REPARATIVE REGENERATION OF THE NEURORECEPTOR APPARATUS OF THE RABBIT EAR SKIN UNDER THE EFFECT OF VARIOUS BIOGENIC STIMULATORS

(UDC 612.794:612.6.03].014.46-019)

A. D. Akramovskaya, I. I. Khvorostukhin,
L. N. Filatovich, and A. L. Liskovich

Department of Histology (Head-Docent I. I. Khvorostukhin), Grodno Medical Institute
(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 61, No. 4,
pp. 97-101, April, 1966
Original article submitted July 13, 1964

The problem of the regeneration of the neuroreceptor apparatus in the newly formed tissues of a skin wound is not only of theoretical interest but is also of value for practical medicine. It is known that when various stimuli, mechanical, thermal, and chemical, act on undamaged skin, various changes are observed in nerve elements leading to reorganization of skin innervation [2, 3]. Regeneration of the nerve structures in granulation tissue of a skin wound has been studied without the effect of a stimulator [1, 4]. We did not find in the literature any experimental histological works with testing of biogenic stimulators on regeneration of the nervous system of the skin.

In the present work, we undertook to study regeneration of the peripheral nervous system in the presence of penetrating wounds in rabbit ears under the effect of the following biogenic stimulators: ASD preparation (Dorogov's antiseptic stimulant) fractions II and III, Borislav ozokerite, and Dukor ozokerite. Dukor ozokerite, manufactured in Belorussia, has not been used in therapeutic practice and is investigated for the first time.

EXPERIMENTAL METHOD

Two penetrating wounds were made in each ear by a large metal stamp (7-mm in diameter). The following day we applied to the wound a 15% aqueous solution of ASD (II fraction), 15% ASD (III fraction on a base of cod-liver oil), Borislav and Dukor ozokerite with a temperature of 50° and as a 20% ointment on mineral oil. The exposure was 30 min. The effect of the solvent of a given stimulator: water, cod-liver oil, mineral oil, was the control in each series of experiments. The regenerates of wounds not subjected to the effect of the stimulator showed as the general control for all series of experiments. In all, we set up ten series of experiments.

Biopsies of the wound (170) were made by a large stamp (12-mm in diameter) at two periods—after 15 and 30 days. The material was fixed in a 12% solution of neutral formalin and impregnated with silver nitrate by the technique of Bielschowsky-Gros and Campos as modified by Rasskazova.

EXPERIMENTAL RESULTS

A study of the histological preparations of the near-wound areas of the skin made it possible to elicit that the skin of the rabbit ear in the norm is innervated by nerve bundles consisting of medullated and nonmedullated fibers with predominance of the latter which are mainly accompanied by vessels and send out branches to all skin derivatives. At the base of the ear are 8-10 nonmedullated nerve fibers surrounded by a rather wide common Schwann sheath which form powerful "cable" systems. In the connective-tissue part of the skin, epidermis, on the vessels, root sheaths, sebaceous glands are found only primitive receptors: intraepithelial and dendritically branched endings in the connective tissue, primarily of a polyvalent type. No encapsulated receptors were found.

The degree of intensity of regeneration of nerve elements in the various series of experiments was noted with consideration of the following indices: number of young nerve fibers with signs of regeneration, caliber of the nerve

