DEGREE OF COMPLETENESS OF REGENERATION OF SKIN IN RATS

(UDC 616.5-003.93-092.9 + 612.6.03 : 612.79)

E. A. Efimov

Laboratory of Growth and Development (Head—Professor L. D. Liozner)
Institute of Experimental Biology (Director—Professor I. N. Maiskii)
Academy of Medical Sciences of the USSR, Moscow
(Presented by Active Member of the Academy of Medical Sciences of the USSR N. A. Kraevskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 9, pp. 102-106, September, 1965
Original article submitted November 30, 1964

According to generally accepted opinion, if all the layers of mammalian skin are damaged the process of healing is accompanied by the formation of a connective tissue scar. The scar tissue does not undergo further change and does not acquire any structural similarity to normal skin layers. It is believed that the attainment of a specific structure by the skin during regeneration is only possible in those cases when the damage is confined to the superficial layer, and the hair follicles in the more deep-seated layers remain intact. The possibility of regeneration of the hair follicles and cutaneous glands after the destruction of all layers of the skin has beeen categorically denied and is still denied by many research workers [4].

In contrast to what has been stated above, in recent years certain investigators have considered the possibility of re-establishing the typical structure of the skin,following damage which involves all the cutaneous layers. A. A. Braun and his colleagues suggest that scars forming at the site of a wound may, in course of time, acquire the structure of normal skin [3, 5, 6]. Of particular interest is the work of those authors who hold the opinion that regeneration of the skin is possible without formation of scar tissue, even when all the layers of the skin have suffered damage [1, 7-9]. However, the facts set forth in the above-mentioned works are open to some doubt [10, 11] and most authors hold the view that healing of cutaneous wounds can only take place through the medium of scar formations.

The problem of cutaneous regeneration after injury to skin tissue is still inadequately solved. The possibility of regenerating tissue in the structural form typical of undamaged skin therefore requires more detailed study.

The aim of the present work was to study the dynamics of connective tissue scar formation during the process of cutaneous wound healing in rats and also to investigate the possibility of new specific structures forming during regeneration, i.e., to investigate the complete regenerative process in the skin.

EXPERIMENTAL

In our experiments we used 150 white male rats of no specific breeds, 120-150 g in weight. The experiments were set up in the following way. The animals ware fastened to the bench dorsal side up, and hair was shaven off the mid-dorsum over an area corresponding to that of the future wound. After depilation the skin was tattooed (in the form of a square) with India ink using two fine needles attached together; alternatively the tattoo mark consisted of a rectangle of constant dimensions. Each tattoo prick penetrated into the dermis. The purpose of the tattooing was to delimit the area of freshly formed, regenerated skin from that of old skin adjacent to the wound. Cuts were then made within the areas (squares or rectangles) defined by the tattoo marks, the cuts running parallel to the sides of the figures and 1-2 mm from them. The strips of skin were separated by the cuts right down to the fascia. All manipulations were carried out under ether narcosis. The wounds were

Areas of Scars Resulting from the Healing of Wounds of Various Sizes

Series No.	Area of skin removed (in cm^2)	Ratio of area of scar to area of skin removed (in %)
I	2,25	25
II	2,25	22
III	2,25	18
IV	4,00	23
V	21—25,2	42

