INTENSITY OF RNA AND PROTEIN SYNTHESIS IN FIBROBLASTS DURING WOUND HEALING IN MICE

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RNA and protein synthesis in fibroblasts was studied by a double-labeling method on the 3rd and 7th days of wound healing in mice by electronmicroscopic autoradiography. The ratio of protein synthesis in the cytoplasm and RNA synthesis in the nucleus was shown to differ significantly in individual cells, with the result that fibroblasts with relative predominance of one of the two types of synthesis were found at both stages of wound healing. On average fibroblasts showed a higher level of protein synthesis on the 3rd day of healing. This high mean level was maintained by an increase in the proportion of fibroblasts in which protein synthesis predominated over RNA synthesis.

KEY WORDS: fibroblast; RNA synthesis; protein synthesis; electronmicroscopic autoradiography; wound healing.

Fibroblasts form the intercellular substance of connective tissue and are considered to be the principal cells responsible for the repair of tissue defects [3, 7, 8]. Hence the great interest of the study of changes in the key processes of biosynthesis which take place in the fibroblasts after trauma and lead to the formation of new connective tissue in the zone of injury.

This paper describes the results of a study of RNA and protein synthesis in the fibroblasts of a healing wound obtained by electron-microscopic autoradiography, with the use of a double labeling method described previously [6].

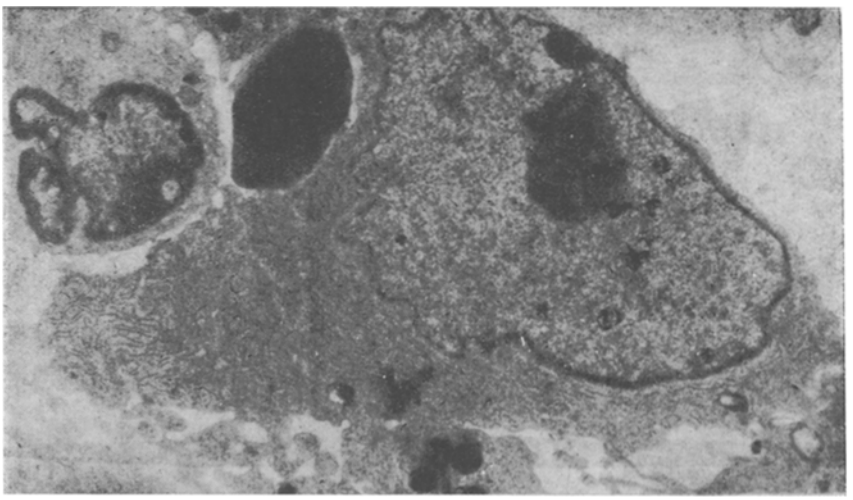

Fig. 1. Fibroblast of mouse granulation tissue on 3rd day of wound healing. [^{3}H]Uridine injected into animals (group 1). Grains of silver located above nucleus, 14,000 ×.

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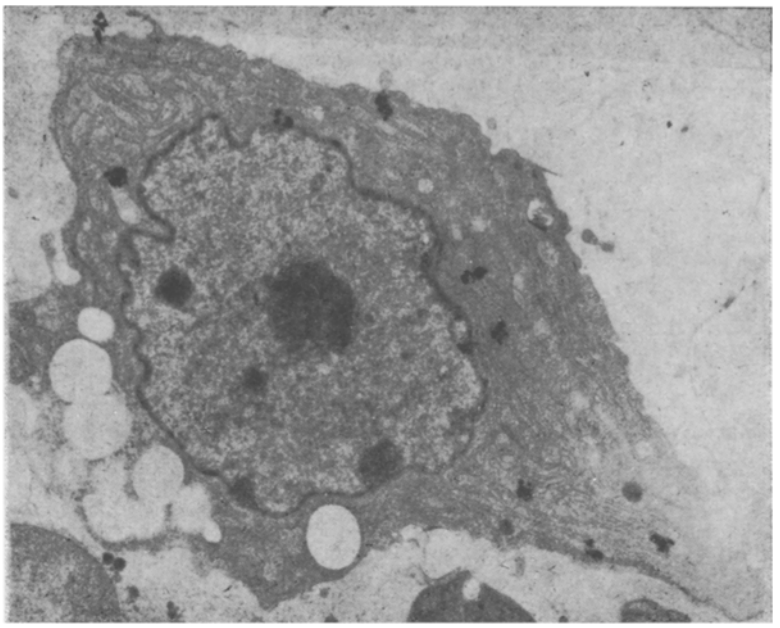

Fig. 2. Fibroblast of mouse granulation tissue on 7th day of wound healing. [^{3}H]Proline injected into animals (group 2). Grains of silver above cytoplasm, 14,000 ×.

