

## Structural alterations of nerve during cuff compression

(nerve compression/shear forces/axonal degeneration/segmental demyelination)

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**ABSTRACT** Whether compression nerve injury is due to ischemia, direct mechanical injury, or both remains unsettled. To assess structural changes of nerve during compression, peroneal nerves of rats were compressed at various pressures for different times, and the structural alterations were stopped by simultaneous *in situ* and perfusion fixation. The structural changes observed during a few minutes of compression cannot be explained by ischemic injury because the pathologic alterations characteristic of ischemia take many hours to develop and in any case are different from the ones found here. The pressure- and time-related structural changes observed in the present study under the cuff were (i) decrease in fascicular area and increase in fiber density due to expression of endoneurial fluid; (ii) compression and expression of axoplasm, sometimes to the point of fiber transection; (iii) lengthening of internodes; and (iv) obscuration of nodes of Ranvier due to cleavage and displacement of myelin and overlapping of nodes by displaced loops of myelin. At the edges of the cuff the changes were (i) increase of fascicular area probably from expressed endoneurial fluid; (ii) widening of nodal gaps, perhaps mainly from translocated axonal fluid; and (iii) disordered structure of axoplasm. We suggest that the process of paranodal demyelination and axonal transection are linked, occur during the act of compression, and are due to shear forces. The initial event is expression of endoneurial fluid, followed by compression and expression of axoplasm and cleavage and displacement of layers of myelin. Conceivably, with prolonged cuff compression ischemic injury might be found to be superimposed on mechanical injury.

In humans, nerve compression injury may follow use of a tourniquet at too high a pressure, for too long a time, or from improper application; lying in one position without moving for a long time (as may occur during anesthesia, inebriation, or coma) with a limb nerve compressed against bone by a protruding ridge or hard surface; or prolonged sitting with the legs crossed or prolonged leaning on the elbows (1, 2). Excellent recovery is expected after compression injury, whereas it is usually delayed and faulty after nerve transection. This difference in outcome is usually explained by conduction block and demyelination in the former and complete fiber degeneration and faulty regeneration in the latter (3–5).

The mechanisms underlying nerve compression injury have usually been attributed to ischemia (6–8), to mechanical forces (9), or to both.

In the present study the structural alterations of nerve during compression were stopped by simultaneous *in situ* and perfusion fixation, usually during short periods of compression. We found nodal lengthening and other acute structural changes after only a few minutes of compression, which appear to explain the paranodal demyelination and axonal degeneration that are characteristic of nerve compression

injury. The structural alterations that develop during acute compression are different in their time of appearance and in type from those induced by ischemia (10–14). We infer that the structural changes are due to stress forces rather than ischemia. Although the conditions and effects of cuff compression (especially the effect of a given pressure or duration) cannot be directly extrapolated to tourniquet or compression injury in humans (there might be great differences due to species, age, amount of overlying tissue, amount of endoneurial connective tissue, and area of nerve compressed), the mechanisms should be similar and this study is relevant to understanding human nerve compression injury.

### MATERIALS AND METHODS

The cuff (for illustration, see ref. 15) consisted of two balloons held in fixed apposition to one another by metal frames, one under and the other over the mobilized peroneal nerve of rat. When the cuff was inflated, the nerve was compressed between the two balloons. The balloons were made of nondistensible Silastic sheeting with subdermal implants (no. 501-1, Dow) and were fitted over two rectangular box-like metal frames with the open sides (bottom or top) of each frame facing the nerve. The inner surfaces of the balloons were glued to each of the outer surfaces of the sides with the closed side (top or bottom) of the metal frames facing away from the nerve. When just inflated at 0 pressure, the apposing surfaces of the balloons would just meet without folds in the balloons for the width of the cuff (8 mm).

