Percussive injury to peripheral nerve in rats

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Weights were dropped on rat sciatic nerves. Teased fibers and light and electron micrographs of nerves removed between 10 minutes and 2 weeks later were examined. Axonal alterations were seen 10 minutes after injury, and subsequently interruption of axonal continuity with preservation of the basal lamina was apparent. Dissolution of myelin began within 30 minutes and progressed. At 4 days, a segment of some large fibers was devoid of myelin and, by 2 weeks, remyelination had commenced. Demyelination of a significant number of fibers was always accompanied by Wallerian degeneration of other fibers of the same nerve. Percussive injury of nerves caused a mixed lesion in which the early and late pathological features were clearly distinguishable from those following crush or compression by a cuff. Any explanation of the transient interruption of function that has been described following such an injury is at present speculative.

Key Words: peripheral nerve, nerve compression syndrome, nerve degeneration, sciatic nerve, myelin sheath, nerve conduction

Blunt injuries comprise crushing, compression, percussion, or stretching of nerves. In clinical practice, the result of a combination of more than one of these forces is usually observed; in the laboratory they can be studied in isolation.

Seddon outlined a classification of nerve injuries which was intended as a guide in clinical work. "Neurotmesis" implies discontinuity of all essential structures (but not necessarily of the epineurium) of the nerve; "axonotomy" indicates severance of axons within intact endoneurial sheaths and can, as Ramón y Cajal knew, be produced by crushing a nerve with a pair of forceps. The third term, "neurapraxia," describes a localized conduction block in which the motor paralysis (either complete or incomplete) is generally greater than the loss of sensation, and in which the speed of recovery of motor function precludes, as an explanation of its mechanism, Wallerian degeneration followed by regeneration. Seddon distinguished between two types of neurapraxia: that which recovers in a matter of weeks he correctly predicted to be caused by demyelination; that which lasts less than a day or so he thought unlikely to show any profound structural change. The pathological substratum of this briefer form of neurapraxia remains unknown.

The effects on a nerve of crush and of prolonged compression by a cuff have both been described ultrastructurally. In the former injury, axoplasm and myelin are displaced away from the crushed region, and expand the basal lamina on either side. Haftek and Thomas showed that the basal lamina remained intact in the crushed zone. Ochoa, et al., used light and electron microscopy to examine teased fibers of the baboon tibial nerve after compression by a cuff. They demonstrated, under each end of the cuff, intussusception of the terminations of the myelin lamellae on the axolemma in the larger myelinated fibers at the nodes of Ranvier, and they inferred that the axoplasm had been displaced longitudinally relative to its myelin sheath. Paranodal demyelination occurred later. When compression was more prolonged or at higher pressure, some fibers underwent Wallerian degeneration, and a few underwent segmental demyelination. Conduction through the lesion was blocked for up to 2 months, with little impairment of function distally.

The effects of percussion on peripheral nerve have been studied less intensively than have those of crush or compression by a tourniquet. Mitchell noted that: "In some cases where I struck the nerve sharply with a smooth broad whalebone slip, allowing a thin layer of muscle to intervene, the paralysis which ensued, although temporary, was in degree complete. Within a few days, the rabbit showed no discernible paralysis." He believed that this transient palsy was associated with a mechanical disturbance of the semifluid content of the nerve that was rapidly repaired. In other in-
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stances, changes in nerve fibers distal to the contusion were identical to those after section. In an article devoted to the effect of percussion on the cat sciatic nerve, Denny-Brown and Brenner experimented with blows over a wide range of intensity. They found a motor paralysis, complete for dorsiflexion and partial for plantar flexion, when the cat was examined the next day. The deficit persisted 3 to 4 days, and subsequently disappeared over a period of 48 hours. No histological abnormality was detected in the first 24 hours. By 3 days the nerve was swollen with interstitial edema and cellular infiltration, and, by 5 days, many of the myelin sheaths had disappeared. At 14 days, longitudinal sections, stained with silver, showed axons that were devoid of myelin for a distance of 0.01 to 1.1 mm. Some fibers appeared normal. With osmium tetroxide staining, distal degeneration was demonstrated in less than 2% of the fibers. The transient paralysis was attributed to demyelination, in spite of the admitted disparity between the duration of the palsy (less than 1 week) and the time of remyelination (not less than 13 days).

