AUTORADIOGRAPHIC ANALYSIS OF THE EFFECT OF POTASSIUM OROTATE ON THE INTENSITY OF [5-^{3}H]URIDINE AND [^{3}H]PROLINE INTO WOUND FIBROBLASTS

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The intensity of incorporation of [5-^{3}H]uridine into the nucleus and cytoplasm of fibroblasts during wound healing in control mice and in mice receiving potassium orotate and the intensity of incorporation of [^{3}H]proline into the same cells and into the intercellular spaces between them were studied. Incorporation of [^{3}H]proline was shown to take place much more intensively than that of [5-^{3}H]uridine. A considerable increase was observed in RNA synthesis in the fibroblasts under the influence of potassium orotate, accompanied by a less marked increase in synthesis of proline-containing proteins under analogous conditions. The dynamics of the nucleo-cytoplasmic ratios revealed by autoradiographic investigation correlated with the ultrastructural changes in the fibroblasts in the course of their differentiation.

KEY WORDS: proline-containing proteins; fibroblasts; stimulation of wound healing.

Proline is a component not only of proteins which are synthesized by cells "for export" but also of proteins that are constituents of the nucleus and cytoplasm and take part in the structural organization of cells [3-6]. The manner in which the intensity of synthesis of these proteins changes during stimulation of repair processes is not only of theoretical, but also of practical importance.

Previous investigations [1, 2] showed that potassium orotate stimulates RNA synthesis in the nuclei of fibroblasts and nucleo-cytoplasmic transport of newly formed RNA. A problem not yet solved is the extent to which these processes are linked with protein synthesis in fibroblasts during stimulation of wound healing.

The object of the present investigation was to determine correlation between the intensity of RNA synthesis and nucleo-cytoplasmic transport of newly formed RNA and to study the intensity of synthesis of proline-containing proteins in fibroblasts under the influence of potassium orotate.

EXPERIMENTAL METHOD

Experiments were carried out on 72 noninbred albino mice weighing 20-25 g. Standard incised wounds of skin and muscle, 1 cm long, were inflicted on the animals in the thigh region. The mice were divided into two groups, one of which was the control. The mice of the second group received potassium orotate per os from the first postoperative day (in doses of 0.2 ml of the 2% solution daily). Each group consisted of two subgroups. The intensity of protein synthesis was determined in the animals of one subgroup, and of RNA synthesis in the other. [^{3}H]Proline in a dose of 10 μCi/g was used as the precursor of proteins and [5-^{3}H]uridine in a dose of 20 μCi/g body weight as the precursor of RNA. Portions of wound tissue were fixed 3, 5, 7, 11, 13, 15, 18, and 21 days after the operation, 6 h after administration of the isotope. Autoradiographs were obtained on sections 1-2μ thick, by means of type M photographic emulsion by the usual method. The length of exposure was 4 weeks at 4°C. After development, the sections were stained with hematoxylin-eosin, 1% methylene blue solution, and by Masson's method. The intensity of incorporation of the isotope was determined by counting tracks above the nuclei and cytoplasm of the fibroblasts and intercellular space in the immediate vicinity of the fibroblasts. The results were subjected to statistical analysis by Wilcoxon's method. The results were recorded graphically to show not only the total number of tracks above the cells and intercellular space, but also the ratio between the number of tracks above the cytoplasm and their number above the nuclei

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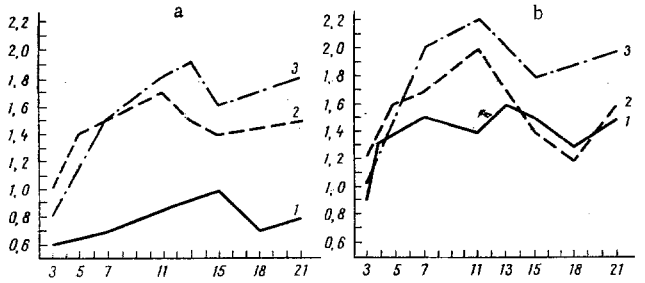

Fig. 1. Effect of potassium orotate on incorporation of [^{3}H]proline and [5-^{3}H]uridine into wound fibroblasts. Nucleo-cytoplasmic ratios: a) control, b) experiment. Abscissa, time after operation (in days); ordinate, nucleocytoplasmic ratios in fibroblasts. 1) Ratio of number of grains of silver above cytoplasm of 100 fibroblasts to their number in nuclei of same cells after administration of [5-^{3}H]uridine; 2) the same after administration of [^{3}H]proline; 3) ratio of number of grains of silver above intercellular space close to 100 fibroblasts to their number in cytoplasm of same cells after administration of [^{3}H]-proline.

