from survival of the animals until the time of their death from bone marrow damage (from the seventh day after irradiation). It can be concluded from analysis of mortality of the rats in this period that their survival, as a result of reimmigration following screening of one limb and an interval of 3 h between irradiations, is only half of that found in mice: The percentage of surviving rats did not exceed 20, whereas the percentage of surviving mice under similar conditions was considerably greater, namely 46%. Approximately the same survival rate (36%) as a result of screening of the previously irradiated limb was achieved in rats when this was done, not 3 h beforehand, as in mice, but 24 h beforehand. In rats, just as in mice, reimmigration of stem cells thus takes place, but it plays a significant role only if a larger volume of marrow is previously irradiated and later screened, or if the time interval between previous irradiations of the region of bone marrow and subsequent irradiations of the rest of the body is considerably longer. The limited importance of migration of bone marrow stem cells in rats compared with mice is in agreement with observations obtained earlier by other methods [3, 5]. The comparison of reimmigration of bone marrow stem cells in rats and mice made in this paper confirms previous observations and characterizes migration of bone marrow stem cells from a new aspect.

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CHANGES IN THE CYCLIC NUCLEOTIDE CONTENT IN WOUND TISSUES DURING HEALING

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Changes in the content of cyclic nucleotides (cyclic AMP and cyclic GMP) in wound tissues (muscle and granulation tissue) were investigated in rats. The experimental model was a wound with a skin defect in the dorsal region with pulping of the underlying muscle. The cyclic AMP level in muscle tissue was shown to rise to two peaks: on the first day and more especially on the 7th day. The cyclic GMP content rose a little on the 1st-4th days, fell on the 7th day, and rose again until the 14th day. The cyclic AMP concentration in granulation tissue followed a similar course to that in the muscle tissue: a rise on the 7th day and a fall on the 14th day. On the other hand, the curve of the cyclic GMP content in the granulation was more uniform. Only a small increase toward the 7th day was observed.

KEY WORDS: cyclic nucleotides; regeneration; cell; wound.

In the modern view, cyclic nucleotides play the leading role in the regulation of many different processes in the cell, including those processes of cell division and differentiation that are directly related to wound healing [1, 2, 3, 6, 7, 9-13]. Meanwhile, investigations of cyclic nucleotides during regeneration have been undertaken chiefly on tissue cultures [8]. It was therefore decided to study the dynamics of the cyclic nucleotide content in wound tissue at different stages of healing, for the information so obtained, it was considered, could shed some light on the basic principles of healing, and so could lead to the formulation of recommendations aimed at promoting or accelerating healing.

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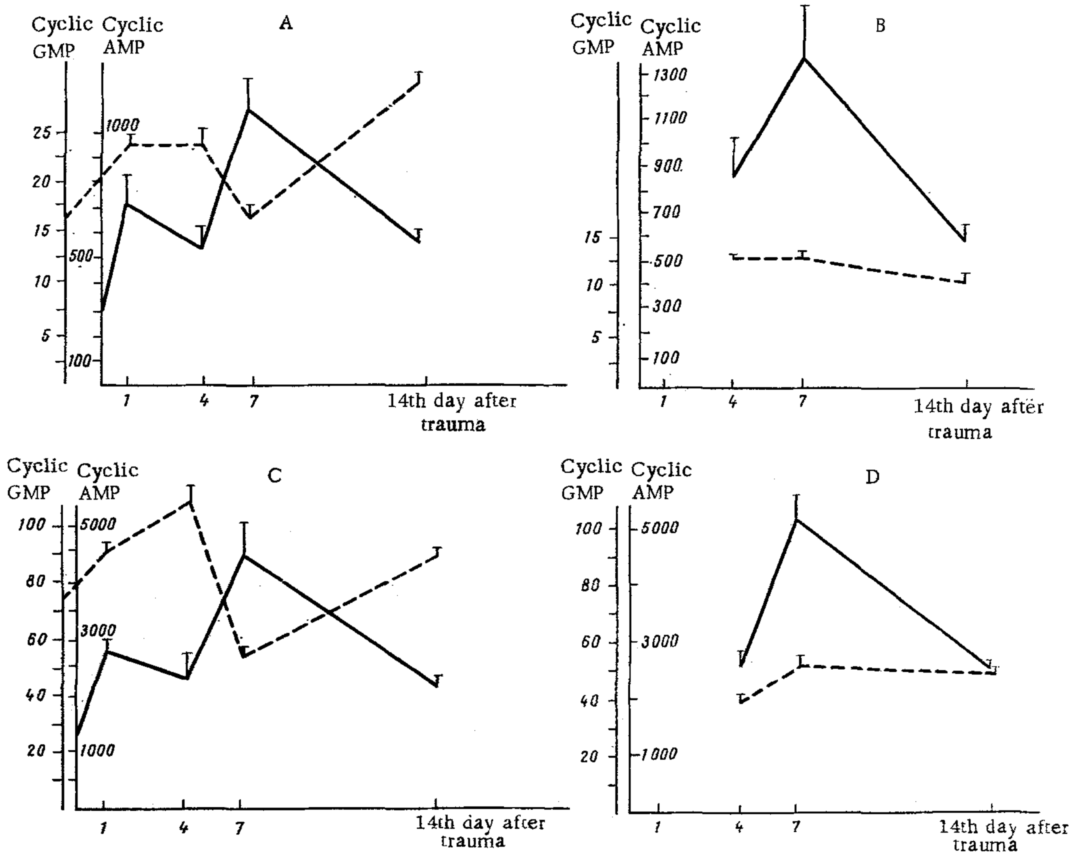

