EFFECT OF MEASURED TRACTION ON REGENERATION OF ENDS OF AN INJURED NERVE: NEUROHISTOLOGIC STUDY

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The shortcomings of autoplasty, which has established its place as the method of choice in the treatment of peripheral nerve injuries, are responsible for the interest shown in nongrafting methods of replacement of their defects. Attempts at gradual elongation of segments of divided nerves beyond the terminal neuroma and glioma have been described [1]. However, the loose structure of these latter disturbed the fixation of the traction system to the distal segment, making it impossible to lengthen it [2], and this situation accordingly required doubling of the period of traction, and this led to an even greater increase in the period of denervation: this is an extremely adverse factor, for the time limit on the regenerative powers of the nerve allowed by nature is very small, and in surgical practice the optimal time for a nerve repair operation has to be within 2-3 weeks after trauma [3]. Under these conditions there appeared to be good grounds for using the technique of distraction of segments of the nerve trunk, developed at the All-Union Research Center for Restorative Traumatology and Orthopedics (Kurgan) [4], to be used for the treatment of recent injuries. The morphological characteristics of regenerative phenomena taking place under these circumstances are described below.

METHODS

A defect of the sciatic nerve measuring on average 32% of the length of the thigh was produced in 25 adult mongrel dogs proximally to the level of separation of the femoral nerve into its anterior and posterior divisions an Ilizarov's apparatus was applied to the limb and the joints were fixed in the mid-physiological position. Special attachments were mounted on the supports of the apparatus [5]. The nerve ends were fixed by epiperineural sutures to the conical tips of the traction pins. Seven dogs constituted the control series (without traction). Measured traction was applied to each nerve stump in the remaining animals from the 5th day after the operation by 0.25 mm twice a day The control animals and 10 dogs of the experimental series were withdrawn from the experiment on the 5th, 12th, 19th, 26th, and 33rd days. After replacement of the nerve defect, neurorhaphy was performed, and these animals also were sacrificed between 2 and 12 months later. The ends of the stumps of the injured nerves after fixation in 12% neutral formalin solution, were cut into transverse and longitudinal frozen sections for impregnation by the methods of Rasskazova and Bielschowsky-Gros, followed by microscopic study. Some of the material was fixed with glutaraldehyde and paraformaldehyde, postfixed in osmium tetroxide, and embedded in Epon—Araldite by the standard method. Ultrathin sections cut on LKB ultrotomes (Sweden), were stained with lead citrate and uranyl acetate and examined in the JEM-100B transmission electron microscope (JEOL, Japan).

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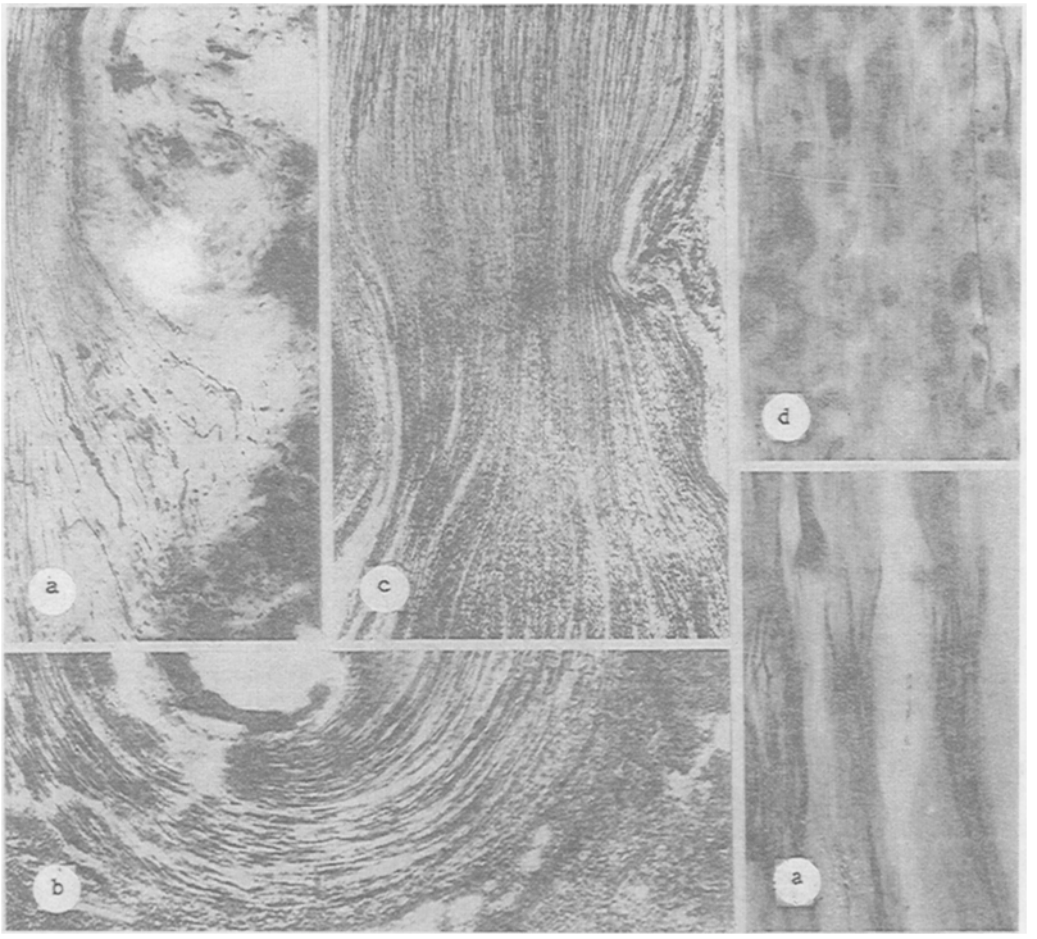

