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# TREATMENT OF BURNS WITH CULTURED FIBROBLASTS

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**Key words:** fibroblast; culture; burns; transplantation.

An important problem in the treatment of burns is the search for effective methods of treatment of extensive burns, for which skin autografting is difficult because of a deficiency of the patient's skin. New opportunities in this field have been opened up by recent achievements in biotechnology, making the obtaining of skin substitutes a real possibility. There have been several independent reports of positive results of the use of skin substitutes based on cultures of epidermis [3-5, 8]. However, a long time (over 3 weeks) is needed to obtain the principal component of substitutes of this kind, namely a cultured layer of epidermis [5], and its use does not exclude the possible development of a graft rejection reaction [6]. Wound infection, which is the rule with extensive burns, greatly reduces the efficacy of skin substitutes [7]. The use of autologous epithelial cells for the production of cultured epidermal layers makes it virtually impossible to create "banks" of these substitutes.

For the reasons mentioned above, the search for new approaches to the solution of this problem is necessary. We therefore studied the possibility of using cultured fibroblasts for the treatment of extensive burns. By using a culture of fibroblasts to treat burns, we took into account the regulating effect of fibroblasts on wound healing [2] and also Sarkisov's hypothesis [1] of the polypotency of the properties of certain cells of mesenchymal origin and, in particular, the cells known as pericytes.

## EXPERIMENTAL METHOD

Fibroblasts obtained by culture were transplanted to 11 patients at the All-Union Burns Center. The area of the burn wounds varied from 30 to 75% of the body surface. No special preparation of the wounds was carried out before transplantation of fibroblasts. The treatment program was worked out in the department. In nine cases transplantation was carried out on burns of the IIIa-IIIb and IIIb degree, with fine pink granulations and with no evidence of suppuration, bleeding, edema, or hypergranulation, and in two cases on skin donation sites slow to heal. The area of the grafts varied from 28 to 280 $cm^2$. A graft of fibroblasts was applied to the wound at a distance of 0.5-1 cm from its edges, and the graft was then covered with petrolatum

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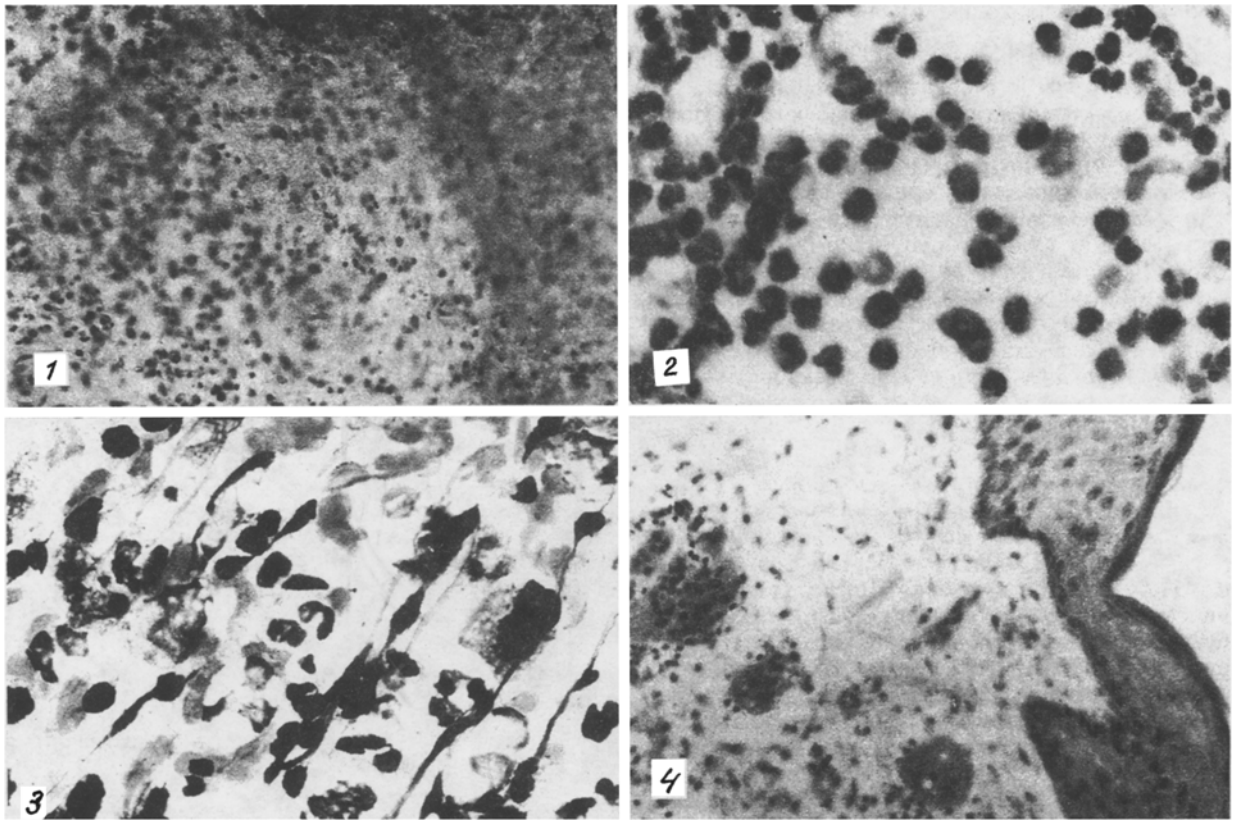

