SKIN MICROAUTOGRAFTING. CHANGES IN AREA AND MORPHOLOGY OF THE NEW SKIN

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Since Tanner [5] unsuccessfully experimented with transplantation of skin fragments on the surface of a human skin wound by Reverdin's (1869) method the opinion has been held that the method is unpromising. During the last three years, however, the method of skin microautografting (SMAG) has attracted the attention of research workers once again [1, 2, 6] in connection with the search for possible bioengineering approaches to the treatment of extensive skin burns. The SMAG method differs from existing methods of autodermoplasty in that skin microfragments are distributed randomly on the wound surface: dermis to dermis, epidermis to dermis, or in the lateral position. In the development of the SMAG technique much still remains unknown. The aim of this investigation was to study changes in the area and histological structure of new skin formed after SMAG on the wound surface in rats of different ages.

EXPERIMENTAL METHOD

Male rats weighing 200-220 g and 450-500 g were used. Under hexobarbital anesthesia and after removal of the hair and disinfection, a full-thickness skin graft with an area of 5 cm^2 was excised. To prevent contractions of the wound a ring (thickness of its wall 1 mm) 30 mm in diameter, made of polyethylene or Teflon, was fixed along the edges of the wound by means of a surgical suture. A strip measuring 1 × 25 mm was cut from the excised piece of skin, and pieces of cubical shape and measuring about 1 mm^2 (25 pieces) were obtained from it with the end of a microtome blade. The skin fragments were kept for 5-10 min in physiological saline containing 500 U each of penicillin and streptomycin, after which they were evenly distributed on the recently excised wound. The SMAG was covered with a fresh full-thickness skin allograft taken from another rat. To give the allograft elasticity and to create a drainage system, it was pricked with a scalpel over its whole areas. The allograft was kept for 5-10 min in the same physiological saline. Initially the ratio of the area of SMAG to the area of the wound was 1:20. Altogether 42 rats of different ages were used in this group, and they were withdrawn from the experiment 7, 9, and 17 weeks after the operation (7 rats at each point). Wounds in another group of animals with fixed rings were left open for 4 and 6 days, when granulation tissue with a varied degree of maturity was formed. At these times, a strip of skin measuring 3 × 10 mm was excised from the back of these rats in the distal direction downward from the wound (under general anesthesia), and 25 fragments for SMAG were prepared from it in the same way and were applied to the granulating wound. The SMAG was covered with a skin allograft as described above. Altogether in this series of experiments 72 rats of different ages were used, and were killed 7, 9, and 17 weeks after SMAG (6 rats at each point). On the first day after SMAG the wound surface was covered with gauze, soaked in 0.5% dioxidine. Later, "Dermazan" ointment was used, followed by Solcoseril. After removal of the ring from the wound, the area of the neoepidermis was measured by applying a piece of cellophane to it. The outline of the neoepidermis was then traced on squared paper and the area calculated. At the end of the experiment the new skin was completely removed and subjected to histological treatment with fixation in 10% formalin solution or Carnoy's solution followed by embedding in paraffin wax. Histological sections 4-5 μm thick were prepared perpendicularly to the skin surface, stained with hematoxylin and eosin and by the PAS reaction, and then examined under the microscope.

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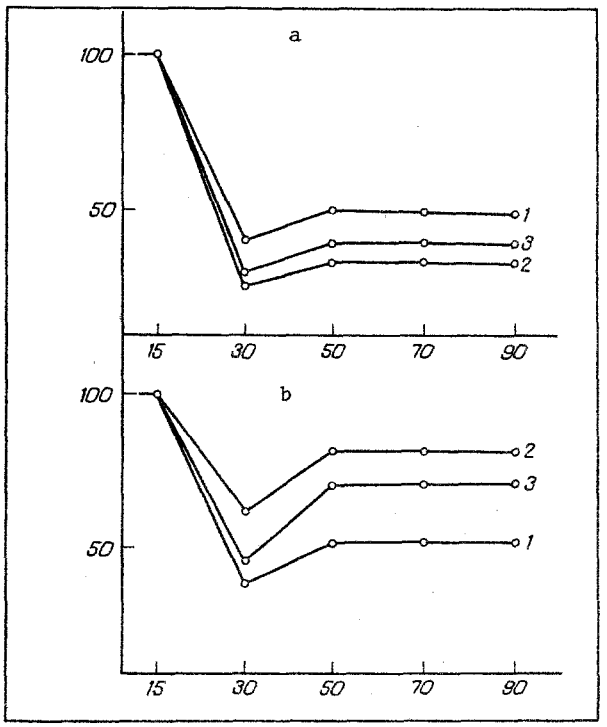

Fig. 1. Change in area of neoepidermis formed during SMAG in young (a) and old (b) rats, on recently excised (1), and 4-day- (2) and 8-day-old (3) granulating wound. Abscissa, time of experiment (in days); ordinate, area of neoepidermis (in per cent). Area of primary wound taken as 100% (5 cm^2).