Under pentobarbital anesthesia, the left peroneal nerve of a 300-g Sprague-Dawley rat was compressed for a given pressure and time and then fixed *in situ* and by perfusion. Fixation was begun by pouring 4% (vol/vol) glutaraldehyde in 0.025 M sodium cacodylate (pH 7.38 and 37°C) into the tissue well made by elevating the cutaneous edges of the surgical incision and simultaneously perfusion fixation was begun through the left ventricle of the heart. To begin perfusion fixation at the end of the predetermined time of compression, the chest operation started 1 min before both fixation schedules were to begin. As soon as the perfusion fixative reached the lower limbs, indicated by muscle quivering and beginning stiffness (15–20 sec), the cuff was deflated and removed from the nerve. For the first minute, perfusion by pump was at a rate of 100 ml/min and then at a rate of 60 ml/min for a total of 1 liter of fixative solution.

After *in situ* and perfusion fixation, the nerve was removed from a point 5 mm above the proximal edge of the cuff (transverse cut) to a point 5 mm distal to the distal edge of the cuff (oblique cut). The nerve was fixed overnight in 2.5% glutaraldehyde in 0.025 M sodium cacodylate, washed, and osmicated, and serially cut tissue blocks were embedded in epoxy so that transverse and longitudinal semithin and thin sections could be cut. Other nerves were fixed for shorter times in aldehyde; after osmication and glycerination they

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Abbreviations: MF, myelinated fiber; FA, fascicular area.  
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were teased apart for the evaluation of at least 100 individual myelinated fibers (MFs) from proximal (proximal end of the nerve to distal margin of the cuff) and distal (distal margin of the cuff to the distal end of the nerve) segments of nerve.

Transverse 0.75- $\mu\text{m}$  epoxy-embedded sections of the serial, 1- to 2-mm-long blocks of peroneal nerve were stained with phenylenediamine, and the number of MFs per nerve and the size distribution of MF diameters (diameter of a circle of equivalent area) were measured by using described techniques and our imaging system for nerve morphometry (16).

## RESULTS

**Testing the Assumption That *in Situ* and Perfusion Fixation Stop the Pathologic Alteration.** In a series of preliminary studies, we determined that the *in vivo* indentation of the nerve by the cuff was maintained with this fixative schedule. Low-pressure and short-duration compression resulted in mild indentations, whereas high-pressure and long-duration compression resulted in severe flattening of the nerve between the balloons; both indentations were maintained after fixation. Differences in teased fibers and in ultrastructural alterations, to be described below, also provided evidence that fixation was rapid enough to reflect structural alterations induced by the cuff. Fixation was not instantaneous but presumably was rapid enough to maintain approximate structural alterations even after the cuff was removed during fixation. We know that tissue alterations described below were due to physical distortion of nerves and not due to poor fixation for the following reasons. (i) The changes were stereotyped, graded with increasing pressure, and reproducible—having been observed in  $\approx 100$  nerves. (ii) The changes did not occur in sham-compressed (cuff in place without pressure) nerves. (iii) Ultrastructural preservation of mitochondria, neurofilament, and microtubules and of myelin lamellae and periodicity was very good—in poor fixation, good mitochondrial preservation is not expected.

**Effect on Fascicular Area (FA) or on MF Density with Increasing Pressures or Durations of Compression During Cuff Compression.** The FA and MF density (number of MFs per  $\text{mm}^2$ ) were assessed in sections from 16 consecutive blocks in each peroneal nerve compressed at a pressure of 150 mg for various times (0, 10, 30, 60, 120, or 240 min) or compressed

