Effect of Thermal Burns on Wound Healing *

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AMONG THE VARIED biochemical changes which occur soon after injury is an abrupt, and often sustained, drop in blood and urinary ascorbic acid, and an apparent decrease in tissue ascorbic acid saturation as judged by "load" tests.[1, 9, 11, 13] The seriously injured patient, then behaves biochemically like a scorbutic. Does this represent functional scurvy? If the injured individual is truly physiologically scorbutic, alterations in the wound healing characteristic of ascorbic acid deficiency should be observed. Since the guinea pig, like monkey and man, requires exogenous vitamin C, it has been the experimental animal used by us.

METHODS

Young male guinea pigs of an inbred strain (WRAMC) were used in this study. For those animals allowed food *ad libitum*, a Rockland † diet was offered: it is complete nutritionally for guinea pigs except for a total lack of ascorbic acid. The amount of food eaten was measured daily. When ascorbic acid was given, it was administered by pharyngeal tube. Water was offered *ad libitum* to all animals and, in some, constituted their sole nutrient intake except for ascorbic acid. In some experiments, cortisone was injected subcutaneously at a dosage of 10 mg. daily, beginning on the day of operation.

The guinea pigs were housed in individual cages, randomly distributed by groups, in an air-conditioned room, the temperature of which was kept at 78° ± 1° C. They were weighed daily. Preparatory to wounding, the skin of the abdomen was shaved; a midline 4 cm. laparotomy incision was made in all animals under light ether anesthesia; the incision was closed with interrupted, through and through fine #36 stainless steel wire sutures placed about 4 mm. apart.

Burns were produced under light ether anesthesia shortly after laparotomy by dipping the guinea pigs' backs into water containing wetting agent ("BRIJ 35") ‡ at 85° centigrade for 30 seconds after the method of McCarthy.[12] The burns involved about one-third of the body surface and were third degree as judged grossly and by histologic examination. Isotonic sodium chloride solution, containing 5 per cent glucose, was given by stomach tube immediately after burning, in an amount equivalent (by weight) to 5 per cent of the animal's body weight.

At days seven, 10 and 14 postoperatively, animals of each group (a total of 200 ani-

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† Specially prepared by the Arcady Farms Milling Co., 223 West Jackson Blvd., Chicago 6, Illinois.

‡ Obtained from the Atlas Powder Co., Wilmington, Delaware.

mals) were sacrificed by overdosage with ether. Sutures were removed, the wounds excised and fixed in 10 per cent buffered formalin. After fixation, blocks taken from the center of the wound were imbedded in paraffin. Sections were stained by a number of technics [10] in an attempt to evaluate the degree and rate of healing.

The general appearance as well as the cellular features of the wounds were studied in slides stained with hematoxylin and eosin. The reticulum fibers were visualized by silver impregnation. Collagen fibers were stained by the van Gieson technic; as the collagen fibers are formed and "mature," they become brilliant red, while "immature" collagen stains yellow. The formation and state of the "ground substance" was followed by staining with toluidine blue and colloidal iron. The former dye stains metachromatically part of the "ground substance" which appears in the early granulation tissue of the wound. The intensity of this staining reaction is thought to vary inversely with the degree of polymerization of the "ground substance," a more intense metachromasia (purple-red) being indicative of depolymerization. As wound healing progresses, the staining reactivity to toluidine blue of the "ground substance" gradually decreases, so that by the seventh day a normally healed wound contains little metachromatic material.

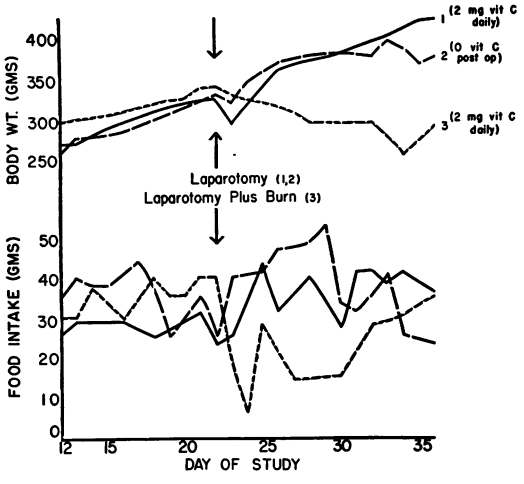
The "ground substance" was also followed by a collodial iron stain (Rinehart's modification of the Hale [14] technic). By this method a portion of the ground substance appears blue. Here, as with toluidine blue, the stain is strongly positive early in normal healing, but by the seventh day little blue staining material is evident.

