PROLIFERATIVE ACTIVITY OF REGENERATING SKIN IN CHRONIC HYPOXIA

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DNA synthesis in different regions of regenerating skin is inhibited during chronic exposure to hypoxia. The decrease in the number of cells in the S-period of the mitotic cycle is evidence of inhibition of proliferation of the epithelial and connective-tissue cells, and this accounts for the longer time required for the healing of wounds in chronic oxygen deficiency.

KEY WORDS: regeneration of the skin; hypoxia; proliferation.

The slower rate of post-traumatic regeneration of the skin during chronic hypoxia has been demonstrated by several investigators [2, 3, 7, 9]. The harmful effect of hypoxia is brought about by activation of the lysosome system, the enzymes of which destroy proteins and nucleic acids forming the cell structures [8]. Meanwhile proliferation and differentiation of the tissues after trauma are associated with increased synthesis of these substances [10]. The investigation of the intensity of these structural processes is thus of great importance to the analysis of the causes of the disturbance of reparative regeneration of the skin during hypoxia.

The object of this investigation was to study DNA synthesis in experimental wounds during chronic hypoxia.

EXPERIMENTAL METHOD

Sixty male albino rats were used. To produce intermittent hypoxia the animals were kept in a pressure chamber under the pressure of 250 mm Hg for 8-10 h daily throughout the period of observation (15 days). Full-thickness skin grafts, 225 mm^2 in area, were taken from the left lateral surface of the trunk. During healing the area of the tissue defect was measured; material was taken by biopsy from the wound edges after 5, 10, and 15 days and sections were stained with hematoxylin and eosin. Thymidine (Soviet manufacture) with a specific activity of 1.4 Ci/mmole, was used as the DNA precursor. The isotope was injected intraperitoneally in a dose of 0.5 μCi/g body weight, 5 and 10 days after the operation and 3, 6, and 24 h before decapitation. Autoradiographs were prepared by the NIKFI method (1959) using type R emulsion. The index of labeled cells was calculated in per cent in different zones of the regenerating skin.

EXPERIMENTAL RESULTS

Complete healing of the defects took place in the control animals 14-15 days after trauma (Fig. 1a).

Under chronic hypoxic conditions repair was delayed: the inflammatory response was inhibited and granulation formation and epithelization were retarded. A well-developed layer of leukocytes and necrotic tissue remained in the center of the wound for a long time. Granulation tissue was formed as foci between the cells of the adipose tissue and it contained solitary histiocytes and fibroblasts. The granulations showed features of plasmorrhagia, hemorrhages, and destruction of the cells, usually manifested as pycnosis of the nuclei. The blood vessels were well developed, extremely dilated, and engorged with red cells; their walls were thin and their continuity frequently disturbed. A layer of horizontally arranged fibroblasts formed slowly, and contraction of the wound edges was thus delayed. Fiber formation was inhibited and some of the collagen fibers were fragmented. Hypertrophy of the boundary zone of the epithelium was slight. The

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TABLE 1. Percentage of Labeled Epithelial and Connective-Tissue Cells (24 h after injection of thymidine-H^3)

Group of animals	Epithelial wedge	Boundary zone of epithelium	Intact epidermis	Young connective tissue	Granulation tissue
5 days after operation (M ± m)					
Control	5,9±0,38	24,5±1,28	14,2±0,47	26,2±0,82	18,0±,051
Experimental	4,8±0,44	13,0±0,83	8,8±0,65	—	12,1±0,75
P	>0,05	<0,05	<0,05		<0,05
10 days after operation (M ± m)					
Control	6,2±0,58	24,2±1,01	14,4±1,04	15,7±0,87	16,5±0,34
Experimental	5,3±0,63	17,6±0,89	10,8±0,51	12,8±0,49	13,3±0,47
P	>0,05	<0,05	<0,05	<0,05	<0,05

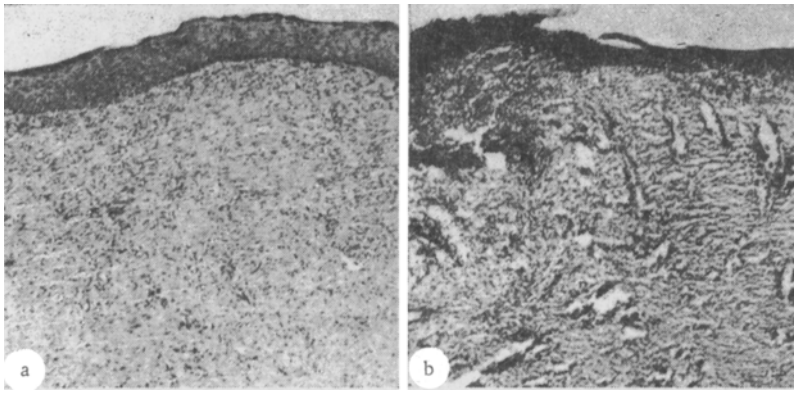

Fig. 1. General view of region of injury 15 days after operation: a) control; b) chronic hypoxia. Hematoxylin–eosin, 120×.

thin layer of the generating epithelium developed slowly, and in the early stages marked destructive changes were present in the epithelial cells, as reflected by perinuclear edema and pycnosis of the nuclei. The area of the wounds of the experimental animals decreased slowly and by the end of the period of observation (15 days) there was still a large (46 mm^2) area of wound surface still present beneath the scab (Fig. 1b).

