Experimental study of the regenerative potential of perineurium at a site of nerve transection

JAUDON E. BEHRMAN, M.Sc., AND ROBERT D. ACLAND, M.D.

Department of Anatomy, and Microsurgery Laboratory, University of Louisville, Louisville, Kentucky

A single fascicle of sciatic nerve was transected in a series of rats. The nerve was repaired by one of three experimental models: 1) perineurial suturing with bulging axons untrimmed, 2) perineurial suturing with bulging axons trimmed, and 3) perineurial suturing to produce a misaligned fascicle. Nerves were excised at 0 to 42 days and were examined in thin, longitudinal section. The complete absence of perineurial regeneration was observed at all time intervals and in all models. Regeneration of axons within the fascicle was disordered. Axonal regeneration extended into the surrounding connective tissue and infiltrated both the proximal and distal perineurium.

Key Words: perineurium • microsurgery • fibroblast • nerve regeneration

PERINEURION is a thin, membrane-like, cellular layer surrounding each peripheral nerve fascicle. It is thought to be centrally continuous with the pia-arachnoid, and sheaths all peripheral nerve branches to their terminal axons.

The function of perineurium is not clear. It has been suggested that it maintains pressure within the fascicle to aid axoplasmic flow. It has also been theorized that perineurium functions as a diffusion barrier isolating the axons from potentially harmful substances in the surrounding mesodermal tissue. Recent studies have emphasized the role of the perineurium in peripheral nerve repair. Hudson, et al., showed that axons thrived only when they regenerated into a perineurial sheath. Consequently, it has been claimed that the passive role of the perineurium as a ducting system is critical to successful nerve repair.

Several studies have investigated the regenerative capacity of perineurium. Morris, et al., described a process of fascicular "compartmentation" that occurred during axonal regeneration. Compartmentation was described as a process by which "mini-fascicles" surrounded by perineurium develop within the larger fascicle during regeneration. Nesbitt and Acland demonstrated the regeneration of perineurium following a stripping injury, in which regeneration occurred uniformly along the defect rather than from the cut ends of the perineurium. In both of these studies, it was concluded that perineurium regenerated from multipotential cells that could differentiate into Schwann cells, endoneurial fibroblasts, or perineurial cells. We have investigated the regenerative nature of perineurium in order to answer the following questions: 1) does perineurium regenerate in such a way as to interpose a barrier to axonal "outgrowth," and remodel a deliberately misaligned fascicle? and 2) since axonal bulging compromises close approximation of perineurium, does the removal of the bulging axons facilitate perineurial regeneration?

This paper describes the histopathological changes of perineurium in three experimental situations following fascicular transection, as follows: In Group A the perineurium was sutured without trimming the bulging axons, in Group B the perineurium was sutured after trimming the bulging axons, and in Group C the perineurium was sutured in deliberate misalignment without trimming the bulging axons.

Materials and Methods

Experimental Technique

Adult male Sprague-Dawley rats weighing between 250 and 300 gm were anesthetized with intraperitoneal sodium pentobarbital (Nembutal). The sciatic nerves on both right and left sides were exposed. With a Zeiss dissecting microscope and microsurgical techniques, the sciatic nerve was dissected from surrounding tissue for a length of 2-cm. Distal to the branch to
FIG. 1. Rat sciatic nerve photographed in vivo, X 25. Upper Left: Group A. The axons can be seen bulging between the sutures. Upper Right: Group B. The axons have been trimmed, producing an intrafascicular space. Lower: Group C. The transected fascicle is misaligned with a single perineurial suture.

Each experimental group contained 25 animals. Within each group, animals were sacrificed at intervals of 0, 2, 5, 10, 21, and 42 days. At these intervals, the animals were reanesthetized and the nerves removed for study by light microscopy. A group of control animals in which no injury was performed was also studied at the same intervals.

Preparation for Light Microscopic Study
The nerves were fixed in situ for 15 minutes with 2.5% glutaraldehyde buffered with Millonig's phosphate buffer. The segment of nerve to be studied was placed in a double microvascular clamp under normal longitudinal tension and was excised. The excised nerve was then fixed, still held in the clamp, for an additional 2 hours in 2.5% buffered glutaraldehyde at 4°C. The 1-cm length of nerve was then removed from the clamp and stored in Millonig's phosphate buffer at 4°C. Specimens were postfixed for 1½ hours in 1% osmium tetroxide buffered with Millonig's phosphate buffer. Post-fixation was followed with dehydration in graded alcohols (50%, 70%, 80%, 95%, and 100%) and transitions in propylene oxide. In embedding the specimen, the side of the nerve bearing the repaired fascicle was placed downward. Care was taken to keep the length of the nerve parallel to the base of the embedding mold in order to assist accurate longitudinal section. Tissue was infiltrated with Maraglas embedding medium and polymerized overnight at 60°C. Blocks were cut with a glass knife on a Sorvall JB-4 ultramicrotome,* in 1-μ sections, stained

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with 1% phosphate-buffered toluidine blue, and examined on a Zeiss light microscope.

