AUTORADIOGRAPHIC INVESTIGATION OF STIMULATION OF RNA SYNTHESIS IN FIBROBLASTS

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UDC 612.751.3:612.398.145.1:087.347

The rate of uptake of uridine-5-^{3}M into fibroblasts from skin and muscle wounds of control and experimental animals and also into fibroblasts of loose connective tissue of intact animals was studied. The character of uptake and the distribution of the isotope in the nuclei and cytoplasm of loose connective tissue fibroblasts from intact mice was shown to differ from that in wound fibroblasts only in their intensity. Stimulation of RNA synthesis by potassium orotate in the fibroblast nuclei was closely linked with an increase in the rate and intensity of migration of labeled RNA from the cell nuclei to the sites of collagen synthesis.

KEY WORDS: RNA synthesis; nucleo-cytoplasmic transport; fibroblasts; potassium orotate.

Recent investigations have led to considerable progress in the study of the synthesis and posttranscription conversions of RNA in cells. It has now been shown that all types of RNA are formed in the cell nuclei as high-molecular-weight precursors, which subsequently migrate through the nuclear membrane into the cytoplasm to the sites of protein synthesis. The migration of RNA from the cell nucleus into the cytoplasm is one of the mechanisms which regulates the rate of protein synthesis. It has been shown that the nuclear membrane contains a number of enzymes that participate in the migration of RNA from nucleus into cytoplasm [1-3]. Another mechanism regulating the rate of nucleo-cytoplasmic transport of materials is a change in the number and diameter of the functioning pores in the nuclear membrane [4]. The problem of the extent to which stimulation of RNA synthesis in the cell nuclei is linked with changes in the rate and intensity of RNA migration into the cytoplasm still remained unexplained.

The object of this investigation was to undertake an autoradiographic study of the rate of incorporation of uridine-5-^{3}M into nuclei of fibroblasts of intact loose connective tissue and skin and muscles, to study the character of migration of labeled RNA precursor from nucleus into cytoplasm, and to determine the influence of a pyridime-base derivative (potassium orotate) on this process.

EXPERIMENTAL METHOD

Experiments were carried out on 102 noninbred albino mice weighing 20-25 g. The animals were divided into three groups containing equal numbers of mice. Group 1 consisted of 34 intact mice. Standard incised skin and muscle wounds 1 cm long were inflicted on the mice of groups 2 and 3. Six days later uridine-5-^{3}M was injected intraperitoneally simultaneously into all the wounded and intact animals in a dose of 20 μCi/g body weight. Simultaneously with the isotope, the mice of group 3 received 2 ml of a 2% solution of potassium orotate per os.

The areas of the wound and skin-muscle flaps from intact mice were fixed in a 10% solution of neutral formalin every 15 min during the first 2 h of the experiment and at intervals of 30 min during the next 4 h.

Autoradiographs were obtained on paraffin sections in the usual way with type M photographic emulsion. The duration of exposure was 4 weeks at 4°C. After development, the sec-

Department of Pathological Anatomy, A.V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Smol'yaninov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 10, pp. 488-491, October, 1978. Original article submitted March 23, 1978.

 0007-4888/78/8610-1390$07.50

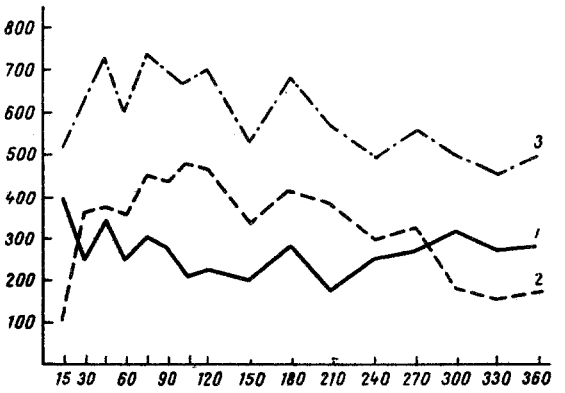

Fig. 1. Intensity of incorporation and distribution of uridine-5-^{3}M in wound fibroblasts of animals receiving potassium orotate. Here and in Figs. 2 and 3: abscissa, circulation time of uridine-5-^{3}M in animals (in min); ordinate, number of grains of reduced silver per 100 fibroblasts. 1) Number of labels above fibroblast nuclei; 2) number of labels above fibroblast cytoplasm; 3) total number of labels above fibroblasts.

tions were stained with hemotoxylin and eosin and the number of grains of reduced silver was counted above the nuclei and cytoplasm of 100 fibroblasts located in the granulation tissue of the wounds and also in the skin and subcutaneous areolar tissue of intact animals.

EXPERIMENTAL RESULTS

By the sixth day after the operation the inflammatory phase was largely finished. The wounds were filled with well developed granulations. Nonepithelized areas of the wounds were covered with a scab, under which the leukocyte barrier separating the zone of necrosis from the granulation tissue was situated. The cells of the granulations consisted of numerous proliferating fibroblasts with large nuclei, containing 2, 3, or sometimes 4 nucleoli. The area of the cytoplasm in these cells was very small. In the lower layers of the granulations and in the intermuscular spaces mature fibroblasts with comparatively small oval nuclei, containing 1 or 2 nuclei, and long outgrowths of cytoplasm predominated.

