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# THE COMPARATIVE STUDY OF DEGENERATIVE AND REGENERATIVE PROCESSES IN FLEXOR AND EXTENSOR BRANCHES OF SCIATIC NERVE AFTER THE CRUSH IN CONDITIONS OF PROTECTION BY PARATHYROID HORMONE, PROLINE-RICH PEPTIDE (PRP-1) AND CENTRAL ASIAN COBRA VENOM *NAJA NAJA OXIANA*

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***Abstract***

*Comparative morphological-and-histochemical studies on development dynamics of degenerative and regenerative processes in the damaged sciatic nerve (SN) area at different time windows after the crush were carried out in rats by method of detecting $Ca^{2+}$-dependent acid phosphatase (AP) activity. For improvement and acceleration of injured nerve rehabilitation, the systemic administration of parathyroid hormone (PTH), hypothalamic proline-rich peptide-1 (PRP-1) and central Asian cobra venom Naja naja oxiana (NOX) were applied. Under effect of PTH, the proliferation of endoneurial and Schwann cells (SC) was revealed, in the nerves G (n. Gastrocnemius) and P (n. Peroneus communis) already at the 3rd day after crush followed by full recovery of AP activity at days 7-9.Under the influence of PRP-1 at the 5th day after SN crush there was a sharp fall in activity of AP on the site of injury, here and there with negligible changes in direction of loose, but arranged in parallel nerve fibers and damage of their wavy lamination, swelling, with a strongly swelled Schwann hollow tubes. On the 7th day at the site of injury there was discovered a large number of SC nuclei and endoneurium, which is associated with their proliferation, enabling the restoration of nerve fibers, characterized by fusiform thickening and irregularity of the caliber. In the proximal part of extensor bundle (P) in the swelled Schwann tubules axons were already traced. At the same time faster recovery of flexor bundle (G) was significant, but without the characteristic waviness of the intact nerve. At the 9th day there was a tendency for restoration of waviness for both flexor and extensor nerve bundles with a few proliferating elements of the endoneurium and SC, with a full manifestation of the activity of AP also in the extensor bundle. In 17 days, as a result of the successful regeneration, a pronounced wavy lamination of the nerve and, due to preservation of perineurium, restoration of the nerve fibers were revealed. Under the action of venom NOX at the 7th day after crushing the enhanced proliferation of cell nuclei endoneurium and SC was observed. After 10 days the progressive schwannosis became weaker, and the enhanced vascularization of large vessels was observed overall. At the 17th day wavy lamination of the nerve is fully restored. As a result of the active proliferation of endo- and perineurium under the influence of the NOX venom flexor and extensor bundles were usually separated by wide connective tissue layers.*

*The results suggest the presence of regenerative processes in combination with a rare picture of the degeneration of certain nerve fibers. During the regeneration of the SN under the influence of NOX venom phosphatase activity was characterized by high levels.*

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**INTRODUCTION**

During environmental disasters the urgency of successful rehabilitation of separate organs and systems, especially the nervous system, increases associated with the crush syndrome. The processes of degeneration and regeneration are intensively and comprehensively investigated under conditions of the peripheral nerve (PN) crush or compression. At this pathology several mechanisms are activated, the knowledge of which contributes to an objective assessment of the changes taking place not only at the source of compression, but also far beyond it [*Gupta R., Steward O., 2003*]. Among them are interesting features of regeneration of sensory/motor neurons, the importance of sensory input in the control of axonal sprouting of motoneurons (MN) in the posterior columns of the spinal cord, changes in presynaptic afferent depolarization (PAD) and presynaptic inhibition, the role of growth factors and neurotrophins, glial reaction, etc. [*Minasyan A. et al., 2008*]. However, despite intensive studies the mechanisms of development and neurode- and regenerative processes at the site of PN crushing much more remain poorly understood. In particular, it was shown that chronic crush of the PN evokes competitive apoptosis and proliferation of Schwann cells (SC) with minimal axonal damage, demyelination, and elevation of the conduction velocity, which reinforces the idea of a direct mitogenic effect of mechanical stimuli on SC [*Gupta R., Steward O., 2003*]. In addition, crush damage draws complete loss of function, which was restored to the control level at the 4th week [*George L. et al., 2003; Gupta R., Steward O., 2003*].

Even in early studies it was shown that germination of regenerating nerve fibers from the proximal stump promotes "downregulation" of myelin genes, dedifferentiation and proliferation of SC, which are arranged in a band of Büngner, express the surface molecules that provide nerve fibers (NF) growth in the range of 3-4 *mm* per day. Favorable conditions are created also in the distal stump through the "upregulation" of neurotrophins, cytokines, molecules adhesion, nerve cells, etc. [*Stoll G., Müller H., 1999*]. It is also revealed that after the PN crush among the regenerated fibers the number of large fibers was not sufficient due to their germination in a changed direction, without restoring the functional reinnervation, with retrograde atrophy and degeneration, as well as disproportional recovery of small-diameter fibers and finally by cellular alterations at the site of injury inhibiting the nerve growth [*Giannini C. et al., 1989*]. Recent studies have shown that in acrylamide-induced neuropathy the key role belongs to the resident endoneural macrophages that only complemented by hematogenous macrophages in the distal margins of more pronounced damage [*Müller M. et al., 2008*]. The nerve crush damage prevents the ability to transmit impulse, and the minimal damage to myelin may significantly affect the activity of ion channels and the subsequent generation of the pulse [*Mert T. et al., 2005*].

