EXPERIMENTAL BIOLOGY

PRINCIPLES GOVERNING REGENERATION OF THE SKIN IN MICE AFTER REMOVAL OF A FULL-THICKNESS GRAFT

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The view is commonly held that regeneration of full-thickness areas of the skin in mammals takes place by the formation of a connective-tissue scar. The scars thereafter remain unchanged and do not acquire a structure similar to that of normal skin; the specific structures of the skin, hairs and glands, are never formed in them [5,12]. Recently, observations have been made during the healing of skin wounds showing that new hairs and glands may be formed [1,8,9,10]. It has even been suggested that the typical structure of the skin may be restored after removal of full-thickness grafts [2,3,6].

The object of the present investigation was to study the structure of the tissue formed during regeneration after removal of full-thickness skin grafts from mice, to examine the possibility that new specific structures, hair and glands, may form in the regenerating tissue, and to investigate the relationship between the rate of regeneration and the experimental conditions.

EXPERIMENTAL METHOD

Experiments were carried out on 140 male mice of the CC57BR line, weighing 18-20 g, and on 60 noninbred male albino mice weighing 18-20 g.

Before the operation on the skin, the animals were tattooed in the dorsal region in the form of a dotted square or rectangle of given area (the ink was injected into the dermis). Incisions were then made inside the area demarcated by the dots, parallel to the sides of the rectangles or squares, and at a distance of 1-2 mm from the lines of dots. The flaps of skin were removed down to the fascia. Before the tattooing was done, the hair was shaved around the future wound, except in the case of 30 noninbred albino mice, in which the hair was not removed. All the manipulations on the animals were carried out under ether anesthesia. The wound healed readily. The areas of the defects and of the regenerating skin were measured at successive periods of the experiment, by observing the position of the ink dots.

Pieces of tissue for histological analysis were taken at definite periods from the region of the wound and from the adjacent areas of intact skin. The material was fixed in 12% formalin, passed through celloidin, and embedded in paraffin wax. Sections were cut to a thickness of 7-10 μ, and stained with hematoxylin-eosin and by Mallory's method.

Altogether, three series of experiments were performed. In series 1, 100 mice of the CC57BR line were used (a square piece of skin measuring 1 cm^2 was removed); in series 2, 40 mice of the CC57BR line (a rectangular piece of skin was removed, measuring 3.2-3.8 cm^2); and in series 3, 60 noninbred albino mice were used (a rectangular area of skin measuring 1 cm^2 was removed). In 30 animals, the hair was clipped around the future wound before the operation, while in the remaining animals, the hair was not removed.

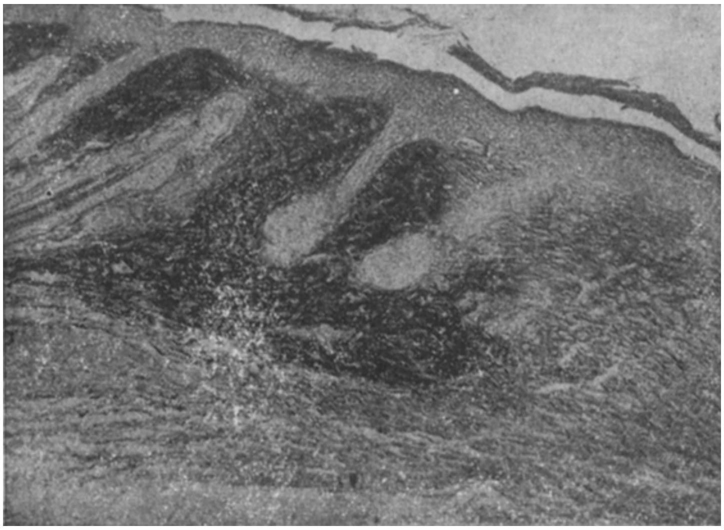

Fig. 1. Section through the skin of a mouse in the region of a wound defect 8 days after operation (series 1): 1) wedge of connective tissue of the old corium. Formalin, Mallory. Photomicrograph. Magnification 90 ×.

