DNA SYNTHESIS BY FIBROBLASTS OF GRANULATION TISSUE DURING STIMULATION OF WOUND HEALING

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The dynamics of DNA synthesis in fibroblasts of granulation tissue of wounds was investigated in adult mice, some of which were given potassium orotate. Regions of the wound in animals receiving 0.2 ml of a 2% solution of potassium orotate and in control animals were investigated autoradiographically daily. Under the influence of potassium orotate fibroblasts took up DNA synthesis more frequently: Increased proliferative activity of these cells was found during stimulation of wound healing.

KEY WORDS: *DNA synthesis; fibroblast; stimulation of wound healing.*

The use of derivatives of the pyrimidine bases to stimulate regeneration first began almost 20 years ago. However, not until very recently have attempts been made to evaluate the intensity of the synthesis and metabolism of nucleic acids and protein in granulation tissue. Histochemical methods of investigation have been used for this purpose [5, 2]. The results of these investigations showed that Lazarev [4] was correct when he postulated that derivatives of the pyrimidine series can stimulate the synthesis of nucleic acids and proteins. The problem of which cellular mechanisms participate in the intensification of cellular proliferation and of biosynthesis in granulation tissue cells during stimulation of wound healing remains unexplained. By the use of autoradiography information concerning these processes can be obtained. DNA is known to be synthesized in cell nuclei in the synthetic period of the mitotic cycle. DNA synthesis thus reflects the state of preparedness of the cells for reproduction.

With this in mind it was decided to study the dynamics of DNA synthesis in fibroblasts during the healing of experimental wounds under ordinary conditions and during administration of potassium orotate, a derivative of the pyrimidine series.

EXPERIMENTAL METHOD

Experiments were carried out on 74 noninbred albino mice weighing 20-25 g. Standard wounds ofthe skin and subcutaneous cellular tissue 1 cm long were inflicted on the anterolateral surface of the animals' thigh. All the animals were divided into two groups, with 32 mice in each group; group 1 was the untreated control; the mice of group 2 received potas-

TABLE 1. Density of Distribution of Fibroblasts in Regions of Injury

Group of animals	Day after operation																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
	number of fibroblasts in 20 fields of vision																				
Control	8	13	25	31	35	47	44	51	50	64	70	68	70	76	70	72	91	68	64	76	60
Experimental	13	52	54	60	68	50	74	70	76	62	102	76	68	60	52	66	76	96	78	52	48

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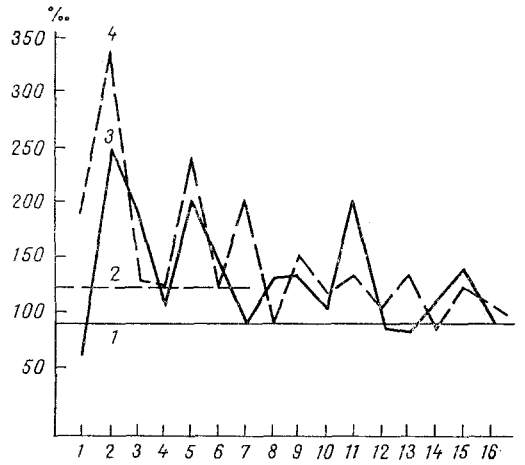

Fig. 1. Dynamics of DNA synthesis by fibroblasts during wound healing. Ordinate, ILN of fibroblasts (in °/₀₀); abscissa, days after operation. 1) ILN of fibroblasts in intact skin and subcutaneous cellular tissue of mice not receiving stimulator; 2) ILN in mice receiving potassium orotate for 5 days; 3) ILN of fibroblasts in wound of control group; 4) the same in experimental group of animals.

sium orotate in a dose of 1.5 g/kg body weight by mouth daily immediately after the operation as a stimulator. DNA synthesis in the fibroblasts was compared in 5 mice receiving potassium orotate for 5 days by the same scheme and in 5 mice not receiving potassium orotate. The cell density in these same areas of the skin and subcutaneous cellular tissue of the animals was determined by counting the number of cells in 20 fields of vision.