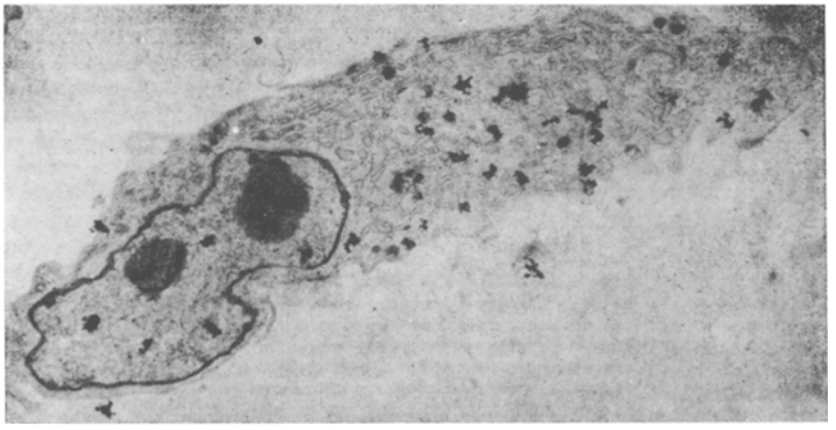

Fig. 3. Fibroblast of mouse granulation tissue on 3rd day of wound healing. Mixture of [^{3}H]uridine and [^{3}H]proline injected into animals (group 3). Many grains of silver above cytoplasm and cell nucleus, 12,000 ×.

METHODS

Fibroblasts from mouse granulation tissue were studied on the 3rd and 7th days after infliction of a wound to the soft tissues of the leg with removal of a piece of subcutaneous cellular tissue, fascia, and muscle measuring 3 × 3 × 4 mm. Animals 3 and 7 days after wounding were divided into three groups: 1) animals receiving a subcutaneous injection of [5-^{3}H]-uridine in a dose of 4 μCi/g (specific activity 22.5 Ci/mmole) only; 2) animals receiving [3,4,5-^{3}H]proline in a dose of 20 μCi/g (specific activity 15 mCi/mmole) only; 3) animals receiving a mixture of [5-^{3}H]uridine and [^{3}H]proline in the same doses as the first two groups. Differences in the distribution of label in the fibroblasts of mice of groups 1-3 are shown in Figs. 1-3. Altogether six groups, each containing four mice, were used in the experiments. Wound tissue was fixed 1 h after injection of the precursors with 2.5% glutaraldehyde solution in phosphate buffer, pH 7.4, then washed in phosphate buffer for 24 h and postfixed in 1% osmium tetroxide solution; the material was then embedded in Epon. To begin with an autora-

TABLE 1. Mean Density of Label in Nucleus and Cytoplasm of Fibroblasts on 3rd and 7th days of Wound Healing (M ± m)

Index	Time of wound healing, days					
	3rd			7th		
	group of animals†					
	1	2	3	1	2	3
Density of label above nucleus	0,012±0,009	0,004±0,003	0,008±0,006	0,011±0,006	0,003±0,003	0,012±0,013
Density of label above cytoplasm	0,003±0,003	0,007±0,005	0,009±0,006	0,001±0,001	0,005±0,003	0,009±0,006
Ratio of density of label cytoplasm to density of label in nucleus*	0,25	1,75	1,125	0,09	1,67	0,75
Number of cells investigated	103	94	350	63	77	118

*Values obtained by division of mean values given in table and, consequently, deviations are not shown.

†Animals of group 1 received [^{3}H]uridine, those of group 2 received [^{3}H]proline, and those of group 3 received both [^{3}H]uridine and [^{3}H]proline.

Legend. Significance of differences in density of label above nucleus: for group 1 $P_1 > 0.05$, for group 2 $P_2 < 0.05$, for group 3 $P_3 < 0.01$; in density of label above cytoplasm: $P_1 < 0.001$, $P_2 < 0.01$, P_3 no difference.

diographic investigation was made of semithin (1-2 μ) sections stained with toluidine blue and azure. An area containing granulation tissue cells was selected in light-microscopic autoradiographs, and a pyramid was cut out of this region for ultrathin sectioning. Electron-microscopic autoradiographs were prepared by the method described by Sarkisov et al. [4]. All fibroblasts occurring in areas of the sections free from technical defects were photographed. The number of grains above the nucleus and above the cytoplasm of the cell was counted on negatives and the area of cross section of nucleus and cytoplasm (the product of length and width in millimeters) measured in conventional units. The labeling density was determined as the ratio of the number of grains above a given part of the cell to its cross-sectional area.

