Progression of partial experimental injury to peripheral nerve

Part 2: Light and electron microscopic studies

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Biopsies from partially lacerated nerves were taken at the sites of proximal stimulus, laceration, and distal recording, and from stimuli and recording sites of control nerves. Electron microscopic examination of the partially lacerated major fasciculus revealed three zones of injury. The laceration zone showed neurotetic changes, the adjacent or intermediate zone, partial degeneration, and the zone most peripheral to the laceration, changes in ground substance. Progression of the original injury is apparently due to ongoing changes in the intermediate and peripheral zones while much of the relative early recovery is due to reversal of changes in these zones. Regeneration through the laceration or neurotetic zone is limited but does account for a small amount of late recovery of function.

KEYWORDS • partial nerve injury • electron microscopic changes

LIGHT and electron microscopic observations on partially lacerated and uninjured nerves of the monkey were made and correlated with the electrophysiological data reported in Part 1 of this study.6

Method

After the last set of electrical evaluations had been completed, each tibial nerve was partially encircled with aluminum wrap and covered with hyaluronidase. In vivo fixation was then achieved with 3% gluteraldehyde and 1% paraformaldehyde. After 10 minutes of fixation, the nerve was removed from the extremity. We carefully avoided trauma to the fixed portion in order to minimize artifacts. Specimens were placed in a trough hollowed out of a paraffin block containing more fixative. The nerve was then cut with a fresh razor blade to provide biopsies from the sites of proximal stimulus, injury, and distal recording. Half of the biopsies were placed in 10% buffered formalin for light microscopic studies. These biopsies were embedded in paraffin and cut into sections 5 μ thick. Alternate sections were stained with Masson, modified Bodian, and Luxol fast-blue techniques. Entire cross sections of the nerves were examined by light microscopy. The remaining biopsies were placed in buffered
sodium bicarbonate. Secondary fixation was accomplished with 1% osmium tetroxide. These specimens were stored in toluene and sent to the University of Toronto for final processing, sectioning for electron microscopic photography, and evaluation. Low-power (× 8000) photographs of fascicular topography were made so that regions of abnormality could be identified.

Cross sections of the nerve had a mature histological structure. At the level of the laceration the usual pattern was that of a large fasciculus with three or four smaller fasciculi in the neighboring epineurium. This anatomical arrangement is analogous to the human ulnar nerve at the elbow, the radial nerve in the spiral groove, the lateral popliteal nerve in the distal fourth of the thigh, and the axillary nerve. Due to normal fascicular branching, the anatomy at the distal recording site was that of several smaller fasciculi. High-power magnification was used for accurate identification of cell types, which is often difficult by light microscopy. Fixation was occasionally suboptimal in the center of large fascicles, and we were careful not to draw false conclusions from such artifacts.

Results

Control Nerves

Epineurial thickening with proliferation of connective tissue elements was the striking abnormality in the control nerves. Eight-week and subsequent biopsies showed this change most clearly, although it was seen also in earlier specimens. The multilayered perineurium, however, maintained its normal architecture. Tight junctions were observed between the flattened perineurial cells. Collagen fibril orientation and density were within normal limits between the perineurial cell layers. Neither extrafascicular nor intraperineurial axons were seen. Endoneurial fibroblast density appeared normal. Orientation of the endoneurial collagen remained in a longitudinal plane. There was minimal condensation of collagen around the nerve fibers. The basement membrane was closely applied to Schwann cells; neither serpentine basement membrane nor basement membrane substance was observed lying free in the endoneurial space. The electron density of the endoneurial ground substance was unaltered, and 98% of the nerve fibers appeared to be normal. There was considerable variation in the density of the Golgi apparatus, endoplasmic reticulum, mitochondria, and ribosomes, depending on the plane of section through the Schwann cells. Neurotubule and neurofilament orientation was normal in both myelinated and nonmyelinated axons. Axonal organelle concentration was within normal limits. Epineurial and intrafascicular longitudinal vascular anastomoses were undisturbed. Tight junctions between capillary endothelial cells persisted.

