:tion 60 minutes after subjection to
y stressful condition (208 mg glu-
ion by intravenous administration)
and alloxan-injected groups was
/ identical; and significantly lower
tion than control values were ob-
The results were discussed as further
of the problem whether adrenal as-
d concentration may be considered
guide of hyper- or hypofunction of
al cortex. A tentative explanation of
ased ascorbic acid concentration in
n-diabetic groups was also discussed.

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Effect of Vitamin C Deficiency on Healed Wounds.* (20034)

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portance of vit. C in wound healing
nown. In advanced ascorbic acid
/ wound healing is impaired(1-8).
pairment is characterized histologi-
an abundant proliferation of fibro-
ich remain immature, by failure of
n of extracellular material, and by a
reduction of histochemically detecta-
ne phosphatase. The small amount of
ular material which may be produced
vere cases does not become organized
gen fibers, and argentaffine reticulum
re-collagen) persist. In addition, the
illaries which form in this abnormal
ion tissue are defective, and lead to
ages in the wound area. The tensile
of the wounds is markedly reduced.
ject has been extensively reviewed by
and Prescott(8) and Levenson *et al.*
t. C has also been reported as essential for the maintenance of scar tissue even when this had formed many years previously. In Walter's account(10) of Lord Anson's voyage around the world (1769) it is stated that, "the scars of wounds which had been for many years healed were forced open again by this virulent distemper" (scurvy, editor). From this description it would appear that wound scar tissue is particularly susceptible to a prolonged ascorbic acid deficiency. Experimental proof of this susceptibility of scar tissue has been reported and commented upon briefly by Hunt(11) and Hartwell and Stone (12). On the other hand Robertson(13), in a recent study, measured chemically the amount of collagen present in healed wounds using the method of Lowry *et al.*, but failed to observe a significant reduction of its concentration in scorbutic guinea pigs.

It is the purpose of this study to present and discuss the nature of the gross and histologic changes taking place in healed wounds during ascorbic acid deficiency. The altera-

pinions expressed in this paper are those thors and do not necessarily represent the ews of any governmental agency.