In the present study, the structural lesion in peripheral nerves, caused by percussion, has been re-examined.

Materials and Methods

Observations were made in 46 adult Sprague-Dawley rats. The sciatic nerve was isolated in the anesthetized animal and a weight (2.5, 5, or 10 gm) was dropped from a height (20, 10, or 5 cm) onto the nerve immediately above its bifurcation. Figure 1 is a photograph of the weight-drop apparatus. At intervals between 10 minutes and 2 weeks later, the damaged nerves were removed after local and/or systemic perfusion with a solution of 0.5% paraformaldehyde and 1.5% glutaraldehyde in 0.2 M phosphate buffer. The common peroneal and tibial divisions were separated from their common epineurial sheath before tissue processing.

From 24 rats, nerves injured by a 50 gm-cm blow were prepared for light and electron microscopic examination. Specimens were left 3 hours in fixative, washed in buffer, post-fixed 2 hours in osmium tetroxide, dehydrated in graded concentrations of ethanol, transferred to propylene oxide and embedded in Epon. Sections 1 μm thick were mounted on glass slides, stained with toluidine blue, and examined with a light microscope. Ultrathin sections were stained with lead citrate and uranyl acetate and examined with a Siemens Elmiskop 102.

Teased fibers from the tibial nerves of 21 rats were studied after injuries of 12.5 to 50 gm-cm. Teased fiber preparations permit the demonstration of several internodes of the same fiber, which is not usually possible with conventional longitudinal sections. The processing schedule before fiber teasing was similar to that for microscopic study. However, nerves were immersed for 5 hours rather than 3 hours in osmium tetroxide and passed through toluene rather than propylene oxide. The nerve was placed on a glass slide and examined under a dissecting microscope.

The epineurium was removed and the nerve divided into quarters from each of which a bundle containing 25 to 50 large myelinated fibers was selected. Each of these fibers was isolated and characterized. In addition, some single teased fibers were transferred to another glass slide so that they could be studied at higher power with the light microscope. Finally, four individual teased fibers were embedded in Epon, sectioned transversely with an ultramicrotome and examined by electron microscopy.

Histograms of the size of the fibers in one tibial nerve at three levels in the thigh were prepared by counting all myelinated fibers in a cross-sectional light micrograph (× 1000) and measuring the diameter of at least 500 fibers under a Zeiss particle-size analyzer TGZ3.

At intervals between recovery from general anesthesia and sacrifice, the ability of the rats to flex and extend the ankle and to spread the toes was determined by examination.
Results

First 4 Hours after Injury

Light Microscopy. After each percussive blow, blood was present in either the epineurial or endoneurial space. The hemorrhage was not always seen with the naked eye but was obvious with the operating or light microscope. The nerve became swollen within minutes of injury. Usually the perineurial sheaths were intact, but occasionally one sheath was breached and a part of its contents had herniated.

In cross-sectional light micrographs of the damaged nerve soon after injury, the endoneurial space was increased in area and contained extravasated red blood cells and extensive fluid (Fig. 2). Injury to blood vessels must therefore have occurred but most of the capillaries that were seen appeared normal.

Teased Fibers. As early as 10 minutes after injury, discrete segments of the teased fibers showed pale and irregular staining. The fiber diameter in these abnormal zones was either normal or decreased. By 90 minutes, in many large fibers one or two entire internodal segments were abnormally pale. Although the transition between abnormal pallor and normal intensity of staining often occurred abruptly at a node of Ranvier, changes in the node itself were minimal.