Along with known therapeutic agents in the PN crush, which will be considered in the discussion part of this manuscript, the parathyroid hormone (PTH), proline-rich peptide (PRP-1) and the Central Asian cobra venom *Naja naja oxiana* (NOX) are considered as promising therapeutic agents.

Of particular interest is PTH, since attributable to him peptide - parathyroid hormone-related peptide (PTHrP) dramatically increases the number of undifferentiated SC, their migration along the axonal membrane and axonal regrowth in explant [*Macica C. et al., 2006*]. In other words, PTHrP acts through the activation of immature SC, critically needed for successful nerve regeneration [*Macica C. et al., 2006*]. By the example of specific neurodegeneration we have shown its successful protective effect [*Khudaverdyan D. et al., 2008*]. A wide spectrum of biological activity of proline-rich peptide (PRP-1), neurotrophine-like peptide, an immunomodulator produced by neurosecretory cells of hypothalamic nuclei NPV and NSO was revealed [*Galoyan A., 2001; 2008*]. Neuroprotective effects of PRP-1 in contrast to the complexities of multi-component therapy in acute and chronic non-specific neurodegeneration of toxic (poisonous animal poisoning) and traumatic (spinal cord hemisection, PN transection) origin stimulates re-growth of transected spinal tract, promotes survival of neuronal elements of the gray matter in the damaged area and below it, through the formation of anti-scar proliferation, migration and accumulation of glial elements in the area of damage, with subsequent recovery of motor function of lower extremity on the side of injury [*Galoyan A. et al., 2000, 2001; 2005a; b; 2007; 2010; Sarkissian J. et al., 2005; Sarkisyan S. et al., 2008; Abrahamyan S.*

*et al., 2001; 2003*]. PRP-1 could be a potential therapeutic agent for specific neurodegenerative diseases as well [*Galoyan A. et al 2004; 2008*]. In general, its therapeutic benefit is related to the prevention of neurodegenerative processes by modulating the apoptotic cascade, regulation of anti-inflammatory and neuroprotective events. Finally, the increasing importance for neuroprotection acquires venoms of animal origin, among which an important place is occupied by snake venoms (SV). The interest in them is conditioned by the unlimited perspective to use SV toxins and enzymes due to their high specificity and selective irreversible effects that determine their prolonged action, which in turn determines the necessity of their combination with medication [*Bowman W., Sutherland G., 1986; Cook N., 1990*]. In addition, based on dendrotoxines (DTX) of vipers (*Elapidae* family), to which belongs the NOX, conjugations were elaborated adaptively controlling the excitability of the damaged neurons at neurodegenerative diseases [*Cook N., 1990; Rudy B., 1988* ]. The neurotrophic growth factor (NGF) of SV is also of interest. NGF bioactivity was revealed for Chinese cobra *Naja naja atra* SV promoting fibers growth without enzyme, toxicological and teratogenic activities. the protective effect of SV was previously shown in non-specific neurodegeneration of the peripheral and central origin [*Sarkissian J. et al., 2006 a, b; Chavushyan V. et al., 2006; Abrahamyan S. et al., 2007; Galoyan A. et al., 2010*] and specific neurodegeneration [*Sarkissyan J. et al., 2007*].

In conclusion, it is interesting that not enough attention was paid to the regeneration of the flexor nerve during the crush, including the development of neurode- and regenerative processes under the segmental and supra segmental control. The flexor nerve as an evolutionarily newer structure, naturally retarding in regeneration compared the extensor nerve by the level of intensity and rate, due to their evolutionary lag and greater commitment to supra-segmental control thus delays in the development of regenerative processes. In other words, the segmental structure of the flexor compared with extensor ones are more susceptible to suprasegmental control, and more vulnerable and lagging with respect to regeneration, the accounting of which is of great practical importance, especially in conditions of directed use of endo-and exogenous modulators with a broad spectrum of action.

In the present work the morphological and histochemical study of the dynamics of restoration of the hindlimbs flexor (*n. Gastrocnemius* - G) and extensor (*n. Peroneus communis* - P) nerves after sciatic nerve (SN) crush without and with the application of PTH, PRP-1 and NOX venom is presented.

