PERIPHERAL NERVE REGENERATION IN THE LUMEN OF IMPLANTED BLOOD VESSELS

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The problem of regeneration of fibers of the peripheral nervous system and restoration of the functions of denervated organs and tissues in man and animals is of great interest to neurobiologists and clinicians [1-5, 7]. New methods of investigation and new experimental models have been developed to study some of these problems. A promising method is by guided control of the growth of nerve fibers along artificial pathways and channels. Attempts to create the conditions for guided regeneration of peripheral nerves have begun in the last decade. Golub et al. [3, 4] discovered that internal organs can be reinnervated by gangliopexy and neuropexy. In the operation to reinnervate the tongue [6] the proximal end of the patient's injured lingual nerve was guided into the lumen of the intact lingual artery. The authors cited interpreted restoration of lingual function as a result of reinnervation of the organ by the nerve regenerating along the vessel, but morphological confirmation of this phenomenon was not presented. Attempts have been made to use artificial sleeves made from silicone tubes [10] and also sleeves made of natural animal tissue [9] to assist regeneration of nerve trunks. In the last of these investigations, in experiments on rats a "mesothelial tube" was created around a stainless steel spring, implanted beneath the dorsal skin in experiments on rats, and this was then transplanted into the thigh and the proximal and distal ends of the divided sciatic nerve were introduced into it. However, the many stages of such an operation, the creation of the "mesothelial sleeve" around the foreign object, and the repeated transplantations required make this method cumbersome.

The most convenient and adequate method, in the writers' view, is guided regeneration of the nerve trunk in the lumen of an implanted blood vessel. Recently [8] an autogenous graft of femoral vein 1-1.5 cm long was used; the ends of the divided sciatic nerve of the rat were sutured into it. However, division of the main limb vein may give rise to complications in the animal, and even more so, in man.

The object of this investigation was to develop a model of guided regeneration of peripheral nerves in the lumen of an artery, removed from another animal, implanted into the tissues of the thigh (allogeneic transplantation).

EXPERIMENTAL METHOD

Experiments were carried out on 26 Wistar rats weighing 200-250 g; 12 animals served as the control. The channel for guided regeneration of the divided sciatic nerve consisted of large veins and arteries taken from donor rats. Preliminary experiments showed that the most convenient substrate for these purposes was the descending part of the aorta. This vessel differs from veins in its elasticity, the fact that its lumen does not collapse, and that somewhere along its length it is easy to find a segment of the required diameter, corresponding in thickness to the injured nerve. At the beginning of the experiment, under ether anesthesia the descending aorta was dissected in the donors and placed in Hanks' solution at 37°C. In the other animal, under pentobarbital anesthesia (5 mg/100 g body weight) the sciatic nerve was divided 2 cm above the knee. The proximal end of the nerve was introduced for 2-3 mm into the lumen of a segment of the implanted aorta 1.5-2 cm long and fixed to the vessel wall with

