MORPHOLOGIC AND CYTOLOGICAL FEATURES OF HEALING OF BURNS TREATED BY DIFFERENT METHODS

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Burn wounds, even if resulting from comparatively small but deep burns, differ from ordinary incised or surgical wounds by causing severe degenerative changes in the internal organs, leading to the development of what may be called burn sickness. Burn toxins are one of the chief causes of toxic manifestations in the patient [1].

The method of treatment of burns in a controlled germfree environment, which has been used in recent years, has considerably reduced the toxic complication of burns. A sharp decline in exudation and loss of protein, a decrease in proteolytic enzyme activity in the wound, more rapid epithelization in the case of superficial burns, and in the case of deep burns, the possibility of earlier plastic repair of the burn wound have been found as a result [4, 5]. With the open method of treatment, regeneration takes place much more rapidly in the burn wound [3]. Regeneration of tissues in an extensive burn wound treated beneath dressings is delayed. Evidently during treatment in a controlled environment, changes take place in the cell composition of the tissues of the burn wound compared with those of wounds treated under dressings. Accordingly, it was decided to study the time course of cytological changes in burn wounds treated by open and closed methods, by examination of squash preparations, and to compare it with the morphological data, so that optimal times for skin autografting could be determined.

EXPERIMENTAL METHOD

The cytological picture of wound healing in a controlled germfree environment was studied in 25 patients with burns of the III-IV degree covering 10 to 25% of the body surface. Three-stage treatment was given in local isolators, the parameters of the sterile environment (temperature, pressure, and humidity) being changed in accordance with the following scheme: 1) drying of the burn wound until the formation of a scab on its surface (48 h after the beginning of treatment) followed by necrectomy and skin autografting (3-5 days after the beginning of treatment); 2) the period after performance of necrectomy with skin autografting with a split skin graft perforated 1:3 (5-7 days); 3) the period of rejection of the crust at the site of perforation of the autograft, starting with the 8th day after the beginning of treatment.

Squash preparations were taken for cytological study before treatment in the controlled environment and beneath dressings (patients of group 1), and also at the first and second stages of the scheme of treatment described above (3rd-4th and 5-th-7th days of treatment; patients of group 2). On the 5th-7th day of treatment biopsy specimens also were taken for histologic study of the burn wound. Group 3 (control) consisted of 15 patients with burns of identical area, treated by application of dressings for 26-32 days. At this stage the burn wounds were cleansed by necrectomy in stages. Squash preparations were obtained from the 3rd through the 32nd days after the beginning of treatment and biopsy specimens

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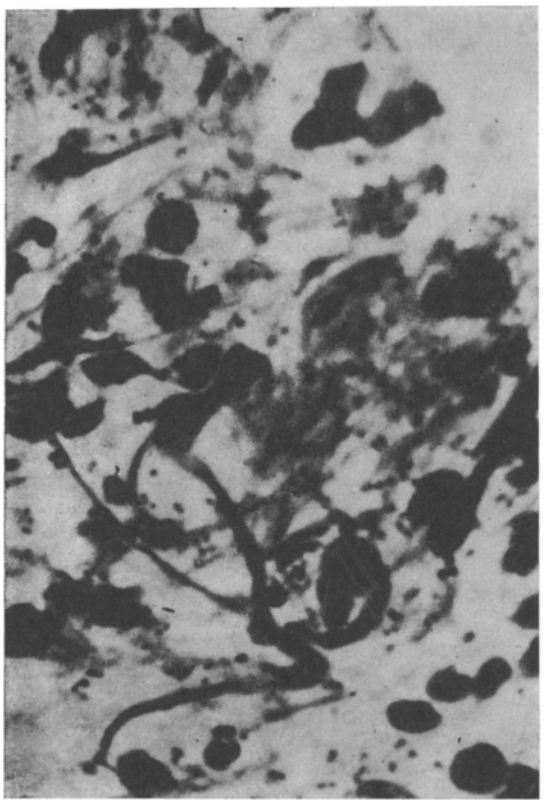