## MATERIAL AND METHODS

Experiments were performed in Albino male rats (250 ± 30 *g*): intact (n = 7) subjected to unilateral crush of SN (control, n = 11) and those under conditions of PTH application (n = 7), PRP-1 (n = 6) and NOX (n = 4). PTH was injected *i/m* on the next day after the SN crush daily for 7 days with 0.35 *mL* ($10^{-9}$ M solution). PRP-1 was injected on the next day after the SN crush daily for 3 days at 0.1 *mg/kg i/m*. NOX was administered *i/m* on the next day after the SN crush daily for 3 days (5% LD50 = 1 *mg/kg*). All procedures were performed in accordance with “Regulations for the care of laboratory animals” (NIH publication for № 85-23 as revised in 1985), as well as specific guidance provided care for the animals and the Committee of the National Health Service and Health. Crushing of the SN was produced under the nembutal anesthesia (40 *mg/kg/w*) in the upper third of the femur (4 *mm* above the trifurcation) within 60 *sec* by means of compression by hemostatic forceps in the position of the first tooth [*Bridge P. et al., 1994*]. In the morphological and histochemical series of experiments the nerve was taken in condition of deep narcotic sleep after 2 hours and 5-70 days after crushing and fixed from 3 days to a week in 10% neutral formalin. Prolonged compaction of the material in a fixative provides better results. Frozen longitudinal sections, thickness of 30-40 *µM*, was transferred into the freshly prepared mixture for identification of the activity of $Ca^{2+}$-dependent acid phosphatase (AP) [*Meliksetyan I., 2007*].

To detect enzyme activity, we recommend the incubation mixture of the following composition:

- 20 *mL* of 0.40% solution of lead acetate;
- 5 *mL* of 1 M acetate buffer (pH 5.6);
- 5 *mL* of 1% solution of ß-glycerophosphate sodium.

The given mixture is brought to 100 *mL* by 3% solution of calcium chloride. Incubation is carried out in an oven at 37$^{o}$ for 3-4 hours. After washing, the sections are developed in 3% solution of sodium sulfide, and enclosed in balsam

**RESULTS**

Comparative studies of rats SN at different times after its crush under the impact of PTH, hypothalamic PRP-1 and NOX venom were conducted using morphohistochemical method of detecting the activity of $Ca^{2+}$-dependent acid phosphatase (AP).

Under the action of PTH at the first days after the SN crush an abrupt decrease in activity of AP is recorded,on the background of which can be hardly traced waveform course of nerve fibers (NF) (Figure 1 *b*) in the form of stumps, thickened or broken (Figure 1 A). Often along their course areas of periodic sharp downturn of AP are observed, due to which NFs are fragmented (Figure 1 B). Apparently, this fact can be associated with reactive changes in NFs associated with the crush. On the third day, there is enhanced proliferation of the SC nuclei and cells of endoneurium (Figure 1 C-F). Against this background thin NFs are traced (Figure 1 C-F). In control animals, a weak proliferation of cellular elements will not start until the 13th day after the crush. It can be assumed that the protection of the PTH provided by proliferation of endoneurium cell, as well by SC as key elements necessary for recovery of the nerve. It is important to note that under the influence of PTH in the course of NFs react blood vessels (Figure 1 C). Under the crush blood vessels can be clamped, leading to local anoxia of axons and thereby violate their functions. Therefore, increased vascularization under the influence of PTH is a factor favoring the rapid recovery. At the 7-9th days after crush proliferation of cellular elements decreases and there is early onset of regeneration: it appears in the shape of thin, intensely stained NFs with wavy layered appearance (Figure 1 G, H).

Under the influence of PRP-1 during the first 5 days after the nerve crush at the site of injury has been a sharp fall in AP activity (Figure 2a) and on the background of NFs staining degree recession in some places a negligible changes in their direction, reducing of the location density and stratification was observed (Figure 2 A, b). NFs at crush site are subjected to reactive changes, in most cases they are swollen, although almost unidirectionality of the fibers is preserved and they are arranged in parallel rows, typical of the nerve trunks (Figure 2 B). Among the fiber spaces between fibers bundles can be traced much swollen hollow Schwann tubes with low enzyme activity (Figure 2 C). At the same time at the site of injury there is a reaction of blood vessels. On the 7th day at the site of nerve crush large number of SC nuclei and endoneurium are revealed which, presumably, is due to their proliferation (Figure 2 d). The nuclei have increased sizes, more rounded, are scattered about, contain a nucleotid and granulation with moderate phosphatase activity (Figure 2 D). It can be assume that the intense proliferation of SC is an ensemble of conditions conducive to the restoration of NFs. Downregulation of myelin genes, dedifferentiation, proliferation of SC, creating favorable conditions for germination of regenerating fibers was found in studies of G. Stoll and H. Müller [*Stoll G., Müller H., 1999*]. As it is known, SC lining up bands of Büngner express the surface molecules, conductive regeneration fibers. At the same time in the distal stump happens upregulation of neurotrophins, cytokines, adhesion of nerve cells molecules, etc. [*Stoll G., Müller H., 1999*]. As a result of metabolic changes in the myelin of NFs on the border of the proximal and distal parts of SN a fusiform thickening and irregularity of the NF caliber are revealed (Figure 2 E). It should be noted that myelin is more fragile than the axon, so often in the proximal extensor nerve bundle in the swollen Schwann tubes in the given terms axons and separate thin fibers that connect the distal and proximal sections are already traced. Flexor bundle recovers much faster, although the characteristic wavy pattern of the nerve is absent and looks like rectified. At the 9th day there is a tendency to restore the wavy form of both flexor and extensor nerve bundles. It should be noted that in this period enzyme activity reveals in full also in extensor bundle. Along the course of proximal NFs there are recorded regular intensely colored swellings that resemble beads, funnel-shaped formation, but between the two axon is always clearly traced (Figure 2 F, G). The characteristic pattern resembling a sponge-like net arises due to the fact that at the level of Schmidt Lantermann cuts, where Golgi craters are formed, a dense boundary between the clear cells is created. In 17 days after the crush as a result of intense phenomena of regeneration under the influence of PRP-1, the absence of swellings is revealed, due to conservation of the perineurium and course of NFs (Figure 2 H). Moreover, in contrast to the nerve of intact rats,