Fig. 1. Morphological changes in the proximal nerve stump at different stages of experiment. Impregnation by Rasskazova's method: a) zonal distribution of morphological changes of nerve fibers, 5 days after resection, 25 ×; b) regenerating axons at end of stump 19 days after resection. Control series, 15 ×; c) zone of free Bungner;s bands. Experimental series, 12th day of experiment, traction for 7 days, 15 ×; d) infiltration of free Bungner's bands by single nerve axons. Experimental series, 12th day of experiment, 7th day of traction. Objective 40, ocular 3.2, attachment 1.25; e) infiltration of free Bungner's bands by nerve axons. Experimental series, duration of experiment 19 days, 14th day of traction, 150 ×.

RESULTS

By the 5th day of the experiment a zone of injury 0.5-1 mm high, containing only fragmented nerve fibers and inhibited by erythrocytes, was located on the ends of the injured nerve. Proximally, in the central nerve stump a zone of retrograde degeneration extending for 2-3 mm could be seen (Fig. 1a), where corkscrew-like twisting, swelling, and sometimes fragmentation of the axons could be found. More proximally still, in the zone of reactive changes, the integrity of the nerve fibers was undisturbed, and they characteristically showed signs of arborization of the old axis cylinders. Thin young axons appearing here toward the 12th day of the experiment in animals of the control series grew as far as the lamina occludans, formed on the boundary between the end of the nerve stump and the fibrin clot filling the tip of the traction pin, and after turning through an arc of 180°, grow in the opposite direction. With an increase in the duration of the experiment the tendency for axons to turn in such an arc and grow in the recurrent