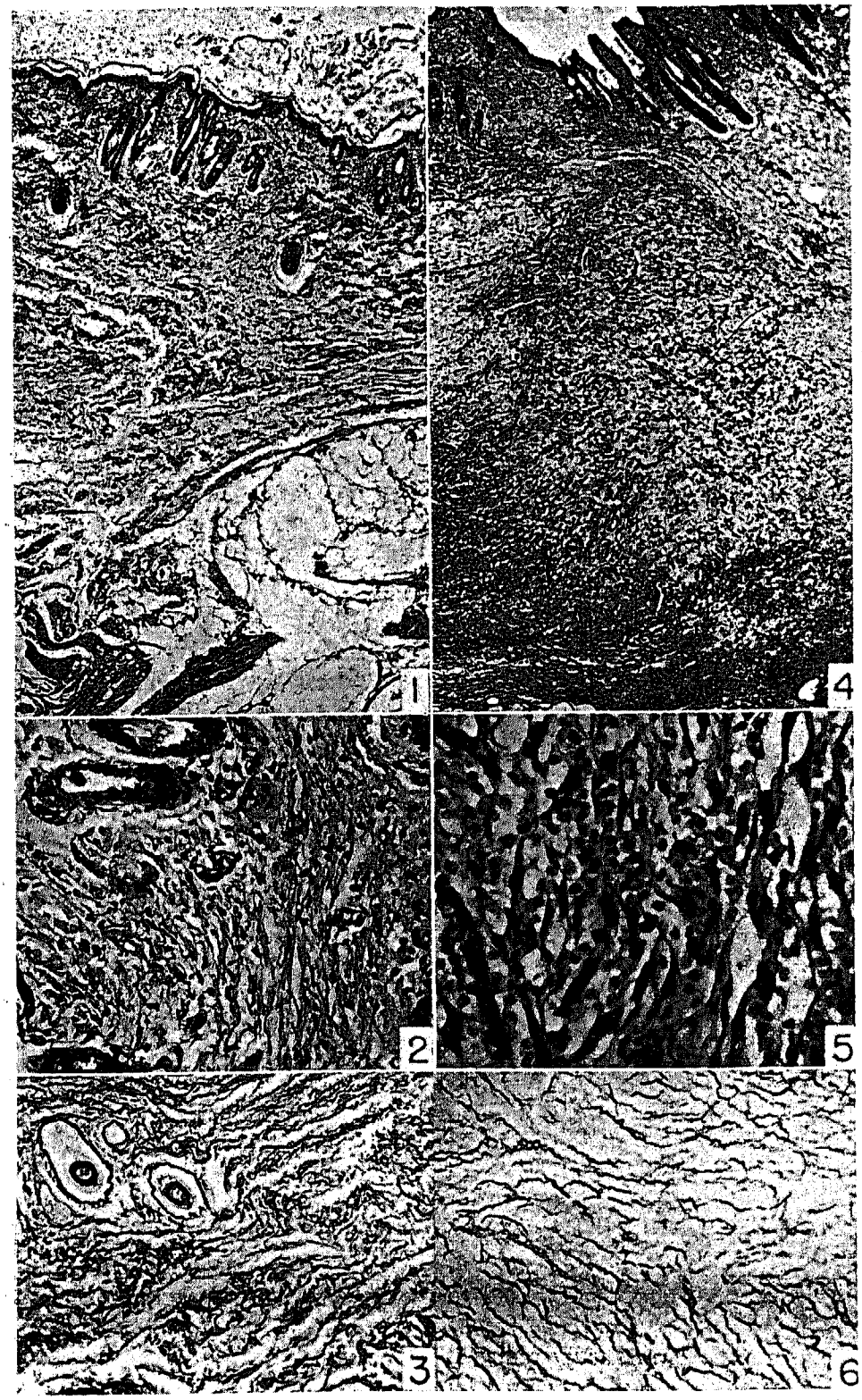

FIG. 1. GP 761. Appearance of healed wound in a control guinea pig maintained for 6 wk postoperatively on a complete ration and then pair-fed for 26 days with a scorbutic animal. The scar consists of a thin band of connective tissue recognizable by the absence of accessory skin structures. H and E, × 50.

FIG. 2. GP 761. Detail of Fig. 1, showing broad, well-formed, collagen bands and a relatively small number of fibrocytes. H and E stain, × 265.

FIG. 3. GP 761. Argentaffine black reticulum fibers are few and scattered. Foot's stain, × 200 (approx.).

FIG. 4. GP 743. Appearance of the wound in a guinea pig maintained for 6 wk post-operatively on a complete ration and then fed for 26 days a diet totally deficient in ascorbic acid. The scar is replaced by abundant immature granulation tissue containing numerous capillaries and small hemorrhagic areas. H and E, × 50.

FIG. 5. GP 743. Detail Fig. 4. The granulation tissue consists of numerous immature fibroblasts separated by extravasated red blood cells and a few collagen fibers. Few defective capillaries can be recognized (left upper corner). H and E, × 500 (approx.).

FIG. 6. Numerous argentaffine reticulum fibers are present in many areas. Foot's stain, × 250 (approx.).

tions observed are consistent and characteristic.