Biopsies of control nerves fixed between 24 hours and 8 weeks after the initial exposure showed very few abnormal nerve fibers. These constituted less than 2% of the total fiber count and lay interspersed between normal fibers in the peripheral rim of the major fascicle where they exhibited axonal and Schwann cell changes characteristic of traumatic and Wallerian degeneration. The severity of these changes was closely related to the number of operative exposures prior to sacrifice. Biopsies taken less than 24 hours after initial exposure showed the anticipated epineurial hemorrhage. There was no intrafascicular hemorrhage, however, nor disturbance of the microarchitecture within the nerve bundles.
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**Partially Lacerated Nerves**

The light and electron micrograph photographs of lacerated nerves were evaluated using the same criteria as with the control nerves. The histological appearances at the proximal stimulus site in the lacerated and control nerves were identical. Study of the sites of injury confirmed partial destruction of the circumferential perineurium at the margin of the fascicle. Underlying this there was complete disruption of the intrafascicular architecture over a varying proportion of the cross-sectional diameter (Fig. 1). Examination of the fibers within the fascicle remote from the region of the injury showed no abnormality. An intermediate zone existed between injured and normal areas. The extent of this zone varied from case to case but was most marked in specimens removed between the fourth and eighth week after injury (Fig. 2).

Lacerated fibers underwent predictable traumatic degenerative changes extending varying distances proximally, usually beyond the nearest node of Ranvier. Marked concentration of organelles within the axons occurred and preceded fragmentation and disappearance of all axonal elements (Fig. 3 upper left). Myelin lamellae were progressively dismantled within their Schwann cells and subsequently intracytoplasmic fat inclusions were observed. The detailed morphology of myelin degradation was exactly as observed by Morris, et al., in the lacerated sciatic nerve of the rat and followed a similar time course so that the majority of Schwann cells related to fibers immediately proximal to this laceration had completed their digestive cycle within 2 weeks. These changes are known to occupy the terminal 2 or 3 mm of the nerve proximal to the laceration; biopsies taken as far back as the proximal stimulus site showed no evidence of degeneration or subsequent regeneration.

Terminal and collateral sprouting caused formation of regenerative units immediately proximal to the site of injury. Biopsies taken 2 weeks after the laceration disclosed remyelination of axons within the regenerative units, known to occur 8 days after injury. Dense compartmentation around these regenerative units appeared after 1 month. Walls of the compartments consisted of endoneurial fibroblasts and altered Schwann cells, a configuration similar to that of perineurium. The 1-week specimens showed degeneration of nonmyelinated axons immediately proximal to the laceration (Fig. 3 upper right). Nonmyelinated regenerating axon sprouts could not be distinguished from the regenerative units derived from myelinated fibers in this mixed nerve until they assumed a characteristic pattern around the remyelinated fibers.

Thus, the changes in fascicular microtopography immediately proximal to the laceration of the partially divided fascicle were identical to those of a fascicle that has been totally divided. Despite the use of sharp instruments and delicate technique and despite the fact that the remaining intact fascicles prevented gross retraction, rapidly proliferating fibroblasts laid down dense collagen within the laceration. This scar extended through the breech in the perineurium to the epineurial tissues (Fig. 1). At 2 weeks,
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Fig. 3. Laceration zone. Upper Left: Nerve fiber showing axonal shrinkage and organelle concentration with early myelin disruption (bottom). Cell at top has almost completed myelin digestive cycle $\times 6982$. Upper Right: Schwann cell containing five nonmyelinated axons. Dense organelle accumulation in swollen axon indicates degenerative change of axon derived from another Schwann cell system. $\times 6982$. Lower Left: Fibroblasts surround regenerative clusters. $\times 4250$.

