the detailed structure of bacteria and of the phagocyte and, because of this, enables conclusions to be drawn regarding the mechanisms of bactericidal activity (for example, the presence or absence of marked degranulation). However, in cases where only the degree of intra- and extracellular bactericidal activity needs to be determined, without going into more details, the method of light-microscopic autoradiography with a fine-grain developer actually has a definite advantage over electron-microscopic autoradiography, for, with much less work, it enables an investigation to be conducted on a large number of objects (an essential condition for obtaining reliable data).

The suggested method and the results obtained by it show that, in a certain sense, electron-microscopic autoradiography and even the intermediate variant described here (light-microscopic autoradiography with electron-microscopic development) may actually prove to be more sensitive than the traditional light-microscopic autoradiography. Of course, in its standard meaning, sensitivity (minimal detectable concentration of radioactivity) of light-microscopic autoradiography is higher than that of electron-microscopic autoradiography. However, the possibility of finding minimal differences between concentrations of radioactivity in objects such as bacteria, which evidently must also be called sensitivity, is significantly higher in electron-microscopic autoradiography or in our suggested method of light-microscopic autoradiography with the use of a fine-grain developer.

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POSSIBLE EFFECT OF A MECHANICAL FACTOR ON COMPLETENESS OF DORSAL SKIN RESTORATION IN MICE

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Completeness of skin restoration during healing of full-thickness wounds may vary from a scar to perfect regeneration. This will depend on a number of experimental conditions: the species of animal, the location of the wound defect, and the intensity of contraction of the wound [2]. We know that healing of full-thickness wounds of the dorsal skin in mice and rats usually ends with the formation of a small connective-tissue scar [1, 2]. However, it has been shown experimentally that completeness of skin restoration in mammals can be deliberately influenced. For instance, by restraining wound contraction or by stimulating wound healing with small doses of the antioxidant dibunol, the outcome of skin regeneration can be changed and instead of an epithelized connective-tissue scar, it is possible to obtain regeneration of the correct skin type with the formation of skin derivatives: hairs and sebaceous glands [3, 4]. Such changes in the repair process in the skin have been shown to be possible if action is confined to young regenerating tissues, before granulation tissue has been converted into fibrous tissue [2].

We also know that severe trauma to the tissues of the regenerating amphibian limb by means of needles can stimulate regeneration and affect its completeness [5]. However, the influence of such a mechanical factor on the course and outcome of skin regeneration has not been studied in mammals. In our view, thanks to such procedures, the regenerating tissues

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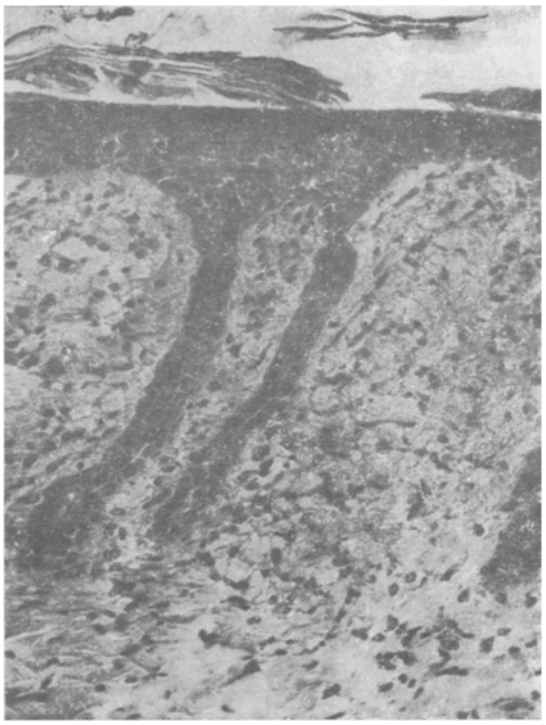

Fig. 1. Stage of formation of hair follicles 8 days after MMI of regenerating tissue. Hematoxylin and eosin. 160 ×.

formed can be approximated to those of normal skin with respect to several of its morphological features.

The aim of this investigation was to study the effect of measured mechanical injuries (MMI) to young regenerating skin at different stages of its formation in the place of a full-thickness wound by the dorsal skin in mice on the course of the repair process.

EXPERIMENTAL METHOD

Experiments were carried out on 100 male (CBA × C57BL)F_1 mice weighing 20-22 g. After epilation, full-thickness square wounds measuring 1 × 1 cm were inflicted in the center of the dorsal region, 1 cm caudally to the interscapular region. After complete epithelization of the wound (on the 17th-20th day after the operation) the mice were divided into three groups in order to choose regenerating tissues with different degrees of maturity. In group 1 the regenerating skin was subjected to MMI after 4-6 days, in group 2 — 14-16 days after complete epithelization of the wound, and in control group 3, the regenerating tissue was not exposed to MMI.

MMI was applied to the regenerating tissue by means of a dissecting needle. Full-thickness multiple punctures were made with this needle over the whole area of regenerating skin. At the time of the procedure the area of the epithelized surface of the regenerating skin was 0.19-0.22 cm^2. All operations were performed under ether anesthesia. The animals of the experimental groups were killed 10 at a time, and those of the control group five at a time, on the 5th, 8th, and 14th days after MMI. To determine the stages of maturity of the regenerating skin, five control animals also were killed on the day of MMI. Regenerating skin with the surrounding area was fixed in Carnoy's fluid and embedded in Paraplast. Histological sections 8-12 μ thick were stained with hematoxylin and eosin and with picrofuchsine by Van Gieson's method.

