TECHNICAL DIFFICULTIES IN DETERMINING THE ORIGIN OF HAIR AND SEBACEOUS GLANDS FOUND IN REGENERATING SKIN

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Technical difficulties in demonstrating new hairs and glands in regenerating skin formed at the site of a whole-thickness skin defect in laboratory animals (rabbits, rats, and mice) are analyzed. To determine the origin of the accessory structures of the skin not only must the wound edges be marked, but histological investigations also are required at successive stages of healing of the wounds, with attention concentrated on the size of the defect, its situation, and the age and species of the animal.

In some cases during the healing of whole-thickness skin wounds in mammals new hairs and glands are formed in the regenerating skin [1, 2, 5, 7, 8, 10]. This is a regular feature of regeneration of the skin on the external auricle of the rabbit where the skin is fixed [1, 5, 10]. Problems regarding the restoration of specific skin structures in parts of the body with mobile skin are not yet settled [1, 6, 8]. The contradictory nature of the observations is associated with technical difficulties in the analysis of the results.

When even comparatively large skin defects heal in rabbits, rats, and mice a small scar is formed. The scar developing after small wounds occupies a negligibly small part of the whole area of the animal's skin, for the wound edges of the original defect contract and come into contact in the center of the defect. A small area of young connective tissue, which is converted into scar tissue, remains between them and is covered by regenerating epithelium. Because of its small size, in some cases this area of new tissues can be detected only by tagging the edges of the original wound defect with ink [4, 5].

Visual examination of the sites of such defects reveals hairs at the edges of the hairless epithelized surface, which is very small in size, their ends directed toward the defect and often depigmented. Sometimes single hairs are also found in the central part of the epithelized surface. The origin of these hairs may vary: either they are formed from residues of hair follicles destroyed during wounding, or they are "old" follicles of the upper layers of the corium which have moved toward the defect as a result of contracture of the wound or, finally, they are hairs arising de novo from regenerating young epithelium. It is a difficult matter to determine their precise origin.

Marking the wound edges with ink to determine the origin of the hairs has proved to be unsatisfactory for the following reasons: first, the tags are not always exactly placed at the edge of the wound, and second, the concentration of the ink tag in some cases persisted only in the deep layers of the dermis and was absent in the upper layers actively concerned with closure of the wound [5, 9].

The writer has attempted to differentiate between old hairs and new by a simpler method, the essence of which is as follows: before wounding or immediately thereafter the hairs lying next to the defect were stained with picric acid or eosin. It was considered that it would be possible to distinguish the new hairs by their color. However, this method also proved unsatisfactory as a means of determining the origin of the hairs, for hairs next to the wound were trapped under the scab and disappeared.

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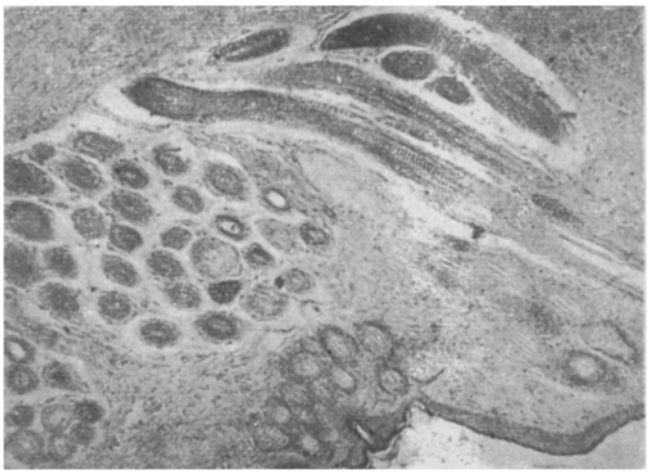

Fig. 1

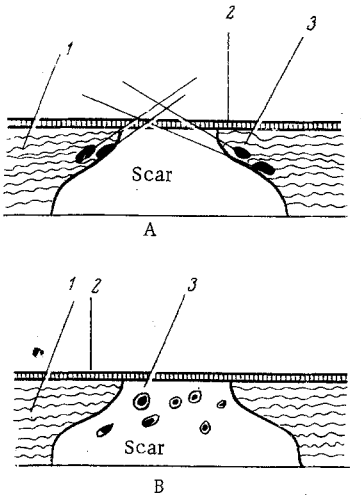

Fig. 2

Fig. 1. Atypically arranged hair follicles in regenerating skin on the external auricle of a rabbit. Hematoxylin-eosin, 90×.

Fig. 2. Diagram showing position of hairs next to a wound defect in the late stages of healing: A) longitudinal section through hair follicles; B) transverse section through hair follicles; 1) intact corium; 2) epithelium; 3) hair follicles.