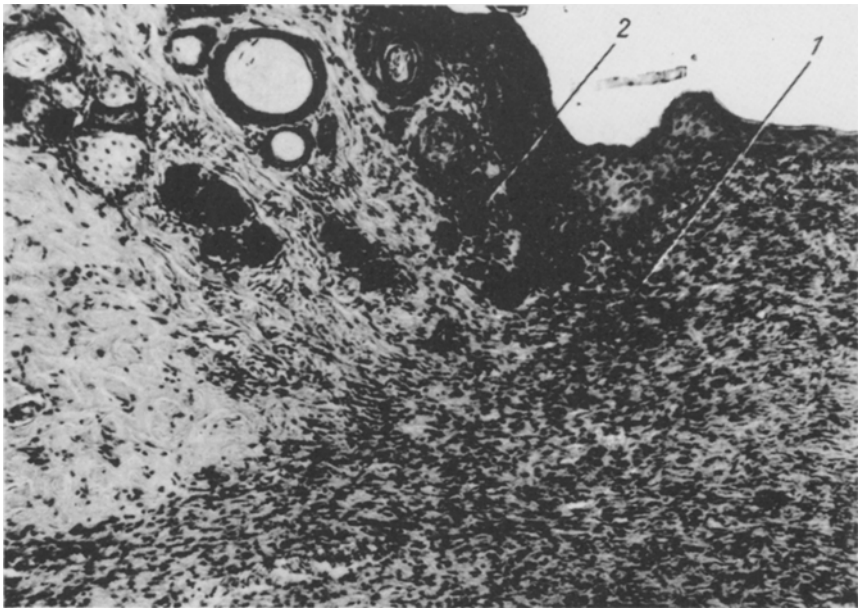

Fig. 1. Section through skin of white rat in the region of cutaneous wound 10 days after operation (series I). 1) Granulation tissue; 2) hair rudiment. Microphotograph. Fixative—formalin. Stain—hematoxylin-eosin. Magnification 90 X.

Fig. 2. Section through skin of white rat in the region of cutaneous wound 20 days after operation (series I). 1) Hairs. Microphotograph. Fixative—formalin. Stain—hematoxylin-eosin. Magnification 90 X.

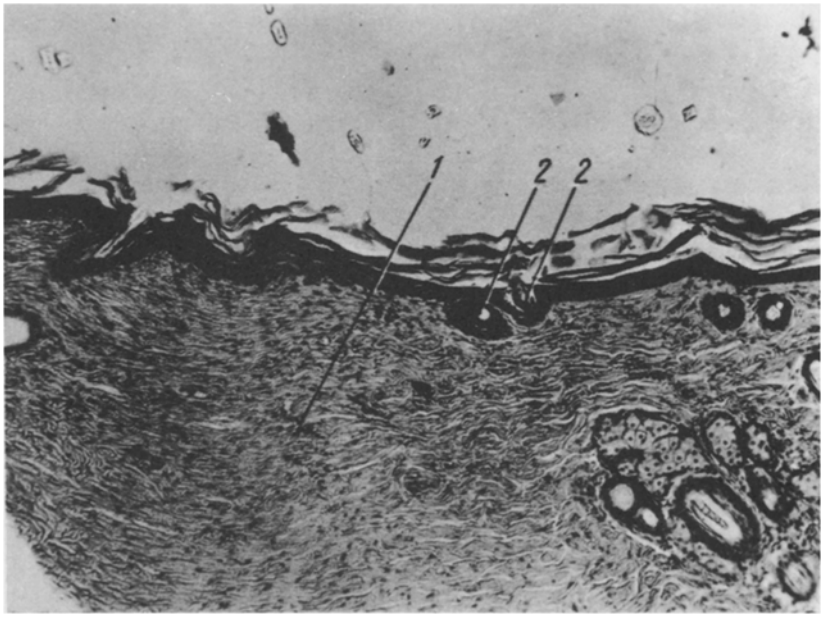

Fig. 3. Section through skin of white rat in region of cutaneous wound 56 days after operation (series I). 1) Scar tissue; 2) Hairs. Microphotograph. Fixative—formalin. Stain—hematoxylin-eosin. Magnification 90 X.

allowed to heal without dressings. The area of the wound and of the regenerating structures was measured at various times throughout the experiment in the following way: a transparent celloidin film was superimposed on the wound or scar and the outline of the latter was drawn on the film in India ink; the outline was then transferred from the celloidin film to paper of standard thickness. Later, a shape was cut from the paper corresponding to the outline of the wound or scar and this shape was weighed. Knowing the weight of 1 cm^2 paper, it was possible to calculate the exact area of regeneration of the wound. Pieces of tissue were removed from the area of the wound and from adjacent areas of undamaged skin at set intervals of time and subjected to histological analysis. The material was fixed in 12% formalin solution, taken through celloidin, and embedded in paraffin wax. Later, sections 7-10 μ thick were prepared and these were stained in hematoxylin-eosin according to Mallory's method.

A total of 5 series of experiments were set up. Each series consisted of 30 animals. In series I, II, and III, square patches of skin measuring 2.25 cm^2 were removed from the dorsal side of the trunk of the experimental animals; in series IV the patches which were removed measured 4 cm^2 and in series V the patches were rectangular in shape and measured from 21.3 to 25.2 cm^2. In series V we removed extensive strips of skin so that the site of their removal could be more readily marked.

