DEVELOPMENT OF METHOD OF UNITING SEVERED NERVES WITH THE AIM OF RESTORING THEIR INTEGRITY

E. I. Chumasov, K. M. Svetikova, and V. I. Gusikhina

UDC 616.833-001.4-089.84

KEY WORDS: regeneration; peripheral nerves; implantation of vessels.

Definite progress has been made with the study of regeneration of nerve trunks [3, 5, 12], but it still remains an urgent task in medical practice. New techniques and approaches for its solution are currently the subject of intensive research. Methods of reinnervating the internal organs by means of gangliopexy and neuropexy, by heterogeneous regeneration [2, 5], and by autoneurotransplantation [1, 5] are being developed with some success. One promising direction of the study of nerve regeneration is the development of methods directing and regulating growth of axons along artifically created pathways. Attempts to create guiding pathways by means of decalcified bone, muscle, formalinized nerves, and guides made of metal, resin, gelatin, and other materials, have not given positive results [4, 7, 14]. Defects of many of these investigations are, as the authors themselves admit, the incompleteness of regeneration of nerves as a result of neuroma formation and the development of an inflammatory reaction and fibrosis.

In neursurgical practice cases of severe injury to nerve trunks with the formation of wide gaps between the fragments are of frequent occurrence. These lesions arise both during operations necessitating wide resection of a proximal neuroma or a dense scar (schwannoma) on the distal stump [3, 12]. These gap defects can be replaced only by continuous union of the injured ends of the nerves. This is an important, but technically very difficult, problem in nerve regeneration.

Attempts have begun both in the USSR and elsewhere to use guiding sheaths made of biologically inert materials to assist regenration of nerve trunks: millipore, silicone, and acrylic copolymer membranes [10, 13], and also sheaths of natural materials — mesothelial sheaths [8], and transplanted veins [9] and arteries [6].

The aim of this investigation was to develop a sutureless method of uniting the ends of a severed nerve by means of implanted vessels, and to study reparative regeneration of a nerve along artificially created pathways.

EXPERIMENTAL METHOD

Experiments were carried out on 120 male Wistar and noninbred rats weighing 180-200 g. Large arteries of rats were used as channels for directing growth of nerve fibers and for uniting the ends of a severed nerve. Preliminary data showed that the most convenient vessel for these surfaces is the descending aorta, which possesses high elasticity, and along the whole of its length it is easy to select a segment of the required diameter, corresponding in thickness to the nerve. The aorta was dissected in donor rats, placed in Hanks' solution, and freed from adventitial tissue. A length (5-6 mm) of the sciatic nerve was resected in the recipient animals under pentobarbital anesthesia, and the proximal and distal ends of the nerve were fitted in turn inside the implanted vessel for a distance of 1-2 mm. To prevent the ends of the nerves from spontaneously falling out of the vessel, the edges of the vessel wall were fixed to the epineurium by means of MK-7 surgical glue. A diagram showing how the nerve ends were joined by means of a blood vessel is given in Fig. 1. The wound was sutured in layers. As a control, in some animals pieces of nerve were resected, in other animals (six rats) empty fragments of blood vessels were transplanted

Laboratory of Experimental Histology, Department of Morphology, Institute of Experimental Medicine, Academy of Medical Sciences of the USSR, Leningrad. translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 9, 374-377, September, 1986. Original article submitted July 11, 1985.

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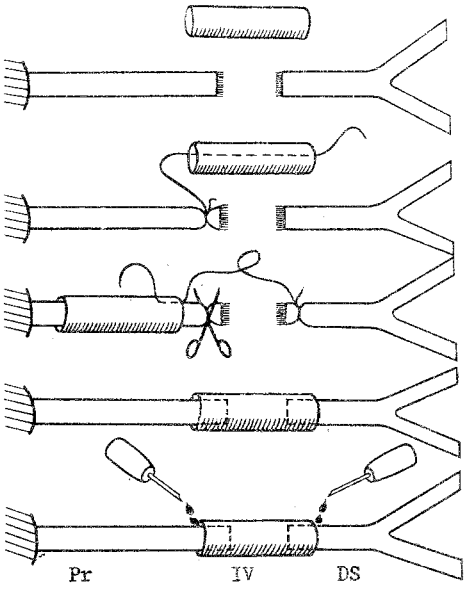

Fig. 1. Diagram showing successive stages of union of proximal (PR) and distal (DS) ends of severed nerve with an implanted blood vessel (IV).

