AUTORADIOGRAPHIC INVESTIGATION OF DNA SYNTHESIS IN SKIN SURROUNDING A WOUND IN MICE

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Full-thickness skin wounds 8 mm in diameter were inflicted on the dorsum of hairless mice. A single injection of thymidine-H^3 was given to the animals from the 7th to the 60th day after the operation, 1 h before sacrifice, and the index of labeled nuclei and mitotic activity were determined in the epithelial and connective-tissue cells in the wound and for a distance of 8 mm from its edge. The number of DNA-synthesizing cells was shown to be considerably increased throughout the area of skin studied, including the most distant parts. Great differences were found in the labeled fibroblasts, especially in the early periods of healing, both in the wound and in the uninjured skin.

During the healing of skin wounds, besides processes taking place in the wound itself, inward growth of the skin around the wound also takes place [1, 2-4, 6, 7]. Increased mitotic activity in the epithelial cells of mouse skin has been found to extend for more than 8 mm away from the wound. This increased proliferative activity persists in the epithelium around the wound for a long time after epithelization of the defect is complete [5]. However, little attention has been paid to the study of proliferative processes taking place during inward growth of the skin around the wound.

The object of this investigation was to study DNA synthesis in the tissues filling in the wound and in the skin around the wound during the period of epithelization and after its completion, namely: on the 7th, 14th, 21st, and 60th day after the operation.

EXPERIMENTAL METHOD

Experiments to determine the activity of DNA synthesis in the epithelial cells and fibroblasts were carried out on 26 female hairless mice weighing 25-27 g. A circular full-thickness excised wound, 8 mm in diameter, was inflicted on the dorsum of 24 animals in the interscapular region. Two intact mice acted as controls. The mice were decapitated at midnight at a time of maximal DNA synthesis in the epithelium [8, 9]. All the mice received an intraperitoneal injection of thymidine-H^3 1 h before sacrifice in a dose of 0.7 μCi/g body weight. Biopsy was carried out on the 7th, 14th, 21st, and 60th days after the operation, 6 animals being killed at each time. Pieces of skin, taken for histoautoradiographic study included the region of the wound and the adjacent intact skin for a distance of 8 mm. The piece of skin for study was divided into five zones. Each zone contained 10 fields of vision of the microscope (objective 90×, ocular 5). The pieces were fixed in Carnoy's mixture and embedded in paraffin wax. Sections, 5μ in thickness, were coated with type-R liquid emulsion. The specimens were developed and stained with hematoxylin, with eosin and lithium carmine, 21 days later. The number of labeled and dividing cells were counted in the specimens among 5000 cells of the stratum germinativum of the epithelium and the number of labeled fibroblasts was counted among 3000 young connective tissue cells. The fibroblasts were counted only in areas of their greatest concentration. The index of labeled nuclei (ILN) was expressed as a percentage and the mitotic index (MI) per thousand cells. Statistical analysis of the numerical results was carried out by the Fisher–Student method.

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TABLE 1. Percentage of DNA-Synthesizing Cells in Epithelium and Dermis of the Skin of Hairless Mice at Various Times after Operation (M ± m)

Time after operation (in days)	Object studied	ILN of cells in skin areas investigated (in percent)					
		wound	zone 1	zone 2	zone 3	zone 4	zone 5
0	E	3,1±0,2	—	—	—	—	—
	F	0	—	—	—	—	—
7	E	—	32,2±2,1	15,9±2,4	6,5±1,3	4,4±0,7	2,8±0,8
	F	26,6±1,8	13,7±0,4	2,8±0,5	—	—	—
14	E	18,6±1,7	16,6±0,9	13,0±1,1	11,2±1,4	6,1±0,9	6,2±0,9
	F	6,5±0,4	5,2±0,5	2,3±0,7	1,1±0,2	—	—
21	E	11,3±1,4	8,0±1,1	7,4±0,9	7,6±0,8	7,7±1,1	8,2±1,2
	F	1,8±0,5	1,1±0,2	—	—	—	—
60	E	5,1±0,7	5,1±0,4	5,4±1,1	5,1±0,9	5,0±0,6	6,8±0,5
	F	<1	—	—	—	—	—

Legend: E) epithelium; F) fibroblasts.

