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EFFECT OF TREATMENT IN A CONTROLLED GERM-FREE ENVIRONMENT ON MORPHOLOGY OF HEALING OF EXTENSIVE SUPPURATING WOUNDS

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A new open method of treatment of suppurating wounds in a controlled germ-free environment (GFE) is now being successfully developed. The method of treatment in a GFE, described by the writers previously [2], includes the following stages: 1) active surgical treatment of the purulent focus; 2) placing the affected part of the body in a plastic isolator with GFE until healing of the wound is complete; 3) intensive pre- and postoperative treatment.

Under the influence of the microclimate in the isolator the wound surface quickly becomes covered with a loose scab of dried exudate. Both the wound exudate and the surface areas of the tissues are exposed to the drying action of a current of air. During the first 24 h of treatment, the classical signs of inflammation regress, and by the end of the 2nd and beginning of the 3rd day, granulation tissue can be detected macroscopically in the wound, covering the wound defect whatever its area by the 6th-7th days. The number of microorganisms in the wound decreases from 10^8-10^9 to 10^2-10^3/g tissue, or the wound becomes sterile. In other words, the clinical course of wound healing during treatment in a GFE is speeded up. It was decided to confirm the clinical features of the course of wound healing by the results of morphological studies of healing of extensive suppurating wounds during treatment in a GFE.

EXPERIMENTAL METHOD

A morphological study was made of wound healing in a GFE by histochemical and cytological investigation of biopsy specimens from an extensive suppurating wound and of squash preparations from wounds.

The duration of treatment of 25 patients with extensive (from 1000 to 1500 cm^2 in area) suppurating wounds in an isolator with GFE was 17-26 days. Biopsy material was studied before treatment and after treatment in the GFE for 1, 3, 5-7, and 10-15 days. Material was fixed in 10% neutral formalin, absolute alcohol, and Carnoy's fluid and embedded in paraffin wax. Sections were stained with hematoxylin and eosin and by Van Gieson's method and the following histochemical tests were carried out: for glycogen and neutral polysaccharide (PAS reaction), for glycosaminoglycans (with alcian blue and toluidine blue), for RNP (by Brachet's method), for lipoids and lipids (by Goldman's method and with Sudan III). Acid phosphatase was determined by Gomori's method. Staining for microorganisms was carried out by the Gram–Weigert method. Squash preparations from wounds were stained by the Romanovsky–Giemsa method.

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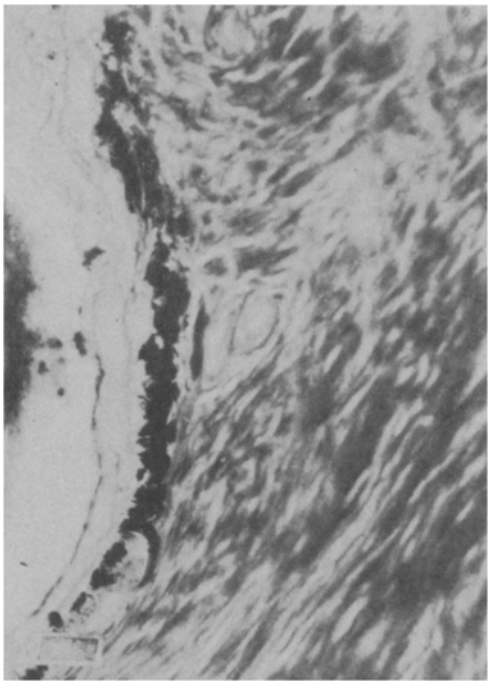

Fig. 1

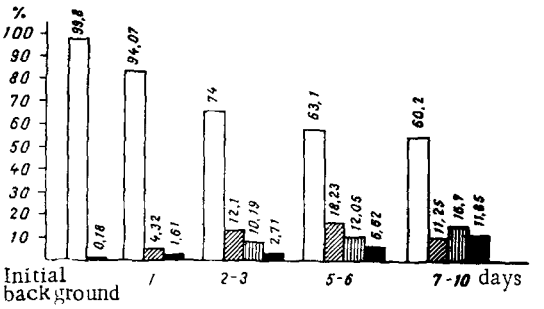

Fig. 2

Fig. 1. Histochemical picture of suppurating wound before treatment in GFE. Accumulation of Gram-positive microflora in necrotic surface layer of wound. Hematoxylin-eosin, 160 ×.

Fig. 2. Changes in cell counts of extensive suppurating wounds during treatment in GFE. Abscissa, duration of treatment in GFE (in days); ordinate, cell composition of wound exudate (in %). Unshaded columns represent polymorphonuclear neutrophils, obliquely shaded columns undifferentiated polyblasts, vertically shaded columns macrophages, black columns pro- and fibroblasts.