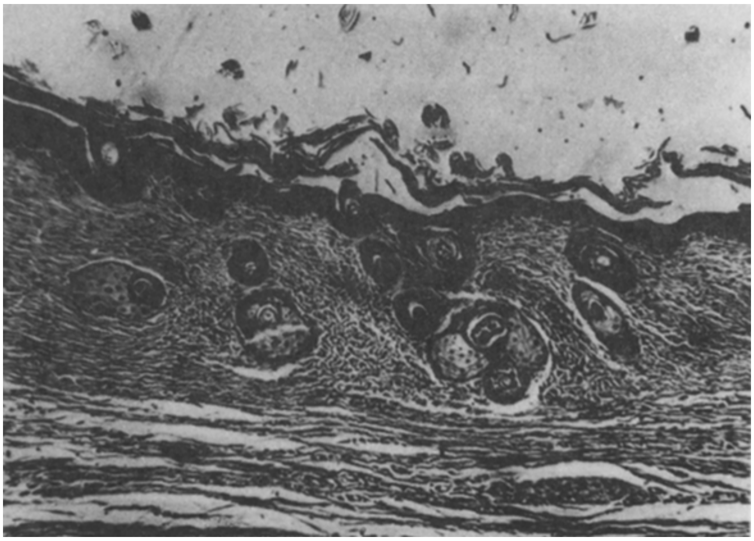

Fig. 2. Sections through the skin of a mouse in the region of a wound defect 17 days after operation (series 1). Formalin, hematoxylin-eosin. Photomicrograph. Magnification 90 ×.

EXPERIMENTAL RESULTS

In all the animals of series 1, in the early stages of regeneration, besides the filling of the defect with granulation tissue and the epithelization of the wound surface, the deep layers of the corium lying adjacent to the wound, which, with their covering epithelium had the appearance of a wedge, were partly replaced by granulation tissue.

At later periods of regeneration (6th-10th day), the wedge of connective tissue had enlarged and invaded the upper layers of the peripheral part of the defect. This penetration evidently took place as a result of two mutually associated processes: the drawing together of the wound edges, and accessory growth outside the wound. This invasion of the defect by the wedge of connective tissue from the corium lying adjacent to the wound ceased at the moment of conversion of the granulation tissue into scar tissue (11th-13th day). During this period, as a rule, the

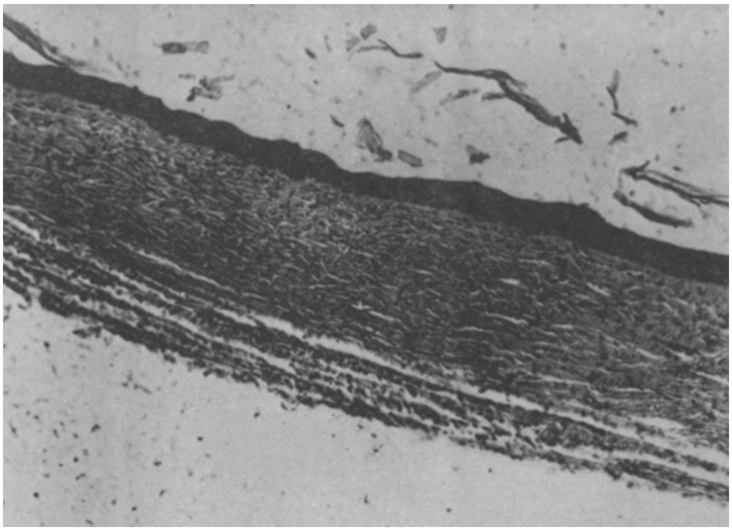

Fig. 3. Section through the skin of a mouse in the region of a wound defect 3.5 months after the operation (series 2). Formalin, hematoxylin-eosin. Photomicrograph. Magnification 90 ×.

wedge had not reached the central part of the defect. By the early stages of regeneration (5th-6th day) in the region of the wedge, besides regeneration of hairs and glands from injured follicles, the development of the rudiments of specific skin structures was observed, and these subsequently developed into hairs and glands. These rudimentary structures appeared in the pointed uppermost part of the wedge as it invaded the defect (Fig. 1). Only after the movement of the connective-tissue wedge had ceased were new rudiments of the specific skin structures no longer seen.