EXPERIMENTAL RESULTS

The allografts on all experimental rats took quickly, but then underwent necrosis at various times and sloughed. During this time (10th-15th days) a new epidermis, pale pink in color, formed beneath the allograft and covered the whole surface of the wound. After complete rejection of the allograft, the ring was removed from the animal. At the previous site of the ring, no epithelium had developed. However, 2 or 3 days after removal of the ring, the marginal epidermis of the wound had joined the epidermis of the new skin. From that moment the area of the new skin was measured, and the results are given in Fig. 1. Clearly by the 30th day after the operation or in the course of two weeks after removal of the ring, the area of the new skin decreased sharply. It then began to increase and stabilize at that level in all versions of the experiment. This character of the change in area of the new skin was evidently determined by contraction of the wound. Contraction of wounds in rats is known to pass through three phases: phase I develops during the 5 days after wounding and is characterized by slow contraction (the lag-phase); phase II lasts from the 5th to the 10th day and is characterized by rapid contraction, during which the wound edges draw together; phase III, in which the rate of contraction diminishes slowly [3, 4]. Contraction of the new skin has two phases which, unlike that of the open wound, are shifted in time because of the preceding mechanical fixation, and it is characterized by a phase of rapid contraction and a phase of stabilization. Contraction of the new skin was found to depend on the age of the rats and the degree of maturation of the granulation. For instance, contraction of the new skin was much more marked in young animals (Fig. 1). This is evidently associated with the more rapid rate of collagen formation in young growing animals.

Contraction of the new skin also depended on the wound bed on which it formed. If the microallograft was transplanted on a recently excised wound the area of new skin in the second phase of contraction was reduced by half compared with its primary area, and this was independent of the rats' ages. In the case of grafting of the microallograft on a 4- or 8-day-old granulating wound the area of new skin in the old rats was reduced by only 20-30%, but in the young rats by 60-70% relative to the area of the original wound. Thus the degree of contraction of the wound depends on age and ultimately determines the area of new skin.

These results must be taken into account during treatment of a burned surface by this method in persons of different ages.

