Aminopeptidase Activity of Granulation Tissue in the Scorbutic Guinea Pig. (30257)

BENITO MONIS*
Department of Pathology, Sinai Hospital, Baltimore, Md.

Granulation tissue during repair of healing wounds in the rat showed histochemical and biochemical evidence of increased aminopeptidase activity assayed with chromogenic substrates: L-leucyl-2-naphthylamide (LNA) and D-L-alanyl-2-naphthylamide (ANA). These changes were due to: a) numeric increase of active cells and b) activation of enzymes in proliferating connective tissue cells(1).

It was pertinent to inquire into the functional significance of the increased enzymatic activity. It was postulated that if enzymatic activity was solely related to proliferation of fibroblasts, no significant histochemical changes should appear if guinea pigs subjected to a scorbutogenic diet were wounded when they showed clinical manifestations of scurvy. On the other hand, if aminopeptidase of fibroblasts plays a role in collagen formation, variations of aminopeptidase activity may occur in fibroblasts of scorbutic guinea pigs.

Materials and methods. Thirty-six female guinea pigs weighing from 300 to 400 g, all were subjected to a diet deficient in ascorbic acid.† Ten animals, while fed the same diet, received 1 ml of a commercial preparation of a sodium salt of ascorbic acid (500 mg/ml) intramuscularly twice a week. They were weighed weekly. Two weeks later, the animals from both groups were shaved in the back. Under light ether anesthesia, a cutaneous wound was performed after delineating an area of the skin with a cork borer No. 11(1). The skin and underlying subcutaneous tissues were excised with a scissors. No suture or dressing was added. At the third and seventh days, animals from scorbutic and control groups were killed with an excess of ether after excising tissues from the wound, normal skin and the proximal end of the tibia. Blocks of tissue from these areas were quickly frozen in isopentane in dry-ice-acetone or in a mixture of propane and isopentane cooled in liquid nitrogen and transferred to a cryostat. Blocks were cut 4 and 6 μ thick. Aminopeptidase activity was demonstrated with L-leucyl-2-naphthylamide, L-leucyl-4-methoxy-2-naphthylamide and D-L-alanyl-2-naphthylamide, following procedures reported elsewhere. (Reference in 1.) Other blocks were dehydrated, embedded in paraffin and sections were stained with hematoxylin and eosin or with the Gomori's modification of the Bielchowsky's silver procedure for demonstration

FIG. 1. Scorbutic guinea pig. Proximal metaphysis of tibia. Bone spicules have been replaced by abnormal connective tissue. Scant spindle-shaped cells are seen enmeshed in abundant loose extracellular substance in which no reticulum fibers were shown in sections stained with a silver procedure. There are hemorrhagic areas. H. and E. × 150.

FIG. 2. Scorbutic guinea pig. Cutaneous wound, 7th day. Granulation tissue in which fibroblasts are surrounded by loose amorphous substance. H. and E. × 400.

FIG. 3. (Like Figure 2). A consecutive section incubated at 37°C for one hour in a medium containing 2-leucyl-4-methoxy-2-naphthylamide, Diazo Blue B, phosphate buffer pH 7.0 for demonstration of aminopeptidase activity. Fibroblasts are delineated by the azo-dye produced by enzymatic activity. × 400.

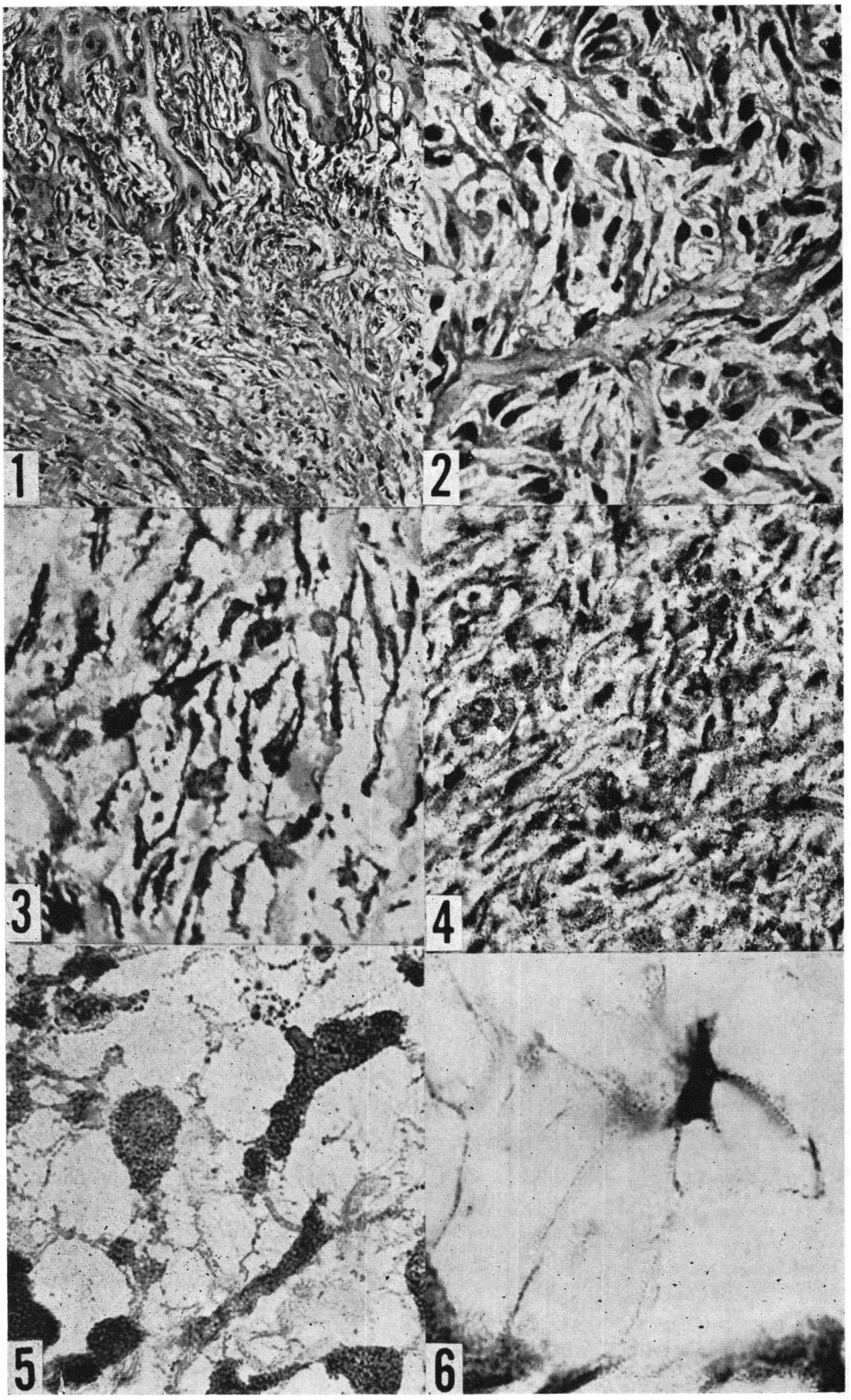
FIG. 4. Guinea pig (control). Cutaneous wound, 7th day. Aminopeptidase activity. Granulation tissue shows numerous *active* fibroblasts. Granulation tissue of the scorbutic guinea pig contains in this area a larger number of cells than in control. Aminopeptidase. × 400.