Fig. 1

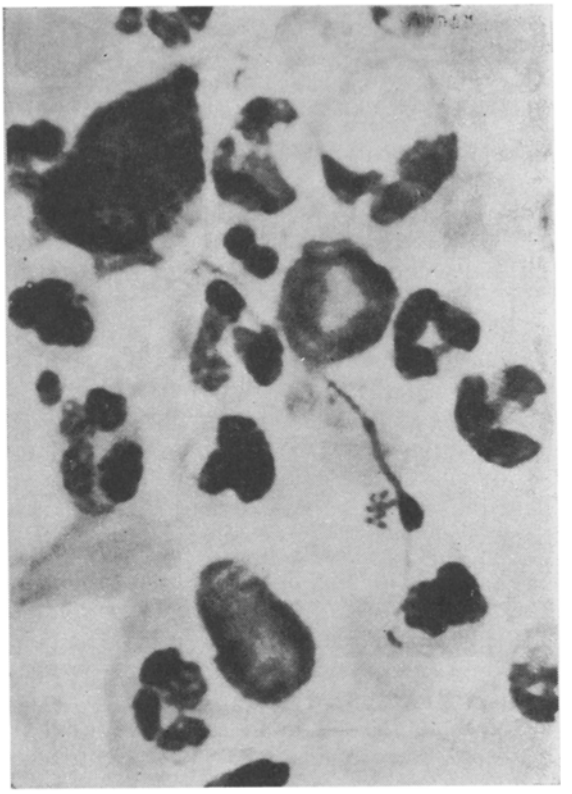

Fig. 2

Fig. 1. Initial cytological picture of wound exudate before treatment in controlled environment and beneath dressings. Greatly contracted and disintegrating neutrophils, abundant microflora. Here and in Figs. 2 and 4: Romanovsky's stain, 1200×.

Fig. 2. Squash preparation from wound surface on 3rd day of treatment in controlled environment: mature polymorphonuclear neutrophils and undifferentiated polyblasts in wound exudate, solitary groups of cocci.

between the 12th and 30th days after the beginning of treatment. The squash preparations were stained by the method of Pokrovskaya and Makarov (1942) using the Romanovsky–Giemsa strain. Biopsy specimens were fixed in 10% neutral formalin solution and in absolute alcohol and embedded in paraffin wax. Some sections were cut on a freezing microtome. Sections were stained with hematoxylin and eosin and with picrofuchsine by Van Gieson's method; the Gram-positive flora was identified by Weigert's method. Activity of alkaline and acid phosphatases (Gomori's and the azo-coupling method) and of ATPase (after Padykula and German) was demonstrated in frozen sections, RNA was determined by Brachet's method, and glycogen by the PAS reaction.

EXPERIMENTAL RESULTS

The cytological picture of the squash preparations from the wounds before treatment under dressings and in a germfree environment was similar. Neutrophils in different stages of degeneration and destruction, in the form of karyopycnosis, karyorhexis, and cytolysis, made up 98% of the tissue exudate. Mononuclear cells (lymphocytes, monocytes, undifferentiated polyblasts) accounted for the other 2% of cells. In most cases a well-marked microflora was discovered (mainly coccal) in a state of incomplete and distorted phagocytosis (Fig. 1). This cytological picture reflected depression of the protective cell reactions of the animal and absence of repair processes in the burn wound. The histologic picture of biopsy specimens taken before treatment also was identical. In 10 cases a scab formed on the wound surface. At the periphery of the scab was a region of moist necrosis; some neutrophils in this case infiltrated an extensive zone of the scab, insinuating themselves between the collagen fibers and causing their lysis. In the thickness of the scab colonies