The study of the proliferative activity of the tissues after 5 days of regeneration in the control animals showed that the labeling index was highest 24 h after injection of the isotope (Table 1). The largest number of epithelial cells incorporating thymidine-H^3 was found in the stratum basale and stratum spinosum of the boundary zone of the epidermis (Fig. 2a). This fact indicates that the intensity of cell proliferation in this zone determined the rate of epithelization of the region of injury. Solitary cells synthesizing DNA were found in the regenerating epithelium; they were absent in the region of the free edge of the epithelium, and their number gradually increased as the boundary zone of the epidermis was approached. The intact epidermis surrounding the wound played an active part in the regeneration processes. Many labeled cells were found in areas of this tissue lying 1–1.5 mm away from the wound edge. Counting the number of labeled connective-tissue cells showed that they were rather more numerous in the young connective tissue covered by regenerating epithelium than in the granulation tissue. In the newly formed connective tissue thymidine-H^3 was incorporated by fibroblasts and endothelial cells, in the granulation tissue adventitial cells and, much less frequently, polyblasts were labeled as well as fibroblasts, and in the uppermost layers of the granulations lymphocytes were labeled (Fig. 2b).

Under conditions of chronic hypoxia the cells in all parts of the regenerating skin were less able to incorporate thymidine-H^3. The inhibitory action of hypoxia on the intensity of DNA synthesis was most marked in the boundary zone of the epidermis, where the number of labeled cells was reduced by almost half compared with the control (Fig. 2c). Mitoses were rare. The first labeled mitoses did not appear until 6 h after injection of the thymidine-H^3, whereas in the control they were found after 3 h. No unlabeled mitoses were observed after 24 h. With a sharp decrease in the number of DNA-synthesizing cells in the

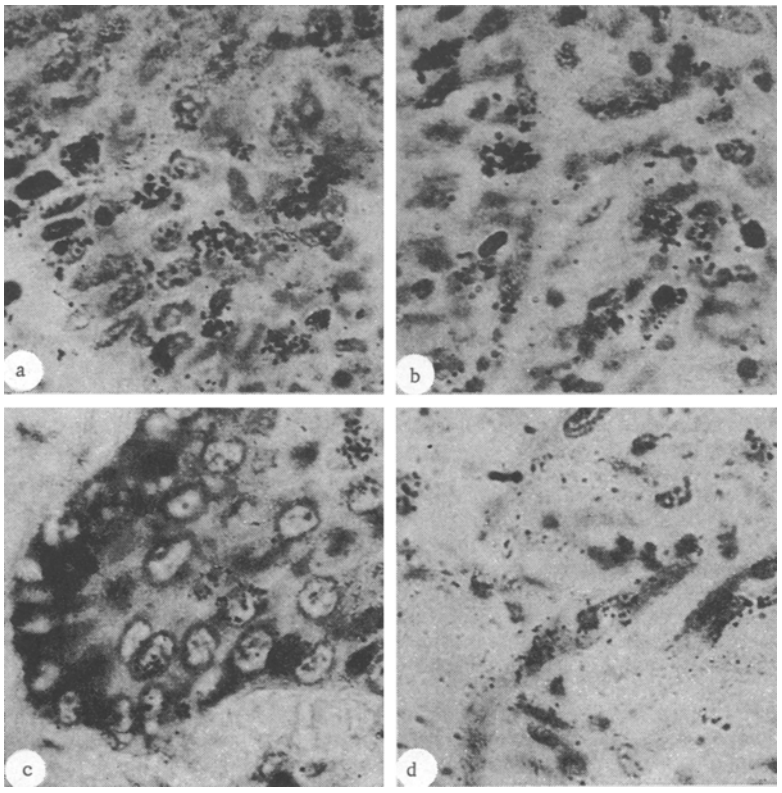

Fig. 2. Incorporation of thymidine-H^3 into cell nuclei of boundary zone of epithelium (a, c) and granulation tissue (b, d); 5 days after operation (24 h after injection of isotope): a, b) control; c,d) chronic hypoxia. Hematoxylin, 1200 ×.

boundary zone of the epidermis, regeneration of the epithelium took place more slowly. The proliferative activity of the connective-tissue cells was much lower than at the same time in the control series; of the cells incorporating thymidine in the granulation tissue most were adventitial cells along the course of the blood vessels, together with polyblasts and lymphocytes (Fig. 2d).

The pattern of distribution of the label discovered after 5 days was still observed in the control animals 10 days after the operation. The percentage of labeled cells in the boundary zone of the epidermis remained high, as before, as a result of the continuing intensive epithelization of the wound surface. By contrast, proliferation of the connective tissue at this period was reduced as a result of its differentiation and maturation; the number of labeled cells in the granulation tissue was only a little higher than their number in the newly formed connective tissue.

The number of DNA-synthesizing cells in the boundary zone and in the intact epidermis of the experimental animals at this period was a little higher than at the previous period, but it still remained much lower than in the control. In the region of regenerating epithelium the decrease in the number of DNA-synthesizing cells was not significant. This autonomy of the free end of the epithelial wedge can be explained by a disturbance of its blood supply and innervation [5]. The number of labeled connective-tissue cells 10 days after the operation increased mainly on account of adventitial cells and endothelium; this could be connected with increased development of the blood vessels in the regenerating skin coupled with its deficient oxygen supply.

The inhibition of proliferation under hypoxic conditions may be associated not only with the deficiency of energy supplied by anabolic processes, but also with the excessive discharge of glucocorticoids into the blood stream [1, 4, 11, 12]; this leads to an increase in the duration of all phases of the cell cycle and, in particular, it blocks the passage of the cells from the postmitotic phase to the phase of DNA synthesis [6, 9].

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