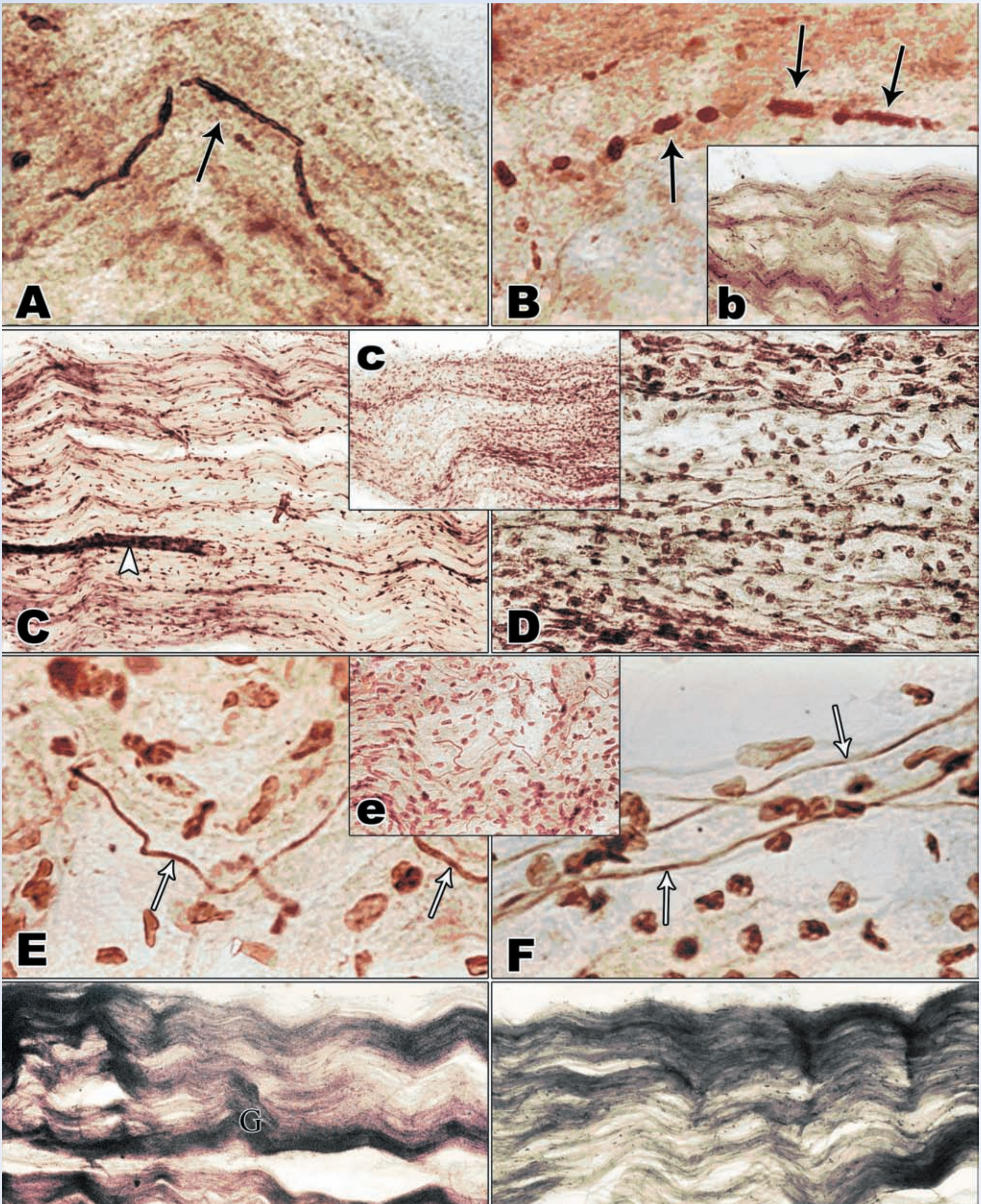


**Figure 1.** *Micrographs of longitudinal sections of SN: 1 (A-B), 3 (C-F), 7 (G) and 9 (H) days after compression under the influence of PTH (black arrows - some thick nerve fibers with high activity of AP; head arrows - a blood vessel; white arrows - some thin nerve fibers; Per - n. Peroneus communis, G - n. Gastrocnemius). Magnification: Oc. 10; Ob. 6.3 (G, H); 10 (b); 16 (C, c); 40 (D, e); 100 (A, B, E, F).*

