Original article

Folia Neuropathologica

The evaluation of the influence of a high injury to brachial plexus elements on the condition of neurons of the anterior horns of the spinal cord – experimental research

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Folia Neuropathol 2009; 47 (4): 347-353

Abstract

The aim of the experimental research was to assess the impact of a high damage to brachial plexus elements on the condition of neurons of spinal cord anterior horns. The research was conducted on 12 rabbits, in which the ventral branches of spinal nerves C5-Th1 were severed. During dissections carried out 7, 30, 60, 180 days after the operation the cervicothoracic segment of the spinal cord was collected. The material was subjected to microscopic histological and ultrastructural examinations, which showed that where brachial plexus elements had been severed some of the neurons of spinal cord anterior horns had died and that the process intensity depended on the time that had passed after the injury.

Key words: *neurotmesis, nerve cell body degeneration, spinal cord, histological examination.*

Introduction

An action of a traumatic factor on a nerve may cause damage of a varying degree of severity [3,18]. Damage to Schwann cells only brings about a segmental disintegration of the myelin sheath [18]. Breaking the continuity of the axon (axial fibre), isolated or with accompanying damage to other structural elements of the nerve, results in the disintegration of the axon and the myelin sheath in the peripheral part of the nerve fibre [8]. The process is referred to as Waller degeneration [19]. The closer section of the damaged nerve experiences a segmental demyelinization of the axon and the process usually encompasses about two internodes [8]. The afferent section of the axon above the retrograde degeneration boundary produces cytoplasmic processes, which TEND towards Schwann tubes regenerated in favorable conditions. It enables proper nerve regeneration, but this process is possible only when the nerve cell is still viable [5,8,17]. In high injuries to peripheral nerves the nerve cell body (perikarion) and its processes may degenerate [14]. The

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extensiveness of this phenomenon, referred to as retrograde degeneration, is not entirely known.

Material and Methods

The aim of the research was to assess the impact of a high cut of brachial plexus elements on the condition of neurons of the anterior horns of the spinal cord. The experiments were done at the Section for Experimental Surgery and Research into Biomaterials of Wrocław Medical University. The microscopic examinations were conducted at the Section for Anatomy and Embryology of Wrocław University of Environmental and Life Sciences. The research proposal was approved by the Local Ethical Committee for Experiments on Animals (Opinion No. 35/05 of 16 Nov 2005).

Operating technique

The experimental research was carried out on 12 New Zealand rabbits, weighing about 2,500-4,000 g each, of both sexes, aged 6-10 months. The rabbits were anaesthetized intramuscularly with xylazine at a dose of 5 mg/kg (preparation: Rometar 2% – Spofa) and Zoletil at a dose of 15 mg/kg (preparation: Zoletil forte – Virbac). Full analgesia was achieved 10-15 minutes after administration of the preparations, and the condition lasted for 60-80 minutes. The cutting area was additionally injected with 1% solution of Lignocaine. After becoming fully analgesic the rabbits were immobilized on the operating table while lying on their right side. The previously shaved operating area was disinfected with an alcoholic preparation (Prevacare – Johnson & Johnson). In all animals the operation was carried out on the left side of the neck. A cut in the supraclavicular area was used to expose the sternocleidomastoid muscle and then, after preparation, the brachial plexus (Fig. 1). After dissecting its elements, the ventral branches of the spinal nerves C5-C6-C7-C8-Th1 were severed (Fig. 2). The wound margins were closed with simple interrupted sutures (5-6), using polyamid non-absorbable suture Amifil M 3/0 (Sinpo – Poznań).

Autopsy examinations

The animals were dissected 7, 30, 60, 180 days after the operations, after intravenous administration of pentobarbitral (preparation: Morbital – Biowet). During the examination the cervicothoracic segment of the spinal cord was exposed and cut out (Fig. 3, 4). The material taken for histopathological examination was fixed in 4% buffered formalin and the specimens for ultrastructural examinations were transported in a 0.9% NaCl solution.

Histological examinations

The spinal cord parts intended for histological examination were, after being fixed for 48 hours in a 4% solution of buffered formalin, rinsed in water, dehydrated in an alcoholic series and embedded in paraffin wax. 7,0 µm thick transverse and longitudinal serial sections were made, which were then stained with hematoxyline and eosin (acc. to Delafield) and by means of the Azan Novum method (acc. to Geidis).