The wound sections were examined by one of us (J. A. P.) in a uniform manner without previous knowledge of the origin of the slide. The wound area was arbitrarily defined as that area limited by the epidermis and the peritoneal surface in one axis and by the width of two low power (× 35) fields in the other. Each specific histologic detail being investigated was graded in arbitrary units (0 to 4+) in an attempt to obtain a quantitative evaluation of possible changes.

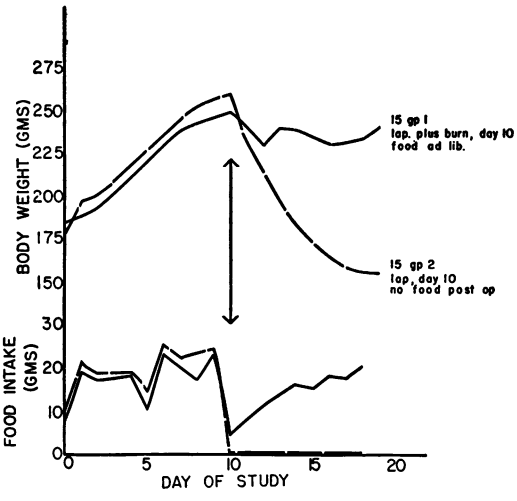
RESULTS

A. *Effect of Abrupt Withdrawal of Ascorbic Acid Postoperatively on Healing of Laparotomy Wounds in Unburned Guinea Pigs.* The first problem we approached was whether the sudden withdrawal of vitamin C postoperatively would result in an acute impairment of the healing of incisional wounds in otherwise healthy animals. Surprisingly enough, despite the very many investigations of the influence of ascorbic acid on wound healing, we were unable to find such data. Accordingly, midline laparotomy incisions were made in two groups of young guinea pigs eating a commercial vitamin C free diet *ad lib.* Each animal received 2 mg. of vitamin C daily by pharyngeal tube during the control period; one group continued to receive vitamin C postoperatively; the other did not. (Two mg. of ascorbic acid daily is the minimal amount which will support normal growth and normal healing of incisional wounds in the otherwise healthy guinea pig). In the former group, healing progressed normally; in contrast, healing was grossly impaired in the animals receiving vitamin C up to operation, but none postoperatively.

Figure 1 represents a 7-day wound in a guinea pig receiving 2 mg. of vitamin C pre and postoperatively. Fibroblasts are plentiful, the amount of reticulum is moderate, and collagen formation has begun. There is little metachromatic (toluidine blue) or blue (colloidal iron) staining "ground substance." In contrast Figure 2 is of a seven-day wound in a guinea pig receiving 2 mg. of vitamin C up to operation but none following. Fibroblasts are present in good number, but their orientation is irregular, hemorrhagic areas are frequent, there is considerable reticulum and very little col-



GRAPH 1.



GRAPH 2.

lagen. There is considerable "ground substance" staining blue with colloidal iron and metachromatically with toluidine blue. These changes are similar to those seen in wounds made in animals with established scurvy.[6]

B. *Effect of Severe Burn on Healing of a Laparotomy Wound.* With these findings, we felt that if the "biochemical" scurvy associated with a severe burn had physiologic significance, it should be demonstrable. Accordingly, we followed the healing of laparotomy wounds in burned and unburned guinea pigs receiving normal maintenance amounts of ascorbic acid and eating the commercial C-free diet *ad lib.* Third degree burns involving about one third the body surface were produced just after laparotomy in the group of burned animals.

The abdominal wounds in the burned guinea pigs healed in a grossly abnormal fashion. Seven days after laparotomy, fibroplasia was ample, but hemorrhages were frequent, reticulum was present in amounts greater than normal and collagen production was scanty. There was considerable "ground substance" stainable metachromatically by toluidine blue and blue by colloidal iron (Fig. 3). These changes were indistinguishable from those seen in unburned animals which received no ascorbic acid postoperatively (Fig. 2). The abnormalities were still present, but to a lesser extent, at ten days. By the 14th day, the wounds were little different from normal.

C. *Effect of Intake of Food (Other than Ascorbic Acid and Water) on Wound Healing.* The burned laparotomized guinea pigs ate less of the ascorbic acid-free Rockland diet offered *ad libitum* than their laparotomized controls. This difference in food intake was most marked in the first few postoperative days (Graph 1). As expected, the burned guinea pigs gained weight briefly, but then fell below their controls. To detect a possible effect of the varying amounts of food consumed, animals fed 2 mg. of vitamin C daily and offered water *ad libitum* but otherwise starved postoperatively were studied. In these animals, healing was not noticeably different from those animals fed *ad libitum* (Fig. 1), although body weight fell sharply and to a greater extent than the burned animals eating *ad libitum* (Graph 2).