Methods. Young adult male guinea pigs about 10 weeks of age and weighing on the average 450 g were placed in single wire-screen bottom cages and adjusted to laboratory conditions for about a week. They were fed ascorbic acid-free pellets† supplemented by adequate greens and fresh tap water *ad libitum*. A linear midline laparotomy wound, 4 cm in length, was then performed in each animal under nembutal anesthesia (3 mg/100 g body weight, intraperitoneally). Clean, but not aseptic, technic was employed. The wound was closed in one layer by 4 or 5 through and through fine stainless steel sutures which were removed 2 weeks later. The wounds were allowed to heal for 6 weeks, the animals being kept on a nutritionally complete diet. Following this period the guinea pigs were divided in 3 groups: Group 1 (13 animals "deficient") was fed the pellet ration *ad libitum* without supplements; Group 2 (6 animals "pair-fed controls") was pair-fed with an equal number of animals of Group 1, and received in addition 2.5 mg ascorbic acid per day orally; Group 3 (7 animals "*ad libitum* controls") was fed *ad libitum* the pellets plus 2.5 mg per day of ascorbic acid. When the guinea pigs of the first group had developed clinical signs of severe scurvy (average 26 ± 3 days) representative animals of all groups were sacrificed by exsanguination under nembutal anesthesia. Complete autopsies were performed. The wounds were resected immediately after death and fixed in 10% neutral formalin. Blocks from the center of the wound were embedded in paraffin. Sections cut at 6 μ were stained with hematoxylin eosin, Van Gieson (collagen), toluidine blue (acid mucopolysaccharides) and Foot (reticulum) technics. Small blocks of the wounds were also fixed in cold 80% alcohol for alkaline phosphatase. Details of histological methods used are given elsewhere(14).

† Rockland guinea pig diet especially prepared *without ascorbic acid* by the Arcady Mills Co., Chicago.

Results. All guinea pigs gained on the average 40% in body weight in the 6 weeks following operation and were in excellent condition at the beginning of the experimental period. During the following 26 days of experimental regimen the scorbutic animals lost on the average 23% and their pair-fed controls 12.5% of their body weight, while the *ad lib.* controls gained 19%. The food consumption of the ascorbic acid deficient guinea pigs and of the pair-fed controls was on the average 32% less than that of the *ad lib.* animals during the same period. Obvious signs of scurvy developed in the majority of the "deficient" animals after 17 to 18 days and were apparent in all of them about 21 to 22 days following the withdrawal of ascorbic acid. At the time of sacrifice (26 days) the degree of scurvy was severe, although most of the animals were not in a terminal condition.

Gross findings. During the first 6 weeks the wounds of all animals healed normally. At the end of this period the wound scar was reduced to a thin line which was considerably shorter than the original wound and in many instances was hardly visible. Approximately 3 weeks following the withdrawal of ascorbic acid from the diet, changes were observed in many of the scorbutic animals. These consisted of swelling of the wound accompanied by a few small hemorrhages. Herniation of the wound developed in 3 cases. However, complete disruption did not occur and the epidermis appeared intact in all cases. No changes were noted in the wound scars of the *ad libitum* and pair-fed controls.

Microscopic findings. In both groups of control animals the wounds appeared entirely

normal. The epidermis appeared somewhat thinner than that of the adjacent areas with flat or absent projections. The underlying connective tissue had a normal appearance consisting of rather thick fibers staining brilliant red with Van Gieson and a relatively small number of fibrocytes. Hyalinization was slight. Reticulum fibers were few in numbers (Fig. 1, 2, 3) and metachromasia was minimal. No sites of alkaline phosphatase activity were detected except at the periphery of a few hair follicles.

In the scorbutic guinea pigs the epidermis in the wound area appeared essentially similar to that of the controls and did not seem to be affected in any way by the deficient diet. In all cases the underlying connective tissue showed distinct changes. These changes varied considerably in severity from animal to animal, and were generally in accord with the severity of scurvy as judged by the gross and microscopic lesions of bones and joints. The changes were in the main limited to the immediate wound area extending laterally only for a short distance. The connective tissue appeared somewhat loose and was much more cellular than that of the controls. A large number of fibroblasts, immature mesenchymal cells and a few fibrocytes were present (Fig. 4). As a result, there was rather abundant granulation tissue in the wound area. This contained numerous capillaries some of which appeared to be defective and there were several areas of hemorrhage (Fig. 5). In no case did the collagen fibers stain brilliant red with Van Gieson's technic; the color, instead, varied from pale red to pink and, in a few animals, to either a diffuse or patchy yellow-pink color. Reticulum was present in much greater amount than in the controls and appeared either as a fine network or as groups of coarse irregular fibrils (Fig. 6). The degree of metachromasia, as judged by the toluidine blue stain, was markedly increased when compared to that of the controls. It was felt that this was due to an enhanced reactivity to toluidine blue rather than to an increased amount of mucopolysaccharides, probably on the basis of depolymerization of the ground substance which is thought to occur in scurvy as well as in other conditions (15,16). No alkaline phosphatase activity could be detected in the wounds of these animals. As a rule, a thin layer of connective tissue immediately beneath the epidermis showed either minimal or no regressive changes.