Microscopic ultrastructural examinations

The material for ultrastructural examination was fixed in a 3.5% solution of glutaraldehyde in 0.1 M phosphate buffer with pH at 7.2-7.4 for three hours; after that the material was rinsed in the above buffer and fixed in a 1% solution of osmium tetroxide. Next it was dehydrated in an alcohol-acetone series and embedded in Epon 812. Semithin sections were stained with a 1% solution of toluidine blue and ultrathin sections were contrasted with uranyl acetate and lead citrate. The material was analysed in a Tesla BS 500 transmission electron microscope. A total of 700 preparations were made and examined.

Results

Histological examinations result

7 days after operation areas of increased translucence were visible around the nucleus. In the peripheral area of perikarions and at the point of protrusion of the processes varied staining was noticed, which indicated separation of cytoplasmic zones. Near to the degenerating perikarions of nerve cells the accumulation of glial cells was slightly higher. In the slide there were astrocytes, oligodendrocytes and microglial cells. The microglial cells and macrophages were to be found principally around blood vessels (Figs. 5, 6).

30 days after operation increased nerve cells degeneration was observed, with the presence of microglial cells and macrophages (Fig. 7).

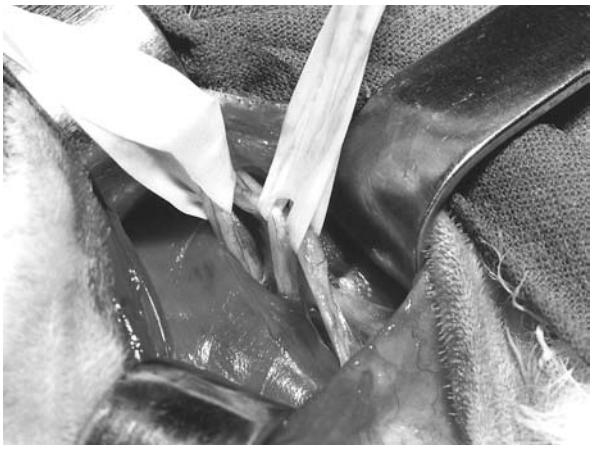

Fig. 1. Intraoperative view: exposition of the brachial plexus elements (upper, middle, lower trunk)

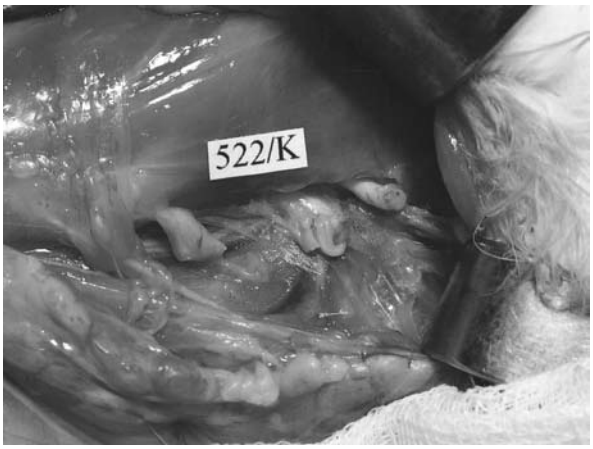

Fig. 2. Intraoperative view: stubs of the transected elements of the brachial plexus.

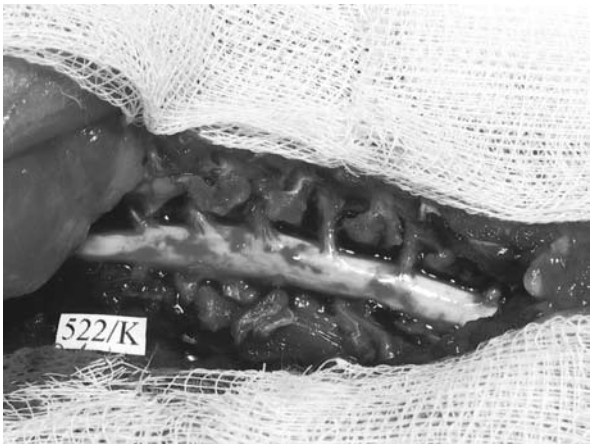

Fig. 3. Autopsy view: a part of the spinal cord with spinal nerves of both sides still attached at bony frame.