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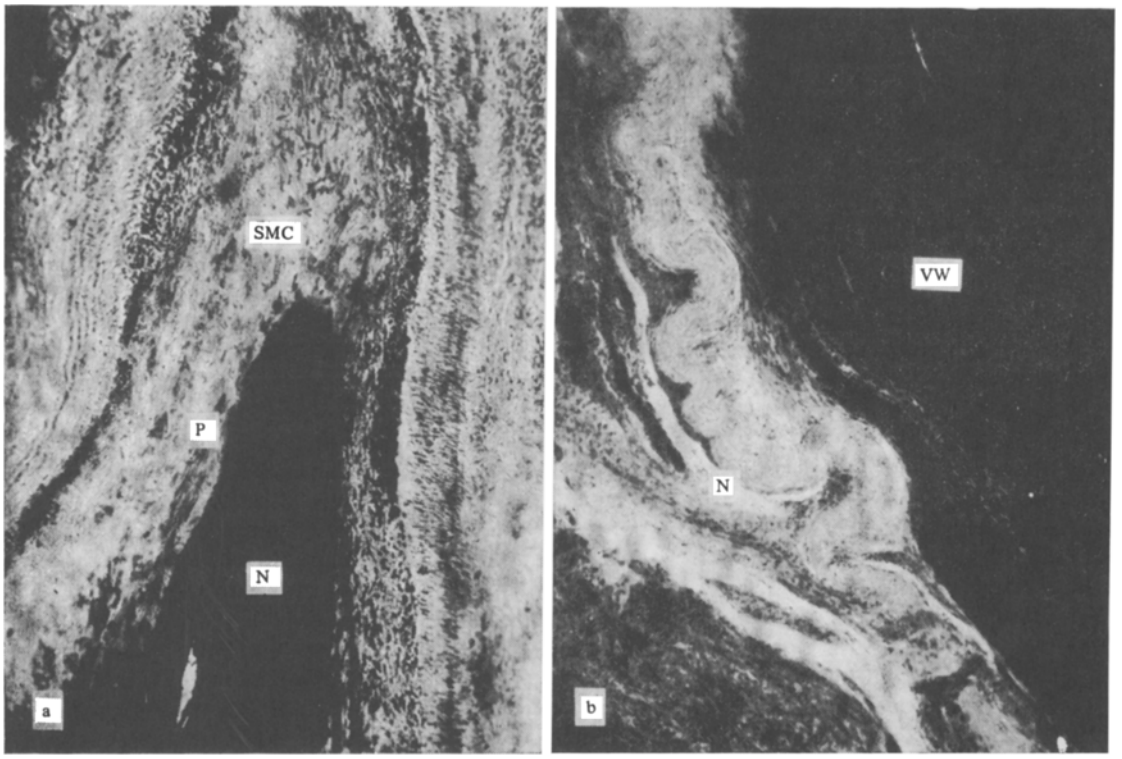

Fig. 1. Regenerating nerve trunk in lumen of aorta: a) tangential section through vessel after 1.5 months of regeneration: P) perineurium on boundary with endothelium, SMC) transverse and longitudinal bundles of smooth muscle cells of vessel wall, 130 ×; b) longitudinal section through vessel after 3 months of regeneration: VW) vessel wall. 90 ×. N) Nerve. Here and in Figs. 2 and 3, impregnation with silver by Bielschowsky–Gros method.