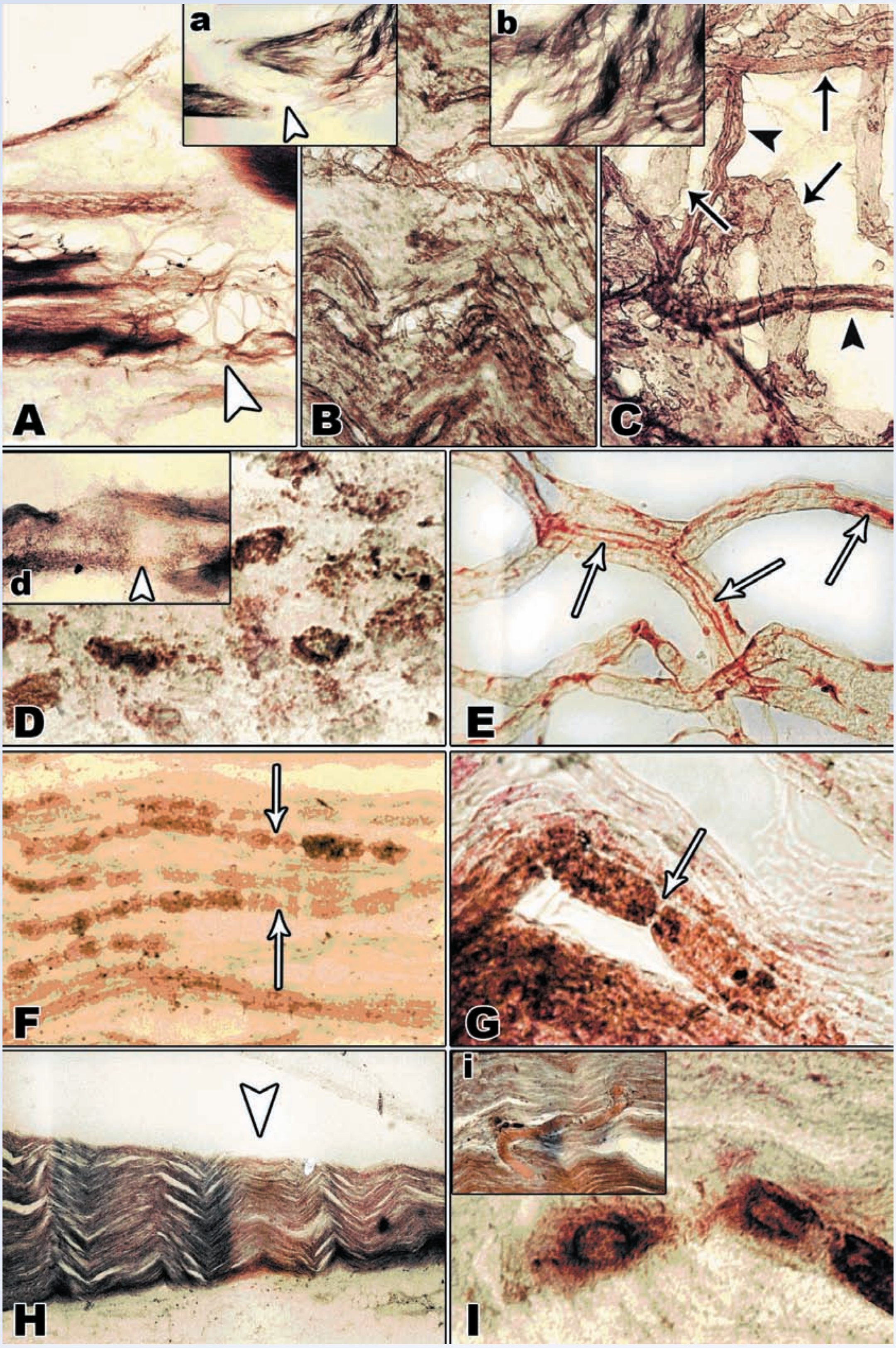


**Figure 2.** *Micrographs of longitudinal sections of SN obtained on days 1-5 (A-C), 7 (D, E), 9 (F, G) and 17 (H, I) after the crush and under the influence of PRP-1; A, a – delamination of nerve fibers and a sharp drop in enzyme activity at the site of crush; b - the absence of wavy lamination; B - swollen nerve fibers; D - irregularity in the caliber of nerve fibers; E - SC nuclear proliferation and endoneurium with moderate AP activity; F, G - funnel-shaped swelling with a clearly traceable axons between them; H, I - complete restoration of the nerve wavy lamination (white arrow head - the place of the crush, black arrow head - the blood vessels, hollow Schwann tubes (black arrows) with an axon clearly seen (white arrows). Magnification: Oc. 10; Oc. 2.5 (a) 6.3 (A, d, H); 16 (b, h); 40 (B, F); 100 (C, D, E, G, I).*

