in RV and 1.0/1.5 in LV, indicating a significant shift of metabolism toward biosynthesis in the myocardium of both ventricles. This conclusion is confirmed by elevation of levels of NADPH-diaphorase and G6PDH (Fig. 2) in both ventricles, the latter being regarded as a marker of the phosphogluconate pathway [3], activation of which is accompanied by accumulation of NADPH.

Thus, whereas in compensated MEPA the structural-metabolic reactions in the ventricular myocardium are on the whole adequate to meet their hemodynamic loads [4], in MEPA accompanied by the development of decompensation changes are found in the myocardium of RV which do not correlate with the increase in the after-load on this part of the heart, namely: a decrease in activity of enzymes involved in catabolism (including enzymes of cellular respiration), a decrease in the number of mitochondria, an increase in the fraction of injured mitochondria. In acute MEPA accompanied by the development of irreversible decompensation a marked shift of metabolism is observed toward biosynthesis, with a significant rise of the NADPH-diaphorase and G6PDH levels in the myocardium of both ventricles.

LITERATURE CITED

1. M. Burstone, Enzyme Histochemistry [Russian translation], Moscow (1965).
2. A. O. Virganskii, M. S. Tverskaya, and R. V. Rogulenko, Byull. Éksp. Biol. Med., **110**, No. 12, 577 (1990).
3. T. B. Zhuravleva and R. A. Prochukhanov, Introduction to Quantitative Histochemistry [in Russian], Moscow (1978).
4. V. V. Karpova, M. S. Tverskaya, A. O. Virganskii, et al., Byull. Éksp. Biol. Med., No. 2, 130 (1991).
5. A. Lehninger, Biochemistry [Russian translation], Moscow (1976).
6. Z. Lojda, R. Gossrau, and T. Schiebler, Enzyme Histochemistry [Russian translation], Moscow (1982).
7. B. Alberts, D. Bray, J. Lewis, et al., Molecular Biology of the Cell [Russian translation], Vol. 1, Moscow (1986).
8. L. H. Opie, Physiology and Pathophysiology of the Heart [Russian translation], Vol. 2, Moscow (1988), pp. 7-23.
9. A. G. E. Pearse, Histochemistry: Theoretical and Applied [Russian translation], Moscow (1962).

EFFECT ON NUMBER AND ORIENTATION OF MICROGRAFTS ON WOUND EPITHELIZATION IN RATS

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Deep wounds can be epithelized by the aid of skin microautografts (MAG) [1-4]. Can the MAG method be used in the case of extensive burns, when the donor's reserves of skin are depleted? An indicator of the efficacy of the skin grafting method is the transplantation coefficient (the ratio of the area of the graft to the area of the wound). We know that the transplantation coefficient attainable by the use of UAG is up to 1:30 in rabbits [3, 4] and up to 1:40 in rats [2]. These values, however, may perhaps not be limiting. The MAG method also differs from traditional grafting of a skin flap in that the pieces of skin are transplanted arbitrarily. The question naturally arises of the ability of MAG to epithelize the wound if they are strictly oriented relative to the wound surface (to the dermis, the epidermis, or laterally). The aim of this investigation was to study these problems.