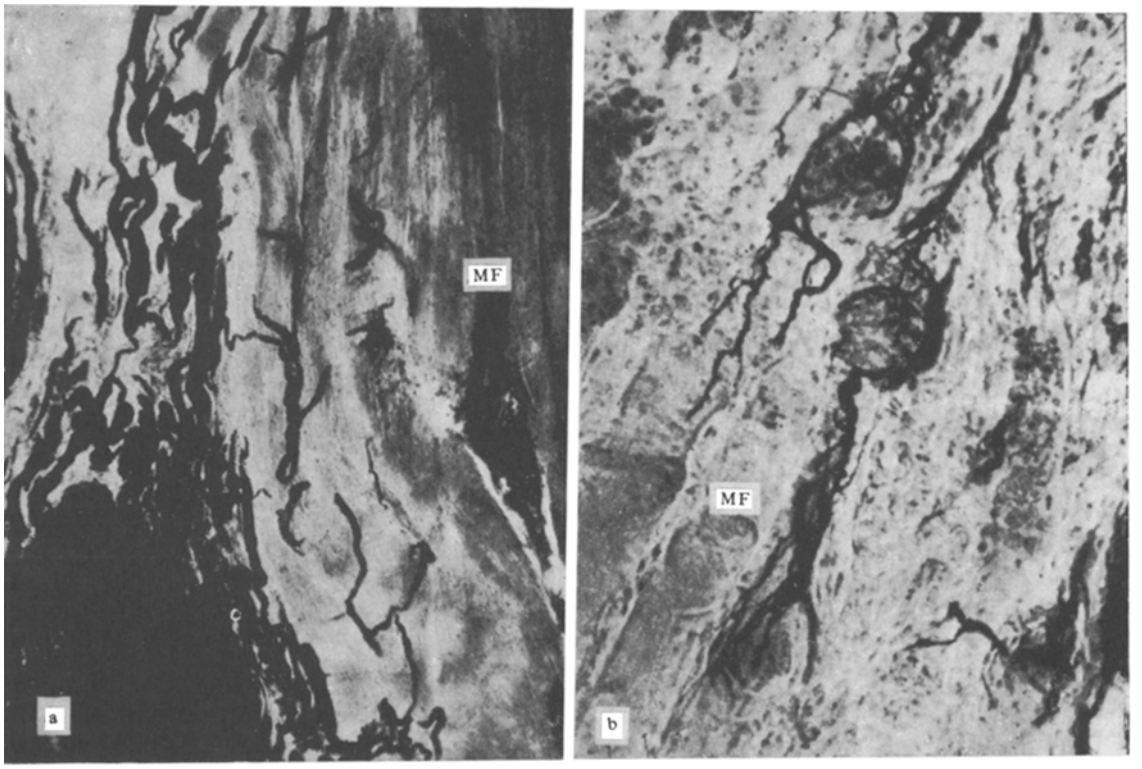

Fig. 2. Distal part of regenerating nerve, emerging from lumen of vessel. a) Bundles of regenerating nerve fibers among muscle fibers, 130 ×; b) helical windings of regenerating nerve fibers on muscle fibers, 340 ×. MF) Muscle fibers.

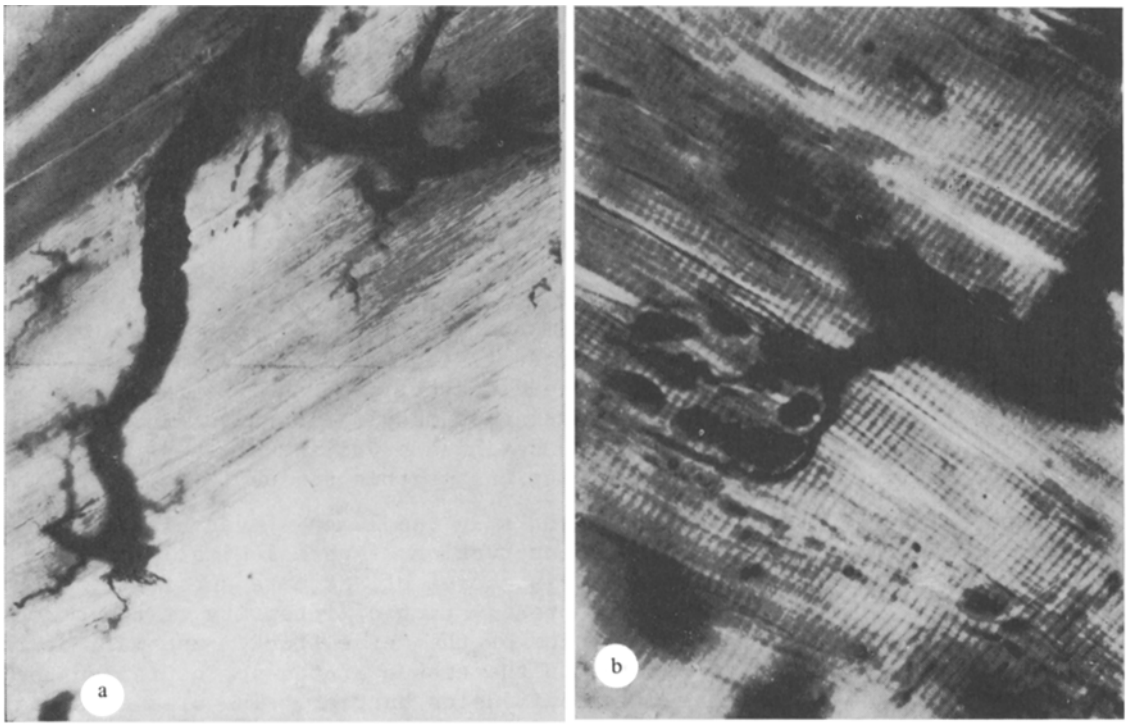

Fig. 3. Endings of regenerating nerve fibers on striated muscles: a) arborization ramifications, 90 ×; fragments of neuromuscular junction, 580 ×.

surgical glue MK-7. The aortic implant with the nerve was placed among the denervated thigh muscles and the wound was sutured in layers. The nerve was simply divided in the control animals and its ends diverged in different directions. The experimental and control animals were killed after 1, 1.5, 2, 3, and 4 months. The nerve with the aortic implant and the surrounding tissues were removed for morphological investigation and the material was treated by the usual histological and neuromorphological techniques.