wavy lamination of the picture is significant and cavity are deeper. In all animals studied after administration of PRP-1 at the given periods we observed no more than 3-4 large cells, in the bodies of which Hamori-positive granulation was revealed. Their nuclei are round, large, most intensely colored perinuclear zone of cytoplasm, and closer to the periphery of bodies the enzyme activity is decreased (Figure 2 H). These cells are always interrelated to each other and often difficult to distinguish the boundaries of neighboring cells. They probably belong to the population of progenitor cells that through the bloodstream enter into the affected organ or tissue and in the site turn into a desired specialized cells and replace those of dead [*Galoyan A., 2004*]. However, to clarify the nature of these cells additional immunocytochemical studies are required, although one can say that these cells have high phosphatase activity and always are located in close proximity to blood vessels (Figure 2 h). Morfological and histochemical study of the NOX effect after SC crush revealed that during the first 3 days at the site of injury has been a sharp fall in phosphatase activity and the blood clots are presented (Figure 3 a). In the proximal part of SC phosphatase activity is significantly higher in the flexor bundle (Figure 1 A). Not all of NFs lying in these areas are modified and some of them, especially the thin ones can remain intact. Proliferation of the cells of endo- and perineurium as well as nuclei of SC starts on the fifth day. As a result of metabolic changes NFs are subjected to reactive changes (Figure 1 B). On the seventh day there is enhanced proliferation of the endoneurium cells' nuclei and SC (Figure 3 c). NFs represent the axis of hyperplastic development of SN, interlacing without anastomosis and always preserving between each other separating narrow space (Figure 3 D). The small blood vessels react overall. Summarizing the histopathological picture for 7 days period after the crush it should be mentioned that in axons is observed the pattern of beads (Figure 3 c) and fibers are often found in the regeneration stage. After 10 days the phenomenon of progressive schwannosis becomes more calm. The nuclei of SC looks flattened, located along the NFs, and chromatin is fragmented (Figure 3 E, F). Phosphatase activity of the SC nuclei and NFs is different. Occasionally the residual pattern of fibers swelling is observed. As a result of increased vascularization arround NFs revealed large vessels (Figure 3 B).

At the 17th day the wavy lamination pattern of the nerve is completely restored (Figure 3 H). Importantly, the enzyme activity is distributed unevenly in the NFs bundles: among the majority of the fibers with moderate phosphatase activity can be traced intensively stained bundles. At these periods the flexor and extensor bundles as a rule are always separated by broad layers of connective tissue, probably as a result of active proliferation of endo- and perineural cells under the influence of NOX venom (Figure 3 G). Among the recovered NFs the expressive individual lesions with disruption of myelin are observed. Such fragmentary defeat are found only in the myelin sheath, not scattered along the fibers and there are few on short segments. In some fibers, the axon is separated from the myelin sheath by narrow light mautnerian space. Perhaps, as a manifestation of functional activity in these areas are observed the swelling of NFs with irregular thickening, appearance of wavy changes with fragmentation and grain. Thus, the changes described after the SN crush under the influence of NOX venom allow to assume a manifestation of regenerative processes in combination with a rare picture of the rebirth of individual NFs. Histochemical data indicate that throughout the dynamics of the SN study under the influence of NOX venom the high levels of phosphatase activity was always observed.

## DISCUSSION

In contrast to control animals, in animals treated with PTH in the early stage after nerve crush wavy lamination pattern is not broken. Enhanced proliferation of cellular elements begins already on the third day after the crush, while in control animals weak proliferative processes were revealed on day 13. Another favorable factor is the increased vascularization. At the 7-9th days proliferative processes cease and, inherent to the intact nerve, the early recovery of the morphological pattern is observed. Flexor and extensor bundles are recovered at the same time. In other words, PTH has been successfully operating on the periphery.

It is important to emphasize the fact PRP-1 influence. Expressive proliferation of the endoneurium cells nuclei and SC observed on the 7th day after the crush. Flexor bundle recovers much faster than the