D. *Effect of Cortisone and Food Intake on Wound Healing.* The guinea pig is less susceptible to exogenous cortisone than the rat. Whereas 1 to 2 mg. of cortisone daily will result in impaired healing in rats, we found little effect on food intake, body

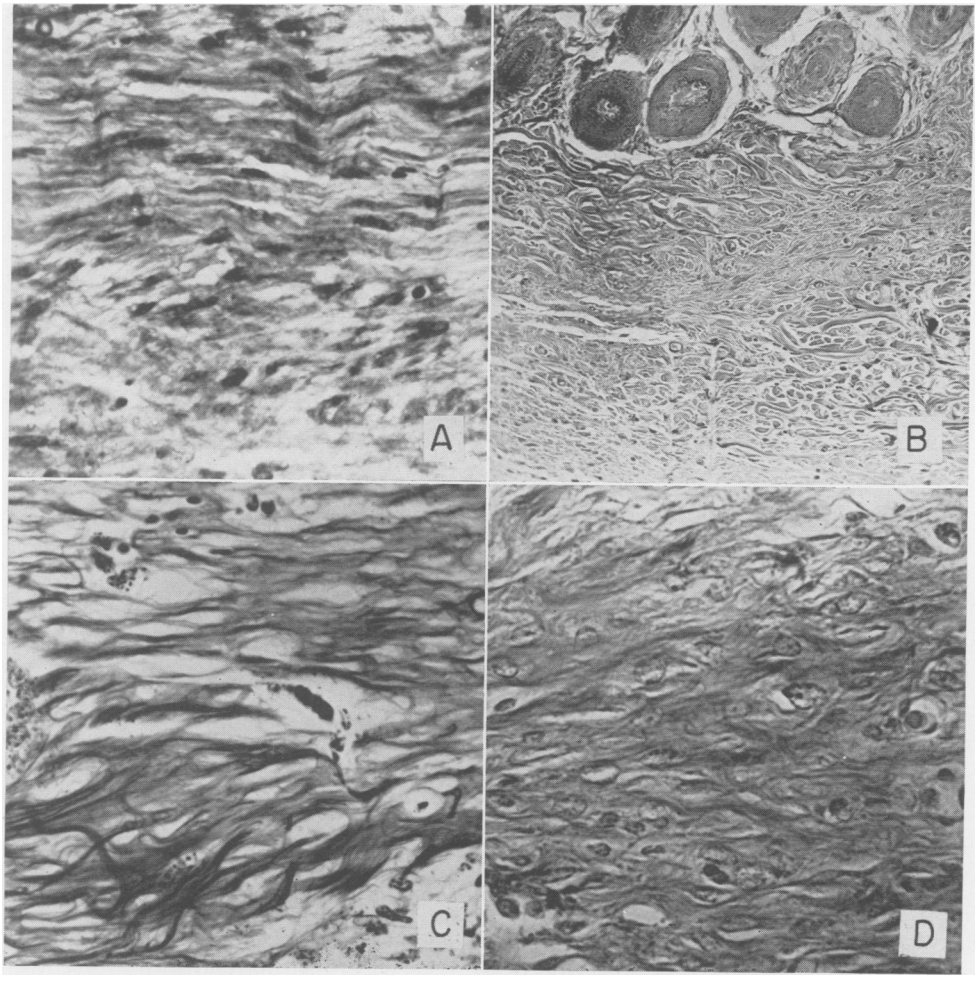


FIG. 1. Laparotomy wound, 7 days postoperatively in an unburned guinea pig receiving 2 mg. ascorbic acid daily. See text for description. A. Hematoxylin-eosin stain, 460 × B. Colloidal iron stain, 120 × C. Silver stain, 460 × D. van Gieson's stain, 460 ×

weight and wound healing when 10 mg. were injected subcutaneously daily beginning on the day of laparotomy in guinea pigs. (This level approximates the amount calculated to be produced daily by the maximally stimulated adrenals of guinea pigs.) These animals ate the commercial ascorbic acid-free diet *ad libitum* and received 2 mg. ascorbic acid daily (Graph 3). Further, no effect on these parameters was noted when the cortisone was given to animals from whom all food (other than the water and ascorbic acid) was withdrawn (Graph 4).