Three of the lesions that might have been expected in teased fibers were sought, but were seen infrequently or not at all. Following a crush injury, myelin and axoplasm are displaced longitudinally so that a focal attenuation with globular enlargement to either side is seen. This change was observed infrequently with blows of 50 gm-cm. The intussusception of nodes and paranodal demyelination that Ochoa, et al., described following prolonged compression with a cuff were not detected. Finally, after injury of 50 gm-cm impact, it was very unusual to see complete interruption of fibers ("neurotmesis" in Seddon’s terms).

The axon was shrunken in all four teased fibers that were examined ultrastructurally, and in two fibers there was, in addition, splitting of the outer myelin lamellae. One axon was followed through its zone of injury (about 0.5 mm in length), and although it dwindled to one-quarter of its normal diameter, discontinuity was not demonstrated. In serial cross-sectional electron micrographs, as in the intact teased fiber, the zone of transition from abnormal to normal was localized at a node of Ranvier.

Electron Microscopy. Changes indicative of interruption of axonal continuity were detected soon after injury. The precise point of disruption was sometimes seen (Fig. 3 upper). By 30 minutes, and particularly by 90 minutes, segregation of organelles was apparent in some axons. Filaments and tubules were concentrated centrally and capped by vesicles and mitochondria which were peripheral and terminal (Fig. 3 lower left). Within 90 minutes, the axoplasm of some fibers had a granular appearance (Fig. 3 lower right). Occasional myelin sheaths contained no axon whatsoever. Neither empty basal laminal tubes nor tubes distended by a mixture of axonal and myelin debris were commonly seen. Both of these are prominent features of crush injury.

A common early change was failure of the axon to fill its myelin sheath (Fig. 4 left). Spaces adjacent to the axon were observed as early as 10 minutes after injury, and became larger and more frequent over the next 90 minutes. They were consistently identified, in cross sections through the zone of injury in more than 40% of large myelinated fibers, whereas they were present in less than 10% of large myelinated fibers of a control segment in an uninjured part of the same nerve, or in the contralateral nerve. This was true with either local or systemic fixation, despite variations in the concentration of sucrose (and therefore the osmolality) of the fixative and buffer. In some instances, the space was between the axolemma and inner Schwann cell cytoplasm, that is, a dilatation of the normal periaxonal space. In other fibers, the split was at the intraperiod line of one of the inner myelin lamellae, and the inner cytoplasmic tongue of the Schwann cell remained adherent to the axolemma (Fig. 4 right). The adventitial space was either electron-lucent or was partially filled with vesicular or scattered granular material. The organelles in the attenuated axons were often well preserved, although highly concentrated to an abnormal degree.

Axons demonstrating any of these appearances
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Fig. 3. Posttraumatic electron micrographs. **Upper:** Four hours after injury. The myelin sheath and possibly the axon appear interrupted but not the basal lamina. × 6000. **Lower Left:** Ninety minutes after injury. Tubules and filaments are concentrated centrally in the axon; mitochondria and lysosomes are peripheral. × 7600. **Lower Right:** Four hours after injury. The axon contains only granular debris. × 7200.

(shrinkage, absence, granular degeneration, or segregation of organelles) could be surrounded by normal myelin or by myelin with vesicular dissolution. The basal lamina was almost always intact. The axons of large myelinated fibers were more commonly and more grossly abnormal than those of smaller fibers. Unmyelinated axons were the least affected; however, a few unmyelinated axons were swollen by numerous mitochondria and vesicles containing only granular material. These two changes were interpreted as indicative of axonal section.