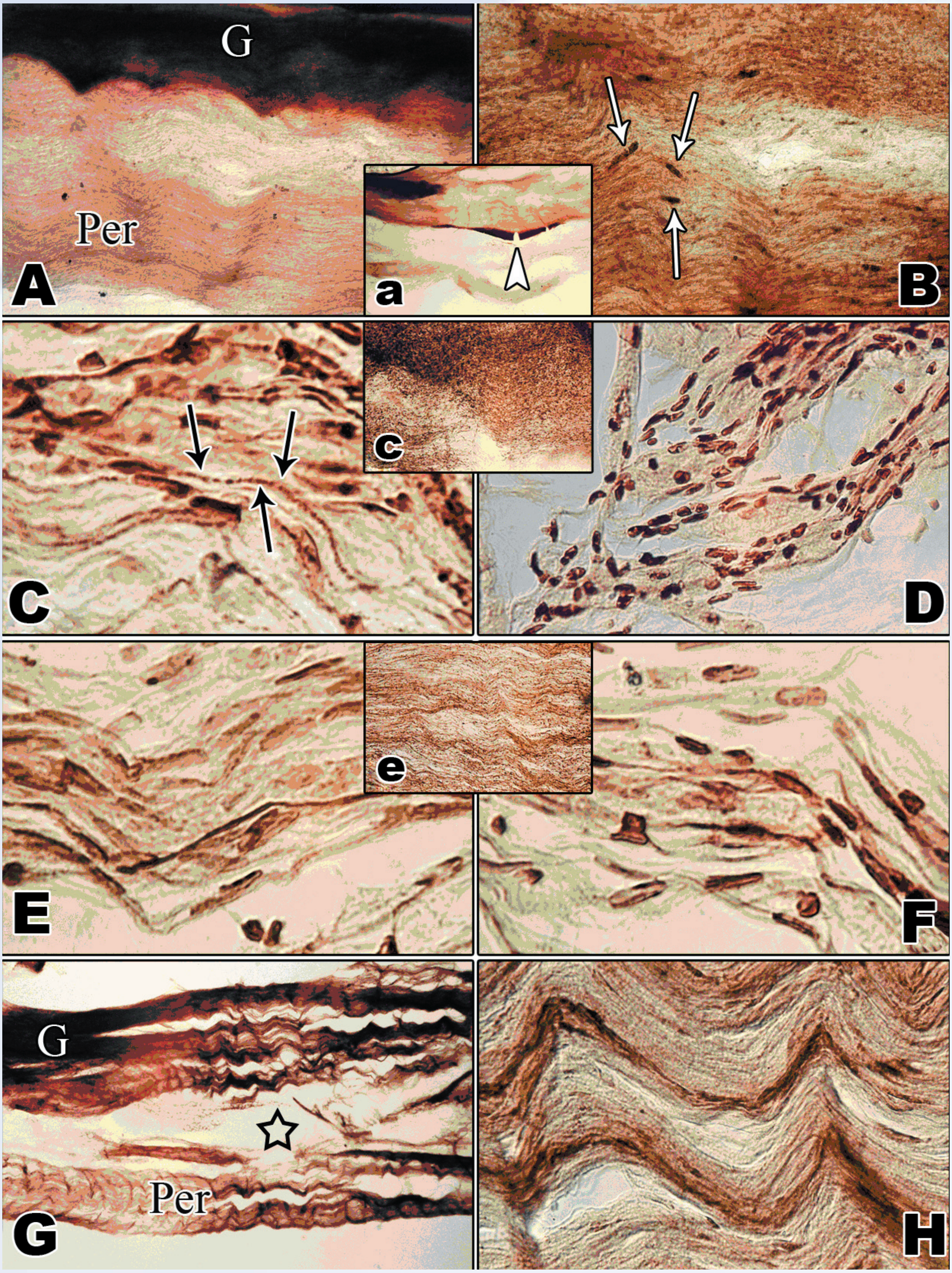


**Figure 3.** *Micrographs of SN longitudinal sections at the 3rd (A, B), 7th (C, D), 10th (E, F), 17th (G, H) days after the crush under the influence of NOX venom. Reduced phosphatase activity at the site of injury, the presence of blood clots (head arrows) and the difference in enzymatic activity of the flexor (G - n. Gastrocnemius) and extensor (Per - n. Peroneus communis) bundles of SN; reactive changes of the nerve fibers (white arrows), irregular swellings along the nerve fibers (black arrows), connective tissue layers between the flexor and extensor bundles (asterisk). Magnification: Oc. 10; Ob. 2.5 (a, G); 10 (c, e); 16 (A, B) 40 (D, H); 100 (C, E, F).*

extensor. Picture of the restored nerve is completed on the 17th day after the crush.

The following positive factors are of interest:

1. During SN crush in condition of three-fold introduction, the same thickness of the nerve was macroscopically observed along its entire length, including the injury site, proximal and distal parts. The site of crush visually does not differ from other parts of the nerve;
2. Typically, when a nerve is cut on a freezing microtome, due to its inherent structure is somewhat difficult to get clear cuts. Often they are exfoliate and nerve bundles diverge, so to avoid this we take small pieces of nerve with a length of approximately less than 1 *cm*. With the injection of PRP-1 2 *cm* long nerve slices were cut on a freezing microtome very smoothly over the entire length, which presumably is associated with improved trophic of nerve under the influence of PRP-1;
3. Wavy lamination of the picture is considerable and hollows looks deeper.
4. Connective tissue streaks almost negligible, indicating a less aggressive endoneurium cell proliferation.

Under the influence of NOX venom, as well as under the PRP-1, an enhanced proliferation of cellular elements begins at the 7th day after the crush. The vascularization is also intensified. Fibers are found in the regeneration stage. Data obtained after the SN crush under the influence of the NOX venom, allow to assume the manifestation of regenerative processes in combination with a rare picture of the rebirth of individual NFs. Histochemical data indicate that throughout the dynamics of changes of the SN under the influence of the NOX venom phosphatase activity was always high. Full restoration is revealed on the 17th day. Proliferation in the flexor and extensor bundles occurs at the same time. However, in these terms as a rule, the latter,is always separated by wide connective tissue layers, so under the influence of venom proliferation of cellular elements is aggressive and along with the SC nuclear proliferation, the endoneurium cells proliferation is also enhanced. In view of this, the nerve macroscopically looks slightly thicker than those in intact rats or, for example, under the influence of PRP-1. Moreover, because of increasing connective tissue layers the nerve is difficult to cut with microtome. The over-activation under the influence of NOX venom also testifies the high phosphatase activity of extensor and, especially, the flexor bundles of SN.