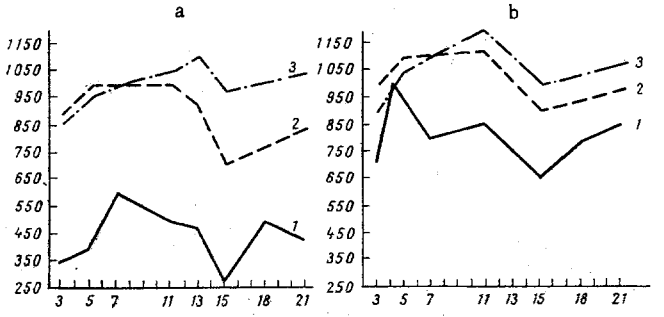

Fig. 2. Effect of potassium orotate on incorporation of [^{3}H]proline and [5-^{3}H]uridine into wound fibroblasts. Total incorporation: a) control, b) experiment. Abscissa, time after operation (in days); ordinate, number of grains of silver per 100 fibroblasts and above intercellular space close to 100 fibroblasts; 1) dynamics of [5-^{3}H]uridine incorporation into fibroblasts, 2) dynamics of incorporation of [^{3}H]proline into fibroblasts, 3) dynamics of [^{3}H]proline incorporation into intercellular spaces.

of 100 fibroblasts and the ratio between the number of tracks above the intercellular space close to 100 fibroblasts and their number above the cytoplasm of these cells.

EXPERIMENTAL RESULTS

The autoradiographic investigation showed that on the 3rd-5th days of wound healing the highest concentration of [^{3}H]proline was found in the cytoplasm of the fibroblasts of both groups of animals, and the nuclei and intercellular spaces contained less of the isotope (Fig. 1a, b). The concentration of the isotope in the wounds of the experimental animals was a little higher than in the control. The localization of [^{3}H]proline in the nuclei of the fibroblasts differed from that of [5-^{3}H]uridine. For instance, most [^{3}H]proline was found in the peripheral zones of the nuclei, immediately adjacent to the nuclear membrane, in the chromatin masses, in the center of the nuclei, and in the nucleoplasm around the nucleoli. The nucleoli themselves as a rule contained no [^{3}H]proline, whereas the highest concentration of [5-^{3}H]uridine was always found in the nucleoli

and adjacent zones of the nucleoplasm. This suggests that the nucleolus "collects" RNA in the form of a complex with proteins containing proline in very small quantities.

The ratio between the quantity of [^{3}H]proline incorporated into the nuclei and cytoplasm of the cells in the course of 5 days in the wounds of the experimental animals and in the course of 7 days after the operation in the wounds of the control mice revealed an increase in its concentration in the cytoplasm of the fibroblasts, and this could be regarded as a result of cell differentiation. Differentiation of fibroblasts evidently took place more rapidly under the influence of potassium orotate. This was proved by the more intensive increase in the number of tracks above the intercellular space on the following days.

Investigation of the ultrastructure of fibroblasts [1, 2] showed that during the 4 or 5 days after the operation fibroblast-like cells and young fibroblasts were predominant in the wounds of the control animals. Mature forms of fibroblasts began to predominate over the rest only on the 7th-8th day. Mature fibroblasts in moderate numbers were found in the wound contents of the experimental mice as early as on the 3rd-4th day after the operation, and on the 5th-6th day they predominated over the other cells in the granulation tissue. Starting from the 8th-10th day after the operation in the experimental mice and the 11th-13th day in the control mice, the number of fibroblasts which had completed their differentiation in the granulation tissue gradually increased. In the period of predominance of mature forms of fibroblasts in the wounds the intensity of incorporation of the RNA precursor and protein precursor into the nuclei, cytoplasm, and intercellular space showed little change. Appreciable fluctuations in the intensity of incorporation of [^{3}H]proline into the above-mentioned structures and of [5-^{3}H]uridine into the nuclei and cytoplasm of the cells were observed during conversion of the fibroblasts into the inactive state (fibrocytes). Under these circumstances the intensity of incorporation of the isotopes decreased both in the nuclei and in the cytoplasm of the cells. The increase in the number of young fibroblasts was accompanied by an increase in the intensity of incorporation of the isotopes into the cytoplasm of the cells and a subsequent increase in incorporation of proline into the intercellular spaces.

Comparison of the intensity of incorporation of [^{3}H]proline and [5-^{3}H]uridine into the nuclei and cytoplasm of the fibroblasts of the two groups of animals showed that the character of incorporation of [^{3}H]proline repeats the stepwise character of incorporation of the RNA precursor, the only difference being that incorporation of [5-^{3}H]uridine was at a lower level of intensity throughout the period of wound healing. The reason was evidently that the mRNA molecules function over and over again in the process of protein synthesis and, for that reason, one molecule of mRNA is sufficient to form scores of protein molecules. The most intensive incorporation of [5-^{3}H]uridine into the nuclei and cytoplasm of the fibroblasts was observed during the period of structural organization of the cytoplasmic network. The reason is evidently that the fibroblasts synthesized at that time the whole range of proline-containing proteins required for the structural organization of the cells and secreted by them into the extracellular space, each of which has its own specific RNA.

It must be specially emphasized that the intensity of incorporation of [^{3}H]proline into the fibroblasts and intercellular spaces during wound stimulation differed less from its intensity in the control than the intensity of [5-^{3}H]uridine incorporation under analogous conditions (Fig. 2a, b). Differences between the control and experimental series were statistically significant. Consequently, despite the fact that RNA synthesis in the fibroblasts increased considerably under the influence of potassium orotate, the synthesis of secretory proteins by these cells did not reach such a high level under analogous conditions.

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