The possibility cannot be ruled out that the increase in the absolute number of mature viable activated AM in the respiratory part of the lung during inhalation of "inert material" (coal particles) is a key factor in the initiation of interstitial fibrosis. The population of activated AM, in our opinion, may play a direct part in the pathogenesis of anthracosis.

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TIME COURSE OF STRUCTURAL AND FUNCTIONAL RESTORATION OF THE SCIATIC NERVE AND OF SKIN RECEPTORS FOLLOWING REINNERVATION OF THE ALBINO RAT HIND LIMB

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KEY WORDS: regeneration; nerve; skin receptors; morphology; function

The problem of restoration of limb function after trauma to a nerve trunk is at the center of attention of morphologists, pathophysiologists, and clinicians. An abundance of experimental data has been gathered and is evidence of differences in the degree of effective repair of nerves depending on the techniques and materials used for reposition of the injured nerve [2-5, 14]. Data on the morphological features of regeneration of nerve fibers in different regions of the nerve after its injury and subsequent neuroplasty [1, 4] with replacement of the gap by various conducting sheaths [1, 6-8, 11-13], have been described in several publications. Attempts at morphometric analysis have been undertaken in order to explain the nature of the regenerating fibers after crushing and division [9, 10]. Chiu and co-workers [8], who used a method of joining together ends of a nerve by means of a venous autograft, carried out morphological investigations of the regenerative process together with electrophysiological observations. The authors cited studied and characterized the development of functional activity of the muscles in the injured animal limb by recording M responses. However, no comprehensive study of morphological and functional restoration of the conducting part of nerve trunks and of their receptor endings in the skin has yet been carried out during reparative

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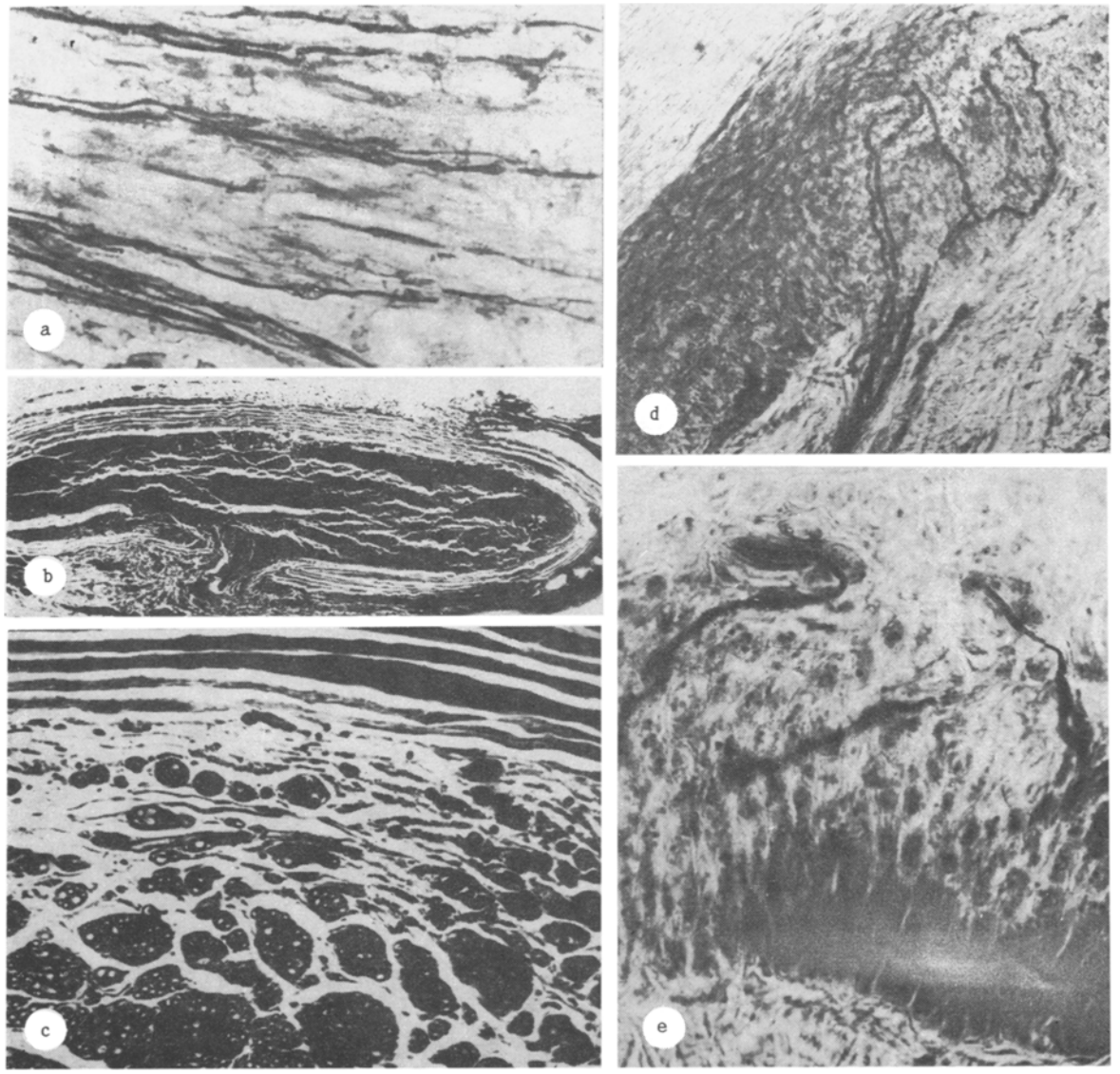
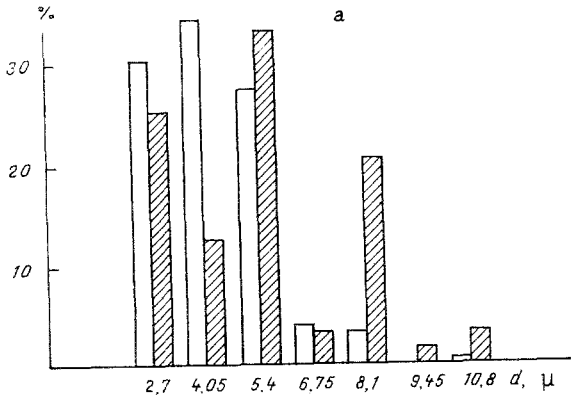


