

EFFECT OF IMIDAZOLE ON COLLAGEN FORMATION IN GRANULATION TISSUE  
OF EXPERIMENTAL WOUNDS

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Much attention has recently been paid to the study of the role of cyclic nucleotides in the course of repair processes, and also to substances modulating their activity *in vivo*. Imidazole is known to be a component of the biologically active substance carnosine, which has a stimulating action on wound healing. Under certain conditions imidazole *in vivo* can double 3',5'-AMP phosphodiesterase activity and so facilitate hydrolysis of cyclic AMP, the products of which stimulate proliferation and biosynthesis in various organs and tissues [3].

The object of this investigation was to study the pattern of collagen formation in granulation tissue in rats receiving imidazole.

EXPERIMENTAL METHOD

Experiments were carried out on 60 noninbred male albino rats weighing 150-170 g. Standard incised wounds of skin and muscle measuring  $2 \times 2.5$  cm were inflicted on the dorsum of the animals. The rats were divided into control and experimental groups. The wounds in both groups healed under a scab. Imidazole in a dose of 5 mg in 0.5 ml of 0.14 M NaCl solutions was injected intraperitoneally into the experimental animals 30 min after the operation, and 24 h later the same dose was repeated. The dynamics of wound healing was assessed on the 3rd, 5th, 7th, 10th, 14th, and 29th days after the operation.

Regions of the wounds were fixed for histological study in Zenker's fluid and embedded in paraffin wax. Sections were stained with hematoxylin and eosin and with picrofuchsin by Van Gieson's method. To obtain autoradiographs pieces of granulation tissue weighing 50 mg were incubated for 8 h at 37°C in the presence of 24  $\mu$ Ci D,L-proline-3,4,5- $^3$ H (specific activity 3.5 Ci/mole). Autoradiographs were obtained with the aid of type "M" photographic emulsion on sections 1-2  $\mu$  thick. Exposure lasted 7 days at 4°C. The number of grains of reduced silver was counted in about 100 fibroblasts and in the intercellular space in the immediate vicinity of those cells. The numerical data were subjected to statistical analysis by Wilcoxon's method. The remaining regions of the wounds were used to determine the content of hydroxyproline in the granulation tissue [4].

EXPERIMENTAL RESULTS

By the 3rd day after the operation the wounds of the control and experimental rats were covered by a scab, beneath which there was a thick barrier of leukocytes and necrotic material. In the region of the wound defect, against the background of well-marked inflammatory phenomena, islets of granulation tissue were formed. The predominant cells in the wounds at this time were neutrophilic leukocytes, macrophages, and polyblasts. A very few fibroblasts also were located around the edges and in the floor of the wounds. No significant histological differences could be found between the control wounds and wounds of animals receiving imidazole during this period.

On the 5th day after the operation signs of inflammation in wounds of the experimental animals were rather less well marked than in the wounds of the control rats. Granulation and epithelization of the wound edges took place more actively. Foci of granulations showed a

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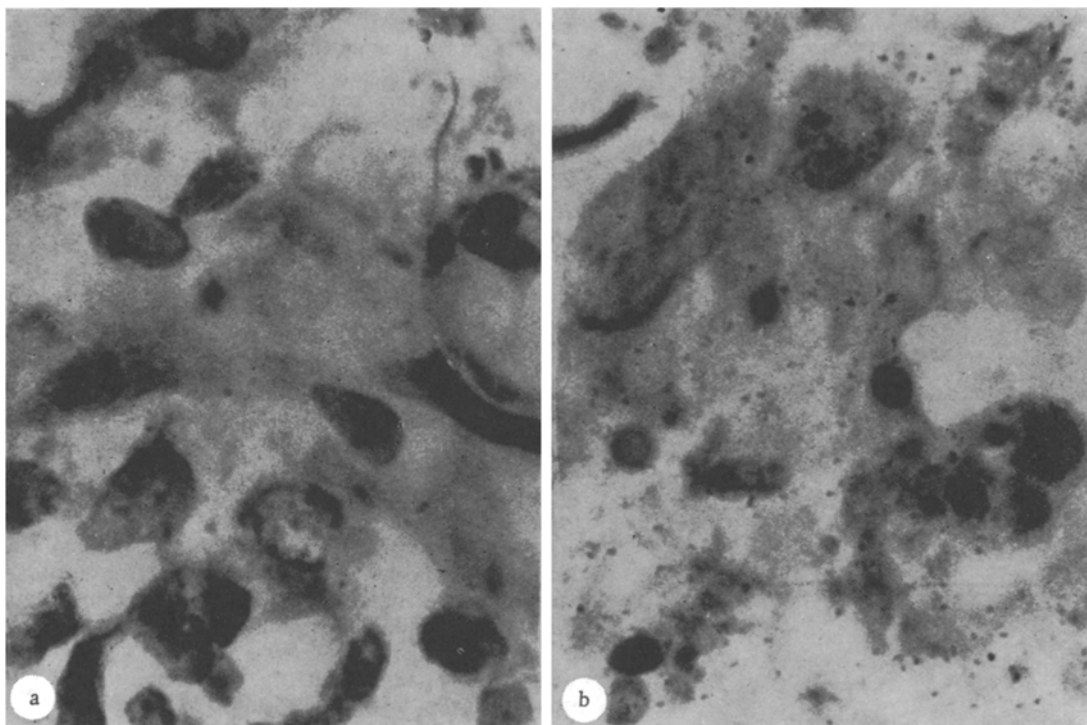