The significance of results obtained in this study can be evaluated after a brief review of recent therapeutic advances in this field

Despite numerous studies over the past two decades, covering the mechanisms of development of this disease and its prevention, existing means and promising strategic targets of successful therapy, certain thematic limitations of such studies still remain. To determine the relative importance of the results of this study a brief overview of advance in PN damages should be provided .

Prevention of disability and the the optimal therapeutic strategy, in particular, during PN crush [*Reyesarmijo E.,1964*], are intensely studied at the interdisciplinary level testing the physical influences, hormones, growth factors, neurotrophins, exogenous peptides and other physiologically active compounds [*Bervar M., 2005; Kato N. et al., 2005; 2010; Aydin M. et al., 2006; Mohri T. et al., 2006; Fleming C. et al., 2007; Fargo K. et al., 2008*].

The role of androgen was shown in the regulation of mRNA neuritin levels, by which increases the axon regeneration and neurite outgrowth in motoneurons [*Fargo K. et al., 2008*]. Neuritin was shown to be involved in the responses when central and peripheral lesions as a common effector molecule for neurotrophic and neurothearpeuitic agent [*Fargo K. et al., 2008*]. It was established that in the case of PN crush neuroactive steroid dihydroprogesterone significantly reduces the density of myelinated fibers up-regulation, and its interaction with progesterone reduces pain and provides the protection [*Roglio I. et al., 2008*]. The important role of glucocorticoids in the myelination through relative receptors of SC was shown [*Morisaki S. et al., 2010*]. The neuroprotective effect of testosterone on spinal cord lumbar motoneurons was recorded [*Tehranipour M., Mooghimi A., 2010*].

An important role in the regeneration of crushed PN belongs to growth factors and neurotrophins. In particular, the regeneration of the axon accelerates by the application of insulin-like growth factor (IGF-I) and platelet-rich plasma to the nerve [*Emel E. et al., 2011*]. As an additional support of regeneration further expression of the glial cell line-derived neurotrophic factor (GDNF) at the 4th week surves [*Cheng F. et al., 2010*]. In addition, the therapeutic prospects of early regeneration created by peripheral (but not central) delivery of GDNF [*Magill C. et al., 2010*]. Further, it encourage SC myelination, potentiating the regrowth and maturation of myelin and significant expression of brain-derived neurotrophic factor (BDNF), provoked by low-frequency electrical stimulation [*Zhang S. et al., 2010; Wan L. et al., 2010 a, b*]. In turn, essential meaning of the SC proliferation was immunohistologicaly shown to support axonal regeneration [*Zhang P. et al., 2008; Zhang S. et al., 2010*]. The key role of meltrin-beta (disintegrin and metalloproteases) in remyelination was revealed and shown its functions as a modulator of a signal from the axon, activating transcription factor of myelination (Krox-20) prior to SC differentiation [*Wakatsuki S. et al., 2009*]. Upregulation of vascular endothelial growth factor (VEGF) also promotes the rehabilitation of the nerve, being activated by vasoactive agent (alprostadil) [*Tang J. et al., 2009*]. In the early stages of myelination for survival of SC neurotrophin 3 (NT-3) is crucial [*Sahenk Z. et al., 2008*]. Finally, it was revealed that after the crush damage of PN in mature and rapidly aging mice VEGF is not locally upregulated, which presupposes an evidence of the interdependent relationship between age, VEGF, angiogenesis and nerve regeneration. [*Pola R. et al., 2004*].

At PN crush distal to the injury a significant increase in the number of myelinated axons is shown and as a serious factor counteracting its optimal recovery – the change in direction of the regenerating motor axons of [*De Ruiter G. et al., 2008*]. Under these conditions, other authors show the increase and the number of the large diameter myelinated fibers (up to 80% of the control level) [*Fugleholm K. et al., 2000*]. There is evidence in favor of the same levels of growth and maturation during regeneration of motor and sensory myelinizated fibers on example of regeneration (up to 140 days) of cat tibial nerve [*Moldovan M. et al., 2006*]. Moreover, the ability of sensory synaptic inputs to MN was demonstrated to affect intermuscular sprouting; only in the presence of sensory input the formation of regenerated axons distal to the fracture occured with an increase in number of motor axons innervating extrafusal fibers (*extensor digitorum longus*) proximal to the fracture [*Cuppini R. et al., 2002*]. However, the mechanism, by which regeneration is not impaired in a long crush-damaged nerve trunk is unknown, while a large number of mature myelinated axons is formed, i.e. sprout is facilitated [*Xu Q. et al., 2008*]. Moreover, damage to the trunk of the nerve-resistant endoneural ischemia and regenerative sprout maintained despite prolonged alterations in the epineural circulation [*Xu Q. et al., 2010*]. It is shown that certain stem cells can differentiate not only to somatic, but also to vascular cells [*Ii M. et al., 2009*].

The above mentioned indicate the obvious protective possibilities of means used in this study and suggests the need for further search for those of clinical practice.

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