Splitting of the myelin sheath and an increase in diameter were seen in fibers at 30 minutes and these changes later became more pronounced (Fig. 5). The contents of the myelin cleft were either electron-lucent or vesicular. Dissolution of myelin lamellae could be seen at any radial distance. The point of separation of myelin lamellae was usually at the intraperiod line between two major dense lines. In other words, the extracellular space had expanded. The dilatation of the periaxonal space described previously was perhaps another manifestation of this phenomenon of enlargement of the extracellular space. This "extracellular vesicular dissolution" of myelin was distinct from opening of the Schmidt-Lantermann clefts, which is seen in an inadequately fixed nerve and in nerve adjacent to crush injury. The axon within an abnormal myelin sheath usually displayed at least one of the pathological alterations described previously, but was sometimes normal.
Fig. 4. Left: Electron micrograph of a nerve fiber 30 minutes after injury. The axon does not fill its myelin sheath. × 17,000. Right: A higher power electron micrograph of another fiber. The inner cytoplasmic tongue of the Schwann cell has separated from the rest of the myelin and is adherent to the axolemma. × 105,000.

Fig. 5. Electron micrographs 4 hours after injury. Left: Focal dissolution of myelin sheath is seen. × 16,000. Right: Gross vesicular dissolution of myelin has occurred. × 4500.
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Four Days after Injury

At 4 days, the number of red blood cells in the endoneurial space had decreased, but the nerve was still swollen with excessive endoneurial fluid and inflammatory cells. Numerous lymphocytes and fibroblasts were present in the endoneurium. Few of the attenuated axons or vacuolated myelin sheaths seen in the acute phase persisted in the same form. Myelin debris abounded, some of it phagocytosed by macrophages which were found free in the endoneurial space and also within basal laminae of the nerve fibers (Fig. 6 left). The contents of the preserved basal laminal tubes varied. Some contained normal myelinated or unmyelinated nerve fibers and some, as already mentioned, contained only myelin debris. In others, Schwann cell processes were the only contents. Small axons, presumed to be sprouts, were found adjacent to degenerating myelin sheaths, or more commonly in association with Schwann cell processes. Figure 6 right shows a large axon completely devoid of myelin and surrounded only by a crenated basal lamina. Such structures were interpreted as demyelinated axon segments.

Fiber diameter histograms from a slightly later stage (6 days) are shown in Fig. 7. The number of large myelinated fibers is greater distal to the site of injury than at the site of injury. The most likely explanation is that segmental demyelination has occurred.

Two Weeks after Injury

Light Microscopy. The injured segment of nerve was still swollen 2 weeks after injury, but less so than at 4 days. Most, but not all, of the myelin debris had been removed at the site of injury, although much of it persisted in distal segments. The extensive rearrangement of the endoneurial architecture that Morris, et al., described following nerve section was not seen. Instead, the normal topography was maintained. The perineurium in most instances was intact. The density of large myelinated fibers was decreased, and many thinly myelinated axons were seen.

Teased Fibers. Two weeks after injury, it was impossible to characterize each large myelinated fiber as being normal, showing focal segmental demyelination (Fig. 8), or having undergone Wallerian degeneration. Paranodal demyelination was very infrequent, and was ignored in the semi-quantitative assessment. The amount of degeneration and demyelination varied in different nerves struck with the same weight dropped from the same height. However, in a moderate range of intensity of injury, a mixed lesion resulted, with a significant number of fibers in any one nerve showing each of the three states: Wallerian degeneration, segmental demyelination, and normalcy (Fig. 9). In no nerve did more than one-half of the large fibers contain a focally demyelinated segment.

Electron Microscopy. Many unmyelinated fibers and small myelinated fibers appeared normal. Un-
myelinated large fibers and sprouts, commonly seen at 4 days, were now infrequent. A hypomyelinated fiber (such as that seen alongside a normal fiber in Fig. 10) possibly contained an axon that had lost its myelin sheath and was undergoing remyelination. However, 7 to 10 days after nerve transection, Morris, et al., described myelinated axonal sprouts in the proximal stump. In the present experiments it was difficult to say, therefore, whether any particular thinly myelinated fiber seen in a cross-sectional electron micrograph had regenerated and was being myelinated or was simply being remyelinated. These thinly myelinated fibers were often surrounded by two basal laminae. An outer crenated lamina was presumed to be the lamina that originally surrounded the fiber; an inner lamina was adherent to and probably formed by the Schwann cell which had myelinated the axon. Occasionally, two thinly myelinated fibers were surrounded by one basal lamina.