The experimental results showed that the first labels appeared above the nucleoli and surrounding nucleoplasm. Their number increased at different rates in different groups of mice, for the time during which an approximately equal concentration of isotope was formed in the nuclei of the fibroblasts in the wounds of the experimental and control animals differed. It was shorter in animals receiving potassium orotate at the same time as the uridine-5-^{3}M. It did not exceed 30 min (Fig. 1). In wound fibroblasts of the control mice this time was about 2 h. After the lapse of this time, in both groups of animals there was a sharp decrease in the number of labels above the nuclei of the fibroblasts and a simultaneous increase in their number above the cell cytoplasm, evidence of migration of a large amount of labeled RNA from the cell nuclei into the cytoplasm. During this period a gradual increase was observed in the concentration of the isotope in the nuclei of the wound fibroblasts of both groups of animals and the corresponding gradual increase in its content in the cell cytoplasm. However, in the wounds of the control mice this process took place more smoothly (Fig. 2). This suggests that small portions of labeled RNA entered the cytoplasm even before migration of any significant quantity of labeled RNA was observed. It may be that RNA can enter the cytoplasm in large quantities only when its concentration in the cell nuclei reached a critically high level. The experiment showed that the level of labeled RNA in the nuclei of the fibroblasts necessary for a large portion of labeled RNA to be transported into the cytoplasm was about the same in the control and the experimental animals. Only the time taken for this level to be reached was different.

Consequently, administration of potassium orotate to the animals led to an increase in the rate of RNA synthesis in the fibroblast nuclei and intensification of nucleo-cytoplasmic transport.

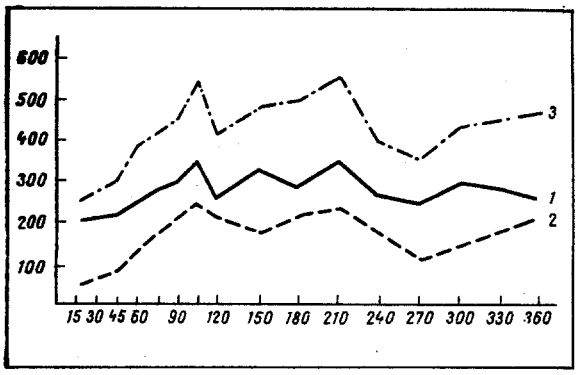

Fig. 2

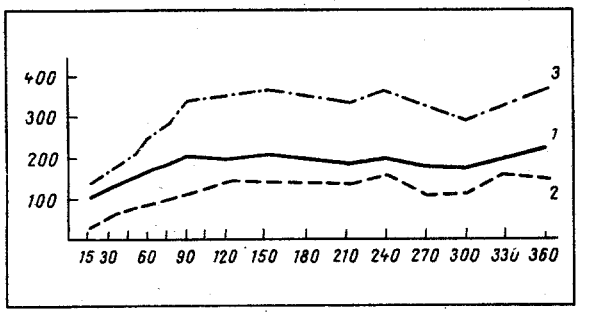

Fig. 3

Fig. 2. Intensity of incorporation and distribution of uridine-5-^{3}M in wound fibroblasts of control animals.

Fig. 3. Intensity of incorporation and distribution of uridine-5-^{3}M in loose connective tissue fibroblasts of intact animals.

During the migration of labeled RNA from nucleus into cytoplasm to the sites of protein synthesis, the intensity of its formation in the nuclei increased again. The most intensive incorporation of uridine-5-^{3}M took place in the nucleoli. In animals receiving potassium orotate the intensity of labeling of the nucleoli was greater than in the control. Often two or three nucleoli containing a large quantity of isotope were located very close to the nuclear membrane. However, much of the label also could be found above the nucleoli located in the central areas of the nucleus.

Subsequent quantitative analysis of the autoradiographs showed that the intensity of RNA synthesis in the fibroblast nuclei depends on the concentration of functioning RNA in the cytoplasm of these cells. When the concentration of uridine-5-^{3}M in the cytoplasm was maximal, the quantity of labeled RNA in the nuclei was minimal. A decrease in the concentration of labeled RNA precursor in the cytoplasm led to subsequent activation of RNA synthesis in the nuclei. This gradual change in the concentration of labeled uridine in the nuclei and cytoplasm of the fibroblasts was observed throughout the experiment (see Figs. 1 and 2).

The stimulating effect of potassium orotate on RNA synthesis in the cell nuclei and the rate of nucleo-cytoplasmic transport of newly formed RNA continued, incidentally, for 4.5 h. During the next 1.5 h of the experiment the intensity of RNA synthesis and of its transport into the cytoplasm of the fibroblasts in the animals of group 3 no longer differed from that in the mice of group 2; in all probability this was due to utilization of the stimulator administered to the animals of group 3.

Comparison of the intensity of uptake of uridine-5-^{3}M into the fibroblasts of all three groups of mice showed that this intensity was lowest in the fibroblasts from the skin and subcutaneous areolar tissue of intact mice. With an increase in the duration of contact between the cells and uridine-5-^{3}M the intensity of labeling of the fibroblasts increased in this group of animals also. The character of incorporation of the isotope into the cells and of the distribution of labels above the nuclei and cytoplasm of fibroblasts from the skin and subcutaneous areolar tissue of intact animals differed from that in the wound fibroblasts of the control mice only in the intensity of uptake of uridine-5-^{3}M into the corresponding cell structures (Fig. 3).

It can accordingly be concluded from these results that stimulation of RNA synthesis in fibroblasts nuclei was closely linked with an increase in the rate and intensity of nucleo-cytoplasmic RNA transport to the sites of collagen synthesis.

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