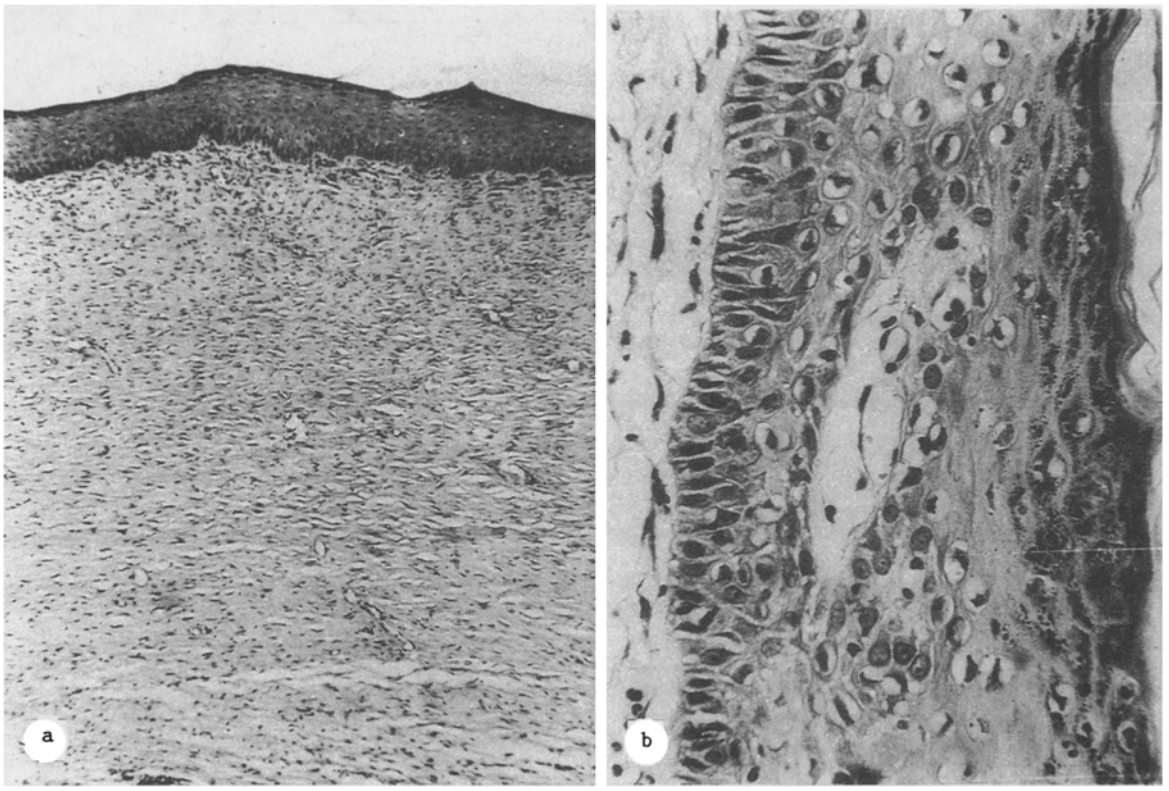

Fig. 2. Structure of 7-week-old new skin: a) hyperplasia of epidermis, basal layer consists of cubical and cylindrical cells. Dermis consists of young connective tissue, with no skin appendages. Stained with hematoxylin and eosin. 32.2×; b) the same preparation, 205×.

On histological examination of the new skin (7 weeks) hyperplasia of the neoepidermis was observed in both age groups (Fig. 2), and it contained all layers of cells characteristic of the epidermis of normal skin. Basal and spinous cells of the neoepidermis were represented by cells with cubical and cylindrical shape. This type of structure of the basal layer probably determines the elasticity of the neoepidermis. Testing for glycogen revealed a well marked basement membrane, evidence of trophic interaction between the neoepidermis and the underlying connective tissue. In the dermis (conventionally, in the reticular layer) epithelioid cysts were found, their cavity filled with horny substance (Fig. 3). These cysts were evidently microorgans, for the walls of the cysts are similar in structure to the epidermis, and the presence of horny material in the cavity of the cyst is the result of increased function of the keratinocytes of the epidermis of the cysts. Besides cysts, sebaceous glands, often forming fistulous tracks in the neoepidermis, also were observed in the dermis. Some glands had cavities, indicating lysis of the gland cells and functional activity of these glands. No skin appendages were present in any of the specimens. Close to the cyst were concentrations of multinuclear cells, resembling foreign body giant cells. This was evidently associated with rupture of the cysts and their subsequent absorption. Later until the 17th week the number of these "artefactual" structures decreased significantly, i.e., the dermis was as it were rid of its extraneous structures. The thickness of the individual layers of the neoepidermis decreased proportionally, but still exceeded that of normal epidermis. Rejection of the neoepidermis was not observed in the late period.

Thus by skin microautografting, using fragment about 1 mm^2 in area, transplanted randomly to the wound surface, a neoepidermis formed in 100% of cases, and corresponded in its structure to that of the epidermis in situ. This epidermis was not rejected (at least before 17 weeks) and preserved its structure during contraction of the wound, evidence of a strong connection of the neoepidermis with the underlying connective tissue, resembling the dermal–epidermal junction under normal conditions. It is important to emphasize that the formation of neoepidermis from SMAG takes place not only on a granulating, but also on a recently excised wound, and it depends essentially on the age of the experimental animal. By contrast with autodermoplasty, the SMAG method can be regarded as organotypical culture in vivo.

Because of its simplicity and economy in donated autologous skin the SMAG method may find widespread application in clinical practice with the aim of securing epithelization of extensive burns.

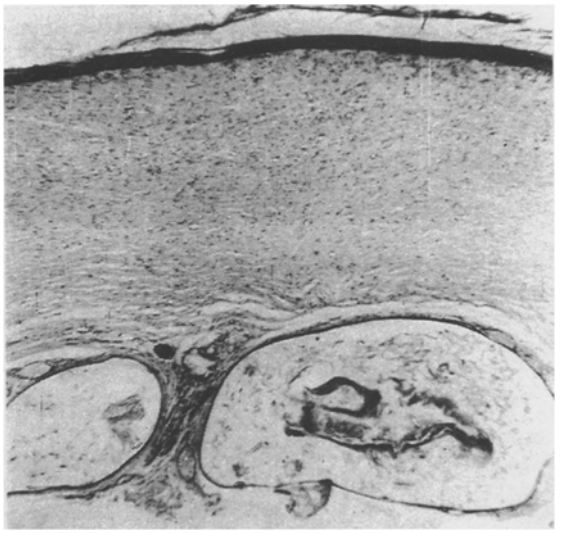

Fig. 3. Structure of 7-week-old new skin. Epithelioid cysts are present in the dermis, their cavity filled with horny material. Stained with hematoxylin and eosin. 16.4×.

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