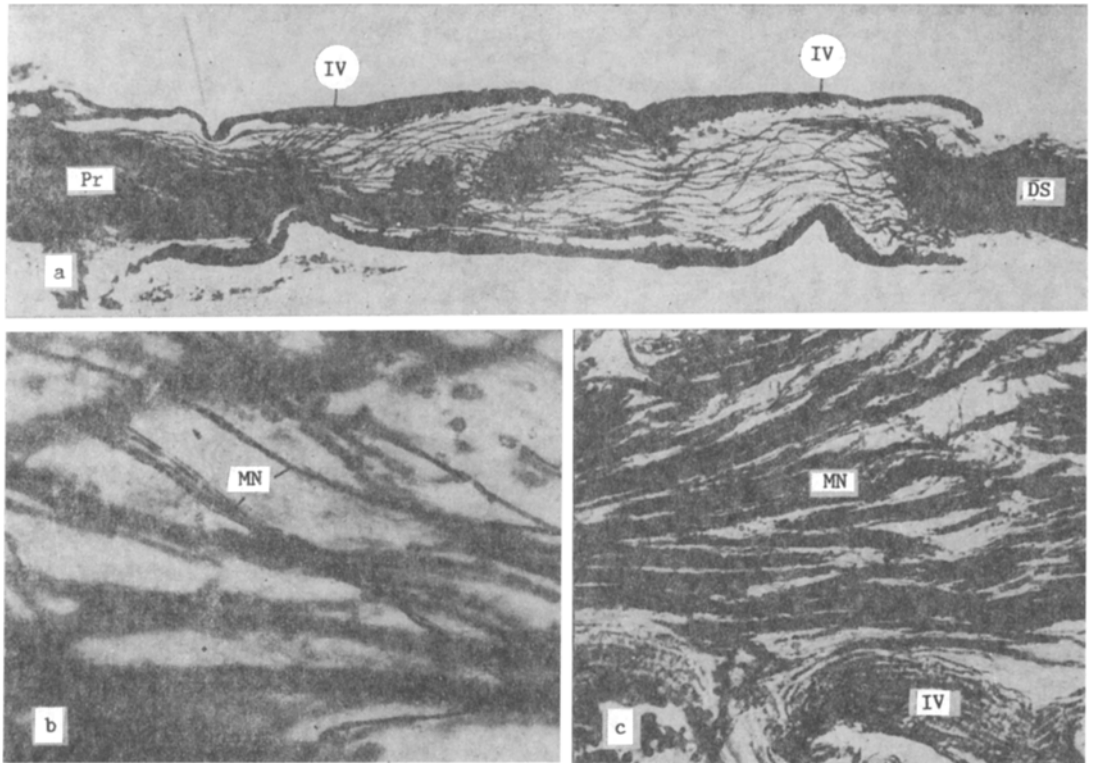

Fig. 2. Regenerating nerve fibers in lumen of implanted vessel: a) longitudinal section through implanted vessel and nerve (1 month after operation, general view). Impregnation with silver. 50 ×; b) Myelinated nerve fibers (MN) in lumen. Osmium-bichromate method. 560 ×; c) Numerous bundles of myelinated fibers (MN) in lumen of vessel 3 months after operation. Sudan black. 130 ×. Remainder of legend as to Fig. 1.