RESULTS

The experimental results are given in Tables 1-3. Analysis of these tables reveals the following structural and functional differences between the fibroblasts studied on the 3rd and 7th days of wound healing: 1) The concentration of labeled RNA in the cytoplasm of the fibroblasts was higher on the 3rd day than on the 7th day of healing, whereas its concentration in the nucleus was roughly the same as on the 7th day (animals of group 1; Table 1); 2) the intensity of uptake of [^{3}H]proline into the cytoplasm of the fibroblasts on the 3rd day of healing was higher (although not significantly) than on the 7th day (animals of group 2; Table 1); 3) in the animals of group 3 the ratio of the densities of label in the cytoplasm and the nucleus on the 3rd day of wound healing was higher in favor of the former than on the 7th day. This observation is complementary to the data in Table 2, which show that in the animals of group 3 fibroblasts with a predominantly "nuclear" or "cytoplasmic" type of concentration of label were present on both the 3rd and the 7th days of healing. However, whereas on the 7th day there were equal numbers of cells of the two types, on the 3rd day there were more fibroblasts with a higher concentration of label in the cytoplasm; 4) on the 3rd day of healing the nucleus and cytoplasm of the fibroblasts were larger than on the 7th day (Table 3).

The higher density of label in the cytoplasm than in the nucleus on the 3rd day of healing (animals of group 3) indicates increased protein production by fibroblasts at this time. This conclusion is confirmed and supported by data showing an increase in size of the cell itself and the more rapid migration of RNA from the nucleus into the cytoplasm on the 3rd day of healing, possibly reflecting hyperplasia of the granular cytoplasmic reticulum.

Ideas regarding increased production of protein in inflammation, put forward on the basis of biochemical [5, 9], morphological [1], and cytochemical [2] investigations can be carried a stage further as a result of the observations now described. The electron-autoradiographic data show differences in the role of individual cells in the process of post-traumatic intensification of protein synthesis. After injection of a mixture of precursors of RNA and protein into animals at both times of wound healing studied, fibroblasts with a

TABLE 2. Distribution of Fibroblasts in Animals of Group 3 by Ratio of Density of Label in Nucleus and Cytoplasm

Time of wound healing, days	Number of cells			Total number of cells studied
	of type I	of type II	of type III	
3	59	100	191	350
7	33	33	52	118

TABLE 3. Mean Area of Cross Section (in mm^2) of Nucleus and Cytoplasm of Fibroblasts (magnification 5000 ×) on 3rd and 7th Days of Wound Healing (M ± m)

Time of wound healing, days	Nucleus	Cytoplasm	Number of cells studied
3-	697±309,42	1584±649,71	275
7-	608±280,45	1265±611,03	258
P	<0,001	<0,001	

higher concentration of label both in the nucleus and in the cytoplasm were observed in the wound. Consequently, there are two types of fibroblasts in wounds: 1) those with relative predominance of RNA synthesis over protein synthesis and 2) those with relative predominance of protein synthesis over RNA synthesis. The relative numbers of cells of these two types vary in the course of wound healing. For instance, on the 3rd day the number of type 2 fibroblasts was increased, thus enabling forced protein synthesis to take place in the wound as a whole. In other words, a high total level of protein production, observed at a definite stage of wound healing, is produced not by an increase in the intensity of this process in all fibroblasts present in the tissue, as may be indicated by the results of biochemical investigation, but simply by a greater degree of synchronization of the phase of increased protein synthesis in individual cells.

LITERATURE CITED

1. M. K. Vasil'tsov and A. I. Radostina, Arkh. Anat., No. 2, 80 (1977).
2. G. G. Kruglikov and L. L. Shimkevich, in: Connective Tissue in Health and Disease [in Russian], Novosibirsk (1968), p. 162.
3. A. A. Makismov, Principles of Histology [in Russian], Part 2, Petrograd (1918), p. 624.
4. D. S. Sarkisov, A. A. Pal'tsyn, and B. V. Vtyurin, Adaptive Modifications of Biorhythms [in Russian], Moscow (1975).
5. V. N. Smirnov, V. I. Mazurov, V. P. Goncharova, et al., Vopr. Med. Khim., No. 3, 305 (1964).
6. A. A. Pal'tsyn and E. G. Kolokol'chikova, Byull. Éksp. Biol. Med., No. 11, 626 (1978).
7. E. Kulonen, in: Biology of Fibroblasts, London (1973), p. 3.
8. R. Ross, Biol. Rev., 43, 51 (1968).
9. J. F. Woessner, jr., and R. J. Boucek, Arch. Biochem., 93, 85 (1961).