It follows from the experiments of series 1 that the full-thickness wound of the skin was closed not merely by scar formation; besides a scar, the connective tissue of the corium lying adjacent to the defect, and the epithelium covering it, also took part in the covering of the peripheral upper layers of the defect. In the tissues filling the peripheral part of the defect, hairs and glands were found (Fig. 2).

The structure of the tissues filling the defect was studied 3 months after the operation. The scar tissue filling the central part of the defect was not converted into normal skin during this period; neither hairs nor glands were formed in it. In the tissues filling the uppermost layers of the peripheral part of the defect, hairs and glands which appeared in the early stages of regeneration were observed for a period of 3 months, but no new hairs or glands were seen to be formed.

The area of the tissues filling the defect in the animals of series 1 was 10.8% of the area of the removed skin graft.

Unlike series 1, in series 2 the drawing together of the wound edges was much less obvious, and it was evidently prevented by the presence of a thick scab, which covered the wound for a long time and even extended beyond its edges. The area of the tissues filling the defect averaged 38.8% of the area of the skin graft removed. The greater part of the defect was filled with scar tissue, which was not converted into normal skin even after 3.5 months; neither hair nor glands were formed in it (Fig. 3).

Solitary hairs and glands were found in the tissues filling the smaller, peripheral parts of the defect, responsible for covering approximately 2% of the total area of the defect.

In the experimental animals of series 3, whose skin around the region of the future wound was depilated before the operation, the process of regeneration terminated by the 10th-12th day, while in the mice not subjected to depilation, the process was complete only by the 32nd day after the operation.

Hence, in mice a full-thickness skin wound closes as the result of scar formation and of the activity of the connective tissue of the corium lying next to the wound, which invades the upper layers of the peripheral part of the defect. Under these circumstances, the connective tissue of the intact corium, lying next to the wound, invaded the upper layers of the region of the defect to approximately the same distance in the different series of experiments, so that the part which it played in closing the defect in the different series of experiments was dependent on the size of the scar formed. When the approximation of the wound edges was delayed, the defect was closed mainly by the formation of an extensive scar and the part played by the invading connective tissue of the corium lying next to the wound, and by the epithelium covering it, in the closing of the defect was insignificant. Conversely, in the experiments in which contraction of the wound was well marked, as a result of healing a small scar was formed and the connective tissue of the corium lying next to the wound and the epithelium covering it, by invading the defect, played an important role in closing it.

In the tissues invading the peripheral part of the wound from the skin lying next to the defect, individual hairs and glands were formed in the early stages of regeneration and they were observed for a long period. The area of the tissues filling the defect, in proportion to the area of the removed skin graft, varied within wide limits from one series to another. The scar tissue filling the central part of the defect was not subsequently converted into skin with a normal structure; no specific skin structures (hairs and glands) were formed in it. Depilation of the skin of the animals around the future defect considerably accelerated the regeneration process. There are reports in the literature that the deep layers of the skin adjacent to the wound take part in regeneration (experiments on sheep [7]). The invasion the defect by connective tissue from the intact corium adjacent to the wound was observed by other authors [7] although, admittedly, in experiments performed under slightly different conditions. The authors cited above make no mention of the formation of hairs or glands in the tissue filling the peripheral part of the defect, nor did they make a detailed histological analysis of the successive stages of regeneration.

It is concluded from the results obtained in mice, and also from analogous data obtained in rats [4] that the principles governing the regeneration of full-thickness skin grafts, as revealed in these animals, apply also to other species of mammals.

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