TABLE 2. Mitotic Activity of Epithelium of Skin of Hairless Mice at Various Times after Operation (M ± m)

Time after operation (in days)	MI of epithelium in skin areas investigated					
	wound	zone 1	zone 2	zone 3	zone 4	zone 5
0	1,2±0,2	—	—	—	—	—
7	—	10,6±0,8	5,7±0,9	3,1±0,7	2,8±0,4	2,5±0,9
14	5,7±0,9	7,5±0,8	7,8±0,8	7,0±0,7	4,2±0,4	3,9±0,8
21	4,6±0,9	3,7±0,6	5,7±0,6	6,5±1,1	4,7±0,8	3,4±0,5
60	2,2±0,4	4,4±1,2	4,8±0,7	4,8±0,6	4,4±0,9	5,7±0,9

EXPERIMENTAL RESULTS

The experimental results are given in Tables 1 and 2. On the 7th day after the operation a sharp increase in ILN was found in the epithelium of the skin around the wound (Figs. 1 and 2). In zone 1, immediately next to the wound edge, ILN was 10 times higher than in intact skin, namely 32.2 ± 2.1%, whereas in zone 2 it was increased to 15.9 ± 2.4%. As the distance from the wound edge increased, the increase in the number of DNA-synthesizing cells became smaller. MI also was high in zone 1 (10.6 ± 0.8‰), i.e., 8 times higher than initially.

On the 7th day the wound was filled with granulation tissue, mostly consisting of fibroblasts. Many labeled fibroblasts were found, chiefly in the uppermost layers of the young connective tissue (Fig. 3). In some parts they were much less frequent. Occasionally, in addition, nuclei of the endothelium of the young blood vessels also were labeled. ILM for the fibroblasts in the 7-day wound was 26.6 ± 1.8%. Labeled fibroblasts also were found in the dermis of the skin next to the wound edge. In this case ILM was 13.7 ± 0.4%.

In most of the mice 14 days after the operation epithelization was not yet complete. ILN of the cells of the regenerating epithelium was high. DNA synthesis in the skin around the wound at this period was increased at all distances studied. In the first 3 zones ILN was 4-5 times higher than in the intact epithelium, and in the most distant part from the edge of the wound it was twice as high.

MI of the regenerating epithelium was 4.5 times higher than initially, whereas in the skin around the wound it was 5-6 times higher.

The number of cells in the connective tissue filling the wound was reduced and the quantity of fibrous structures was increased. However, there was a large concentration of fibroblasts beneath the scab and under the regenerating epithelium. ILN was 6.5 ± 0.4% in the wound and 5.2 ± 0.5% at the wound edge; away from the wound individual labeled fibroblasts were seen mainly in the lower third of the dermis.

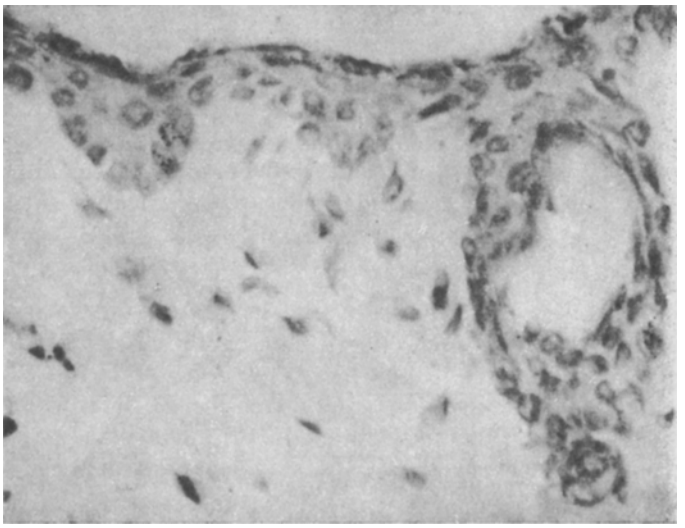

Fig. 1. Labeled cells in epithelium of intact mice. Hematoxylin-eosin, 600×.