FIG. 5. Scorbutic guinea pig. Cutaneous wound, 7th day. Aminopeptidase activity. At higher magnification, cytoplasm of fibroblasts contains granules of azo-dye. × 1,000.

FIG. 6. Control guinea pig. Dermis. Aminopeptidase in fibroblasts. × 1,000.

* Present address: Instituto de Patología General, Facultad de Medicina, U.N.N.E., Corrientes, Argentina.

† This diet was purchased from Nutritional Biochemicals Co., Cleveland, Ohio.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of reticulum and collagenous fibers(2).

Results. All animals in the control group continued to grow and to gain weight. All animals on the scorbutogenic diet with no supplement of ascorbic acid, stopped growing, with weight loss progression. Soon after the animals were subjected to the diet, clinical signs of scurvy were seen in the diseased group. Scorbutic animals appeared quiet, and sat on the hind legs with complete flexion of the leg over the thigh. Evidence of fractures was found and movements seemed painful.

At autopsy, wounds from control animals showed various but definite degrees of healing, whereas the wounds of scorbutic animals were wide open and contained scant granulation tissue which was less cellular and showed spindle shaped fibroblasts (Fig. 2). In this group the classical findings in scurvy were observed: extensive hemorrhage in the soft parts of posterior extremities, fractures (Fig. 1) and marked fragility of bones, easy separation of chondrocostal junctions and swelling of epiphyseal heads of long bones. The characteristic histologic derangement in scurvy, *i.e.*, lack of collagen fibers in the loose granulation tissue of wounds in scorbutic animals was also observed.

Fibroblasts in the wounds of both groups of animals showed very intense aminopeptidase activity, (Fig. 3, 4, and 5). This activity was not significantly different in scorbutic from control animals.

A similar finding was noticed in the newly formed connective tissue in areas of fractures in tibia. In addition, the wound in both groups of animals showed histiocytes, mast cells, capillary and small vessel endothelium which stained for aminopeptidase.

Discussion. A histochemical study has disclosed a marked parallelism between aminopeptidase activity and fibroblastic proliferation in cutaneous wounds in rats(1). These changes were seen in the histochemical preparation and were also assessed by quantitative procedures. A comparison of substrates was made and the results were identical: fibroblasts in the intact skin as well as in the wound showed aminopeptidase activity. Evidence that aminopeptidase activity is found in proliferating connective tissue cells in man has been presented(3).

Because of lack of knowledge of the role of the enzyme in normal conditions we proposed to investigate whether vit C deficiency would lead to changes in histochemically demonstrable aminopeptidase in fibroblasts. Such findings would suggest that the enzyme is under vitamin control and that aminopeptidase may play a role in synthesis of collagen.

The present study seems to indicate that blocking the mechanisms of synthesis of collagen by vit C deprivation does not coexist with qualitative changes of cellular aminopeptidase activity in the scorbutic guinea pig. Scurvy does not lead to significant variations of aminopeptidase activity of cells in granulation tissue. This conclusion would support the hypothesis that protein synthesis in general is independent of ascorbic acid(4).

Aminopeptidase (LNA and ANA) activity in fibroblasts would be related to processes of cell proliferation and growth, as has been shown for other proteolytic enzymes in neoplastic cells(5).

Summary. Histochemical assay of aminopeptidase (LNA and ANA) of granulation tissue in healing wounds of scorbutic and control guinea pigs did not show any significant difference of aminopeptidase activity in fibroblasts in both conditions. This would indicate that the enzymes involved in the histochemical reaction would be related to processes of cell growth and proliferation rather than to the characteristic secretory activities of these cells (collagen precursors and mucopolysaccharides of the ground substance).

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