E. *Effect of Abrupt Withdrawal of Ascorbic Acid for the First Week Post Laparotomy in Unburned Guinea Pigs Followed by Its Administration in the Second Postoperative Week.* Normal guinea pigs were given 2 mg. of vitamin C daily up to the time of laparotomy, no vitamin C for first week postoperatively, and then 2 mg. vitamin C daily for the next week. The healing of the wounds in these animals presented precisely the same histologic pictures as seen in the burned laparotomized guinea pigs on a constant daily intake of 2 mg. of vitamin C, i.e., at seven days healing was abnormal

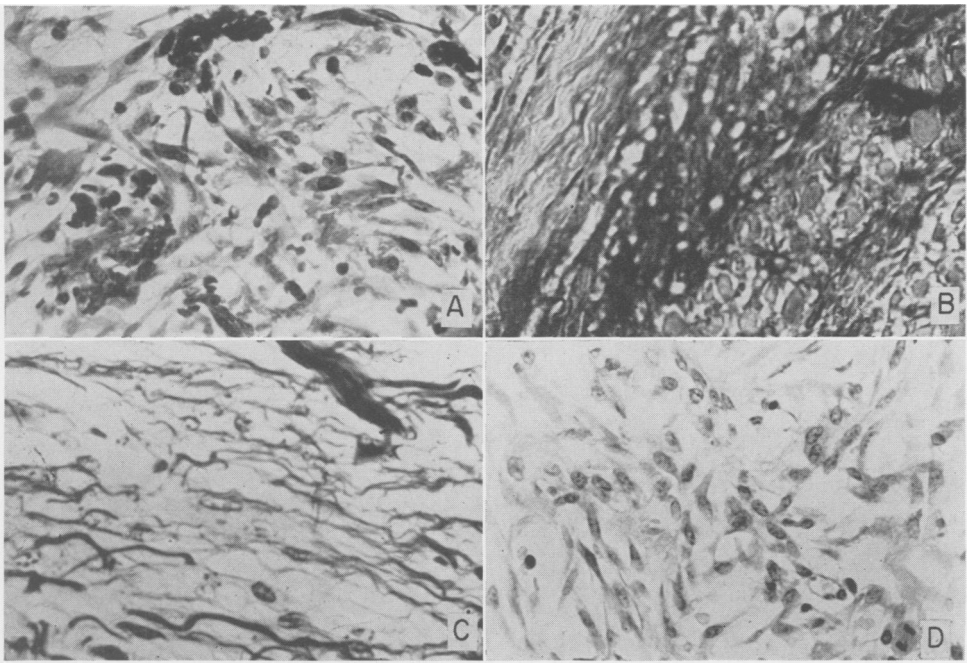


FIG. 2. Laparotomy wound, 7 days postoperatively, in an unburned guinea pig which received 2 mg. ascorbic acid daily prior to operation but none thereafter. See text for description. A. Hematoxylin-eosin stain, 460 × B. Colloidal iron stain, 460 × C. Silver stain, 460 × D. van Gieson's stain, 460 ×

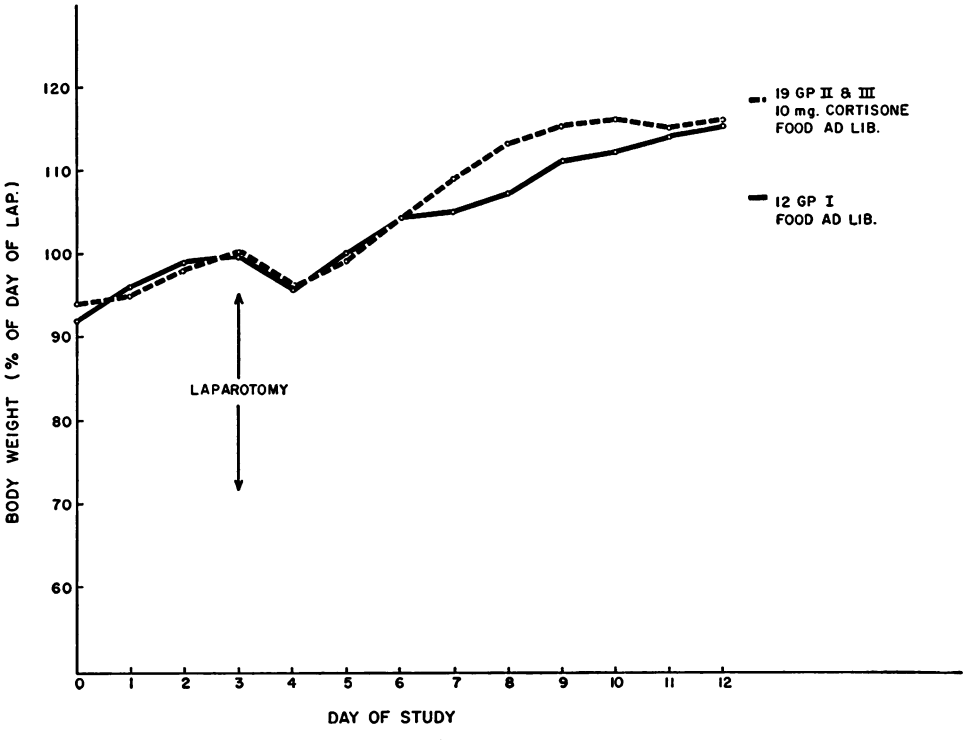
and indistinguishable from that seen in nutritionally scorbutic animals, while at 14 days healing was near normal.