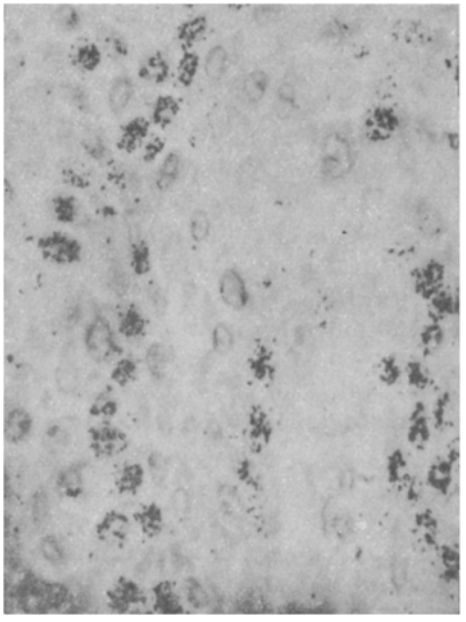

Fig. 2

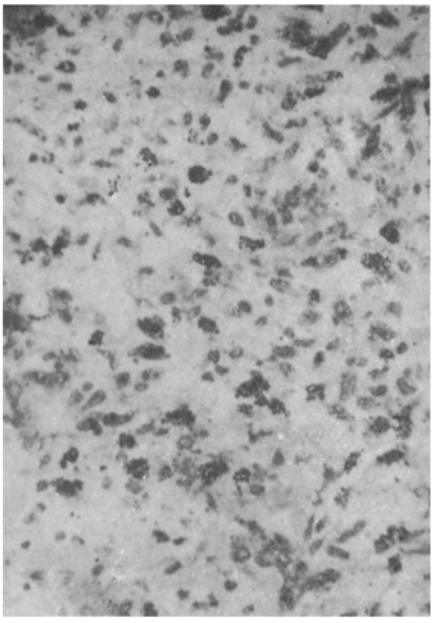

Fig. 3

Fig. 2. Labeled cells in epithelium of wound edge (zone 1) 7 days after operation. Hematoxylin-eosin, 600×.

Fig. 3. Labeled fibroblasts of young connective tissue in wound 7 days after operation. Hematoxylin-eosin, 400×.

All the wounds were completely epithelized 21 days after the operation and the young connective tissue was converted into scar tissue. The number of cells synthesizing DNA in the epitheluim was rather less than in the 14-day wound, but it was still 2-3 times higher than in the intact epithelium. In the part furthest from the wound edge ILN was not reduced but, instead, it was slightly increased to $8.2 \pm 1.2\%$. This evidently shows that the increase in DNA synthesis spread to areas of skin more than 8 mm away from the wound margin (the distance chosen for investigation).

Individual labeled fibroblasts could be found in the connective tissue, chiefly in its lower layers.

On the 60th day after the operation the activity of DNA synthesis was considerably reduced but, as before, it remained above the control level. ILN for cells of the regenerating epithelium was 5.1 ± 0.7%, i.e, 1.5 times higher than in intact epithelium.

In the epithelium of the skin around the wound ILN was identical in all zones studied and was 1.5 times higher than ILN for the cells in the intact skin. MI in the regenerating epithelium was now 2.2 ± 0.4‰, whereas in the skin around the wound, in all five zones studied it was only very slightly smaller than previously, with a mean value of 4.8‰. Labeled fibroblasts were found only occasionally in the connective tissue 60 days after the operation.

Consequently, during regeneration of the skin there is a prolonged increase in the intensity of DNA synthesis in the epithelium, not only in the wound itself, but also in the skin around the wound for a considerable distance. During regeneration the number of grains of silver in the labeled epithelial cells was much greater than their number in the epithelium of the intact zone, indicating an increase in the intensity of DNA synthesis. It is essential to note that an increase in DNA synthesis was found not only in the epithelium, but also in the fibroblasts of the wound and of the adjacent dermis.

Activity of DNA synthesis in the connective-tissue cells, which initially was high, fell rapidly as the granulation tissue matured.

The increase in the intensity of DNA synthesis coupled with proliferative changes thus take place in the skin surrounding the wound also, both in the epithelium and in the dermis.

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