

Wound Healing in Rats with Biotin, Pyridoxin, or Riboflavin Deficiencies.

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Retardation of wound healing due to deficiency of vitamin C has long been recognized and the cytologic alterations in wounds consequent to such deficiency have been described. Hunt¹ demonstrated tinctorially that the primary defect of wounds in scorbutic guinea pigs is the immaturity of the intercellular matrix produced by fibroblasts. The role which vitamin deficiencies other than that of vitamin C may play in wound healing has not been adequately studied.

It is the purpose of the present communication to record qualitative and quantitative differences in the healing of 240 wounds in 60 young rats of uniform strain, evenly distributed to include normal controls and those with deficiency of either riboflavin, pyridoxin, or biotin.

Methods: Care of Animals. Weanling, male, albino rats from the Sprague-Dawley strain were divided into 4 groups of 15 each. One group, serving as *controls*, was fed a basal diet of the following composition: sucrose 76, Labco casein ("vitamin-free") 18, salts² 4, and corn oil 2. Each rat received the following vitamins daily in a supplement dish: thiamin HCL 30γ, pyridoxin HCl 30γ, riboflavin 30γ, nicotinic acid 250γ, calcium pantothenate 100γ, choline chloride 10 mg, and *i*-inositol 3 mg. The *riboflavin*- and *pyridoxin-deficient* groups received the same basal diet and supplements as the controls, except for the omission of riboflavin and pyridoxin, respectively. For the *biotin-deficient* group the basal diet was modified by substituting 56% of the casein by powdered egg white. The vitamin supplements were identical with those of the control group.

The animals were housed in individual

cages with wide-mesh screen bottoms and the basal diets were fed *ad libitum*. Each rat was given 2 drops of Abbott's haliver oil (plain) containing 1.3 mg of added d.1α-tocopherol acetate, weekly, by mouth. The rats were weighed each week.

Production and Characterization of Wounds. After 5 weeks on the respective diets, standard wounds (Series I) were produced on the lateral aspects of each thigh in the following manner: By means of a special stamp a circle 13 mm in diameter was outlined upon the shaved skin with carbolfuchsin. Under ether anesthesia, by carefully following the outline of this circle with an electric knife, a button of skin extending down to the loose fascia was removed. The intensity of the current was such that the cautery effect did not extend more than one mm beyond the line of excision. No attempt was made to establish aseptic conditions or to cover the wounds. The resulting ulcers, together with a rim of apparently normal skin, were excised under ether anesthesia 3, 6, 14, or 20 days later. The skin edges were approximated with cotton sutures after excision of the ulcers. The excised ulcers were fixed in formalin.

A second series of wounds was made in the same animals 3 weeks following the production of the first series. The procedure was identical with that of the first series, but the site of the wounds was now on both lateral sides of the chest. The lower border of the wounds was over the last rib. Three animals from each group were sacrificed at 3 and at 6 days, respectively. The animals were placed in formaldehyde solution, and the ulcers excised after fixation. Five of the remaining animals in each group were sacrificed at 14, and the other 4, at 20 days, and the ulcers similarly excised.

¹ Hunt, A. H., *Brit. J. Surg.*, 1941, **28**, 436.

² Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1941, **138**, 459.

Paraffin sections were prepared from a block which was cut as nearly as possible through the center of the ulcer. These sections were stained with hematoxylin and eosin, a combination of the Van Gieson and the Weigert elastic tissue stains, and the Wilder reticulum stain.

The healing process in each section was studied with regard to the following characteristics:

1. Demarcation and separation of devitalized tissue.
2. The epithelialization of the ulcer.
3. The relative amount of collagen in the scar tissue, as indicated by pink fibers in the Van Gieson, and non-argyrophilic fibers in the reticulum stain.
4. The relative amount of precollagen in the scar tissue, as indicated by yellow fibers in the Van Gieson, and argyrophilic fibers in the reticulum stain.
5. The density of the scar tissue.
6. The vascularity of the scar tissue.
7. The cellular infiltration of the scar tissue.

Results. Observations on wounds of Series I and II are combined since significant differences between the two series were not observed.