Fig. 1. Intensity of incorporation of proline- $^3\text{H}$  into cells and intracellular space of granulation tissue on 5th day after operation. a) Control, b) experiment. Hematoxylin-eosin, 1600  $\times$ .

marked tendency to merge and consisted of large, juicy fibroblasts with oval or round nuclei, containing two or three eccentrically arranged nucleoli. The basophilic cytoplasm of these cells had outgrowths of varied length. The fibroblasts themselves were arranged in parallel rows near the newly formed capillaries. Mast cells, many of them in a state of degranulation, were concentrated there, also in groups. A few neutrophilic leukocytes and lymphocytes and many more polyblasts were observed. All these cells, like those of the adventitia of the newly formed capillaries and cells of the regenerating epithelium, incorporated labeled proline. However, the intensity of its incorporation was much greater in the animals receiving imidazole than in the controls. Quantitative analysis of the autoradiographs showed that the label in wounds of control rats was mainly distributed above the cells, with only a very small proportion of it above the intercellular space (Fig. 1). Above the fibroblasts and close to them, in the granulation tissue of the rats receiving imidazole, the quantity of label was 2.5 times greater than in the control rats. Most of the label lay directly above the intercellular space, and in conjunction with the greater total number of grains of silver in the experimental animals, this could indicate a modification of the intracellular rhythm of protein synthesis, with the result that the rate of protein synthesis in the cytoplasm of the fibroblasts and the rate of their outflow into the extracellular space were increased. A similar increase in the rate of synthesis and outflow of proline-containing proteins was observed by the writers previously in granulation tissue fibroblasts in wounds of mice receiving potassium orotate [1].

On the subsequent days granulation and epithelization of the wounds of the rats receiving the stimulator followed a rather more active course than in wounds of the control animals. No significant difference was found in the density of distribution of fibroblasts in the wounds of the control and experimental rats. Complete epithelization of the wounds in the rats receiving imidazole ended about 2 days earlier than in the controls. This observation was confirmed by the results of planimetry of the wound surfaces.

Biochemical determination of the hydroxyproline concentration in the granulation tissue of wounds of the two groups of animals revealed to significant quantitative differences. As Fig. 2 shows, graphs reflecting the hydroxyproline concentration in granulation tissue during wound healing in the experimental and control groups were similar in character. However, the fact will be noted that despite the increased rate of protein synthesis by fibroblasts in the wounds of rats receiving imidazole, some increase in the hydroxyproline concentration in them

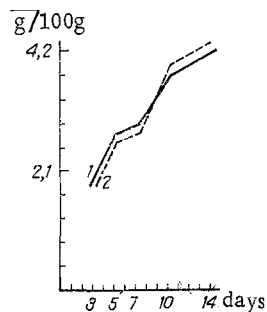


Fig. 2. Hydroxyproline concentration in granulation tissue during wound healing. 1) Control wound; 2) wound in rats receiving imidazole. Abscissa, days after operation; ordinate, hydroxyproline concentration (in g/100 g dry defatted tissue).

compared with the control animals could be observed only 9 days after the operation. In the writers' view, the high rate of protein synthesis in the fibroblasts, and the higher rate of outflow of newly formed proteins into the intercellular space than in the control, were accompanied by an increase in the rate of their metabolism during fibrillogenesis, when wound healing was stimulated. The somewhat higher concentration of hydroxyproline in the wounds of the stimulated animals in the later stages of wound healing may have been due both to slowing of collagen breakdown in connection with the formation of more stable collagen fibers and also to the fact that a certain margin is always allowed in the progress of repair reactions.

Histological investigation of scars on the 29th day after the operation revealed no appreciable differences between the control and experimental rats.

A comparative study of histological preparations of the control wounds and of wounds of animals receiving imidazole and comparison of the results with those of the writers' previous study of the effect of cyclic GMP on healing of similar wounds [2], showed that endogenous cyclic GMP has a stronger stimulating action on wounds. However, no excessive formation of collagen and fibrils in the granulation tissue was observed when it was used.

The results thus indicate that administration of imidazole promotes more rapid healing of experimental wounds. An increase in the rate of protein synthesis is observed in the cytoplasm of fibroblasts and other cells of the granulation tissue and regenerating epithelium, but excessive fibril formation does not take place under these conditions.

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