of partial deficiency of vitamin C.

The condition of the vitamin C deficient animals used in this study may be held analogous to asymptomatic scurvy in man. Wound healing in these partially scorbutic animals progresses in marked contrast to that observed in the controls. This contrast was manifested in inferior tensile strength, in a disposition of these wounds to gape before rupturing, and in the livid appearance of their granulation tissue and its soft consistency. Microscopic study provided what seems to be an adequate explanation for these differences. The deficiency in collagen was undoubtedly reflected in the inferior tensile strength and soft consistency of the scorbutic granulation tissue. Its lividity was probably a reflection of poor vascularization and hemorrhage.

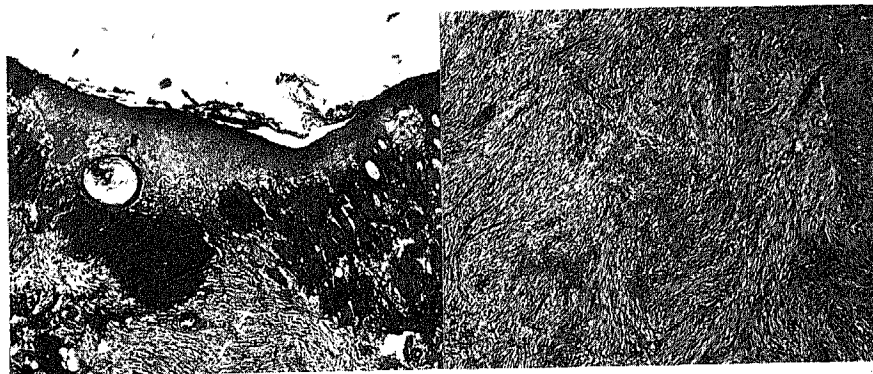


FIG. 5.—Photomicrograph of skin and subcutaneous tissue of scorbutic animal, showing the defect in the corium (low power), aniline blue stain.

FIG. 6.—Photomicrograph of the subcutaneous tissues of the same animal as Fig. 5, showing the great cellularity and irregular arrangement of fibroblasts, which are laying down very little intercellular substance—collagen (low power).

The initial gaping observed in the wounds of the scorbutic animals following abdominal distention can be ascribed to the defect found regularly in the corium. This defect was not seen in the control animals in which a new layer of collagen was laid down in the first ten days after operation. The importance of the corium in repair of the abdominal defect is also indicated by the fact that one normal animal with a postoperative hernia withstood an intra-abdominal pressure of 135 Mm. of mercury before rupture occurred (Table II). The inferior tensile strength of the wounds of the scorbutic animals 30 days postoperatively has an added importance in the light of Harvey and Howes' demonstration that the greater part of the final tensile strength of a normal wound (as measured in the rat's stomach) is attained within ten days after operation.

Vitamin C does not, of course, play the only rôle in wound healing. Indeed its part among the other vitamins may not be the leading one. The progress in the early recognition of the several vitamin deficiency states has been reported by Blackfan¹⁰ (1937). Vitamins A, B₁, C and D can now

be determined by quantitative chemical tests. The individual bearing of all these vitamins on the healing process demands further study. It seems clear, however, that there exists a degree of vitamin C deficiency in human beings that is not recognizable by methods ordinarily used in physical examination. This degree of deficiency, or asymptomatic scurvy, may well have an important bearing on wound healing. The incidence of asymptomatic scurvy is undoubtedly more frequent than is generally realized. Its existence has been proved histologically and chemically in the age group encountered at this hospital. Its existence in older patients, though not yet proved, undoubtedly occurs. If the average daily intake of ascorbic acid falls below the minimal protective dose, the consequences of this increasing increment of deficiency, after depletion of the body depot, are evident.

In estimating the degree of vitamin C deficiency clinically, the plasma determination of ascorbic acid, by the indophenol test, serves as a very useful guide. When the figure falls much below .45 mg. per 100 cc. of plasma, it may be inferred (Table I) that the vitamin C depot is approaching dangerous depletion. In such circumstances it would seem advisable to administer about five to ten 200 mg. doses of ascorbic acid, as an initial step in preparing the patient for operation. This can be given by mouth or with intravenous fluids, and should not in the least complicate or conflict with other steps that may be indicated as a part of preoperative care.

It is evident that further work on quantitative determination of plasma ascorbic acid must be done, not only in the age group here considered but more especially in adults. Further work along this line is now under way at the Children's Hospital. It is hoped that this paper may stimulate similar studies in hospitals for adults. For it seems reasonable to suppose that in older patients, such as those suffering from prolonged obstruction due to cancer of the pylorus, there may well be a dangerously low ascorbic acid depot.

SUMMARY.—Guinea-pigs were partially depleted of their ascorbic acid depot, and were subsequently maintained on approximately one-fifth of the minimal protective daily dose of ascorbic acid. The healing of the operative incisions of these animals, both histologically and physiologically, was inferior to that of a group of control animals.

A normal wound is considered to attain the greater part of its final strength within the first ten days after operation, but the partially scorbutic animals had greatly inferior tensile strength, when compared to their controls, ten, 20, and 30 days postoperatively.

The abdominal wounds of the scorbutic group ruptured at a pressure averaging approximately one-third that required to rupture the wounds of the normal animals.

The scar tissue in these wounds was distinctly abnormal, being livid and soft in consistency. Histologic study of the wounds in the partially scorbutic group showed defective repair of the corium, and a poor production of collagen in the scar.

The clinical application of these facts is discussed, and a case in point is

cited, in which the presence of asymptomatic scurvy is thought to have been a factor in the failure of an operative wound to heal.

CONCLUSIONS

Vitamin C plays an important rôle in the healing of experimentally produced wounds in guinea-pigs.

There may exist in human beings a degree of vitamin C deficiency that cannot be recognized by methods ordinarily used in physical examination.

It has been proved, histologically and chemically, that asymptomatic scurvy is far more common in infants and children than has been realized. Its existence in older patients, though not yet proved, undoubtedly occurs.

Evidence is presented that a partial vitamin C deficiency is of more importance in the healing of surgical wounds in human beings than has hitherto been appreciated. When a low ascorbic acid depot in a patient is found, or suspected, the giving of ascorbic acid, as an aid to wound healing, seems amply justified.

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