STIMULATION OF FIBRILLOGENESIS IN GRANULATION TISSUE

G. N. Dudnikova, Yu. D. Batsura,
and A. A. Paltsyn

UDC 616-003.9-07:616-008.939.629-074

Synthesis of RNA and its transfer from nucleus into cytoplasm of fibroblasts was investigated during the healing of skin wounds in control mice and mice receiving potassium orotate. Under the influence of this compound, the transfer of newly synthesized RNA from nucleus into cytoplasm took place more rapidly. Its role of collagen synthesis was judged from the appearance and maturation of collagen fibers on the wound surface. These data were obtained by means of a scanning electron microscope.

KEY WORDS: fibroblast; RNA synthesis; scanning electron microscope.

In the last 10 to 15 years the problem of collage formation has been studied intensively by various specialists [3-8, 10] but many unexplained and disputed problems still remain, on which some light could be shed by the study of nucleic acid synthesis with the aid of radioactive precursors.

Collagen formation takes place as a result of the activity of fibroblasts mainly through a universal biosynthetic process [9-12]. It is now generally accepted that RNA synthesis in cells and the level of protein biosynthesis are directly dependent on one another. It is also known that RNA is synthesized in the nucleus and then transferred to the cytoplasm. Hence, it follows that the intensity of RNA metabolism and, consequently, the intensity of protein synthesis can be judged from the rate at which RNA is transferred from nucleus into cytoplasm.

It was accordingly decided to investigate the synthetic activity of fibroblasts during wound healing under normal conditions and when the process was stimulated by means of potassium orotate, a derivative of the pyrimidine series capable of intensifying the source of regenerative processes [1, 2].

EXPERIMENTAL METHOD

Experiments were carried out on noninbred mice weighing 25 g. An incision was made in the skin and subcutaneous cellular tissue in the region of the anterolateral surface of the right thigh, 1 cm long, after which a piece of muscle measuring 2 × 2 × 2 mm was removed. The wound healed beneath a scab. The animals were divided into two groups. Immediately after the operation one group began to receive potassium orotate as a 2% aqueous solution in a dose of 0.2 ml by mouth daily; the second group acted as the control.

The mice received uridine-H^3 (specific activity 18 Ci/mmole) by subcutaneous injection in a dose of 20 μCi/g 2 h before sacrifice. Pieces taken from the wound were fixed in 10% neutral formalin solution and embedded in paraffin wax. Sections 3-4 μ in thickness were first treated with 5% TCA solution, then coated with type M emulsion and exposed at −4°C

Department of Pathological Anatomy, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR. Department of Pathomorphology, Institute of Work Hygiene and Occupational Diseases, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. I. Strukov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 80, No. 12, pp. 96-99, December, 1975. Original article submitted March 7, 1975.

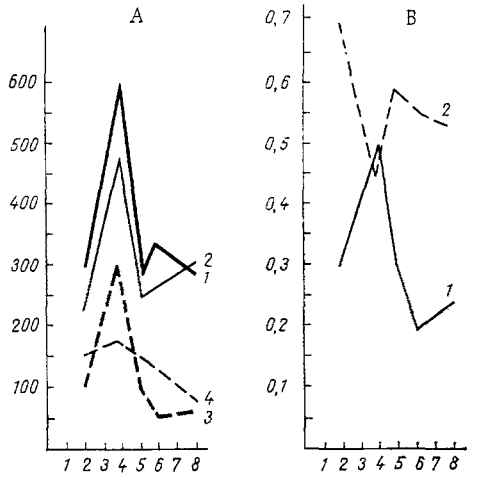

Fig. 1. Incorporation of uridine-H^3 into fibroblasts of granulation tissue: A) distribution of grains of silver in fibroblasts, nuclei (1 and 2), and cytoplasm (3 and 4) in control (1 and 3) and experimental (2 and 4) groups; B) ratio between number of silver grains in cytoplasm and their number in nucleus in control (1) and experiment (2). Abscissa, days after operation; ordinate: in A) number of grains of silver per 100 fibroblasts, in B) ratio between number of grains of silver in cytoplasm and number in nucleus (C:N).

for 6 weeks, developed, and stained with hematoxylin–eosin and picrofuchsin. The number of grains of reduced silver was counted separately in the nucleus and cytoplasm of 200 fibroblasts located in the region of the floor and edges of the wound.

