EFFECT OF CYCLIC GUANIDINE MONOPHOSPHATE ON EXPERIMENTAL WOUND HEALING

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The role of cyclic nucleotides in metabolic processes *in vivo* is well known. The polyvalent character of their action is largely due to their participation in the regulation of cell proliferation and differentiation [2-4]. However, the action of exogenous cyclic nucleotides on repair processes *in vivo* has been inadequately studied.

This paper describes a continuation of the study of the effect of cyclic guanidine monophosphate (GMP) on proliferative processes in an experimental wound [1]. Special attention was paid to the discovery of histobiochemical parallels of collagen formation in granulation tissue.

EXPERIMENTAL METHOD

Experiments were carried out on 50 noninbred male rats weighing 150-170 g. Standard incised wounds measuring 2 × 2.5 cm were inflicted on the animals in the dorsal region. Wounds healed beneath a scab. The animals were divided into control the experimental groups. The experimental rats were given cyclic GMP by intraperitoneal injection 30 min after the operation in a dose of 3.3 μg/g body weight. The course of wound healing was studied, 5, 7, 10, 14, and 29 days after the operation. For histological examination the area of the wound was fixed with 15% neutral formalin solution and embedded in paraffin wax; sections were stained with hematoxylin-eosin and with picrofuchsin by Van Gieson's method. The intensity of migration of fibroblasts into the region of injury was assessed from the number of cells in 20 fields of vision under a magnification of the microscope of 280×. Statistical analysis of the results was carried out by Wilcoxon's method. The remaining parts of the wounds were used to determine the hydroxyproline concentration and specific radioactivity of collagen of the granulation tissue by the usual biochemical methods. Glycine-^{14}C, given in a dose of 0.6 μCi/g body weight, was used as precursor of collagen proteins.

EXPERIMENTAL RESULTS

By the 5th day after the operation the wounds on the control rats were covered by a scab, beneath which there was a barrier of leukocytes and necrotic tissue of considerable thickness. In the region of the wound defect, against a background of marked inflammatory changes, islets of granulation tissue can be seen to be forming. The predominant cells in the wound at this period were neutrophils, macrophages, and polyblasts. A few fibroblasts were distributed around the edges and in the floor of the wound. Their number in 20 fields of vision did not exceed 40. The wounds of the experimental animals differed from those of the controls by the less intensive inflammatory changes and the more active course of the proliferative reaction. This was shown by the larger number of granulations, the cell composition of which was richer than in the control. The number of fibroblasts increased to 68. Differences between control and experiment were statistically significant ($T > T_{05} > 11$).

On the 7th day after the operation a massive barrier of leukocytes and necrotic tissue was present on the surface of the wound in the control animals. The floor and edges of the wound were filled with granulations, consisting of thin-walled capillaries, surrounded by

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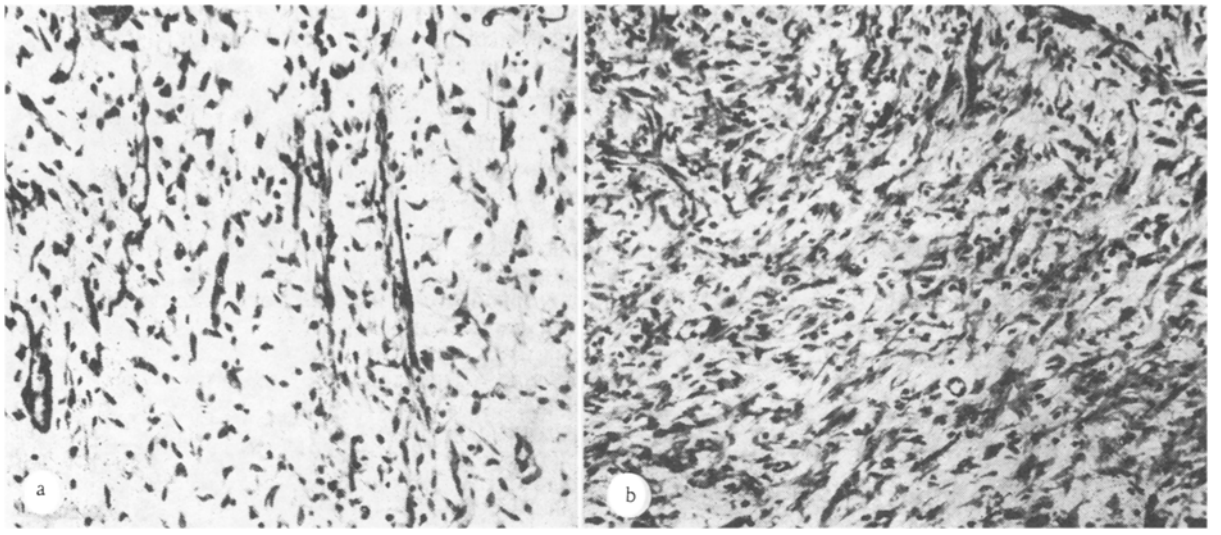

Fig. 1. Wound on 7th day after operation: a) control, b) experiment. Hematoxylin-eosin, 200 ×.