Fig. 1. Regenerating nerve fibers in lumen of implanted blood vessel into nerve endings in plantar skin of a rat at various times after operation. a) Myelinated regenerating axons after 30 days, Sudan black, 450 ×; b) lumen of implanted vessel filled with a dense mass of myelin fibers (after 9 months), transverse section stained with osmium, 75 ×; c, d) receptor endings in depth of plantar epidermis (9 months); c) Sudan black, 250 ×; d) silver impregnation, 560 ×.

regeneration, after division of a nerve and its subsequent sutureless union by means of an implanted artery. The investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on a model of sutureless joining of nerve fragments with the aid of an artery [6, 7]. In male Wistar rats and noninbred animals weighing 180-250 g a segment of the sciatic nerve 5-6 mm long was resected under pentobarbital anesthesia (4 mg/100 g body weight) at the level of the upper third of the thigh. To join the ends of the injured nerve fragments of the aorta of a donor rat, 1.2-1.5 cm long, were used. The distal and proximal ends of the divided nerve were introduced inside the implanted vessel, which was fixed to the epineurium by means of MK-7 glue. Material for histological investigation was taken 20 days and 1, 3, 6, 9, 11, 12, and 13 months after the operation and studied by the Bielschowsky-Gros and Kulchitsky methods, stained with Sudan black, and examined under the electron microscope. Quantitative analysis of the diameters of the regenerating myelin fibers was undertaken on serial sections impregnated with osmium. Parallel with the morphological investigations, electrophysiological studies also were undertaken. The nerve was divided