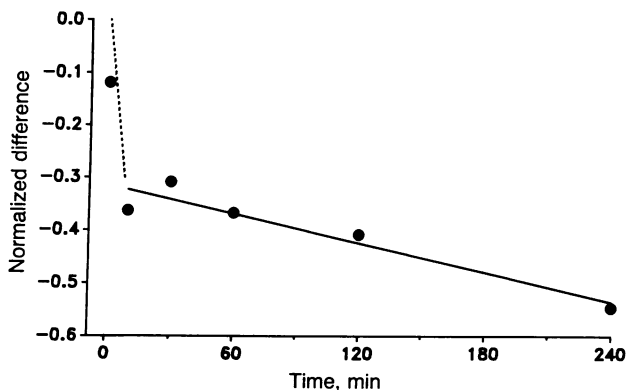


FIG. 1. Mean FA of sections of tissue blocks of peroneal nerve under the cuff expressed as a difference from 1 (the average of the FA of all tissue blocks of the nerve) was calculated for nerves compressed at 150 mm Hg for various times as shown. For the nerve marked 0 time, the compression and the *in situ* and perfusion fixation began at 0 time but the structural alterations were actually stopped somewhat later. The point is plotted as if it occurred at 0 time. The change in FA probably has two phases: an initial rapid one and a later slow one as discussed in the text. Similar but opposite effects (not shown) were demonstrated for the number of MFs per  $\text{mm}^2$ .

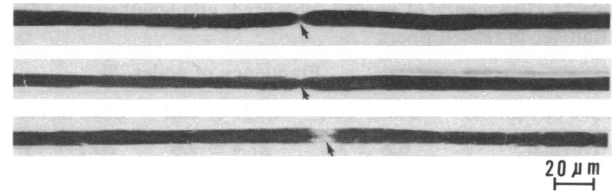


FIG. 2. Lengths of teased fibers from the ends showing a normal node (arrow) of Ranvier (Top), from under the cuff showing an obscured node (Middle), and from the edge of the cuff showing a lengthened node (Bottom) from rat peroneal nerve that was fixed *in situ* and by perfusion during compression at 300 mm Hg for 2 min.

for a constant time (120 min) at various pressures (0, 20, 50, 150, or 300 mm Hg) (11 nerves  $\times$  16 sections = 176 sections).

During compression the FA was decreased under the cuff and was increased at the edges of the cuff, whereas the MF density was increased under the cuff and decreased at the edges of the cuff. Fig. 1 shows the decrease in FA under the cuff when nerves were compressed at 150 mm Hg for various times relative to the average FA of tissue blocks of the nerve. There was an initial rapid and then a slower decrease of FA with increasing durations of compression. When the MF density was assessed, the opposite effect was found. Nerves compressed for 120 min with various pressures showed a decrease in FA (or increase in MF density) under the cuff and an opposite effect at the edges of the cuff.

**Alterations of Nodes of Ranvier and Internodal Length During Cuff Compression in Teased Fibers.** In an initial study of 100 teased fibers from nerves compressed at 50, 150, and 300 mm Hg for 2 min, reproducible alterations of nodes of Ranvier were recognized under the cuff, at the edges of the cuff, and at the ends of the nerve; these were quantitated in seven nerves compressed at 300 mm Hg for 2 min. Typically, at

