Histopathological changes following removal of the perineurium

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Using microsurgical techniques, the perineural sheath was stripped off the sciatic nerves of rats over a 0.5 cm length at a point where the nerve consists of a single fascicle. The nerves were excised 0 to 84 days after the injury, and were examined in semi-thin transverse section. A new sheath, closely resembling normal perineurium, became organized during the first 10 days; it appeared uniformly over the length of the injured segment. The new perineurial sheath was probably formed by endoneurial fibroblasts migrating from within the fascicle. In undamaged specimens, the axons immediately beneath the excised perineurium underwent no degenerative changes.

KEY WORDS □ microsurgery □ fibroblasts □ regeneration □ peripheral nerve □ perineurium

The perineurium is a thin but well defined membrane-like layer that invests each fascicle of a peripheral nerve.8 Electron microscopy has shown that perineurium consists of a multilayered arrangement of flattened cells, normally one to 10 cells thick.5,17,20 Traced peripherally, the perineurium continues as a thinning, but persistent sheath that invests every nerve branch, even to the terminal axons.18,19 It is also thought to be continuous with the pia arachnoid.18

The functional significance of the perineurium is not yet clear. It is known that it has the cytological properties of a diffusion barrier, and that these properties can be altered in certain disease states.19 The perineurium is currently regarded as contributing to the blood-nerve barrier, but the physiological consequences of removing this barrier have received little attention. Spencer, et al.,19 observed that when a small opening is made in the perineurium, degenerative changes in the form of demyelination and remyelination occur. The suggestion has been made that unidentified biochemical factors present in mesodermal tissue are directly harmful to axons.12

Recently, some attention has been given to the significance of the perineurium in repair of the damaged nerve. Hudson, et al.,4 showed that axons that regenerate outside the perineurium do not thrive, but those that find their way into a perineurial sheath regenerate well.

The restoration of perineurial continuity after nerve injury may well prove to be a matter of clinical significance. In a study of transection followed by microsurgical nerve repair, Millesi, et al.,9 demonstrated the re-establishment of a fascicular sheath described as "perineurium." The histological identity of this layer was not clearly shown. Hudson and Kline,6 in a study of partial nerve transection, reported the formation of a continuous layer similar to perineurium that re-established the continuity of the fascicular sheath. The origin and method of growth of the new layer were uncertain.

Apart from the restoration of perineurial continuity outside the fascicle, a process of intrafascicular compartmentation has been described.7,13,14 Compartmentation is the subdivision of the fascicular contents into numerous mini-fascicles by newly formed tissue closely resembling perineurium. The physiological significance of compartmentation in re-establishing a protective surrounding for damaged axons is a matter for speculation.

This paper describes a study of the histopathological changes occurring upon removal of the perineurium alone, with the least possible disturbance of fascicular contents.

Materials and Methods

Twenty-one young adult male Sprague-Dawley rats were anesthetized with intraperitoneal pentobarbital

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(Nembutal). Through an incision on the dorsal aspect of the thigh, the sciatic nerve was exposed on each side. Under a Zeiss MK 6 operating microscope at × 10 magnification, the nerve was mobilized in the upper thigh for 2 cm. Care was taken to avoid dividing the blood vessels supplying the nerve. The epineurium was dissected off the nerve for a distance of 1 cm, beginning at the origin of the branch to the biceps femoris muscle and proceeding distally. The nerve is composed of a single fascicle at this point. In the center of the segment from which epineurium had been removed, the perineurial sheath was excised over a length of 0.5 cm. This was carried out at a magnification of × 40, with highly modified jeweller's forceps and microscissors. Extreme care was taken to avoid damage to the fascicular contents. Nonetheless, due to the instrumentation involved, it is probable that damage to peripherally situated axons occurred in some cases. A 10-0 nylon suture was placed in the perineurium to mark the proximal and distal limits of the perineurial excision. The wounds were closed and the rats were returned to normal caging and care.