EXPERIMENTAL RESULTS

At the time of infliction of MMI on the animals of group 1, cells of the connective-tissue basis of the regenerating skin consisted of fibroblasts, mainly oriented parallel to the surface of the defect. As a result of secondary healing after MMI, complete epithelization of the defect occurred on the 5th day in these animals; the epidermis was hypertrophied in

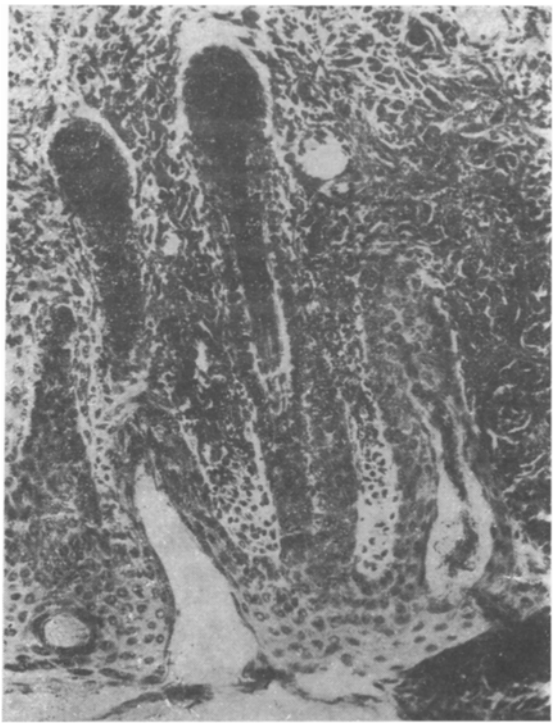

Fig. 2. New hair follicles 14 days after MMI of regenerating tissue. Picrofuchsine, Van Gieson. 160 ×.

places and gave short invaginations into the underlying connective tissue. Residual leukocytic infiltration was observed in the deep layers of the regenerating skin.

At different levels thin-walled blood vessels with a wide lumen were seen. On the 8th day the structure of the regenerating skin became regular, infiltration almost disappeared, but the type of regeneration taking place could not yet be easily identified. In three of the 10 animals stages of development of hair follicles were observed in the regenerating skin (Fig. 1). On the 14th day after MMI regeneration was of the dermal type, with the appearance of hairs and sebaceous glands at a relatively shallow depth (Fig. 2); fibers of its connective-tissue basis formed a complex structure, closely resembling normal skin. In four animals regeneration of dermal type was observed, in which fibrous structures formed a tightly looped network, very different from the structure of a scar. In three animals regeneration consisted of the formation of a connective-tissue scar.

At the time of infliction of MMI on the animals of group 2 the connective-tissue basis of the regenerating tissues consisted of thin collagen fibers, mainly arranged parallel to the surface of the defect, and fibroblasts with the same orientation. In the early stages of the investigation (5th and 8th days), just as in the animals of group 1, the type of regeneration could not be determined but the main differences from group 1 were that in group 2 the regenerating tissues were relatively richer in ground substance, and on the 8th day no rudiments of hairs could be found in any single animal. On the 14th day a connective-tissue scar formed in six mice, but regeneration of dermal type was found in only four animals.

In all the animals of the control group connective-tissue scars were observed at stages of formation corresponding to the times of investigation (Fig. 3).

The use of MMI of regenerating tissues at different stages of maturity thus changed the course of regeneration in the skin in 55% of cases and directed it along the path of organotypical regeneration. Pooled data for the two experimental groups were used in the calculations for the 14th day after MMI, because at earlier times the types of regeneration were still indistinguishable. When MMI was applied to regenerating tissues at the fibroblast stage of development (group 1), regenerating skin could be formed with the presence of hairs and sebaceous glands.

It is suggested that one of the factors influencing the outcome of the regeneration process is the changes induced by MMI in the tension lines in the regenerating tissue, which in

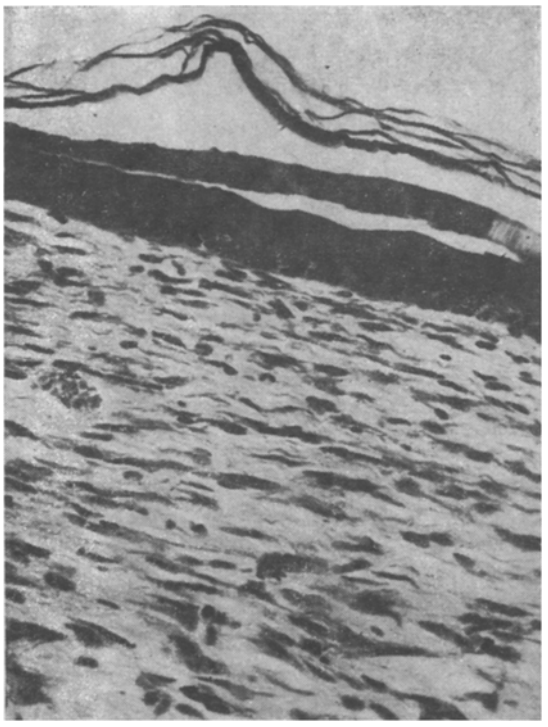

Fig. 3. Regeneration in the form of a connective-tissue scar, 26 days after wounding. Hematoxylin and eosin. 250 ×.

turn leads to reorientation of the fibroblasts and fibers, which form a reticular structure. As a result, interaction essential for the formation of skin derivatives becomes possible between the epidermis and connective tissue of the regenerating skin. In addition, the regenerative process in the skin is also undoubtedly influenced by the release of biologically active substances, activating the repair process, in response to multiple measured injuries [5].

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