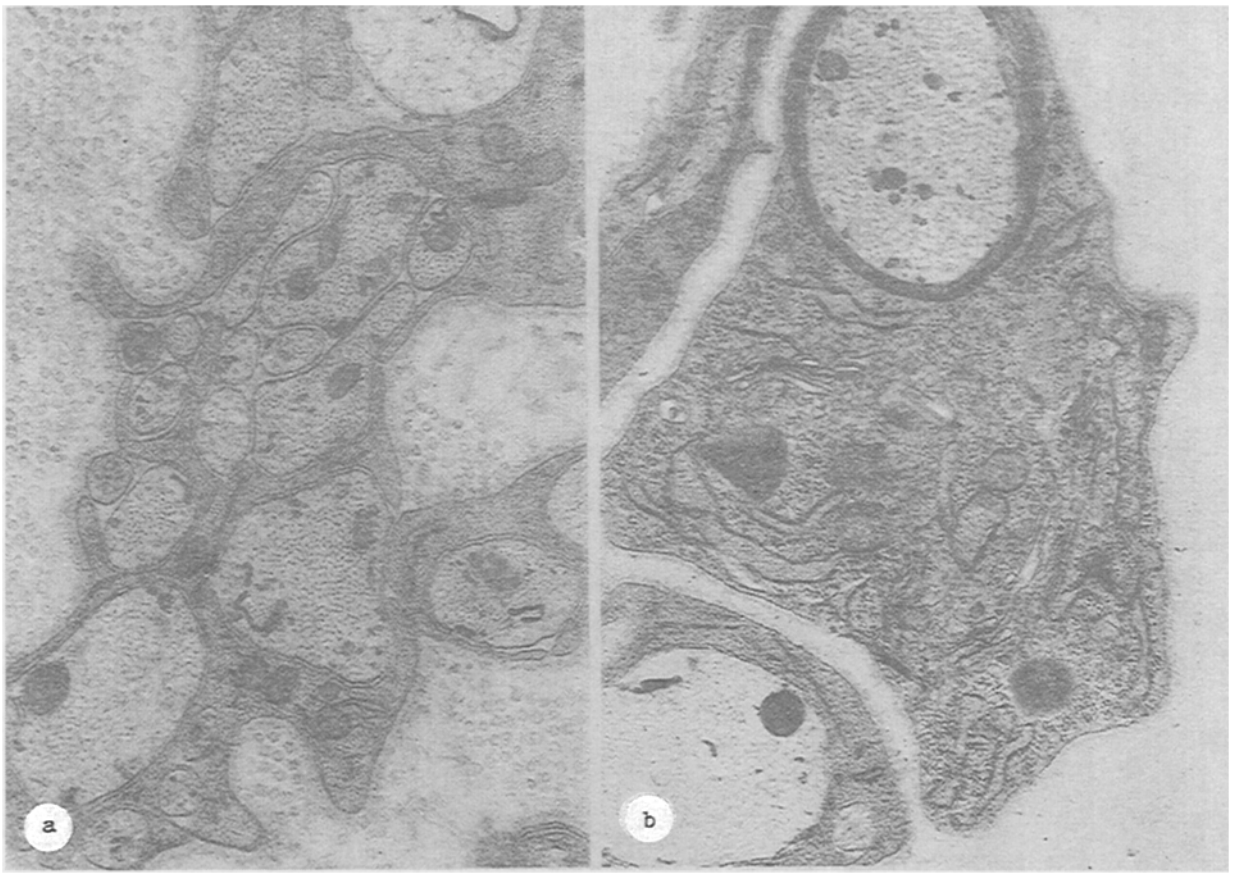

Fig. 2. Ultrastructure of regenerating nerve fibers in proximal stump before neurorrhaphy (47th day of experiment, 21st day of traction, 21st day of fixation): a) axon—lemmocyte relations, 22,000 ×; b) fiber undergoing myelination, 16,500 ×.

direction is intensified (Fig. 1b). This unique manifestation of excessive growth of nerve fibers, characteristic of injured nerves and due to our particular experimental conditions, leads in the usual case to the formation of a terminal neuroma.

Meanwhile, in animals of the experimental series, by the 12th day of the experiment a zone of free Bungner's bands appears as a result of 7 days of traction in the proximal nerve stump (Fig. 1c). This zone lies between the zones of retrograde degeneration and of reactively changed nerve fibers described above. Its longitudinal dimensions correspond to the elongation of the nerve stump achieved by traction until that time.

At subsequent stages of lengthening the number of axons growing into each Bungner's band increases significantly (Fig. 1e); most of them, moreover, are present in groups which are partly or completely surrounded by the cytoplasmic outgrowths of lemmocytes (Fig. 2a). Only a few axons with the largest caliber lie singly in their own mesaxon, and begin to undergo myelination toward the end of the experiment (Fig. 2b). Toward this time some young axons have reached the end of the stump, and a few of them turn back, but as in the control series, they do not form structures typical of terminal neuromas.