EXPERIMENTAL RESULTS

The histological picture of biopsy material taken before treatment was typical of a slowly healing suppurating wound. In 20 cases masses of fibrin and leukocytes could be detected on the surface of the wound, with necrotic foci beneath them. In half of the cases many colonies of Gram-positive microorganisms were found on the surface of an extensive suppurating wound, covered with a film of fibrin (Fig. 1). In two cases the Gram-positive microflora infiltrated necrotic tissues in the deep layers of the wound. The necrotic layer was separated from underlying tissues by a well-developed demarcation barrier. Beneath the demarcation barrier there were bundles of thick collagen fibers, as a rule infiltrated with neutrophils. The neutrophils were distinguished by low alkaline phosphatase activity, the granules in their cytoplasm were poorly developed, and their nuclei were rod-shaped. In five cases the wound cavity was filled with edematous granulation tissue, containing many blood vessels and cells: stab cells, degenerating fibroblasts, plasma cells, lymphocytes, and macrophages. The macrophages were small in size, free from PAS-positive granules, and had low acid phosphatase activity. Such macrophages can be described as having low activity. The macrophages often formed lacunae and plasma cells were scattered near the vessels. Collagen fibers were almost completely absent in the edematous granulation tissue.

The predominant feature in the cytological picture of squash preparations from wounds before treatment in the GFE was polymorphonuclear neutrophils, many of which had degenerative changes (99.82%). The rest of the cells (0.18%) of the wound exudate were lymphocytes and monocytes (Fig. 2). The abundance of microflora, both intracellular and extracellular, in a state of abnormal and incomplete phagocytosis, was a regular feature of the cell counts made on patients with extensive and indolent suppurating wounds before treatment in the GFE. Before treatment, inhibition of physiological wound cleansing, regeneration, and epithelization was thus observed in the wound.

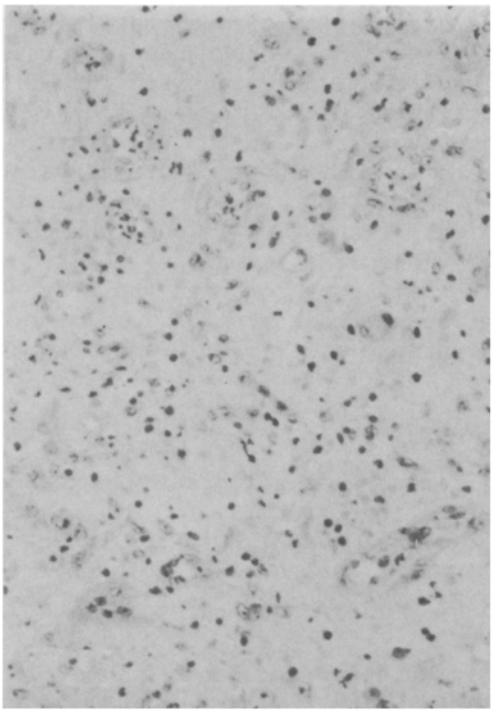

Fig. 3

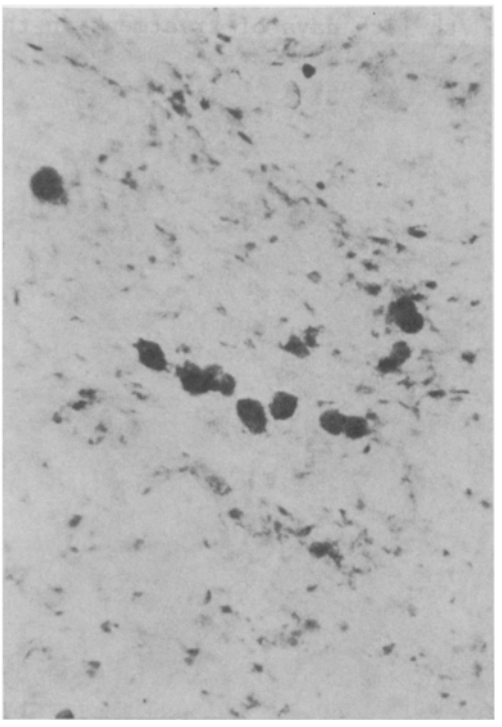

Fig. 4

Fig. 3. Young granulation tissue rich in blood vessels and cells on 5th day of treatment in GFE. Lumen of vessels filled with polymorphs. Hematoxylin–eosin, 260 ×.

Fig. 4. High acid phosphatase activity in macrophages of granulation tissue on 5th day of treatment in GFE. Reaction by Gomori's method, 260 ×.

