REGENERATION OF SKIN AFTER ITS PRELIMINARY TREATMENT WITH CHEMICAL IRRITANTS (OZOKERITE OINTMENT)

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The work of A. N. Kruglov and N. A. Bychenkov [1] on rabbits showed that ultraviolet irradiation accelerates the healing of skin wounds, and that preliminary irradiation of the skin leads to accelerated healing of wounds subsequently inflicted. It is also known, from the work of A. D. Troitskaya [2], that the epithelium and connective tissue of skin which has been subjected to the action of chemical irritants retain their irritated condition after application of the chemical agent has been discontinued.

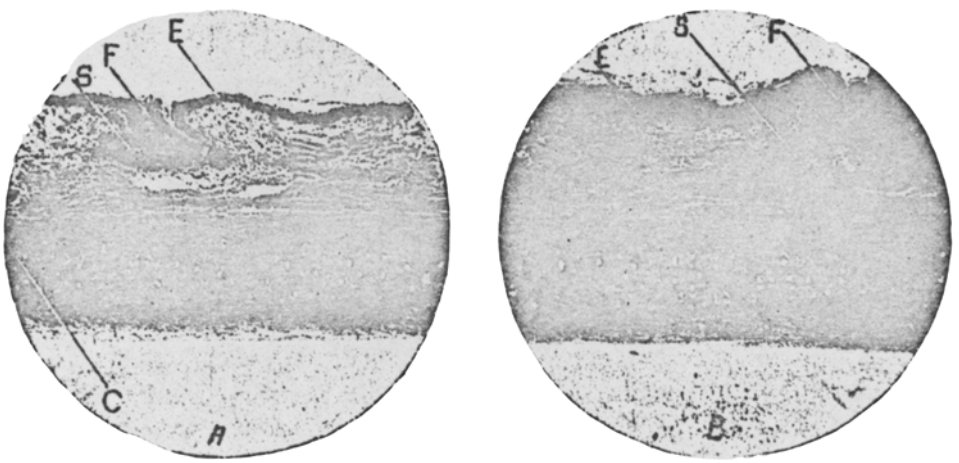

Fig. 1. Skin of the inner surface of the pinna of Rabbit No. 17 (A) control, (B) after daily inunction of ozokerite ointment for a week.
E) epidermis; F) hair follicle; S) sebaceous gland; C) cartilage. Photomicrograph, enlargement x 40.

The object of the present research was to investigate the effect on time of healing of a skin lesion of a preliminary treatment of the skin with a chemical stimulant of regenerative processes, for which purpose we used ozokerite ointment.

The experiments were performed on rabbits, and we studied the healing of penetrating wounds of the pinnae.

The histological material was fixed in 20% formalin (8% formaldehyde). Sections were prepared on a freezing microtome, and were stained with hematoxylin-eosin. Measurements were made with the aid of an ocular micrometer. Three series of experiments were performed on 20 rabbits.

In the first series of 9 rabbits one ear was treated a number of times with ozokerite ointment, after which circular pieces of tissue were taken from symmetrical sites of each ear by means of a punch. Histological study of the samples enabled us to assess the state of the skin at the time of infliction of the lesions. We then observed the healing of the lesion, and made a histological study of the regenerating tissues.

Histological study of the samples showed that the tissues of the treated rabbits were in a state of irritation at the time of wounding. Whereas the epidermal layer of the skin of the inner surface of the pinna of the untreated ear (Fig. 1,A) was made up of 2-3 rows of cells of the germinative layer and of an incomplete sheet of cells of the stratum granulosum, and was usually about 12-18μ thick, after 6-7 applications of ozokerite ointment it attains a thickness of 40μ, and consists of 6-7 rows of cells of the stratum germinativum and of a complete layer of granulosum cells.

No difference in the nature of the healing process could be observed between the treated and untreated skin. There were, however, striking differences in the rate of healing; the wound on the ear previously treated with ozokerite ointment healed much faster than the untreated ear. Thus, samples were taken for histological study from rabbit No. 4 nine days after wounding. Healing had proceeded very rapidly in the treated ear; the wound (diameter 3 mm) was fully healed at the time of taking the specimen, whereas the regenerating tissues in the other ear had barely made contact from opposite sides by that time. A wound 6 mm in diameter (Rabbit No. 3) was fully healed by the 21st day for the treated ear, but only by the 28th day for the control side, and the corresponding figures for rabbit No. 1 were 25 and 44 days. Whereas the regenerating skin of the control ear had barely closed the gap, the upper layers of the regenerated skin of the treated ear had already taken on the qualities of the dermis, and formation of hairs had commenced (Fig. 2).

Fig. 2. Early stage of fusion of regenerating skin growing in to the center of a skin defect of the untreated ear of Rabbit No. 16 on the 44th day after infliction of the wound (A). The skin defect was fully healed by the 25th day in the other ear, which had had 8 applications of ozokerite ointment before wounding; a hair is seen growing in the regenerated skin(B). Photomicrograph, magnification x 40.

In the second series of experiments we studied the effect on the rate of healing of applying ozokerite ointment before and after infliction of the wound.

We first applied the ointment a given number of times to one ear of a rabbit, and then inflicted identical wounds on both ears, after which the same treatment was given to the previously untreated ear. Specimens for histological study were taken simultaneously from both ears. Seven rabbits were taken for this series.

