TIME COURSE OF MORPHOLOGICAL CHANGES IN BURNS DURING TREATMENT

IN A MONITORED ABACTERIAL ENVIRONMENT

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UDC 716-001.17-003.9-02:614.48

KEY WORDS: wound healing; thermal burns; granulations; abacterial environment.

The treatment of burns is an urgent problem in modern surgery. The most widespread method of treatment, under a dressing, in the case of deep and extensive burns often does not guarantee the conditions for early autoplastic closure of the wounds. Nowadays modern methods of open treatment in a monitored abacterial environment are beginning to be introduced on a wide scale into practice in the USSR and elsewhere.

It was therefore deemed interesting to study the time course of healing of burn wounds in biopsy material, depending on the method of treatment used (beneath dressings or the open method).

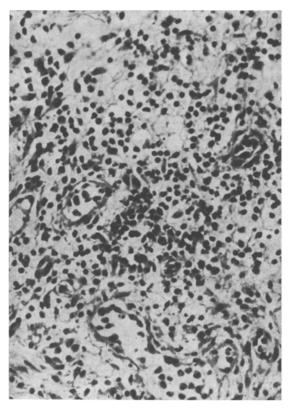
EXPERIMENTAL METHOD

The morphology of wound healing in a monitored abacterial environment was studied in 30 patients with III-IV degree burns covering between 25 and 50% of the body surface. Most of the area of deep burn wounds was covered at operations within 15 and 20 days after trauma. Biopsy material was taken in the course of 5-10 days after the beginning of treatment. The control group consisted of 10 patients of the same age group with burns of identical depth and area, treated by application of dressings. The time taken to cleanse the burn wounds in these patients by necrectomy in stages was 25-32 days. Biopsy was performed between the 12th and 30th days after the beginning of treatment. Material was fixed in 10% neutral formalin solution and in absolute alcohol and embedded in paraffin wax. Some material was used to cut frozen sections. Sections were stained with hematoxylin and eosin, with picrofuchsin by van Gieson's method, and the gram positive flora was determined by Weigert's method. Activity of alkaline and acid phosphatases (by Gomori's and the azo-coupling methods) and of ATPase (after Padykula and German) was determined in frozen sections. Glycosaminoglycans was determined by staining with alcian blue and toluidine blue. Ribonucleoproteins (RNP) were determined by Brachet's method and glycogen by the PAS reaction.

EXPERIMENTAL RESULTS

In the control group granulations were observed on the 12th day in only seven patients, and three patients had no granulations. The granulation tissue was covered by a scab, and in some parts of the wound the scab had separated. Colonies of microorganisms were visible in the scab and were diffusely distributed in its substance. The necrotic region was separated from the underlying tissues by a well-developed demarcation barrier, the leukocytes in which were rich in glycogen and contained high alkaline phosphatase activity. The granulation tissue at these times of treatment contained masses of amorphous material with many cells, among which lymphoid and plasma cells predominated. There were few capillaries in the granulations and only some of them contained neutrophils (Fig. 1). Neutrophils in the amorphous substance of the granulation tissue were single, with an ill-defined granular structure and a rod-shaped nucleus. Fusiform fibroblasts with a thickened nucleus and a narrow rim of cytoplasm also were distributed singly. No RNP could be seen in these fibroblasts. These were evidently degenerating fibroblasts, incapable of synthesizing collagen. However, bands of collagen fibers were just perceptible in the substance of the granulation tissue.

All-Union Burns Center and Laboratory of Histochemistry and Autoradiography, 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 M. I. Kuzin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 4, pp. 108-111, April, 1982. Original article submitted December 22, 1981.



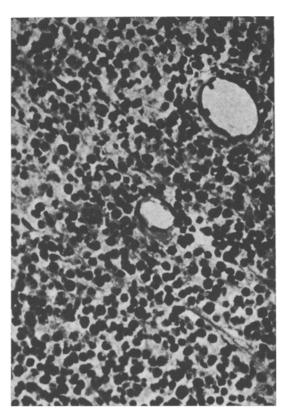


Fig. 1 Fig. 2

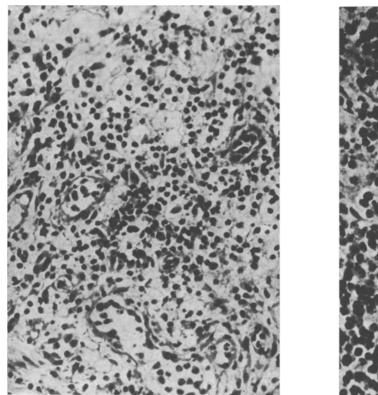
Fig. 1. Granulation tissue of burn wound on 12th day of treatment under dressings. Many lymphoid cells in substance of granulation. Deformed vessels with neutrophils in their lumen. Here and in Figs. 2 and 3: hematoxylin—cosin, $300 \times$.

Fig. 2. Considerable concentrations of plasma cells in granulation tissue of burn wound on 30th day of treatment beneath dressings.

On the 19th-20th days after trauma separation of the scab was observed or it was removed at operation. In this case the wound surface was covered with a thick layer of finely granular debris, beneath which was the fatty areolar tissue with islets of granulation tissue. Areas of necrosis with a mild macrophagal reaction around them could often be seen in the latter. The macrophages were small, with low acid phosphatase activity, and there were no glycogen grains in their cytoplasm. These cells can be classified as weakly phagocytic macrophages.

In seven patients treated by the closed method skin autografting was carried out on the 25th-30th days after trauma, during treatment under dressings. In biopsy material taken before skin autografting the whole wound surface was occupied by granulation tissue, with vertically running vessels and horizontally oriented fibroblasts concentrated in it. A noteworthy feature was the large number of plasma cells and collagen fibers in the substance of the granulations. Plasma cells formed large concentrations near the vessels (Fig. 2).