During the first day of treatment in the GFE the wound surface became covered with a thin film of dried exudate, without scab formation. Histological study of the deep layers of the wound showed no significant changes compared with the initial histological picture. Marked regenerative processes were found in the wound tissues on the 3rd-5th day of treatment in the GFE. Fine granulation containing many capillaries and cells could be seen in the wound (Fig. 3). The capillary walls reacted strongly for ATPase and nuclei of the endothelial cells did so for RNP. Signs of leukostasis could be observed in the capillaries in the granulation tissue. Segmented neutrophils in the lumen of the vessels still contained granules consisting of glycogen. Single neutrophils could be seen in the stroma of the granulation tissue. Cells, mainly fibroblasts and macrophages, were concentrated around the vessels in granulation tissue. High alkaline phosphatase activity, characteristic of actively proliferating fibroblasts, capable of synthesizing collagen intensively [3, 5], was found in the cytoplasm of the fibroblasts [3, 5]. Most macrophages were in a state of increased phagocytic activity, they were large, but their outlines were indistinct. High acid phosphatase activity and an increase in PAS-positive granulation were found in the cell cytoplasm (Fig. 4). In foci of granulation tissue formed on the 3rd-5th days after treatment in the GFE, young fibroblasts and macrophages were thus the principal cells.

The cytological data confirmed the results of the morphological studies. On the 3rd-5th days of treatment in the GFE the cytological picture showed a decrease in the number of polymorphs from 74.0 ± 4.76% to 63.1 ± 4.35% ($P < 0.001$), and these cells were functionally active. Toward the end of the 3rd day an increase was seen in the number of polyblasts to 25%, with their differentiation toward active, mature macrophages (10.19 ± 1.94%; $P < 0.001$). In occasional fields of vision profibroblasts and fibroblasts appeared (up to 2.71 ± 1.22%). By the 5th day the number of mature macrophages increased to 12.05 ± 2.16% ($P < 0.001$) and the number of profibroblasts and fibroblasts to 6.62 ± 0.99% ($P < 0.02$). This cytological picture indicates active cleansing of the wound from necrotic tissues and pus and it signals the beginning of formation of young granulation tissue (the phase of regeneration).

On the 7th-10th days of treatment in the GFE mature granulation tissue containing many thin collagen fibers was formed. The number of vessels, on the other hand, decreased and the fibroblasts became horizontal in position. Some of them were converted into fibrocytes. Alkaline phosphatase activity in the cytoplasm of the fibroblasts remained high, whereas the intensity of the reaction for RNP showed some decrease. In the deep layers of granulation tissue circular structures were formed from collagen fibers. Their center was occupied by infiltrating lymphocytes and plasma cells. These areas of lymphocyte–plasma cell infiltration were rich in blood vessels with thin walls, and also in macrophages with high functional activity. The presence of infiltrating lymphocytes and plasma cells during healing of the wound is evidently a sign of growth of the immune response in the body [4]. Complete cleansing of the wound was marked by the formation of these foci of infiltration with lymphocytes and plasma cells, but the macrophagal response still continued at a high level.

Correspondingly, in the cytological study of squash preparations from wounds on the 7th-9th days of treatment in the GFE a further decrease was observed in the number of polymorphs (to 60.2 ± 4.21%; $P < 0.001$), the number of undifferentiated polyblasts decreased (to 11.25 ± 2.77%; $P < 0.05$), the number of macrophages continued to rise (to 16.7 ± 2.74%; $P < 0.001$), and the number of fibroblasts increased (to 11.85 ± 4.28%; $P < 0.02$). The cytological picture as described above also correlated with the results of histological investigations of biopsy material taken from granulations of extensive suppurating wounds, and indicated the formation of mature granulation tissue.

A distinguishing feature of the healing of extensive suppurating wounds in the GFE is thus the more rapid course of repair processes in the wound. The number of neutrophils, especially stab cells, decreases first of all and the number of mononuclear cells, especially macrophages, increases. Macrophages mature from small cells with low acid phosphatase activity into large cells with high activity of this enzyme. These mature macrophages have high phagocytic activity. It was concluded in [6, 7] that macrophages produce a substance which stimulates fibroblast proliferation. It has been suggested that this substance is a hormone or enzyme factor. In the present experiments intensive fibroblast proliferation correlated with the appearance of numerous mature macrophages. It is these cells which cleanse the wound. There is no doubt that intensive wound cleansing of this type is possible when the neutrophilic reaction in the wound is sharply depressed as a result of treatment in the GFE. Depression of the neutrophil response was accompanied by granulation tissue formation. These granulations, rich in blood vessels and cells (macrophages, fibroblasts), are very reminiscent of granulations formed during the healing of clean population wounds.

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