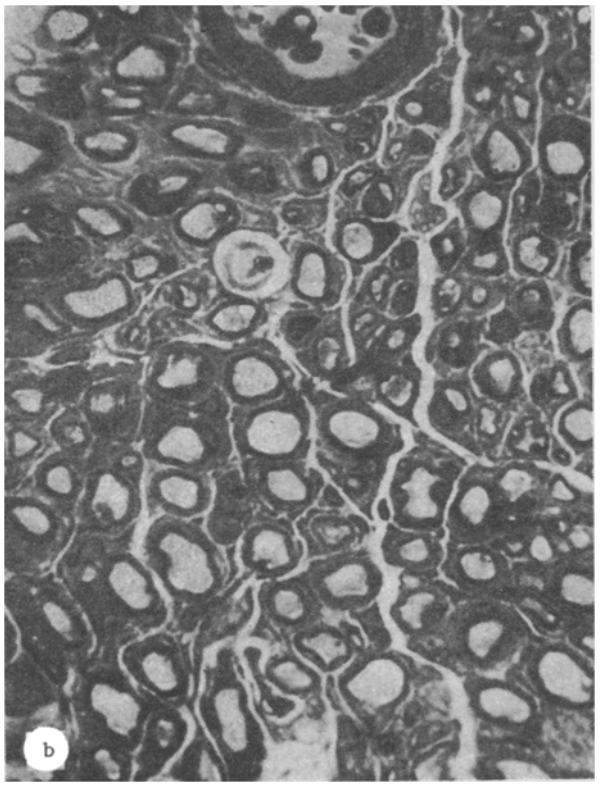


Fig. 2

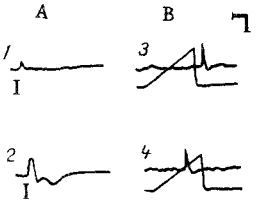


Fig. 3

Fig. 2. Distribution of diameters of regenerating myelin fibers in distal segment of nerve at various times. a) Histogram; unshaded columns — 3 months, shaded columns — 9 months; b) profiles of myelinated fibers in semithin (1 μ) section (9 months), azure-2-methylene blue. 621 ×.

Fig. 3. Combined action potential of sciatic nerve (A) and spike activity (B) from receptor endings of plantar skin during their regeneration. 1) 6 months, 2) 11 months, 3) 9 months, 4) 13 months later. Time marker: for 1 and 2) 1 msec, for 3 and 4) 10 msec.

proximally to the vascular anastomosis. The distal segment of the nerve was placed on a platform consisting of two isolated cells with silver recording electrodes and filled with Hanks' solution. Under a binocular microscope individual fibers from the nerve trunk were dissected with needles and carried over the partition into the neighboring cell. The skin surface was stimulated by means of an electrodynamic mechanical stimulator. The duration of the stimulus was 50 msec and its amplitude from 100 to 2000 μ. The complex action potential (AP) from the nerve was recorded from the segment distal to the vascular anastomosis. The duration of the electrical stimulus was 0.25 msec. Spike activity (SA) was recorded from single fibers from the region of the nerve proximally to the implanted vessel. After preamplification the bioelectrical signals were led to the input of a cathode-ray oscilloscope for subsequent photographic recording.

EXPERIMENTAL RESULTS

Connective tissue grew from the surface of the nerve 20 days after the operation on to the surface of the implanted vessel, guaranteeing their firm union. In the lumen of the vessel macrophages, fibrin, and granulocytes accumulated, and this was accompanied by intensive growth of capillaries. Numerous fine (0.8-1 μ), diffusely arranged nerve fibers, aiming for the distal segment of the nerve, appeared from the proximal segment of the nerve. Abundant myelin breakdown products, macrophages, and newly formed Buengner's bands of lemmocytes were present in the distal segment of the nerve.

After 30 days nerve fibers inside the implanted vessel were loosely arranged, some of them running in a straight line, others in a spiral along the wall of the vessel. Before emerging from the vessel they joined into bundles of nerve fibers and entered the distal trunk. The diameter of the nerve fibers was increased to 1.5-2 μ due to the formation of myelin sheaths around the regenerating axons (Fig. 1a).

After 3 months the number of myelinated nerve fibers in the lumen of the vessel was considerably increased and they formed bundles of varied thickness. The diameter of the fibers at this period was increased to 2.5-4 μ. However, wide intercellular spaces, in which blood vessels (capillaries, venules, and arterioles), macrophages, and mast cells and plasma cells could be seen, were still present between individual bundles. After 6 months the diameter of the fibers within the vascular anastomosis reached 4-6 μ and their number per unit area increased sharply. In the 9th month the gap was replaced by a dense mass of newly formed myelinated and unmyelinated fibers (Fig. 1b), i.e., part of the nerve trunk located in the lumen of the implanted vessel constituted a single entity with the proximal and distal segments of the nerve. Thus by this time complete anatomical repair had taken place.