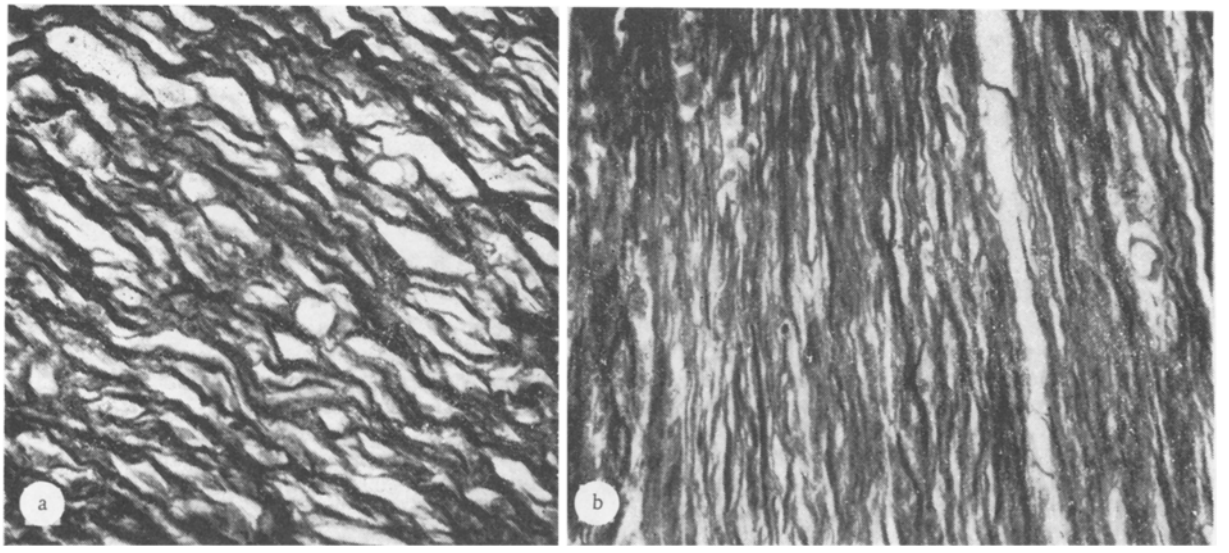

Fig. 2. Scar on 29th day after operation: a) control, b) Van Gieson, 400 ×

neutrophils, polyblasts, and cells resembling fibroblasts. The wound edges were covered by outgrowths of epithelium composed of four or five rows of cells. In the center of the wound foci of granulation tissue alternated with areas of edema, hemorrhages, and infiltrating leukocytes. The inflammatory changes were more marked in the upper layers of the wounds. In the experimental rats at this time, the barrier of leukocytes and necrotic tissue on the surface of the wounds was very thin. The regenerating epithelium, advancing over the granulations, formed projections consisting of 6 or 7 rows of cells, in which figures of mitotic division could be seen. Granulation tissue, with ill-defined signs of inflammation in the upper layers, filled the whole wound defect. Its cell composition consisted of a few neutrophils, polyblasts, and young forms of fibroblasts, with large oval nuclei containing two or three nucleoli and with long processes of basophilic cytoplasm. These processes interwove with thin twisted fibrils, staining a delicate pink color with picrofuchsin. The fibroblasts in the granulation tissue were more numerous than in the control (Fig. 1).

By the 10th day after the operation the thickness of the barrier of leukocytes and necrotic tissue on the wounds in the control rats was greater than that of the experimental animals on the 7th day after the operation. The granulation tissue remained focal in character,

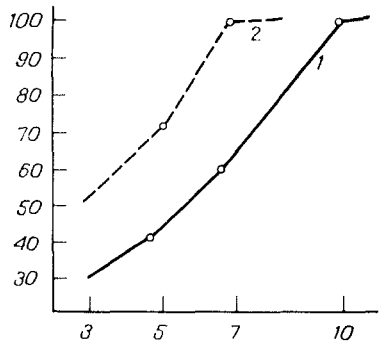

Fig. 3. Intensity of migration of fibroblasts into region of injury: 1) control, 2) experiment. Abscissa, days after operation; ordinate, number of cells in 20 fields of vision.

but the inflammatory changes in it were less abundant than on the 7th day. The number of fibroblasts in 20 fields of vision increased to 100. The cells had the appearance of young fibroblasts. The barrier of leukocytes and necrotic tissue was almost completely absent in the experimental animals at this time. The wounds were filled with granulation tissue containing numerous vertically oriented capillaries and mature fibroblasts, interweaving with fuchsinophilic fibers of varied thickness. The number of these cells showed no significant change compared with the previous period (Fig. 2). The layer of granulation tissue was thinner than in the control. Epithelization of the wound surface was more active than on the previous days.