For examination in the scanning electron microscope the material was fixed in 2.5% glutaraldehyde solution and 1% osmium tetroxide solution, then dehydrated in alcohols of increasing concentration and in propylene oxide. Scrapings of the wound surface were prepared in a medium of liquid nitrogen and sprayed with metallic gold. The objects were examined in the Kwikscan 100 electron microscope with a resolving power of 90 Å.

EXPERIMENTAL RESULTS

The number of fibroblasts increased rapidly between the second and fifth days after injury. During wound healing in the control mice incorporation of isotope in the nuclei of the fibroblasts was maximal on the fourth day after the operation (Fig. 1A), and later (fifth, sixth, and eighth days) their uridine-H^3 content remained almost stable. The dynamics of incorporation of the labeled precursor also was similar in fibroblast nuclei of animals receiving potassium orotate. In the cytoplasm, however, the concentration of uridine-H^3 was higher on the fourth day in the fibroblasts of the experimental mice than in the controls.

These results suggest that the most intensive RNA synthesis in the nuclei of the fibroblasts during wound healing in the mice of both groups took place

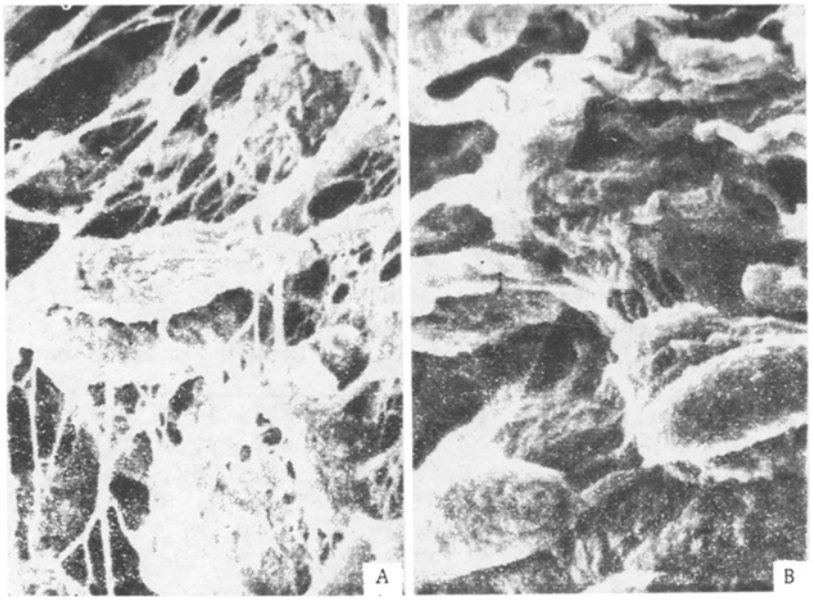

Fig. 2. Wound surface on 5th day after operation (5000×): A) group of fibroblasts with thin network of collagen fibrils; B) mature collagen fibers during stimulation of repair process.

on the fourth day and that later it evidently took place at a constant rate. The lower uridine-H^3 content in the fibroblast nuclei of the experimental animals can be explained by the fact that repair processes were stimulated by means of a salt of orotic acid, which is related to uracil (uracil-4-carboxylic acid), and this could lead to dilution of the label in the fibroblast nuclei of the experimental group of mice.