Fig. 1. Changes in cyclic nucleotide concentration in wound tissues at various times after trauma. A, C) underlying muscle; B, D) granulation. Cyclic AMP (continuous line) and cyclic GMP (broken line) concentrations expressed in picomoles/g wet weight of tissue (A, B) and in picomoles/g dry weight of tissue (C, D). Mean values (M ± m) given, for n = 10.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 120-150 g. The experimental model consisted of a wound with a skin defect in the dorsal region (measuring 2.5 × 2 cm) with pulping of the underlying muscle. The content of cyclic adenosine-3',5'-monophosphate (AMP) and cyclic guanosine-3',5'-monophosphate (GMP) was determined by a radioimmunologic method using special kits (from "Amersham"). Tissues for determination of cyclic AMP and cyclic GMP were fixed in liquid nitrogen. Protein was precipitated with ethanol. The precipitate was washed with aqueous ethanol (2 : 1). The pooled supernatant was evaporated to dryness, washed off with Tris-EDTA buffer, pH 7.5, and centrifuged; the concentrations of cyclic AMP and cyclic GMP in the supernatant were determined. Values were calculated per gram wet and dry weight of tissue. The dry weight in percent was determined by drying to constant weight. The investigations were carried out on granulation and muscle tissue from the region of the wound on the 1st, 4th, 7th, and 14th days after injury. Intact animals served as the control.

EXPERIMENTAL RESULTS

The cyclic AMP level in the muscle tissue throughout the period of investigation rose to 2 maxima: on the first day (+121.3%, $P < 0.05$) and more especially on the 7th day (+301.1%, $P < 0.01$). By the 14th day the cyclic AMP concentration fell, although it was still higher than in intact animals ($P < 0.01$). The cyclic GMP concentration on the first day also rose a little (+33.1%, $P > 0.001$), remained at the same level on the 4th day, and then fell to normal by the 7th day, after which it rose again until the 14th day (+164%, $P < 0.05$; Fig. 1A).

The cyclic AMP concentration in the granulation tissue followed a parallel course to the corresponding changes in muscle tissue during the period of wound healing studied (Fig. 1B): It rose until the 7th day (+57%,

$P < 0.02$) and then fell until the 14th day. Conversely, the cyclic GMP concentration in granulation and muscle tissues did not change in accordance with a consistent pattern. Whereas in muscle tissue the changes in the concentration of this nucleotide were more marked with maxima on 1st-4th and 14th day, its concentration in granulation tissue remained steady.

Since processes of inflammation and proliferation are closely bound with changes in the relative water content in the tissues, it was considered that the concentrations of the cyclic nucleotides should be expressed not only relative to wet weight, but also to dry weight of the tissues (Fig. 1C, D). The graph shows that the character of changes in the concentration of cyclic nucleotides in the wound tissues was basically similar during the period of observation by both methods of calculation, but when expressed relative to dry weight the changes stood out in greater relief. Reciprocity of the relations between cyclic AMP and GMP in the muscle tissue was particularly demonstrative: A decrease in the cyclic AMP level on the 4th day corresponded to an increase in the cyclic GMP concentration. Conversely, an increase in the cyclic AMP concentration on the 7th day was accompanied by a decrease in the cyclic GMP level, so that a second "scissors" appeared on the graph: By the 14th day the level of two cyclic nucleotides showed changes of the opposite pattern – a decrease in the cyclic AMP and an increase in the cyclic GMP levels. In granulation tissue the cyclic AMP concentration on the 7th day was doubled (+100.48%, $P < 0.05$) when expressed per gram wet weight of tissue. The cyclic GMP level also showed a significant increase by the 7th day (+27.27%, $P < 0.05$). By the 14th day the levels of both cyclic nucleotides had returned almost to their values on the 4th day. The investigations thus revealed a characteristic correlation between the nucleotide concentration and the course of wound healing.

Cyclic GMP is known to be a transmitter of proliferation signals and cyclic AMP a transmitter of differentiation signals [1-3]. In leukemia and neoplastic processes the cyclic GMP/cyclic AMP ratio in the cell is reversed and high [2]. The development of wound healing processes by the 14th day under the experimental conditions used also was accompanied by a sharp increase in the cyclic GMP concentration and a fall in the cyclic AMP level. In the muscle of intact animals, for instance, the cyclic GMP/cyclic AMP ratio was 0.053, on the 4th day after trauma it was almost unchanged (0.047), by the 7th day it was sharply reduced (0.011), and by the 14th day it was increased (0.086). This ratio can probably be used as an index of proliferative activity and, consequently, as an index of the efficacy of stimulation of wound healing by various agents. In addition, the results confirm Goldberg's hypothesis [4] on the existence of reciprocal antagonistic relations between these two cyclic nucleotides, on the basis of evidence obtained for various tissues [4, 5]. In the present investigation this can be seen particularly clearly in the case of muscle tissue.

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