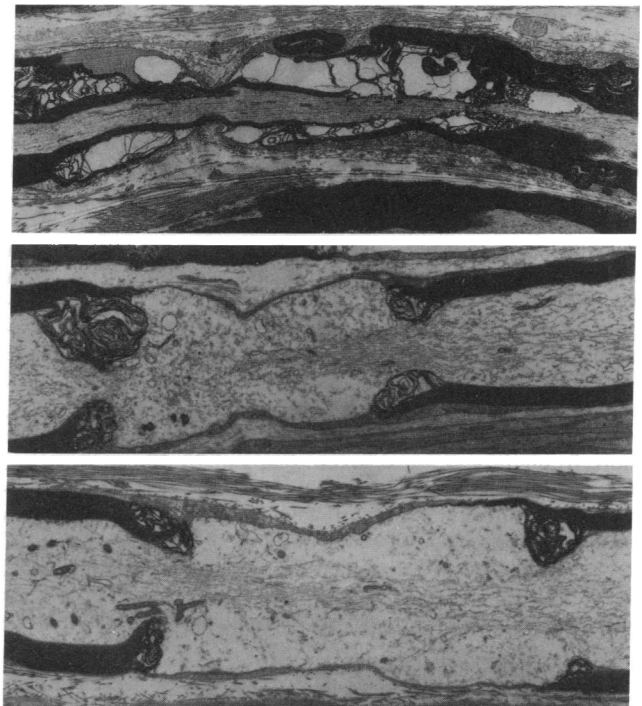


FIG. 3. Electron micrographs of obscured node (Top) ( $\times 2000$ ) and lengthened nodes [Middle ( $\times 3000$ ) and Bottom ( $\times 3600$ )] from peroneal nerve of rat compressed at 300 mm Hg for 4 min. The obscured node came from peroneal nerve near the edge of the cuff; the lengthened node also came from near the edge of the cuff. The features and mechanisms of these structural changes are given in text.

Table 1. Frequency of normal, obscured, and lengthened nodes of Ranvier in teased fibers

Nodes of Ranvier	Frequency of nodes, %		
	Under cuff	Edge of cuff	Ends of nerve
Normal	6.8 ± 5.0	27.4 ± 9.6	80.2 ± 11
Obscured	70.8 ± 8.9	36.6 ± 6.3	9.6 ± 7.1
Lengthened	22.4 ± 5.1	36.0 ± 8.1	10.2 ± 5.5

Data are from seven rat peroneal nerves compressed at 300 mm Hg for 2 min. The frequency of nodes is expressed as mean ± SD. Considering the frequency of the node condition among the three levels of peroneal nerves evaluated, statistically significant differences ( $P < 0.004$ ) were found for all nine comparisons.

the proximal and distal ends of the nerve normal nodes of Ranvier were found. Under the cuff the nodes were frequently obscured (Figs. 2 and 3). At the edges of the cuff, normal, obscured, or lengthened nodes were found (Table 1). Internodal length was measured in teased fibers from nerve under the cuff, at the edges of the cuff, and at the ends of the nerve. The average internode lengths of teased segments with obscured nodes were estimated by first determining the number of internodes in this segment (the nearest whole number obtained by dividing the length of the segment with obscured node by the average internode length of the same teased fiber in proximal and distal ends of the nerve) and dividing the measured length of the fiber segment with obscured node by the number of calculated internodes. The average internode length was significantly longer under the cuff than at the ends of the nerve (Table 2).

**Ultrastructural Alterations During Cuff Compression.** Semithin and thin transverse and longitudinal sections were cut from peroneal nerves compressed at 50 mm Hg for 2 min, at 150 mm Hg for 2 min, at 300 mm Hg for 2 min, and at 300 mm Hg for 2 hr (8 nerves × 15 blocks = 120). Four control (without compression) peroneal nerves were also studied. In transverse sections from under the cuff the axons were usually compressed, with cytoskeletal components and mitochondria closely packed together (Fig. 4). The degree of compression occurred in more fibers and was more severe at higher pressures. In the nerve compressed at 300 mm Hg for 2 hr, axonal contents frequently could not be seen (Fig. 4) and if present were often severely attenuated with components matted together. Complete disappearance of axoplasm components was also sometimes observed when lower pressures were used. At low pressures and for short times the normal circular outline of the myelin sheath was altered to form irregular-shaped profiles. At high pressure, myelin was severely split and distorted. Myelin periodicity was not appreciably altered from normal. "Obscured" nodes of Ranvier were explained by excessive cleavage of myelin and overlapping of nodes by redundant loops of myelin. At cuff edges, lengthened nodes were commonly encountered. These were more frequent and more severe (gaps of 20–50 μm were not uncommon) at increased pressures or durations of cuff application. Paranodal myelin was frequently displaced—in both directions from what we think were the former sites of nodes of Ranvier. Precise identification of the original nodal site was frequently not possible with certainty. Sometimes one paranode had a relatively normal appearance and the

Table 2. Internodal lengths of teased fibers

	Length of internodes, μm		
	Under cuff	Edge of cuff	Ends of nerve
	1392 ± 153	1370 ± 128	1287 ± 145

Data are from seven rat peroneal nerves compressed at 300 mm Hg for 2 min and are presented as mean ± SD.  $P < 0.001$  for under cuff vs. ends of nerve and for edge of cuff vs. ends of nerve.  $P > 0.05$  for under cuff vs. edge of cuff.