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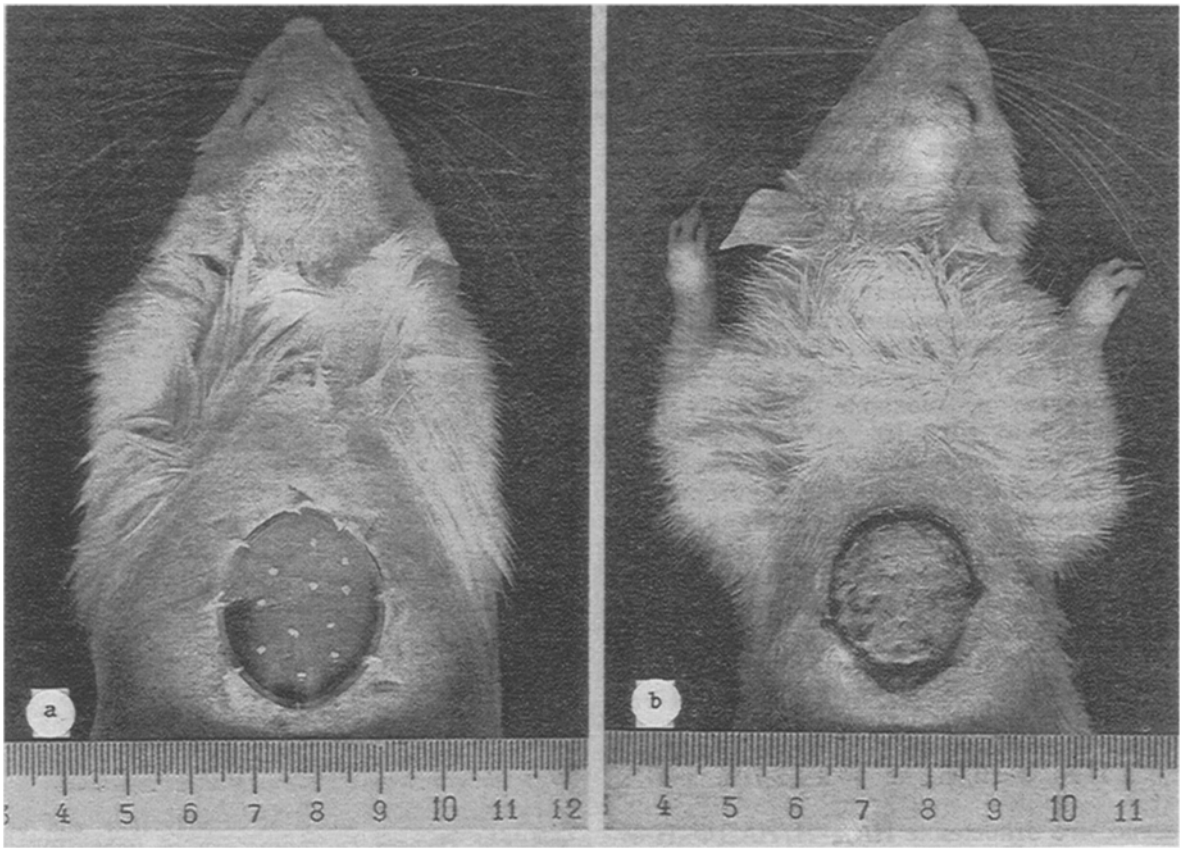

Fig. 1. Healing of skin wound: a) distribution of MAG on wound bed with transplantation coefficient of 1:100, wound edges fixed with a polyethylene ring to prevent contraction and marginal epithelization; b) epithelized wound (2 weeks after transplantation of MAG, immediately after removal of ring).

EXPERIMENTAL METHOD

Noninbred male rats weighing 250-300 g were used. The model of the wound, transplantation of MAG onto it, and evaluation of the new skin were carried out as described previously [1, 2]. In the experiment with orientation of MAG on the wound the epidermis of the transplanted fragments was first labeled with Brilliant green. In all cases the experiment ended 6 weeks after transplantation of MAG measuring 0.5 × 1.0 mm on to a wound with an area of 5.4 cm^2. All the experiments were repeated twice. The MAG technique is illustrated in Fig. 1. To study the character of growth of MAG, the method of organ culture was used. For this purpose pieces of skin measuring 0.5 × 1.0 mm were applied with the dermis underneath to plastic Petri dishes measuring 3.5 cm in diameter. The skin was cultured in Ham's F-12 medium containing 10% fetal calf serum and antibiotics. The CO_2 concentration in the air phase was 10%. The culture was fixed after 2 weeks in 10% formalin. Total preparations were stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

The use of the MAG method demonstrated its high efficacy with a transplantation coefficient of up to 1:100. A transplantation coefficient of this magnitude eliminates the problem of a shortage of donor's skin in cases of extensive burns. It will be clear from Table 1 that the thickness of the neoepidermis was independent of the number of MAG transplanted. This indicates that the frequency of seeding of MAG on a given wound area did not affect the rate of histogenesis of the neoepidermis. This was confirmed by the fact that epithelization of the wound was complete at the same time (2-3 weeks after transplantation), irrespective of the value of the transplantation coefficient. It can accordingly be postulated that epithelization of the wound took place, not on account of the total length of the line of marginal epithelization of all the fragments (with differing transplantation coefficients, the length of this line will vary), but rather on account

TABLE 1. Thickness of Neoepidermis Corresponding to Different Values of Transplanation Coefficient (M ± m)

Transplantation coefficient	Number of rats	Number of MAG	Thickness of neopidermis (without stratum corneum), mm
1:50	18	21	0,1±0,02
1:70	19	16	0,11±0,02
1:80	18	13	0,1±0,01
1:100	17	11	0,1±0,008

Legend. Thickness of epidermis of intact rat skin is 0.02 ± 0.002 mm without stratum corneum.