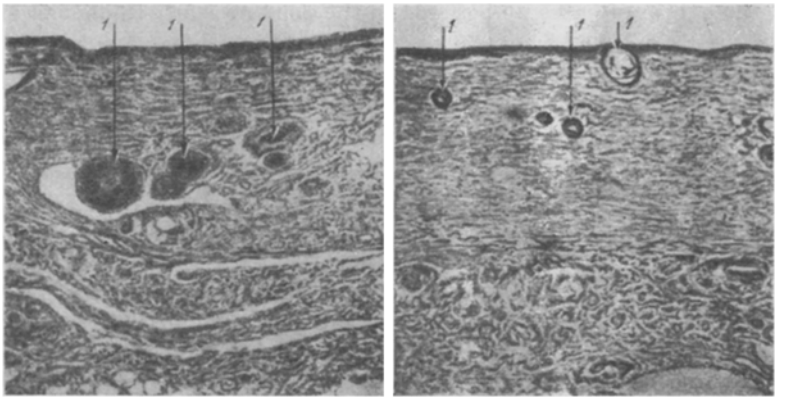

Fig. 3. Central part of wound defect of a rat 60 days after operating (a, b): 1) transverse sections through hair follicles considered to be new. Hematoxylin-eosin, 120×.

Consequently, by the use of the methods indicated above it is impossible to differentiate exactly between newly formed hairs and hairs arising from parts of damaged "old" follicles, and also those arising from follicles in the upper layers of the corium growing into the defect.

It would evidently be much easier to determine the origin of new hairs by the use of serial histological sections through the zone of the defect and the wound edges at consecutive periods of healing. However, there are difficulties in the way of histological treatment of the material. As a histological material the skin is difficult to cut and it is not always possible to obtain serial sections of the required thickness. Because of the sebaceous glands in the skin the hair follicles do not lie at a definite angle, and in atypically arranged bundles of hairs in the zone of the defect each follicle may lie at a different angle, so that it is difficult to obtain sections of any size which would pass vertically through the follicles (Fig. 1).

For the reasons just given, the results of investigations into the formation of new hairs and glands are frequently illustrated by photomicrographs taken from histological sections in which the hair follicles in the zone of the defect are not cut strictly vertically, but at an angle or even transversely [3, 8]. In the writer's opinion the origin of the hairs cannot be determined from such preparations because hair follicles next to the wound have their position altered by the developing scar, and the hair shaft emerges on the

surface in the zone of the defect although the bulb of the follicle itself lies in intact dermis (Fig. 2). Hairs on such transverse or oblique sections through follicles which appear in the zone of the defect in such sections are often taken for new hairs [8] (Fig. 3). If, however, vertical sections are obtained through the newly formed hair follicles and it becomes clear that they belong to the peripheral zone of the defect, it still cannot be concluded that these are new follicles, i.e., that they arise from invaginations of regenerating epithelium into the underlying young connective tissue. Most probably these follicles developed from residues of follicles destroyed during wounding.

In the writer's opinion the only reliable evidence of the formation of new hairs and sebaceous glands in the zone of a defect is the demonstration of the consecutive stages of development of these structures. However, in papers describing investigations into this problem there are no photomicrographs with consecutive stages of development of the hairs and glands. Observations on the early stages of development of hairs and glands with conclusions deduced from them that they are formed de novo, could admittedly be erroneous. For example, during the healing of full-thickness skin wounds on the back and head of newborn rats and mice invaginations of regenerating epithelium into the underlying young connective tissue have frequently been observed. These invaginations are similar in structure to the early stages of development of hairs and sebaceous glands. However, these invaginations subsequently disappeared and no hairs or glands developed.

During the healing of extensive defects on mobile areas of skin of an animal's body the newly formed tissues occupy a larger area than after smaller defects. In that case it is easier to determine the origin of the new hairs, especially if they are found in the center of the epithelized surface, for the probability of migration of old follicles or their parts into the central zone of the defect is less. If, therefore, in histological sections through the central part of an extensive epithelized surface the bulbs of hair follicles are found cut through at any angle it can be reliably concluded that these hairs have developed de novo.

In order to study the origin of hairs and glands in the zone of a full-thickness skin defect healing chiefly by contraction of the wound, and where newly formed tissues are represented by only a very small area, it is essential to examine consecutive stages of the formation of new hairs and glands. Only thus can it be decided which hairs and glands have formed from the remnants of old hairs and glands and which formed from follicles located in the small area of the upper layers of the corium adjacent to the wound and carried into the zone of the defect through contraction of the wound.

Histological sections through hair follicles in the zone of the defect must be cut strictly vertically in order to avoid the mistaken conclusion that old follicles lying next to the wound are new. For the reasons given above skin defects must be made as large as possible.

To estimate the number of new hairs and glands arising after treatment with various stimulators and inhibitors of regeneration it is better to use areas of the animal's body with fixed skin and, consequently, with a minimal degree of wound contraction. In that case the wound defect will be covered chiefly by the formation of new tissues and under those conditions the migration of follicles or parts of follicles into the central zone of the defect will not take place. In order to prove the formation of new hairs and glands in such wounds the stages of their development must be described.

To sum up, no methods which could be used to differentiate cells of hair follicles damaged at operation and incorporated into regenerating epithelium from the cells of the surface epithelium are known at the present time. It is possible that under certain conditions new hairs and glands may arise later from the epithelial cells of hair follicles.

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