F. *Effect of Large Doses of Ascorbic Acid on the Healing of Laparotomy Wounds in Burned Guinea Pigs.* Two groups of laparotomized animals, burned and unburned, were given 100 mg. of ascorbic acid daily subcutaneously beginning the day of operation. Histologic examination revealed identical findings (Figs. 4 and 5) in both groups, findings which were indistinguishable from those seen in unburned animals receiving 2 mg. of ascorbic acid daily (Fig. 1).

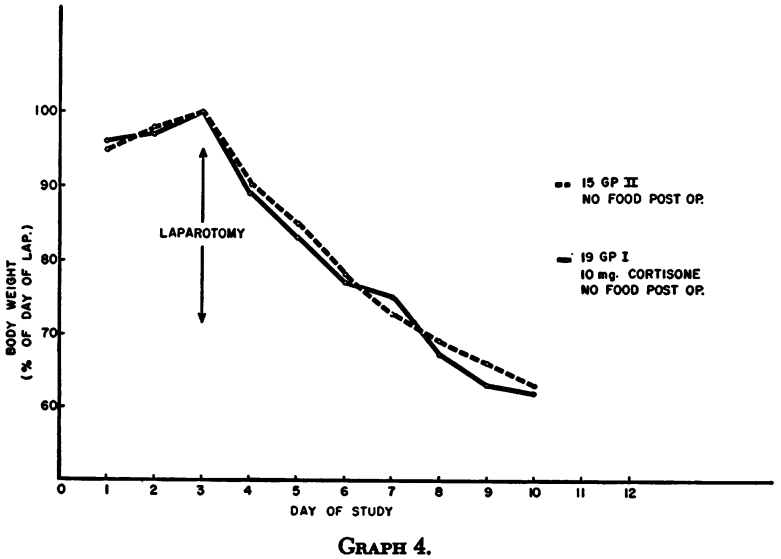
DISCUSSION

Although there are innumerable descriptions of the varied biochemical changes which occur soon after injury, little objective data of the clinical effects attributable to these changes are available. There are conflicting opinions as to the significance of these early metabolic derangements, and, depending on the viewpoint taken, attempts are, or are not, made to modify the response. In this regard, Selye,[16] discussing the so-called "therapeutic vitamin stress formulae," states: "In perusing the world literature on stress, we have been unable to find any reports of systematic investigations which would demonstrate the improvement in the response to stress that may be obtained with such remedies."

It has been postulated that no attempt be made to modify the metabolic reaction to injury because it represents part of a "defense mechanism" to supply metabolites to the wounded area to enable satisfactory healing at a time when, ordinarily, the injured animal or patient would not be eating much. There is no concrete evidence to support this concept. The observations of Young and his associates [19] that secondary



GRAPH 3.



GRAPH 4.

and tertiary wounds heal faster than primary wounds have been cited in support of the theory. However, the initial wounds made in this study were very small, and it is questionable whether a period of significant metabolic imbalance occurred. Further, several other groups of later investigators [17, 18] have not found the healing of