Each ear of rabbits Nos. 11 and 12 were given 7 inunctions, over a week. Specimens were taken from the regenerating areas and from undamaged areas of both ears, on the 16th day after wounding. Up till then, the skin of both ears was seen to be in an irritated state, which was more pronounced for the ear which had been treated

after wounding (as far as could be judged from the morphological state of the epidermis). The irritated condition of the tissues of the ear which had been treated before wounding appeared to have subsided, to a certain extent, 8 days after discontinuing the application of the ointment. Whereas the thickness of the epidermis of rabbit No. 11 was 9μ before application of the ointment, and of rabbit No. 12 12μ, and the epidermis consisted of 2 layers of stratum germinativum cells and an incomplete layer of granulosum cells, the thickness of the epidermis of the first ear at the time when the course of inunctions of the second ear was completed had risen to 22μ, and the number of rows of cells in the germinative layer to 4, and in the granulosum layer to one complete row. In the ear treated after wounding, the thickness of the epidermis was 28μ, and the number of rows of cells in the germinative layer was 5, and in the granulosum layer one. The thickness of the epidermis for rabbit No. 12 was 20μ, and the number of rows of cells of the germinative layer was 3-4, and of the granulosum 1 full row, for the ear which had been treated before wounding; for the ear treated after wounding the thickness of the epidermis was 24μ, and the germinative and granulosum layers consisted of 4-5 and 1 rows of cells, respectively. Epithelization had, however, proceeded further in the pretreated ear than in that treated after wounding; thus the scab on the pretreated ear of rabbit No. 12 had already been discarded, while at the same time that on the other ear was still adherent.

The later stages of healing of the pretreated ear were somewhat retarded, in comparison with the other ear; this is ascribable to subsidence of the irritated state.

Seven applications of ointment were made to ear of rabbit No. 8 before wounding, and the same number to the other ear after wounding. Sixteen days after infliction of the wound the remaining defect of the ear treated before wounding had a diameter of 3 mm, as compared with 1 mm for the ear treated after wounding. Rabbit No. 10 similarly received 7 applications of ointment. The wound in the ear treated after wounding was fully healed when the specimen was taken on the 49th day, at which time the margins of the regenerating epithelium of the pretreated ear had not yet made contact. The pretreated ear of rabbit No. 9 (7 inunctions) was fully healed 63 days after wounding, whereas the other ear had healed by the 44th day, and the corresponding times for rabbit No. 7 (10 inunctions) were 100 and 45 days.

It appears from the results of this series of experiments that stimulation of the tissues while regeneration is in progress is more effective, in the long term, than when the treatment is given before wounding. This effect may be ascribed to the relatively short duration of action of the stimulating agent after its application was discontinued. In these experiments the process of full healing of the skin defect extended over a considerable time (over a month). Hence, when the stimulating agent was applied before inflicting the wound its action was exerted only during the first part of the regenerative process. Since epithelization of the margins of the wound took place during the first few days of healing, regeneration of the epithelium fell within the period of subsequent action of the stimulating agent. For this reason, the process of epithelization was achieved sooner in the ear which had been treated before wounding, and whose tissues were already in a stimulated condition. Where the ozokerite ointment had been applied after wounding, the stimulating action of the agent was inadequate during the first days of healing; the process of filling up of the defect, which began after epithelization of the margins of the wound, and which depends on new formation of connective tissue, was in this case prolonged by the presence of the stimulant. This led to the more rapid conclusion of the process of healing of the wound defect in the ear which was treated with ozokerite ointment after wounding. Thus, identical treatment of the skin tissues with a chemical stimulant before or after wounding produced different effects. Application of the stimulant before inflicting a penetrating wound has a greater effect on the rate of epithelization of the margins of the wound, whereas its application after wounding leads to the more rapid filling up of the skin defect.

The third series of experiments was undertaken in connection with the results of the first two series, from which it appeared that induction of an irritated state of the tissues at the time of infliction of the wound accelerated subsequent regenerative processes, and that the action of the stimulant was of short duration after its application had been discontinued. In this connection it seemed necessary to perform experiments in which the action of the stimulant was continued until the wound was fully healed, beginning with different initial states of the tissues. Such experiments were performed on 4 rabbits, the left ear of two of which was treated daily with ozokerite ointment for a week, and the right ear of the other two. Holes were then made at corresponding locations of both ears, using a round (6 mm diameter) punch, and both ears were thereafter treated daily until healing was completed.

Histological examination of specimens taken on the 8th day of the experiment showed that the tissues of the ear which had been treated with the ointment were in a stimulated state, whereas those of the other, untreated ear had a normal appearance.

The pretreated ear of rabbit No. 17 was fully healed on the 28th day after infliction of the wound, whereas there still remained a barely visible opening in the other ear at this time. The wound in the pretreated ear of rabbit No. 18 was fully healed on the 26th day, and in the other ear on the 31st day; the corresponding times for rabbits Nos. 19 and 20 were 22 and 34 days for the pretreated ear, and 29 and 35 days for the other ear, respectively.

It thus appears that preliminary stimulation with the chemical agent, followed by its application during the whole of the healing process, leads to accelerated healing, as compared with treatment begun only after infliction of the wound.

In connection with this finding, we think it would be of advantage in operative surgery if treatment of the skin, where an incision is to be made, with substances stimulating regenerative processes were to be adopted as one of the preoperative procedures. This simple procedure might be carried out by the patient himself, on the instructions of the surgeon, or by a member of his household, while he awaits admission to a surgical ward for operation.

LITERATURE CITED

[1] N. A. Bychenkov and A. N. Kruglov, Vestnik Khir. i progr. Obl. 1929, No. 53, pp. 24-34.

[2] A. D. Troitskaya, Experimental and Clinical Studies of the Leningrad Dermatological-Venereological Institute * (Leningrad, 1949), Vol. 7, pp. 206-218.

*In Russian.