MORPHOLOGICAL AND BIOCHEMICAL INVESTIGATION OF STIMULATED COLLAGEN FORMATION

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There have been many clinical and experimental studies of the effects of various stimulators on wound healing. Nevertheless, the problem of stimulation of wound healing still remains one of great practical and theoretical importance, for the basic cellular and intracellular principles governing the course of reparative regeneration are still largely unexplained.

The object of this investigation was to study activation of proliferation of fibroblasts and the relative and absolute intensities of RNA synthesis by fibroblasts and collagen during stimulation of healing of wounds involving skin and muscles.

EXPERIMENTAL METHOD

Experiments were carried out on 166 noninbred albino mice weighing 20-25 g with a standard incised wounds involving skin and muscles, 1 cm long. The wounds healed beneath a scab. After the operation all animals were divided into four groups of equal numbers, one of which served as the control. The mice of group 2 received 0.04 g potassium orotate as a 2% solution once a day per os through a metal tube; this was the daily dose of the compound, calculated per kilogram body weight. The mice of groups 3 and 4 received the same dose of stimulator twice and 3 times a day respectively, with an interval of 4 h. Daily for 2 weeks a histological investigation was made of the areas of the wounds and autoradiographs prepared in the usual way with type M photographic emulsion on paraffin sections 3-4 μ thick were analyzed. Uridine-5-^{3}H (specific activity 18 Ci/mmole) in a dose of 20 μCi/kg body weight was used as RNA precursor. The isotope was injected intraperitoneally into animals of all groups 6 h before fixation of tissues from the wounds. Pieces of the wounds were fixed in Zenker's fluid.

Proliferative activity of the fibroblasts in wounds of all groups of animals was assessed from the number of these cells in 20 fields of vision under magnification of the microscope by 280 times. All numerical data were subjected to statistical analysis by Wilcoxon's method. The intensity of collagen production was judged from histological sections stained by Van Gieson's method and the hydroxyproline concentration in the granulation tissue of the wounds [3] on the 2nd, 4th, 7th, and 10th days after the operation.

EXPERIMENTAL RESULTS

Wound healing in mice receiving potassium orotate once a day took place more rapidly and ended 3 days earlier than in the control [2]. After administration of orotate twice a day, the wounds healed 4 days earlier than in the control. Disappearance of inflammatory phenomena in this case was complete by the 2nd postoperative day. Granulation and epithelization of the wounds took place sooner and more actively. By contrast with the first two groups of animals, in mice receiving potassium orotate twice a day the wounds were filled with well-developed granulation tissue, with no signs of inflammation, containing many young and mature fibroblasts, intensively incorporating the labeled RNA precursor, as early as on the 3rd day after the operation. The number of grains of silver above fibroblasts after a single injection of the stimulator at this period of wound healing, incidentally, was 50% greater than in

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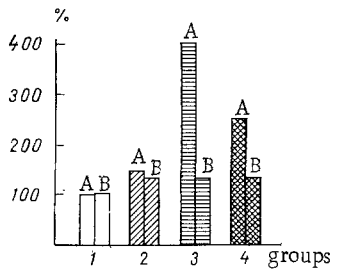

Fig. 1

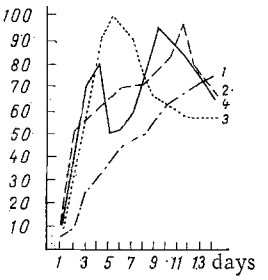

Fig. 2

Fig. 1. Intensity of incorporation of uridine-5-^{3}H by fibroblasts.

Fig. 2. Intensity of proliferation of fibroblasts in wounds of different groups of animals.

the control, whereas after its administration twice a day it was three or four times greater (Fig. 1). The high level of RNA synthesis was accompanied by an increase in the rate of nucleo-cytoplasmic transport of newly formed RNA.

Complete epithelization of the wounds in animals receiving potassium orotate twice a day was accompanied by more rapid maturation of granulation tissue than in the control or in animals receiving the compound once a day, and the process ended with a thin, delicate scar at the site of the previous wound defect.

Wound healing in animals receiving potassium orotate three times a day differed only a little from its course in animals receiving the compound twice a day during the first 3-4 days. The inflammatory changes disappeared just as rapidly, activation of fibroblast proliferation was observed in the region of the wound defect, intensive epithelization of the wound edges took place and granulation tissue was formed. By contrast with the animals of group 3, in the mice of group 4 the granulations of the wounds contained many more mature forms of fibroblasts. The density of distribution of these cells in the region of injury at this period was somewhat smaller in animals receiving potassium orotate three times a day than in those receiving it twice a day (Fig. 2). A sharp decrease in the density of distribution of fibroblasts in the mice of group 4 on the following days took place simultaneously with the change of many fibroblasts into an inactive state, evidently due not only to their more rapid maturation, but also to the appearance of trophic disturbances. Some cells decreased in size and acquired the morphological features of fibrocytes, whereas the nuclei of another group of cells became pycnotic and varied numbers of vacuoles appeared in their cytoplasm. The intensity of incorporation of labeled uridine decreased in these and other fibroblasts. Areas of leukocytic infiltration and small abscesses appeared during this period in the granulation tissue. The animals of this group began to be retarded in weight compared with those of all other groups including the control and they became lethargic and untidy. This was interpreted as a manifestation of poisoning from an overdosage of potassium orotate. The subsequent course of wound healing in the mice of this group was protracted, and the time taken for final healing was the same as in the control. The number of fibroblasts increased once more in the granulation tissue of animals receiving orotate three times a day, toward the 7th-8th day after the operation, but it did not exceed the number on the 5th day in the wounds of mice receiving orotate twice a day. The maximal density of distribution of fibroblasts in the region of the wound defect was the same in the animals of all groups, but it occurred at different times after the operation (Fig. 2). Detailed analysis of autoradiographs of wounds of the group 4 mice showed that the fraction of fibroblasts which did not undergo trophic changes in response to "excessive stimulation" retained a high level of biosynthetic activity. This was shown by the general high level of incorporation of radioactive uridine. The concentration of grains of silver above the cytoplasm was higher than above the nucleus of these cells, and coupled with the higher total level of incorporation of isotope by fibroblasts in the control, which preserved their intracellular homeostasis, was the result of an increase in the rate of nucleo-cytoplasmic transport of newly formed RNA under these conditions.

Determination of the hydroxyproline concentration in granulation tissue of wounds of the control mice revealed a steady rise from the 2nd to the 7th postoperative day; on subsequent days the hydroxyproline level increased to reach a maximum by the time of complete epithelization of the wound surface. The increase in the hydroxyproline concentration followed a simi-

lar course in the wounds of all animals receiving the stimulator. Its level was higher in these groups than in the control, but it was approximately the same in all groups of mice receiving potassium orotate (Fig. 1).

These results indicate that although intensification of RNA synthesis during stimulation by orotate can increase threefold or more compared with the control with an increase in the dose of stimulator, excessive collagen production does not arise under these conditions. The fact that no change was observed in the rhythm of DNA synthesis with an increase in the dose of potassium orotate in the writers' previous investigations [1] suggests that the quantity of DNA-like RNA controlling collagen synthesis under analogous conditions evidently does not change significantly. On the other hand, the excessive quantity of RNA formed during intensive stimulation of wound healing is evidently utilized for synthesis of various proteins, including enzymes, responsible for the high rate of collagen synthesis in the cytoplasm of the fibroblasts and of fibril formation.

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