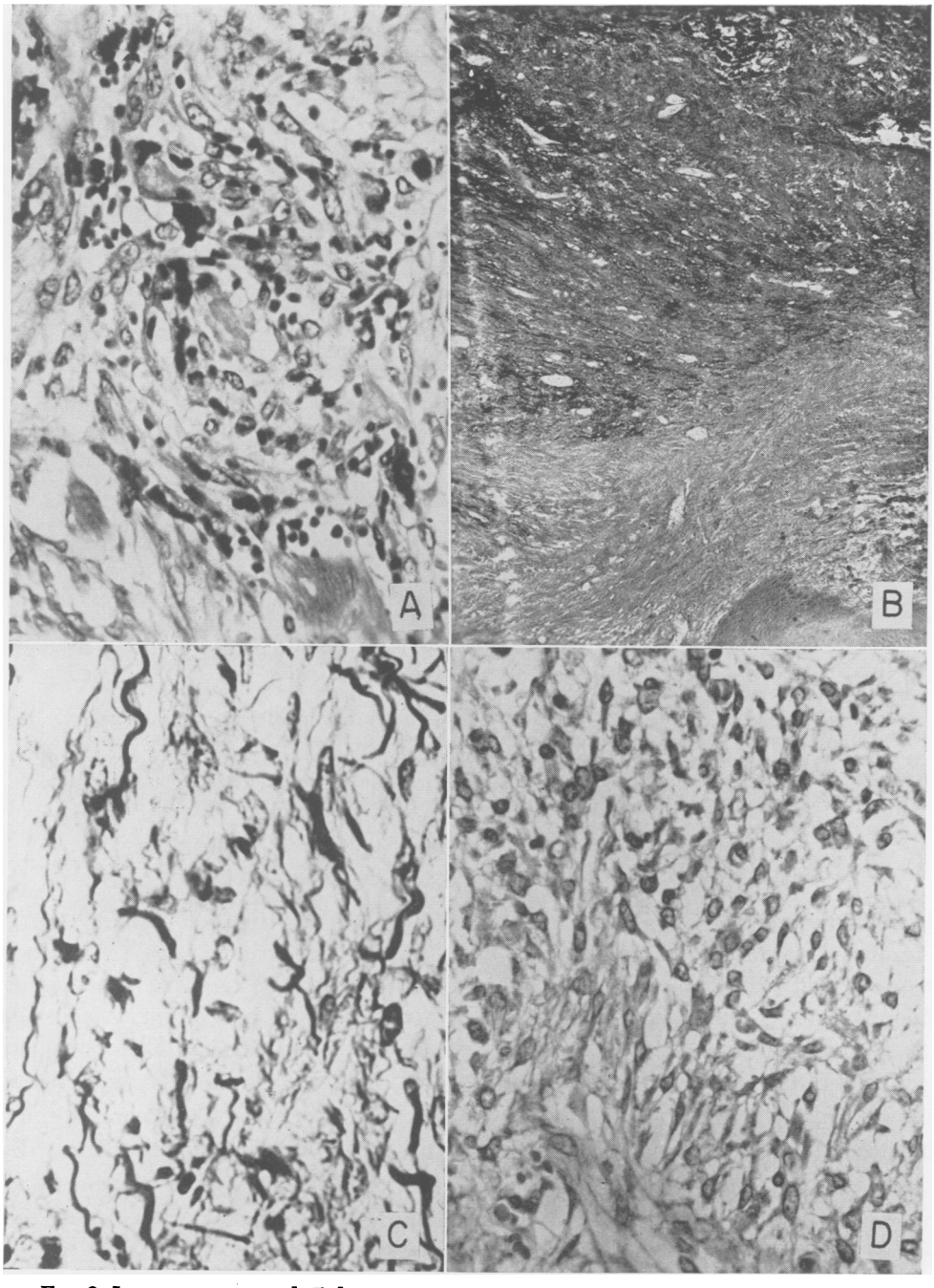


FIG. 3. Laparotomy wound, 7 days postoperatively, in a burned guinea pig receiving 2 mg. ascorbic acid daily. See text for description. A. Hematoxylin-eosin stain, 460 × B. Colloidal iron stain, 120 × C. Silver stain, 460 × D. van Gieson's stain, 460 ×

secondary wounds to be accelerated unless the second wounds were made in the identical area of the primary, e.g., resutured ruptured laparotomy wounds.[15]

A few years ago, one of us (S. M. L.)[8] studied the healing of experimental incisional wounds in the early postinjury period in severely burned rats. The healing of