Three rats were sacrificed at each of the following postoperative intervals: immediately, and at 5, 10, 21, 42, and 84 days. In addition, normal undamaged sciatic nerves in three rats were removed for comparison. At sacrifice, the animal was anesthetized, and each nerve was exposed and dissected free from its surrounding tissues. Where the nerve was adherent to surrounding muscle, a thin layer of the muscle was taken with the nerve. The nerve was then in a trough formed by the semitendinosus and biceps femoris muscles. Into this trough was dripped a 3% solution of Millonig's phosphate buffered glutaraldehyde. After the nerve had been covered with the solution for 15 minutes, it was excised and fixed for 1 hour in buffered glutaraldehyde. The nerves were then cut into 1.5-cm lengths for cross section or about 0.5-cm lengths for longitudinal sectioning and fixed for an additional hour in the same fixative. The nerves were then postfixed in phosphate-buffered 1% osmium tetroxide for 2 hours at 4°C, rinsed in 50% alcohol, and continued through a graded series of ethyl alcohols (50%, 70%, 95%, and 100%) at room temperature for dehydration. Dehydration was followed by transition through propylene oxide. The tissue was infiltrated with Maraglas 665. During embedding, care was taken to insure proper tissue orientation and to retain the original relationship to the site of the surgical injury.

After polymerization for 24 to 36 hours, the blocks were sectioned on a Sorvall Porter-Blum ultramicrotome.* Semi-thin sections of 1 μ were stained with phosphate-buffered toluidine blue and examined by light microscopy.

FIG. 1. Sciatic nerve of a rat photographed in vivo. × 35. The epineurium has been removed. The coarse cross bands (bands of Fontana) are reflections from the axons inside the nerve fascicle. The fine cross bands (unnamed) are reflections from the perineurium.

Results

Normal Appearance of The Perineurium

When a peripheral nerve fascicle is examined in situ under the operating microscope at × 25, two different and superimposed patterns of cross-banding are seen, one coarse (the bands of Fontana²) and one fine (Fig. 1). The coarser pattern has a periodicity of about 200 μ. The finer pattern has a periodicity of about 50 μ. The coarser pattern is due to the communal zig-zag course of the axons within the fascicle; the finer pattern arises from the perineurium itself. When the fascicle is stretched by about 15%, the coarse pattern of cross-bands disappears, but the fine pattern persists. When the perineurium is excised, as in this experiment, the fine pattern disappears but the coarse pattern persists.

In transverse sections, the perineurium is seen to be a multi-layered sheath of flattened cells surrounding each fascicle. In the rat sciatic nerve, this layer is two to five cells thick. It stains more intensely with toluidine blue than epineurial tissue. The flattened cells appear notably fusiform with long processes. The nuclei stain lightly, and up to two nucleoli are usually visible. These cells can be seen in close approximation, forming a sheath around the nerve fascicle.

Appearance Immediately Following Injury

Nerves were examined immediately after excision of the perineurium to confirm that it had been complete. On transverse section, no perineurium was seen, although in other respects the fascicle appeared normal. The absence of the perineurium on transverse section correlated well with the absence of the fine pattern of cross-banding.

Regeneration of perineurium

Five Days After Excision

At 5 days, a laminated zone of flattened cells was seen around the periphery of the fascicle. Characteristically, these cells had flattened oval nuclei and markedly extended and flattened cytoplasm. The layer of flat cells was two to 10 cells thick, but the cells did not produce distinct layers, being discontinuous and interspersed with connective tissue elements. The flat cells of this new perineurium-like layer bore a close resemblance to the nearby endoneurial fibroblasts in that the cells were fusiform, with long tapering processes, and possessed elliptical nuclei with one or two nucleoli. Pale-staining globular inclusions were seen in the new perineurium-like cells as well as in the endoneurial fibroblasts (Fig. 2 left). At 5 days, there appeared to be a marked increase in the number of endoneurial fibroblasts. These were most numerous around endoneurial blood vessels in the peripheral part of the nerve. In longitudinal sections, the layer of newly forming perineurium-like tissue extended uniformly over the whole of the injured length of the nerve.

Some specimens showed no degenerative changes of the axons. The perineurium-like cells were seen at the periphery of the fascicle with no architectural disruption apparent. These were apparently undamaged during the surgical procedure (Fig. 2 right).