Discussion

Experimental Method

Injuries inflicted in the laboratory by the dropping of weights mimic, to some degree, those encountered in clinical experience, yet are amenable to quantitative analysis. This experimental model has been used for more than 60 years to study spinal cord trauma. Dohrmann, et al., described some of the biomechanical aspects that must be taken into consideration in order to inflict a reproducible spinal cord injury. In the present experiments, the lesion varied in the peripheral nerves of different animals when the same weight was dropped from the same height. With uniform animal size, avoidance of stretch during injury, and precise positioning of the nerve in the apparatus, it is anticipated that the lesion could be standardized.

The early changes in the present experiments resembled those described by Dohrmann, et al., that took place soon after spinal cord trauma. In both in-
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Fig. 9. Block graph showing the percentage of large myelinated fibers that have undergone no change (white blocks), segmental demyelination (dotted blocks), or Wallerian degeneration (black blocks).

stances there was axonal shrinkage, dilatation of the periaxonal spaces, and dissolution of the myelin sheath. Despite the structural differences between axons and neuroglia in the central and peripheral nervous system, the early reaction of fibers to at least one form of injury appears to be similar in the spinal cord and peripheral nerve. It is suggested that peripheral nerves can be used to advantage in the elucidation of basic biophysical processes of nerve-fiber trauma.

Axonal Changes

The single most common lesion seen after percussive nerve injury was interruption of axons with preservation of the basal laminae that surround the myelin sheaths ("axonotmesis" in Seddon's terms). Soon after injury, segregation of axonal organelles and granular degeneration of axoplasm were seen, both of these pathological processes indicating that axonal section had occurred. The segregation of organelles resembled that which has previously been described in axonal stumps after nerve crush or transection. Granular degeneration of axoplasm with destruction of microtubules and filaments was seen near the site of injury within 90 minutes. This change has previously been described in distal segments during the course of Wallerian degeneration, occurring at 24 to 48 hours after injury. It is concluded that shrinkage of axons and dilatation of the periaxonal spaces was a direct consequence of injury rather than a nonspecific artifact. The possibility that these changes were exaggerated during tissue processing has not, however, been eliminated. The axonal shrinkage appeared to extend as far as the nodes of Ranvier. It is envisaged that the stumps of interrupted axons retract as far as a node, where the terminal cytoplasmic rim of the myelin lamellae is firmly adherent to the axolemma (paranodal axoglial junction). In the present study it is not determined whether all the abnormally narrow axons were divided at some point in their course or whether they were merely attenuated over a distance. Morgan-Hughes and Engel, after crushing guinea-pig nerves, concluded that axons were not always severed immediately, and that for 24 hours after injury axonal stumps reeled from the borders of the crushed zone as far as a node of Ranvier. Shrunk axons that fail to fill their myelin sheath are seen adjacent to nerve crush or transection. Young postulated that cut nerve ends retract because of the disorientation of longitudinally arranged filaments in the axoplasm.

Fig. 10. Electron micrograph 2 weeks after injury, showing one thinly myelinated fiber and one normally myelinated fiber. ×8000.
Wallerian degeneration of some fibers was seen in all nerves with any structural changes following injury. In this respect, our results differed from those of Denny-Brown and Brenner, who observed extensive demyelination with little or no Wallerian degeneration. This discrepancy is perhaps due to differences in the animal species and in the precise method of injury, or perhaps to the more sensitive histological methods for the detection of Wallerian degeneration that are now available.

Myelin Changes

The structure of the myelin sheath was altered rapidly and greatly by percussive injury. The extracellular space expanded, causing myelin lamellae to separate at the intraperiod line. A similar "extracellular vesicular dissolution of myelin" has been described in peripheral nerve fibers after irradiation, after immersion in hypotonic solution, after systemic intoxication with triethyl tin, lead, or hexachlorophene, and in some cases of idiopathic inflammatory polyneuropathy. This acute change may also in some way be related to the myelin "bubbles" seen in amputation neuromata of long standing.