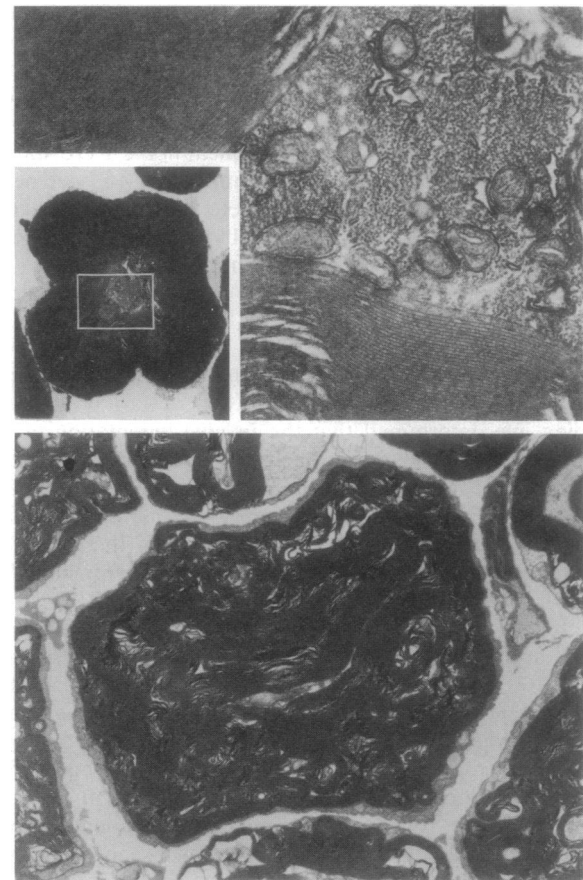


FIG. 4. Electron micrographs of transverse section of peroneal nerve of rat to show the compaction of cytoskeleton and organelles of axons that occurred under the cuff when peroneal nerve was compressed at 300 mm Hg for 2 min (Upper) and at 300 mm Hg for 2 hr (Lower). (Inset) Low-power view (×4800) of a transverse section of a myelinated fiber showing the rectangular area at a higher magnification (Upper) (×38,300). The ultrastructural preservation of microtubules and of cristae of mitochondria provides evidence that fixation is adequate. (Lower) In the transverse section (×7700) of the fiber, myelin is split and crumpled and axoplasm is not seen. The pressure that induced these changes and their sites and mechanisms of the changes are described in text.

other one was partially or wholly shifted away from the node. Paranodal Schwann cell cytoplasm was often watery. The axon caliber at nodes was often enlarged. Not infrequently at center of axons there was a core of relatively normal-appearing axoplasm surrounded by watery fluid (Fig. 3).

**Axonal Degeneration After Cuff Compression.** In teased fibers obtained 1–2 weeks after compression the frequency of teased fiber abnormalities was estimated in proximal and distal nerve segments (Table 3). Demyelination and axonal degeneration occurred at all pressures and times studied. Demyelination and remyelination were essentially confined to the former region of the cuff, whereas axonal degeneration was the only change in the distal segment. The frequency of all pathologic alterations of teased fibers (demyelination, remyelination, and axonal degeneration) was higher under the cuff than in the distal segment.