TABLE 2. Thickness of Neoepidermis with Different Orientation of MAG (M ± m)

Orientation of MAG* relative to wound surface	Number of rats	Thickness of neopidermis (without stratum corneum), nm
With dermis	20	0,087±0,008
With epidermis	18	0,073±0,007
Laterally	17	0,078±0,009

Legend. *Transplantation coefficient 1:40 (26 fragments).

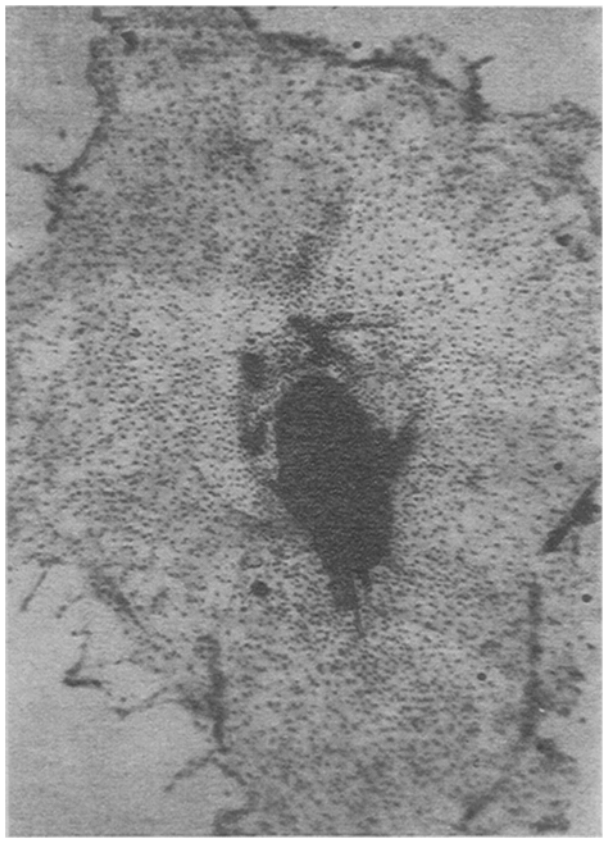

Fig. 2. Photomicrograph of piece of skin forming zone of growth of epithelial cells. Total preparation. Hematoxyin-eosin 13×.

of "insular" epithelization, implying that each fragment of skin is an independent microorgan. It can be tentatively suggested that under certain conditions (addition of cell growth factors etc.), a separate piece of skin can theoretically give rise to an unlimited zone of growth, in the same way as a culture of epidermocytes in vitro. Our organ culture experiments in vitro in fact showed that pieces of skin can form "islets" of epithelization (Fig. 2). This property of the organ culture is evidently preserved on the wound surface also. However, whereas in culture the fragments were arranged with the dermis underneath, in order to attach them to the substrate, MAG were grafted on to the wound arbitrarily. By analogy with organ culture, it can be postulated that epithelization of the wound also can take place only on account of those MAG which were arranged with the dermis toward the wound surface. In fact it was found that epithelization of the wound took place whatever the orientation of MAG, and at the same speed. The parameters of the neoepidermis, moreover, were identical with one another (Table 2).

In all the experiments the neoepidermis consisted of layers characteristic of the normal epidenmis with no visible evidence of dystrophy, regardless of the marked hyperplasia.

It can thus be concluded that complete epithelization of a wound can be achieved by the use of the MAG method with a transplantation coefficient of 1:100, and with fragments of skin measuring 0.5 mm^2. Under these circumstances the rate of epithelization is independent of the orientation of the MAG relative to the wound surface.

LITERATURE CITED

1. V. N. Shvets, Z. E. Vnukova, and B. B. Egorov, Byull. Éksp. Biol. Med., No. 5, 470 (1990).
2. V. N. Shvets and Z. E. V'nukova, Byull. Éksp. Biol. Med., No 5, 473 (1990).
3. S. D. Blair, J. Nanchahal, C. M. Backhouse, et al., Lancet, **2**, 483 (1987).
4. M. L. Zhang, Z. Chang, X. Han, et al., Burns, **12**, No. 8, 540 (1986).