To investigate DNA synthesis, [^{3}H]thymidine (specific activity 15 Ci/mmole) was injected intraperitoneally in a dose of 1 μCi/g body weight 2 h before fixation. Areas of the wound were fixed daily in 10% neutral formalin. The material was embedded in paraffin wax and sections cut to a thickness of 4-5 μ were coated with type M photographic emulsion and exposed for 5 weeks at 4°C. After development the sections were stained with hematoxylin–eosin. To calculate the index of labeled nuclei (ILN) of the fibroblasts the number of labeled cells was counted among 200 cells. Their density in the focus of injury was estimated as the number of fibroblasts in 20 fields of vision. The results were subjected to statistical analysis by Wilcoxon's method.

EXPERIMENTAL RESULTS

In mice not receiving potassium orotate, ILN of the fibroblasts was 95°/₀₀, and the number of fibroblasts in 20 fields of vision did not exceed 30, whereas in the mice receiving the stimulator these indices increased to 125°/₀₀ and 35 respectively. Under the influence of potassium orotate ILN of the fibroblasts in the intact skin and subcutaneous cellular tissue thus increased by 31% and the cell density by 15%.

During the 1st and 2nd days after the operation the wound exudate contained large numbers of leukocytes and undifferentiated cells. Moderate numbers of mature fibroblasts were found. Fibroblasts were more numerous in the wounds of the animals which received the stimulator (Table 1). DNA synthesis was observed in these cells during the 1st day. It rose sharply on the 2nd day. These observations agree with those of Helpap and Cremer [7], who used [^{3}H]thymidine to study cellular proliferation in experimental skin wounds. In the present experiments ILN of the fibroblasts on the 2nd day after the operation in the experimental animals exceeded the control value by 37%. Compared with ILN of fibroblasts in intact skin and subcutaneous cellular tissue it was twice as high in the mice of the control group and four times as high in those of the experimental group. Fibroblasts took up DNA synthesis in waves. For instance, an increase in ILN of the fibroblasts in the control group of mice was observed on the 2nd, 5th, 8th, 11th, and 15th days after the operation, and in the experimental groups on the 2nd, 5th, 7th, 9th, 11th, 13th, and 15th days. The period of most active DNA synthesis in the fibroblasts of the control group of animals continued for 11 days, whereas in the experimental group of mice it was shortened to 7 days (Fig. 1). Control counts of the number of fibroblasts in 20 fields of vision showed that on the 5th-7th day in the experimental animals it was the same as on the 11th day in the control group (Table 1). Differences between the experimental and control groups as regards the density of distribution of the fibroblasts and their ILN were statistically significant.

By the 16th day of wound healing the number of DNA-synthesizing fibroblasts fell sharply in both groups of animals. A tendency was observed for an increase in the number of fibro-

blasts completing their cycle of development and passing into an inactive state, which was accompanied by a decrease in size of the nuclei and cytoplasm of the cells. ILN of fibroblasts in the newly formed connective tissue did not exceed its level in intact skin and subcutaneous cellular tissue.

It can be concluded from the fact that the increase in the number of DNA-synthesizing fibroblasts and the rapid increase in the density of their distribution in the focus of injury were observed earlier in the mice of the experimental group than in the control that the rate of proliferation of these cells was increased after stimulation. No figures of mitotic division were found in the fibroblasts in the animals of either group. This can be explained not only by the fact that mitoses are in general infrequent in fibroblasts, but also on the grounds that the material was always fixed in the morning when, as Arend and Torps [1] have observed, the number of mitoses in fibroblasts of newly formed connective tissue is minimal. Since accelerated differentiation of fibroblasts under the influence of potassium orotate was observed by the writer previously [3], the possibility of more rapid transformation of undifferentiated wound cells into fibroblasts under similar conditions cannot be ruled out.

The present investigations showed that during stimulation of healing of experimental wounds the reaction of adaptation to the constant action of potassium orotate took the form of a change in the rhythm of commencing DNA synthesis by the fibroblasts, so that the pool of wound fibroblasts was replenished by the more frequent entry of these cells into the mitotic cycle.

As regards the mechanisms of the stimulant action of potassium orotate on fibroblast proliferation one possibility is that it could have been due to shortening of the duration of the inflammatory phase. A reduction in the intensity of the destructive and exudative phenomena and of activation of the phagocytic reaction can be produced by activation of pituitary and adrenocortical function by means of pyrimidine derivatives. On the other hand, proliferative activity of fibroblasts can evidently be increased through the more rapid transformation of the undifferentiated cells of the focus of injury into fibroblasts. The increase in the number of DNA-synthesizing fibroblasts under stimulation conditions, however, is evidence of the increased mitotic activity of these cells.

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