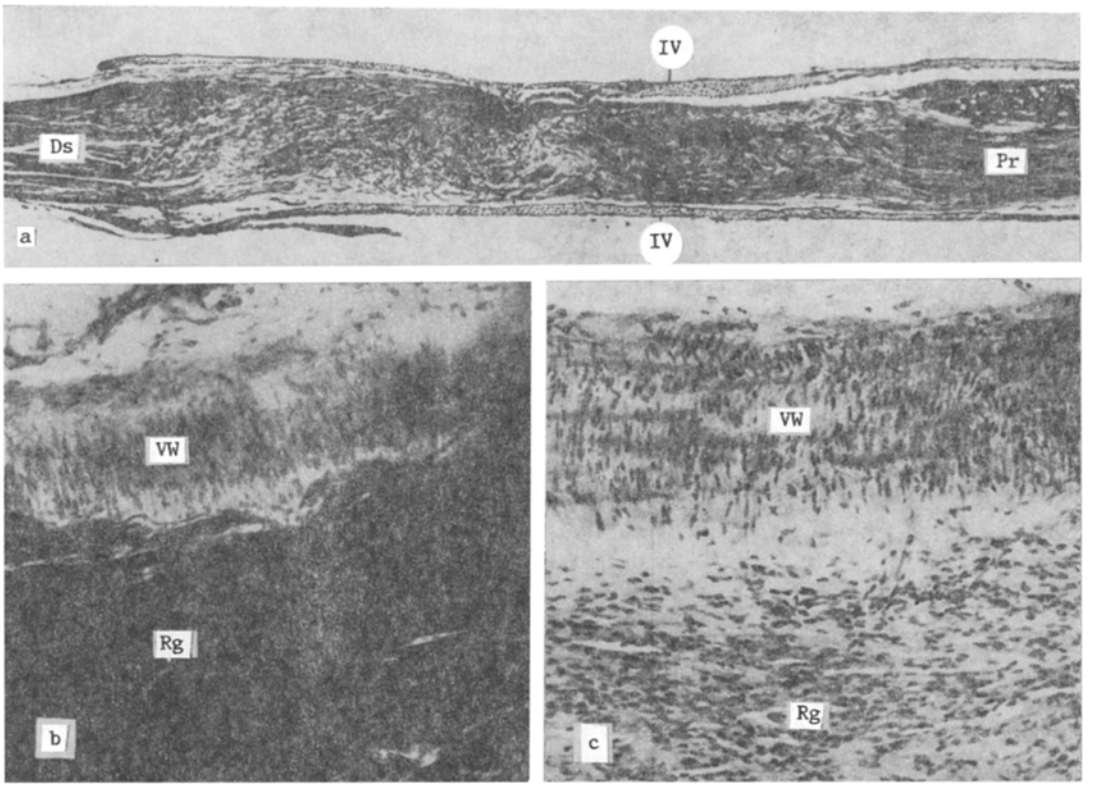

Fig. 3. Regenerating nerve in lumen of implanted blood vessel 9 months after operation. a) General view of longitudinal section. Sudan black. 35 ×; b) Bundles of myelin fibers filling lumen of vessel and firmly apposed to its wall. Silver impregnation, counterstaining with hematoxylin. 430 ×; c) Structure of smooth-muscle vascular wall and of regenerating nerve in its lumen. Hematoxylin and eosin, 430 ×. VW) Vessel wall, RG) regenerating tissue. Remainder of legend as to Fig. 1.

into the region of the intact sciatic nerve from noninbred animals in order to discover the time of their rejection by the recipient. The rats were killed with ether 20 days and 1, 3, 6, and 9 months after the operation. The nerves were removed together with the implanted blood vessels and investigated by neurohistologic and general histologic methods: silver impregnation by the Bielschowsky–Gros method, staining with hematoxylin and eosin, with azure II-eosin, and with Sudan black.

EXPERIMENTAL RESULTS

After 20 days continuity between connective tissue and the surface of the nerve was formed on the surface of the vessel. In most cases the vessel wall was well preserved and contained no inflammatory cells. Negligible infiltration by lymphocytes was found only at the site of junction of the nerve with the implant, where glue was applied during the operation. Migration of lemmocytes, fibroblastlike cells and macrophages from the proximal stump and growth of capillaries were observed in the lumen of the vessel. In sections impregnated with silver, thin (0.5-1 μ) diffusely arranged regenerating unmyelinated nerve fibers could be seen among the cells, along the path of growth from the proximal pole to the distal pole, and some of them entered the distal pole.