Results

Immediately After Injury

Photographs of representative nerves immediately after injury are shown in Fig. 1. In Group A (sutured with axons untrimmed), there were axons bulging in between the perineurial sutures. In Group B (sutured with axons trimmed), the perineurium had collapsed into the intrafascicular space left by the trimming of the axons. In Group C (misaligned fascicle), the misaligned fascicle could be seen with axons bulging past the cut ends of perineurium.

Two Days After Injury

All three groups showed early signs of axonal degeneration. In Groups A and B, the normally compact layers of perineurium appeared separated for a distance of 0.5 to 1.0 mm, both proximally and distally. In Group C, the perineurium showed slight thickening at the injury site.

Five Days After Injury

In all three groups, characteristic Wallerian degeneration was present in both the proximal and distal stumps. In none of the groups was there any evidence of regeneration of the divided ends of perineurium. In Group A, the perineurium appeared thickened because of separation of its layers for a distance of less than 0.5 mm proximally and distally at the injury site. Numerous fibroblasts surrounded the exposed axons, but with no apparent organization. In Group B, the perineurium appeared thickened because of separation of its layers for a distance of 1.0 mm proximally and distally. The cut ends of perineurium of the proximal and distal stumps were in close contact and well aligned. Increased numbers of fibroblasts were present within the fascicle between the cut ends of the axons. In Group C, the perineurium remained minimally thickened at the injury site. A layer of fibroblasts, oriented parallel to the nerve, covered the exposed axons at the injury site (Fig. 2).

Ten Days After Injury

Specimens in all three groups showed early axonal regeneration. In longitudinal section, the axonal regeneration appeared to be in cord-like groups. The cords contained thinly myelinated, regenerating axons surrounded by cells resembling fibroblasts. These longitudinal cords may correspond to the compartments or mini-fascicles described by Morris, et al., in transverse section. As at 5 days, regeneration of the perineurium was conspicuously absent.

In Group A, longitudinally-directed regeneration was present within the distal stump for a distance of 1 mm. Regenerating axons were also seen external to the distal perineurium in the connecting tissue. In Group B, the regenerating axons that were located peripherally in the fascicle followed a predominantly longitudinal orientation. The centrally located axons, however, regenerated in a random direction (Fig. 3). In Group C, the direction of regeneration was predominantly longitudinal. Regenerating axons at this time were within the confines of the perineurium or of the fibroblastic covering around the injury site.

Twenty-One Days After Injury

In all three groups, axonal regeneration was marked in the distal stump. In addition, there was extensive infiltration of the separated layers of perineurium by regenerating axons (Fig. 4). This perineurial infiltration was present in all three groups, although to different degrees (greatest in Group C, and least in Group B).

In Group A, regeneration showed minimal disorganization compared to Group B, being primarily longitudinally directed. There was extensive regeneration not only in the distal stump, but also in the external connective tissue. In Group B, as at 10 days, the regeneration of the axons located centrally within the fascicle appeared randomly directed and chaotic. Regeneration of the peripherally-located axons remained longitudinally oriented. There was no apparent regeneration into the external connective tissue. In Group C, there was extensive axonal regeneration, not only into the distal stump but also...
through the fibroblastic covering at the repair site and external to the distal perineurium. The parallel arrangement of fibroblasts covering the injury site was clearly not acting as a barrier to the outgrowth of regenerating axons (Fig. 5).

Forty-Two Days After Injury

In all three groups, the perineurium was markedly infiltrated with regenerating axons both proximal and distal to the injury site. Axonal regeneration into the external connective tissue was present in all three groups, and was extensive in Groups A and C.

In Group A, regeneration into the distal stump was primarily longitudinal in direction. In Group B, regeneration of the centrally located axons remained highly disorganized. Axonal regeneration into the distal stump was not primarily in a longitudinal direction. In Group C, the axons which had regenerated into the distal stump showed restoration of the normal, longitudinal "zig-zag" pattern.

In none of the three experimental groups at any time interval postinjury was any evidence of active perineurial regeneration observed. We did not observe the divided ends of the perineurium uniting in such a way as to impede the outgrowth of axons. There was no remodeling of the misaligned fascicle and no bridging of the gaps between divided perineurial ends with any tissue resembling perineurium.

Discussion

The principal finding of this study was a complete absence of regeneration of the perineurium. The gap between the proximal and distal cut ends of perineurium remained the same at 42 days as at the time of repair.

Although it did not regenerate, the perineurium underwent two striking changes: separation of its layers, and infiltration with regenerating axons. Separation and fanning out of the layers of perineurium proximal and distal to the repair site were marked in Groups A and B. Because of this process, the perineurium at the injury site appeared to lose its identity as a discrete sheath. In Group C, the separation was less marked, but perineurium was thickened and hypercellular.
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The findings of this study indicate that perineurium fails to regenerate when a nerve fascicle is completely transected. This finding is not altered by the presence or absence of axonal bulging or by the accuracy of perineurial approximation.

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References


Address reprint requests to: Robert D. Acland, M.D., Director, Microsurgery Laboratory, University of Louisville, Health Sciences Center, Louisville, Kentucky 40201.

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