On the 14th day activation of epithelization of the wound surface was observed in the control rats. Nonepithelized areas were covered with a scab, beneath which the barrier of leukocytes and necrotic tissue was preserved. The wounds were completely filled with granulation tissue, in the upper layers of which areas of edema and hemorrhages and infiltrating lymphocytes and leukocytes still remained. The principal cells of the granulation tissue were young and mature fibroblasts, the number of which was not increased at this time. In the experimental rats on the 14th day the wounds were largely epithelized. The layer of granulation tissue was much thinner than in the control. In the deep layers of the wound, emptying of the capillaries had begun. Thick collagen fibers formed horizontal rows, between which fibroblasts and fibrocytes were distributed.

On the 29th day the wounds of the control and experimental animals were completely epithelized. The young connective tissue of the experimental rats was thinner than that of the control, it contained fewer cells, and the arrangement of its collagen fibers was more compact (Fig. 3).

Biochemical determination of the hydroxyproline concentration in the granulation tissue revealed more intensive accumulation of this substance in the experimental animals. Its accumulation also took place earlier in the experimental animals than in the control. For instance, the maximal concentration of hydroxyproline in the wounds of the control animals was observed on the 10th day after the operation, compared with the 7th day for wounds of the experimental animals.

Determination of specific radioactivity of glycine-^{14}C showed that collagen synthesis in the granulation tissue took place most actively on the 7th day in the experimental rats and on the 10th day in the control. On subsequent days the radioactivity of the granulation tissue diminished. This may have been due to "dilution" of the label as a result of accumulation of collagen. The maximal hydroxyproline concentration, like maximal incorporation of glycine-^{14}C, in the granulation tissue differed only slightly in the experimental rats from the control.

The results of measurement of the areas of the wounds in the two groups of animals showed that the surface area of the wound on the experimental rats decreased in size almost twice as fast as those on the controls.

Under the influence of cyclic GMP there was thus no change in the phasic nature of the course of wound healing, but granulation tissue and the surface epithelium were formed somewhat faster and cell proliferation and differentiation were activated. Compared with the control wounds there was no increase in the number of fibroblasts, but their number reached a maximum sooner after the operation. This led to an increase in the rate of collagen and fibril formation.

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QUATERNARY STRUCTURE OF MOUSE AMYLOID FIBRILS: DISSOCIATION OF FIBRILS INDUCED BY SODIUM DODECYLSULFATE AND ALKALI

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Among the main problems in the pathogenesis of amyloidosis that still remain unsolved are the mechanism of formation of amyloid fibrils and the directly connected problem of the character of the bonds responsible for maintaining the fibrillary structure. The chemical nature of the elementary subunits of amyloid fibrils has been studied intensively, and considerable progress has been achieved in this field [3, 8, 10-12, 14, 15]. However, the quaternary structure of fibrils, i.e., the character of the bonds between the subunits and the method of their packing in the fibril has not yet been studied. Some workers have suggested that the fibril contains S—S bonds between the subunits [1, 6, 7]. There is also evidence of the essential role of noncovalent bonds in fibrillary structure [14].

Some results of a study of the quaternary structure of the amyloid fibril obtained during an investigation of dissociation of fibrils under the influence of denaturing agents are described in this paper.

EXPERIMENTAL METHOD

Experimental amyloidosis was induced in CBA mice weighing 18-20 g by injection of 5% casein in 0.25% NaOH subcutaneously in a dose of 1 ml daily for 35-40 days. The mice were then killed and their spleens treated by methods described in the literature [9-13]. The spleens were homogenized in a knife homogenizer in physiological saline. The homogenate was centrifuged at 19,600g for 15 min and the supernatant poured off; the residue was again homogenized and centrifuged for 15 min as long as the supernatant contained protein, judging from its extinction at 280 μ. The final residue was homogenized in a glass homogenizer in distilled water and centrifuged at 48,000g on an ultracentrifuge for 1.5 h four times. The first supernatant was discarded and the rest were collected, pooled, and lyophilized. The resulting material was investigated under the electron microscope by the negative staining method.

Partially dissociated fibrils were obtained by treating the amyloid fibrils with 0.1 N NaOH with a protein concentration of between 1 and 10 mg/ml. Dissociation was carried out in a cold room on a magnetic mixer for 30 min and 2 and 6 h. The resulting solutions were adjusted to neutral pH with 1 N HCl.

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