During treatment by the open method in an abacterial environment, on the 5th-6th day after the beginning of treatment finely granular bright red granulations rich in capillaries and cells (neutrophils, macrophages, fibroblasts) appeared beneath the mummified scab. The scab consisted of coagulated collagen fibers, among which there were small colonies of microorganisms, apparently immured in the substance of the collagen. On the 6th-9th day after trauma the burn scab was removed by operation, in half of the cases by surgical and in the rest by chemical necrectomy. On the 2nd day after removal of the scab many newly formed capillaries could be seen in the granulation tissue; the endothelium of the capillaries was swollen and contained high ATPase activity. Near the surface of the granulations the capillaries formed loops. Their lumen was dilated and filled with neutrophils; many neutrophils also could be seen in the stroma of the granulation tissue (Fig. 3). The neutrophils had segmented nuclei in which fine grains of glycogen could be clearly distinguished (Fig. 4).



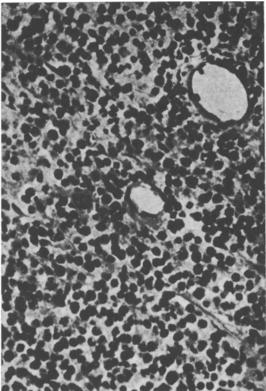


Fig. 3 Fig. 4

Fig. 3. Granulation tissue of burn wound rich in blood vessels and cells on 8th day after treatment in a monitored abacterial environment.

Fig. 4. Large quantity of glycogen in neutrophilic leukocytes of granulation tissue on 8th day after treatment in monitored abacterial environment. PAS reaction, $320 \times .$

These are what are called mature neutrophils, with high phagocytic activity. They phagocytose bacteria, lyse nonviable tissues, and secrete mediators of inflammation [4]. Migration of neutrophils facilitates fibroblast proliferation in the region of injury. Fibroblasts were concentrated mainly near the vessels; sometimes they could be seen to be budding from the vessel wall. The cells were elongated in shape, with round or oval nuclei and basophilic RNP-rich cytoplasm, i.e., in their histological and histochemical features they resembled the so-called active fibroblasts, capable of intensive collagen synthesis [1, 3, 6]. In fact, a dense network of collagen fibers was found near the vessels. Signs of high functional activity also were found in the macrophages, which were considerably enlarged, and which had high acid phosphatase activity in their cytoplasm. Phagocytosed particles could be seen in some macrophages.

In biopsy material taken 8-10 days after the beginning of treatment in a monitored abacterial environment, development of granulation tissue had proceeded further. The number of fibroblasts was increased, and in the depth of the wound they occupied a horizontal position. In the layer of horizontally oriented fibroblasts bands of thick collagen fibers were concentrated. Many polymorphs and macrophages, with high phagocytic activity, were still present in the superficial layers of the granulation tissue. Meanwhile there were only single plasma cells in the substance of the granulations. This completes the description of the microscopic structure of granulation tissue in a burn wound treated in an abacterial environment before skin autografting.

This morphological and histochemical investigation of biopsy material from the region of burn wounds during treatment under dressings and in a monitored abacterial environment thus revealed a significant difference in the character of healing of these wounds treated by these methods. Granulations formed under dressings, especially in the early stages of treatment (12-14 days), greatly resembled the granulation tissue formed during the slow healing of gunshot

wounds and indolent trophic ulcers [1, 2]. A common feature of all these wounds was the presence of many plasma cells in their tissues. Their large number in granulation tissue formed under dressings is undoubtedly attributed to the considerable microbial contamination of these wounds. Plasma cells are carriers of local immunity, and their presence in large numbers in granulations of wounds treated under dressings often leads ultimately to the rejection of an autograft. Granulations formed in a burn wound during treatment in an abacterial environment are rich in blood vessels and cells even in the early stages of treatment (5th-6th day) and they resemble the granulation tissue formed during healing of surgical wounds. The low bacterial contamination of such wounds and the mild degree of inflammation lead to the presence of a small number of plasma cells in the granulations, and these provide the best conditions for survival of an autograft. Healing of burn wounds treated in a monitored abacterial environment occurred 10-12 days sooner than that of burns treated under dressings. A sterile airflow, drying the scab, evidently inhibits the microflora, reduces the degree of absorption of breakdown products of the tissues of burn necrosis into the body, reduces the severity of the toxic manifestations, and thereby stimulates the processes of cleansing and regeneration of the wound.

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A MORPHOMETRIC TECHNIQUE FOR MEASURING DEFECTS OF THE GASTRIC

AND INTESTINAL WALL

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UDC 616.33.342-002.44:616-018.001.8

KEY WORDS: technique; morphometry; defects; stomach; intestine.

Morphometric methods are used comparatively rarely in histological investigations of the healing of wounds, ulcers, and other injuries to the wall of the stomach and intestine. Usually, after a very detailed description of the process of tissue reconstruction in the region of the defect, the authors concerned mention only the times of separation of necrotic masses, proliferation of connective tissue, epithelization, and the appearance of the first newly formed glands, villi, and so on [1-4]. Other workers, who have concentrated their attention on proliferation in the tissues at the edges of the defect, mention only values of the index of labeled nuclei or the mitotic index [5-7]. Yet the simultaneous measurement of several general parameters of the defect in histological preparations would allow a more integrated idea to be obtained of structural changes in the region of injury to the stomach or intestine, and this is particularly important when studying the dynamics of healing of defects and assessing the efficacy of different methods used to correct them. This was the aim of the present investigation.

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