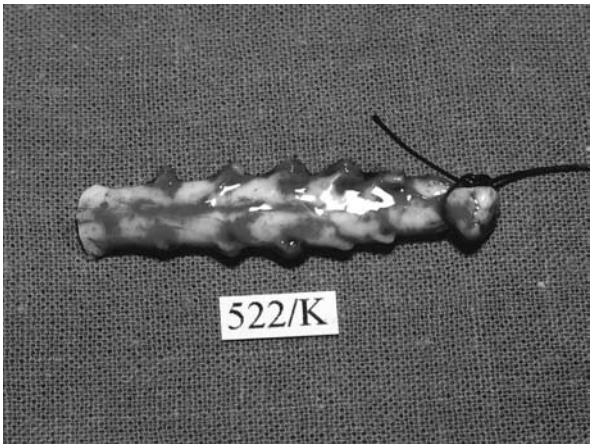

Fig. 4. Autopsy view: the spinal cord removed from bony frame and prepared for microscopic examinations.

60 days after operation more neurons were degenerated. Separation of degenerating nerve cells from the glial stroma was also observed. This was accompanied by infiltration of microglial cells and macrophages. Near degenerating neurons a characteristic foamy texture of neuropile was discovered (Fig. 8).

180 days after operation numerous nerve cells were distinctly degenerated. Near degenerating and degenerated nerve cells cellular infiltration composed of neutrophilic granulocytes and macrophages was observed. Phagocytes were present near such foci (Fig. 9).

Ultrastructural examinations result

7 days after operation the cytoplasm of neurons was found to be vacuolized. Furthermore, initial symptoms of separation of the myelin sheath were discovered (Fig. 10). Some nerve cells were subject to autophagy. Also glial cells underwent degradation; chromatin in such cells was visibly thickened (karyopyknosis).

30 days after operation nerve cells were even more degenerated, which was reflected in autophagy

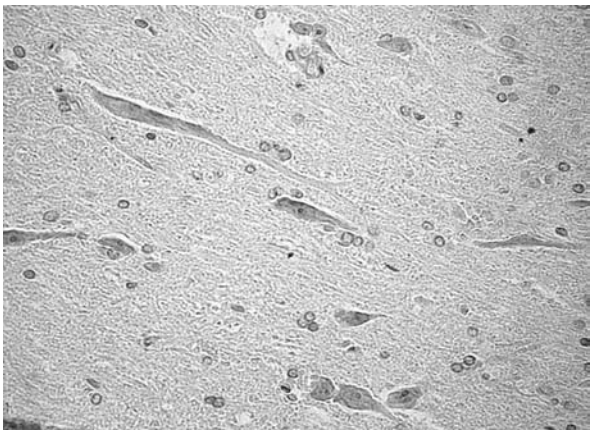

Fig. 5. Histological view 7 days after operation: increased translucence visible around the nucleus and at the point of protrusion of the processes. Separation of cytoplasmic zones is noticed at the peripheral area of perikarions. Stain. Azan-Novum. ×250.

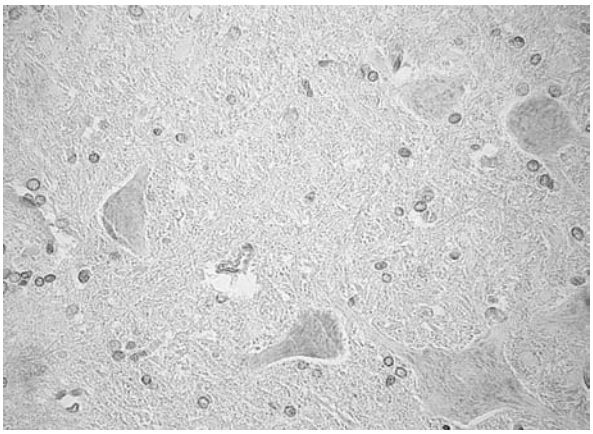

Fig. 6. Histological view 7 days after operation. Degeneration of some neurons. Proliferation of glial cells. Stain. Azan-Novum. ×400.

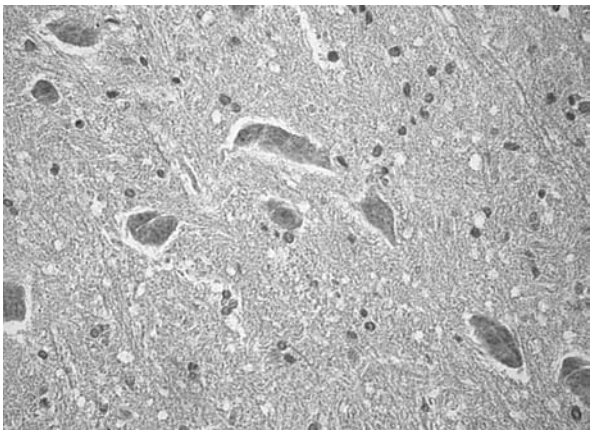

Fig. 7. Histological view 30 days after operation: degeneration of some neurons. Proliferation of glial cells. Stain. HE. ×250.