Quantitative analysis of the composition of the regenerating fibers in the distal segment revealed a definite time course of their structural restoration (Fig. 2a). Until 2.5 months after the operation most of the myelinated nerve fibers did not exceed 2.5 μ in thickness. After 3 months 92.4% of fibers had a diameter of 2.7-5.4 μ. After 9 months 69.8% of them attained a diameter of 4.05-8.1 μ; the number of fibers 2.7 μ in diameter showed a small decrease (by 5%) whereas the number with a diameter of 6.75-8.1 μ was increased more than threefold. The appearance of a population of thick fibers 9.45-10.8 μ in diameter was also noted (4.8%). It will be clear from Fig. 2b that most regenerating nerve fibers after 9 months were already quite well differentiated, although their myelin sheaths were still thinner than in the control.

At the periphery of the limb, just as in the sciatic nerve after its division, destruction of nervous apparatuses was observed. Terminal portions of receptors and their myelinated conducting fibers were the first to degenerate (after 5-7 days), and this was followed by disintegration in the large bundles and trunks in the dermis and subcutaneous cellular tissue. Myelin breakdown products were found in them sometimes after 1.5 months or more. Regeneration of axons took place along conducting pathways which remained, i.e., trunks and bundles, surrounded by perineural sheaths, and filled with bands of lemmocytes. The first, very thin regenerating myelin fibers began to appear in the plantar tissues of the injured limb after 2.5 months. After 6 months their number increased sharply and they ranged in diameter from 1.5 to 4 μ. At these times a few encapsulated and diffuse receptor endings could be clearly seen in the plantar skin (Fig. 1c, d). After 11 months the density of the nerve plexuses was increased and the number of receptors rose sharply.

The electrophysiological investigations showed that until 6 months there was no bioelectrical activity in the nerve. Combined action potentials were first recorded from the nerve trunk 6 months after the operation. They occurred 0.3 msec after electrical stimulation. Unlike the multiphase action potential characteristic of the intact nerve, AP in the regenerating nerve at this time consisted of a positive phase only (Fig. 3). After 9-11 months the configuration of the response and its amplitude and temporal parameters changed. The AP assumed the characteristic picture of AP of the nerve of an intact animal. However, to obtain it, the intensity of the electrical stimulus had to be increased. Finally, after 12-13 months the temporal and amplitude parameters and also the composition of AP were similar to those of intact nerves.

Recording of spike activity showed that this can be done for the first time 9 months after the operation. Under these circumstances the stimulus had to be applied through a needle 150 μ in diameter, with a maximal amplitude of displacement of the needle of 2000 μ. A distinguishing feature of the response was that it consisted of a series of spikes and its duration was

30-40 msec. Characteristically, the duration of each spike in the response was longer (3 msec) than that obtained by recording AP from single fibers in intact animals. After 11 months the sensitivity of the receptors was increased. Responses were recorded to application of the stimulus to the plantar skin surface by means of a mechanostimulator with rod 1.5 mm in diameter, although the amplitude of its displacement remained quite high (2000 μ). Spikes were observed with a considerable delay after application of the stimulus (54 ± 3 msec), but the duration of each spike was reduced to 1-2 msec. It was only 13 months after the operation on the rats that responses with the minimal latent period (28 ± 4 msec) were recorded, the sensitivity of the receptors was restored to its initial level, and the amplitude of displacement of the glass rod was 100 μ.

Comparison of the morphological and electrophysiological data shows that the process of nerve regeneration after bridging of the gap in it by means of a blood vessel continued for at least 9-13 msec and was characterized by differences in the time of structural and functional recovery. Growth and differentiation of axons, the formation and myelination of the nerve fiber, and formation of receptor endings took place initially. Next followed a period of structural maturation, and only after certain qualitative and quantitative changes had occurred did a period of their near-normal functional activity supervene. The results can be regarded as an objective criterion for the evaluation of completeness of reinnervation of the limb tissues, and the proposed model, based on the use of an arterial implant as a conducting pathway for regenerating axons, can be introduced into neurosurgical practice for use during operations for replacement of extensive gaps in a nerve.

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