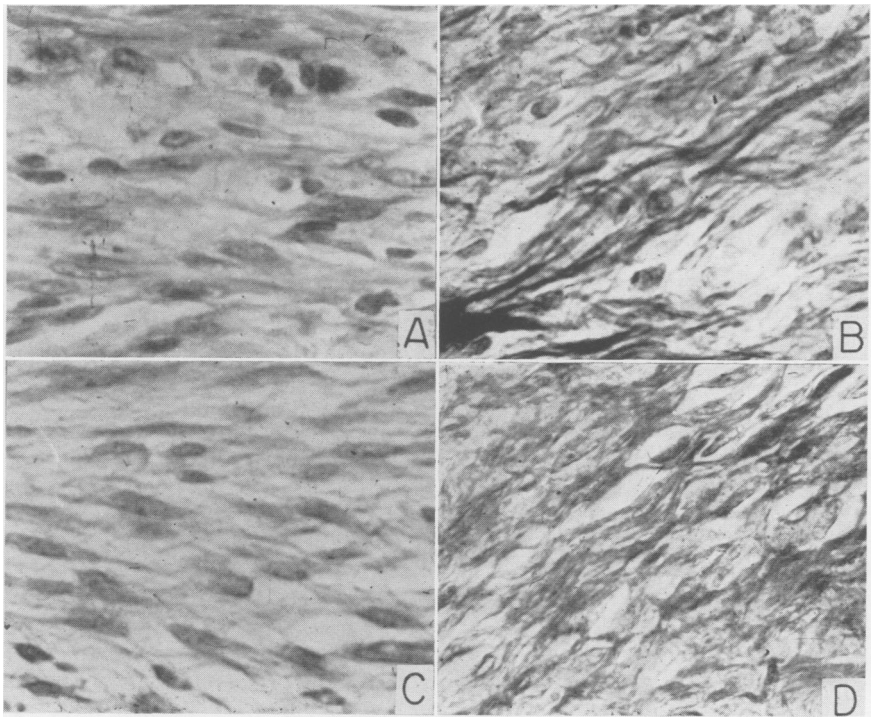


FIG. 4. See text for description. *A and B.* Laparotomy wound, 7 days postoperatively, in an unburned guinea pig receiving 100 mg. ascorbic acid daily after operation. A. Hematoxylin-eosin stain, 460 × B. van Gieson's stain, 460 × *C and D.* Laparotomy wound, 7 days postoperatively, in a burned guinea pig receiving 100 mg. ascorbic acid daily after operation. A. Hematoxylin-eosin stain, 460 × B. van Gieson's stain, 460 ×

the laparotomy wounds in the burned rats was significantly retarded as compared with the controls. Epithelization was not affected, but there was a definite delay in the appearance and maturation of the fibroblasts and collagen of the connective tissue in the burned animals.

Chassin and his associates [4] studied tensile strength of laparotomy wounds in young rats which had been subjected to a variety of stresses four to nine days (usually four) prior to laparotomy. Among the preoperative stresses were a linear sutured skin incision, excision of an 18 sq. cm. patch of skin from the back, a burn, and fractures of femur, knee joint, tibia and fibula bilaterally. The animals were fed *ad libitum.* Except for those animals with mild stresses (e.g., the skin incision), there was a statistically significant decrease from the control level of the bursting pressure of the laparotomy wounds of the various experimental groups on the fifth postoperative day. (This was the only day on which measurements were made.) When multiple fractures were employed as the stress, depression of the fifth day bursting pressure persisted even 45 days after the fractures were produced.

This impairment of healing associated with serious injury elsewhere is in keeping with the observations made by Carrel [3] over a quarter of a century ago; he demonstrated delayed healing of open wounds in animals with distant abscesses. But what of the mechanisms underlying this impairment of

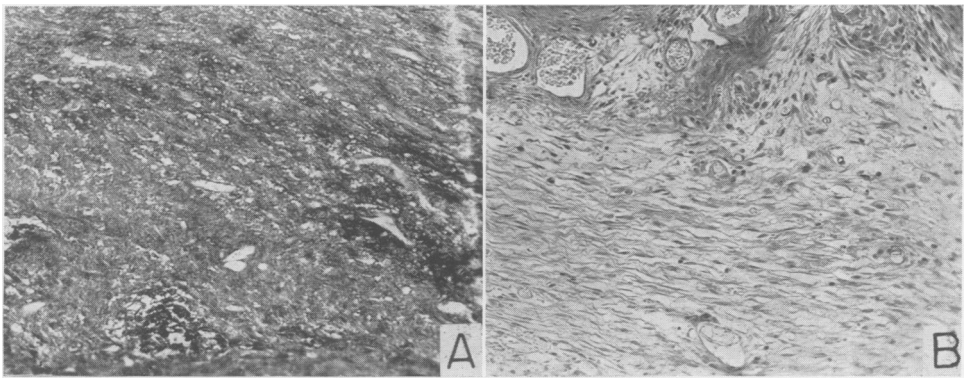


FIG. 5. Colloidal iron stains of 7 day laparotomy wounds in burned guinea pigs. See text for description. A. This burned animal received 2 mg. ascorbic acid daily. 100 × B. This burned animal received 100 mg. ascorbic acid daily postoperatively. 100 ×

healing? Since wound healing involves a complicated series of biochemical, physiologic, and physio-chemical reactions, it seems likely that the alterations in healing associated with severe injury are related to the abrupt generalized metabolic changes characteristically seen after trauma. Studies of wound healing under various controlled experimental conditions, should yield data for the objective evaluation of certain aspects of the metabolic reaction to injury. It was from this point of view that the present study was undertaken.

Our experiments were planned to determine whether the "biochemical scurvy" associated with severe injury represents "functional scurvy" as judged by the healing of laparotomy wounds. Such is the case. Wound healing was dramatically impaired in severely burned guinea pigs receiving an amount of ascorbic acid adequate for normal growth and healing in unburned guinea pigs. The changes in the healing were indistinguishable histologically from those seen in unburned scorbutic animals.