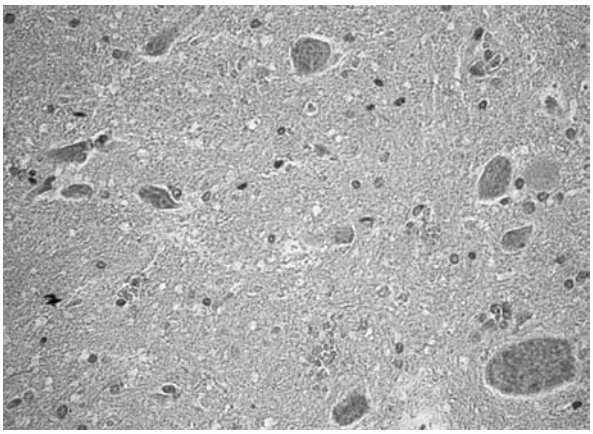

Fig. 8. Histological view 60 days after operation: increased progressive degeneration of some nerve cells with glioproliferation. Stain. HE. ×250.

concerning perikarions of neurons and the cytoplasm of their processes (axoplasm). The mitochondria in axoplasm were distinctly swollen (Figs. 11, 12).

60 days after operation, apart from degeneration of neurons, retrograde changes in the myelin sheath and glial cells were even more pronounced. More advanced separation of myelin sheath lamellae was discovered. Glial cells showed symptoms of karyopyknosis and their mitochondria were swollen (Fig. 13).

180 days after operation some cells were significantly degenerated. In some neurons signs of autophagy were observed. The separation of the myelin sheath was characteristically networked (Figs. 14, 15).

Discussion

Changes occurring in the perikarions of nerve cells after a high cut of the nerve include: swelling of the cell body, translocation of the nucleus to the periphery and disintegration of basophile granules (chromatolysis). Such changes indicate the readiness of the cell to regenerate [7]. The atrophy of Nissl gra-

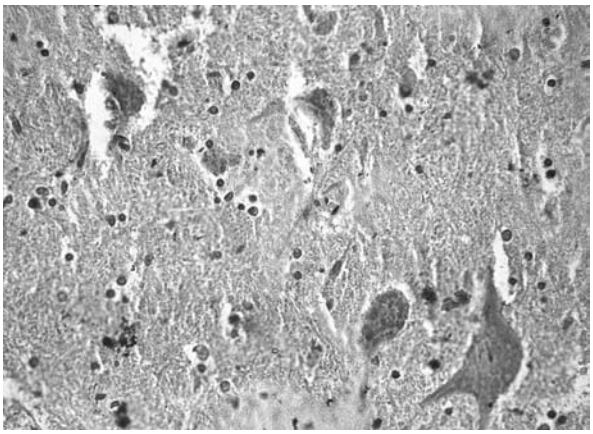

Fig. 9. Histological view 180 days after operation: degeneration of motoneurons. Astrocytic gliosis. Stain. HE. ×400.

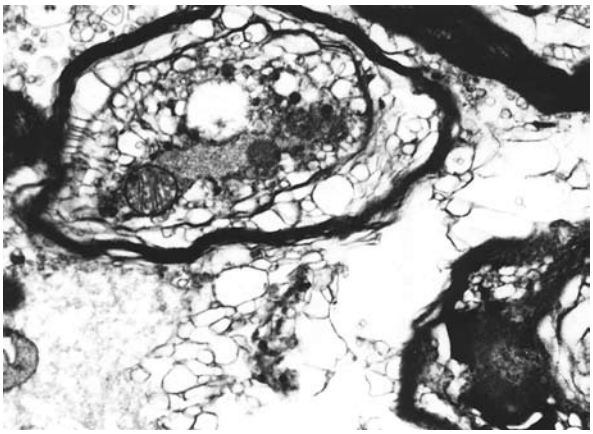

Fig. 10. Ultrastructural view 7 days after operation: separation of the myelin sheath, axonal degeneration. ×11 000.