The implanted vessel 1 month after the operation appeared normal, and histologic investigation of its wall revealed clearly distinguishable, viable smooth muscle cells and elastic fibers. Numerous thin bundles of nerve fibers, infrequently distributed, could be seen uniformly in the lumen, over its whole length from the proximal to the distal end of the nerve, and some of them grew along the vessel wall with a helical course, while others could be traced centrally (Fig. 2a). The diameter of the majority of the nerve fibers at this period had increased, compared with the previous period, to 1.5-2 μ on account of the beginning of formation of myelin sheaths around the axons (Fig. 2b). Thin myelinated fibers could be traced for a considerable distance in the distal segment of the nerve also. Thin

newly formed vessels were present at this stage between the bundles of nerve fibers: arterioles, venules, and capillaries.

The lumen of the vessel from the proximal to the distal end of the nerve 3 months after the operation was filled with a mass of myelinated fibers from 2 to 4 μ in diameter. The nerve fibers were gathered into numerous interweaving bundles (Fig. 2c). Between the bundles of myelin fibers, wide spaces were observed at this time, evidence that regeneration was not yet sufficiently complete.

The mass of regenerating nerve bundles in the lumen of the vessel had grown rapidly 6 months after the operation and was much more dense on account both of the formation of new myelinated fibers and of an increase in the diameter of the myelin sheaths, the thickness of which in the overwhelming majority of cases amounted to 4 μ.

After 9 months the whole volume of the lumen of the implanted vessel was so densely packed with bundles of thick myelinated and unmyelinated nerve fibers (Fig. 3a, b) that under the light microscope it was difficult to discover any free intercellular spaces, such as were present in the earlier stages. Well developed capillaries, arterioles, and venules were frequently found between the bundles, evidence of the rich vascularizatin of the regenerating nerve. As will be clear from Fig. 3a, the regenerating tissue in the lumen of the vessel consisted of continuous, organically united proximal and distal ends, and the nerve trunk and regenerating tissue formed a single entity. Thus by the 9th month the integrity of the severed nerve trunk was completely restored. However, histologic examination of the material still showed definite structural differences between the proximal and distal segments of the nerve trunk, on the one hand, and the regenerating tissue in the lumen of the vessel on the other hand. These differences amounted to the fact that the nerve fibers in the proximal and distal trunks had an undulating course and parallel distribution typical of the normal case, whereas nerve fibers in the implant were gathered into numerous bundles, arranged in the form of plaited hair. However, the fact that the whole mass of nerve bundles, on leaving the implant in the distal segment of the nerve, recombined into a single trunk and acquired the normal structure, is sufficiently convincing proof of successful regeneration and of restoration of the integrity of the whole nerve.

Implanted arteries, from both inbred and noninbred animals, in whose lumen the nerve fibers regenerated, were not absorbed and had not died even 9 months after the operation (Fig. 3c). Smooth-muscle and elastic cells were well preserved in their wall. Meanwhile empty, control implants, transplated in the region of intact nerves in the same areas of the limb, were rejected by the recipient after only 1-2 months.

The results of this investigation showed that regeneration of a nerve in the lumen of an implanted artery takes place in a purposive manner and without the formation of neuromas, which always occurred in the control. Often the nerve fibers succeeded in covering the distance between proximal and distal ends of the divided nerve under these conditions in 20 days. Myelination of the regenerating fibers in the lumen of the vessel began after 1 month, and after 3 months the whole lumen of the vessel was filled with bundles of myelinated fibers; after 9 months the structure of the regenerating nerve was similar to a mature nerve trunk of the animal. It is suggested that this experimental model can be used to study the reparative histogenesis of tissues of the peripheral nervous system. The results of such research may be capable of application in neurosurgical practice.