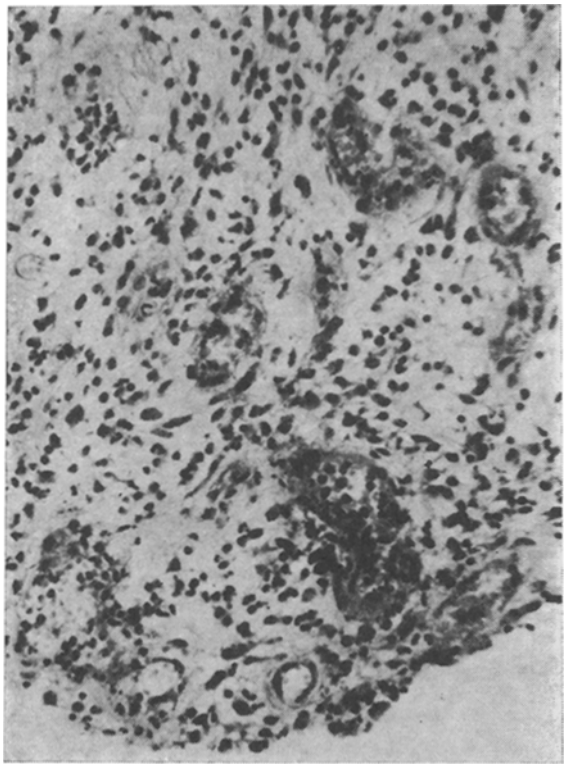

Fig. 3. Developed granulation tissue in cavity of burn wound on 7th day of treatment in controlled environment: Numerous fibroblasts and macrophages are concentrated in the substance of the granulations. Hematoxylin—eosin, 260×.

of microorganisms, chiefly coccal forms, were constantly observed. Beneath the focus of necrosis a leukocyte barrier was formed, and leukocytes infiltrated the muscle fibers. Viable regions of the wound consisted of edematous granulation tissue containing masses of amorphous material. This tissue contained deformed vessels with a few neutrophils in their lumen. Many degeneratively changed neutrophils, containing no glycogen granules, were discovered in the thickness of the granulation tissue, although lipoid granules, detectable by Goldman's stain, still remained even after destruction of the cells. Besides lysed neutrophils, many lymphoid and plasma cells were concentrated in the amorphous substance of the granulation tissue.

On the 3rd-4th day after treatment in a chamber with controlled germfree environment, cells indicating the beginning of regeneration of the tissues of the burn wound appeared in the wound exudate. These were polyblasts and active macrophages, which accounted for 27% of the total number of cells. In two patients, at these times, fibroblasts and squamous epithelium were found in squash preparations of the wounds. Among cells of the wound exudate there were many preserved polymorphonuclear neutrophils with distinct neutrophilic granules (Fig. 2). Degenerating neutrophils accounted for only 25% of the total number of cells.

In the second stage of treatment in a local isolator fine-grain granulations with many vessels and cells appeared in the region of the wound. Cells of young granulation tissue predominated in the wound exudate, chiefly large fibroblasts and macrophages (Fig. 3). A thin network of collagen fibers could be identified in the substance of the granulation tissue. Collections of polymorphonuclear neutrophils with distinct granules could be seen in the lumen of the vessels and in the surface layers of the granulation tissue. Glycogen granules were concentrated in their cytoplasm. Granulations of this kind are most favorable for skin autografting, and accordingly the third stage of treatment was later successfully carried out in local isolators, during which the transplanted skin autografts healed.

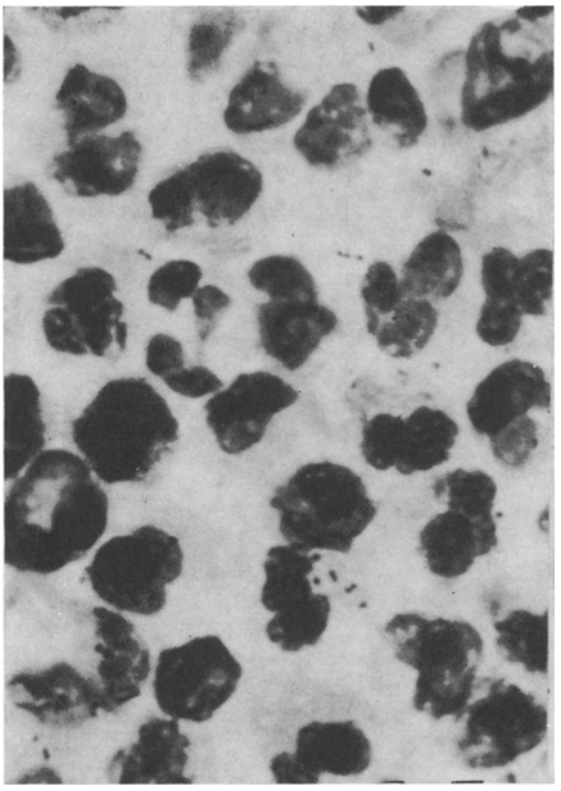

Fig. 4. Squash preparation from surface of burn wound on 14th day of treatment under dressings: degenerative changes in neutrophils, intracellular coccal microflora.