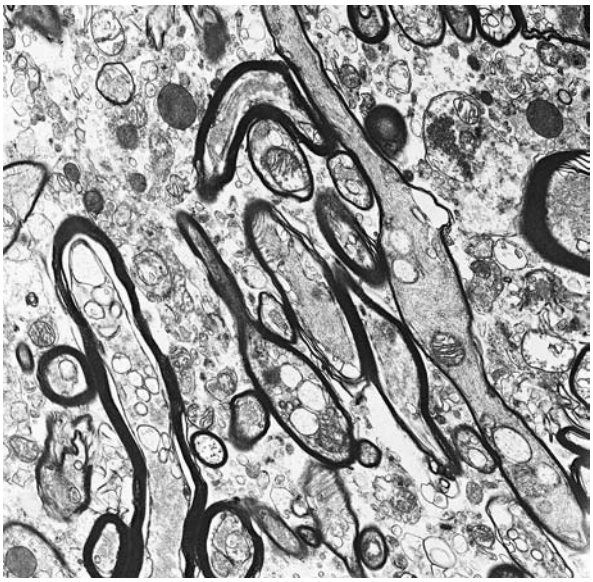

Fig. 11. Ultrastructural view 30 days after operation: vacuolization of axoplasm, separation of the myelin sheath. ×7000.

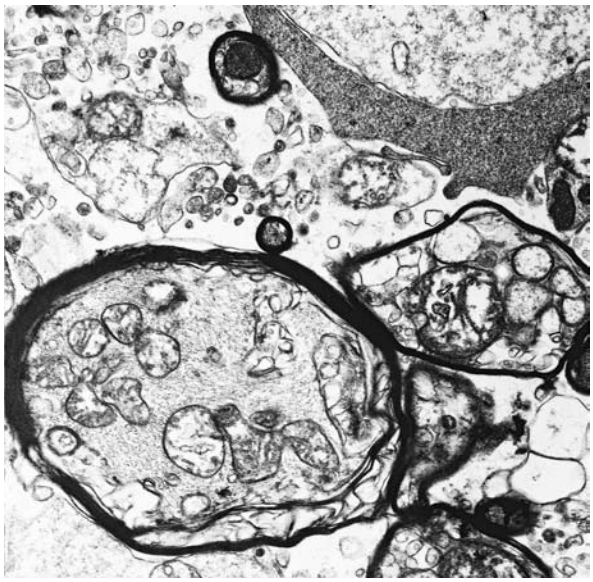

Fig. 12. Ultrastructural view 30 days after operation: vacuolization of axoplasm, swelling of mitochondria. ×8000.

nules containing stored RNA is related to the cell's increased metabolism and protein production [7,8]. This means that chromatolysis is the beginning of repair processes. At the same time, the regrouping of organelle indicates a serious injury to the nerve cell [5]. In some circumstances the nerve cell may die despite undertaking regenerating efforts [5,7,17].

Linxi and co-workers in an experimental study did not find any connection between a high neurotomy and degeneration of motoneurons [9,10]. Neurotomy of the spinal nerve C7 at an intervertebral foramen failed to produce any degenerative changes in the motoneurons of the spinal cord in mice. The authors found, however, massive degradation of motoneurons in adult mice (70% within 3 weeks and 100% within 6 weeks after the procedure) as a result of the avulsion of the C7 spinal nerve motor root [9,10]. Behnam-Rasouli and co-workers, who carried out experiments

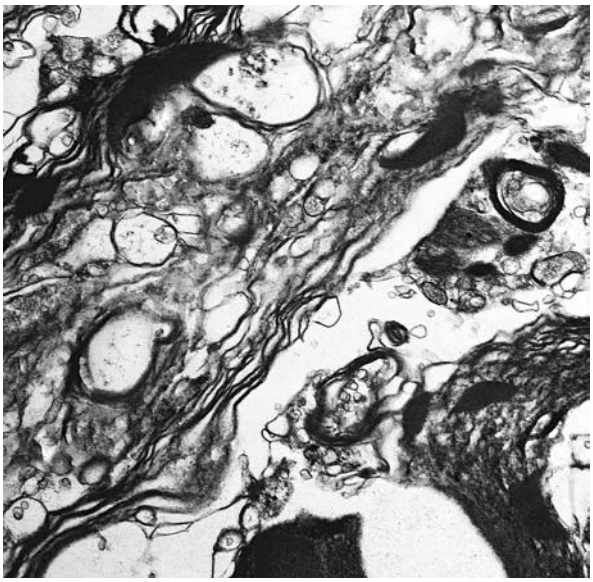

Fig. 13. Ultrastructural view 60 days after operation: delamination of the myelin sheath. ×12 000.