The ratio between the number of grains of silver in the cytoplasm of the cell and the number in its nucleus characterizes the rate of RNA migration from the nucleus into the cytoplasm. As is clear from Fig. 1B, the curve reflecting this ratio in the fibroblasts of animals of the experimental group was considerably higher throughout almost the whole period of observation than the corresponding curve in the control. The difference between the control and the experimental groups was particularly marked starting with the fifth day of wound healing and later (Fig. 1B), confirming the more rapid transfer of newly synthesized RNA from the nucleus into the cytoplasm under the influence of potassium orotate.

The results of the autoradiographic investigation were reflected in data obtained by the study of fibroblasts with the scanning electron microscope. For instance, in the first 2 or 3 days after the operation no typical fibroblasts were yet visible in the animals of the control group in the surface layers of the wound. In this period cells with a rough surface, 6.5-8 μ long, appeared. In view of the very thin collage fibrils at the point of contact of these cells with the wound surface, it seems that they were fibroblast-like cells. Toward the fifth day, mainly fibroblasts were found on the wound surface. Their length was 8-10 μ, their surface was nodular, the cells were flat or cylindrical in shape, and they had rounded poles. The cells were loosely connected with each other by collagen fibrils, 0.1-0.12 μ in diameter and with a spacing of 0.09-0.1 μ. Occasionally collagen fibrils were gathered into filaments 0.2-0.3 μ thick, or into flat, fanshaped structures (Fig. 2A).

In mice receiving potassium orotate on the second to third day after the operation not only fibroblast-like cells, but also typical fibroblasts, surrounded by thin fibrils of collagen, were found in the surface layers of the wound. By the fifth day after wounding large, spindle-shaped fibroblasts 18-20 μ long and with a nodular surface could be seen. At the same time numerous twisted collagen fibers, 1 μ in diameter and with a spacing of 0.068-0.084 μ, were found on the wound surface (Fig. 2B). These results indicate a correlation between the rate of morphological differentiation of fibroblasts (as shown by scanning electron microscopy) and the level of the biosynthetic activity of these cells. Activation of RNA synthesis, observed from the fourth day of wound healing, coincided in time with the appearance of differentiated fibroblasts, collagen fibrils, and the first collagen fibers on the wound surface. The more rapid transfer of RNA from the nucleus into the cytoplasm in the experimental animals evidently reflects intensification of collagen formation. This is confirmed by the fact that many mature collagen fibers were found on the wound surface in animals receiving potassium orotate as early as on the fifth day after wounding.

LITERATURE CITED

1. A. L. Bandman, in: Abstracts of Proceedings of a Scientific Conference on the Regulation of Inflammatory and Regenerative Processes [in Russian], Leningrad (1959), p. 5.
2. E. E. Belen'kii, "Pyrimidine and purine derivatives as stimulators of regneration in acute and chronic experimental myocardial pathology," Author's Abstract of Doctoral Dissertation, Moscow (1974).
3. S. G. Kolaeva, in: Abstracts of Proceedings of a Scientific Conference of Morphologists of Eastern Siberia [in Russian], Irkutsk (1961), p. 155.
4. A. A. Tustanovskii and G. V. Orlovskaya, Arkh. Pat., No. 3, 32 (1953).
5. B. B. Fuks, Dokl. Akad. Nauk SSSR, 135, 747 (1960).
6. B. Goldberg and H. Green, J. Cell Biol., 22, 227 (1964).
7. R. H. Kretsinger, G. Manner, B. S. Goüld, et al., Nature, 202, 438 (1964).
8. D. A. Lowther, N. M. Green, and J. A. Chapman, J. Biophys. Biochem. Cytol., 10, 373 (1961).
9. K. R. Porter, Biophys. J., 4, No. 1, Part 2, 167 (1964).
10. R. Ross, Biol. Rev., 43, 51 (1968).
11. R. Ross and E. P. Bendit, J. Cell Biol., 27, 83 (1965).
12. M. L. Stearns, Am. J. Anat., 67, 55 (1940).