That focal segmental demyelination occurred in at least some fibers was substantiated by the presence at 4 days after injury of large demyelinated axons, by the fiber diameter histograms at 6 days posttrauma, which showed more large myelinated fibers distal to than at the site of injury, and by the appearance of teased fibers at 2 weeks after injury. It is likely that demyelination, when it does occur, is the consequence of the acute process described in the previous paragraph. Demyelination can, of course, only be observed in fibers in which the axon is preserved. More severe trauma causes axonal interruption and Wallerian degeneration.

Remyelination started at about 7 to 10 days after injury. The time course of demyelination and remyelination was similar to that described after a "perineurial window" is fashioned. In some of the present experiments, the perineurium was breached but demyelinated fibers were also frequently observed within an intact perineurium so that simple opening of the perineurium cannot be the only pathogenetic mechanism involved.

Comparison with Cuff and Crush Injury

After a localized crush injury, the tubes of the basal lamina directly under the forceps blades are empty, and to either side are distended with disorganized myelin and axoplasm which are of semi-fluid composition and have been displaced. After percussive injury, axons or their myelin sheaths are not physically displaced to the same extent, and neither empty basal laminal tubes nor masses of disorganized axoplasm and myelin are seen. However, in both instances, the essential axonal lesion is interruption of continuity within an intact basal lamina (in Seddon's terms, "axonotmesis"). Axons are able to return to their previous termination, and much better return of function is expected than after nerve transection and suture. With excessive crush or percussion injury, the basal lamina can, of course, be severed but it is more resistant to these forms of trauma than is the axon. After cuff compression of the sciatic nerve at 1000 mm Hg for 1 hour, no Wallerian degeneration was detected.

Weights dropped on a nerve cause myelin breakdown and, later, focal segmental demyelination. Demyelination also occurs after cuff compression, but it is predominantly paranodal and is related to nodal intussusception. After crush injury, the axons are all severed so that demyelination is not observed, although undoubtedly the Schwann cell and myelin sheath are also injured.

In summary, percussive injury to the nerve is a mixed lesion with Wallerian degeneration (axonotmesis), focal segmental demyelination, and undamaged fibers. Crush leads exclusively to the former process, and cuff compression causes a different form of demyelination. Although similarities do exist, it is preferable, in experimental work, to distinguish these three forms of blunt trauma from one another.

Neurapraxia

Except in injuries that disrupted more than 90% of the large myelinated fibers, no weakness was detected in the muscles innervated by the sciatic nerve. We can only speculate, then, on the cause of the transient interruption of nerve function observed by Mitchell and by Denny-Brown and Brenner. The sole proven basis for any neurapraxic lesion is demyelination, which accounts for impairment of function of more than 1 week's duration, but not for that with resolution in a few days. Three explanations of this brief type of neurapraxia require consideration: 1) The blood-nerve barrier was damaged, and the endoneurial milieu changed as witnessed by the fact that hemorrhage and edema were consistently seen. A grossly abnormal biochemical environment, such as potassium at 20 mM concentration, can block saltatory conduction, but it is unlikely that changes of such magnitude occurred; 2) The observed axonal attenuation and myelin dissolution would lead respectively to increased axial resistance and decreased membrane impedance, both of which would cause increased current decrement between nodes and perhaps failure to excite the succeeding node of Ranvier. It has not been proved that these two structural alterations are reversible; 3) Changes in the nodal properties sufficient to cause failure of impulse transmission might be associated with minimal morphological changes which we failed to detect.

Elucidation of the type of neurapraxia that lasts less than a few days awaits further electrophysiological study.

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Acknowledgments

The authors wish to thank Miss Ursula McGuinness who did almost all the technical and photographic work, Dr. Rosalind King who made numerous helpful suggestions, and Dr. Alan Hudson who reviewed the manuscript.

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This work was supported in part by a grant from the Medical Research Council of Canada.

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