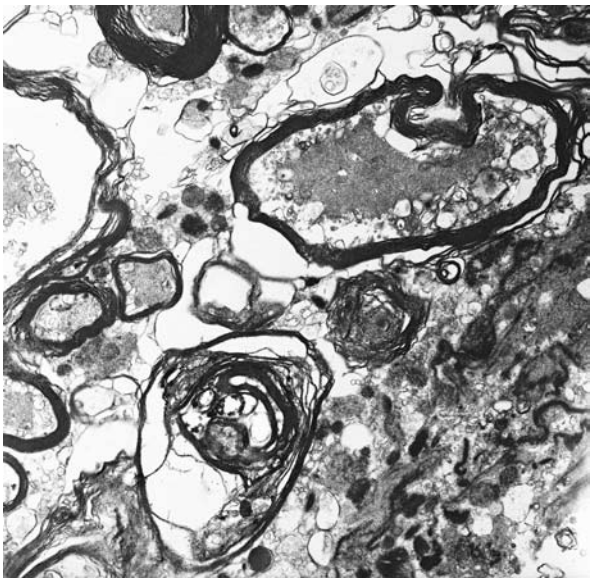

Fig. 14. Ultrastructural view 180 days after operation: advanced degeneration of axoplasm. Clear separation of myelin sheath. ×9000.

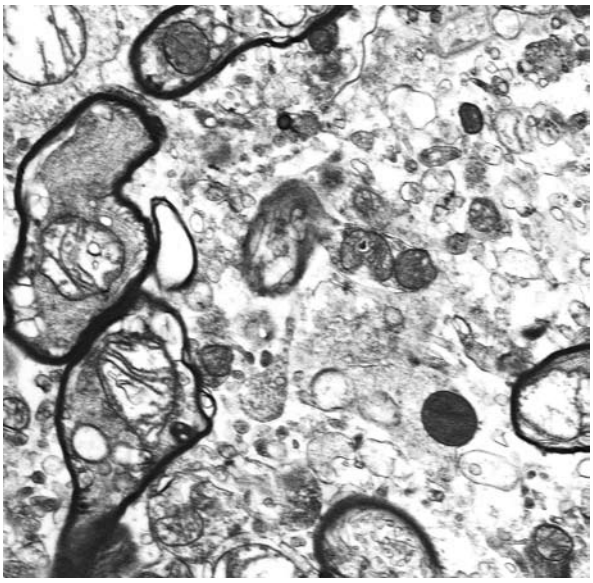

Fig. 15. Ultrastructural view 180 days after operation: advanced degeneration of neurons. ×12 000.

aimed at determining retrogressive changes in the anterior horns of the spinal cord in rats after a crush of the sciatic nerve, noticed other phenomena [1]. As part of the experimental model the sciatic nerve in 12 rats was crushed on the left side for 30 seconds. Dissections were carried out 3 and 8 weeks after the operation. The number of cells on the left and the right side was compared. The team found a considerable difference in the number of motoneurons on either sides of the spinal cord, while the time factor played no important role in the case of 3- and 8-week observation [1]. In own experiment the occurrence of motoneurons bodies degradation after a high injury to peripheral nerves was confirmed. However, in own research the team found that the intensity of the process depended on the time that passes after the injury. It was also discovered that in a short-term observation (7 days after the operation) it was impossible to establish on the basis of a histological examination whether the changes in nerve cells would result in their regeneration or necrosis. Because the motoneurons on the uninjured part receive stimuli via crossed interneurons, also the cell bodies of the motoneurons on the opposite side may be subject to degeneration [1]. In their experiment Behnam-Rasouli and co-workers observed a decrease in the number of normal motoneurons in the anterior horns of the spinal cord on the side which had not been operated on [1]. In the own material no atrophic changes in the anterior horns of the spinal cord on the unoperated-on side was reported, and the observed changes in the perikarion density of motoneurons was of a physiological nature and did not indicate any pathology of the nervous system (7, 30, 60, 180 days after operation).

The outcome of operative treatment of traumatic damage to the brachial plexus are in many cases unsatisfactory [11-13,15]. Treatment results may be affected by various factors, such as the timing of the operation, the choice of the adequate operating technique and its properly application, and the condition of the muscle effector [2,4,16]. In the case of such injuries the long way of axon regeneration is prognostically unfavourable [6]. The experimental model presented by us indicates that a cut to brachial plexus elements leads to pathological changes of the nerve cells of the anterior horns of the spinal cord, including necrosis. In our opinion good treatment outcome following brachial plexus reconstruction may be dependent, among other factors, on the number of healthy mononeurons whose cell bodies have not degenerated as a result of a high injury to brachial plexus elements.

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