LITERATURE CITED

1. S. P. Galich, Yu. B. Chaikovskii, V. P. Yatsenko, et al., Klin. Khir., No. 12, 11 (1983).
2. D. M. Golub, Regeneration of Nerves and Reinnervation of Organs [in Russian], Minsk (1981).
3. K. A. Grigorovich, Surgical Treatment of Nerve Innuries [in Russian], Leningrad (1981).
4. E. A. Gromova, in: Disease, Treatment, and Recovery [in Russian], Moscow (1952), pp. 49-76.
5. V. V. Kaverina, Regeneration of Nerves after Neuroplastic Operations [in Russian], Leningrad (1975).
6. E. I. Chumasov and N. I. Chalisova, Byull. Éksp. Biol. Med., No. 11, 104 (1983).
7. S. Ramon-y-Cajal, Degeneration and Regeneration of the Nervous System, London (1928).
8. D. T. W. Chiu, I. Ianecka, T. I. Krizek, et al., Surgery, 91, 226 (1982).
9. G. Lundborg, L. V. Dahlin, N. P. Danielsen, et al., J. Neurosci. Res., 6, 265 (1981).

10. G. Lundborg, L. V. Dahlin, N. P. Danielsen, et al., Exp. Neurol., 76, 361 (1982).
11. S. Sunderland, Nerves and Nerve Injuries, Edinburgh (1978).
12. B. G. Uzman and G. M. Villegas, J. Neurosci. Res., 9, 325 (1983).
13. P. Weiss, J. Neurosurg., 1, 400 (1944).

DETERMINATION OF ACETYLCHOLINESTERASE ACTIVITY BY GAS-LIQUID CHROMATOGRAPHY

A. D. Ado, R. M. Zolotareva, and B. M. Zolotarev

UDC 616.153.1:577.152.311/-074:543.544.45

KEY WORDS: gas-liquid chromatography; acetylcholinesterase; acetyl-β-methylcholine.

Under ordinary conditions of work of the gas-liquid chromatograph the temperature of the column and vaporizer is always kept above the boiling point of the least volatile component of the test mixture, and in that way all components of the mixture are converted into the vapor phase. Under these conditions, however, it is impossible to introduce a large volume of the test mixture into the chromatograph, because the chromatograph column will cease to function normally due to overloading. If a small volume of sample is introduced into the chromatograph, however, the presence of a trace component cannot be detected. This drawback is not found in Deans' method, the essence of which is that the column and vaporizer temperature is lower than the boiling point of the principal component (in our case – water). Under these conditions of work by introducing an adequate volume of the sample, it is possible to fill virtually the whole of the column with test liquid, and in that case, when the flow of carrier gas passes through the column, the liquid sample to be tested will perform the role of stationary phase, whereas the trace component will be concentrated toward the "tail" of the main component.

Deans studied this method in detail and used two columns, connected together in sequence, the first of which was fitted with a thermal conductivity detector (katharometer), the second with a flame-ionization detector (FID) [3, 4]. He found that the peak of the trace component always comes out in the "tail" of the principal component, and its intensity is proportional to the concentration of the trace component, given equal volumes of sample. The characteristics of the columns, the material with which they are packed, and the nature of the stationary phase of the solid carrier and gas carrier do not play an essential role. A change of column temperature, as usual, affects only the retention time.

We have modified Deans' method and have used it to determine acetylcholinesterase (AChE) activity.

EXPERIMENTAL METHOD

The proposed method is based on the reaction of enzymic hydrolysis of acetyl-β-methylcholine by AChE with the formation of acetic acid:

$$[CH_3{=}COO{=}(CH)CH_3{=}CH_2{=}N^+(CH_3)_3]Cl^- \xrightarrow{-H_2O}$$
$$\longrightarrow CH_3COOH + [HO{=}(CH)CH_3){=}CH_2{=}N^+(CH_3)_3]Cl^-.$$

Acetic acid (the trace component) is determined on a chromatograph fitted with an FID. Since the column and vaporizer temperature must be below the boiling point of the principal component (water), we chose 90°C. Lowering the temperature below 90°C leads to an increase in retention time of the trace component (acetic acid). Unlike Deans, we used one column

Research Group for the Study of Bronchial Asthma, N. I. Pirogov Second Moscow Medical Institute. Department of Physical Chemistry, Timiryazev Agricultrual Academy, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 102, No. 9, pp. 377-379, September, 1986. Original article submitted February 13, 1986.

 0007-4888/86/0009-1312$12.50