Discussion. The changes which develop in the connective tissue of a *healed* wound when ascorbic acid is completely removed from the diet are of the same type as those which occur in *healing* wounds of scorbutic animals and man. The appearance of a large number of immature mesenchymal cells which replace the relatively few fibrocytes, and the reversion of mature or immature collagen fibers simultaneously with reappearance of argentaffine reticulum and depolymerization of the ground substance, can be explained on the basis of our knowledge of the fundamental phenomena of scurvy. In this disease similar regressive changes occur in bone and in other tissues of mesenchymal origin, particularly where mechanical stress is applied as in muscles, tendons, cardiac valves, etc.(2,17,18). A thin layer of connective tissue immediately under the healed epidermis showed minimal changes. This can possibly be explained either by the very close approximation of the wound margins superficially which resulted in the formation of little granulation tissue in this area during the process of healing or perhaps by a supporting action of the epidermis, which was not visibly affected by ascorbic acid deficiency.

It is difficult to explain satisfactorily why a deficiency of vit. C should markedly affect normally healed granulation tissue (scar tissue) while it does not produce significant morphologically detectable changes in the adjacent normal connective tissue. There are however, well known definite gross and histologic differences between the 2 types of connective tissue: "old" scar tissue is less elastic, less vascularized, relatively acellular and contains denser collagen which frequently undergoes hyalinization. Although normal scar tissue appears morphologically "stronger" and has a tensile strength identical or greater to that of normal connective tissue, it is apparent that it is more susceptible to changes when subjected to metabolic stress. Our results clearly indicate that scar tissue is more sensi-

tive than normal connective tissue to the lack of vit. C. The effect of vit. C deficiency is probably not mediated through a diminution of histochemically detectable alkaline phosphatase since a healed wound does not exhibit any stainable activity of this enzyme. In this respect an old scar differs from young granulation tissue. Our findings are in fundamental agreement with the recent studies of Gould and Gold(19) and throw considerable doubt on the importance of alkaline phosphatase in the wound healing process in general and on the maintenance of mature collagen in particular. A healed wound because of its retraction is probably subject to greater mechanical stress (traction from adjacent healthy tissues) and this may explain at least in part the type of changes observed. The relatively young age (6 wk) of the healed wounds studied may also have been a factor. In this respect it would be of interest to investigate whether older scars behave in a similar fashion. The presence of a normal concentration of collagen in healed wounds of scorbutic guinea pigs reported by V. B. Robertson(13) is not inconsistent with the histologic features previously described since such measurements obviously do not rule out the possibility of physical or architectural alterations in wounds.

Summary. When young adult guinea pigs bearing linear laparotomy wounds which had healed under normal conditions for 6 weeks were subjected to an ascorbic acid-free dietary regimen for approximately 26 days, severe changes were observed in the scar tissue area. The changes consisted of fibroblastic proliferation, regression of connective tissue elements, and of hemorrhages. The wounds of pair-fed and *ad lib.* control animals presented normal healing. The results indicate that adequate ascorbic acid intake is required not only for normal healing in the early post wound period but also for the maintenance of the scar tissue which has formed in healed wounds over a period of many weeks.

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