regenerating axon clusters invaded the scar tissue haphazardly, and subsequent biopsies from this area showed remyelination of regenerating fibers (Fig. 3 lower left). The continuity of the perineurium was eventually restored by lamellae of cells on the inner aspect of this scar. These cells showed the characteristics of perineurial cells and some contained myelin debris. At no time during this response to injury were white blood cells seen. Macrophages, presumably derived from the circulating blood and containing myelin debris, were apparent during the first 2 weeks. The intermediate zone was most distinct in the 2-, 4-, and 8-week specimens and became less easy to find as time went on. Fiber density was reduced, and by 12 hours there was a distinct increase in the electron dense ground substance in this zone. Fibers within this zone exhibited varying degrees of injury. Normal fibers mingled with others showing evidence of minimal injury by way of abnormal axonal organelle concentrations of mitochondria, quasimembranous bodies, dense bodies,
dense-cored vesicles, and alveolated vesicles. In more severely injured fibers, the Schwann cells showed evidence of myelin digestion at varying stages of completeness. In contrast to the laceration zone, the essential histological feature was preservation of the basement membrane tubes. Some segmental demyelination occurred (Fig. 4 upper left); in this situation the axon continues intact to the periphery and is supported in its peripheral course by surrounding normal Schwann cells. Subsequent remyelination of the injured internodal segments was noted (Fig. 4 upper right). In a more severe degree of fiber injury, axons died and Wallerian degeneration occurred throughout the peripheral course of that fiber, but the resulting band of Bungner remained as a guide to the regrowing axonal tips (Fig. 4 lower left). These partially damaged fibers retained their overall longitudinal relationship; proliferation of fibroblasts and collagen was not marked. Macrophages stuffed with myelin were seen but the presence of active myelin degeneration in this zone as
late as Week 24 suggested that fiber degeneration can either continue or be initiated at a point in time later than the original injury.

Examination of myelinated and nonmyelinated fibers in the peripheral zone of the fascicle opposite the injury showed no abnormality; however, a distinct increase in electron-dense ground substance occurred in this region by 24 hours (Fig. 5 left). This newly visible material was observed in the extracellular space between the normal fibers in specimens taken up to 8 weeks after injury. The intensity of the change eventually receded, and normal fiber density was restored by Week 24 (Fig. 5 right). The initiation of the disappearance of the interfiber substance paralleled restoration of the perineurium.

In the majority of animals some of the small fasciculi escaped damage completely. The caliber of these uninjured fascicles was normal and they did not appear to be compressed by epineurial scar tissue. No perineurial thickening was seen. Caliber of the individual fibers within these undamaged fascicles was normal, and there was no excessive axonal organelle concentration at the nodes of Ranvier to suggest a damming-up process.

Distal recording-site biopsy specimens contained more numerous smaller fascicles, the result of normal fascicular branching. Some fascicles were totally degenerated, while others had a mixture of degenerative and regenerative changes, and still others were totally normal. In the 24-, 36-, and 52-week animals, only 8% of the fascicular area exhibited mixed fiber populations. In each instance, the appearance of the distal biopsies coincided with that anticipated from studies of the injury site.

Discussion

Although epineurial thickening was present in the control nerves, there was no reduction in fascicular caliber nor was there any evidence of Schwann cell pathology or axonal flow interruption in 98% of the fibers. Damage to some of the superficial fibers at the site of repeated exposure and recording was ascribed to operative trauma, and is reflected in the electrical data. These changes were most marked in nerves remobilized after relatively short intervals of 1, 2, or 4 weeks and were less marked in those remobilized after longer intervals of 12 to 52 weeks. The histological methods described showed no evidence of transspinal fiber injury. Epineurial thickening per se did not alter the histology of the functioning nerve fibers. The mobilization required to obtain the electrical data did not appear to injure the two

Fig. 5. Peripheral zone. Left: Normal myelinated and nonmyelinated fibers separated by increased ground substance. × 4845. Right: Normal relationship between myelinated and nonmyelinated fibers is restored. × 12,825.
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anatomical sites of the blood nerve barrier, namely, the perineurium and the endoneurial capillaries, and hypoxic damage was not observed. The endoneurial environment, in which the nerve fibers lay, was undisturbed. No attempt was made to give functional interpretation to varying organelle density in Schwann cells and fibroblasts, since experience has shown wide variation among ultrathin sections examined in the normal state. Review of the detailed histological analysis of control nerves indicated that mobilization of peripheral nerve for the purpose of stimulation and recording can be performed with relative safety and without inducing significant permanent histological or electrical deterioration of function, provided surgical technique is gentle and accurate and the nerve is not repeatedly disturbed. Furthermore, the failure of epineurial scarring to alter fiber appearance or function suggests that treatment of this state in humans is not usually indicated.