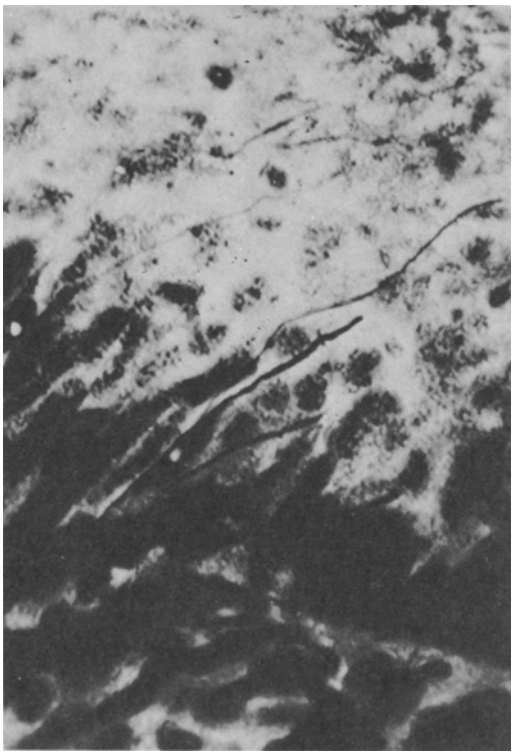

Fig. 1. Intraepithelial receptors in the form of pointed whisker-like endings in the newly formed epidermis. Rabbit No. 4. Fifteen days regeneration (after 12 applications of 20% ointment of Dukor ozokerite). Bielschowsky-Gros silver technique. Photomicrograph. Objective 60x, ocular 15x.

fibrils and bundles, density of their interweaving, depth of penetration of nerve elements into the regenerated tissue of the skin, branching and spatial distribution of terminals in the connective tissue, epithelium, hair bulbs, and on the vessels.

In the control experiments (without the effect of stimulators) individual(2-3 in the preparation) very thin, short fibrils with growth bulbs or points were often found on the 15th day after inflicting the penetrating wounds in the young granulation tissue near the old cartilage of the ear. Sluggish regeneration of individual fibrils was noted in the control with the effect of the solvents of the biostimulators, in particular water. In the experiments with the effect of mineral oil, and especially of cod-liver oil, regeneration of the nerve fibers was somewhat activated in comparison with the control. The number of solitary nerve fibrils increased slightly in the granulation tissue. In certain series we observed small bundles consisting of 3-4 fibrils with a tortuous course. The nerve fibers with the growth bulbs sometimes branched into 1-2 short twigs.

In the experiments with the effect of the biostimulators ASD (II, III fractions), Borislav, and Dukor ozokerite the process of regeneration was appreciably enhanced 15 days after the operation, i.e., the number of nerve elements increased and their morphology became complicated. In the specimens of the biopsies obtained in the experiments with the effect of 15% ASD (II, III fractions), applications of Borislav ozokerite with a temperature of 50° and as a 20% ointment on mineral oil, the number of nerve elements increased in the regenerating bundles. Along with the thin nerve fibers we found thickened fibers which extended far from the distal segment of the wound along the course of the proliferating capillary-type vessels under the newly formed epithelium where, on branching, they formed free nerve endings.

The application Dukor ozokerite with a temperature of 50° and especially as a 20% ointment lead to an even greater increase in the number of regenerating nerve fibers and their complication. Here, along with small bundles of nerve fibers we found large nerve bundles primarily along the course of the vessels of the granulation tissue. Here and there, the thick nerve fibers in the granulation tissue branched dichotomously into 2-3 thin,long branches and penetrated the newly formed epithelium, ending as pointed whisker-like terminals in the surface layers of the epidermis (Fig. 1). Thus, on the 15th day postoperation intraepithelial receptors were distinctly noted only in experiments with the effect of Dukor ozokerite.

On the 30th day of the experiment regenerative processes of the nerve elements were more strongly evidenced. The degree of regeneration of the nerve elements depended on the stimulators used, just as at the 15th day of the experiment. In the control (without the effect of stimulators) we could note nerve fibers of appreciable length and thickness which singly or as small bundles extended up to the newly formed epithelium and formed under the epidermis a few primitive short branches. In the preparations of the regenerates obtained in experiments with the effect of water, no particular deviations were observed in comparison with that described above. The number of nerve fibers and their ramifications increased under the effect of mineral oil and cod-liver oil.

Upon stimulation with preparation ASD (II and III fractions) and Borislav ozokerite we noted in the granulation tissue nerve fibers primarily with pointed ends or curved loops. Here and there in the nerve fibers we clearly noted neurofibrillation. Often immediately under the newly formed epidermis a few nerve fibers dendritically ramifying, formed simple receptor endings which sometimes penetrated the newly formed epidermis. In comparison with the regeneration processes of nerve elements in the above-described series of experiments, these processes were noticeably enhanced upon stimulation with Dukor ozokerite with a temperature of 50° and especially as a 20% ointment.