RESULTS

In all the series of experiments there was an increase in the area of skin lying between the wound margin and the India ink marking drawn 1-2 cm from the edge of the wound during the process of healing. A connective-tissue scar formed in the animals of all five experimental series as a result of the healing of the wound. The ratio of the area of this scar to the area of skin removed varied within considerable limits (from 18 to 42%) for the various experimental series (cf. table).

Animals of one and the same experimental series gave uniform results. It is possible to explain the variation in the dimensions of connective-tissue scars between different experimental series in the following way. In the opinion of many research workers, healing of the skin involves three processes: a drawing together of the edges of the wound; intercalary growth of those tissues which are immediately adjacent to the wound (regenerative hypertrophy); and an outgrowth of tissue from the margin of the wound. The processes of drawing together of the edges and of intercalary growth are interrelated and difficult to separate from each other. The result of healing evidently depends on the extent to which one or the other process is made manifest.

In series V experiments, in which large fragments of skin (from 21 to 25.2 cm^2) were removed, the drawing together of the wound margins was rather feebly expressed and the damaged surface was covered over to a large extent, as a result of the formation of scar tissue. This was confirmed by the behavior of the India ink marks: they only moved inwards from the edges of the original wound. The area of skin lying between the marks and the scar margin increased less than in those experimental series in which smaller fragments of skin had been removed, which indicates a comparatively slight drawing together of the wound edges and little intercalary growth of the skin surrounding the wound. In series I, II, III, and IV [experiments involving the removal of small fragments of skin (from 2.25 to 4 cm^2)], the main process leading to the covering over of the wound appeared to consist of the drawing together of the wound margins and intercalary growth in those areas of skin immediately adjacent to the wound. In these cases the India ink marks moved a considerable distance towards the center.

In all cases, animals in the early stages of wound healing showed both an epithelialization and filling of the wound cavity with granulation tissue and also the formation of a wedge of connective tissue in those places where the epithelium had grown over the granulation tissue. This wedge appears to have arisen as a result of elements in the corium having moved to the edges of the wound. Even during the early stages of healing it was possible to observe the development of some hair follicles and gland rudiments. The rudiments appeared on the upper part of the wedge near the point of its entry into the granulation tissue (Fig. 1). After the granulation tissue had given rise to the scar tissue no further increase in size of the wedge was noticed and no new rudiments of either hairs of glands appeared to form. By this time the rudiments of specific cutaneous structures—hairs and glands—which were observable during the early stages of regeneration, had developed into fully formed hairs and glands. This process can be followed in sections taken over a period of time (Figs. 2 and 3). As a rule, the wedge has not by this time reached the central part of the original wound, and in all the experimental series its area was insignificant compared with that of the scar as a whole.

The histological structure of the forming scar was studied for different periods of time in all the experimental series (up to 8 months). It was not possible to observe any conversion of scar tissue to normal skin furnished with hairs and glands.

Thus, one of the main conclusions which can be drawn from our experiments is that scar tissue does not later convert to skin of normal structure. Specific cutaneous structures—hairs and glands—are not capable of being derived from it. The area of the scar in relation to the area of skin removed may vary considerably from one series to another, evidently depending on the nonuniformity of the conditions. In addition to the scar, separate hairs and glands were also formed, and their development was traced over a considerable period of time.

LITERATURE CITED

1. M. E. Aspiz, In the book: Problems of the Regeneration of Organs and Tissues in Vertebrate Animals [in Russian], Moscow (1954), p.92.
2. A. A. Braun, Izv. AN SSSR, Ser. Biol., No. 6 (1945), p. 695.
3. A. A. Braun and V. V. Raivid, Paper Presented at 2nd Conference on the Problems of Regeneration and Cellular Reproduction [in Russian]. Moscow (1960), p. 16.
4. L. D. Liozner, Editor, Regeneration of Organs in Mammals, Moscow (1960), p. 66.
5. V. V. Raivid, Arkh. anat., No. 12 (1961), p. 64.
6. S. Tezekbaev, Byull. éksper. biol., No. 2 (1955), p. 57.
7. G. V. Khomyllo, Doklady Akad. Nauk SSSR, 90, 2 (1953), p. 313.
8. R. E. Billingham and P. S. Russell, Ann. Surg., 144 (1956), p. 961.
9. C. Breedis, Cancer Res., 14 (1954), p. 575.
10. W. Montagna and E. J. van Scott, In the book: The Biology of Hair Growth. New York (1958), p. 39.
11. W. Straile, Ann. New York. Acad. Sci., 83, Art. 3 (1959), p. 499.