Fig. 1. Zone of necrosis and leukocytic infiltration in floor of a burn wound in patient of control group. Here and in Fig. 4: hematoxylin and eosin. 100×.

Fig. 2. Many polymorphs in control squash preparation from a burn wound. Here and in Fig. 3: azure-eosin. 600×.

Fig. 3. Many fibroblasts in squash preparation from wound 5 days after transplantation of fibroblasts.

Fig. 4. Stratified squamous keratinizing epithelium formed on burn wound 8 days after transplantation of fibroblasts.

gauze, which in turn was covered by a medicated dressing. The graft was examined during redressings, starting with the 3rd-4th day after transplantation and continuing until 8 months. The control of the efficacy of treatment consisted of 10 patients with burns similar in depth and area to those of the experimental group, and treated in the usual way, as well as burned areas on patients of the experimental group which were not covered with fibroblast grafts. The state of the wounds in all groups was assessed at intervals by morphological and cytological methods, including visible inspection of the wound, analysis of biopsy material, stained with hematoxylin and eosin, and a study of squash preparations from the wounds.

## EXPERIMENTAL RESULTS

The morphological picture of the burn wound and its duration. The wound surface consisted of fine red granulations. Microscopically, an outer zone of necrosis was clearly visible in the floor and at the edges of the wounds of the IIIa-IIIb and IIIb degree, beneath which lay a zone of granulation tissue the maturity of which depended on the stage of burn treatment (Fig. 1). In burns of the IIIa and IIIa-IIIb degree fragments of skin appendages were found among the granulations. These were not found in burns of the IIIb degree. Cells most frequently observed in squash preparations of wounds of the control group were neutro-

philic leukocytes and eosinophils, which accounted for 80-90% of the total. This reflected predominance of an inflammatory process with a marked allergic component in the wounds (Fig. 2).

Burns of the IIIb degree treated conservatively did not epithelize spontaneously, and plastic operations with split perforated grafts of autologous skin were an essential component of the treatment.

The use of fibroblast grafts for the treatment of burns gave variable results. The first group consisted of patients in whom transplantation of fibroblasts caused epithelization of the burn wound, the second group of patients in whom transplantation produced partial epithelization of the wound, and the third group of patients with no effect from transplantation.

Transplantation induced spontaneous healing of the burn wound in six of nine patients (66%). The area of the burn in these cases was 30-80%. In four patients of this group the burns had tissue damage of the IIIa-IIIb degree in depth, and only in one, of the IIIb degree. The area of the epithelized wounds was 28-280 $cm^2$. Epithelization of the wound in the patients of this group began 3 days after transplantation in the case of burns of the IIIa-IIIb degree, and 5 days after transplantation in burns of the IIIb degree. A thin semitransparent grayish film was formed on the wound surface by these times, and small round projections up to 1 cm in diameter appeared on the surface of a white focus. The epithelium at the wound edge acquired a festooned appearance, evidence of the beginning of marginal epithelization. In squash preparations taken from the wounds at these times the number of fibroblasts was greatly increased compared with the initial pattern and with the control: from 0-4 to 40% of the total number of cells. The number of eosinophils was sharply reduced, or they disappeared completely (Fig. 3). These changes pointed to a switch from inflammatory processes in the wound to regenerative. In the next 5-11 days the film covering the wound grew thicker and had the appearance of thin stratified keratinizing epithelium, in which the characteristic layers could be differentiated histologically (Fig. 4). Under these circumstances mature granulation tissue was formed beneath the epidermis. Epithelization of burns of the IIIa-IIIb degree took 8 ± 2 days irrespective of their area, and in the case of a burn of the IIIb degree, 18 days. Observations on the patients of this group for 8 months showed that in five patients areas of the wounds covered with the transplant were covered with stratified squamous keratinizing epithelium, and scar changes in this case were slight in degree. Only in one case was a graft of fibroblasts with an area of 28 $cm^2$ rejected on the 21st day after transplantation.