In other specimens, a small area of Wallerian degeneration and architectural disorganization was seen in part of the periphery of the fascicle (Fig. 3). It was presumed that this was caused by accidental damage to the fascicular contents when the perineurium was excised. In these areas, cells resembling endoneurial fibroblasts were occasionally seen partially or completely surrounding small groups of axons. This appearance probably represents an early stage of compartmentation.

Ten Days After Excision

By 10 days, formation of the new layer of perineurium had progressed further. The flat cells of the layer lay close together, and the innermost cells touched each other to form continuous sheets (Fig. 4).
left). Endoneurial fibroblasts were still present in increased numbers, although there were fewer than in the 5-day specimens.

As at 5 days, some specimens showed no damage to the fascicular contents (Fig. 4 left). No compartmentation was apparent at 10 days in damaged or undamaged specimens (Fig. 4 right).

Twenty-One Days After Excision

At 21 days, the perineurial layer was more condensed and more clearly defined in both damaged and undamaged sections. Its appearance resembled that of normal perineurium, except that the nuclei were more prominent, and the layer was thicker than normal (Fig. 5 left). The damaged specimens showed small peripheral areas of compartmentation. The minifascicles in these areas were more distinct and complete than at 10 days (Fig. 5 right). Each minifascicle consisted of two to five axons, enveloped in a single or double layer of cells with the appearance of perineurium. Peripheral to the areas of axonal damage, the main perineurial sheath extended continuously so that some of the compartmented axons in this area were in places doubly surrounded with perineurium.

There was a marked reduction, almost to normal, in the number of endoneurial fibroblasts seen.

Forty-Two Days After Excision

At 42 days, the cells of the perineurial layer were more densely stained and thinner than at 21 days, and...
Regeneration of perineurium

Fig. 6. Photomicrograph 84 days after injury showing apparently normal perineurium. × 400.

the nuclei were less prominent. Compartmentation was only indistinctly present in the sections showing damage.

Eighty-Four Days After Excision

At 84 days, the perineurium appeared entirely normal (Fig. 6). No compartmentation was seen in any of the specimens. There were no areas in which residue of axonal damage was apparent.

Discussion

The findings of this study indicate that, after being stripped from a nerve, perineurium regenerates rapidly. It appears to regenerate from cells that migrate outward from within the fascicle. These cells probably are endoneurial fibroblasts. The following findings support this account of events. First, the formation of new perineurium appears simultaneously throughout the length of the perineurial defect, and does not proceed from the ends of the defect toward the center. Second, there is an increase in the number of endoneurial fibroblasts in the periphery of the fascicle at 5 to 10 days, when the new perineurial layer is forming, followed by a return to normal numbers when formation of the new layer is complete. Third, the cells in the new layer at 5 days closely resemble endoneurial fibroblasts.

The possibility that endoneurial fibroblasts may become perineurial cells is supported by the work of Morris, et al.,12 whose findings indicate that Schwann cells, endoneurial fibroblasts, and perineurial cells probably are interconvertible.

There was surprisingly little damage of the fascicular contents at the site of the perineurial stripping; indeed, in many specimens there was none. In specimens were damage was seen, it was confined to small localized areas on the periphery of the fascicle. These areas never occupied more than a quarter of the circumference of the fascicle, and are believed to have been produced in the course of microsurgical dissection with scissors. The generally undamaged state of peripheral axons conflicts with the observation by Spencer, et al.,19 that myelin degeneration regularly occurs at a site of perineurial injury. In Spencer's study, the perineurium was not removed but was incised, producing a window through which axons were able to bulge. Damage could have occurred at the time of injury. It seems more likely that the damage observed by Spencer was due to strangulation pressure at the edge of the perineurial window, rather than to the simple absence of perineurial protection. According to Spencer's observations, demyelination and remyelination would be expected throughout all of the present specimens.

Compartmentation or minifascicle formation was seen in this study only in those areas where there was evidence of damage. In areas where axonal structure was undisturbed, there was no organized partitioning of the endoneurium. The conditions necessary for compartmentation to occur must include factors other than the mere absence of perineurium. Such factors may include not only the presence of damaged axons, but also the presence of numbers of Schwann cells which have been liberated from their original function.

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References


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