Neither postoperative reduction of food intake (all food other than water *ad lib.* and 2 mg. of ascorbic acid daily, was removed) nor the administration of cortisone (10 mg. subcutaneously daily) singly, or in combination, led to discernible abnormalities in healing. These latter studies were prompted by available evidence of an abrupt, marked increase in adrenocortical activity in the early period after injury. The view that adrenocortical hormones liberated in excessive amounts might be partially responsible for the connective tissue changes seen in the wound of injured animals has been championed by Chassin and his colleagues.[5] They found that in young adrenalectomized rats maintained on a small fixed dosage of aqueous adrenal cortical extract, skin excision (four days prior to laparotomy) did not result in depression of the bursting pressure of fifth day laparotomy wounds, as was the case with non-adrenalectomized rats subjected to the same preoperative stress. This observation is contrary to the view that the increased adrenal cortical activity after injury results in a "homeostatic" state rather than a "hyperadrenal" state.

In the burned guinea pigs studied by us, wound healing was strikingly altered during the first postoperative week. These abnormalities were still present, but to a lesser extent, at ten days. By the 14th day, the wounds were little different from normal. An explanation we offer for the findings of

disturbed wound healing observed by us in the burned guinea pigs is as follows: beginning very soon after the burn, there is an alteration in ascorbic acid metabolism such that the injured guinea pigs become physiologically scorbutic despite their receiving an intake of vitamin C adequate for growth and healing in normal animals. This intensely altered state persists for about a week; thereafter, a more nearly normal condition obtains so that by the end of the second week healing is apparently unimpaired. It was to test this hypothesis that normal guinea pigs were given 2 mg. of vitamin C daily up to the time of laparotomy, no vitamin C for the first week postoperatively ,and then 2 mg. of vitamin C daily for the next week. The healing of wounds in these animals presented precisely the same histologic findings as seen in the burned laparotomized guinea pigs on a constant daily intake of 2 mg. of vitamin C, i.e. at seven days healing was abnormal and indistinguishable from that seen in nutritionally scorbutic animals, while at 14 days healing was normal.

Further, if the changes we have observed in the healing of the laparotomy wounds of the burned guinea pigs are due to a physiologic deficiency of ascorbic acid (despite the administration of that amount of ascorbic acid adequate for normal growth and healing in unburned animals), these changes should be prevented by the administration of large doses of ascorbic acid in the post burn and postoperative period. Such were our findings when 100 mg. of ascorbic acid were given daily postoperatively.

The question that arises immediately is: "What happens to the vitamin C in these burned animals?" It seems to us that there are several possibilities which merit investigation.

1) Accumulation of the ascorbic acid in the burned area. (Bartlett's [2] experiments lend support to this possibility.)

2) Increased "utilization" or "destruction" or "inactivation" of the vitamin C locally in the burn or systemically as part of the general metabolic reaction to injury. (Studies with radioisotopically labeled ascorbic acid would be useful.)

3) Increased excretion of ascorbic acid metabolites, not in the ordinarily measured forms, but in some as yet undetermined manner. Here again, the use of labeled ascorbic acid would be helpful. It should be pointed out that increased adrenal activity induced by injection of ACTH or cortisone leads to an increased urinary excretion of ascorbic acid in its common forms.[7] This is in contrast to the reduced excretion of these metabolities following injury.

SUMMARY AND CONCLUSIONS

Among the varied biochemical changes which occur after injury is an abrupt, and often sustatined, drop in urinary, blood and tissue ascorbic acid. The seriously injured patient, then, behaves biochemically like a scorbutic. Does this represent functional scurvy? If the injured individual is truly scorbutic, alterations in healing characteristics of scurvy should be observed. Since the guinea pig, like man, requires exogenous vitamin C, it has been our experimental animal.

Healing of laparotomy wounds was followed by a variety of histologic technics. Abdominal wounds in burned guinea pigs healed abnormally: fibroplasia was ample, but hemorrhages were frequent, reticulum and "ground substance" were excessive, while collagen production was scanty. These changes are indistinguishable from those of unburned scorbutic animals. Large doses of vitamin C given in the postoperative period prevented the wound changes in the burned animals.

Our intent was to gain some objective evidence of the clinical significance of the metabolic reaction to injury; we have shown

a distinct physiologic effect which correlates with an observed biochemical change.

ACKNOWLEDGMENT

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DISCUSSION

PRESIDENT DAVIS: The Hand Clinic at the Cook County Hospital tells me they have seen a number of cases there at that hospital with abrasions, redness and swelling, and subcuticular undermining of the skin. They found that the most effective care in those people is to give them Vitamin C, to dress them cleanly, and then splint their hands. In about 48 hours the wound is healed, they say.

They also have a rather large clinic with burned patients at County Hospital, and they routinely give those patients a high Vitamin C intake