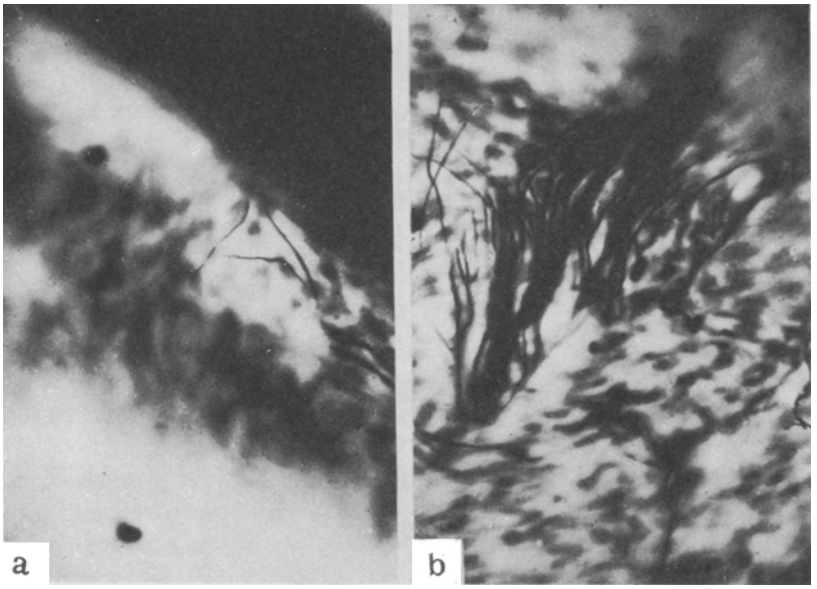

Fig. 2. Growth bulbs of regenerating nerve fibers in granulation tissue of ear skin. a) Several regenerated nerve fibers under newly formed epidermis. Rabbit No. 12. Thirty days regeneration (after 25 applications of 20% ointment of Borislav ozokerite); b) numerous nerve fibers with growth bulbs. The branches of the fibers penetrate the newly formed epidermis. Rabbit No. 2. Thirty days regeneration (after 25 applications of 20% ointment of Dukor ozokerite). Bielschowsky-Gros silver technique. Photomicrograph on comparison microscope. Objective 40×, ocular 7×.

As a rule, in the granulation tissue we noted many new fibers of different caliber. Some of them were very thin, hardly noticeable, or thick with well evidenced growth bulbs, and others had characteristics meandering and a tendency toward multiple branching, penetrating the epidermis (Fig. 2).

In the preparations of this series of experiments the intraepithelial receptors were represented as multiple dense branches of thin nerve fibrils spread over cells of the epidermis. In certain preparations of this series we noted in granulation tissue regenerated hair bulbs around which were numerous nerve fibers with growth bulbs.

Thus, reparation of the nerve elements upon regeneration of the skin in experiments with the effect of biostimulators occurs more intensely quantitatively and qualitatively in comparison with all control series of experiments of the same period (on the 15th and 30th day). In this case, the newly formed nerve fibers grow primarily parallel to the vessels of the granulation tissue, developing as vascularization progresses. The degree of regeneration of the nerve elements in the connective-tissue part of the skin and epithelium under the effect of various stimulators is different. The best regeneration was observed when we used Dukor ozokerite as a 20% ointment on mineral oil, it was somewhat less pronounced under the effect of 15% solution of ASD (II and III fractions) and Borislav ozokerite with a temperature of 50°.

LITERATURE CITED

1. G. I. Grishkun, Arkh. pat., No. 3 (1962), p. 53.
2. E. D. Gryazeva and I. G. Egorov, Transactions of the Fifth All-Union Congress of Anatomists, Histologists, and Embryologists [in Russian], Leningrad (1951), p. 596.
3. N. I. Zazybin, Transactions of the Fifth All-Union Congress of Anatomists, Histologists, and Embryologists [in Russian], Leningrad (1951), p. 597.
4. K. K. Sergeev, Exterceptors of Human and Mammal Skin [in Russian], Author's Abstract of Doctoral Dissertation, Rostov-on-Don (1963).