## DISCUSSION

Compression injury was, at first, attributed to ischemia because (i) nerve function was known to be dependent on blood supply and perfusion was impaired during compression, (ii) nerve conduction failure did not occur even when nerve *in vitro* was compressed under high pressures (in this

Table 3. Mean frequency of teased fiber abnormalities in proximal and distal segments of peroneal nerves compressed 7–14 days earlier

Nerves, no.	Pressure, mm Hg	Duration		Frequency of abnormalities, %					
		Compression, min	To harvesting, days	Proximal			Distal		
				AB	CDFG	EH	AB	CDFG	EH
1	50	2	10	70	18	12	82	0	18
1	150	2	10	30	39	31	65	1	34
9	150	30	14	*	*	*	61.8 ± 13.4	0.3	37.9 ± 13.7
7	300	2	7–10	*	*	*	84.5 ± 14.2	0	15.5 ± 14.2

A, normal; B, myelin irregularity; C, demyelination; D, demyelination and remyelination; E, axonal degeneration; F, remyelination; G, focal myelin thickening; H, axonal degeneration and regeneration. \*, Difficulty, due to breakage, made judgment of teased fiber abnormalities problematic. Some data are presented as mean ± SEM.

experimental situation shear forces are obviated) (8, 17), (iii) nerve injury was greater with increased durations of compression, and (iv) large MFs were especially vulnerable to ischemic injury. Today mechanisms other than ischemia (mechanical shear forces) may explain the greater nerve injury that develops with prolonged cuff compression and recent work suggests that ischemia injures all classes of fibers, not large fibers selectively (10, 11, 13) [for a report that suggests that myelinated and unmyelinated fibers are selectively vulnerable, see Parry and Brown (18)].

An alternative hypothesis, namely, that mechanical forces cause the structural alterations after cuff compression came from the observation by Ochoa *et al.* (5, 9). They found that myelin was intussuscepted into the adjacent paranodes, in opposite directions at either end of the cuff. The resultant redundant loops of myelin overlying nodes of Ranvier later degenerated and caused paranodal demyelination (refs. 5 and 9, and Jose L. Ochoa, personal communication). Others, accepting the role of mechanical injury to fibers from compression, explained the selective involvement of large-diameter fibers, especially at the edges of the cuff, by the expected greater effect of shear forces on cylinders of large size (19). Making the leg ischemic before and during compressing the nerve with a ligature did not appear to produce more severe conduction block or pathologic abnormality than when the nerve was similarly compressed without having made the leg ischemic (20)—further evidence that ischemia is unlikely to be responsible for the structural nerve alterations. Despite these compelling studies, Powell and Myers (8) again invoked ischemia as the mechanism underlying compression injury. In part they based this opinion on finding necrosis of paranodal Schwann cell cytoplasm. They explained the sub-perineurial involvement by ischemia as mediated by the transperineurial vessels.

The structural changes that we have described herein are likely to be due to cellular events related to mechanical forces and not to ischemic injury because the duration of compression that produce acute alterations was too short to induce ischemic alterations (21), and the pathologic alterations were of the type consistent with mechanical injury and were different from those found after ischemia. Experimental models of ischemia produce structural alterations that have a different time course, distribution, and type of fiber change than described here.

The structural changes that occur during compression are unlike those occurring after ischemia in the following respects. (i) They develop during compression, not many hours later. (ii) Fascicular edge fibers tend to be more affected than central fascicular fibers. (iii) Large fibers are preferentially affected. (iv) Paranodal demyelination occurs at the edge of the cuff, not at regions of lowest blood perfusion. (v) Ultrastructural alterations typical of ischemia are not seen.

The structural changes we have demonstrated confirm the correctness of the conception of Ochoa *et al.* (9) that mechanical forces are responsible for many, perhaps most, of the structural alterations after cuff compression. We have

extended their observations to what happens during compression. Ochoa *et al.* (5, 9) emphasized the primacy of myelin intussusception into adjacent paranodes—we find evidence for more complex changes in axon and myelin and find that it happens during compression not later. Our studies also appear to link demyelination and axonal degeneration.