In patients treated under dressings the first signs of regeneration did not appear until the 6th-7th day, but degenerating neutrophils predominated; only a few intact polymorphonuclear leukocytes still remained (Fig. 4). The microflora after 14 days of treatment under dressings could not longer be detected cytologically. A period of wound cleansing began at that time. The number of undifferentiated polyblasts rose from 5 to 10% and the number of macrophages with low activity was under 5%. The undifferentiated polyblasts consisted of monocultures of cells a little larger than lymphocytes, with a round or oval palely stained nucleus, a delicate chromatin structure, and a narrow border of pale blue cytoplasm, with irregular outlines. Their cytoplasm was rich in RNA. Most cells of the wound exudate were neutrophils in a state of destruction. Neutrophils accounted for 85 to 95% of the total number of cells, i.e., the differential cell count at these times can be described as inflammatory. Not until 21-24 days after the beginning of treatment under dressings did granulation tissue cells containing many preserved, functionally active macrophages and differentiated fibroblasts appear in the wound exudate.

This cytological picture reflected a regenerative type of cell count. Meanwhile, the wound exudate contained many plasma cells — carriers of local immunity, which could lead to rejection of the autograft. Many plasma cells also were found in biopsy specimens taken before skin autografting, concentrated around the vessels.

The results of these cytological investigations can thus serve to predict the course of wound healing after burns. During treatment in a germfree environment, even in the first stage (3rd-4th day) wound cleansing is accompanied by the appearance of preserved polymorphonuclear neutrophils with distinct neutrophilic granules. The appearance of polymorphonuclear neutrophils in the wound exudate is an indication of the beginning of regeneration of the burn wound tissues and is accompanied by the appearance of functionally active macrophages and polyblasts. According to results of recent investigations, polymorphonuclear leukocytes saturated with neutrophilic granules synthesize RNA intensively and possess high phagocytic activity [2]. They also bring about rapid cleansing of the burn wound and, in the second stage of treatment, they form mature granulation tissue. The cleansing process in the burn

wound during treatment under dressings is sharply retarded and not until 21-24 days after the beginning of treatment do polymorphonuclear neutrophils predominate in the wound exudate. Correspondingly, skin autografting is carried out at a later stage than in patients treated in a germfree environment.

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METHODS

QUANTITATIVE ESTIMATION OF AORTIC WALL PERMEABILITY

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KEY WORDS: permeability; aorta; fluorescence microscopy; photometry; experimental hypertension and atherosclerosis.

Permeability of the walls of large blood vessels, including the aorta, is studied experimentally on quite a wide scale in models of various pathological states, notably atherosclerosis, hypertension, and so on [3, 5, 10-12]. Substances labeled with radioactive isotopes, or vital dyes are used for this purpose [2, 6, 8, 9]. Adams and Bayliss [4] proposed and tested a fluorescence-microscopic method of studying permeability of the aortic wall, using the dye trypan blue. The method is based on the ability of trypan blue to give red fluorescence in monochromatic green light (570 nm). When injected into the blood stream of an animal the dye forms stable complexes with plasma albumin, and in the composition of these complexes it penetrates into the vascular wall. Entry of the dye into the aortic wall is judged from the intensity and distribution of fluorescence, and on that basis the permeability of its membranes is determined. The state of permeability is assessed on the basis of a purely descriptive characteristic of luminescence in the aortic wall.

The object of this investigation was to develop a method of quantitative estimation of permeability of the aortic wall using the dye trypan blue. The technique included photometry and a method of quantitative analysis of scanograms obtained by means of it, developed by the present writers. Minor changes were introduced into the method of preparing the material for investigation as suggested by Adams and Bayliss [4].

EXPERIMENTAL METHOD

Chinchilla rabbits were used. Trypan blue was injected into the marginal vein of the animals' ear in a dose of 20 mg/kg body weight as a 1% solution made up in physiological saline. The animals were killed by air embolism 90 min after injection of the dye. The aorta was removed, washed with ice-cold physiological saline, and freed from loose connective tissue. Tubular specimens about 5 mm high were cut from different parts of the vessel.

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