Analysis of the lacerated nerves indicated that, as with human injury, inspection and palpation of partially injured experimental nerves at the operating table yields few clues as to the degree of damage present and one could not predict the number of fascicles involved nor define the relative size of the three zones of involvement within a major fascicle. The laceration zone was the site for a sequence of degenerative and regenerative events known to occur after total disruption of both perineurium, nerve fibers, and their basement membrane tubes. Many of the regenerating units were blocked by dense scar tissue and some were apparently deflected. Only the regenerating fibers that gain the endoneurial environment of the distal fascicles are transmitted to the periphery where the fibers subsequently increase in caliber and in myelination.

Intensity of the fibroblast and collagen reaction in these studies greatly exceeded that experienced in other experiments where sharply lacerated nerves were immediately washed of hematoma and sutured or placed in Silastic cuffs. Indeed, regeneration through the zone of laceration and into the distal fascicles subtended by that zone was not very effective. The few fibers that did gain the distal fascicle were responsible in part for the later improvement reflected by the electrical studies.

Fiber changes in the intermediate zone were those of partial fiber injury, with regeneration of Schwann cells at sites of segmental demyelination allowing rapid restoration of function. The basement membrane system remained intact and guided regenerating sprouts from fibers which had undergone Wallerian degeneration. Endoneurial scarring was minimal; thus, degenerative changes were reversible since regeneration was unimpeded. Some of the fibers adjacent to the laceration zone were injured by the initial physical disruption, with its consequent crushing force. However, the late appearance of segmental and Wallerian degeneration suggested an ongoing noxious process affecting fibers in a nonuniform manner so that normal fibers intermingled with damaged axons and Schwann cells.

Conclusions

In the experimental mode reported in these two papers, the entire spectrum of nerve fiber damage was revealed within the confines of a major fascicle. Comparison of the changing histology in the zones of injury with the electrical data leads to the following conclusions:

The initial reduction in electrical activity is related to destruction of nerve fibers in the immediate region of the laceration. Further electrical deterioration is probably due to two factors, namely, continuing degeneration of nerve fibers in the intermediate zone in the region adjacent to the site of laceration, and a decrease in function of the fibers in the peripheral zone. Although they appear normal, the presence of the electron-dense ground substance that might be due to a proteinaceous, edema fluid suggests that they are in an abnormal environment. Reconstitution of the blood-nerve barrier by the reconstruction of the perineurium leads to the restoration of a stable endoneurial environment and probably causes the subsequent return of electrophysiological activity toward that recorded immediately after laceration. The undamaged peripheral fibers and those undamaged fibers of the intermediate zone thus resume their normal function. The later improvement in electrical function reflects regeneration of a portion of the cross section of the nerve; this is most marked in the intermediate zone where the damage is non-
neurotometric \(^4\) and scarring minimal. It also occurs to a lesser extent in the laceration zone where the injury is neurotmetic and subsequent scarring great.

Correlation of the time sequence of the histological observations with that of the electrophysiological observations suggests that in this model the electrical parameters give a reasonably accurate picture of the changing histology in the partially injured nerve.

Acknowledgments

The authors wish to thank Miss A. Drury, Mr. D. Hunter, Mr. William Coleman, and Miss Jackie Valentin for technical help.

References


This study was funded by DADA Grant 17-69-C-9133, Surgical Directorate, U.S. Army Research and Development Command, Washington, D.C., and M. R. C. Grant MA 3393.

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