The initial decrease of FA is undoubtedly due to expression (proximally and distally) of endoneurial fluid from beneath the cuff. A second slower phase of decrease of FA and increase in MF density at the point of maximal compression might theoretically be due to (i) further expression of endoneurial fluid; (ii) compression of axonal contents with expression of fluid from axoplasm or translocation of axoplasm, or both; (iii) paranodal disruption and extrusion of cytoplasm of Schwann cells; (iv) compression of myelin; and (v) displacement of other tissue elements from beneath the cuff. Our studies provide unequivocal evidence for hypothesis *ii* and evidence against hypothesis *iv*. Compression of axoplasm resulted in close packing of cytoskeleton elements and of organelles. When high pressures were maintained for longer times in some MFs, axoplasm could not be recognized even at high magnification, suggesting that it had been squeezed out into adjacent parts of the same fiber. It is possible that some or all of the axonal contents remained in these compressed fibers but were so compacted that their recognition as axoplasm was not possible. Myelin profiles became irregularly shaped and myelin cleavage planes developed. Nodes of Ranvier became obscured mainly because of cleavage of paranodal myelin and redundant loops of myelin overlapped nodes.

In contrast to the changes under the cuff, at the edges of the cuff the axons were not compressed and actually appeared to be increased in volume. At the edges of the cuff nodes were frequently lengthened. In such lengthened nodes inner cores of axoplasm appeared relatively normal and surrounded by clear fluid. Myelin was frequently retracted, in both directions from the node.

Lengthened nodes of Ranvier may represent the effect of three processes: (i) lengthening of the axon due to compression and expression of axonal fluid or displacement of axonal contents, or both; (ii) detachment of myelin at nodes of Ranvier; and (iii) cleavage and longitudinal displacement of layers of partial- or full-thickness myelin. Lengthening of fibers during compression is inferred from our findings of frequent lengthened nodes of Ranvier at the edges of the cuff without finding shortening of internode length beneath the cuff (actually lengthening of internodes under the cuff was demonstrated). Displacement of axoplasm is of two kinds—expression of fluid and of structural components. Our term “obscured” nodes may reflect the same change represented by the term “intussuscepted” nodes used by Ochoa *et al.* (5, 9), but for which we find a somewhat different explanation (described above). We also find a different explanation for demyelination than surmised by Ochoa *et al.* (5, 9). Present evidence is unequivocal that paranodal demyelination occurs during compression of a few minutes, is frequent at the edges

of the cuff, and does not require degeneration of redundant loops of myelin to explain them.

The series of events that we have described was seen with low and high pressures and when they were applied for short times and for long times. The structural alterations were at similar sites and of similar kind and varied only in severity. This suggests that the mechanism underlying segmental demyelination and axonal degeneration are linked.

As emphasized by others, it is the differential forces (shear modulus) along the length of nerve that are responsible for the structural events. As shown (17), if compression is equal along the length of the nerve (as occurs with compression of excised segments in a chamber), no deleterious effect on nerve conduction ensues. Our work suggests that fluid movements (initially, expression of endoneurial fluid; then, compression and expression of axoplasm; and shearing and displacement of myelin) are important manifestations of the differential forces under and at the edges of the cuff and explain the structural alterations seen during the process of nerve compression.

The present suggested events also provide an explanation for why fiber injury may worsen with increasing time and severity of compression. Whereas expression of endoneurial fluid is rapid, expression of fluid from axoplasm or of axoplasm itself is slow because of the higher viscosity of axoplasm (22) than of endoneurial fluid and the resistance to fluid movement in small-caliber cylinders (19); additional factors (e.g., presence of cytoskeletal network) may account for worsening with pressure and time. These results are not unexpected since the severity of conduction block in caudal nerve of rat is known to be longer with greater pressure and duration of compression (23).

Does ischemia have a role in nerve compression injury? It is conceivable that it does when compression is maintained for long times, but lesions similar to those induced by ischemia have, in our opinion, not been demonstrated in cuff compression injury.

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