General Effects of Dietary Deficiencies. After 5 weeks on the respective diets (the time at which the first wounds were inflicted) the average weights were as follows: biotin-deficient group, 127 g; pyridoxin-deficient group, 82 g; riboflavin-deficient group, 59 g; and control group, 176 g. The average weights of the vitamin-deficient groups remained fairly constant thereafter, whereas that of the control group continued to increase throughout the experiment. Besides the retardation in growth, the animals receiving vitamin-deficient diets developed the symptoms characteristic of the respective deficiencies. These symptoms were more severe during the healing of the second wounds than they were while the first wounds were healing.

Gross Observations of Wounds. At no time was there gross evidence of infection. All wounds remained dry and clean. The

ulcers of the control and biotin-deficient groups appeared to heal equally rapidly, while the healing of the ulcers of the pyridoxin- and riboflavin-deficient groups was strikingly delayed. There was also a considerable difference in the thickness of the skin of the rats, detectable by palpation. The skin of the control and biotin-deficient groups was moderately thick, in contrast to the thin skin of the other two groups (confirmed by microscopic measurement).

Toward the end of the first week following the production of the wounds, the ulcers of the control and biotin-deficient groups were definitely smaller than those of the other two groups. Since microscopic section at this time showed very little epithelial or connective tissue growth, the difference in the size of the ulcers could be explained only on the basis of a difference in the contraction of the skin about the ulcers.

The ulcers of the control and biotin-deficient groups were healed at 14 days and those of the pyridoxin- and riboflavin-deficient groups, at 20 days.

Microscopic Observations of Wounds (see Fig. 1). An early reaction in the wounds is a narrow zone of leukocytic infiltration which forms at the junction of devitalized tissue (from the cautery effect of the cutting current) with viable tissue. This leukocytic zone of demarcation extends from the epidermis to the base of the ulcer. Epithelium regenerating from the surface and from sweat glands and hair-follicles adjacent to the leukocytic zone grows immediately subjacent to the latter. Later a cleft develops in the leukocytic zone which marks the beginning separation of this devitalized tissue. Because of the similarity of this process to the sequestration of necrotic bone, it will hereafter be referred to as sequestration. The separating, necrotic, burned tissue becomes an integral component of the eschar, together with dried serum, condensed fibrin, blood, and cellular exudate.

Biotin-deficient group. During the first 2 weeks there is some retardation in collagen production and in the density of the granulation tissue, while the amount of precollagen

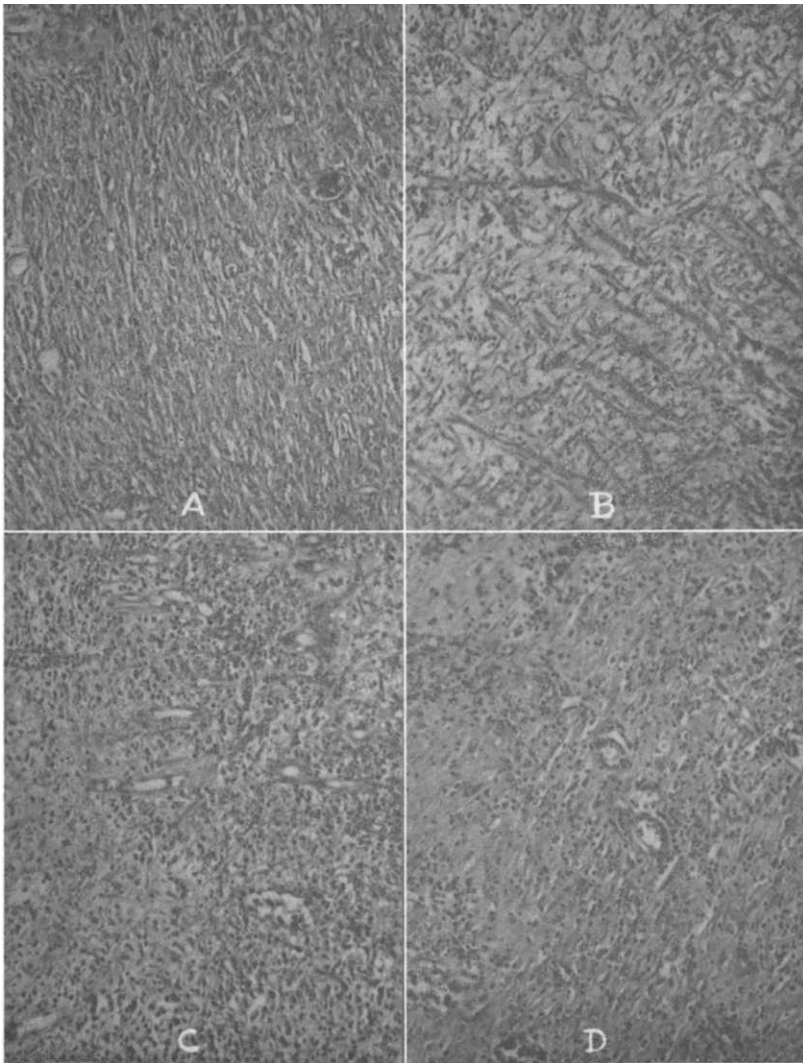


FIG. 1.