Transplantation of fibroblasts was successful in the treatment of skin donation sites which had not healed after more than 2 months. In the course of 7 days after transplantation of fibroblasts, parts of such wounds were epithelized. The course of healing of these skin donation sites did not differ from that of burns of the IIIa-IIIb degree. Changes in the cell composition of squash preparations from the surface of the skin donation site after transplantation were characteristic. Whereas before transplantation the predominant cells in the squash preparations were neutrophilic leukocytes, accounting for up to 85% of total, and the number of fibroblasts did not exceed 1%, by the 5th-7th day after transplantation the proportion of neutrophilic leukocytes had fallen to 30%, whereas the number of fibroblasts had risen to 33%, and sheets of young epithelium appeared.

These results, as a whole, demonstrate the prospective value of the suggested method of treatment of burns. The efficacy of fibroblast transplantation depends primarily on the depth of the burn. In deep burns of the IIIa-IIIb degree transplantation of fibroblasts in all cases led to spontaneous epithelization of extensive wounds in the course of 8 ± 2 days, which is at least 3 or 4 times faster than with the ordinary methods of treatment. Transplantation of fibroblasts proved effective in the treatment of indolent wounds at skin donation sites. Efficacy of transplantation of fibroblasts in the treatment of burns of the IIIb degree must be emphasized: in one patient spontaneous healing of wounds of the IIIb degree with an area of 28 and 56 $cm^2$ was observed, whereas in another patient transplantation reduced the area of the wound in the course of 7 days by 15%. In our opinion these results can be attributed to the marked stimulating effect of the cultured fibroblasts on processes of regeneration. The intimate mechanisms of this effect require elucidation and detailed study.

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# CHANGES IN STRUCTURE AND FUNCTION OF CHROMATIN OF CEREBRAL CORTICAL NEURONS IN THE EARLY POSTRESUSCITATION PERIOD IN RATS

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Changes in the CNS after severe hypoxia, arising during systemic circulatory arrest, constitute one of the main pathogenetic mechanisms of postresuscitation sickness [9]. Previous investigations have showed that to understand the mechanisms of formation of postresuscitation brain pathology it is essential to study the state of the different components of the protein-synthesizing system of nerve cells. It has been found [2], for instance, that after clinical death, of varied etiology and duration, the nucleus and cytoplasm of the neurons enlarge and their dry weight increases. In the postresuscitation period enlargement of the nucleolus of nerve cells has been observed [8], and the intensity of this process correlates with the duration of previous ischemia and the degree of recovery of the animals' neurological status.

The aim of this investigation was to evaluate the structural and functional state of the transcription apparatus of neurons located in different functional layers of the cerebral cortex of rats in the early stages after systemic circulatory arrest.

## EXPERIMENTAL METHOD

The sensomotor cortex of six noninbred male albino rats weighing 160-180 g was studied after a 10-min period of systemic circulatory arrest caused by compression of the vascular bundle of the heart [7], and in three intact rats (control). The medium-sized pyramidal cells of layer III, stellate neurons of layer IV, and large pyramidal cells of layer V of the cortex were investigated 1 and 24 h after resuscitation. To assess the state of transcription in the neurons a histoautoradiographic method was used to demonstrate activity of endogenous RNA-polymerases in fixed cells [13]. The state of transcription was assessed by the intensity of labeling of the nucleolus and the extranucleolar zone, by counting the number of grains of reduced silver separately in pale and dark neurons in each layer of the cortex (staining with methylene blue). By the term dark cells were understood normal, morphologically unchanged neurons with darker staining of their nucleus and cytoplasm [1]. The intensity of labeling of 50 cells was counted in each layer for one animal. The results were subjected to statistical analysis by the Kolmogorov-Smirnov $\lambda$ test and the $\varphi$ test [6].

To assess the structural state of the chromatin the method of differential staining of lysine- and arginine-rich histones with ammoniacal silver was used [12]. Although this method was suggested for staining histones, the pattern revealed by ammoniacal silver is essentially determined by the specific nature of interaction of histones with other components of DNP in nuclei of one type or another and, consequently, it reflects differences in the structure of chromatin. It is important to note that according to the existing data [4], there is no unambiguous correlation between characteristics revealed by ammoniacal silver and the level of template activity of the chromatin, determined by Moore's method. Thus the methods which we used characterize different aspects of the state of the neuronal transcription apparatus.

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