EXPERIMENTAL RESULTS

After division of the sciatic nerve disturbances of the motor function of the affected limb were observed in animals of both control and experimental groups. The rat drew the limb up toward the abdomen, could not bear weight on it, and limped while running. After 1-2 weeks trophic disturbances appeared in the form of loss of hair from the limb with ulcers on the leg and foot. After 3 months restoration of motor function in the experimental animals was almost complete: they could draw the limb up toward the abdomen and use the flexed limb for walking and running. In the control rats the disturbances of gait persisted until 4 months, although they were less marked than immediately after the operation. In the experimental animals the trophic disturbances disappeared sooner, but in the control rats they lasted until 3 months.

The results of the morphological investigations showed that the nerve, placed inside an aortic implant, does not form neuromas, its axons regenerate well and, having grown along the whole length of the segment of the vessel, emerge from its distal end into surrounding muscles. Examination of preparations impregnated with silver shows clearly that the overwhelming majority (up to 90%) of the nerve fibers are present in the lumen of the aorta, and are arranged in the form of a single nerve trunk (Fig. 1a, b). Axons of the degenerating nerve trunk are separated from the vascular endothelium by a newly formed perineurium, through which single thin capillaries pass in certain places. A few nerve fibers (10%) separated from the common nerve trunk as thin branching bundles and grew out toward the perineurium. In this way the pattern of formation of a loose subperineural plexus around the nerve trunk was formed. Some nerve fibers of this plexus, in the form of thin bundles, even grew into the folds of the elastic membrane of the implanted vessel and terminated there in arborization-like ramifications and diffuse bush-like endings. However, the nerve fibers did not penetrate beyond the elastic membrane.

Around the implanted vessel, after the first few days inflammatory infiltration by neutrophilic granulocytes, monocytes, macrophages, and mast cells appeared. During the first 2

months the zones of infiltration were located in the adventitia and smooth-muscle membrane of the vessel as far as the elastic membrane, but outside the membrane and inside the lumen of the vessel they were absent. During this time a new perineural sleeve formed around the regenerating nerve. Only single inflammatory cells were observed inside the vessel.

From 1.5 to 2 months after the operation regenerating axons, both inside the vessel itself and emerging from it into muscle tissue, became myelinated and grew to 3-4 μ in diameter. On emerging from the vessel the nerve trunk divided into separate thin bundles, running in different directions between bundles of muscle fibers (Fig. 2a). The course of some of them could be traced for a long distance in the surrounding muscle tissue as far as their endings. Each bundle consisted of myelinated fibers surrounded by a thin perineurium. On approaching the muscles the nerve fibers formed either characteristic spiral coils on them (Fig. 2b) whose thinner twigs could be traced for a considerable distance, or bush-like ramifications. After 2-3 months bundles of myelinated fibers terminating on muscle fibers in arborization ramifications could be seen (Fig. 3a). Examination under high power showed that their terminal portions form the characteristic neuromuscular junctions of this tissue (Fig. 3b).

Guided regeneration of peripheral nerve trunks in the lumen of vascular implants thus provides facilities for rapid and effective reinnervation of pathologically changed tissues and organs. The decisive factor in such cases is choice of adequate substrate, which must satisfy several demands: first and foremost, preservation of integrity of the structural organization and maintenance of the internal medium of the nerve trunk. The wall of the guiding sleeve must also correspond in its properties to the tissue of the perineural epithelium, which performs the functions of a nerve-tissue diffusion barrier. The sleeve must also perform a protective function against penetration of surrounding inflammatory cells into the nerve. The wall of arteries and, in particular, of the aorta satisfied these demands most closely. The endothelial lining of the vessel helps to maintain the internal microenvironment at a constant level long enough for the new perineurium to form around the regenerating nerve trunk. The absence of a neuroma at the end of the nerve introduced into the lumen of the vessel allows unimpeded growth of nerve fibers guided in one direction. Normal differentiation of regenerating nerve fibers takes place, followed by myelination, perineurium formation, and vascularization. All these create good conditions for rapid reinnervation of the surrounding tissues to an adequate extent.

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