Photomicrographs of representative areas of 14-day-old wounds stained with a combination of the Van Gieson and the Weigert elastic tissue stains, $\times 130$. A is from a control rat; B, from a riboflavin-deficient rat; C, from a pyridoxin deficient rat; and D, from a biotin-deficient rat.

and the vascularity are greater than is seen in the normal animal. At 20 days the scar is identical with that of the control.

Pyridoxin-deficient group. Initially there is retardation of leukocytic reaction and in the development of granulation tissue. Epithelialization is delayed throughout. Although terminally these ulcers are epithelialized as well as the control ulcers, the scars contain less collagen and a considerable amount of precollagen. The latter is absent

at this time in the control and biotin-deficient groups. The scar tissue is less dense, more vascular, and more cellular than that of the control group. During the course of healing there is retardation of four to seven days in the evolution of the lesion, as compared with that of the control group.

Riboflavin-deficient group. There is delay not only in the evolution of the granulation tissue, but also in the epithelialization of the ulcer. Granulation tissue is tardy in appear-

ance and terminally it contains a decreased amount of collagen with considerable admixture of precollagen. The scar is less dense than that of the control group, but more dense than that of the pyridoxin-deficient group. The vascularity and cellularity are increased.

Discussion. Disturbance in the production of collagen is generally considered characteristic of scurvy, but is also known to occur in hypoproteinemia. The results obtained in the present experiments indicate that a similar disturbance is associated with deficiency of biotin, pyridoxin, or riboflavin in rats. However, the retardation of collagen production in these vitamin-deficiencies is less pronounced and less persistent than in scurvy.

The pyridoxin- and riboflavin-deficient groups exhibit, in addition, delayed contraction of the wound as observed grossly, and tardy exudation; the latter resulting in delayed sequestration and production of granulation tissue. The residual increased vascularity and correlative decreased density of the scars of the pyridoxin- and riboflavin-deficient groups are further indications of retardation in wound healing which are logically associated with the terminally decreased collagen and increased precollagen.

The wound of biotin-deficient animals exhibits a somewhat diminished density and decreased collagen content of the scar. There is no retardation of exudation or of granulation tissue production. Terminally the scar is indistinguishable from that of a normal animal.

The role which inanition plays in the retardation of wound healing is difficult to assess. It may or may not be coincidence that the greatest retardation in wound healing occurs in animals where inanition is also maximal—in the riboflavin- and pyridoxin-deficient groups. However, while the retardation in wound healing is greater in pyridoxin-deficient rats than in the riboflavin-deficient animals, inanition is greater in the latter than in the former.

Summary. The process of wound healing has been observed in rats exhibiting severe deficiency of biotin, pyridoxin, or riboflavin. Marked impairment of rate and quality of healing was noted in the pyridoxin- and riboflavin-deficient groups. The biotin-deficient group showed only mild delay in healing.

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Peptidases in Human Serum.*

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Although the existence of proteolytic enzymes in blood serum and leucocytes has long been recognized, no definitive work has been done in this field until recent years (cf. review of the early literature, Opie¹).

With the development of the use of synthetic di- and tri-peptides as substrates, a

quantitative microtitration technique was devised for the determination of peptidase hydrolysis (Grassmann and Heyde,² Abderhalden and Hanson,³ Maschmann,⁴ Fruton,⁵

² Grassmann, W., and Heyde, W., *Z. physiol. Chem.*, 1929, **183**, 32.

³ Abderhalden, E., and Hanson, H., *Fermentforsch.*, 1937, **15**, 382.

⁴ Maschmann, E., *Biochem. Z.*, 1941, **308**, 359.

⁵ Fruton, J. S., *J. Biol. Chem.*, 1946, **166**, 721.

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¹ Opie, E. L., *Physiol. Rev.*, 1922, **2**, 552.