No signs of formation of a terminal glioma can be seen in the distal nerve stumps. Products of Wallerian degeneration (Fig. 3a) in the experiments with measured traction are evacuated more rapidly (Fig. 3b). Also, in the experimental series, a zone of Bungner's bands free from breakdown products is formed during traction at the level of the sutures fixing the nerve to the tip of the traction pin (Fig. 3c); just as in the proximal stump, this is a zone of traction-induced growth, where under the influence of the tension of traction, intercalation of the tissue structures of the nerve, including its membranes, takes place [7]. As a result, the fascicular structure of the nerve is reproduced

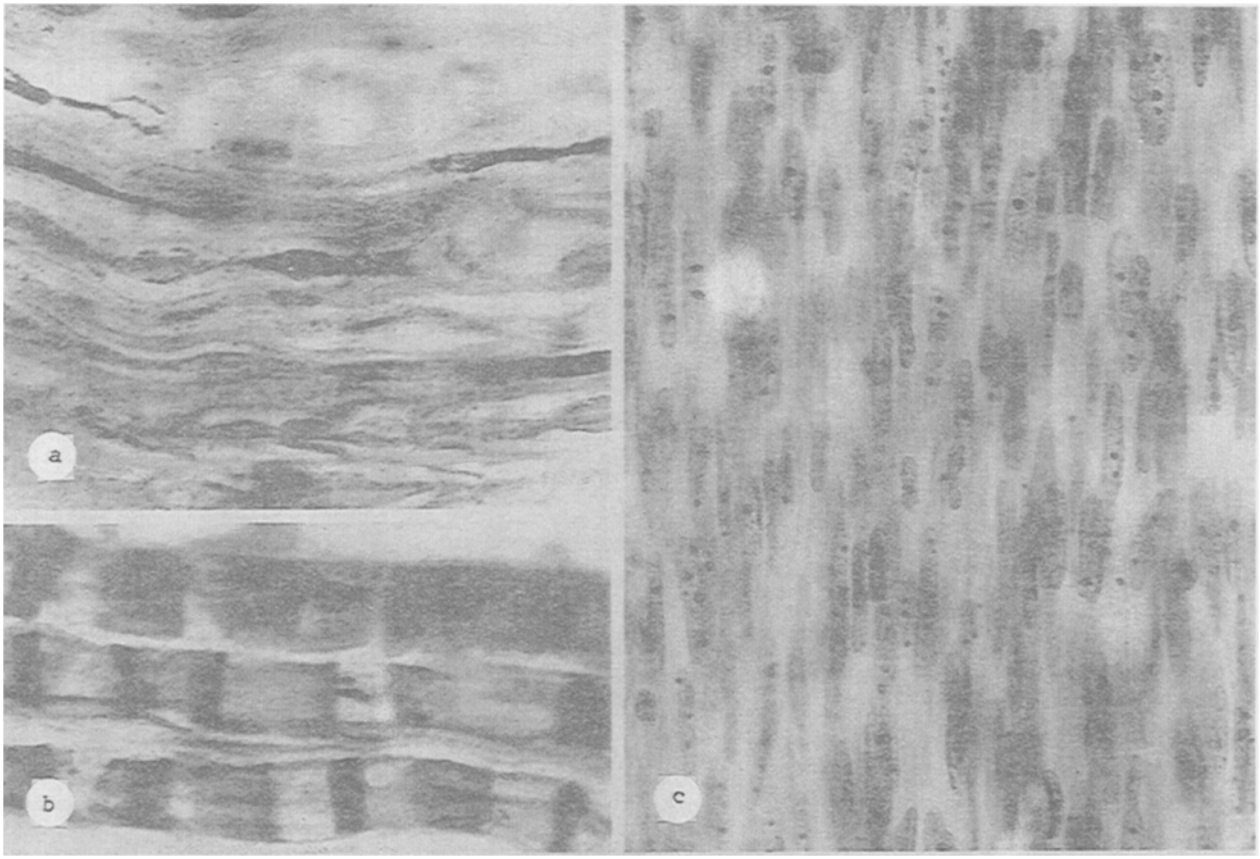

Fig. 3. Morphological changes in distal nerve stump at stages of experiment. Impregnation by Rasskazova's method: a) myelinated nerve fibers packed with products of Wallerian degeneration. Control series, 26th day of experiment; b) neurilemmal tubules of pre-existing myelinated nerve fibers, freed from axon breakdown products. Experimental series, 26th day of experiment, 21st day of traction; c) zone of traction-induced nerve growth. Experimental series, duration of experiment 33 days, 28th day of traction.

in the outgrowing segments, and strictly longitudinally oriented Bungner's bands fill the whole volume of the perineural tubules.

Lengthening of the stumps of a damaged nerve by the method described above is thus accompanied by intercalated growth of its membranes and Bungner's bands. The newly grown Bungner's bands have a strictly longitudinal orientation, and are free from breakdown products, and they therefore provide the best substrate for regenerating axons.

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